

**Supplemental Information**

**High-Density Amplicon Sequencing Identifies  
Community Spread and Ongoing Evolution  
of SARS-CoV-2 in the Southern United States**

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## **Supplemental Information**

**Includes**

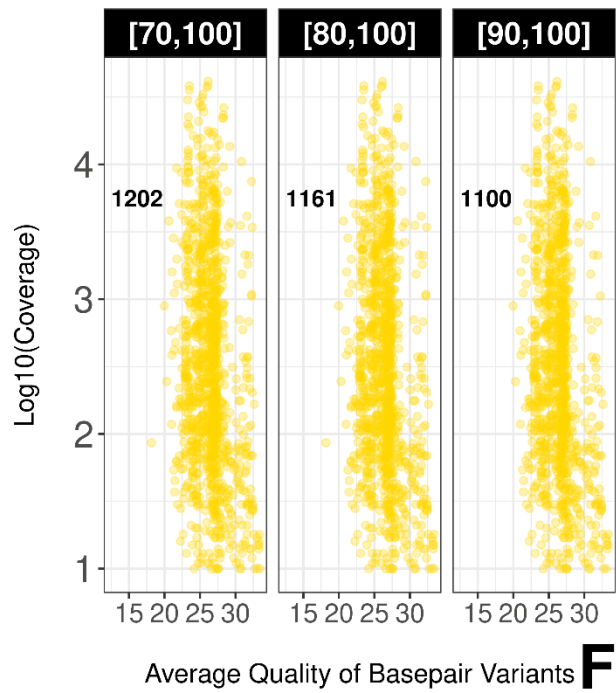
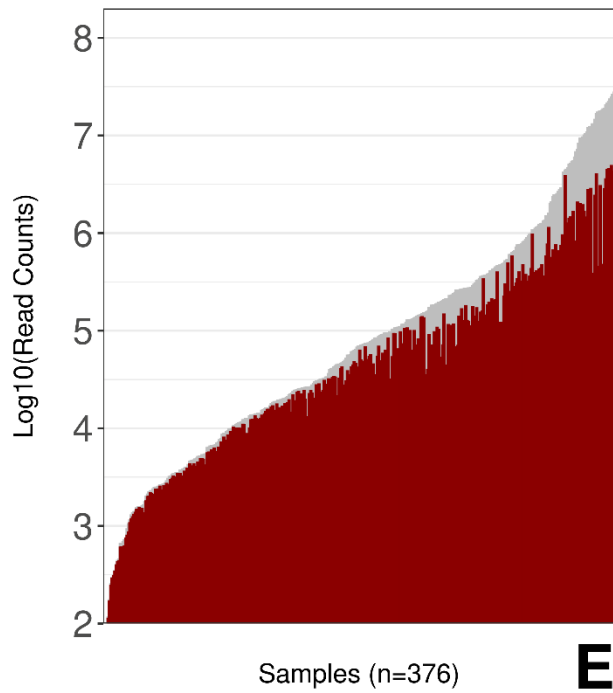
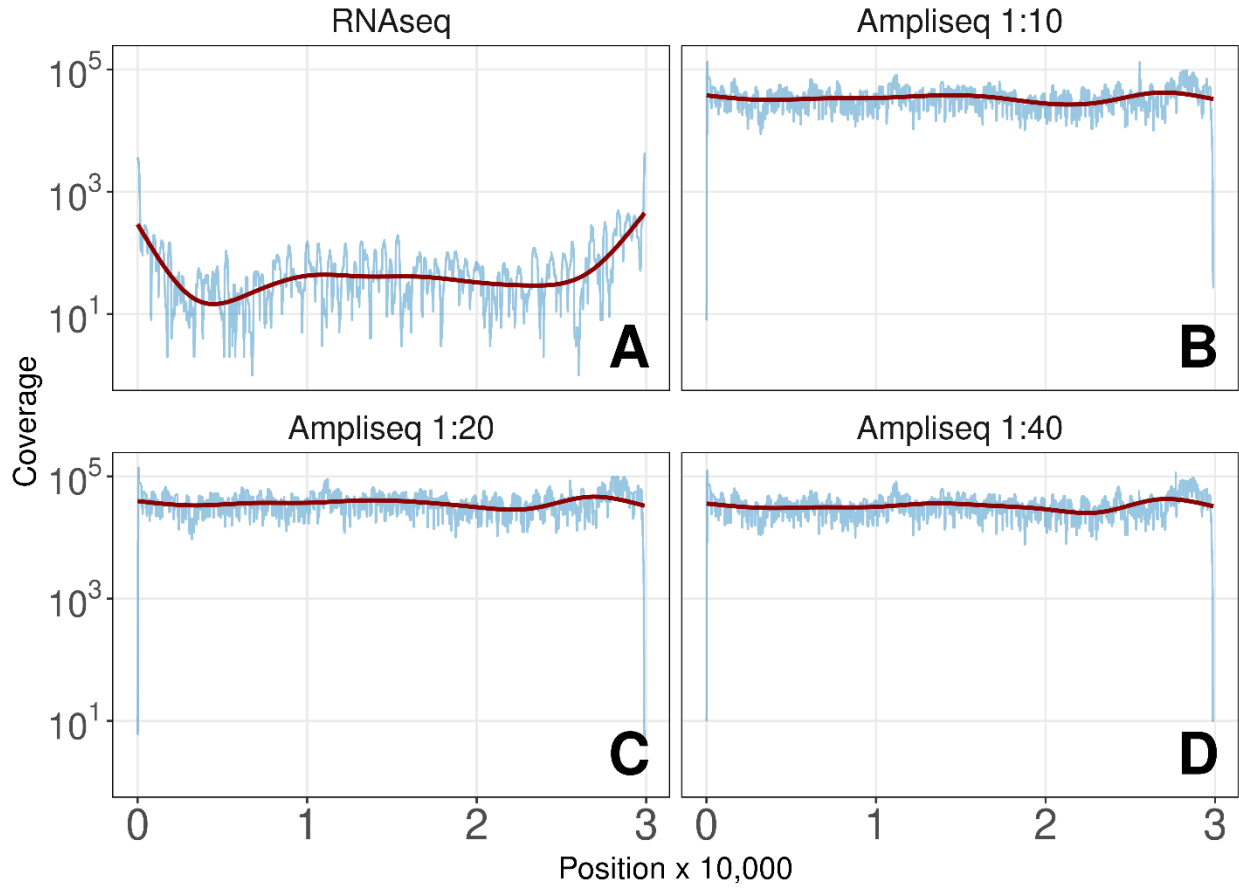
**Supplemental Figure S1-3**

