Supplementary Materials

Utilizing cost-effective portable equipment to enhance COVID-19 variant tracking both on-site and at a large scale

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Control Run

The genomic RNAs of NY1-PV08001 (GenBank: MT370904.1), WI-CDC-03041142-001 (GenBank; MT039887.1), and CA-CDC-03039618-001 (GenBank: MT027062.1) were used for a control run. These genomic RNAs were provided by BEI Resources. In addition, remnant RNAs from specimens USA/CA-LAC-USC1 (GISAID: EPI_ISL_569664) and USA/CA-LAC-USC2 (GISAID: EPI_ISL_569665) were included in the control run, and both Pacbio HiFi sequencing and Sanger sequencing data were available for these specimens (1). We used the RT-PCR primers 1_LEFT/Rev-c for the first amplicon (31-10,267), 31.5_LEFT/67_RIGHT for the second (9,634-20,572), and 67_LEFT /98_RIGHT for the third (20,173-29,866) (Supplementary Table S3). The samples were then incubated at 50 °C for 10 min, followed by 98 °C for 2 min, then 40 cycles of 98 °C for 10 sec, 55 °C for 10 sec, and 72 °C for 5 min, with a final extension of 72 °C for 5 min, and 4 °C hold until the next step. The RT-PCR products were subsequently subjected to index PCR (1).

Each amplicon of NY1-PV08001, WI-CDC-03041142-001, and CA-CDC-03039618-001 were equimolarly pooled, size-selected using E-Gel™ 1% Agarose with SYBR™ Safe dye (Thermo Fisher Scientific) and purified by pre-made washed AMPure® XP Beads (Beckman Coulter). Each amplicon of USA/CA-LAC-USC1 and USA/CA-LAC-USC2 were purified in the same manner and equimolarly pooled. A total of 450 ng of the size-selected and purified genomic RNA pool was then added to 100 ng of USA/CA-LAC-USC1 and USA/CA-LAC-USC2 pool, yielding a total of 550 ng in 47 µl. The pooled library was then subjected to Oxford Nanopore MinION library preparation using a Ligation Sequencing kit V14 (Oxford Nanopore Technologies) and NEBNext® Companion Module (NEB) as described above and sequenced via the MinION Mk1b sequencer using the R10.4.1 flow cell (Oxford Nanopore Technologies) for 72 hours.

The reference sequences of three genomic RNA strains differed from the sequences in GenBank, with NY1-PV08001 having one mutation, WI-CDC-03041142-001 having two mutations and 9 deletions, and CA-CDC-03039618-00 having one mutation. We confirmed these differences through Sanger sequencing (Supplementary Figure S1). The reference sequence of USA/CA-LAC-USC2 had two mutations in comparison to the Pacbio HiFi sequencing data. These mutations were verified by the presence of double peaks in the Sanger chromatogram data (Supplementary Figure S2).

Phylogenetic tree analysis

The phylogenetic trees were built using MAFFT (version 7.392)(2) and FastTree (version 2.1.8) (3). The sequence obtained by our portable system (TU5327) was aligned with 813 sequences collected in December 2022 in Los Angeles, California, USA using MAFFT (version 7.392) (2).The Los Angeles sequences were retrieved from the GISAID (4), filtered by location "North America / USA / California / LOS ANGELES" and collection date between December 1st, 2022 and December 31st, 2022. The resulting alignment was used as input for FastTree (version 2.1.8) (3) to construct the maximum likelihood phylogenetic tree, which was then visualized using FigTree (version 1.4.4).

We aligned the 289 SARS-CoV-2 whole genome sequences with the reference sequence Wuhan-Hu-1 (MN908947), as well as with representative sequences of the Alpha (GISAID: EPI_ISL_751801, EPI_ISL_802601, EPI_ISL_802614, EPI_ISL_802617 and EPI_ISL_802618) Beta (EPI_ISL_1011702, EPI_ISL_890360, EPI_ISL_911874, EPI_ISL_911912 and EPI_ISL_955107), Gamma (EPI_ISL_1493886, EPI_ISL_1555711, EPI_ISL_1557003, EPI_ISL_1576115 and EPI_ISL_1613185) Delta (EPI_ISL_1562503 EPI_ISL_1614854,

EPI_ISL_1666166, EPI_ISL_1666372 and EPI_ISL_1666373) and Omicron variants (EPI_ISL_10084178, EPI_ISL_10084186, EPI_ISL_10722651, EPI_ISL_11342896 and EPI_ISL_8290981) and TU5327 using MAFFT (2). The maximum likelihood of all these sequences was also obtained by FastTree (3) and FigTree. The variant composition was compared with that of 1,918 sequences which were downloaded from the GISAID database (4). Samples were filtered by location "North America / USA / California / LOS ANGELES" and collection date between December 1st, 2020 and December 31st, 2020.

