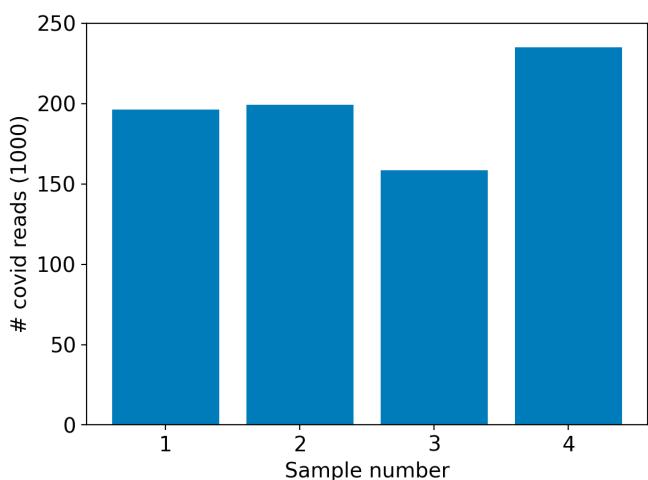


WASTEWATER SARS-COV2 ANALYSIS REPORT

Summary

Sample#	Sample name	Total #reads	Reads aligned PF*	Genomic coordinates 0X	Genomic coordinates <10X
1	SRR18910147	343476	196284 (57%)	18nt (0%)	554nt (1%)
2	SRR18910148	344810	199154 (57%)	85nt (0%)	1166nt (3%)
3	SRR18910149	321719	158671 (49%)	82nt (0%)	1776nt (5%)
4	SRR18910150	427492	234986 (54%)	10nt (0%)	1317nt (4%)



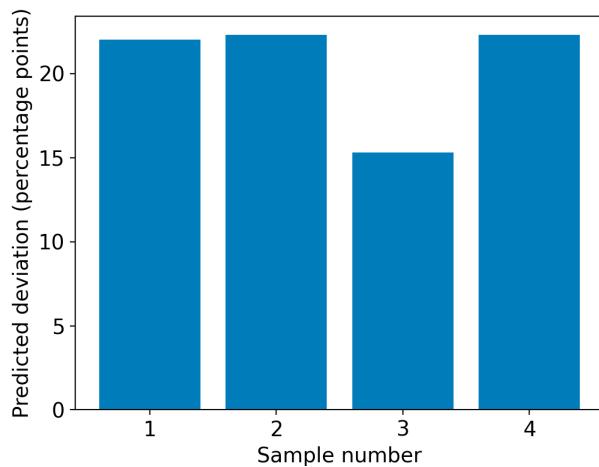
*Quantity of raw reads that align to the reference sequence and pass filter, i.e. the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4. SNR refers to the ratio of SC2-mapping reads aligned that pass filter in the sample vs. that in the auto-detected negative control samples (if any). The dashed line represents the baseline level of covid reads detected from the negative control or their average if multiple negative controls were included.

QC-bot (Experimental)

QC category	Subjective definition	Objective metrics
A	No QC issues evident	0x coordinates <1% 10x coordinates <5% average coverage > 1000X average quality score >35 for Illumina, >15 if ONT, >70 if PacBio HiFi most abundant taxon is coronovirinae
B	Some QC issues, but accurate variant calling possible	0x coordinates <20% 10X coordinates < 40% >80% of diverse SNPs covered average coverage > 100X average quality score >35 for Illumina >15 if ONT, >70 if PacBio HiFi
C	Some QC issues, and accurate variant calling impossible	0x coordinates <99% 10X coordinates <95%

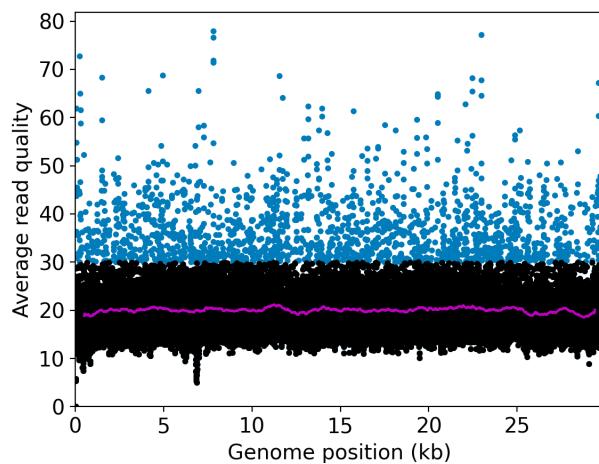
F	Significant QC/study design issues	Contamination (SNR<50) No/negligible coverage (< 1X) Biological/technical replicates' results are irreconcileable.
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Sample Number	Suggested category	Suggested QC flags
1	A	None
2	A	None
3	A	None
4	A	None

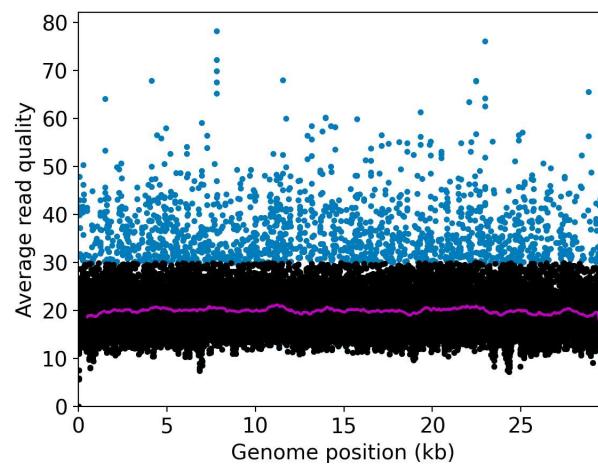


Machine-learning based prediction of the SC2 variant calling accuracy of Freyja of this dataset. The model is a random forest trained on FDA/CFSAN's experimental wastewater WGS data obtained in January 2022 and aims to assess the impact of the potential coverage gaps on the variant abundance estimates. The plotted values represent the predicted deviation of the omicron percentage points from the value that would have been obtained if the coverage was near-complete.

[SRR18910147](#)

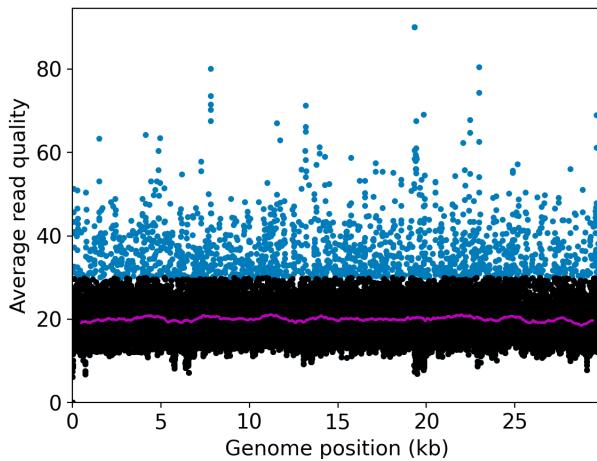
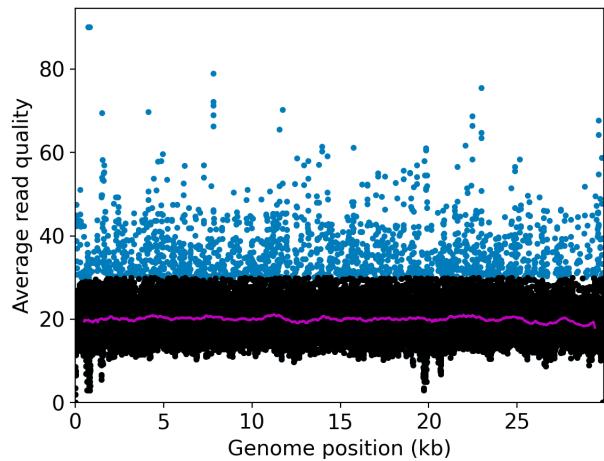


[SRR18910148](#)



[SRR18910149](#)

[SRR18910150](#)



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WASTEWATER SARS-COV2 ANALYSIS REPORT

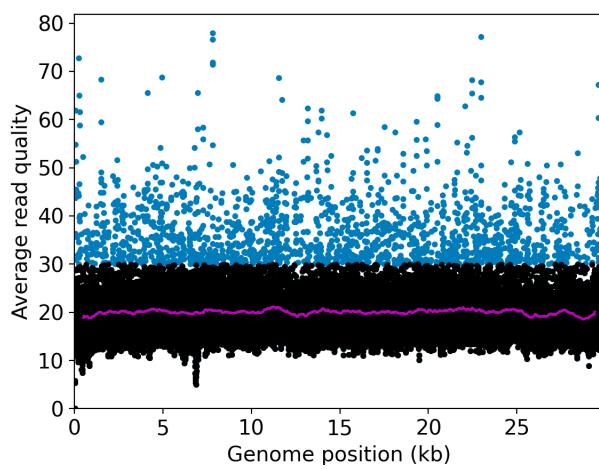
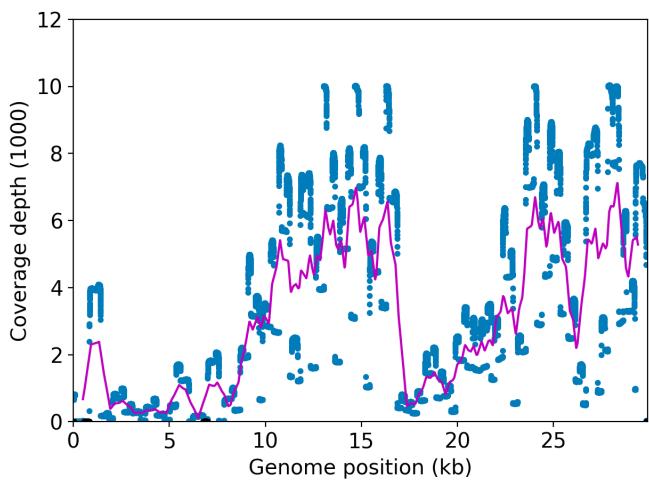
Sample name:	SRR18910147
Date generated:	2022-07-27, 10:43:43 EDT
Timestamp of C-WAP version used:	Tue Jul 26 10:08:16 2022 -0400
Executed by:	Tunc Kayikcioglu (Tunc.Kayikcioglu@fda.hhs.gov)
Executed on:	172.20.44.227 (aka n227.raven.cfsan)

