Electronic Supplementary Information

Rapid PCR kit: lateral flow paper strip with Joule heater for SARS-CoV-2 detection

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Experimental Section

Materials: CA membranes (pore size: 0.8 μm, thickness: 0.11 mm, max water flow rate [L min^{-1} cm^{-2}]) were obtained from ADVANTEC (Tokyo, Japan). Absorbent pads were obtained from Vericel Co. (Ann Arbor, MI, USA). The Vivid plasma separation-GX membrane (T9EXPPA0200S00X) and asymmetric membrane MMM0.45 (T9PA045W000M) were purchased from Pall Corporation (Port Washington, NY, USA). The NC membrane (FF170HP) was purchased from GE Healthcare (Chicago, IL, USA). Glass fiber 8964 grade was purchased from Ahlstrom-Munksjö (Helsinki, Finland). The hydrophilic PES membrane filter was purchased from EMD Millipore (Burlington, MA, USA). OneTaq® DNA polymerase was purchased from New England Biolabs (Ipswich, UK). The TOPreal™ One-step RT PCR kit and TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) were purchased from Enzynomics (Daejeon, South Korea). cDNA of the N gene region of the 2019 novel coronavirus DNA was purchased from Integrated DNA Technologies (IDT; Coralville, IA, USA). Heat Inactivated SARS-CoV-2 viral particle (2019 Novel Coronavirus ATCC VR-1986HK™) was purchased from ATCC (Manassas, VA, USA). Oligonucleotide primers were synthesized by Genotech (Daejeon, South Korea). A 50 bp DNA ladder (TaKaRa, Japan) was used as a DNA molecular marker. Agarose was purchased from Roche (Basel, Switzerland). Bovine serum albumin (BSA), streptavidin-AP conjugate, RNA/RNAse-free water, and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). NiCr (80:20 wt%) was obtained from iTASCO (Seoul, South Korea). Anti-fluorescein polyclonal antibody was purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Forty-nanometer gold nanoparticle was obtained from Boreda (Gyeonggi-do, Korea).

Membrane selection: All membranes — CA, PES, GF, NC, Vivid, and mixed matrix membranes — were cut to 2 mm × 2 mm to measure their absorption and inhibitory

capacities. The absorption capacity was measured with a micropipette. After dropping 10 μL of solution onto the membrane, the rest was aspirated from the membrane to measure the amount of absorbed solution.

Membrane-induced PCR inhibition was tested by immersing each piece of membranes (2 mm × 2 mm) into PCR solution (20 μL)-containing microtubes. Each membrane was fully immersed in each PCR solution. We compared PCR results of those. PCR was also conducted without the membranes as a control. The PCR solution consisted of TOPreal™ qPCR 2X PreMIX and 0.1 μM primer with 200 copies μL^{-1} cDNA. After PCR, the products were analyzed using gel electrophoresis.

Fabrication and characterization of Joule heaters: NiCr thin films were deposited onto 1 cm × 1.5 cm glass slides using an e-beam evaporator. The thickness of the deposited films was 600 nm, and the deposition rate was 1 Å s^{-1} during deposition. Surface and element analysis of the NiCr thin film was performed using an ultra-high-resolution field-emission scanning electron microscope and energy-dispersive X-ray spectroscopy (Verios 5 UC, Thermo Fisher Scientific, MA, USA). In addition, the sheet resistance of the film was measured using a four-point probe system (CMT-SR2000N, Advanced Instrument Technology, Suwon, South Korea). A programmable power supply (E36312A, Keysight Technologies, Santa Rosa, CA, USA) was used to apply a voltage on the NiCr thin film to generate Joule heat. The temperature of the NiCr thin film was measured using a thermocouple (5SC-TT-K-40-36, OMEGA Engineering, Norwalk, CT, USA).

