SPECIES CLASSIFICATION FROM SHORT GENOMIC READS USING FEEDFORWARD NEURAL NETWORKS

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Species Classification from Short Genomic Reads using Feedforward Neural Networks

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ABSTRACT

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With the cost of Next Generation Sequencing technologies in decline, the need for fast and efficient classification of genomic findings has become of utmost importance. Due to the output length limitations of most Second Generation Sequencing techniques, it is important that we are able to classify short reads of DNA. In this research, we trained a basic Artificial Neural Network model with three hidden layers on short reads(50-500 bp) taken from two species' reference genomes. We selected Escherichia Coli and Saccharomyces Cerevisiae for their short and well-studied reference genomes. Their taxonomic difference makes them ideal candidates for ascertaining the viability of using the whole genome for species classification. We then classified these short reads. We achieved moderate success with a classification accuracy of $80\% - 91\%$ corresponding to differing hyperparameters and read lengths. We documented the encountered issues and considered future directions.

Keywords: Bioinformatics, Classification, Machine Learning, Neural Networks

ÖZ

BESLEMELİ SİNİR AĞLARI KULLANARAK GENOMİK KISA OKUMALARDAN TÜR SINIFLANDIRMASI

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Yeni Nesil Dizileme teknolojilerinin maliyetlerinin düşmesi ile genomik bulguların hızlı ve verimli sınıflandırılmasının önemi artmıştır. İkinci Nesil Dizileme tekniklerinin çıktı uzunluklarındaki kısıtlamalardan dolayı, kısa DNA okumalarını sınıflandırabilmemiz önem taşımaktadır. Bu çalışmada iki türe ait referans genomlardan alınan kısa okumalar(50-500 bp) ile üç saklı katmana sahip temel bir Yapay Sinir Ağı modelini eğittik. Kısa ve iyi çalışılmış referans genomlara sahip olduklarından Escherichia Coli ve Saccharomyces Cerevisia türlerini seçtik. Bu türlerin taksonomik olarak farklı olması, sınıflandırmada tüm genomu kullanma üzerine bir araştırmanın konusu olmak bakımından onları ideal adaylar haline getirdi. Daha sonra referans genomlardan alınan bu okumaları sınıflandırdık. Çeşitli üst değişkenler ve okuma uzunlukları kullanarak elde ettiğimiz modellerin eğitim süreçleri sonunda uygulanan sınıflandırma işlemlerinde $\%80 - \%91$ aralığında doğruluğa eriştik. Karşılaştığımız sorunları belgeledik, geleceğe yönelik önerilerde bulunduk.

Anahtar Kelimeler: Biyoenformatik, Sınıflandırma, Makine Öğrenmesi, Sinir Ağları

To my beloved family and friends...
Aileme ve dostlarıma...

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LIST OF ABBREVIATIONS

WGS	Whole Genome Sequencing
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
FNN	Feedforward Neural Networks
ANN	Artificial Neural Networks
NN	Neural Networks
RNN	Recurrent Neural Networks
CNN	Convolutional Neural Networks,
PCR	Polymerase Chain Reaction
NGS	Next Generation Sequenci
LSTM	Long Short Term Memory
GRU	Gated Recurrent Unit
GPU	Graphics Processing Unit
PCR	Polymerase Chain Reaction
ddNTP	Dideoxyribonucleotide
NaN	Not a Number

CHAPTER 1

INTRODUCTION

Identifying species has been around since there were cave paintings. Primitive classification criteria included; size, colour, number of limbs, mobility etc. It also was limited to the species the people encountered, which was not much. The first great generalizer in classification was Aristotle, who virtually invented the science of logic, of which classification was a part for 2,000 years. The Aristotelian method dominated classification until the 19th century. Carolus Linnaeus, who is usually regarded as the founder of modern taxonomy and whose books are considered the beginning of modern botanical and zoological nomenclature, drew up rules for assigning names to plants and animals and was the first to use binomial nomenclature consistently. [1] There was something that differentiated all the species but it was yet unknown.

Discovery of DNA was a big step in uncovering this unknown factor. This was followed by the discovery of the central dogma. Genes were identified and linked to proteins. These proteins were observed inside and outside of the cells and were found to be responsible for almost every single function any living organism has.

In later years, the world continued to embrace digital technologies and the field of bioinformatics emerged. Computers offered analysis humankind could not perform by hand, and provided visualization that made concepts easier to understand. As the sequencing technologies evolved and costs reduced, getting a species' whole genome became a regular task. This led to the collection of a large amount of genomic data.

The advancement in digital technologies in other fields entailed the collection of large amounts of data in those fields as well. This led to the introduction of the term *big data*. The sheer size of big data led to the development and refinement of machine learning methods. Genomic data, despite not being macro-scale, has been classified as big data due to its immense size. Advancement in the classification and compression of such data is critical to allow scientific progress in bioinformatics.

1.1 Motivation

The task of species classification has been tackled in the past in a few ways. The most basic solution was the alignment of sequences to compare differences and to determine a species. In order to be deterministic, this required the use of a large sequence.

Then Hebert et al.[2] proposed a system where one would select a specific position in the sequence where the nucleotides differed interspecies but were mostly unchanged intraspecies. Then this gene could be used as a DNA barcode. Some genes that fit this criterion were found for animals(COI[3], Cytob[4], 12S[5], 16S[5]). Most of these were mitochondrial genes and were selected because they lacked intronic regions, were haploidically inherited, and had limited recombination. For some kingdoms other than the animal kingdom, a combination of genes was selected to successfully apply this technique. For some others, no such combination was found. The weakness of using DNA barcoding was the requirement of a reliable reference library, which was extremely difficult to create in a comprehensive manner. It also lacked a standard protocol to use.

The use of deep learning allowed the classification task itself to evolve from basic alignment of the sequences to computing distances and training neural network models. These models were trained most commonly on the genes that were formerly selected to be used in DNA barcoding, since such genes were already shown to be present in multiple species and to be mostly unique interspecies. However, this method was still held back by the limitations of DNA barcodes, because they were performing the classification by using only certain parts of the DNA.

DNA barcoding requires either annotated data or large sequences to work, so that an alignment can be performed to determine the barcode area in the sample. But second generation sequencing machines can output sequences ranging from 75 to 400 base long sequences. Therefore we need a network that can classify reads of these length.

1.2 Scope and Goal

In this study we are taking the whole reference genomes for two species: Saccharomyces Cerevisiae (Baker's Yeast) [6] and Escherichia Coli (E. Coli) [7] and training a deep learning model to identify short reads(50-500 bases) taken from the reference genomes using an artificial neural network. The goal is to successfully identify the species of any short read we have sampled out of the reference genomes themselves.

1.3 Contributions of the Study

This study provides a neural network model that can handle the task of binary classification using short(50-500 bp) reads taken from the reference genomes of two species to successfully($\sim 83\%$ accuracy for 50 bp,$\sim 91\%$ accuracy for 500 bp) classify similarly generated reads.

This network can be extended to classify more than two species. In which case, it can prove to be a strong tool to classify certain reads where other methods in the literature fall short. Classifying regions where comprehensive DNA barcode information is lacking has not been explored extensively. Research on short reads of non-barcode regions is even less common. For some domains viable DNA barcodes have not yet been discovered.

1.4 Structure of the Thesis

Chapter 2 contains the background information for the subjects included in the thesis. Chapter 3 considers the specifications of the neural network model and the details of the training process. Chapter 4 presents the test results. Chapter 5 discusses the results, compares this study to other similar studies and offers the conclusions of the thesis and possible future directions. In Appendices A and B are the python code files used to prep the data and build, train and test the neural network, respectively. In appendix C is the table with software versions installed in the conda environment.

CHAPTER 2

BACKGROUND

2.1 Discovery of DNA

The origins of the discovery of hereditary similarities date back as far as Ancient Greece, where Hippocrates built a foundation for what is now known as Charles Darwin's Pangenesis(1868) [8]. Mendel's work(1866) [9] solidified the existence of a genetic structure housed within living creatures and is passed down using a dual encoded method to offsprings. Around the same time, Meisner discovered an irregular phosphorus-rich compound, unlike any protein, calling it *nuclein*. This nuclein would later go on to be called DNA(Deoxyribonucleic acid). With the help of X-ray crystallography and former various theories, Watson and Crick theorized a shape for what this genetic material could be, giving birth to the double-helix structure DNA is known to be today [10].

2.2 Sequencing

Sequencing is the process of experimentally determining the sequence of bases that construct an organism's genetic materials. There are different methods used to sequence the genetic materials, an extensive list of used machines can be found in Table 1.

2.2.1 PCR

It is a commonly used molecular biology technique that aims to make multiple copies of a region of DNA. A double-stranded DNA molecule is broken apart and is built back together using the primers that are provided. Therefore effectively replicating the DNA molecule.

2.2.2 Sanger sequencing

Also known as the chain termination method, the dideoxynucleotide method, or the sequencing by synthesis method. First discovered by Frederick Sanger[11] and his colleagues in 1977. It played an important role in the Human Genome Project.

As illustrated in Figure 1[12], Sanger Sequencing is composed of three steps.

Generation	**Platform**	**Instrument**	**Reads per run**	**Avg Read Length (pb)**	**Year**
First Generation	ABI Sanger	3730xl	96	**400–900**	2002
Second Generation	454	GS20	200	**100**	2005
	454	GS FLX	400	**250**	2007
	454	GS FLX Titanium	1M	**450**	2009
	454	GS FLX	1M	**700**	2011
	454	Titanium+	1M	**700**	2011
	454	GS Junior	100	**400**	2010
	454	GS Junior+	100	**700**	2014
	Illumina	MiniSeq	25M (max)	**150**	2013
	Illumina	MiSeq	25M (max)	**300**	2011
	Illumina	NextSeq	400M (max)	**150**	2014
	Illumina	HiSeq	5B (max)	**150**	2012
	Illumina	HiSeq X	6B (max)	**150**	2014
	SOLiD	5500 W	3B	**75**	2011
	SOLiD	5500xl W	6B	**75**	2013
	Ion Torrent	PGM 314 chip v2	400,000-550,000	**400**	2011
	Ion Torrent	PGM 316 chip v2	2M-3M	**200**	2011
	Ion Torrent	PGM 318 chip v2	4M-5.5M	**400**	2013
	Ion Torrent	Ion Proton	60M-80M	**200**	2012
	Ion Torrent	Ion S5/S5XL 520	3M-5M	**400**	2015
	Ion Torrent	Ion S5/S5XL 530	15M-20M	**400**	2015
	Ion Torrent	Ion S5/S5XL 540	60M-80M	**400**	2015
Third Generation	PacBio	RS C1	432	**1300**	2011
	PacBio	RS C2	432	**2500**	2012
	PacBio	RS C2 XL	432	**4300**	2012
	PacBio	RS II C2 XL	564	**4600**	2013
	PacBio	RS II P5 C3	528	**8500**	2014
	PacBio	RS II P6 C4	660	**13500**	2014
	PacBio	Sequel	350	**10000**	2016
	Oxford Nanopore	MinION Mk	100	**9545**	2015
	Oxford Nanopore	PromethION	NA	**9846**	2016

