Supplementary Information for

Detection of SARS-CoV-2 with Solid-State CRISPR-Cas12a-Assisted Nanopores (SCAN)

Reza Nouri [1], Yuqian Jiang [2,3], Zifan Tang [1], Xiaojun Lance Lian [2,3,4] and Weihua Guan [1, 2, *]

[1] Department of Electrical Engineering, Pennsylvania State University, University Park, Pennsylvania 16802, United States

[2] Department of Biomedical Engineering, Pennsylvania State University, University Park, Pennsylvania 16802, United States

[3] Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, Pennsylvania 16802, United States

[4] Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802, United States

*Correspondence: Weihua Guan (w.guan@psu.edu)

Table of Contents

Supplementary Methods S-2

Materials and chemicals S-2

SARS-CoV-2 RT-PCR assay S-2

SARS-CoV-2 Cas12a assay S-3

Glass nanopore fabrication and characterization S-3

Nanopore sensing and data analysis S-3

Supplementary Figures S-4

Supplementary References S-10

Supplementary Methods

Materials and chemicals

A.s.Cas12a Ultra (#10001272) and primers were purchased from Integrated DNA Technologies (IDT). crRNA was synthesized by IDT. qScript™ XLT One-Step RT-qPCR Tough Mix was purchased from Quantabio. M13mp18 ssDNA (#N4040S) was purchased from NEW ENGLAND Biolabs Inc. (NEB). The heat-inactivated SARS-CoV-2 viral RNA was from ATCC (ATCC® VR-1986HK™). The synthetic human coronavirus RNA controls were purchased from Twist Bioscience (Twist Bioscience, 103011, 103012, and 103013). DNA gel loading dye (6X) was from NEB (#B7024S). 10X Cas12a reaction buffer (200 mM HEPES, 1 M NaCl, 50 mM $MgCl_2$ and 1 mM EDTA, PH6.5@25°C) was made at the lab. $MgCl_2$, NaCl, KCl, and Tris-EDTA-buffer solution (10 mM Tris-HCl and 1 mM EDTA) were purchased from Sigma-Aldrich. HEPES was from Gibco #15630-080. Agarose was from Bio-rad (#1613102). Ethidium Bromide (EB) was from Life Technologies (#15585011). DNA Ladder was from Thermo Scientific (#SM0311). Ag/AgCl wires electrodes were fabricated by using 0.2 mm Ag wires (Warner Instruments, Hamden, USA). Micro injectors of 34 gauge were purchased from World Precision Instruments. Piranha solution was made by mixing concentrated sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2). Quartz capillaries with inner and outer diameters of 0.5 mm and 1 mm were purchased from Sutter Instrument.

SARS-CoV-2 RT-PCR assay

We used the CDC primers targeting the N2 region of the SARS-CoV-2 (Forward primer: TTACAAACATTGGCCGCAAA, Reverse primer: GCGCGACATTCCGAAGAA) with a target product length of 67 bp. The 20 µl of the RT-PCR master mix consist of 5 µl of heat-inactivated SARS-CoV-2 viral RNA (for SARS-CoV-2 Cas12a assay validation 2×10^5 copies/µl, and for analytical sensitivity test different concentrations ranging from 2 to 200 copies/µl were used), 1.5 µl of primer/probe mix (50 µM forward primer, 50 µM reverse primer, 20 µM probes), 10 µl of qScript™ XLT One-Step RT-qPCR Tough Mix, and 3.5 µl of H_2O. It should be noted that different concentrations of RNA were prepared by diluting the stock concentration (2×10^5 copies/µl) with ultra-pure DNase/RNase-free distilled water. The RT-PCR was performed for 45 cycles in a Bio-

Rad CFX96 Deep Well Real-Time PCR detection system. Each cycle consists of 3 seconds denaturation step at 95 °C and 30 seconds annealing step at 55 °C.