Sample	Collection date	Cycle threshold	PANGO lineage
JI4231	12/30/20	14.32	B.1.429
WT9794	12/5/20	15.54	B.1.429
HP7395	12/30/20	19.49	B.1.2
DI1136	12/22/20	18.41	B.1.240
HW8248	12/30/20	17.78	B.1.232
JB3588	12/18/20	19.97	B.1.2
WM7065	12/30/20	17.7	B.1.2
BT9716	12/23/20	17.19	B.1.2
HK5693	12/23/20	12.62	B.1.599
VL0369	12/23/20	14.1	B.1.401
JY5514	12/23/20	19.63	B.1.36.8
BJ9010	12/23/20	16.4	B.1.2
SD0040	12/24/20	19.47	B.1.399
AY5172	12/26/20	14.34	B.1.2
PA1164	12/26/20	15	B.1.369
DY5573	12/27/20	18.15	B.1.2
DC9155	12/26/20	14.44	B.1.427
RP9716	12/29/20	17.28	B.1.2
VX9670	12/29/20	14.98	B.1.429
PI0188	12/29/20	19.54	B.1.243
ZY3992	12/29/20	21.39	B.1.429
ZB9727	12/29/20	18.24	B.1.400
JX2247	11/30/20	16.02	B.1.558
LP5275	11/22/20	15.81	B.1.2
ER2730	11/23/20	21.05	B.1.429
IO7000	12/18/20	17.94	B.1.429
AM7038	12/20/20	20.56	B.1.429
OI1405	12/23/20	13.94	B.1.429
ST2570	12/21/20	21.64	B.1.429
DR5866	11/30/20	20.1	B.1.558
CZ6308	12/22/20	13.73	B.1.2
VI9997	12/22/20	16.3	B.1.429
UV5412	12/13/20	15.65	B.1
BK9318	1/2/21	15.59	B.1.1.368
NU1669	12/14/20	15.22	B.1.427
XC3154	12/14/20	15.17	B.1.1.416
YG2693	12/14/20	14.66	B.1.1.1
NJ3527	12/21/20	17.58	B.1.429

LQ2216	12/22/20	21.49	B.1.2
GU3382	1/5/21	15.71	B.1.2
IE8550	1/5/21	12.47	B.1
MD0767	12/22/20	19.36	B.1.1.519
XT7543	1/5/21	19.51	B.1
SF8699	1/5/21	15.23	B.1.429
UK3603	1/4/21	19.04	B.1.2
AJ3420	1/2/21	19.49	B.1.2
IG1625	12/18/20	15.87	B.1.232
TG0776	12/8/20	16.43	B.1.609
TX1840	12/17/20	19.53	B.1.349
EM5221	12/20/20	17.72	B.1.429
PP4204	12/18/20	14.02	B.1.1
FW3867	12/20/20	18.71	B.1.1
UZ0290	12/20/20	19.21	B.1.2
VI5908	12/21/20	12.08	B.1.429
AQ6479	12/20/20	13.74	B.1.1.368
AE8847	12/23/20	15.68	B.1.2
FT0593	1/5/21	21.23	B.1.1.222
PU3006	12/14/20	15.45	B.1.599
OH7436	12/28/20	16.84	B.1.609
ZY8808	12/26/20	19.47	C.23
BT5819	11/23/20	22.33	B.1.429
BA9193	11/30/20	17.21	B.1.596
SJ9593	11/30/20	20.69	B.1.509
AQ1668	12/29/20	18.72	B.1.429
OL0708	12/29/20	22.41	B.1.429
QR8696	1/3/21	19.41	B.1.2
AZ9519	12/31/20	12.89	B.1.429
SH0093	12/24/20	14.02	B.1.429
OF5260	12/14/20	14.18	B.1.429
RC7357	1/5/21	13.2	B.1.429
TL0675	1/5/21	15.87	B.1.427
CZ0687	12/14/20	16.98	B.1.1
JB6682	12/14/20	16.2	B.1.429
HB7777	12/14/20	19.54	B.1.2
MV2051	12/25/20	15.11	B.1.240
NZ1546	12/14/20	19.2	B.1.240
BE3032	1/1/21	19.68	B.1.2
HF6439	12/22/20	19.53	B.1.243

AC7433	12/14/20	12.44	B.1.551
TL1929	12/25/20	17.07	B.1.243
XB2178	12/6/20	19.24	B.1
AU4629	12/5/20	21.88	B.1.2
GM1395	12/19/20	14.93	B.1.561
WK0961	12/30/20	18.97	B.1.2
SG7359	12/24/20	20.95	B.1.243
DN9420	12/27/20	19.27	B.1.429
ZR6613	12/24/20	17	B.1.429
LZ3639	12/18/20	15.89	B.1.2
DH6456	12/29/20	24.61	B.1.369
JF9721	12/18/20	19.86	B.1.239
FP1386	12/28/20	15.65	B.1.561
ZD3133	12/31/20	21.97	B.1.429
MA6919	1/5/21	17.92	B.1.2
YT2631	12/28/20	18.43	B.1.427
JS2952	1/5/21	17.61	B.1.1.519
WG5887	1/5/21	16.11	B.1.429
XB2794	12/18/20	15.26	B.1.2
VD6385	11/25/20	13.33	B.1.243
CB3852	11/30/20	18.77	B.1.2
VW7371	12/28/20	15.08	B.1.429
JV9281	12/29/20	18.04	B.1.429
AE4035	12/28/20	22.05	B.1.609
IT0677	12/21/20	16.24	B.1.369
VJ4966	12/21/20	16.92	B.1.232
XZ4820	12/21/20	16.87	B.1.2
YU9159	12/22/20	17.19	B.1.429
YC7172	12/21/20	19.85	B.1.427
TH3913	12/22/20	15.85	B.1.1.368
TH6141	12/22/20	20.97	B.1.427
KO6851	12/17/20	16.01	B.1.369
LL2086	12/15/20	19	B.1.509
WV6072	12/20/20	20.49	B.1.243
SY3098	12/21/20	21.44	B.1.2
XS8393	12/15/20	14.45	B.1.239
ZT2608	12/29/20	14.24	B.1.427
JG6758	11/25/20	14.92	B.1.429
JR7525	12/29/20	19.53	B.1.429
BM0491	12/14/20	17.64	B.1.232

