Integrated Deep Learning and Bayesian Classification for Prioritization of Functional Genes in Next-Generation Sequencing Data

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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Acknowledgements

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Abstract

The advent of next-generation sequencing technology has enabled large-scale interrogation of the genome to identify variants in patient samples. The accurate identification of functional variants can provide critical insights into the disease process to guide diagnosis and treatment. However, the use of clinical genomics remains limited as (i) the accurate identification of variants remains suboptimal, and (ii) the large number of variants identified may be difficult to interpret without a systematic approach of ranking by functional importance.

Here, we describe the development of a deep learning neural network to improve the accuracy of variant-calling, and a Bayesian classification method for the probabilistic ranking of functionally relevant genes. We show that an optimised neural network can call variants more accurately than single variant callers or concordant callers, with F1 score improvements of 6.5 percent in simulated datasets and 4.5 percent in real datasets over the best concordant methods. Following the identification of high confidence variants, we further demonstrate that a Bayesian classification system can rank functionally relevant genes in a Diffuse Large B-Cell Lymphoma (DLBCL) patient sample.

We propose that the combined use of deep learning and Bayesian network analysis could be extended to build an analytical pipeline for clinical use to augment diagnosis and treatment of diseases by identifying high-confidence variants and ranking them systematically.

1 Introduction

1.1 Next Generation Sequencing (NGS) for Clinical Genomics

There has been a growing interest in using a patient's genome to guide the diagnosis and treatment of diseases (Rehm, 2017; Angrist, 2016), based on the fundamental intuition that variants and mutations in the genome alter gene functions that drive the initiation and progression of the disease. In oncology, for example, identification of the key driver mutations has been shown to be useful in stratifying cancer subtypes (Stratton, Campbell & Futreal, 2009), and identifying mutations for targeted therapy (Janitz, 2011). Furthermore, the development of next generation sequencing (NGS) technologies has dramatically reduced sequencing costs (Metzker, 2010; Mardis, 2008), enabling the adoption of genomic sequencing in clinical labs.

Although clinical genomics holds great promise, there are still two critical issues that limit its use in a clinical setting. Firstly, it is often difficult to obtain high-confidence variant calls from sequencing data, and secondly, the large number of variant calls for patient samples makes interpretation difficult for clinical decision-making.

1.2 Variant Calling of NGS Data

In variant calling, genomic DNA is fragmented, and the short reads are sequenced in a massively parallel manner using next generation sequencing technologies such as sequencing by synthesis. These reads are aligned to the reference genome, and variations in the DNA sequence, such as single nucleotide variants (SNV) and insertions/deletions (indels) are identified by comparing the different reads aligned to the reference genome (Figure 1).

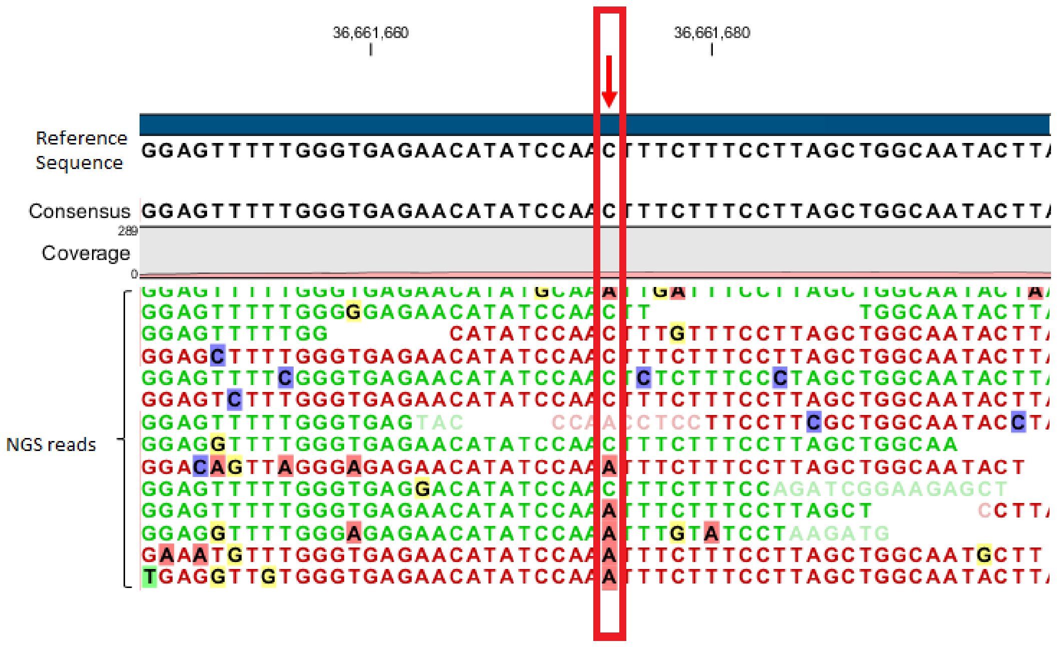


Figure 1: **Illustration of Variant Calling Pileup**. Due to noise and errors in sequencing and read mapping, it can be difficult to call variants accurately. At the position of interest, there seems to be a possible mutation from the original base of Cytosine to the base Adenine, but not all reads agree with this mutation call. Figure adapted from CLC Genomics Workbench 9.5, Figure 29.8.

Variant calling with NGS data primarily involves the use of various statistical and algorithmic methods to identify variants in the genome (Nielson et al., 2011). These variants represent the deviations and differences between the genome of interest and a reference human genome. This analysis is non-trivial as each variant call requires the integration of multiple sequence reads (e.g. millions of reads) that contain experimental noise and errors (Zook et al., 2014). The calling of variants can be further complicated by errors in mapping the reads to a reference genome.

To account for these errors, variant callers employ a variety of algorithms and statistical models to determine the existence and type of variation/mutation (Zook et al., 2014; Davey et al., 2011). Because of the differences in assumptions and models employed by different variant callers, certain calling algorithms are more sensitive and accurate in calling specific classes of variants but do not perform well in calling other variant types (O'Rawe et al., 2013). To address these problems, ongoing efforts have focused on improving current variant calling algorithms, including optimisation of variant calling for different classes of mutations, as well as the reduction in the number of false positive calls (Mohiyuddin et al., 2015; Gézsi et al., 2015).

Despite the variety of approaches used for identifying variants and mutations, the accuracy and precision of single variant callers remain suboptimal (Cornish and Guda, 2015; O'Rawe et al., 2013). Each variant caller can differ greatly in accuracy depending on the type of sequencing methodology and the statistical algorithm used (Figure 2), making it difficult to identify true high-confidence variant calls.

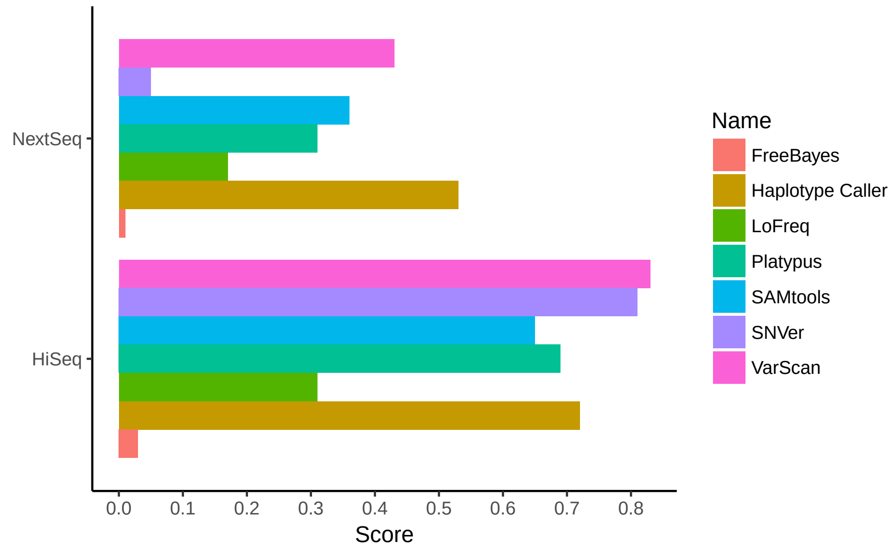


Figure 2: **Performance of Variant Calling Tools on Patient Data using the HiSeq and NextSeq Illumina Sequencing Platforms** . The F1 score indicates how well a caller can predict true positives (See Appendix 5.3 for more details). Notably, the F1 score for the same variant calling tool can differ greatly. Figure adapted from Sandmann et al. (2017)

1.3 Ensemble Methods for Improving the Accuracy of Variant Calling

While it is clear that single variant callers may not perform well across a variety of variant classes, the combination or ensemble of several callers can be used to augment the accuracy of variant callers beyond what can be achieved with a single caller. By aggregating the calls from each different variant caller, the relatively weak prediction calls from each caller can be combined to provide a better aggregate prediction for a variant call.

One simple approach to aggregating variant calls is by concordance, where the likelihood of an accurate call depends on multiple variant callers identifying the same variant or mutation (Lam et al., 2012; Wei et al., 2011). While straightforward and intuitive, the recall rates of such a tool is poor with a high number of false negative calls. This is because a high concordance of variant calls will reciprocally decrease the number of true variants calls that are identified by specific variant callers (O'Rawe et al., 2013).

Beyond concordance, supervised machine learning approaches have been used to combine the calls from different variant callers to improve the accuracy. In these approaches, machine learning algorithms have been used predict the accuracy of a variant call by integrating different features of each variant call (e.g. variant frequencies, mapping quality). For example, the support vector machine (SVM) algorithm was used successfully to improve the accuracy of variant calls (Gézsi et al., 2015) over concordance-based methods.

Recent advances in machine learning, in particular, deep learning neural networks, have increased the accuracy of predictions from complex multi-modal data (Ng et al., 2015) beyond traditional algorithms such as SVM and Random Forests. The ability of deep learning networks to learn from complex high-dimensional data suggests that they may be useful in improving the accuracy of high-confidence variant calls derived from the complex features from each variant caller.

1.4 Deep Learning for Improving the Accuracy of Variant Calling

Deep learning is a machine learning approach based on artificial neural networks (LeCun et al., 2015) that are built on artificial neurons. Each artificial neuron is analogous to a biological neuron where weighted input signals are integrated to produce an output once the signals cross a threshold for activation (Figure 3).

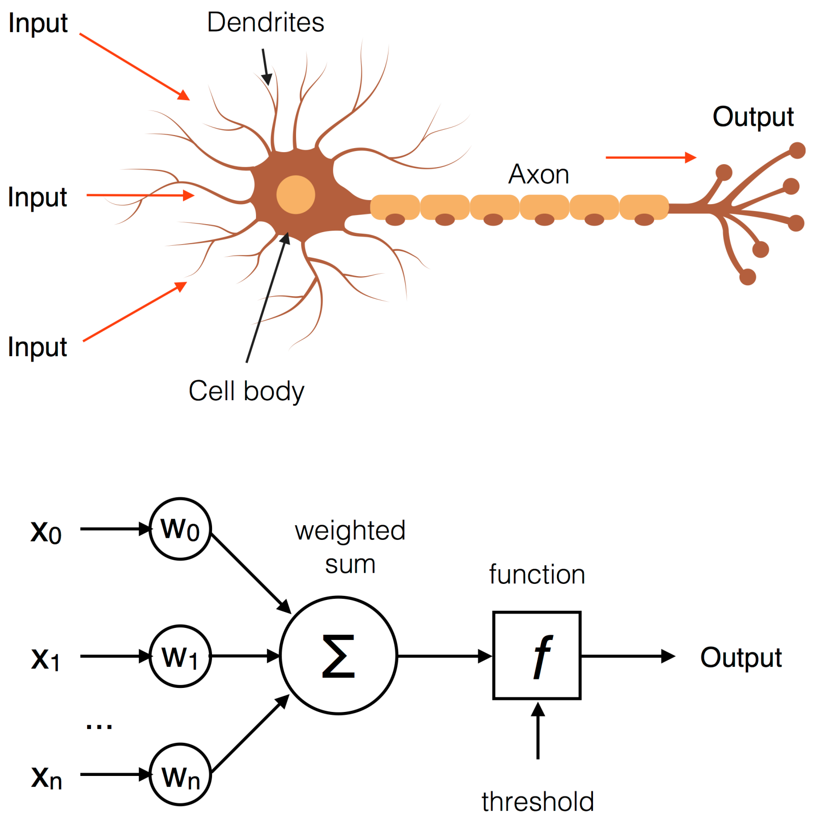


Figure 3: **Artificial Neurons as Building Blocks of Neural Networks**

In a deep learning network, the artificial neurons are connected together in layers, comprising an input layer, an output layer, and a variable number of intermediate hidden layers (Figure 4). Here, the output of the neurons of one layer are connected to the input of next layer of neurons, with the weights of the connections determining the strength of signal propagation from one neuron to another.

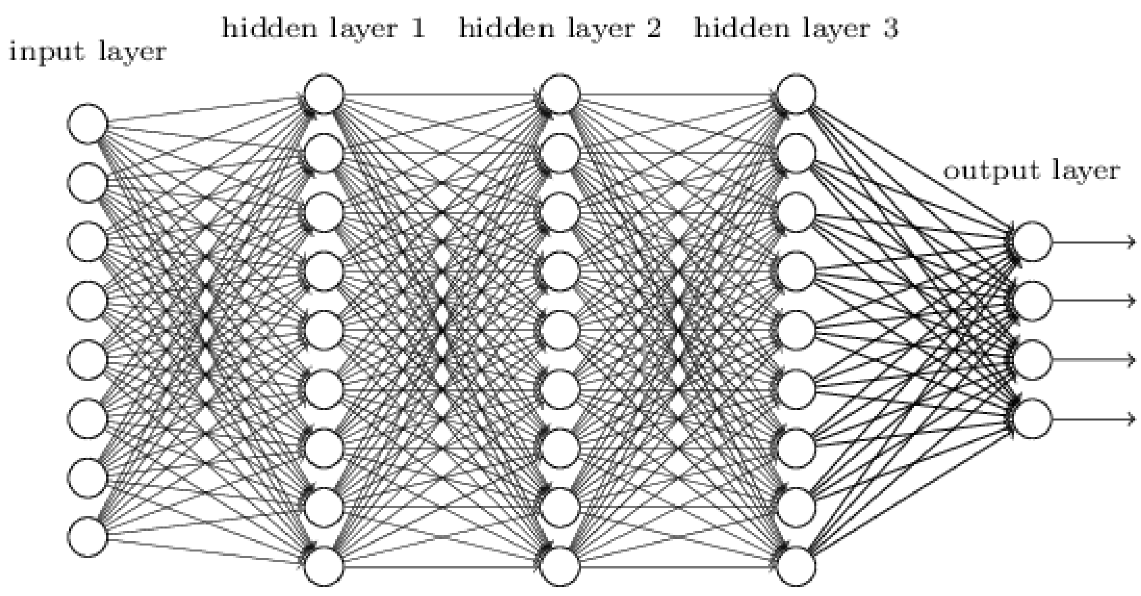


Figure 4: **Sample Neural Network with Five Layers, Including Three Hidden Layers**. This represents a densely connected neural network, where each node is connected to every node of the preceding and subsequent layers.

Deep learning networks can be trained by providing labeled data, where features of the dataset are fed into the input layer to produce the output prediction. By comparing the output to the actual labels, the network can be trained by adjusting the weights that determine the propagation of a signal from one neuron to the next. In this way, a trained neural network can predict the output when given new data (for a more detailed explanation, see Appendix 5.1).

Because deep learning networks contain multiple layers, they are able to learn different features in a hierarchical manner. This allows the network to solve complex non-linear decision boundary problems, including drug molecule solubility (Lusci et al., 2013), facial recognition (Sun et al., 2014) and even predicting the best move in the Japanese board game, Go (Silver et al., 2016). Given this ability to learn from complex features, deep learning networks provide a possible approach to improve the prediction high confidence variant calls from an ensemble of different variant calling algorithms.

1.5 Prioritisation of Variants with Bayesian Networks

Once high-confidence variant calls can be established, there remains the second problem of identifying the functional importance of each variant/mutation, given that there are multiple variants in a typical genome (Shen et al., 2013). The ability to systematically prioritise and rank clinically significant variants and mutations would allow clinicians to focus their attention on relevant candidate mutations that can guide decision-making on diagnosis and treatment.

The problem of prioritisation of genetic variants and mutations arises from the multiplicity and complexity of data sources that can be utilised to determine the clinical and functional relevance of a variant or mutation in a gene (Moreau & Tranchevent, 2012). Several approaches have been used, including characterizing variants and their phenotypes in clinical cases, and determining if a variant/mutation will alter protein function based on amino acid changes in conserved regions. Although these functional annotations of variants and mutations can be performed with tools such as ANNOVAR (Wang, Li, & Hakonarson, 2010), the fundamental problem of integrating the information in a systematic manner remains.

One possible approach to addressing this problem is by using Bayesian networks to probabilistically integrate diverse information sources to predict the likelihood of an outcome. This approach has been successfully used in solving a variety of decision making problems (Pourret et al.,2008; Jensen et al., 1996), including medical treatment decision making (Windecker et al., 2014), ecological studies (Johnson et al., 2014) and predictive epidemiology (Su et al., 2014).

In a Bayesian network, the probabilities of different events can be linked so that the final likelihood of an outcome can be evaluated. An example of a simple Bayesian network is shown in Figure 5. Here, the likelihood of the outcome, which is rain, is dependent on the probabilities of thunder, cloudy day and the weather forecast. The probabilities of the variables can be use to update the final likelihood of the outcome, based on the conditional probabilities of thunder, cloudy day, and weather forecast. In a similar way, the probability of a functionally important variant/mutation can be evaluated based on the conditional probabilities of the functional annotation and confidence of a true variant call.

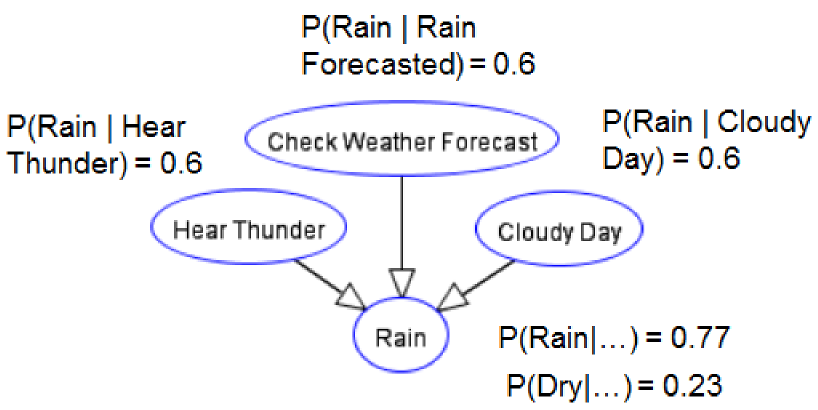

P(Rain | Rain Forecasted) = 0.6
P(Rain | Hear Thunder) = 0.6
Check Weather Forecast
P(Rain | Cloudy Day) = 0.6
Hear Thunder
Cloudy Day
Rain
P(Rain|…) = 0.77
P(Dry|…) = 0.23

Figure 5: **Sample Bayesian Network for Rain Prediction.**

One advantage of Bayesian network analysis is that it approximates how humans reason about relationships between events and outcomes - we observe events and update our probabilistic estimates of the likelihood of an outcome. Additionally, because Bayesian networks require the explicit specification of relationships between the events and the outcome, the model is transparent and can built based on known relationships between events, based on existing knowledge.

Bayesian networks are well suited to ranking the importance of functionally important variants/mutations because the model can built using explicit specification of the probabilities accounting for the high confidence calls and the predicted functional effects of the variants/mutations. This explicit specification permits better understanding of the ranking process, which is important for clinical decision-making.

1.6 Aims and Approach

The overall goal of this project is to address the two major issues limiting the utility of clinical genomics through the following aims:

1. To develop and validate a deep learning network model for improving the accuracy of variant calling.
2. To develop a Bayesian network model for ranking functionally important variants/mutations from high confidence calls identified by the deep learning network.

We describe the development of (i) a deep learning network to identify high-confidence variant calls (focusing on SNVs and short indels) and (ii) a Bayesian network to probabilistically prioritise their functional importance. As a first step, we developed and optimised a deep learning network to identify true variants in both synthetic and real-world datasets. Following the identification of high-confidence variant calls, we will built a Bayesian network ranking system based on functional annotations to prioritise mutations and used it to identify functionally important mutations in a cancer sample.

2 Materials and Methods

2.1 Overall Experimental Approach

As a first step in the development of deep learning networks for variant calling, we built two main computational pipelines: (i) a training pipeline for training and the optimisation of the neural network, and (ii) an analysis pipeline that uses a trained neural network to perform variant prediction and validation (Figure 6).

In the training pipeline, training datasets from simulated and real sequencing data, were used for performing the processing steps of alignment, variant calling and training of the deep learning network. Briefly, FASTQ sequence reads were first mapped to the reference genome before variant calling was performed using an ensemble of callers. The different variant calls were used to generate the feature vectors used for the inputs to the deep learning network. The predictions by the neural network were compared to the ground truth variant calls in order for adjustments to be made to the weights of the connections

In the analysis pipeline, the trained optimized network from the training pipeline is then used to predict high confidence variant calls in naive samples without ground truth variant calls. In brief, the FASTQ sequence reads are aligned and variant calling performed in a similar fashion as in the training pipeline. The feature vectors from the ensemble of callers is used to predict high confidence calls using the trained optimized deep learning network.

Finally, we applied the Bayesian network analysis to rank the functionally important variants/mutations from the high confidence calls identified from naive samples in the analysis pipeline.

