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Feature selection of microarray data using genetic algorithms and artificial neural networks

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Feature Selection of Microarray Data Using Genetic Algorithms and Artificial Neural Networks

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ABSTRACT

Microarrays, which allow for the measurement of thousands of gene expression levels in parallel, have created a wealth of data not previously available to biologists along with new computational challenges. Microarray studies are characterized by a low sample number and a large feature space with many features irrelevant to the problem being studied. This makes feature selection a necessary pre-processing step for many analyses, particularly classification. A Genetic Algorithm -Artificial Neural Network (ANN) wrapper approach is implemented to find the highest scoring set of features for an ANN classifier. Each generation relies on the performance of a set of features trained on an ANN for fitness evaluation. A publically-available leukemia microarray data set (Golub et al., 1999), consisting of 25 AML and 47 ALL Leukemia samples, each with 7129 features, is used to evaluate this approach. Results show an increased performance over Golub's initial findings.

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Introduction

Central Dogma

To fully understand this study, a basic knowledge of molecular biology and the data used is necessary. At the heart of gene expression and cell biology is the Central Dogma. The central dogma has influenced molecular biology since it was first described by Crick in 1956 (Watson, et al. 2004). Simply, information is stored in DNA which passes through an intermediate molecule named mRNA to construct a protein as in Figure 1.

Figure 1 A simplified view of the Central Dogma of molecular biology.

DNA which has been described as the 'blueprint' of life is the initial source of information. In eukaryotic organisms DNA is located in the nucleus of cells. A DNA sequence can be thought of as a string of characters consisting of the letters, A, T, C and G. These are representations of the bases, Adenine, Thymine, Cytosine, and Guanine. An organization of these bases that expresses a polypeptide results in a *gene*. While the exact definition of a gene is still debated amongst experts in the field, it can be understood as a substring of the entire DNA sequence that serves as a set of instructions for building a protein. These instructions are read in

groups of three bases, together which code for an amino acid. However the DNA does not leave the nucleus; it serves as a "permanent" memory that is accessed for its information and returned for storage. In order to convert the information contained within a string of DNA to a functional protein, the sequence must first be converted into an intermediate molecule known as mRNA. Chemically mRNA differs in its backbone structure and its base usage (DNA uses Thymine, mRNA uses Uracil). The "m" in mRNA stands for *messenger*, which accurately describes its purpose. A series of enzymes read the DNA sequence and transcribe the DNA sequence into an mRNA molecule. The mRNA molecule serves as an inverse mobile copy of the DNA sequence. The mRNA exits the nucleus of the cell where it is translated into a polypeptide by complexes of molecules in the cytoplasm called ribosomes. These polypeptides go on to perform different functions throughout the cell. One of the important roles these polypeptides play is in complex feedback loops that regulate the amount of themselves or other polypeptides; making cellular processes and regulation a complex web of non linear interactions.

Cancer

Cancer has afflicted humans throughout recorded history, with some of the earliest documented cases found in ancient Egyptian mummies (American Cancer Society, 2009). Cancer arises through several small accumulated mutations or a few large disruptions within the genetic material of cells (Hagemeijer & Grosveld, 1996). These mutations can arise from DNA replication errors or through environmental effects such as radiation and chemical exposure. These mutations disrupt the normal functioning of a cell.

Cellular division is the process that converts a single fertilized egg into an organism with billions or trillions of cells. Normally cellular division is a tightly controlled process, where several systems ensure that cells divide only when appropriate. However, if a cell collects enough mutations in these control systems over time, problems will arise and the cell will no longer possess the ability to control its cellular division. This results in an increase in aberrant, often non-functional cells, which can form a tumor. The different subtypes of cancer derive from their tissues of origin. For examples lung cancers arise from mutated lung cells and skin cancer from skin cells. While all cells within an organism originally arise from a single cell, these cells undergo differentiation that result in physiologically different cells. When these cells of different lineages become diseased they can become unique forms of cancer.

Leukemia is a common cancer of the blood caused by diseased cells from an organism's immune system. Approximately 231,641 people in the United States suffer from this disease (Leukemia and Lymphoma Society, 2009). The two predominate forms of leukemia are Acute Myelogenous Leukemia and Acute Lymphoblastic Leukemia. Both Acute Myelogenous Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) are characterized by increases in circulating non functional immune precursor cells.

Leukemia cells arise from progenitor cells that normally undergo hematopoiesis. Hematopoiesis is the differentiation process that results in the different cell types of the immune system. As a cell further divides in a differentiation pathway, its function becomes more specialized. All of cells of the immune system start as a multipotential hematopoietic stem cell and then further differentiate into their specific cell types. Stem cells retain the ability to differentiate into a specific type of cell. While they can also renew themselves the hematopoietic cells follow two major lineages: myeloid and lymphoid as seen in Figure 2.

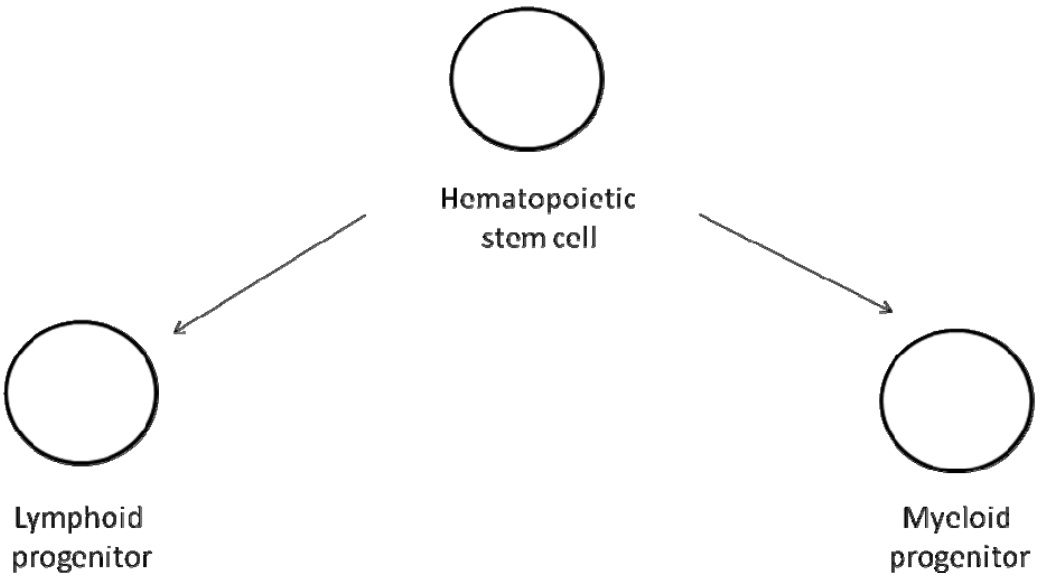


Figure 2 Hematopoiesis two major lineages, Lymphoid and Myeloid.

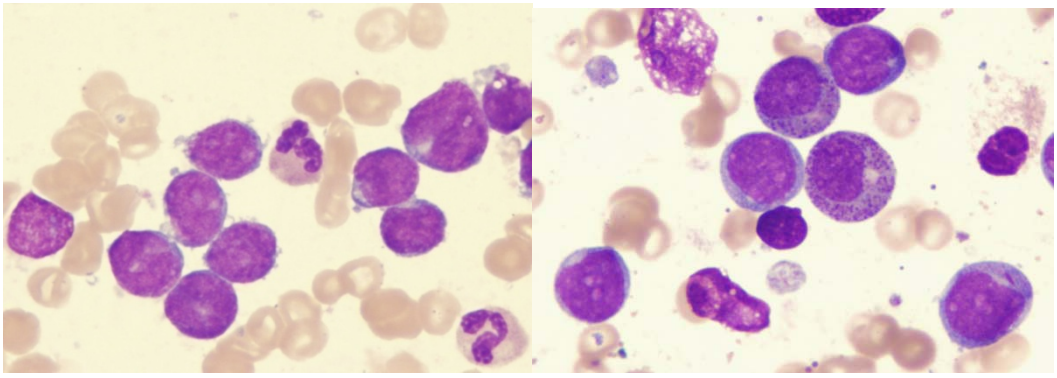
The myeloid lineage gives rise to cells such as granulocytes, phagocytes and monocytes (Parham, 2005). These cells primarily function as part of the innate immune response. Cells of the innate immune response serve as the first responders to threats to the body. This is contrasted to the lymphoid lineage which differentiates into B cells and T cells which are the key components of the adaptive immune response, which tailors antibodies to fight infections. AML and ALL, respectively, refer to unregulated division of a myeloid lineage cell or a lymphoid lineage cell.

Once these cells have lost the ability to control their division, they rapidly dominate the blood stream. This is contrasted with CML and CLL which are the chronic forms of disease that typically have a slower progression, and often arise from cancerous cells that are further differentiated.

Importance of Diagnostic Tests

In prescribing treatment for these cancers, accurate diagnostic tests play an important role. Without knowing the specific type of cancer, important decisions about treatment regimes are difficult for a doctor to make. Due to the difference in origin, the cancerous cells respond differently to treatments and clinical outcome can vary (Poi & Evans, 1998). Therapies that are tailored for AML do not work as well against ALL and *vice versa*, resulting in differences in patient outcome. In the information age and with the future vision of personalized medicine, knowing specific details about a cancer can result in a much different prognosis and treatment.

Leukemia cells are traditionally difficult to classify by morphology alone as seen in Figure 3, and require a series of immunological tests to determine their nature. However these tests remain inaccurate by making diagnosis a subjective task. Microarray profiling of cancer cells has been suggested as a more informative approach to diagnosis (Golub et al., 1999)



ALL AML

Figure 3 Very similar morphologies between cancer cells

Microarrays

Biologists are particularly interested in the types and amounts of proteins present within the cell under given conditions. Because proteins act as the functional molecules of a cell, having different types and quantities changes the behavior of the cell. In cancer this is of even greater interest, as cancers have different expression patterns. However, measuring the amount of a specific protein remains a complicated process. This is due to the complex, three-dimensional structures of proteins. A protein structure can be flexible and can vary greatly in shape and size, making high throughput measurement difficult (Primrose & Twyman, 2004). To help infer the proteins present in a cell under given conditions, researchers measure mRNA. Since mRNA is a precursor for protein, it can be assumed with some confidence that the quantity of mRNA is proportional to the quantity of protein present. The relationship is not always 1-to-1 requiring further validation using protein studies.

Although it is a labile macromolecule, the structure of mRNA lends it to much easier measurement than protein. Every protein is unique, making it difficult to design a tool that can measure thousands of proteins simultaneously. Nucleic acids are able to hybridize to their complementary sequence. Hybridization is the binding of complementary molecules in a low energy state that creates a double stranded molecule that does not easily separate. In hybridization, Adenine binds with Thymine and Cytosine binds with Guanine (Figure 4). This highly specific molecular interaction allows for massive parallel comparison of thousands of sequences (Bevilacqua, 2006).

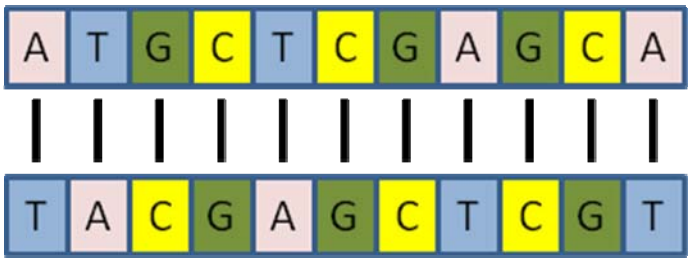


Figure 4 Hybridization between two DNA molecules

When preparing a microarray, first mRNAs are isolated from a given sample representing a state of interest (cancer, non-cancer etc.) and reverse transcribed and labeled with a fluorescent tag. Reverse transcription is the process of converting mRNA back into DNA, a more stable information carrier, using mRNA dependent DNA polymerase or reverse transcriptase.

This solution of reverse transcribed cDNA, known as the target, is then washed over the array and sequences are allowed to hybridize to the probes. After hybridization, the remaining single stranded sequences are washed away, leaving only sequences that have found a complement among the probe sequences bound to the array. The amount of double stranded DNA is then measured by the fluorescence intensities of the hybridized sequences for each location on the array.

There exist several forms of microarray, one of the most popular being Affymetrix GeneChip®. An Affymetrix GeneChip® microarray contains thousands of individual DNA sequences called *probes*, affixed to the surface of a quartz wafer using photolithographic techniques (Draghici, 2003). These probes contain the known complement to thousands of genes of interest. The array uses multiple probes to interrogate a given gene to ensure a robust signal.