SARS-CoV-2 Cas12a assay

The crRNA was synthesized by Integrated DNA Technologies, and its sequence was: 5'-UAAUUUCUACUCUUGUAGAUCCCCCAGCGCUUCAGCGUUC-3'. The crRNAs were resuspended in pH 7.5 buffer and stored in -80 ºC. Cas12a and crRNA were mixed in 1×PBS to form the non-activated Cas12a/crRNA at room temperature for 20 min and stored at -80ºC. In the cleavage reaction, the 2 µl of non-activated Cas12a/crRNA complex was mixed with the 1 µl of cDNA target in 14.5 µl of water, and 3.5 µl of a buffer consisting of 100 mM NaCl, 5 mM MgCl2, 20 mM HEPES, and 0.1 mM EDTA and incubated at 37ºC for 10 min for Cas12a activation. Afterward, ssDNA reporters were added and incubated at 37ºC for cleavage. After the reaction, results were examined both in agarose gel and in the nanopore sensor. For gel imaging, reactions were terminated with 4 µl of DNA loading dye (6X). The 24 µl mixture was loaded to EB-stained 1% (wt/vol) agarose gel for electrophoresis analysis. For nanopore analysis, reactions were terminated by adjusting the salt concentrations to 1 M KCl.

Glass nanopore fabrication and characterization

The quartz capillaries were cleaned in Piranha solution for 30 minutes, then rinsed with DI water, and dried in a vacuum oven at 120 ºC for 15 min. This process was performed to remove organic residues from quartz capillaries. A two-line recipe, (1) Heat 750, Filament 5, Velocity 50, Delay140, and Pull 50; (2) Heat 710, Filament 4, Velocity 30, Delay 155, and Pull 215, was used to pull the capillaries with a laser pipette puller (P-2000, Sutter Instruments, USA). A current-voltage experiment was performed to characterize the nanopores by changing the voltage from -400 to 400 mV. A typical nanopore used in this study has a diameter around 10 nm.

Nanopore sensing and data analysis

A constant voltage was applied across the glass nanopore by a 6363 DAQ card (National Instruments, USA). A transimpedance amplifier (Axopatch 200B, Molecular Device, USA) was used to amplify the resulting current. The amplified voltage was digitalized by the 6363 DAQ card at a 100 kHz sampling rate. Finally, a customized MATLAB (MathWorks) software was used to

analyze the current time trace and extract the single molecule translocation information (dwell time, current drop, and ECD).

Supplementary Figures

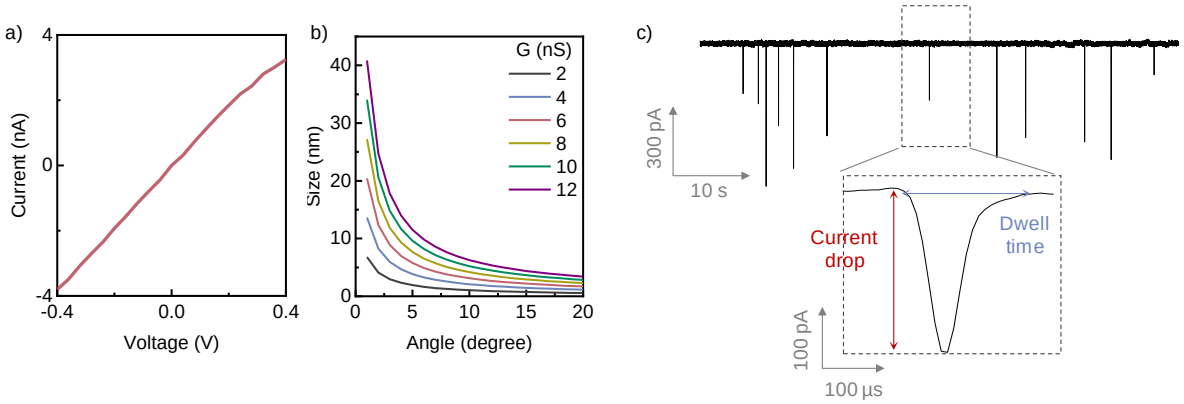


Figure S1. a) A typical I-V characterization of the glass nanopore. Based on the result, the average conductance of the nanopore was around 9.2 nS b) Estimation of the nanopore size based on its conductance (G) and half-cone angle (θ). While it is well known that there are variations in each fabrication due to the temperature and humidity changes, SEM and TEM characterization is needed to ensure the pore size and shape. However, since the TEM and SEM characterization of the glass nanopore is destructive in nature (high energy beams will melt the delicate structures at the tip), we often rely on the *in-situ* I-V characterization to infer the size of the nanopore. Based on the findings of Kowalczyk *et al.*[1] and Makra *et al.*[2], the conductance of a pore has a linear relationship with the pore diameter (D_p) as: $D_p = \frac{2G}{\sigma}\frac{4+\pi\,tan\theta}{4\pi\,tan\theta}$, where σ is the conductivity of the 1 M KCL solution (10.86 S/m). Therefore, one can estimate the nanopore size based on the angle and the conductance of the pore measured by the I-V characterization. In our system, for a pore with a half-cone angle between 3 to 6 degrees, the nanopore size would be between 5 to 10 nm. b) Demonstration of the event duration and blockage measurements from a typical ionic current trace of the nanopore experiment.