**Supplemental Figure S1-3 Legends**

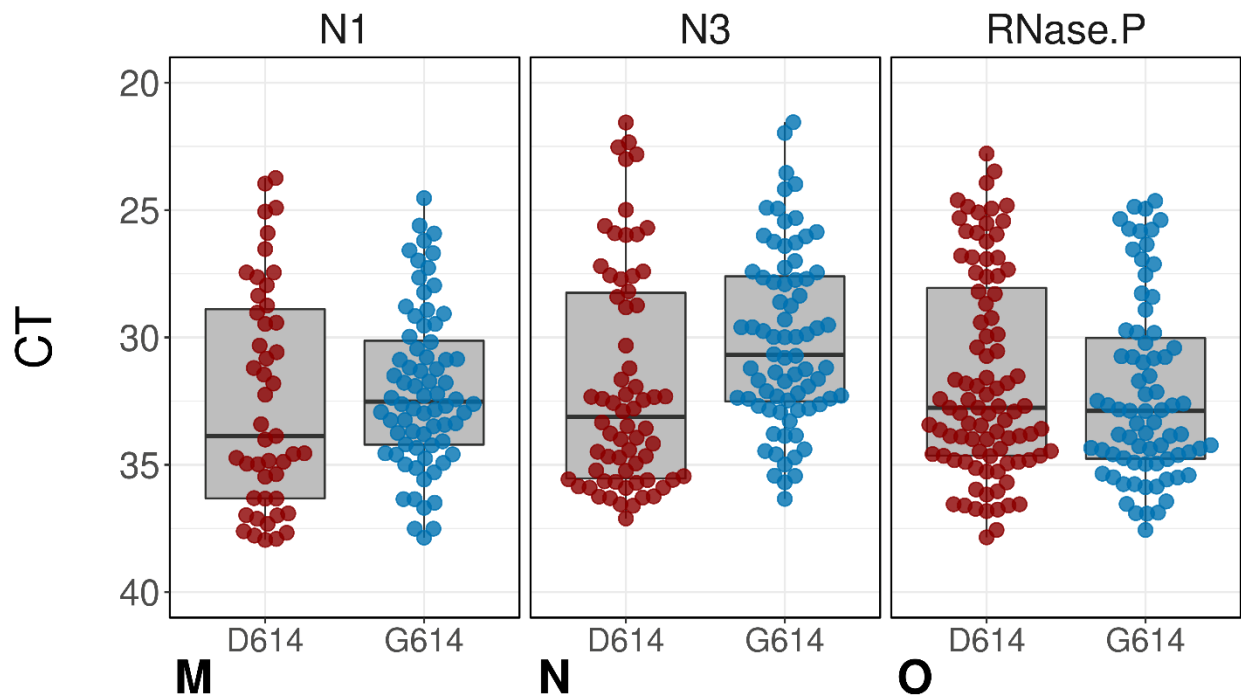