The expression values for a gene from Affymetrix® data actually represent an average of 20 different probes pairs. A probe pair consists of one perfect match (PM) probe and one mismatch (MM) probe. The perfect match regions are sequences that are perfectly complementary to a target sequence. A mismatch contains the same sequence as the PM, except that it contains exactly one base difference in the middle of the probe and is considered to be a measurement of non-specific hybridization (Draghici, 2003). This technique allows for correction of intensities to determine a more accurate measurement of the true amount of cRNA present. Ideally the PM intensity is much greater than the MM intensity, allowing for a clear signal.

$$Average\ Difference = \frac{\sum_N PM - MM}{N}$$

Equation 1

PM represents the perfect match probe intensity, MM the mismatch probe intensity, N the number of probe pairs.

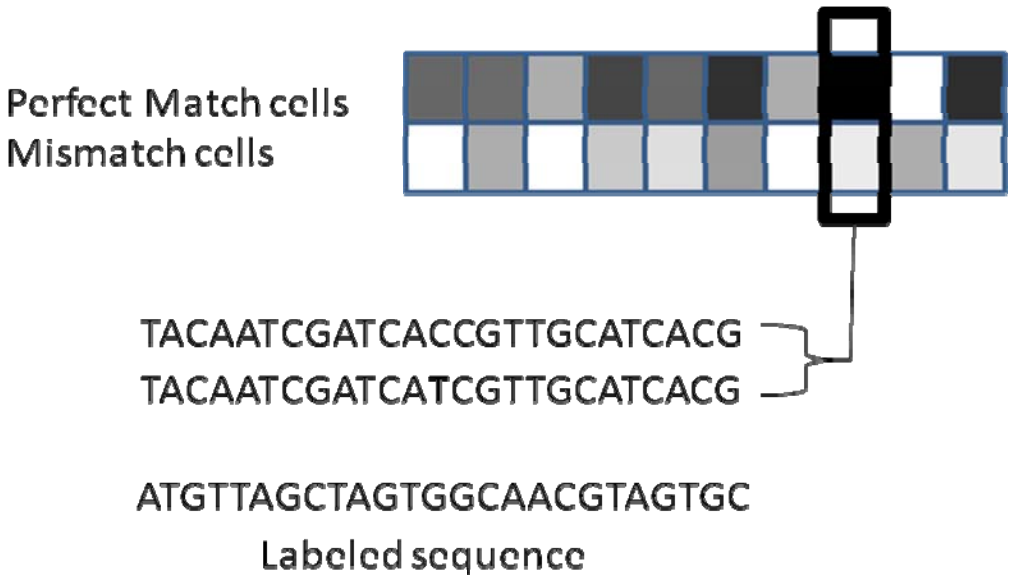


Figure 5 Affymetrix® PM MM strategy

The main benefit from microarray technology is the high throughput measurement of thousands of genes in parallel. In the past, technologies such as the Northern blot analysis and real time PCR could only be used to quantify the expression of a handful of genes at a given time (Kuo, et al., 2004). However a common problem in microarray data analysis is the small number of replicates. This stems from the huge imbalance between the number of features (probes) and the number of samples.

Traditionally microarray data has been analyzed using clustering techniques. From this, a visual approximation is typically taken to determine if there is indeed a pattern present that warrants further study. As microarray data is noisy, it can sometimes be difficult to determine where these patterns exist. The clustering results are typically represented in what is called a heat map. In Figure 6 the color and intensity represents the expression value across several

samples, and the y axis represents the genes analyzed. This representation allows for quick visual interpretation of intensities that is easily interpretable by the human eye.

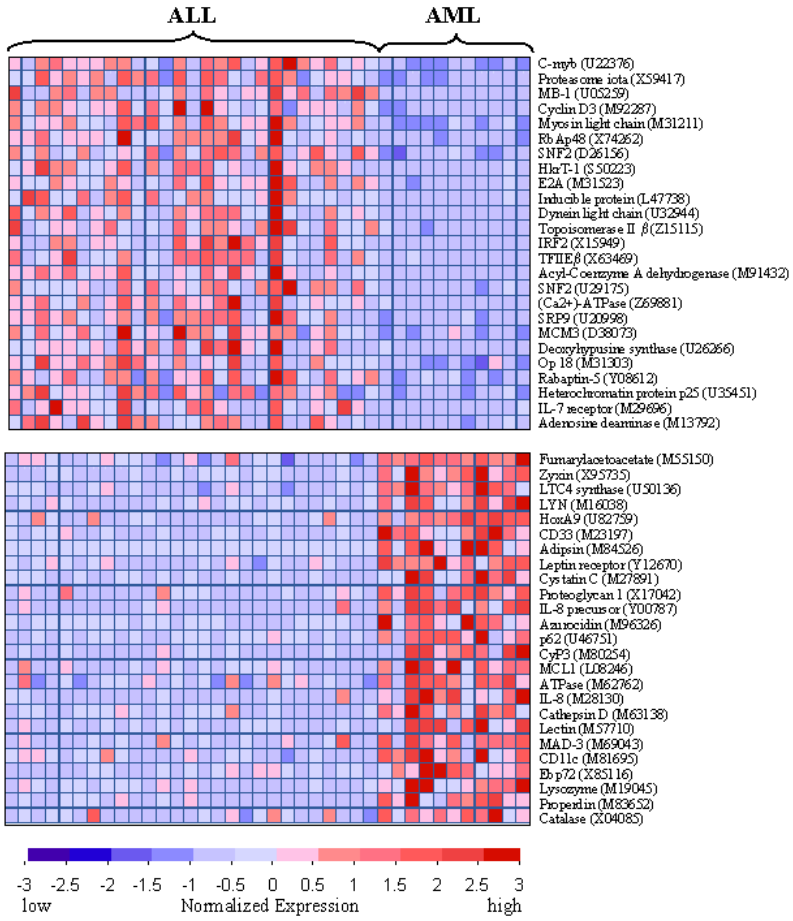


Figure 6. Normalized gene expression values for 50 genes, represented as a heatmap from (Golubet al., 1999).

Feature Subset Selection

Feature subset selection (FSS) is a well studied problem within the machine learning community. This problem is characterized by a dataset with a large number of features. Within this set of features there are a few features that contain relevant information, with the rest of the features being irrelevant or of low information content. The highly informative features are typically used to build a classification model while the non informative are disregarded. By

reducing the number of features that are ultimately used for classification, an increase in performance in the algorithm can be seen (Yang & Honavar, 1998). Filter and wrapper approaches are the two primary methods that researchers utilize to tackle this problem. The important difference between filter and wrapper methods is their use of univariate and multivariate analysis, respectively.

The filter approach performs feature selection as a preprocessing step before the use of a classification algorithm (Yang & Honavar, 1998). Here each feature is evaluated independently using a test statistic and acceptable features are used for the classification algorithm. Studies of feature reduction of microarray data most commonly use this approach (Inza et al. 2004). A commonly used test statistic in microarray studies is the t-test to isolate significantly differentially expressed genes.

The wrapper approach is any method that incorporates a classifier to select relevant features as a group (Yang & Honavar, 1998). Groups of features are evaluated together using an algorithm, and the best set of features is retained before modifying the feature set. This approach, while generally more accurate than the filter approach, comes at a computational cost (Inza et al. 2004). The wrapper method relies on many evaluations of the data while the filter approach uses a single evaluation.

Dataset

Within the microarray data mining community the Golub dataset (Golub et al., 1999) is often used as a standard for evaluating new algorithms. The data is presented as a training set of 38 samples and an independent testing set of 34 samples. Each of these is represented in an n x m *expression matrix*, of 7129 rows and 38 and 34 columns respectively. The expression matrix

contains the expression values for each sample at each feature. The samples represent two types of leukemia, acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).

Table 1

Cancer Type	Training	Testing
AML	11	14
ALL	27	20

Original data division from Golub 1999

Golub et al. (1999) developed a class prediction algorithm that achieved an accuracy of 85% for samples presented and 100% for samples that were above a prediction strength threshold. This method first identified samples that were significantly differentially expressed by a signal to noise statistic. This statistic compares the average expression between two classes and looks for a high difference, representing high correlation between the gene and an idealized expression pattern of high expression in one class and low expression in the other.

$$Signal\ to\ Noise = \frac{\mu_1 - \mu_2}{\sigma_1 + \sigma_2}$$

Equation 2

μ_1=mean of genes in class 1, μ_2=mean of genes in class 2, σ_1= standard deviation of genes in class 1,σ_2=standard deviation of genes in class 2

A positive value reflects gene expression that is high in class 1 and low in class 2, whereas a negative value represents gene expression that is high in class 2 but low in class 1. The magnitude of the value represents the strength of the correlation. This value is not bound

between -1 and 1 like a Pearson correlation. The genes were then ranked based on this statistic. The set of n informative genes is constructed from $n/2$ genes that are most highly correlated to high expression in class one and $n/2$ genes that are most highly correlated to high expression in class two.

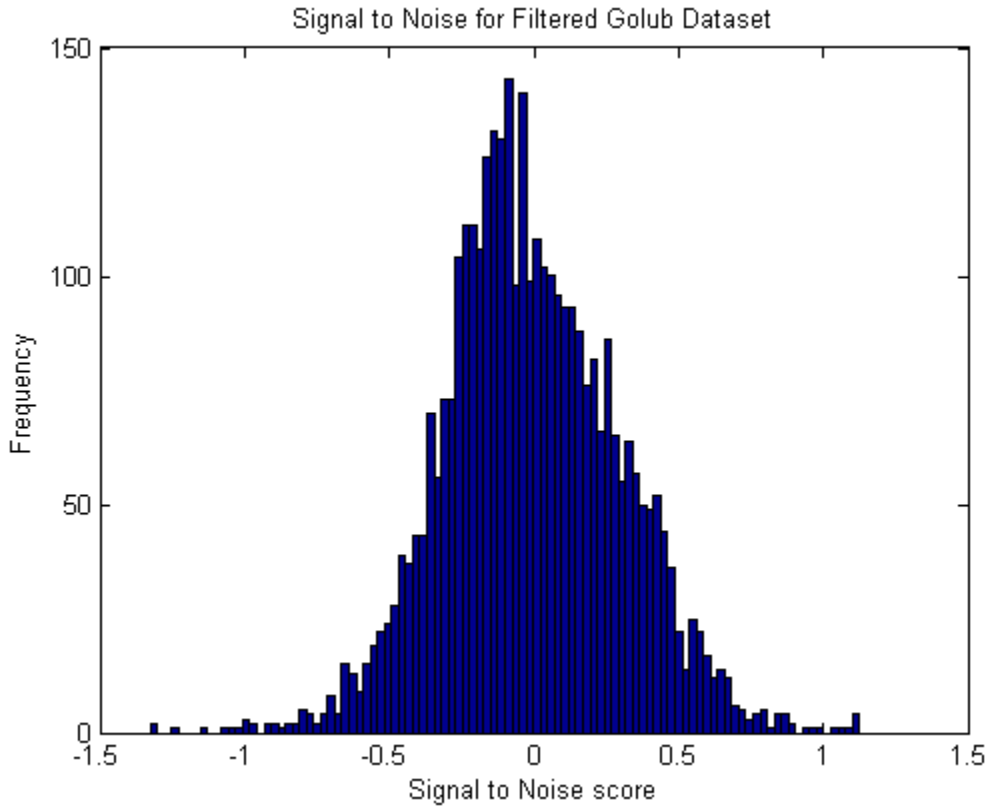


Figure 7 Signal to noise distribution. Negative values indicate a strong correlation to high expression in ALL samples one and low expression in AML samples. Positive values indicate a strong correlation to low expression ALL samples and high expression in AML samples.

From the set of informative genes from the training class a classifier was constructed. This classifier calculates a weighted vote for each informative gene.

$$Vote_g = a_g(x_g - b_g)$$

Equation 3

a_g=Correlation, x_g=Expression of gene, $b_g=\frac{[x_1+x_2]}{2}$

The sum of the absolute values of the vote for each class is calculated to give the final prediction. The winning class is determined by the most votes. A larger difference in votes yields higher prediction strengths.

$$Prediction\ Strength = \frac{(Votes_{win} - Votes_{lose})}{(Votes_{win} + Votes_{lose})}$$

Equation 4

This model made strong predictions about most of the independent samples presented; it did not classify five of the samples above the set prediction strength threshold of 0.3, resulting in samples labeled "no call". Of the 34 independently tested samples, only 29 met or exceeded the threshold for prediction. Therefore, it would be more accurate to claim that this technique classified correctly for ~85% of the samples that were presented to it. Of the five samples that did not meet the prediction threshold, two did not correctly classify. Golub et al.'s 1999 report of 100% accuracy is misleading and should be more properly reported as 100% accuracy of samples that exceeded the threshold.

While they showed that there was sufficient information contained within the data to classify, they did not attempt to determine the minimum number of features for accurate prediction. Ultimately Golub chose a subset of 50 genes to use as predictive features. However, they state that this number is somewhat arbitrary as they found that predictors from 10 to 200

genes obtained the same percent accuracy (Golub et al., 1999). Whether this represents a classification ability higher than the original 85%, remains unreported.

