

Development and validation of a high throughput SARS-CoV-2 whole genome sequencing workflow in a clinical laboratory

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SUPPLEMENTARY MATERIALS

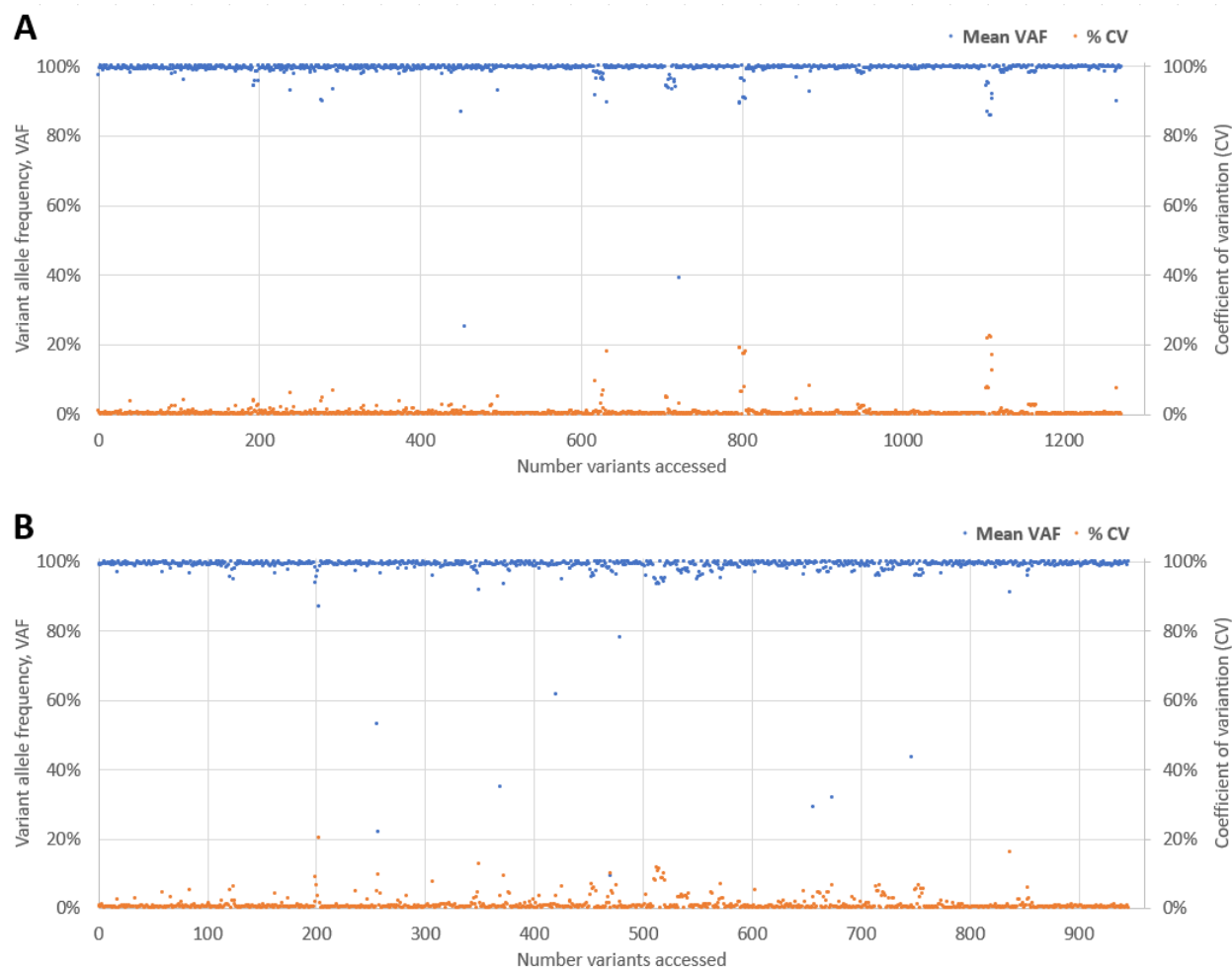


Figure S1. Precision studies using the automated, high-throughput SARS-CoV-2 NGS workflow. (A) Intra-assay precision of 1,272 variants from 175 specimens repeated 3 times. The mean of the detected variant frequencies (blue dot) and coefficients of variations (orange dot) are shown. (B) Inter-assay precision of 946 variants from 160 specimens replicated 3 times. The mean of the detected variant frequencies (blue dot) and coefficients of variations (orange dot) are shown.

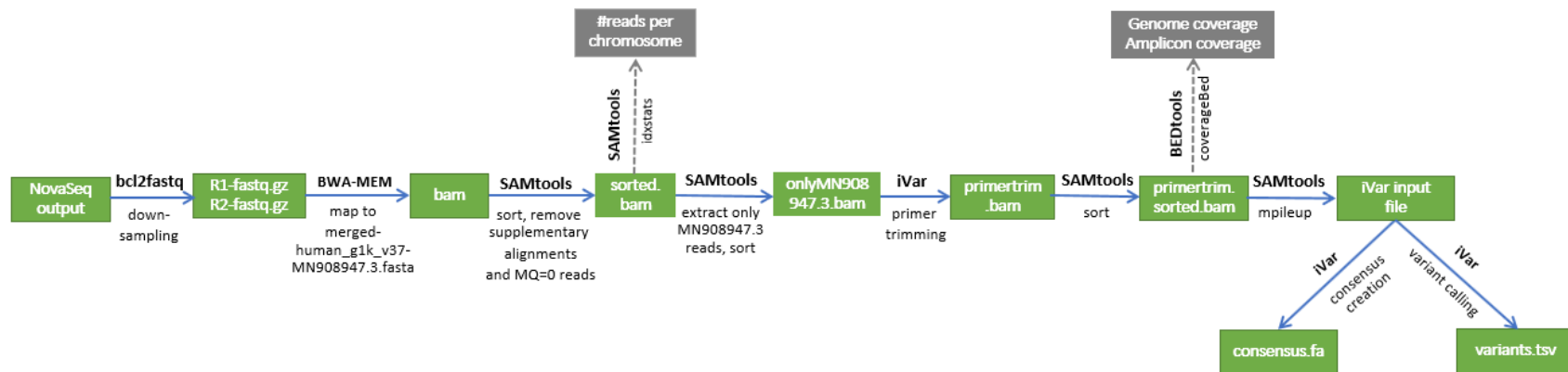


Figure S2. Bioinformatics pipeline for the analysis of SARS-CoV-2 tiled amplicon Illumina sequencing data.

Following mapping with “bwa mem”, “samtools view” is run with the parameters -q 1 to filter out multi-mapping reads and with -F 2048 to filter out supplementary alignment reads. “samtools mpileup” is run with the iVar-recommended parameters -A -B -d 600000 -Q 0. To generate a PCR primer-trimmed bam file, the iVar program is used to trim the ARTIC V3 primers with “ivar trim -b <primers.bed>”. For Illumina MiSeq runs, iVar is used for consensus sequence generation with the minimum depth set to 10 reads (-m 10), minimum quality value 10 (-q 10) and minimum variant frequency threshold of 50% (-t 0.5). To create consensus sequence for Illumina NovaSeq runs, we use a dynamic read depth cutoff (-m) as described in the Methods section. “samtools idxstats” is used to calculate the number of reads mapped to the viral and human reference sequence chromosome. To calculate minimum and average amplicon coverage from the primer-trimmed bam file, the “bedtools coverage” command is used from the BEDtools package.