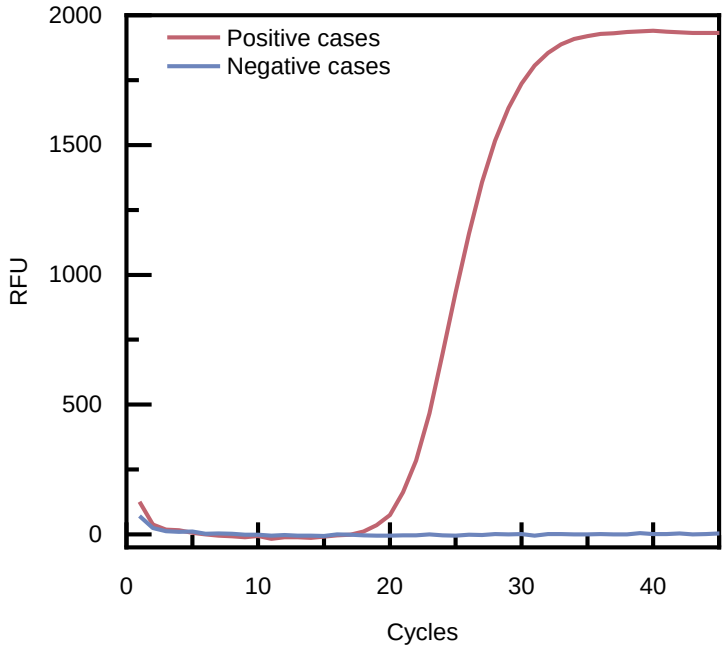


Figure S2. Relative fluorescence units (RFU) at different cycles of the RT-PCR. The RT-PCR was performed with an input of 2×10^5 copies/µl of SARS-CoV-2 viral RNAs for a duration of 45 cycles. The result of the fluorescent signal indicated the amplification after 20 cycles

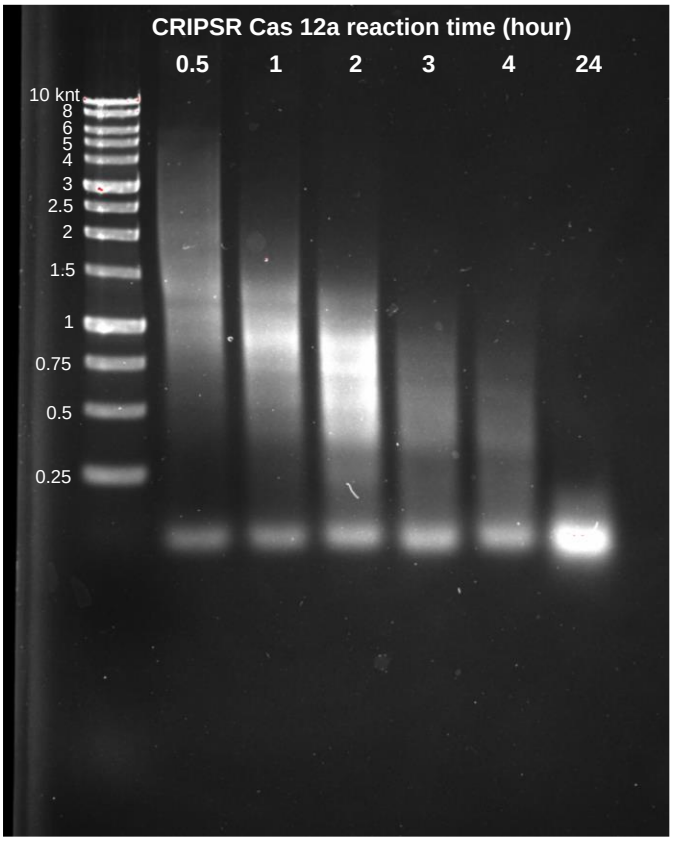


Figure S3. Gel electrophoresis results of the Cas12a assay products at five reaction times from 0.5 to 24 hours. All mother reporters were completely cleaved to be less than 250 nt after 24 hours. This suggests the trans-cleavage activities indiscriminately and continuously affect both the mother reporters and the partially cleaved daughter reporters.