The number of samples within this dataset is adequate for accurate prediction according to Dobbin (Dobbin, Zhao, Simon, 2008) who created a new method to determine required sample sizes based on the maximum standardized fold change of the dataset. Fold change is the ratio of the experimental group expression to the baseline group expression. Dobbin uses Equation 5 to determine fold change for a gene.

$$Fold\ change = \frac{E}{B}$$

Equation 5

B=Baseline group mean and E=Experimental Group mean

Using this method only 28 samples are required for a training set to produce an accurate classifier (Dobbin et al. 2008).

Samples that are vastly different will require fewer features to classify on, and samples that are most similar will require more complex feature subsets to classify on. The signal intensity patterns of the Golub dataset are expected to have a large number of features to classify upon. (Dobbin, Zhao, Simon, 2008).

Normalization

To correct for intensity differences, Golub et al. (1999) used several normalization steps. First a rescaling method based on regression was used to rescale the samples. This method fits a linear regression model of genes that are present for a reference sample and another

sample. The inverse of the slope is used as a multiplicative rescaling factor by which the data is multiplied by. The closer a rescaling factor is to 1 the more similar it was to the reference sample. For all samples within the Golub dataset, the greatest rescaling factor is 3.091.

Additional preprocessing steps include (Dudoit, Fridlyand, & Speed, 2002):

1) $$f(x) = \begin{cases} 100, & x < 100 \\ 16{,}000, & x > 16000 \end{cases}$$

Equation 6

Setting a minimum value of 100 and maximum value of 16000 for all expression measurements

2) $$f(x) = \begin{cases} \{\ \}, & \frac{max}{min} \leq 5 \text{ or } max - min \leq 500 \\ x_i & \end{cases}$$

Equation 7

Removing features that the maximum /minimum expression values for a gene are less than or equal to 5 or where difference between the maximum and minimum values is less than 500.

$$f(x) = \log_{10} x$$

Equation 8

Log transformation of expression values

After performing these steps, 3051 features remained (Dudoit, Fridlyand & Speed, 2002). These were then used to calculate the signal to noise statistic (Dudoit, Fridlyand, & Speed, 2002). Max and min refer to the maximum and minimum expression value respectively, for an individual gene.

Neural networks

Artificial Neural Networks (ANNs) are an attempt to model the power of the brain (Baldi & Brunak, 2001). The brain has evolved many efficient ways to store and process information that we attempt to model through artificial neural networks.

The Neuron

Artificial neural networks had their start relatively recently in the 1940's. The basic processing unit of a neural network is the *neuron*. The first model of the neuron was published by McCullough and Pitts in 1943 (Trappenberg, 2002). At the highest level a neuron receives a series of inputs and depending upon the strength of the input and the connection determines whether the neuron will fire or not. The inputs are multiplied by their synaptic connection and summed. This sum is then used as input for a *transfer function* which calculates the output of the neuron. This function is represented in Equation 9. The basic conceptual framework for a single neuron is show in Figure 8.

$$r^{out} = g\left(\sum_i w_i r_i^{(in)}\right)$$

Equation 9

w represents the weight of the synaptic connection between the input and the neuron, r represents the input value. g represents the transfer function of the neuron.

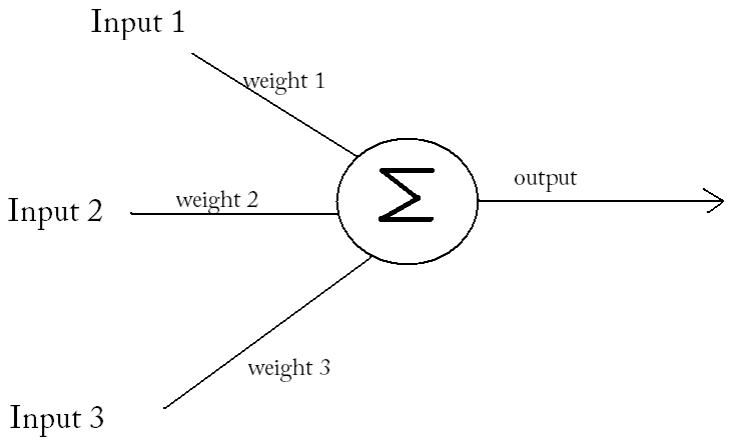


Figure 8 A Simple diagram of a perceptron. Lines represent connections to other neurons (synapses).

Each neuron utilizes a transfer function that determines a neurons response to the sum of its inputs. Early neuronal models such as the McCullough Pitts neuron utilized a hard limit transfer function that produced a binary response if a threshold was met. However newer models utilize continuous functions that allow for finer adjustments in neuronal output. Some commonly used transfer functions are shown in Figure 9

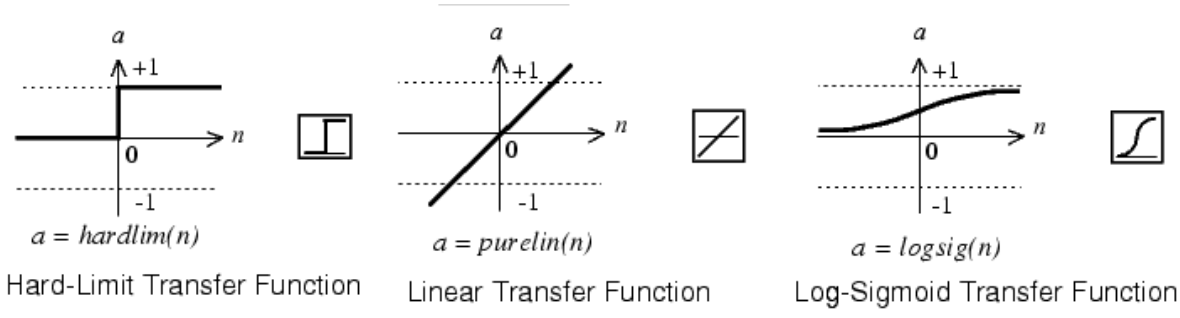


Figure 9 Sample transfer/activation functions. Different usage will give the network different dynamics. (From http://www.mathworks.com/)

These different transfer functions result in different neuron output. For example a hard limit function will only propagate a 1 or a 0, revealing little information to how accurate the neuron is but resulting in very clear propagation of signal. Whereas a continuous transfer function is much more precise with outputs but can potentially propagate irrelevant signals.

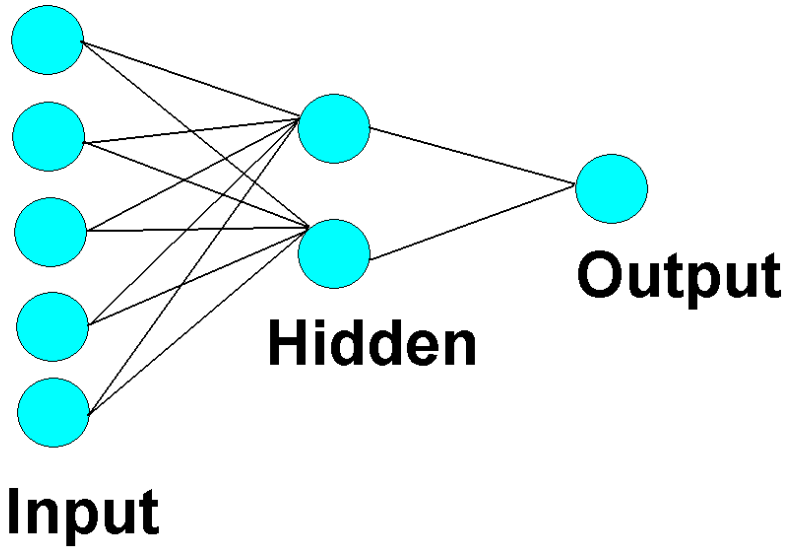


Figure 10 Basic architecture of an artificial neural network. Input neurons take in supplied values and connect to one or more hidden layers. This hidden layer can connect to another hidden layer or to an output layer depending on the architecture.

The basic architecture of an artificial neural network is seen in Figure 10. Each circle represents a single neuron as seen in Figure 8, where the output of each serves as an input into the next layer. This connection of simple neurons was first implemented in the perceptron model by Rosenblatt in 1958. The connections between neurons represent a weighted connection called a *synapse*. Like the single neuron seen the inputs are multiplied by the synaptic weight and passed through a transfer function. A typical ANN consists of an input layer, k hidden layers, and an output layer. This network topology is determined by the user and is based on the type and complexity of the problem space.

Training

Training is an iterative process that seeks to modify the network through numerous presentations of data. There are many different methods to train neural networks, the two main distinctions are unsupervised and supervised learning. An unsupervised neural network only uses the input data to adjust its synaptic weights. Supervised learning however relies on a set of training data with known target values. In other words, the training data consists of a set of input patterns and output values. The goal of training is to optimize a function that will map the inputs to the outputs that can be used to correctly approximate unseen inputs.

Constructing an ANN using a supervised learning methodology requires the initialization of a network with random synaptic weights between neurons. At this point an input signal presented to the network would result in no meaningful output. To derive a meaningful output the network synapses must be adjusted. The method to adjust the many weights of the network requires a calculation of error of the network for an input pattern at each epoch. An epoch represents an iteration of measuring the output error and updating the synaptic weights in

response. The error of the network for a given input pattern is described as the difference between the network output and the desired output value. A standard error measure, such as mean squared error is often used to describe this distance.

$$Error = (target\ value - network\ output)^2$$

Equation 10

While this error value shows how far the network output is from a desired value, it does not reveal anything about how to correct the network to more closely match the desired value. To minimize the error value, the error is used in a learning function to update the synaptic connections of the network. A simple synaptic learning function is shown in Equation 11

$$w_{new} = w_{old} + \alpha(Error) * input$$

Equation 11

w_{new} represents the new weight of the input neuron, w_{old} the old weight of the neuron, alpha the learning rate, targetValue-output is the error function and the input is the input that goes through the synapse

A learning rate is often used to control how quickly the weights are updated. If a large value is used the weights of the network will oscillate wildly if set too low it will take more epochs to adjust the weights.

Several learning paradigms exist to train networks, one of the most commonly used is backpropagation. Backpropagation originally described in 1974 by Paul Werbos (Chester, 1993) is an extension of Equation 11. Simple learning methods are not able to directly compute the error from hidden layers of multilayered networks; however with the discovery of backpropagation, weights between hidden layers could be adjusted thereby unlocking many new

topologies that were previously unavailable. Backpropagation is accomplished through a process where error correction flows through the network in a reverse direction (Swamy, 2006). Backpropagation attempts to minimize the amount of error by modifying the synapses with the strongest connections. The weights of these synapses are then modified to a greater degree than their weaker counterparts using a method similar to Equation 11.

Testing

After training has been completed, usually signaled by a lack of further decrease in the error or after a set number of epochs, the weights of the network are set and testing of new samples begins. During testing the testing data is presented to the network to obtain a measure of performance. This performance is measured by a similar method that is used to determine the error of the network during training.

Cross validation

An important part in evaluation of any classifier is the use of cross validation. However with microarray data this is difficult, as typically there are a statistically small number of samples, making over fitting of the model a real possibility. Some basic cross validation techniques include, leave one out, hold out and k-fold.

Leave one out cross validation is one of the most extreme cross validation methods. In this case, 1 sample is used for testing and n-1 samples are used for training. This is repeated n times, until all individuals have taken a turn as the testing sample. This is a nearly unbiased estimate of the true error (Simon et al., 2003). As expected the computational cost of this

method rapidly grows with larger sample sizes. With 72 samples, this would take 72 repeat evaluations to build a fitness value.

The n-fold cross validation method is a scaled down version of the leave one out method, where the dataset is divided into n divisions. Each of these divisions is used as a testing set while the classifier is trained on the remaining samples.

Hold out cross validation is commonly used for classifiers that have a low number of samples. Typically in this case, 90% of the samples are used for training and the remaining 10% are used for testing procedures.

While supervised artificial neural networks are powerful tools by themselves they can only sort what is presented to them. The typical structure of microarray data has too many features and not enough replicates. To alleviate this problem, a *Genetic Algorithm* (GA) can be used to evaluate combinations of genes.

Genetic Algorithms

Genetic algorithms comprise a search algorithm that guides its search based on a model of evolution (Mitchell, 1999). Evolution is the process by which organisms continually improve over generations, through, selection, crossover and mutation. Evolution derives its power by evaluating many possible solutions at once, and propagating the fittest.