Table S1. Primer pools for the automated, high-throughput SARS-CoV-2 NGS workflow

Primer Name (nCoV-2019_)	Sequence (5' to 3')	Pool	Ratio ¹
1_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT ACCAACCAACTTTTCGATCTCTTGT	1A	1X
1_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGTGCCATCTTTAAGATGTTGACG	1A	1X
2_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT CTGTTTACAGGTCGCGACGT	2A	1X
2_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAAGGATCAGTGCCAAGCTCGT	2A	1X
3_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT CGGTAATAAAGGAGCTGGTGGC	1B	1X
3_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGGTGTCTGCAATTCATAGCTCT	1B	1X
4_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT GGTGTATACTGCTGCCGTGAAC	2B	1X
4_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACAAGTAGTGGCACCTTCTTTAGT	2B	1X
5_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT TGGTGAAACTTCATGGCAGACG	1A	1X
5_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATTGATGTTGACTTTCTCTTTTGGAGT	1A	1X
6_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT GGTGTTGTTGGAGAAGGTTCCG	2A	1X
6_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGCGGCCTTCTGTAAACACG	2A	1X
7_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT ATCAGAGGCTGCTCGTGTGTA	1B	1X
7_LEFT_alt0	ACACTCTTCCCTACACGACGCTCTCCGATCT CATTTGCATCAGAGGCTGCTCG	1A	1X
7_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCACAGGTGACAATTTGTCCA	1B	1X
7_RIGHT_alt5	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTGACAATTTGTCCACCGAC	1A	1X
8_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT AGAGTTTCTTAGAGACGGTTGGGA	2B	1X
8_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCTTCAACAGCTTCACTAGTAGGT	2B	1X
9_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT TCCCACAGAAGGTGTTAACAGAGGA	1B	1X
9_LEFT_alt4	ACACTCTTCCCTACACGACGCTCTCCGATCT TCCCACAGAAGGTGTTAACAGAGG	1A	1X
9_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTATGACAGCATCTGCCACAA	1B	1X
9_RIGHT_alt2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GACAGCATCTGCCACAACACAG	1A	1X
10_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT TGAGAAGTGCTCTGCCTATACAGT	2A	1X
10_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCATCTAACCAATCTTCTTCTTGCTCT	2A	1X
11_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT GGAATTTGGTGCCACTTCTGCT	1B	1X
11_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCATCAGATTCAACTTGCATGGCA	1B	1X
12_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT AAACATGGAGGAGGTGTTGCAG	2B	1X
12_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTAATCTTCTTTTCAAAAAGCTTGA	2B	1X
13_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT TCGCACAAATGTCTACTTAGCTGT	1A	1X
13_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATAACCACAGCAGTTAAACAC	1A	1X
14_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT CATCCAGATTCTGCCACTCTTGT	2A	1X
14_LEFT_alt4	ACACTCTTCCCTACACGACGCTCTCCGATCT TGGAATCTTCATCCAGATTCTGC	2B	1X
14_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTTTCCACACAGACAGGCATT	2A	1X
14_RIGHT_alt2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCCTGTTTCTTCTGCATGTGC	2B	1X
15_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT ACAGTGCTTAAAAAGTGAAAAAGTGCC	1B	1X
15_LEFT_alt1	ACACTCTTCCCTACACGACGCTCTCCGATCT AGTGCTTAAAAAGTGAAAAAGTGCTT	1A	1X
15_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGAAACAGAACTGTAGCTGGC	1B	1X
15_RIGHT_alt3	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTGTAGCTGGCACTTTGAGAGA	1A	1X
16_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT AATTTGGAAGAAGCTGCTCGGT	2A	1X
16_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACAACCTGCGTGTGGAGGTTA	2A	1X
17_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT CTCTTTCTTTGAGAGAAGTGAGGACT	1B	1X
17_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTGTTGGAGTGTTAAACATGCAGT	1B	1X
18_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT TGGAAATACCCACAAGTTAATGGTTTAAAC	2B	1X
18_LEFT_alt2	ACACTCTTCCCTACACGACGCTCTCCGATCT ACTTCTATTAATGGGCAGATAACAACCTGT	2A	1X

18_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGCTTGTTTACCACACGTACAAGG	2B	1X
18_RIGHT_alt1	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GCTTGTTTACCACACGTACAAGG	2A	1X
19_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GCTGTTATGTACATGGGCACACT	1A	1X
19_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGTCCAACCTAGGGTCAATTTCTGT	1A	1X
20_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACAAAGAAAACAGTTACACAACAACCA	2B	1X
20_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACGTGGCTTTATTAGTTGCATTGTT	2B	1X
21_LEFTv2	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGGTGGCTATTGATTATAAACACTACACA	1B	1X
21_LEFT_alt2	ACACTCTTTCCCTACACGACGCTCTCCGATCT GGCTATTGATTATAAACACTACACACCCT	1A	1X
21_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TAGATCTGTGTGGCCAACCTCT	1B	1X
21_RIGHT_alt0	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GATCTGTGTGGCCAACCTCTTC	1A	1X
22_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTACCGAAGTTGTAGGAGACATTATACT	2A	1X
22_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACAGTATTCTTTGCTATAGTAGTCGGC	2A	1X
23_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACAACTACTAACATAGTTACACGGTGT	1B	1X
23_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACCAGTACAGTAGTTGCAATAGTG	1B	1X
24_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGGCATGCCTTCTTACTGTACTG	2B	1X
24_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACATTCTAACCATAGCTGAAATCGGG	2B	1X
25_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GCAATTGTTTTTCAGCTATTTTGCACT	1A	1X
25_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACTGTAGTGACAAGTCTCTCGCA	1A	1X
26_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TTGTGATACATTCTGTGCTGGTAGT	2A, 2B	1X
26_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TCCGCACTATCACCAACATCAG	2A, 2B	1X
27_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTACAGTCAGCTTATGTGTCAACC	1B	1X
27_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AATACAAGCACCAAGGTCACGG	1B	1X
28_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACATAGAAGTTACTGGCGATAGTTGT	2B	1X
28_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGTTTAGACATGACATGAACAGGTGT	2B	1X
29_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTTGTTCTCTTTTGTGCTGC	1A, 1B	1X
29_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGTGTACTCTATAAGTTTTGATGGTGTGT	1A, 1B	1X
30_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GCACAACTAATGGTGACTTTTTGCA	2A	1X
30_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACCACTAGTAGATACACAAACACCAG	2A	1X
31_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TTCTGAGTACTGTAGGCACGGC	1B	1X
31_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACAGAATAAACACCAGGTAAGAATGAGT	1B	1X
32_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGGTGAATACAGTCATGTAGTTGCC	2B	1X
32_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GGTAATAGCACATCACTACGCAAC	2B	1X
33_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTTTTGAAGAAGCTGCGCTGT	1A	1X
33_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGGACAGTAACTACGTCATCAAGC	1A	1X
34_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCCCATCTGGTAAAGTTGAGGGT	2A	1X
34_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGTGAAATTGGGCCTCATAGCA	2A	1X
35_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTTGCGATTCAACCAGGACAG	1B	1X
35_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACTTCATAGCCACAAGGTTAAAGTCA	1B	1X
36_LEFTv2	ACACTCTTTCCCTACACGACGCTCTCCGATCT ATTACAGTTAATGTTTAGCTTGGTTGTA	2B	1X
36_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GAACAAAGACCATTGAGTACTCTGGA	2B	1X
37_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACACACCACTGGTTGTTACTCAC	1A	1X
37_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GTCCACACTCTCCTAGCACCAT	1A	1X
38_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTGTGTATGTATGCATCAGCTGT	2A	1X
38_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ATCATAAACACCAAGAGTCAGTCTAAA	2A	1X
39_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGTATTGCCCTATTTTCTTCATAACTGGT	1B	1X
39_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGTAAGTGGACACATTGAGCCC	1B	1X
40_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGCACATCAGTAGTCTTACTCTCAGT	2B	1X
40_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CATGGCTGCATCACGGTCAAAT	2B	1X
41_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GTTCCCTTCCATCATATGCAGCT	1A	1X
41_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGGTATGACAACCATTAGTTTGGCT	1A	1X

