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Project Final

Submitted to:

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Introduction

Ever since the corona outbreak, many activities were limited which affected a wide variety of businesses and economies. And just as the corona outbreak began to subside, the new variant going by the name omicron came to exist which is called variant since it's actually a mutated version of corona. Consequently, trying to know the origins of these viruses and how they are related became a top priority if the world is going to go back to how it was. For that, we did this simplified research by selecting a specific number of sequences for *SARS-Cov-2*. In addition, we chose 10 sequences for the SARS-Cov-2 Omicron variant in order to make a simple comparison between them and deduce the differences and mutations that took place for the original mutant.

The 10 sequences for *SARS-Cov-2* for *Italy*:

Original 1	hCoV-19/Italy/PIE-SLL-MS45/2020|EPI_ISL_569866|2020-02-25
Original 2	hCoV-19/Italy/PIE-SLL-MS46/2020|EPI_ISL_569867|2020-02-22
Original 3	hCoV-19/Italy/TUS-C44/2020|EPI_ISL_738147|2020-02-25
Original 4	hCoV-19/Italy/TUS-C198/2020|EPI_ISL_738194|2020-02-24
Original 5	hCoV-19/Italy/LOM-UniMI05/2020|EPI_ISL_779704|2020-02-24
Original 6	hCoV-19/Italy/LOM-UniMI07/2020|EPI_ISL_779709|2020-02-24
Original 7	hCoV-19/Italy/LOM-UniMI08/2020|EPI_ISL_779712|2020-02-24
Original 8	hCoV-19/Italy/LOM-UniMI09/2020|EPI_ISL_779713|2020-02-24
Original 9	hCoV-19/Italy/LOM-UniSR2/2020|EPI_ISL_1499504|2020-02-28
Original 10	hCoV-19/Italy/EMR-UA-02_00108/2020|EPI_ISL_5687737|2020-02-29

The 10 sequences for SARS-Cov-2 Omicron variant for *Italy*:

variant 1	hCoV-19/Italy/UMB-IZSGC-318786.1.36/2021|EPI_ISL_7952669|2021-12-14
variant 2	hCoV-19/Italy/UMB-IZSGC-318786.1.33/2021|EPI_ISL_7952666|2021-12-14
variant 3	hCoV-19/Italy/UMB-IZSGC-318786.1.37/2021|EPI_ISL_7952670|2021-12-14
variant 4	hCoV-19/Italy/UMB-IZSGC-318786.1.32/2021|EPI_ISL_7952665|2021-12-14
variant 5	hCoV-19/Italy/UMB-IZSGC-318786.1.35/2021|EPI_ISL_7952668|2021-12-14
variant 6	hCoV-19/Italy/UMB-IZSGC-318786.1.34/2021|EPI_ISL_7952667|2021-12-14
variant 7	hCoV-19/Italy/UMB-IZSGC-318786.1.68/2021|EPI_ISL_7952701|2021-12-14
variant 8	hCoV-19/Italy/UMB-IZSGC-318786.1.38/2021|EPI_ISL_7952671|2021-12-14
variant 9	hCoV-19/Italy/UMB-IZSGC-318786.1.69/2021|EPI_ISL_7952702|2021-12-14
variant 10	hCoV-19/Italy/CAL-AOCatanzaro-12171195_CZ/2021|EPI_ISL_7952114|2021-12-17

Information About Data Used

	Gender	Patient Age	Patient status
Original 1	Female	83	Deceased
Original 2	Male	41	Live
Original 3	Male	49	Deceased
Original 4	Male	63	Released
Original 5	Male	43	Hospitalized
Original 6	Female	72	Hospitalized
Original 7	Male	60	Hospitalized
Original 8	Female	61	Hospitalized
Original 9	Male	58	Not Hospitalized
Original 10	Male	66	Deceased

	Gender	Patient Age	Patient status
variant 1	Female	22	Live
variant 2	Male	40	Live
variant 3	Male	24	Live
variant 4	Male	34	Live
variant 5	Male	56	Live
variant 6	Female	23	Live
variant 7	Male	25	Live
variant 8	Female	57	Live
variant 9	Female	49	Live
variant 10	Male	37	Live

methodology and findings

We Construct a consensus sequence from the reference sequences.by using UGENE software we get at each sequence location, the nucleotide/amino acid of the consensus sequence will be the most dominant one across all the sequences at that location.