In a genetic algorithm, a population of organisms is represented by a population of short strings called *chromosomes*. Each chromosome represents a different portion of the possible search space that is to be evaluated. A search space is all of the possible combinations or values

of features for a given problem. One could evaluate every possible solution for a problem, known as a brute force approach, but as the number of parameters increases the search space grows in dimensionality, resulting in problem spaces that are too large to search with exhaustive methods. Genetic algorithms have been shown to be a robust search method for problems with extremely large search spaces (Goldberg, 1989). Within a search space there are often many *local minima/maxima* and one *global minima/maxima*. Local minima/maxima are solutions that are close but are not the best solution. Minima or maxima are substituted depending on the direction that function is being optimized. Many optimization algorithms go to great lengths to avoid or escape local solutions. The global solutions represent the best possible solution and are often difficult to find.

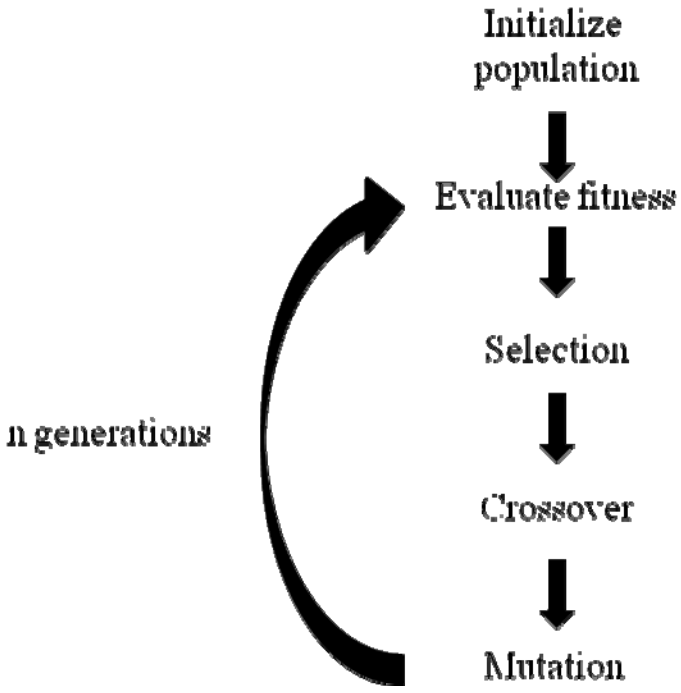


Figure 11 Overview of simple genetic algorithm

Representation

The choice of representation in a genetic algorithm is of utmost importance; this process involves mapping parameters into a string that the algorithm can manipulate. Poor choice of representation can limit how the algorithm works (Reeves & Wright,1995).

Many Genetic algorithms utilize a binary representation of the data (Sivanandam and Deepa). In a feature selection problem this would consist of a string the length of the feature set, where each character was a binary value that represented the presence (1) or absence (0) of a feature.

Representation is also important due to linkage. Linkage is the probability that genes will be inherited together. This probability is based directly upon how far apart the genes are located from each other. Two genes that are close together are less likely to separate than two genes that are located at either end of the chromosome (Goldberg, 1989). This is because there are more possible crossover events that could separate the two genes when the genes are farther apart, than if the genes were adjacent to each other.

Fitness Function

The fitness function represents the problem that the genetic algorithm seeks to solve. At each generation the performance of the individuals within the population must be measured against this function (Srinivas & Patnaik, 1994). The performance of an individual with the fitness function is used to determine which organisms are allowed to reproduce.

Selection

Selection is the method by which GAs determine which chromosomes should propagate. The scores from the fitness function are evaluated by the selection function. Individuals reproduce proportionately to their fitness. There are several types of selection methods including ranked and tournament among others. There is no correct answer for which selection method to use. The method implemented here is that of a roulette wheel.

To implement a roulette wheel the fitness of the entire population must be evaluated at each generation. Next the probability of selection for each individual is calculated by dividing the individual's fitness by the sum of the population's fitness (Zhong et al., 2005).

$$Probability(i) = \frac{f_i}{\sum_{i=1}^{n} f_i}$$

Equation 12

Individuals are then ranked in descending order and a vector is constructed of accumulating probabilities.

$$Selection\ Vector = \sum_{i=1}^{n} probability(i) + probability(i-1)$$

Equation 13

The sum of all probabilities must equal one. For example if the probabilities from a 4 member population were (.50, .25, .14, .11), the resulting accumulated probability vector would be: (.50, .75, .89, 1). Next a random number is generated between zero and one. Where the value falls indicates which individual is selected. Continuing with the example, .453 is the random number generated. This number is below .5 in the accumulated probability vector indicating that

individual 1 is selected. Because the individuals with the highest contribution to the population fitness occupy a greater range of values between 0 and 1, they are selected more frequently into mating pairs. However this does not prevent an individual who has a low probability from being selected, gives them as much opportunity as they provide fitness. This allows genes that potentially have fitness in other combinations to remain in the pool, but with low chance of being selected. There is nothing to stop a high scoring individual from being selected to mate with itself, producing no new offspring.

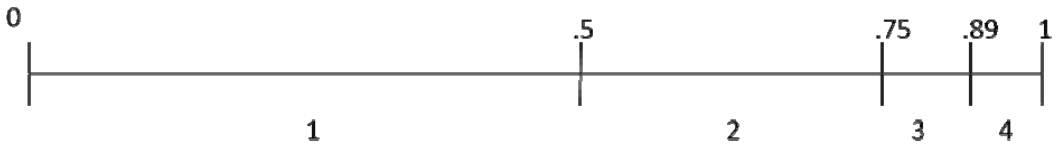


Figure 12 Probability vector. Genes with higher fitness occupy a greater range of the vector.

Crossover

Crossover is the process of swapping genes between individuals at each generation. The mating pairs selected by the selection function will determine which individuals will cross with each other. For an individual mating pair utilizing a single point of crossover, a random point is used and the strings are swapped at that index. The offspring then replace the parents within the population. For example if the crossover point was 5 and the length of the chromosome was 10, each child would contain half of the chromosome from each parent. However, this process should not occur for every individual at each generation, as the GA will quickly converge to a solution that is not necessarily close to the global optimum. To limit the amount of crossover, the crossover probability is left as a free parameter.

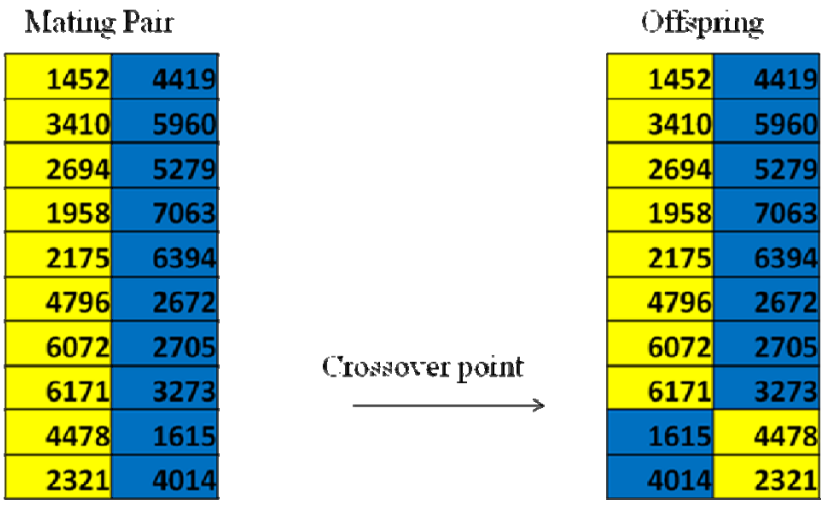


Figure 13 Overview of crossover operator

Mutation

The mutation operator is used as a source of genetic variation in the population. This increases the features evaluated beyond the features within the initial population and allows the algorithm to escape local minima. Without allowing for the addition of new genes the GA would quickly converge. However if the mutation rate is set to high, the system becomes unstable and any performance increases are immediately wiped out by mutation. Therefore the mutation rate must be set to an intermediate value.

Statistics

The central limit theorem is the backbone of statistical theory. From Introductory Statistics by N. Weiss (page 346)

"For a relatively large sample size, the variable x is approximately normally distributed regardless of the distribution of the variable under consideration. The approximation becomes better with increasing sample size."

The basis of this theorem is that from a population, if a statistic, of all possible samples is calculated; for example the mean of these sample means will resemble a normal distribution. A histogram of sample means is called a *sampling distribution*, and it is useful to show how probable a sample statistic is. In building a sampling distribution one finds that as the number of samples increases, the closer the sampling distribution resembles a normal curve.

Methods

A genetic algorithm that optimizes inputs for a neural network was constructed in MATLAB. This system evaluated the classification ability of feature combinations using an artificial feed forward neural network. Each set of features was used to train a neural network and then the classification ability of those features was evaluated. High scoring features were preserved by the GA while low scoring features and feature combinations were discarded.

The feature subset size was determined by the smallest subset size reported by Golub et al. (1999). Golub reports that results from 10 to 50 features achieved the same classification ability. To isolate a small number of features that could be useful for diagnostic purposes 10

features were used. A fixed feature size was used because the neural network requires a fixed number of input features.

The initial population of chromosomes was created by randomly generating a 10 x *m* matrix. The value 10 represents the number of features within a chromosome and *m* the population size. The ceiling of each of the random values is multiplied by the maximum number of genes in the dataset, which returns a matrix of integers between 1 and the maximum number of features. A chromosome containing the numbers 3780, 2387, 1816, etc. refer to the rows 3780, 2387 and 1816 from the original expression matrix.

Table 2

Chromosome 1	Chromosome 2	Chromosome 3	Chromosome 4	Chromosome 5
3780	5519	3779	4856	5876
2387	6532	4235	4808	1199
1816	6751	3378	5348	2089
2288	6837	5923	3287	2702
6576	3316	396	59	4011
1580	3601	2743	4051	5335
1176	5968	756	2889	4515
2791	1330	6765	687	5753
3304	1854	5439	3920	3027
5678	23	4576	7123	321

Population of 5 chromosomes

At each generation, selection was performed using roulette wheel selection to construct mating pairs. Of the 50 mating pairs generated, approximately 75% crossed at each generation. For the pairs that did mate, a single crossover point was used to swap features. The mutation

operator was called at each generation and it randomly changed 1% of the indices to a randomly generated index.

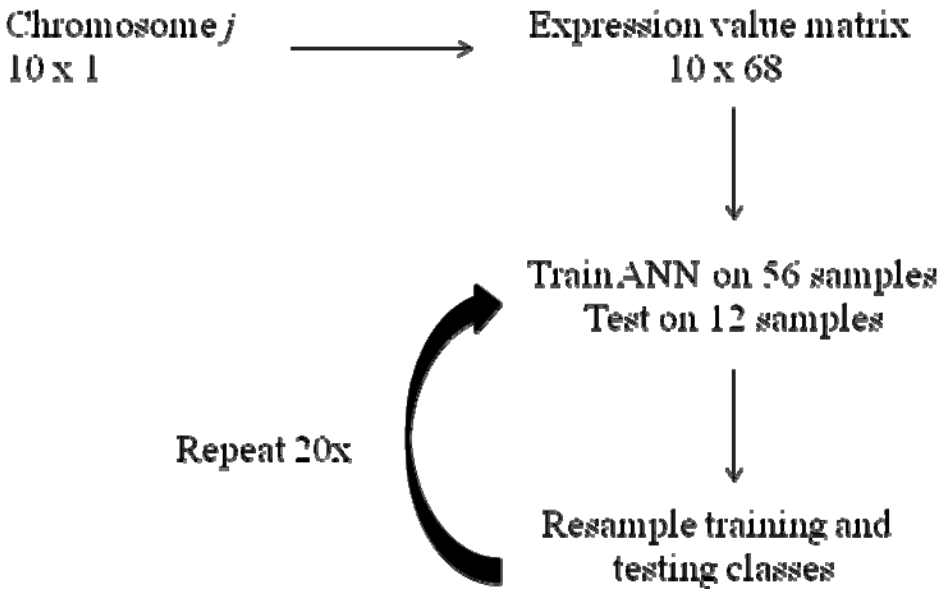


Figure 14 Decoding of chromosomes. Chromosome values are used to construct a matrix of expression values that are used as input for an artificial neural network.

Data Division

In most supervised learning studies, the dataset is divided into a training set and a testing set. The original data division of 38 training and 34 testing samples was changed in this study to increase the number of samples that could be included within the training dataset. By increasing the number of samples in the training set, the ANN is able to perform at a higher level due to having a more robust training set.

The testing samples from the original division samples are reported to be from independent sources that sometimes used different sample preparation techniques (Golub et al.,

1999). Given the noisy nature of microarray data this is an important consideration. Training on samples from one laboratory and validating on independently collected samples could result in a classifier that is biased towards sample preparation methods from the training set. To help diminish this effect the data was divided into 68 samples for training and testing the model, and 4 samples were reserved for final validation. Each leukemia type was represented by two samples within the validation data. The 68 samples were further divided into a training set and a testing set, with 56 samples in the training and 12 samples for testing.

