Extracting and Evaluating Features from RNA Virus Sequences to Predict Host Species Susceptibility Using Deep Learning

Kevin Sutanto

kevin.sutanto@uottawa.ca

School of Electrical Engineering and Computer Science,
University of Ottawa
Ottawa, Ontario, Canada

Marcel Turcotte*

marcel.turcotte@uottawa.ca

School of Electrical Engineering and Computer Science,
University of Ottawa
Ottawa, Ontario, Canada

ABSTRACT

Identifying and monitoring hosts of zoonotic RNA viruses, that is, RNA viruses which can be transmitted from one species to another, including the recent SARS-CoV-2 causing the COVID-19 pandemic, is paramount to control their spread. However, efforts to control such spread may be affected if there are unmonitored or unknown hosts. To help identify potential hosts that may harbour such zoonotic viruses, we propose a pipeline that extracts features from sequences of RNA viruses, then uses the extracted features with deep learning to predict host species susceptibility. In addition to using sequence-related features, our method also extracts and uses features derived from the RNA secondary structures that can be formed by the viral sequences, since RNA secondary structures are known to take part in virus-host interaction. We evaluated the performance of our method and the different extracted features with a dataset containing RNA virus sequences and the host they infect, regardless of the viral species, from the NCBI Virus database. Using 10-fold cross validation, we found that a combination of the extracted features yielded the highest overall prediction accuracy of 86.89%.

CCS CONCEPTS

• **Applied computing** → **Bioinformatics**; *Computational biology*; • **Computing methodologies** → *Machine learning*.

KEYWORDS

RNA virus, host susceptibility, deep learning

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1 INTRODUCTION

Monitoring and surveillance of reservoir hosts have been an important strategy to anticipate and control the spread of viruses [28].

*Corresponding author: Marcel Turcotte (marcel.turcottet@uottawa.ca)

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This is especially true for RNA viruses; as they are capable of re-assorting themselves during replication, allowing them to “jump” between different host species in a short period of time [44], in addition to their ability to rapidly adapt to novel hosts [7, 8, 26, 43, 46]. Their high mutation rates compared to DNA replication also allows them to have a wider range of susceptible host species [10].

However, such surveillance and control effort may be impacted when there are unknown, unmonitored hosts. This may be the case with the SARS-CoV-2 virus causing the ongoing COVID-19 pandemic, in which researchers believe there may be unknown intermediate hosts between bats and humans [11, 25, 49]. Testing a wide variety of hosts for their susceptibility to the virus, in order to identify these unknown hosts, is demanding. But computational techniques, such as deep neural networks, could be utilized to narrow down the possible unknown hosts, reducing the required tests only to the likely hosts. Deep learning techniques have previously been used to identify viruses from their sequences [39], protein interactions between viral and host proteins [50], and carrier hosts for sequences of “influenza A”, “rabies lyssavirus” and “rotavirus A” [31].

Prior work in host identification has mostly relied on the sequences of the viruses themselves [14, 31], the encoded proteins [9], and “word frequency” or k-mers [2, 48]. However, in the case of single-stranded RNA viruses, secondary structure often takes part in their biological and replication processes; and as a result, there may be parts of the viral genomes in which secondary structures are fairly conserved despite the high mutation rate of the nucleotides themselves [17, 41]. For example, alphaviruses were found to have a secondary structure motif whose function is to evade the host viral recognition mechanism [18]. In addition, a recent study has also found conserved structures, “hinting” conserved functions, among the coronaviruses including the recent SARS-CoV-2 [29]. Finally, when it comes to SARS-CoV-2 relative to other viruses in the SARS family, another study has found both structural and nucleotide conservation as well [37]. Due to these roles and conservation of secondary structures in RNA viruses, including their roles in virus-host interaction, we decided to investigate if features derived from secondary structures can be used to improve predictions of virus-host relationship, in addition to the nucleotide-based features that have been commonly used in previous studies.

Several related host prediction studies focused on certain species of viruses [9, 31], while another focused on the type of host (bacterial hosts) and included hosts and viruses with adequate numbers of sequences [48]. Due to the prevalence of zoonotic RNA viruses [32, 35, 45] (including SARS-CoV-2 [30] and HIV [15]) that can spread from non-human animals to humans, we have decided

to focus on RNA viruses regardless of the virus species or the host species.

2 METHODS

2.1 Dataset

Our dataset contains sequences of RNA viruses and their host species. It was obtained from NCBI Virus [3] on September 12, 2020. Only entries that are annotated as being complete (as opposed to partial nucleotides) were included, and duplicate entries were removed. Due to the large memory requirements of matching overly long sequences with our curated RNA secondary structure motifs, we have excluded entries whose viral sequences are longer than 40,000 nucleotides. Furthermore, sequences containing unknown nucleotides and sequences with unknown hosts were also excluded. In addition, as some of the host species have only small numbers of viral sequences in the dataset, we have further filtered the dataset to include only host species with at least 100 viral sequences – similar to how a prior study predicting host genus and virus relationship included only genera with a sufficient number of viral entries, in order to ensure that the learning process has reasonable amount of data points per genus [48].

