Evaluating COVID-19 Sequence Data Using Nearest-Neighbors Based Network Model

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Abstract—The SARS-CoV-2 coronavirus is the cause of the COVID-19 disease in humans. Like many coronaviruses, it can adapt to different hosts and evolve into different lineages. It is well-known that the major SARS-CoV-2 lineages are characterized by mutations that happen predominantly in the spike protein. Understanding the spike protein structure and how it can be perturbed is vital for understanding and determining if a lineage is of concern. These are crucial to identifying and controlling current outbreaks and preventing future pandemics. Machine learning (ML) methods are a viable solution to this effort, given the volume of available sequencing data, much of which is unaligned or even unassembled. However, such ML methods require fixed-length numerical vectors in Euclidean space to be applicable. Similarly, euclidean space is not considered the best choice when working with the classification and clustering tasks for biological sequences. For this purpose, we design a method that converts the protein (spike) sequences into the sequence similarity network (SSN). We can then use SSN as an input for the classical algorithms from the graph mining domain for the typical tasks such as classification and clustering to understand the data. We show that the proposed alignment-free method is able to outperform the current SOTA method in terms of clustering results. Similarly, we are able to achieve higher classification accuracy using well-known Node2Vec-based embedding compared to other baseline embedding approaches.

Index Terms—Sequence Classification, KNN, K Nearest Neighbors, Spike Sequence, COVID-19, Sequence Clustering, k-mers

I. INTRODUCTION

Because of the COVID-19 pandemic, a lot of sequence data is publicly available on databases such as GISAID. This motivated the researchers to evaluate the data using multiple tools for the purpose of advanced analysis. Networks are one tool to this end and have found many applications in bioinformatics. More specifically, sequence similarity networks are useful for the analysis of biological data using well-established classification and clustering methods. Clustering the sequences based on the lineages, locality, and time could help us understand the evolution and spread of different lineages, which indeed helps the biologists and relevant government authorities to take appropriate measures in advance.

Some effort has been made recently to classify and cluster sequences based on hosts [25], [4] and lineages [9], [35], [7]. Clustering approaches can help in identifying novel and rapidly growing lineages, while classification can assist in keeping the track of existing ones. Since the spike (S protein) sequence (see Figure 1) of a coronavirus is the point of contact to the host cell, it is a vital characteristic of this type of virus [26], [37]. Therefore, the spike the region is often the focus of the literature (rather than the entire genome) for studying the behavior of coronaviruses, both in terms of host and lineage specificity [25], [12]. However, for a spike sequence to be a compatible input to machine learning (ML) models, it must be transformed into a fixed-length numerical representation, known as the feature vector. There are many methods proposed in the literature to produce such a representation of a spike sequences, such as one-hot encoding [25], k-mers based encoding [7], and position weight matrix based encoding [4].

Fig. 1: The full-length genome sequence contains structural and non-structural parts. We are interested in spike (s) region as mutations related to coronavirus happen in that region.

Our contributions to this paper are the following:
1) A new method to convert the biological sequences into a sequence similarity graph is proposed, which can be used to study the sequences efficiently.
2) Using the clustering algorithms, we show that the proposed model outperforms the current SOTA approach using different internal clustering evaluation metrics.
3) Using different embedding representation methods for nodes, we show that node classification using Node2Vec gives higher accuracy than other embedding approaches.

The remaining paper is structured as follows. Section II contains related work. Section III contains the proposed approach. Section IV contains experimental setup and dataset statistics. Section V contains results and discussion. Section VI concludes this paper.

II. RELATED WORK

In the biology domain, sequence analysis using the Phylogeny-based method [21] is an important task. The
method that uses substring (called mers) of length $k$ (hence $k$-mers) counts was first explored in [15] for phylogenetic applications. Authors in [15] proposed a phylogenetic tree-based method from the sequences (non-coding and coding regions). In recent years, since we have had a huge availability of sequencing data due to the coronavirus pandemic, the sequence analysis problem attracts the attention of researchers [23], [18], [17]. Few methods proposed for supervised tasks are alignment-free. Similarly, some approaches depend on the alignment of sequences for both supervised and unsupervised tasks.