Table 1: Sequencing Technologies and Specifications

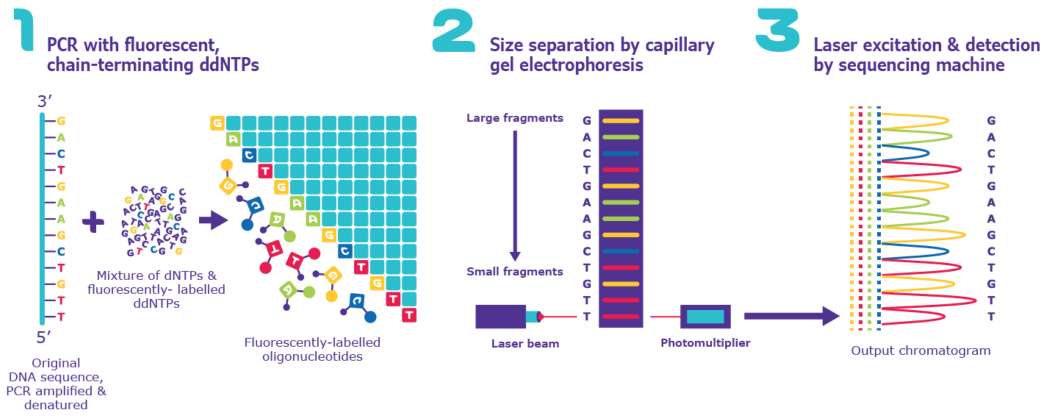

1 PCR with fluorescent, chain-terminating ddNTPs
2 Size separation by capillary gel electrophoresis
3 Laser excitation & detection by sequencing machine
3'
5'
Original DNA sequence, PCR amplified & denatured
Mixture of dNTPs & fluorescently- labelled ddNTPs
Fluorescently-labelled oligonucleotides
Large fragments
Small fragments
Laser beam
Photomultiplier
Output chromatogram

Figure 1: Sanger Sequencing Pipeline

- 1) PCR with fluorescent, chain-terminating ddNTPs

  Chain-terminating PCR is applied. Similar to standard PCR the goal is to replicate the sequence, but with one major difference: the addition of modified nucleotides (dNTPs) called dideoxyribonucleotides (ddNTPs). In the extension step of standard PCR, DNA polymerase adds dNTPs to a growing DNA strand by catalyzing the formation of a phosphodiester bond between the free 3'-OH group of the last nucleotide and the 5'-phosphate of the next Figure 1.

- 2) Size separation by capillary gel electrophoresis

  The oligonucleotides are put in a gel matrix and applied an electrical current. Because DNA is negatively charged, the oligonucleotides are pulled, at different speeds due to their size. This helps order them by size.

- 3) Laser excitation & detection by sequencing machine

  Since DNA polymerase starts its work from the provided primer, by reading the gel bands from smallest to largest, the 5' to 3' sequence of the original DNA strand can be determined.

This method was revolutionary at its time but proved too slow and too expensive as alternative approaches emerged.

2.2.3 Next Generation Sequencing(NGS)

2.2.3.1 Second Generation Sequencing

Second Generation Sequencing saw the introduction of massively parallel sequencing with Illumina. Soon followed by Ion Torrent. They excelled at sequencing many small parts quickly and at a low cost. For this reason, they excelled at resequencing projects and SNP calling. Their shortcoming was their short reads of $\sim 50 - 200$bp.

2.2.3.2 Third Generation Sequencing

Third generation sequencing technologies allow for much longer read lengths.

2.2.4 Gene sequence file types

Gene data has different formats it can be stored in depending on the specific information it contains. It can contain sequence data, annotation data, quantitative data and read alignments.

- FASTA[13]

  Can be used to represent either nucleotide or amino acid sequences. Can have the following extensions: .fasta, .fna, .ffn, .faa, .frn, .fa.

```
>BK006935.2 TPA_inf: Saccharomyces cerevisiae S288C chromosome I, complete sequence
ccacaccacacccacacacccacacaccacaccacacaccacaccacacccacacacacacatCCTAACACTACCCTAAC
ACAGCCCTAATCTAACCCTGGCCAACCTGTCTCTCAACTTACCCTCCATTACCCTGCCTCCACTCGTTACCCTGTCCCAT
```

Figure 2: Example FASTA file

- FASTQ:

  This is an extension of FASTA. Also includes the quality score for the elements indicating how confident the measurement is of that value.

```
@2fa9ee19-5c51-4281-abdd-eac8663f9b49 runid=f53ee40429765e7817081d4bcdee6c1199c2f
91d sampleid=18S_amplicon read=109831 ch=33 start_time=2019-09-12T12:05:03Z
CGGTAGCCAGCTGCGTTCAGTATGGAAGATTTGATTTGTTTAGCGATCGCCATACTACCGTGACAAGAAAGTTGTCAGTCT
TTGTGACTTGCCTGTCGCTCTATCTTCCAGACTCCTTGGTCCGTGTTCAATCCCGGTAGTAGCGACGGGCGGTGTATGTAT
TATCAGCGCAACAGAAACAAAGACACC
+
%&&-&%$%%$$$#)33&0$&%$''*''%$#%$%#+-5/---*&&%$%&())(&$#&,'))5769*+..*&(+28./#&122
8956:7674';:;80.8>;91;>?B=%.**==?(/'($$$$*'&'**%&/));807;3A=;88>=?9498++0%"%%%%'#
&5/($0.$2%&0'))*'%**&)(.%&&
```

Figure 3: Example FASTQ file

2.3 DNA Barcoding

With the idea put forward by Hebert et al.[2], the mitochondrial gene cytochrome c oxidase I (COI) was established as the core of a global bio identification system for animals. Finding such an identifier for plants wasn't as easy, since mutation in plants was a much rarer occurrence. For other species, there are systems using multiple genes to create a barcode.

2.4 Machine Learning

With the increase in processing power of computers, it became possible to analyze whole genomes. Pattern recognition, anomaly detection, clustering,and classification were tasks that could be automated. Which led to even more information to be extracted from genetic materials.

The goal of most machine learning models is to construct a model and fit that model to the task/input at hand in a way that can produce the desired outcome. This fitting process needs to be adjusted according to the results it produces with both its original training set and similar yet non-identical test set. According to its scores on these tests, its fit is determined.

2.4.1 Underfitting

If the machine learning model can not successfully learn, it can be seen on its scores. If the model underperforms in both the training and the test set, it is said to be **underfit**. Underfitting can be due to an insufficient learning time, a simple model structure or over regularization. It can be overcome by changing these.

2.4.2 Overfitting

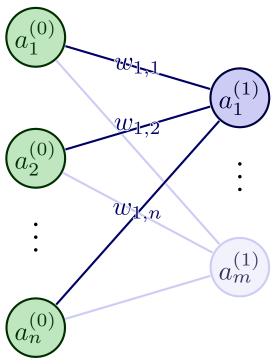
If the machine learning model performs well specifically on the set it was trained on, and underperforms in the test set; it is said to be **overfit**, meaning that it captured the specifications of the training set too well when it was desired to be more accommodating of a wider range of data. The training set could be enriched with more diverse data points.

2.5 Neural Networks

As the technology advanced, humankind analyzed what makes intelligence, or even consciousness, and tried to mimic it. The trials of simulating the biological neural networks of the brain tissue led researchers to Artificial Neural Networks. The core structure of Neural networks is as follows:

- There is an input layer and an output layer. There may be varying numbers of "hidden layers" in between.
- Each layer contains a certain number of nodes. These nodes are connected to other nodes in adjacent layers. This enables the flow of information.
- These connections each have weights and associated *activation functions*. This manipulates the flow of information to, hopefully, extract, store and infer a certain piece of information that belongs to the input. (This piece of information can be positive/negative for a sentence, containing a cat for an image, belonging to a specific species for DNA in our case)
- These weights are the main holders of information. The other specifications of the model are called "hyperparameters" and are configured according to the specific model structure and input data type used to extract the intended information out of the input data.

- Since numbers and mathematical operations are used throughout the model, the values of the output layer can be represented in terms of the values of the input layer, the weights of the connections between nodes, the *activation function* and a correcting value called *bias*. By feeding the desired outcome and the input to the model, one can tell the model to resemble that outcome.
- In this case, a function to calculate resemblance/difference/distance is needed. This is where the *loss function* comes in. Using the *loss function*, one can determine how *alike* the model is to the ideal model. The weights can then be adjusted in a way so that the model is more similar to this ideal model. How much change each learning step makes is called the *learning rate*.



$$\begin{pmatrix} a_1^{(1)} \\ a_2^{(1)} \\ \vdots \\ a_m^{(1)} \end{pmatrix} = \sigma \left[\begin{pmatrix} w_{1,0} & w_{1,1} & \cdots & w_{1,n} \\ w_{2,0} & w_{2,1} & \cdots & w_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ w_{m,0} & w_{m,1} & \cdots & w_{m,n} \end{pmatrix} \begin{pmatrix} a_1^{(0)} \\ a_2^{(0)} \\ \vdots \\ a_n^{(0)} \end{pmatrix} + \begin{pmatrix} b_1^{(0)} \\ b_2^{(0)} \\ \vdots \\ b_m^{(0)} \end{pmatrix} \right]$$

$$a^{(1)} = \sigma \left(\mathbf{W}^{(0)} a^{(0)} + \mathbf{b}^{(0)} \right)$$

Figure 4: Neural Network Activation

2.5.1 Encoding

Encoding is used to represent categorical(specifically nominal in our case) data so that it can be used in a mathematical fashion.

- **One-hot Encoding**

$$\text{Blonde} \rightarrow \{1,0,0\} \qquad \text{Brown} \rightarrow \{0,1,0\} \qquad \text{Grey} \rightarrow \{0,0,1\}$$

Figure 5: One-hot Encoding for Hair Color Data

  Useful for nominal data where one cannot quantify the difference between the classes. Can also be used for ordinal data with ease, however, capturing the actual distances between values will be out of the question. Increases the dimensions of the data significantly. Easy to quantify differences and calculate distances due to its high dimensional nature. Every class increases the dimensions, which can lead to issues, so not very useful when the classes are high in number. A dummy case can be added where all the values are 0 which would reduce the total number of dimensions by 1.

- **Label/Ordinal Encoding**

  Useful for ordinal data where you can order the classes. Even though the difference/distance between the points will not be accurate when calculated, they will be comparable to each other and will be statistically indicative.

Blonde $\longrightarrow$ $\{1, 0\}$ Brown $\longrightarrow$ $\{0, 1\}$ Grey $\longrightarrow$ $\{0, 0\}$

Figure 6: Dummy Encoding for Hair Color Data

Underweight $\longrightarrow$ 1 Healthy weight $\longrightarrow$ 2 Overweight $\longrightarrow$ 3 Obese $\longrightarrow$ 4

Figure 7: Label Encoding for Weight Data

Although it is best suited for ordinal data, label encoding can also be used on non-ordinal data. In such a case it is prudent to be aware that the distance between *Blonde-Brown* and *Brown-Grey* will be identical and will be the half of *Blonde-Grey*. This is nonsensical in terms of the data used, so some metrics should not be given attention.

- **Target-Mean Encoding**

  Compared to other encoding types, Target Encoding encodes the data with values that are indicative of the target. Most commonly the **mean** of an indicative column is used. Can be used in a way where the value is calculated without using that specific value, called the Leave one out method.

- **Frequency/Count Encoding**

  Can be useful in displaying the distribution of a set. Every value is encoded with the count of instances it has in the dataset, or the overall *frequency(count/total count)*.

2.5.2 Gradient Descent

A gradient is the partial derivatives of a function at a given point. Gradient descent is an optimization algorithm that tries to minimize the gradient, achieving a local minimum. There are some issues that can be encountered regarding this algorithm:

2.5.2.1 Vanishing Gradients Problem

During backpropagation, gradients are used to update the weights. In this step, if the gradients keep getting smaller where they are taken as zero, the weights will not be updated since the algorithm believes it has reached a local minimum point, when in fact it has not. As a result, the gradient descent algorithm never converges to the optimal solution. Can be spotted by slow or non-existent learning process[14]

Blonde $\longrightarrow$ 1 Brown $\longrightarrow$ 2 Grey $\longrightarrow$ 3

Figure 8: Label Encoding for non-ordinal Hair Color Data

2.5.2.2 Exploding Gradients Problem

On the other hand, if the gradients become so large they can make the gradient descent diverge instead. This can occur when the denominator approaches zero, overflowing the gradient, making it NaN at which point it can no longer be worked on. Can be spotted by large changes at every step or even NaN valued losses.