ZP1354	12/14/20	15.94	B.1.429
UY1184	1/2/21	18.2	B.1.429
IR5477	12/14/20	13.78	B.1.599
MU8683	12/13/20	18.12	B.1.599
RY9761	1/1/21	19.34	B.1.234
BB6627	12/13/20	19.22	B.1.429
DK0381	12/24/20	11.97	B.1.2
ZD7446	12/24/20	19.21	B.1.429
LH8066	12/22/20	18.76	B.1.2
FH3885	12/24/20	17.39	B.1.2
UQ0627	12/29/20	19.47	B.1.429
WP7246	12/21/20	12.16	B.1.429
UI5090	1/4/21	14.4	B.1.429
KQ8979	12/19/20	16.37	B.1.429
LS1200	12/19/20	14.03	B.1.429
GD7333	12/5/20	16.34	B.1.1.222
BY6561	12/19/20	15.3	B.1.427
GW7911	12/31/20	15.01	B.1.1.432
GE5311	1/1/21	14.47	B.1.243
LG9653	12/15/20	18.34	B.1.2
JG8980	12/28/20	16.62	B.1.429
UH0983	1/5/21	18.31	B.1.558
LM6779	12/28/20	17.98	B.1.241
ZR9776	12/21/20	15.83	B.1.2
XE8272	12/15/20	21.48	B.1.429
YP5963	12/24/20	17.81	B.1.429
BU6467	12/21/20	18.48	B.1.1.239
VE8901	12/21/20	23.06	B.1.599
VY0153	12/18/20	16.72	B.1.429
WU7415	12/21/20	16.1	B.1.2
JX8470	12/21/20	19.46	B.1.2
ER9825	12/14/20	16.5	B.1.36.8
HX9272	12/24/20	16.33	B.1.126
GE6816	12/29/20	14.01	B.1.427
SY0553	12/29/20	18.87	B.1.240
TN1225	12/23/20	17.51	B.1.401
RT5697	11/23/20	16.73	B.1.1.228
TL6974	12/28/20	20.17	B.1.2
BK9278	12/29/20	16.57	B.1.429
YM6123	12/22/20	14.92	B.1.429

GA6643	12/14/20	15.69	B.1.602
EI0462	12/29/20	21.67	B.1.400
KM8670	12/15/20	21.15	B.1.429
SB8937	12/29/20	21.38	B.1.429
WB5319	11/30/20	18.64	B.1.234
LG1147	12/6/20	16.22	B.1.243
EQ4052	12/25/20	16.77	B.1.599
RT8658	12/23/20	18.57	B.1.126
YE0245	1/1/21	11.63	B.1.2
JY7656	12/24/20	15.47	B.1.2
CC3805	12/15/20	11.96	B.1.427
BG0216	12/23/20	18.25	B.1.429
RZ5476	12/23/20	21.19	B.1.429
FH1865	12/25/20	19.56	B.1.429
OY4586	12/5/20	20.64	B.1.2
OK0835	12/24/20	18.08	B.1.2
CA3649	12/23/20	20.23	B.1.240
FQ3697	12/29/20	16.9	B.1.243
JA4776	12/20/20	17.08	B.1.429
GU2976	12/24/20	17.36	B.1
RY2179	12/5/20	19.81	B.1.2
WE4882	12/22/20	16.73	B.1.2
PV3046	12/15/20	17.24	B.1.427
NH4027	12/22/20	13.86	B.1
EG7205	12/22/20	15.03	B.1.2
IY0010	12/21/20	16.46	B.1.427
EI9700	12/24/20	15.04	B.1
LH4400	12/18/20	15.83	B.1.609
ZT8055	12/25/20	15.33	B.1.429
OM4070	12/23/20	18.53	B.1.429
KP9073	1/5/21	18.06	B.1.427
SG2753	12/20/20	17.18	B.1.429
YF7950	11/22/20	14.97	B.1.429
CL5555	12/21/20	18.51	B.1.609
DD8748	1/3/21	17.84	B.1.2
HV1419	12/29/20	23.23	B.1.561
TR3003	12/24/20	17.32	B.1.2
CZ6426	12/29/20	13.99	B.1
KF5703	1/5/21	22.77	B.1.429
ZI1686	12/25/20	21.88	B.1.429