AML samples were labeled with a target value of -1 and ALL samples with a target value of 1.

Neural Network

The neural network used to evaluate fitness consisted of two hidden layers and can be seen in Figure 15. The first hidden layer contained two neurons, while the second contained a single neuron.

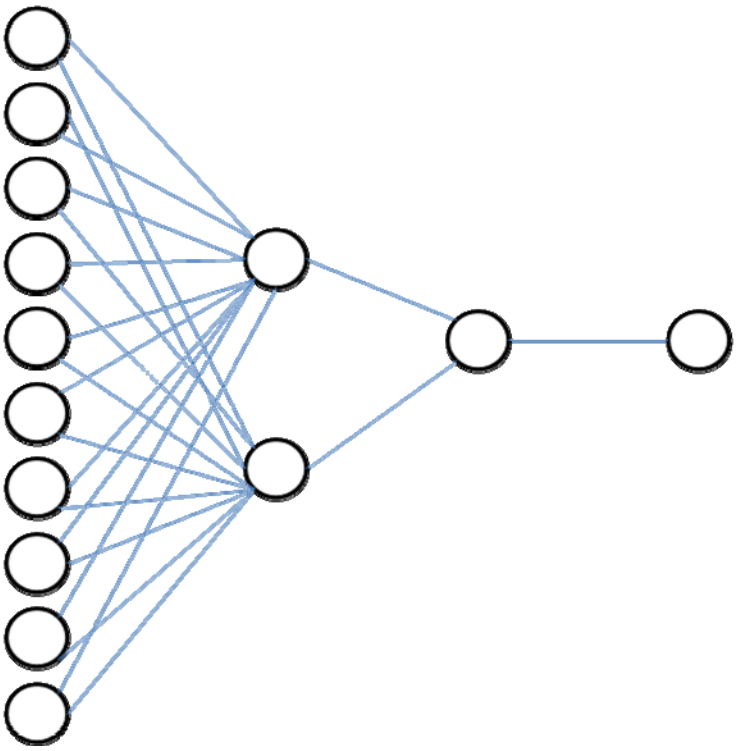


Figure 15 Structure of ANN used to evaluate feature sets

Each neuron used a tangent sigmoid transfer function as seen in Equation 14.

$$g^{sig}(x) = \frac{1}{1 + \exp(-x)}$$

Equation 14

Sigmoid transfer function (Trappenberg, 2002)

At every generation, each chromosome was decoded into its values and 56 samples were used to train a neural network using the MATLAB adapt function. The adapt function uses incremental training, resulting in an update of weights after presentation of each input value.

Each network was initialized to the same weight values to decrease fluctuations in performance caused by randomized starting weights. Each network was trained for 1000 epochs, after which classification ability was evaluated on the 12 testing samples. For each network this process was repeated 20 times with different samples of training and testing classes. This sampling method helped to build an accurate representation of the mean performance. The mean of the scores was taken as the fitness of the chromosome. This process was repeated for each chromosome in the population at each generation. As can be seen in Figure 16, as more samples of the training and testing sets are taken, the closer the histogram resembles a normal curve, making the mean more representative of the majority of the samples.

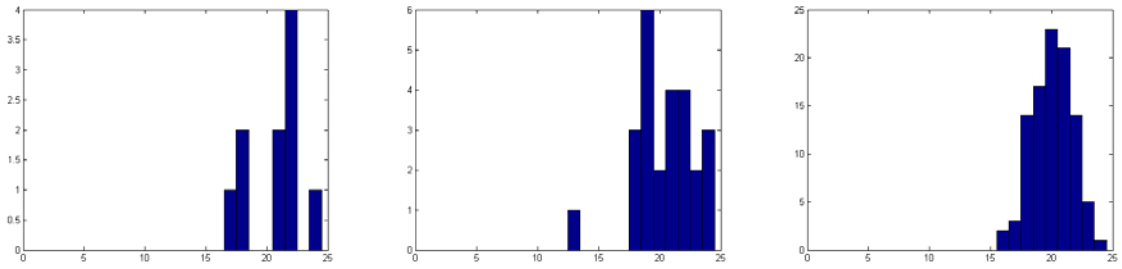


Figure 16 Sampling distributions of ANN performance, 10, 25 and 100 samples

Performance score

The classification ability of each network was measured by the amount of error in the classification. For each of the 12 testing samples, the error was measured by the following function.

$$Performance(x) = 2 - |Desired\ value - Network\ output|$$

Equation 15

The difference between the actual target and network output is a standard measure of error that is used to train the network. However, here the absolute value of this difference was subtracted from 2, as 2 is the largest error that performance could incur. A perfect score would result in a value of 2 and a completely wrong classification would result in a score of 0. While a binary "pass/fail" measure could be used to determine the performance, it would not be representative of prediction strength. Acceptable prediction strength was set at 1.5, and any performance greater than 1.5 was set to 2, as shown in Equation 16. This was done so that the performance measure could distinguish between performances that were weak on all testing samples and performances that were all correct except for a few samples. These scores are then summed to create a single value of performance. Thus over 12 samples a perfect score is represented by a performance of 24.

$$f(x) = \begin{cases} 2, & x \geq 1.5 \\ x, & x < 0 \end{cases}$$

Equation 16

Thresholding equation for performance scores.

To determine a satisfactory solution that met the GA stopping criteria, every 5 generations the top two performing sets of features were isolated and trained on a network for 2000 epochs. If one of the fitness scores was perfect, then the GA was halted and the high scoring features were reported.

The Affymetrix® analysis strategy today implements a more superior method than the method available in 1999. However the Golub dataset utilizes methods from 1999 where

negative values are present in many of the rows within the expression matrix. These values indicate that the mismatch probes, on average, bound more labeled transcript than the perfect match probes. However in modern analysis, negative values are treated as noise and are corrected. However Li et al. (2003) state that "...MM responses do contain information on the gene expression levels and that this information can be better recovered by analyzing the PM and MM responses separately." Moreover, Li and Wong (2001) implemented a new model that corrected for probe-specific differences that they measured. These researchers raise questions about what exactly is represented by the PM-MM difference values from the Golub study.

If the original raw files from the Golub et al. (1999) study were available, the raw data could be analyzed more thoroughly; however only average probe differences were released to the public. Due to the inability to compute expression differences using newer methods, the data was left un-normalized to preserve as much signal as possible. If there is no true signal within the negative of the dataset, then any chromosome containing a feature with uninformative negative values will be at a disadvantage by having a feature with no difference between classes. However if there is any signal within these values, the ANN will detect it.

Logging

Population values are logged at each generation, and used to create graphs summarizing the performance of the population. The x-axis represents the generation while the y-axis represents the fitness score as seen in Figure 17. Each chromosome is represented by a single point at each generation. The mean fitness of the population is calculated and plotted on this graph as well. This graph is useful for showing average fitness and the variance of the

population over generations. It also helps to quickly analyze the results while the GA continuously runs. At each generation a log file is updated with the fitness scores and the chromosomes present within the population. This is used as a method to restart the GA if a power failure or computer crash should occur before saving. Retaining the population at each generation also allows for analysis of the appearance of features within the generation. Finally a log of the gene names within the final population, with frequencies and accession numbers, is created. These numbers help provide insight into which genes are most important.

Parallel Computing

Parallel computing is used to distribute workloads over multiple nodes. In the most basic terms this is a division of computational processing that allows independent tasks to be run on multiple machines simultaneously. The fitness evaluation procedure for the GA is scalable to a parallel architecture. While the GA eventually needs to compare the fitness of the individual with respect to the rest of the population, the computation of fitness of a given individual does not require information from any of the other individuals. In parallel computing this type of function is referred to as "embarrassingly parallel."

To optimize this code, the fitness function was converted to a parallel *for* loop. The population was divided into 4 groups, one for each processor. The processors then independently calculated the fitness for each individual and reported the values back to a central array. The scores in this array were then used for the selection method.

Results

The GA/ANN isolated a high scoring solution on the training data after 131 generations. This simulation took approximately a week and a half to complete, and in total 262,000 neural networks were trained and tested. Of all the 7129 features, only approximately 27% percent of these features were represented within the population. The mutation rate was set at 1% for all features present and 75% of the selected mating pairs were crossed. The population size was set at 100 individuals. These parameters are summarized in Table 3

Table 3

Number of Features	10
Population Size	100
Number of Epochs	1000
Max Generation	2000
Size of Hidden Layer	2
Size Hidden Layer 2	1
Mutation Rate	0.01
Crossover Probability	0.75

Genetic Algorithm parameters.

Figure 17 shows the individual fitness scores along with the mean of the population over 131 generations. Each circle represents the fitness of an individual chromosome at a specific generation and the solid blue line represents the population mean. This figure helps to illustrate the diversity of the population and the overall performance of the system. Over time, the population's mean steadily increased and the variance decreased slowly. In the first generations the variance was very high, however as the algorithm converged on high scoring solutions the variance decreased (Appendix 7). A small number of chromosomes retained a low level of

fitness at each generation. The largest increase in performance occurred over the first 20 generations.

To show that the genetic algorithm became more specific at each generation, a histogram of the features at generation 1 and generation 131 was generated and can be seen in Figure 18. This histogram shows the frequency of all of the features within the population. At generation 1, the frequency of any given feature was very low, as expected due to random initialization. However by generation 131 a select number of features reached a very high frequency.

The appearance of a feature within the population can help determine how useful the GA/ANN found that feature for classifying. Features that appeared in generation 1 were part of the initial random population that survived until the last generation. Features that appeared in later generations arose through the mutation operator. The longer a feature has been in the population the more ways it has been represented and tested. For example, if a feature appeared in generation 130, that feature did not receive the same fitness evaluation as a feature that appeared in generation 1 and would be less likely to contain meaningful information.

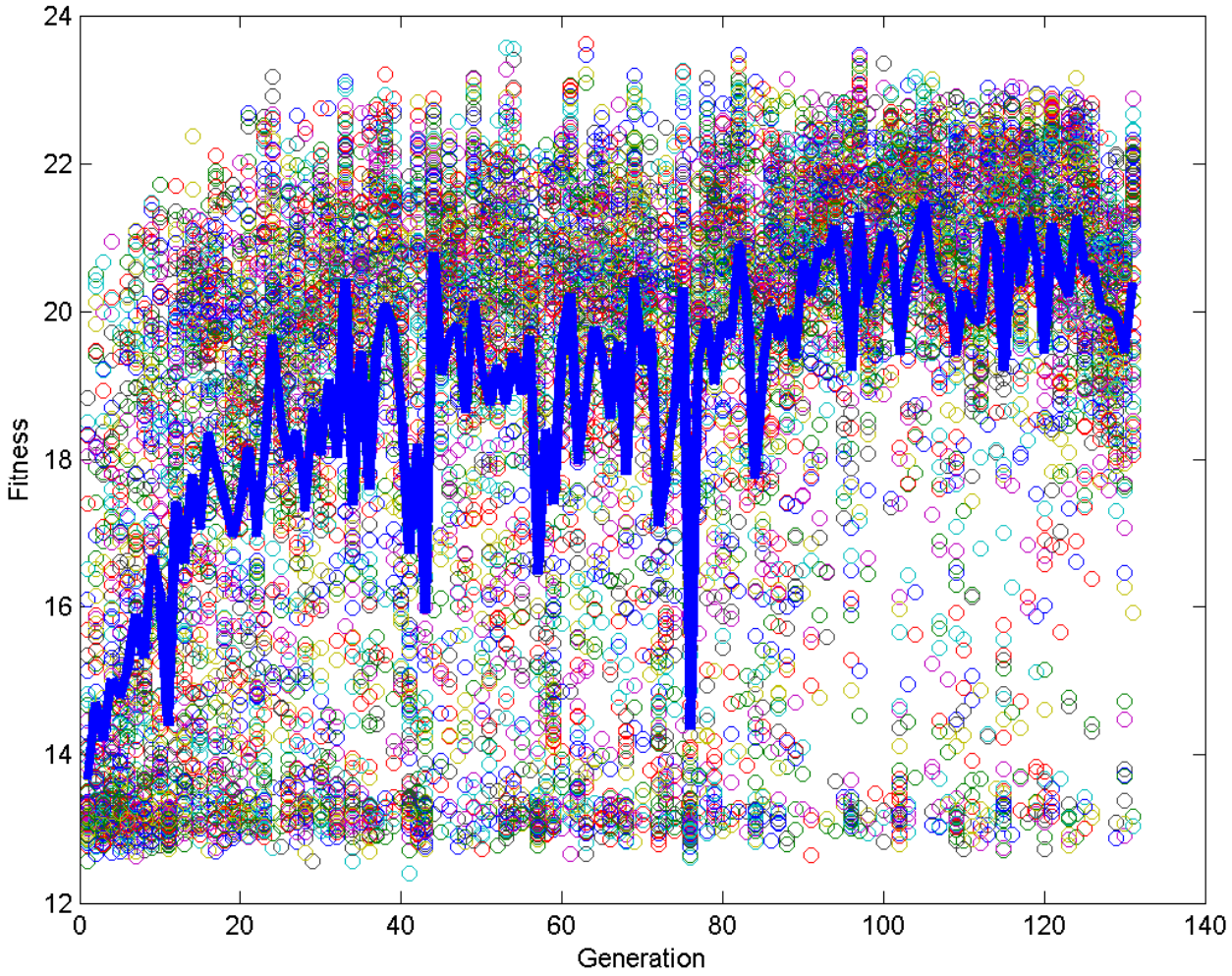


Figure 17 Fitness of population over 131 generations. Fitness increased with occasional dips in performance.

