fortune Journals

Research Article

ARCHIVES OF MICROBIOLOGY & IMMUNOLOGY

ISSN: 2572-9365

Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency

Eunhee Kang[1], TaeJin Ahn[*, 1] and Taesung Park[*, 2]

Abstract

Coronavirus disease 19 (COVID-19), currently prevalent worldwide, is caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Similar to other RNA viruses, SARS-CoV-2 continues evolving through random mutations, creating numerous variants, including Alpha, Beta, and Delta. It is, therefore, necessary to predict the mutations constituting the dominant variant before they are generated. This can be achieved by continuously monitoring the mutation trends and patterns. Hence, we sought to design a dominant variant candidate (DVC) selection algorithm in the current study. To this end, we obtained COVID-19 sequence data from GISAID and extracted position-nucleotide (POS-NT) frequency ratio data by country and date through data preprocessing. We then defined the dominant dates for each variant in the USA and developed a frequency ratio prediction model for each POS-NT. Based on this model, we applied DVC criteria to build the selection algorithm, which was verified for Delta and Omicron. Using Condition 3 as the DVC criterion, 69 and 102 DVC POS-NTs were identified for Delta and Omicron an average of 47 and 82 days before the dominant dates, respectively. Moreover, 13 and 44 Delta- and Omicron-defining POS-NTs were recognized 18 and 25 days before the dominant dates, respectively. We identified all DVC POS-NTs before the dominant dates, including rapidly and gently increasing POS-NTs. Considering that we successfully defined all POS-NT mutations for Delta and Omicron, the DVC algorithm may represent a valuable tool for providing early predictions regarding future variants, helping improve global health.

Abbreviation:

BAM	Binary alignment/map
COVID	Coronavirus disease 19
DVC	Dominant variant candidate
GISAID	Global Initiative for Sharing All Influenza Data
POS-NT	Position-nucleotide
SAM	Sequence alignment/map
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Background

The recent Coronavirus disease 19 (COVID-19) pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has had severe implications worldwide. Continuous mutations in the genome generate new variants, enabling the virus to thwart disease control

Affiliation:

[1]Department of Life Science, Handong Global University, Pohang, Republic of Korea

[2]Department of Statistics, Seoul National University, Seoul, Republic of Korea

***Corresponding author:**
Taesung Park, Department of Statistics, Seoul National University, Seoul, Republic of Korea.

TaeJin Ahn, Department of Life Science, Handong Global University, Pohang, Republic of Korea

Citation: Eunhee Kang, TaeJin Ahn and Taesung Park. Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency. Archives of Microbiology and Immunology. 8 (2024): 101-117.

Received: February 29, 2024
Accepted: March 08, 2024
Published: March 27, 2024

measures. Next-generation sequencing technology is widely employed to characterize the genetic SARS-CoV-2 variants. Owing to the contributions of many researchers, SARS-CoV-2 genomic data has been collected from infected individuals worldwide. GISAID is a database that stores and provides sequenced SARS-COV-2 genomes along with basic metadata, including the sequencing date and location. GISAID also visually presents the status of SARS-CoV-2 variant spread in a geographical and time-dependent manner. In particular, predicting the emergence of a novel variant is critical to identifying new potential outbreaks capable of evading the current diagnostic and vaccine strategies.

In this study, we provide a prediction model that estimates whether a single SARS-COV-2 mutation is a prominent factor in determining disease severity in infected patients. This functionality is helpful in disease control in several aspects. First, single mutations may be associated with known clinical characteristics, such as symptom severity, incubation period, and morbidity rate. Second, mutations in PCR primer binding regions can be used to estimate if an infectious virus evades diagnostic methods. Third, single mutations help assess the vaccination efficacy of the designed epitope.

SNPs defining Delta and Omicron variants

Based on the WHO nomenclature system (GISAID, Pango lineage, Nextstrain clade): Alpha (GRY, B.1.1.7, 20I (V1)), Beta (GH/501Y. V2, B.1.351, 20H (V2)), Gamma (GR/501Y. V3, P.1, 20J(V3)), Delta (G/478K.V1, B.1.617.2, 21A-21I-21J), and Omicron (GR/484A, B.1.1.529, 21K-21L-21M-22A-22B-22C-22D) (WHO: https://www.who.int/activities/tracking-SARS-CoV-2-variants) SARS-CoV-2 strains have arisen due to mutations in the genomic sequence. The SARS-CoV-2 genome comprises 29,903 nucleotides, encoding 12 proteins (ORF1a/1ab, S, ORF3a, ORF3b, E, M, ORF6, ORF7a, ORF7b, ORF8, and ORF10*). These mutations are caused by single nucleotide changes, i.e., replacement, insertion, or deletion, leading to changes in the amino acid sequence (Fig 1; GISAID: https://gisaid.org/). Figure 1 presents a genome sequence map of SARS-Cov-2 and the major mutational positions of several variants. In this study, we attempted to predict the Delta and Omicron variants, i.e., the most recent dominant SARS-CoV-2 variants. According to Pango (Pango cov-lineages: https://cov-lineages.org/), the Delta and Omicron variants have 13 and 47 defining SNPs, respectively (Tables 1 and 2).

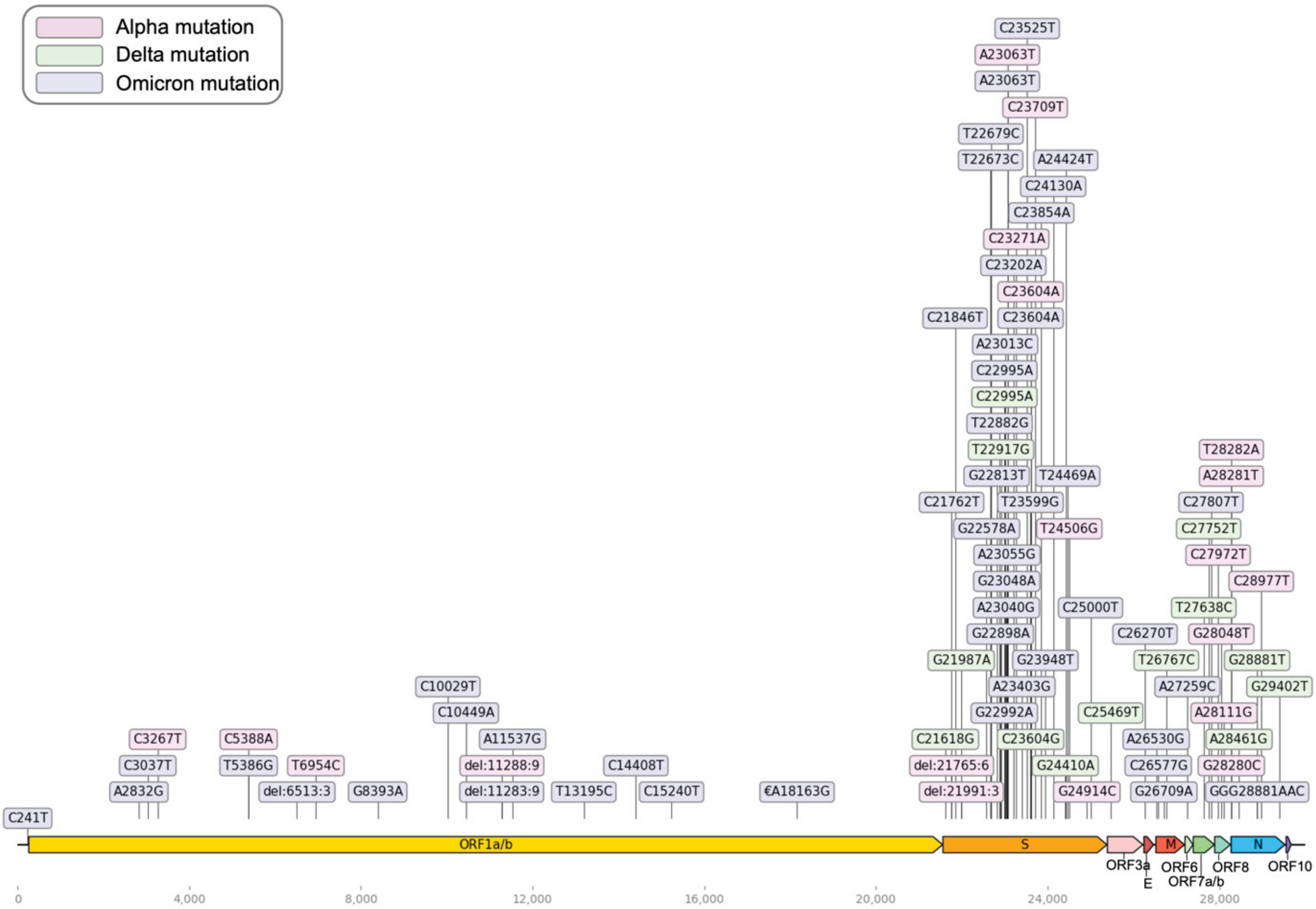


Figure 1: SARS-CoV-2 genome sequence map. The 29,903 nucleotide positions are shown in the context of the 12 encoded proteins. The main mutations of each dominant variant are shown; pink: Alpha mutation, green: Delta mutation, blue: Omicron mutation.

Table 1: Delta defining position-nucleotides.

	Amino acid	Nucleotide
1	T19R	C21618G
2	T478K	C22995A
3	L452R	T22917G
4	D950N	G24410A
5	P681R	C23604G
6	D377Y	G21987A
7	S26L	T26767C
8	I82T	T27638C
9	V82A	C27752T
10	R203M	C25469T
11	T120I	G29402T
12	G142D	A28461G
13	D63G	G28881T

Table 2: Omicron defining position-nucleotides

	Amino acid	Nucleotide
1	**6513>3**	
2	**11283>9**	
3	C241T	C241T
4	K856R	A2832G
5	C3037T	C3037T
6	T5386G	T5386G
7	A2710T	G8393A
8	T3255I	C10029T
9	P3395H	C10449A
10	I3758V	A11537G
11	T13195C	T13195C
12	P314L	C14408T
13	C15240T	C15240T
14	I1566V	A18163G
15	A67V	C21762T
16	T95I	C21846T
17	G339D	G22578A
18	S371L	T22673C
19	S373P	T22679C
20	K417N	G22813T
21	N440K	T22882G
22	G446S	G22898A
23	S477N	G22992A
24	T478K	C22995A
25	E484A	A23013C
26	Q493R	A23040G
27	G496S	G23048A
28	Q498R	A23055G
29	N501Y	A23063T
30	T547K	C23202A
31	D614G	A23403G
32	H655Y	C23525T
33	N679K	T23599G
34	P681H	C23604A
35	N764K	C23854A
36	D796Y	G23948T
37	N856K	C24130A
38	Q954H	A24424T
39	N969K	T24469A
40	C25000T	C25000T
41	T9I	C26270T
42	D3G	A26530G
43	Q19E	C26577G
44	A63T	G26709A
45	A27259C	A27259C
46	C27807T	C27807T
47	**RG203KR**	**GGG28881AAC**

COVID-19 sequence data from GISAID

The Global Initiative for Sharing All Influenza Data (GISAID) provides a database of nucleotide sequence information and related epidemiological information for all influenza viruses and COVID-19-causing coronaviruses. GISAID provides multiple SARS-CoV-2 sequence data analyses collected worldwide, as well as sequence alignments, diagnostic primer and probe coordinates, 3D protein models, drug targets, and phylogenetic trees. In this study, global SARS-CoV-2 sequence data were obtained from GISAID on February 22, 2022; 8,474,962 sequence data were obtained from December 1, 2019, to February 22, 2022 (GISAID: https://gisaid.org/).