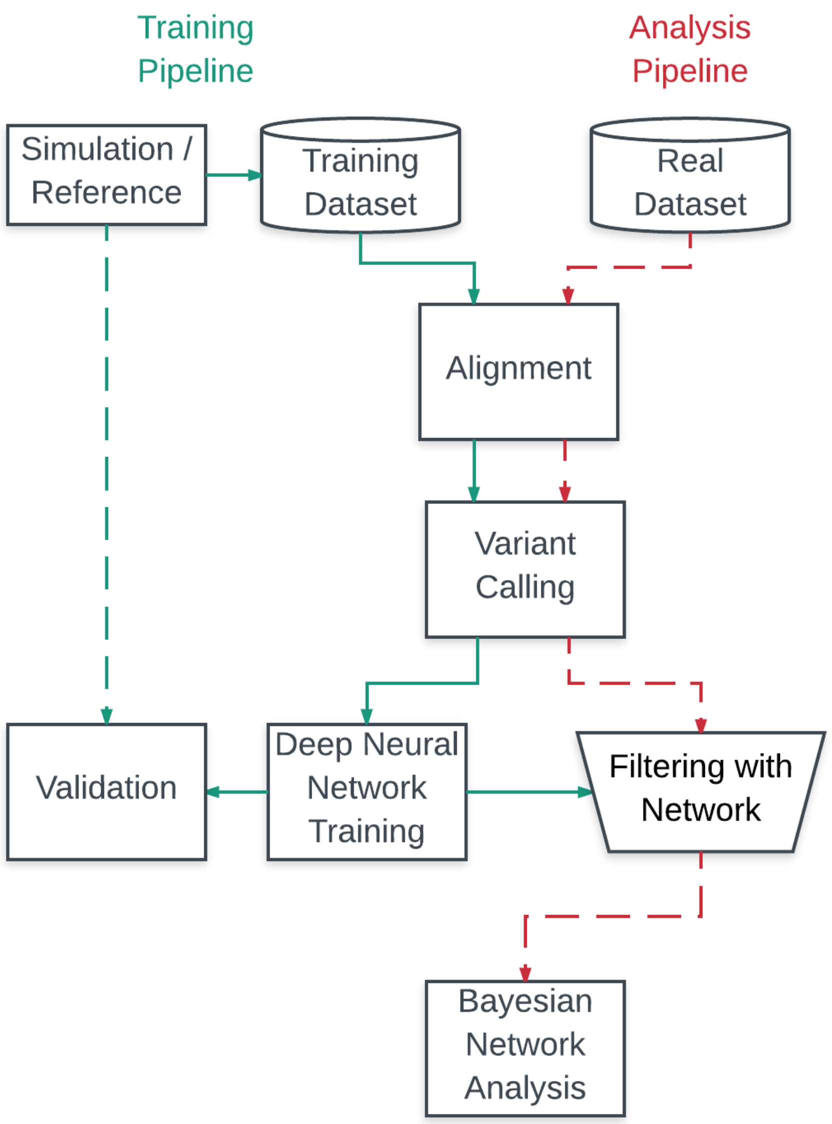


Figure 6: **Overall Structure of Computational Pipelines.** The pipelines were implemented using NextFlow, a domain-specific language for workflows

2.2 Implementation of Computational Pipelines

2.2.1 Workflow Management of Pipelines

The workflows in the training and analysis pipelines were managed using NextFlow,(v0.21.3.3990) a Groovy based Domain Specific Language (DSL) that provides easy management of parallel pipelines consisting of dependent tasks organized as a directed acyclic graph (Tommaso et al., 2014). Nextflow was used to manage and coordinate the different steps in the pipelines to ensure reproducibility and scalability.

2.2.2 Preprocessing and Analysis

The preprocessing and analytical components were implemented using Python (v2.7) (Van Rossum, 2007) and the following Python libraries: NumPy, scikit-Learn, Pomegranate and PyVCF. Briefly, NumPy (v1.11.3) was used to prepare feature vectors for deep learning training, scikit-learn (v0.18.1) was used to perform Principal Component Analysis (PCA) and Synthetic Minority Oversampling Technique (SMOTE) Methods (See Appendix 5.3 for details). PyVCF (v0.6.8) was used to parse the VCF files to facilitate the comparison of variants efficient in O(1) time using hash-based dictionary lookups.

2.2.3 Implementation of Deep Learning Networks

Deep learning networks were implemented using the Keras library (v1.1.1) with a TensorFlow backend (v0.11.0). TensorFlow, from Google (Abadi et al., 2015), was used for better network training performance due to its distributed computation and queue management system. The code used to generate the feature vectors and train the neural network can be found in Relevant Code – Section 7.1 and 7.2 respectively. For details on the algorithms used in deep learning are detailed in Appendix 5.1.

2.2.4 Bayesian Network Ranking of Mutations

For the Bayesian ranking of mutations, the high confidence calls from the deep learning network were annotated using ANNOVAR (v2015Jun17) (Wang, Li, & Hakonarson, 2010). The annotated features for each variant were used as inputs to the Bayesian network, which was implemented using Pomegranate (v0.6.1), a Python library for Bayesian analysis. The code for the the Bayesian network can be found in Relevant Code – Section 7.3.

2.3 Generation of Synthetic Dataset

Synthetic datasets were used for the initial training and optimization of the deep learning network. Variants based on the hg19 human reference genome were first simulated using Mason (v2.3.1) with an indel rate of 0.00002 and a SNP rate of 0.00008, resulting in 229253 SNPs and 57257 indels. The sequence reads were next simulated with Mason using the ground truth variants and sequencing error rates (Figure 7). For error rates, we used published data from Schirmer et al. (2016) – the general substitution error rate used was 0.0004 per base in the genome, and the insertion and deletion error rate per base was $5 * 10^{-6}$.

2.4 Alignment and Variant Calling of Sequence Reads

The sequence reads (FASTQ) from simulated or real datasets were first aligned to the hg19 human reference genome using BWA (0.7.13) (Li, 2013) using the default settings. Following alignment, the alignment files (BAM) were used for variant calling with the following callers with their default settings: FreeBayes (v1.0.2-16); GATK Haplotype Caller (v3.7-0) and Unified Genotyper (v3.7-0); Samtools (v1.3.1); Pindel (v2.3.0) (Garrison & Marth, 2012; McKenna et al. 2010, DePristo et al. 2011; Li H, et al., 2009; Ye et al., 2009). The overall process is shown in Figure 7.

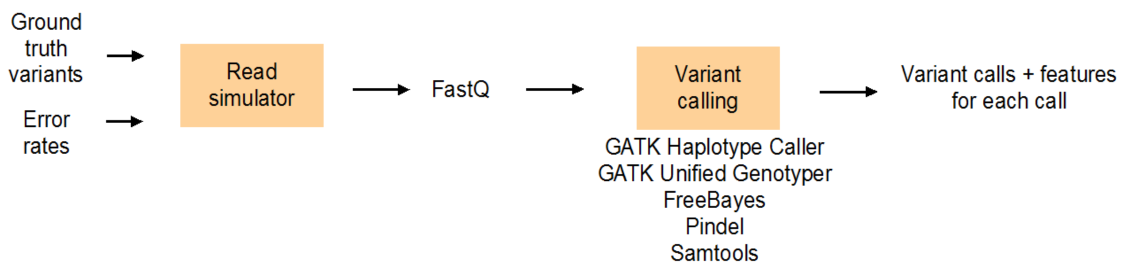


Figure 7: **Pipeline for Simulation of Artificial Genomes for Analysis**

2.5 Feature Engineering for Deep Learning

The output of the variant callers was used to generate features in the form of numerical vectors for the input layer of the deep learning network. These features can be broadly categorized into 3 sets: (i) base-specific information; (ii) sequencing error and bias information; and (iii) calling and mapping quality.

The computation of the features were performed as described below. For an in-depth explanation of their usage and interpretation, see Appendix 5.2.

Base Information

Shannon Entropy

Shannon Entropy captures the amount of information contained inside the allele sequences. It is calculated using the equation:

$$H(X) = -\sum_{i=1}^{n} P(x_i) \log_2 P(x_i) \tag{1}$$

where $P(x_i)$ is the prior probability of finding each base at each position. This prior probability is calculated in two ways – over the entire genome and over a region of space around the allele (10 bases plus the length of the allele in our calculations).

Kullback Leibler Divergence

The Kullback-Leibler Divergence feature is similar to Shannon entropy, but instead, we use this to measure the informational gain from the reference to the allele sequence. The Kullback-Leibler Divergence is calculated as follows:

$$D_{KL}(P||Q) = -\sum_{i=1}^{n} P(x_i) \log_2 \frac{P(x_i)}{Q(x_i)} \tag{2}$$

where $Q(x_i)$ is the prior probability of finding each base at each position based on the genomic region around the allele, while $P(X_i)$ is the posterior probability of finding a specific base inside the allelic sequence.

Base Quality

Base quality refers to the Phred score probability that the called allele is wrong. It is given by the equation:

$$P = 10^{\frac{-Q}{10}}$$

Where P is the Base Quality, and Q is the probability that the allele called is wrong. This is a number computed by the sequencing machine based on the quality of the base samples provided.

Sequencing Biases and Errors

GC content

This feature comprises the GC content of the reference genome for at least ten bases around the mutation site.

Longest homozygous run

This feature comprises the longest similar string of bases in the reference genome, for at least ten bases around the mutation site.

Allele Count and Allele Balance

This feature is an output from Haplotype Caller and Unified Genotyper, and describes the total number of alleles contributing to a call and the balance between reference and alternate alleles reads.

Calling and Mapping Qualities

Genotype Likelihood

The genotype likelihood score provides the Phred-scaled likelihood scores of how confident the caller is in determining that it is a homozygous or heterozygous call, and is provided by all variant callers.

Read Depth

Mapped read depth refers to the total number of bases sequenced and aligned at a given reference base position. It is provided by all variant callers.

Quality by Depth

Quality by depth is computed by dividing the quality score against allele depth, to obtain an average score of allele quality. This is provided by Haplotype Caller and Unified Genotyper.

Mapping Quality

Mapping quality is a score provided by the alignment method and gives the probability that a read is placed accurately. It is provided by all variant callers except Pindel.

2.6 Patient Derived Xenograft Mouse Model Development and Sequencing

A patient-derived xenograft model of diffuse large B-cell lymphoma DLBCL was performed by the Mouse Models of Human Cancer Unit (MMHCU) at the Institute of Molecular and Cell Biology (IMCB), in accordance with the approved protocols by the Institutional Review Board (IRB). In brief, a sample of the DLBCL tumour was implanted into NOD-SCID-gamma mice and serially propagated as a xenograft. The DNA from the xenograft was extracted for high throughput sequencing using the Illumina HiSeq platform (Genotypic Technology, India). The sequence reads from the xenograft was used to validate deep learning prediction of the variant calls and the Bayesian network ranking of important functional mutations.

3 Results

3.1 Generation of Artificial Datasets

Using a genome mutation software, we generated a mutated genome using the hg19 genome from UCSC (Karolchik et al., 2014) as a reference. The mutated genome contains over 300,000 random mutations spread over the chromosomes as can be seen below in Figures 8 and 9. Artificial genomes are a good method to analyse deep learning networks on as the ground truth, which are the truth variants inside the genome, are already known. This allows accurate verification of prediction schemes and is a commonly used method to test next generation sequencing related software (Escalona, Rocha & Posada, 2016). This is primarily because it is difficult to obtain complete truth datasets for real genomes as due to the inhibitory cost of checking every variant called via Sanger sequencing. Thus, artificial genomes present a simple way to simulate NGS data with perfectly known ground truth variants to test our validation platform.

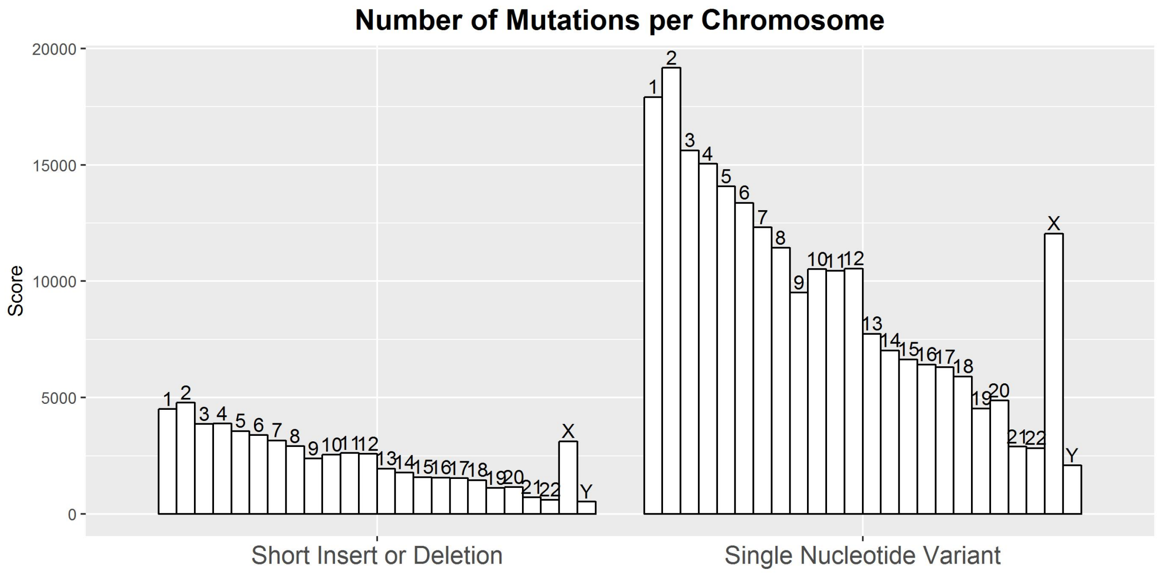


Figure 8: **Number of Ground Truth Mutations (Variants) Created in Each Chromosome**

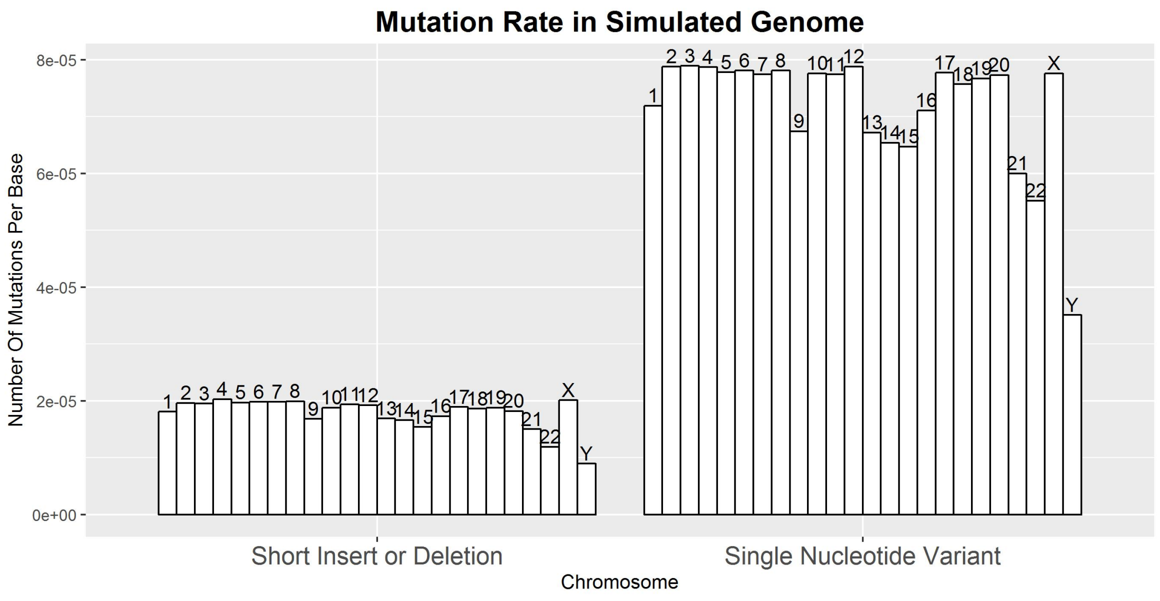
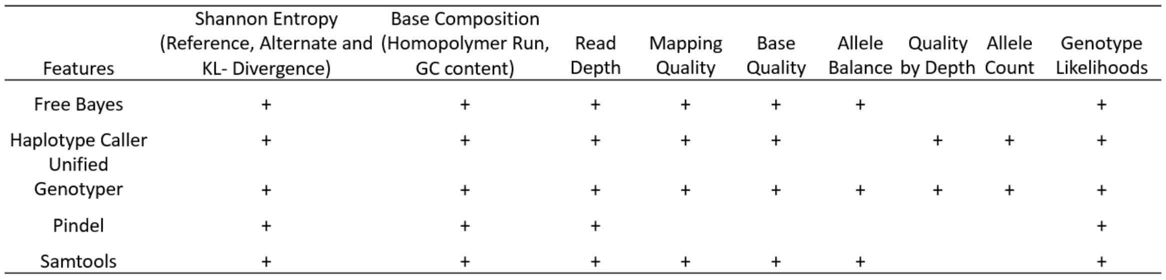


Figure 9: **Mutation Rate per Base in Each Chromosome**

3.2 Feature Engineering

Subsequently, we engineered a set of 19 features to use as input data for our variant callers, using data obtained from the variant callers themselves as well as engineering other features from the dataset. A summary of the features used in training can be found in Table 1, and a description of the full list of features can be found in Appendix 5.2. Features were engineered based on obtaining information on the main aspects of variant calling, which includes the information contained in the sample bases (Base Quality, Entropy, Kullback–Leibler divergence, etc.), the confidence we have in the calling and alignment (Read Depth, Mapping Quality etc) and finally possible biases in the sequencing machine (Allele Balance, Allele Count, GC content).

Table 1: **Feature Engineering Table**



Features	Shannon Entropy (Reference, Alternate and KL- Divergence)	Base Composition (Homopolymer Run, GC content)	Read Depth	Mapping Quality	Base Quality	Allele Balance	Quality by Depth	Allele Count	Genotype Likelihoods
Free Bayes	+	+	+	+	+	+			+
Haplotype Caller	+	+	+	+	+		+	+	+
Unified Genotyper	+	+	+	+	+	+	+	+	+
Pindel	+	+	+						+
Samtools	+	+	+	+	+	+			+

3.3 Variant Callers

Variant callers were chosen for our deep learning neural network based on their orthogonal calling and reference methodologies – we wanted to optimise the information that the neural network receives (See Table 2). We used two haplotype-based callers, FreeBayes (Garrison & Marth, 2012) and GATK Haplotype Caller (McKenna et al. 2010, DePristo et al. 2011), two position based callers GATK Unified Genotyper and Samtools (Li H, et al., 2009) and finally Pindel, a pattern growth based caller (Ye et al., 2009). When we analysed the concordance rates of the callers on the simulated dataset, we found a high amount call discordance (Figure 10).

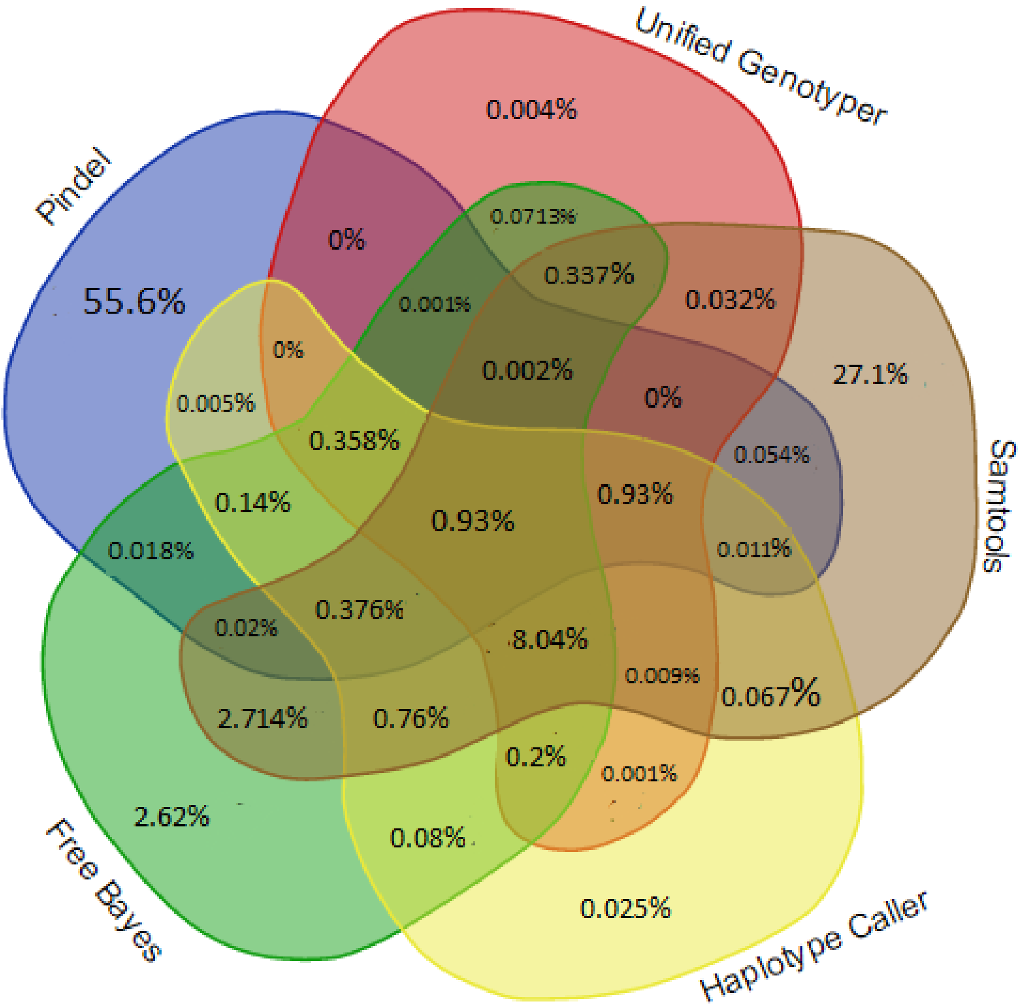


Figure 10: **Concordance of Callers on Simulated Dataset**

Of all the callers, Pindel was the most discordant caller, with over 1.6 million (55.6%) unique calls that are different from other calls. Samtools was also very discordant, with over 800 thousand unique calls

(27.1%) that were unique from the other callers, followed by FreeBayes at 80,000 calls. Interestingly, a high amount of calls (about 100,000) also exists in the intersection of only two callers. Discordance in the variant callers can be explained by the different methodologies that they use to call variants (Table 2). Due to implementation and design choices, as well as statistical methods, each variant caller has a different calling profile. Discordance in the callers provides a strong argument using deep learning to integrate the information from all the callers in a sophisticated manner.