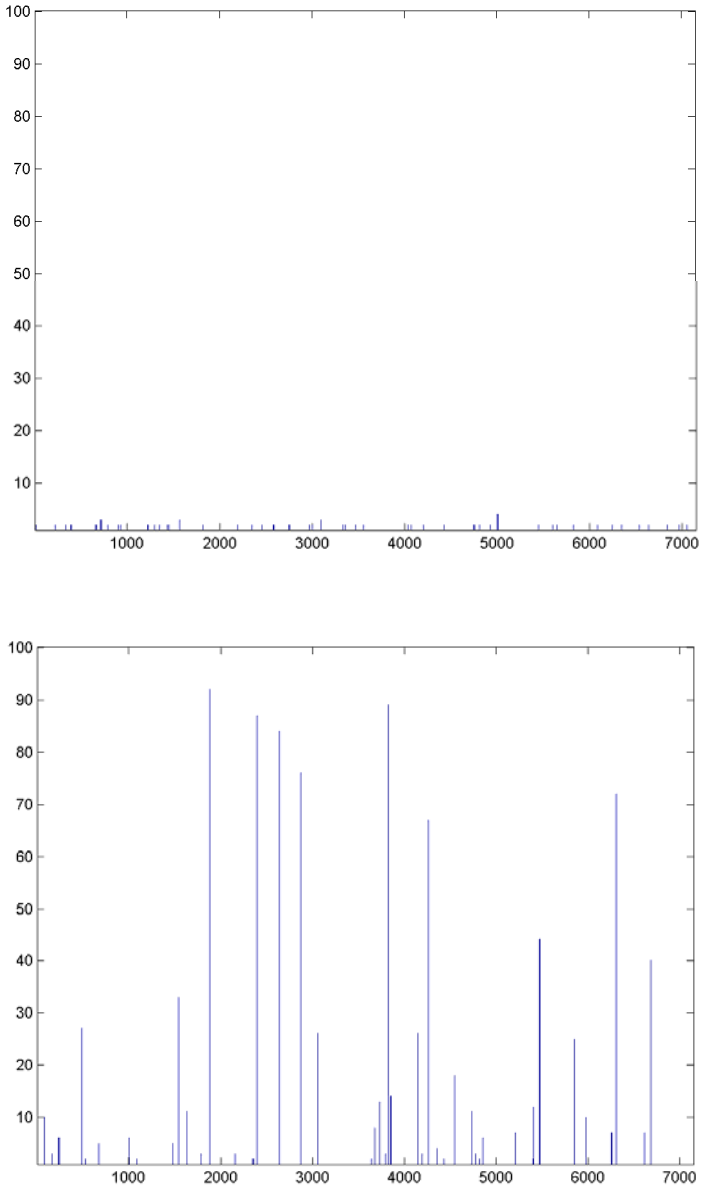


Figure 18 Histogram of the final population frequencies. The x-axis shows the index of a feature and the y-axis the frequency of that feature within the population (a) Generation 1, almost uniform for genes that are present(b) Generation 131, several genes have frequencies greater than 50.

Validation

To determine how accurate the isolated features were in classifying, a final network was trained on all of the training data (68 samples) and tested on the 4 reserved validation samples. The GA/ANN solution correctly classified the 4 validation samples with 100% accuracy.

Each time a new neural network is initialized within MATLAB it starts with different weights. To ensure no bias in the selected features towards the locked-in weights of the ANN, the feature set was tested on 20 different networks. As stated previously, samples with performance values greater than 1.5 were set to 2. On the validation samples a value of 8 represents perfect classification. While a value below 8 does not mean that it did not make an accurate prediction, it means that a sample did not accurately predict at a satisfactory level.

Table 4

Probe Name	**Class correlation**	**Pass Golub filter**	**Rank |S/N|**	**Golub classifier**	**First Gen.**
CST 3 Cystatin C	ALL	Yes	3	Yes	58
MPO from human myeloperoxidase	ALL	Yes	140	No	25
MB-1 gene	AML	Yes	7	yes	1
PLCB2 Phospholipase C, beta 2	ALL	Yes	247	No	1
KIAA0128 gene partial cds	AML	Yes	158	No	1
MYBPC1 Myosin-binding protein C, slow-type	ALL	Yes	1555	No	42
Mucin 1 epithelial, Alt. Splice 6	-	No	-	-	1
Phosphatidylinositol 4-kinase	ALL	Yes	2341	No	5
PZP pregnancy-zone protein	-	No	-	-	31
Triadin mRNA	-	No	-	-	1

Ten genes isolated by the GA/ANN after 131 generations. Golub Filter is in reference to preprocessing equations 6, 7 and 8. The rank of the signal to noise score represents the absolute value of these scores. Golub classifier shows which features found were part of the original set of features used by Golub et al. (1999). First gen refers to the first instance of that feature within the population.

Ten genes were isolated by the GA/ANN and are shown in Table 4. To better understand how the GA/ANN compared to the classifier from Golub 1999, the signal to noise scores (Equation 2) for each feature was reported. For features that did not pass the preprocessing steps (Equations Equation 6Equation 7 and Equation 8) the signal to noise statistic was not calculated. The absolute value of the signal to noise statistic was calculated and the features were ranked by value to determine the most informative genes regardless of class correlation. A high score on this statistic represents a feature containing a large difference between the two sample means.

Evaluating the top ten GA/NN isolated features, it was found that Cystatin C was the third most informative gene, while MB-1 was the 7^{th} and MPO was the 140^{th} when using the signal/noise filtering statistic. Of the 10 features only Cystatin C and MB-1 gene were included in the original Golub classifier.

Architecture Performance

Neural network architecture plays a large role in how the neural network performs. To evaluate the effect of the architecture chosen, additional architectures were evaluated. Along with this the effect of epoch number on these topologies was evaluated and can be seen in Table 5. Architectures are denoted by the number of hidden layers present. Each integer represents a hidden layer, and the value of the integer represents the number of neurons within that hidden layer. Each architecture was trained for 6 different epoch lengths of 1, 100, 1000, 2000, 5000 and 10000 epochs. This was done because varying the structure of the network modifies the amount of time required to sufficiently fit the data. As more hidden layers are added, it takes longer to train the network. These networks were trained on the ten features isolated by the GA/ANN

Two effects can be measured in Table 5, showing the effect of different ANN architectures and the required training time to reach an acceptable performance. Each value is the average of 20 networks under the same conditions. All conditions eventually reached an acceptable performance threshold, but as expected, required different training lengths.

As shown in Table 5, increasing the number of neurons within a single layer did not have as large of an impact as increasing the number of hidden layers. The architecture used by the GA/ANN of two hidden layers with three total nodes reached sufficient training performance by epoch 1000. In networks with a third hidden layer, such as the [2,2,1] and [3,2,1] networks, the number of epochs required to reach a satisfactory performance score increased from 1000 to 10,000 epochs.

Table 5

Hidden Layers	1 epoch	100 epochs	1,000 epochs	2,000 epochs	5,000 epochs	10,000 epochs
[2,1]	4.66	5.55	8	8	8	8
[1,2]	4.41	4.80	8	8	8	8
[3]	3.85	4.98	8	8	8	8
[5]	4.25	5.56	7.89	8	8	8
[10]	4.01	5.04	8	8	8	8
[20]	4.91	6.94	8	8	8	8
[2,2]	4.07	4.39	7.07	7.80	8	8
[3,2,1]	3.74	5.16	7.2	7.2	7.47	8
[2,2,1]	4.35	4.37	6.29	7.20	7.74	8

Performance of different ANN architectures with different training lengths. The notation [x,y] represents two hidden layers with x number of neurons in the first layer and y number of neurons in the second layer.

Feature Combinations

Because the feature number was locked at 10 features for the GA/ANN, a number of other combinations of features were used to train and validate a [2,1] network for 1000 epochs. For each of the features that passed the filtering statistic, the signal to noise statistic was

calculated (Golub, 1999). The top 2 features, according to the signal/noise statistic, were tested and resulted in a classification score of 7.6. However this did not meet the acceptable threshold of 8. Using the top 4 features (Signal/Noise rank < 300) did not increase the performance of the classifier. Using features that would not have passed a preprocessing step resulted in a classification score close to random. Using only the features that passed the preprocessing steps in equations Equation 6,Equation 7Equation 8, resulted in a classification score of 7. The top ten features ranked by the Golub 1999 signal to noise statistic resulted in a perfect classification.

Table 6

Combination	**Score**
Top 2 (Signal/Noise) MB-1 & CST3	7.6
Eliminated in preprocessing PZP, Mucin, Traidin	3.98
Signal/Noise rank<300 MB-1, CST3, KIAA, & PLCB2	7.6
Passed preprocessing all but PZP, Mucin, Triadin	7
Golub top 10	8
GA/NN selected	8

Performance of different feature combinations

Discussion

Genetic Algorithm Considerations

The solution found by the combine GA/ANN algorithm performed perfectly when validated. However the algorithm would most likely converge on a different solution if the algorithm were run again as indicated by the performance of the top ten features from Golub et al. (1999). This is due to the high information content within the dataset and differences between the two cancer types. Because the GA only focuses on accurate classification, local minima are acceptable if they reach an acceptable threshold. This is shown by the perfect performance of the 10 features isolated using Golub's method and the GA/ANN method.

The ability of the GA to only represent slightly more than a quarter of the features and find an acceptable solution could be indicative of effective GA exploration or of many high scoring local minima that are easily found.

Linkage most likely plays a role in the preservation of some of the lower scoring features. If two features are close together on a chromosome, it is more probable that they will be inherited together because they are not likely to be separated by the crossover operator. This appears to have resulted in the genetic algorithm holding on to several of the lower scoring features, as the low scoring features were always physically next to high scoring features within the chromosome. This is consistent regardless of fitness measure (Signal/Noise or ANN). The majority of the low scoring features were present within the population from the first generation. It could be that the low scoring features were linked to high scoring features and simply did not

have enough generations to separate. However when only the high scoring features were tested the classification accuracy was less than perfect, signaling some information loss with the exclusion of the low scoring features. Therefore, linkage alone cannot explain the retention of these lower scoring features, and the features most likely contribute some signal.

GA Parameters

Machine learning methods require the specification of several parameters by the user. The changing of these values can greatly alter the efficiency and performance of an evolutionary system. Several pre-runs were conducted to help determine the free parameters of the system. Of interest was an early run that led to the implementation of the sampling method used. In this run a high scoring feature set was found after a lengthy search process. However when attempts were made to validate the accuracy on a differently sampled set the fitness of this solution was found to be substandard. This discovery led to the implementation of the cross-validation technique that ensured that the fitness level used by the genetic algorithm was representative of the feature sets true performance. The sampling method helped to build an accurate assessment for a set of features from 20 samples of the training data. While larger samples will allow for a more accurate mean, it is not computationally feasible to sample excessively. For example, 100 samples of the training data would require 100 neural networks to be trained and tested to evaluate a single individual at each generation. For *n* samples this quickly increases the computational cost. Sample sizes greater than or equal to thirty are traditionally used for accurate estimation (Weiss, 2008). To balance the computation time with accuracy of performance, the average of 20 samples was used. This allows for a more accurate estimate of

the mean, while not requiring excessive computation. Ideally a larger dataset would eliminate the need for this sampling method as more samples could be used for both training and validation.

The population size of 100 resulted in a solution by generation 131. Increasing the population size would most likely have resulted in a longer time to reach convergence, but it would have performed a more thorough search that might have found a high scoring solution sooner. A larger population will increase the number of new features that are introduced by the mutation operator and in the original population. This allows for a greater number of features to be evaluated. By exposing the GA/ANN to more possible features the GA would more likely converge on a solution closer to the global minima. The crossover rate used could have been lowered and have resulted in a more stable increase in the population's average fitness, but would be dependent upon the mutation operator.