42_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGCAAGAGATGGTTGTGTTCCC	2A	1X
42_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CCTACCTCCCTTTGTTGTGTTGT	2A	1X
43_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TACGACAGATGTCTTGCTGC	1B	1X
43_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGCAGCATCTACAGCAAAAGCA	1B	1X
44_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGCCACAGTACGTCTACAAGCT	2B	1X
44_LEFT_alt3	ACACTCTTTCCCTACACGACGCTCTCCGATCT CCACAGTACGTCTACAAGCTGG	2A	1X
44_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AACCTTCCACATACCGCAGAC	2B	1X
44_RIGHT_alt0	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CGCAGACGGTACAGACTGTGTT	2A	1X
45_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TACCTACAACTTGCTAATGACCC	1A	1X
45_LEFT_alt2	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGTATGTACAAATACCTACAACTTGCT	1B	1X
45_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AAATTGTTTCTCATGTTGGTAGTTAGAGA	1A	1X
45_RIGHT_alt7	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TTCATGTTGGTAGTTAGAGAAAGTGTGTC	1B	1X
46_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTCGCTTCCAAGAAAAGGACG	2B	1X
46_LEFT_alt1	ACACTCTTTCCCTACACGACGCTCTCCGATCT CGCTTCCAAGAAAAGGACGAAGA	2A	1X
46_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CACGTTACCTAAGTTGGCGTA	2B	1X
46_RIGHT_alt2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CACGTTACCTAAGTTGGCGTAT	2A	1X
47_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGGACTGGTATGATTTGTAGAAAACCC	1A	1X
47_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AATAACGGTCAAAGAGTTTAACTCTC	1A	1X
48_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTTGACACTGACTTAACAAAGCCT	2B	1X
48_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TAGATTACCAGAAGCAGCGTGC	2B	1X
49_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGGAATTACTTGTGTATGCTGCTGA	1B	1X
49_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGACGATGACTTGGTTAGCATTAAATACA	1B	1X
50_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GTTGATAAGTACTTTGATTGTTACGATGGT	2A	1X
50_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TAACATGTTGTGCCAACCA	2A	1X
51_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCAATAGCCGCACTAGAGGAG	1A	1X
51_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGTGCATTAAACATTGGCCGTGA	1A	1X
52_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT CATCAGGAGATGCCAACCTGC	2B	1X
52_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GTTGAGAGCAAAATTCATGAGGTCC	2B	1X
53_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGCAAAATGTTGGACTGAGACTGA	1B	1X
53_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGCCTCATAAACTCAGGTTCCC	1B	1X
54_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGAGTTAACAGGACACATGTTAGACA	2A	1X
54_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AACCAAAAACTTGTCATTAGCACA	2A	1X
55_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTCAACTTTACTTAGGAGGTATGAGCT	1A	1X
55_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GGTGACTCTCTATTGTACTTTACTGT	1A	1X
56_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACCTAGACCACCACTTAACCGA	2B	1X
56_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AACTATGCGAGCAGAAGGGTA	2B	1X
57_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ATTCTAACTCCAGGGACCACC	1B	1X
57_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GTAATTGAGCAGGGTCGCCAAT	1B	1X
58_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGATTTGAGTGTTGTCAATGCCAGA	2A	1X
58_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CTTTCTCCAAGCAGGGTTACGT	2A	1X
59_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCACGCATGATGTTTCATCTGCA	1A	1X
59_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AAGAGTCCTGTTACATTTTCAGCTTG	1A	1X
60_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGATAGAGACCTTTATGACAAGTTGCA	2B	1X
60_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GGTACCAACAGCTTCTCTAGTAGC	2B	1X
61_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTTTATCACCCGCAAGAAGC	1B	1X
61_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ATCACATAGACAACAGGTGCGC	1B	1X
62_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GGCACATGGCTTTGAGTTGACA	2A	1X
62_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GTTGAACCTTTCTACAAGCCGC	2A	1X
63_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTTAAGCGTGTGACTGGACT	1A	1X
63_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACAAAGTCCACCATCACAAAC	1A	1X