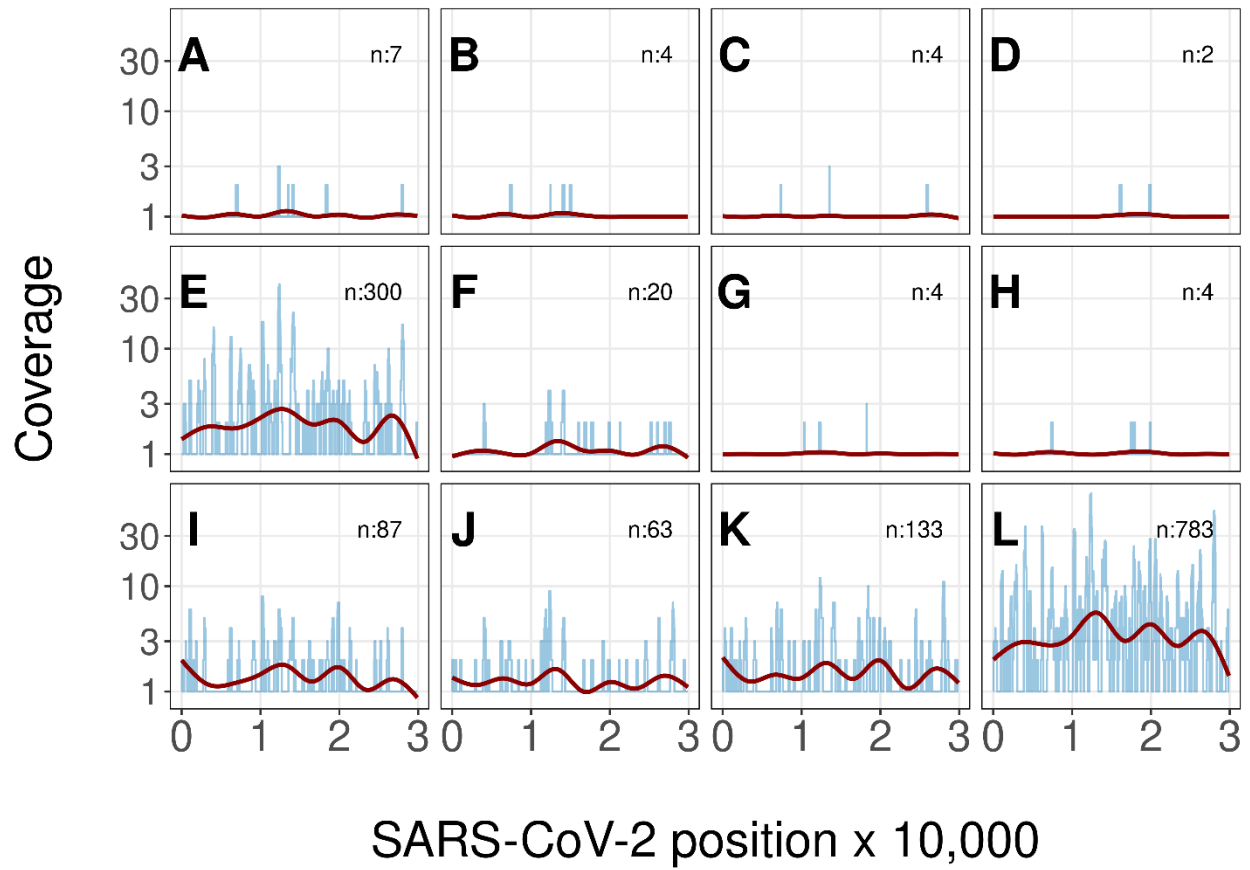
**Table S1**