Throughout the simulation, the algorithm retained a higher degree of variance that only decreased towards the end of the simulation (Appendix 7). This could be due to the crossover and mutation operators. If a high scoring feature set relies on interactions between all of its features for an accurate performance, a disruption of any of those features could radically drop that set's performance. This disruption could occur through the mutation or crossover operator. The mutation operator in general should be the more disruptive operator as it is more likely to introduce a low scoring feature than the crossover operator as the generations increase.

Neural Network Considerations

By limiting the number of epochs to a low value, the GA/ANN isolated features that were able to quickly classify. If the value was increased to 10,000 epochs or beyond, less information carrying genes could have been selected for high scoring feature combinations. This is because as the training epoch's increases the model has more time to fit its weights to minimize error. If one set of features could have classified within 1000 epochs and the other only after 8,000 epochs, there would be no measurable difference between the performances by epoch 10,000. By epoch 10000 the performance of the first set of features may have degraded due to a model that memorized the training data.

The performance score implemented does not allow for searching for the global minima. By accepting values that possibly still contain some error, local minima are accepted. To search for the global minima this threshold could be removed. However the goal of this study was to accurately classify, so local minima that can achieve this result are deemed acceptable. By adjusting the threshold, the specificity of the algorithm is modified. For example, by setting the threshold value very low, combinations of features that were accurate predictors would be included even though these combinations are not the most informative. This would not preclude high-scoring features from being found, but would allow for low-scoring and high-scoring combinations of features to be selected as a solution. Setting a higher threshold would force the algorithm to search for combinations of features that would be easily separable.

Comparisons to Golub et al. (1999)

Several of the features that were isolated in this experiment would have been eliminated by using the preprocessing statistics used by Golub and coworkers. It was hypothesized that

eliminating these features would result in no decrease in the performance score, as the preprocessing steps should ideally only eliminate features that contain no significant signal. A classifier built only using the features that passed the filtering conditions was tested. This classifier performed worse than the full set of features isolated by the GA/ANN. Several factors could account for this. First, there could be a small signal within the features that the filtering methods deemed insignificant. Another possibility is that a complex interaction between the features allows for a small signal to convey enough information for the ANN to detect. Complex non-linear relationships are common in gene expression patterns and possibly could have allowed for a small set of weakly interacting signals to convey a significant signal.

As shown previously in Table 6, the highest non-perfect combination score was obtained by using only the two highest signal to noise scoring features isolated by the GA/ANN. This performance did not decrease when the set was enlarged to include the top 4 features (overall rank less than 300). This indicates that the inclusion of two more features within the feature set did not increase the average performance. This could be because the expression profiles contained redundant information to the features already present. The features by themselves showed high information content.

Evaluating the top ten selected features from Golub's signal to noise statistic (5 highly correlated to each class) show that several combinations of features exist that have perfect classification ability. This also highlights the potential use of a filtering statistic that could be used to eliminate a large number of features from the data set before applying the GA/ANN search. However this comes at the cost of not finding novel combinations of features that allow for a perfect classification score.

An interesting note is that the isolated features included more features that were closely correlated to high ALL expression than AML expression. This could account for some of the increased performance of the classifier over those used in Golub's original study. Golub's study relied on using an equal number of features from each class (top 25). By doing this, features that contained a greater class difference (by having a highly correlated expression pattern to one type of leukemia) could have been excluded. However the weighted voting method relies on equal votes from each class to not tip the scales in favor of one class. Thus the weighted voting method is must use samples that have strong correlation patterns in both classes.

The architecture of the ANN played a large role in the classification ability of features. As expected for each network, training after one epoch resulted in a classification score close to random and as the epoch number increased the classification accuracy increased. If a larger neural network had been used, the epoch number would have to be adjusted accordingly to achieve accurate classification. Increasing the number of hidden layers while not increasing the epochs would most likely result in finding features that contained only a very high average difference that the model could quickly fit. A good balance was found by using the [2,1] architecture. However using a [1,2] or a [3] architecture resulted in the same classification accuracy. Increasing the number of hidden layers required a greater number of generations to achieve accurate performance. This is because a greater number of weights must be adjusted to achieve acceptable performance.

An interesting result is found in the overall frequency of the features within the final population. The full table is presented in Appendix 4. For ten features that were isolated, only 8 were among the most frequent in the population.

A very interesting finding is that MPO, one of the highest scoring features on the absolute Golub ranking, only occurred 4 times in the final population. However, this gene had been present from generation 25. In Appendix 1, it can be seen how the frequencies of the individual features varied through the generations. This low presence in the final generation indicates that it was very close to being completely lost from the population.

Cursory analyses of the features selected by the GA/ANN reveals a diverse set of cellular processes. EntrezGene summaries are presented in Appendix 1. While an attempt to build a unifying theory for their expression pattern could be attempted, the scientific validity of any theory would be highly questionable. The found feature set represents a set of ten features that can accurately classify these leukemia types. Because this was pre-set at ten features, no greater number of potentially important features can be included. Because many acceptable local minima also exist, there is no guarantee that the GA/ANN would return the same set of ten features after every run as this simulation only encountered 27% of the possible features. The frequency of features through several GA/ANN runs would be needed to verify the results of this one simulation. Only then would it be prudent to attempt to understand why these features play a biological role in classification. These ten features could also be artifacts of many different methods, including the preparation techniques used in 1999 or of the array technology utilized in 1999. While these features are able to accurately classify on the training data from this dataset, including a greater training and testing set would add further validation to this data.

Conclusions

The GA/ANN hybrid system helped to isolate interesting combinations of features that were able to accurately classify Leukemia samples. This system could be of great supplemental use to physicians attempting to make an accurate diagnosis between leukemia samples. From the isolated features, a diagnostic test could also be built around this minimal set of measurements.

The ability of the wrapper method to isolate interesting combinations of features exceeds that of the filter method. Neural networks remain a powerful tool for building classifiers. While the implementation of a GA/ANN wrapper method comes at a high computational cost, the discovery of high scoring feature combinations that would have been ignored using a filter method justifies its use. Genetic Algorithms are able to effectively explore large search spaces without doing an exhaustive search. However they run into difficulties when attempting to find global minima. This problem is compounded within this study due to the scoring and sampling methods. As the same chromosome can take on different scoring values each time it is evaluated, the global minima remain an elusive target. The algorithm usually had no difficulty reaching a high scoring solution. However running the algorithm several times would help to isolate consistently high scoring feature combinations

For researchers looking for a quick answer with highly probable results, the filter method is the best choice. However in methods that seek to isolate interesting combinations that can build robust and thorough classifiers, the wrapper approach provides a more thorough examination.

The GA/ANN approach could be scaled to run much faster if distributed over a greater number of nodes. Since the evaluation process is not dependent upon the other members in the

population, distribution of chromosome evaluations saved a great deal of computational time in this study but could have run faster by further distribution.

Because the GA/ANN has a large number of free parameters, an evolutionary approach could be applied to optimize these parameters after or concurrent with the feature selection process. Allowing an evolvable neural network architecture would help to locate an ideal architecture and the best inputs for that architecture. However, because the Golub dataset contains a large difference between the classes, this method would not add much value. A major genetic algorithm parameter change would be to increase the population size. By increasing the population size, the algorithm would perform a more thorough search of the solution space and would be more likely to locate the global minima. Another beneficial measure would be to re-run the simulation several more times and compare the solutions found. There is no guarantee that the algorithm would settle on the same set of features, yet features that appeared more often over many runs most likely would be the most informative.

More subtle combinations of features could be detected by the GA/ANN method by eliminating the strong signals prior to implementation of this algorithm. By eliminating the features with strong signals, the GA/ANN would be forced to search for more complex and subtle interactions that may be passed over by less sensitive methods.

High throughput technologies are generating datasets that are becoming increasingly larger and more complex. To extract meaningful information from these data sets remains a complex task. The ability of machine learning methods that can detect complex signal patterns can help research and modern personalized medicine advance at a much faster rate.

References

American Cancer Society. (April 2009) History of Cancer from [http://www.cancer.org/docroot/CRI/content/CRI_2_6x_the_history_of_cancer_72.asp?sitearea=]

Baldi P., Brunak S.(2001). Bioinformatics: the machine learning approach 2nd ed. Cambridge: The MIT Press

Bevilacqua V. Mastronardi G, Menolascina F., Paradiso A., Tommasi S. (November 2006) Genetic Algorithms and Artificial Neural Networks in Microarray Data Analysis: A Distributed Approach, Engineering Letters 13(3)

Chester M. (1993) Neural Networks a Tutorial, Englewood Cliffs, NJ, Prentice-Hall.

Dobbin K. K., Zhao Y., Simon R. M. (2008) How Large a Training Set is Needed to Develop a Classifier for Microarray Data? Clinical Cancer Research, 14(1), 108-114.

Draghici, S. (2003) Data analysis tools for DNA microarrays. Boca Raton: Chapman & Hall

Dudoit S., Fridlyand J., Speed T.P. (2002). Comparison of discrimination methods for the classification of tumors using gene expression data. *Journal of the American Statistical Association*, Vol. 97, No. 457, p. 77-87.

Goldberg, D.,(1989). Genetic algorithms in search, optimization, and machine learning. Reading: Addison-Wesley

Golub T. R., Slonim D.K., Tamayo P., Huard C., Gaasenbeek M., Mesirov J.P., Coller H., Loh M. L., Downing J.R., Caligiuri M.A., Bloomfield C.D, Lander E.S. (1999) Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring, 286, 531-537

Hagermeijer A., Grosveld G.,Molecular (1996) Cytogenetics of Leukemia. In Henderson E.S., Lister, T.A. Greaves M.F. (Eds.) Leukemia (6^{th} ed.) (pp. 131-159) Philadelphia: W.B Saunders Company a Division of Harcourt Bruce & Company

Inza I., Larranaga P., Blanco R., Cerrolaza A. J. (2004) Filter versus wrapper gene selection approaches in DNA microarray domains, Artificial Intelligence in Medicine, 31, 91-103.

Kuo W. P., Kim E. Y., Trimarchi J., Jenssen T. K., Vinterbo S. A., Ohno-Machado L. O. (2004) A primer on gene expression and microarrays for machine learning researchers. Journal of Biomedical Informatics, 37, 293-303.

Leukemia and Lymphoma Society. (April 2009) Leukemia Facts and Statistics from

[http://www.leukemia-lymphoma.org/all_page.adp?item_id=9346]

Li C. Tseng. G, Wong WH. Model based analysis of oligonucleotide arrays and issues in cDNA microarray analysis. in Terry Speed (2003) Statistical Analysis of Gene Expression MicroarrayData. Boca Raton, FL, Chapman and Hall.

Li C., Wong WH - Proceedings of the National Academy of Sciences, 2001 - National Acad Sciences, 98(1), 31-36.

Mattiussi C. & Floreano D (2007) Analog Genetic Encoding for the Evolution of Circuits and Networks, Transactions on Evolutionary Computation, 11(5)

Mitchell M. (1998) An Introduction To Genetic Algorithms, A Bradford Book The MIT press, Cambridge: MA.

Parham P. (2005) The Immune System second edition. New York: Garland Science, Taylor & Francis group

Poi C.H. & Evans W. (1998) Acute Lymphoblastic Leukemia, New England Journal of Medicine, 339(9), 605-615

Primrose S , Twyman R. (2004) Genomics: Applications in Human Biology. Malden MA: Blackwell Publishing

Reeves C., C. Wright (1995) An Experimental Design Perspective On Genetic Algorithms published in Whitley D. ,Vose M. (Eds.) Foundations of Genetic Algorithms 3. Morgan Kaufmann, San Mateo, C.A.

Rosenblatt F., (1958) The Perceptron: a probabilistic model for information storage and organization in the brain. Psychological Review, 65(6), 386-408

Simon R., Radmacher M. D., Dobbin K., McShane L. M. (2003) Pitfalls in the Use of DNA Microarray Data for Diagnostic and Prognostic Classification, Journal of the National Cancer Institute, 95(1)

Sivanandam S. N., Deepa S. N. (2008) Introduction to Genetic Algorithms by, Springer-Verlag Heidelberg

Srinivas M. & Patnaik LM. (1994) Genetic Algorithms: A Survey. Computer, 27(6)17-26
Swamy M.N.S. (2006) Neural Networks in a Softcomputing Framework Springer-Verlag London Limited.

Trappenberg, T. (2002) Fundamentals of Computational Neuroscience. New York: Oxford University Press

Watson J., Baker T., Bell S., Gann A., Levine M., Losick R. (2004) Molecular Biology of the Gene Fifth Edition. San Francisco: Benjamin Cummings Pearson Education

Weiss, N. A. (2008). Introductory Statistics. Boston: Pearson Education

Yang J., Honavar V (1998) Feature subset selection using a genetic algorithm. In Liu H., Motoda H., Feature Extraction, Construction and Selection: a Data Mining Perspective, Kluwer Academic Publishers, Norwell MA.