The conversion of sequences into embeddings is an important step in the machine-learning pipeline while working with supervised and unsupervised tasks. Numerical embedding generation is important in many fields like time series forecasting [5], [2], analysis of graphs [11], [28], electromyography [36], clinical data analysis [13], and network security [3]. A method to convert the aligned sequences into fixed-length embeddings using a position weight matrix is proposed in [4]. Their method shows improvement in terms of predictive performance. However, the sequence alignment step in their pipeline makes it difficult to use for a large number of sequences. A method based on $k$-mers spectrum for supervised analysis of biological sequences is proposed in [7], [10]. For unsupervised analysis, many recent studies proposed embedding methods for the biological sequences [12], [35], [8].

Using kernel matrix for supervised sequence analysis is another domain explored in the literature. A kernel (gram) matrix is generated in this case that corresponds to pairwise distances between sequences [8], [24]. This gram matrix is then used with kernel-based classifiers for supervised analysis. A kernel matrix-based method is proposed in [9] for biological sequence classification. Although the proposed method shows promising results, it is not applicable in real-world scenarios due to the expensive storage cost of the kernel matrix.

III. PROPOSED APPROACH

In this section, we describe the overall approach that we are using in this paper. Our proposed method is divided into different steps described below.

A. Generating Fixed-Length Numerical Representation

Given a set of spike sequences and attributes (coronavirus lineages) related to them, we want to generate sequence similarity network (SSN). SSNs is a network in which nodes are sequences and edges show the top $K$ nearest neighbors to any given sequence. Since the KNN algorithm works in euclidean space, the first step is to convert the biological sequences into a fixed-length numerical representation. For this purpose, we use a method called $k$-mers [9] (also called n-gram in the NLP domain) as given in Figure 2.

Definition 3.1 ($k$-mers): Given some sequence $\sigma$ on alphabet $\Sigma$, we generates substrings (also called mers) of length $k$, i.e., $k$-mers (using sliding window approach. In $\Sigma$, we have the following 20 characters (amino acids) “ACDEFGHIKLMN-PQRSTVWY”.

For any sequence of length $N$, the total $k$-mers are:

$$\text{total} \ k\text{-mers} = (N - k) + 1 \quad (1)$$

![Fig. 2: $k$-mers example for $k = 3$.](image)

We use $k = 3$ for the $k$-mers, computed using the validation set. After generating the $k$-mers for a given sequence, we create the frequency vector (numerical representation) of length $\Sigma^k$ (i.e., length comprised of all possible $k$-mers in $\Sigma$ of length $k$), which contains the count/frequencies of $k$-mers.

The pseudocode to compute the frequency vectors is given in Algorithm 1.

Algorithm 1 ComputeFrequencyVector

1: Input: Set $S$ of $k$-mers on alphabet $\Sigma$, the size of mers $n$
2: Output: Frequency Vector $V$
3: combos = GenerateAllKmersCombinations($\Sigma$, n)
4: $V = [0] * |\Sigma|^n$  \ $\triangleright$ Total length of (zero) vector
5: for $i$ from 1 to $|S|$ do
6: \ $\triangleright$ Find index of $i^{th}$ k-mer
7: $V[\text{idx}] \leftarrow V[\text{idx}] + 1$  \ $\triangleright$ Increment bin by 1
8: return($V$)

B. Generating Sequence Similarity Network (SSN)

After generating the $k$-mers-based numerical representation, we use the $K$-Nearest Neighbors-based approach to generate the Sequence Similarity Network (SSN), where nodes are the sequences and edges between them show the similarity.

Remark 1: Note that we generate an unweighted SSN. We use $K = 20$ in this case (decided using standard validation set approach [20]). The resultant SSN graph is given in Figure 3.

After generating the SSN, we use several supervised (classification) and unsupervised (clustering) methods to perform sequence analysis.