Table 2: **Comparison of Different Methods and Features of Variant Callers.**

	GATK Unified Genotyper	Samtools	GATK Haplotype Caller	Free Bayes	Pindel
Calling Method	Uses a list of mapped reads, calling model is probabilistic with increased priors at regions with known SNPs	Uses a list of mapped reads, calling model is probabilistic. Does not assume sequencing errors are independent and has less hard filters compared to Unified Genotyper	Uses Hidden Markov Models to build a likelihood of haplotypes which are then used to call variants	Uses a posteriori probability model to build a set of haplotypes to represent mutations, calling model is probabilistic with population based priors	Locates regions which were mapped with indels or only one end was mapped, and then performs a pattern growth to find inserts and deletions. Shown to be able to identify medium length indels missed by other callers in real samples (Spencer et al., 2013)
Reference and Mapping Method	Position based caller that realigns fragments and analyses each position to call SNPs and indels	Position based caller that uses mapped sequences to call SNPs and indels.	Analyses regions where there is high likelihood of mutation based on activity score, and builds a De Bruijn-like graph that reassembles reads (Haplotypes) in that region	Dynamic sliding window based reference frame, using algorithms to determine window size for analysis. Does not require precise alignment, unlike other callers	Focuses on Unmapped regions, regions known to have insert and deletions or regions with only one end mapped.

3.4 Network Architecture

Before training our deep learning network, we tested out various neural network architectures to see which architecture would perform the best for our set of input features. We first explored the flat architecture (Figure 11), which contains stacks of fully connected layers with multiple nodes (initially seven layers, with 80 nodes per layer). This is the simplest architecture, where all the features are loaded onto a single vector, and this entire vector is used as an input to train the neural network. We next explored the PCA + flat architecture which had the same neural network architecture but before the input data was fed into the network, a Principal Components Analysis was done to reduce the dataset to 8 principal components which were then used as input data for the neural network (please see Appendix 5.3 for more details of the PCA analysis). Principal components analysis is a dimensionality reduction technique that enables a compressed representation of data. Each principal component is a linear summation of the original features (X) in the form

$$PC_1 = \beta_{1,1} * X_1 + \beta_{2,1}X_2 + ... + \beta_{n,1}X_n$$

$$...$$

$$...$$

$$PC_i = \beta_{1,i} * X_1 + \beta_{2,i}X_2 + ... + \beta_{n,i}X_n$$

which enables a few principal components to capture a high amount of variance in the dataset. Finally, the last architecture we tested was the merged network this network had a set of layers (initially five layers, 24 nodes per layer) that learns from each caller alone, and then the outputs from each of these layers are subsequently merged and used to make a prediction.

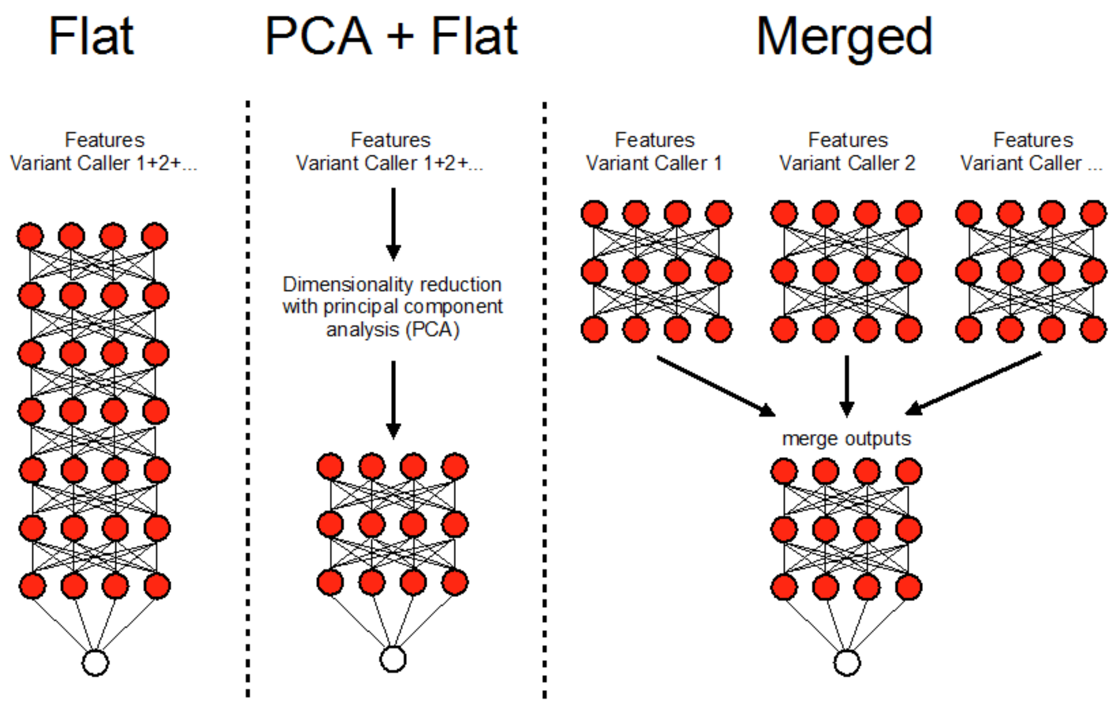


Figure 11: **Different Designs for Neural Network Architecture**

To study how well each architecture is able to perform, we use the metrics of precision, recall and F1 score. Precision measures how many mistakes the predictor makes (the ratio of true positives over false positives and true positives), recall measures what portion of the truth class a predictor can discover (the ratio of true positives over true positives and false negatives) and finally the F1 score is composite function of both precision and recall. The derivations of the metrics can be found in Appendix 5.3.1. Figure 12 shows the precision, recall and F1 score of the three different architectures.

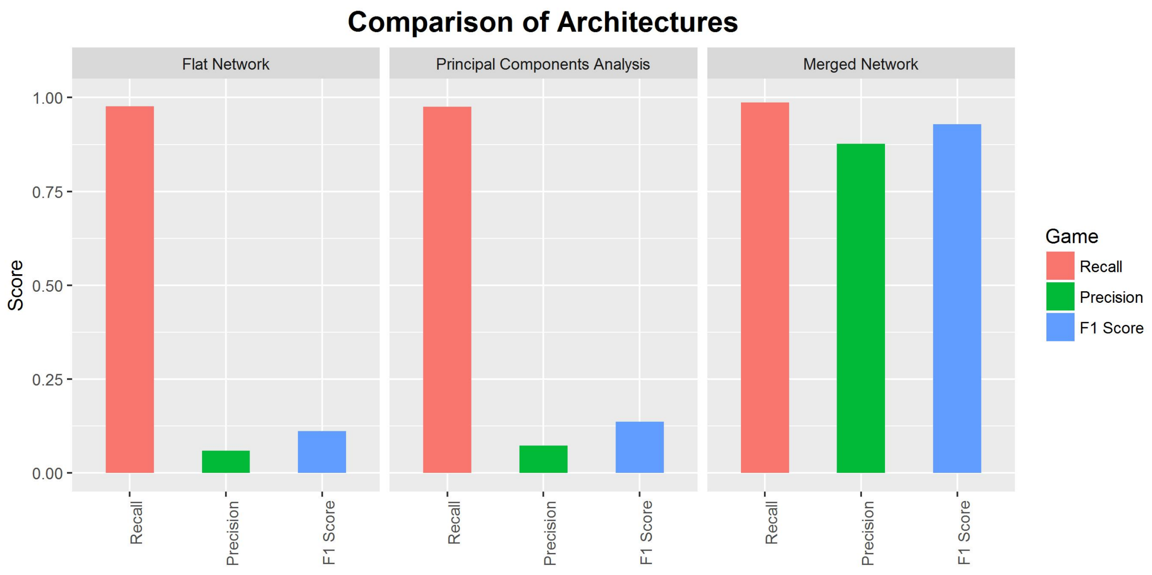


Figure 12: **Analysis of Different Neural Network Architecture**

Initially, with the flat network, the precision rate was very low at 0.059 with an F1 score of 0.112, indicating that the neural network was unable to learn from the input feature set. We suspected that this was due to high dimensionality in the dataset, which led to our second architecture design, the PCA with flat analysis. Principal components analysis has been shown to be able to successfully improve learning in high-dimensionality datasets (Chen et al., 2014; Van Der Maaten, Postma & Van den Herik, 2009). However, the precision and F1 score for the PCA architecture was also low at 0.0735 and 0.137 respectively. Ultimately both failed to learn, indicating to us that perhaps the features from each of the callers had to be analysed separately before being passed into a separate neural network that did the final output integration. With this merged network, we managed to obtain a precision score(0.877) and an F1 score(0.929) that was far better than the previous two architectures. Interestingly, the recall scores for all three architectures were around the same (±0.01), indicating the main difference for the neural network was in its ability to remove false positive calls.

3.5 Network Tuning and Optimisation

Next, we systematically optimised and tuned the deep learning neural network to maximise its predictive ability. In tuning our network, we also sought to study how the various hyperparameters as well as the data structure affected our network's ability to learn from the data. In particular, we focused on four issues – the number of layers, optimiser choice, learning rate choice and finally sample balancing. These four issues are known to be critical in deep learning networks (Ruder et al., 2016; LeCun, Bengio & Hinton, 2015; Yan et al., 2015; Sutskever et al., 2013) and would likely be critical to the success of a deep learning neural network.

3.5.1 Number of Layers

Firstly, we studied how many layers should be in the neural network. The number of layers is critical as it determines what kind of information and the representation of data that can be captured by the neural network. Choosing the number of layers is important as sufficient layers are needed to obtain the complex data representation needed for learning, but too many layers might result in the vanishing gradient problem (Sutskever et al., 2013; Bengio et al., 1994). Our initial neural network architecture is shown below (Figure 13), and then we varied the number of layers in at each point.

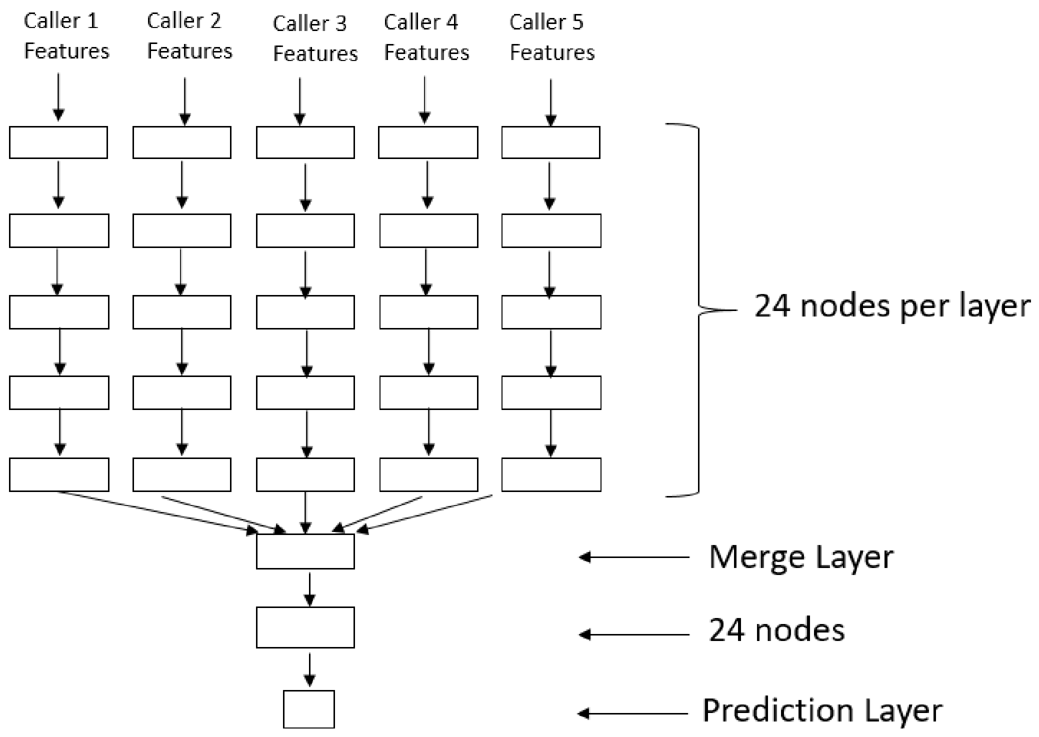


Figure 13: **Basic Merge Network Structure.** Each individual pre-merge layer takes in an input from a single caller. The information is then integrated in a set of merge layers to give a prediction.

For all layers, the LeakyReLU activation function was used. The LeakyReLU is a refinement of the

ReLU activation function which minimises the ”dying ReLU” problem, and both are well-documented activation functions that have been shown to work well in deep neural networks (Anthimopoulos et al., 2016; LeCun, Bengio & Hinton, 2015; Maas, Hannun & Ng, 2013). We noticed that changing the number of layers after the merge layer did not significantly vary the output, and so we focused on changing the number of layers before the merge layer. We studied 6 different neural network structures (4 layers to 9 layers). Accuracy was used as the main metric to compare the neural network architectures, and is defined as the fraction of all samples that the neural network is able to correctly predict.

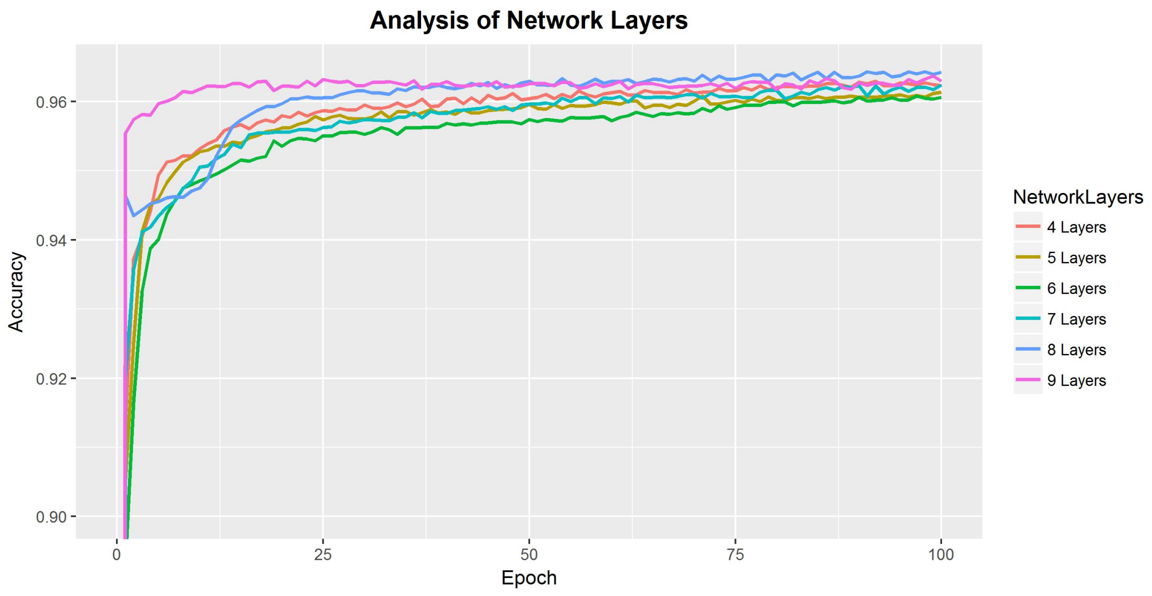


Figure 14: **Analysis of Different Number of Layers On Training Accuracy**

From Figure 14, we find that the 8 layer neural network seem the best at learning from the input data, with a final accuracy of 0.964 that is about 0.001 higher than other layers. We note that all the layers follow the same rough trend of accuracy, indicating they are all able to learn from the dataset. A final design feature used was to add two dropout filters at the last two layers before merging in order to prevent overfitting in data. Dropout filters have been shown to be an effective in preventing overfitting of data (Srivastava et al., 2014).

3.5.2 Optimiser and Learning Rates

Next, we sought to choose the best optimiser and learning rate for our dataset. Both optimisers and learning rates have been well studied and known to be important in neural network training (Ruder et al., 2016; Sutskever et al., 2013). Optimiser choice is critical as the optimisers determine how the weights and gradients are updated in the network, thus playing an integral part in learning. We studied 3 well-known optimisers for use in our network, ADAM, RMSprop and Stochastic Gradient Descent (SGD). ADAM is an adaptive learning rate optimiser that is known to be well suited in large dataset and parameter problems(Kingma & Ba, 2014). RMSprop is another adaptive learning rate optimiser that is unpublished, but has been shown to work well for real experimental datasets (Tieleman & Hinton, 2012). SGD is the simplest learning model with no adaptive learning rate but is a useful model because it is the easiest to understand mathematically and has also been shown to solve deep learning problems (Kingma & Ba, 2014). For more information on the mathematical foundations of optimisation and backpropagation, please see Appendix 5.1. For the three optimisers, we ran tests to study the accuracy of the neural network running on each optimiser to predict true variants (Figure 14).

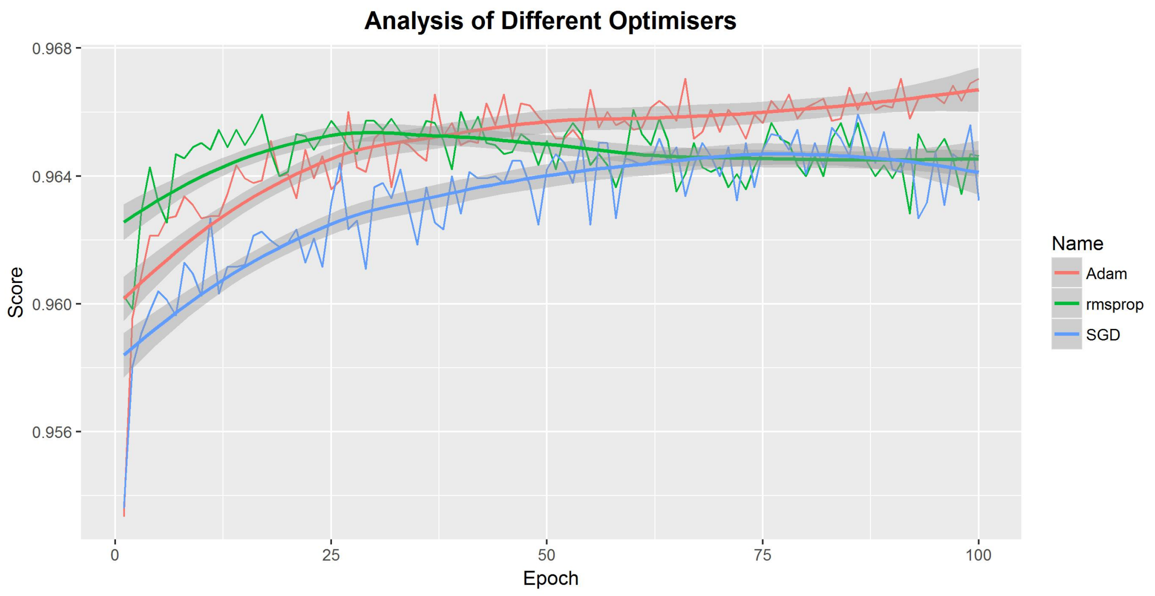


Figure 15: **Optimiser Accuracies for Training at each Epoch.** Due to the noise in accuracies, the overall momentum of the dataset, calculated as a sliding window average is shown. The 95% confidence interval is also shown.

Adam obtained the highest accuracy of 0.9670, while RMSprop and SGD reached maximum accuracies of 0.9660 and 0.9569 respectively. Interestingly the adaptive rate optimisers seemed to have complex learning trajectories, while SGD has a very stable learning rate. This makes sense as adaptive learning rates allow greater gradient descents when the error is high, and decreasing the learning rate at smaller errors (Kingma and Ba, 2014; Zeiler, 2012). This allows Adam and RMSprop to learn at variable rates based on the current gradients. For SGD, it appears that while it takes a while to learn the true minima, it eventually still reaches about the same minima as RMSprop. Ultimately, we chose Adam as our optimiser as the final accuracy discovered by Adam was noted to be higher than RMSprop and SGD, and we note a stable learning curve for Adam, indicating it is able to learn and update the gradients in the neural network to learn from input data at all epochs. Subsequently, we also looked at various initial learning rate for Adam (Figure 16) and found that the most stable learning could be found at a learning rate of 10^{-5}. This initial learning rate is critical as it determines the first few gradient descents which enable stable adaptive learning throughout the epochs (Sutskever et al., 2013). At any larger learning rates (10^{-4} and below), a very high amount of noise was observed, indicating that the learning rate was too high resulting in minima finding errors. At smaller learning rates (10^{-6} and above), the final accuracy after 100 epochs (0.9639 for 10^{-6}) was lower than the learning rate at 10^{-5} (0.9672). Thus, we chose 10^{-5} to be our learning rate.

