Characterizing SARS-CoV-2 Spike Sequences

Based on Geographical Location

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Abstract: With the rapid spread of COVID-19 worldwide, viral genomic data is available in the order of millions of sequences on public databases such as GISAID. This Big Data creates a unique opportunity for analysis towards the research of effective vaccine development for current pandemics, and avoiding or mitigating future pandemics. One piece of information that comes with every such viral sequence is the geographical location where it was collected — the patterns found between viral variants and geographical location surely being an important part of this analysis. One major challenge that researchers face is processing such huge, highly dimensional data to obtain useful insights as quickly as possible. Most of the existing methods face scalability issues when dealing with the magnitude of such data. In this paper, we propose an approach that first computes a numerical representation of the spike protein
sequence of SARS-CoV-2 using \( k \)-mers (substrings) and then uses several machine learning models to classify the sequences based on geographical location. We show that our proposed model significantly outperforms the baselines. We also show the importance of different amino acids in the spike sequences by computing the information gain corresponding to the true class labels.

1 Introduction

The adaptability of viruses like SARS-CoV-2, when coupled with a variety of selection pressures from the various ecosystems, host immunities and approaches to pharmaceutical intervention provide an evolutionary environment that leads to the emergence of strains and variants in different geographical locations. While SARS-CoV-2 has spread rather quickly to many parts of the globe since the initial outbreak in Wuhan at the end of 2019, which led to the COVID-19 pandemic [Wu et al. 2020], it continues to raise global concerns as the virus persistently evolves and accumulates new mutations. Consequently, new variants of SARS-CoV-2 have emerged in different parts of the world: the Alpha variant (B.1.1.17) emerged in the UK, Beta (B.1.351) in South Africa, Gamma in Brazil, Epsilon in California, Iota (B.1.526) in New York, Delta (B.1.167.2) and Kappa (B.1.167.1) in India, to name a few. All of these variants possess some mutations that confer increased transmissibility or higher binding affinity of their spike protein (see Figure 1) to human host ACE2 receptors [Farinholt et al. 2021, Huang et al. 2020].

It is concerning that the longer SARS-CoV-2 has to propagate, its exposure to wider ranges of immune response attacks across diverse communities and geographically diverse environments may be incubating the virus to evolve new variants and strains that are dangerous and extremely immunologically evasive.
Figure 1: The SARS-CoV-2 genome codes for several proteins, including the surface, or spike protein. The spike protein is composed of 3821 (25384 – 21563) nucleotides (and one “stop” character ‘*’). Therefore, the final length of the spike protein is $3822/3 = 1274$ (we divide by 3 because each amino acid corresponds to 3 DNA characters, or codons) [Huang et al. 2020].

Both locally and globally, as the pandemic prolongs. From the point of view of evolution, this is like giving the virus robust evolutionary room and time to learn, to evolve adaptations, gain of function, and escapes from host immune arsenal and attacks. Sadly, this is gradually the case already, as the original Wuhan strain is now almost completely replaced by new variants with different characteristic behaviors and are hence less responsive to the currently available vaccines [Korber et al. 2020, Hu et al. 2021]. This is why it is important to characterize different strains and variants of SARS-CoV-2 based on geographical location, to understand the patterns of spread in hopes to contain, or at least cope with this virus.

All viruses mutate with time — RNA viruses particularly do so at a faster rate. The SARS-CoV-2 is an RNA virus, it however exhibits a moderately lower rate compared to other RNA viruses like HIV and influenza due to the possession of a genetic proof-reading mechanism for correcting errors. The SARS-CoV-2 genome typically accrues 1 or 2 point mutations (SNVs) in a month. According to a review, some 12,706 such mutations have so far been detected by researchers since the advent of the COVID-19 pandemic. While some changes have neu-
tual effects, a few that occur in major proteins — be it, addition, substitution or deletion — are critical to viral evolution, genomic stability, transmissibility, antigenicity, virulence, adaptation and escape from host immune response [2020, 2020, 2020]. The SARS-CoV-2 Spike (S) Protein is a key player in the virus life cycle. The protein is composed of 1274 amino acids encoded by the S gene of the virus (see Figure 1). It is the major target of the neutralizing antibodies from host immune response and currently available vaccines for COVID-19. The virus uses the spike protein to bind the host ACE2 receptor on the cell surface (found abundantly in airways, lungs, mucous lines and the intestine) which facilitates the uptake of the virus into host cells [2020, 2021]. Thus, mutations in the S gene have reportedly imparted viral pathogenesis, binding activity of the spike protein to the host, as well as causing conformational changes in the protein molecule. For instance, mutation D614G — that is, a substitution of Glycine (G) for Aspartate (D) at position 614 — was found to enhance the viral infectivity and stability of the SARS-CoV-2 genome, which has been attributed to spike protein assembly on the virion surface [2020].