**Table S1 Legend**

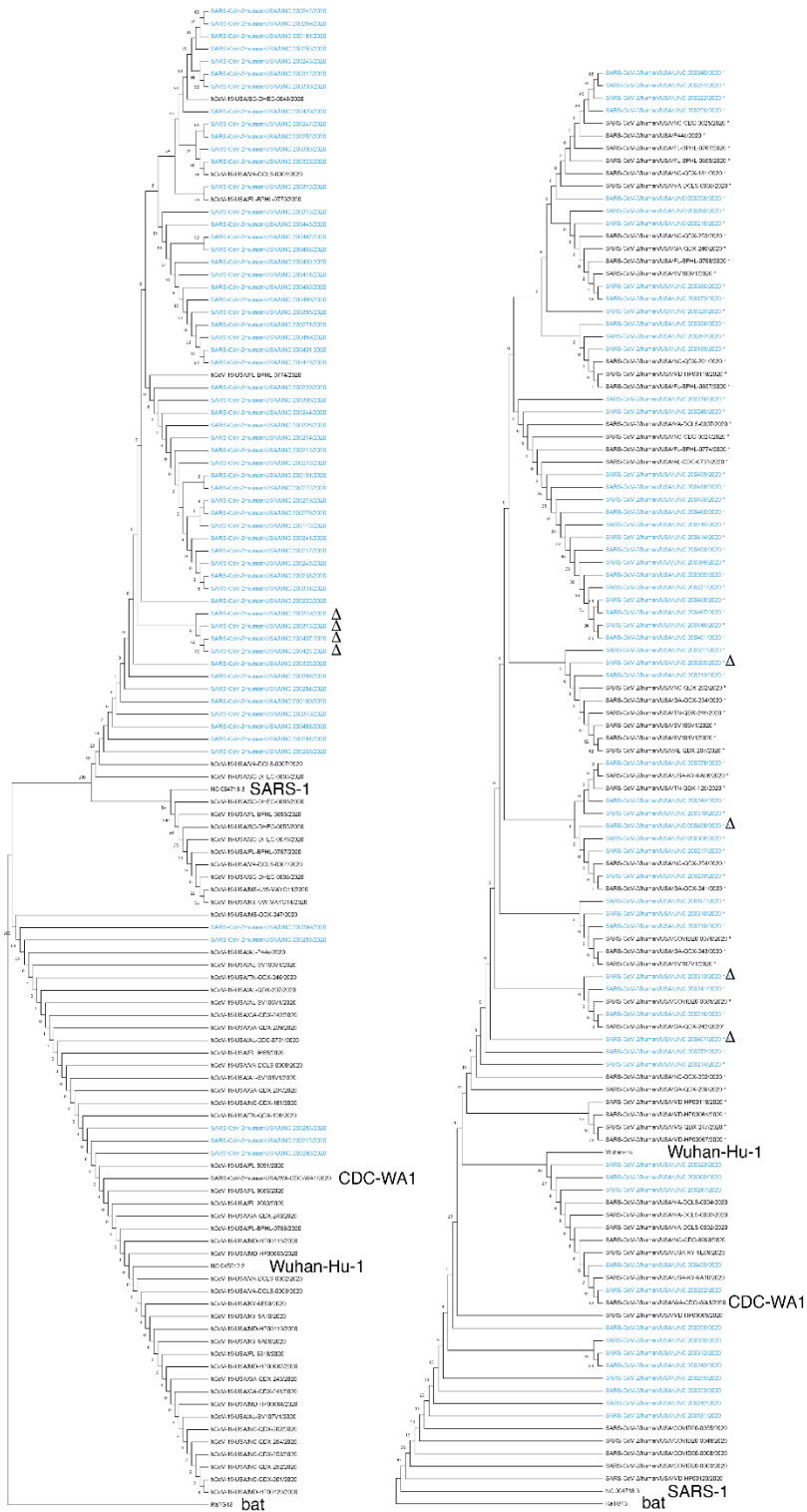
Supplemental Figure S1



Supplemental Figure S2



# Supplemental Figure S3



## Supplemental Figure Legends

### Supplemental Figure S1

Panels A-D show coverage comparison of targeted (AmpliSeq) and non-targeted sequencing of the BEI reference material (NR-52285, strain SARS-CoV-2/human/USA-WA1/2020). Sample types, RNA seq or dilutions of input RNA, are listed on top. Coverage is shown on the vertical and genome position on the horizontal axis; Loess-regression line is shown in red. (E) Log-base 10 of the total read counts of all samples used in this study. (F) The average quality of base-pair variants called by sequencing was plotted based on increasing coverage reads. Related to **Figure 1**.

### Supplemental Figure S2

Panels A-N shows the coverage analysis of unknown cases that had at least one read mapped to the reference sequence NC\_045512. The number of reads is shown on the vertical axis and genome position on the horizontal axis; Loess-regression line is shown in red. The insert label indicates the total number of reads. Panels M-O depict PCR cycle CT of D614 and G614 isolates using the SARS-CoV-2 N1 and N3 primers. RNaseP was used as an internal control for total RNA. Shown is a beehplot of Raw CT numbers obtained by real-time RT-qPCR using CDC primers N1 and N3, as well as RNaseP, which serves as a control for reverse transcription. Lower CT values signify a higher genome copy number per sample. CT values are shown on the vertical and the SNV variant G614D (ancestral, red) or G614G (recent, blue) on the horizontal axis. Related to **Figure 1** and **Figure 2**.

### Supplemental Figure S3

Phylogenetic analysis. A. Neighbor-joining tree of whole SARS-CoV-2 genomes from the southeastern United States (from GenBank and GISAID) The bat coronavirus genome strain RaTG13 was used as an outlier to root the tree. Average nucleotide difference is shown for the two major branches and the difference between SARS (NC\_004718.3) and SARS-CoV-2. B. Neighbor-joining tree based on amino acids for S protein. Support values are listed at the major branch points. Colors indicate geographic regions. Additional genome sequences and protein sequences are from GISAID and GenBank. Related to **Figure 2**.