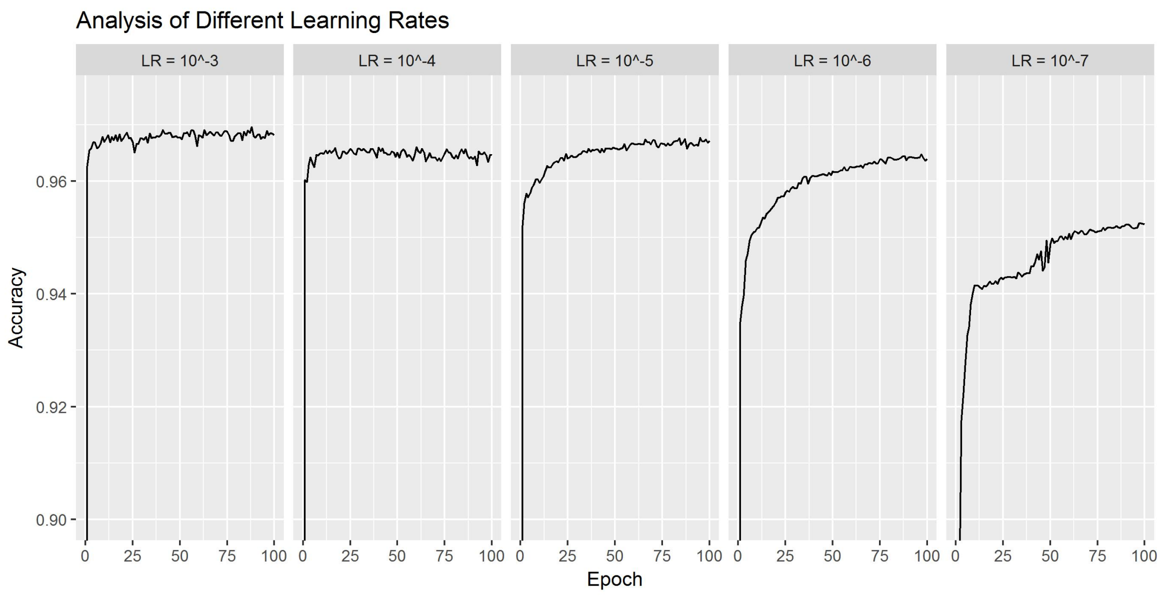


Figure 16: **Training Accuracies over Each Epoch for Different Learning Rates**

3.5.3 Sample Balancing

Our final concern was sample balancing – the simulated dataset contained an imbalance of positive training examples versus negative training examples. In total, there were 286510 positive training examples and 4547919 negative training examples, which is ar0a 15-fold difference. Such a sample imbalance has been known to affect learning adversely (Yan et al., 2015; López et al., 2012). Thus, we sought to implement two methods of sample balancing, undersampling and oversampling. Undersampling was implemented by removing negative training examples until the number of negative training examples was equal to the number of positive training examples. In oversampling, the Synthetic Minority Oversampling Technique(SMOTE) was done, which uses nearest neighbours to create more data points for the positive training example. Specifically, SMOTE looks at two nearby positive class examples, and creates a new synthetic example in the middle of these two examples (see Appendix 5.3 for more details). Figure 17 shows the metrics for each sampling technique.

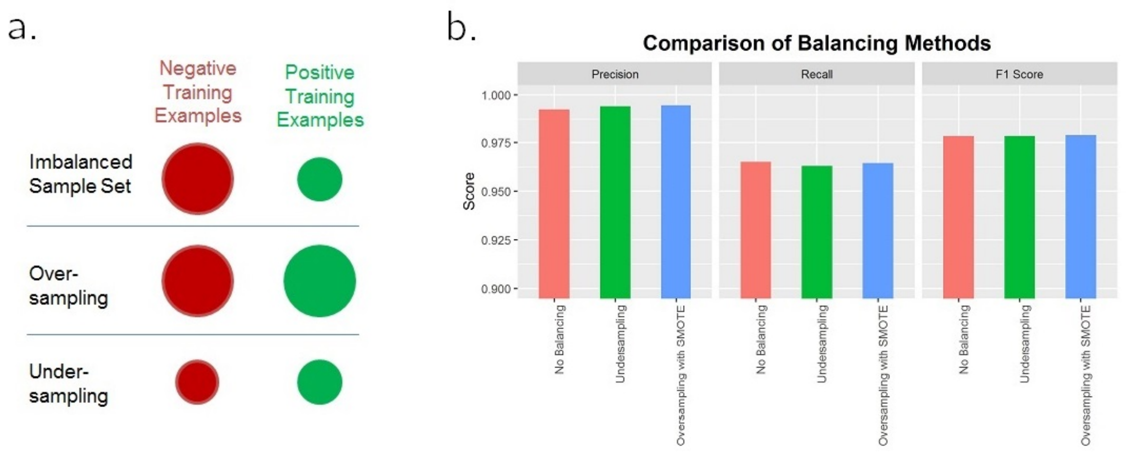


Figure 17: **a) Graphical Illustration of Sample Balancing. b) Effect of Sample Balancing Techniques on Prediction Ability.**

Interestingly, we note that overall, undersampling, oversampling and no sampling at all had very small effects on precision, recall and the final F1 score. Specifically, all three metrics were within a range of 0.003 for the different techniques. This could be due to clear boundary separation within positive and negative class examples as well as good representative datapoints within the positive training example class. This prevents the imbalanced data from having too much of an effect on variant prediction and classification. Still, we note that oversampling techniques resulted in a marginally higher F1 score (0.001 higher than undersampling and no sampling), and since ensuring that datasets are balanced is a recommended protocol to prevent further bias downstream (Chawla, 2005), we used SMOTE oversampling to produce extra positive training class examples for all analysis pipelines.

3.6 Benchmarking of Optimised Network with Mason Dataset

From optimisation steps, we finalised the network architecture as seen in Figure 9, but with 8 layers before the merge layer. We chose the learning rate to be 10^{-5}, and the optimiser used was Adam. With this network, we benchmarked the neural network against the single variant callers, as well as concordance callers, which are an integration of the outputs of the 5 variant callers. Specifically, the n-concordance variant caller is defined as the set of calls that any n callers agree upon – so 1-concordance includes all the calls made by all callers and 4-concordance includes all the calls made by any 4 callers.

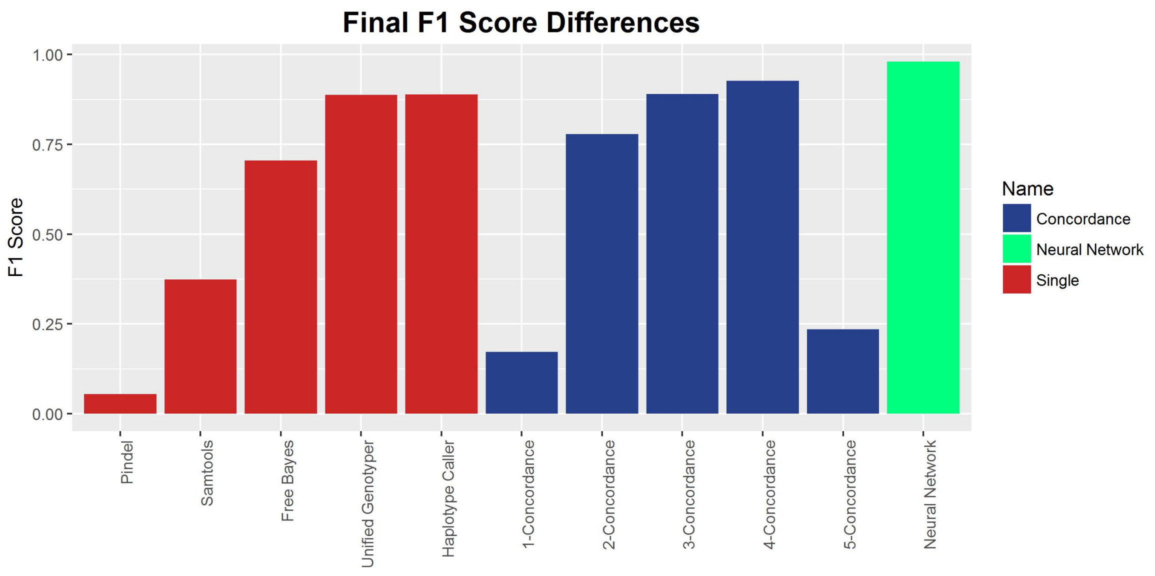


Figure 18: **Overall Comparison of Variant Callers**

In terms of overall F1 score (Figure 18), we see that the neural network was able to outperform single and concordance-based callers. This provides strong evidence that the neural network is able to learn from the input features whether the variant call is real or not, validating its usage in variant calling. The final F1 score obtained by the best single variant caller was Haplotype caller at 0.888, the best concordance caller had an F1 score of 0.927 while the neural network achieved an F1 score of 0.980. To study whether the increase in F1 score is due to improvements in precision or recall, we studied the exact precision, recall and F1 scores of the top 2 variant callers as well as the best single variant caller versus the neural network. We find that the neural network is more precise than both, but the recall is rather similar (Figure 19).

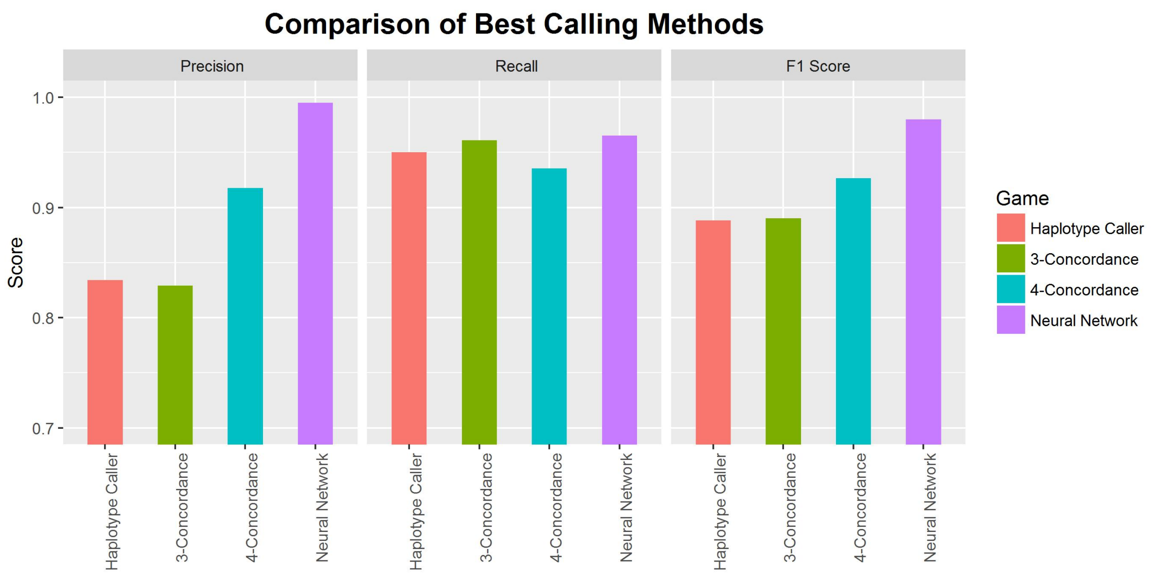


Figure 19: **Comparison of Best Variant Callers in terms of Precision, Recall and F1 Score**

From Figure 18, we see that the neural network is more precise than all four callers, and had the highest precision of 0.995 compared to only 0.917 for 4 concordance. Specifically, this is a 20 fold decrease in the number of false positives or about 23,000 more false positive calls in the 4 concordance network compared to the neural network. Interestingly, the recall of all the callers was high in the range of 0.90 to 0.95, indicating that while all were able to pick out most of the truth class variables, the main errors came from a high number of false positives. Ultimately, the neural network had an F1 score that was 11% above the best single caller and 6% above the best concordance caller. Thus, this provides strong evidence that the neural network is able to sieve out false positives within the dataset and stably predict whether a mutation is true.

3.7 Benchmarking of Optimised Network with NA Dataset

After verification of the optimised neural network on a simulated dataset, we sought to analyse a real dataset to test the validity of the neural network in validating variants. We studied the NA12878 Genome In a Bottle dataset (Zook et al., 2014), which has been used in other variant calling validation pipelines (Talwalkar et al., 2014; Linderman et al., 2014) and contains a set of high-confidence variant calls which we can use as ground truth for training and validation. This set of high-confidence variant calls is obtained from multiple iterations of orthogonal sequencing methods (using Solid, Illumina platforms, Roche 454 sequencing and Ion Torrent technologies). The usage of multiple platforms enables an intersection of variants that can be considered as the ground truth. We then sought to see if our neural network can predict the ground truth better than single or concordance based variant callers.

We applied the same methodology to the sequences as with the simulated data and then used our neural network to predict the true variants. Validation was done with 47971 high-confidence variant calls in total.

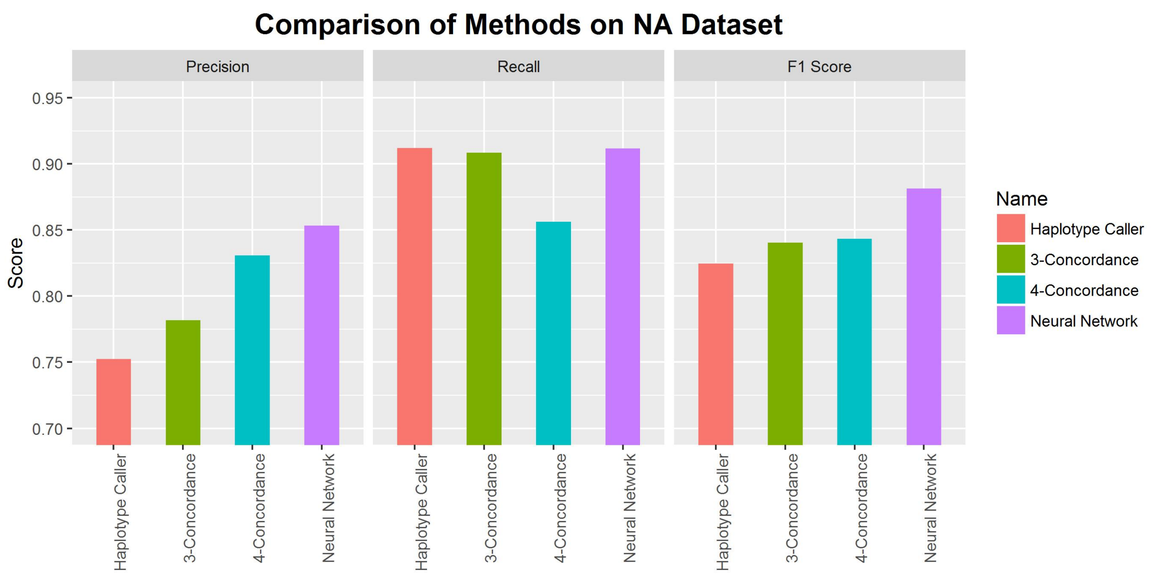


Figure 20: **Comparison of Variant Callers**

As can be seen from Figure 20, the neural network was able to predict with the highest precision (0.859) when compared the best single caller, haplotype caller (0.752) and the 2 best concordance callers, 3-concordance (0.782) and 4-concordance (0.830). In terms of recall, the neural network had a higher recall (0.911) compared to the 4 concordance caller (0.856). Thus, we see that in the NA dataset, the neural network compared with the 4 concordance network is able to call 2650 true variants that were missed by 4 concordance and still had 1228 less false positives. This means the neural network was more aggressive in making calls, yet more of the calls were correct. Compared to the three concordance caller, the neural network had 4253 less false positives. Ultimately when we looked at the F1 score, the neural network was able to outperform concordance variant callers by at least 0.04 and single callers by 0.06. This validates our neural network pipeline in a real genomic dataset and indicates that network is able to learn from the input features.

3.8 Analysis of Gene Importance using Bayesian Ranking systems

After validation of high confidence calls using a deep learning network, we proceeded on to designing a Bayesian network for the clear and understandable ranking of genes. We first build a Bayesian network using known functional annotations from ANNOVAR (Figure 21).

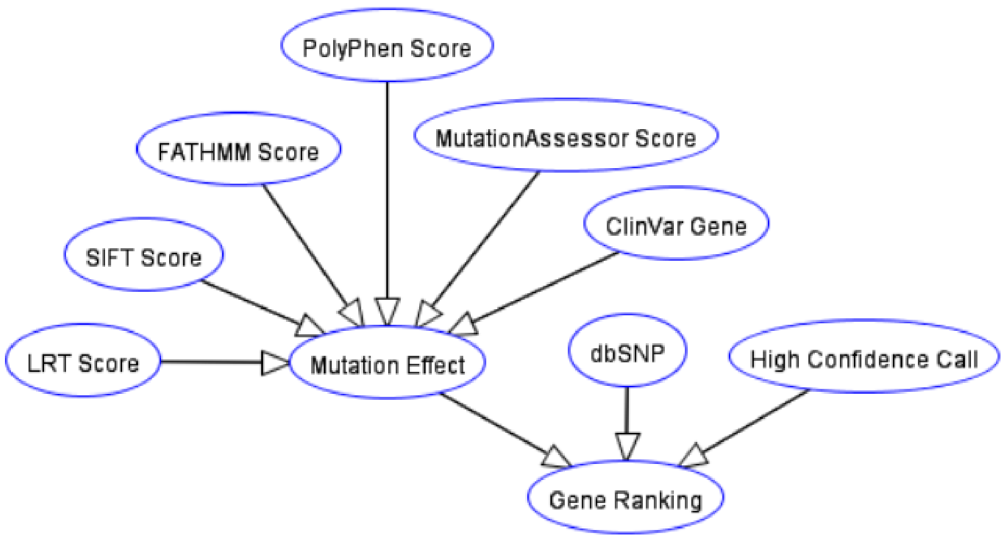
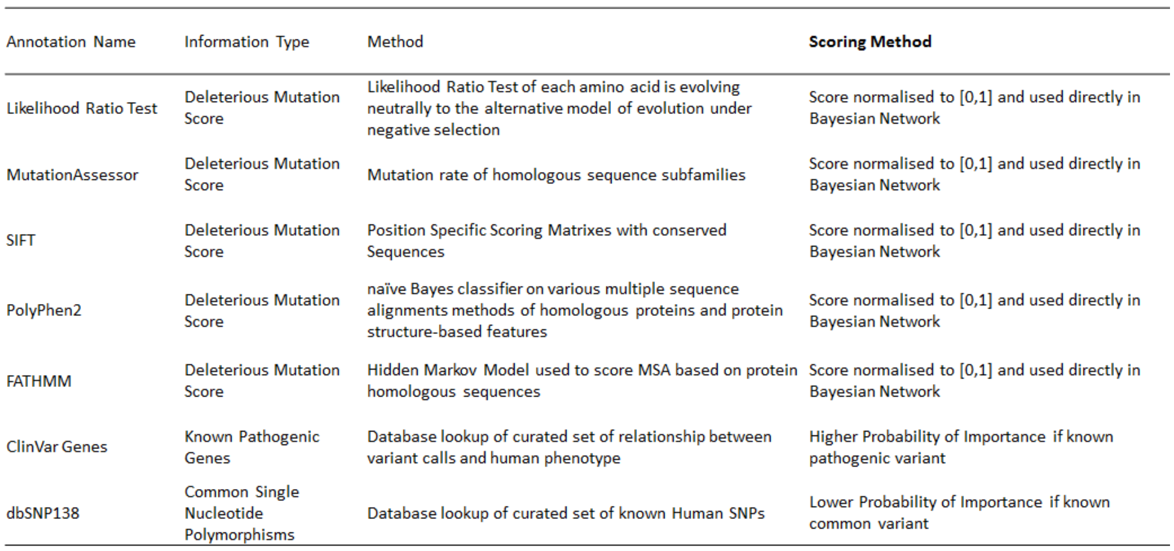


Figure 21: **Final Bayesian Network used in Analysis**

This network structure was chosen as we wanted to use three different sets of information to update the probability of the gene being important. Firstly, the confidence of the call should matter in how important it is – the more likely a gene is real, the more important it should be. Secondly, the rank should also be determined by how common the variation is, based on studying known SNP polymorphism rates. If it is a common SNP, then the ranking should be downgraded as it is less likely to be a driver mutation (Schork et al., 2009). Finally, we sought to predict the overall effect of mutations via an ensemble of mutation effect predictors. These predictors use different methods to predict the average effect of that mutation – based on statistical methods like position-specific substitution matrixes and Hidden Markov Models to study the effect of a mutation on protein structure and function. We also used the ClinVar database, a curated repository of known Human variants and their resulting phenotypes (Landrum et al., 2014). These scores were then aggregated to update the probability of the mutation effect. To obtain these functional annotations, the informatics tool ANNOVAR (Wang, Li, & Hakonarson, 2010) was used. Table 3 shows the functional annotations obtained from ANNOVAR and how they were computed.

Table 3: **Functional Annotations obtained from ANNOVAR**



Annotation Name	Information Type	Method	**Scoring Method**
Likelihood Ratio Test	Deleterious Mutation Score	Likelihood Ratio Test of each amino acid is evolving neutrally to the alternative model of evolution under negative selection	Score normalised to [0,1] and used directly in Bayesian Network
MutationAssessor	Deleterious Mutation Score	Mutation rate of homologous sequence subfamilies	Score normalised to [0,1] and used directly in Bayesian Network
SIFT	Deleterious Mutation Score	Position Specific Scoring Matrixes with conserved Sequences	Score normalised to [0,1] and used directly in Bayesian Network
PolyPhen2	Deleterious Mutation Score	naïve Bayes classifier on various multiple sequence alignments methods of homologous proteins and protein structure-based features	Score normalised to [0,1] and used directly in Bayesian Network
FATHMM	Deleterious Mutation Score	Hidden Markov Model used to score MSA based on protein homologous sequences	Score normalised to [0,1] and used directly in Bayesian Network
ClinVar Genes	Known Pathogenic Genes	Database lookup of curated set of relationship between variant calls and human phenotype	Higher Probability of Importance if known pathogenic variant
dbSNP138	Common Single Nucleotide Polymorphisms	Database lookup of curated set of known Human SNPs	Lower Probability of Importance if known common variant

These were subsequently used to compute the Bayesian probability ranking, which is shown in the equation below. Based on scores provided, we report the update the conditional probabilities using the probabilities chain rule – for the first level; this is given as

$$\begin{aligned} P(Impt|(Del\ \cap\ Uncom \cap High\ Conc)) = \quad & P(Impt \cap Del\ \cap\ Uncom \cap High\ Conc) \\ & * P(Del \cap Uncom \cap High\ Conc) \end{aligned} \tag{3}$$

P(Impt) refers to the probability of the gene being important,
P(Del) refers to the probability of the gene being deleterious,
P(Uncom) refers to the probability of the gene being uncommon and
P(High Conc) refers to the probability of the gene being a high confidence call.
Further calculations can be found, and derivations can be found in Appendix A

To compute the final probabilities, the software library Pomegranate was used. This simplifies the node drawing and probabilistic updates of the final ranking scores (see Relevant Code – Section 7.3).

