Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY METHODS

SARS-COV-2 TESTING
SARS-CoV-2 infected individuals were identified in Iceland through targeted testing and population screening. A flowchart outlining the study is shown in Figure 1.

The targeted testing began on January 31 2020 and focused on individuals deemed at high-risk of being infected by SARS-CoV-2. These were mainly symptomatic (cough, fever, aches, and shortness of breath) individuals returning to Iceland from countries/regions classified by the health authorities as high-risk, or who had been in contact with infected individuals. Individuals that met the criteria for targeted testing were encouraged by the health authorities to contact the health care system for testing. They could call a specific phone number, connect through a specific website or contact their primary health care provider. A contagion tracker team tracked down contacts of infected individuals and arranged testing if they had symptoms. Sample for testing were taken in most local primary health care clinics and hospitals. In addition, a number of individuals with symptoms were tested at the international airport on arrival from high-risk areas.

As of March 19, all travel outside Iceland was designated high-risk. As of March 31, 9,199 individuals had been targeted for testing.

The population screening for SARS-CoV-2 was initiated by deCODE on March 13. The screening was open to all Icelandic residents who were symptom free or with mild symptoms of common cold that is highly prevalent in Iceland at this time of the year. The registration for the test was online and during sample collection information on recent travels, contacts with infected individuals, and symptoms compatible with COVID-19 were registered. The sample collection was performed in Reykjavik, the capital of Iceland. On April 1, after twenty days of population screening, 10,797 Icelanders had been screened for SARS-CoV-2.
To evaluate the sampling method of the population screening, we also invited 6,782 randomly chosen Icelanders between ages 20 and 70 to participate through a phone text message sent on March 31 and April 1. Of these, 2,283 had participated by April 4 (33.7%). Of the invited, 41.2% were males and of the participants 37.8% were males.

Those who tested positive for SARS-CoV-2 through any means were required to self-isolate, and those who had been in contact with them, to self-quarantine, for 2 weeks. In addition to isolating positives and quarantining those at high risk of infection, on March 16 the Icelandic authorities initiated a ban on gathering of over 100 people and stated that a social distancing of at least 2 meters should be maintained. On March 24, the gatherings were restricted to 20 people. To protect the elderly and other groups that are at greater risk of serious illness should they develop COVID-19 health authorities have promoted self-isolation and banned visits to nursing homes and hospitals. Although universities and colleges have been closed since March 16, daycares and elementary schools have been open. For SARS-CoV-2 testing, it was recommended to take both nasopharyngeal and oropharyngeal samples. RNA from all samples was isolated within 24 hours.

**TRACKING OF SARS-COV-2 INFECTIONS**

All individuals who tested positive for SARS-CoV-2 were contacted by phone by a contagion tracking team designated by the authorities. They were instructed to enter isolation at home. They were asked about their symptoms and onset, recent travels and previous contacts with infected individuals. They were also asked to identify everyone whom they had been in contact with 24 hours before noticing their first symptom and for how long they interacted with each individual and how intimate the interaction was. All registered contacts were contacted by phone, requested to go into 2 weeks quarantine and asked about symptoms.
Those with symptoms and those who developed them in quarantine were tested for SARS-CoV-2.

Isolation places more severe restrictions on the individual than quarantine. The Icelandic surgeon general provides detailed instructions about quarantine and isolation on the webpages [https://www.covid.is/categories/how-does-quarantine-work](https://www.covid.is/categories/how-does-quarantine-work) and [https://www.covid.is/categories/how-does-isolation-work](https://www.covid.is/categories/how-does-isolation-work). Quarantine last for fourteen days. Isolation ends 10 days after fever subsides or when the individual tests negative for presence of the virus.

**RNA EXTRACTION**

Viral RNA samples were extracted either at the Department of Clinical Microbiology laboratory at Landspitali - the National University Hospital of Iceland (LUH) or at deCODE. Both extraction methods are based on an automated magnetic bead-purification procedure, which includes cell lysis and Proteinase K treatment. RNA from samples at LUH were extracted (32 samples per 60 min run) using the MagNA Pure LC 2.0 or MagNA Pure Compact instruments from Roche LifeScience, with 200/100 µL input/output volume(s), respectively. Samples at deCODE were extracted from swabs (96 samples per 70 min run) using the Chemagic Viral RNA kit on the Chemagic360 instrument from Perkin Elmer, with 300/100 µL input/output volume(s), respectively. Each step in the workflow was monitored using an in-house LIMS (VirLab) with 2D barcoding (Greiner, 300 µL tubes) of all extracted samples.