Currently, quite a number of novel variants are being identified by the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) SARS-CoV-2 Variant Classifications and Definitions. The variants are divided into categories such as Variants of Concern (VOCs), Variants Being Monitored (VBM), Variants of interest (VOI), and Variants of High Consequence (VOHC). At the time of this study, the VOC was the Delta variant (B.1.617.2 and AY.1 sublineages) SARS-CoV-2 Variant Classifications and Definitions. Since all of these variants are characterized by different spike protein content [2021, 2020], classification can help us to discover also patterns in the geographic distribution of these variants. At the
time of this study, the VBMs comprised Alpha (B.1.1.7, Q.1-Q.8 pango lineage), Beta (B.1.351, B.1.351.2, B.1.351.3 pango lineage), Gamma (P.1, P.1.1, P.1.2 pango lineage), Epsilon (B.1.427 B.1.429 pango lineage), Eta (B.1.525 pango lineage), Iota (B.1.526 pango lineage), Kappa (B.1.617.1 pango lineage), Zeta (P.2 pango lineage), and MU (B.1.621, B.1.621.1 pango lineage) SARS-CoV-2 Variant Classifications and Definitions. There are no VOIs or VOHCs at the time.

The SARS-CoV-2 still circulates among human populations in different locations, weather conditions and epidemiological descriptions. It is important to investigate how this regional diversity contributes to viral evolution and emergence of new variants in these regions. Research suggests possible selective mutations in the SARS-CoV-2 genome — specific sites which appear more subject to selective mutation. Some mutational sites in ORF1ab, ORF3a, ORF8 and N regions of SARS-CoV-2 reportedly exhibit different rates of mutation [Wang et al. 2020]. A study involving the analysis and characterization of samples from COVID-19 patients in different parts of the world identify 8 novel recurrent mutational sites in the SARS-CoV-2 genome. Interestingly, the studies also note changes at sites 2891, 3036, 14408, 23403, and 28881 to be common in Europe, while 17746, 17857, and 18060 are common in North America [Pachetti et al. 2020]. A recent study also identified the ongoing evolution of SARS-CoV-2 to involve purifying selection, and that a small number of sites appear to be positively selected. The work also identifies the spike protein receptor binding domain (RBD) and a region of nucleocapsid protein to be also positively selected for substitutions. The work also highlighted trend in virus diversity with geographic region and adaptive diversification that may potentially make variant-specific vaccination an issue [Rochman et al. 2021].

Given all of the novel SARS-CoV-2 variants and strains that have emerged
from different geographical regions of the world, we need to investigate this connection to the spread of the virus, e.g., weather factors possibly play a systematic role [Segovia-Dominguez et al. 2021, Pezzotti et al. 2016]. There is also diversity of immune system across the human population. Genomic variations only cause 20–40% of this immune system variation, while the rest 60–80% is accounted for by age, environmental factors, such as where we live and our neighbors, cohabitation and chronic viral infections, etc. Immune response is also known to show intra-species variation [Liston et al. 2016]. There is an ongoing evolutionary arms-race between host and pathogens they are exposed to which constantly changes the host anti-pathogen attack and in turn causes the pathogen to refine or adjust its escape from host immune attack [Liston et al. 2016, Brodin et al. 2015]. This is constantly taking place, with the virus under evolutionary pressure and natural selection to propagate the virus with the highest fitness. It may be complex to characterize how each factor contributes to this variation. The immune system variation is possibly an important driver on how new variants of SARS-CoV-2 are regionally emerging with positive selections for escaping immune neutralization, increased infectivity and transmissibility, as observed recently.

