U.S. FOOD & DRUG ADMINISTRATION

CFSAN/OAO
BIOSTATISTICS AND BIOINFORMATICS STAFF

WASTEWATER SARS-COV2 ANALYSIS REPORT

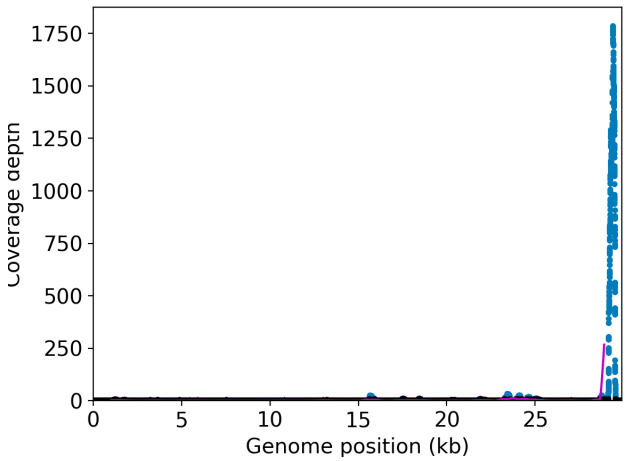
Sample name:	SRR16828013
Date generated:	2022-03-15, 14:47:48 EDT
Executed by:	Tunc Kayikcioglu (Tunc.Kayikcioglu@fda.hhs.gov)
Executed on:	172.20.44.145 (aka n145.raven.cfsan)

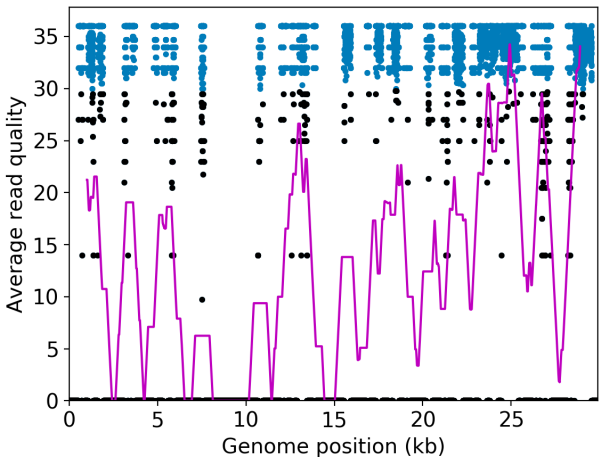
Sequencing summary

Sequencing chemistry:	AMPLICON with NextSeq 500
Source site:	USA: Washington (?,?)
Sampling date:	2021-01-11
Collected by:	Aquavitas
Sequenced by:	Missing
Total number of reads:	906676
Reads aligned:	3476 (0%)
Average read quality:	34.6
Average read length:	141
Reads passing filter:	3399 (0%)
Average read quality passing filter:	34.7
Average read length passing filter:	141
Average coverage passing filter:	16X

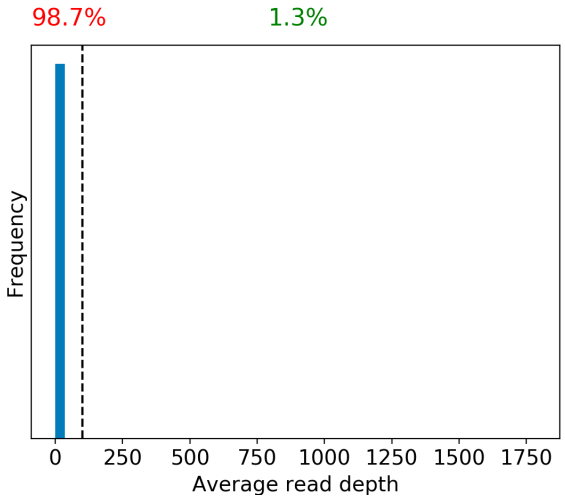
A read passes filter if the read length after adaptor trimming ≥ 30 and minimum read quality ≥ 20 within a sliding window of width 4.