The 10 SARS-Cov-2 sequences:

original all.fasta

We have a lot of *Consensus types*:

- *Strict* — specifies that a set of species must appear in all input trees to be included in the strict consensus tree.
- *Majority Rule (extended)* — specifies that any set of species that appears in more than 50% of the trees is included. The program then considers the other sets of species in order of the frequency with which they have appeared, adding to the consensus tree any which are compatible with it until the tree is fully resolved. This is the default setting.
- *M1* — includes in the consensus tree any sets of species that occur among the input trees more than a specified fraction of the time (see the *Fraction* parameter below). The *Strict* consensus and the *Majority Rule* consensus are extreme cases of the MI consensus, being for fractions of 1 and 0.5 respectively.
- *Majority Rule* — specifies that a set of species is included in the consensus tree if it is present in more than half of the input trees.

In our example we choose the Strict type and we specify the threshold by 39% but before that we will do the multiple sequence alignment.

Now the file contains the consensus sequence.

Multiple alignment_consensus_3 (1).fa

The 10 sequences for the SARS-Cov-2 Omicron variant:

variant all.fasta

WE choose to Apply the align with MAFFT:

In bioinformatics, MAFFT is a program used to create multiple sequence alignments of amino acid or nucleotide sequences. MAFFT use an algorithm based on progressive alignment, in which the sequences were clustered with the help of the Fast Fourier Transform.

A progressive alignment method is described that utilizes the Needleman and Wunsch pairwise alignment algorithm iteratively to achieve the multiple alignment.

It has parameters in which you should choose to begin the alignment

1. Gap opening penalty we used 3
2. Offset (works like gap extension penalty) we used 1
3. Maximum number of iterative refinements we used the default which is 2

file after apply MAFFT:

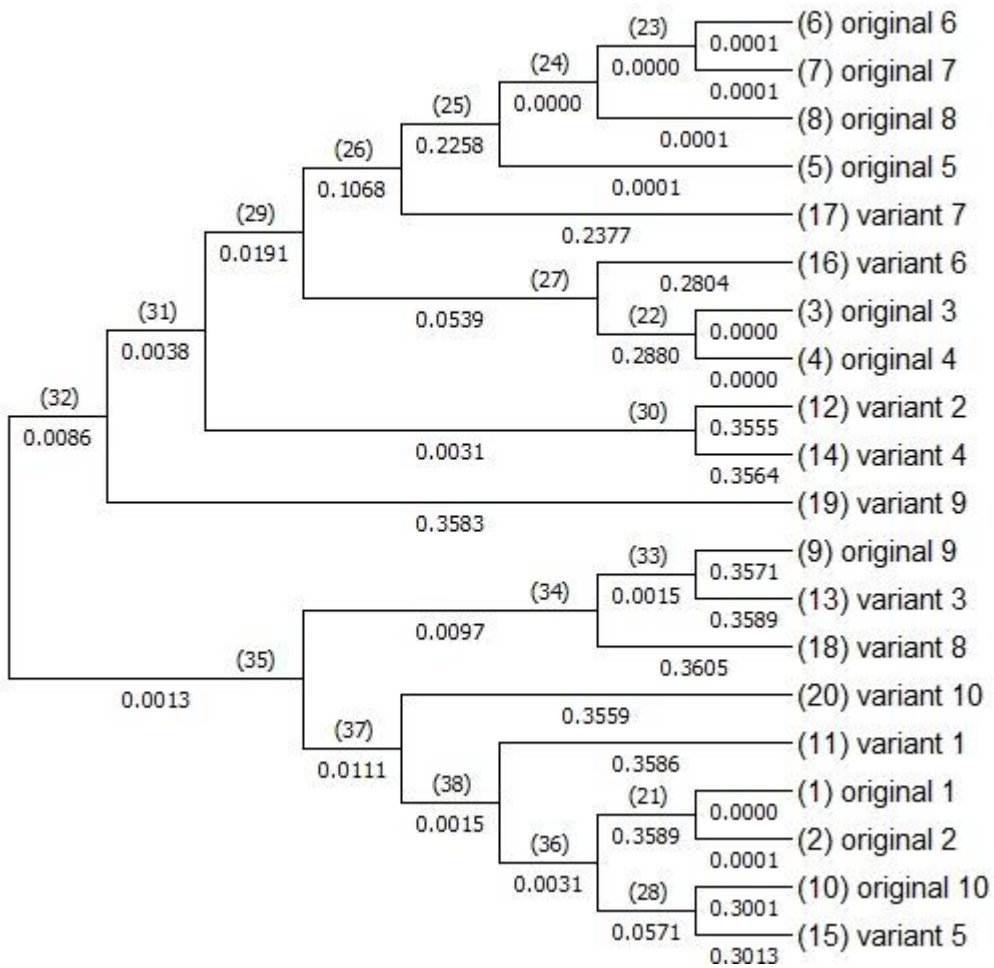
Multiple_alignment.fa

We Construct a phylogenetic tree between all the above 20 sequences:

We constructed the phylogenetic tree using the statistical method neighbor-joining.