Classification of the SARS-CoV-2 Spike protein sequences based on geographical location of emergence is therefore an important and informative exploration for possible unique patterns, trends and distribution. The SARS-CoV-2 spike protein must interact chemically with the host receptor molecule, ACE2 for cellular uptake. Since millions of spike sequences are available now on public databases such as GISAID, classifying those sequences becomes a Big Data problem. When dealing with big data, scalability and robustness are two important challenges. Some algorithms are robust while other scale well, but give poor predictive performance on larger datasets. The author of
Son [2021] proposed a scalable approach, called Spike2Vec, which is scalable to larger sized datasets. When there is some structure (natural clustering) in the data, Spike2Vec is proven to be useful as compared to one-hot embedding Ali and Patterson [2021]. However, we show in this paper that Spike2Vec does not always work in all types of scenarios. To further improve the results of Spike2Vec and that of one-hot embedding, we use a neural network (NN) model.

In this paper, we propose to use a simple sequential convolutional neural network along with a $k$-mers based feature vector representation for classifying the geographical locations of COVID-19 patients using spike protein sequences only. Our contributions in this paper are the following:

1. We show that the neural network model is scalable on a high volume of data and significantly outperforms the baseline algorithms.

2. We show the importance of different amino acids within the spike sequence by computing information gain corresponding to the class label.

3. We show that given the complexity of the data, our model is still able to outperform the baselines while using only 10\% of the training data.

4. We show that preserving the order of amino acids using $k$-mers achieves better predictive performance than the traditional one-hot encoding based embedding approach.

5. Our approach allows us to predict the geographical region of the COVID-19 infected patient while accounting for important local and global variability in the spike sequences.

The rest of the paper is organized as follows: Section 2 contains the related work. The proposed approach is given in Section 3. Dataset detail and experimental setup are in Section 4. The results of our method and comparison with baselines is shown in Section 5. Finally, we conclude our paper in Section 6.
2 Related Work

Sequence classification is a widely studied problem in domains like sequence homology (shared ancestry) detection between a pair of proteins and Phylology based inference Dhar et al. [2020] of disease transmission Krishnan et al. [2021]. Knowledge of variants and mutations can also help in identifying the transmission patterns of different variants, which will help to devise appropriate public health interventions so that the rapid spread of viruses can be prevented Ahmad et al. [2016, 2017], Tariq et al. [2017], Ahmad et al. [2020]. This will also help in vaccine design and efficacy. Previous studies on working with a fixed length numerical representation of the data successfully perform different data analytics tasks. It has applications in different domains such as graphs Has-san et al. [2020, 2021], nodes in graphs Ali et al. [2021a], Grover and Leskovec 2016, and electricity consumption Ali et al. [2019, 2020b]. This vector-based representation also achieves significant success in sequence analysis, such as texts Shakeel et al. [2020b,a, 2019], electroencephalography and electromyography sequences Atzori et al. [2014], Ullah et al. [2020], Networks Ali et al. [2020a], and biological sequences Ali et al. [2021c]. However, most of the existing sequence classification methods require the input sequences to be aligned. Although sequence alignment helps to analyze the data better, it is a very costly process.

In the evolution of the SARS-CoV-2 genome, it is well-known that a disproportionate amount (in terms of its length) of the variation takes place in the spike region. Kuzmin et al. in Kuzmin et al. [2020] show that viral-host classification can be done efficiently using spike sequences only and applying different machine learning (ML) models. They use one-hot encoding (OHE) to obtain a numerical representation for the spike sequences and then apply traditional ML classifiers after reducing the dimensions of the data using the principal compo-
nent analysis (PCA) method [Wold et al. 1987]. Although OHE is proven to be efficient in terms of predictive performance, it does not preserve the order of amino acids in the spike protein if we want to take the pair-wise Euclidean distance [Ali et al. 2021d]. Another problem with the one-hot encoding based approach is that it deals with aligned sequential data only.