**TESTING OF SAMPLES FOR SARS-COV-2**

Testing for SARS-CoV-2 was performed either at LUH or deCODE using similar quantitative real-time PCR (qRT-PCR) methods. The assay at LUH is based on the WHO recommended
screening method (https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf), which involves a single probe pan-screening assay for betacoronaviruses, followed by confirmatory measurements for all positive samples using an nCoV-2019 specific assay. The broad betacoronavirus assay is based on probes for a conserved region of the E-gene, whereas confirmatory testing assays were done using either nCoV-2019 specific probes for the RdRp gene or the TaqMan™ Fast Virus 1-step Master Mix, 2019-nCoV Assay kits v1 from Thermo Fisher (three probes, see below for details). All labelled probes and primers for the E-and -RdRP genes were from TAG (Copenhagen, Denmark). Superscript™ III One-Step RT-PCR assay mix with Platinum™ Taq DNA polymerase was from ThermoFisher. 2019 E gene control and SARS-CoV Frankfurt 1 positive controls were obtained from EVAg (https://www.european-virus-archive.com/bundle/diagnostics-controls-wuhan-coronavirus-2019-2019-ncov). Each assay was done in a 25 µL total sample volume with FAM™ dye labelled probes in addition to VIC™ dye labelled probes for human RNase P as internal control. Plates (96 well) were scanned in an AB-7500 Fast real-time PCR thermocycler for 40 cycles of amplification following the manufacturer´s instructions (ThermoFisher). Samples in the E-gene screening assay with C_t <35 were considered strong positive and went for confirmatory testing using RdRp, whereas samples with C_t values between 35-37 were considered weak positive and were confirmed using the TaqMan™ Fast Virus method. Samples with C_t values from 37-40 were classified as inconclusive and were tested again to confirm their status. The sensitivity of the WHO assays are 5.2 RNA copies/reaction for the non-specific E-gene and 3.8 RNA copies/reaction for the 2019- nCoV specific confirmatory RdRp gene. Specificity of the WHO recommended assays were assessed against a number of known viruses, including alphacoronaviruses, non-asian strains of betacoronaviruses, influenza and MERS. No cross-reactivity was observed.
deCODE used exclusively the three probe TaqMan™ Fast Virus 1-step Master Mix, 2019-nCoV v1 assay described above. Both the assay kit and 2019-nCov control kits were obtained from Thermo Fisher. Assay mix A, B and C were prepared containing FAM™ dye labelled probes for the SARS-CoV-2 specific genes ORF1ab, S-protein and N-protein, respectively. In addition, each assay mix contained VIC™ dye labelled probes for human RNase P as internal control. Samples from 96-well RNA sample plate(s) were dispensed into three wells each in a 384 plate layout, in addition to three negative (no template) and three positive controls. Assay mix was added in a total reaction volume of 12.5 µL per sample. All sample aliquoting and mixing at deCODE was performed with an automated Hamilton STARlet 8-channel liquid handler and the assay plates were scanned in an ABI 7900 HT RT-PCR system following manufacturer’s instructions with a total of 40 cycles of amplification. Samples with FAM™ dye C\text{t} values <37 in at least two of three assays were classified as positive. Samples with FAM™ dye C\text{t} values between 37 and 40 were classified as inconclusive and their testing repeated. If repeated testing gave the same result with at least two probes the sample was classified as positive. If repeated testing gave positive results for only one probe the test was considered inconclusive and a new sample from the subject was requested. The frequency of inconclusive results was 0.04% (data not shown). Samples with undetected FAM™ dye C\text{t} values or values equal to 40 in all three assays were classified as negative if the human RNaseP assay was positive (VIC™ dye C\text{t} <40). The sensitivity of the assay was evaluated by serial dilution of the positive control and was estimated at 6 copies per reaction (data not shown). Validation of the RNA extraction and the qRT-PCR method(s) at deCODE was performed using 124 samples that had previously tested positive (n=104) or negative (n=20) with the qRT-PCR assay at LUH. All of the negative samples tested negative at deCODE and 102 of the 104 positive tested at LUH were also positive at
deCODE. Two samples that tested positive at LUH were negative at deCODE. Upon subsequent sequencing (see below) viral genome could not be detected in these two samples, probably because very few viral particles were present. Samples from 643 individuals that tested positive using either the deCODE or the LUH qPCR assays were also submitted for viral genome sequencing (see below). Viral RNA (cDNA) from six samples (0.9 %) yielded no sequence data mapping to the viral reference genome. The success of generating sequencing libraries with good coverage is highly dependent on the amount of viral RNA in the samples as assessed by the C_t values from the qRT-PCR assays. Figure S2 shows the relationship between measured C_t values and the consensus coverage of the sequenced samples. These data show that the qRT-PCR assay is more sensitive in detecting viral RNA than the amplicon sequencing method.

**SAMPLE PREPARATION FOR SEQUENCING**

Reverse transcription (RT) and multiplex PCR was performed based on information provided by the Artic Network initiative (https://artic.network/) to generate cDNA. In short, extracted viral RNA was pre-incubated at 65 °C for 5 min in the presence of random hexamers (2.5 µM) and dNTP’s (500 µM). Sample cooling on ice was then followed by RT using SuperScript IV (ThermoFisher) in the presence of DTT (5 mM) and RNaseOUT inhibitor (ThermoFisher) for 10 min at 42°C, followed by 10 min at 70 °C. Multiplex PCR of the resulting SARS-CoV-2 cDNA was performed using a tiling scheme of primers, designed to generate overlapping amplicons of approximately 800 bp (Table S1). The primers were generously provided by Dr. David Stoddard at Oxford Nanopore Technologies. Two PCR reactions were done for each sample using primer pools A and B, respectively (Table S1). PCR amplification was done using the Q5® Hot Start High-Fidelity polymerase (New England Biolabs) with primers at 1 µM concentration. The reactions were performed in a MJR thermal cycler with a heated lid at 105 °C, using 35 cycles
of denaturation (15 sec at 98 °C) and annealing/extension (5 min at 65 °C). The resulting PCR amplicons were purified using Ampure XP magnetic beads (Beckman Coulter) and quantified using the Quant-iT™ PicoGreen dsDNA assay kit (Thermo Fisher). Amplified samples (20-500 ng) were randomly sheared using focused acoustics in 96-well AFA-TUBE-TPX plates (Covaris Inc.) on the Covaris LE220plus instrument with the following settings: Sample volume, 50 µL; temperature, 10 °C; peak incident power, 200W; duty factor, 25%; cycles per burst, 50; time, 350 sec. Sequencing libraries were prepared in the 96 well Covaris plates, using the NEBNext® Ultra II kit (New England Biolabs) following the manufacturer’s instructions. In short, end repair and A-tailing was performed in a combined reaction per sample (plate) for 30 min at 20 °C, followed by thermal enzyme inactivation at 65 °C for 30 min. Adaptor ligation was done using the NEBNext® ligation master mix plus enhancer and the TruSeq unique dual indexed IDT adaptors (Illumina, Table S2). Ligation reactions were incubated for 15 min at 20 °C. Ligated sequencing libraries were purified on a Hamilton STAR NGS liquid handler, using two rounds of magnetic SPRI bead purification (0.7X volume).