C. Supervised Analysis

Given the SSN, we perform lineage classification using different machine learning (ML) algorithms in this setting. To
apply the ML algorithms on graphs, we need to get the feature embeddings for the nodes, which can be used as input to the ML classifiers. To get the numerical embeddings, we use the following methods:

1) **Locally Linear Embedding (LLE) [32]**: Given a node \( v \) from the graph along with its neighbors \( N(v) \), this method assumes that \( v \) is a linear combination of \( N(v) \) in the embedding space (where this linear combination property must hold for all nodes in the graph). It uses the objective function to minimize the distances between the embeddings of two nodes that are neighbors to each other. The embeddings are normalized in the end to get the final representation.

2) **Laplacian Eigenmaps [14]**: Using the graph Laplacian, it generates the embedding for each node while preserving the local neighborhood information for that node. Similar to LLE, it also solves the objective function to keep the embeddings close to each other for the nodes that are neighbors. However, it involves graph Laplacian.

3) **Higher-Order Proximity preserved Embedding (HOPE) [29]**: Given the similarity matrix, it preserves the higher order proximity between the embedding of nodes by minimizing the objective function in which we minimize the squared differences between the similarity matrix and the pairs of given embeddings.

4) **Graph Factorization (GF) [1]**: Given the adjacency matrix of the graph, this method factorizes that matrix. The GF minimizes the objective function (reducing the distance between the neighboring nodes in the corresponding embeddings) to give an approximate embedding representation for a given node. Since it does not give an exact solution, the embeddings may contain some noise.

5) **DeepWalk [30]**: Given a pair of nodes, the goal of this method is to preserve higher-order proximity. This goal is fulfilled by maximizing the probability of observing in the random walk (previous and next top \( k \) nodes).

6) **Node2Vec [22]**: It is similar to DeepWalk. The difference between the two is that this approach adopts biased-random walks. This bias behavior gives a trade-off between depth-first graph search and breadth-first graph search. Therefore it results in efficient embeddings compared to DeepWalk.

### D. Unsupervised Analysis

In this setting, we perform clustering using different unsupervised clustering algorithms. We use the following clustering algorithms.

1) **MiniBatch KMeans**: It is based on the simple idea of KMeans with mini-batch optimization. The idea of using a mini-batch is to reduce the runtime cost of the algorithm.

2) **Affinity Propagation**: Given measures of similarity between data points, it uses the concept of message passing to cluster the data. It does not require the number of clusters as input. However, it has a quadratic runtime.

3) **Mean Shift**: It is a centroid-based method that updates the candidates for centroids so that they can be the mean of the points within a given region.

4) **Spectral Clustering**: This method uses the spectrum (eigenvalues) of the kernel matrix (also called gram/similarity matrix), which is computed from the input data to perform clustering (after performing dimensionality reduction) in fewer dimensions.

5) **Ward**: It uses agglomerative hierarchical clustering along with an objective function so that merging two clusters at any iterations should satisfy the objective function (minimizing error sum of squares ESS).

6) **Agglomerative Clustering**: This method combines the pairs of clusters recursively from the data points. It uses the dendrogram-based approach.

7) **Density-Based Spatial Clustering of Applications with Noise (DBSCAN)**: Given the input data, DBSCAN groups together data points that are close to each other (nearest neighbors). It also keeps track of the outliers data points, which lies alone in low-density regions (points that do not have nearby neighbors).

8) **Ordering Points To Identify the Clustering Structure (OPTICS)**: This method searches for the core sample of high density and expands clusters from them. It is similar to DBSCAN. One of the main differences OPTICS has as compared to the DBSCAN is that it keeps cluster hierarchy for a variable neighborhood radius.

9) **BIRCH**: It works by constructing a tree data structure. In that tree, the cluster centroids behave as the leaf. Using the threshold value, clusters could be extracted from the tree. Similarly, other algorithms can be used in combination to get the final clusters, such as agglomerative clustering.

10) **Gaussian Mixture**: It contains a mixture of multiple gaussian distributions. It is similar to the KMeans algorithm. However, it is considered a Soft clustering approach (overlapping allowed), while KMeans is considered a hard clustering algorithm (no overlapping). Given the data, this approach assigns the data points to the multivariate normal components. This assignment should maximize the component posterior probability.