The model used is the p-distance.

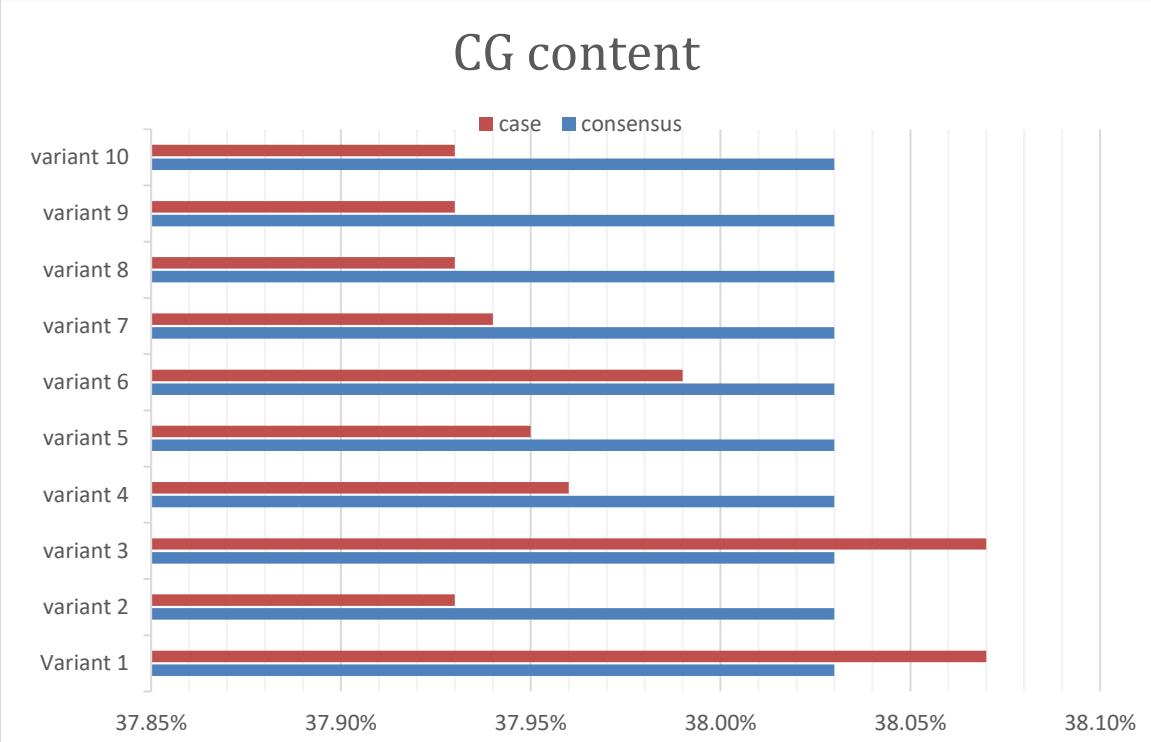
This distance is the proportion (p) of nucleotide sites at which two sequences being compared are different. It is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared



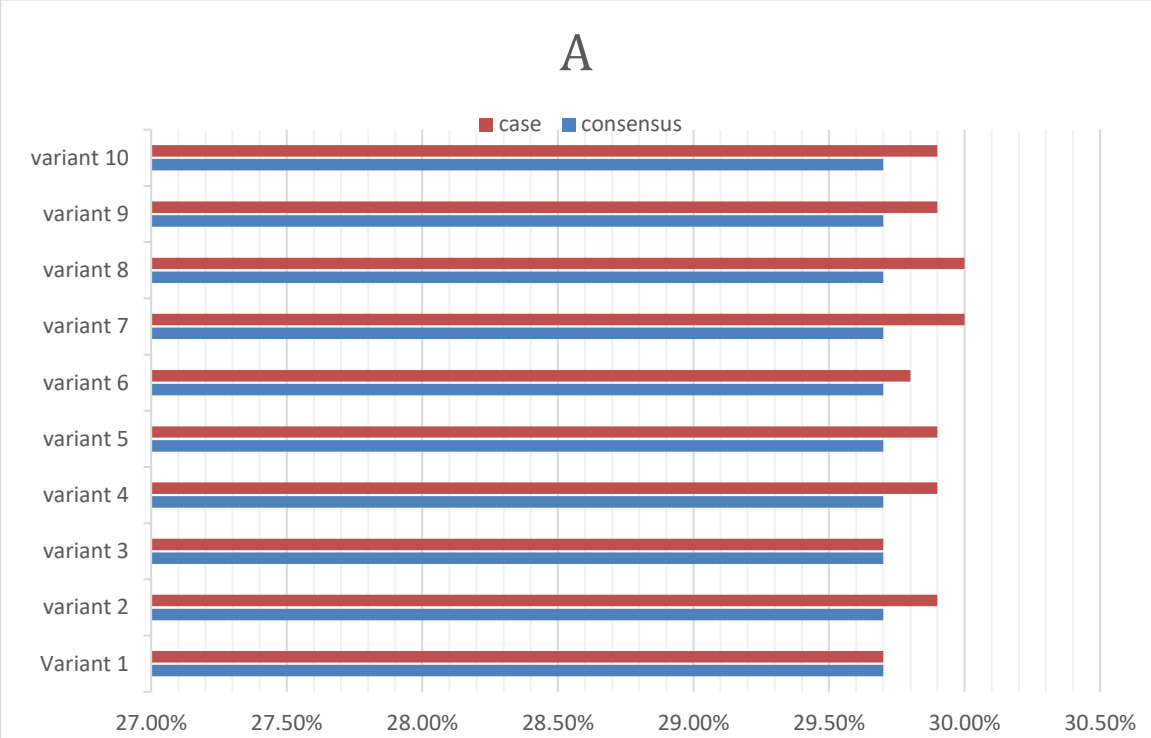
This table shows the correlation between each node and the other and the length of each branch:

AncId	Desc1	Desc2	Branch Length 1	Branch Length 2
21	original 1	original 2	4.18E-05	5.88E-05
22	original 3	original 4	3.27E-05	3.47E-05
23	original 6	original 7	7.03E-05	6.40E-05
24	23	original 8	7.42E-06	9.33E-05
25	24	original 5	1.58E-05	0.000119
26	25	variant 7	0.22584	0.237745
27	variant 6	22	0.280379	0.287968
28	original 10	variant 5	0.300108	0.301322
29	26	27	0.106773	0.053892
30	variant 2	variant 4	0.355503	0.356402
31	29	30	0.019106	0.00313
32	31	variant 9	0.003826	0.358349
33	original 9	variant 3	0.357081	0.358899
34	33	variant 8	0.001494	0.360514
35	34	37	0.00971	0.011143
36	21	28	0.35889	0.057082
37	variant 10	38	0.355892	0.001529
38	variant 1	36	0.358615	0.003127
39	32	35	0.008608	0.001311

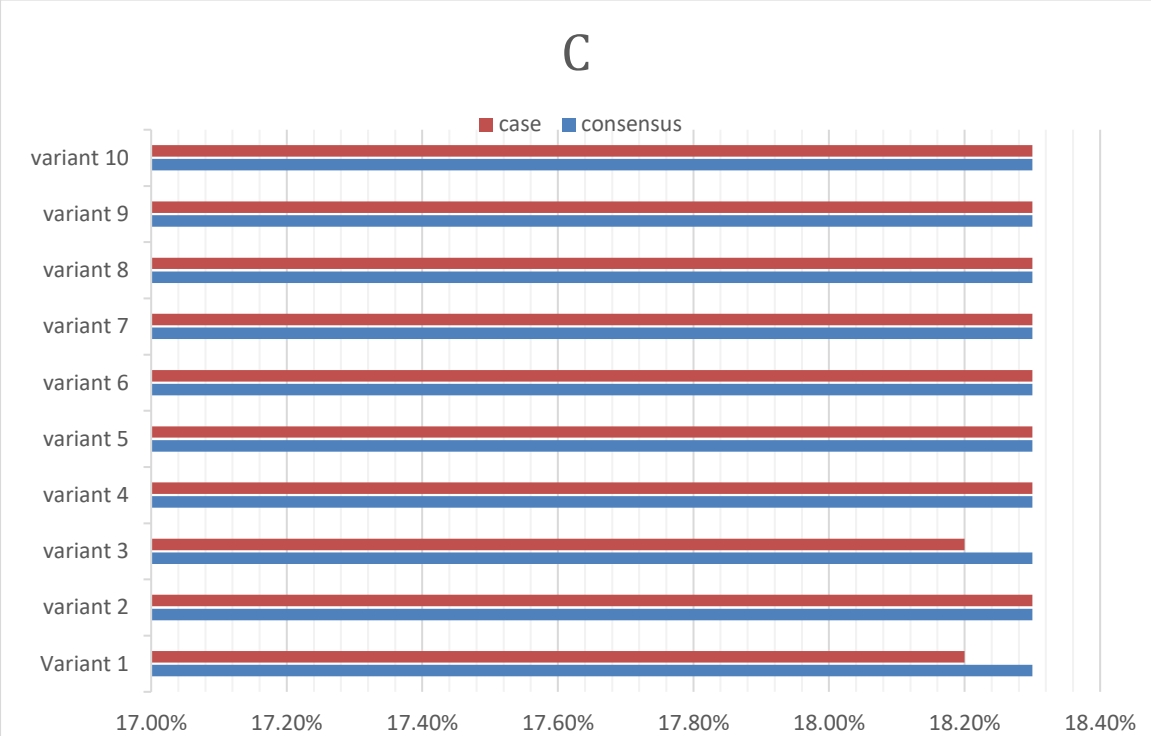
The average percentage of the chemical constituents (C, G, T, and A) and the CG content, between the reference sequences and the case sequences.