64_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCGATAGATATCCTGCTAATTCCATTGT	2B	1X
64_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGTCTTGTAAGTGTCCAGAGGT	2B	1X
65_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GCTGGCTTAGCTGTGGGTTT	1B	1X
65_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGTCAGTCATAGAACAAACACCAATAGT	1B	1X
66_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GGGTGTGGACATTGCTGCTAAT	2A	1X
66_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TCAATTTCCATTGACTCCTGGGT	2A	1X
67_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GTTGCCAACAATTACCTGAAACTTACT	1A, 1B	1X
67_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CAACCTTAGAACTACAGATAAATCTGGG	1A, 1B	1X
68_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACAGGTTTCATCTAAGTGTGTGTGT	2A, 2B	1X
68_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CTCCTTTATCAGAACCAGCACCA	2A, 2B	1X
69_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGTCGCAAAATATACTCAACTGTGTCA	1B	1X
69_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TCTTTATAGCCACGGAACCTCCA	1B	1X
70_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AAAAAAGAAAATGACTCTAAAGAGGGTTT	2B	1X
70_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGACCTTCTTTAAAGACATAACAGCAG	2B	1X
71_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACAAATCCAATTGAGTTGCTTCTCTATTC	1A	1X
71_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGGAAAAGAAAGGTAAGAACAAGTCCT	1A	1X
72_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACACGTGGTGTATTACCCTGAC	2B	1X
72_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACTCTGAACCTCACTTCCATCCAAC	2B	1X
73_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT CAATTTTGAATGATCCATTTTGGGTGT	1B	1X
73_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CACCAGCTGTCCAACCTGAAGA	1B	1X
74_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACATCACTAGGTTTCAAACCTTACTTGC	2A, 2B	2X
74_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GCAACACAGTTGCTGATTCTCTTC	2A, 2B	2X
75_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGAGTCCAACCAACAGAATCTATTGT	1A, 1B	1X
75_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACCACCAACCTTAGAATCAAGATTGT	1A, 1B	1X
76_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT AGGGCAAACCTGGAAAGATTGCT	2B	1X
76_LEFT_alt3	ACACTCTTTCCCTACACGACGCTCTCCGATCT GGGCAAACCTGGAAAGATTGCTGA	2A	1X
76_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TCTCTGCCAAATTTGTTGGAAAGGCA	2B	1X
76_RIGHT_alt0	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACCTGTGCCTGTTAAACCATTGA	2A	1X
77_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT CCAGCAACTGTTTGTGGACCTA	1B	1X
77_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CAGCCCTATTAAACAGCCTGC	1B	1X
78_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT CAACTTACTCCTACTTGCGTGT	2B	1X
78_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGTGTACAAAACCTGCCATATTGCA	2B	1X
79_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GTGGTGATTCAACTGAATGCAGC	1A, 1B	1X
79_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT CATTTTCATCTGTGAGCAAAGGTGG	1A, 1B	1X
80_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TTGCCTTGGTGATATTGCTGCT	2A	1X
80_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGGAGCTAAGTTGTTAACAAGCG	2A	1X
81_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GCACTTGGAAAACCTCAAGATGTGG	1B	1X
81_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT GTGAAGTTCTTTTCTGTGCAGGG	1B	1X
82_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT GGGCTATCATCTTATGTCCTTCCCT	2B	1X
82_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TGCCAGAGATGTACCTAAATCAA	2B	1X
83_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCCTTTGCAACCTGAATTAGACTCA	1A	1X
83_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT TTTGACTCCTTGAGCACTGGC	1A	1X
84_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TGCTGTAGTTGTCTCAAGGGCT	2A	1X
84_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT AGGTGTGAGTAACTGTTACAAACAAC	2A	1X
85_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT ACTAGCACTCTCCAAGGGTGTT	1B	1X
85_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACACAGTCTTTTACTCCAGATTCCC	1B	1X
86_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT TCAGGTGATGGCACAACAAGTC	2B	1X
86_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACGAAAGCAAGAAAAAGAAGTACGC	2B	1X
87_LEFT	ACACTCTTTCCCTACACGACGCTCTCCGATCT CGACTACTAGCGTGCCTTTGTA	1A	1X
87_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT ACTAGGTTCCATTGTTCAAGGAGC	1A	1X

88_LEFT	ACACTCTTCCCTACACGACGCTCTCCGATCT CCATGGCAGATTCCAACGGTAC	2A	1X
88_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGTCAGAATAGTGCCATGGAGT	2A	1X
89_LEFTv2	ACACTCTTCCCTACACGACGCTCTTCCGATCT CGCGTACGCGTTCCATGTGGTC	1B	1X
89_LEFT_alt2	ACACTCTTCCCTACACGACGCTCTCCGATCT CGCGTTCATGTGGTCATTCAA	1A	1X
89_RIGHTv2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATAGTAACCTGAAAGTCAACGAGAT	1B	1X
89_RIGHT_alt4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACGAGATGAAACATCTGTTGTCACT	1A	1X
90_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT ACACAGACCATTCCAGTAGCAGT	2B	1X
90_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGAAATGGTGAATTGCCCTCGT	2B	1X
91_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT TCACTACCAAGAGTGTGTTAGAGGT	1B	1X
91_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCAAGTGAGAACCAAAAGATAATAAGCA	1B	1X
92_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT TTTGTGCTTTTGTAGCCTTTCTGCT	2A	1X
92_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTTCTGGCAATTAATTGTAAAAGG	2A	1X
93_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT TGAGGCTGGTTCTAAATCACCCA	1A	1X
93_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTCTTCTTGCCATGTTGAG	1A	1X
94_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT GGCCCCAAGGTTTACCAATAA	2B	1X
94_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTGGCAATGTTGTTCTTGAGG	2B	1X
95_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT TGAGGGAGCCTTGAATACACCA	1B	1X
95_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAGTACGTTTTTGCCGAGGCTT	1B	1X
96_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT GCCAACAACAACAAGGCCAAAC	2A	1X
96_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGGCTCTGTTGGTGGGAATGT	2A	1X
97_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT TGGATGACAAAGATCCAAATTTCAAAGA	1A	1X
97_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACACACTGATTAAGATTGCTATGTGAG	1A	1X
98_LEFT	ACACTCTTCCCTACACGACGCTCTTCCGATCT AACAAATTGCAACAATCCATGAGCA	2B	1X
98_RIGHT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCTCCTAAGAAGCTATTAATAACACATGG	2B	1X

¹Relative primer concentration in each pool is indicated under Ratio.

SARS-CoV-2 specific sequences are from the ARTICv3 primer set (Quick 2020).

Primers from Itokawa et al (2020) are marked in bold.

Quick J, Loman NJ. hCoV-2019/nCoV-2019 Version 3 Amplicon Set. March 24, 2020. Available: <https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>.

Itokawa K, Sekizuka T, Hashino M, Tanaka R, Kuroda M (2020) Disentangling primer interactions improves SARS-CoV-2 genome sequencing by multiplex tiling PCR. PLoS ONE 15(9): e0239403.

Table S2. Modified primer concentration for each pool for a standard ARTIC v3 workflow