**Remark 2**: Note that all clustering methods except ward and agglomerative clustering take \( k \)-mers-based numerical
embeddings as input. The ward and agglomerative clustering approaches take SSN as input.

IV. EXPERIMENTAL SETUP

We split the data into 70-30\% for training and testing (held out) sets, respectively, for experimentation. We use 5 fold cross-validation on the training data for hyperparameter tuning. The final results are computed for 30\% held-out testing set. We use 5 random initialization of data to avoid biases in the results. All experiments are performed on Windows 10 operating system, having a Core i5 processor and 32 GB RAM. The code is written in Python, which is available online.\(^2\)

A. Dataset Statistics

We extracted spike sequence data from GISAID website (as given in [6]), which consists of 7000 sequences of length 1274 from 22 lineages. The proportion of lineages in the dataset is given in Table I.

TABLE I: Dataset statistics for 22 lineages [6]. The character '-' means that information is not available.

| Lineage  | Region of First Time Detection | Variant Name | No. Mut. S/Gen. | No. of Sequences |
|----------|--------------------------------|--------------|-----------------|-----------------|
| B.1.1.7  | UK                             | Alpha        | 8/17            | 3369            |
| B.1.617.2| India                          | Delta        | 8/17            | 875             |
| AY.4     | India                          | Delta        | -               | 593             |
| B.1.2    | USA                            | -            | -               | 333             |
| B.1      | USA                            | -            | -               | 292             |
| B.1.177  | Spain                          | -            | -               | 243             |
| P.1      | Brazil                         | Gamma        | 10/21           | 194             |
| B.1.1    | UK                             | -            | -               | 163             |
| B.1.429  | California                     | Epsilon      | 3/5             | 107             |
| B.1.526  | New York                       | Jota         | 6/16            | 104             |
| AY.12    | India                          | Delta        | -               | 101             |
| B.1.160  | France                         | -            | -               | 92              |
| B.1.351  | South Africa                   | Beta         | 9/21            | 81              |
| B.1.427  | California                     | Epsilon      | 3/5             | 65              |
| B.1.214  | Japan                          | -            | -               | 64              |
| B.1.1519 | USA                            | -            | -               | 56              |
| D.2      | Australia                      | -            | -               | 55              |
| B.1.221  | Netherlands                    | -            | -               | 52              |
| B.1.277.21| Denmark                       | -            | -               | 47              |
| B.1.258  | Germany                        | -            | -               | 46              |
| B.1.243  | USA                            | -            | -               | 36              |
| R.1      | Japan                          | -            | -               | 32              |
| Total    | -                              | -            | -               | 7000            |

B. Elbow Method for Optimal Number of Clusters

We use a data-driven method called Elbow, to compute the optimal number of clusters [33], [12]. In this method, we evaluate the trade-off between runtime and the sum of squared error and get the number of clusters where these two metrics are smaller. In this paper, we selected 4 as the optimal cluster number (see Figure 4).

C. Baseline Model

As an unsupervised baseline, we use a method, called PWM2Vec, which is proposed in [4]. This approach works by computing weights for each \(k\)-mers using the position weight matrix[34] and then concat all those weights for all \(k\)-mers within a sequence to get the final embedding.

\(^2\)https://github.com/sarwanpasha/Spike2Network
\(^3\)https://www.gisaid.org/

D. Evaluation Metrics for Clustering

For the analysis of the unsupervised task, we use \(k\)-means clustering. The following evaluation metrics are used to measure the quality of clusters:

Silhouette Coefficient [31]: This metric measures the similarity of a vector with its own cluster (cohesion) and with vectors in other clusters (separation). The value of this metric is between \(-1\) (worst) to 1 (best).

Calinski-Harabasz Score [16]: This metric works by analyzing inter-cluster dispersion and the between-cluster dispersion (computing ratio between the two). A lower score of this metric means bad clustering and vice versa.