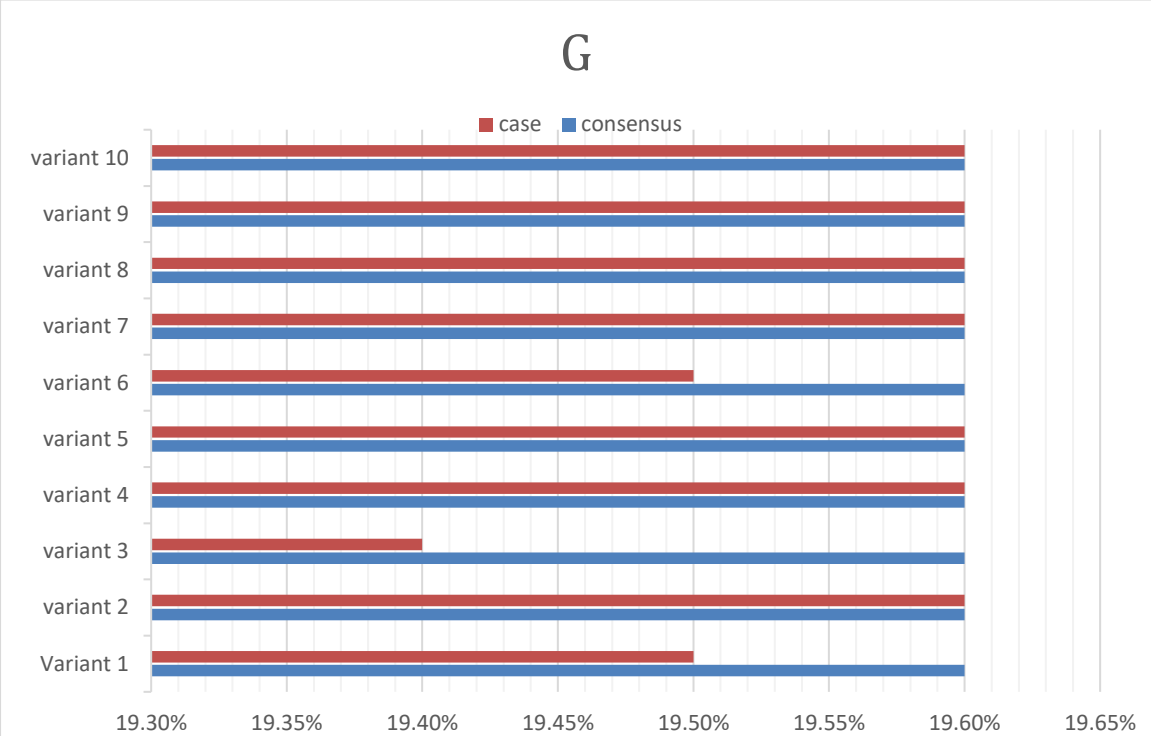
	Variant 1	variant 2	variant 3	variant 4	variant 5	variant 6	variant 7	variant 8	variant 9	variant 10
case	38.07%	37.93%	38.07%	37.96%	37.95%	37.99%	37.94%	37.93%	37.93%	37.93%
consensus	38.03%	38.03%	38.03%	38.03%	38.03%	38.03%	38.03%	38.03%	38.03%	38.03%