2.5.3 Hyperparameters in Deep Learning

A big part of the machine learning process is fine-tuning everything. Split into two model structure parameters and training (learning) parameters

2.5.3.1 Model (Structure) Related Parameters

- **Number of hidden layers/neurons**

  A neural network model with more than three layers(one input layer, one output layer, one or more hidden layers) is considered a deep learning method. The number of hidden layers may depend on the number of features desired to be captured from the input data. Also increasing the number of hidden layers and neurons tend to increase the accuracy, but can eventually cause overfitting, just as too few layers can cause underfitting.[15]

- **Dropout**

  The models are trained on specific data, but often times are expected to be effective on similar yet not identical data. For this reason, it is essential not to overfit. Dropout is a method to overcome overfitting. Some nodes in the model are temporarily removed from the model so that some randomness(noise) can be added into the training process. The goal is to break up situations where network layers co-adapt to correct mistakes from prior layers, in turn making the model more robust.[16]

- **Momentum**

  Momentum allows the learning process to easily get to where it needs to. It achieves this by adding the previous update vector into the next batch. This allows for easier convergence.

- **Weight initialization**

  There a few options that can be used to initialize the weights between the nodes. Assigning all zeros or ones is an option, or to add some noise, like before, random values can be assigned. But the the general vicinity of this randomness can be controlled, using Normal or Uniform distributions to select the random values. The specifications of the distributions(mean, standard deviation, min, max) are then become the hyperparameters to tune the models

- **Activation Function**

  As can be seen on Figure 4 the activation function(σ) is used in the calculation of the nodes throughout the learning process. They are used to introduce non-linearity to the model. Different activation functions used:

  - Tanh

    $tanh(x)$. Provides values between (-1,1).
  - Sigmoid

    $\frac{1}{1+e^{-x}}$
  - Rectified Linear Units (ReLU)

    Offers a non-negative value by taking the $max(0, x)$. Produces values between $[0, \inf)$

- **Loss function**

  The loss function determines the fit of the model. The goal of training the model is to minimize the difference between the desired output and the actual output of the model. The loss function helps us achieve that. During training the loss function eventually leads into a local minimum. The local minimum is desired to be a global minimum. Commonly used loss functions are as follows:

  - Mean Squared Error

    MSE is calculated by taking a mean of the squares of the distances between points in the data.
  - Cross-entropy

    Useful for calculating the distance(difference) between categorical data points. Cross-entropy loss is also called logarithmic loss, log loss, or logistic loss. Each predicted class probability is compared to the actual class desired output 0 or 1 and a score/loss is calculated that penalizes the probability based on how far it is from the actual expected value. The penalty is logarithmic in nature yielding a large score for large differences close to 1 and small score for small differences tending to 0. [17] Cross-entropy is expressed by the equation;

    $$l(p, q) = -\sum p(x) log q(x) \tag{1}$$

    Where *x* represents the predicted results by ML algorithm, *p(x)* is the probability distribution of the "true" label from training samples, and *q(x)* depicts the estimation of the ML algorithm.

    * Categorical Cross-entropy is used for classification tasks where multiple classes are present.
    * Binary Cross-entropy is used in binary classification tasks.

2.5.3.2 Training(Optimization) Related Parameters

- **Learning rate**

  Learning rate indicates how much correction will be made in response to the error calculated by the loss function after each epoch.

- **Number of epochs**

The number of epochs determine how many times the training process will be repeated on the training data. To achieve convergence, the training process may need to be repeated a number of times. The rule of thumb to determine the correct number of epochs is to stop when the validation error starts to increase, which can be an indicator of overfitting.

- **Batch size**

  The batch size determines how many data runs the model will go through before it updates its weights.

2.5.4 Specialized Neural Networks

In addition to the core design of neural networks explained above, there are more specialized versions that are more adept at extracting/storing certain patterns in data.

- **Convolutional Neural Networks (CNN)**

  As neural networks tried to mimic the process of learning, Fukushima(1980)[18] tried the same for the human visual pattern recognition capability. The previous models lacked the ability to handle inputs of varying shapes. But by moving the learning process through the input this obstacle was overcome. Like the animal visual cortex of the human body, the neurons correspond to multiple points of the input that are adjacent to them and store them as a single representative value. By doing this with multiple neurons, the whole data can be covered.

  A further simplification process called pooling usually follows. In pooling multiple points are reduced to one neuron mainly by *max()* or *avg()* functions. By repeating these steps multiple times it is possible for different layers to represent different properties in the input.

  CNNs excel at pattern recognition in a variety of data types, specifically image and video data.

  Most common example encountered when CNNs are concerned is the MNIST database of handwritten digits[19]. In this example, the power of CNNs is obvious. By having multiple convolutional layers, it is possible to capture lines->connected lines->numbers in order.

- **Recurrent Neural Networks (RNN)**

  RNNs use sequential or time series data. These deep learning algorithms are commonly used for ordinal or temporal problems, such as language translation, natural language processing (NLP), speech recognition, and image captioning. They are distinguished by their “memory” as they take information from prior inputs to influence the current input and output. The output of recurrent neural networks depend on the prior elements within the sequence. There are bidirectional RNNS that also take into account the outputs that would follow this output in order.

  RNNs share parameters across each layer of the network which need to be tuned similarly to feedforward networks. The weights on the RNNs are adjusted through the processes of backpropagation and gradient descent to facilitate reinforcement learning.

  RNNs utilize a process called backpropagation through time (BPTT) where the errors it has is summed throughout the training process. Due to this method, RNNs can easily encounter two problems, known as vanishing gradients and exploding gradients. These issues are defined by the size of the gradient, which is the slope of the loss function along the error curve. Where the weights are either too small or too large. In which case the weights become insignificant or not a number(NaN) respectively. One solution to these issues is to reduce the number of hidden layers within the neural network, eliminating some of the complexity in the RNN model.[20]

  – Long-Short Term Memory (LSTM) [21]

    Developed to combat the vanishing/exploding gradient problems, LSTMs contain a forget gate, which can help the model learn better. [22]

  – Gated Recurrent Unit (GRU)[23]

    GRUs are simpler versions of LSTMs, they have fewer gates(just a reset and update gates instead of the forget, input, and output gate of LSTM) therefore fewer parameters but they are less powerful and adaptable.

- **Autoencoders**[24]

  Autoencoders are similar to Artificial Neural Networks in that they essentially have the same architecture. The difference in Autoencoder architecture is that their network always resembles an hourglass like shape, with layers getting smaller closer to the middle.

  The first half of the network is called the encoding part and the second part the decoding part. Backpropagation is used to train the network. They are symmetrical and during the training process the error is calculated as the difference of the output to the input trying to achieve true symmetry. This means the goal is to encode(compress) the input to fit the smallest middle hidden layer(s) to then be able to recreate the input as the output.

  – Variational Autoencoders[25] VAEs have the same architecture as AEs but they use the approximated probability distribution to train.

2.6 Related Work

Abd–Alhalem et al.[26] compares DNA sequence classification algorithms that use deep learning. They split the algorithms in three: alignment based methods, alignment free methods and ML-DSP(Machine Learning - Digital Signal Processing). They point out the rise in popularity of deep learning in DNA sequence classification due to their remarkable performance and reduction of challenges. They emphasize the need for a high amount of data for deep learning to perform successfully.

Rizzo et al.[27] use 16S gene sequences to taxonomically classify bacteria species down to the genus level. They randomly select 1000 sequences from each of the three most common bacteria phyla, Actinobacteria, Firmicutes, Proteobacteria, collecting in total 3000 sequences. All the sequences have length greater than 1200 bp, They use short reads of length 500 bases. They use a Convolutional Neural Network on spectral representations of the genomes. They have very high accuracy on higher (Phylum, class) taxonomic levels, but as they approach the genus level, they require the use of full length sequences to achieve $> 50\%$ accuracy. But when using full length sequences, they achieve $> 85\%$.

Busia et al.[28] also use 16S gene sequence but for a much wider range of species. They also use a CNN. They have $19,851$ sequences with $13,838$ from $2,768$ genera. They tested their model on read lengths $L = 25, 50, 100, 150, 200$. They used 16S mock community sequencing data and they also test on synthethic data they simulate base-flipping noise on. Their results suggests that their approach of injecting random base-flipping noise at train time allows the DNNs to learn a more robust noise model than the one implicitly incorporated by aligners like BLAST and BWA by allowing gaps and mismatches to be tolerated. Overall, this study acts as a first step towards their long-term goal of developing a general-purpose deep learning model that can successfully perform any task framed as the assignment of labels to short biological sequences.

Weitschek et al.[29] introduce BLOG(Barcoding with LOGic) 2.0 it uses a supervised machine learning approach that extracts species-specific positions of the DNA Barcode sequences from the training set and formulates rules on these positions. Since it is learning its rules on specific positions of the data, it requires a complete reference library of polymorphisms for each species in the training set to avoid false negatives. They use DNA barcode sequence files in FASTA format that are either of the same region or have been pre-aligned. They achieve 92% accuracy on fungi and 100% accuracy on algae.

Yang et al.[30] use DNA barcode sequences to classify species. They use convolutional methods, treating one-hot encoding as its own dimension and convoluting and pooling in 2D. Afterwards taking the flattened pooling results through hidden layers. Using ReLu as its general activation function and softmax in the last layer to compute the probabilities of the species.

Cui et al.[31] propose a genomic sequence compression algorithm based on a deep learning model and an arithmetic encoder. Their deep learning model is structured as a convolutional layer followed by an attention-based bi-directional long short-term memory network, which predicts the probabilities of the next base in a sequence. The arithmetic encoder employs the probabilities to compress the sequence.

CHAPTER 3

MATERIAL AND METHOD

The neural network model was implemented using the Keras toolkit, which is based on TensorFlow libraries in Python programming language.[32] [33] [34] The data is prepared and dumped into files using *pickle*. This preparation step will especially be useful should there be new genes added to the dataset. Because, since it won't be possible to fit all the data in the memory at once, batches will need to be implemented due to the increase in the space required. As the species selected had exceptionally small DNA lengths, any addition to the dataset (which would likely involve species with longer DNA) will significantly increase the size.

A virtual environment was used to perform the training and testing using Anaconda[35]. We used its package and environment manager for the command line interfacing tool Conda(version 4.12.0). The list of installed relevant packages and their versions can be found on Table C.

3.1 Data Preparation

Two reference genomes were used to train and test the model: Saccharomyces Cerevisiae(Baker's Yeast)[6] and Escherichia Coli(E. coli)[7]. These species were selected mainly due to their small sized and more importantly well-studied DNA sequences. They are also taxonomically very different which makes them ideal candidates for ascertaining the viability of using the whole genome for species classification. Saccharomyces cerevisiae is a eukaryote whereas eschericia coli is a prokaryote. We wanted to span a wider range of species. The genomes were extracted out of their FASTA files and preprocessed to contain nothing but the genome sequences.

E. coli reference genome consists of 4,699,673 nucleotides and is 4.48 MB in size.

Baker's Yeast reference genome consists of 12,309,078 nucleotides and is 11.7 MB in size.