Sequencing summary

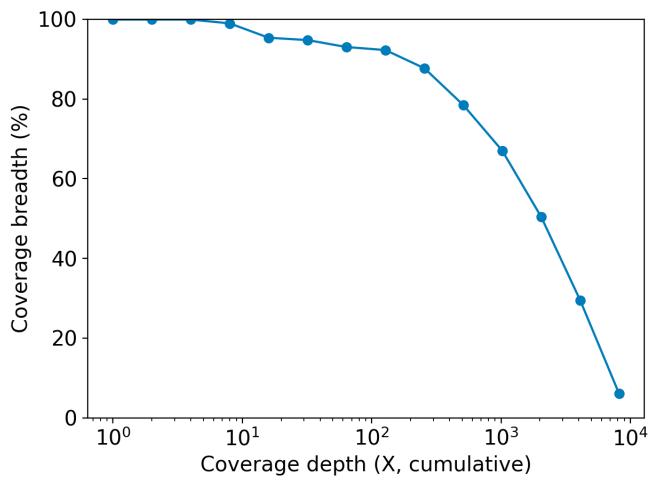
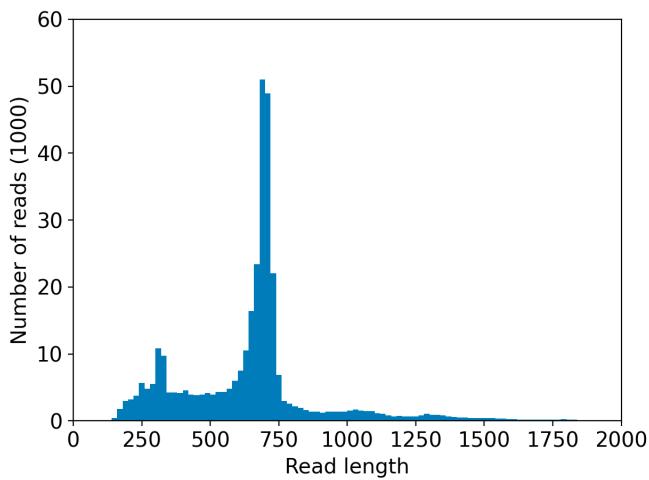
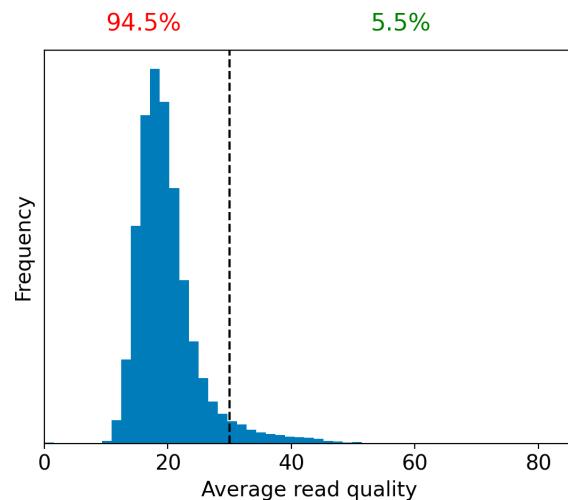
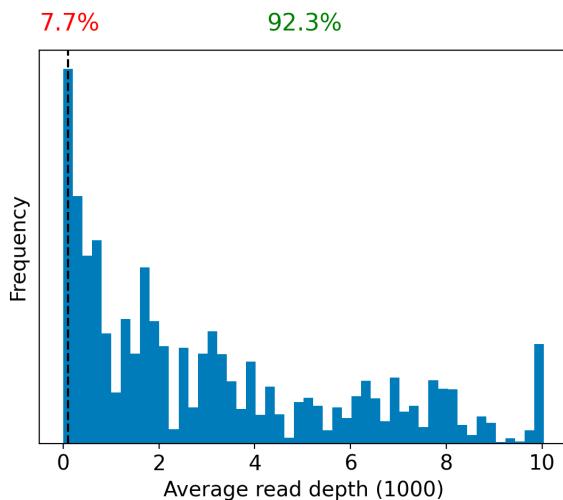
Sequencing chemistry:	WGS with MinION
Source site:	USA: Maryland (missing.?)
Sampling date:	2022-03-16
Collected by:	FDA Center for Food Safety and Applied Nutrition
Sequenced by:	Missing
Total number of reads:	343476
Reads aligned:	196284 (57%)
Average read quality:	19.0
Average read length:	672
Reads passing filter:	196284 (57%)
Average read quality passing filter:	19.0
Average read length passing filter:	672
Average coverage passing filter:	4411X

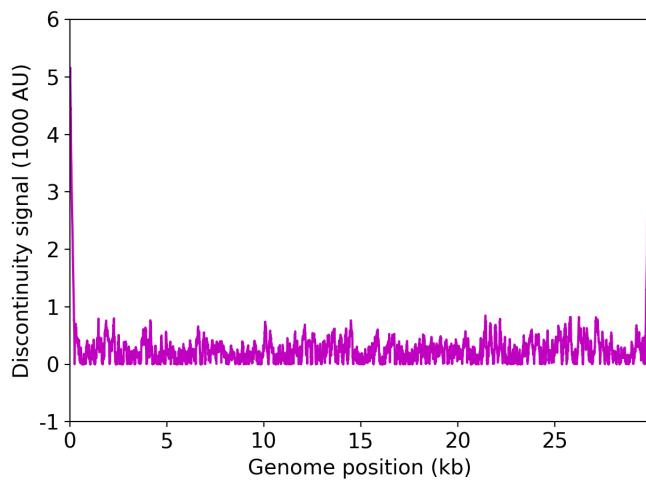
A read passes filter if the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4.

Overall sequence characteristics



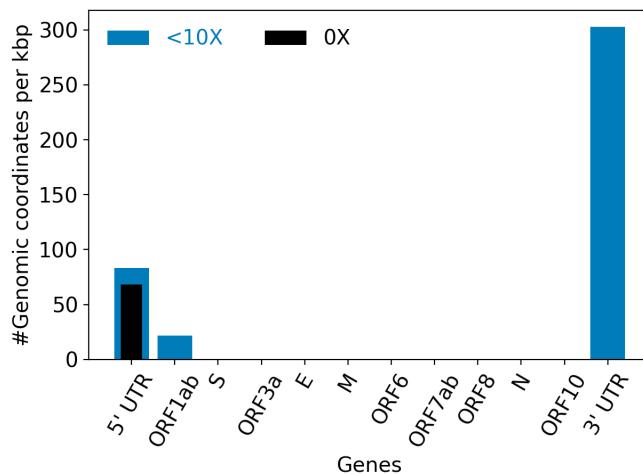
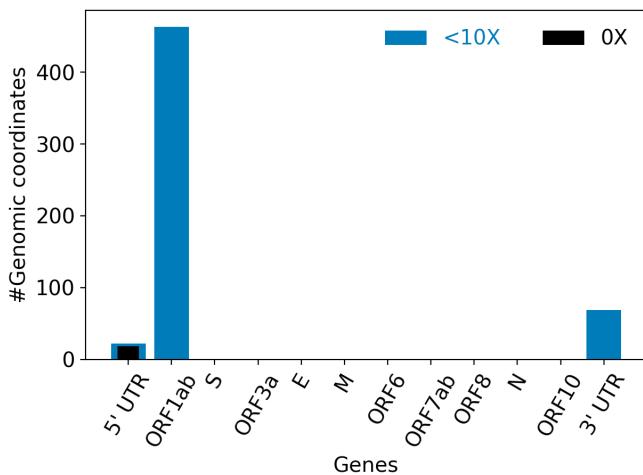
NOTE: The red shaded areas marked with a (*) are not covered by the design of the library preparation kit and hence excluded from analyses. Magenta curves represent moving average with a window width of 1kb.





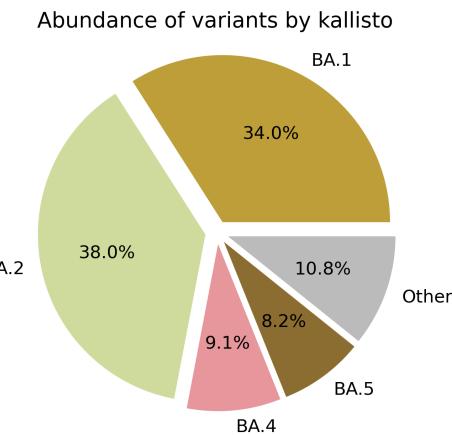
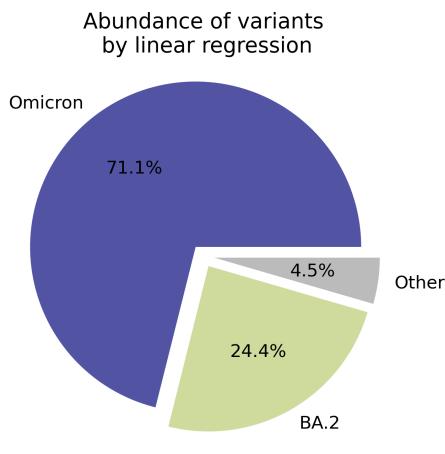
	Uncovered coordinates (0X)	Poorly covered coordinates (<10X)
# Inaccessible genomic coordinates by kit design:	-1nt (0%)	-1nt (0%)
All genomic coordinates:	18nt (0%)	554nt (1%)
Common SNPs:	0nt (0%)	0nt (0%)
Diverse SNPs:	0nt (0%)	29nt (6%)
Rare SNPs:	0nt (0%)	10nt (1%)

SNPs refer to the polymorphic sites currently in circulation that were detected out of recent GISAID entries. The sites that differ from the SC2 reference sequence are denoted as "common" if [90%, 100%] of the submissions carry this mutation, whereas those that are prevalent in [0%, 10%] of the submissions are grouped under the "rare" category. The population is still diverse at the mutation sites that are observed in (10%, 90%) of the entries and these coordinates are grouped under the "diverse" category.



Hits to SARS-CoV2 genome (kraken2):	193664 reads (56.38%)
Hits to human genome (kraken2):	2401 reads (0.70%)
Hits to synthetic sequences (kraken2, taxid 28384):	0 reads (0.00%)
Most abundant organisms (kraken2, family level):	Coronaviridae (56.38%) Arcobacteraceae (18.65%) Pseudomonadaceae (2.53%)

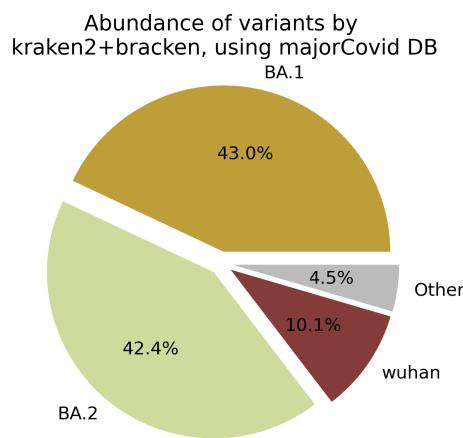
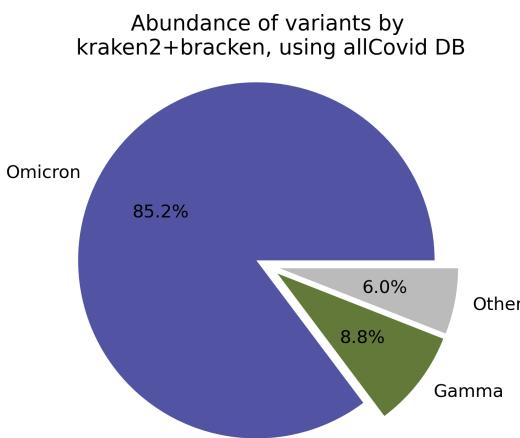
Detected variants (Experimental)



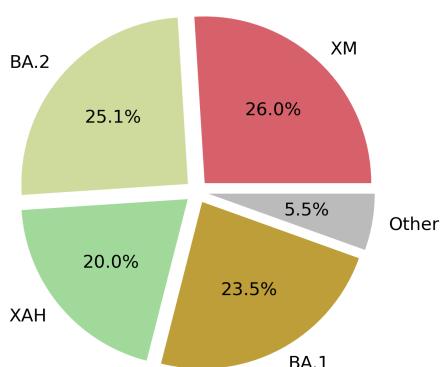
Based on deconvolution, [B.1.1.529](#) is estimated to constitute 71.06% of the viral particles and hence is the most abundant variant in the sample. The R^2 for the linear regression was 0.62. Variants that were detected less than 5% were grouped under "Other"

Based on the consensus sequence of the observed reads, the "ensemble-averaged sequence" most closely resembles the [B.1.1.529](#) lineage. If this is a sample consisting of a single source of pathogens or an overwhelming majority of the different sources are infected with the same variant, the sample is dominated by this variant.