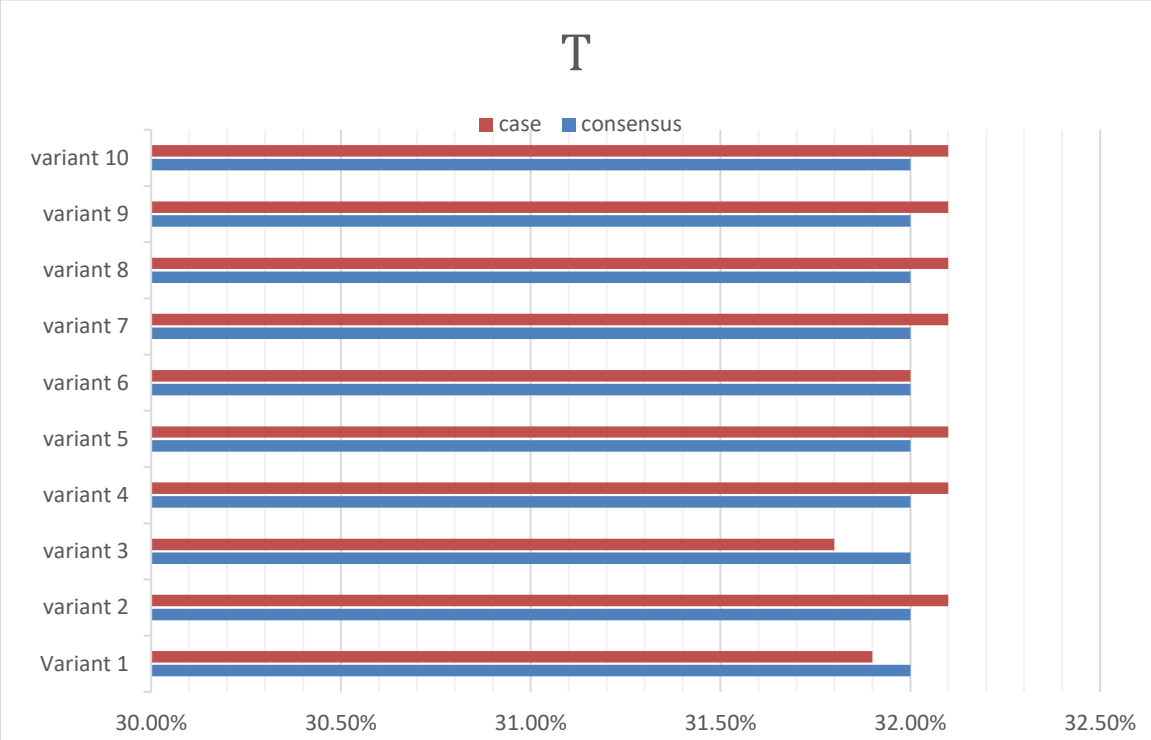
	Variant 1	variant 2	variant 3	variant 4	variant 5	variant 6	variant 7	variant 8	variant 9	variant 10
case	29.70%	29.90%	29.70%	29.90%	29.90%	29.80%	30.00%	30.00%	29.90%	29.90%
consensus	29.70%	29.70%	29.70%	29.70%	29.70%	29.70%	29.70%	29.70%	29.70%	29.70%



	Variant 1	variant 2	variant 3	variant 4	variant 5	variant 6	variant 7	variant 8	variant 9	variant 10
case	18.20%	18.30%	18.20%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%
consensus	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%	18.30%



	Variant 1	variant 2	variant 3	variant 4	variant 5	variant 6	variant 7	variant 8	variant 9	variant 10
case	19.50%	19.60%	19.40%	19.60%	19.60%	19.50%	19.60%	19.60%	19.60%	19.60%
consensus	19.60%	19.60%	19.60%	19.60%	19.60%	19.60%	19.60%	19.60%	19.60%	19.60%