Materials and Methods

Data preprocessing and formatting

COVID-19 sequence data obtained in a FASTA file format from GISAID were converted from a multiline to a single-line format; only complete sequences corresponding to > 29,000 bp were extracted. We secured sequence data by country and date through the GISAID unique ID, country, collection date, and sequence information in the header of the sequence data. In this study, countries with the most sequencing data, namely, the USA (2,702,068), UK (1,936,958), and Germany (415,309), as well as Korea, were analyzed. The sequence data obtained by country were mapped to the original sequence (NC_045512) to obtain a sequence alignment/map

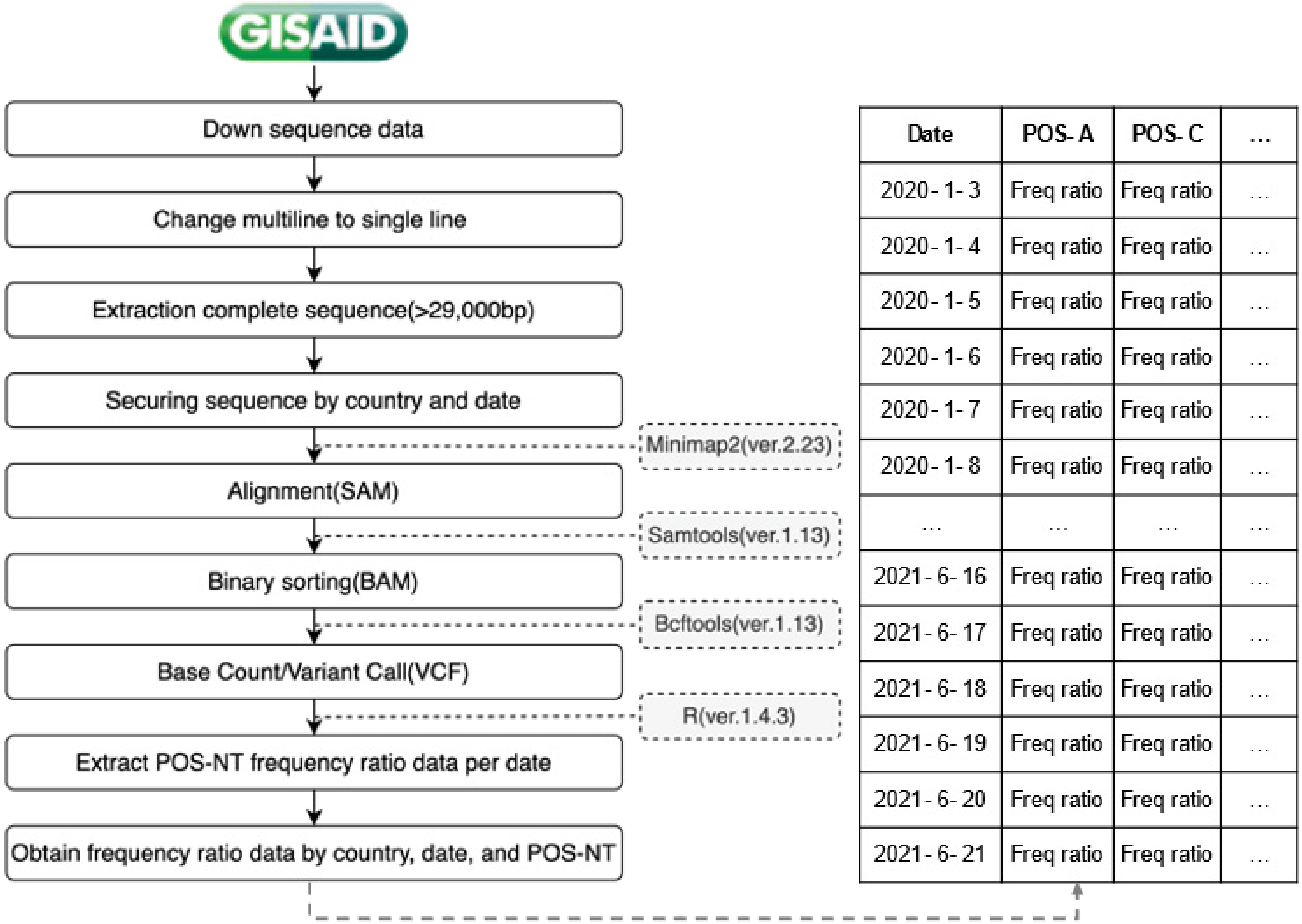
Citation: Eunhee Kang, TaeJin Ahn and Taesung Park. Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency. Archives of Microbiology and Immunology. 8 (2024): 101-117.

(SAM) file. The binary alignment/map (BAM) file was then converted to a binary format using SAM tools to reduce the file size. From the generated BAM file, sequencing reads were synthesized for each position of the original sequence to determine whether bases differed from the original data; the mutation data was extracted in a variant call format. The obtained data were used to extract information on the number of mutations and mutation frequency ratio information for each position in the sequence and to confirm the mutation trend by securing the frequency ratio data by country, date, and POS-NTs (total 29,903 positions × 4 nucleotides × 4 countries; Fig. 2).

As it is overly computationally intensive to determine the trend for all mutations in SARS-COV-2 (i.e., a combination of 29,903 positions and 3 SNP mutations), each nucleotide position was subjected to additional preprocessing to remove those without mutations (reference frequency = 100), those with no information on the time point of the dominant variant, and those where the change in reference allele frequency was < 10%. Next, we created continuous data for date information for which frequency ratio information did not exist and the position where the total data date was < 50 days removed. Subsequently, cubic spline interpolation was used to fill in the data for which the frequency ratio information did not exist. We removed the reference allele from the four nucleotides as we were interested in mutations. An additional preprocessing step is shown in Figure 3.

DVC selection for the prediction model

We attempted to predict the mutations comprising the dominant variants by analyzing and predicting the Delta and Omicron variants. To confirm the trend of a specific POS-NT, a dominant variant selection time point was required. Moreover, we aimed to confirm whether the developed algorithm could identify all mutations constituting the Delta and Omicron variants at the dominant variant time point after determining the DVC POS-NT until the variant became dominant. Therefore, we attempted to define the dominant variant time-point (i.e., dominant date) for Delta and Omicron. We defined the strains that accounted for > 50% of all new COVID-19 cases as the dominant variants. However,


GISAID
Down sequence data
Change multiline to single line
Extraction complete sequence(>29,000bp)
Securing sequence by country and date
Minimap2(ver.2.23)
Alignment(SAM)
Samtools(ver.1.13)
Binary sorting(BAM)
Bcftools(ver.1.13)
Base Count/Variant Call(VCF)
R(ver.1.4.3)
Extract POS-NT frequency ratio data per date
Obtain frequency ratio data by country, date, and POS-NT

Date	POS- A	POS- C	...
2020- 1- 3	Freq ratio	Freq ratio	...
2020- 1- 4	Freq ratio	Freq ratio	...
2020- 1- 5	Freq ratio	Freq ratio	...
2020- 1- 6	Freq ratio	Freq ratio	...
2020- 1- 7	Freq ratio	Freq ratio	...
2020- 1- 8	Freq ratio	Freq ratio	...
...	...	...	...
2021- 6- 16	Freq ratio	Freq ratio	...
2021- 6- 17	Freq ratio	Freq ratio	...
2021- 6- 18	Freq ratio	Freq ratio	...
2021- 6- 19	Freq ratio	Freq ratio	...
2021- 6- 20	Freq ratio	Freq ratio	...
2021- 6- 21	Freq ratio	Freq ratio	...

Figure 2: Frequency ratio data acquisition process by country, date, and POS-NT. Freq, frequency.