After all of these filtering steps, we ended up with 47,266 entries (which maximum sequence length is 31,473 instead of our original limit). In total, there are 47 different host species in the included entries. They are as follows: *Allium sativum*, *Anas carolinensis*, *Anas clypeata*, *Anas platyrhynchos*, *Anatidae*, *Apodemus agrarius*, *Aves*, *Bos taurus*, *Canis lupus familiaris*, *Capra hircus*, *Capsicum annum*, *Columbidae*, *Corvus brachyrhynchos*, *Cricetulus griseus*, *Culex*, *Culex pipiens*, *Culex quinquefasciatus*, *Culicidae*, *Culiseta melanura*, *Cyanocitta cristata*, *Equus caballus*, *Felis catus*, *Gallus gallus*, *Glycine max*, *Homo sapiens*, *Macaca mulatta*, *Malus domestica*, *Meleagris gallopavo*, *Melogale*, *Mus musculus*, *Oryza sativa*, *Ovis aries*, *Procyon lotor*, *Prunus*, *Prunus avium*, *Prunus persica*, *Pyrus communis*, *Rattus norvegicus*, *Rosa sp.*, *Solanum lycopersicum*, *Solanum tuberosum*, *Sus scrofa*, *Sus scrofa domesticus*, *Triticum aestivum*, *Vitis vinifera*, *Vulpes vulpes*, and *Zea mays*.

2.2 Secondary Structure Motifs Fingerprinting

We have previously curated a set of common RNA secondary structure motifs, in order to form secondary structure “fingerprints” to be used as deep learning features [42]. The steps to produce the secondary structure “fingerprints” are as follows: finding matches of the curated RNA secondary structure motifs within the sequence in question; getting free energy values of the matches; substituting non-matches with the maximum free energy value (representing a highly unstable or weak structure); rescaling the values so that maximum free energy for a motif is represented by 0 (poorest or no match), while the minimum free energy for a motif is represented by 1 (structurally most stable match), and the free energy values in between is represented by values between 0 and 1; and finally, concatenating the rescaled values together, forming the fingerprint [42]. These steps are illustrated by Figure 1. The secondary structure matching and free energy calculations were performed using the RNAMotif program (the built-in “*efn2*” method was used to calculate the free energy of matches) [27].

Figure 1: Steps to produce secondary structure fingerprints, using our curation of known RNA secondary structure motifs [42].

In our prior work, for a single secondary structure motif and a single sequence, only the minimum free energy was used (representing the most thermodynamically stable match, if there are multiple matches) [42]. However, for this study, we decided to also include the average free energy (of all matches in single a sequence) and maximum free energy (least stable match if there are multiple match) values as additional features; and investigate if combining them with the minimum free energy values would improve accuracy, compared to using only the minimum free energy by itself.

2.3 K-mers

K-mer representations can be considered “signatures” of the corresponding sequences [38]. Using k-mers allows us to infer nucleotide similarities in different sequences, even if the sequences are not

aligned [20]. They are widely used in bioinformatics, and their usefulness as representations of viral sequences has been observed in previous related studies [2, 48].

In this study, k -mers of $k = 4, 5$, and 6 were used. For a single sequence and a k , the k -mers are formed by taking the occurrence counts of each possible nucleotide combination (of length k) divided by the total of all of the aforementioned occurrences. Therefore, the k -mer distribution using a specific k for a sequence adds up to 1 [42]. Similar to our previous approach of using k -mers as features for deep learning, k -mers were both used alone and in conjunction with other types of features to compare the differences in results.

2.4 Skip-mers

Using k -mer representations with larger values of k in order to capture distributions of longer subsequences is CPU and memory intensive – for a length of k with 4 possible nucleotides at each position, a k -mer representation of a sequence will contain 4^k values. In other words, simply increasing the value of k will result in exponential increase in the dimension of the feature vectors and the memory requirements to train the neural network.

Therefore, in order to capture longer patterns that may be present in the dataset while still keeping the memory requirements reasonable, we have used “skip-mers” [6] as additional features. “Skip-mers” [6] are based on the “gapped q-grams” concept [4], in which matches are made with non-consecutive strings. The sparsity of the matching strings can be defined by the number of consecutive characters that have to match (matching characters), and the number of consecutive characters that do not have to match (skip characters) following the matching characters. For the purposes of describing the “skip-mers” [6] used in our study, we define the total number of matching and skip characters to be the length of the “skip-mer” [6]. Therefore, its length would be equal to the length of the strings that fit its criteria. All of the “skip-mers” [6] we used start with matching characters. Matching characters are followed by skip characters, while the latter is followed by the former. In other words, a “skip-mer” [6] alternates between consecutive matching characters and consecutive skip characters until the total number of characters matches its required length. A “skip-mer” [6] representation of a single sequence consists of the distribution of all possible combinations of the matching characters (in their corresponding positions) – that is, 4^m values, where m is the total number of matching characters, in which each matching character can be one of the four possible nucleotides.