RE5900	12/14/20	19.31	B.1.1.368
BQ1903	12/21/20	11.17	B.1.429
MP9758	12/29/20	21.95	B.1.400
IZ7944	12/23/20	15.6	B.1.126
QI9870	12/13/20	14.76	B.1.2
HK8609	12/24/20	13.72	B.1.2
VH6355	12/14/20	14.26	B.1.429
GC0577	1/3/21	12.96	B.1.429
AN4673	12/18/20	14.55	B.1.2
CO5183	12/25/20	14.69	B.1.126
TN0136	12/25/20	13.15	B.1.429
SB9300	12/24/20	14.72	B.1.1.316
AP1804	12/29/20	19.8	B.1.2
MJ2769	12/28/20	17.17	B.1.2
ZW9807	12/27/20	15.15	B.1.429
DV4052	12/26/20	15.05	B.1.429
ZY7185	12/26/20	20.34	B.1.429
RM9602	1/5/21	24.24	B.1.429
YZ4434	12/31/20	18.45	B.1.1.192
WX7713	12/22/20	16.2	B.1.429
NF7093	12/23/20	19.05	B.1.1
GO2530	12/22/20	18.49	B.1.349
SS5859	1/5/21	20.84	B.1.2
WJ1864	12/22/20	17.68	B.1.2
WQ5202	12/22/20	14.37	B.1.429
OW1668	12/22/20	16.83	B.1.429
NJ1057	12/22/20	17.04	B.1.561
QN1684	12/23/20	14.7	B.1.240
LF5356	12/23/20	16.56	B.1.429
HW7458	12/28/20	14.62	B.1.429
IY3432	11/23/20	15.96	B.1.1.207
MF6738	12/25/20	13.86	B.1.234
KR9652	12/29/20	14.51	B.1.397
TX5558	12/20/20	20.32	B.1.2
RO7972	11/23/20	17.36	B.1.561
WO4840	1/4/21	16.82	B.1.243
NB3224	11/30/20	17.44	B.1.2
IR7746	12/24/20	19.94	B.1.1.518
FH0323	12/23/20	14.57	B.1.240
WU1409	12/15/20	17.89	B.1.1

KF9092	12/26/20	14.98	B.1.429
SJ7550	12/26/20	18.84	B.1.429
NB3353	12/26/20	16.64	B.1.2
FK2454	12/27/20	19.38	B.1.429
CS9782	12/18/20	18.65	B.1.1.186
LA0793	12/18/20	15.41	B.1.429
YF3796	12/22/20	18.66	B.1.1.207
OB9346	1/5/21	17.35	B.1.2
VZ6626	12/15/20	14.34	B.1.2
WO8999	12/23/20	11.79	B.1.1.519
LB5198	12/23/20	14.74	B.1.429
CX9666	12/15/20	19.68	B.1.429
JS3494	12/15/20	19.31	B.1.427
GS2239	12/17/20	12.73	B.1.126
YV9735	12/22/20	16.41	B.1
FS8456	12/22/20	12.07	B.1.1
SA0118	12/31/20	17.19	B.1.429
KI6491	12/24/20	19.37	B.1.577
KB9241	12/8/20	21.94	B.1.429
IZ4921	1/5/21	15.49	B.1.400
VX0120	12/20/20	15	B.1.2
ZU5381	1/2/21	16.63	B.1.2
LB1380	12/8/20	17.65	B.1.2
UQ7244	1/5/21	16.28	B.1.429
BQ7044	12/21/20	14.97	B.1
XZ0118	12/24/20	15.58	B.1.2
BU9246	12/18/20	14.42	B.1.2
RS1309	12/26/20	17.2	B.1.126
BA2093	12/30/20	19.39	B.1.2
ZN5315	12/26/20	20.21	B.1.429
NY0181	12/15/20	17.88	B.1.429
JB8183	12/23/20	18.91	B.1.240
DC1144	12/20/20	17.57	B.1.2
ME8933	12/24/20	19.49	B.1.421
UO1382	12/22/20	18.05	B.1.2
NN8987	12/29/20	14.52	B.1
MD0278	12/29/20	18.64	B.1.2
KG5869	12/29/20	14.5	B.1.429
NA5237	12/29/20	16.36	B.1.429
NG5256	12/29/20	17.89	B.1.429

AI7563	12/29/20	16.73	B.1.400
DA4871	12/22/20	20.69	B.1.2
XU6832	12/21/20	21.02	B.1.429
TZ2082	12/5/20	20.6	B.1.1.432
PF7567	12/13/20	14.67	B.1.2
AQ3327	12/14/20	16.4	B.1.429
TZ9744	12/29/20	16.63	B.1.429
XT4197	12/13/20	14.85	B.1.2
TN6172	12/29/20	24.2	B.1.2
RE3176	12/29/20	22.96	B.1.429
PF5717	12/21/20	17.17	B.1.429

Supplementary Table S1. COVID-19 remnant NO/OP specimens' collection date, cycle threshold, and PANGO lineage (5).