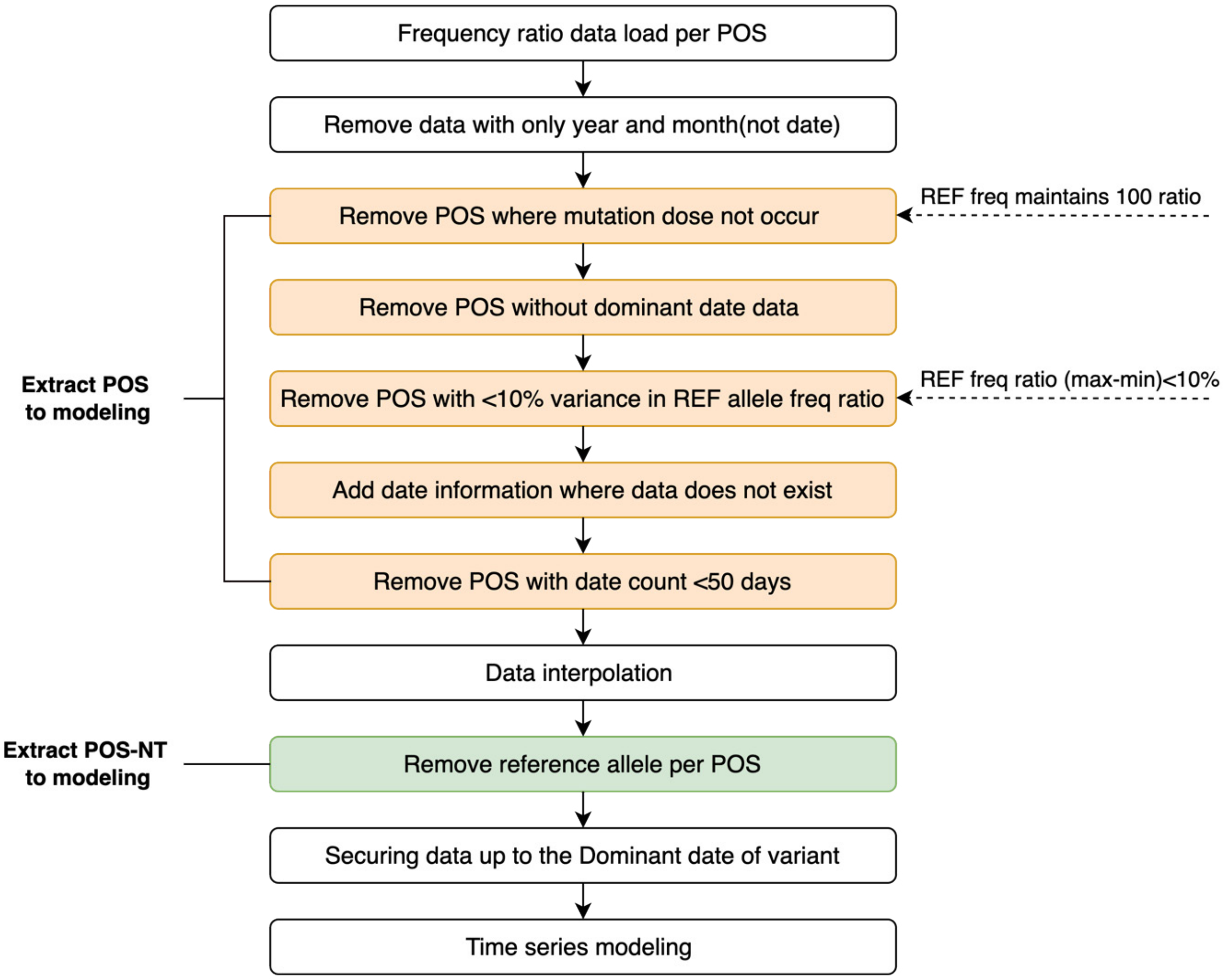

Frequency ratio data load per POS
Remove data with only year and month(not date)
Remove POS where mutation dose not occur
REF freq maintains 100 ratio
Remove POS without dominant date data
Extract POS to modeling
Remove POS with <10% variance in REF allele freq ratio
REF freq ratio (max-min)<10%
Add date information where data does not exist
Remove POS with date count <50 days
Data interpolation
Extract POS-NT to modeling
Remove reference allele per POS
Securing data up to the Dominant date of variant
Time series modeling

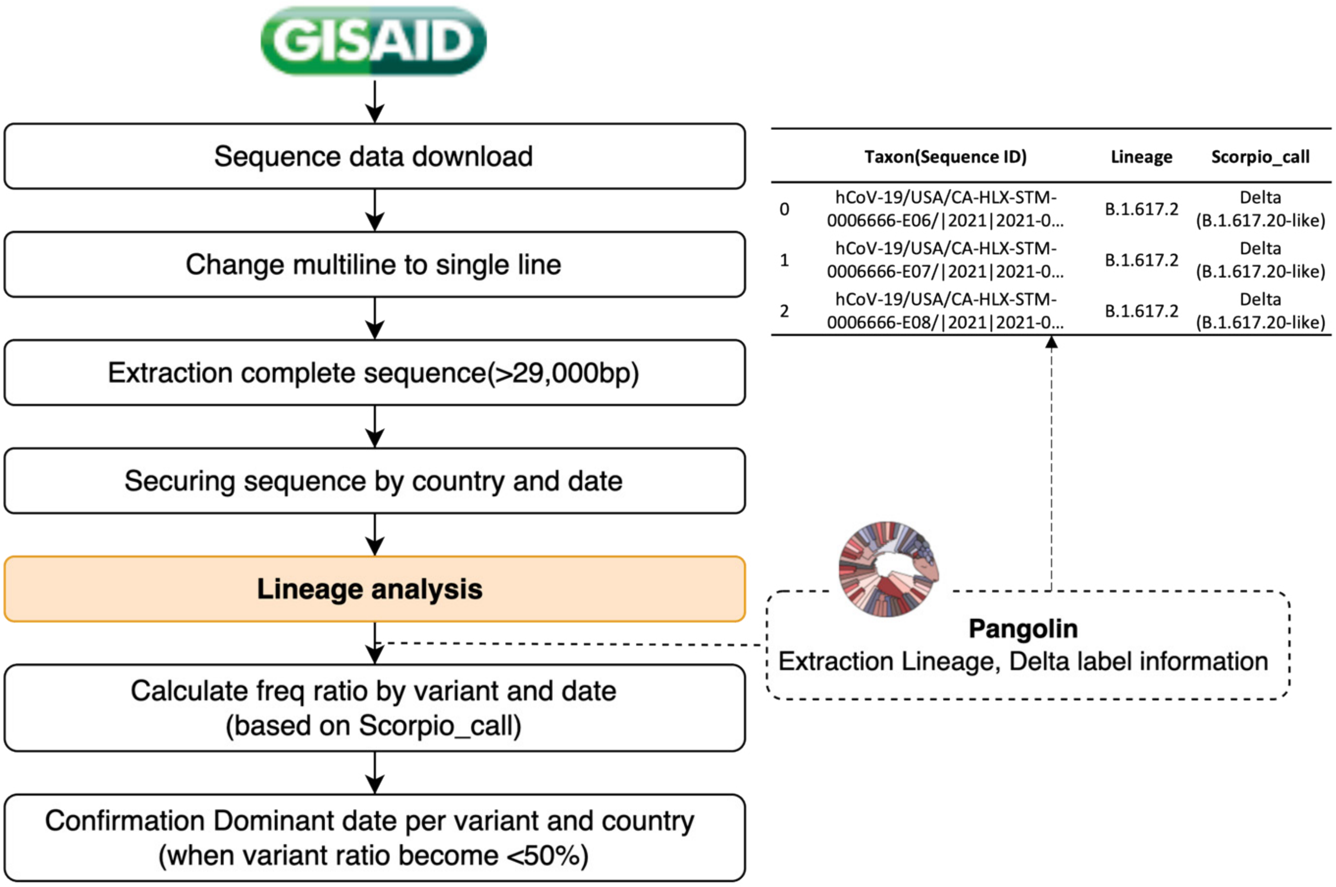
Figure 3: Additional preprocessing step to select POS-NTs for modeling.

information on the strain and lineage of the sequences was not available in the data provided by GISAID. Therefore, we proceeded with the lineage analysis provided by Pangolin, assigned a strain label, including Delta and Omicron, for each sequence, and secured the daily frequency ratio data of the strain. The strain that accounted for > 50% of all new COVID-19 cases was defined as the dominant variant and the corresponding time point was defined as the dominant date. Figure 4 illustrates the scheme determining the dominant date for each country and its variants. The dominant date was used as the time point for selecting the dominant variant using this algorithm and as a criterion for learning and prediction date windows for each variant. For the analysis and prediction of Delta mutations, the extracted data, including the alpha-dominant to the delta-dominant dates, were employed for each POS-NT. The analysis and prediction of Omicron mutation data from the Delta dominant to Omicron dominant date.

Results

POS-NT frequency ratio prediction model

A POS-NT frequency ratio prediction model was developed to confirm the trend in each POS-NT frequency ratio. Gaussian Process Regression (GPR) is a powerful Bayesian-based, non-parametric kernel-based probabilistic model for regression analyses applied in exploration and utilization scenarios. It predicts the output of a new test set considering the novel input vectors of the test and training sets [1–3]. The most prominent advantage of GPRs is their ability to obtain the forecast uncertainty with the forecast value. In addition, GPR boasts computational efficiency and high accuracy and is suitable for other time series forecasting, such as weather forecasting [4]. Recently, the GPR model was used widely in predicting COVID-19 spread and deaths, exhibiting improved performance compared with other models [3, 5–7].



	Taxon(Sequence ID)	Lineage	Scorpio_call
0	hCoV-19/USA/CA-HLX-STM-0006666-E06/|2021|2021-0...	B.1.617.2	Delta (B.1.617.20-like)
1	hCoV-19/USA/CA-HLX-STM-0006666-E07/|2021|2021-0...	B.1.617.2	Delta (B.1.617.20-like)
2	hCoV-19/USA/CA-HLX-STM-0006666-E08/|2021|2021-0...	B.1.617.2	Delta (B.1.617.20-like)

GISAID
Sequence data download
Change multiline to single line
Extraction complete sequence(>29,000bp)
Securing sequence by country and date
Lineage analysis
Calculate freq ratio by variant and date (based on Scorpio_call)
Confirmation Dominant date per variant and country (when variant ratio become <50%)
Pangolin
Extraction Lineage, Delta label information

Figure 4: Dominant variant time point (dominant date definition process). After detecting the sequence data for each country, the strain and lineage information for each sequence was allocated through lineage analysis provided by Pangolin. After securing daily rate data for each strain, those accounting for > 50% of all new COVID-19 cases were defined as dominant, and the corresponding time point was defined as the dominant variant time point (dominant date). The dominant date was used as the dominant variant selection time point and as a criterion for learning and prediction date windows for each variant in the algorithm.

The following four patterns were identified for Delta and Omicron variant-defining mutations: (1) a gentle increase from a ratio of 0 (Fig. 5A); (2) consistent high-frequency ratio values (Fig. 5B); (3) a gentle increase through the dominant date, with a high-frequency ratio, of the previous variant (Fig. 5C); (4) soaring pattern (Fig. 5D). To identify the trend of a gently increasing pattern and soaring pattern, it was necessary to select optimal training and prediction dates. Therefore, to learn the soaring pattern trend, we applied the latest information to predict the future and modeled each learning and prediction combination until the dominant date for each variant (i.e., learn for 10 and 20 days and predict 3, 5, 8, and 10 days later; Fig. 6A). In the case of the Delta mutation, data from the Alpha-dominant to Delta-dominant dates were employed for the analysis window based on the variant. In the case of the Omicron mutation, data from the Delta-dominant date to the Omicron-dominant date were modeled (Fig. 6B).

DVC selection algorithm

Based on the frequency ratio prediction model for each POS-NT, a dominant variant candidate selection algorithm (DVC selection algorithm) was developed by applying the dominant variant candidate criteria (DVC criteria; Fig. 7). We then determined whether all POS-NTs met the DVC criteria for each prediction time point; upon failing to meet the DVC criteria, the corresponding POS-NT was reanalyzed the next day. If it met the DVC criteria at that time point, the corresponding POS-NT was classified as DVC POS-NT. The DVC POS-NT was identified up to the dominant date of the variant, and then the identified DVC POS-NT was compared with the actual variant definition POS-NT list. Eight conditions were simulated to select the optimal DVC criteria based on the criteria for outliers in which the frequency of the DVC increased the next day (i.e., Criterion 2), and the measured value was higher than the predicted value (i.e., Criterion 4; Table 3). The DVC criteria defined the corresponding POS-NT as DVC POS-NT when all four detailed criteria were satisfied.

Citation: Eunhee Kang, TaeJin Ahn and Taesung Park. Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency. Archives of Microbiology and Immunology. 8 (2024): 101-117.