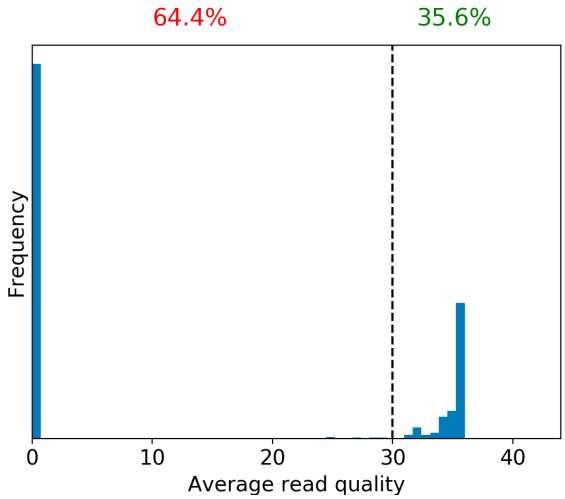
Overall sequence characteristics

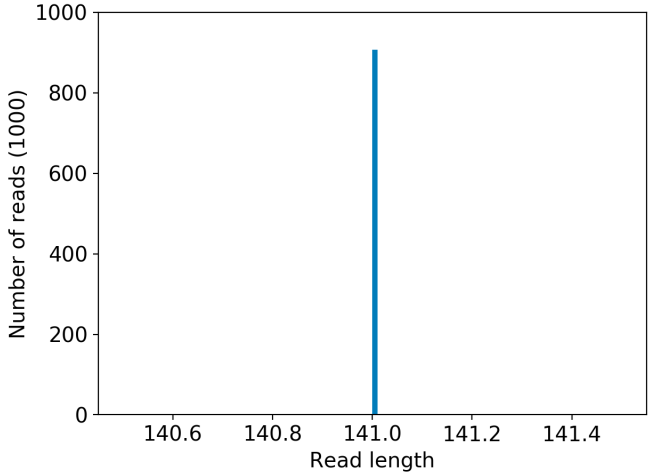


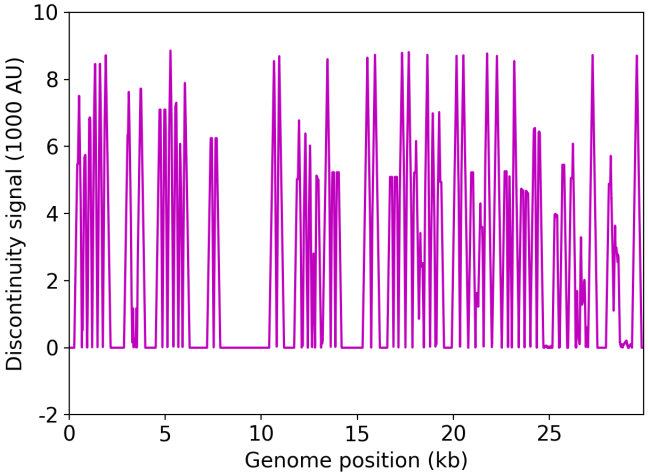


NOTE: The red shaded areas marked with a (*) are not covered by the design of the library preparation kit and hence excluded from analyses. Magenta curves represent moving average with a window width of 1kb.









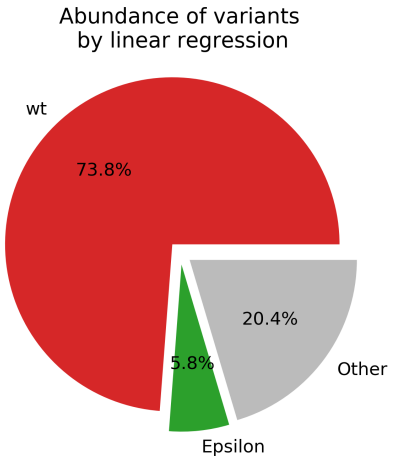
	Uncovered coordinates (0X)	Poorly covered coordinates (<10X)
# Inaccessible genomic coordinates by kit design:	-1nt (0%)	-1nt (0%)
All genomic coordinates:	18832nt (62%)	28129nt (94%)