Uniform distribution is used to randomly select short reads of length 50 to 500 out of the DNA data. Equal number of samples are collected out of both samples. This decision is discussed further in 5.2.

The files were reformatted to contain only lowercase letters for a unified representation of each nucleotide (thus disregarding the information for repeating sequences indicated by the uppercase letters). Then the data is encoded into a one-hot encoding format. With the special case of the character "N" which can be used to represent the existence of a non-specific nucleic acid, 5 categories were used for the encoding process. The transformation matrix is shown on Figure 9.

a-A $\rightarrow$ $\{1, 0, 0, 0, 0\}$

t-T $\rightarrow$ $\{0, 1, 0, 0, 0\}$

c-C $\rightarrow$ $\{0, 0, 1, 0, 0\}$

g-G $\rightarrow$ $\{0, 0, 0, 1, 0\}$

n-N $\rightarrow$ $\{0, 0, 0, 0, 1\}$

Figure 9: Encoding of the Data

3.2 Model structure

The model consists of six layers as can be seen on Figure 10. One input layer, one flatten layer, three hidden dense layers, and one output layer.

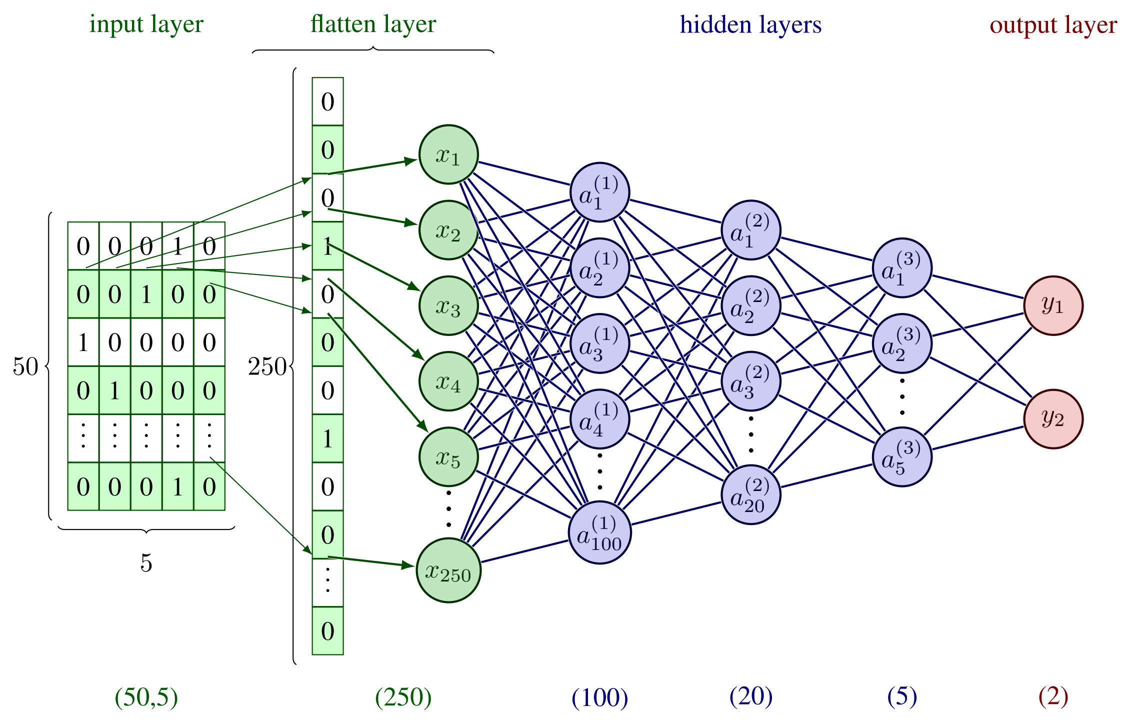


Figure 10: Overview of the Neural Network

The data is taken through a Flatten() layer to reformat the data back into a 1D format. The model consists of three fully connected hidden layers. The hidden layers are separated by activation layers.

3.3 Hyperparameters

The number of layers determines the information that can be extracted from the data. We initially selected three hidden layers for our model. When we tested whether lower number of layers was feasible, we saw that three layers gives us the best results(Figure 11).

We selected our output layer to have two nodes, one for each species. After every test case we check the output layer and select the highest value to be the result of our classification. We can also compare the outputs of our network to determine how confident the classification has been.

We use initializers to assign certain values to the weights in the network to jump-start the learning. We used Keras' initializer that samples Random Normal distribution. Its default specifications are *mean=0.0, stddev=0.05*, further tests on these values were performed. The values that gave the best results were 0.09 for standard deviation. The mean did not seem to affect the model in a sensible way. For this reason the mean was kept as 0.0 since it gave as good a result as any value (4.1.6).

The activation functions determine the flow of information in the network. The activation functions we considered for our network are as follows: Tanh, Sigmoid, ReLU, Exponential. We opted to use Tanh activation function as trying different activation functions proved difficult with our setup(4.1.4). We tried different activation functions for the last layer, the one that improved the accuracy best was ReLU.(4.1.5)

Loss functions help the network learn by providing a metric with which can be optimized to get the desired outcome. Because we have categorical data, the loss functions we considered were: Categorical Cross-entropy and Binary Cross-entropy. We used categorical cross-entropy initially since it allowed us to later add new classes. We then found out that using categorical cross-entropy for binary classification resulted in us getting inconsistent results(further discussed in 5.1.1).

The learning rate determines how much each batch is going to affect the model. It can be adjusted to overcome certain convergence issues. We used Keras' Adam optimizer which defaulted to 0.001 learning rate. As we tested multiple learning rates, 0.0006 was seen to provide better results(4.1.3).

3.4 Training

Initially, we set a seed to achieve reproducibility of the results. We used *tf.keras.utils.set_random_seed (seed)* since it sets seeds for Python, Numpy and TensorFlow's randomized operations. Despite setting these seeds, some variance between runs persisted. Further investigation revealed that using the GPU introduced some randomness due to order of operations. To circumvent this issue *tf.config.experimental .enable_op_determinism()* was used. This option makes the training process deterministic, eliminating some parallel qualities therefore reducing the overall performance of the training process.

The time required to train the model ranges from 15 seconds to 250 seconds with five epochs. The main factor affecting the training times is *Read Length*. We take our reads in a way that the total length of the reads will be double the size of the original DNA. Therefore as the read length increases number of reads decrease. With less reads to train, the training process naturally takes shorter. We were unable to find a direct correlation of anything else with the training time. This could be due to the noise in the training time data since the other processes running in the device can alter the training times.

We tried three devices throughout the project. Even though the devices had similar setups, the training times were changed tenfold. Rather than investigate the cause of this issue, we continued using the fastest device.

3.5 Testing

The testing process is aimed towards adjusting the hyperparameters in the network. We used Accuracy Score(Equation 2) and F1 Score(Equation 3) to measure test success. F1 score is effective in indicating a class imbalance in some cases where accuracy seems to have increased.

We take the Baker's Yeast as the positive outcome in our tests. This is due to it being the first species in the species list.

$$Accuracy = \frac{TruePositives + TrueNegatives}{TruePositives + FalsePositives + TrueNegatives + FalseNegatives} \tag{2}$$

$$F1 = \frac{2 * Precision * Recall}{Precision + Recall} = \frac{2 * TruePositives}{2 * TruePositives + FalsePositives + FalseNegatives} \tag{3}$$

We use *sklearn.model_selection.train_test_split(test_size=0.20)* to reserve 20% of our initial data to use for validation and testing. The validation set is fed to the network during its training. After the training is done, we run this test data through Keras' *predict()* function to get its predictions. By comparing these predictions to the actual classes we can judge the performance of our model.

After all of our tests was performed, it was pointed out that our validation test being the same as our test set could lead to overfitting. So we compared the results of an 80/20/20 split and an 80/10/10 split. The results can be seen on Table 2. It is important to point out that in these cases when splitting the data into 80/20/20 the validation set and the test set becomes identical but when splitting the data into 80/10/10 the validation set and the test set are separate. The results of this adjustment didn't prompt us to perform all of our previous tests again since it had a minimal effect on the network results where accuracy and F1 scores are concerned.

Train Set	Test Set	Validation Set	Accuracy	F1 Score
80%	20%	20%	0.894	0.886
80%	10%	10%	0.896	0.888

Table 2: Result Comparison on Different Test and Validation data setups

CHAPTER 4

RESULTS

4.1 Experiments

4.1.1 Testing Number of Hidden Layers

As an initial decision, the number of hidden layers was set to be three. In this experiment we tested different numbers of hidden layers in a similar model structure. In Figure 11 we can see their highest performing specifications' accuracy values.

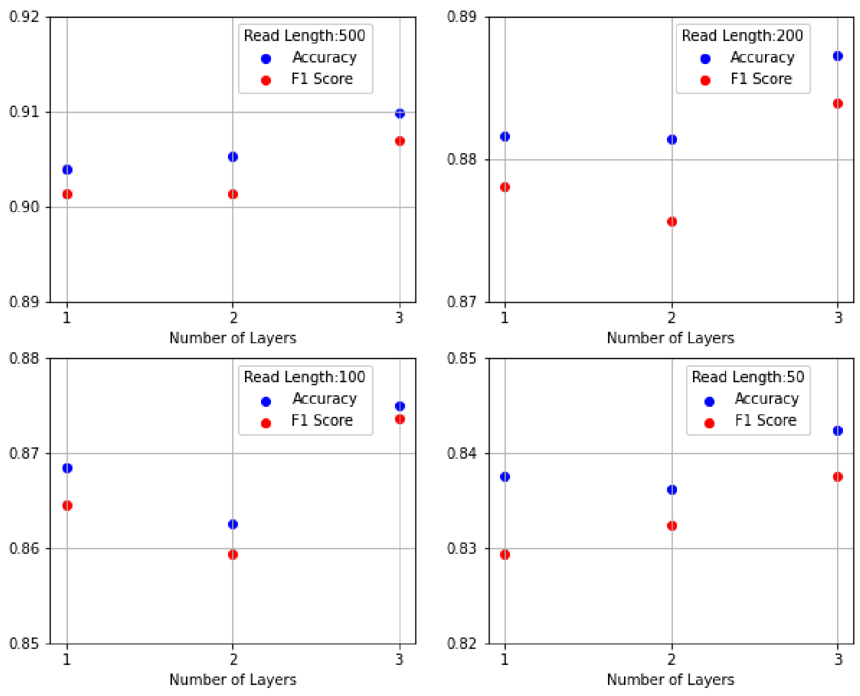


Figure 11: Testing Number of Hidden Layers

4.1.2 Testing Read Length and Layer Sizes

As can be seen on Figure 12, the increase in the length of the reads of DNA we used correlates with an increase in accuracy, which was to be expected. However, since the goal was to find solutions for short reads of data, reads of 50 were kept in all other tests.

Layer sizes of 200/100/20 was found to be the best performing for read length 50. 1000/100/20 for 500 read length.