Number	Name	Company (Country)
1	Biohazard waste container	ULINE (USA)
2	Filtered pipette tips (1 to 20 µL)	USA Scientific (USA)
2	Filtered pipette tips (1 to 200 µL)	USA Scientific (USA)
2	Filtered pipette tips (100 to 1000 µL)	USA Scientific (USA)
3	Nitrile Exam Gloves	Fisher Scientific (USA)
4a	96-well PCR Plate	ThermoFisher Scientific (USA)
4b	DynaMag™-2 Magnet	ThermoFisher Scientific (USA)
5	Magnetic Stand-96	ThermoFisher Scientific (USA)
6	Nuclease-Free Water (not DEPC-Treated)	ThermoFisher Scientific (USA)
7	Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit	ThermoFisher Scientific (USA)
8	80-well 1.5 mL tube rack	VWR International (USA)
8	DNA LoBind® 1.5 mL tubes	Eppendorf (Germany)
8	Qubit™ Assay Tubes	ThermoFisher Scientific (USA)
8	QIAamp Viral RNA Mini Kit	Qiagen (Maryland, USA)
8	Ethyl alcohol, Pure, 200 proof, for molecular biology	Sigma-Aldrich (Missouri, USA)
8	Mineral Oil, BioReagent, for molecular biology, light oil	Sigma-Aldrich (Missouri, USA)
8	RT-PCR Primers	Integrated DNA Technologies (New Jersey, USA)
8	Agencourt AMPure® XP Beads	Beckman Coulter (California, USA)
9	Cooling Chamber	Diversified Biotech (Massachusetts, USA)
9	Nordic Ice Gel Pack	Nordic Cold Chain Solutions (Pennsylvania, USA)
9	0.2 mL PCR tubes	USA Scientific (Florida, USA)
9	SuperScript™ IV One-Step RT-PCR System	ThermoFisher Scientific (Massachusetts, USA)
10	Westinghouse iGen300s Portable Power Station	Westinghouse Electric Corporation (Pennsylvania, USA)
11	Tabletop Centrifuge	Fisher Scientific (Massachusetts, USA)
12	Tabletop Vortex Mixer	Fisher Scientific (Massachusetts, USA)
13	Qubit™ 4 Fluorometer	ThermoFisher Scientific (Massachusetts, USA)
14	Laptop (Alienware m15 R7 i9 12900H 3070Ti(8GB) 32GB DDR5 1TB SSD)	Dell Technologies (Texas, USA)
15	Finnpipette™ F2 (1 to 10 µL)	ThermoFisher Scientific (Massachusetts, USA)
15	Finnpipette™ F2 (10 to 100 µL)	ThermoFisher Scientific (Massachusetts, USA)
15	Finnpipette™ F2 (100 to 1000 µL)	ThermoFisher Scientific (Massachusetts, USA)
16	PocketPCR Machine	GaudiLabs LLC (Luzern, Switzerland)

16	USB Type-C to USB-A cable	Amazon (Washington, USA)
17	Ligation Sequencing Kit V14	Oxford Nanopore Technologies (Oxford, UK)
17	NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing	New England Biolabs (Massachusetts, USA)
17	UltraPure™ BSA (50 mg/mL)	ThermoFisher Scientific (Massachusetts, USA)
18	Flow Cell Wash Kit	Oxford Nanopore Technologies (Oxford, UK)
19	MinION Sequencing Device Mk1b	Oxford Nanopore Technologies (Oxford, UK)
19	Flow Cell (R10.4.1)	Oxford Nanopore Technologies (Oxford, UK)

Supplementary Table S2. Components of portable laboratory for COVID-19 variant tracking.

Name	Direction	Position	Sequence
1_LEFT-IF11	Forward	31-54	AGTTAGTGATTAACCAACCAACTTTCGATCTCTTGT
Rev-c-IR1	Reverse	10,244-10,267	GGTGTCTTTGTGACCAGCCTGTACCAAGAAATTATG
31.5_LEFT-IF11	Forward	9,634-9,658	AGTTAGTGATTAAGCACATATTCAGTGGATGGTTATG
67_RIGHT-IR1	Reverse	20,543-20,572	GGTGTCTTTGTGCAACCTTAGAAACTACAGATAAATCTTGGG
67_LEFT-IF11	Forward	20,173-20,200	AGTTAGTGATTAGTTGTCCAACAATTACCTGAAACTTACT
98_RIGHT-IR1	Reverse	29,837-29,866	GGTGTCTTTGTGTTCTCCTAAGAAGCTATTAAAATCACATGG
1_LEFT	Forward	31-54	ACCAACCAACTTTCGATCTCTTGT
For-c	Forward	381-404	TATCAGAGGCACGTCAACATCTTA
Rev-c	Reverse	10,244-10,267	ACCAGCCTGTACCAAGAAATTATG
31.5_LEFT	Forward	9,634-9,658	AGCACATATTCAGTGGATGGTTATG
67_RIGHT	Reverse	20,543-20,572	CAACCTTAGAAACTACAGATAAATCTTGGG
67_LEFT	Forward	20,173-20,200	GTTGTCCAACAATTACCTGAAACTTACT
98_RIGHT	Reverse	29,837-29,866	TTCTCCTAAGAAGCTATTAAAATCACATGG
91_LEFT-mod	Forward	27,449-27,469	ACTACCAAGAGTGTGTTAGAG
39_LEFT-mod	Forward	11,562-11,584	GCCCTATTTTCTTCATAACTGGT
73_LEFT-mod	Forward	21,969-21,991	GTAATGATCCATTTTTGGGTGTT
36_LEFT	Forward	10,666-10,688	ACACACCACTGGTTGTTACTCAC
78_LEFT	Forward	23,443-23,466	CAACTTACTCCTACTTGGCGTGT
34_LEFT	Forward	10,076-10,099	TCCCATCTGGTAAAGTTGAGGGT

Supplementary Table S3. Primers' direction, position and sequence.