Primer Name (nCoV-2019_)	Sequence (5' to 3')	Pool	Ratio ¹
1_LEFT	ACCAACCAACTTTCGATCTCTTGT	1	1X
1_RIGHT	CATCTTTAAGATGTTGACGTGCCTC	1	1X
2_LEFT	CTGTTTTACAGGTTGCGACGT	2	1X
2_RIGHT	TAAGGATCAGTGCCAAGCTCGT	2	1X
3_LEFT	CGGTAATAAAGGAGCTGGTGGC	1	1X
3_RIGHT	AAGGTGTCTGCAATTCATAGCTCT	1	1X
4_LEFT	GGTGTATACTGCTGCCGTGAAC	2	1X
4_RIGHT	CACAAGTAGTGGCACCTTCTTTAGT	2	1X
5_LEFT	TGGTGAACTTCATGGCAGACG	1	7X
5_RIGHT	ATTGATGTTGACTTTCTCTTTTGGAGT	1	7X
6_LEFT	GGTGTGTTGGAGAAGGTTCCG	2	2.5X
6_RIGHT	TAGCGGCCTTCTGTAAACACG	2	2.5X
7_LEFT	ATCAGAGGCTGCTCGTGTGTA	1	1X
7_LEFT_alt0	CATTTGCATCAGAGGCTGCTCG	1	1X
7_RIGHT	TGCACAGGTGACAATTTGTCCA	1	1X
7_RIGHT_alt5	AGGTGACAATTTGTCCACCGAC	1	1X
8_LEFT	AGAGTTTCTTAGAGACGGTTGGGA	2	1X
8_RIGHT	GCTTCAACAGCTTCACTAGTAGGT	2	1X
9_LEFT	TCCCACAGAAGTGTTAACAGAGGA	1	1X
9_LEFT_alt4	TTCCCACAGAAGTGTTAACAGAGG	1	1X
9_RIGHT	ATGACAGCATCTGCCACAACAC	1	1X
9_RIGHT_alt2	GACAGCATCTGCCACAACACAG	1	1X
10_LEFT	TGAGAAGTGCTCTGCCTATACAGT	2	1X
10_RIGHT	TCATCTAACCAATCTTCTTCTGCTCT	2	1X
11_LEFT	GGAATTTGGTGCCACTTCTGCT	1	1X
11_RIGHT	TCATCAGATTCAACTTGCATGGCA	1	1X
12_LEFT	AAACATGGAGGAGGTGTTGCAG	2	7X
12_RIGHT	TTCACTCTTCATTTCCAAAAAGCTTGA	2	7X
13_LEFT	TCGCACAAATGTCTACTTAGCTGT	1	7X
13_RIGHT	ACCACAGCAGTTAAAAACCCCT	1	7X
14_LEFT	CATCCAGATTCTGCCACTCTTGT	2	1X
14_LEFT_alt4	TGGCAATCTTCATCCAGATTCTGC	2	1X
14_RIGHT	AGTTTCCACACAGACAGGCATT	2	1X
14_RIGHT_alt2	TGCGTGTTTCTTCTGCATGTGC	2	1X
15_LEFT	ACAGTGCTTAAAAAGTGTAAGTGCC	1	1X
15_LEFT_alt1	AGTGCTTAAAAAGTGTAAGTGCCCT	1	1X
15_RIGHT	AACAGAACTGTAGCTGGCACT	1	1X
15_RIGHT_alt3	ACTGTAGCTGGCACTTTGAGAGA	1	1X
16_LEFT	AATTTGGAAGAAGCTGCTCGGT	2	1X
16_RIGHT	CACAACCTGCGTGTGGAGGTGA	2	1X

17_LEFT	CTTCTTTCTTTGAGAGAAGTGAGGACT	1	7X
17_RIGHT	TTTGTTGGAGTGTTAACAATGCAGT	1	7X
18_LEFT	TGGAAATACCCACAAGTTAATGGTTTAAAC	2	1X
18_LEFT_alt2	ACTTCTATTAATGGGCAGATAACAACCTGT	2	1X
18_RIGHT	AGCTTGTTTACCACACGTACAAGG	2	1X
18_RIGHT_alt1	GCTTGTTTACCACACGTACAAGG	2	1X
19_LEFT	GCTGTTATGTACATGGGCACACT	1	2.5X
19_RIGHT	TGTCCAACCTAGGGTCAATTTCTGT	1	2.5X
20_LEFT	ACAAAGAAAACAGTTACACAACAACCA	2	1X
20_RIGHT	ACGTGGCTTTATTAGTTGCATTGTT	2	1X
21_LEFT	TGGCTATTGATTATAAACACTACACACCC	1	7X
21_LEFT_alt2	GGCTATTGATTATAAACACTACACACCTT	1	7X
21_RIGHT	TAGATCTGTGTGGCCAACCTCT	1	7X
21_RIGHT_alt0	GATCTGTGTGGCCAACCTCTTC	1	7X
22_LEFT	ACTACCGAAGTTGTAGGAGACATTATACT	2	1X
22_RIGHT	ACAGTATTCTTTGCTATAGTAGTCGGC	2	1X
23_LEFT	ACAACACTAACATAGTTACACGGTGT	1	7X
23_RIGHT	ACCAGTACAGTAGGTTGCAATAGTG	1	7X
24_LEFT	AGGCATGCCTTCTTACTGTACTG	2	1X
24_RIGHT	ACATTCTAACCATAGCTGAAATCGGG	2	1X
25_LEFT	GCAATTGTTTTTCAGCTATTTTGCAGT	1	1X
25_RIGHT	ACTGTAGTGACAAGTCTCTCGCA	1	1X
26_LEFT	TTGTGATACATTCTGTGCTGGTAGT	2	7X
26_RIGHT	TCCGCACTATCACCAACATCAG	2	7X
27_LEFT	ACTACAGTCAGCTTATGTGTCAACC	1	1X
27_RIGHT	AATACAAGCACCAAGGTCACGG	1	1X
28_LEFT	ACATAGAAGTTACTGGCGATAGTTGT	2	2.5X
28_RIGHT	TGTTTAGACATGACATGAACAGGTGT	2	2.5X
29_LEFT	ACTTGTGTTCTTTTTTGTGCTGC	1	2.5X
29_RIGHT	AGTGTACTCTATAAGTTTTGATGGTGTGT	1	2.5X
30_LEFT	GCACAACATAATGGTGACTTTTTGCA	2	1X
30_RIGHT	ACCACTAGTAGATACAAAACACCAG	2	1X
31_LEFT	TTCTGAGTACTGTAGGCACGGC	1	1X
31_RIGHT	ACAGAATAAACACCAGGTAAGAATGAGT	1	1X
32_LEFT	TGGTGAATACAGTCATGTAGTTGCC	2	1X
32_RIGHT	AGCACATCACTACGCAACTTTAGA	2	1X
33_LEFT	ACTTTTGAAGAAGCTGCGCTGT	1	1X
33_RIGHT	TGGACAGTAAACTACGTCATCAAGC	1	1X
34_LEFT	TCCCATCTGGTAAAGTTGAGGGT	2	1X
34_RIGHT	AGTGAAATTGGGCCTCATAGCA	2	1X
35_LEFT	TGTTTCGATTCAACCAGGACAG	1	1X
35_RIGHT	ACTTCATAGCCACAAGGTTAAAGTCA	1	1X
36_LEFT	TTAGCTTGGTTGTACGCTGCTG	2	2.5X