We used a set of “skip-mers” [6] with 1 consecutive matching character followed by 1 consecutive skip character, and another set with 2 consecutive matching characters followed by 1 consecutive skip character. The rationale behind the latter set is to capture any possible biases on the first two nucleotides of codons (based on the fact that the third nucleotide is often redundant in amino acid translation [16]), which, if present and not undermined by false matches from non-coding regions or matches with incorrect open reading frames [33], may be useful for the neural network to learn and use as additional features.

Each of the sets contains “skip-mers” [6] with different lengths such that the total number of matching characters are 4, 5, and 6 (which are the same as the numbers of characters that have to

match in the k -mer features). To summarize, the “skip-mers” [6] we used are as follows:

- Match 1 Skip 1 with a length of 7 (4 matching characters in total),
- Match 1 Skip 1 with a length of 9 (5 matching characters in total),
- Match 1 Skip 1 with a length of 11 (6 matching characters in total),
- Match 2 Skip 1 with a length of 6 (4 matching characters in total),
- Match 2 Skip 1 with a length of 7 (5 matching characters in total), and
- Match 2 Skip 1 with a length of 9 (6 matching characters in total).

Similar to the two other types of features, the “skip-mers” [6] are both used alone and combined with the other feature types. When it comes to using them along with k -mers, those with the same number of matching characters were paired together. For example, k -mer with $k = 4$ was used together with the match 1 skip 1 “skip-mer” [6] of length 7.

For each of the above configurations of “skip-mer” [6] and for a single sequence, similar to our approach with k -mers, the number of matches with each of the possible strings is counted. Each of these counts is then divided by the sum of the counts, forming a probability distribution of matches with the possible strings within the sequence. This distribution of matches is then used as the neural network input vector representing the sequence.

2.5 Deep Learning Models

Deep learning was used in a related study on host prediction from sequences of specific viral species [31], in which the authors had found that their deep learning based approach outperformed a preceding study of similar datasets using a different approach [21] [31]. Other studies involving RNA or DNA sequences have also found deep learning based approaches to be dependable [23], likely due to the ability of deep neural networks to recognize and uncover subtle patterns [40].

For each set of features (of a single type or multiple/combined types, as previously discussed), 3 deep neural network architectures differing only in depths were used, as we found that certain types or combination of features worked better with shallow networks while others worked better with deeper networks. The size of the input vector and the width of the network depend on the feature set being used. For sets consisting only of a single feature type, the input vector length and the network width are equal to the number of values representing an input sequence (e.g. 4^k for a feature set consisting only of k -mer for a specific k). Meanwhile, for sets with more than one feature type in it, the input vector length and network width are equal to the total number of values from all of the included features, as the values from the different features are concatenated. In all of the network architectures; each layer, except the last/output layer, is fully connected to the next layer (i.e. densely connected).

The 3 different neural network architectures are as follows:

- 2 consecutive dense layers with the “relu” activation function [34] (each of the layers is n in width, where n is the

number of values in the feature set being used), followed by a final dense layer with the “softmax” activation function (total depth = 3);

- 3 consecutive “relu”-activated [34] dense layers (each is n in width), followed by a “softmax”-activated dense layer (total depth = 4); and
- 4 consecutive “relu”-activated [34] dense layers (each is n in width), followed by a “softmax”-activated dense layer (total depth = 5).

The best of the 3 results from the different network architectures is then considered as the performance of the feature set. These architectures and other configurations, such as the number of epochs and learning rates, were determined by trying out different configurations, initially with early stopping estimating the optimal number of epochs and learning rates. This was done using 80% of the dataset as training set, 10% as validation set (for early stopping), and the last 10% as final test set. However, the training and evaluation of the final models were performed without early stopping, with 90% of entries used as training set, and the remaining 10% used as test set. These final models used to evaluate the performance of each feature set were trained using the “Adam” optimizer [19] for 300 epochs, starting with the default learning rate of 0.001 for the first 100 epochs, then reduced by half for each of the next 100 epochs (i.e. 0.0005 for epochs 101-200, then 0.00025 for epochs 201-300). Keras [5] with the Tensorflow backend [1] was used to build, train, and evaluate the deep neural networks. The sparse categorical crossentropy function built into Keras [1] was used as the loss function.

3 RESULTS AND DISCUSSION

Similar to our previous study on RNA secondary structure fingerprints [42], we evaluate the performance of the classifier trained using the different feature sets with 10-fold cross validation [13]. This means that the training and evaluation processes for each feature set were performed 10 times, in which the training and evaluation sets in each repetition differed from the others. In addition, as per our previous approach [42], the data splitting into different folds takes the distribution of hosts into account, which means that each fold has the same distribution of host species in it.