Many previous studies propose the use of $k$-mers (substrings of length $k$), which is an alignment-free approach, instead of the traditional OHE based embedding to obtain the numerical vector representation for the genomic data [Ali et al. 2021b,d, Ali and Patterson 2021]. After computing substrings of length $k$, a fixed-length feature vector is generated, containing the count of each unique $k$-mer in a given sequence. This $k$-mers based method has been used for phylogenetic applications [Blaisdell 1986] and has shown success in constructing accurate phylogenetic trees from DNA sequences. Authors in [Ali et al. 2021d] argue that better sequence classification results can be achieved using $k$-mers instead of OHE because $k$-mers tends to preserve the order of amino acids within a (e.g., spike) sequence.

After obtaining the numerical representation, a popular approach is to compute the kernel matrix and provide that matrix as input to traditional machine learning classifiers like support vector machines (SVM) [Leslie et al. 2003, Farhan et al. 2017, Kuksa et al. 2012]. Farhan et al. in [Farhan et al. 2017] propose an approximate kernel (Gram matrix) computation algorithm, which uses the $k$-mers based feature vector representation as an input to the kernel computation algorithm.

3 Proposed Approach

In this section, we present our proposed model for classifying population regions based on spike sequences only. We start by explaining the basic MAJORITY
based model for the classification. We then show the one-hot encoding (OHE) based feature vector generation approach. After that, we show how we generate $k$-mers based frequency vectors. Then, we introduce our models, which we are using for the purpose of classification. Finally, we give brief details on the experimental setup, before reporting the results of these experiments in the following section.

3.1 MAJORITY

We start with a simple baseline model called MAJORITY. In this approach, we simply take the class with majority representation in the training data and declare it as the class label for all data points in the test set. We then measure the performance of this baseline model using different evaluation metrics.

3.2 One-Hot Encoding [Kuzmin et al., 2020]

In order to obtain a numerical representation for the sequence-based data, one of the popular methods is using one-hot encoding (OHE) [Kuzmin et al., 2020], [Ali et al., 2021b,d], [Ali and Patterson, 2021]. Note that the length of each spike sequence in our dataset is 1274, which contains characters (amino acids) from a set of 21 unique alphabets “ACDEFGHIKLMNPQRSTVWXY”. For OHE, since we need to have a 21 dimensional sub-vector for each amino acid, the length of the OHE based feature vector for each spike sequence will be $21 \times 1273 = 26,733$ (we take the length of spike protein as 1273 instead of 1274 because we have the stopping character ‘*’ at the 1274th position). After obtaining the OHE for the whole data, since the dimensionality of the data will be high, authors in [Kuzmin et al., 2020] use the typical principal component analysis (PCA) approach for dimensionality reduction. Since the size of the data is much larger in our case, we simply cannot use PCA because of high
3.3 Random Fourier Features (RFF) Based Embedding \cite{Rahimi2007}

computational cost \cite{Ali2021}. For this purpose, we use instead an unsupervised approach for low dimensional feature vector representation, called random Fourier features (RFF) \cite{Rahimi2007}.

3.3 Random Fourier Features (RFF) Based Embedding \cite{Rahimi2007}

To compute the pair-wise similarity between two feature vectors, a popular method is to compute the kernel (similarity) matrix (Gram matrix) and give it as input to popular classifiers such as support vector machine (SVM) \cite{Farhan2017}. However, exact kernel methods are expensive in terms of computation (scale poorly on training data \cite{Rahimi2007}), and they require huge space to store an $n \times n$ matrix (where $n$ is the total number of data points). To overcome this problem, we can use the so-called kernel trick.

**Definition 1** (Kernel Trick). *The kernel trick is a fast way to compute the similarity between feature vectors using the inner product. The kernel trick’s main goal is to avoid the explicit need to map the input data to a high-dimensional feature space.*

The kernel trick relies on the assumption that any positive definite function $f(a,b)$, where $a, b \in \mathcal{R}^d$, defines an inner product and a lifting $\phi$ so that we can quickly compute the inner product between the lifted data points \cite{Rahimi2007}. It can be described in a formal way as $\langle \phi(a), \phi(b) \rangle = f(a,b)$.