Paper chip preparation for amplification and detection zones: The CA membrane was used for the paper chips as an amplification membrane, and one was prepared at a size of 20 mm × 3.2 mm to construct an amplification zone. Then, one side of the 20 mm × 3.2 mm CA

membrane was cut to make a narrow arm shape with a width of 1 mm. Double-sided Kapton tape (polyimide matrix with silicone adhesive) was used to seal the CA membrane. The tape was cut to a size of 24 mm × 10 mm, and a 2 mm hole was made on the tape at the site of PCR solution injection. The tape was blocked with 1% BSA in phosphate-buffered saline (PBS). Then, the CA membrane was sealed with BSA-blocked thermal tape. The other side of the CA membrane, which had a width of 3.2 mm, was folded at 45° and unsealed to allow contact with the NC membrane after PCR. To make a detection zone in the NC membrane, 0.5 μL streptavidin solution (1 mg μL^{-1}) was dropped onto the 3.2 mm × 10 mm NC membrane, resulting in the formation of a streptavidin spot after the solution dried.

Preparation of a conjugate pad for visual detection: One hundred microliter of borate buffer (0.1 M, pH 8.5) was added to 1 mL of AuNP solution, and then, 0.25 mg mL^{-1} of anti-fluorescein antibody was added into the solution. After 30 min, 100 μL of 1% BSA solution in 1 × PBS was added to the solution for blocking of AuNP/antibody conjugates. Then, the conjugated was collected by centrifuging (6448 x*g*, 15 min) and resuspending for three times (for the last resuspension, the AuNP/antibody conjugates were resuspended in 100 μL PBS). Conjugate pads were fabricated by dropping and drying of the AuNP/antibody conjugates on glass fiber membranes that had been blocked by 10% BSA and washed.

Fabrication of Joule heater-integrated paper chips for PCR and detection: Cases for the Joule heater-integrated paper chips for PCR were made using a three-dimensional printer (Ultimaker3, Ultimaker, Utrecht, Netherlands). The cases were composed of bottom and top cases. The top case had a hole for solution injection and a push button, which formed a junction between the end point of the amplification membrane and the start point of the conjugate pad by pushing the junction area after PCR was completed. The bottom case had a

rectangular hole to embed a Joule heater. The detailed sizes of the cases are described in Figure S7. A NiCr thin film heater was embedded into the bottom case, and both edges of the thin film were connected with copper tape. The overlap of each side of the thin film was 4.5 mm; thus, the active heating area was 6 mm × 10 mm (length × width). A silver paste was applied at the contact area between the thin film and the copper tapes to ensure proper electrical contact. A prepared CA membrane sealed with Kapton tape was placed on the thin film heater as an amplification membrane (center-aligned). A sample pad (3 mm × 3.2 mm) was attached to the side of the CA membrane with the 2 mm hole. The NC membrane was then attached to the bottom case as a detection membrane. The conjugate pad was attached to the side of the NC membrane, and the start point of conjugate pad was located just below the folded area of the CA membrane. Then, the NC membrane was connected to an absorbent pad and covered with clear tape to prevent evaporation. Lastly, a top case was fixed onto the bottom case.

PCR solution preparation: Two target genes, the open reading frame of 1ab (ORF1ab) and the nucleocapsid protein (N), are the main components used to detect SARS-CoV-2 RNA.[1,2] The primer used in this study for the amplification and detection of SARS-CoV-2 nucleic acid was designed to target the nucleocapsid protein (N) and was obtained from the National Center for Biotechnology Information (NCBI), and the sequences are displayed in Figure S1. Designed forward primer contain FAM or Cy5 at 5′ end, and biotin is tagged at 5′ end of reverse primer. TOPreal™ qPCR 2X PreMIX and TOPreal™ One-step RT PCR kits were used to amplify cDNA (N gene region of the 2019 novel Coronavirus DNA) and the RNA, respectively. The SARS-CoV-2 viral particle was heated at 95°C for 5 min prior to use. The reaction solution for DNA amplification contained qPCR 2X PreMIX with 0.1 μM forward and reverse primers, 0.1% Tween 20, and 5× Onetaq. The RT-PCR mixture for RNA

amplification was prepared with 0.5 μM primers, 0.1% tween 20, and 5× Onetaq. Reaction solutions contained 10% template.

