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Supplementary information

Sensitive fluorescence detection of SARS-CoV-2 RNA in clinical samples via one-pot isothermal ligation and transcription

In the format provided by the authors and unedited

Table of contents

Supplementary Note 1

Probe design protocol. SENSR detection relies on two single-stranded DNA probes, i.e., the promoter and reporter probes. The former consists of an upstream hybridization sequence (UHS) and a stem-loop T7 promoter. The latter is composed of a downstream hybridization sequence (DHS) and a template sequence for a dye-binding RNA aptamer. We generated UHS and DHS candidates using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

1. UHS design for the promoter probe. First, we analyzed nucleic acid sequences of target pathogens to identify hybridization sites using the following parameters for Primer-BLAST.

a. PCR product size: 40-60 bps
(to find best candidate sites that ensure specific binding of both probes which are 20-30-nt long and adjacent to each other)
b. Primer pair specificity checking parameter
Database: Refseq mRNA
Organism: *Homo sapiens*
(to guarantee specific binding of the probes to the target pathogenic RNA in the presence of human RNA as a background)
c. Other parameters were unchanged from the default values set by Primer-BLAST

With the above parameters, we ran Primer-BLAST to obtain ten primer pairs for each target RNA. From each primer pair, we took a primer that has the same sequence as the target RNA and generated its reverse complement to get the UHS candidate sequence. If the UHS candidate sequence contains dC or dG at the 5'-end, we further inspected +2 position of it. If the +2 base was also dC or dG, the UHS candidate was withdrawn from consideration immediately because the activity of SplintR ligase is inhibited by 5'-dC:G or 5'-dG:C base pair at the phosphorylated donor junction, particularly when +2 position of the probe also contains dC:G or dG:C base pair[1]. When the primer contains dA or dT at the 5'-end, we directly proceeded to the next step.

To the 3'-end of UHS, we sequentially added the T7 promoter complementary sequence (5'-CCCTATAGTGAGTCGTATTA-3'), a loop sequence (5'-ATTTCGCGACAACACGCGAA AT-3'), and the T7 promoter sequence (5'-TAATACGACTCACTATAGGG-3') to complete a promoter probe. Then, we used NUPACK to predict the secondary structure of the promoter probe. If the stem-loop structure for the T7 promoter is intact and the UHS shows minimal predicted secondary

structure, we used the entire nucleotide sequence as a promoter probe. For ligation, we phosphorylated the 5'-end of the promoter probe.

2. DHS design for the reporter probe. The DHS of the reporter probe should be located immediately upstream to the 5'-end of the UHS (i.e., downstream in the target RNA). With the 3'-end fixed, the length of DHS was decided to have a melting temperature similar to that of the UHS. To the 5'-end of DHS, we added a fluorogenic aptamer template sequence, either malachite green aptamer (5'-GGATCCATTCGTTACCTGGCTCTCGCCAGTCGGGATCC-3') or broccoli aptamer with a linker (5'-GTATGTGGGAGACGGTCGGGTCCAGATATTC GTATCTGTCGAGTAGAGTGTGGGCTCCCACATAC-3'). Next, we used NUPACK to predict the probe structure. If the DHS exhibits minimal predicted secondary structure, we used it as a reporter probe.

Finally, we ran NUPACK to inspect the full-length transcript (RNA) structure produced when the ligation and transcription were successful. If the fluorogenic aptamer structure in the full-length transcript was unchanged from that when the aptamer was unaccompanied by any additional sequence, we finalized the probe sets.

Supplementary Note 2

SENSR reaction optimization. United reaction buffer for all component reactions of SENSR was termed SENSR buffer. SENSR buffer was optimized based on the T7 RNA polymerase buffer, which has the most inclusive composition of four reaction buffers (i.e., probe annealing, ligation, transcription, and aptamer fluorescence reaction buffers). First, dithiothreitol (DTT) was removed due to the negative effect on the aptamer fluorescence reaction (Supplementary Fig. 2a). Likewise, potassium chloride (KCl) contained only in the aptamer fluorescence reaction buffer was also eliminated because the fluorescence of the sequential ligation-transcription-fluorescence reaction decreased as the concentration of potassium chloride increased (Supplementary Fig. 2b). Potassium chloride is known to inhibit SplintR ligase at high salt concentrations[2]. Similarly, the concentrations of Tris-HCl and $MgCl_2$ were also adjusted to 50 mM and 10 mM, respectively, for higher fluorescence intensity (Supplementary Figs. 2c and 2d). Collectively, the composition of the initial SENSR buffer was 50 mM Tris-HCl, 10 mM $MgCl_2$, and 1 mM NTPs each.

Once the SENSR buffer was formulated, we further tested several reaction conditions and additives to establish the optimal condition for one-pot isothermal SENSR reaction. First, we optimized the reaction temperature. All reaction components were added to the SENSR buffer, and the reaction was performed at 25-40 °C (Supplementary Fig. 3a). The fluorescence intensity was highest at 37 °C, following the optimal temperature for the enzymes. We further optimized other reaction components. First, a wide concentration range of the fluorescent dye, malachite green, was tested, and the maximum fold activation was achieved at 16 μM (Supplementary Fig. 3b). Next, the amount and ratio of the enzymes, SplintR ligase and T7 RNA polymerase, were adjusted. A high amount of enzymes can accelerate the reactions, while unnecessarily high volume can inhibit the overall reaction rate because of glycerol and other potential inhibitors in the storage buffers[3]. When various combinations of the enzyme amounts were tested, the highest fold activation was achieved with 10 μL SplintR ligase and 5 μL T7 RNA Polymerase in 100 μL SENSR reaction (Supplementary Fig. 3c). Then, we introduced the single-stranded DNA binding protein that reduces complex structures[4] in the target RNA[5]. In SENSR, hybridization between the probes and target RNA requires exposure of the hybridizing regions in both the probes and target RNA in isothermal condition. To achieve better hybridization in isothermal condition, we added an ssDNA-binding protein, ET-SSB (extreme thermostable single-stranded DNA

binding protein), to SENSR reaction. When 400 ng of ET-SSB was added to the SENSR reaction, the fluorescence and the fold activation were maximized (Supplementary Fig. 3d). Finally, the NTP concentration was adjusted to 2.5 mM NTPs each for higher transcription efficiency (Supplementary Fig. 3e). In summary, the 100 µL of 1× optimized SENSR reaction mixture contained 200 nM promoter probe (PP), 220 nM reporter probe (RP), 16 µM malachite green, 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 2.5 mM NTPs each, 0.8 µL ET-SSB (500 ng/µL), 0.5 µL Recombinant RNase inhibitor (40 U/µL), 10 µL SplintR ligase (25 U/µL), and 5 µL T7 RNA polymerase (50 U/µL).

Supplementary Note 3

Development of chemical lysis workflow for SENSR. Chemical lysis workflow was developed for one-pot, isothermal SENSR assay for a clinical sample. First, we screened the reagent for the chemical lysis of the virus. We chose five detergents as candidates based on two criteria: 1) non-ionic, non-denaturing detergent to ensure the compatibility with enzymatic reactions of SENSR, and 2) documented evidence of viral inactivation or lysis. The candidate detergents and their working concentrations are as follows: 0.1% Triton X-100[6], 0.5% IGEPAL CA-630[7], 1% Tween 80[8], 0.5% Tween 20[7], and 0.1% Nonidet P-40[9]. Then, we measured each detergent's fluorescence in the 100 μL SENSR background mixture (1× SENSR buffer, 10 μM DFHBI-1T, and 5 μL UTM). Measured fluorescence was subtracted by that from the SENSR background mixture without the detergent (Supplementary Fig. 6a). Nonidet P-40 was selected for the lowest fluorescence.

Next, we confirmed the capability of Nonidet P-40 as a lysis reagent for SENSR and optimized its concentration. We utilized the MRSA cell as a proxy for the viral particle. Five CFU of MRSA cells were suspended in 5 μL UTM. Then, the cell was mixed with 5 μL Nonidet P-40 of different concentrations (0, 2, 10, and 20% (v/v)). After 5-min incubation at room temperature, the SENSR mixture was added to the samples to produce a 100-μL SENSR reaction (10 μM DFHBI-1T, MRSA-BR probe pair). Fluorescence was measured after a 50-min incubation at 37 °C. We observed that Nonidet P-40 was compatible with the SENSR assay and that the final concentration of 0.1% was sufficient (Supplementary Fig. 6b).

Supplementary Note 4

1. SENSR protocol for synthetic RNA

① Synthetic RNA preparation

i. Amplify template DNA containing the target RNA sequence by PCR with primers. (All primers are listed in Supplementary Table 7)

ii. Purify template DNA from the PCR reaction mixture.

iii. Assemble *in vitro* transcription as follows.

Reagent	Concentration	Amount
RNase-free water		up to 20 μL
T7 RNA Polymerase Reaction Buffer	10×	2 μL
NTPs	25 mM each	0.8 μL
Template DNA		1 μg
Recombinant RNase Inhibitor	40 U/μL	0.5 μL
DTT	100 mM	1 μL
T7 RNA Polymerase	50 U/μL	2 μL
Total		20 μL

iv. Incubate the mixture at 37 °C for 16 h in a water bath.

v. After the incubation, add 1 μL DNase I (RNase-free) and incubate at 37 °C for 1 h.

vi. Purify DNase I-treated sample using Riboclear^{TM} (plus!) RNA kit.

vii. Measure the optical density of the RNA solution at 260 nm by the BioDrop DUO UV/Vis spectrophotometer (BioDrop, Cambridge, UK)

viii. Calculate the volume for 22 pmoles of RNA and add RNase-free water up to 100 μL to produce a 220-nM solution.

ix. Using a new pipette tip, mix 40 μL 220-nM RNA solution with 48 μL RNase-free water to produce a 100-nM RNA solution.

x. Using a new pipette tip, add 50 μL 100-nM RNA solution to 450 μL RNase-free water.

xi. Mix thoroughly using a new 200-μL pipette tip to yield a 10-nM RNA solution.

xii. Repeat the dilution steps x.-xi. eleven times more to produce 1-nM to 0.1-aM RNA solutions.

② SENSR for synthetic RNA

i. Prepare purified RNA as target RNA for SENSR.

ii. Assemble SENSR reaction as follows.
(10× SENSR buffer: 500 mM Tris-HCl (pH 7.4) and 100 mM $MgCl_2$)

Single SENSR assay		
Reagent	Concentration	Amount
RNase-free water		53.72 μL **(for 1)** 38.72 μL **(for 2)**
SENSR buffer	10×	10 μL
NTPs	25 mM each	10 μL
1. Malachite green <u>or</u> **2. DFHBI-1T**	320 μM 50 μM	5 μL 20 μL
Promoter probe (PP)	10 μM	2 μL
Reporter probe (RP)	10 μM	2.2 μL
Recombinant RNase inhibitor	40 U/μL	0.5 μL
ET-SSB	500 ng/μL	0.8 μL
SplintR ligase	25 U/μL	10 μL
T7 RNA polymerase	50 U/μL	5 μL
Synthetic target RNA		0.78 μL
Total		100 μL
Dual SENSR assay		
Reagent	Concentration	Amount
RNase-free water		29.52 μL
SENSR buffer	10×	10 μL
NTPs	25 mM each	10 μL
Malachite green	320 μM	5 μL
DFHBI-1T	50 μM	20 μL
Promoter probe 1 (PP1)	10 μM	2 μL
Reporter probe 1 (RP1)	10 μM	2.2 μL
Promoter probe 2 (PP2)	10 μM	2 μL
Reporter probe 2 (RP2)	10 μM	2.2 μL
Recombinant RNase inhibitor	40 U/μL	0.5 μL
ET-SSB	500 ng/μL	0.8 μL
SplintR ligase	25 U/μL	10 μL
T7 RNA polymerase	50 U/μL	5 μL
Synthetic target RNA		0.78 μL
Total		100 μL

iii. Incubate the mixture at 37 °C for 0.5–2 h in a water bath.

③ Fluorescence measurement

i. Load 100 μL SENSR mixture to a 96-well clear flat-bottom black microplate or 20 μL SENSR mixture to a 384-well clear flat-bottom black microplate.

ii. Place the microplate to a microplate reader (Hidex Sense).

iii. Run measurement with the following setup.
(ex: 616 nm / em: 665 nm for malachite green,
ex: 460 nm / em: 520 nm for DFHBI-1T)

2. SENSR protocol for cell lysate

① Cell lysate preparation

i. Culture MRSA and MSSA in Luria-Bertani (LB) liquid medium at 37 °C for 24 h with shaking (300 rpm).

ii. Transfer the culture broth to a PCR tube, heat at 95 °C for 2 min, and place it on ice.

② SENSR for cell lysate

i. Prepare the cell lysate.

ii. Assemble SENSR reaction as follows.

(10× SENSR buffer: 500 mM Tris-HCl (pH 7.4) and 100 mM $MgCl_2$)

Reagent	Concentration	Amount
RNase-free water		53.72 μL
SENSR buffer	10×	10 μL
NTPs	25 mM each	10 μL
Malachite green	320 μM	5 μL
Promoter probe (PP)	10 μM	2 μL
Reporter probe (RP)	10 μM	2.2 μL
Recombinant RNase inhibitor	40 U/μL	0.5 μL
ET-SSB	500 ng/μL	0.8 μL
SplintR ligase	25 U/μL	10 μL
T7 RNA polymerase	50 U/μL	5 μL
Cell lysate		0.78 μL
Total		100 μL

iii. Incubate the mixture at 37 °C for 2 h in a water bath.