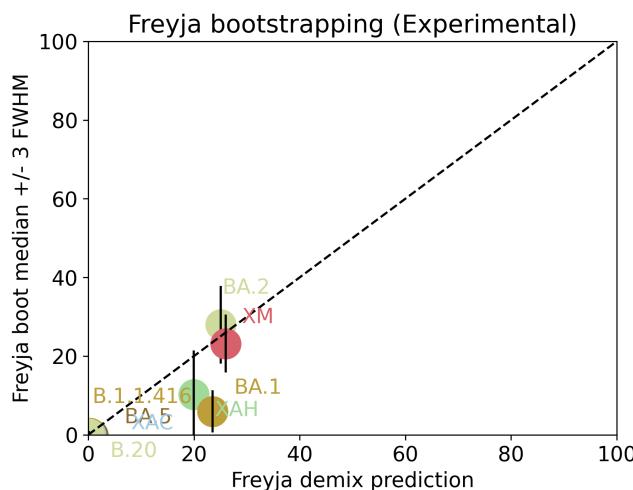
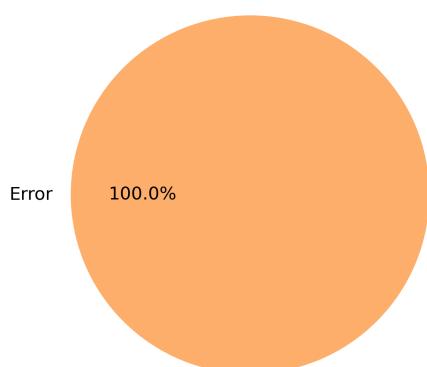
Based on mapping individual reads to the variant consensus sequences in the reference database, kallisto predicts that the sample is dominated by [BA.2](#) lineage. Accuracy of this measure is expected to improve if the input data consists of long reads as opposed to convolution.



Abundance of variants by Freyja



Abundance of variants by LCS



Under the assumption that the presence of a variant requires the detection of all respective mutations of the variant, the characteristic mutations which support the presence of the respective variant are indicated in the respective column of the table. Numbers show the number of mutations detected, if any, and the number of mutations expected to be present based on the variant definitions.

VOC	<u>B.1.617.2</u>	<u>BA.1</u>	<u>BA.2</u>	<u>BA.3</u>	<u>BA.4</u>	<u>BA.5</u>
Characteristic mutations detected	(2 of 13) S:G142D S:T478K	(13 of 26) NUC:C15240T NUC:C25000T NUC:C25584T NUC:T13195C NUC:T5386G ORF1AB:A2710T ORF1AB:I3758V S:G446S S:G496S S:L981F S:N856K S:Q493R S:T547K	(22 of 31) N:S413R NUC:A20055G NUC:A9424G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:T13195C NUC:T5386G ORF1AB:G1307S ORF1AB:L3027F ORF1AB:L3201F ORF1AB:T3090I ORF1AB:T842I S:D405N S:G446S S:Q493R S:R408S S:S371F S:T19I	(11 of 21) N:S413R NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:D405N S:G446S S:Q493R S:S371F	(18 of 31) N:S413R NUC:A20055G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I ORF1AB:T842I S:D405N S:G446S S:Q493R S:S371F S:T19I S:T376A S:V213G	(17 of 28) N:S413R NUC:A20055G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I ORF1AB:T842I S:D405N S:G446S S:Q493R S:S371F S:T19I S:T376A S:V213G

Jaccard Index is a measure of similarity between two sets A and B, reaching the maximum value of 1 if A=B and minimum value of 0 if A ∩ B = {}. In the c(d) representation below, c represents the Jaccard index of the set of mutations that were experimentally detected for this sample as listed above, whereas d refers to the ideal value of the Jaccard index expected from complete genome coverage without any sequencing errors.

	B.1.617.2	BA.1	BA.2	BA.3	BA.4	BA.5
B.1.617.2	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.02)	0.00 (0.03)
BA.1	0.00 (0.00)	1.00 (1.00)	0.09 (0.10)	0.09 (0.21)	0.07 (0.08)	0.07 (0.08)
BA.2	0.00 (0.00)	0.09 (0.10)	1.00 (1.00)	0.43 (0.33)	0.74 (0.63)	0.70 (0.59)
BA.3	0.00 (0.00)	0.09 (0.21)	0.43 (0.33)	1.00 (1.00)	0.45 (0.30)	0.40 (0.29)
BA.4	0.00 (0.02)	0.07 (0.08)	0.74 (0.63)	0.45 (0.30)	1.00 (1.00)	0.94 (0.84)
BA.5	0.00 (0.03)	0.07 (0.08)	0.70 (0.59)	0.40 (0.29)	0.94 (0.84)	1.00 (1.00)

Detected mutations

Excluded from this pdf version due to file size limitations.

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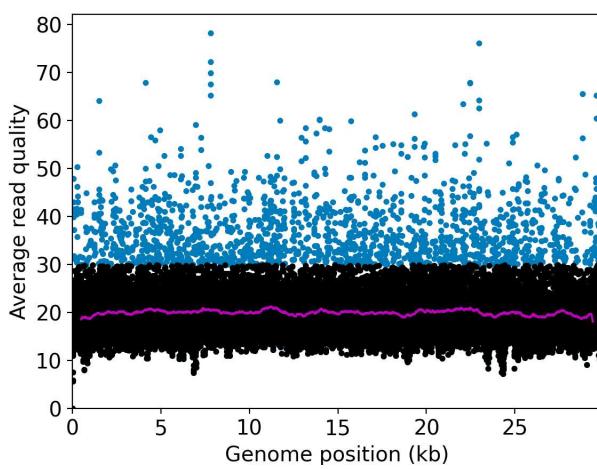
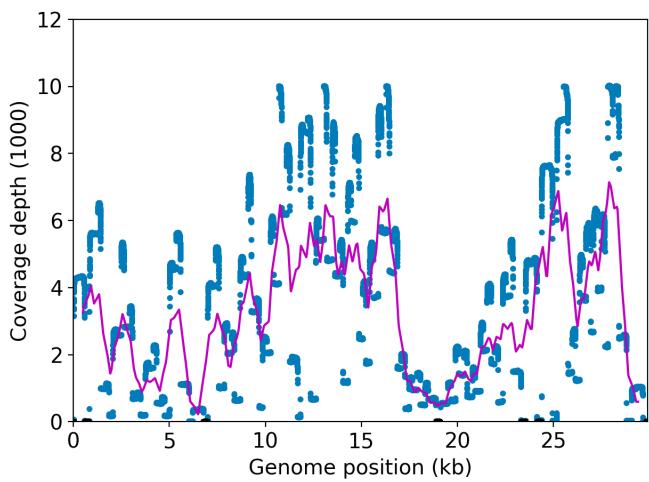
Sample name:	SRR18910148
Date generated:	2022-07-27, 10:41:10 EDT
Timestamp of C-WAP version used:	Tue Jul 26 10:08:16 2022 -0400
Executed by:	Tunc Kayikcioglu (Tunc.Kayikcioglu@fda.hhs.gov)
Executed on:	172.20.44.227 (aka n227.raven.cfsan)

Sequencing summary

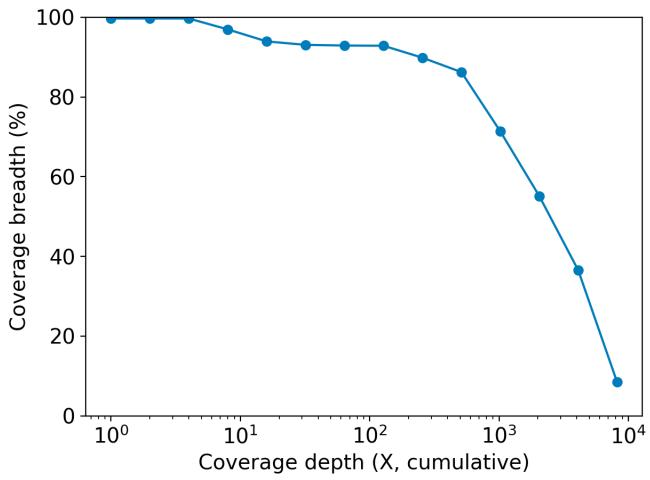
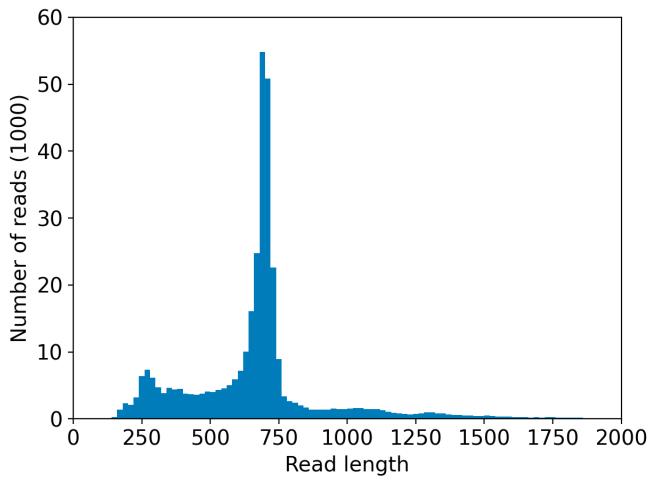
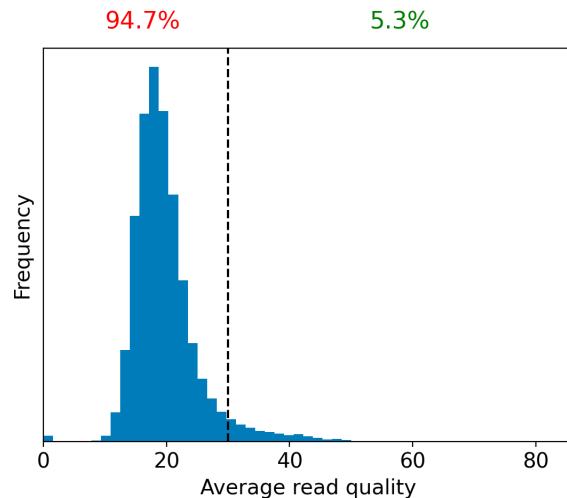
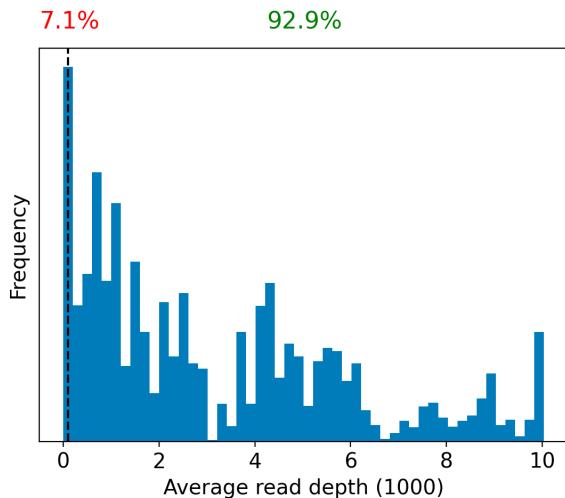
Sequencing chemistry:	WGS with MinION
Source site:	USA: Maryland (missing.?)
Sampling date:	2022-03-16
Collected by:	FDA Center for Food Safety and Applied Nutrition
Sequenced by:	Missing
Total number of reads:	344810
Reads aligned:	199154 (57%)
Average read quality:	19.1
Average read length:	679
Reads passing filter:	199154 (57%)
Average read quality passing filter:	19.1
Average read length passing filter:	679
Average coverage passing filter:	4522X