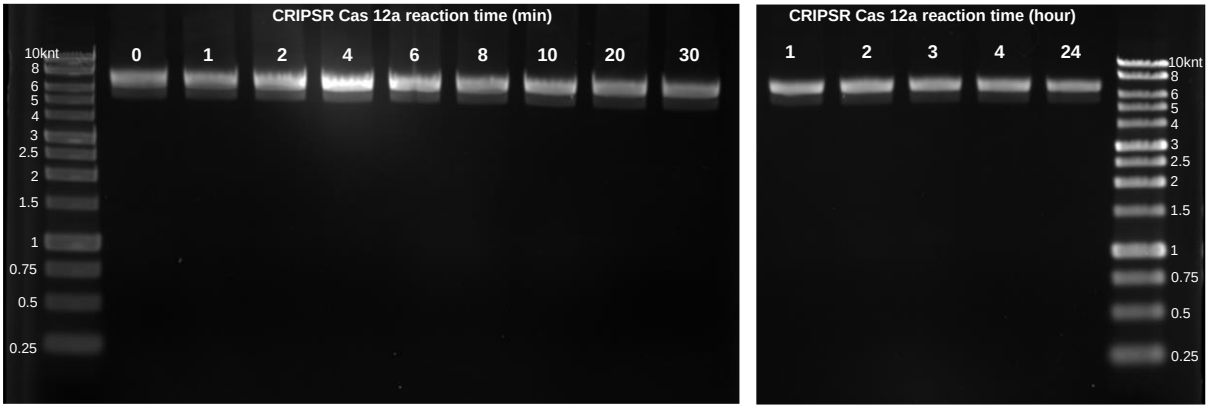


Figure S4. Gel electrophoresis results of the no target negative control samples at different Cas12a reaction times from 0 min to 24 hours. No Cleavage was observed in all reaction times, which confirms that daughter and granddaughter reporters would not be generated in no target negative control samples.