③ Fluorescence measurement

i. Load 100 μL SENSR mixture to a 96-well clear flat-bottom black microplate or 20 μL SENSR mixture to a 384-well clear flat-bottom black microplate.

ii. Place the microplate to a microplate reader (Hidex Sense).

iii. Run measurement with the following setup. (ex: 616 nm / em: 665 nm)

3. SENSR protocol for proxy clinical sample

① Proxy clinical sample preparation

i. Culture MRSA and MSSA in LB liquid medium at 37 °C for 24 h with shaking (300 rpm).

ii. Add the culture broth to human serum, which was diluted at a 1/7 ratio in RNase-free water.

iii. Transfer the proxy clinical sample to a PCR tube, heat at 95 °C for 2 min, and place it on ice.

② SENSR for proxy clinical sample

i. Prepare the proxy clinical sample.

ii. Assemble SENSR reaction as follows.

(10× SENSR buffer: 500 mM Tris-HCl (pH 7.4) and 100 mM $MgCl_2$)

Reagent	Concentration	Amount
RNase-free water		53.72 μL
SENSR buffer	10×	10 μL
NTPs	25 mM each	10 μL
Malachite green	320 μM	5 μL
Promoter probe (PP)	10 μM	2 μL
Reporter probe (RP)	10 μM	2.2 μL
Recombinant RNase inhibitor	40 U/μL	0.5 μL
ET-SSB	500 ng/μL	0.8 μL
SplintR ligase	25 U/μL	10 μL
T7 RNA polymerase	50 U/μL	5 μL
Proxy clinical sample		0.78 μL
Total		100 μL

iii. Incubate the mixture at 37 °C for 2 h in a water bath.

③ Fluorescence measurement

i. Load 100 μL SENSR mixture to a 96-well clear flat-bottom black microplate or 20 μL SENSR mixture to a 384-well clear flat-bottom black microplate.

ii. Place the microplate to a microplate reader (Hidex Sense).

iii. Run measurement with the following setup. (ex: 616 nm / em: 665 nm)

4. Experimental protocol using clinical sample

① Clinical sample preparation

i. Obtain a nasopharyngeal swab from a suspected patient and collect it in Universal Transport Media (UTM).

ii. Prepare the clinical samples for SENSR by either chemical or thermal lysis.

Chemical lysis. Add 2.5 μL nasopharyngeal swab samples into a PCR tube pre-loaded with 2.5 μL 2% Nonidet P-40. Mix and wait for 5 min at room temperature.

Thermal lysis. In a PCR tube, heat nasopharyngeal swab samples at 95 °C for 5 min, and place it on ice.

iii. For each assay, include four 2.5 μL-reference samples, each containing a single copy of the synthetic target RNA. For the chemical lysis workflow, add 2.5 μL 2% Nonidet P-40 to each reference sample.

② SENSR for clinical sample

i. Prepare the clinical sample.

ii. Assemble SENSR reaction as follows.

(10× SENSR buffer: 500 mM Tris-HCl (pH 7.4) and 100 mM $MgCl_2$)

Reagent	Concentration	Amount
RNase-free water		14.75 μL **(for 1)** 17.25 μL **(for 2)**
SENSR buffer	10×	5 μL
NTPs	25 mM each	5 μL
DFHBI-1T	50 μM	10 μL
Promoter probe (PP)	10 μM	1 μL
Reporter probe (RP)	10 μM	1.1 μL
Recombinant RNase inhibitor	40 U/μL	0.25 μL
ET-SSB	500 ng/μL	0.4 μL
SplintR ligase	25 U/μL	5 μL
T7 RNA polymerase	50 U/μL	2.5 μL
1. Chemical lysis sample <u>or</u> **2. Thermal lysis sample**		5 μL 2.5 μL
Total		50 μL

iii. Incubate the mixture at 37 °C for 30–50 min in a water bath.

③ Fluorescence measurement

i. Load 20 μL SENSR mixture to a 384-well clear flat-bottom black microplate.

ii. Place the microplate to a microplate reader (Hidex Sense).

iii. Run measurement with the following setup. (ex: 460 nm / em: 520 nm)

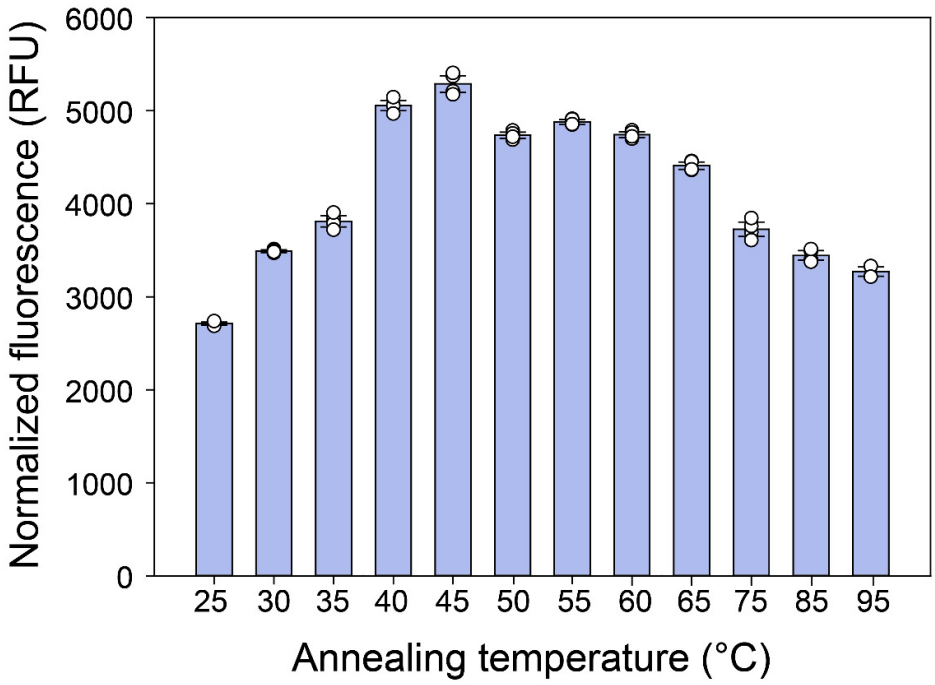
5. Data analysis

Fluorescence normalization. The fluorescence values from 1× SENSR reaction mixture containing only chemical components were taken as backgrounds: reaction buffer, fluorescent dye (malachite green or DFHBI-T1), UTM (optional for clinical samples), and detergent (optional for chemical lysis of clinical samples). Therefore, the background values differ between experiments depending on the chemical components. Then, the background values were subtracted from the fluorescence values measured from the SENSR assay to get the final values presented throughout the manuscript.

Regression analysis. All regressions were performed using Sigmaplot 12.0.

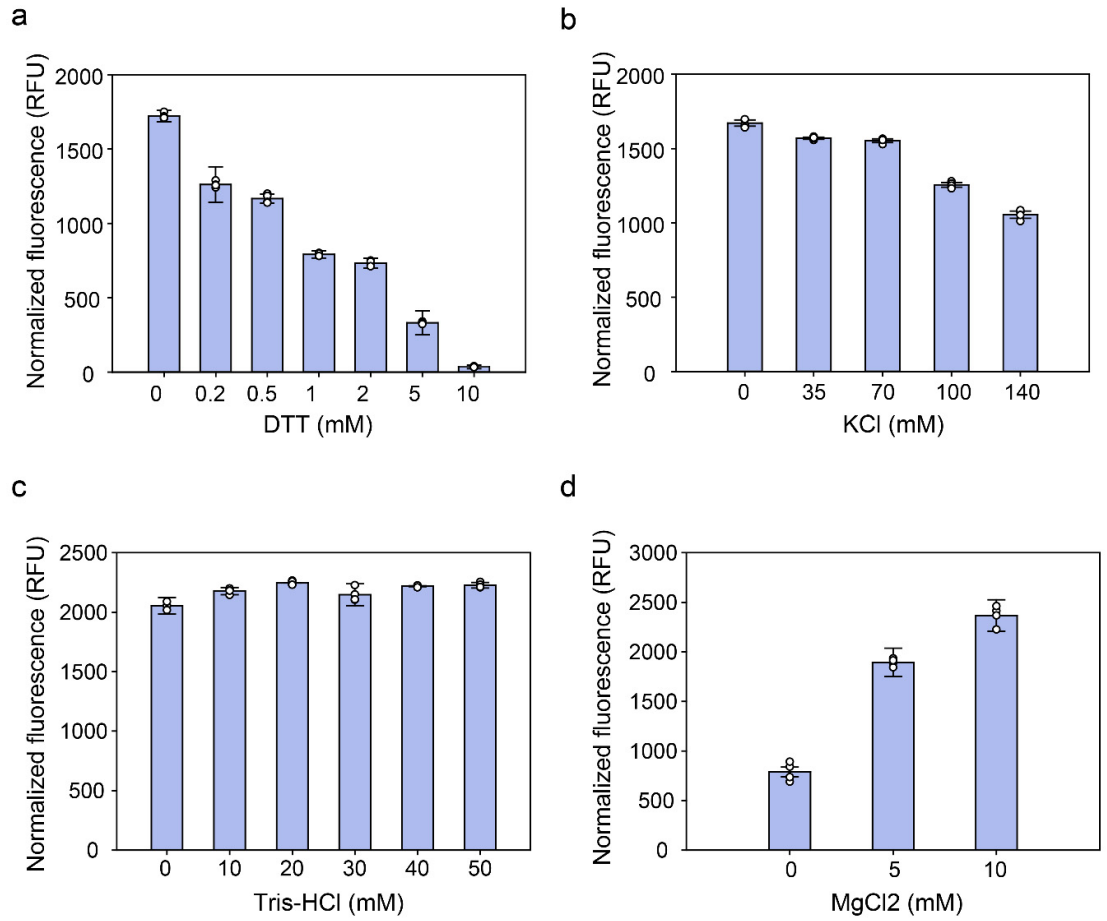
Statistical analysis. All data were obtained by measurements from distinct samples. Two-tailed Student's t-tests were performed using Microsoft Excel 2016.

Supplementary Fig. 1



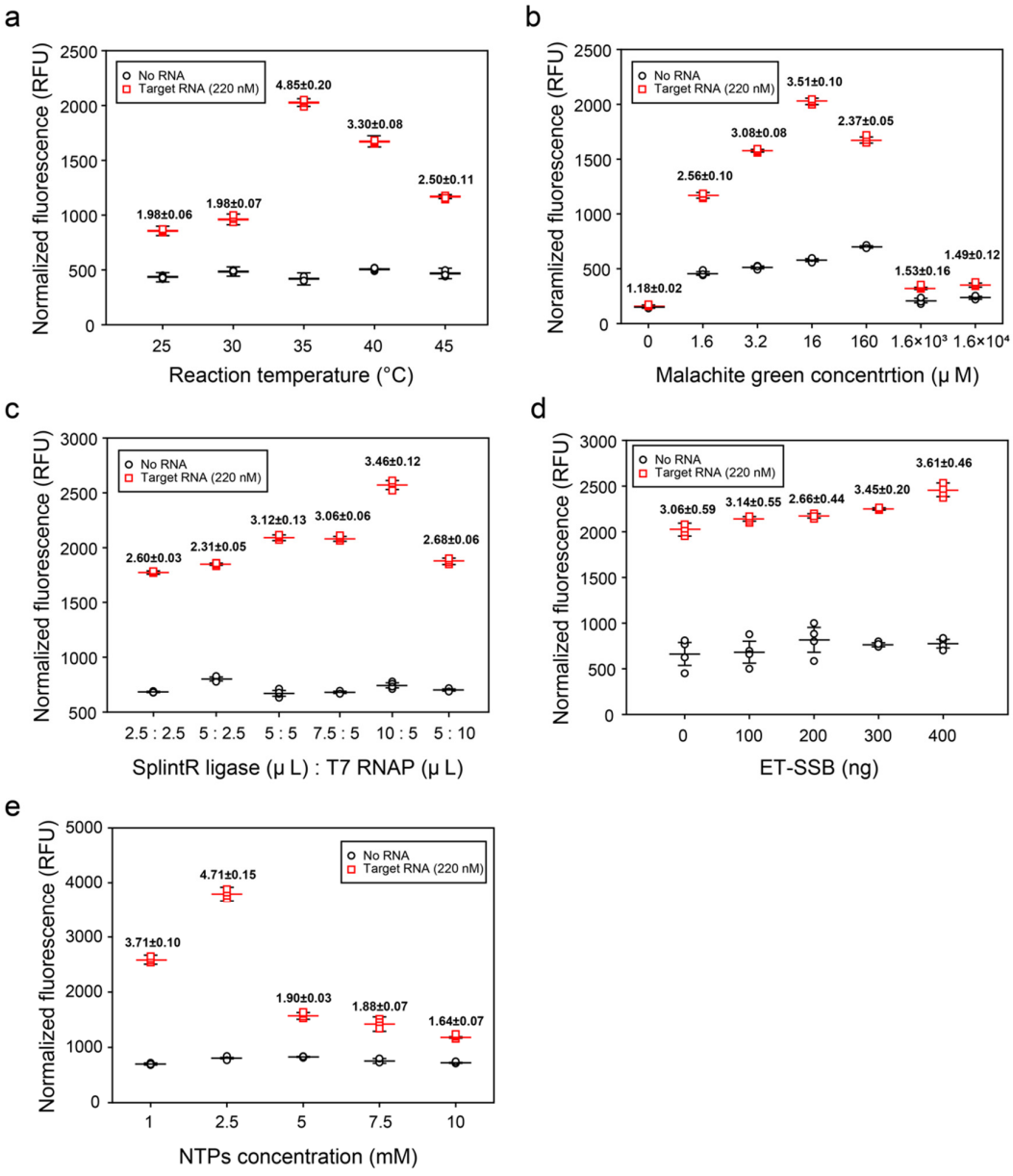
Supplementary Fig. 1 | Annealing temperature test for SENSR. Among the wide range of annealing temperatures, around 35 °C to 45 °C exhibited similar or higher fluorescence intensity compared to other temperatures after SENSR reaction was completed. All tests are four biological replicates. Bars represent mean ± s.d.