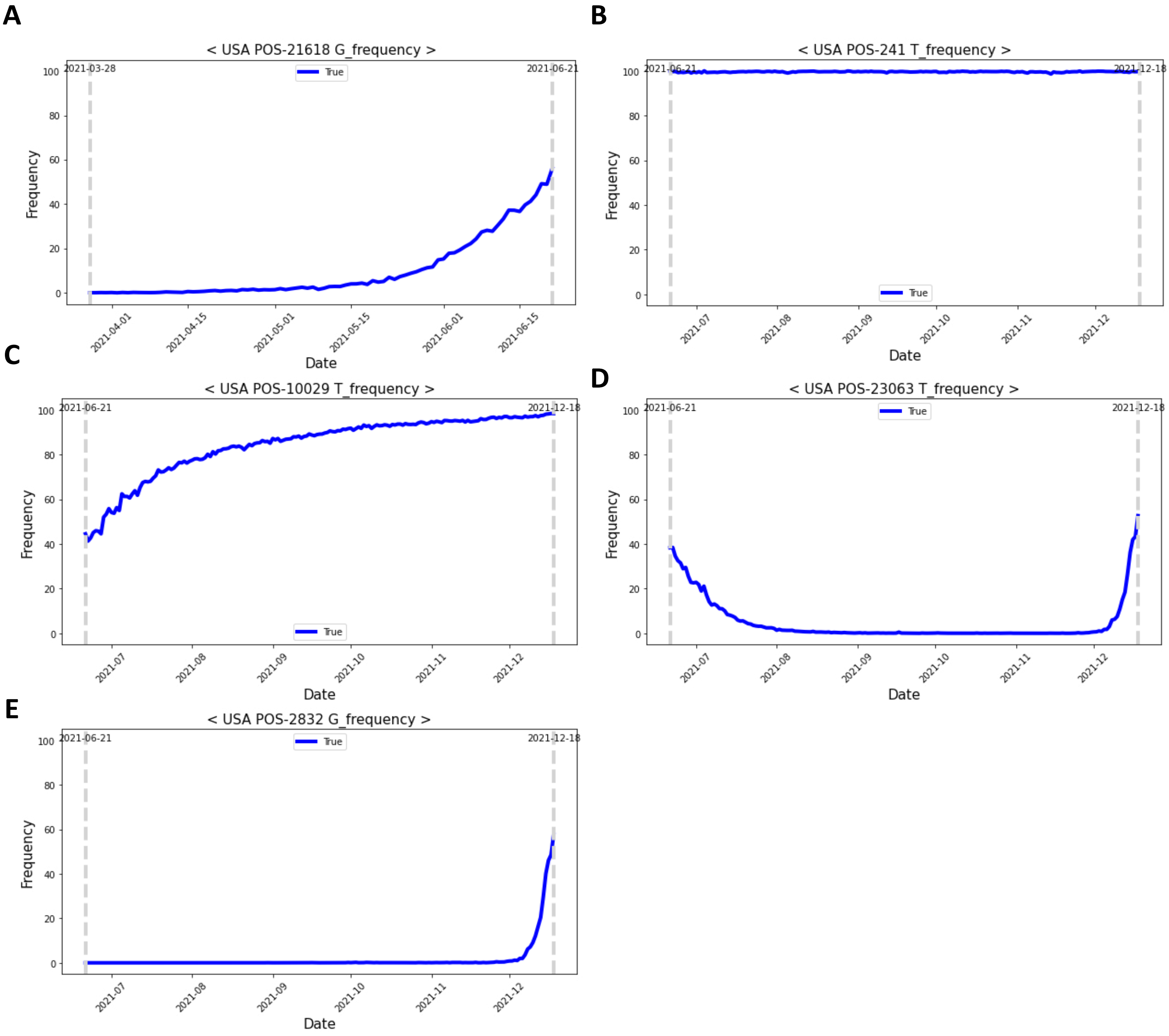


Figure 5: Time-dependent patterns of delta- and omicron-defining mutations. (A) Delta variant pattern: gentle increase from a ratio value of 0. (B–E) Omicron variant pattern; (B) high-frequency ratio values are consistently present; (C) gentle increase through the dominant date (with a high-frequency ratio) of the previous variant; (D) soaring pattern.

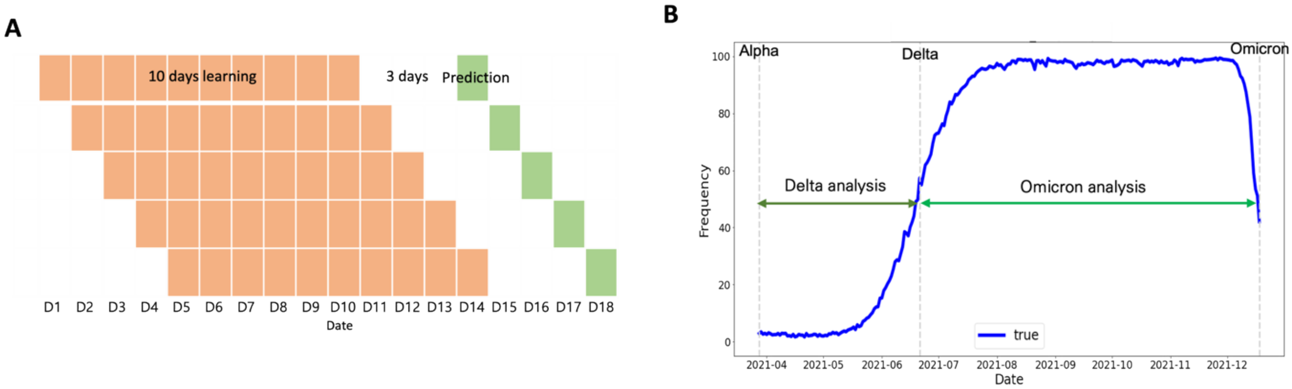


Figure 6: Learning and prediction window selection. (A) Example training and prediction window for one POS-NT (training for 10 days and predicting after 3 days) and (B) Delta and Omicron analysis date time.

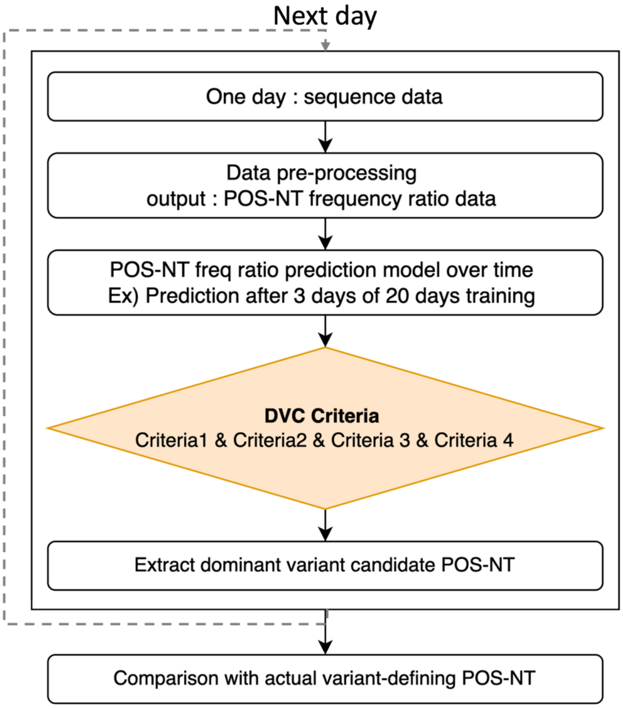

Next day
One day : sequence data
Data pre-processing
output : POS-NT frequency ratio data
POS-NT freq ratio prediction model over time
Ex) Prediction after 3 days of 20 days training
DVC Criteria
Criteria1 & Criteria2 & Criteria 3 & Criteria 4
Extract dominant variant candidate POS-NT
Comparison with actual variant-defining POS-NT

Figure 7: DVC POS-NT Selection Algorithm and Combined DVC Criteria. When POS-NT ratio data at a specific point occur, predictions for the future can be made. If the DVC criteria are met, the corresponding POS-NT is identified as the DVC POS-NT. If it does not meet the DVC criteria at that time point, the POS-NT moves to the next point, and the analysis continues. Criteria 1: number of days in which all dominant variant candidate criteria were satisfied; Criterion 2: whether the observed frequency ratio increased the next day compared with the previous day; Criterion 3: threshold of the predicted frequency ratio; Criterion 4: Observed value greater than the predicted value.

A visual summary of the methodology is shown in Figure 8. The SARS-CoV-2 sequence data from GISAID were formalized to secure frequency ratio information for each POS-NT by country and date. A time-series forecasting model was developed using the time-series frequency ratio data obtained using POS-NT. Over time, learning and prediction progressed to the dominant date for each variant. For each prediction date, the DVC POS-NT selection algorithm was applied to all POS-NTs to secure DVC POS-NT for each variant. When all DVC POS-NTs were selected until the dominant date for each variant, they were compared with the actual variant-defining POS-NT to determine the number of days preceding the dominant date of the average number of variant-defining POS-NTs. We also compared the prediction results with the actual variant-defining POS-NT, to determine how many variant-defining POS-NTs could be identified, on average, how many days ago.