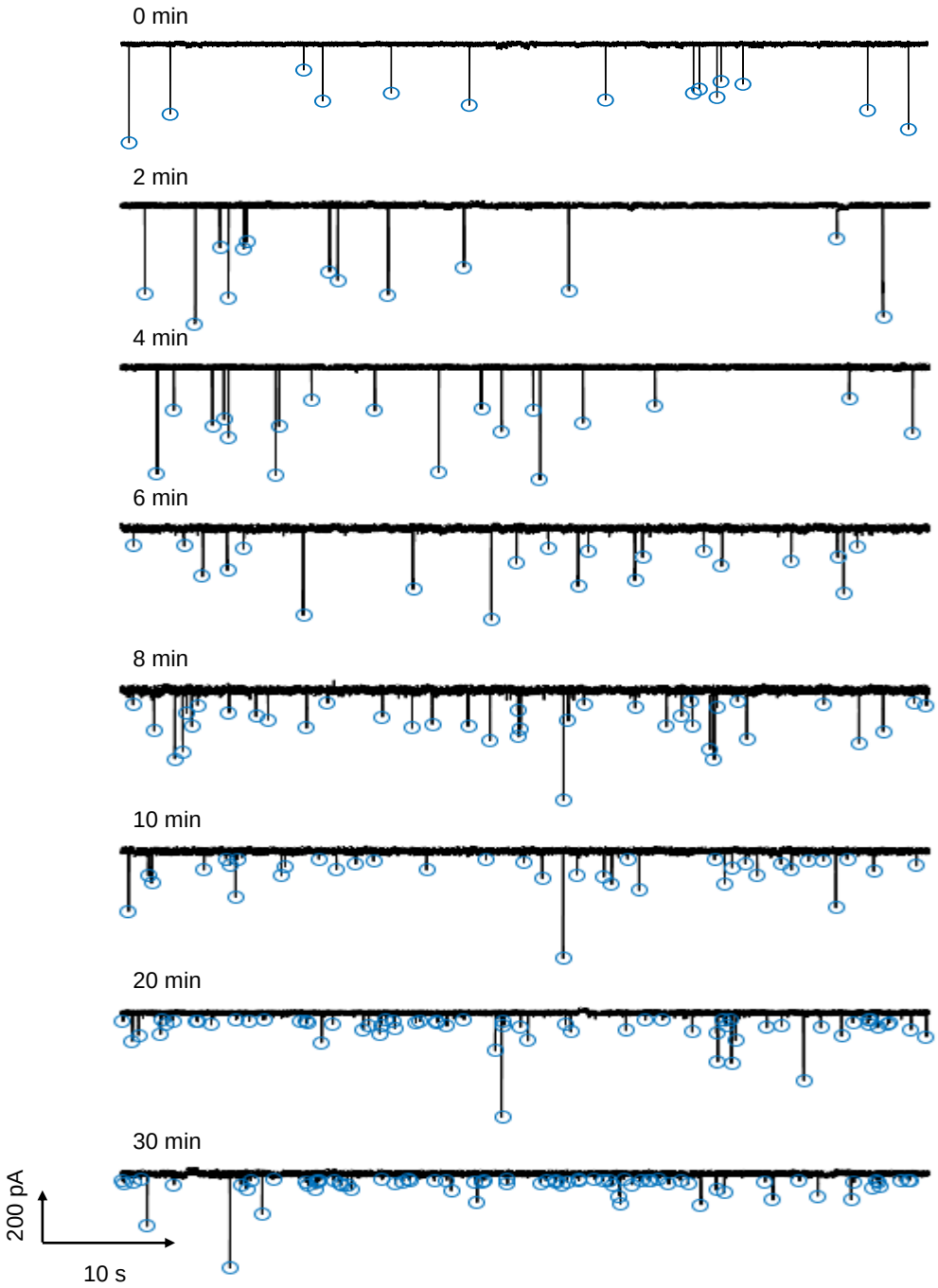


Figure S5. Translocation recording of ssDNA reporters at different reaction times from 0 to 30 minutes through the glass nanopore under 400 mV bias. The Cas12a and buffer salt concentrations were fixed as 30 nM and 1 M, respectively. As we increased the reaction time, more events were observed; however, the magnitude of the current drop of events become smaller.

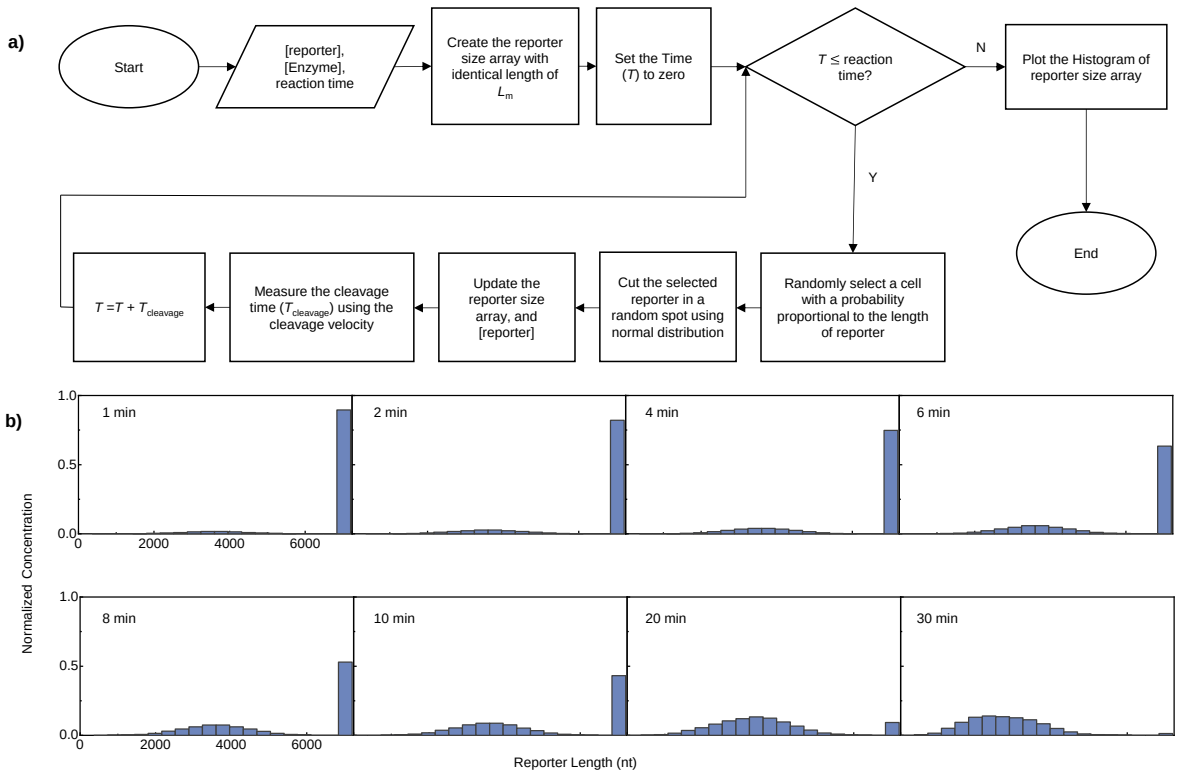
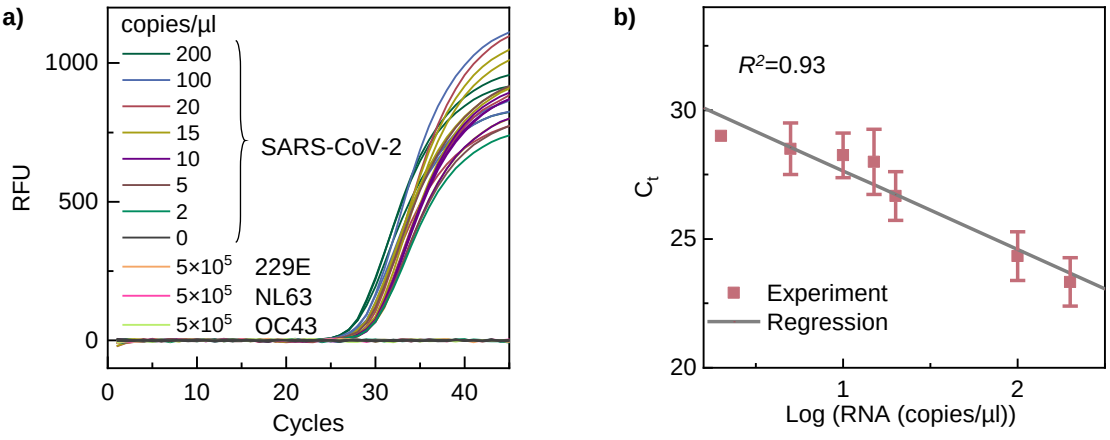