Supplementary Fig. 2



Supplementary Fig. 2 | SENSR buffer optimization. a, Effect of DTT. The highest fluorescence was obtained when DTT was absent in the reaction buffer. **b,** Effect of potassium chloride (KCl). The highest fluorescence was obtained when KCl was absent in the reaction buffer. **c,** Effect of Tris-HCl. Fluorescence was mostly unchanged with all tested Tris-HCl concentrations. **d,** Effect of $MgCl_2$. Fluorescence from SENSR was highest with 10 mM $MgCl_2$. All tests are four biological replicates. Bars represent mean ± s.d.

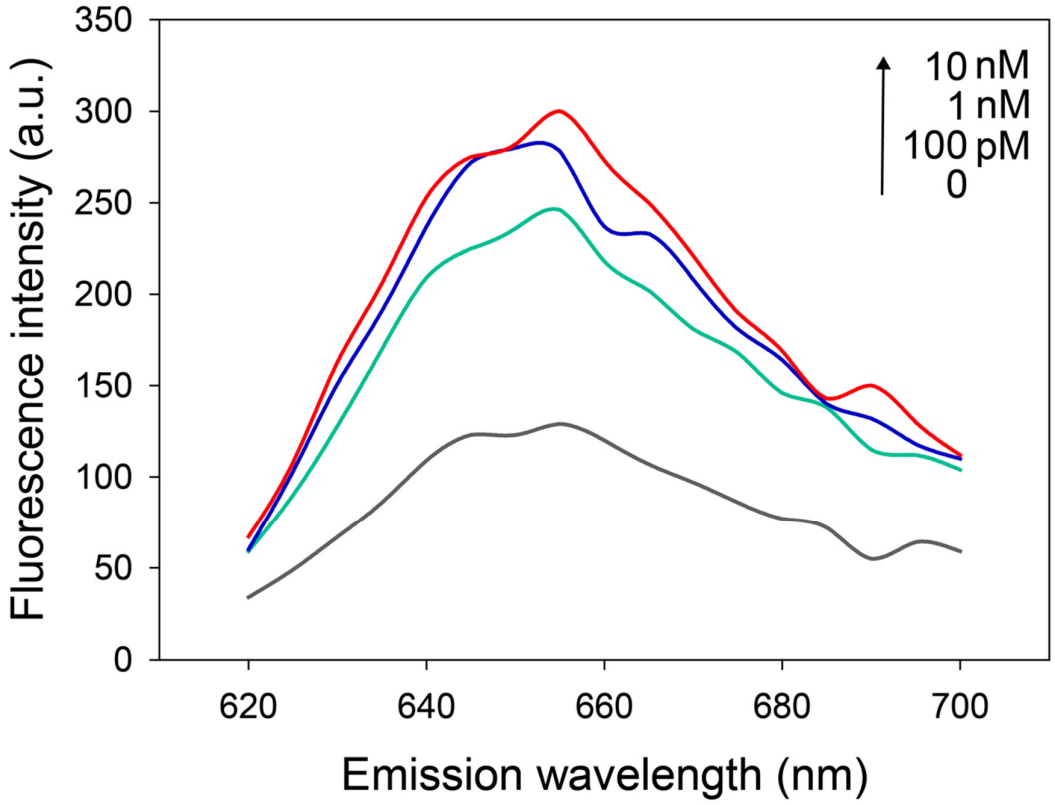
Supplementary Fig. 3



Supplementary Fig. 3 | SENSR reaction condition optimization. a, Optimization of reaction temperature. SENSR reaction was isothermally performed at various temperatures. The optimal temperature for SENSR reaction was 37 °C. **b,** Optimization of malachite green concentration. Maximum fold change was obtained with 16 μM malachite green. **c,** Optimization of the quantity and ratio of SplintR ligase to T7 RNA polymerase. The fluorescence fold change was greatest with 10 μL SplintR ligase and 5 μL T7 RNA polymerase in 100 μL reaction. **d,** Effect of ET-SSB. Fluorescence intensity and fold change

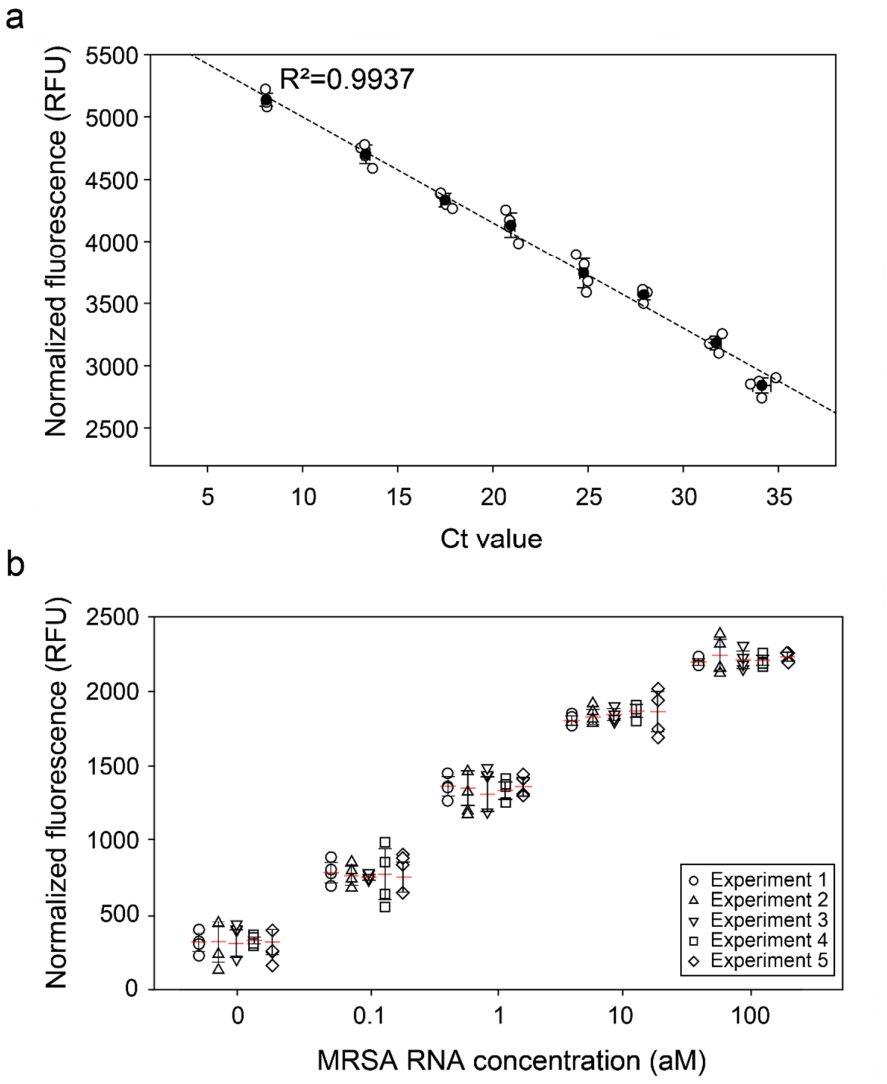
were highest when 400 ng of ET-SSB was added to the SENSR reaction. **e,** Optimization of NTPs concentration. Fluorescence and fold change were highest with 2.5 mM NTPs each. Dots indicate normalized fluorescence from individual SENSR reaction. Numbers above dots indicate the fold activations, obtained by dividing the fluorescence intensity with the target RNA by that without the target RNA. All tests are four biological replicates. Horizontal lines represent mean ± s.d.

Supplementary Fig. 4



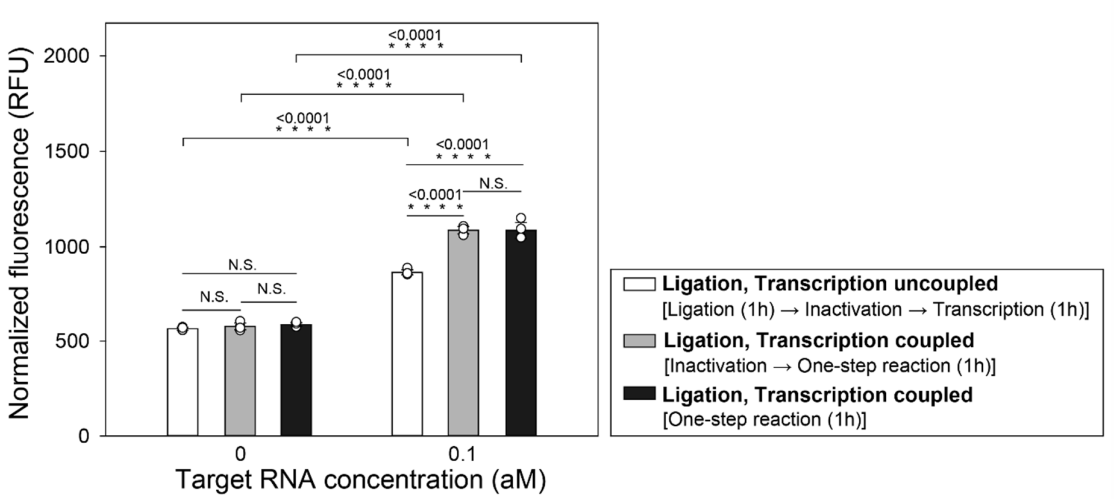
Supplementary Fig. 4 | RNA detection under the optimized one-step SENSR condition. The *mecA* RNA was detected using the optimized SENSR assay. The target RNA was added to the SENSR mixture to produce a 100-µL reaction and incubated at 37 °C for 2 h. After that, the SENSR reaction mixture was transferred to a 96-well clear flat-bottom black polystyrene microplate (Corning Inc., Corning, NY, USA) for measurement. The fluorescence spectra were collected at 24.6 °C, using a Spark 10M multimode microplate reader (Tecan Group Ltd., Switzerland). The excitation wavelength was 570 nm, and the emission wavelengths were in the range from 620 to 700 nm by 1 nm step size with a bandwidth of 20 nm for both excitation and emission.

Supplementary Fig. 5



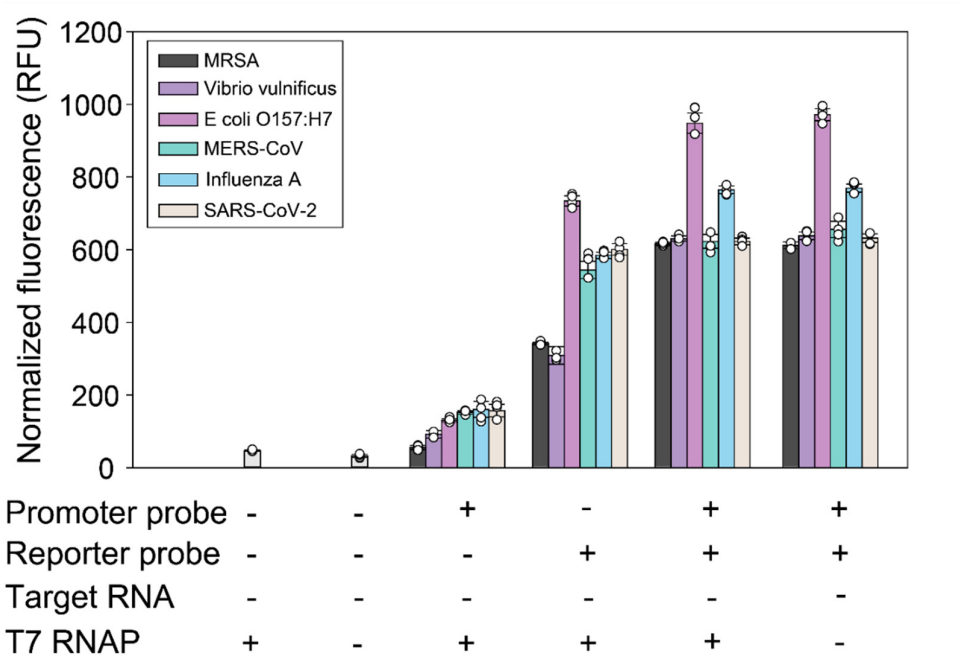
Supplementary Fig. 5 | Validation of SENSR. a, Validation of SENSR with rRT-PCR. Samples of a target RNA (*mecA*) of concentrations ranging from 100 nM to 10 fM were analyzed by either SENSR or rRT-PCR. The fluorescence intensity from SENSR and the C_t value from rRT-PCR showed a linear correlation (R^2 = 0.9937). All tests are four experimental replicas. White circles indicate the results for individual samples. Black circles represent mean ± s.d. **b,** Validation of the reproducibility of SENSR. Samples of a target RNA (*mecA*) of concentrations ranging from 0 to 100 aM were analyzed by SENSR in five independent experiments. Intra- and inter-assay coefficient of variation were calculated in Supplementary Table 4. All tests are four biological replicates. Horizontal lines represent mean ± s.d.