**ILLUMINA SEQUENCING**

Sequencing libraries were pooled (24-36 samples/pool) and quantified using the Qubit dsDNA assay (ThermoFisher). Samples were diluted appropriately and denatured to a final loading concentration of 10 pM. All samples were sequenced on Illumina MiSeq sequencers using 300-cycle MiSeq v2 reagent kits (Illumina). Each pool was sequenced using dual indexed paired-end sequencing of 150*8*8*150 bp cycles of data acquisition and imaging with a run time of approximately 24 hrs. Basecalling was done in real time using MCS v3.1 and FASTQ files were generated using MiSeq Reporter. At least 15M PF reads (>4.5Gb) with base qualities of >Q30 for at least 90% of bases were collected for each run.
**SEQUENCING DATA ANALYSIS**

Amplicon sequences were aligned to the reference genome of the SARS-CoV-2 (NC_045512.2) using bwa mem, possible PCR duplicates were marked with markDuplicates from Picard tools and reads with less than 50 bases aligned were omitted from the alignment. The resulting aligned filtered reads were used for variant calling with bcftools. For consensus sequence generation only variants reported as homozygous were used. In regions targeted with primers we allowed variants to have allele frequency below one in individual. The consensus sequence was masked with ambiguous nucleotides (N) at positions if the depth of coverage was strictly less than 5 reads after restricting to bases of quality 20 or higher. Consensus sequences with more than 10,000 ambiguous nucleotides were discarded from analysis. The SARS-COV-2 consensus sequences with less than 10,000 ambiguous nucleotides were uploaded to GISAID (https://www.gisaid.org), with virus names hCov/Iceland/1/2020-hCov/Iceland/604/2020, and EPI accession numbers, EPI_ISL_417481, EPI_ISL_417535-EPI_ISL_417876, and EPI_ISL_424367-EPI_ISL_424624. The mutations in Table S3 were used to define haplogroups/clades.

In addition to calling consensus sequence of the samples independently, we jointly called variants across the samples using a modified version of Graphtyper. In this modified version we excluded the termini of paired end reads overlapping a primer region. Further we supplied the following flags to Graphtyper:

```bash
--no_filter_on_read_bias, --no_filter_on_strand_bias  --no_filter_on_coverage  --impurity_threshold=1.0  --primer_bedpe={primer_file}  --is_only_cigar_discovery  --genotype_aln_min_support_ratio=0.30  --genotype_aln_min_support=5  --is_discovery_only_for_paired_reads  --no_filter_on_begin_pos
```
In this joint calling, we restricted to genotypes with coverage of 5 or more. We then define a carrier matrix, where the cell in matrix was 1 if the fraction of reads supporting the alternative allele (AB) was greater than 85% (carrier), and 0 if the AB was less than 5% (non-carrier). With this carrier matrix, we manually curated list of mutations in Table S3 to define haplotypes. We then assigned a haplotype per sample, by aggregating the haplotype informative mutations of the sample and constructing a consensus haplotype.

Variants were annotated using Variant Effect Predictor (VEP) version 99.2 in custom mode. As a gene map we used Uniprot mature gene track downloaded from UCSC. A single VEP annotation was assigned to each sequence variant according to our previously described scheme (Sveinbjornsson et al 2015) using gorpipe scripts.

For network analysis of haplogroups a median-joining network of SARS-CoV-2 sequences was generated using data from our sequencing effort in Iceland and from GISAID available on March 22 (Table S4). Only sequences with start positions <=200 and stop positions >=29750 were included in the analysis. For the GISAID sequences, only those with <=1% missing nucleotides were used, whereas for the Icelandic sequences a more permissive threshold of <=5% was imposed. To reduce noise in the network, an imputation step was implemented for sequences with missing nucleotides at sites where other sequences varied, whereby the missing nucleotide was imputed to the consensus variant for the clade it was assigned to, based on non-missing sites.