36_RIGHT	GAACAAAGACCATTGAGTACTCTGGA	2	2.5X
37_LEFT	ACACACCACTGGTTGTTACTCAC	1	1X
37_RIGHT	GTCCACACTCTCCTAGCACCAT	1	1X
38_LEFT	ACTGTGTTATGTATGCATCAGCTGT	2	2.5X
38_RIGHT	CACCAAGAGTCAGTCTAAAGTAGCG	2	2.5X
39_LEFT	AGTATTGCCCTATTTTCTTCATAACTGGT	1	1X
39_RIGHT	TGTAAGTGGACACATTGAGCCC	1	1X
40_LEFT	TGCACATCAGTAGTCTTACTCTCAGT	2	1X
40_RIGHT	CATGGCTGCATCACGGTCAAAT	2	1X
41_LEFT	GTTCCCTTCCATCATATGCAGCT	1	1X
41_RIGHT	TGGTATGACAACCATTAGTTTGGCT	1	1X
42_LEFT	TGCAAGAGATGGTTGTGTTCCC	2	1X
42_RIGHT	CCTACCTCCCTTTGTTGTGTTGT	2	1X
43_LEFT	TACGACAGATGTCTTGTGCTGC	1	1X
43_RIGHT	AGCAGCATCTACAGCAAAAGCA	1	1X
44_LEFT	TGCCACAGTACGTCTACAAGCT	2	1X
44_LEFT_alt3	CCACAGTACGTCTACAAGCTGG	2	1X
44_RIGHT	AACCTTTCCACATACCGCAGAC	2	1X
44_RIGHT_alt0	CGCAGACGGTACAGACTGTGTT	2	1X
45_LEFT	TACCTACAACCTTGCTAATGACCC	1	1X
45_LEFT_alt2	AGTATGTACAAATACCTACAACCTGTGCT	1	1X
45_RIGHT	AAATTGTTTCTTCATGTTGGTAGTTAGAGA	1	1X
45_RIGHT_alt7	TTCATGTTGGTAGTTAGAGAAAGTGTGTC	1	1X
46_LEFT	TGTCGCTTCCAAGAAAAGGACG	2	1X
46_LEFT_alt1	CGCTTCCAAGAAAAGGACGAAGA	2	1X
46_RIGHT	CACGTTACCTAAGTTGGCGTA	2	1X
46_RIGHT_alt2	CACGTTACCTAAGTTGGCGTAT	2	1X
47_LEFT	AGGACTGGTATGATTTTGTAGAAAACCC	1	1X
47_RIGHT	AATAACGGTCAAAGAGTTTTAACCTCTC	1	1X
48_LEFT	TGTTGACACTGACTTAACAAAGCCT	2	1X
48_RIGHT	TAGATTACCAGAAGCAGCGTGC	2	1X
49_LEFT	AGGAATTACTTGTGTATGCTGCTGA	1	1X
49_RIGHT	TGACGATGACTTGGTTAGCATTAAATACA	1	1X
50_LEFT	GTTGATAAGTACTTTGATTGTTACGATGGT	2	1X
50_RIGHT	TAACATGTTGTGCCAACCA	2	1X
51_LEFT	TCAATAGCCGCCACTAGAGGAG	1	1X
51_RIGHT	AGTGCATTAACATTGGCCGTGA	1	1X
52_LEFT	CATCAGGAGATGCCACAACCTGC	2	1X
52_RIGHT	GTTGAGAGCAAAATTCATGAGGTCC	2	1X
53_LEFT	AGCAAAATGTTGGACTGAGACTGA	1	1X
53_RIGHT	AGCCTCATAAAACTCAGGTTCCC	1	1X
54_LEFT	TGAGTTAACAGGACACATGTTAGACA	2	1X
54_RIGHT	AACCAAAAACCTGTCCATTAGCACA	2	1X

55_LEFT	ACTCAACTTTACTTAGGAGGTATGAGCT	1	1X
55_RIGHT	GGTGTACTCTCCTATTTGTACTTTACTGT	1	1X
56_LEFT	ACCTAGACCACCACCTTAACCGA	2	1X
56_RIGHT	ACACTATGCGAGCAGAAGGGTA	2	1X
57_LEFT	ATTCTAACTCCAGGGACCACC	1	1X
57_RIGHT	GTAATTGAGCAGGGTCGCCAAT	1	1X
58_LEFT	TGATTTGAGTGTTGTCAATGCCAGA	2	1X
58_RIGHT	CTTTTCTCCAAGCAGGGTTACGT	2	1X
59_LEFT	TCACGCATGATGTTTCATCTGCA	1	1X
59_RIGHT	AAGAGTCCTGTTACATTTTCAGCTTG	1	1X
60_LEFT	TGATAGAGACCTTTATGACAAAGTTGCA	2	1X
60_RIGHT	GGTACCAACAGCTTCTCTAGTAGC	2	1X
61_LEFT	TGTTTATCACCCGCGAAGAAGC	1	1X
61_RIGHT	ATCACATAGACAACAGGTGCGC	1	1X
62_LEFT	GGCACATGGCTTTGAGTTGACA	2	1X
62_RIGHT	GTTGAACCTTTCTACAAGCCGC	2	1X
63_LEFT	TGTTAAGCGTGTTGACTGGACT	1	1X
63_RIGHT	ACAAACTGCCACCATCACAAACC	1	1X
64_LEFT	TCGATAGATATCCTGCTAATTCATTGT	2	7X
64_RIGHT	AGTCTTGTAAGAGTGTCCAGAGGT	2	7X
65_LEFT	GCTGGCTTTAGCTTGTTGGGTTT	1	1X
65_RIGHT	TGTCAGTCATAGAACAACCAATAGT	1	1X
66_LEFT	GGGTGTGGACATTGCTGCTAAT	2	7X
66_RIGHT	TCAATTTCCATTTGACTCCTGGGT	2	7X
67_LEFT	GTTGTCCAACAATTACCTGAACTTACT	1	1X
67_RIGHT	CAACCTTAGAACTACAGATAAATCTTGGG	1	1X
68_LEFT	ACAGGTTTCTAAGTGTGTGTGT	2	1X
68_RIGHT	CTCCTTTATCAGAACCAGCACCA	2	1X
69_LEFT	TGTCGCAAAATATACTCAACTGTGTCA	1	1X
69_RIGHT	TCTTTATAGCCACGGAACCTCCA	1	1X
70_LEFT	ACAAAAGAAAATGACTCTAAAGAGGGTTT	2	7X
70_RIGHT	TGACCTTCTTTAAAGACATAACAGCAG	2	7X
71_LEFT	ACAAATCCAATTCAGTTGTCTTCCTATTC	1	7X
71_RIGHT	TGGAAAAGAAAGGTAAGAACAAGTCCT	1	7X
72_LEFT	ACACGTGGTGTTTATTACCCTGAC	2	1X
72_RIGHT	ACTCTGAACTCACTTTCCATCCAAC	2	1X
73_LEFT	CAATTTTGTAATGATCCATTTTGGGTGT	1	2.5X
73_RIGHT	CACCAGCTGTCCAACCTGAAGA	1	2.5X
74_LEFT	ACATCACTAGGTTTCAAACCTTACTTGC	2	7X
74_RIGHT	GCAACACAGTTGCTGATTCTCTTC	2	7X
75_LEFT	AGAGTCCAACCAACAGAATCTATTGT	1	7X
75_RIGHT	ACCACCAACCTTAGAATCAAGATTGT	1	7X
76_LEFT	AGGGCAAACCTGGAAAGATTGCT	2	2.5X