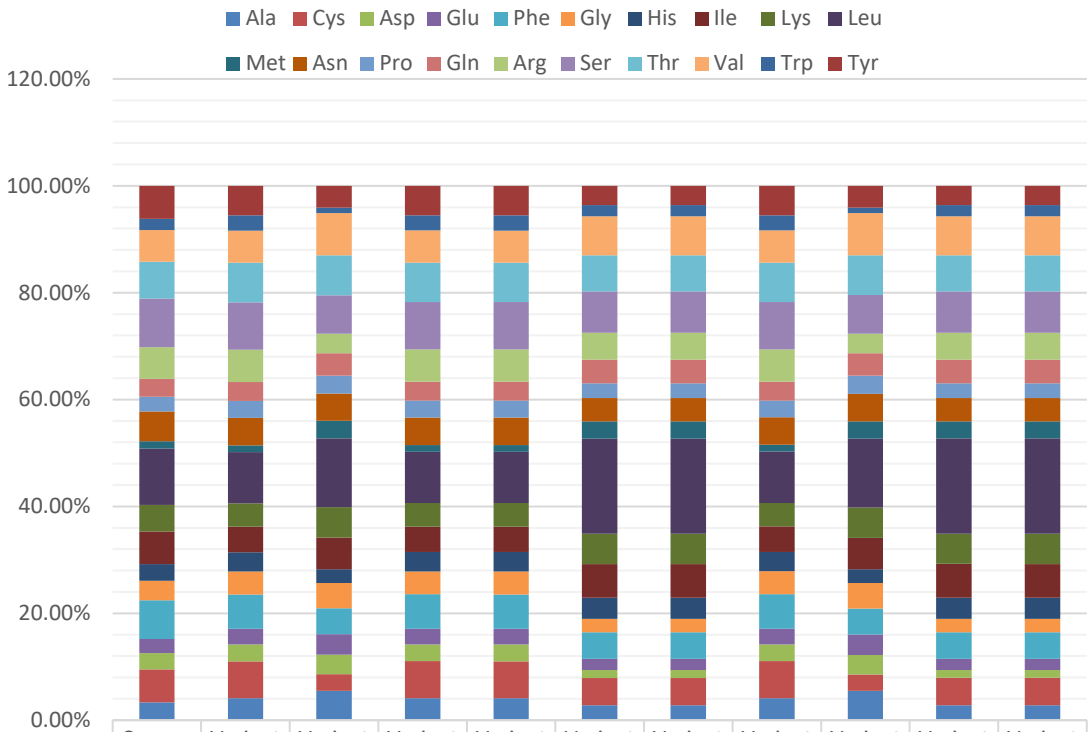


	Variant 1	variant 2	variant 3	variant 4	variant 5	variant 6	variant 7	variant 8	variant 9	variant 10
case	31.90%	32.10%	31.80%	32.10%	32.10%	32.00%	32.10%	32.10%	32.10%	32.10%
consensus	32.00%	32.00%	32.00%	32.00%	32.00%	32.00%	32.00%	32.00%	32.00%	32.00%

In [12]:
```
fasta_sequences = SeqIO.parse(open('variant_1.fasta'),'fasta')
for fasta in fasta_sequences:
    name, sequence = fasta.id, str(fasta.seq)

print(len(sequence))
#setting up variables
A = 0
C = 0
G = 0
T = 0
for i in sequence:
    if i=='A':
        A+=1
    elif i=='C':
        C+=1
    elif i=='G':
        G+=1
    else:
        T+=1
print(A)
print(C)
print(G)
print(T)
print(A+C+G+T)
```

```
29783
8845
5426
5811
9701
29783
```



	Consensus	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6	Variant 7	Variant 8	Variant 9	Variant 10
Tyr	6.19%	5.54%	4.05%	5.53%	5.54%	3.59%	3.59%	5.53%	4.08%	3.60%	3.60%
Trp	2.09%	2.84%	1.02%	2.83%	2.83%	2.09%	2.09%	2.83%	1.02%	2.09%	2.11%
Val	5.94%	6.01%	7.95%	6.01%	6.01%	7.32%	7.32%	6.01%	7.92%	7.32%	7.27%
Thr	6.89%	7.40%	7.44%	7.39%	7.38%	6.77%	6.77%	7.37%	7.41%	6.76%	6.77%
Ser	9.05%	8.86%	7.23%	8.85%	8.83%	7.72%	7.72%	8.86%	7.24%	7.71%	7.72%
Arg	5.97%	6.07%	3.65%	6.06%	6.06%	5.04%	5.04%	6.05%	3.67%	5.04%	5.06%
Gln	3.34%	3.54%	4.18%	3.55%	3.56%	4.42%	4.42%	3.55%	4.18%	4.41%	4.43%
Pro	2.77%	3.15%	3.38%	3.15%	3.15%	2.77%	2.77%	3.14%	3.41%	2.77%	2.75%
Asn	5.53%	5.13%	5.09%	5.13%	5.13%	4.36%	4.36%	5.12%	5.17%	4.35%	4.34%
Met	1.39%	1.27%	3.25%	1.26%	1.26%	3.21%	3.21%	1.27%	3.22%	3.21%	3.20%
Leu	10.53%	9.61%	12.90%	9.62%	9.63%	17.83%	17.83%	9.62%	12.84%	17.83%	17.84%
Lys	4.99%	4.38%	5.70%	4.40%	4.40%	5.66%	5.66%	4.40%	5.72%	5.66%	5.69%
Ile	6.10%	4.75%	5.89%	4.75%	4.76%	6.29%	6.29%	4.77%	5.88%	6.29%	6.27%
His	3.13%	3.62%	2.57%	3.63%	3.63%	3.95%	3.95%	3.63%	2.54%	3.96%	3.97%
Gly	3.64%	4.29%	4.75%	4.29%	4.29%	2.53%	2.53%	4.29%	4.79%	2.53%	2.53%
Phe	7.22%	6.39%	4.88%	6.42%	6.41%	4.98%	4.98%	6.43%	4.88%	4.98%	4.96%
Glu	2.65%	2.95%	3.79%	2.95%	2.95%	2.09%	2.09%	2.95%	3.79%	2.09%	2.08%
Asp	3.02%	3.17%	3.70%	3.16%	3.16%	1.46%	1.46%	3.16%	3.66%	1.46%	1.43%