Contact tracking information was obtained from the Chief Epidemiologist, Directorate of Health, for 1317 confirmed cases. The information includes travel abroad, confirmed transmissions, contact with other confirmed cases as well as demographic information.
study was approved by the National Bioethics Committee of Iceland (Approval no. VSN-20-070).
**SUPPLEMENTARY FIGURES**

**Figure S1** Coverage of sequenced viral genomes shown as size of the consensus sequence as a function of measured Ct values from the qRT-PCR screening assays. A total of 643 samples were sequenced, thereof 581 and 605 samples with coverage for at least 90% (27Kb) and 67% (20 Kb) of the genome, respectively. Six samples yielded no coverage.
Figure S2 The number of individuals tested per day (A) in the targeted testing, (B) the open invitation part of the population screening, and (C) the random sample from the population screening.
**Figure S3** Fraction of participants in (A) the targeted testing and (B) population screening that had recently traveled outside Iceland.
Figure S4 The fraction of participants in the population screening reporting symptoms by study date.
Figure S5 The fraction of individuals that tested positive before age 20 in the targeted testing stratified by age and sex. The results for males are shown in blue and females in red. Vertical bars indicate 95% confidence intervals. The solid curves indicate logistic regression fits of a model with a sex effect and an age effect. The dashed lines indicate 95% confidence intervals for the male and female logistic regression fits. The age odds ratio is 1.08 per year (95% CI: 1.05-1.12) and the male sex odds ratio is 1.45 (95% CI: 1.04-2.0).
Figure S6 The fraction of individuals that tested positive in the targeted testing stratified by sex. The results for males are shown in blue and females in red. Vertical bars indicate 95% confidence intervals. The solid curves indicate logistic regression fits of a model with a sex effect, an age effect and an effect for those under 10 years of age. The dashed lines indicate 95% confidence intervals for the male and female logistic regression fits. As discussed in the main text, the fraction of positives is lower in those under 10 years of age compared to those older, but the age effect is not significant.
Figure S7 The distribution of SARS-CoV-2 haplotypes depending on sampling and travel status. The counts of each of the eight haplotypes seen in Iceland is shown depending on whether the positive was found through early targeted testing, population screening, or later targeted testing and whether the positive had recently traveled outside Iceland.
### Table S1 Primers used for PCR amplification of viral cDNA for sequencing