76_LEFT_alt3	GGGCAAAGTGGAAAGATTGCTGA	2	2.5X
76_RIGHT	ACACCTGTGCCTGTAAACCAT	2	2.5X
76_RIGHT_alt0	ACCTGTGCCTGTAAACCATGA	2	2.5X
77_LEFT	CCAGCAACTGTTTGTGGACCTA	1	1X
77_RIGHT	CAGCCCCTATTAACAGCCTGC	1	1X
78_LEFT	CAACTTACTCCTACTTGGCGTGT	2	1X
78_RIGHT	TGTGTACAAAACTGCCATATTGCA	2	1X
79_LEFT	GTGGTGATTCAACTGAATGCAGC	1	1X
79_RIGHT	CATTTTCATCTGTGAGCAAAGGTGG	1	1X
80_LEFT	TTGCCTTGGTGATATTGCTGCT	2	1X
80_RIGHT	TGGAGCTAAGTTGTTAACAAGCG	2	1X
81_LEFT	GCACTTGGAAAACTCAAGATGTGG	1	2.5X
81_RIGHT	GTGAAGTTCTTTCTTGTGCAGGG	1	2.5X
82_LEFT	GGGCTATCATCTTATGCTTCCCT	2	1X
82_RIGHT	TGCCAGAGATGTCACCTAAATCAA	2	1X
83_LEFT	TCCTTTGCAACCTGAATTAGACTCA	1	2.5X
83_RIGHT	TTTGACTCCTTGAGCACTGGC	1	2.5X
84_LEFT	TGCTGTAGTTGTCTCAAGGGCT	2	1X
84_RIGHT	AGGTGTGAGTAACTGTTACAACAAC	2	1X
85_LEFT	ACTAGCACTCTCCAAGGGTGTT	1	2.5X
85_RIGHT	ACACAGTCTTTTACTCCAGATTCCC	1	2.5X
86_LEFT	TCAGGTGATGGCACAACAAGTC	2	2.5X
86_RIGHT	ACGAAAGCAAGAAAAAGAAGTACGC	2	2.5X
87_LEFT	CGACTACTAGCGTGCCCTTGTGA	1	1X
87_RIGHT	ACTAGGTTCCATTGTTCAAGGAGC	1	1X
88_LEFT	CCATGGCAGATTCCAACGGTAC	2	1X
88_RIGHT	TGGTCAGAATAGTGCCATGGAGT	2	1X
89_LEFT	GTACGCGTTCCATGTGGTCATT	1	2.5X
89_LEFT_alt2	CGCGTTCCATGTGGTCATTCAA	1	2.5X
89_RIGHT	ACCTGAAAGTCAACGAGATGAAACA	1	2.5X
89_RIGHT_alt4	ACGAGATGAAACATCTGTTGTCACT	1	2.5X
90_LEFT	ACACAGACCATTCCAGTAGCAGT	2	1X
90_RIGHT	TGAAATGGTGAATTGCCCTCGT	2	1X
91_LEFT	TCACTACCAAGAGTGTGTTAGAGGT	1	7X
91_RIGHT	TTCAAGTGAGAACCAAAAGATAATAAGCA	1	7X
92_LEFT	TTTGTGCTTTTGTAGCCTTTCTGCT	2	1X
92_RIGHT	AGGTTCTTGCAATTAATTGTAAAAGG	2	1X
93_LEFT	TGAGGCTGGTTCTAAATCACCCA	1	1X
93_RIGHT	AGGTCTTCCTTGCCATGTTGAG	1	1X
94_LEFT	GGCCCCAAGGTTTACCCAATAA	2	1X
94_RIGHT	TTTGGAATGTTGTTCTTGAGG	2	1X
95_LEFT	TGAGGGAGCCTTGAATACACCA	1	7X
95_RIGHT	CAGTACGTTTTTGCCGAGGCTT	1	7X

96_LEFT	GCCAACAACAACAAGGCCAAAC	2	1X
96_RIGHT	TAGGCTCTGTTGGTGGGAATGT	2	1X
97_LEFT	TGGATGACAAAGATCCAAATTTCAAAGA	1	7X
97_RIGHT	ACACACTGATTAAAGATTGCTATGTGAG	1	7X
98_LEFT	AACAATTGCAACAATCCATGAGCA	2	2.5X
98_RIGHT	TTCTCCTAAGAAGCTATTAAAATCACATGG	2	2.5X

Primer sequences are from the ARTICv3 primer set (Quick 2020).

¹ Relative primer concentration in each pool is indicated under Ratio.

Quick J, Loman NJ. hCoV-2019/nCoV-2019 Version 3 Amplicon Set. March 24, 2020. Available:

<https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>.

Table S3. SARS-CoV-2 primer binding site mutations found in samples (n=3,506) collected between January and February of 2021

Position ¹	Reference >Variant	Prevalence in the study cohort, % ²	ARTICv3 Primer Name	Primer binding site mutation ³ (5' to 3')
1,578	T>C	1.3	nCoV-2019_6_LEFT	GGTGcTGTTGGAGAAGGTTCCG
2,258	G>A	2.7	nCoV-2019_7_RIGHT	TGCACAGGTGA ^t AATTTGTCCA
2,258	G>A	2.7	nCoV-2019_7_RIGHT_alt5	AGGTGA ^t AATTTGTCCACCGAC
4,683	C>T	1.0	nCoV-2019_15_RIGHT_alt3	ACTGTA ^a CTGGCACTTTGAGAGA
5,011	A>C	1.7	nCoV-2019_16_RIGHT	CACAACgTGC GTGGAGGTTA
5,869	C>T	2.5	nCoV-2019_20_LEFT	AtAAAGAAAACAGTTACACAACAACCA
6,730	C>T	1.0	nCoV-2019_23_LEFT	ACA ACTACTAAtATAGTTACACGGTGT
8,264	G>T	1.7	nCoV-2019_28_LEFT	ACATAGAAGTTACT ^t GCGATAGTTGT
9,204	A>G	2.7	nCoV-2019_31_LEFT	TTCTGAGTACTGTAGGCACGGC
10,369	C>T	1.3	nCoV-2019_35_LEFT	TGTTCG ^t ATTCAACCAGGACAG
10,741	C>T	2.3	nCoV-2019_35_RIGHT	ACTTCATAGCCACAAGGTTAAaTCA
12,484	C>T	1.4	nCoV-2019_41_RIGHT	TGGTATaACAACCATTAGTTTGGCT
12,789	C>T	4.2	nCoV-2019_42_RIGHT	CCTACCTCCCTTTaTTGTGTTGT
13,019	C>T	2.3	nCoV-2019_44_LEFT	TGCCACAGTACGT ^t TACAAGCT
13,019	C>T	2.3	nCoV-2019_44_LEFT_alt3	CCACAGTACGT ^t TACAAGCTGG
16,500	A>C	1.3	nCoV-2019_54_RIGHT	AACCAAAAACgTGTCCATTAGCACA
21,364	C>T	1.9	nCoV-2019_71_LEFT	ACAAAT ^t CAATTCAGTTGTCTTCCTATTC
22,018	G>T	17.7	nCoV-2019_72_RIGHT	ACTCTGA ACTCACTTTCCATaCAAC
22,335	G>T	1.9	nCoV-2019_73_RIGHT	CACCAGCTGTCaAACCTGAAGA
23,191	C>T	1.4	nCoV-2019_76_RIGHT	ACACCTGTGCCTGTTAAACCAT
25,907	G>T	41.6	nCoV-2019_86_LEFT	TCAG ^t TGATGGCACAACAAGTC
26,305	C>G	1.3	nCoV-2019_86_RIGHT	ACGAAAGCAAcAAAAAGAAGTACGC
28,087	C>T	2.7	nCoV-2019_93_LEFT	TGAGG ^t TGGTTCTAAATCACCCA
29,362	C>T	17.9	nCoV-2019_96_RIGHT	TAGGCTCTGTTGGTGGaAATGT
29,377	T>A	1.5	nCoV-2019_96_RIGHT	T ^t GGCTCTGTTGGTGGGAATGT

¹ Variant position is shown against a SARS-CoV-2 reference genome OMN908947.3.