Davies-Bouldin Score [19]: This metric computes the similarity between clusters by measuring the ratio of distances within-cluster to between clusters. In this case, a lower value corresponds to better clustering and vice versa.

E. Evaluation Metrics For Classification

We use multiple classifiers to test the performance of embeddings. The resultant feature vectors of length 200 (using parameters tuning) are fed to different classifiers as input. We use Multi-Layer Perceptron (MLP), SVM, Decision Tree (DT), Naive Bayes (NB), Random Forest (RF), K-Nearest Neighbour (KNN), and Logistic Regression (LR) algorithms. The evaluation metrics used to test the performance are recall, precision, accuracy, weighted F1, macro F1, and ROC area under the curve (AUC). To use the metrics designed for the binary classification task, we use the one-vs-rest approach to use them for multi-class classification.

V. RESULTS AND DISCUSSION

In this section, we show the results of classification and clustering methods.

A. Clustering Results

1) Subjective Evaluation: We use t-distributed stochastic neighbor embedding (t-SNE) [27] to represent the data in 2-dimensions so that we can use scatter plots to visualize the data and analyze the patterns. The t-SNE method maps input data

Fig. 4: Elbow method using different value of \(k\) to get optimal value. The notation \(k\) represents the number of clusters.
to 2D real vectors (low dimensions) while preserving the pairwise distance between the points from high dimensions. The subjective evaluation of different clustering methods (using t-SNE) is shown in Figure 5. The t-SNE-based plot for the data with original labels is also shown in Figure 5k for comparison purposes. Compared with the original plot, we can conclude that the gaussian mixture and ward give the clustering that is closely related to the original plot in some sense.

2) Objective Evaluation: The results for objective evaluation are given in Table II. In general, we can observe that there is no clear winner for the goodness of clustering. For the Silhouette coefficient, the Ward method performs the best. In the case of the Calinski-Harabasz score, the Gaussian Mixture model performs the best. Similarly, Agglomerative clustering shows the best performance in the case of Davies Bouldin score. PWM2Vec (the SOTA method) performs best in terms of clustering runtime because of lower dimensional embeddings.

### TABLE II: Objective evaluation using internal clustering quality metrics for different clustering algorithms. The best values are shown in bold.

| Algorithm       | Evaluation Metrics | Silhouette Coefficient | Calinski Harabasz Score | Davies-Bouldin Score | Clustering Run-time (Sec.) |
|-----------------|--------------------|------------------------|-------------------------|----------------------|---------------------------|
| PWM2Vec         |                    | 0.477                  | 1762.983                | 1.007                | 1.45                      |
| MiniBatch KMeans|                   | 0.133                  | 1505.001                | 2.064                | 53.43                     |
| Affinity        |                   | -0.600                 | 0.111                   | 1.901                | 511.47                    |
| Mean Shift      |                   | -0.768                 | 0.874                   | 5.175                | 1326.34                   |
| Spectral Clustering |               | -0.613                 | 402.117                 | 1.984                | 219.44                    |
| Ward            |                   | 0.593                  | 2657.689                | 5.152                | 322.17                    |
| Agglomerative Clustering |         | 0.290                  | 95.723                  | 0.455                | 155.35                    |
| DBSCAN          |                   | 0.216                  | 23.174                  | 1.351                | 14.55                     |
| OPTICS          |                   | -0.196                 | 285.435                 | 1.548                | 4229.51                   |
| BIRCH           |                   | 0.524                  | 2242.030                | 4.757                | 227.01                    |
| Gaussian Mixture |                  | -0.418                 | 2764.134                | 1.463                | 1030.63                   |

### B. Classification Results

The classification results for different embedding methods and classification algorithms are given in Table III. We can observe that Node2Vec significantly outperforms all other embeddings for all evaluation metrics using SVM and KNN classifiers. This behavior shows that the embeddings generated using Node2Vec were able to preserve the structure of the network more efficiently than the other embedding approaches.