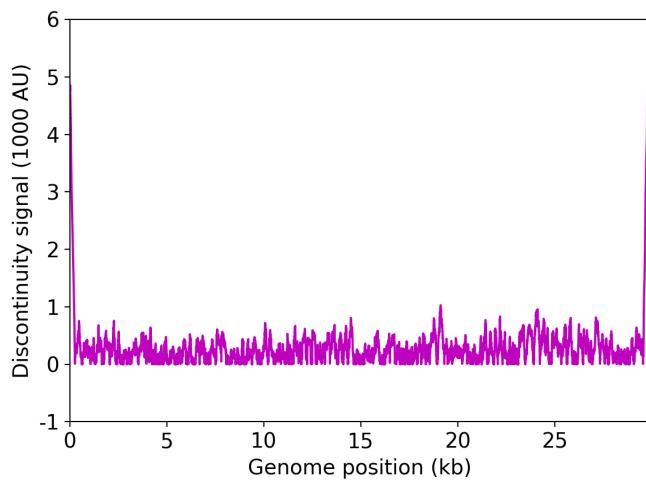
A read passes filter if the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4.

Overall sequence characteristics



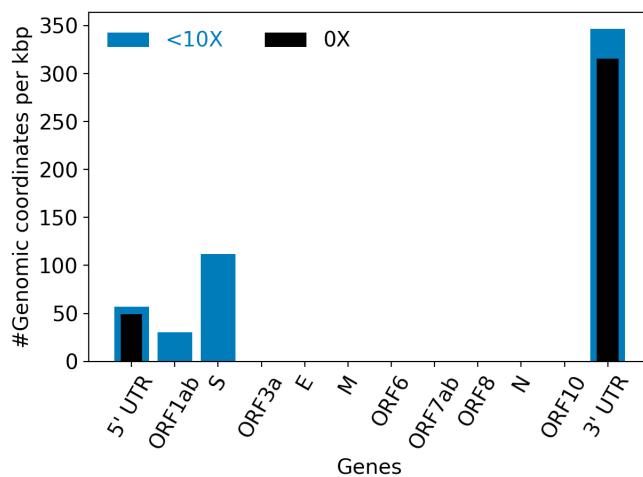
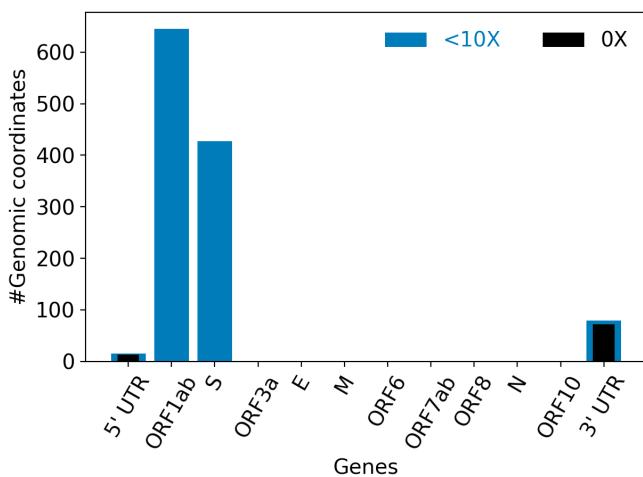
NOTE: The red shaded areas marked with a (*) are not covered by the design of the library preparation kit and hence excluded from analyses. Magenta curves represent moving average with a window width of 1kb.





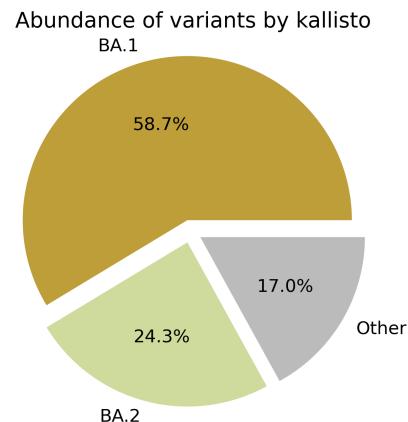
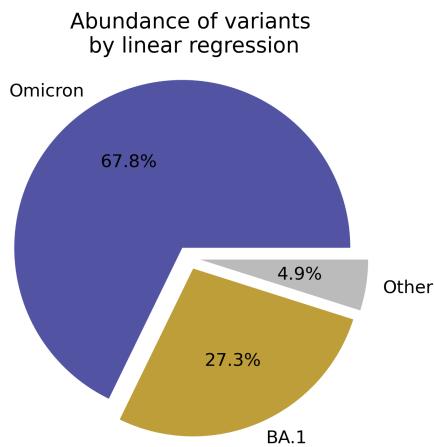
	Uncovered coordinates (0X)	Poorly covered coordinates (<10X)
# Inaccessible genomic coordinates by kit design:	-1nt (0%)	-1nt (0%)
All genomic coordinates:	85nt (0%)	1166nt (3%)
Common SNPs:	0nt (0%)	2nt (5%)
Diverse SNPs:	27nt (5%)	28nt (6%)
Rare SNPs:	13nt (1%)	17nt (1%)

SNPs refer to the polymorphic sites currently in circulation that were detected out of recent GISAID entries. The sites that differ from the SC2 reference sequence are denoted as "common" if [90%, 100%] of the submissions carry this mutation, whereas those that are prevalent in [0%, 10%] of the submissions are grouped under the "rare" category. The population is still diverse at the mutation sites that are observed in (10%, 90%) of the entries and these coordinates are grouped under the "diverse" category.



Hits to SARS-CoV2 genome (kraken2):	196951 reads (57.12%)
Hits to human genome (kraken2):	2481 reads (0.72%)
Hits to synthetic sequences (kraken2, taxid 28384):	0 reads (0.00%)
Most abundant organisms (kraken2, family level):	Coronaviridae (57.12%) Arcobacteraceae (18.60%) Pseudomonadaceae (3.03%)

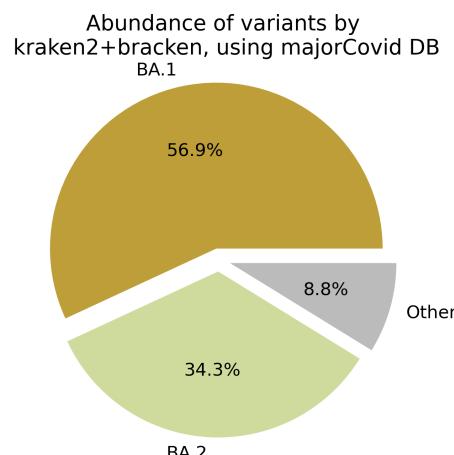
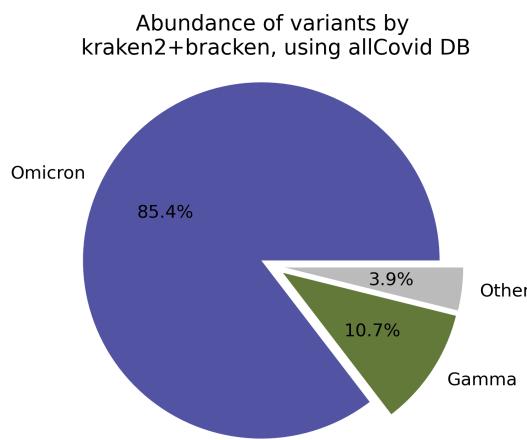
Detected variants (Experimental)



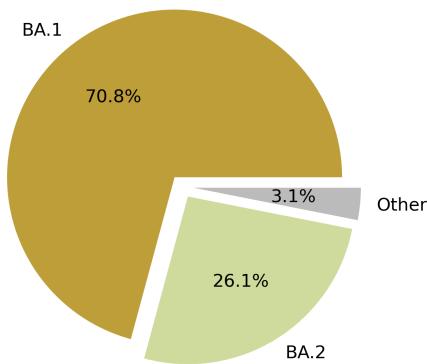
Based on deconvolution, [B.1.1.529](#) is estimated to constitute 67.80% of the viral particles and hence is the most abundant variant in the sample. The R^2 for the linear regression was 0.54. Variants that were detected less than 5% were grouped under "Other"

Based on the consensus sequence of the observed reads, the "ensemble-averaged sequence" most closely resembles the [BA.1.1](#) lineage. If this is a sample consisting of a single source of pathogens or an overwhelming majority of the different sources are infected with the same variant, the sample is dominated by this variant.

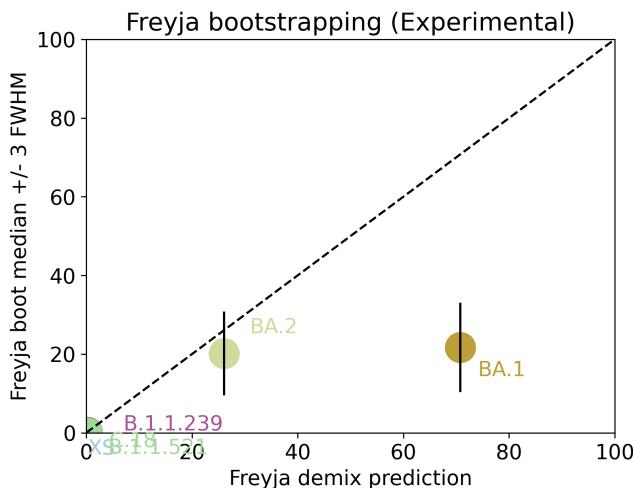
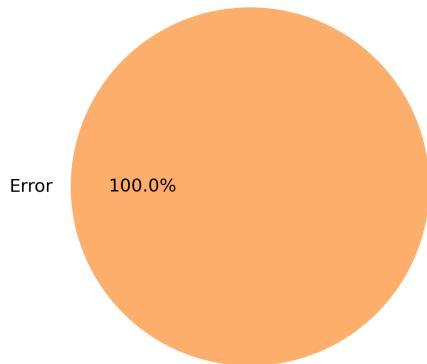
Based on mapping individual reads to the variant consensus sequences in the reference database, kallisto predicts that the sample is dominated by [BA.1](#) lineage. Accuracy of this measure is expected to improve if the input data consists of long reads as opposed to convolution.