Country	A.2.5 sequences in GISAID(4)	Sequenced cases (4)	Confirmed cases (6, 7)	Estimated A.2.5 cases with 95% confidence interval	Proportion of A.2.5 sequences
Czech Republic	1	3,398	924,616	272 [0 – 805]	0.03%
Guyana	1	3	11,737	3,912 [0 – 10,173]	33.33%
Honduras	1	29	122,721	4,232 [0 – 12,382]	3.45%
Japan	1	28,568	532,868	19 [0 – 55]	0.00%
Netherlands	1	28,225	857,953	30 [0 – 90]	0.00%
New Zealand	1	217	527	2 [0 – 7]	0.46%
Rwanda	1	124	19,093	154 [0 – 455]	0.81%
Sint Maarten	2	180	794	9 [0 - 21]	1.11%
Australia	3	883	1,775	6 [0 – 13]	0.34%
Luxembourg	3	5,288	23,568	13 [0 – 29]	0.06%
Portugal	3	5,774	432,870	225 [0 – 479]	0.05%
Suriname	3	72	11,522	480 [0 – 1,012]	4.17%
Colombia	4	382	2,039,827	21,359 [537 – 42,181]	1.05%
Dominican Republic	4	36	134,882	14,987 [1,140 – 28,834]	11.11%
Spain	4	20,437	1,835,956	359 [7 – 712]	0.02%
Germany	6	118,506	1,949,303	99 [20 – 178]	0.01%
Paraguay	6	133	279,338	12,602 [2,748 – 22,455]	4.51%
Chile	7	1426	853,090	4,188 [1,093 – 7,282]	0.49%
United Kingdom	9	261,973	1,958,705	67 [23 – 111]	0.00%
Argentina	9	874	2,463,496	25,368 [8,880 – 41,856]	1.03%
Ecuador	14	338	222,608	9,220 [4,492 – 13,949]	4.14%
Mexico	17	7875	1,011,635	2,184 [1,147 – 3,221]	0.22%
Canada	41	21,551	812,077	1,545 [1,073 – 2,017]	0.19%
Italy	45	24,610	2,102,281	3,844 [2,722 – 4,966]	0.18%
Cuba	59	620	144,013	13,705 [10,378 – 17,031]	9.52%
Costa Rica	132	356	138,526	51,364 [44,413 – 58,314]	37.08%
Panama	138	160	136,536	117,762 [110,477 – 125,048]	86.25%
US	529	359,148	13,146,210	19,364 [17,715 – 21,021]	0.15%