Supplementary Fig. 6



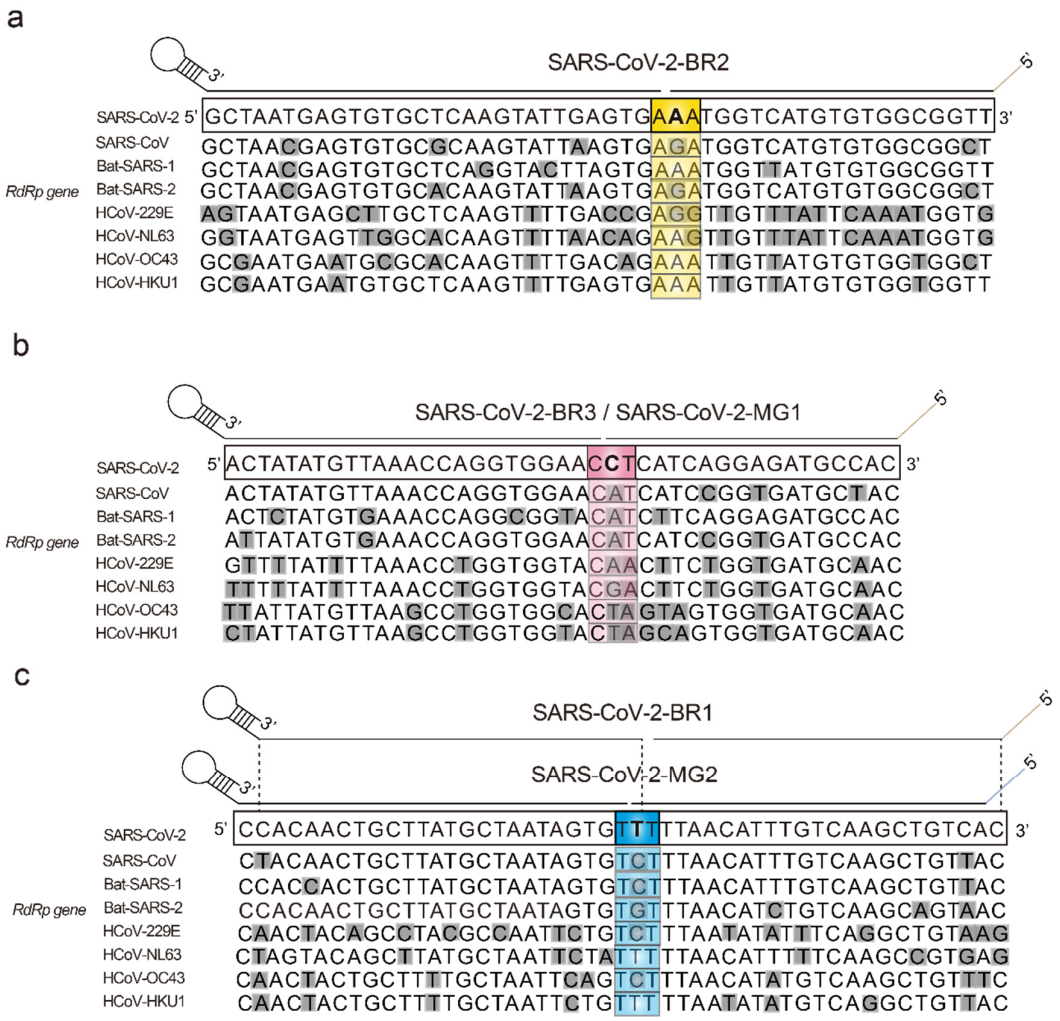
Supplementary Fig. 6 | Signal amplification in one-step SENSR. One-step, one-pot SENSR reaction enabled signal amplification compared to the two-step uncoupled reaction. For the uncoupled reaction, ligation was first conducted with an 89.5 μL of 1× SENSR mixture excluding the malachite green, T7 RNA polymerase, and recombinant RNase inhibitor. After a 1-h incubation at 37 °C, the reaction mixture was heated to 95 °C for 10 min to inactivate the SplintR ligase and then cooled on ice for 3 min. After that, 5 μL malachite green (320 μM), 5 μL T7 RNA polymerase (50 U/ μL), and 0.5 μL recombinant RNase inhibitor (40 U/ μL) was added to the mixture and incubated at 37 °C for 1 h for transcription. For the one-step reaction, the heat inactivation step was either included or excluded to confirm the thermal stability of the chemical components in the SENSR mixture. For the one-step reaction after the heat inactivation, 78.7 μL of 1× SENSR mixture was assembled excluding the SplintR ligase, ET-SSB, malachite green, T7 RNA polymerase, and recombinant RNase inhibitor. The mixture was incubated at 95 °C for 10 min and then cooled on ice for 3 min. After that, 10 μL SplintR ligase (25 U/μL), 0.8 μL ET-SSB (500 ng/μL), 5 μL malachite green (320 μM), 5 μL T7 RNA polymerase (50 U/ μL), and 0.5 μL recombinant RNase inhibitor (40 U/ μL) was added to the mixture and incubated at 37 °C for 1 h. For the one-step reaction without the heat inactivation, 100 μL of 1× SENSR mixture containing all components was incubated at 37 °C for 1 h. All tests are four biological replicates. (two-tailed Student's t-test; N.S., not significant ($P > 0.5$), **** $P < 0.0001$; bars represent mean ± s.d).

Supplementary Fig. 7



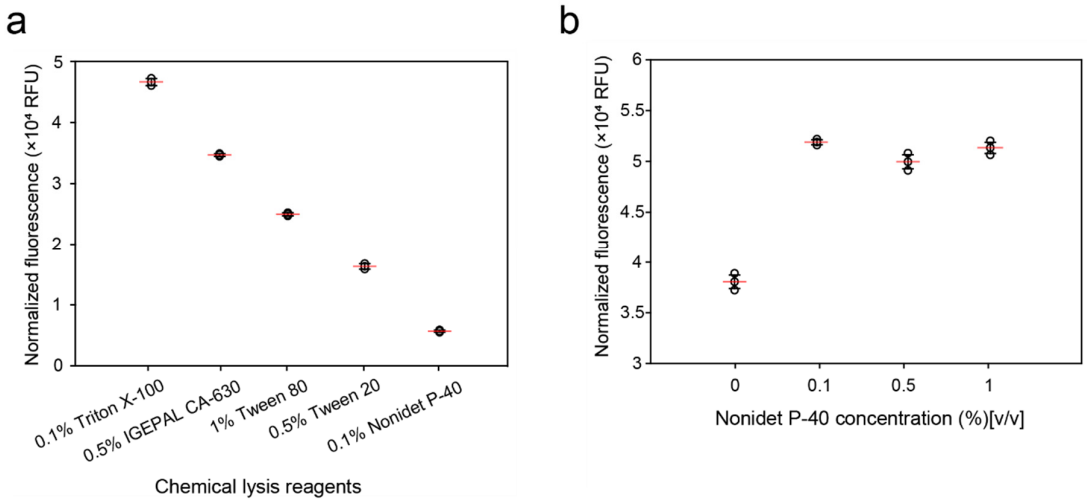
Supplementary Fig. 7 | Source of the background fluorescence. The source of the background fluorescence was identified by SENSR reactions without the target RNAs. The background fluorescence was affected mainly by the DNA probes. The reporter probes had a larger effect than the promoter probes. The background fluorescence was also dependent on the sequence of the reporter probes. All SENSR reactions were run for 2 hours. All tests are four biological replicates. Bars represent mean ± s.d.

Supplementary Fig. 8



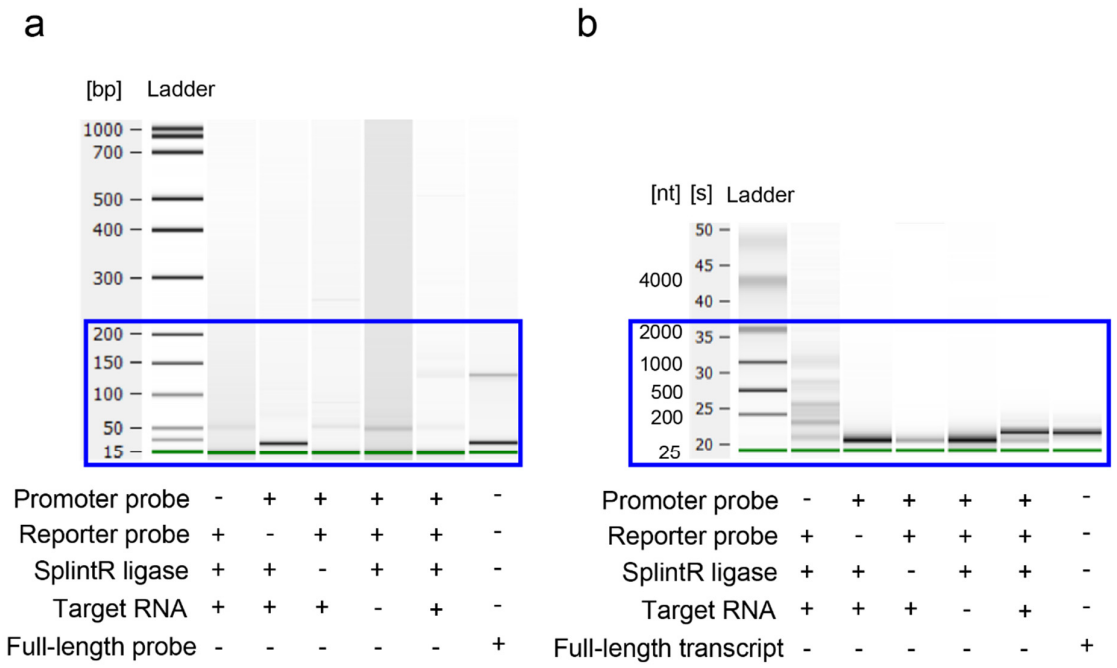
Supplementary Fig. 8 | Probe-binding sites on the SARS-CoV-2 genome and sequence alignment. a, Probe-binding site for SARS-CoV-2-BR2. **b,** Probe-binding site for SARS-CoV-2-BR3 and SARS-CoV-2-MG1. **c,** Probe-binding site for SARS-CoV-2-BR1 and SARS-CoV-2-MG2. Discriminatory bases that enable specific detection of SARS-CoV-2 against other viruses with highly similar sequences are marked by bold letters. Grey shades indicate mismatches between the sequences of SARS-CoV-2 and other viruses.

Supplementary Fig. 9



Supplementary Fig. 9 | Optimization of chemical lysis reagent. a, Screening for chemical lysis reagent. Five non-ionic detergents for viral inactivation were added at their respective working concentrations sufficient for the inactivation of the virus to the 100 µL SENSR background mixture (1× SENSR buffer, 10 µM DFHBI-1T, and 5 µL UTM). Measured fluorescence was subtracted by that from the SENSR background mixture without added detergent. Nonidet P-40 showed the lowest fluorescence. **b,** Validation of Nonidet P-40, and optimization of the concentration. Five CFU of MRSA cells in 5 µL UTM were mixed with an equal volume of Nonidet P-40 of different concentrations. After 5-min incubation at room temperature, the SENSR mixture was added to the lysed cell samples to produce a 100-µL SENSR reaction. Fluorescence was measured after a 50-min incubation at 37 °C. Nonidet P-40 enabled the chemical lysis workflow for SENSR, and the final concentration of 0.1% was sufficient. All tests are two biological replicates. Horizontal lines represent mean ± s.d.

Supplementary Fig. 10



Supplementary Fig. 10 | Full scan Bioanalyzer images for SENSR's ligation and transcription reactions. a, Ligation reaction. **b,** Transcription reaction. Blue boxes indicate cropped images used for Fig. 2.