3.1 Primary Evaluation

Table 1 shows the features used in each set, and their 10-fold cross validation overall accuracy followed by the standard errors. The accuracy reported takes class imbalances into account. For each set, the highest accuracy achieved is indicated in bold.

When using only features of a single type at a time, sequence-based features (i.e. k-mers and “skip-mers” [6]) outperformed secondary structure based features in this study, with the longest “skip-mer” [6] matching 2 nucleotides then skipping 1 nucleotide performed best. When it comes to the secondary structure fingerprints, it’s also noticeable that combining the different scores derived from the minimum free energy (representing the most thermodynamically stable match of the secondary structure in the sequence), average free energy, and maximum free energy (least thermodynamically stable) of matches produced better results than using only the scores derived from the minimum free energy alone.

The highest accuracy at 86.89% was obtained by combining 6-mer with the match-2-skip-1 “skip-mer” [6] of length 9. None of the combined features of all 3 types managed to exceed this accuracy rate.

3.2 Additional Training and Evaluation with Limited Dataset

In order to test if the best performing feature set is consistent in datasets of different sizes, we have also evaluated our pipeline when trained with a limited amount of data. To perform this additional evaluation, we produced 2 different limited datasets: one is produced by keeping only sequences that are at most 80% similar using the CD-hit tool [12, 22], while the other only has sequences that are at most 90% similar. The lowest similarity threshold we used has been chosen by other studies involving RNA sequences as well [24, 36, 42, 47]. We used the same set of hosts/classes with our 80% and 90% datasets, but different than the set of hosts used with our primary dataset. This is due to the limited number of samples per class in these limited datasets. In addition, we reduced the minimum number of entries per class from 100 to 50, counted on our 80% dataset. In other words, only hosts that have at least 50 entries in the 80% dataset are used. We ended up with a set of 14 hosts, and they are as follows: *Allium sativum*, *Arthropoda*, *Astacoidea*, *Bos taurus*, *Culicidae*, *Gallus gallus*, *Hexapoda*, *Homo sapiens*, *Octopus*, *Odonata*, *Sarcosphaera coronaria*, *Solanum lycopersicum*, *Sus scrofa*, and *Vitis vinifera*. In the 80% dataset, 3,163 entries are of those hosts and therefore used; while in the 90% dataset, 5,781 entries are used.

Unlike the primary dataset evaluation, 10-fold cross validation was not used in this preliminary evaluation with the limited datasets (the standard errors of the 10-fold cross validation in the primary evaluation were found to be small as well). For each of the training and evaluation processes, 80% of the entries were used as training set, 10% used as validation set (for early stopping), and another 10% used as test set (to evaluate the final accuracy). Other than the aforementioned differences, the architectures of the deep learning models are the same as the models we used for the primary evaluation.

The resulting evaluation accuracy of each feature set for the 80% dataset is listed in table 2, while table 3 lists the results for the 90% dataset. Our findings indicate that the best performing set of features differed between each of the datasets. 5-mer combined with the match-2-skip-1 “skip-mer” of length 7 [6] performed best with the 80% dataset at 60.54% test set accuracy, while 6-mer alone performed best with the 90% dataset at 80.51%. These suggest that the best performing set of features on our primary evaluation may still not be the best when used with a more complete, future dataset.

4 CONCLUSIONS

To help control the spread of zoonotic RNA viruses, we proposed and tested a deep learning based pipeline to predict susceptible hosts (which may act as reservoirs for the viruses) from the viral sequence. Different types of features were extracted from the viral sequences, and their performance as inputs to the deep neural network were evaluated. These different types of features evaluated in this study are k-mers, “skip-mers” [6], and secondary structure fingerprints [42]. Unlike prior studies in host predictions [9, 21, 31],

Table 1: 10-fold cross validation results (accuracy and standard errors) for each feature set