**Table S1****Information on complete genomes**

| <u>Name</u>  | <u>Accession</u> | <u>Collection Date</u>         | <u>GISAID clade</u> |
|--|------------------|--------------------------------|---------------------|
| <b>Batch 1</b>                                     |                  |                                |                     |
| BankIt2330137 SARS-CoV-2/human/USA/UNC_200173/2020 | MT300186         | March 19 <sup>th</sup> , 2020, | B.1 (GH),           |
| BankIt2331121 SARS-CoV-2_Human_UNC_200191_2020     | MT308702         | March 23 <sup>rd</sup> , 2020, | B.1.2 (GH)          |
| BankIt2331639 SARS-CoV-2_human_UNC_200181_2020     | MT308703         | March 23 <sup>rd</sup> , 2020, | A.3 (S)             |
| BankIt2331651 SARS-CoV-2_human_UNC_200189_2020     | MT308704         | March 23 <sup>rd</sup> , 2020, | B.1.2 (GH)          |
| <b>Batch 2</b>                                     |                  |                                |                     |
| SUB7503826 SARS_CoV-2_Human_UNC_200213_2020        | MT522852         | March 25 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7503826 SARS_CoV-2_Human_UNC_200214_2020        | MT522853         | March 30 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7503826 SARS_CoV-2_Human_UNC_200215_2020        | MT522854         | March 31 <sup>st</sup> , 2020, | B.1 (GH)            |
| SUB7503826 SARS_CoV-2_Human_UNC_200216_2020        | MT522855         | March 30 <sup>th</sup> , 2020, | B.1 (GH)            |
| <b>Batch 3</b>                                     |                  |                                |                     |
| SUB7504586 SARS_CoV-2_Human_UNC_200217_2020        | MT528598         | March 25 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200219_2020        | MT528599         | March 30 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200229_2020        | MT528631         | March 31 <sup>st</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200238_2020        | MT528600         | April 1 <sup>st</sup> , 2020,  | B.1.3 (GH)          |
| SUB7504586 SARS_CoV-2_Human_UNC_200241_2020        | MT528601         | March 21 <sup>st</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200242_2020        | MT528602         | March 20 <sup>th</sup> , 2020, | A.3 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200243_2020        | MT528603         | March 20 <sup>th</sup> , 2020, | A.3 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200244_2020        | MT528604         | March 19 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200245_2020        | MT528605         | March 31 <sup>st</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200247_2020        | MT528606         | March 26 <sup>th</sup> , 2020, | A.1 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200252_2020        | MT528627         | March 23 <sup>rd</sup> , 2020, | A.1 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200253_2020        | MT528628         | March 22 <sup>nd</sup> , 2020, | A.3 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200254_2020        | MT528607         | March 28 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200255_2020        | MT528608         | March 28 <sup>th</sup> , 2020, | A.3 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200256_2020        | MT528609         | March 27 <sup>th</sup> , 2020, | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200259_2020        | MT528610         | March 30 <sup>th</sup> , 2020, | A.3 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200264_2020        | MT528611         | April 6 <sup>th</sup> , 2020,  | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200271_2020        | MT528612         | April 6 <sup>th</sup> , 2020,  | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200272_2020        | MT528613         | April 1 <sup>st</sup> , 2020,  | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200278_2020        | MT528614         | April 2 <sup>nd</sup> , 2020,  | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200290_2020        | MT528615         | April 3 <sup>rd</sup> , 2020,  | B.1 (GH)            |
| SUB7504586 SARS_CoV-2_Human_UNC_200300_2020        | MT528616         | March 18 <sup>th</sup> , 2020, | A.1 (S)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200306_2020        | MT528617         | March 24 <sup>th</sup> , 2020, | B.1 (G)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200310_2020        | MT528618         | March 26 <sup>th</sup> , 2020, | B.1 (G)             |
| SUB7504586 SARS_CoV-2_Human_UNC_200312_2020        | MT528619         | March 25 <sup>th</sup> , 2020, | A.3 (S)             |