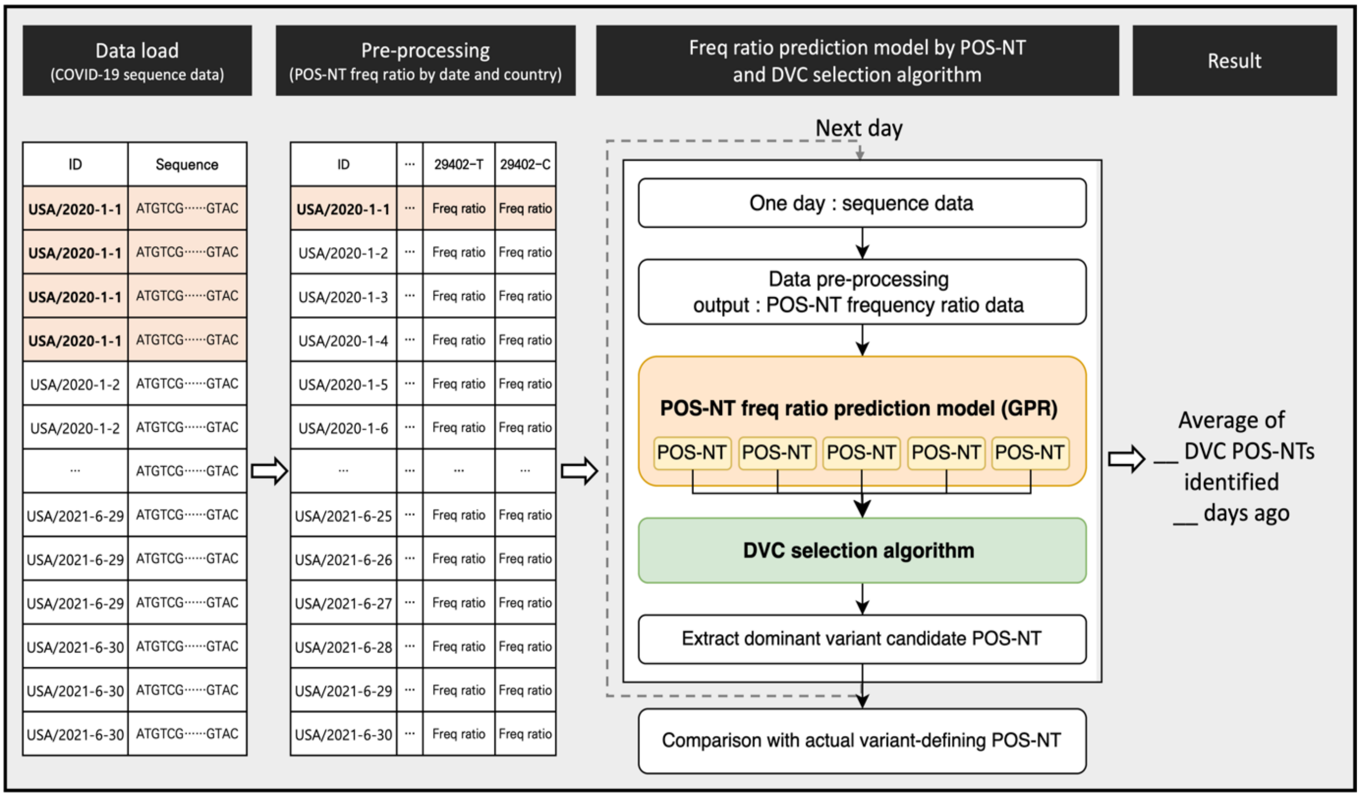
Confirmation metric for the results

The following four metrics were used to confirm the results: (1) number of DVC POS-NTs identified by the algorithm developed in this study, i.e., candidate count; (2) average number of days for identification; (3) number of POS-NTs corresponding to the POS-NTs that define the actual variant (candidate∩actual) among the identified DVC POS-NTs; (4) ratio of the number of POS-NTs corresponding to the actual variant-defining POS-NTs among the identified DVC POS-NTs (Eq. (1)). Upon identifying all actual variant-defining POS-NT, the Candidate∩Actual value will be incremented. The algorithm can sensitively identify the DVC POS-NT as the ratio value increases.

Table 3: Eight DVC criteria combinations

	Criteria 1	Criteria 2	Criteria 3	Criteria 4
Condition 1	3 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 30	Actual > Pred
Condition 2	3 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 20	Actual > Pred
Condition 3	3 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 10	Actual > Pred
Condition 4	3 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 5	Actual > Pred
Condition 5	2 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 30	Actual > Pred
Condition 6	2 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 20	Actual > Pred
Condition 7	2 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 10	Actual > Pred
Condition 8	2 days in a row	Freq ratio_D – Freq ratio_{D-1} > 0	Pred freq ratio ≥ 5	Actual > Pred

The DVC criteria define the corresponding POS-NT as the DVC POS-NT when all four detailed criteria are satisfied. Criteria 2 and 4 are fixed, and Criteria 1 and 3 are manipulated to simulate each combination. Freq ratio_D: frequency ratio at time point D (current); Freq ratio_{D-1}: frequency ratio at time point D-1 (previous day); Actual: actual frequency ratio; Pred: predicted frequency ratio.


Data load
(COVID-19 sequence data)
Pre-processing
(POS-NT freq ratio by date and country)
Freq ratio prediction model by POS-NT
and DVC selection algorithm
Result
Next day
One day : sequence data
Data pre-processing
output : POS-NT frequency ratio data
POS-NT freq ratio prediction model (GPR)
POS-NT
POS-NT
POS-NT
POS-NT
POS-NT
DVC selection algorithm
Extract dominant variant candidate POS-NT
Comparison with actual variant-defining POS-NT
Average of
__ DVC POS-NTs
identified
__ days ago

ID	Sequence
USA/2020-1-1	ATGTCG······GTAC
USA/2020-1-1	ATGTCG······GTAC
USA/2020-1-1	ATGTCG······GTAC
USA/2020-1-1	ATGTCG······GTAC
USA/2020-1-2	ATGTCG······GTAC
USA/2020-1-2	ATGTCG······GTAC
···	ATGTCG······GTAC
USA/2021-6-29	ATGTCG······GTAC
USA/2021-6-29	ATGTCG······GTAC
USA/2021-6-29	ATGTCG······GTAC
USA/2021-6-30	ATGTCG······GTAC
USA/2021-6-30	ATGTCG······GTAC
USA/2021-6-30	ATGTCG······GTAC

ID	···	29402–T	29402–C
USA/2020-1-1	···	Freq ratio	Freq ratio
USA/2020-1-2	···	Freq ratio	Freq ratio
USA/2020-1-3	···	Freq ratio	Freq ratio
USA/2020-1-4	···	Freq ratio	Freq ratio
USA/2020-1-5	···	Freq ratio	Freq ratio
USA/2020-1-6	···	Freq ratio	Freq ratio
···	···	···	···
USA/2021-6-25	···	Freq ratio	Freq ratio
USA/2021-6-26	···	Freq ratio	Freq ratio
USA/2021-6-27	···	Freq ratio	Freq ratio
USA/2021-6-28	···	Freq ratio	Freq ratio
USA/2021-6-29	···	Freq ratio	Freq ratio
USA/2021-6-30	···	Freq ratio	Freq ratio

Figure 8: Visual summary of the methodology.

Table 4: Number of nucleotides removed during the preprocessing process and the number of POS-NTs to be modeled.

	Delta				Omicron			
	USA	UK	Korea	Germany	USA	UK	Korea	Germany
POS where mutation do not occur	193	2399	15019	2375	193	2399	15019	2374
POS without dominant date data	1008	3478	6063	2292	99	327	274	240
POS with < 10% variance in REF allele freq ratio	26,382	19,962	4035	8461	27,281	23,110	9730	10,507
POS with date count < 50 days	3	0	89	1	0	0	22	0

Table 4-1: Number of positions removed during the preprocessing process

	USA	UK	Korea	Germany	USA	UK	Korea	Germany
Number of POS for modeling	2317	4064	4697	16773	2330	4067	4858	16782
Number of POS-NTs for modeling	6951	12,192	14,091	50,319	6990	12,201	14,574	50,346

Table 4-2: Number of POS-NTs to be modeled (the number of total models)

Position (POS), Frequency (freq).

$$Ratio = \frac{Candidate \cap Actual}{Identified\ DVC\ POS-NT} \qquad (1)$$

$$Candidate \cap Actual = Number\ of\ (Identified\ DVC\ POS - NT\ \cap Actual\ variant - definin$$

POS-NT frequency ratio data for modeling

The number of nucleotide positions for the final model and the total number of models (POS-NT) are listed in Table 4. In this study, the USA data were analyzed for the first time. Prediction modeling was performed with the frequency ratio data for 6951 POS-NTs of Delta and 6990 of Omicron variants.

Dominant date by country and variant

In the USA, the Delta and Omicron variants were confirmed as the dominant variants on June 21 and December 18, 2021, respectively (Fig. 9A). In the UK, Delta emerged as the dominant variant on May 15, 2021, and Omicron on December 14, 2021 (Fig. 9B). In Korea, Delta emerged as the dominant variant on July 4, 2021, and Omicron on January 5, 2022 (Fig. 9C). In Germany, the Delta mutation was defined as the dominant variant on June 13, 2021, while the Omicron mutation accounted for > 50% of new COVID-19 cases on December 28, 2021 (Fig. 9D). We used the dominant date as the dominant variant selection time point for this algorithm and as the criterion for the learning and prediction date windows for each variant.

POS-NT frequency ratio prediction model

The prediction results for each learning and prediction date combination, i.e., 10- and 20-day training and prediction after 3, 5, 8, and 10 days, were confirmed. Figures 10 and 11 show the Delta and Omicron predictions for a model trained for 20 days and predicted three days after the learning period. The results for the learning and prediction for other combinations are shown in Figures S1–14. It was confirmed that as the number of forecast days decreased, the forecast trend improved (after 3 days > after 5 days > after 8 days > after 10 days).

POS-NT identification with the algorithm

Based on the developed frequency ratio prediction model for each POS-NT, eight combinations of DVC criteria were applied to identify DVC POS-NT until the dominant date of each variant and were compared with the actual variant-defining POS-NT. In addition, the number of days ago, on average, that the POS-NT was identified as a DVC POS-NT and the ratio of the identified POS-NT corresponding to the actual variant-defining POS-NT to the identified DVC POS-NT was confirmed (Eq. (1)). Table S1 provides the learning dates, prediction dates, number of POS-NTs recognized as DVC POS-NTs by condition and the average number of days for identifying Delta mutation for all combinations of learning and prediction dates and the eight DVC conditions. Table S2 shows Delta-like information for Omicron.

The optimal DVC criterion was specified when two conditions were satisfied: (1) identify all variant-defining POS-NTs in Delta and Omicron, and (2) have the highest ratio (Eq. (1)). In the case of Delta mutation, all Delta-defined POS-NTs were identified in 39 model-specific and DVC criteria combinations and showed the highest ratio values in prediction using Condition 2, 3 days after the 20-day learning period. In the case of the Omicron mutation, all Omicron-defined POS-NTs were identified in 11 model-specific and DVC criteria combinations and showed the highest ratio values in prediction using Condition 3, 3 days after the 20-