K-mer	“Skip-mer” [6]		Skip	Secondary Structure Fingerprints	10-Fold Cross Validation Averaged Accuracy		
	Length	Match			3-Layers Model	4-Layers Model	5-Layers Model
4-mer		-		-	62.48% ± 0.51%	64.86% ± 0.76%	62.09% ± 0.77%
5-mer		-		-	77.29% ± 0.22%	75.24% ± 0.53%	74.31% ± 0.46%
6-mer		-		-	84.56% ± 0.28%	83.55% ± 0.48%	83.55% ± 0.57%
-	6	2	1	-	61.74% ± 0.31%	61.85% ± 0.94%	59.45% ± 1.0%
-	7	1	1	-	55.89% ± 0.34%	54.38% ± 0.99%	48.39% ± 1.86%
-	7	2	1	-	77.32% ± 0.5%	75.76% ± 0.8%	71.74% ± 1.83%
-	9	1	1	-	75.16% ± 0.41%	73.23% ± 0.46%	65.53% ± 4.57%
-	9	2	1	-	84.92% ± 0.25%	84.0% ± 0.36%	82.2% ± 1.13%
-	11	1	1	-	84.08% ± 0.21%	81.78% ± 0.98%	81.15% ± 0.88%
-		-		min. free energy	35.91% ± 0.42%	36.75% ± 0.76%	35.94% ± 0.51%
-		-		min., avg. free energy	50.65% ± 0.57%	52.04% ± 0.86%	52.6% ± 0.65%
-		-		min., avg., max. free energy	57.37% ± 0.52%	59.39% ± 0.58%	59.42% ± 0.76%
4-mer	6	2	1	-	71.57% ± 0.4%	71.69% ± 0.41%	71.15% ± 0.49%
4-mer	7	1	1	-	70.52% ± 0.39%	71.91% ± 0.38%	69.63% ± 1.01%
5-mer	7	2	1	-	82.14% ± 0.29%	82.08% ± 0.46%	80.1% ± 0.65%
5-mer	9	1	1	-	81.77% ± 0.47%	81.13% ± 0.39%	80.39% ± 0.69%
6-mer	9	2	1	-	86.89% ± 0.28%	86.09% ± 0.21%	84.68% ± 0.83%
6-mer	11	1	1	-	86.7% ± 0.38%	86.17% ± 0.61%	84.73% ± 1.58%
4-mer		-		min. free energy	67.96% ± 0.56%	70.97% ± 0.54%	72.6% ± 0.63%
5-mer		-		min. free energy	78.93% ± 0.24%	80.49% ± 0.62%	81.05% ± 0.46%
6-mer		-		min. free energy	84.33% ± 0.51%	84.05% ± 0.71%	77.7% ± 5.36%
4-mer		-		min., avg. free energy	69.92% ± 0.56%	72.28% ± 0.52%	75.38% ± 0.6%
5-mer		-		min., avg. free energy	74.93% ± 1.66%	81.28% ± 0.43%	81.02% ± 0.33%
6-mer		-		min., avg. free energy	83.42% ± 0.39%	83.73% ± 0.32%	82.23% ± 0.32%
4-mer		-		min., avg., max. free energy	71.14% ± 0.49%	74.63% ± 0.54%	75.85% ± 0.54%
5-mer		-		min., avg., max. free energy	79.28% ± 0.75%	80.74% ± 0.52%	81.23% ± 0.75%
6-mer		-		min., avg., max. free energy	83.21% ± 0.37%	83.53% ± 0.13%	81.87% ± 0.44%
-	6	2	1	min. free energy	66.94% ± 0.58%	69.98% ± 0.65%	71.02% ± 0.83%
-	7	1	1	min. free energy	66.83% ± 0.22%	69.69% ± 0.48%	71.23% ± 0.34%
-	7	2	1	min. free energy	78.66% ± 0.55%	80.35% ± 0.41%	80.72% ± 0.59%
-	9	1	1	min. free energy	77.78% ± 0.27%	80.05% ± 0.29%	79.43% ± 1.73%
-	9	2	1	min. free energy	84.58% ± 0.34%	79.17% ± 3.18%	80.65% ± 1.28%
-	11	1	1	min. free energy	83.61% ± 0.52%	83.88% ± 0.32%	77.77% ± 5.17%