Abundance of variants by Freyja



Abundance of variants by LCS



Under the assumption that the presence of a variant requires the detection of all respective mutations of the variant, the characteristic mutations which support the presence of the respective variant are indicated in the respective column of the table. Numbers show the number of mutations detected, if any, and the number of mutations expected to be present based on the variant definitions.

VOC	<u>B.1.617.2</u>	<u>BA.1</u>	<u>BA.2</u>	<u>BA.3</u>	<u>BA.4</u>	<u>BA.5</u>
Characteristic mutations detected	(2 of 13) S:G142D S:T478K	(15 of 26) M:D3G NUC:C15240T NUC:C25584T NUC:T13195C ORF1AB:A2710T ORF1AB:I3758V ORF1AB:K856R S:A67V S:G446S S:G496S S:L981F S:N856K S:Q493R S:T547K S:T95I	(13 of 31) NUC:C12880T NUC:C15714T NUC:C25584T NUC:C26858T NUC:C4321T NUC:G10447A ORF1AB:G1307S ORF1AB:L3201F ORF1AB:T3090I ORF1AB:T842I S:Q493R S:S371F S:T19I	(10 of 21) NUC:C12880T NUC:C15714T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:A67V S:G446S S:Q493R S:S371F	(12 of 31) NUC:C12880T NUC:C15714T NUC:C25584T NUC:C26858T NUC:C4321T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I ORF1AB:T842I S:S371F S:T19I S:V213G	(11 of 28) NUC:C12880T NUC:C15714T NUC:C25584T NUC:C4321T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I ORF1AB:T842I S:S371F S:T19I S:V213G

Jaccard Index is a measure of similarity between two sets A and B, reaching the maximum value of 1 if A=B and minimum value of 0 if A ∩ B = {}. In the c(d) representation below, c represents the Jaccard index of the set of mutations that were experimentally detected for this sample as listed above, whereas d refers to

the ideal value of the Jaccard index expected from complete genome coverage without any sequencing errors.

	B.1.617.2	BA.1	BA.2	BA.3	BA.4	BA.5
B.1.617.2	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.02)	0.00 (0.03)
BA.1	0.00 (0.00)	1.00 (1.00)	0.08 (0.10)	0.14 (0.21)	0.04 (0.08)	0.04 (0.08)
BA.2	0.00 (0.00)	0.08 (0.10)	1.00 (1.00)	0.53 (0.33)	0.79 (0.63)	0.71 (0.59)
BA.3	0.00 (0.00)	0.14 (0.21)	0.53 (0.33)	1.00 (1.00)	0.47 (0.30)	0.40 (0.29)
BA.4	0.00 (0.02)	0.04 (0.08)	0.79 (0.63)	0.47 (0.30)	1.00 (1.00)	0.92 (0.84)
BA.5	0.00 (0.03)	0.04 (0.08)	0.71 (0.59)	0.40 (0.29)	0.92 (0.84)	1.00 (1.00)

Detected mutations

Excluded from this pdf version due to file size limitations.

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BIOSTATISTICS AND BIOINFORMATICS STAFF

WASTEWATER SARS-COV2 ANALYSIS REPORT

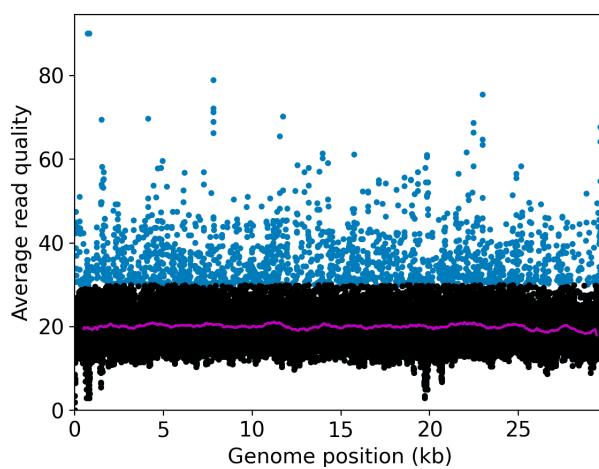
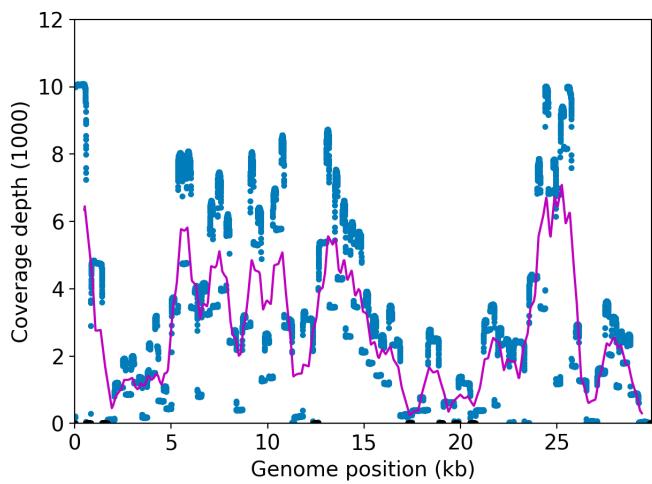
Sample name:	SRR18910149
Date generated:	2022-07-27, 10:41:28 EDT
Timestamp of C-WAP version used:	Tue Jul 26 10:08:16 2022 -0400
Executed by:	Tunc Kayikcioglu (Tunc.Kayikcioglu@fda.hhs.gov)
Executed on:	172.20.44.227 (aka n227.raven.cfsan)

Sequencing summary

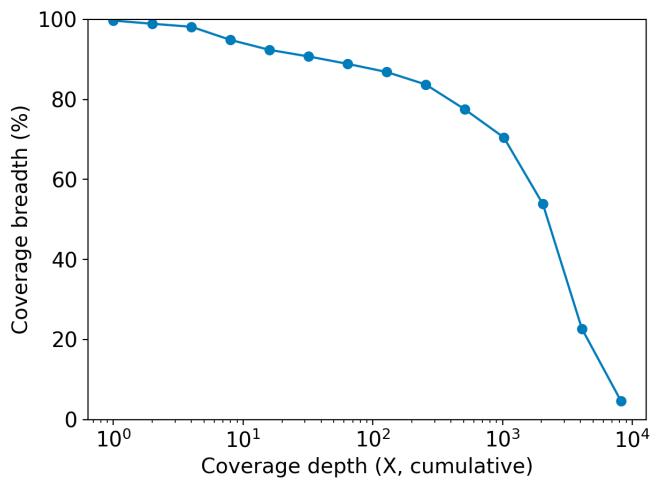
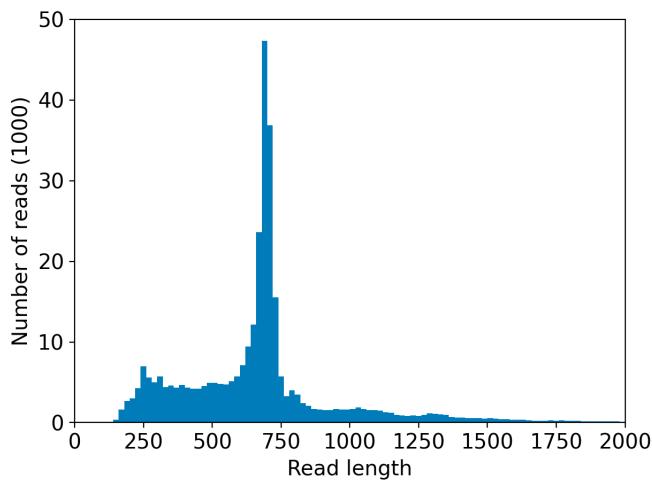
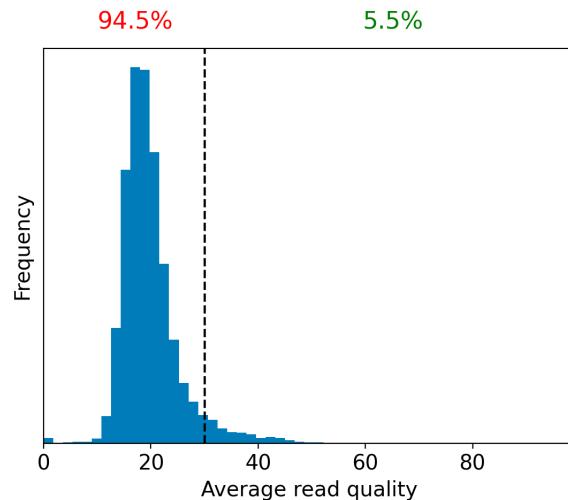
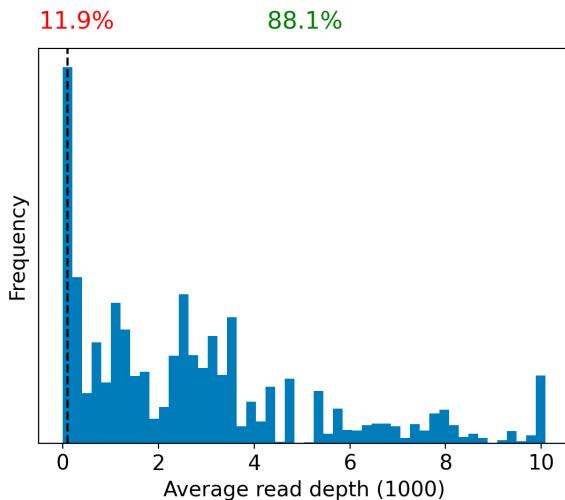
Sequencing chemistry:	WGS with MinION
Source site:	USA: Maryland (missing,?)
Sampling date:	2022-03-16
Collected by:	FDA Center for Food Safety and Applied Nutrition
Sequenced by:	Missing
Total number of reads:	321719
Reads aligned:	158671 (49%)
Average read quality:	19.4
Average read length:	673
Reads passing filter:	158671 (49%)
Average read quality passing filter:	19.4
Average read length passing filter:	673
Average coverage passing filter:	3571X

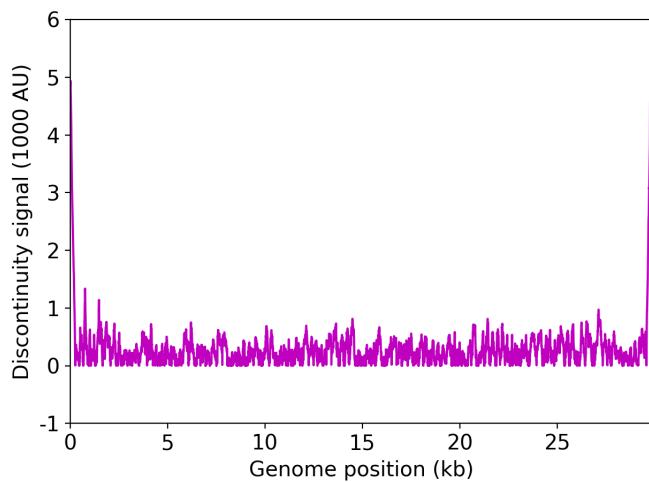
A read passes filter if the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4.