Supplementary Table S4. Countries with A.2.5 cases between January and June 2021.
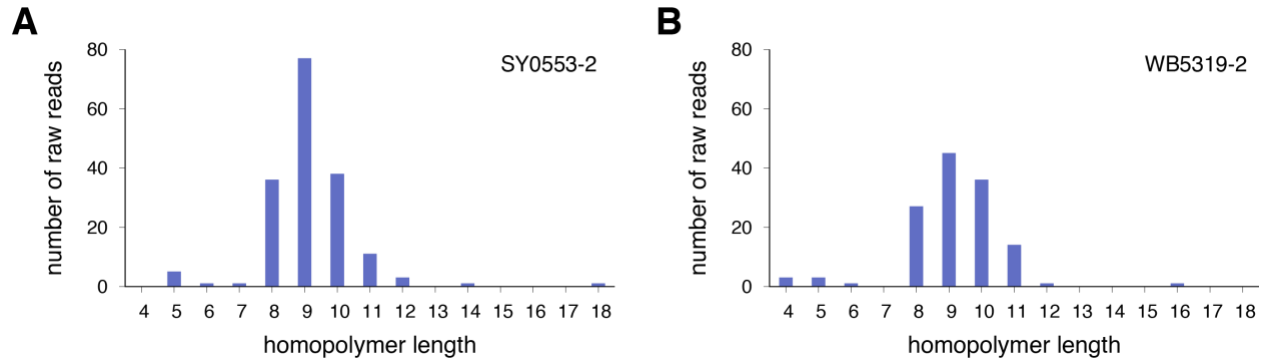
Supplementary Figure S1. Homopolymer length distributions and error corrections
(A) For the second segment of SY0553, the homopolymer sequence 'TCTTTTTTTTTATG', with a length of 9, was the most frequently observed, occurring in 77 raw reads. The number of raw reads with homopolymer lengths 10, 11, 12 were 38, 11, and 3, respectively. Given that the total for these lengths, 52 (sum of 38, 11, 3), exceeds 55% of 77 (=42.4), the homopolymer length of the consensus sequence was assigned as 10, resulting in the sequence 'TCTTTTTTTTTTATG'. (B) The most frequent length the same homopolymer 'TCTTTTTTTTTATG' within the second segment WB5319 was 9 with 45 raw reads. The number of raw reads with homopolymer lengths 10, 11, 12 were 36, 14, and 1, respectively. Therefore, the homopolymer length was assigned as 10, resulting in the sequence 'TCTTTTTTTTTTATG'.
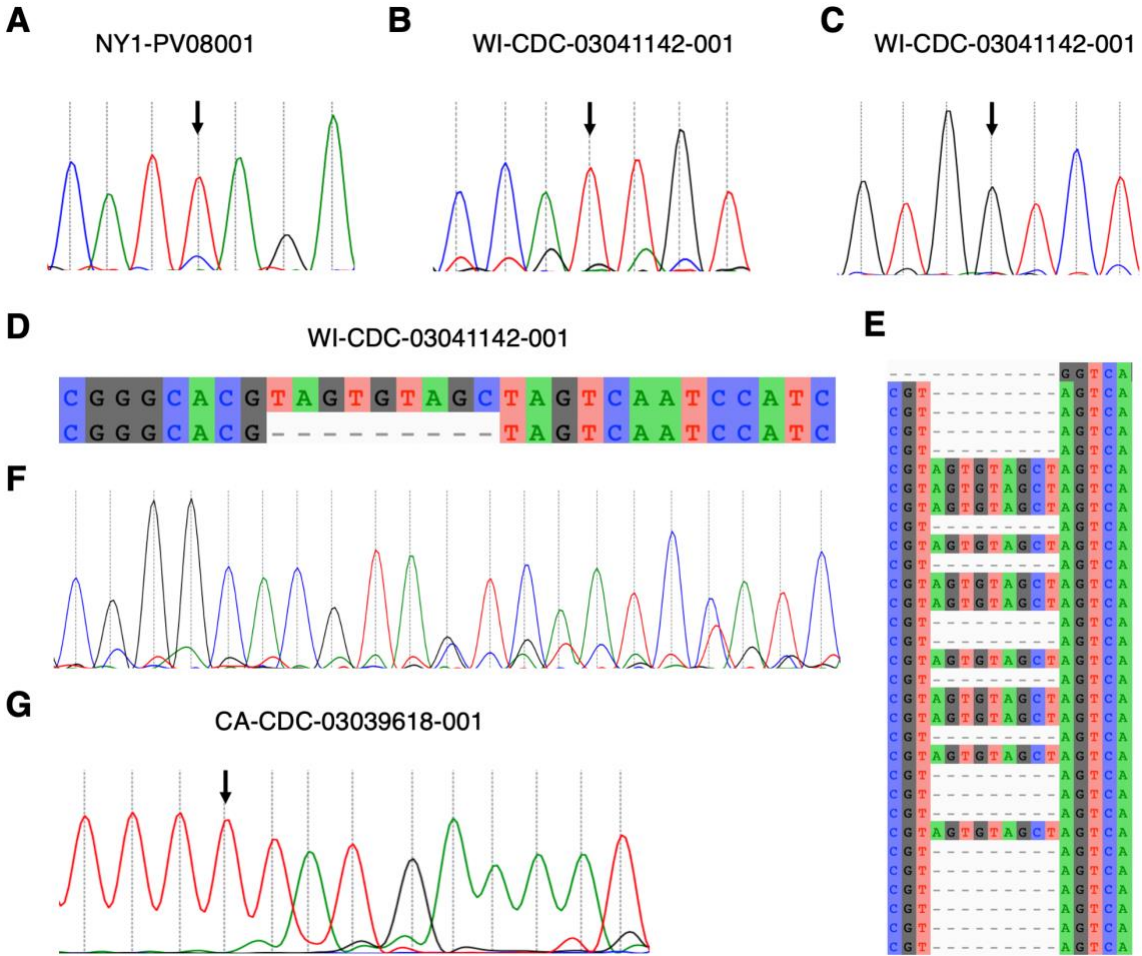
Supplementary Figure S2. Sanger chromatogram of control genomic RNAs
(A) Sanger sequencing chromatogram data for the genomic RNA of NY1-PV08001 (GenBank: MT370904.1), plotted by UGENE. Each of nucleotide bases, A, T, G, and C is represented by green, red, black and blue, respectively. The nanopore sequencing data showed a single base substitution (CAT**T**AGA) in comparison to the GenBank reference sequence (CATCAGA). The observed base substitution was confirmed by Sanger sequencing, as indicated by the arrow on the chromatogram. (B) The nanopore sequencing data showed a base substitution (CCA**T**TGT) in comparison to the GenBank reference sequence (CCACTGT) for the genomic RNA of WI-CDC-03041142-001 (GenBank; MT039887.1). This substitution was confirmed by Sanger sequencing (black arrow). (C) Base substitution (GTG**G**TCT), compared to the GenBank reference (GTGATCT) for the genomic RNA of WI-CDC-03041142-001. This substitution was also confirmed by Sanger sequencing, as shown by the arrow. (D) The nanopore sequencing data (bottom) showed 9 nucleotide base deletions (TAGTGTAGC) in comparison with the GenBank reference sequence (top) for the genomic RNA of WI-CDC-03041142-001, plotted by AliView. (E) The nanopore sequencing data of WI-CDC-03041142-001 showed a mixture of raw reads with and without the 9 bases deletions. (F) Sanger sequencing chromatogram peaks confirmed the mixture of the 9 nucleotide deletions (CGGGCACGTAGTCAATCCATC) and non-deletions (CGGGCACGTAGTGTAGCTAGT). (G) Sanger sequencing confirmed the nanopore sequencing data's base substitution (TTT**T**TATGAAAAT) in comparison to the GenBank reference (TTTTGTATGAAAAT) for the genomic RNA of CA-CDC-03039618-001 (GenBank; MT039887.1).
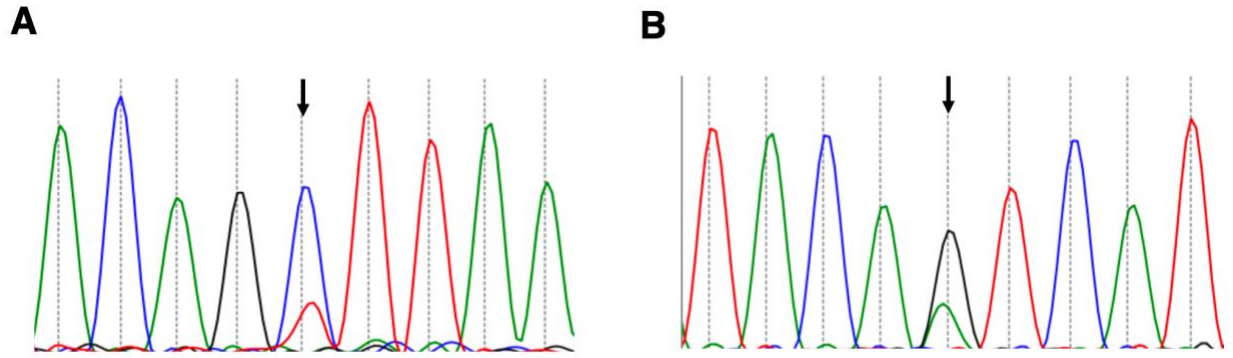
Supplementary Figure S3. Sanger chromatogram of USA/CA-LAC-USC2
(A) The nanopore sequencing data showed a base substitution (ACAG**T**TTAA) in comparison to the Pacbio HiFi reference sequence (ACAGCTTAA) for specimen USA/CA-LAC-USC2 (GISAID: EPI_ISL_569665). Sanger chromatogram data showed the mixture of C and T bases at this position, as marked by the arrow. (B) Second base substitution (TACA**G**TCAT), compared to the Pacbio HiFi reference (TACAATCAT) for specimen USA/CA-LAC-USC2. These mixed bases were also confirmed by Sanger sequencing, as indicated by the arrow.