-	6	2	1	min., avg. free energy	69.62% ± 0.49%	71.83% ± 0.74%	74.16% ± 0.6%
-	7	1	1	min., avg. free energy	68.11% ± 0.99%	71.45% ± 0.73%	73.93% ± 0.59%
-	7	2	1	min., avg. free energy	78.64% ± 0.35%	79.75% ± 0.85%	80.9% ± 0.32%
-	9	1	1	min., avg. free energy	78.48% ± 0.59%	79.58% ± 0.55%	81.29% ± 0.32%
-	9	2	1	min., avg. free energy	83.2% ± 0.54%	83.37% ± 0.43%	82.45% ± 0.57%
-	11	1	1	min., avg. free energy	82.83% ± 0.41%	82.75% ± 0.44%	82.3% ± 0.45%
-	6	2	1	min., avg., max. free energy	70.77% ± 0.42%	74.04% ± 0.75%	75.38% ± 0.36%
-	7	1	1	min., avg., max. free energy	69.28% ± 0.75%	74.32% ± 0.52%	74.74% ± 0.75%
-	7	2	1	min., avg., max. free energy	79.02% ± 0.58%	80.43% ± 0.52%	81.16% ± 0.52%
-	9	1	1	min., avg., max. free energy	78.73% ± 0.45%	80.42% ± 0.7%	81.3% ± 0.33%
-	9	2	1	min., avg., max. free energy	83.93% ± 0.2%	83.38% ± 0.42%	82.34% ± 0.63%
-	11	1	1	min., avg., max. free energy	83.04% ± 0.4%	83.0% ± 0.18%	82.47% ± 0.35%
4-mer	6	2	1	min. free energy	74.26% ± 0.46%	76.11% ± 0.66%	78.78% ± 0.3%
4-mer	7	1	1	min. free energy	74.22% ± 0.29%	77.44% ± 0.83%	78.54% ± 0.47%
5-mer	7	2	1	min. free energy	83.74% ± 0.39%	83.54% ± 0.39%	83.65% ± 0.18%
5-mer	9	1	1	min. free energy	82.17% ± 0.47%	83.21% ± 0.42%	83.07% ± 0.45%
6-mer	9	2	1	min. free energy	85.9% ± 0.28%	84.37% ± 0.71%	83.1% ± 0.73%
6-mer	11	1	1	min. free energy	85.86% ± 0.35%	84.94% ± 0.34%	82.39% ± 0.57%
4-mer	6	2	1	min., avg. free energy	75.06% ± 0.53%	77.33% ± 0.66%	78.89% ± 0.48%
4-mer	7	1	1	min., avg. free energy	74.78% ± 0.46%	77.11% ± 0.39%	78.47% ± 0.48%
5-mer	7	2	1	min., avg. free energy	82.52% ± 0.38%	82.77% ± 0.41%	82.68% ± 0.27%
5-mer	9	1	1	min., avg. free energy	81.26% ± 0.38%	82.59% ± 0.6%	82.37% ± 0.41%
6-mer	9	2	1	min., avg. free energy	84.39% ± 0.52%	84.2% ± 0.3%	82.54% ± 1.01%
6-mer	11	1	1	min., avg. free energy	84.33% ± 0.53%	84.19% ± 0.65%	82.06% ± 0.7%
4-mer	6	2	1	min., avg., max. free energy	75.73% ± 0.57%	79.56% ± 0.2%	79.65% ± 0.67%
4-mer	7	1	1	min., avg., max. free energy	75.77% ± 0.61%	77.99% ± 0.38%	79.23% ± 0.44%
5-mer	7	2	1	min., avg., max. free energy	82.54% ± 0.39%	82.92% ± 0.28%	82.16% ± 0.61%
5-mer	9	1	1	min., avg., max. free energy	81.41% ± 0.34%	83.13% ± 0.17%	81.55% ± 1.31%
6-mer	9	2	1	min., avg., max. free energy	84.73% ± 0.32%	83.71% ± 0.16%	82.33% ± 0.64%
6-mer	11	1	1	min., avg., max. free energy	84.61% ± 0.26%	83.33% ± 0.68%	80.4% ± 2.18%