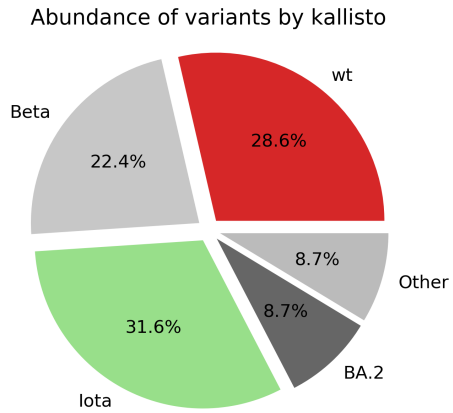
Common SNPs:	14nt (43%)	26nt (81%)
Diverse SNPs:	117nt (23%)	504nt (99%)
Rare SNPs:	1987nt (79%)	2495nt (99%)

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):	3577 reads (0.79%)
Hits to human genome (kraken2):	1968 reads (0.43%)
Hits to synthetic sequences (kraken2, taxid 28384):	113 reads (0.02%)

Detected variants (Experimental)

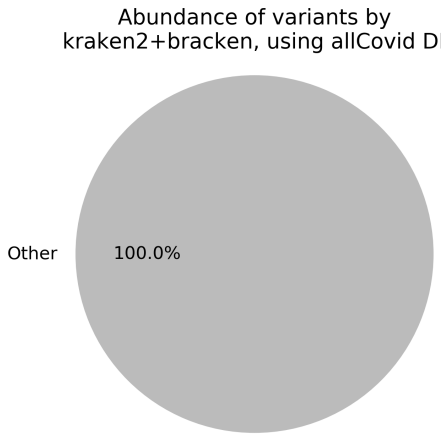


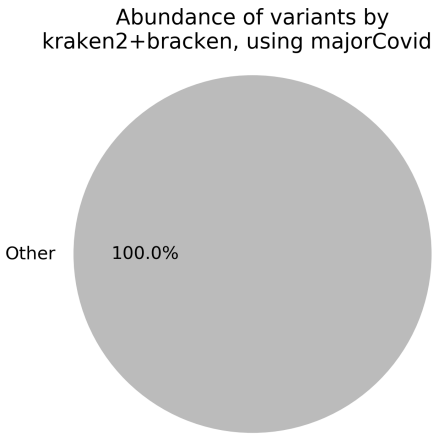


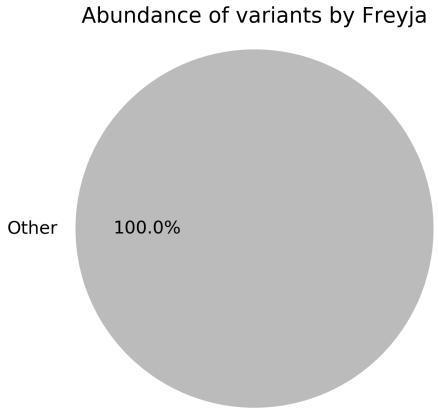
Based on deconvolution, wt is estimated to constitute 72.54% of the viral particles and hence is the most abundant variant in the sample. The R^2 for the linear regression was 0.21. Variants that were detected less than 5% were grouped under "Other"

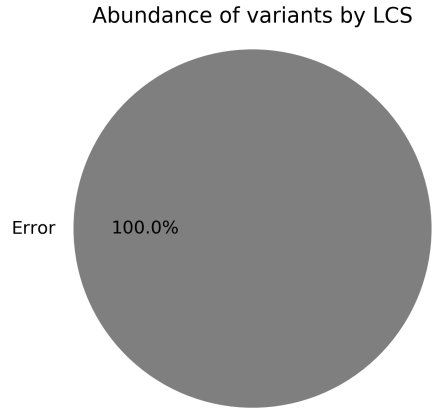
Based on the consensus sequence of the observed reads, the "ensemble-averaged sequence" most closely resembles the B lineage. If this is a sample consisting of a single source of pathogens or an overwhelming majority of the different sources are infected with the same variant, the sample is dominated 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 Iota lineage. Accuracy of this measure is expected to improve if the input data consists of long reads as opposed to convolution.