To evaluate whether the computed results are statistically significant, we used the student t-test and evaluated the p-values for the results using the average and standard deviation results of 5 runs. The standard deviation results are reported in Table IV. We noted that the p-values were < 0.05 in the majority of the cases and for all embedding methods (because standard deviation values are very low), hence confirming the statistical significance of the reported results. Note that we have not reported the p-values in this paper due to space constraints. However, we believe that they can easily be computed by anyone using the reported average and standard deviation results.

### VI. Conclusion

In this study, we propose a method to convert the protein (spike) sequences into a graph to use well-established graph mining algorithms to analyze the biological sequences in both a supervised and unsupervised manner. We show that clustering the nodes gives better qualities of the clusters as compared to the feature engineering-based approach. For the supervised analysis, we show that Node2Vec could be used to generate the feature embeddings for the nodes, which can give better classification accuracies compared to the other embedding methods. In the future, we will work towards testing the proposed model on more number of sequences to test the scalability. Using a neural network-based model such as GNN for the classification is another interesting future work. We will also consider other attributes, such as locality information and calendar values for computing richer embeddings for sequences.
Fig. 5: Subjective evaluation from different clustering approaches using t-SNE.

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TABLE IV: Standard Deviation values of 5 runs for Classification results on the proposed and SOTA methods. The average results are reported in Table III.

| Embed Method | ML Alg | Acc  | Prec  | Recall | F1 score | MAC  | ROC AUC | Time (sec.) |
|--------------|--------|------|-------|--------|---------|------|---------|-------------|
| SVM          | NB     | 0.00139 | 0.00037 | 0.00010 | 0.00089 | 0.01899 | 0.02130 |
|              | MLP    | 0.01977 | 0.03472 | 0.02301 | 0.01414 | 0.02141 | 0.02085 |
|              | KNN    | 0.00208 | 0.00789 | 0.00730 | 0.00755 | 0.01821 | 0.01808 |
|              | LR     | 0.00650 | 0.00505 | 0.00560 | 0.00531 | 0.01425 | 0.01730 |
|              | RF     | 0.00904 | 0.00476 | 0.00390 | 0.00434 | 0.01624 | 0.01625 |
|              | SVM    | 0.03442 | 0.01075 | 0.00812 | 0.01647 | 0.02786 | 0.02787 |
|              | MLP    | 0.02596 | 0.00786 | 0.00730 | 0.00799 | 0.01920 | 0.01921 |
|              | KNN    | 0.00620 | 0.00415 | 0.00359 | 0.00409 | 0.01600 | 0.01600 |
|              | LR     | 0.00658 | 0.00275 | 0.00250 | 0.00265 | 0.01580 | 0.01580 |
|              | RF     | 0.00957 | 0.00189 | 0.00190 | 0.00200 | 0.01720 | 0.01720 |
|              | SVM    | 0.03485 | 0.01330 | 0.01300 | 0.01350 | 0.02700 | 0.02700 |
|              | MLP    | 0.04772 | 0.00800 | 0.00730 | 0.00799 | 0.02200 | 0.02200 |
|              | KNN    | 0.01235 | 0.01079 | 0.01079 | 0.01110 | 0.01710 | 0.01710 |
|              | LR     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |
|              | RF     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |
|              | SVM    | 0.03938 | 0.01330 | 0.01300 | 0.01350 | 0.02700 | 0.02700 |
|              | MLP    | 0.04772 | 0.00800 | 0.00730 | 0.00799 | 0.02200 | 0.02200 |
|              | KNN    | 0.01235 | 0.01079 | 0.01079 | 0.01110 | 0.01710 | 0.01710 |
|              | LR     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |
|              | RF     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |
|              | SVM    | 0.03938 | 0.01330 | 0.01300 | 0.01350 | 0.02700 | 0.02700 |
|              | MLP    | 0.04772 | 0.00800 | 0.00730 | 0.00799 | 0.02200 | 0.02200 |
|              | KNN    | 0.01235 | 0.01079 | 0.01079 | 0.01110 | 0.01710 | 0.01710 |
|              | LR     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |
|              | RF     | 0.00979 | 0.00479 | 0.00430 | 0.00459 | 0.01600 | 0.01600 |