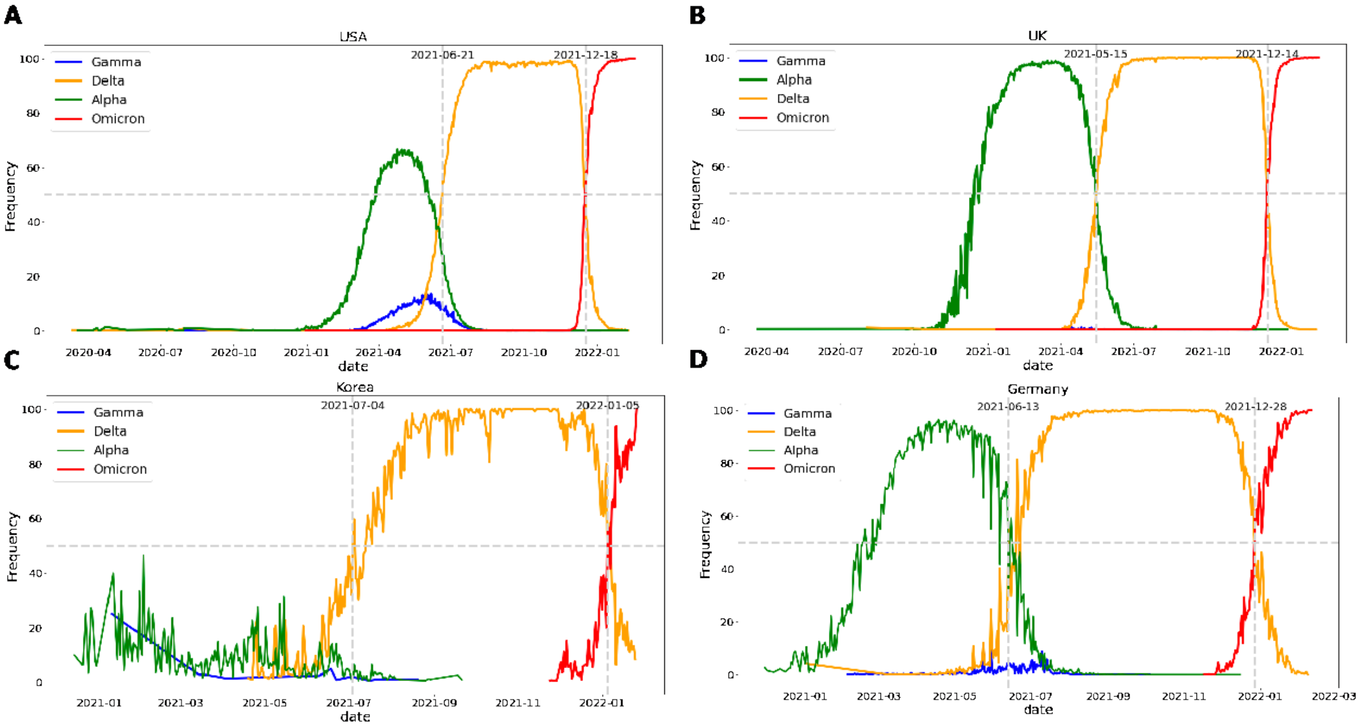


Figure 9: Definition of dominant dates for Delta and Omicron by country. (**A**) In the USA, Delta became the dominant variant on June 21, 2021, and Omicron on December 18, 2021; (**B**) in the UK, Delta became the dominant variant on May 15, 2021, and Omicron on December 14, 2021; (**C**) in Korea, Delta became the dominant variant on July 4, 2021, and Omicron on January 5, 2022; (**D**) in Germany, Delta was defined as the dominant variant on June 13, 2021, and Omicron accounted for more than 50% of all new COVID-19 cases on December 28, 2021.

day learning period. As a result, when using the frequency ratio prediction model that learns for 20 days and predicts 3 days later and the DVC selection algorithm using Condition 3 (3 days in a row, difference between the frequency ratio of the current and previous day is ≥ 0, predicted frequency is > 10%, and measured value exceeds the predicted value), all variant-defining POS-NTs are identified for Delta and Omicron with the highest ratio (Eq. (1), Table 5).

Through the optimal ratio prediction model (i.e., learning for 20 days and prediction 3 days later) and DVC selection algorithm (i.e., Condition 3), 69 DVC POS-NTs were identified for Delta mutation, an average of 47 days before the dominant date. Among them, 13 Delta variant-defining POS-NTs were recognized 18 days before the dominant date. Similarly, 102 DVC POS-NTs were identified for Omicron mutation an average of 82 days before the dominant date, of which 44 Omicron variant-defining POS-NTs were recognized 25 days before the dominant date.

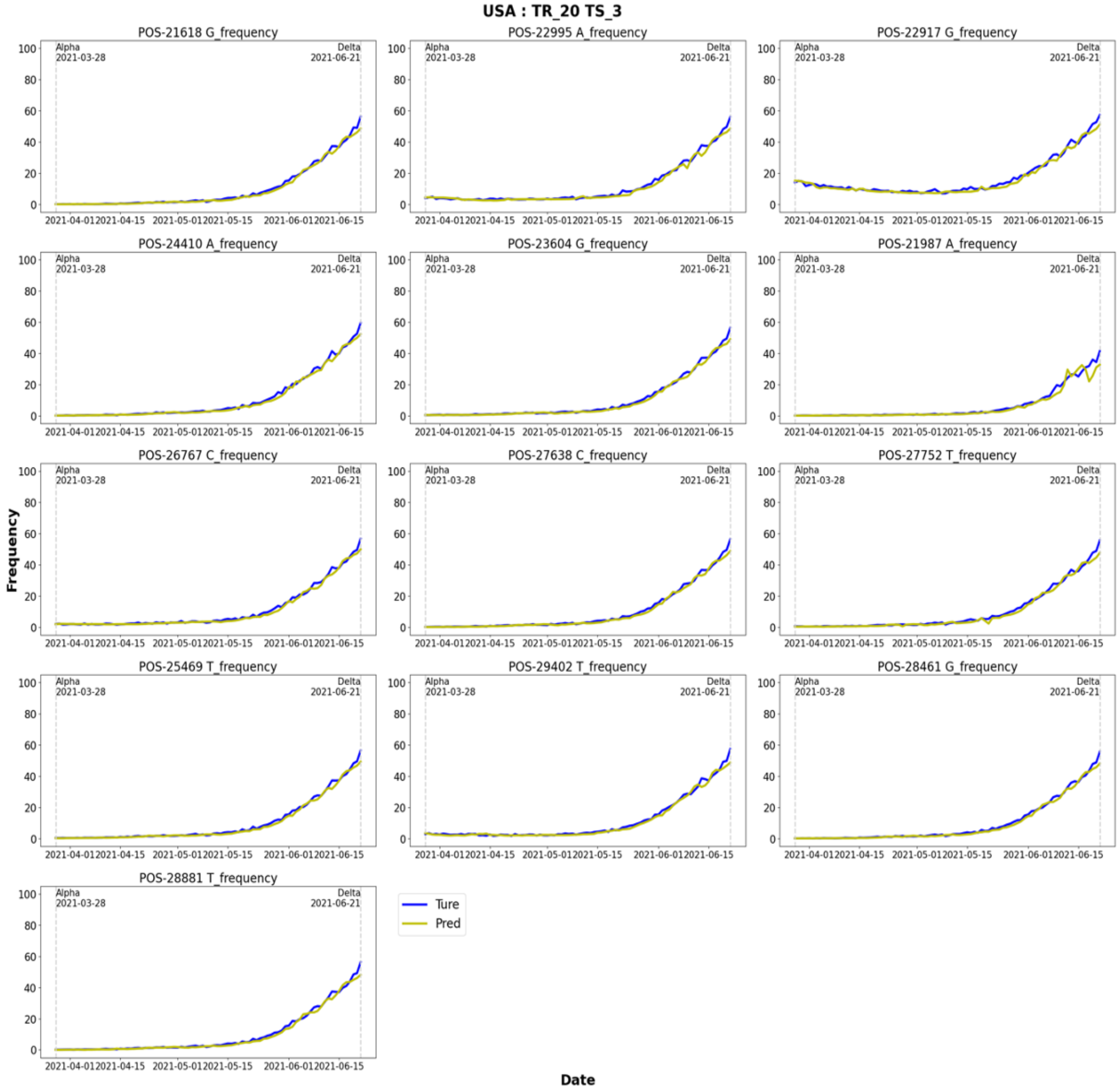
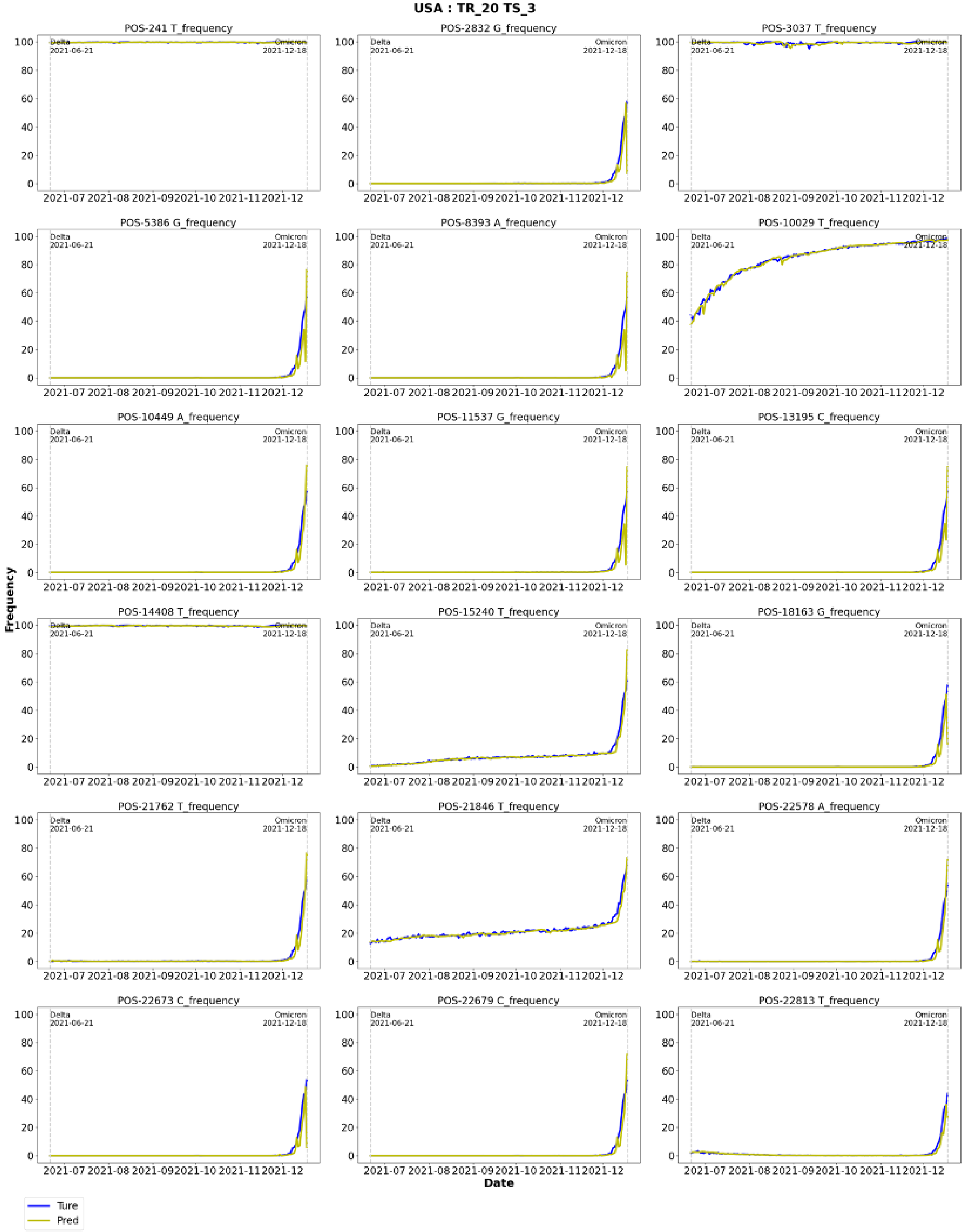
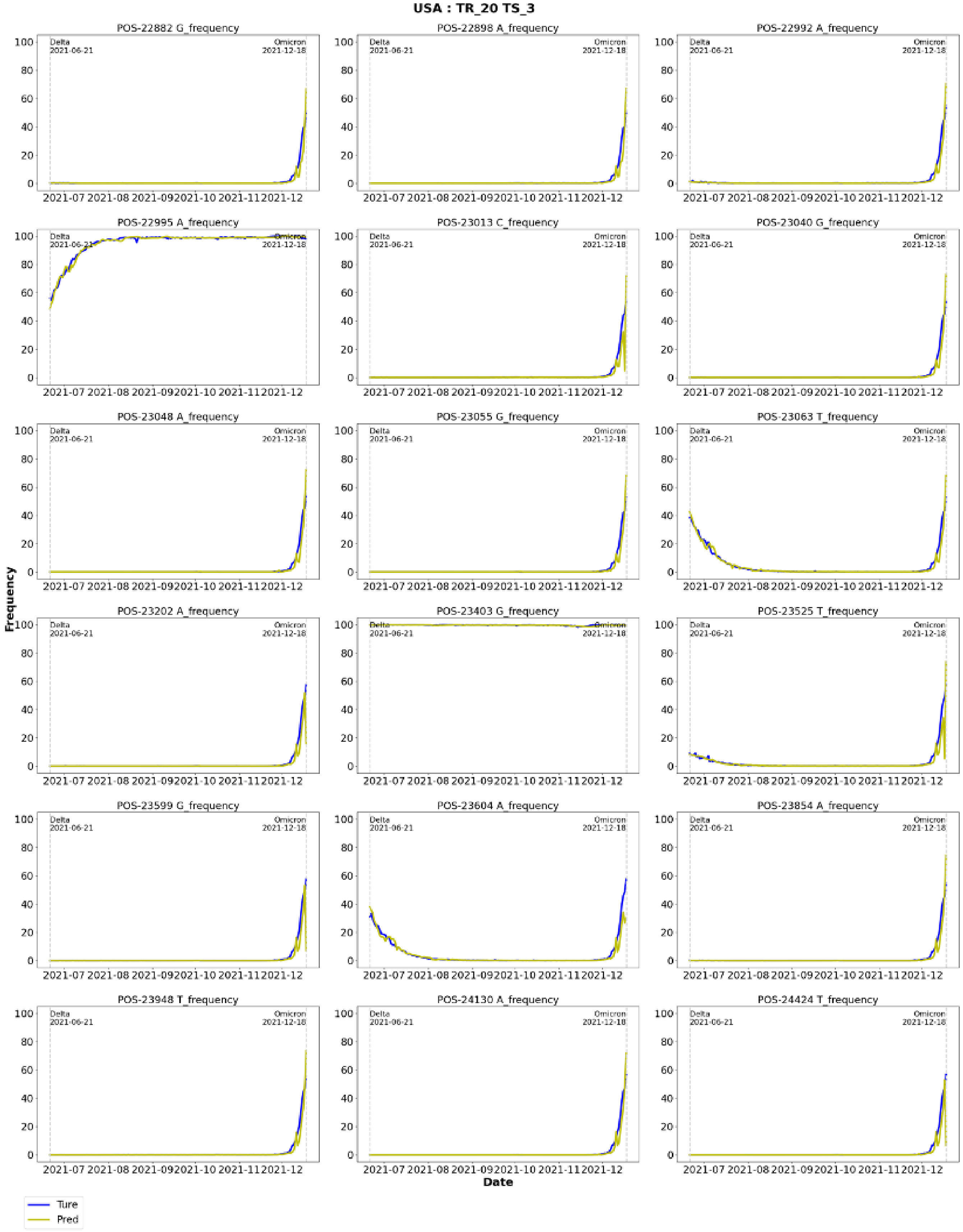