Although the kernel trick is effective in terms of computational complexity, it is still not scalable to millions of data points. To overcome these computational and storage problems, we use RFF \cite{Rahimi2007}, an unsupervised approach that maps the input data to a randomized low dimensional feature space (Euclidean inner product space). It can be described in a formal way as $z: \mathcal{R}^d \to \mathcal{R}^D$. In RFF, we approximate the inner product between a pair of
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transformed points, which is almost equal to the actual inner product between the original data points. More formally: \( f(a, b) = \langle \phi(a), \phi(b) \rangle \approx z(a)'z(b) \).

Here, \( z \) is a (transformed) low dimensional (approximate) representation of the original feature vector (unlike the lifting \( \phi \)). Since \( z \) is the approximate low dimensional representation of the original feature vector, we can use \( z \) as an input for different machine learning (ML) tasks such as classification.

3.4 Spike2Vec [Ali and Patterson 2021]

Spike2Vec is a recently proposed method that uses \( k \)-mers and RFF to design a low dimensional feature vector representation of the data and then perform typical ML tasks such as classification and clustering [Ali and Patterson 2021]. The first step of Spike2Vec is to generate \( k \)-mers for the spike sequences.

3.4.1 \( k \)-mers Computation

The main idea behind \( k \)-mers is to preserve the order of amino acids within spike sequences. The \( k \)-mers is basically a set of substrings (called mers) of length \( k \). For each spike sequence, the total number of \( k \)-mers is \( N - k + 1 \), where \( N \) is the length of the spike sequence (1274), and \( k \) is a user-defined parameter for the size of each mer. An example of \( k \)-mers (where \( k = 3, 4, \) and 5) is given in Figure 2. In this paper, we are using \( k = 3 \) (selected empirically).

3.5 Machine Learning Models

To both feature engineering based embeddings, namely OHE and Spike2Vec, we apply three ML classifiers downstream, namely Naive Bayes (NB), Logistic Regression (LR), and Ridge Classifier (RC). For all these classifiers, default parameters are used for training. To measure the performance, we use average accuracy, precision, recall, weighted and macro \( F_1 \), receiver operating charac-
3.6 Neural Network Model

Although the Spike2vec embedding allows the downstream ML models to scale to datasets with millions of sequences, and is proven to outperform the typical OHE, it is not always effective in terms of overall predictive performance in certain scenarios. To further increase the predictive performance, we move to a neural network (NN) architecture, which takes OHE or $k$-mers based vectors as input. Note that no dimensionality reduction step (e.g., PCA, RFF) is applied beforehand — the NN model takes the OHE or $k$-mers based vectors directly.

Our NN architecture comprises a sequential constructor. We create a fully connected network with one hidden layer that contains 9261 neurons (which is equal to the length of the feature vector i.e., one neuron for every feature at the beginning). The activation function that we are using is “rectifier”. In the output layer, we use the “softmax” activation function. At the end, we use the efficient Adam gradient descent optimization algorithm \cite{zhang2018} with the

Figure 2: Example of different length $k$-mers in spike sequence “MDPEG”.

characteristic area under the curve (ROC-AUC). We also show the training runtime (in seconds) for all methods.
“sparse categorical crossentropy” loss function (used for multi-class classification problems), which computes the crossentropy loss between the labels and predictions. The batch size that we are taking is 100, and we take 10 as the number of epochs for training the model. Note that we use OHE and $k$-mers based frequency vectors (separately) as input to the NN.

Remark 1. Note that we are using “sparse categorical crossentropy” rather than simple “categorical crossentropy” because we are using integer labels rather than the one-hot representation of labels.

4 Experimental Evaluation

In this section, we provide some statistics and visualization on the data that we use, and then the precise details of the experimental setup used to produce the results.

4.1 Dataset Statistics

We use a set of 2,384,646 spike amino acid sequences obtained from the GISAID Website along with metadata on geographical location (continent, country, and in the case of the USA, states). This data, organized by country, is given in Table 1.