| name             | pool           | seq                             | length | %gc  | tm  |
|------------------|----------------|---------------------------------|--------|------|-----|
| nCoV-2019_1_LEFT| nCoV-2019_1    | ACCAACCACTTTGCTACTTTGGT     | 24     | 41.67| 60.69 |
| nCoV-2019_2_RIGHT| nCoV-2019_2   | TAAGGATCAGTGGCAAGCTCTG        | 22     | 50   | 61.74 |
| nCoV-2019_3_LEFT | nCoV-2019_1    | CGTAAATAAAGAGCTGAGCCAG        | 22     | 54.55| 61.32 |
| nCoV-2019_4_RIGHT | nCoV-2019_2 | CACAAGTACTGAGCTTCTTTTGT      | 25     | 44   | 60.97 |
| nCoV-2019_5_LEFT | nCoV-2019_1    | TGGTGAAACTCTATGGCCAGCAG       | 22     | 50   | 61.39 |
| nCoV-2019_6_RIGHT | nCoV-2019_2 | TACCCGGCTTCTGTAACACAGCG       | 22     | 50   | 61.18 |
| nCoV-2019_7_LEFT | nCoV-2019_1    | ATCAGAGGCTGCTGGTGGT          | 22     | 50   | 61.73 |
| nCoV-2019_8_RIGHT | nCoV-2019_2  | GCTCAACAGCTTCACTAGGTG        | 24     | 45.83| 60.56 |
| nCoV-2019_9_LEFT | nCoV-2019_1    | TCCCAAGAAGTGTTAAGAGGGAGG     | 24     | 45.83| 61.18 |
| nCoV-2019_10_RIGHT | nCoV-2019_2 | TCATTCTACAATCTTCTTGTCTC     | 27     | 37.04| 60.31 |
| nCoV-2019_11_LEFT | nCoV-2019_1   | GGAATTTGGTGCCACTTCTGCT       | 22     | 50   | 61.66 |
| nCoV-2019_12_RIGHT | nCoV-2019_2 | TTCATTCTTCTTTCCAAGGTTGTA     | 27     | 33.33| 60.36 |
| nCoV-2019_13_LEFT | nCoV-2019_1    | TCAGCAAAATGTCTAATCTGCT       | 24     | 41.67| 60.56 |
| nCoV-2019_14_RIGHT | nCoV-2019_2 | AGTTTCCACACAGACGCCATT        | 22     | 45.45| 60.42 |
| nCoV-2019_15_LEFT | nCoV-2019_1    | ACAGTGCTTTAAAAAGGTAAAAGTGCC  | 27     | 37.04| 61.32 |
| nCoV-2019_16_RIGHT | nCoV-2019_2 | CACAACCTGGTGCTGGAGGTTA      | 22     | 50   | 61.32 |
| nCoV-2019_17_LEFT | nCoV-2019_1    | CTCTTCTTGGAGAGAAGGTGAGACT    | 27     | 40.74| 60.74 |
| nCoV-2019_18_RIGHT | nCoV-2019_2 | AGCTTGTGTTACACAGCTGAAAGG   | 24     | 45.83| 61.51 |
| nCoV-2019_19_LEFT | nCoV-2019_1    | GCTTTATGCACTTGGCCACACT       | 23     | 47.83| 61.18 |
| nCoV-2019_20_RIGHT | nCoV-2019_2  | ACCTGGCTTTATATTGCATGATTG      | 25     | 36   | 60.28 |
| nCoV-2019_21_LEFT | nCoV-2019_1    | TGGCATTTGATTATAAACACTACACACCC | 29    | 37.93| 61.49 |
| nCoV-2019_22_RIGHT | nCoV-2019_2 | ACAGTATTCTTGTATAGTGATCGCG   | 27     | 40.74| 60.73 |
| nCoV-2019_23_LEFT | nCoV-2019_1    | ACAACTAATCAATGTTACACGGTGT    | 27     | 37.04| 60.26 |
| nCoV-2019_24_RIGHT | nCoV-2019_2  | ACATTCTAACCACAGCTGAAATCCGG   | 26     | 42.31| 61.19 |
| nCoV-2019_25_LEFT | nCoV-2019_1    | GCATTGTTTTTTTCAGCTATTTTGCGT | 27     | 33.33| 60.73 |
| nCoV-2019_26_RIGHT | nCoV-2019_1  | TCGCGACTATCAACACACACACT      | 22     | 50   | 60.42 |
| nCoV-2019_27_LEFT | nCoV-2019_1    | ACTACAGTGCAGGTATGTCAGACC     | 25     | 44   | 60.8  |
| nCoV-2019_28_RIGHT | nCoV-2019_2 | TGTCTAGACATGACATGACAGCAGTGT | 26     | 38.46| 60.91 |
| nCoV-2019_29_LEFT | nCoV-2019_1    | ACTTGTTGCTCTTTTGCTGGCTG      | 24     | 41.67| 61.39 |
| nCoV-2019_30_RIGHT | nCoV-2019_2   | ACCACATAGTAGACACAAACACAG     | 26     | 42.31| 60.3  |
| nCoV-2019_31_LEFT | nCoV-2019_1    | TTCTGAGACTGCTGACAGGCG        | 22     | 54.55| 62.03 |
| nCoV-2019_32_RIGHT | nCoV-2019_2  | ACAGCACTACAGACACATTTAGA      | 24     | 41.67| 60.56 |
| nCoV-2019_33_LEFT | nCoV-2019_1    | ACCTTTGAAGAGCTGGCTGT         | 22     | 45.45| 61.58 |
| nCoV-2019_34_RIGHT | nCoV-2019_2 | AGTGAAGATGGGCTCTATAGCAGCA    | 22     | 45.45| 60.03 |
| nCoV-2019_35_LEFT | nCoV-2019_1    | TGTCACACATCCACGACAGCAG       | 22     | 50   | 61.39 |
| nCoV-2019_36_RIGHT | nCoV-2019_2   | GAACAAAGACATTGAGCTTCTGGTA    | 26     | 42.31| 60.74 |