Figure 10: Delta: Results of learning for 20 days and predicting 3 days later. TR: learning dates (training dates), TS: test dates.


USA : TR_20 TS_3
POS-241 T_frequency
POS-2832 G_frequency
POS-3037 T_frequency
POS-5386 G_frequency
POS-8393 A_frequency
POS-10029 T_frequency
POS-10449 A_frequency
POS-11537 G_frequency
POS-13195 C_frequency
POS-14408 T_frequency
POS-15240 T_frequency
POS-18163 G_frequency
POS-21762 T_frequency
POS-21846 T_frequency
POS-22578 A_frequency
POS-22673 C_frequency
POS-22679 C_frequency
POS-22813 T_frequency
Delta
2021-06-21
Omicron
2021-12-18
0
20
40
60
80
100
2021-07
2021-08
2021-09
2021-10
2021-11
2021-12
Frequency
Date
Ture
Pred


USA : TR_20 TS_3
POS-22882 G_frequency
POS-22898 A_frequency
POS-22992 A_frequency
POS-22995 A_frequency
POS-23013 C_frequency
POS-23040 G_frequency
POS-23048 A_frequency
POS-23055 G_frequency
POS-23063 T_frequency
POS-23202 A_frequency
POS-23403 G_frequency
POS-23525 T_frequency
POS-23599 G_frequency
POS-23604 A_frequency
POS-23854 A_frequency
POS-23948 T_frequency
POS-24130 A_frequency
POS-24424 T_frequency
Delta 2021-06-21
Omicron 2021-12-18
0
20
40
60
80
100
2021-07
2021-08
2021-09
2021-10
2021-11
2021-12
Frequency
Date
Ture
Pred

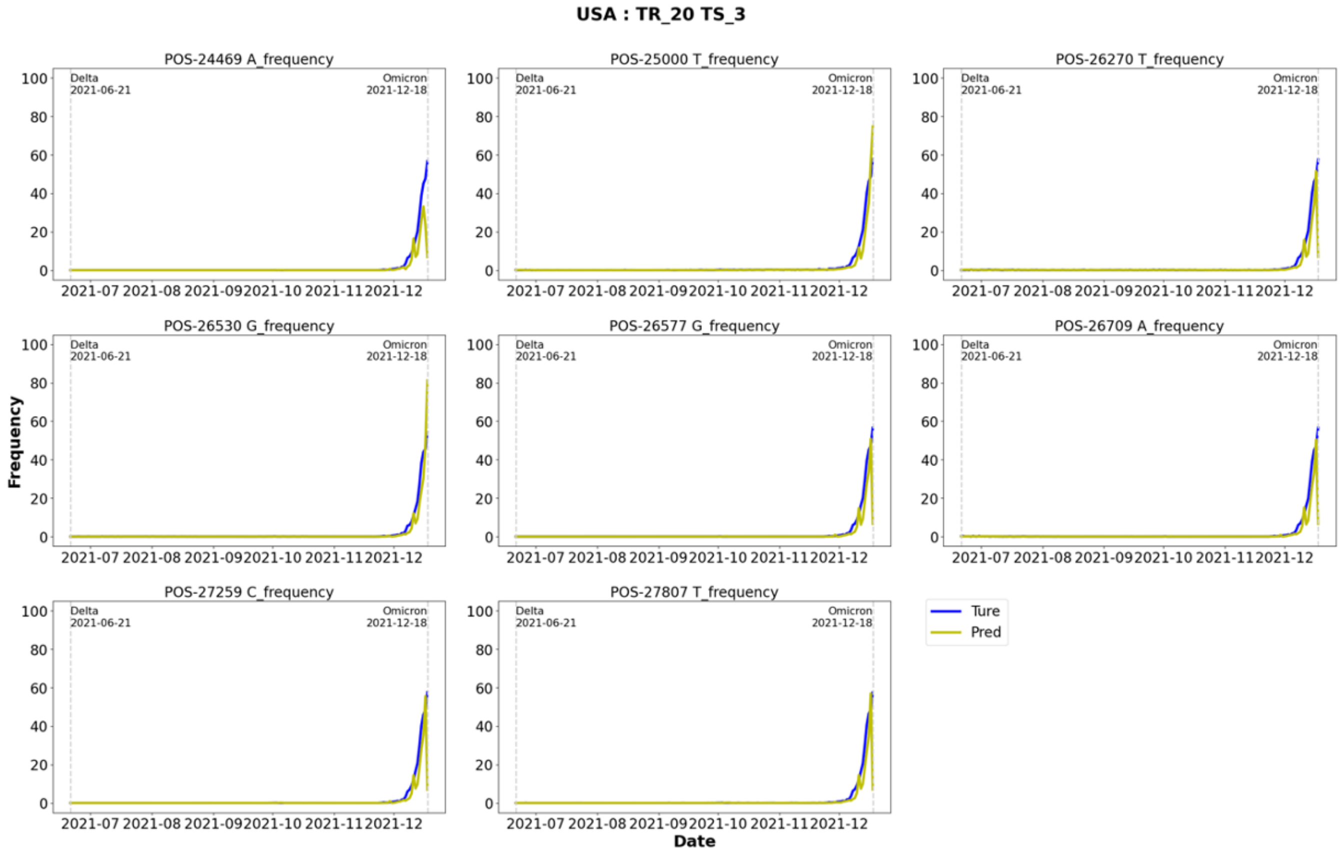


Figure 11: Omicron: Results of learning for 20 days and predicting 3 days later. TR: learning dates (training dates), TS: test dates.

Table 5: Combination results of POS-NT identification model and DVC criteria for all variant definitions.

	TR_TS date	Condition	Candidate Count	Candidate∩Actual	Ratio
1	TR_20_TS_3	Condition 2	45 (49 days ago)	13 (10 days ago)	0.288889
2	TR_20_TS_10	Condition 2	49 (44 days ago)	13 (7 days ago)	0.265306
3	TR_10_TS_8	Condition 6	56 (48 days ago)	13 (11 days ago)	0.232143
4	TR_20_TS_8	Condition 3	58 (33 days ago)	13 (16 days ago)	0.224138
5	TR_10_TS_10	Condition 6	60 (46 days ago)	13 (9 days ago)	0.216667
6	TR_10_TS_10	Condition 3	61 (43 days ago)	13 (14 days ago)	0.213115
7	TR_10_TS_3	Condition 6	61 (47 days ago)	13 (13 days ago)	0.213115
8	TR_20_TS_10	Condition 6	61 (47 days ago)	13 (8 days ago)	0.213115
9	TR_20_TS_3	Condition 6	61 (47 days ago)	13 (12 days ago)	0.213115
10	TR_20_TS_10	Condition 3	63 (44 days ago)	13 (15 days ago)	0.206349
11	TR_10_TS_8	Condition 3	63 (40 days ago)	13 (15 days ago)	0.206349
12	TR_10_TS_5	Condition 3	67 (47 days ago)	13 (18 days ago)	0.19403
13	TR_20_TS_5	Condition 3	67 (47 days ago)	13 (18 days ago)	0.19403
14	**TR_20_TS_3**	**Condition 3**	**69 (47 days ago)**	**13 (18 days ago)**	**0.188406**
15	TR_10_TS_3	Condition 3	69 (46 days ago)	13 (18 days ago)	0.188406
16	TR_10_TS_10	Condition 4	80 (43 days ago)	13 (19 days ago)	0.1625
17	TR_10_TS_8	Condition 4	83 (42 days ago)	13 (23 days ago)	0.156627
18	TR_10_TS_10	Condition 7	97 (47 days ago)	13 (16 days ago)	0.134021