Supplementary Table 1. Probe sequence

Pathogen (Probe pair)	Type	Sequence (5′-3′)	Modification	Note
MRSA (MRSA-MG)	MG-PP	TTCTCCTTGTTTCATTTTGAGTTCTGCAGccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 2,3,5,6 Supplementary Fig. 1,2,3,4,5,6,7
	MG-RP	*ggatccattcgttacctggctctcgccagtcgggatcc*ACCACCCAATTTGTCTGCCAGT		
MRSA (MRSA-BR)	BR-PP	TTCCACAACATACACCCCCTCccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Supplementary Fig. 9
	BR-RP	*gtatgtgggagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcccacatac*GGGCGATAAACTCTAGTATGCCA		
Vibrio vulnificus	MG-PP	TTCTTGTGCGCCAACCTGTAccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 4 Supplementary Fig. 7
	MG-RP	*ggatccattcgttacctggctctcgccagtcgggatcc*CTTCTCAACAATCGGCACATA		
E. coli O157:H1	MG-PP	TCAACTCCCCAACGCCTTTTccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 4 Supplementary Fig. 7
	MG-RP	*ggatccattcgttacctggctctcgccagtcgggatcc*CGCACCGCTATTTGACTCCC		
MERS-CoV	MG-PP	AAGAGGAACTGAATCGCGCGccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 4 Supplementary Fig. 7
	MG-RP	*ggatccattcgttacctggctctcgccagtcgggatcc*GAGCTCGGGGCGATTATGTG		
Influenza A (Influenza A-MG)	MG-PP	TCCCCTGCTCATTGCTATGGccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 4 Supplementary Fig. 7
	MG-RP	*ggatccattcgttacctggctctcgccagtcgggatcc*TTTGTCTGCAGCGTATCCAC		
Influenza A (Influenza A-BR)	BR-PP	TTCCACAACATACACCCCCTCccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 6
	BR-RP	*gtatgtgggagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcccacatac*GGGCGATAAACTCTAGTATGCCA		

SARS-CoV-2 (SARS-CoV-2-MG1)	MG-PP1	GTTCCACCTGGTTTAACATATAGTccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 4, 7 Supplementary Fig. 7
	MG-RP1	*ggatccattcgttacctggctctcgccagtcgggatcc*GTGGCATCTCCTGATGAG		
SARS-CoV-2 (SARS-CoV-2-MG2)	MG-PP2	ACACTATTAGCATAAGCAGTTGTGGccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 7
	MG-RP2	*ggatccattcgttacctggctctcgccagtcgggatcc*TGACAGCTTGACAAATGTTAAAA		
SARS-CoV-2 (SARS-CoV-2-BR1)	BR-PP1	AACACTATTAGCATAAGCAGTTGTGCcctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 7, 8
	BR-RP1	*gtatgtgggagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcccacatac*GTGACAGCTTGACAAATGTTAAA		
SARS-CoV-2 (SARS-CoV-2-BR2)	BR-PP2	TCACTCAATACTTGAGCACACTCATTccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 7
	BR-RP2	*gtatgtgggagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcccacatac*AACCGCCACACATGACCATT		
SARS-CoV-2 (SARS-CoV-2-BR3)	BR-PP3	GTTCCACCTGGTTTAACATATGTccctatagtgagtcgtattaatttcgcgacaacacgcgaaattaatacgactcactataggg	5′-Ph	Fig. 8
	BR-RP3	*gtatgtgggagacggtcgggtccagatattcgtatctgtcgagtagagtgtgggctcccacatac*GAGTAGTCCTCTACGGTG		

PP: Promoter probe

MG-RP: Reporter probe with malachite green aptamer sequence

BR-RP: Reporter probe with broccoli aptamer sequence

Upper case: Hybridization sequence

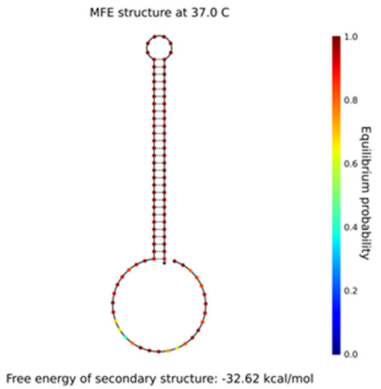
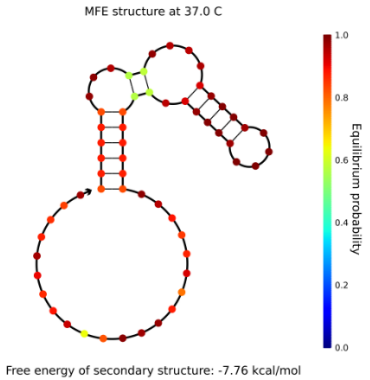
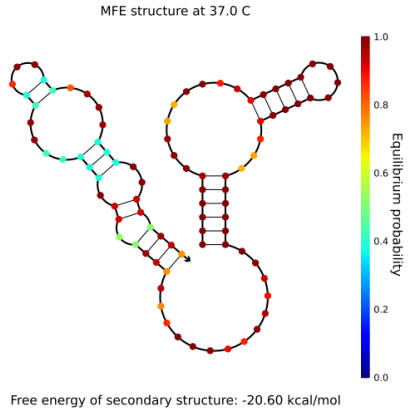
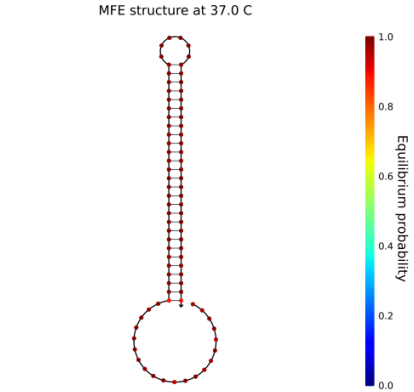
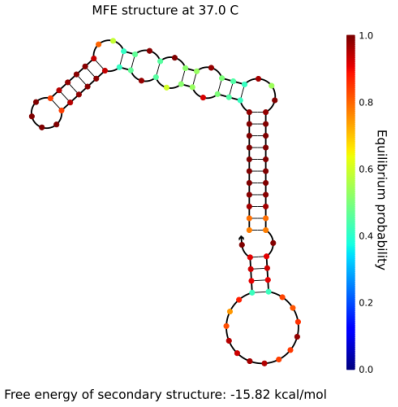
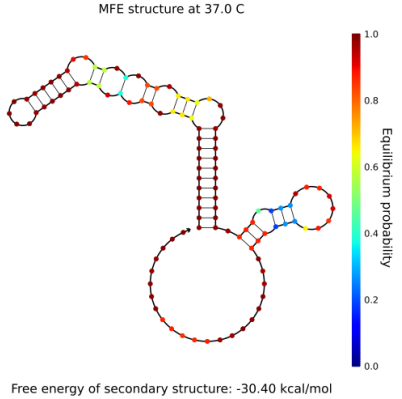
Lower case: T7 promoter complementary sequence + loop sequence + T7 promoter sequence

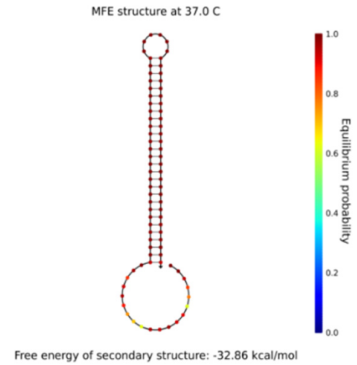
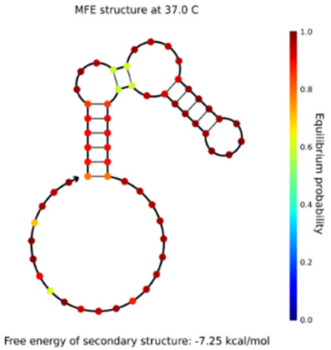
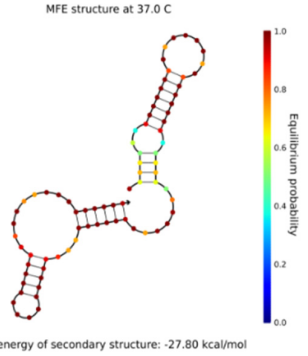
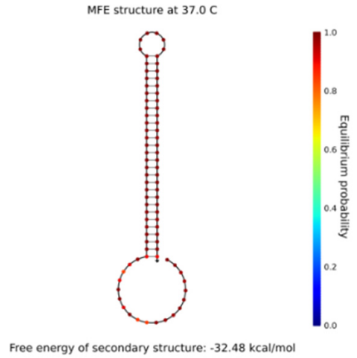
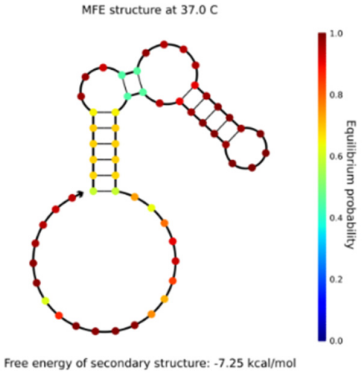
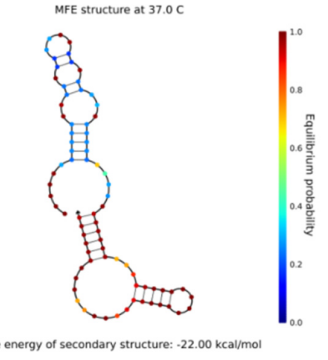
Red, italicized: Malachite green aptamer sequence

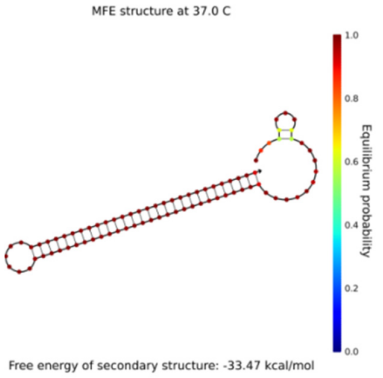
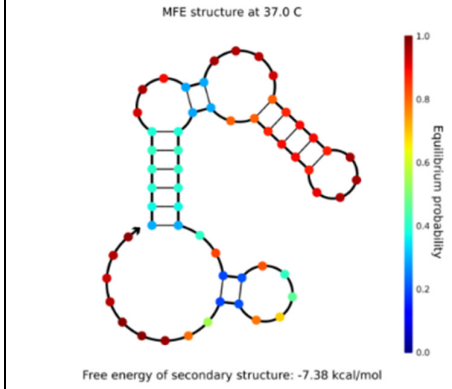
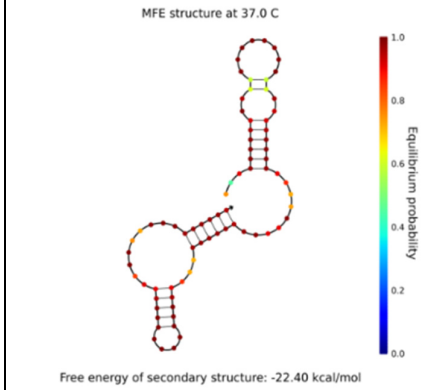
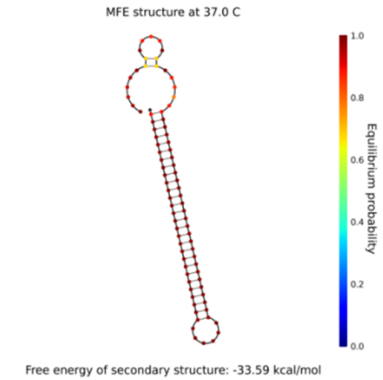
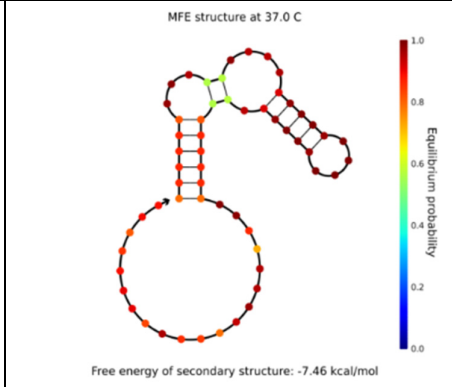
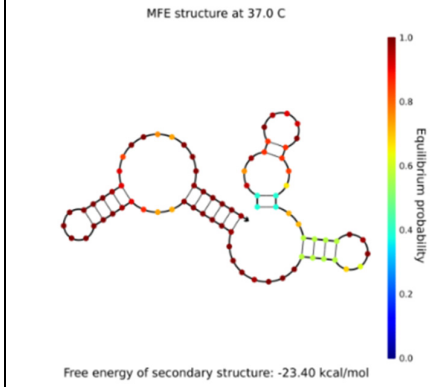
Green, italicized: Broccoli aptamer sequence, including F-30 arm sequence[10–13]