| nCoV-2019_37_LEFT | nCoV-2019_1    | ACACACACTGTTTGCTACCTC        | 23     | 47.83| 60.93 |
| nCoV-2019_38_RIGHT | nCoV-2019_2  | CACCAAGACTGACCTTAAATGAGC     | 25     | 48   | 61.13 |
| nCoV-2019_39_LEFT | nCoV-2019_1    | AGATGCTTCCCACTTTCTGACACTG    | 29     | 34.48| 61    |
| nCoV-2019_40_RIGHT | nCoV-2019_2 | CATGGCCTGACTACGGTCAAAT       | 22     | 50   | 62.09 |
| nCoV-2019_41_LEFT | nCoV-2019_1    | GTCCCTTACATCATACAGCCT        | 23     | 47.83| 60.75 |
| nCoV-2019_42_RIGHT | nCoV-2019_2   | CCTACCTCCCTTGGCTGGTGT       | 23     | 47.83| 60.69 |
| nCoV-2019_43_LEFT | nCoV-2019_1 | TACGACAGATGTCCTTGTCCTG | 22 | 50 | 60.93 |
| nCoV-2019_44_RIGHT | nCoV-2019_2 | AACCTTTCCACATACCGCCAGAC | 22 | 50 | 60.87 |
| nCoV-2019_45_LEFT | nCoV-2019_1 | TACCTACAACCTTGCTAATGAACC | 25 | 44 | 60.57 |
| nCoV-2019_46_RIGHT | nCoV-2019_2 | CACGTTCACCTAAGTTGGCTGA | 22 | 50 | 60.86 |
| nCoV-2019_47_LEFT | nCoV-2019_1 | TAGATTACCAAGACCCAGCTGC | 22 | 50 | 60.74 |
| nCoV-2019_48_RIGHT | nCoV-2019_2 | AGGAATTACTTGTGTATGCTG | 25 | 40 | 60.57 |
| nCoV-2019_49_LEFT | nCoV-2019_1 | TACCTACAACTTGTGCTAATG | 25 | 44 | 60.57 |
| nCoV-2019_50_RIGHT | nCoV-2019_2 | CAACATGTTGTGCCAACCAC | 22 | 45.45 | 60.95 |
| nCoV-2019_51_LEFT | nCoV-2019_1 | TCAATAGCCGCACTAGAGGAG | 22 | 54.55 | 61.34 |
| nCoV-2019_52_RIGHT | nCoV-2019_2 | GTTGAGAGCAAAATTCATGAC | 22 | 50 | 60.74 |
| nCoV-2019_53_LEFT | nCoV-2019_1 | AGCAAAATGTTTGAACTGAACTGA | 24 | 41.67 | 60.69 |
| nCoV-2019_54_RIGHT | nCoV-2019_2 | ACACAAAAACTTGTTCCATAGGCA | 25 | 36 | 60.11 |
| nCoV-2019_55_LEFT | nCoV-2019_1 | ACTCAACTTTACTTAGGAGGT | 28 | 39.29 | 61.43 |
| nCoV-2019_56_RIGHT | nCoV-2019_2 | ACACAAAAACTTGTTCCATAGGCA | 25 | 36 | 60.11 |
| nCoV-2019_57_LEFT | nCoV-2019_1 | TCAATAGCCGCACTAGAGGAG | 22 | 54.55 | 61.34 |
| nCoV-2019_58_RIGHT | nCoV-2019_2 | CTAACACTCCGACAGGGACACC | 22 | 54.55 | 61.16 |
| nCoV-2019_59_LEFT | nCoV-2019_1 | TATACTGACGAGCAGGAAGGTA | 22 | 50 | 61.21 |
| nCoV-2019_60_RIGHT | nCoV-2019_2 | ATPCTAATGCTTTACATGCTGCA | 23 | 43.48 | 61.42 |
| nCoV-2019_61_LEFT | nCoV-2019_1 | GTTTATACCCCGCCGAAAGGC | 22 | 50 | 60.44 |
| nCoV-2019_62_RIGHT | nCoV-2019_2 | GTGTGCCCTTTTAAGCTTG | 22 | 50 | 60.35 |
| nCoV-2019_63_LEFT | nCoV-2019_1 | TGTTAAGCGCTTGATTGGACTGAGT | 22 | 45.45 | 60.16 |
| nCoV-2019_64_RIGHT | nCoV-2019_2 | AGCTCTGTTAAAAGGTGTCCAGAGGT | 25 | 40 | 60.1 |
| nCoV-2019_65_LEFT | nCoV-2019_1 | GTGACATCAATGCTTG | 22 | 50 | 61.92 |
| nCoV-2019_66_RIGHT | nCoV-2019_2 | TCAATTTCCATGCTTCCCTG | 24 | 41.67 | 60.45 |
| nCoV-2019_67_LEFT | nCoV-2019_1 | GTTGCCCAAATAATAGTTCCAGAGGT | 28 | 35.71 | 60.43 |
| nCoV-2019_68_RIGHT | nCoV-2019_2 | CTCTTTATCAAGAACCAGCAC | 23 | 47.83 | 60.31 |
| nCoV-2019_69_LEFT | nCoV-2019_1 | TGTCGCAAAATATACTCAACTG | 27 | 37.04 | 61.43 |
| nCoV-2019_70_RIGHT | nCoV-2019_2 | TGACCTTCTTTTTAAAGCTCCAG | 28 | 35.71 | 60.27 |
| nCoV-2019_71_LEFT | nCoV-2019_1 | ACAATCCAATATCGTCTTCATTTC | 29 | 34.48 | 60.54 |
| nCoV-2019_72_RIGHT | nCoV-2019_2 | ACCTCGAATCTCATTCTCAGAAC | 25 | 44 | 60.97 |
| nCoV-2019_73_LEFT | nCoV-2019_1 | CAATT TTGAATGATCCTTATG | 29 | 31.03 | 60.29 |
| nCoV-2019_74_RIGHT | nCoV-2019_2 | GCAACACAGTTGCTTTCTCTC | 24 | 45.83 | 60.85 |
| nCoV-2019_75_LEFT | nCoV-2019_1 | AGATCGCAACCACAGAACATCTATTG | 26 | 38.46 | 60.24 |
| nCoV-2019_76_RIGHT | nCoV-2019_2 | ACACCTGTGCCTGTATTTACCAT | 22 | 45.45 | 60.42 |
| nCoV-2019_77_LEFT | nCoV-2019_1 | CCAGCAACTGTTGGGCAC | 22 | 50 | 60.75 |
| nCoV-2019_78_RIGHT | nCoV-2019_2 | TGGTACAAACCTGGCCATATTGCA | 25 | 36 | 60.22 |