Supplementary Figure S4. Portable laboratory for COVID-19 variant tracking
The portable laboratory's components for SARS-CoV-2 whole genome sequencing within a within a 1.5×0.5-meter area. Biohazard waster container (1), pipette tips (2), gloves (3), PCR plate (4a), magnets (4b and 5), nuclease-free water (6), fluorescent dye (7), room-temperature reagents and 1.5mL tubes (8), aluminum block with ice pack, heat-sensitive reagents, and PCR tubes (9), battery (10), tabletop centrifuge (11), vortex mixer (12), fluorometer (13), laptop (14), pipettes (15), miniaturized thermocycler (16), end repair, ligation, and sequencing reagents (17), flow cell wash kit (18), and MinION Mk1B portable sequencer and flow cell (19).
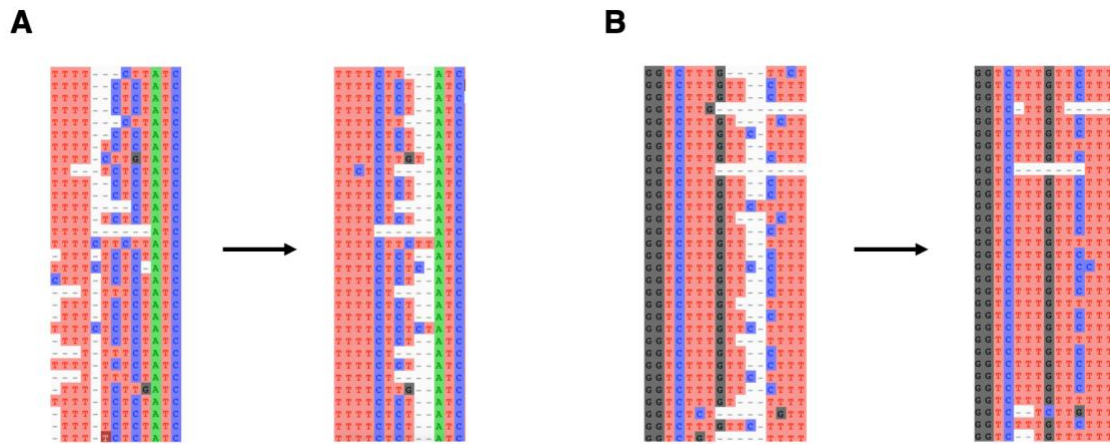
Supplementary Figure S5. Insertion and mutation error corrections by local realignment
(A) An insertion error ('T' in 'TTTTTCTCTATC') in the consensus sequence of specimen GO2530's reads on the left was corrected by performing a local realignment, resulting in improved alignment of reads on the right. The resulting consensus sequence for this region was 'TTTTCTCTATC', which matched the Pacbio HiFi reference sequence. (B) A mutation error ('T' in 'GGTCTTTGTTTTTT') in the consensus sequence of specimen ZI1686's alignment on the left was corrected by conducting a local realignment, resulting in the accurate consensus sequence 'GGTCTTTGTTCTTT'.

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