4.2 Data Visualization

To evaluate any natural clustering in our data (if any exist), we use the t-distributed stochastic neighbor embedding (t-SNE) approach. The t-SNE approach maps the data into a 2-dimensional (2D) real vector, which can then be visualized using a scatter plot. Since applying t-SNE on the whole data is very costly and time consuming, we randomly sampled a
4.2 Data Visualization

| Region    | Country | No. of sequences | Region    | Country | No. of sequences |
|-----------|---------|------------------|-----------|---------|------------------|
| Europe    | England | 568202           | North America | USA   | 663527           |
|           | Germany | 146730           |           | Canada | 91193            |
|           | Denmark | 138574           |           | Mexico | 20040            |
|           | Sweden  | 78810            |           |        |                  |
|           | Scotland| 69387            | USA       |        |                  |
|           | France  | 56247            |           |        |                  |
|           | Netherlands | 49938 | Japan    | 75423 |
| Europe    | Spain   | 48830            | Asia      | India  | 37943            |
|           | Switzerland | 48516 | Israel   | 14361 |
|           | Wales   | 46851            |           |        |                  |
|           | Italy   | 44728            | Australia |        | 20985            |
|           | Belgium | 28758            |           |        |                  |
|           | Ireland | 23441            |           |        |                  |
|           | Poland  | 16061            |           |        |                  |
|           | Norway  | 14684            |           |        |                  |
|           | Lithuania | 13586 |            |        |
|           | Luxembourg | 12713 |            |        |
|           | Finland | 11254            |           |        |                  |
|           | Slovenia | 17135            |           |        |                  |
|           | Total   | 19               | Total     | 3      | 127727           |
|           |         |                  | Total     | 1      | 20985            |

Table 1: The set of 2,384,646 SARS-CoV-2 spike sequences used in this study, labeled by country of origin.

subset (≈80K sequences) from the data (of Table 1) and generated a 2D real vector using the t-SNE approach (see Figure 3).

**Remark 2.** The reason for (randomly) selecting ≈80K sequences is because the t-SNE method is computationally very expensive (runtime is \( O(N^2) \), where \( N \) is the number of data-points [Pezzotti et al. (2016)] and is infeasible in terms of runtime on 2.3 million sequences.

The rate of spread of the 3 most common variants of SARS-CoV-2 (in the USA) from March 2020 to July 2021 from our data are given in Figure 4. We can see that the Alpha variant was clearly the variant of concern when it reached its peak in April 2021. We can see a drop from this peak for all variants after April 2021. This is likely because a significant proportion of the population were
Figure 3: A t-SNE plot from the frequency ($k$-mers based feature) vectors along with the country information for $\approx$80K randomly sampled sequences from the set of 2,384,646 sequences (Table 1) used in this study.

vaccinated by this point, hence the total number of cases started decreasing [All and Patterson 2021].

Figure 4: The rate of spread of the 3 most common SARS-CoV-2 variants (in the USA) from March 2020 till July 2021.
4.3 Experimental Setup

All experiments are conducted using an Intel(R) Xeon(R) CPU E7-4850 v4 @ 2.10GHz having Ubuntu 64 bit OS (16.04.7 LTS Xenial Xerus) with 3023 GB memory. The implementation of our algorithms is done in Python and the code is available online for reproducibility\(^1\) We obtain a set of 2,384,646 spike amino acid sequences from the GISAID Website\(^2\) The GISAID provides many different metadata for these sequences, such as collection date, geographical location, and sometimes variant information, which can be used after agreeing to the terms and conditions of GISAID\(^2\) For the classification algorithms, we use 10% of the data for training and 90% for testing. The purpose of using a smaller training dataset is to show how much performance gain we can achieve while using minimal training data.

5 Results and Discussion

In this section, we present results for three different granularities of class labels, namely continents, countries, and finally states in a case study of the United States of America (USA).