| nCoV-2019_79_LEFT | nCoV-2019_1 | GTGGTGATAGTCCATAAGC | 23 | 47.83 | 60.92 |
| nCoV-2019_80_RIGHT | nCoV-2019_2 | TGGAGCTAGTTGTTATCAACAGCG | 24 | 41.67 | 60.02 |
| nCoV-2019_81_LEFT | nCoV-2019_1 | GACCTTGAAAACCTTCAAGTGTCGG | 25 | 44 | 61.24 |
| nCoV-2019_82_RIGHT | nCoV-2019_2 | TGCCAGAGATGTCACCTTAATACTCA | 24 | 41.67 | 60.02 |
| nCoV-2019_83_LEFT | nCoV-2019_1 | TCTTTGTCAACCTGTAATGACT | 25 | 40 | 60.46 |
| nCoV-2019_84_RIGHT | nCoV-2019_2 | AGGTGAGTAACATGTTGTTACAAAC | 27 | 37.04 | 60.36 |
| nCoV-2019_85_LEFT | nCoV-2019_1 | ACTAGACTCCTCCAGGCTGTT | 22 | 50 | 61.03 |
| nCoV-2019_86_RIGHT | nCoV-2019_2 | AGCAAGCAAGAAAGAAGTAGACGC | 25 | 40 | 61.01 |
| nCoV-2019_87_LEFT | nCoV-2019_1 | CGACTACTACGCTGCTTGTGA | 22 | 50 | 60.16 |
| nCoV-2019_88_RIGHT | nCoV-2019_2 | TGGTCAGAATAGTGCCATGGAGT | 23 | 47.83 | 61.4 |
| nCoV-2019_89_LEFT | nCoV-2019_1    | GTACGCGTCCATGTCATT | 22  | 50  | 61.5 |
|------------------|----------------|-------------------|-----|-----|------|
| nCoV-2019_90_RIGHT | nCoV-2019_2    | TGAAATGGTAATGCCCTCGT | 22  | 45.45 | 60.82 |
| nCoV-2019_91_LEFT  | nCoV-2019_1    | TCACTACCAAAGAGGTGTAGAGT | 25  | 44  | 60.93 |
| nCoV-2019_92_RIGHT  | nCoV-2019_2    | AGGTTCCTGGCAATTATTTGTAAAAGG | 27  | 37.04 | 60.53 |
| nCoV-2019_93_LEFT  | nCoV-2019_1    | TGAGGCCTGGTCTAAATCACCCA | 23  | 47.83 | 61.59 |
| nCoV-2019_94_RIGHT  | nCoV-2019_2    | TTTGGCAATGTGTCTTGTGAGG | 23  | 43.48 | 60.18 |
| nCoV-2019_95_LEFT  | nCoV-2019_1    | TGAGGGAGCCCTTGAATACACCA | 22  | 50  | 61.1 |
| nCoV-2019_96_RIGHT  | nCoV-2019_2    | TAGGCTCTGTTGGTGGGAATG | 22  | 50  | 61.36 |
| nCoV-2019_97_LEFT  | nCoV-2019_1    | TGGATGACAAAGATCCAAATTTCATAAAGA | 28  | 32.14 | 60.22 |
| nCoV-2019_98_RIGHT  | nCoV-2019_2    | TTCTCCTAGAAGCTTAAATACATGGA | 30  | 33.33 | 60.01 |
| Well i7 index | Index            |
|--------------|-----------------|
| 1. A1        | CCGCGGTTC       |
| 2. A2        | AGTTCAGG        |
| 3. A3        | TAATACAG        |
| 4. A4        | ACTAAGAT        |
| 5. A5        | TACCGAGG        |
| 6. A6        | ATATGGAT        |
| 7. A7        | ATATCTCG        |
| 8. A8        | TCGGCGGT        |
| 9. A9        | ACACTAAG        |
| 10. A10      | CAATTAAC        |
| 11. A11      | AACTGTAG        |
| 12. A12      | TATCGCAC        |
| 1. B1        | TTATAACC        |
| 2. B2        | GACCTGAA        |
| 3. B3        | CGGCGTGA        |
| 4. B4        | GTCGGAGC        |
| 5. B5        | CGTTAGAA        |
| 6. B6        | GCGCAAGC        |
| 7. B7        | GCGCTCTA        |
| 8. B8        | CATAATAC        |
| 9. B9        | GTGTGGGA        |
| 10. B10      | TGGCCCGGT       |
| 11. B11      | GGTCACGA        |
| 12. B12      | CGCTATGT        |
| 1. C1        | GGACTTTGG       |
| 2. C2        | TCTCTACT        |
| 3. C3        | ATGTAAGT        |
| 4. C4        | CTTGGGTAT       |
| 5. C5        | AGGCTCTA        |
| 6. C6        | AAGATACT        |
| 7. C7        | AACAGGTT        |
| 8. C8        | GATCTATC        |
| 9. C9        | TTCTTGTT        |
| 10. C10      | AGTACTCC        |
| 11. C11      | CTGCTTCC        |
| 12. C12      | GTATGTTC        |
| 1. D1        | AAGTCCAA        |
| 2. D2        | CTCTCGTC        |
| 3. D3        | GCACGGAC        |
| 4. D4        | TCCAACGC        |
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AGCGCTAG CCAACAGA ATATTAC AACCACGG CCTGAAC CTGTATTA ATCTTAGT CCTCGGTA
ATCCATAT CGAGATAT ACGCCGCA CTTAGTGT GATATCGA TTGGTGAG GCGCCTGT GGTTATAA
TTCAAGTC TCACGCGG GCTCGGCT GGTTACCT GATATCGT