now we Extract the dissimilar regions/columns between the alignment of the case sequences and the consensus sequence:

disSimilar table.csv

and we Extract the similar regions/columns between the alignment of the case sequences and the consensus sequence:

Similar table.csv

the numbers of similar for each Nucleotide:

Nucleotide	Number of similar
A	2693
C	987
G	1044
T	3004

the numbers of dissimilar for each Nucleotide:

Nucleotide	Number of dissimilar
A	6228
C	4504
G	4816
T	6608

Most converted to nucleotides in dissimilar case:

Nucleotide	Number of Change
A	6170
C	4449
G	4772
T	6525

Table showing the amount of similarity between each variant and case sequence:

Similar table

Variants / Case sequence	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6	Variant 7	Variant 8	Variant 9	Variant 10
# Of similar	7771	7749	7699	7769	7759	7735	7761	7742	7762	7762
Percentage%	26%	26%	26%	26%	26%	26%	26%	26%	26%	26%

Table showing the amount of dissimilarity between each variant and case sequence:

Dissimilar table

Variants / Case sequence	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6	Variant 7	Variant 8	Variant 9	Variant 10
#Of dissimilar	22121	22143	22193	22123	22133	22157	22131	22150	22130	22130
Percentage%	74%	74%	74%	74%	74%	74%	74%	74%	74%	74%

In [6]:
```
fasta_sequences = SeqIO.parse(open('last.fasta'),'fasta')
sequences = []
for fasta in fasta_sequences:
    sequences.append(str(fasta.seq))

#seprating the sequences for easr of access
seq_1 = sequences[0]
seq_2 = sequences[1]
seq_3 = sequences[2]
seq_4 = sequences[3]
seq_5 = sequences[4]
seq_6 = sequences[5]
seq_7 = sequences[6]
seq_8 = sequences[7]
seq_9 = sequences[8]
seq_10 = sequences[9]
#reference sequence
seq_11 = sequences[10]
# #setting up variables
similarities = 0
dissimilarities = 0

#compareing elements in same index for all the sequences
for i in range(len(seq_1)):
    if seq_1[i]==seq_2[i]==seq_3[i]==seq_4[i]==seq_5[i]==seq_6[i]==seq_7[i]==seq_8[i]==seq_9[i]==
        similarities+=1
    else:
        dissimilarities+=1

print("The number of similarities is: {}".format(similarities))
print("The number of dissimilarities is: {}".format(dissimilarities))
```

```
The number of similarities is: 7623
The number of dissimilarities is: 22249
```

Conclusion:

- In conclusion, we observed that CG contented, which represents the stability of a sequences, was pretty low in the corona sequences which led to the Inevitable mutations resulting in the omicron variant.

- As the excel sheet shows, the differences that occur between SARS-Cov-2 Omicron variant and the Consensus sequence are large, but we note that the percentage of amino acid change was not that large, and this shows that most of the differences that occur are nothing but silent mutation

  Ex:

  GAA ⟶ GLU

  GAG ⟶ GLU

  Both ***GAG*** and ***GAG*** refers to the same Amino acid (***GLU***)

- With the same idea, we note that there are slight changes in some amino acid present in SARS-Cov-2 Omicron variant compared to the Consensus sequence, an example of this (***Tyr***)
  We note that the ***Tyr*** has decreased in the variant 1 compared to the Consensus sequence, it has decreased by 0.65 % and this Missense *mutation*.

- It can be seen that the omicron variant is quite aggressive, so one of the best ways if not the best is to take the corona vaccine seeing that omicron originated from corona. It might not be the optimal solution, but it is sure to help reduce the adverse effects that omicron bears.