5.1 Continent Classification

In this section, we show classification results for 5 different continents, namely Europe, North America, South America, Asia, and Australia (see Table \[1\]). The classification results (average ± standard deviation of 5 runs) are given in Table\(^2\). In terms of predictive performance, we can observe that the NN model with the \(k\)-mers based embedding performs best compared to the baselines. While comparing the two embedding methods (i.e., OHE and \(k\)-mers), we can

\(^1\)https://github.com/sarwanpasha/COVID-19-Country-Classification  
\(^2\)Available at https://www.gisaid.org/
see that \(k\)-mers is better than OHE for the NN model. Since \(k\)-mers can preserve
the order of amino acids better as compared to the OHE, it is able to give richer
information in the feature vector. In terms of runtime, RC with the Spike2Vec
embedding is performing best. The NN model will take longer to train the
models compared to simple ML classifiers because of the tuning of different
parameters.

| Approach | Embed Method | Algo. | Accuracy | Precision | Recall | \(F_1\) weigh | \(F_1\) Macro | ROC-AUC | Training runtime (sec.) |
|----------|--------------|-------|----------|-----------|--------|---------------|--------------|---------|------------------------|
| MAJORITY |              |       | 0.60 ± 0.000 | 0.36 ± 0.000 | 0.60 ± 0.000 | 0.40 ± 0.000 | 0.15 ± 0.000 | 0.50 ± 0.000 | - |
| Feature Engineering | | OHE | Ne | 0.49 ± 0.005 | 0.63 ± 0.006 | 0.49 ± 0.006 | 0.50 ± 0.007 | 0.38 ± 0.006 | 0.63 ± 0.005 | 1837.2 ± 0.023 |
| Spike2Vec | Ne | 0.48 ± 0.007 | 0.63 ± 0.006 | 0.48 ± 0.006 | 0.48 ± 0.007 | 0.36 ± 0.006 | 0.63 ± 0.006 | 1141.9 ± 0.072 |
| NN | Neural | 0.75 ± 0.007 | 0.76 ± 0.008 | 0.75 ± 0.008 | 0.72 ± 0.000 | 0.67 ± 0.007 | 0.67 ± 0.008 | 1622.4 ± 0.031 |
| Neural | 0.77 ± 0.000 | 0.79 ± 0.008 | 0.77 ± 0.009 | 0.74 ± 0.008 | 0.69 ± 0.008 | 0.65 ± 0.009 | 1329.1 ± 0.029 |

Table 2: Continent classification results (average ± standard deviation of 5
runs) for 5 continents comprising 2,384,646 spike sequences (10% training set
and 90% testing set). Best average values are shown in bold.

### 5.2 Country Classification

After classifying the continents, we take countries as the class label and train all
ML and NN models again with the same parameter settings. The classification
results (average ± standard deviation of 5 runs) for countries is given in Table 3.

In terms of predictive performance, we can observe that the NN model is per-
forming better than all baselines. In terms of runtime, RC with the OHE is the
best classifier. An important observation here is the drop in overall performance
of all classification models as compared to the continent classification. The rea-
son for this behavior is likely due to any natural clustering or other information
in the spike sequences corresponding to the location of patients breaking down
at this level of granularity. This lack of knowledge in the data makes country
classification a difficult task. However, we can see that the NN model can still
classify the countries better than the baselines.

| Approach | Embed. Method | Algto. | Accuracy | Precision | Recall | F1 weight | F1 macro | ROC-AUC | Training runtime (sec.) |
|----------|---------------|--------|----------|-----------|--------|-----------|----------|---------|------------------------|
| MAJORITY |               |        | 0.27 ± 0.000 | 0.10 ± 0.000 | 0.22 ± 0.000 | 0.12 ± 0.000 | 0.16 ± 0.000 | 0.06 ± 0.000 |                      |
| Feature Engineering | OHE | LR | 0.40 ± 0.000 | 0.40 ± 0.000 | 0.40 ± 0.000 | 0.53 ± 0.007 | 0.15 ± 0.008 | 0.55 ± 0.009 | 1358.4 ± 0.098 |
|                   | IC | LR | 0.40 ± 0.000 | 0.38 ± 0.007 | 0.40 ± 0.008 | 0.31 ± 0.008 | 0.31 ± 0.007 | 0.54 ± 0.006 | 716.4 ± 0.095 |
| Spike2Vec | OHE | LR | 0.50 ± 0.000 | 0.50 ± 0.000 | 0.40 ± 0.000 | 0.51 ± 0.006 | 0.55 ± 0.005 | 0.55 ± 0.007 | 335.5 ± 0.085 |
|            | IC | LR | 0.50 ± 0.000 | 0.37 ± 0.007 | 0.30 ± 0.006 | 0.31 ± 0.008 | 0.11 ± 0.006 | 0.54 ± 0.007 | 779.4 ± 0.074 |
| NN | OHE | Neural Network | 0.40 ± 0.000 | 0.53 ± 0.006 | 0.40 ± 0.009 | 0.43 ± 0.009 | 0.24 ± 0.007 | 0.6 ± 0.006 | 2001.8 ± 0.453 |
|  | IC | Neural Network | 0.51 ± 0.005 | 0.57 ± 0.004 | 0.53 ± 0.005 | 0.45 ± 0.006 | 0.28 ± 0.006 | 0.60 ± 0.007 | 198936.6 ± 0.745 |