1. E1  ATCCACTG
2. E2  CCAAGTCT
3. E3  GGTACCTT
4. E4  CCGTGAAG
5. E5  TGCTAGTG
6. E6  ATGGCATG
7. E7  CAACAATG
8. E8  CGGAACGT
9. E9  GCCACAGG
10. E10 TGCGAGAC
11. E11 AGGTATTA
12. E12 TACTCATA

1. F1  GCTTGTCA
2. F2  TTGGACTC
3. F3  AACGTTCC
4. F4  TTACAGGA
5. F5  CTACGACA
6. F6  GCAATGCA
7. F7  TGGTGCCA
8. F8  TAAGGTCA
9. F9  ATGGTGAA
10. F10 CATAGACT
11. F11 GAACCGGC
12. F12 CGTCTGCG

1. G1  CAAGCTAG
2. G2  GGCTTAAG
3. G3  GCAGAATT
4. G4  GCCATTCT
5. G5  TAAGTGGA
6. G6  GTTCCAAT
7. G7 AGGCAGAG
8. G8 TTGCCTAG
9. G9 ACTCGTGT
10. G10 ACAGGCGC
11. G11 CTCAACAA

GACGAGAG GTCCGTGC GCGTTGGA GCAGAATC GACGCTCC GGTTCACC AGCGAGCT GGTGAAGG
AGGTGCGT AAGGATGA AAGACGTC CAGTGGAT AGACTTGG AAGGTACC CTTCACGG CACTACGA
CATGCCAT CATTGTTG CAGTTCCG CCTGTGAC GAACATAAC GGAAGCAG GGAGTACT TGACAAGC
GAGTCCAA GGAACGGT TTCCTGAA TGTCTTAG TGCATTGG TGCCACCA TGACCTTA TGCACAAT
ACATAGCG TCGTGACC ACCGGCCA CTAGCTTG CTTAAGCC AATTCTGC AGAATGCC ACCACTTA
ATTGGAAC CTCTGCCT CTAGGCAA

G12 TCGATATC

1. H1 TGGATCGA
2. H2 AATCCGGA
3. H3 ATGAGGCC
4. H4 AATGCCTC
5. H5 CGGAACAC
6. H6 ACCTTGCC
7. H7 GAATGAGA
8. H8 CCATTGCA
9. H9 GTCTACAC
10. H10 GTGAATAT
11. H11 TCTTTGGA
12. H12 CTAGCGCT

ACACGAGT GTGCGATA CTACAGTT GTTAATTG TCGATCCA TCCGGATT GCCCTCAT GAGGCATT
GTGTCCCG GCCAAGGGT TCTCATCC TCGAATGG GTGTAAGC
| Clade | Pos   | Ref | Alt |
|-------|-------|-----|-----|
| B     | 28144 | T   | C   |
| B1    | 18060 | C   | T   |
| B1a   | 17858 | A   | G   |
| B1a1  | 17747 | C   | T   |
| B1a1a | 24694 | A   | T   |
| B1a1a1| 9445  | T   | C   |
| B1a1a1a| 17531 | T   | C   |
| B1a1a1a| 18756 | G   | T   |
| B1a1a1b| 29140 | G   | T   |
| B4    | 28878 | G   | A   |
| B4    | 29742 | G   | A   |
| B2    | 29095 | C   | T   |
| A     | 20229 | C   | T   |
| A     | 13064 | C   | T   |
| A     | 18483 | T   | C   |
| A     | 8017  | A   | G   |
| A1a   | 11083 | G   | T   |
| A1a   | 26144 | G   | T   |
| A1a1  | 14805 | C   | T   |
| A1a1a | 17247 | T   | C   |
| A1a1a1| 5142  | C   | T   |
| A1a1a2| 1321  | A   | C   |
| A1a1a3| 3034  | T   | C   |
| A1a1a3| 16054 | C   | T   |
| A1a1a3| 17859 | T   | C   |
| A1a1a3| 29751 | G   | C   |
| A1a1a4| 1515  | A   | G   |
| A1a1a5| 7479  | A   | G   |
| A1a1b | 2558  | C   | T   |
| A1a2  | 7876  | T   | A   |
| A3    | 1397  | G   | A   |
| A3    | 11083 | G   | T   |
| A3    | 28688 | T   | C   |
| A3    | 29742 | G   | T   |
| A6    | 514   | T   | C   |
| A7    | 9924  | C   | T   |
| A8    | 1440  | G   | A   |
| A8    | 2891  | G   | A   |
| A8a   | 28851 | G   | T   |
| A8b   | 4140  | A   | G   |
| A8c   | 27661 | C   | T   |
| A9    | 1604  | ATGA| A   |
| A9    | 20270 | C   | T   |
| A10   | 1218  | C   | T   |
| Clade | Position | Nucleotide 1 | Nucleotide 2 |
|-------|----------|--------------|--------------|
| A10   | 27806    | G            | T            |
| A10   | 29711    | G            | T            |
| A2    | 241      | C            | T            |
| A2    | 3037     | C            | T            |
| A2    | 23403    | A            | G            |
| A2a   | 14408    | C            | T            |
| A2a1  | 28881    | G            | A            |
| A2a1  | 28882    | G            | A            |
| A2a1  | 28883    | G            | C            |
| A2a1a | 27046    | C            | T            |
| A2a1a1| 25958    | A            | G            |
| A2a1a2| 28344    | C            | A            |
| A2a1a3| 23086    | C            | T            |
| A2a1b | 10097    | G            | A            |
| A2a1b | 23731    | C            | T            |
| A2a1c | 19839    | T            | C            |
| A2a1d | 27430    | G            | A            |
| A2a2  | 25563    | G            | T            |
| A2a2a | 1059     | C            | T            |
| A2a3  | 20268    | A            | G            |
| A2a3a | 10323    | A            | G            |
| A2a4  | 2455     | C            | T            |
| A2a4  | 10450    | C            | T            |
| A2a5  | 26530    | A            | G            |
| A2a6  | 24862    | A            | G            |
| A2a7  | 25429    | G            | T            |
| A2a8  | 15324    | C            | T            |
| A2a9  | 187      | A            | G            |
| A2a10 | 25350    | C            | T            |
| A2a11 | 20275    | G            | A            |
| A2a12 | 24077    | G            | T            |
| A2a13 | 28836    | C            | T            |

The clade nomenclature at ncov-NextStrain (https://academic.oup.com/bioinformatics/article/34/23/4121/5001388) was used as a basis for our clade definitions and we added clades in accordance with the phylogenetic tree at NextStrain (at 2020-04-04).
Table S4 GISAID sequences 23.03.2020: List of contributing labs. See excel document.
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