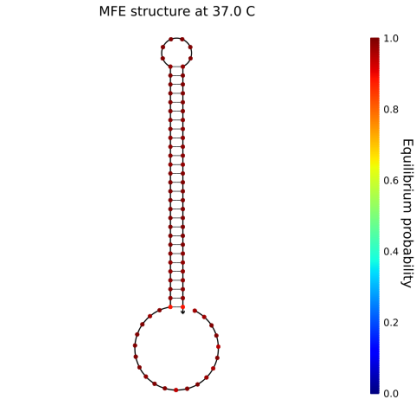
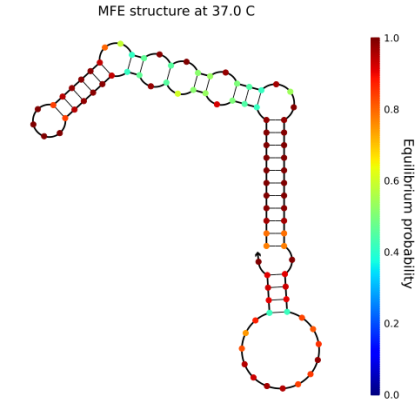
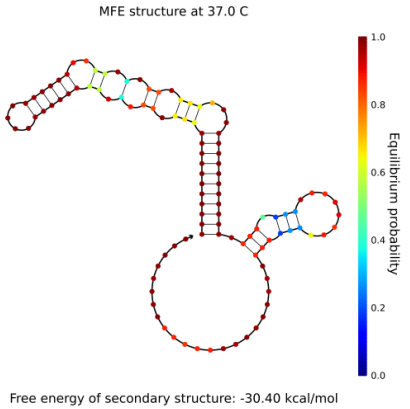
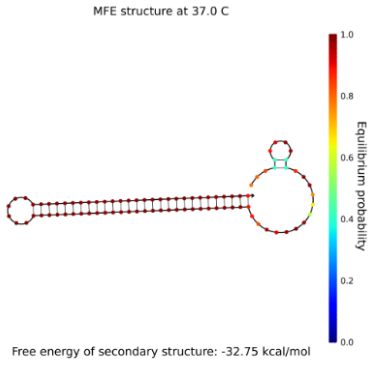
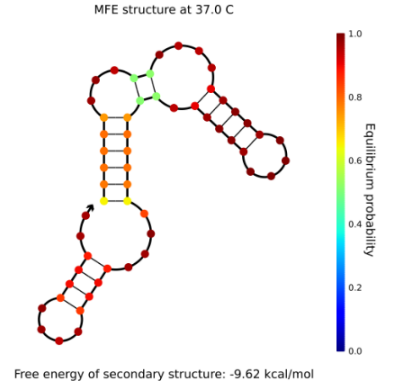
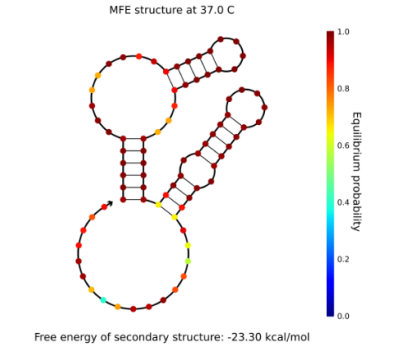
5′-Ph: Phosphate group at the 5′-end

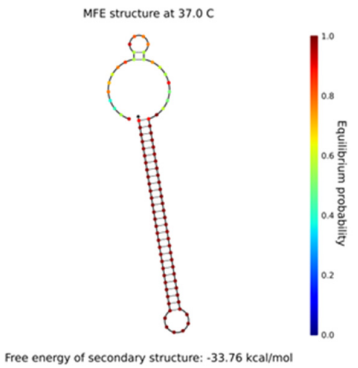
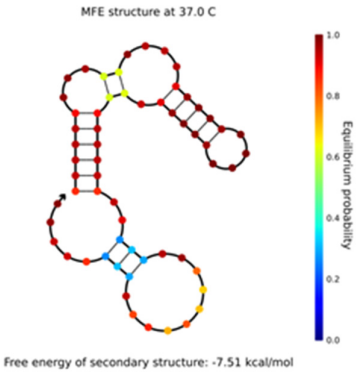
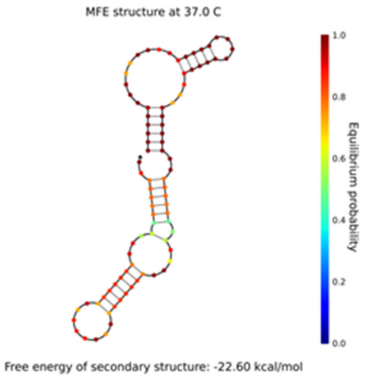
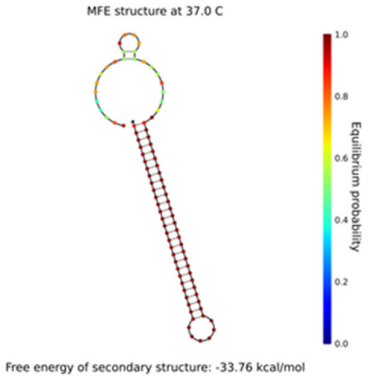
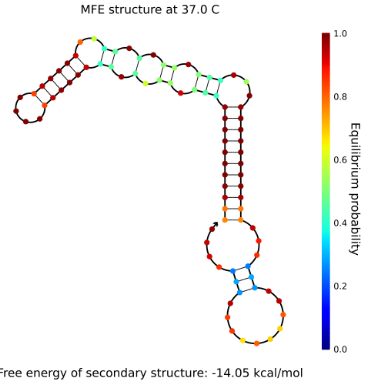
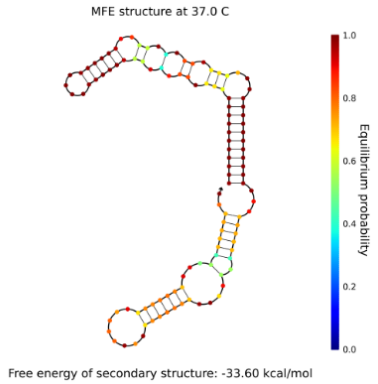
Supplementary Table 2. Secondary structures of the probes predicted by NUPACK

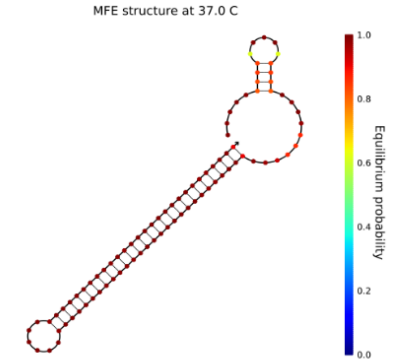
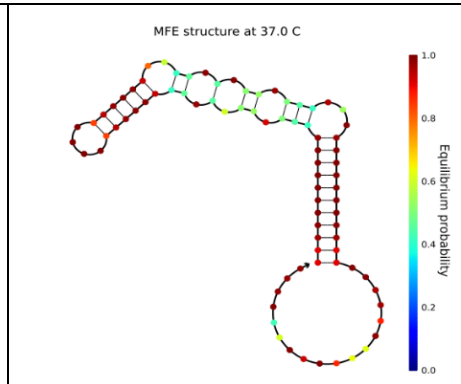
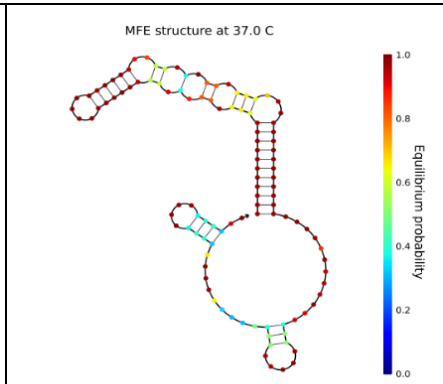
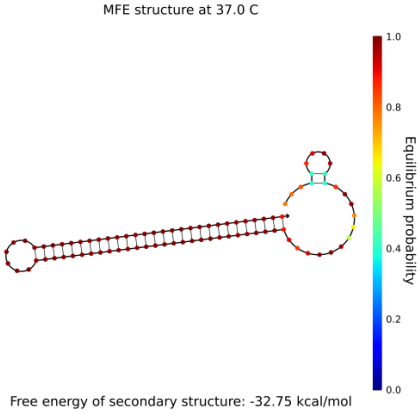
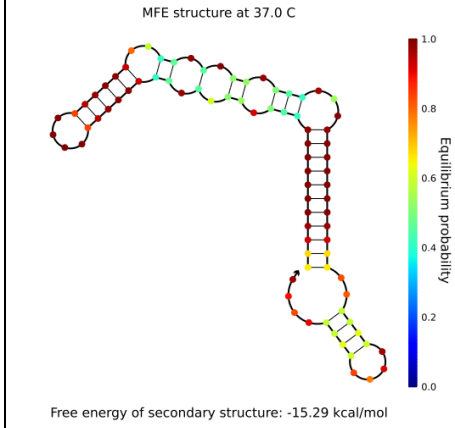
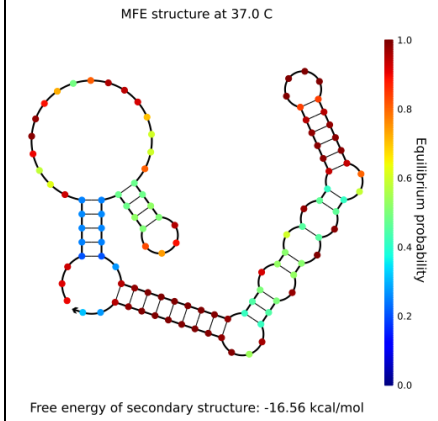
Probe pair	Promoter probe	Reporter probe	Full-length transcript
MRSA-MG	 MG-PP	 MG-RP	
MRSA-BR	 BR-PP	 BR-RP	

Vibrio vulnificus	 MG-PP	 MG-RP	
E. coli O157:H7	 MG-PP	 MG-RP	

MERS-CoV	 MG-PP	 MG-RP	
Influenza A-MG	 MG-PP	 MG-RP	

Influenza A-BR	 BR-PP	 BR-RP	
SARS-CoV-2-MG1	 MG-PP1	 MG-RP1	

SARS-CoV-2-MG2	 MG-PP2	 MG-RP2	
SARS-CoV-2-BR1	 BR-PP1	 BR-RP1	

SARS-CoV-2-BR2	 BR-PP2	 BR-RP2	
SARS-CoV-2-BR3	 BR-PP3	 BR-RP3	

Supplementary Table 3. Target RNA sequence

Pathogen	Target gene	Target RNA sequence (5′-3′)
MRSA	*mecA*	UAUCAAUCUAUUAACUGAUGGUAUGCAACAAGUCGUAA AUAAAACACAUAAAGAAGAUAUUUAUAGAUCUUAUGCA AACUUAAUUGGCAAAUCCGGUACUGCAGAACUCAAAAU GAAACAAGGAGAAACUGGCAGACAAAUUGGGUGGUUUA UAUCAUAUGAUAAAGAUAAUCCAAACAUGAUGAUGGCU AUUAAUGUUAAAGAUGUACAAGAUAAAGGAAUGGCUAG CUACAAUGCCAAAAUCUCAGG
Vibrio vulnificus	*vvhA*	AUGAAAAAAAUAACUCUGUUUACCCUUUCUCUUUUAGC UACCGCGGUACAGGUUGGCGCACAAGAAUAUGUGCCGA UUGUUGAGAAGCCUAUUUAUAUCACCAGCUCAAAAAUU AAGUGCGUGCUACACACAAGUGGUGAUUUCAACGCCAC ACGAGACUGGUGUAAUGCGGGCGCUUCCAUCGAUGUUC GCGUCAAUGUGGCACAGAUGCGCUCGGUGCAAUCAGCA ACGUCAGAUGGUUUUACUCCUG
E. coli O157:H7	*tir*	GCAUGCUAUGGUCACCGUUGCUUCAGAUAUCACGGAAG CCCGCCAAAGGAUACUGGAGCUGUUAGAGCCCAAAGGG ACCGGGGAGUCCAAAGGUGCUGGGGAGUCAAAAGGCGU UGGGGAGUUGAGGGAGUCAAAUAGCGGUGCGGAAAACA CCACAGAAACUCAGACCUCAACCUCAACUUCCAGCCUUC GUUCAGAUCCUAAACUUUGGUUGGCGUUGGGGACUGUU GCUACAGGUCUGAUAGGGUUG
MERS-CoV	*upE*	UGCAGCUGUUCUCGUUGUUUUUAUUUGCACUCUUCCAC UUAUAUAGAGUGCACUUAUAUUAGCCGUUUUAGUAAGA UUAGCCUAGUUUCUGUAACUGACUUCUCCUUAAACGGC AAUGUUUCCACUGUUUUCGUGCCUGCAACGCGCGAUUC AGUUCCUCUUCACAUAAUCGCCCCGAGCUCGCUUAUCG UUUAAGCAGCUCUGCGCUACUAUGGGUCCCGUGUAGAG GCUAAUCCAUUAGUCUCUCUUUGGACAUAUGGAAAACG AACUAUGUUACCCUUUGUCCAAGAACGAAUAGGGUUGU UCAUAGUAAACUUUUUCAUUUUUACCGUAGUAUGUGCU AUAACACUCUUGGUGUGUAUGGCUUUC
Influenza A	*HA*	AGCUAUAGCAGGUUUUAUAGAGGGAGGAUGGCAGGGAA UGGUAGAUGGUUGGUAUGGGUACCACCAUAGCAAUGAG