3.9 Validation of Bayesian Network Ranking on PDX dataset

To study the effectiveness of our Bayesian network ranking system, we sequenced and analysed a patient-derived xenograft (PDX) tumour genome. This tumour genome was grafted onto the immunocompromised mouse from a patient with a known cancer subtype – Diffuse Large B-Cell Lymphoma (DLBCL). We chose to analyse lymphoma as it is a well-known and studied disease model with a well-defined disease progression (Knudson et al., 2001; Alizadeh et al., 2000). The patient-derived xenograft model also allows *in vivo* studies of the tumour in its environment and serves as a good model for sequencing and analysis (Tentler et al., 2012). After sequencing the PDX genome, we put it through our full analysis pipeline, which involves identifying high-confidence mutations using the neural networks and then ranking these genes using the Bayesian network ranking. Figure 22 shows the top 30 genes by probability.

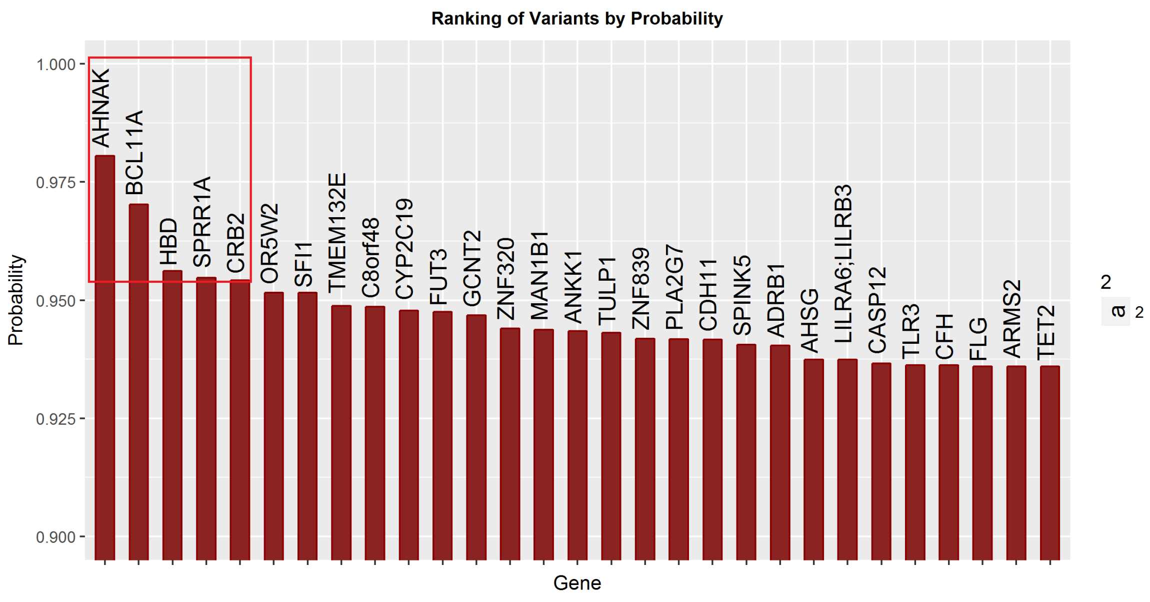


Figure 22: **Top 30 genes from Bayesian Ranking Algorithm**

Studying the top 5 genes, we found that four of these five genes have been implicated in lymphomas or other cancers (Table 4). AHNAK is a known tumour suppressor and has been known to be downregulated in lines of Burkitt Lymphoma (Lee et al., 2014; Amagai et al., 2004; Shtivelman et al., 1992). BCL11A is a known proto-oncogene in DLBCL and has been found to be overexpressed in 75% of primary mediastinal B-cell Lymphomas, a subset of DLBCL (Weniger et al., 2006; Satterwhite et al., 2001). SPRR1A, the fourth gene ranked in terms of importance, has been shown to be expressed in DLBCL (Zhang et al., 2014) and its expression has been shown to strongly correlate with 5-year survival rate (Figure 23). Finally, development of B-cell lymphoma has been noted in CRB2 related syndrome, which is a bi-allelic mutation of CRB2 (Slavotinek, 2016; Lamont et al., 2016). Interestingly, the last of the high ranked genes was noted to be a subunit of Hemoglobin. While there is no strong evidence for the role of Haemoglobin in DLBCL, it has been shown to be expressed in aggressive glioblastomas lines, indicating a possible previously unknown role in cancer (Emara et al., 2014). This gives us high confidence that the Bayesian ranking method can pick up important and relevant mutations. Without such a ranking system, we would have to look through over 70 thousand genes, without a way to systematically study their likelihood of being important.

Table 4: **Highest Ranked Genes from Bayesian Ranking**

Gene	Full Name	Known Involvement in Lymphoma or Cancer	Evidence	Mutation Location	Predicted Mutation Type
AHNAK	Neuroblast Differentiation-Associated Protein (Desmoyokin)	• Known **tumour suppressor** via modulation of TGFβ/Smad signalling pathway • Known to be **downregulated** in cell lines of **Burkitt lymphomas**	Lee et al., 2014; Amagai et al., 2004; Shtivelman et al, 1992	chr11 - 62293433 T -> C	**non synonymous SNV**
BCL11A	B-Cell CLL/Lymphoma 11A	• Known **proto-oncogene** in **DLBCL** • **Overexpression** of **BCL11A** was found in **75% of primary mediastinal B-cell lymphomas** (a subset of DLBCLs)	Weniger et al., 2006; Schlegelberger et al. 2001; Satterwhite et al., 2001	chr2 - 60688580 C -> G	**non synonymous SNV**
HBD	Hemoglobin Subunit Delta	• Shown to be **expressed** by aggressive **glioblastoma** cell lines	Allalunis-Turner et al., 2013	chr11 - 5255274 G -> A	**stop-gain**
SPRR1A	Small Proline Rich Protein 1A (Cornifin-A)	• Known to be **expressed** in **DLBCL** and **expression** has been shown to correlate with **5 year survival rate**	Liu et al., 2014	chr1 - 152957961 G -> C	**non synonymous SNV**
CRB2	Crumbs 2, Cell Polarity Complex Component	• **Cell polarity and cytoskeletal reorganisation** is known to affect B-cell lymphoma **migration and invasiveness** • **Development of B-cell lymphoma** has also been noted in **Crb2-related syndrome** (bi-allelic mutation of Crb2)	Slavotinek, 2015; Gold et al., 2010	chr9 – 126135887 T -> C	**non synonymous SNV**

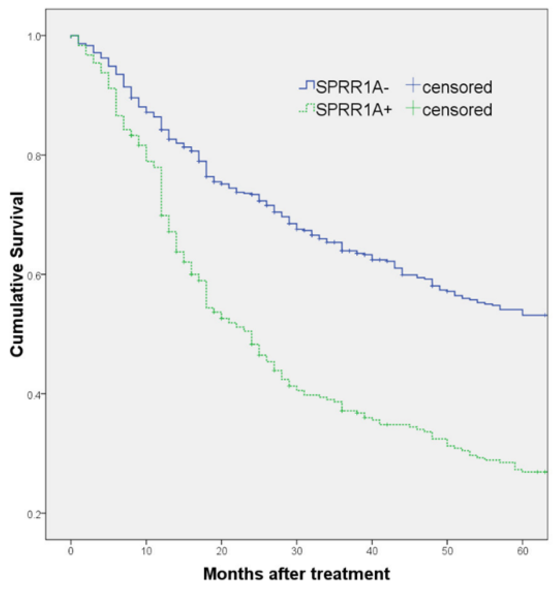


Figure 23: **5 Year Survival Curve of Patients with SPRR1A+ and SPRR1A- DLBCL.** Source : Zhang et al. (2014), Figure 2.

To aggregate the data from our Bayesian Ranking system, we did a Circos plot for the top 300 genes picked up by our gene ranking system (Figure 24). A Circos enables easy visualisation and analysis of large genome datasets, enabling quick understanding and comprehension of results.

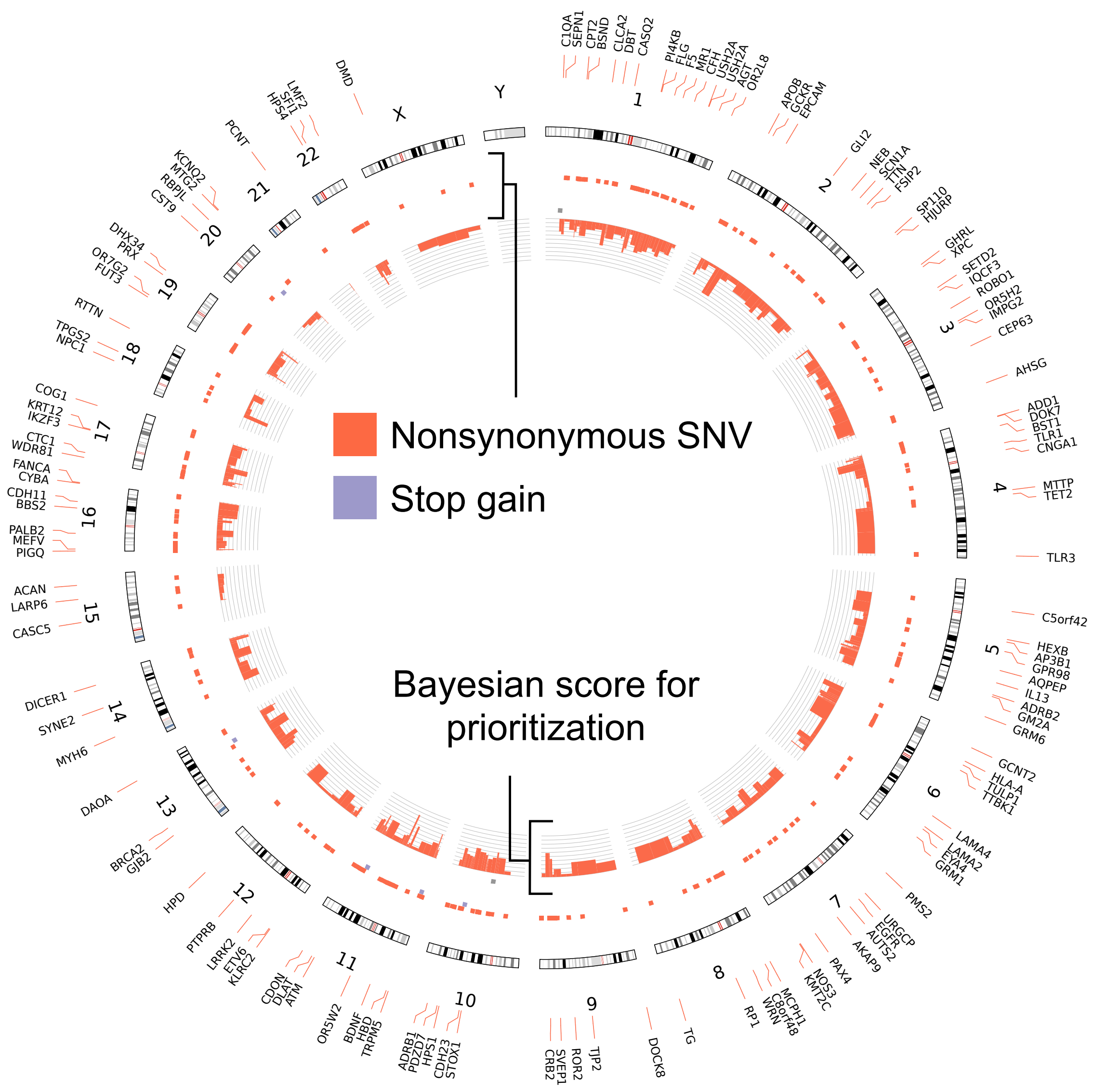


Figure 24: **Circos Plot of Top 300 Ranked Genes from Bayesian Network Ranking.** In this Circos plot, the outer track indicates the top ranked genes and their positions on the chromosome. The inner track describes the type of mutation that was observed – most mutations were non-synonymous SNVs, with a few stop-gain mutations. The innermost track shows the relative probabilities of each ranked gene.

From the Circos Plot (Figure 24), we find several interesting gene families that might also be relevant in B-Cell Lymphoma. These include several Toll-Like Receptors(TLRs), TLR3 (chr4,rank 26) and TLR1(chr4,rank 77) as well as interleukin receptors IL4R (chr16,rank 37) and IL1β (chr2,rank 196). TLRs are of significant interest in cancer due to their involvement in the caspase pathway (Kelly et al., 2006), and have been implicated in B-Cell Lymphomas (Marron, Joyce, & Cunningham-Rundles,2012). Interleukins are also important in cancer due to their importance in mediating inflammation and immune response (Balkwill & Mantovani, 2001). Thus, we show that our Bayesian network can be used by clinicians to quickly interrogate the information from functional annotations and database lookups to report important genes.

4 Discussion

We demonstrate the validation of high-confidence variant calls using an optimised deep learning neural network on both real and simulated datasets, and we also show that a Bayesian network can rank and prioritise genes in a systematic way so as to obtain important genes. We show that four of the top five genes had published findings that linked them with lymphoma. Looking at the top 300 genes ranked, we also found interesting families of genes that are known to be involved in Lymphoma progression, including the Toll-Like receptor and Interleukin receptors families. To benchmark these results, we compared our variant calling results with other methods like VariantMetaCaller and BAYSIC (Gézsi et al., 2015; Cantarel et al., 2014), and we also looked at other methods of gene prioritisation to see how our ranking system compares.

4.1 Comparison of Deep Learning with other Integration Methods

First, we looked at other methods of integrating variant call information, including VariantMetaCaller, which uses Support Vector Machines (a decision making machine learning technique) and BAYSIC, a method that uses a Bayesian probabilistic model to integrate variant call information. Both methods were also used to analyse and predict variants for NA12878 data (Gézsi et al., 2015). VariantMetaCaller increased SNP prediction by 0.04 and indel prediction by 0.07 in terms of the Area Under Prediction Recall Curve (AUPRC) metric when compared to their best single variant caller. The AUPRC measures the precision differences at all levels of recall. BAYSIC also noted a 0.03 increase in SNP prediction and 0.05 increase in indel prediction compared to the best single variant caller. Numerically, this seems comparable to our results of a 0.06 increase in both indel and SNP prediction for the NA dataset compared to the best single variant caller. However, since we used the F1 score metric, instead of the AUPRC metric, a relative quantitative comparison is also not so simple. While the AUPRC metric provides evidence of precision and recall improvements at all levels of threshold (Fawcett et al., 2006), it does not provide evidence for a predictors performance at the best threshold. To measure this, the measurement of the F1 score at the best threshold is required – since it is the F1 score that looks at precision and recall for a specific threshold. Instead, what they have shown is that looking at all thresholds, there is an overall increase in precision and recall, but it is unclear what the improvements are at the optimised thresholds. Fawcett (2006) also mentions this problem, as he notes that 'It is possible for a high-AUC classifier to perform worse in a specific region of ROC space than a low-AUC classifier'. Here, AUC refers to the Area

Under Curve, another term for the AUPRC, and ROC refers to the Receiver Operator Characteristics graph (Egan, 1976) which is the curve that the AUPRC uses. Thus, a higher AUPRC does not mean that one caller will outperform another when considering only the optimised threshold. Measurement of the F1 score is more relevant in clinical practice as we are mainly interested in the optimal operating conditions where precision and recall are maximised and not the fringe conditions. Our results provide specific evidence that at the optimal recall threshold for each specific type of caller, we can show a significant F1 score improvement.

Thus, one definite step moving forward is to incorporate VariantMetaCaller and BAYSIC into our pipelines as negative controls, and measure using the same dataset and same processes whether deep learning can outperform these two methods using the same comparison methods and metrics. Intuitively, we believe that deep learning will be able to edge out improvements as deep learning can form complex representations of the data to learn from that Support Vector Machines are unable to do and ultimately have been shown to outperform Support Vector Machines in decision problems (LeCun et al., 2015; Schmidhuber, 2015) Furthermore, evidence from our flat network architecture shows that putting all the features in a single vector and using that to performing machine learning might not be the best method as it is difficult to learn features from it.

However, one large limitation in the overall approach of measuring each of the methods against the NA12878 dataset is that the high confidence calls provided is not the ground truth. Zook et al.(2014) themselves estimate a possible false negative or positive for every 30 million bases in the NA12878 dataset. This is due to variants that are not inside the high confidence dataset because of errors in one sequencing machine, or genomic regions that cause all sequencing machines to have similar biases and noise. Hence, this would result in misclassification and wrongly called false negative and false positive results, thus skewing the classification results. To solve this problem, a lot of effort has to be put in to obtain a set of verified truth variants via gold standard Sanger sequencing (Tsiatis et al., 2014), but this might be prohibitively expensive for a large number of mutations. Still, this would have to be done for us to have a good set of truth variables to test prediction software with before such software can be considered for use in actual treatment and diagnosis.

4.2 Analysis of Bayesian Network

For the Bayesian Network analysis, it is more difficult to numerically benchmark our results for gene prioritisation with current platforms. This is because currently used platforms are qualitative methodologies like gene panels (Olek and Berlin, 2002) or manual literature look-ups of disease-related genes, such as using the ClinVar database. While gene panels work well in a clinical setting, with NGS data it is hoped that as much information about a person's genome as possible can be used in treatment and diagnosis (Meldrum et al., 2011). Using a gene ranking system instead of just looking at a set of implicated genes might allow doctors to find out tease out possible homologs or interacting agents that might be related to the known deleterious genes (perhaps in the gene panel) and integrate that into their treatment and diagnosis.

4.3 Future Directions

One interesting extension we would like to move into in the future is to be able to integrate a druggable genome into the network, enabling the prioritisation of genes which have possible candidate drug targets. This method would look up a drug-gene interaction database, for example, DGIdb (Griffith et al., 2013), and use the results to inform the importance of a gene. This would enable doctors to notice further possible drug candidates that would work very well on the gene profile of the patient that they might not have considered previously, thus increasing their scope of possible treatment options and augmenting their skills. Other directions in the future include being able to include extra variant callers which will provide it with even more feature data, enabling it to make better predictions. We also hope to build structural variant calling neural networks, as this is a current set of variants that our neural network does not take into account. Finally, we would also like to move everything onto a web interface such that it is accessible for use to perform variant validation and gene prioritisation. This would enable easy access to both the validation and prioritisation pipelines.

Thus, in this paper, we have shown the use of deep learning neural networks to validate variants in both real and simulated datasets successfully. We also show that using a Bayesian network can identify important genes within a lymphoma disease sample. Ultimately, we hope to be able to put these networks to use in a clinical setting to augment treatment and diagnosis of diseases.

5 Appendixes

5.1 Neural Network Learning

Machine learning with deep neural networks is underpinned by two key phases, the feed-forward phase and the backpropagation phase.

5.1.1 Feedforward Phase

The feedforward phase describes the computation of a prediction, and during this phase, the input features are used to compute the final output prediction. For a simple network below:

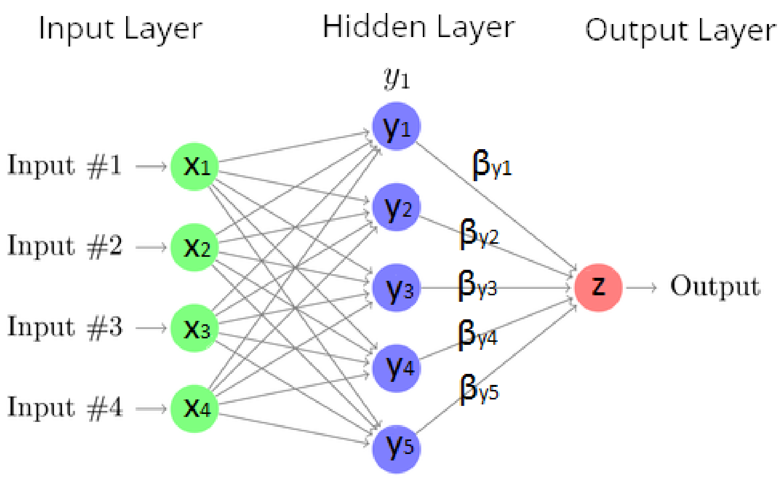


Figure 25: **Sample Neural Networks with Labelled Nodes and Weights.**

The final prediction, z is computed with the equation:

$$z = \beta_{y1} * y_1 + \beta_{y2} * y_2 + \beta_{y3} * y_3 + \beta_{y_4} * y_4 + \beta_{y_5} * y_5 \quad (4)$$

Where β indicates, the weights linking each output to the input of z and each of the y_i terms are computed in the same manner from the x_i layer. At each node (x,y,z), there is also the existence of an activation function that modifies the input of the node to compute an output. Commonly used activation functions include the rectified linear unit (ReLU), sigmoid functions like hyperbolic tangent and logistic function,$S(T) = \frac{1}{1+e^{-t}}$.Thus, the final prediction can be seen as a summation of all weights multiplied by the activation output of each node. In theory, we can expand each of the y_i terms in equation (2) to include the y_i layer activation function as well as rewrite the y_i layer inputs in terms of the sum of outputs and weights from the x_i layers. This complex integration of terms allows for the neural network to form complex continuous decision boundaries as the neural networks can compute sophisticated non-linear prediction functions despite being a fundamentally linear model.

5.1.2 Backpropagation Phase

After a prediction is made, we then have to check whether it is correct and change our weights if an erroneous prediction was made (Figure 26).