Operation of Joule heater-integrated paper chips for PCR: Twenty microliters of the reaction solution was injected into the hole in the top case, and the hole was sealed to prevent evaporation. After the solution reached the end of the CA membrane, 5.18, 0, and 1.92 V were applied to a NiCr thin film for 1.65, 11.2, and 17 s, respectively, to generate Joule heat and control the thermal cycling process for PCR (unless otherwise specified). A step was added to apply 1.5 V for 10 min before the thermal cycling process for reverse transcription (note that 2 μL of PCR solution was evaporated during PCR). Then, the push button on the top case was pushed to allow contact between the end of the amplification membrane and the start of the detection membrane. The overlapping area of the two membranes was 2 mm. After 5 min, the colorimetric intensity of gold nanoparticles at the streptavidin-spotted region was measured using a ChemiDoc MP system (Bio-Rad, Hercules, CA, USA).

Numerical simulation: FEA-based numerical simulation was performed using COMSOL Multiphysics v5.4 (COMSOL Inc.) with AC/DC heat transfer modules to determine the distribution of solution heating and cooling in a PCR microtube and membrane. The general heat equation is given by PDE and was solved in a time-dependent solver for a solid body, as follows:

$$\rho C\frac{\partial T}{\partial t} = -k\nabla T + Q \qquad \text{Equation (1)}$$

where ρ is the solution density, C is the heat capacity, T is the temperature, k is the thermal conductivity of the solution, and Q is the heat source per unit volume and time that is absorbed on the surface of the solution from the side exposed to the heat source. For heating, Q = 10 kW/m^2 assumes a uniform distribution on the bottom of the PCR tube and membrane surface. For cooling, the surface-to-ambient radiation of the solution surface to air was assumed and solved as follows:

$$-n \cdot q = \varepsilon\sigma \left(T_{amb}^{4} - T^4\right) \qquad \text{Equation (2)}$$

where n is the normal vector, q is the heat flux, ε is the surface emissivity, σ is the Stefan-Boltzmann constant, and T_{amb} is the ambient (room) temperature.

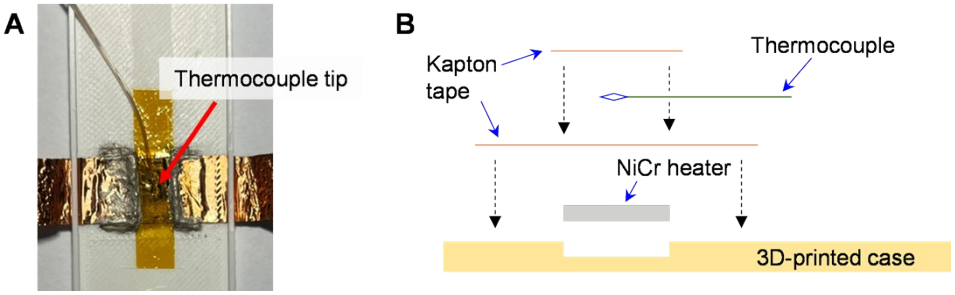
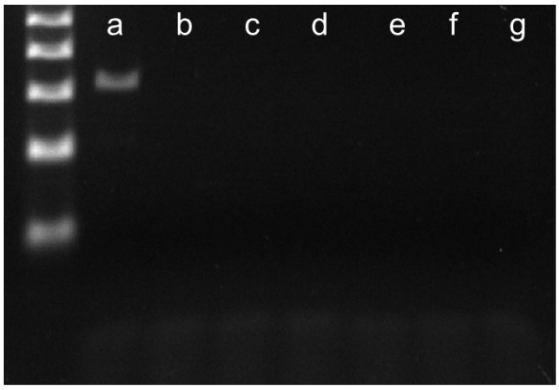


Figure S1. (A) A digital image (top view) and (B) a schematic illustration (side view) of thermocouple-attached NiCr thin film.