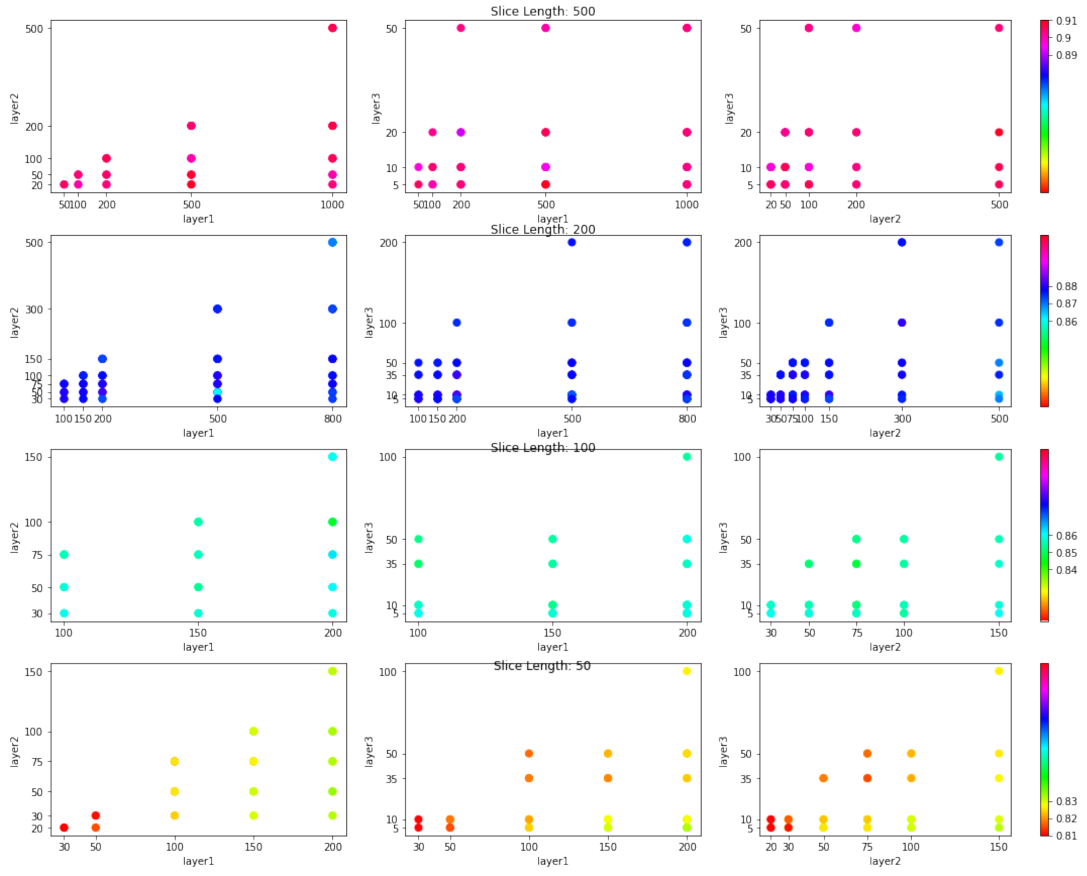


Figure 12: Test of Layer Sizes

4.1.3 Testing Learning Rates

Figure 13 clearly shows an ideal range of learning rate values to use($[0.005, 0.01]$). As the values differ from this range the performance of the model decreases.

On a network of input size(read length) 500, the optimal learning rate seemed to be much lower than the initial values as Figure 14 shows. We selected 0.00003 to be the optimal value, since it was surrounded by more consistent results.

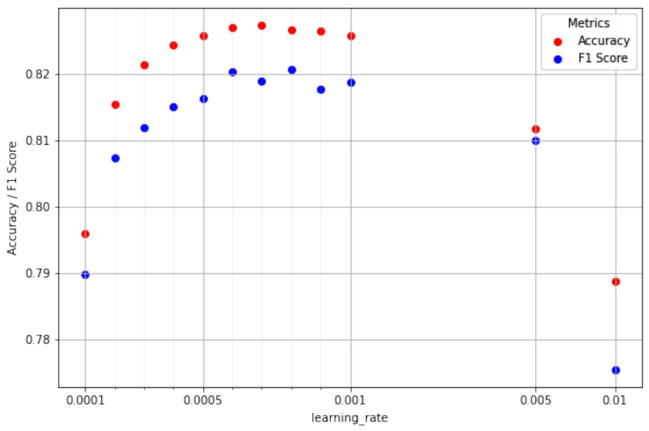


Figure 13: Test of Learning Rates on Read Length 50

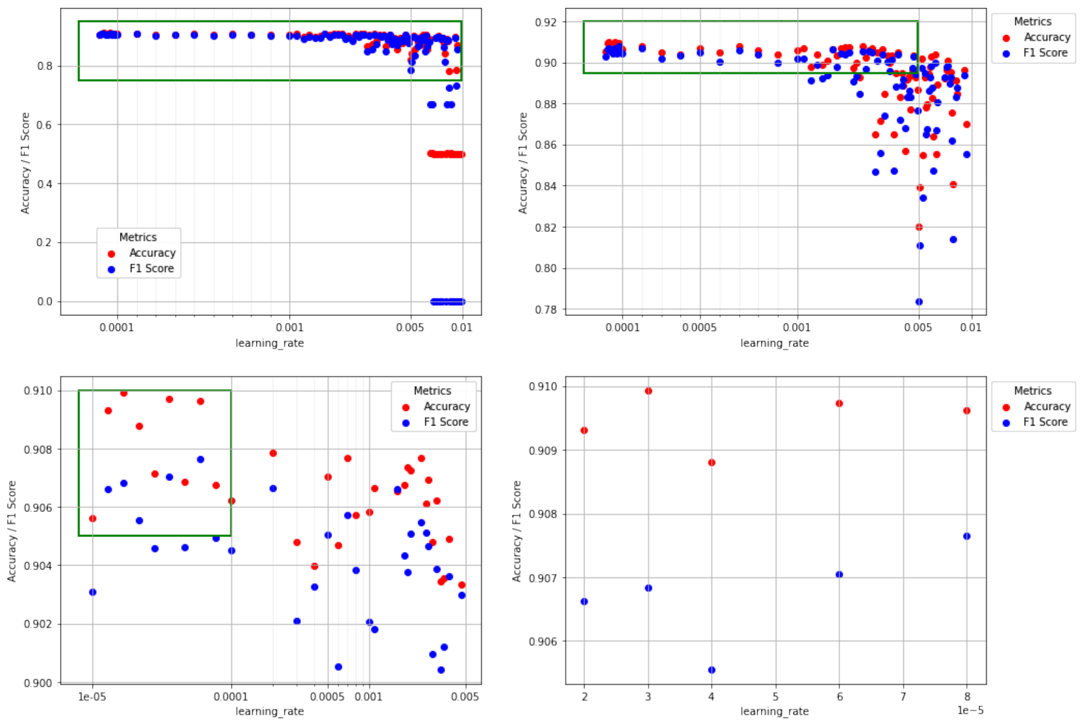


Figure 14: Test of Learning Rates on Read Length 500

4.1.4 Testing Different Activation Functions

Table 3 has the results of the different activation function test. The only viable option here is tanh. The other cases are due to vanishing and exploding gradients. This is further discussed in chapter 5.

Function	Accuracy	F1		Epoch 1	Epoch 2	Epoch 3	Epoch 4	Epoch 5
Tanh	**0.8361**	**0.8266**	Loss	0.5786	0.5485	0.5390	0.5353	0.5330
			Val Loss	0.5568	0.5442	0.5426	0.5359	0.5362
Sigmoid	0.4989	0.6657	Loss	0.6931	0.6931	0.6931	0.6931	0.6931
			Val Loss	0.6932	0.6932	0.6932	0.6932	0.6932
ReLU	0.5017	0.6682	Loss	0.6268	0.6134	0.6087	0.6064	0.6050
			Val Loss	0.6172	0.6123	0.6089	0.6078	0.6074
Exponential	0.5002	0.6668	Loss	NaN	NaN	NaN	NaN	NaN
			Val Loss	NaN	NaN	NaN	NaN	NaN

Table 3: Accuracy Test for Different Activation Functions

4.1.5 Testing Different Activation Functions for the Output Layer

Activation Function	Accuracy	F1 Score
tanh	0.827	0.821
sigmoid	0.827	0.826
relu	**0.835**	**0.829**
exponential	0.828	0.826
softmax	0.829	0.826

Table 4: Accuracy Test for Different Activation Functions on Output Layer

Table 4 shows us that a final layer with ReLu activation performs slightly better.

4.1.6 Testing Initializer Specs for Normal Distribution

4.1.6.1 Standard Deviation

The standard deviation values *keras.initializers.RandomNormal(stddev, mean)* uses to set the initial node weights seems to have a range in which it lets the network train the best $[0.07, 0.09]$.(Figure 15)

When testing on read length 500 reads we found $[0.03, 0.05]$ to be the optimal range of values as can be seen on Figure 16.

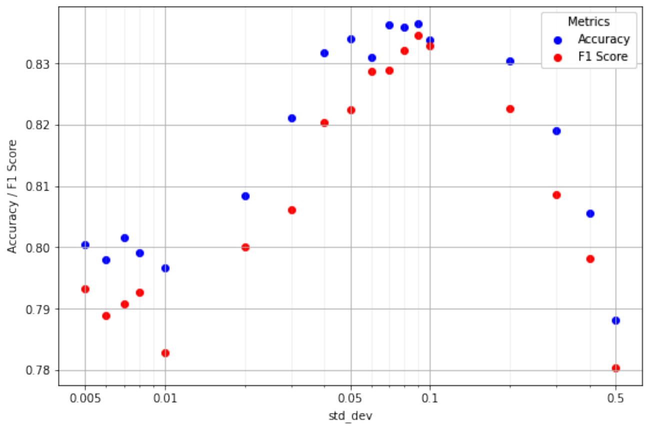


Figure 15: Test of Standard Deviations for Initializer

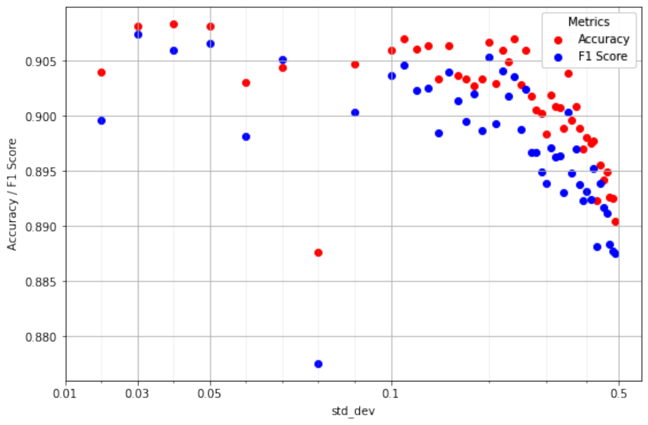


Figure 16: Test of Standard Deviations for Initializer on read length 500

4.1.6.2 Mean

The mean values *keras.initializers.RandomNormal(stddev, mean)* uses to set the initial node weights doesn't seem to have a significant effect on the model's performance.(Figure 17

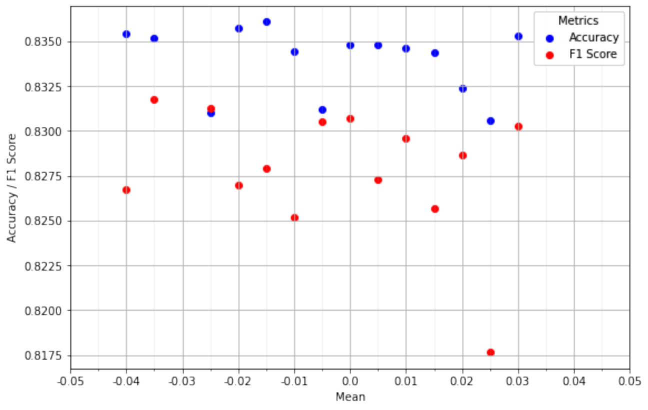


Figure 17: Test of Means for Initializer

4.1.7 Testing Different Sample Species Representation Methods

In Table 5 Different representation of species in the samples were investigated. This test was conducted for two different read lengths: 50 and 500. When the samples are taken proportional to the length of the reference genomes of the species, the accuracies seem to increase. But the decrease in F1 score show us that this is in fact not the case. This leads us to think that there is a big imbalance of results between classes/species.