		CAGGGGAGUGGAUACGCUGCAGACAAAGAAUCCACUCA AAAGGCAAUAGAUGGAGUCACCAAUAAGGUCAACUCGA UCAUUGACAAAAUGAACACUCAGUUUGAGGCCGUUGGA AGGGAAUUUAAUAACUUGGAAAGGAGGAUAGAGAAUU UAAACAAGCAGAUGGAAGACGGA
SARS-CoV-2	*RdRp*	AAACAUACAACGUGUUGUAGCUUGUCACACCGUUUCUA UAGAUUAGCUAAUGAGUGUGCUCAAGUAUUGAGUGAAA UGGUCAUGUGUGGCGGUUCACUAUAUGUUAAACCAGGU GGAACCUCAUCAGGAGAUGCCACAACUGCUUAUGCUAA UAGTGUUUUUAACAUUUGTCAAGCUGUCACGGCCAAUG UUAAUGCACUUUUAUCUACUGAUGGUAACAAAAUUGCC GAUAGUAUGUCCGCAAUUUAC
SARS-CoV	*RdRp*	UCGCAAACAUAACACUUGCUGUAACUUAUCACACCGUU UCUACAGGUUAGCUAACGAGUGUGCGCAAGUAUUAAGU GAGAUGGUCAUGUGUGGCGGCUCACUAUAUGUUAAACC AGGUGGAACAUCAUCCGGUGAUGCUACAACUGCUUAUG CUAAUAGUGUCUUUAACAUUUGUCAAGCUGUUACAGCC AAUGUAAAUGCACUUCUUUCAACUGAUGGUAAUAAGAU AGCUGACAAGUAUGUCCGCAAU
Bat-SARS 1	*RdRp*	UCGCAAACAUAGUACUUGUUGUAACCUUUCACACCGUU UCUACGGGUUAGCUAAUGAGUGUGCUCAGGUACUUAGU GAAAUGGUUAUGUGUGGCGGUUCACUCUAUGUGAAACC AGGCGGUACAUCUUCAGGAGAUGCCACCACUGCUUAUG CUAAUAGUGUCUUUAACAUUUGUCAAGCUGUUACAGCU AAUGUUAAUGCACUUUUGUCUACUGAUGGUAAUAAAAU UGCUGACAAGUAUGUCCGCAAU
Bat-SARS 2	*RdRp*	UCGCAAACAUAGCACUUGUUGUAACUUGUCACACCGUU UCUAUAGAUUAGCUAAUGAGUGUGCACAAGUAUUAAGU GAGAUGGUCAUGUGUGGCGGCUCAUUAUAUGUGAAACC AGGUGGAACAUCAUCCGGUGAUGCCACAACUGCUUAUG CUAAUAGUGUGUUUAACAUCUGUCAAGCAGUAACAGCU AAUGUAAAUGCACUUCUUUCAACUGAUGGUAAUAAGAU UGCUGAUAAGUAUGUCCGCAAC
HCoV-229E	*RdRp*	UUUGACCGAGGUUGUUUAUUCAAAUGGUGGGUUUUAUU UUAAACCUGGUGGUACAACUUCUGGUGAUGCAACUACA GCCUACGCCAAUUCUGUCUUUAAUAUAUUUCAGGCUGU

		AAGUUCUAACAUUAAUUGCGUUUUGAGCGUUAACUCGU CAAAUUGCAAUAAUUUUAAUGUUAAGAAGUUACAGAGA CAACUUUAUGAUAAUUGCUAUAGAAAUAGUAAUGUUGA UGAAUCUUUUGUGGAUGACUUU
HCoV-NL63	*RdRp*	UUUAACAGAAGUUGUUUAUUCUAAUGGUGGUUUUUAUU UUAAGCCAGGUGGUACGACUUCUGGUGACGCUAGUACA GCUUAUGCUAAUUCUAUUUUUAACAUUUUUCAAGCCGU GAGUUCUAACAUUAACAGGUUGCUUAGUGUCCCAUCAG AUUCAUGUAAUAAUGUUAAUGUUAGGGAUCUACAACGA CGUCUGUAUGAUAAUUGUUAUAGGUUAACUAGUGUUGA AGAGUCAUUCAUUGAUGAUUAU
HCoV-OC43	*RdRp*	UUUGAGUGAAAUUGUUAUGUGUGGUGGCUGUUAUUAU GUUAAGCCUGGUGGCACUAGUAGUGGUGAUGCAACUAC UGCUUUUGCUAAUUCAGUCUUUAACAUAUGUCAAGCUG UUUCAGCCAAUGUAUGUGCCUUAAUGUCAUGCAAUGGC AAUAAGAUUGAAGAUCUUAGUAUACGUGCUCUUCAGAA GCGCUUAUACUCACAUGUGUAUAGAAGUGAUAAGGUUG AUUCAACCUUUGUCACAGAAUAU
HCoV-HKU1	*RdRp*	UUUGAGUGAAAUAGUUAUGUGUGGCGGUUGCUAUUAUG UUAAGCCUGGUGGUACUAGCAGUGGUGAUGCAACUACU GCUUUUGCUAAUUCUGUUUUUAAUAUAUGUCAGGCUGU UACUGCUAAUGUUUGUUCUCUUAUGGCCUGUAAUGGCC AUAAGAUUGAAGAUUUAAGUAUACGCAAUUUACAAAAA CGCUUAUACUCUAAUGUUUAUCGUACAGAUUAUGUUGA UUAUACAUUUGUUAAUGAGUAU

Supplementary Table 4. Inter- and Intra-assay coefficient of variation (CV) of the repeated assay shown in Supplementary Fig. 5b

Inter-assay CV

	RNA = 0 aM					RNA = 0.1 aM					RNA = 1 aM					RNA = 10 aM					RNA = 100 aM				
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
Assay 1	562	413	495	480	487.5	909	1073	980	1002	991	1354	1196	1278	1272	1275	1813	1845	1864	1794	1829	2144	2127	2161	2110	2135.5
Assay 2	599	601	333	423	489	899	950	1046	997	973	1186	1413	1295	1167	1265.25	1799	1912	1866	1823	1850	2099	2068	2292	2234	2173.25
Assay 3	510	517	545	345	479.25	965	934	945	974	964	1063	1269	1277	1316	1231.25	1826	1869	1916	1845	1864	2092	2118	2157	2226	2148.25
Assay 4	514	518	471	485	497	1063	809	881	1178	982.75	1272	1259	1167	1304	1250.5	1858	1932	1839	1908	1884.25	2113	2145	2133	2191	2145.5
Assay 5	476	598	481	397	488	1040	981	1019	822	965.5	1225	1216	1313	1339	1273.25	1793	2026	1745	1959	1880.75	2191	2145	2136	2192	2166
	Mean of means				488.15	Mean of means				975.25	Mean of means				1259.05	Mean of means				1861.6	Mean of means				2153.7
	StDev of means				6.298809411	StDev of means				11.52171862	StDev of means				18.30590752	StDev of means				22.8189888	StDev of means				15.50544259
	CV of means				1.290343012	CV of means				1.181411804	CV of means				1.453946032	CV of means				1.225772927	CV of means				0.719944402

Inter-assay CV (Mean of CVs) = 1.174%

Intra-assay CV

	Assay 1					Assay 2					Assay 3					Assay 4					Assay 5				
RNA (aM)	1	2	3	4	CV	1	2	3	4	CV	1	2	3	4	CV	1	2	3	4	CV	1	2	3	4	CV
0	562	413	495	480	12.540812 18	599	601	333	423	27.267211 99	510	517	545	345	18.939692 93	514	518	471	485	4.5735015 75	476	598	481	397	16.969885 75
0.1	909	1073	980	1002	6.8165942 24	899	950	1046	997	6.4756357 67	965	934	945	974	1.9137291 79	1063	809	881	1178	17.138516 24	1040	981	1019	822	10.226167 63
1	1354	1196	1278	1272	5.0627233 28	1186	1413	1295	1167	8.9707912 92	1063	1269	1277	1316	9.2613467 79	1272	1259	1167	1304	4.7013665 59	1225	1216	1313	1339	4.8645174 53
10	1813	1845	1864	1794	1.7179803 58	1799	1912	1866	1823	2.6900605 23	1826	1869	1916	1845	2.0856153 88	1858	1932	1839	1908	2.2890070 63	1793	2026	1745	1959	7.0903949 37
100	2144	2127	2161	2110	1.0277174 26	2099	2068	2292	2234	4.9260633 1	2092	2118	2157	2226	2.7143797 34	2113	2145	2133	2191	1.5418648 05	2191	2145	2136	2192	1.3700844 18
	Mean of CVs = 5.433%					**Mean of CVs = 10.066%**					**Mean of CVs = 6.983%**					**Mean of CVs = 6.049%**					**Mean of CVs = 8.104%**				

* Five replicate experiments each showed the intra-assay coefficients of variation from 5.433 to 10.066%. The inter-assay coefficient of variation of the five experiments was 1.174%, showing high reproducibility of the assay.

Supplementary Table 5. Reaction conditions for SplintR-based RNA detection methods

Reaction component	Ref 17 (Jin, J., Vaud, S., Zhelkovsky, A. M., Posfai, J. & McReynolds, L. A. *Nucleic Acids Res.* **44,** e116 (2016).	Ref 18 (Ying, Z.-M. *et al. ChemComm* **54,** 3010–3013 (2018))	Current work
Donor probe (5′-Ph)	14.3 nM (0.1 μM × 1 μL / 7 μL rxn.)	200 nM	200 nM
Receptor probe	14.3 nM (0.1 μM × 1 μL / 7 μL rxn.)	200 nM	220 nM
SplintR ligase	1 μL / 7 μL rxn. (Units unknown)	2 U/μL rxn. (10 U/μL × 2 μL / 10 μL rxn.)	2.5 U/μL rxn. (25 U/μL × 10 μL / 100 μL rxn.)
T7 RNA polymerase	n.a.	1.55 U/μL rxn. (12 U/μL × 2 μL / 15.5 μL rxn.)	2.5 U/μL rxn. (50 U/μL × 5 μL / 100 μL rxn.)
NTPs	n.a.	0.96 mM each (10 mM each × 1.5 μL / 15.5 μL rxn.)	2.5 mM each (25 mM each × 10 μL / 100 μL rxn.)
Malachite green	n.a.	10 μM	16 μM

Supplementary Table 6. Information of the clinical samples

No	Specimen	rRT-PCR assay date	Region (city)	Sex	Age	Ct value (RdRp gene)	Results
1	Nasopharyngeal swab	20.03.10	Daegu	F	71	12.50	Positive
2	Nasopharyngeal swab	20.03.10	Daegu	M	48	13.33	Positive
3	Nasopharyngeal swab	20.03.10	Daegu	F	26	13.63	Positive
4	Nasopharyngeal swab	20.03.10	Daegu	M	74	14.13	Positive
5	Nasopharyngeal swab	20.03.10	Daegu	M	75	15.57	Positive
6	Nasopharyngeal swab	20.03.10	Daegu	M	79	16.60	Positive
7	Nasopharyngeal swab	20.03.19	Seongnam	F	60	17.26	Positive
8	Nasopharyngeal swab	20.03.10	Daegu	F	90	17.49	Positive
9	Nasopharyngeal swab	20.03.19	Daegu	M	31	17.74	Positive
10	Nasopharyngeal swab	20.03.19	Andong	F	75	19.33	Positive
11	Nasopharyngeal swab	20.03.19	Seongnam	M	55	21.17	Positive
12	Nasopharyngeal swab	20.03.19	Andong	M	91	21.29	Positive
13	Nasopharyngeal swab	20.03.25	Siheung	F	26	21.67	Positive
14	Nasopharyngeal swab	20.03.10	Sangju	M	66	22.96	Positive
15	Nasopharyngeal	20.03.19	Andong	F	84	24.10	Positive

	swab						
16	Nasopharyngeal swab	20.03.19	Gimcheon	F	57	27.04	Positive
17	Nasopharyngeal swab	20.03.24	Daegu	F	43	27.05	Positive
18	Nasopharyngeal swab	20.03.10	Daegu	F	88	27.10	Positive
19	Nasopharyngeal swab	20.03.10	Sangju	F	33	29.67	Positive
20	Nasopharyngeal swab	20.03.12	Sangju	M	63	30.80	Positive
1	Nasopharyngeal swab	20.04.10	Daejeon	F	29	-	Negative
2	Nasopharyngeal swab	20.04.10	Seongnam	M	92	-	Negative
3	Nasopharyngeal swab	20.04.10	Seongnam	M	83	-	Negative
4	Nasopharyngeal swab	20.04.10	Gwang myeong	M	57	-	Negative
5	Nasopharyngeal swab	20.04.10	Sangju	F	31	-	Negative
6	Nasopharyngeal swab	20.05.07	Yongin	F	44	-	Negative
7	Nasopharyngeal swab	20.05.07	Seongnam	M	72	-	Negative
8	Nasopharyngeal swab	20.05.07	Gwang myeong	F	39	-	Negative
9	Nasopharyngeal swab	20.05.07	Incheon	F	36	-	Negative
10	Nasopharyngeal swab	20.05.07	Seongnam	F	28	-	Negative
11	Nasopharyngeal swab	20.05.07	Yongin	M	40	-	Negative

12	Nasopharyngeal swab	20.05.07	Incheon	F	46	-	Negative
13	Nasopharyngeal swab	20.05.07	Seoul	F	21	-	Negative
14	Nasopharyngeal swab	20.05.07	Incheon	F	15	-	Negative
15	Nasopharyngeal swab	20.05.07	Incheon	F	20	-	Negative
16	Nasopharyngeal swab	20.05.07	Incheon	F	19	-	Negative
17	Nasopharyngeal swab	20.05.07	Seoul	M	93	-	Negative
18	Nasopharyngeal swab	20.05.07	Seoul	F	21	-	Negative
19	Nasopharyngeal swab	20.05.07	Seoul	M	88	-	Negative
20	Nasopharyngeal swab	20.05.07	Gwang myeong	M	80	-	Negative