A

	Sequence (5'→3')	5' Tag
Forward	TGGACCCCAAAATCAGCGAA	Biotin
Reverse	GTGAGAGCGGTGAACCAAGA	Cy5

B



a) SARS-CoV-2
b) Influenza A H1N1
c) Influenza A H3N2
d) Influenza B
e) 10x Influenza A H1N1
f) 10x Influenza A H3N2
g) 10x Influenza B

Figure S2. (A) Forward and reverse primer sequences tagged with biotin and Cy5, respectively, for SAR-CoV-2 amplification. (B) Specificity test of the designed primers towards two other epidemic respiratory viruses, influenza A and B.

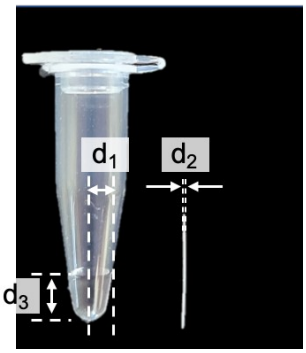


Figure S3. The image and actual size of a PCR microtube containing 20 µL of the solution and an amplification membrane (CA).

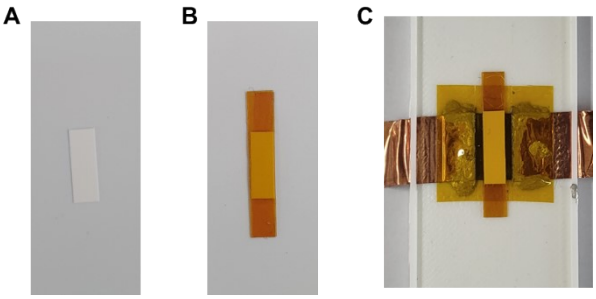


Figure S4. (A) An amplification membrane and (B) an amplification membrane sealed with Kapton tape and (C) its attachment on a NiCr thin film.

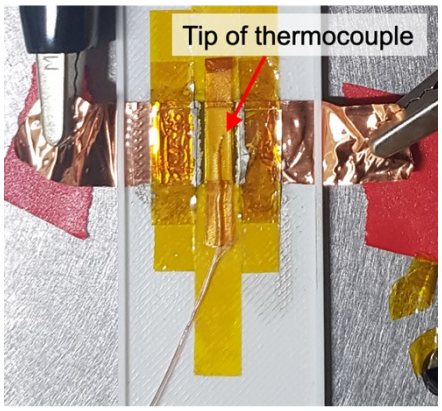


Figure S5. A digital image of a thermocouple tip on an amplification membrane, which was sealed with Kapton tape.

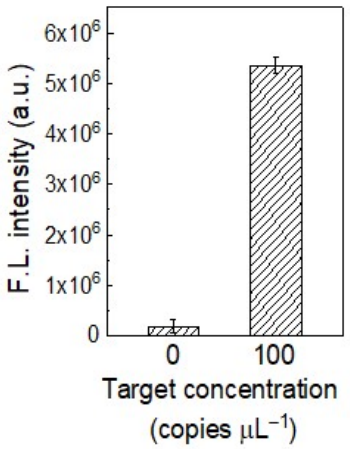


Figure S6. The fluorescence intensities after PCR using the closed-type Joule heater-integrated paper chip for PCR. The intensities were obtained from Fig. 5(e).

Figure S7. An example of the calculation of the fluorescence intensity of amplified products captured on a streptavidin spot on a NC membrane.