Read Length	Number of Samples		Accuracy	F1 Score
	Ecoli	Yeast		
50	185666	486284	0.869	0.746
	486284	486284	0.836	0.826
500	18566	48628	0.919	0.849
	48628	48628	0.903	0.900

Table 5: Accuracy Test for Different Sampling Methods regarding Species Representation

CHAPTER 5

DISCUSSION AND CONCLUSION

We have explained our network and presented our results in the previous chapters. Now we will talk about some of the issues we have encountered throughout the development process and some things to consider in further development.

5.1 Issues Encountered

5.1.1 Loss Selection

We were stuck randomly getting inverted accuracy scores. This issue persisted for a long time. The accuracy would, seemingly randomly, switch between $\sim 80\%$ and $\sim 20\%$.

This looked similar to a labelling issue we previously had where the labels of the classes were mixed and we were getting all the results as $\sim 20\%$. But the differences were that the switch between positive and negative scores were at random and not in batches unlike our previous problem. Also the Accuracy scores weren't exactly complementary. In our previous problem the labels in the test set were mixed so the training process was untainted, which led to accuracy scores that would add exactly up to 1. But that wasn't the case here.

During random tests we discovered that the issue resulted from the use of *tf.keras.losses.Categorical Crossentropy*. We had used Categorical Crossentropy so we could, in the future, add more classes to the network. But as it stands our task is binary classification. Our assumption was that both Binary Crossentropy and Categorical Crossentropy would give us the same results in a binary classification task. Apparently Categorical Crossentropy is not suitable for use with only two classes. A switch to *tf.keras.losses. BinaryCrossentropy* resolved our issue.

5.1.2 Gradients in Activation Function Selection

During our tests to find the most suitable activation function for the network, we encountered some nonsensical results. As can be seen on 4.1.4 Tanh function was the only feasible option. We think these nonsensical results are due to vanishing and/or exploding gradients.

As explained in 2.5.2, a vanishing gradient problem can be spotted by a slow or non-existent learning process. For our test on Sigmoid function, we can see from the losses that the network hasn't learned at all. In case of ReLU function, as illustrated in Table 6 when compared to the first epoch of Tanh function's losses, the reduction in loss (which usually indicates learning) is very low. We can deduct from this slow loss reduction and low accuracy that the model doesn't seem to be learning, $\sim 50\%$ accuracy that the gradients are vanishing. For Exponential function the losses are all NaN which is probably an indicator of integer overflow. This means that the value of the loss has become so high that the value can not be represented by the programming language. This is most probably due to exploding gradients. Gradient clipping can be performed to overcome this issue but since Tanh is a perfectly acceptable option, we did not explore the other options further.

Function	Epoch 1	Epoch 2	Epoch 3	Epoch 4	Epoch 5
		-5%	-2%	-0.7%	-0.4%
Tanh	0.5786	0.5485	0.5390	0.5353	0.5330
ReLU	0.6268	0.6134	0.6087	0.6064	0.6050
		-2%	-0.7%	-0.4%	-0.2%

Table 6: Decreased Loss Reduction in Vanishing Gradient Problem

However, we continued to observe outcomes similar to those of the vanishing gradient problem. These happened mostly on edge cases while we were performing wide range of values on our hyperparameters. We tracked this particular issue to our loss function. Loss functions in Keras/TensorFlow have *from_logit = False* as default. This option makes the loss function assume that all the values it gets will be $(-1, 1)$. But since we are using Tanh activation function for intermediary nodes and ReLU for our output layer, therefore not normalizing our weights, we needed to set *from_logit = True* for our loss function. This makes the loss function apply the softmax function to normalize the output values before it uses them.

5.1.3 Achieving Determinism

As we mentioned before, we used *keras.utils.set_random_seed()* to set seeds for python, numpy and tensorflow. But we still weren't able to get deterministic results. Runs with same specifications were providing similar yet non-identical results. Upon further investigation we found that the randomness was due to the randomness introduced by the order of processes in the GPU. To overcome this we set *tf.config.experimental.enable_op_determinism()*. With this option we achieved true deterministic results. This determinism came at a cost of training time since the GPU was no longer using multiple cores to optimize the process.

5.1.4 Using Keras' Own Metrics

Keras has its own metrics that can be used to track the learning process. We encountered various errors when trying to use them. We later found these errors to be caused by two things: one of them is because we set *tf.config.experimental.enable_op_determinism()*, and the other is due to our use of logits, since these metrics were incompatible with logits. These issues are fixed in newer versions of Tensorflow but due to some dependencies we have, we were unable to upgrade Tensorflow.

5.1.5 Memory/Time Issues in Training

When training our network, our code took a very long time, and in some specifications the device would run out of memory. We then realized that since we were using numpy.append() to read the data into an array, at every step a new array would be created which increased the time and space it took. Creating an empty array first and then filling it resolved this issue.

With the current two species setup, batching isn't required. Should there be more species added to the dataset, there will become a need for batching the data, since it may not be possible to load all of the data into memory all at once.

Similarly, initially we would read the data file, sample, train and test all in the same file. We then separated the files. prep.py(A) file reads and samples the data. It then dumps the sampled reads into a pickle file. test.py(B) reads the sampled reads from the pickled file, creates and trains the network and tests and outputs the specifications of the test and its results.

As pointed out before, we tried three different devices throughout the project. One of the devices was 10 times slower without any apparent differences that could cause this. We continued the tests using the fastest device.

5.2 Representation of Species in the Data

We initially selected the number of samples for each species to be proportional to their length. This decision was due to the possible addition of different species to the data. Because in such a case, the size of the samples would increase exponentially. The sampling size differences in a hypothetical inclusion of the species Drosophila Melanogaster can be found on Figure 18. Drosophila Melanogaster reference genome consists of 145,523,498 nucleotides and is 138 MB in size therefore the lengths of the genomes in the figure closely represent a real-life case.

In Case A, the space the samples take up is $\sum_{i=0}^{n} l_i$ where n is the number of species in the data, and l_i is the length of the genome of the i^{th} species in the data. With the introduction of the third species Drosophila, $3n$ total data size would increase to $31n$.

However, in Case B, the space the samples take up is $l_{max} * n$ where n is the number of species in the data, and l_{max} is the length of the longest genome. $4n$ total data size would increase to $84n$.

This is indicated in the prep.py(A) file with a flag *species_length_equalized*. 0 for Case A and 1 for Case B.

In Table 5 we see Case A results have their accuracy higher but their F1 score lower. This indicates a class imbalance because we are testing the species with test data proportional to their training set. Therefore there are more test cases for Yeast than E. coli. Which makes Yeast more dominant in the overall accuracy result. Since E. coli's training set was smaller, the network was also more overfit on

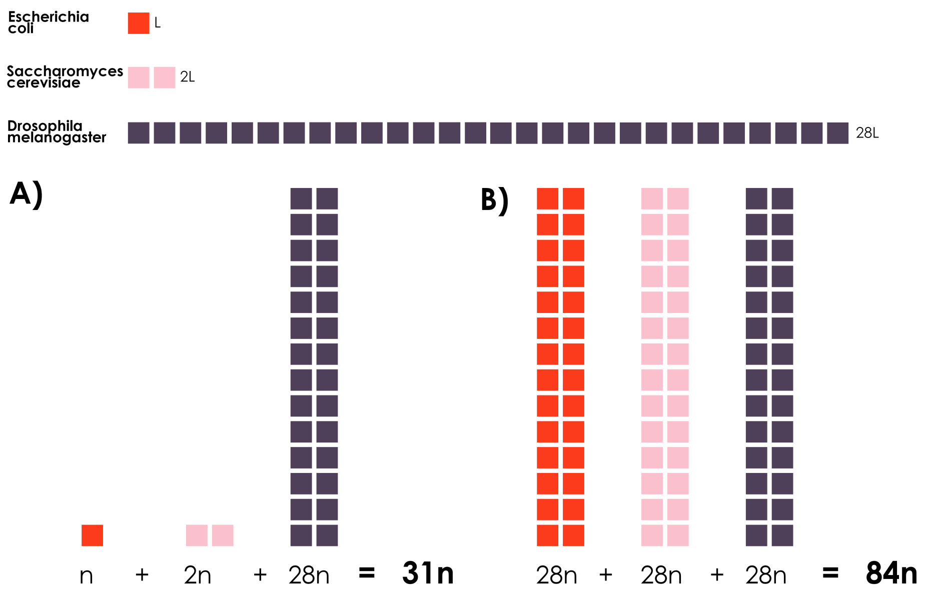


Figure 18: Visual representation of different sampling cases in case of the inclusion of Drosophila melanogaster as a third species

yeast. So when the more trained Yeast has a bigger weight in the overall score the accuracy becomes higher.

F1 score takes into account precision and recall and is more informative in cases of class imbalance. F1 scores clearly show that the Case B is significantly better at classification. That is why we have opted to use the same number of samples for both species.

As we previously stated we took Baker's Yeast as our positive outcome. During the tests we performed, F1 Score was always lower than the Accuracy Score. This can lead us to believe that the negative outcome which means classifying a read as E. coli is more likely. Since we performed these tests using Case B; E. coli is more represented in the training and test set which could cause this. Another reason might be due to the simplicity of the E. coli genome. This can be further investigated by comparing different species with genome simplicity in mind.

5.3 Using Synthetic Short Reads vs. Real-life Sequencing Outputs

We have tested our network on synthetic short reads taken from the reference genomes of the selected species. This was because our initial goal was fast classification of a long genome by taking multiple short reads and taking an average of the results we got. But more tests can be performed on real-life short reads to see how the network performs in such cases. Another option is to introduce base-flipping noise as elaborated on (Busia et al., 2019)[28].

Upon using such real-life sequences, the following topics should be considered:

- **Reads of Different Sizes**

  Most sequencers do not produce fixed length results. The lengths of the reads produced can vary. Due to the nature of non-recurrent neural networks, the read length of the input on this network is fixed. However, there is a fifth category in the one-hot encoding that can be used in such an occasion. All the reads can be aligned to the left and padded with the character "N" In such a case, the model would need to be trained on such padded reads in the first place, or else it wouldn't know how to handle them.

- **Possibly Mutated Sequences**

  All genetic material mutates in some way or another. This is not reflected on this study. Reads that are not exactly present in the trained set can affect the performance of the network. It will however, classify the read as the species it finds it to be closer to. Therefore especially SNPs should not propose a big challenge for this network.

- **Assay Contamination**

  Due to the small nature of the DNA, there is always a possibility of a contamination in the assay. Since we are training our model on labeled data, if the label is incorrect, it could worsen the performance of the model.

- **Sequencing Errors**

  The error rates of NGS technologies have been shown to be around 0.1% [36]. This can obviously affect the performance of the model.

5.4 Specifications of the Output Layer

We have two nodes on our output layer, one for each species the network has trained on. We feed the desired outputs the same way we do our one-hot encoded inputs. $[1, 0]$ for first class(Yeast), $[0, 1]$ for second class(E. coli). Another option we had was to use one layer for binary classification $[0]$ for yeast, $[1]$ for e. coli. But using one node for each species gives us the opportunity to measure how confidently the network has classified a certain read.

We have used ReLU activation function on the output layer since it provided us with the best results. ReLU function maps our tanh function's $[-1, 1]$ range into $[0, \inf)$. Since we have two species that do not have much in common genomewise, almost all of the results provide very similarly symmetric results when tanh function is used on the output layer(eg. 0.91 and -0.89). So even when using ReLU function, we can take the positive output as our confidence score. This would of course not be the case on multi-species classification and possibly not the case on binary classification with more common regions.