Table 2: Performance of each feature set when trained and evaluated with a limited dataset without sequences that are more than 80% similar

K-mer	Length	Skip-mer Match	Skip	Secondary Structure Fingerprints	3-Layers Model	Test Set Accuracy 4-Layers Model	5-Layers Model
4-mer		-		-	44.22%	41.5%	40.14%
5-mer		-		-	46.26%	48.3%	35.37%
6-mer		-		-	53.74%	47.62%	36.73%
-	6	2	1	-	31.97%	46.26%	42.86%
-	7	1	1	-	33.33%	36.73%	35.37%
-	7	2	1	-	54.42%	34.01%	36.73%
-	9	1	1	-	42.18%	35.37%	32.65%
-	9	2	1	-	46.26%	49.66%	19.05%
-	11	1	1	-	47.62%	26.53%	26.53%
-		-		min. free energy	25.17%	25.17%	31.29%
-		-		min., avg. free energy	31.29%	31.97%	32.65%
-		-		min., avg., max. free energy	35.37%	36.05%	33.33%
4-mer	6	2	1	-	45.58%	48.3%	50.34%
4-mer	7	1	1	-	44.9%	44.22%	43.54%
5-mer	7	2	1	-	60.54%	56.46%	39.46%
5-mer	9	1	1	-	59.18%	50.34%	50.34%
6-mer	9	2	1	-	57.14%	50.34%	42.18%
6-mer	11	1	1	-	57.82%	44.9%	42.86%
4-mer		-		min. free energy	30.61%	31.29%	41.5%
5-mer		-		min. free energy	44.22%	46.94%	35.37%
6-mer		-		min. free energy	43.54%	44.22%	42.86%
4-mer		-		min., avg. free energy	37.41%	42.86%	40.82%
5-mer		-		min., avg. free energy	49.66%	48.3%	42.86%
6-mer		-		min., avg. free energy	40.14%	46.26%	44.22%
4-mer		-		min., avg., max. free energy	44.9%	37.41%	35.37%
5-mer		-		min., avg., max. free energy	42.86%	36.73%	36.05%
6-mer		-		min., avg., max. free energy	49.66%	42.18%	27.89%
-	6	2	1	min. free energy	39.46%	35.37%	30.61%
-	7	1	1	min. free energy	36.73%	36.73%	37.41%
-	7	2	1	min. free energy	46.94%	51.02%	36.73%
-	9	1	1	min. free energy	38.1%	34.69%	33.33%
-	9	2	1	min. free energy	51.02%	48.98%	37.41%
-	11	1	1	min. free energy	34.69%	34.69%	25.85%
-	6	2	1	min., avg. free energy	36.05%	33.33%	31.97%
-	7	1	1	min., avg. free energy	32.65%	31.97%	34.01%
-	7	2	1	min., avg. free energy	38.78%	44.9%	44.9%
-	9	1	1	min., avg. free energy	36.73%	34.01%	34.69%
-	9	2	1	min., avg. free energy	42.86%	37.41%	36.73%
-	11	1	1	min., avg. free energy	34.69%	31.97%	33.33%
-	6	2	1	min., avg., max. free energy	27.89%	34.01%	43.54%
-	7	1	1	min., avg., max. free energy	36.05%	29.93%	43.54%
-	7	2	1	min., avg., max. free energy	40.82%	38.1%	35.37%
-	9	1	1	min., avg., max. free energy	38.78%	38.78%	42.18%
-	9	2	1	min., avg., max. free energy	44.9%	43.54%	39.46%
-	11	1	1	min., avg., max. free energy	43.54%	38.1%	35.37%
4-mer	6	2	1	min. free energy	46.94%	45.58%	40.82%
4-mer	7	1	1	min. free energy	35.37%	41.5%	29.93%
5-mer	7	2	1	min. free energy	48.98%	46.94%	45.58%
5-mer	9	1	1	min. free energy	54.42%	48.98%	37.41%
6-mer	9	2	1	min. free energy	36.73%	42.86%	44.22%
6-mer	11	1	1	min. free energy	53.74%	42.86%	36.05%
4-mer	6	2	1	min., avg. free energy	37.41%	43.54%	38.78%
4-mer	7	1	1	min., avg. free energy	47.62%	44.22%	44.9%
5-mer	7	2	1	min., avg. free energy	44.9%	47.62%	37.41%
5-mer	9	1	1	min., avg. free energy	40.82%	40.14%	46.26%
6-mer	9	2	1	min., avg. free energy	53.06%	32.65%	35.37%
6-mer	11	1	1	min., avg. free energy	40.14%	40.14%	36.05%
4-mer	6	2	1	min., avg., max. free energy	46.94%	45.58%	42.86%
4-mer	7	1	1	min., avg., max. free energy	39.46%	45.58%	42.18%
5-mer	7	2	1	min., avg., max. free energy	44.22%	50.34%	47.62%
5-mer	9	1	1	min., avg., max. free energy	48.98%	48.3%	42.86%
6-mer	9	2	1	min., avg., max. free energy	45.58%	42.18%	32.65%
6-mer	11	1	1	min., avg., max. free energy	50.34%	46.26%	40.14%

Table 3: Performance of each feature set when trained and evaluated with a limited dataset without sequences that are more than 90% similar