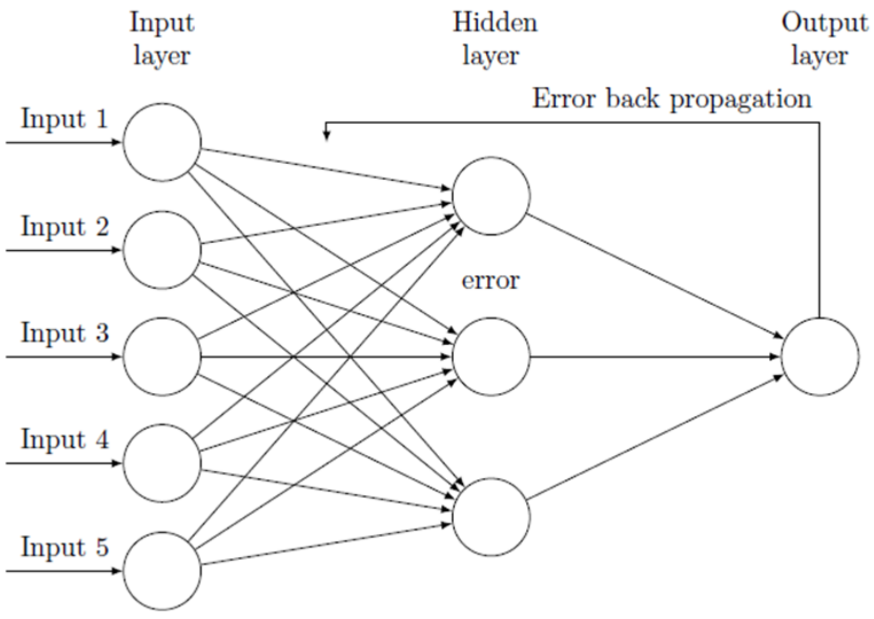


Figure 26: **Backpropagation of Error Terms**

This is the backpropagation step, which involves backpropagating the error terms from the output layers to the input layer and updating the weights at each node based on the differential relationship between the error and each specific gradient. Specifically, this is governed by the optimiser functions which have been mentioned earlier – one example of such a function is the Stochastic Gradient Descent function, which is

$$\beta_{yi}^{n} = \beta_{yi}^{n-1} - \alpha \frac{\partial E_n(\beta)}{\partial \beta_i} \tag{5}$$

Here, each β term indicates a gradient, α is a constant for the learning rate and $\frac{\partial E_n(\beta)}{\partial \beta_i}$ is the term used to modify the weight of the gradient based on the cost function $E_n(\beta)$. The idea used in all backpropagation functions is gradient descent, where the contribution of the gradient term to the error is computed, and the gradient is changed by an amount in order to reduce the future contribution of the gradient to that error.

5.1.3 Cost Function in Gradient Descent

Here it is useful to consider what the cost function $E_n(\beta)$ is. It is essentially the error rate when a set of gradients is used to perform predictions, as it measures how many accurate predictions were made and how many wrong predictions were made. For a binary class predictor (which is what we are using, only true and false), this is given by the equation

$$E(\beta) = -\frac{1}{n}\sum_{i=1}^{n}\sum_{j=1}^{2} y_{ij} log(p_{ij}) \tag{6}$$

where y_{ij} indicates the empirically observed probabilities of each class label while $log(p_{ij}$ is the theoretical probabilities of each class label. This is also known as binary cross-entropy, which is derived from Shannon's entropy (See Appendix 5.2.1). From this term, we see that if the neural network predicts something with a high probability (y_{ij} is high) and it is false (p_{ij} is low) so then $log(p_{ij})$ is a big negative number, and so the cost function will very high. On the other hand, if y_{ij} and p_{ij} is high then the entropy will be close to zero, indicating a correct prediction. Since each of the prediction terms can be rewritten in terms of the gradient(rewrite z in terms βy_i and so on), we can theoretically compute the contribution of each gradient to the cost function to see how the cost function changes as the gradient changes. Thus, this is what gradient descent does – it tries to see how the cost function changes as each gradient changes, then attempts to move the gradient in the direction that minimises the error term.

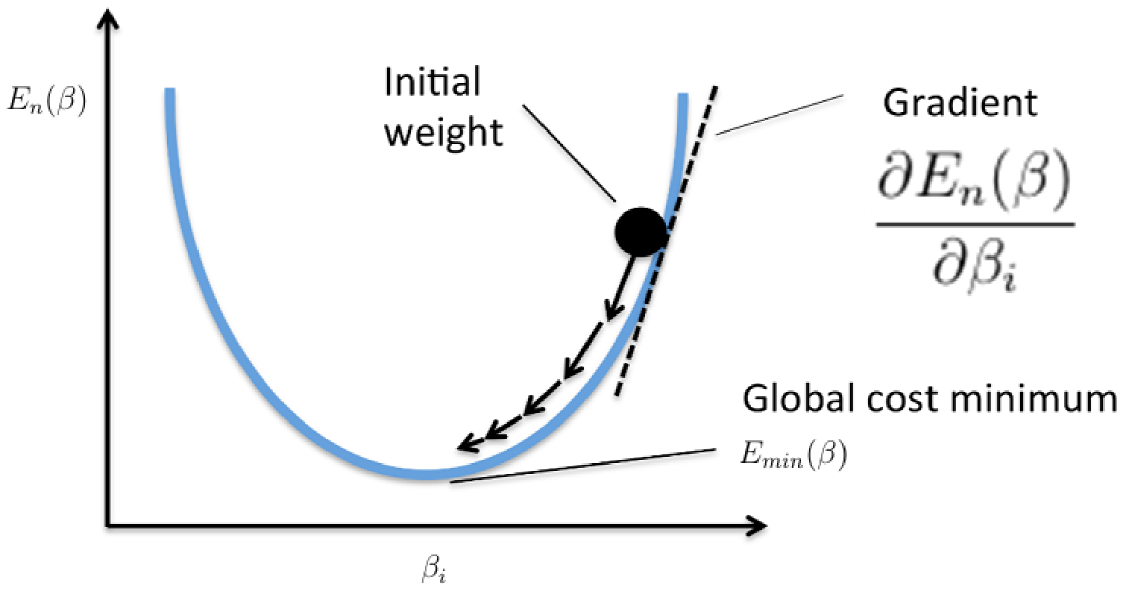


Figure 27: **Graphical Illustration of Gradient Descent.** Gradient descent attempts to find the gradient at which the cost function is minimised (since the cost function depends on the gradient).

This is best seen in Figure 27 above, where the gradient or specifically the partial differentiation of the cost function with regards to each gradient is used to move the gradient to a new position so as to minimise the error term. Thus, machine learning is, in essence, a minimisation problem – we want to find a set of weights that minimises the cost function. Because the cost function describes how many predictions we made correctly, gradient descent essentially trains our network to accurately predict outputs from inputs.

5.2 Feature Engineering

We subset our features into three broad sets, which are base-specific information, sequencing error and bias information features, and calling and mapping quality. Base information tells us base specific properties, including information contained in the base as well as the quality of sequenced bases in the samples. Sequencing error and bias features attempt to tease out potential biases in sequencing, including features such as GC content, longest homopolymer run and as well as allele balances and counts. Finally, calling and mapping quality provides information on the mapping and calling confidence of the variant callers, and includes features such as genotype confidence and mapping quality. In all, these sets of information provide information on the key aspects of variant calling – specifically the properties of the bases in the samples, the characteristics of the sequencing process and finally the variant calling and mapping algorithms.

5.2.1 Base Information

Shannon Entropy

Shannon Entropy captures the amount of information contained in the allele sequences. It is calculated using the equation:

$$H(X) = -\sum_{i=1}^{n} P(x_i) \log_2 P(x_i) \tag{7}$$

where $P(x_i)$ is the probability of finding each base at each position. Thus, we calculate the entropy by summing up the probabilities/log(probabilities) at each position. This prior probability is calculated in two ways, and both are used as features – firstly, the overall genome base probabilities are calculated over the entire genome, and thus the entropy is related to the probability of finding a base at any position in the genome. The second way prior probability is calculated is to take a region of space around the allele (10 bases plus the length of the allele in our calculations) and use those probabilities to calculate the entropy of the allelic sequence. Intuitively, it attempts to find out the amount of information contained within the allelic sequence, and hopefully, the neural network can use the information to determine the validity of a mutation.

Kullback Leibler Divergence

The Kullback-Leibler Divergence feature is similar to Shannon entropy, but instead, we use this to measure the informational change converting from the reference to the allele sequence. The Kullback-Leibler Divergence is calculated as follows:

$$D_{KL}(P||Q) = -\sum_{i=1}^{n} P(x_i) \log_2 \frac{P(x_i)}{Q(x_i)} \tag{8}$$

where $Q(x_i)$ is the prior probability of finding each base at each position based on the genomic region around the allele, while $P(X_i)$ is the posterior probability of finding a specific base inside the allelic sequence. Thus, the KL divergence describes the informational gain when the probabilities from Q is used to describe P. Intuitively, since we know the base probabilities of the region, we can then study the probabilities observed in the reference allelic sequence and see how well $Q(X_i)$ probabilities can approximate $P(X_i)$ probabilities.

Base Quality

Base quality refers to the Phred score probability that the called allele is wrong. It is given by the equation:

$$P = 10^{\frac{-Q}{10}}$$

Where P is the Base Quality, and Q is the probability that the allele called is wrong. This is a number computed by the sequencing machine based on the quality of the base samples provided, and tells us how much confidence the sequencing machine has in calling that base.

5.2.2 Sequencing Biases and Errors

GC content

This feature computes the calculated GC content of reference genome, which may affect sequencing results and accuracy as regions with a GC content are known to be more difficult to sequence. This is because of the greater strength of GC bonds, resulting in errors and biases in sequencing (Benjamini & Speed, 2012).

Longest homozygous run

Homopolymer runs (AAAAAAAA) are known to cause sequencer errors (Quail et al.,2012), and might be a factor in determining whether a variant is true. This because long homopolymers provide the same type of signal to the sequencing machine, resulting in a difficult in estimating the magnitude of the signal or rather how many bases are in that homopolymer, resulting in errors and wrongly called variants. The reference sequence region including the allele was checked for homopolymer runs.

Allele Count and Allele Balance

Allele count gives the total number of alleles in called phenotypes, while allele balance gives the ratio of final allele called over all other alleles called(reference allele for heterozygous calls, or other alleles for homozygous calls). Both these features give us information of possible biases in the sequencing machine.

5.2.3 Calling and Mapping Qualities

Genotype Likelihood

The genotype likelihood provides the Phred-scaled likelihood scores of how confident the caller is in determining that it is a homozygous or heterozygous call, and for the homozygous calls whether it is a more likely to be a bi-allelic mutation or no mutation at all. This feature thus gives us the confidence of the caller in determining if one or two alleles have mutated.

Read Depth

Mapped read depth refers to the total number of bases sequenced and aligned at a given reference base position. The read depth tells us how many reads contributed to a specific call, and thus provides information on how much evidence there is for the variant call

Quality by Depth

Quality by Depth is computed by dividing the quality score against allele depth, to obtain an average score of allele quality. This composite feature provides information on the information provided by each read supporting the call

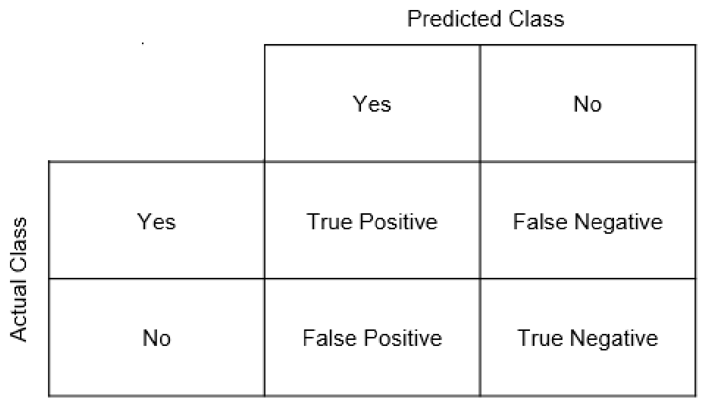
Mapping Quality

Mapping quality is originally a score provided by the alignment method and gives the probability that a read is placed accurately. The variant callers compute an overall mapping quality of the reads that provide evidence for a variant call which is given in this feature. A low mapping quality means that there are multiple positions where the reads contributing to this variant call could have gone, and thus providing evidence that this might not be an accurate call due to poor mapping.

5.3 Mathematical and Statistical Tools

5.3.1 Derivation of F1 Score

The F1 score is a useful measure as it can measure both the precision as well as the recall of a predictor. For a binary predictor with a binary truth class(Figure 28), we can obtain four types of results – true positives, true negatives, false positives and false negatives.



		Predicted Class	
		Yes	No
Actual Class	Yes	True Positive	False Negative
	No	False Positive	True Negative

Figure 28: **Confusion Matrix for a Binary Class Predictor.**

True positives are positive predictions that are made that are positive class labels, while false positives are positive predictions that are made that have negative class labels. Similarly, true negatives are negative predictions that have negative class labels, while false negatives are negative predictions that are positive class labels. From this, we can define two equations, precision and recall. Precision is defined as (8) while recall is defined as (9).

$$Precision = \frac{True\ Positive}{True\ Positive + False\ Positive} \tag{9}$$

$$Recall = \frac{True\ Positive}{True\ Positive + False\ Negative} \tag{10}$$

Precision tells us how likely a positive prediction made will be true, while recall tells us how much of the truth class positive predictions the predictor can classify successfully . Thus, a predictor can have a high precision but low recall (makes few predictions but are very accurate) or a high recall and low precision(makes many predictions that capture all truth variables, but have a lot of false positives as well). In genomics, both types of errors are not desired – we would want all the predictions to be true (precision), while not losing out on any important mutations (recall). Thus, we use the composite metric, the F1 score, that looks at the overall precision and recall of a predictor. It is defined as follows:

$$F1\ Score \quad = \frac{2 * Precision * Recall}{Precision + Recall} \tag{11}$$

5.3.2 Principal Components Analysis (PCA)

Principal Components Analysis (PCA) is a commonly used tool for dimensionality reduction. It was first proposed by Pearson in 1901 (Pearson, 1901) and has been commonplace in many data analytics and signal processing methodologies (Jolliffe, 2002). PCA works by attempting to discover orthogonal principal components (PCs) that are able to represent the original data. Specifically, this means that the PCs can capture variance in the datasets. This is done by finding the Eigenvalues and Eigenvectors of the dataset, with the eigenvectors representing a linear combination of all input variables and the eigenvalues representing the amount of variance that that eigenvector can represent. Ultimately, we select n eigenvectors that can represent a percentage of variance in our dataset. Because each eigenvector is orthogonal, they can capture the variance in the dataset. For our analysis, we decided to use eight principal components – we took the limit as the last principal component that was able to represent at least 0.5% of the variance in the dataset (Figure 29).

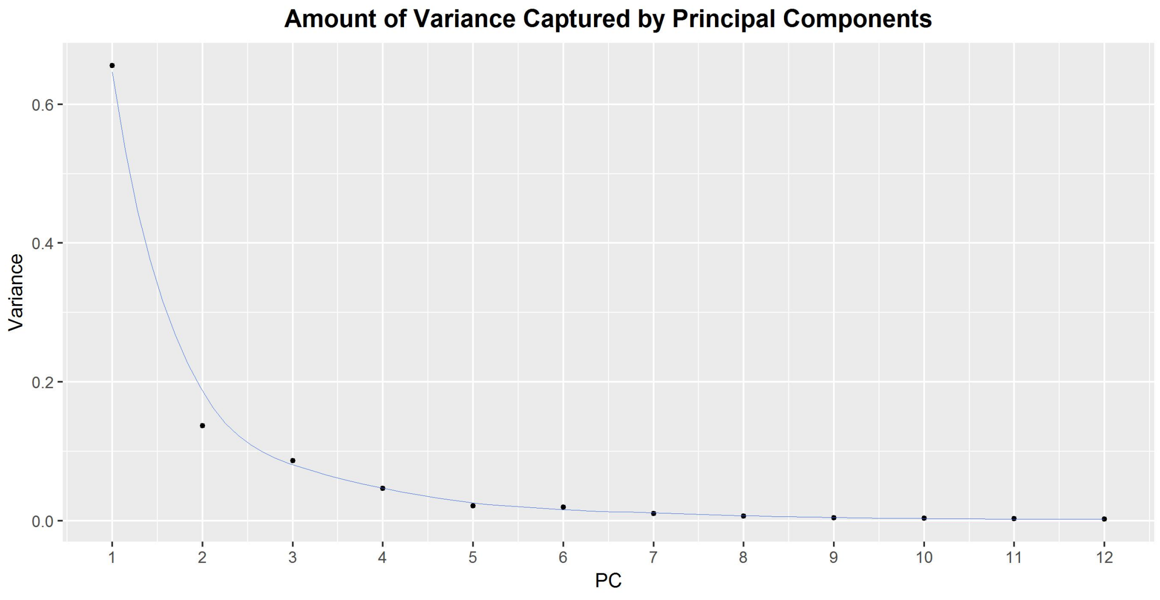


Figure 29: **Variance Captured by First 12 Principal Components**

To carry out PCA, we used the preprocessing step SciPy to normalise all the input vectors to mean 0 and standard deviation 1. Subsequently, we perform principal components decomposition to obtain the eigenvector transformed representation of the dataset and their corresponding eigenvalues. We then fit 8 of the principal components that explained the largest amount of variance into the neural network to study if it can learn from the compressed representation of the input features.

5.3.3 Synthetic Minority Overrepresentation Technique (SMOTE)

SMOTE is a statistical technique described in by Chawla et al. (2002) to overcome problems with imbalanced datasets that are common in machine learning. SMOTE oversamples the training class with fewer variables in a way that tries not to replicate data points (that makes certain data points over-represented) without creating new invalid training examples. It does this by taking the intersection of two nearest data points of the same training class. This can be seen in Figure 30.

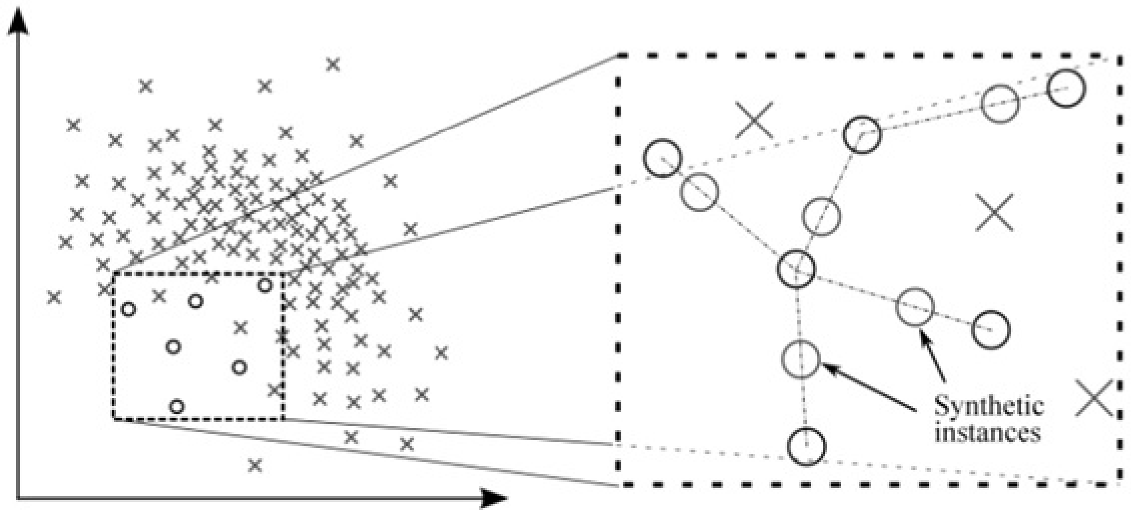


Figure 30: **Illustration of SMOTE Oversampling Algorithm.** Note the use of 2 nearest neighbours to create a new synthetic example. Figure from Chawla et al., 2002

In doing so, it creates a more generalised representation of the sample class with less training examples, without replicating certain datapoints and without creating invalid data. This enables intelligent oversampling of the dataset to balance out the positive and negative feature classes. SMOTE has been shown to be valid for other datasets including sentence boundary detection (Liu et al., 2006) and data mining (Chawla, 2005).

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7 Relevant Code

3 code segments are provided to clarify the implementation of generating the matrixes for deep learning, deep learning networks and finally bayesian networks. Other code segments not shown include the code base for parsing vcf input into features, concordance generators, NextFlow and Bash code used to simulate and process genomic data as well as to control deep learning and analytic pipelines, and other python helper scripts (e.g. comparing two VCF files, analysis with pre-trained network).