19	TR_20_TS_10	Condition 7	97 (46 days ago)	13 (16 days ago)	0.134021
20	TR_10_TS_3	Condition 4	97 (49 days ago)	13 (26 days ago)	0.134021
21	TR_20_TS_8	Condition 4	97 (42 days ago)	13 (26 days ago)	0.134021
22	TR_20_TS_8	Condition 7	98 (47 days ago)	13 (17 days ago)	0.132653
23	TR_10_TS_5	Condition 7	99 (50 days ago)	13 (19 days ago)	0.131313
24	TR_10_TS_5	Condition 4	99 (51 days ago)	13 (26 days ago)	0.131313
25	TR_20_TS_10	Condition 4	100 (46 days ago)	13 (24 days ago)	0.13
26	TR_10_TS_8	Condition 7	101 (47 days ago)	13 (21 days ago)	0.128713
27	TR_20_TS_5	Condition 4	101 (53 days ago)	13 (27 days ago)	0.128713
28	TR_20_TS_5	Condition 7	102 (47 days ago)	13 (20 days ago)	0.127451
29	TR_20_TS_3	Condition 7	102 (51 days ago)	13 (20 days ago)	0.127451
30	TR_20_TS_3	Condition 4	102 (53 days ago)	13 (27 days ago)	0.127451
31	TR_10_TS_3	Condition 7	103 (51 days ago)	13 (20 days ago)	0.126214
32	TR_10_TS_10	Condition 8	122 (51 days ago)	13 (24 days ago)	0.106557
33	TR_10_TS_8	Condition 8	126 (54 days ago)	13 (28 days ago)	0.103175
34	TR_10_TS_5	Condition 8	128 (54 days ago)	13 (28 days ago)	0.101562
35	TR_20_TS_8	Condition 8	131 (55 days ago)	13 (27 days ago)	0.099237
36	TR_10_TS_3	Condition 8	131 (55 days ago)	13 (28 days ago)	0.099237
37	TR_20_TS_10	Condition 8	131 (55 days ago)	13 (26 days ago)	0.099237
38	TR_20_TS_5	Condition 8	132 (55 days ago)	13 (29 days ago)	0.098485
39	TR_20_TS_3	Condition 8	137 (57 days ago)	13 (28 days ago)	0.094891

Twenty-nine model-specific and DVC criterion combinations identified all Delta-defined POS-NTs and showed the highest ratio values in prediction and Condition 2 after three days of 20-day learning. TR: learning dates(training dates), TS: test dates. Bold marks indicate combinations that identified all variant-defining POS-NT. Bold marks indicate the combination of the final DVC selection algorithm proposed in this study.

Table 5-1: Combination results of model and DVC criteria that identify all Delta-defined POS-NTs.

	TR_TS date	Condition	Candidate Count	Candidate∩Actual	Ratio
1	**TR_20_TS_3**	**Condition 3**	**102 (82 days ago)**	**44 (25 days ago)**	**0.431373**
2	TR_10_TS_3	Condition 3	104 (81 days ago)	44 (25 days ago)	0.423077
3	TR_10_TS_3	Condition 7	110 (87 days ago)	44 (29 days ago)	0.4
4	TR_20_TS_3	Condition 4	111 (87 days ago)	44 (29 days ago)	0.396396
5	TR_20_TS_3	Condition 7	113 (89 days ago)	44 (30 days ago)	0.389381
6	TR_20_TS_5	Condition 4	113 (88 days ago)	44 (27 days ago)	0.389381
7	TR_10_TS_3	Condition 4	114 (86 days ago)	44 (29 days ago)	0.385965
8	TR_10_TS_3	Condition 8	122 (94 days ago)	44 (34 days ago)	0.360656
9	TR_20_TS_5	Condition 8	136 (107 days ago)	44 (37 days ago)	0.323529
10	TR_20_TS_3	Condition 8	137 (105 days ago)	44 (38 days ago)	0.321168
11	TR_10_TS_5	Condition 8	141 (109 days ago)	44 (35 days ago)	0.312057

All omicron-defined POS-NTs were identified in 11 model-specific and DVC criterion combinations and showed the highest ratio values in prediction and Condition 3 after 3 days of 20-day learning. TR: learning dates(training dates), TS: test dates. Bold marks indicate combinations that identified all variant-defining POS-NT. Bold marks indicate the combination of the final DVC selection algorithm proposed in this study.

Table 5-2: Combination results of model and DVC criteria that identify all Omicron-defined POS-NTs.

Citation: Eunhee Kang, TaeJin Ahn and Taesung Park. Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency. Archives of Microbiology and Immunology. 8 (2024): 101-117.

Discussion

Many previous studies have predicted the incidence of COVID-19 and the ratio of Delta and Omicron mutations. For example, Pathan and Biswas predicted the COVID-19 time series by analyzing the ratio of 12 base mutations using 3,068 samples and the LSTM model from NCBI GenBank in 2020 to predict the mutation rate for future patients who do not yet exist [8]. Singh et al. obtained COVID-19 case count data for 15 states in India through the Kaggle website and predicted the future spread of SARS-CoV-2 using the Kalman filter [9]. Marzouk et al. collected the COVID-19 data of Engypt from the Flevy open source in 2021 and predicted a COVID-19 outbreak (i.e., cumulative infection) after one week and one month, using LSTM, CNN, and MLP; the prediction results were in excellent agreement with the reported results [10]. Meanwhile, Obermeyer et al. proceeded with clustering using GISAID data on January 20, 2022, and the Pango lineage to infer prevalence for each lineage. Subsequently, they developed a hierarchical Bayesian regression model, PyR0, to detect and predict increases in B.1.1.7, AY.4, and BA.I in England [11]. De Hoffer et al. used 646.697 spike protein sequence data from the UK through GISAID in 2022 to perform clustering on a monthly or weekly basis based on amino acid substitution information and defined the appearance of a major cluster. They defined a new permanent variant as a chain containing clusters that share the same variant three or more consecutive times and designated an early warning for the emergence of a new permanent variant when 1% of the total sequence data was reached. As a result, an early warning was provided for the Alpha cluster as a new permanent variant six weeks before the WHO officially classified it as a VoC [12]. Although a few studies have predicted the occurrence of new mutations [Jankowiak, 12], they used protein-based data, and no studies have confirmed the trend by predicting the POS-NT ratio. Therefore, the current study can provide more detailed information regarding SARS-CoV-2 variants by predicting the trend and aspect of the mutation for each POS-NT.

This study has several limitations. First, the increasing POS-NT ratio was predicted using the DVC candidate selection algorithm, while the decreasing POS-NT ratio remained unanalyzed. Second, given that the dominant variant candidate identification algorithm was developed based on USA data, the algorithm may not apply to other countries in Asia. Hence, as different countries have demonstrated different rates of SARS-CoV-2 transmission and emergence of dominant variants, it is necessary to develop DVC selection algorithms for other countries, such as the UK, Germany, and Korea. Third, only replacement mutations were analyzed in this study, whereas other mutation types, such as insertions and deletions, were not considered.

Conclusions

We obtained SARS-CoV-2 POS-NT frequency ratio data for each country using a large amount of GISAID sequence data and defined the time point of the dominant variants for each mutation in each country. Subsequently, we developed a SARS-CoV-2 POS-NT frequency ratio prediction model and DVC selection algorithm using GPR for the USA and verified them for Delta and Omicron. Using this algorithm, we successfully identified all DVC POS-NTs before the dominant date, regardless of the soaring or gently increasing POS-NT patterns. As we were able to identify all mutation definitions of POS-NT for Delta and Omicron mutations, the algorithm can provide early warnings for other mutations in the future. If sufficient data exists, our model is expected to serve as an early warning algorithm for other viruses, thus improving global health.

Availability of the data and materials

The COVID-19 nucleotide sequence data used in this study can be obtained through GISAID (https://gisaid.org/) and compared with the original nucleotide sequence NC_045512. Correspondence and requests for materials should be addressed to TaeJin Ahn.

Funding

This research was supported by the research grants from Ministry of Science and ICT, South Korea (No.2021M3E5E3081425).

Conflict of interest

The authors have declared that no competing interests exist.

References

1. Rasmussen CE. Gaussian Processes in Machine Learning. In: Bousquet O, von Luxburg U, Rätsch G, editors. Summer School on Machine Learning. Springer (2004): 63–71.
2. Schulz E, Speekenbrink M, Krause A. A tutorial on Gaussian process regression: Modelling, exploring, and exploiting functions. Journal of Mathematical Psychology 85 (2018): 1–16.
3. Jarndal A, Husain S, Zaatar O, Al Gumaei T, Hamadeh A. In: 2020 International Conference on Communications, Computing, Cybersecurity, and Informatics (CCCI) (2020): 1–5.
4. Tolba H, Dkhili N, Nou J, Eynard J, Thil S, et al. GHI forecasting using Gaussian process regression: Kernel study. IFAC-PapersOnLine 52 (2019): 455–460.
5. Velásquez RMA, Lara J VM. Forecast and evaluation of COVID-19 spreading in USA with reduced-space

Gaussian process regression. Chaos, Solitons & Fractals 136 (2020): 109924.

6. Dhamodharavadhani S, Rathipriya R. COVID-19 mortality rate prediction for India using statistical neural networks and Gaussian process regression model. African Health Sciences 21 (2021): 194–206.

7. Lounis M, Khan FM. Predicting COVID-19 cases, deaths and recoveries using machine learning methods. Engineering and Applied Science Letters 4 (2021): 43–49.

8. Pathan RK, Biswas M, Khandaker MU. Time series prediction of COVID-19 by mutation rate analysis using recurrent neural network-based LSTM model. Chaos, Solitons & Fractals 138 (2020): 110018.

9. Singh KK, Kumar S, Dixit P, Bajpai, MK. Kalman filter based short term prediction model for COVID-19 spread. Applied Intelligence 51 (2021): 2714–2726.

10. Marzouk M, Elshaboury N, Abdel-Latif A, Azab S. Deep learning model for forecasting COVID-19 outbreak in Egypt. Process Safety and Environmental Protection 153 (2021): 363–375.

11. Obermeyer F, Jankowiak M, Barkas N, Schafner SF, Pyle JD, et al. Analysis of 6.4 million SARS-CoV-2 genomes identifies mutations associated with fitness. Science 376 (2022): 1327–1332.

12. de Hoffer A, Vatani S, Cot C, Cacciapaglia G, Chiusano ML, et al. Variant-driven early warning via unsupervised machine learning analysis of spike protein mutations for COVID-19. Scientific Reports 12 (2022): 9275.

Please Follow the link for Supplementory file:
https://www.fortunejournals.com/supply/Supply-AMI_10333.zip

Citation: Eunhee Kang, TaeJin Ahn and Taesung Park. Algorithm for Selecting Potential SARS-CoV-2 Dominant Variants based on POS-NT Frequency. Archives of Microbiology and Immunology. 8 (2024): 101-117.