The linear nature of ReLU function may result in some outcomes where the comparison between certain outputs may not make as much sense.

5.5 Multi-species Classification

The network can be extended to go beyond binary classification and be trained on and classify multiple species data. The loss would need to become categorical cross-entropy, the number of nodes on the output layer would need to be adjusted to the number of species in the data.

The classification results' metrics would need to be adjusted to accommodate multiple classes. Accuracy and F1 scores exist also for multi-species classification.

If more similar species are selected to classify, or more species are added to the model, the outputs will need to be closely monitored since more than one positive/negative outputs may be present. In such a case the best scoring classification may be taken or any classification that pass a certain threshold may be shown.

5.6 Comparison to similar work

Rizzo et al.[27] use randomly selected 1000 sequences from each of the three most common bacteria phyla, Actinobacteria, Firmicutes, Proteobacteria, collecting in total 3000 sequences. All the sequences have length greater than 1200 bp. They all belong to 16S genes. We are using all parts of the DNA for two species. They use sequences that are 500 bases long and also experiment with the full-length sequences of the genes. We also use 500 base long sequences but have also experimented with 50 base long sequences.

They use spectral sequence representations of the sequences. They extract k-mers where $k = 5$ from the sequence and list out their frequencies. They use this frequency data to train the model and perform the classifications on the test sets. This approach disregards any long distance correlations the sequence may hold. The frequency data is fixed in size which is important since they are using convolutional layers in their network. They have two convolutional and pooling layers followed by a hidden layer. We use the one-hot encoded genome sequences to train and test. We have three hidden layers.

They use tanh activation function throughout the network. We also use tanh function but on the output layer we switch to ReLU.

They are classifying the sequences down to the genus level. We are only interested in the species level of the classification. But since we have only two species that separate at the domain level, it could be said that we are classifying our sequences at the domain level.

Using 500 base long sequences, they achieve $\sim 100\%$ accuracy on phylum and class level, $\sim 90\%$ on order level, $\sim 75\%$ on family level and $\sim 50\%$ on genus level. We achieve $\sim 91\%$ accuracy on domain level.

Busia et al.[28] use 16S rRNA sequences. They have $19,851$ sequences with $13,838$ from $2,768$ genera. They use read lengths $L = 25, 50, 100, 150, 200$. We use short reads taken from the whole genome that have read length 50 to 500. We use two species.

To test their network, they used 16S mock community sequencing data and also synthetic data they simulated base-flipping noise on. We used synthetic small reads taken from the reference genome to train and test our network. They used one-hot encoding to encode the data similar to us.

They use three convolutional layers followed by two to three fully connected layers depending on the read length. They use an extra average pooling layer afterwards and use softmax activation function on the output layer. We use three hidden layers.

Weitschek et al.[29] use DNA barcodes (COI for animals, rbcL and matK for plants). They require the sequences to be of the same region or at least to be pre-aligned so that the barcode regions can be extracted. We use short reads taken from the whole genome.

Since it is learning its rules on specific positions of the data, it requires a complete reference library of polymorphisms for each species in the training set to avoid false negatives. They achieve 92% accuracy on fungi and 100% accuracy on algae. We achieve 91% accuracy when classifying at the domain level.

Yang et al.[30] use DNA barcode sequences to classify species. They use COI for animals, trnD-trnT and ITS for plants with 621 species in total. We use short reads taken from the whole reference genome for two species.

They use a convolutional network, treating one-hot encoding as its own dimension and convoluting and pooling in 2D. They then take the flattened pooling results through hidden layers, using ReLU as its general activation function and softmax in the last layer to compute the probabilities of the species. We have three hidden layers and use tanh activation function with ReLU function on the output layer.

5.7 Conclusion

Using DNA barcodes to classify DNA sequences is a common practice today. This method is lacking in situations where the sequence might not be accessible as a whole. Since most second generation sequencing machines tend to output reads of around 75 - 400 bp long, we need to be able to classify sequences of this length.

In this study, we set out to develop a deep learning method that could be trained on short reads taken from two reference genomes that can then successfully identify the origin of any short read taken from these reference genomes.

We have selected the two species to be Escherichia Coli and Saccharomyces Cerevisiae, due to their short and well-studied reference genomes. These two species are taxonomically very different, hence lack many identical DNA regions. This makes them ideal candidates to test the viability of using the whole genome for species classification.

We trained the network on short reads we randomly sampled from the reference genomes. The lengths of the reads were 50 to 500 for different networks. We performed our tests with same length reads. The model can accommodate the use of variable length reads. The nature of ANNs do not allow for variable length input but a recurrent layer can be introduced to combat this issue. Also the variable length reads can be padded with the character ’N’ to be made the same length. If variable length test cases will be encountered, the training set should also contain similar padded reads.

We tried different specifications for the neural network and achieved $\sim 83\%$ accuracy when predicting the species of short reads of 50 bases long. As we increased the read length up to 500 bases, we achieved $\sim 91\%$ accuracy.

5.8 Future Work

The inclusion of different deep learning techniques (RNNs, CNNs, transformers) could capture sequential correlations a simple ANN could not and might improve accuracy further.

The network can be extended to classify more than two species.

Finally, once reliable multiple species classification is achieved, further methods can be used to compress the data using the result of the classification. An autoencoder can be used to compress the data using the reference genome that belongs to that species. The compressed data would consist of the model, weights corresponding to the model and the difference the decompression of the compressed data would have with the reference genome.

REFERENCES

[1] A. J. Cain, *taxonomy*. Encyclopædia Britannica, inc., Jan 2011.

[2] P. Hebert, A. Cywinska, S. Ball, and J. DeWaard, “Biological identifications through dna barcodes,” *Proceedings of the Royal Society B: Biological Sciences*, vol. 270, no. 1512, p. 313–321, 2003.

[3] P. D. N. Hebert, S. Ratnasingham, and J. R. deWaard, “Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species.,” *Proceedings of the Royal Society B: Biological Sciences*, vol. 270, p. S96–S99, Aug 2003.

[4] H. A. Yacoub, M. M. Fathi, and W. M. Mahmoud, “Dna barcode through cytochrome b gene information of mtdna in native chicken strains,” *Mitochondrial DNA*, vol. 24, p. 528–537, Oct 2013.

[5] F. Yang, F. Ding, H. Chen, M. He, S. Zhu, X. Ma, L. Jiang, and H. Li, “Dna barcoding for the identification and authentication of animal species in traditional medicine,” *Evidence-based Complementary and Alternative Medicine: eCAM*, vol. 2018, p. 5160254, Apr 2018.

[6] “Saccharomyces cerevisiae organism reference genome.” Available at https://www.ncbi.nlm.nih.gov/datasets/taxonomy/4932/.

[7] “Escherichia coli organism reference genome.” Available at https://ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria/Escherichia_coli/reference/GCF_000005845.2_ASM584v2/.

[8] Y. Liu and X. Li, “Darwin’s pangenesis and molecular medicine,” *Trends in Molecular Medicine*, vol. 18, p. 506–508, Sep 2012.

[9] S. Abbott and D. J. Fairbanks, “Experiments on Plant Hybrids by Gregor Mendel,” *Genetics*, vol. 204, pp. 407–422, 10 2016.

[10] “Pray, l. (2008) discovery of dna structure and function: Watson and crick. nature education 1(1):100.”

[11] F. Sanger, S. Nicklen, and A. R. Coulson, “Dna sequencing with chain-terminating inhibitors,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, p. 5463–5467, Dec 1977.

[12] “Sanger sequencing steps & methods.” Available at https://www.sigmaaldrich.com/TR/en/technical-documents/protocol/genomics/sequencing/sanger-sequencing.

[13] D. J. Lipman and W. R. Pearson, “Rapid and sensitive protein similarity searches,” *Science (New York, N.Y.)*, vol. 227, p. 1435–1441, Mar 1985.

[14] K. Y. Li, “Vanishing and exploding gradients in neural network models: Debugging, monitoring, and fixing,” Jul 2022.

[15] M. Uzair and N. Jamil, “Effects of hidden layers on the efficiency of neural networks,” in *2020 IEEE 23rd International Multitopic Conference (INMIC)*, p. 1–6, Nov 2020.

[16] J. Brownlee, “A gentle introduction to dropout for regularizing deep neural networks,” Dec 2018.

[17] N. S. Chauhan, “Loss functions in neural networks,” Aug 2021.

[18] K. Fukushima, “Neocognitron: A self-organizing neural network model for a mechanism of pattern recognition unaffected by shift in position,” *Biological Cybernetics*, vol. 36, p. 193–202, Apr 1980.

[19] Y. LeCun, C. Cortes, and C. J. Burges, “Mnist handwritten digit database.” Can also be accessed at: https://www.kaggle.com/datasets/hojjatk/mnist-dataset.

[20] “What are recurrent neural networks?.” Available at https://www.ibm.com/topics/recurrent-neural-networks.

[21] S. Hochreiter and J. Schmidhuber, “Long short-term memory,” *Neural Computation*, vol. 9, p. 1735–1780, Nov 1997.

[22] F. V. VEEN, “Neural network zoo.” Available at https://www.asimovinstitute.org/overview-neural-network-zoo/.

[23] K. Cho, B. van Merrienboer, C. Gulcehre, D. Bahdanau, F. Bougares, H. Schwenk, and Y. Bengio, “Learning phrase representations using rnn encoder-decoder for statistical machine translation,” 2014.

[24] H. Bourlard and Y. Kamp, “Auto-association by multilayer perceptrons and singular value decomposition,” *Biological Cybernetics*, vol. 59, p. 291–294, Sep 1988.

[25] D. P. Kingma and M. Welling, “Auto-encoding variational bayes,” 2022.

[26] S. M. Abd –Alhalem, E.-S. M. El-Rabaie, N. F. Soliman, S. E. S. E. Abdulrahman, N. A. Ismail, and F. E. Abd El-samie, “Dna sequences classification with deep learning: A survey,” *Menoufia Journal of Electronic Engineering Research*, vol. 30, p. 41–51, Jan. 2021.

[27] R. Rizzo, A. Fiannaca, M. La Rosa, and A. Urso, “A deep learning approach to dna sequence classification,” in *Computational Intelligence Methods for Bioinformatics and Biostatistics* (C. Angelini, P. M. Rancoita, and S. Rovetta, eds.), Lecture Notes in Computer Science, (Cham), p. 129–140, Springer International Publishing, 2016.

[28] A. Busia, G. E. Dahl, C. Fannjiang, D. H. Alexander, E. Dorfman, R. Poplin, C. Y. McLean, P.-C. Chang, and M. DePristo, “A deep learning approach to pattern recognition for short dna sequences,” p. 353474, Aug 2019.

[29] E. Weitschek, R. Van Velzen, G. Felici, and P. Bertolazzi, “Blog 2.0: a software system for character-based species classification with dna barcode sequences. what it does, how to use it,” *Molecular Ecology Resources*, vol. 13, no. 6, p. 1043–1046, 2013.

[30] C.-H. Yang, K.-C. Wu, L.-Y. Chuang, and H.-W. Chang, "Deepbarcoding: Deep learning for species classification using dna barcoding," *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, vol. 19, p. 2158–2165, Jul 2022.

[31] W. Cui, Z. Yu, Z. Liu, G. Wang, and X. Liu, *Compressing Genomic Sequences by Using Deep Learning*, vol. 12396 of *Lecture Notes in Computer Science*, p. 92–104. Cham: Springer International Publishing, 2020.

[32] F. Chollet *et al.*, "Keras." `https://keras.io`, 2015.