Under the assumption that the presence of a variant requires the detection of all respective mutations of the variant, the characteric 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	AY.4	AY.4.2	B.1.617.2	BA.1	BA.2	BA.3
Characteristic mutations detected	(0 of 19)	(0 of 3)	(0 of 13)	(0 of 23)	(0 of 28)	(0 of 19)

Detected mutations

Only genomic coordinates with at least 10X coverage were considered.

Position	Ref. base	Alt. base	Alt. freq	p-value	Mutation name	Compatible lineages
23403	A	G	0.778	2.03E-07	S:D614G	B.1.1.529, B.1.427, C.37, B.1.429, B.1.526, AV1, B.1.621
24076	T	C	0.278	7.89E-03	NUC:T24076C	None found
29133	G	T	0.320	1.63E-03	N:G287V	None found
29135	G	T	0.342	2.67E-06	N:D288Y	None found
29136	A	G	0.225	7.92E-04	N:D288G	None found
29137	C	T	0.436	1.48E-08	NUC:C29137T	None found

29138	C	T	0.100	7.24E-03	N:Q289*	None found
29140	G	+A	0.203	1.03E-04	NUC:G29140+A	None found
29141	G	T	0.072	9.38E-03	N:E290*	None found
29142	A	T	0.093	2.13E-03	N:E290V	None found
29143	A	T	0.246	1.15E-09	N:E290D	None found
29144	C	T	0.062	3.07E-03	NUC:C29144T	None found
29145	T	G	0.267	7.94E-10	N:L291R	None found
29146	A	T	0.172	7.98E-09	NUC:A29146T	None found
29147	A	G	0.131	2.10E-06	N:I292V	None found
29148	T	A	0.143	3.37E-07	N:I292N	None found
29149	C	G	0.139	6.28E-08	N:I292M	None found
29150	A	T	0.113	3.06E-06	N:R293*	None found
29151	G	+T	0.070	7.52E-04	NUC:G29151+T	None found
29153	C	T	0.120	5.08E-10	N:Q294*	None found
29156	G	+A	0.052	3.45E-03	NUC:G29156+A	None found
29157	G	A	0.053	2.10E-05	N:G295E	None found
29158	A	G	0.050	3.28E-05	NUC:A29158G	None found
29161	T	G	0.056	4.36E-06	NUC:T29161G	None found
29276	G	T	0.052	2.80E-18	N:G335C	None found
29384	G	T	0.543	0.00E+00	N:D371Y	None found
29508	G	+C	0.055	1.61E-07	NUC:G29508+C	None found
29510	A	T	0.087	4.77E-16	N:S413C	None found
29512	T	+C	0.051	1.49E-05	NUC:T29512+C	None found
29513	G	T	0.072	1.31E-12	N:A414S	None found
29516	G	T	0.078	4.81E-11	N:D415Y	None found
29519	T	A	0.096	4.65E-13	N:S416T	None found
29520	C	+TGT	0.057	3.30E-03	NUC:C29520+TGT	None found
29521	A	T	0.132	2.92E-08	NUC:A29521T	None found
29522	A	T	0.487	1.55E-34	N:T417S	None found
29525	C	T	0.111	3.10E-07	N:Q418*	None found
29526	A	T	0.200	1.40E-07	N:Q418L	None found
29527	G	T	0.494	4.83E-23	N:Q418H	None found
29528	G	T	0.139	1.23E-04	N:A419S	None found
29530	C	T	0.404	1.45E-07	NUC:C29530T	None found
29531	T	G	0.303	1.46E-04	N:*420E	None found
29533	A	T	0.667	4.88E-08	N:*420Y	None found
29536	T	A	1.000	9.61E-08	NUC:T29536A	None found