7.1 generate_matrixes.py

```python
#This python script generates the set of matrixes to be used in deep learning, and then calls the main method that trains the deep learning network.
#Input : a directory that contains all the vcf files for processing, as well as a truth file.
#Output : np.arrays of features from generate_matrixes with accompanying truth labels, feature set lengths, a dictionary of vcf object records, as well as the list of relevant sample features for easy reference
#Notes :
#Vcf files should have the "vcf" string in their name and truth file should have a "truth" string in its name.
#No other file should be present in the folder
#Overall Strategy :
#Generate a dictionary of lists, where the keys are mutations, and the value is contains a matrix containing information of all five callers
#Secondly, for each mutation label, check if it is inside the truth file or not. The truth is preloaded into a dictionary
#Finally, pass the set of features with accompanying truth labels to the neural network
#The main datastructure used are python dictionaries, which allows O(1) dictionary lookup times

import os
import time
from ANNgenerateresults import *  #this file contains all the main methods for actual neural network training
from methods import *  #this file contains all the methods for parsing each VCF entry into a numerical list of features

#declare names of useful files that contains processed data to be saved
LIST_OF_INPUTS_NAME = '/ANN/samplelist.p'
```

```
TRUTH_DICTIONARY_NAME = '/ANN/truthdict.p'
CALLER_LENGTH_FILE_NAME = '/ANN/callerlengths.txt'
VCF_LIST_FILE_NAME = '/ANN/vcf_list.p'
SCORES_NAME = '/ANN/scores.txt'
Y_DATA_NAME = '/ANN/myydata.txt'
X_DATA_NAME = '/ANN/myXdata.txt'

#Initialise NUMBER_OF_CALLERS
NUMBER_OF_CALLERS = 5


# This method follows the typical input output processing pipeline
# It takes in the user input, and loads it into local variables.
# It then executes another method, main_analyse_samples_and_truth on the loaded variables
# Finally, it then saves files into a directory determined by the final variables, and calls
↪   the next step of the pipeline
# the neural network training, which is main_gather_input_execute_prep_output

def load_and_save_data(user_input):
    user_input = vars(user_input)
    input_samples, referencepath, output_location = load_references(user_input)   # load
    ↪   user input
    my_x_dataset, my_y_dataset, list_of_samples, truth_dictionary, length_of_caller_outputs,
    ↪   \
    vcf_record_list = main_analyse_samples_and_truth(input_samples, referencepath)
    save_files(output_location, my_x_dataset, length_of_caller_outputs,
               list_of_samples, truth_dictionary, vcf_record_list, my_y_dataset)
    orig_stdout = sys.stdout  #save print statements into stdout
    f = file(str(output_location) + SCORES_NAME, 'w')
    sys.stdout = f
    main_gather_input_execute_prep_output(length_of_caller_outputs, truth_dictionary,
    ↪   my_x_dataset, my_y_dataset, list_of_samples, output_location, vcf_record_list)

# This method first prepares a dictionary of truth to be checked against. It then
↪   initialises
# a dictionary of samples with all the keys, each key being a variant call, and then fills
↪   it up each key with data from each caller
# subsequently, it removes dictionary entries that are the wrong size, and then checks
↪   whether
```

```
# each entry in the dictionary is true or not by looking up the truth dictionary
# subsequently it performs array balancing, and converts the data to np.array, as well as
↪  the dictionary of truth
# and list of called samples

def main_analyse_samples_and_truth(path, referencepath):
    os.chdir(path)
    truthdict = generate_truth_list(path)
    print "truth dictionary generated at time :", time.time() - start
    callerlengths, list_of_called_samples, vcf_list = generate_input(path, referencepath)
    print "samples generated at time :", time.time() - start
    clean_truth_array, cleaned_sample_array =
     ↪  check_predicted_with_truth(list_of_called_samples, truthdict)
    print "samples checked with truth at time :", time.time() - start
    cleaned_sample_array = np.array(cleaned_sample_array, np.float64)
    clean_truth_array = np.array(clean_truth_array)
    return cleaned_sample_array, clean_truth_array, list_of_called_samples, truthdict,
     ↪  callerlengths, vcf_list

# This method generates the truth dictionary, by iterating through the vcf file, parsing all
↪  the vcf entries and appending them all as keys in the dictionary

def create_truth_dictionary(generated_truth_dictionary, truth_file):
    vcf_reader = vcf.Reader(open(truth_file, 'r'))
    for record in vcf_reader:
        if "GL" in record.CHROM:    #Ignore non-regular chromosomes in our dataset
            continue
        templist = []
        for item in record.ALT:
            templist.append(str(item).upper())          #Alternates might be a list, so they
             ↪  have to be saved as a immutable tuple
        generated_truth_dictionary[(str(record.CHROM), str(record.POS),
         ↪  str(record.REF).upper())] = tuple(templist)

# This method generates the input dictionary, by first initialising the keys of the
↪  dictionary by iterating through the vcf file once, and then
# Iterating through the vcf file again and parsing all the entries as input vectors

def generate_input(path, referencepath):
    reference_dictionary = get_reference_dictionary_for_entropy(referencepath)
    base_entropy = get_ref_entropy(referencepath)
    full_dictionary = get_dictionary_keys(path)
    list_of_called_samples, callerlengths, vcf_list = fill_sample_dictionary(base_entropy,
     ↪  full_dictionary, path, reference_dictionary)
    return callerlengths, list_of_called_samples, vcf_list


# This method goes through all the training variant calling files and extracts unique calls
 ↪  as keys in the sample dictionary

def get_dictionary_keys(path):
    sample_dictionary = {}
    for vcf_file in os.listdir(path):
        if ignore_file(vcf_file):
            continue
        vcf_reader = vcf.Reader(open(vcf_file, 'r'))
        sample_dictionary = create_dictionary_keys(vcf_reader, sample_dictionary)
    return sample_dictionary

#This method ensures the feature vector is in the right order - the entries must always be
 ↪  in the order fb, hc, ug, pindel and st.

def create_list_of_paths(path):
    list_of_paths = [0] * NUMBER_OF_CALLERS
    for vcf_file in os.listdir(path):
        if ignore_file(vcf_file):
            continue
        if "fb" in vcf_file:
            list_of_paths[0] = vcf_file
        if "hc" in vcf_file:
            list_of_paths[1] = vcf_file
        if "ug" in vcf_file:
            list_of_paths[2] = vcf_file
        if "pind" in vcf_file:
            list_of_paths[3] = vcf_file
        if "st" in vcf_file:
            list_of_paths[4] = vcf_file
    return list_of_paths
```

```

# This method goes through all the training variant calling files and fills each entry in a
 ↪  sample dictionary
# with data. If it is empty, it returns an array of length n, where n is the number of
 ↪  variables
# that same caller would have provided.
# Each caller has a different amount of variables because it contains different datasets

def fill_sample_dictionary(base_entropy, sample_dictionary, path, reference_dictionary):
    callerlengths = [0] * number_of_callers
    index = 0
    total_mode_value = 0
    list_of_paths = create_list_of_paths(path)
    for vcf_file in list_of_paths:
        index += 1
        opened_vcf_file = vcf.Reader(open(vcf_file, 'r'))
        removaldict = iterate_over_file_to_extract_data(base_entropy, sample_dictionary,
                                                        reference_dictionary,
                                                         ↪  opened_vcf_file, vcf_file)
        mode_value = get_mode_value(removaldict)
        add_length_to_caller_lengths_based_on_file_name(vcf_file, mode_value, callerlengths)
        refill_dictionary_with_zero_arrays_for_each_file(sample_dictionary, index,
         ↪  mode_value)
        total_mode_value += mode_value
    list_of_passed_samples, vcf_list =
     ↪  add_mode_values_into_list_of_samples(sample_dictionary, total_mode_value)
    return list_of_passed_samples, callerlengths, vcf_list


# this method fills the dictionary with empty arrays with the same length as the ones that
 ↪  were supposed to be added

def refill_dictionary_with_zero_arrays_for_each_file(full_dictionary, index,
 ↪  length_of_data_array):
    empty_set = []
    for i in range(length_of_data_array):
        empty_set.append(0)
    for item in full_dictionary:
        checksum = len(full_dictionary[item][0])
```

```python
        if checksum < index:
            arbinfo = empty_set
            full_dictionary[item][0].append(arbinfo)


# this method iterates through all the files to extract data from each sample. It uses methods from the
# methods.py function, which parses each record for data.

def iterate_over_file_to_extract_data(base_entropy, sample_dictionary, recorddictionary, vcf_reader1, vcf_file):
    removaldict = {}
    for record in vcf_reader1:
        if "GL" in str(record.CHROM):
            continue
        sample_name = get_sample_name_from_record(record)
        sample_data = getallvalues(record, recorddictionary, base_entropy, vcf_file)
        sample_dictionary[sample_name][0].append(sample_data)
        sample_dictionary[sample_name][1] = record
        create_removal_dict(sample_data, removaldict)
    return removaldict

# this method counts the mode number of entries in the dictionary. Due to certain vcf files having multiple possible number of entries for a field, this will create an error
# as the size of the input arrays should always be constant. Thus, any sample that does not fit the array should be removed.
# TO-DO See if a better implementation can be done that doesn't reduce data available

def create_removal_dict(sample_data, removaldict):
    count = 0
    count += len(sample_data)
    if count not in removaldict:
        removaldict[count] = 1
    else:
        removaldict[count] += 1


# this method prepares the reference genome dictionary for use in entropy calculations

```

```python
def get_reference_dictionary_for_entropy(reference_path):
    record_dictionary = SeqIO.to_dict(SeqIO.parse(reference_path, "fasta"),
     ↪  key_function=get_chr)
    return record_dictionary

# this method ensures that the files inputed are correct

def ignore_file(vcf_file):
    if "vcf" not in vcf_file or "truth" in vcf_file:
        return True
    return False

# this method creates the set of keys for the dictionary

def create_dictionary_keys(vcf_reader, sample_dictionary):
    for record in vcf_reader:
        if "GL" in str(record.CHROM):
            continue
        sample_name = get_sample_name_from_record(record)
        sample_dictionary[sample_name] = [[], []]  # fullname has become a key in
         ↪  fulldictionary
    return sample_dictionary

# standard method that returns a tuple of the variant call object with the chromosome,
 ↪  position, reference and tuple of alternates

def get_sample_name_from_record(record):
    templist = []
    for item in record.ALT:
        templist.append(str(item).upper())
    sample_name = (str(record.CHROM), str(record.POS), str(record.REF).upper(),
     ↪  tuple(templist))
    return sample_name

# this method sets the length of the input neural networks

def add_length_to_caller_lengths_based_on_file_name(vcf_file, caller_length, callerlengths):
    if "fb" in vcf_file:
        callerlengths[0] = caller_length
    if "hc" in vcf_file:
        callerlengths[1] = caller_length
    if "ug" in vcf_file:
        callerlengths[2] = caller_length
    if "pind" in vcf_file:
        callerlengths[3] = caller_length
    if "st" in vcf_file:
        callerlengths[4] = caller_length

# this method wraps the create truth dictionary method and is used to checking that the
↪  dictionary file has the correct name

def generate_truth_list(path):
    generated_truth_dictionary = {}
    for truth_file in os.listdir(path):
        if "truth" not in truth_file:
            continue
        create_truth_dictionary(generated_truth_dictionary, truth_file)
    return generated_truth_dictionary

# this method takes in the mutation (in a tuple) and checks if that mutation exists in the
↪  truth dictionary
# A mutation exists if the chromosome, reference and position of the variant call is
↪  correct, AND one of the alternate alleles it contains
# is also an alternate allele in the truth dataset

def check_sample_against_truth_dictionary(tuple_name, final_truth_list, truth_dictionary):
    temp_tuple = (tuple_name[0], tuple_name[1], tuple_name[2])
    if temp_tuple in truth_dictionary:
        for alternate in tuple_name[3]:
            if alternate in truth_dictionary[temp_tuple]:
                final_truth_list.append(1)
                return
    final_truth_list.append(0)
    return

# This method loads the paths of the files into local variables

def load_references(user_input):
    file1 = user_input['input'][0]
    referencepath = user_input['reference']
    output_location = user_input['output']
    return file1, referencepath, output_location

# This method saves all the processed data into files that can be used for other purposes
↪  later or loaded natively instead of doing the processing again

def save_files(output_location, x_array, length_of_caller_outputs, sample_list, truth_dict,
↪  vcf_dictionary_file,
               y_array=[]):
    file2 = output_location
    x_data_file_name = str(file2) + str(X_DATA_NAME)
    np.save(x_data_file_name, x_array)
    vcf_file_name = str(file2) + str(VCF_LIST_FILE_NAME)
    caller_length_file_name = str(file2) + str(CALLER_LENGTH_FILE_NAME)
    truth_dictionary_name = str(file2) + str(TRUTH_DICTIONARY_NAME)
    list_of_inputs_name = str(file2) + str(LIST_OF_INPUTS_NAME)
    np.save(caller_length_file_name, length_of_caller_outputs)
    with open(list_of_inputs_name, 'wb') as samplesave1:
        pickle.dump(sample_list, samplesave1)
    with open(truth_dictionary_name, 'wb') as samplesave2:
        pickle.dump(truth_dict, samplesave2)
    with open(vcf_file_name, 'wb') as samplesave3:
        pickle.dump(vcf_dictionary_file, samplesave3)
    if y_array != []:
        y_data_file_name = str(file2) + str(Y_DATA_NAME)
        np.save(y_data_file_name, y_array)

# This method takes in two dictionaries, a dictionary of truth mutations and a dictionary of
↪  sample mutations,
# checks whether each of the sample variables are inside the truth dictionary
# and returns 2 arrays, an array of samples and an array of accompanying truth labels

def check_predicted_with_truth(passed_list_of_samples, dictionary_of_truth=[]):
    final_array_of_samples = []
    final_truth_list = []
    for item in passed_list_of_samples:
        if dictionary_of_truth:
```

```python
            check_sample_against_truth_dictionary(item[0], final_truth_list,
             ↪  dictionary_of_truth)
        temp_array = []
        for row in item[1]:
            temp_array.extend(row)
        final_array_of_samples.append(temp_array)
    if dictionary_of_truth:
        return final_truth_list, final_array_of_samples
    return final_array_of_samples

# This method ensures that only the variables that have the modal number of features are
 ↪  used
# in neural network training to ensure all array sizes are the same

def add_mode_values_into_list_of_samples(full_dictionary, mode_value):
    list_of_passed_samples = []
    vcf_list = []
    for key in full_dictionary:
        second_count = 0
        for item in full_dictionary[key][0]:
            second_count += len(item)
        if second_count != mode_value:
            continue
        list_of_passed_samples.append([key, full_dictionary[key][0]])
        vcf_list.append(full_dictionary[key][1])
    return list_of_passed_samples, vcf_list

# This method gets the modal number of features from a modal dictionary

def get_mode_value(removaldict):
    curr = 0
    mode_value = 0
    for new_key in removaldict:
        if removaldict[new_key] > curr:
            curr = removaldict[new_key]
            mode_value = new_key
    return mode_value

```

```python
# This method iterates through the dataset to create a modal dictionary which contains a
↪  key-value pair of (number of features - number of times seen).
# The mode number of features is kept

def iterate_through_dictionary_to_find_mode_size(full_dictionary):
    removaldict = {}
    samples = 0
    for key in full_dictionary:
        samples += 1
        if samples == sample_limit:
            break
        count = 0
        for item in full_dictionary[key]:
            count += len(item)
        if count not in removaldict:
            removaldict[count] = 1
        else:
            removaldict[count] += 1
    return removaldict


if __name__ == "__main__":
    np.seterr(divide='raise', invalid='raise')
    parser = argparse.ArgumentParser(description="train neural net")
    parser.add_argument('-i', '--input', help="give directories with files", nargs='+')
    parser.add_argument('-d', '--debug', help="look at matrixes built")
    parser.add_argument('-r', '--reference', help="")
    parser.add_argument('-o', '--output', help="")
    paths = parser.parse_args()
    start = time.time()
    load_and_save_data(paths)
```

7.2 train_network.py

```python
#This script is called by the generate_matrixes.py script and contains the implementation of the neural network.
#Input : np.arrays of features from generate_matrixes with accompanying truth labels, feature set lengths, a dictionary of vcf object records, as well as the list of sample features
#Output : A VCF file containing all the filtered entries by the neural network, as well the list of accompanying scores
#Overall Strategy :
#Perform SMOTE oversampling of the input features, and then use the features to train the neural network
#After training, perform validation on test dataset, and subsequently prepare a vcf file with filtered entries


#import all necessary components
import argparse
import cPickle as pickle
import sys
import numpy as np
import vcf
from imblearn.over_sampling import SMOTE
from keras.callbacks import *
from keras.layers import Dense, Dropout, Activation
from keras.layers.advanced_activations import LeakyReLU
from keras.layers.normalization import BatchNormalization
from keras.models import Sequential
from keras.models import load_model
from keras.optimizers import RMSprop
from sklearn.metrics import *
from sklearn.model_selection import train_test_split


#set constants
PCA_COMPONENTS = 8
STEP_INCREMENT = 10
RECURSION_LIMIT = 0.0002
VERBOSE = 1
```

```python
seed = 1337

# Initialise random seed for reproducibility
np.random.seed(seed)

#Prepare file names for saving
vcf_file_name = "/ANN/truevcf.vcf"
keras_model_name = "/ANN/model"
model_truth_name = "/ANN/modeltruths.txt"
model_predictions_name = "/ANN/modelpredictions.txt"
original_vcf_reader =
 ↪  "/data/backup/metacaller/stage/data/version6.3a/hc.vcf.normalisedtrain.vcf"

# this method takes in a path and returns training matrixes for the ANN
# The path should contain n caller vcf files and 1 truth file
# vcf files should be labelled with vcf and truth file should be labelled with truth
# no other file should be present in the folder
def main_gather_input_execute_prep_output(array_sizes, dict_of_truth_input,
 ↪  fullmatrix_sample, fullmatrix_truth, list_of_samples_input, save_location,
 ↪  vcf_dictionary):
    calculated_prediction_actual, calculated_truth_actual = train_neural_net(20, 10,
     ↪  fullmatrix_sample, fullmatrix_truth,
     ↪  save_location, array_sizes)
    get_all_relevant_scores(calculated_prediction_actual, calculated_truth_actual,
     ↪  dict_of_truth_input, list_of_samples_input, vcf_dictionary, save_location)

# This method counts the number of false negatives inside the input sample

def count_false_negative(calculated_prediction_actual, calculated_truth_actual):
    count_false_negative = 0
    for i in range(len(calculated_prediction_actual)):
        if calculated_prediction_actual[i] == 0 and calculated_truth_actual[i] == 1:
            count_false_negative += 1
    return count_false_negative

# this is the wrapper function for the recursive hill climbing algorithm to get the best f1
 ↪  score
# It starts from a low threshold value, and marginally increases the threshold until it is
 ↪  unable to find
```

```
# any better F1 scores. It then reports the threshold, F1 score and produces the filtered
↪  callset

def get_all_relevant_scores(calculated_prediction_actual, calculated_truth_actual,
↪  dict_of_truth_input,
                            list_of_samples_input, vcf_list, outputpath):
    print "Here are some predictions", calculated_prediction_actual[:100]
    print "here are some truths", calculated_prediction_actual[:100]
    f1_score_left = get_scores(calculated_prediction_actual, calculated_truth_actual, 0.0,
    ↪  list_of_samples_input, dict_of_truth_input)
    guess_f1_final_score, guess_f1_final =
    ↪  recursive_best_f1_score(calculated_prediction_actual,   calculated_truth_actual,
    ↪  dict_of_truth_input, list_of_samples_input, 0.0, f1_score_left, 0.2)
    get_scores(calculated_prediction_actual, calculated_truth_actual, guess_f1_final,
    ↪  list_of_samples_input, dict_of_truth_input, VERBOSE)
    produce_vcf_file(calculated_prediction_actual, guess_f1_final, list_of_samples_input,
    ↪  vcf_list, outputpath)

# This method produces the vcf file through filtering with the neural network threshold
↪  calls

def produce_vcf_file(calculated_prediction_actual, guess_f1_final, list_of_samples_input,
↪  vcf_list, outputpath):
    prediction = []
    for item in calculated_prediction_actual:
        if item > guess_f1_final:
            prediction.append(1)
        else:
            prediction.append(0)
    list_of_records = []
    for i in range(len(list_of_samples_input)):
        if prediction[i] == 1:
            list_of_records.append(vcf_list[i])
    vcf_reader = vcf.Reader(filename=original_vcf_reader)
    vcf_writer = vcf.Writer(open(outputpath + vcf_file_name, 'w'), vcf_reader)
    for record in list_of_records:
        vcf_writer.write_record(record)

```

```
# This method is the recursive function that attempts to find the threshold that produces
↪  the best f1 score. It does this
# by iterating through steps of thresholds (0.2, 0.02 and 0.002) until no better F1 score
↪  can be found for a marginal increase in threshold.
# It then returns the best F1 score and the threshold

def recursive_best_f1_score(calculated_prediction_actual, calculated_truth_actual,
 ↪  dict_of_truth_input,
                            list_of_samples_input, guess, guess_score, step):
    if step <= RECURSION_LIMIT:
        return guess_score, guess
    new_guess = guess + step
    new_guess_score = get_scores(calculated_prediction_actual, calculated_truth_actual,
     ↪  new_guess,
                                 list_of_samples_input, dict_of_truth_input)
    if new_guess_score > guess_score:
        return recursive_best_f1_score(calculated_prediction_actual,
         ↪  calculated_truth_actual, dict_of_truth_input,
                                       list_of_samples_input, new_guess, new_guess_score,
                                        ↪  step)
    return recursive_best_f1_score(calculated_prediction_actual, calculated_truth_actual,
     ↪  dict_of_truth_input,
                                   list_of_samples_input, guess, guess_score, step /
                                    ↪  STEP_INCREMENT)

# this method uses pre-loaded data to train the neural network. It is optional and only used
↪  when this python script is called natively and not imported

def load_references(input_paths):
    input_paths = vars(input_paths)
    fullmatrix_sample = np.load(input_paths['input'][0])
    fullmatrix_truth = np.load(input_paths['input'][1])
    with open(input_paths['input'][3], 'rb') as fp1:
        list_of_samples_input = pickle.load(fp1)
    with open(input_paths['input'][4], 'rb') as fp2:
        dict_of_truth_input = pickle.load(fp2)
    array_sizes = np.load(input_paths['input'][5])
    with open(input_paths['input'][6], 'rb') as fp3:
        vcf_dictionary = pickle.load(fp3)
    orig_stdout = sys.stdout
    f = file(str(input_paths['input'][3]) + '.txt', 'w')
    sys.stdout = f
    return array_sizes, dict_of_truth_input, fullmatrix_sample, fullmatrix_truth,
     ↪  list_of_samples_input, input_paths, vcf_dictionary

# this method solves the double false negative problem that is created due to the neural
 ↪  network prediction scheme

def remove_duplicated_false_negative(prediction_list, truth_list, false_negatives):
    count = 0
    removal_list = []
    for i in range(len(prediction_list) - 1, -1, -1):
        if count == false_negatives:
            break
        if prediction_list[i] == 0 and truth_list[i] == 1:
            removal_list.insert(0, i)
            count += 1
    for index in removal_list:
        prediction_list.pop(index)
        truth_list.pop(index)
    return prediction_list, truth_list

# this method takes in the binary truth and predicted samples and calculates the true
 ↪  positive rate, false positive rate, recall, precision and f1 score

def get_scores(actual_predictions, actual_truth, value, sample_list, truth_dictionary,
 ↪  verbose=0):
    temp_actual_truth = list(actual_truth)
    prediction = []
    for item in actual_predictions:
        if item > value:
            prediction.append(1)
        else:
            prediction.append(0)
    false_negatives = count_false_negative(actual_predictions, actual_truth)
    finalpredictionnumbers, finaltruthnumbers = add_negative_data(sample_list,
     ↪  truth_dictionary, prediction, temp_actual_truth)
    finalpredictionnumbers, finaltruthnumbers =
     ↪  remove_duplicated_false_negative(finalpredictionnumbers, finaltruthnumbers,
     ↪  false_negatives)
    final_f1_score = f1_score(finaltruthnumbers, finalpredictionnumbers)
    if verbose:
        print_scores(actual_truth, final_f1_score, finalpredictionnumbers,
         ↪  finaltruthnumbers, prediction, value)
    return final_f1_score

# default method for printing all relevant scores

def print_scores(actual_truth, final_f1_score, finalpredictionnumbers, finaltruthnumbers,
 ↪  prediction, value):
    final_false_positive, final_true_negative = perf_measure(finaltruthnumbers,
     ↪  finalpredictionnumbers)
    print "final false positive rate is :", final_false_positive
    print "final true negative rate is :", final_true_negative
    print "final precision score is :", precision_score(finaltruthnumbers,
     ↪  finalpredictionnumbers)
    print "final recall score is :", recall_score(finaltruthnumbers, finalpredictionnumbers)
    print "threshold is", value
    print "final F1 score is : ", final_f1_score

# This method looks at the set of predicted samples and the set of truths and adds the false
 ↪  negatives to the predicted sample.

def add_negative_data(list_of_samples, dict_of_truth, array_of_predicted, array_of_truth):
    dict_of_samples = generate_sample_dictionary(array_of_predicted, list_of_samples)
    list_of_truth = generate_list_of_truth(dict_of_truth)
    new_array_of_predicted = list(array_of_predicted)
    new_array_of_truth = list(array_of_truth)
    original_length = len(new_array_of_predicted)
    for item in list_of_truth:
        fillnegative(item, dict_of_samples, new_array_of_predicted, new_array_of_truth)
    print "number of false data samples are", (len(new_array_of_predicted) -
     ↪  original_length)
    return new_array_of_predicted, new_array_of_truth