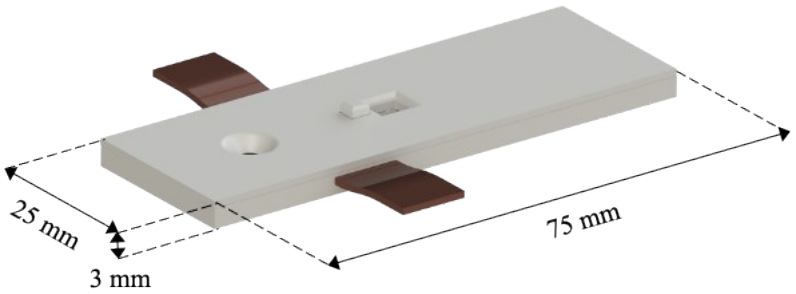


Figure S8. Schematic illustration of the detailed size of a Joule heater-integrated lateral flow paper chip.

Table S1. Comparison of heating (or cooling) surface area per PCR solution volume. Required solution volumes for a PCR microtube and a membrane; and the heating (or cooling) surface areas per volume of those.

PCR-solution container	**Solution volume (mm^3)**	**Heating (or cooling) surface area (mm^2)**	**Heating (or cooling) surface area/solution volume**
PCR tube	20.45	9.945pi=31.243	1.526
CA membrane	3	32	10.667

The volume and surface area of the solution in the PCR tube were calculated using the following formula for the volume and surface area of the rotating body, assuming that interior shape of PCR tube was a parabola (Equation 1):

$$V = \int_a^b \pi[f(y)]^2 dy \quad \text{and} \quad S = \int_a^b 2\pi f(y)\sqrt{1 + [f'(y)]^2}dy \quad . \quad \text{Equation (S1)}$$

Table S2. The estimated cost of our Joule heater-integrated lateral flow paper strip

Component	Cost (USD)
Membranes, pad, Kapton & sealing tapes	0.09
Biomaterials (antibody, DNA, streptavidin, PCR reagent)	0.88
Materials & chemicals (Au nanoparticles, buffer, etc)	0.10
Packaging (3D-printed case)	0.05
Heater (nichrome thin film, copper tape, silver paste, etc)	0.10

The material cost of view for NiCr thin film was estimated as follows. The dimension of the NiCr thin film in this communication was 600 nm × 1.0 cm × 1.5 cm (= 9×10^{-5} cm^3). Considering NiCr density (8.4 g/cm^3) and e-beam source cost (261.70 USD/100 g), one NiCr thin film costs 0.002 USD (if there is no loss during e-beam deposition.). There is always loss during e-beam deposition. Thus, if we assume the loss was 90%, the cost of a single NiCr thin film would be 0.02 USD, approximately. Please note that the loss and price of NiCr e-beam source depend on the physical scale of an e-beam evaporator and purchase scale, respectively.

The material cost of view for biomaterials — used for a single Joule heater-integrated lateral flow paper strip — was estimated as follows. Streptavidin: 0.46 USD; fluorescein-labelled and biotin-labelled primers: 0.01 USD; PCR reagent: 0.16 USD; Anti-fluorescein antibody: 0.25 USD.

Table S3. Comparison of our Joule heater-integrated, lateral flow paper chip with conventional and real-time RT-PCR. (*C-PCR: Conventional PCR, EP: electrophoresis)

Platform	Heating method	RT time (min)	PCR time (min)	Analysis method	Analysis time (min)	Total time (min)	Sensitivity (copies μL^{-1})
C-PCR	Peltier element	30	81	EP	20	131	10
Real-time PCR	Peltier element	30	60	Intercalating dye	Real time	90	1
Our chip	Joule heating	10	15	Lateral flow detection	5	30	1

It should be noted that the times taken for C-PCR and real-time PCR might be varied depending on the operator, PCR reagent, and PCR efficiency.

References

[1] X. Zhang, M. Li, B. Zhang, T. Chen, D. Lv, P. Xia, Z. Sun, X. Shentu, H. Chen, L. Li, W. Qian, *Clin. Chim. Acta*, 2020, **511**, 291.

[2] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, Y. Zhao, Y. Li, X. Wang, Z. Peng, *JAMA*, 2020, **323**, 1061.