Overall sequence characteristics



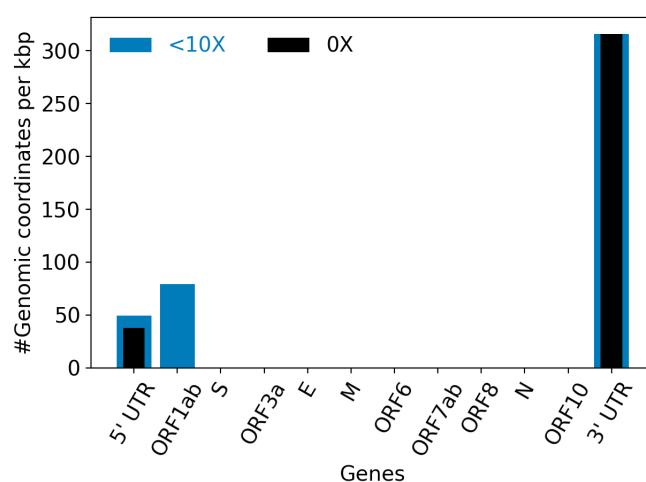
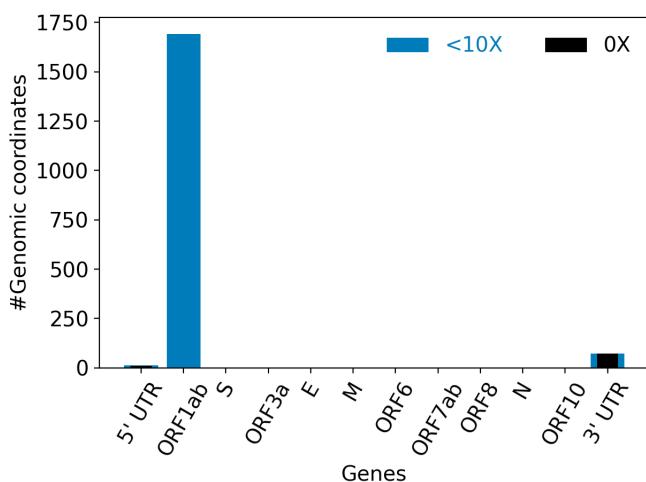
NOTE: The red shaded areas marked with a (*) are not covered by the design of the library preparation kit and hence excluded from analyses. Magenta curves represent moving average with a window width of 1kb.





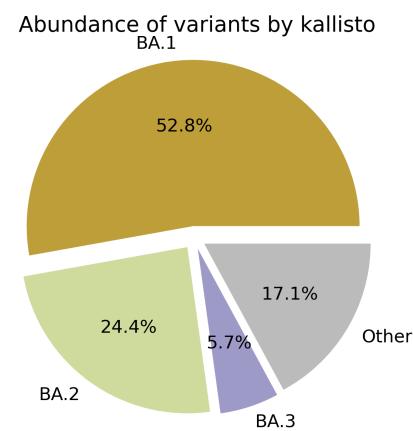
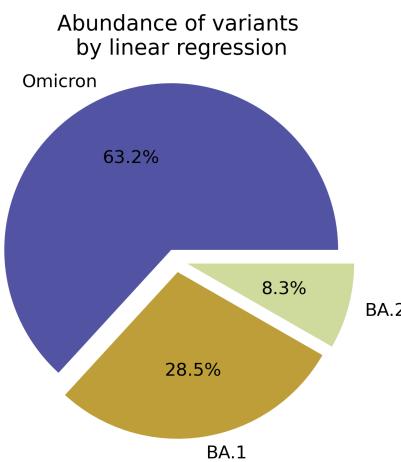
	Uncovered coordinates (0X)	Poorly covered coordinates (<10X)
# Inaccessible genomic coordinates by kit design:	-1nt (0%)	-1nt (0%)
All genomic coordinates:	82nt (0%)	1776nt (5%)
Common SNPs:	0nt (0%)	0nt (0%)
Diverse SNPs:	27nt (5%)	29nt (6%)
Rare SNPs:	13nt (1%)	14nt (1%)

SNPs refer to the polymorphic sites currently in circulation that were detected out of recent GISAID entries. The sites that differ from the SC2 reference sequence are denoted as "common" if [90%, 100%] of the submissions carry this mutation, whereas those that are prevalent in [0%, 10%] of the submissions are grouped under the "rare" category. The population is still diverse at the mutation sites that are observed in (10%, 90%) of the entries and these coordinates are grouped under the "diverse" category.



Hits to SARS-CoV2 genome (kraken2):	156611 reads (48.68%)
Hits to human genome (kraken2):	2502 reads (0.78%)
Hits to synthetic sequences (kraken2, taxid 28384):	0 reads (0.00%)
Most abundant organisms (kraken2, family level):	Coronaviridae (48.68%) Arcobacteraceae (21.97%) Pseudomonadaceae (4.03%) Comamonadaceae (2.14%)

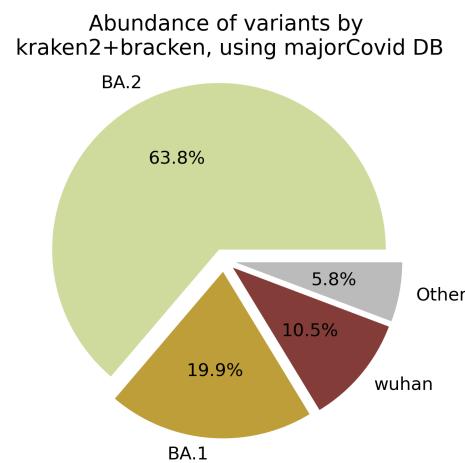
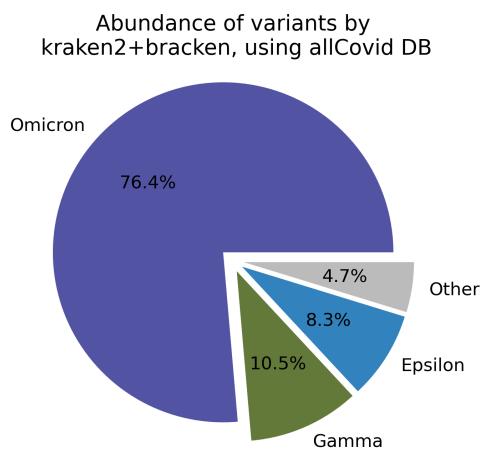
Detected variants (Experimental)



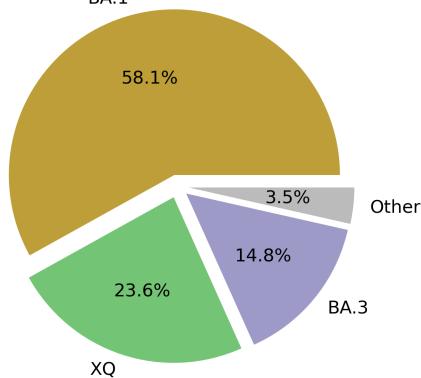
Based on deconvolution, [B.1.1.529](#) is estimated to constitute 63.22% of the viral particles and hence is the most abundant variant in the sample. The R^2 for the linear regression was 0.53. Variants that were detected less than 5% were grouped under "Other"

Based on the consensus sequence of the observed reads, the "ensemble-averaged sequence" most closely resembles the [B.1.1.529](#) lineage. If this is a sample consisting of a single source of pathogens or an overwhelming majority of the different sources are infected with the same variant, the sample is dominated by this variant.

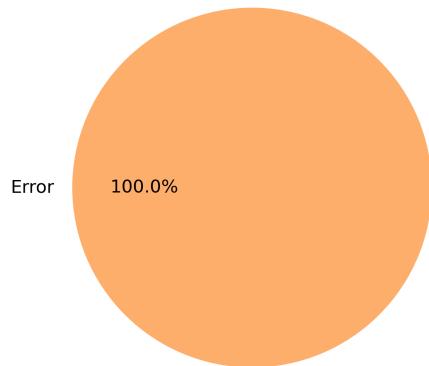
Based on mapping individual reads to the variant consensus sequences in the reference database, kallisto predicts that the sample is dominated by [BA.1](#) lineage. Accuracy of this measure is expected to improve if the input data consists of long reads as opposed to convolution.



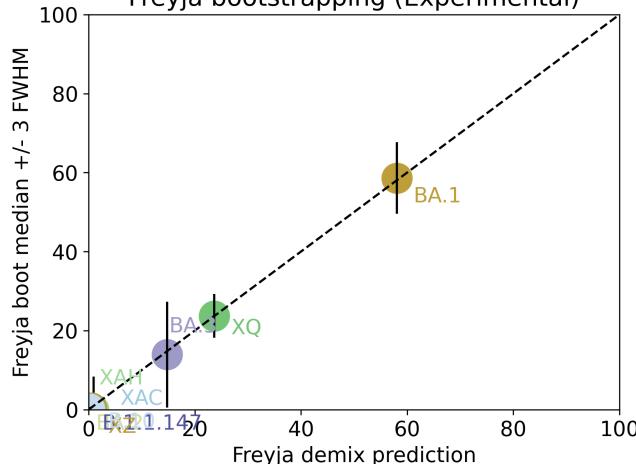
Abundance of variants by Freyja
BA.1



Abundance of variants by LCS



Freyja bootstrapping (Experimental)



Under the assumption that the presence of a variant requires the detection of all respective mutations of the variant, the characteristic mutations which support the presence of the respective variant are indicated in the respective column of the table. Numbers show the number of mutations detected, if any, and the number of mutations expected to be present based on the variant definitions.