[33] M. Abadi, A. Agarwal, P. Barham, E. Brevdo, Z. Chen, C. Citro, G. S. Corrado, A. Davis, J. Dean, M. Devin, S. Ghemawat, I. Goodfellow, A. Harp, G. Irving, M. Isard, Y. Jia, R. Jozefowicz, L. Kaiser, M. Kudlur, J. Levenberg, D. Mané, R. Monga, S. Moore, D. Murray, C. Olah, M. Schuster, J. Shlens, B. Steiner, I. Sutskever, K. Talwar, P. Tucker, V. Vanhoucke, V. Vasudevan, F. Viégas, O. Vinyals, P. Warden, M. Wattenberg, M. Wicke, Y. Yu, and X. Zheng, "TensorFlow: Large-scale machine learning on heterogeneous systems," 2015. Software available from tensorflow.org.

[34] G. Van Rossum and F. L. Drake Jr, *Python reference manual*. Centrum voor Wiskunde en Informatica Amsterdam, 1995.

[35] "Anaconda software distribution," 2020. Available at https://docs.anaconda.com/.

[36] J. J. Salk, M. W. Schmitt, and L. A. Loeb, "Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations," *Nature Reviews Genetics*, vol. 19, p. 269–285, May 2018.

[37] NVIDIA, P. Vingelmann, and F. H. Fitzek, "Cuda, release: 10.2.89," 2020.

[38] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, and E. Duchesnay, "Scikit-learn: Machine learning in Python," *Journal of Machine Learning Research*, vol. 12, pp. 2825–2830, 2011.

APPENDIX A

CONTENTS OF FILE PREP.PY

```
import numpy as np
from pickle import dump
from sys import argv
from os import stat
from math import ceil
import tensorflow as tf
from tensorflow import keras
from numpy.random import randomsample

seed = 33333
keras.utils.set random seed(seed)
tf.config.experimental.enable op determinism()

def prepare string data into encoded 1D( stringdata ):
    result = np.zeros([len(stringdata),5], dtype = np.dtype(np.int8))
    for i in range(len(stringdata)):
        if stringdata[i] ==  A :
            result[i][0] = 1
        elif stringdata[i] ==  T :
            result[i][1] = 1
        elif stringdata[i] ==  C :
            result[i][2] = 1
        elif stringdata[i] ==  G :
            result[i][3] = 1
        else:
            result[i][4] = 1
    return result

read len = int(argv[1])
sample representation = int(argv[2])
is working on small sample = int(argv[3])

species length equalized = 1
data list = [["Bakers Yeast", "Ecoli"],["yeast", "ecoli"],[0,0],[]]
data = []
len samples list = len(data list[0]) [0]

open("pkl/" + str(read len) + is working on small sample " small" + ".pkl
    ","wb")
open("pkl/" + str(read len) + is working on small sample " small" + " y.pkl
    ","wb")

data list[2] = [0] len(data list[0])
data lens = [0 for i in range(len( data list [0]))]
for i in range(len( data list [0])):
    data =   . join (np. genfromtxt (  data /   + data list [0][ i ] +
        is working on small sample   small   +  . fna  , delimiter =  n  , dtype
       = str )) . upper ()
    data = prepare string data into encoded 1D (data)
    data lens [ i ] = len (data)
max data = max( data lens )

for i in range(len( data list [0])):
    data =   . join (np. genfromtxt (  data /   + data list [0][ i ] +
        is working on small sample   small   +  . fna  , delimiter =  n  , dtype
       = str )) . upper ()
    data = prepare string data into encoded 1D (data)
    if( species length equalized ):
        samples indices = (( len (data)  read len )  random sample ( size = int (
            max data    sample representation / read len ))) . astype ( int )
    else :
        samples indices = (( len (data)  read len )  random sample ( size = int (
            len (data)    sample representation / read len ))) . astype ( int )

    samples = np. empty ([ len ( samples indices ) , read len ,5] , dtype = np. dtype (
        np . int8 ))
    for j in range(len( samples indices )):
        index = samples indices [ j ]
        sample = data [ index : index+read len ]
        samples [ j ] = sample
    len samples list [ i ] = len ( samples )

    with open("pkl/" + str ( read len ) + is working on small sample " small"
         + ". pkl" ,"ab") as f:
        dump( samples , f )
    data list [2][ i ] = len ( samples )

    y = np. asarray ([np. array ( i  [0] + [1] + ( len ( data list [0])  i  1)  [0]) for
         j in range( data list [2][ i ])] , dtype = np. dtype (np. int8 ))

    with open("pkl/" + str ( read len ) + is working on small sample " small"
        + " y . pkl" ,"ab") as f:
        dump( y, f)

with open("pkl/ data list " + str ( read len ) + is working on small sample "
    small" + ". pkl" ,"wb") as f:
    dump( data list , f )
```

APPENDIX B

CONTENTS OF FILE TRAIN_TEST.PY

```
import numpy as np
import tensorflow as tf
from tensorflow import keras
from keras.callbacks import LambdaCallback
from keras.layers import Flatten, Dense, Activation
from keras import activations, losses, initializers, metrics
from sklearn.model selection import train test split
from sklearn.metrics import accuracy score, recall score, precision score
from sys import argv
from pickle import load
from time import time
from gc import collect
from math import ceil

tf.config.experimental.enable op determinism()
seed = 0
batch size = 128
std dev = 0.03    0.09 for 50, 0.03 for 500
mean = 0.0
learning rate = 0.00003    0.0006 for 50, 0.00003 for 500
keras.utils.set random seed(seed)
epochs = 5

print("seed: ", seed, ", start time:",time(),end =", ")
is working on small sample = int(argv[1])
read len = 500  int(argv[2])
encoding dim layer 1 = 1000    200 for 50, 1000 for 500
encoding dim layer 2 = 100    100 for both 50 and 500
encoding dim layer 3 = 20    20 for both 50 and 500
activation function = activations.tanh
loss = losses.BinaryCrossentropy(from logits=True)
sample representation = 2
one hot encoding size = 5

with open("pkl/data list " + str(read len) + is working on small sample "
    small" + ".pkl", "rb") as f:
    data list = load(f)
for i in data list[2]:
    print(i, end = ", ")

initializer = initializers.RandomNormal(stddev=std dev, mean = mean)
initializer name = "random normal"
  layers
```

```
flatten = Flatten()
dense1 = Dense(encoding dim layer 1 , kernel initializer=initializer)
activation1 = Activation(activation function)
dense2 = Dense(encoding dim layer 2 , kernel initializer=initializer)
activation2 = Activation(activation function)
dense3 = Dense(encoding dim layer 3 , kernel initializer=initializer)
activation3 = Activation(activation function)
output = Dense(len(data list[0]), kernel initializer=initializer)
activation4 = Activation(activations.relu)
  data flow
input = keras.Input(shape=(read len ,one hot encoding size))
flattened = flatten(input)
encoded1 = dense1(flattened)
activated1 = activation1(encoded1)
encoded2 = dense2(activated1)
activated2 = activation2(encoded2)
encoded3 = dense3(activated2)
activated3 = activation3(encoded3)
encoded triangle = output(activated3)
activated triangle = activation4(encoded triangle)
triangle = keras.Model(input, activated triangle)


x test , y test = np.array([]), np.array([])

lenx = sum(data list[2])
X full = np.zeros( [lenx,read len ,one hot encoding size], dtype = np.dtype(
    np.int8) )
it = 0
filename = "pkl/"+ str(read len) + is working on small sample " small" + ".
    pkl"
with open(filename,"rb") as f:
    for i in range(len(data list[0])):
        sample = load(f)
        for j in sample:
            X full[it] = j
            it += 1
del sample
collect()

Y full = np.zeros( [lenx,len(data list[0])], dtype = np.dtype(np.int8) )
it = 0
y filename = "pkl/" + str(read len) + is working on small sample " small" +
     " y.pkl"
with open(y filename,"rb") as f:
    for i in range(len(data list[0])):
        sample = load(f)
        for j in sample:
            Y full[it] = j
            it += 1

start time = time()

x train, x test, y train, y test = train test split(X full, Y full,
    random state=seed, test size=0.20)   80 train
x val, x test, y val, y test = train test split(x test, y test,
    random state=seed, test size=0.5)   10 val 10 test

```

```
del X full , Y full

printerCallback = LambdaCallback(
    on epoch begin = lambda epoch , logs: print(epoch, end = ", "),
    on epoch end = lambda epoch, logs :
        print(" :.4f ".format(logs[ loss ]), ", ", " :.4f ".format(logs[
            val loss ]), ", ", " :.1f  sec".format(ceil((time()
            train start time) 10)/10), end = ", ")
)
opt = tf.keras.optimizers.Adam( learning rate)
print("optimizer:", "Adam ", ", learning rate: ", learning rate , ",
    batch size: ", batch size , ", initializer: ", initializer name , ",
    std dev: ", std dev , ", Mean: ", " :.5f ".format(mean), end=", ")
triangle.compile(optimizer=opt,
                loss=loss
                )
y true = [0 if x[0]   x[1] else 1 for x in y test ]
temp = triangle.predict(x test , verbose=0)
avgs = np.average(temp, axis=0)
print("untrained output averages: ", "[ :.5f , :.5f ]".format(avgs[0],avgs
    [1]), end = ", ")
train start time = time()
print( sample representation , ", ", read len , ", ", str(loss).split(" ")
    [1], ", ", encoding dim layer 1 , ", ", encoding dim layer 2 , ", ",
    encoding dim layer 3 , ", ", str(activation function).split(" ")[1], ",
    ", epochs, ", ", train start time , end = ", ", )
triangle.fit(x train , y train ,
                epochs=epochs,
                batch size=batch size ,
                shuffle=True,
                validation data=(x val , y val),
                callbacks= [printerCallback],
                verbose = 0
                )
temp = triangle.predict(x test , verbose=0)


y pred = [0 if x[0]   x[1] else 1 for x in temp  ]
y true = [0 if x[0]   x[1] else 1 for x in y test  ]

 with open("output pred real weights.csv", "w") as f:
     for i in range(len(temp)):
         f.write(" :.2f ,  :.2f ,   ,    n".format(temp[i][0], temp[i][1],
    y pred[i], y true[i]))

print(" nend time:",time(),end=", ")
print("Accuracy: ", accuracy score(y true , y pred),
    ", Recall: ", recall score(y true , y pred ,average="binary"),
    ", Precision: ", precision score(y true , y pred ,average="binary"),
        flush=True)

keras.backend.clear session()
```

APPENDIX C

LIST OF INSTALLED PACKAGES AND VERSIONS

Name	Version
_tflow_select	2.1.0
cudatoolkit[37]	11.3.1
cudnn	8.2.1
ipykernel	6.13.0
ipython	8.2.0
ipython_genutils	0.2.0
jupyter_client	7.3.0
jupyter_core	4.9.2
jupyter_server	1.17.0
jupyterlab	3.3.4
jupyterlab_pygments	0.2.2
jupyterlab_server	2.13.0
keras[32]	2.9.0
keras-preprocessing	1.1.2
notebook	6.4.11
notebook-shim	0.1.0

Name	Version
numpy	1.23.4
pdflatex	0.1.3
pickleshare[34]	0.7.5
pip	23.2.1
python[34]	3.9.12
rsa	4.7.2
scikit-learn[38]	1.1.1
scipy	1.9.3
sklearn	0.0
tensorboard[33]	2.9.1
tensorboard-data-server	0.6.0
tensorboard-plugin-wit	1.6.0
tensorflow[33]	2.9.1
tensorflow-estimator	2.9.0
tensorflow-gpu[33]	2.6.0
tensorflow-io-gcs-filesystem	0.26.0

Table 7: Packages in environment tf_gpu