Table 3: Country classification results (average ± standard deviation of 5 runs) for 27 countries comprising 2,384,646 spike sequences (10% training set and 90% testing set). Best average values are shown in bold.

### 5.3 A Case Study of the United States of America (USA)

After classifying continents and countries, we investigate our model with more highly granular class labels. For this purpose, we first take the single country with the highest number of spike sequences in the data. Since the USA contains most of the spike sequences in our data (see Table 1), we took it as a case study to further explore different states within the USA. The pie chart showing the distribution of the sequences over the states of the USA is given in Figure 5. The classification results (average ± standard deviation of 5 runs) for different states are given in Table 4. We can again observe the drop in predictive performance for all models. This again proves that as we increase the granularity of the class labels, it becomes difficult for any model to classify with higher accuracy. We can also observe that the NN model with the k-mers based feature embedding is performing better than all the baselines.
5 RESULTS AND DISCUSSION

Figure 5: Distribution of the 663,527 sequences over the states of the USA, with the top 11 states specified, while the remaining fall into the “others” category.

Table 4: Classification results (average ± standard deviation of 5 runs) for different states of the USA (10% training set and 90% testing set). The best average values are shown in bold.

5.4 Importance of Attributes

To evaluate the importance of the positions in the spike sequences, we find the importance of each attribute with respect to class label (using the Weka tool\(^3\)). For this purpose, a randomly selected subset of spike sequences (≈80K) is taken from the original dataset. We then compute the information gain (IG) between each attribute (P = amino acid position) and the true class label (C = country). More formally, IG can be computed as

\(^3\)Available at [https://www.cs.waikato.ac.nz/ml/weka/](https://www.cs.waikato.ac.nz/ml/weka/)
where $H(C)$ and $H(C|P)$ are entropy and conditional entropy, respectively. The entropy $H$ can be calculated using

$$H = \sum_{i \in C} -p_i \log p_i,$$

where $p_i$ is the probability of the class $i$. The IG values for each attribute are given in Figure 6. The IG values for each attribute are also available online.\(^4\)

Figure 6: Information gain for each amino acid position corresponding to the class.

6 Conclusion

This paper employs several machine learning models using a $k$-mers based representation as input and efficiently classifies SARS-CoV-2 spike sequences based on geographical location. We show that our proposed approach outperforms the

\(^4\)Available at https://github.com/sarvanpasha/COVID-19-Country-Classification/blob/main/attributes_correlation.csv
baselines in terms of predictive performance. Using information gain, we also show the importance of attributes (amino acids) in the spike sequences. Such classification and its analysis can help researchers to study more deeply the connection between geographical location and SARS-CoV-2 variants. In the future, we will explore more sophisticated models like LSTM and GRU, and also use other attributes like months information to increase the predictive performance, and maybe give an idea of the dynamics (spread) of the virus over time. Using other alignment-free methods such as minimizers is another possible future direction.

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Author Contribution Statement

SA, MP: Conceptualization. SA: Methodology. SA: Software. SA, ZT: Validation. SA, MP: Formal Analysis. SA, BB, ZT: Investigation. All: Resources. All: Data Curation. All: Writing - Original Draft. All: Writing - Review & Editing. SA: Visualization. SA, MP: Supervision. MP: Project Administration. MP: Funding Acquisition.

Conflict of Interest

The authors declare no conflict of interest.
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