VOC	B.1.617.2	BA.1	BA.2	BA.3	BA.4	BA.5
Characteristic mutations detected	(3 of 13) ORF3A:S26L S:G142D S:T478K	(15 of 26) NUC:C15240T NUC:C25000T NUC:C25584T NUC:T13195C NUC:T5386G ORF1AB:A2710T ORF1AB:K856R S:A67V S:G446S S:G496S S:L981F S:N856K S:Q493R S:T547K S:T95I	(15 of 31) N:S413R NUC:A9424G NUC:C10198T NUC:C12880T NUC:C25000T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I	(10 of 21) N:S413R NUC:C12880T NUC:C26858T NUC:C25000T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:A67V S:G446S S:Q493R S:S371F	(12 of 31) N:S413R NUC:C10198T NUC:C12880T NUC:C25000T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:S371F S:T19I S:V213G	(11 of 28) N:S413R NUC:C10198T NUC:C12880T NUC:C25000T NUC:C25584T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:S371F S:T19I S:V213G

[Jaccard Index](#) is a measure of similarity between two sets A and B, reaching the maximum value of 1 if A=B and minimum value of 0 if A ∩ B = {}. In the c(d) representation below, c represents the Jaccard index of the set of mutations that were experimentally detected for this sample as listed above, whereas d refers to the ideal value of the Jaccard index expected from complete genome coverage without any sequencing errors.

	B.1.617.2	BA.1	BA.2	BA.3	BA.4	BA.5
B.1.617.2	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.02)	0.00 (0.03)
BA.1	0.00 (0.00)	1.00 (1.00)	0.11 (0.10)	0.14 (0.21)	0.08 (0.08)	0.08 (0.08)
BA.2	0.00 (0.00)	0.11 (0.10)	1.00 (1.00)	0.47 (0.33)	0.69 (0.63)	0.62 (0.59)
BA.3	0.00 (0.00)	0.14 (0.21)	0.47 (0.33)	1.00 (1.00)	0.47 (0.30)	0.40 (0.29)
BA.4	0.00 (0.02)	0.08 (0.08)	0.69 (0.63)	0.47 (0.30)	1.00 (1.00)	0.92 (0.84)
BA.5	0.00 (0.03)	0.08 (0.08)	0.62 (0.59)	0.40 (0.29)	0.92 (0.84)	1.00 (1.00)

Detected mutations

Excluded from this pdf version due to file size limitations.

CFSAN/OAO
BIOSTATISTICS AND BIOINFORMATICS STAFF

WASTEWATER SARS-COV2 ANALYSIS REPORT

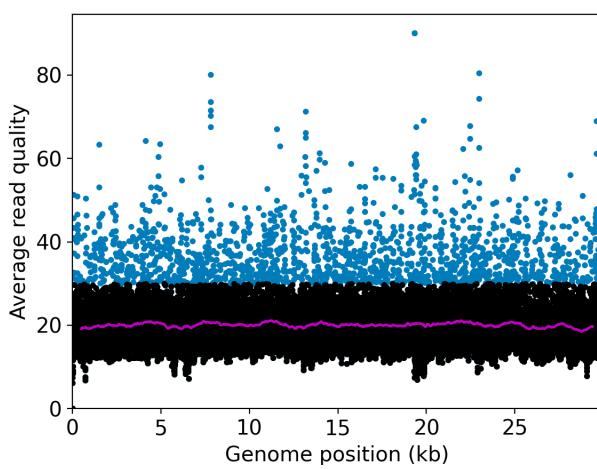
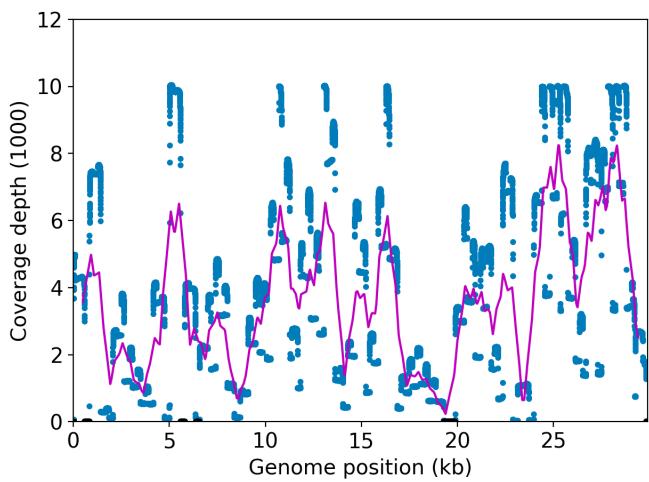
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Date generated:	2022-07-27, 10:43:09 EDT
Timestamp of C-WAP version used:	Tue Jul 26 10:08:16 2022 -0400
Executed by:	Tunc Kayikcioglu (Tunc.Kayikcioglu@fda.hhs.gov)
Executed on:	172.20.44.227 (aka n227.raven.cfsan)

Sequencing summary

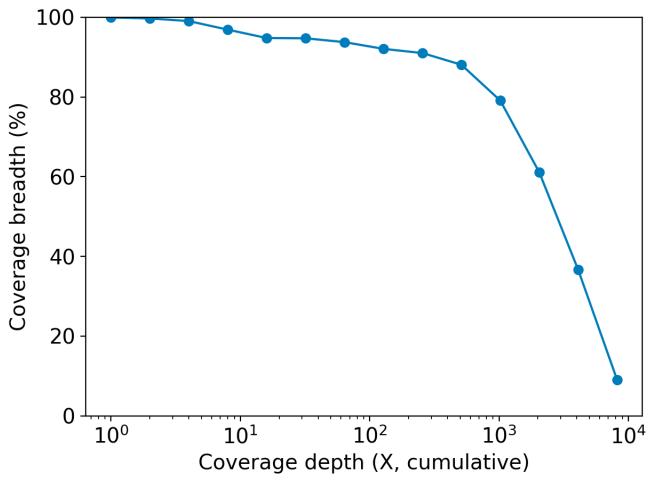
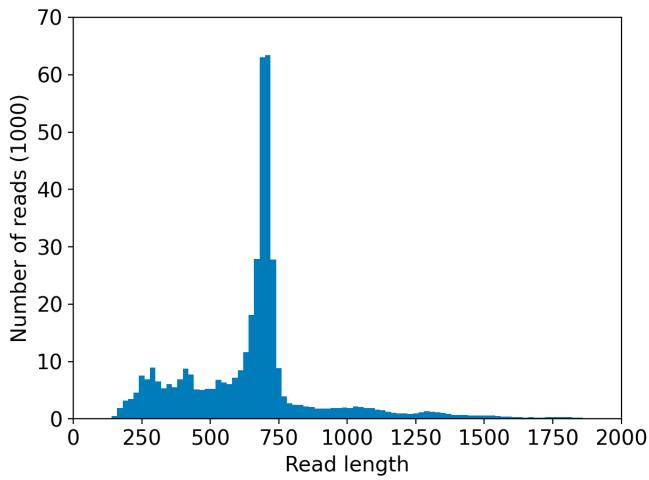
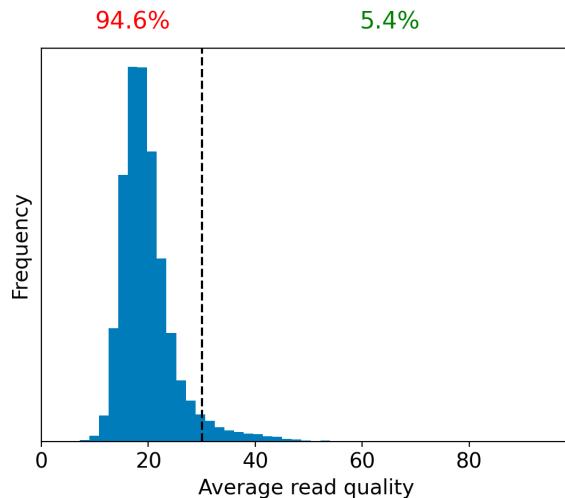
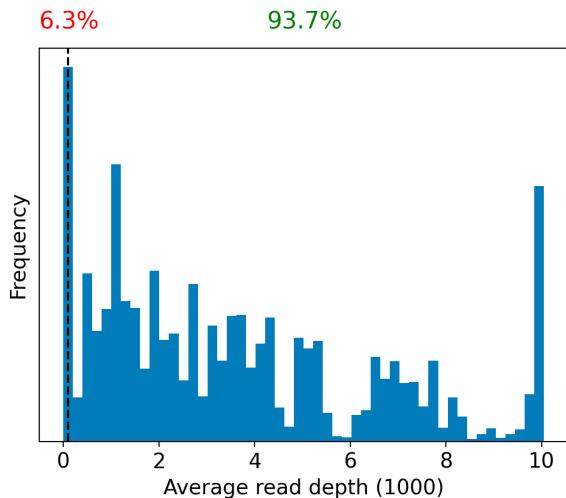
Sequencing chemistry:	WGS with MinION
Source site:	USA: Maryland (missing.?)
Sampling date:	2022-03-16
Collected by:	FDA Center for Food Safety and Applied Nutrition
Sequenced by:	Missing
Total number of reads:	427492
Reads aligned:	234986 (54%)
Average read quality:	18.9
Average read length:	676
Reads passing filter:	234986 (54%)
Average read quality passing filter:	18.9
Average read length passing filter:	676
Average coverage passing filter:	5312X

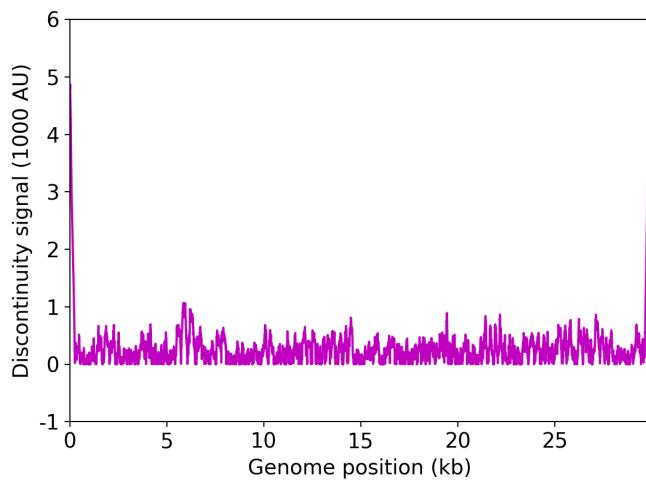
A read passes filter if the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4.

Overall sequence characteristics



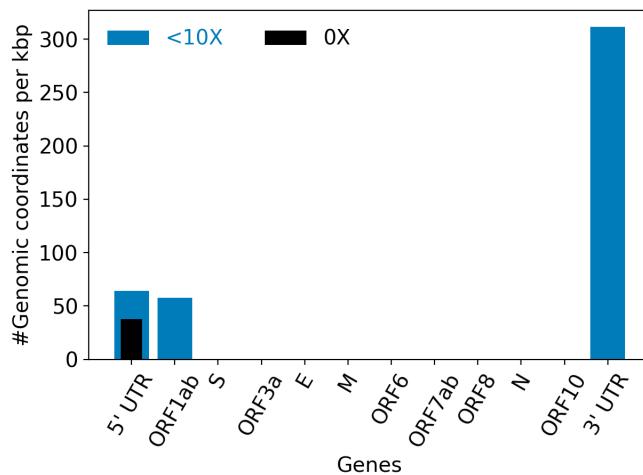
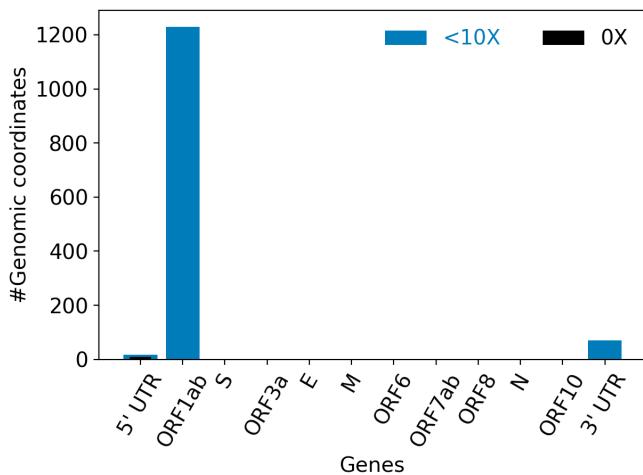
NOTE: The red shaded areas marked with a (*) are not covered by the design of the library preparation kit and hence excluded from analyses. Magenta curves represent moving average with a window width of 1kb.