K-mer	Length	Skip-mer Match	Skip	Secondary Structure Fingerprints	3-Layers Model	Test Set Accuracy 4-Layers Model	5-Layers Model
4-mer		-		-	52.72%	53.67%	59.74%
5-mer		-		-	72.52%	70.29%	70.61%
6-mer		-		-	80.51%	75.08%	71.57%
-	6	2	1	-	56.23%	62.3%	65.81%
-	7	1	1	-	41.21%	44.09%	52.08%
-	7	2	1	-	75.4%	71.25%	71.88%
-	9	1	1	-	48.24%	64.54%	61.98%
-	9	2	1	-	79.87%	71.25%	67.73%
-	11	1	1	-	68.37%	68.37%	66.45%
-		-		min. free energy	39.94%	38.02%	39.94%
-		-		min., avg. free energy	51.44%	48.88%	59.11%
-		-		min., avg., max. free energy	46.33%	48.56%	48.88%
4-mer	6	2	1	-	71.25%	70.93%	52.72%
4-mer	7	1	1	-	72.2%	69.33%	71.57%
5-mer	7	2	1	-	78.27%	77.0%	73.8%
5-mer	9	1	1	-	69.97%	65.81%	69.65%
6-mer	9	2	1	-	76.36%	71.88%	68.69%
6-mer	11	1	1	-	72.84%	71.57%	53.99%
4-mer		-		min. free energy	61.66%	61.98%	49.2%
5-mer		-		min. free energy	67.09%	72.2%	69.01%
6-mer		-		min. free energy	72.2%	69.97%	61.98%
4-mer		-		min., avg. free energy	63.58%	59.42%	63.9%
5-mer		-		min., avg. free energy	64.22%	66.77%	66.77%
6-mer		-		min., avg. free energy	69.65%	63.9%	58.47%
4-mer		-		min., avg., max. free energy	59.74%	60.06%	65.18%
5-mer		-		min., avg., max. free energy	65.81%	64.22%	56.23%
6-mer		-		min., avg., max. free energy	64.86%	63.26%	52.08%
-	6	2	1	min. free energy	60.7%	59.42%	65.5%
-	7	1	1	min. free energy	42.17%	48.24%	47.92%
-	7	2	1	min. free energy	60.06%	69.01%	70.29%
-	9	1	1	min. free energy	44.73%	47.6%	63.26%
-	9	2	1	min. free energy	68.69%	69.01%	68.05%
-	11	1	1	min. free energy	71.25%	68.37%	64.86%
-	6	2	1	min., avg. free energy	53.99%	58.47%	55.91%
-	7	1	1	min., avg. free energy	54.31%	53.04%	52.72%
-	7	2	1	min., avg. free energy	59.11%	67.41%	69.33%
-	9	1	1	min., avg. free energy	63.26%	61.66%	64.86%
-	9	2	1	min., avg. free energy	71.57%	63.58%	65.81%
-	11	1	1	min., avg. free energy	69.33%	66.13%	62.62%
-	6	2	1	min., avg., max. free energy	57.51%	57.19%	59.74%
-	7	1	1	min., avg., max. free energy	53.35%	53.35%	52.08%
-	7	2	1	min., avg., max. free energy	57.83%	62.62%	59.74%
-	9	1	1	min., avg., max. free energy	51.76%	63.26%	65.81%
-	9	2	1	min., avg., max. free energy	67.41%	68.37%	64.22%
-	11	1	1	min., avg., max. free energy	62.3%	63.9%	61.66%
4-mer	6	2	1	min. free energy	67.09%	64.22%	65.5%
4-mer	7	1	1	min. free energy	60.7%	59.74%	47.28%
5-mer	7	2	1	min. free energy	77.0%	72.2%	66.13%
5-mer	9	1	1	min. free energy	70.29%	72.84%	71.88%
6-mer	9	2	1	min. free energy	76.04%	68.69%	66.45%
6-mer	11	1	1	min. free energy	78.59%	77.32%	61.66%
4-mer	6	2	1	min., avg. free energy	60.06%	60.38%	61.02%
4-mer	7	1	1	min., avg. free energy	66.77%	66.45%	65.18%
5-mer	7	2	1	min., avg. free energy	67.73%	68.37%	63.58%
5-mer	9	1	1	min., avg. free energy	70.61%	66.77%	69.33%
6-mer	9	2	1	min., avg. free energy	77.32%	69.97%	63.58%
6-mer	11	1	1	min., avg. free energy	73.48%	69.65%	57.51%
4-mer	6	2	1	min., avg., max. free energy	67.73%	65.18%	68.69%
4-mer	7	1	1	min., avg., max. free energy	59.11%	63.9%	63.9%
5-mer	7	2	1	min., avg., max. free energy	66.13%	61.34%	66.13%
5-mer	9	1	1	min., avg., max. free energy	71.88%	68.37%	66.13%
6-mer	9	2	1	min., avg., max. free energy	77.32%	74.76%	59.42%
6-mer	11	1	1	min., avg., max. free energy	67.09%	62.3%	58.15%

we did not limit our dataset to specific species of RNA viruses. A 10-fold cross-validated highest prediction accuracy of 86.89% was achieved by combining 6-mer with match-2-skip-1 “skip-mers” [6] of length 9. However, preliminary evaluation of the feature sets with artificially limited datasets suggests that this combination may not necessarily be the best performing set for a more complete dataset in the future, as the best performing set of features was found to be different for each of the datasets. In addition, our results indicate that the structure-based features failed to provide a significant advantage to the overall performance of the trained deep neural networks, despite the conservation [17, 41] and known function of RNA secondary structures in certain virus-host interactions [18].

When it comes to limitation, our approach has only been evaluated with datasets from a single source, the NCBI Virus [3]. This means that any possible limitations in the source, such as possibly missing hosts for a sequence of RNA virus, could have impacted the evaluation of our proposed approach. Furthermore, for a single sequence of RNA virus, only the top host predicted by the neural network is currently considered – this study has not investigated the lower-ranked predicted hosts, which may or may not be susceptible or act as reservoir hosts. Finally, the approach has only been developed for and tested with complete sequences of RNA viruses, whereas the majority of RNA virus sequences available from NCBI Virus are annotated as “partial” [3]. A future study could adapt our approach so that host susceptibility can be predicted from partial viral sequences, which may be useful for cases where only incomplete sequences of a specific virus are available.

5 AVAILABILITY

The dataset used in this study (with the secondary structure fingerprints) is available at: <https://www.eecs.uottawa.ca/~turcotte/icbbt2021>.

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