² Shown only prevalence >1%

³ Red lower case indicates mutation in the primer binding site.

Table S4. SARS-CoV-2 clade comparison between the high-throughput 2-step PCR workflow and a standard ARTIC v3 workflow

Clade by 2-step PCR method	Clade by ARTIC v3 method														Total
	19B	20A	20B	20C	20G	20H (Beta, V2)	20I (Alpha, V1)	20J (Gamma, V3)	21A (Delta)	21C (Epsilon)	21D (Eta)	21F (Iota)	21G (Lambda)	21H	
19B	3														3
20A		53													53
20B			105				1								106
20C				98											98
20G					175										175
20H (Beta, V2)						7									7
20I (Alpha, V1)							713								713
20J (Gamma, V3)								44							44
21A (Delta)									1						
21C (Epsilon)										101					101
21D (Eta)											10				10
21F (Iota)												131			131
21G (Lambda)													1		1
21H														3	3
Total	3	53	105	98	175	7	714	44		101	10	131	1	3	1446

¹ SARS-CoV-2 clades were assigned with Nextclade v1.3.0 (<https://clades.nextstrain.org/>).

Table S5. SARS-CoV-2 lineage comparison between the high-throughput 2-step PCR workflow and a standard ARTIC v3 workflow¹

Lineage by ARTIC v3 method	
Lineage*	
A.2.5	3
AZ.3	3
B.1	6
B.1.1	2
B.1.1.207	1
B.1.1.222	8
B.1.1.306	1
B.1.1.316	2
B.1.1.318	4
B.1.1.345	1
B.1.1.348	4
B.1.1.434	2
B.1.1.519	53
B.1.1.7	702
B.1.110.3	1
B.1.189	1
B.1.2	153
B.1.214.2	2
B.1.232	1
B.1.234	15
B.1.239	1
B.1.243	10
B.1.243.1	2
B.1.265	1
B.1.298	1
B.1.311	3
B.1.351	7
B.1.36.29	1
B.1.404	1
B.1.427	19
R.1	22
B.1.429	80
B.1.429.1	1
B.1.475	1
B.1.517	2
B.1.525	10
B.1.526	131
B.1.539	1
B.1.561	6
B.1.568	3
B.1.575	13
B.1.588	3
B.1.595	2
B.1.596	24
B.1.604	1
B.1.609	1
B.1.617.2	1
B.1.621	3
B.1.623	3
B.1.625	2
B.1.628	3
B.1.634	1
B.1.635	1
B.1.637	54
C.37	1
N.5	1
P.1	29
P.1.10	14
P.1.2	1
P.2	1
Q.3	2
Q.4	8
Q.8	1
Total	1446

Lineage by high-throughput 2-step method	
Lineage*	
B.1.429	80
B.1.429.1	1
B.1.475	1
B.1.517	2
B.1.525	10
B.1.526	131
B.1.539	1
B.1.561	6
B.1.568	3
B.1.575	13
B.1.588	3
B.1.595	2
B.1.596	24
B.1.604	1
B.1.609	1
B.1.617.2	1
B.1.621	3
B.1.623	3
B.1.625	2
B.1.628	3
B.1.634	1
B.1.635	1
B.1.637	54
C.37	1
N.5	1
P.1	29
P.1.10	14
P.1.2	1
P.2	1
Q.3	2
Q.4	8
Q.8	1
Total	1446

¹Lineages were assigned with Pangolin v3.1.11 (<https://pangolin.cog-uk.io/>)

Table S6. SARS-CoV-2 primer binding site mutations causing amplicon dropout by a standard ARTIC v3 workflow

Clade	Number Sample	Failed Amplicon	Affected Primer	Primer Sequence ¹ (5' to 3')	Position ²	Reference	Variant
20H (Beta, V2)	7	ARTICv3-74	nCoV-2019_74_LEFT	ACATCACTAGGTTTCAAAC(tttacttgc)	22281	C	-TTTACTTGC
21A (Delta)	1	ARTICv3-72	nCoV-2019_72_RIGHT	ACTC(tgaact)CACTTTCCATCCAAC	22028	G	-AGTTCA
21C (Epsilon)	113	ARTICv3-72	nCoV-2019_72_RIGHT	ACTCTGAACTCACTTTCCAT a CAAC	22018	G	T
21G (Lambda)	1	ARTICv3-97	nCoV-2019_97_LEFT	TGGATGACAAAGATCCAATTT t AAAGA	29311	C	T
			nCoV-2019_97_RIGHT	ACACACTGATTAAAGATT a CTATGTGAG	29675	C	T

¹ Red lower case indicates mutation in the primer binding site; deleted nucleotides are shown in parentheses.

² Variant position is shown against a SARS-CoV-2 reference genome MN908947.3.

Table S7. Proportion of samples generating complete SARS-CoV-2 consensus sequence by the high-throughput workflow and a standard ARTIC v3 workflow

Clade	High-throughput workflow				ARTIC v3 workflow			
	Number complete	Number incomplete	Total assessed	Percent complete	Number complete	Number incomplete	Total assessed	Percent complete
20H (Beta, V2)	4	1	5	80.0	16	69	85	18.8
21A (Delta)	855	42	897	95.3	4	515	519	0.8
21C (Epsilon)	7	0	7	100.0	5	256	261	1.9
21G (Lambda)	15	3	18	83.3	1	29	30	3.3
Total	881	46	927	95.0	26	869	895	2.9

A different sample set was used between the two workflows.

The percent genome coverage was determined by counting the number of nucleotides meeting minimum coverage requirement divided by the total SARS-CoV-2 genome length excluding the 5' (1-54nt) and 3' (29,836–29,903nt) ends not covered by the amplicon panel.

Table S8. SARS-CoV-2 clade distribution of clinical cases analyzed between January and September 2021

Clade ¹	Number of cases	Percent
21A (Delta)	23,239	35.13
20I (Alpha V1)	19,022	28.76
20G	5,665	8.56
21C (Epsilon)	3,475	5.25
21F (Iota)	3,259	4.93
20C	3,103	4.69
20B	2,558	3.87
20J (Gamma V3)	2,540	3.84
20A	1,929	2.92
21H	683	1.03
20H (Beta V2)	156	0.24
19B	141	0.21
21G (Lambda)	140	0.21
21D (Eta)	131	0.2
20D	68	0.1
21B (Kappa)	25	0.04
20E (EU1)	12	0.02
Total	66,146	100

¹ SARS-CoV-2 clades were assigned with Nextclade v1.3.0 (<https://clades.nextstrain.org/>).