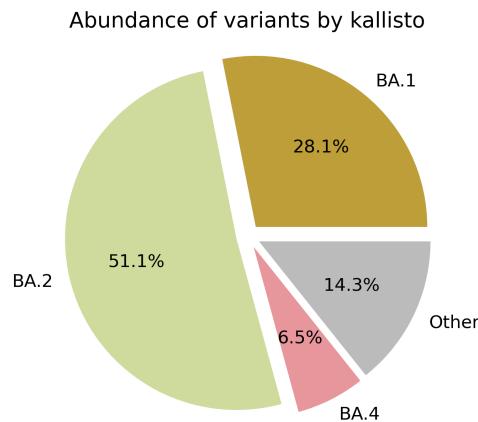
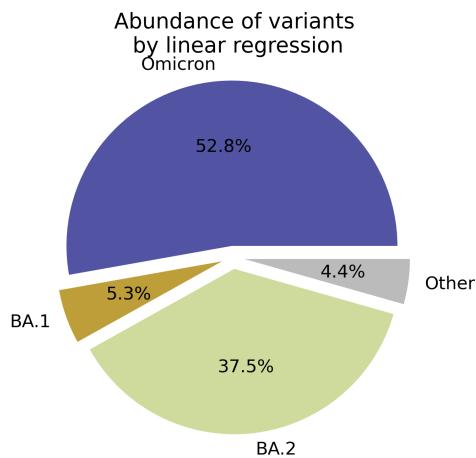
	Uncovered coordinates (0X)	Poorly covered coordinates (<10X)
# Inaccessible genomic coordinates by kit design:	-1nt (0%)	-1nt (0%)
All genomic coordinates:	10nt (0%)	1317nt (4%)
Common SNPs:	0nt (0%)	0nt (0%)
Diverse SNPs:	0nt (0%)	29nt (6%)
Rare SNPs:	0nt (0%)	13nt (1%)

SNPs refer to the polymorphic sites currently in circulation that were detected out of recent GISAID entries. The sites that differ from the SC2 reference sequence are denoted as "common" if [90%, 100%] of the submissions carry this mutation, whereas those that are prevalent in [0%, 10%] of the submissions are grouped under the "rare" category. The population is still diverse at the mutation sites that are observed in (10%, 90%) of the entries and these coordinates are grouped under the "diverse" category.



Hits to SARS-CoV2 genome (kraken2):	232379 reads (54.36%)
Hits to human genome (kraken2):	2750 reads (0.64%)
Hits to synthetic sequences (kraken2, taxid 28384):	0 reads (0.00%)
Most abundant organisms (kraken2, family level):	Coronaviridae (54.36%) Arcobacteraceae (18.57%) Pseudomonadaceae (3.17%)

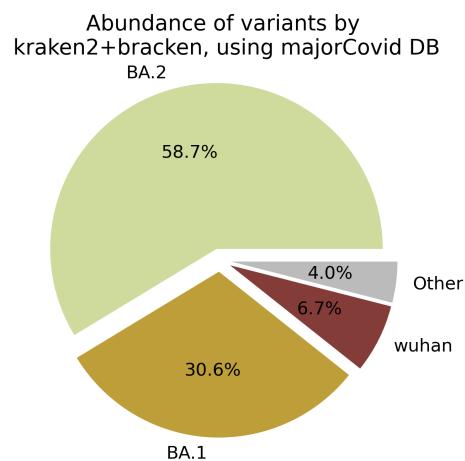
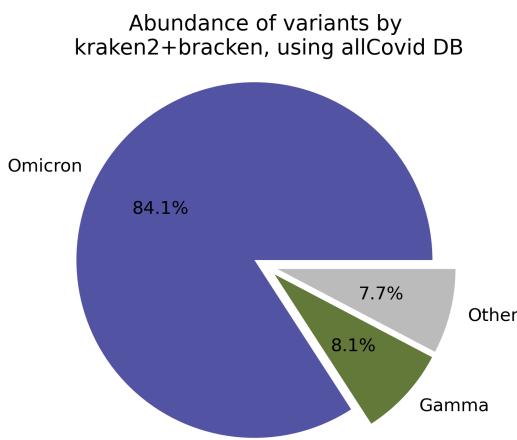
Detected variants (Experimental)



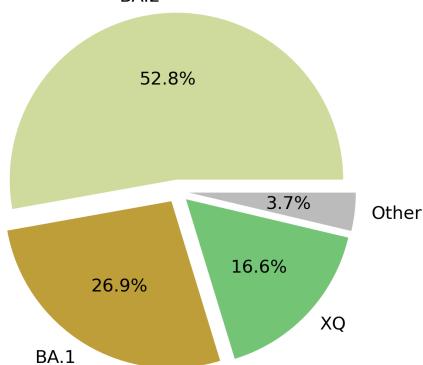
Based on deconvolution, [B.1.1.529](#) is estimated to constitute 52.81% of the viral particles and hence is the most abundant variant in the sample. The R^2 for the linear regression was 0.47. Variants that were detected less than 5% were grouped under "Other"

Based on the consensus sequence of the observed reads, the "ensemble-averaged sequence" most closely resembles the [BA.2](#) lineage. If this is a sample consisting of a single source of pathogens or an overwhelming majority of the different sources are infected with the same variant, the sample is dominated by this variant.

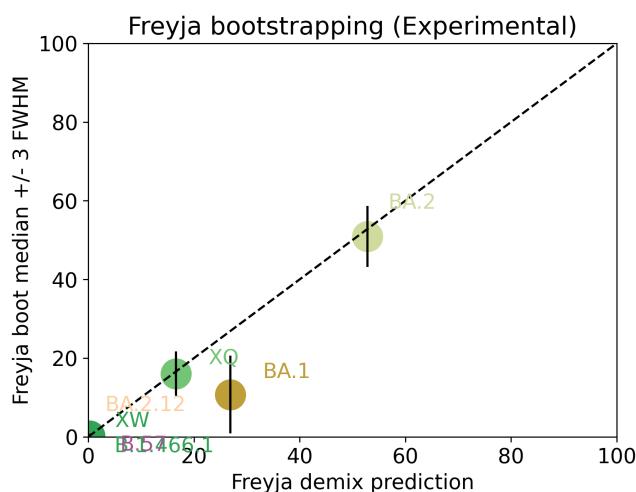
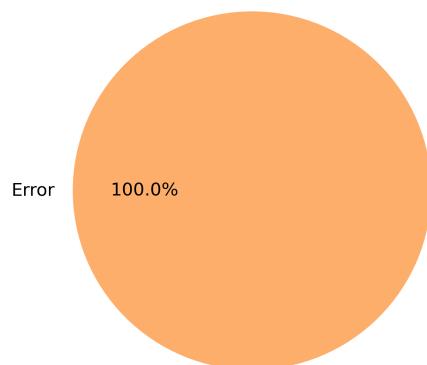
Based on mapping individual reads to the variant consensus sequences in the reference database, kallisto predicts that the sample is dominated by [BA.2](#) lineage. Accuracy of this measure is expected to improve if the input data consists of long reads as opposed to convolution.



Abundance of variants by Freyja BA.2



Abundance of variants by LCS



Under the assumption that the presence of a variant requires the detection of all respective mutations of the variant, the characteristic mutations which support the presence of the respective variant are indicated in the respective column of the table. Numbers show the number of mutations detected, if any, and the number of mutations expected to be present based on the variant definitions.

VOC	<u>B.1.617.2</u>	<u>BA.1</u>	<u>BA.2</u>	<u>BA.3</u>	<u>BA.4</u>	<u>BA.5</u>
Characteristic mutations detected	(1 of 13) S:G142D	(12 of 26) NUC:C15240T NUC:C25000T NUC:C25584T NUC:T13195C ORF1AB:I3758V ORF1AB:K856R S:A67V S:L981F S:N856K S:Q493R S:T547K S:T95I	(22 of 31) N:S413R NUC:A20055G NUC:A9424G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:C26858T NUC:C4321T NUC:G10447A ORF1AB:G1307S ORF1AB:L3027F ORF1AB:L3201F ORF1AB:T3090I ORF1AB:T842I S:D405N S:Q493R S:R408S S:S371F S:T19I	(11 of 21) N:S413R NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:A67V S:D405N S:Q493R S:S371F	(18 of 31) N:S413R NUC:A20055G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:C26858T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I S:D405N S:S371F	(17 of 28) N:S413R NUC:A20055G NUC:C10198T NUC:C12880T NUC:C15714T NUC:C25000T NUC:C25584T NUC:C4321T NUC:G10447A ORF1AB:G1307S ORF1AB:T3090I ORF1AB:T842I S:D405N S:S371F S:T19I S:T376A S:V213G

Jaccard Index is a measure of similarity between two sets A and B, reaching the maximum value of 1 if A=B and minimum value of 0 if A ∩ B = {}. In the c(d) representation below, c represents the Jaccard index of the set of mutations that were experimentally detected for this sample as listed above, whereas d refers to the ideal value of the Jaccard index expected from complete genome coverage without any sequencing errors.

	B.1.617.2	BA.1	BA.2	BA.3	BA.4	BA.5
B.1.617.2	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.02)	0.00 (0.03)
BA.1	0.00 (0.00)	1.00 (1.00)	0.10 (0.10)	0.10 (0.21)	0.07 (0.08)	0.07 (0.08)
BA.2	0.00 (0.00)	0.10 (0.10)	1.00 (1.00)	0.43 (0.33)	0.74 (0.63)	0.70 (0.59)
BA.3	0.00 (0.00)	0.10 (0.21)	0.43 (0.33)	1.00 (1.00)	0.45 (0.30)	0.40 (0.29)
BA.4	0.00 (0.02)	0.07 (0.08)	0.74 (0.63)	0.45 (0.30)	1.00 (1.00)	0.94 (0.84)
BA.5	0.00 (0.03)	0.07 (0.08)	0.70 (0.59)	0.40 (0.29)	0.94 (0.84)	1.00 (1.00)

Detected mutations

Excluded from this pdf version due to file size limitations.