Supplementary Table 7. Primer sequence

Primer name	Sequence (5′-3′)
*mecA*_RNA_F1	GCATAAGATCTATAAATATCTTCTTTATGTGTTTTATTTACGACTTGTTGCATACCATCAGTTAATAGATTGATAT
*mecA*_RNA_R1	CTCCTTGTTTCATTTTGAGTTCTGCAGTACCGGATTTGCCAATTAAGTTTGCATAAGATCTATAAATATCTTCTTTATGTGT
*mecA*_RNA_F2	CCATCATCATGTTTGGATTATCTTTATCATATGATATAAACCACCCAATTTGTCTGCCAGTTTCTCCTTGTTTCATTTTGAGTTC
*mecA*_RNA_R2	CCTGAGATTTTGGCATTGTAGCTAGCCATTCCTTTATCTTGTACATCTTTAACATTAATAGCCATCATCATGTTTGGATTATC
T7_*mecA*_ F	TAATACGACTCACTATAGGGATATCAATCTATTAACTGATGGTATGC
*mecA*_R	CTACAATGCCAAAATCTCAGG
*HA*_RNA_F1	CGTTGGAAGGGAATTTAATAACTTGGAAAGGAGGATAGAGAATTTAAACAAGCAGATGGAAGACGGA
*HA*_RNA_R1	GCAATAGATGGAGTCACCAATAAGGTCAACTCGATCATTGACAAAATGAACACTCAGTTTGAGGCCGTTGGAAGGGAATTTAATAAC
*HA*_RNA_F2	CACCATAGCAATGAGCAGGGGAGTGGATACGCTGCAGACAAAGAATCCACTCAAAAGGCAATAGATGGAGTCACCAATAA
*HA*_RNA_R2	AGCTATAGCAGGTTTTATAGAGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGTACCACCATAGCAATGAGCAGG
T7_*HA*_F	TAATACGACTCACTATAGGGAGCTATAGCAGGTTTTATAGAGGGA
*HA*_R	AAGCAGATGGAAGACGGA
*vvhA*_RNA_F1	ATCGATGTTCGCGTCAATGTGGCACAGATGCGCTCGGTGCAATCAGCAACGTCAGATGGTTTTACTCCTG
*vvhA*_RNA_R1	AGTGGTGATTTCAACGCCACACGAGACTGGTGTAATGCGGGCGCTTCCATCGATGTTCGCGTCAAT
*vvhA*_RNA_F2	GAATATGTGCCGATTGTTGAGAAGCCTATTTATATCACCAGCTCAAAAATTAAGTGCGTGCTACACACAAGTGGTGATTTCAACGCC
*vvhA*_RNA_R2	ATGAAAAAAATAACTCTGTTTACCCTTTCTCTTTTAGCTACCGCGGTACAGGTTGGCGCACAAGAATATGTGCCGATTGTTGA
T7_*vvhA*_F	TAATACGACTCACTATAGGGATGAAAAAAATAACTCTGTTTACCC

*vvhA*_R	ACGTCAGATGGTTTTACTCCTG
*tir*_RNA_F1	ACCTCAACTTCCAGCCTTCGTTCAGATCCTAAACTTTGGTTGGC GTTGGGGACTGTTGCTACAGGTCTGATAGGGTTG
tir _RNA_R1	TTGGGGAGTTGAGGGAGTCAAATAGCGGTGCGGAAAACACCA CAGAAACTCAGACCTCAACCTCAACTTCCAGCCTTC
tir _RNA_F2	AGCTGTTAGAGCCCAAAGGGACCGGGGAGTCCAAAGGTGCTG GGGAGTCAAAAGGCGTTGGGGAGTTGAGGGAGT
tir _RNA_R2	GCATGCTATGGTCACCGTTGCTTCAGATATCACGGAAGCCCGCC AAAGGATACTGGAGCTGTTAGAGCCCAAAGG
T7_*eaeA*_F	TAATACGACTCACTATAGGGCATGCTATGGTCACCGTT
eaeA_R	GCTACAGGTCTGATAGGGTTG
SARS-CoV-2 *RdRp*_RNA_F1	ACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAA CTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTCACG
SARS-CoV-2 *RdRp* _RNA_R1	GTAAATTGCGGACATACTTATCGGCAATTTTGTTACCATCAGTAG ATAAAAGTGCATTAACATTGGCCGTGACAGCTTGACAAATGTT
SARS-CoV-2 *RdRp* _RNA_F2	TAATACGACTCACTATAGGGAAACATACAACGTGTTGTAGCTTG TCACACCGTTTCTATAGATTAGCTAATGAGTGTGCTC
SARS-CoV-2 *RdRp* _RNA_R2	GGTTCCACCTGGTTTAACATATAGTGAACCGCCACACATGACCA TTTCACTCAATACTTGAGCACACTCATTAGCTAATCTATAGAAA
SARS-CoV-2 T7_ *RdRp*_F	TAATACGACTCACTATAGGGAAACATACA
SARS-CoV-2 *RdRP*_R	GTAAATTGCGGACATACTTATCG
Bat-SARS 1 *RdRp*_RNA_F1	TCTATGTGAAACCAGGCGGTACATCTTCAGGAGATGCCACCACT GCTTATGCTAATAGTGTCTTTAACATTTGTCAAGCTGTTACAGCT
Bat-SARS 1 *RdRp*_RNA_R1	ATTGCGGACATACTTGTCAGCAATTTTATTACCATCAGTAGACA AAAGTGCATTAACATTAGCTGTAACAGCTTGACAAATGTTA
Bat-SARS 1 *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTCGCAAACATAGTACTTGTTGTAA CCTTTCACACCGTTTCTACGGGTTAGCTAATGAGTG
Bat-SARS 1 *RdRp*_RNA_R2	CCGCCTGGTTTCACATAGAGTGAACCGCCACACATAACCATTTC ACTAAGTACCTGAGCACACTCATTAGCTAACCCGTAGAAAC

Bat-SARS 1 T7_*RdRp*_F	TAATACGACTCACTATAGGGTCGC
Bat-SARS 1 *RdRp*_R	ATTGCGGACATACTTGTCAGC
Bat-SARS 2 *RdRp*_RNA_F1	ATATGTGAAACCAGGTGGAACATCATCCGGTGATGCCACAACT GCTTATGCTAATAGTGTGTTTAACATCTGTCAAGCAGTAACAGC T
Bat-SARS 2 *RdRp*_RNA_R1	GTTGCGGACATACTTATCAGCAATCTTATTACCATCAGTTGAAA GAAGTGCATTTACATTAGCTGTTACTGCTTGACAGATGTTA
Bat-SARS 2 *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTCGCAAACATAGCACTTGTTGTAA CTTGTCACACCGTTTCTATAGATTAGCTAATGAGTGTGCACA
Bat-SARS 2 *RdRp*_RNA_R2	ATGTTCCACCTGGTTTCACATATAATGAGCCGCCACACATGACC ATCTCACTTAATACTTGTGCACACTCATTAGCTAATCTATAGAA
Bat-SARS 2 T7_*RdRp*_F	TAATACGACTCACTATAGGGTCGC
Bat-SARS 2 *RdRp*_R	GTTGCGGACATACTTATCAGCA
SARS-CoV *RdRp*_RNA_F1	TATATGTTAAACCAGGTGGAACATCATCCGGTGATGCTACAACT GCTTATGCTAATAGTGTCTTTAACATTTGTCAAGCTGTTACAGC
SARS-CoV *RdRp*_RNA_R1	ATTGCGGACATACTTGTCAGCTATCTTATTACCATCAGTTGAAAG AAGTGCATTTACATTGGCTGTAACAGCTTGACAAATGTTAA
SARS-CoV *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTCGCAAACATAACACTTGCTGTAA CTTATCACACCGTTTCTACAGGTTAGCTAACGAGTGTG
SARS-CoV *RdRp*_RNA_R2	GATGTTCCACCTGGTTTAACATATAGTGAGCCGCCACACATGAC CATCTCACTTAATACTTGCGCACACTCGTTAGCTAACCTGTAGA
SARS-CoV T7_*RdRp*_F	TAATACGACTCACTATAGGGTCGC
SARS-CoV *RdRp*_R	ATTGCGGACATACTTGTCAGCTA
HCoV-229E *RdRp*_RNA_F1	GCTGTAAGTTCTAACATTAATTGCGTTTTGAGCGTTAACTCGTC AAATTGCAATAATTTTAATGTTAAGAAGTTACAGAGACAACT
HCoV-229E *RdRp*_RNA_R1	AAAGTCATCCACAAAAGATTCATCAACATTACTATTTCTATAGCA ATTATCATAAAGTTGTCTCTGTAACTTCTTAACATTAAAAT
HCoV-229E	TAATACGACTCACTATAGGGTTTGACCGAGGTTGTTTATTCAAAT GGTGGGTTTTATTTTAAACCTGGTGGTACAACTTCTGGTGATG

*RdRp*_RNA_F2	
HCoV-229E *RdRp*_RNA_R2	CGCAATTAATGTTAGAACTTACAGCCTGAAATATATTAAAGACA GAATTGGCGTAGGCTGTAGTTGCATCACCAGAAGTTGTACCACC
HCoV-229E T7_*RdRp*_F	TAATACGACTCACTATAGGGTTTGACC
HCoV-229E *RdRp*_R	AAAGTCATCCACAAAAGATTCATCA
HCoV-NL63 *RdRp*_RNA_F1	TTTTTCAAGCCGTGAGTTCTAACATTAACAGGTTGCTTAGTGTC CCATCAGATTCATGTAATAATGTTAATGTTAGGGATCTACAACG
HCoV-NL63 *RdRp*_RNA_R1	ATAATCATCAATGAATGACTCTTCAACACTAGTTAACCTATAACA ATTATCATACAGACGTCGTTGTAGATCCCTAACATTAACATT
HCoV-NL63 *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTTTAACAGAAGTTGTTTATTCTAAT GGTGGTTTTTATTTTAAGCCAGGTGGTACGACTT
HCoV-NL63 *RdRp*_RNA_R2	TTAGAACTCACGGCTTGAAAAATGTTAAAAATAGAATTAGCATA AGCTGTACTAGCGTCACCAGAAGTCGTACCACCTGGCTTAA
HCoV-NL63 T7_*RdRp*_F	TAATACGACTCACTATAGGGTTTAACAGA
HCoV-NL63 *RdRp*_R	ATAATCATCAATGAATGACTCTTCAAC
HCoV-OC43 *RdRp*_RNA_F1	TGTCAAGCTGTTTCAGCCAATGTATGTGCCTTAATGTCATGCAAT GGCAATAAGATTGAAGATCTTAGTATACGTGCTCTTCAGAAGC
HCoV-OC43 *RdRp*_RNA_R1	ATATTCTGTGACAAAGGTTGAATCAACCTTATCACTTCTATACAC ATGTGAGTATAAGCGCTTCTGAAGAGCACGTATACTAAGATC
HCoV-OC43 *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTTTGAGTGAAATTGTTATGTGTGGT GGCTGTTATTATGTTAAGCCTGGTGGCACTAGTAGTG
HCoV-OC43 *RdRp*_RNA_R2	ATTGGCTGAAACAGCTTGACATATGTTAAAGACTGAATTAGCAA AAGCAGTAGTTGCATCACCACTACTAGTGCCACCAGGCTTA
HCoV-OC43 T7_*RdRp*_F	TAATACGACTCACTATAGGGTTTGAGTG
HCoV-OC43 *RdRp*_R	ATATTCTGTGACAAAGGTTGAATCAA
HCoV-HKU1	GTCAGGCTGTTACTGCTAATGTTTGTTCTCTTATGGCCTGTAATG GCCATAAGATTGAAGATTTAAGTATACGCAATTTACAAAAAC

*RdRp*_RNA_F1	
HCoV-HKU1 *RdRp*_RNA_R1	ATACTCATTAACAAATGTATAATCAACATAATCTGTACGATAAAC ATTAGAGTATAAGCGTTTTTGTAAATTGCGTATACTTAAATCT
HCoV-HKU1 *RdRp*_RNA_F2	TAATACGACTCACTATAGGGTTTGAGTGAAATAGTTATGTGTGG CGGTTGCTATTATGTTAAGCCTGGTGGTACTAGCAGT
HCoV-HKU1 *RdRp*_RNA_R2	AACATTAGCAGTAACAGCCTGACATATATTAAAAACAGAATTAG CAAAAGCAGTAGTTGCATCACCACTGCTAGTACCACCAGGCT
HCoV-HKU1 T7_*RdRp*_F	TAATACGACTCACTATAGGGTTTGAGT
HCoV-HKU1 *RdRp*_R	ATACTCATTAACAAATGTATAATCAACATAATC
LigChk_F	CGTATTAATTTCGCGTGTTGTCG
LigChk_R	GTTACCTGGCTCTCGCCAG

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