Figure S6. a) Algorithm and the model details for estimating the reporter length distribution over the trans-cleavage process. Initially, the reaction time, reporter, and enzyme concentration were specified. An array with an identical length of L_m (in this study L_m=7200) was created to present the mother reporter size. Time was set as zero, and the cleavage loops were started. In each loop, one cell of the reporter size array was randomly selected proportional to the length of the reporters. Afterward, the reporter in the selected cell was cleaved in a random spot using a normal distribution. After the cleavage, the reporter size array and reporter concentration was updated. The cleavage time was calculated by the cleavage velocity, and the total time was updated. The looped continued while the total time was less than the specified reaction time. Finally, after the cleavage process, the histogram of reporter size was plotted. b) The distribution of reporter length with an initial enzyme concentration of 30 nM over different reaction times from 1 to 30 minutes.



c)

	SARS COV-2								Human Coronavirus		
									229E	NL63	OC43
RNA (copies/µl)	200	100	20	15	10	5	2	0	5×10^5	5×10^5	5×10^5
Amplified Cases	3/3	3/3	3/3	5/5	4/5	2/5	1/5	0/5	0/3	0/3	0/3

Figure S7. a) The relative fluorescence unit (RFU) over different cycles of the RT-PCR process. The fluorescence results indicated that the limit of detection of RT-PCR assay was around 10 copies/µl, where 4 out of 5 cases were amplified. b) The threshold cycle (C_t) measurement at different starting RNA copies revealed that the SARS-CoV-2 could be quantitatively estimated by the real-time RT-PCR. The logarithmic regression for the C_t values over different input copies showed that the SARS-CoV-2 RNA in a sample could be estimated by C_t=*-3.052×log (copies)+32.829*. c) Summary of the amplification process for all the samples to evaluate the analytical sensitivity and specificity of SARS-CoV-2 SCAN.

Supplementary References

S1. Kowalczyk, S. W.; Grosberg, A. Y.; Rabin, Y.; Dekker, C., Modeling the conductance and DNA blockade of solid-state nanopores. *Nanotechnology* **2011,** *22* (31), 315101.

S2. Makra, I.; Gyurcsányi, R. E., Electrochemical sensing with nanopores: A mini review. *Electrochemistry communications* **2014,** *43*, 55-59.