|   |          |                                |                |
|---|----------|--------------------------------|----------------|
| SUB7504586 SARS_CoV-2_Human_UNC_200318_2020 | MT528620 | March 18 <sup>th</sup> , 2020, | B.1.37<br>(GH) |
| SUB7504586 SARS_CoV-2_Human_UNC_200319_2020 | MT528621 | March 23 <sup>rd</sup> , 2020, | B.1.2 (GH)     |
| SUB7504586 SARS_CoV-2_Human_UNC_200323_2020 | MT528622 | March 26 <sup>th</sup> , 2020, | A.1 (S)        |
| SUB7504586 SARS_CoV-2_Human_UNC_200325_2020 | MT528623 | March 23 <sup>rd</sup> , 2020, | B.1 (GH)       |
| SUB7504586 SARS_CoV-2_Human_UNC_200326_2020 | MT528624 | March 30 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7504586 SARS_CoV-2_Human_UNC_200330_2020 | MT528626 | March 20 <sup>th</sup> , 2020, | A.3 (S)        |
| SUB7504586 SARS_CoV-2_Human_UNC_200331_2020 | MT528625 | March 19 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7504586 SARS_CoV-2_Human_UNC_200340_2020 | MT528629 | March 28 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7504586 SARS_CoV-2_Human_UNC_200386_2020 | MT528630 | March 30 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200459_2020 | MT551604 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200460_2020 | MT551605 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200467_2020 | MT551606 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200468_2020 | MT551607 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200486_2020 | MT551608 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7536347 SARS_CoV-2_Human_UNC_200488_2020 | MT551609 | April 10 <sup>th</sup> , 2020, | B.1 (GH)       |
| SUB7539666 SARS_CoV-2_Human_UNC_200222_2020 | MT554052 | March 31 <sup>st</sup> , 2020, | B.1 (GH)       |
| SUB7540664 SARS_CoV-2_Human_UNC_200429_2020 | MT557568 | March 6 <sup>th</sup> , 2020,  | A (S)          |
| SUB7540664 SARS_CoV-2_Human_UNC_200445_2020 | MT557569 | May 14 <sup>th</sup> , 2020,   | B.1 (GH)       |
| SUB7540664 SARS_CoV-2_Human_UNC_200446_2020 | MT557570 | May 14 <sup>th</sup> , 2020,   | B.1 (GH)       |
| SUB7544178 SARS_CoV-2_Human_UNC_200395_2020 | MT559800 | April 8 <sup>th</sup> , 2020,  | B.1 (GH)       |
| SUB7544178 SARS_CoV-2_Human_UNC_200399_2020 | MT559801 | April 8 <sup>th</sup> , 2020,  | B.1 (GH)       |
| SUB7544178 SARS_CoV-2_Human_UNC_200401_2020 | MT559802 | April 7 <sup>th</sup> , 2020,  | B.1 (GH)       |
| SUB7544178 SARS_CoV-2_Human_UNC_200409_2020 | MT559803 | April 8 <sup>th</sup> , 2020,  | B.1 (GH)       |
| SUB7552904 SARS_CoV-2_Human_UNC_200414_2020 | MT565495 | April 8 <sup>th</sup> , 2020,  | B.1 (GH)       |
| SUB7552904 SARS_CoV-2_Human_UNC_200407_2020 | MT565496 | April 8 <sup>th</sup> , 2020,  | B.1.2 (GH)     |
| SUB7552904 SARS_CoV-2_Human_UNC_200428_2020 | MT565497 | April 7 <sup>th</sup> , 2020,  | B.1.2 (GH)     |
| SUB7552904 SARS_CoV-2_Human_UNC_200313_2020 | MT565498 | March 24 <sup>th</sup> , 2020, | B.1.2 (GH)     |
| SUB7552904 SARS_CoV-2_Human_UNC_200265_2020 | MT565499 | April 3 <sup>rd</sup> , 2020,  | B.1.2 (GH)     |

### Supplemental Table S1

Table of complete SARS-CoV-2 genomes deposited in this study. Shown are sequence file names, accession numbers, date of sequencing, and GISAID clade. Related to **Figure 1**, **Figure 2**, and **Figure 3**.