```

```python
# This method generates a list of truth variant calls from a dictionary of truth variant
↪   calls.

def generate_list_of_truth(dict_of_truth):
    list_of_truth = []
    for key in dict_of_truth:
        mytuple = dict_of_truth[key]
        temptuple = []
        for item in mytuple:
            temptuple.append(item)
        list_of_truth.append([key[0], key[1], key[2], temptuple])
    return list_of_truth

# This method generates a dictionary of sample variant calls from a list of sample variant
↪   calls.

def generate_sample_dictionary(array_of_predicted, list_of_samples):
    dict_of_samples = {}
    for i in range(len(list_of_samples)):
        item = list_of_samples[i]
        if array_of_predicted[i] == 0:
            continue
        new_key = (item[0][0], item[0][1], item[0][2])
        new_value = item[0][3]
        if new_key not in dict_of_samples:
            dict_of_samples[new_key] = new_value
        else:
            dict_of_samples[new_key] = list(dict_of_samples[new_key])
            dict_of_samples[new_key].extend(new_value)
            dict_of_samples[new_key] = tuple(dict_of_samples[new_key])
            # print dict_of_samples[new_key]
    return dict_of_samples

# Actual method to calculated false positive, false negative rates

def perf_measure(y_actual, y_hat):
    true_positive = 0
    false_positive = 0
    false_negative = 0
    true_negative = 0

    for i in range(len(y_hat)):
        if y_actual[i] == 1 and y_hat[i] == 1:
            true_positive += 1
    for i in range(len(y_hat)):
        if y_hat[i] == 1 and y_actual[i] == 0:
            false_positive += 1
    for i in range(len(y_hat)):
        if y_actual[i] == 1 and y_hat[i] == 0:
            false_negative += 1
    for i in range(len(y_hat)):
        if y_hat[i] == 0 and y_actual[i] == 0:
            true_negative += 1

    print "true positives :", true_positive
    print "false positives :", false_positive
    print "false negatives :", false_negative
    print "true negatives :", true_negative

    true_positive = float(true_positive)
    false_positive = float(false_positive)
    false_negative = float(false_negative)
    if false_positive == 0 and true_positive == 0:
        false_positive_rate = 0
    else:
        false_positive_rate = false_positive / (false_positive + true_positive)
    if false_negative == 0 and true_positive == 0:
        true_negative_rate = 0
    else:
        true_negative_rate = false_negative / (false_negative + true_positive)

    return false_positive_rate, true_negative_rate


# comparator method that takes a tuple and checks whether it is in the dictionary of
 ↪  samples, if it is not, then add a false negative call to the dataset

def fillnegative(tuple1, sampledict, arrayofsamples, arrayoftruths):
    tuple2 = (tuple1[0], tuple1[1], tuple1[2])
    if tuple2 in sampledict:
        for ALT in tuple1[3]:
            if ALT in sampledict[tuple2]:
                return
    arrayofsamples.append(0)
    arrayoftruths.append(1)

# main method that performs neural network training. This method takes in the sample
↪  matrixes, the truth variables, a save file location, number of epochs,
# size of input arrays and the minibatch training size. It first performs SMOTE on the input
↪  dataset, then splits it into training and test dataset. It then
# initialises the deep learning layers, compiles the neural network and uses the input data
↪  to fit the network. The best set of weights at any point is saved
# to a file and reloaded at the end of the fitting. After training, the neural network is
↪  used to predict the original un-oversampled dataset

def train_neural_net(mybatch_size, mynb_epoch, myX_train, myy_train, location, arraysize):
    fb_size, hc_size, ug_size, pindel_size, st_size = get_sizes(array_sizes)
    X_resampled, y_resampled = do_smote_resampling(myX_train, myy_train)
    X_train, X_test, y_train, y_test = train_test_split(X_resampled, y_resampled,
                                                        test_size=0.33, random_state=seed)
    X_fb, X_hc, X_ug, X_pindel, X_st = prep_input_samples(array_sizes, X_train)
    X_fb_test, X_hc_test, X_ug_test, X_pindel_test, X_st_test =
    ↪  prep_input_samples(array_sizes, X_test)
    batch_size = mybatch_size
    nb_epoch = mynb_epoch

    fb_branch = Sequential()
    develop_first_layer_matrixes(fb_branch, fb_size)

    hc_branch = Sequential()
    develop_first_layer_matrixes(hc_branch, hc_size)

    ug_branch = Sequential()
    develop_first_layer_matrixes(ug_branch, ug_size)

    pindel_branch = Sequential()
    develop_first_layer_matrixes(pindel_branch, pindel_size)

    st_branch = Sequential()
    develop_first_layer_matrixes(st_branch, st_size)

    final_model = Sequential()
    final_model.add(Merge([fb_branch, hc_branch, ug_branch, pindel_branch, st_branch],
     ↪  mode='concat', concat_axis=1))
    final_model.add(Dense(24, activation='linear'))
    final_model.add(LeakyReLU(alpha=0.05))
    final_model.add(Dense(6, activation='linear'))
    final_model.add(LeakyReLU(alpha=0.05))
    final_model.add(Dense(1, activation='linear'))
    final_model.add(Activation('sigmoid'))
    print (final_model.summary())
    adam = Adam(lr=0.00001, rho=0.9, epsilon=1e-08, decay=0.0)
    final_model.compile(loss='binary_crossentropy',
                        optimizer=adam,
                        metrics=['accuracy'])

    filepath = location + "/best_weights.hdf5"
    checkpoint = ModelCheckpoint(filepath, monitor='val_acc', verbose=1,
     ↪  save_best_only=True, mode='max')
    callbacks_list = [checkpoint]
    model_history = final_model.fit([X_train], y_train, batch_size=batch_size,
     ↪  nb_epoch=nb_epoch,
                                    validation_split=0.2, verbose=2,
                                     ↪  callbacks=callbacks_list)
    final_model = load_model(location + "/best_weights.hdf5")
    print model_history.history['val_acc'], model_history.history['val_acc']
    print model_history.history['val_loss'], model_history.history['val_loss']
    np.save(location + "/best_weights.hdf5", model_history.history['val_acc'])
    np.save(location + "/best_weights.hdf5", model_history.history['val_loss'])
    scores = final_model.evaluate([X_test], y_test)
    print scores
    final_prediction_array_probabilities = final_model.predict([myX_train])
    final_prediction_array_probabilities = np.squeeze(final_prediction_array_probabilities)
    save_model_details(final_model, final_prediction_array_probabilities, myy_train,
     ↪  location)

    return final_prediction_array_probabilities, myy_train

# Method to perform SMOTE oversampling

def do_smote_resampling(myX_train, myy_train):
    sm = SMOTE(kind='regular')
    where_are_NaNs = np.isnan(myX_train)
    myX_train[where_are_NaNs] = 0
    X_resampled, y_resampled = sm.fit_sample(myX_train, myy_train)
    return X_resampled, y_resampled

# this method saves the details of the neural network

def save_model_details(final_model, save_model_probabilities, trutharray, location):
    name1 = location + model_predictions_name
    name2 = location + model_truth_name
    name3 = location + keras_model_name
    np.save(name1, save_model_probabilities)
    np.save(name2, trutharray)
    final_model.save(name3)

# this method gets the array size of the features used

def get_sizes(array_sizes):
    fb_size = array_sizes[0]
    hc_size = array_sizes[1]
    ug_size = array_sizes[2]
    pindel_size = array_sizes[3]
    st_size = array_sizes[4]
    return fb_size + hc_size + ug_size + pindel_size + st_size


# this method uses a map function to filter data such that each merge layer gets the correct
 ↪   set of data

def prep_input_samples(array_sizes, x_training_data):
    count = 0
    X_fb = np.array(map(lambda x: x[count:array_sizes[0]], x_training_data))
    count += array_sizes[0]
    X_hc = np.array(map(lambda x: x[count:count + array_sizes[1]], x_training_data))
    count += array_sizes[1]
    X_ug = np.array(map(lambda x: x[count:count + array_sizes[2]], x_training_data))
    count += array_sizes[2]
    X_pindel = np.array(map(lambda x: x[count:count + array_sizes[3]], x_training_data))
    count += array_sizes[3]
    X_st = np.array(map(lambda x: x[count:count + array_sizes[4]], x_training_data))
    count += array_sizes[4]
    return X_fb, X_hc, X_ug, X_pindel, X_st


if __name__ == "__main__":
    parser = argparse.ArgumentParser(description="train neural net")
    parser.add_argument('-i', '--input', help="give directories with files", nargs='+')
    input_path = parser.parse_args()
    array_sizes, dict_of_truth_input, fullmatrix_sample, fullmatrix_truth, \
    list_of_samples_input, paths, vcf_dictionary = load_references(input_path)
    main_gather_input_execute_prep_output(array_sizes, dict_of_truth_input,
     ↪  fullmatrix_sample, fullmatrix_truth, list_of_samples_input, paths, vcf_dictionary)
```

7.3 compute_bayesian.py

```
#This script takes in a VCF file with functional annotation already done, and computes the
 ↪  bayesian network using the annotations. It produces a sorted list of vcf entries in a
 ↪  text file, with accompanying annotation scores
#Input : VCF file with functional annotation
#Output : A sorted list of vcf entries with accompanying annotation scores, redirected from
 ↪  stdout to a file
#Overall Strategy :
#First extract all the features from the vcf files and then perform feature-wise
 ↪  normalisation.
#Subsequently, prepare the bayesian network by creating edges, nodes, preparing prior
 ↪  distritions
#Finally use features to update the bayesian network to obtain final probabilities for
 ↪  importance
#Report a list of sorted probabilites for easy ranking

import matplotlib
import vcf
```

```python

matplotlib.use('Agg')

from pomegranate import *

#main method for loading references into local variables

def load_reference(paths):
    paths = vars(paths)
    input = paths['input']
    opened_vcf_file = vcf.Reader(open(input, 'r'))
    name3 = input + "finalscores.txt"
    # orig_stdout = sys.stdout
    # f = file(name3 + '.txt', 'w')
    # sys.stdout = f
    return opened_vcf_file

#method for getting functional annotation scores

def get_scores(record):
    list_of_important_mutations = [record.INFO['SIFT_score'], record.INFO['LRT_score'],
                                   record.INFO['MutationAssessor_score'],
                                   record.INFO['Polyphen2_HVAR_score'],
                                    ↪  record.INFO['FATHMM_score']]
    if 'NN_prediction' in record.INFO:
        NN_prediction = record.INFO['NN_prediction'][0]
    else:
        NN_prediction = -1
    list_of_important_mutations = map(lambda x: x[0], list_of_important_mutations)
    list_of_important_mutations = map(lambda x: None if x == None else float(x),
     ↪  list_of_important_mutations)
    return NN_prediction, list_of_important_mutations

#main method that controls I/O - it gets the input, applies the main function and then
 ↪  prepares the output

def main(paths):
    vcf_object = load_reference(paths)
    full_list_of_scores = analyse_main(vcf_object)
    prepare_output(full_list_of_scores)

#this method controls the processes applied to the vcf file - for each record, it extract
↪   the list of scores,
# normalises it, compute probabilities, sorts it and then return output

def analyse_main(vcf_object):
    full_list_of_scores = extract_list_of_scores(vcf_object)
    apply_feature_wise_normalisation(full_list_of_scores)
    compute_network_and_probabilities(full_list_of_scores)
    full_list_of_scores.sort(key=lambda x: x[4], reverse=True)
    return full_list_of_scores

# since print is redirected to stdoutput, print function is used to store output

def prepare_output(full_list_of_scores):
    for item in full_list_of_scores:
        print item[2], item, item[2].INFO['Gene.refGene']

# wrapper function used to create bayesian network for all records

def compute_network_and_probabilities(full_list_of_scores):
    for record in full_list_of_scores:
        network = create_network_and_compute_probabilities(record)
        compute_record(network, record)

# this function applies a featurewise normalisation of all features to a range of 0-1, and
↪   flip scores
# for certain features

def apply_feature_wise_normalisation(full_list_of_scores):
    for i in range(6):
        min_num = 1000000
        max_num = -1000000
        for item in full_list_of_scores:
            if item[1][i] != None:
                min_num = min(min_num, item[1][i])
                max_num = max(max_num, item[1][i])
        for item in full_list_of_scores:
            if item[1][i] != None:
                value = ((item[1][i] - min_num) / (max_num - min_num) + 0.2) / 1.3
                item[1][i] = value
            else:
                item[1][i] = 0.5
        if i == 0 or i == 5:
            for item in full_list_of_scores:
                if item[1][i] != None:
                    item[1][i] = -item[1][i]

# extract list of of scores from each record, including all functional annotations, clinvar
 ↪  scores and dbsnp

def extract_list_of_scores(vcf_object):
    count = 0
    full_list_of_scores = []
    for record in vcf_object:
        count += 1
        nn_prediction, list_of_scores = get_scores(record)
        if not list(filter(lambda x: x != None, list_of_scores)):
            continue
        get_clinvar_scores(list_of_scores, record)
        snp_present = get_db_snp_scores(record)
        full_list_of_scores.append([float(nn_prediction), list_of_scores, record,
         ↪  snp_present])
    return full_list_of_scores

# Compute the Bayesian Network by assuming observations and attaching mapped probabilities
 ↪  (0,1) to P(X=True | Y=True)

def compute_record(network, record):
    beliefs = network.predict_proba({'Real Gene': 'True', 'ClinVar': 'True', 'PolyPhen':
     ↪  'True', 'LRT': 'True','MutationAssessor': 'True', 'SIFT': 'True', 'FATHMM_gene':
     ↪  'True', 'rs_gene': 'True'})
    # print "\n".join("{}\t{}".format(state.name, belief) for state, belief in
     ↪  zip(network.states, beliefs))
    # get the probability that the gene is important
    prob_gene_important = beliefs[2].values()[1]
    beliefs = map(str, beliefs)
    record.append(prob_gene_important)
    record.append(record[2].INFO['snp138'])
    record.append(record[3])

# If snp is present in db-snp, attach probability of importance to 0.3, else 0.7

def get_db_snp_scores(record):
    snp_present = 0.7
    if record.INFO['snp138'][0] != None:
        snp_present = 0.3
    return snp_present

# If snp is present in clinvar, attach probability of importance to 0.7, else 0.3

def get_clinvar_scores(list_of_scores, record):
    if record.INFO['clinvar_20150629'][0] != None:
        list_of_scores.append(0.7)
    else:
        list_of_scores.append(0.3)

# wrapper method to create the bayesian network and compute probabilities

def create_network_and_compute_probabilities(record):
    ClinVar_gene, FATHMM_gene, LRT_gene, MutationAssessor_gene, MutationTaster_gene,
     ↪  PolyPhen2_gene, SIFT_gene, functional_gene, importgene, real_gene, rs_gene =
     ↪  initialise_distributions(
        record)
    # set up states
    s1, s10, s11, s2, s3, s4, s5, s6, s7, s8, s9 = generate_states(ClinVar_gene,
     ↪  FATHMM_gene, LRT_gene, MutationAssessor_gene, MutationTaster_gene, PolyPhen2_gene,
     ↪  SIFT_gene, functional_gene, importgene, real_gene, rs_gene)
    # set up network
    network = add_edges_bake_network(s1, s10, s11, s2, s3, s4, s5, s6, s7, s8, s9)
    return network

#  method to create the edges in the network

def add_edges_bake_network(s1, s10, s11, s2, s3, s4, s5, s6, s7, s8, s9):
    network = BayesianNetwork("Gene Prediction")
```

```python
    network.add_states(s1, s2, s3, s4, s5, s6, s8, s9, s10, s11)
    network.add_edge(s1, s3)
    network.add_edge(s2, s3)
    network.add_edge(s4, s2)
    network.add_edge(s5, s2)
    network.add_edge(s6, s2)
    network.add_edge(s7, s2)
    network.add_edge(s8, s2)
    network.add_edge(s9, s2)
    network.add_edge(s10, s2)
    network.add_edge(s11, s3)
    network.bake()
    return network

# method that generates the nodes in the bayesian network

def generate_states(ClinVar_gene, FATHMM_gene, LRT_gene, MutationAssessor_gene,
↪  MutationTaster_gene, PolyPhen2_gene, SIFT_gene, functional_gene, importgene, real_gene,
↪  rs_gene):
    s1 = State(real_gene, name="Real Gene")
    s2 = State(functional_gene, name="Functional Gene")
    s3 = State(importgene, name="Important Gene")
    s4 = State(ClinVar_gene, name="ClinVar")
    s5 = State(PolyPhen2_gene, name="PolyPhen")
    s6 = State(LRT_gene, name="LRT")
    s7 = State(MutationTaster_gene, name="MutationTaster")
    s8 = State(MutationAssessor_gene, name="MutationAssessor")
    s9 = State(SIFT_gene, name="SIFT")
    s10 = State(FATHMM_gene, name="FATHMM_gene")
    s11 = State(rs_gene, name="rs_gene")
    return s1, s10, s11, s2, s3, s4, s5, s6, s7, s8, s9

#methods to initialise prior distributions in bayesian network

def initialise_distributions(record):
    ClinVar_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    PolyPhen2_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    LRT_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    MutationTaster_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    MutationAssessor_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    SIFT_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    FATHMM_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    rs_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    import_cdp = get_cdp(3, [(record[0] + 0.2) / 1.3, record[3], 0.8])
    functional_cdp = get_cdp(6, record[1])
    functional_gene = ConditionalProbabilityTable(functional_cdp, [ClinVar_gene, PolyPhen2_gene, LRT_gene,
                                                                   MutationAssessor_gene,
                                                                   SIFT_gene, FATHMM_gene])
    real_gene = DiscreteDistribution({'True': 0.5, 'False': 0.5})
    importgene = ConditionalProbabilityTable(import_cdp, [real_gene, rs_gene, functional_gene])
    return ClinVar_gene, FATHMM_gene, LRT_gene, MutationAssessor_gene, MutationTaster_gene, PolyPhen2_gene, SIFT_gene, functional_gene, importgene, real_gene, rs_gene


# method that builds the cdp table. n is the number of input variables, probability list gives the probability
# that the i-th X variable is true P(Xi=True).

def get_cdp(n, prob_list):
    temp_list = create_true_false_matrix(n)
    calculate_probabilities(n, prob_list, temp_list)
    return temp_list


# Generates a True False matrix using binary counting logic, critical for input in bayesian network

def create_true_false_matrix(n):
    temp_list = []
    for i in range(0, 2 ** n):
        temp_row = []
        for j in range(n):
            number_2 = i // (2 ** (n - j - 1))
            number_1 = number_2 % 2
            if number_1 == 0:
                temp_row.append('False')
            else:
                temp_row.append('True')
        temp_list.insert(0, temp_row + ['False'])
        temp_list.insert(0, temp_row + ['True'])
    return temp_list


# calculates the probabilities, taking in the true list as well as a list of probabilities. The key here is
# the probability that the mutation is true is related to the scores given by mutation taster etc..
# ie P(X is impt | X is Clinvar) = P(X is Clinvar)

def calculate_probabilities(n, prob_list, temp_list):
    for i in range(0, 2 ** (n + 1), 2):
        true_row = temp_list[i]
        true_probability = 1
        false_probability = 1
        for k in range(0, n, 1):
            if true_row[k] == 'True':
                true_probability *= prob_list[k]
                false_probability *= 1 - prob_list[k]  # probability that mutation is false is 1 minus mutation is true
            else:
                true_probability *= 1 - prob_list[k]
                false_probability *= prob_list[k]
        final_true_probability = true_probability / (true_probability + false_probability)
        final_false_probability = false_probability / (true_probability + false_probability)
        temp_list[i].append(final_true_probability)
        temp_list[i + 1].append(final_false_probability)

if __name__ == "__main__":
    parser = argparse.ArgumentParser(description="train neural net")
    parser.add_argument('-i', '--input', help="give directories with files")
    paths = parser.parse_args()
    main(paths)
```