Zhong J., Hu X., Gu M., Zhang J. (2005) In Comparison of Performance between Different Selection Strategies on Simple Genetic Algorithms, Computational Intelligence for Modelling, Control and Automation, 2005 and International Conference on Intelligent Agents, Web Technologies and Internet Commerce, International Conference on, November 28-30 (pp. 1115-1121) Vienna

Appendix 1

EntrezGene summaries for isolated features

Phosphatidylinositol 4-kinase

Function: Phosphorylates phosphatidylinositol (PI) in the first committed step in the production of the second messenger inositol-1,4,5,-trisphosphate (PIP). May regulate Golgi disintegration/reorganization during mitosis, possibly via its phosphorylation

May play a possible role in Golgi reorganization during mitosis (http://www.genecards.org/cgi-bin/carddisp.pl?gene=PI4KB&search=U81802&suff=txt).

KIAA0128 gene partial cds

This gene is a member of the septin family of GTPases. Members of this family are required for cytokinesis. One version of pediatric acute myeloid leukemia is the result of a reciprocal translocation between chromosomes 11 and X, with the breakpoint associated with the genes encoding the mixed-lineage leukemia and septin 2 proteins. This gene encodes four transcript variants encoding three distinct isoforms. An additional transcript variant has been identified, but its biological validity has not been determined. [provided by RefSeq]

PZP pregnancy-zone protein
UniProtKB/Swiss-Prot: PZP_HUMAN, P20742
Function: Is able to inhibit all four classes of proteinases by a unique 'trapping' mechanism. This protein has a peptide stretch, called the 'bait region' which contains specific cleavage sites for different proteinases. When a proteinase cleaves the bait region, a conformational change is induced in the protein which traps the proteinase. The entrapped enzyme remains active against low molecular weight substrates (activity against high molecular weight substrates is greatly reduced). Following cleavage in the bait region a thioester bond is hydrolyzed and mediates the covalent binding of the protein to the proteinase

MB-1 gene

Function: Required in cooperation with CD79B for initiation of the signal transduction cascade activated by binding of antigen to the B-cell antigen receptor complex (BCR) which leads to internalization of the complex, trafficking to late endosomes and antigen presentation. Also required for BCR surface expression and for efficient differentiation of pro- and pre-B-cells. Stimulates SYK autophosphorylation and activation. Binds to BLNK, bringing BLNK into proximity with SYK and allowing SYK to phosphorylate BLNK. Also interacts with and increases activity of some Src-family tyrosine kinases. Represses BCR signaling during development of immature B cells

EntrezGene summary for CD79A:

The B lymphocyte antigen receptor is a multimeric complex that includes the antigen-specific

component, surface immunoglobulin (Ig). Surface Ig non-covalently associates with two other proteins, Ig-alpha and Ig-beta, which are necessary for expression and function of the B-cell antigen receptor. This gene encodes the Ig-alpha protein of the B-cell antigen component. Alternatively spliced transcript variants encoding different isoforms have been described. [provided by RefSeq]

Mucin 1 epithelial, Alt. Splice 6

EntrezGene summary for MUC1:
This gene is a member of the mucin family and encodes a membrane bound, glycosylated phosphoprotein. The protein is anchored to the apical surface of many epithelia by a transmembrane domain, with the degree of glycosylation varying with cell type. It also includes a 20 aa variable number tandem repeat (VNTR) domain, with the number of repeats varying from 20 to 120 in different individuals. The protein serves a protective function by binding to pathogens and also functions in a cell signaling capacity. Overexpression, aberrant intracellular localization, and changes in glycosylation of this protein have been associated with carcinomas. Multiple alternatively spliced transcript variants that encode different isoforms of this gene have been reported, but the full-length nature of only some has been determined. [provided by RefSeq]

UniProtKB/Swiss-Prot: MUC1_HUMAN, P15941

Function: The beta subunit contains a C-terminal domain which is involved in cell signaling, through phosphorylations and protein-protein interactions. Modulates signaling in ERK, Src and NF-kappaB pathways. In activated T-cells, influences directly or indirectly the Ras/MAPK pathway. Promotes tumor progression. Regulates P53-mediated transcription and determines cell fate in the genotoxic stress response. Binds, together with KLF4, the PE21 promoter element of P53 and represses P53 activity

MYBPC1 Myosin-binding protein C, slow-type

UniProtKB/Swiss-Prot: MYPC1_HUMAN, Q00872
Function: Thick filament-associated protein located in the crossbridge region of vertebrate striated muscle a bands. In vitro it binds MHC, F-actin and native thin filaments, and modifies the activity of actin-activated myosin ATPase. It may modulate muscle contraction or may play a more structural role

CST3 Cystatin C

EntrezGene summary for CST3:
The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity. There are three inhibitory families in the superfamily, including the type 1 cystatins (stefins), type 2 cystatins and the kininogens. The type 2 cystatin proteins are a class of cysteine proteinase inhibitors found in a variety of human fluids and

secretions, where they appear to provide protective functions. The cystatin locus on chromosome 20 contains the majority of the type 2 cystatin genes and pseudogenes. This gene is located in the cystatin locus and encodes the most abundant extracellular inhibitor of cysteine proteases, which is found in high concentrations in biological fluids and is expressed in virtually all organs of the body. A mutation in this gene has been associated with amyloid angiopathy. Expression of this protein in vascular wall smooth muscle cells is severely reduced in both atherosclerotic and aneurysmal aortic lesions, establishing its role in vascular disease. [provided by RefSeq]

UniProtKB/Swiss-Prot: CYTC_HUMAN, P01034
Function: As an inhibitor of cysteine proteinases, this protein is thought to serve an important physiological role as a local regulator of this enzyme activity

MPO from human myeloperoxidase

EntrezGene summary for MPO:
Myeloperoxidase (MPO) is a heme protein synthesized during myeloid differentiation that constitutes the major component of neutrophil azurophilic granules. Produced as a single chain precursor, myeloperoxidase is subsequently cleaved into a light and heavy chain. The mature myeloperoxidase is a tetramer composed of 2 light chains and 2 heavy chains. This enzyme produces hypohalous acids central to the microbicidal activity of netrophils. [provided by RefSeq]

UniProtKB/Swiss-Prot: PERM_HUMAN, P05164
Function: Part of the host defense system of polymorphonuclear leukocytes. It is responsible for microbicidal activity against a wide range of organisms. In the stimulated PMN, MPO catalyzes the production of hypohalous acids, primarily hypochlorous acid in physiologic situations, and other toxic intermediates that greatly enhance PMN microbicidal activity

Triadin mRNA

UniProtKB/Swiss-Prot: TRDN_HUMAN, Q13061
Function: May be involved in anchoring calsequestrin to the junctional sarcoplasmic reticulum and allowing its functional coupling with the ryanodine receptor (By similarity)

PLCB2 Phospholipase C, beta 2

UniProtKB/Swiss-Prot: PLCB2_HUMAN, Q00722
Function: The production of the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) is mediated by activated phosphatidylinositol-specific phospholipase C enzymes

Appendix 2

To implement the mutation operator each generation a random matrix with dimensions matching the population is created. The matrix is searched for values below the user set mutation rate. If a value is found a random gene index is generated and replaces that value in that chromosome

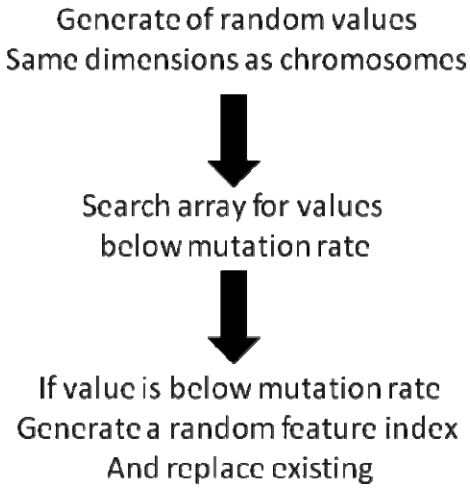


Figure 16. Overview of mutation operator

Appendix 3

Probe Name	**Probe number**
Phosphatidylinositol 4-kinase	3826
KIAA0128 gene partial cds	490
PZP pregnancy-zone protein	4263
MB-1 gene	2642
Mucin 1 epithelial, Alt. Splice 6	5856
MYBPC1 Myosin-binding protein C, slow-type	6688
CST3 Cystatin C	1882
MPO from human myeloperoxidase	6215
Triadin mRNA	2874
PLCB2 Phospholipase C, beta 2	2394

Appendix 4

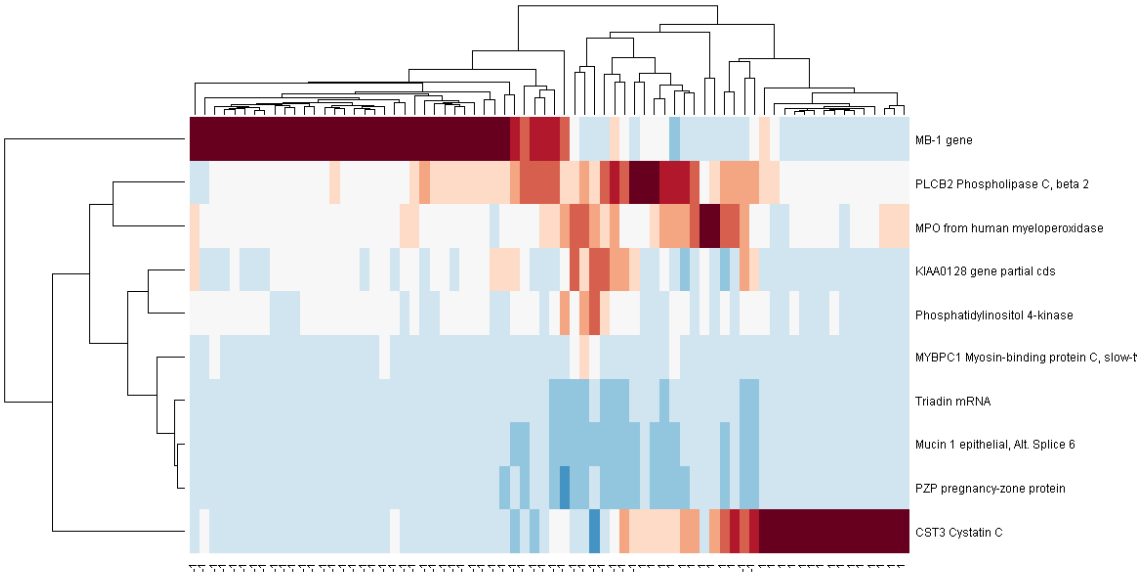
Number of Features: 10
Population Size: 100
Number of Epochs: 1000
Number of Generations: 2000
Size of Hidden Layer: 2
Mutation Rate: 0.01
Crossover Prob: 0.75
Hidden Layer 2: 1
Percentage Covered: 27.4372
Genes and prevalence in population

M27891_at	90	CST3 Cystatin C (amyloid angiopathy and cerebral hemorrhage)
U81802_at	90	Phosphatidylinositol 4-kinase
M95678_at	88	"PLCB2 Phospholipase C, beta 2"
U05259_rna1_at	84	MB-1 gene
U18985_at	78	Triadin mRNA
M57731_s_at	68	GRO2 GRO2 oncogene
X54380_at	60	PZP Pregnancy-zone protein
X66087_at	47	MYBL1 V-myb avian myeloblastosis viral oncogene homolog-like 1
X66276_s_at	35	"MYBPC1 Myosin-binding protein C, slow-type"
D50918_at	30	"KIAA0128 gene, partial cds"
L38820_at	28	"CD1D CD1D antigen, d polypeptide"
U33054_at	26	G PROTEIN-COUPLED RECEPTOR KINASE GRK4
HG371-HT1063_s_at	23	"Mucin 1, Epithelial, Alt. Splice 6"
X14362_at	22	"CR1 Complement component (3b/4b) receptor 1, including Knops blood group system"
M21119_s_at	18	LYZ Lysozyme
X74837_at	16	HUMM9 mRNA
U78107_at	15	Gamma SNAP mRNA
X86163_at	12	BDKRB2 Bradykinin receptor B2
M27533_s_at	12	GB DEF = Ig rearranged B7 protein mRNA VC1-region
L48516_at	11	"GB DEF = Paraoxonase 3 (PON3) mRNA, 3' end of cds"
U35376_at	11	Repressor transcriptional factor (ZNF85) mRNA
U83192_at	9	Post-synaptic density protein 95 (PSD95) mRNA
U68162_cds1_s_at	8	MPL gene (thrombopoietin receptor) extracted from Human thrombopoietin receptor (MPL) gene
HG4390-HT4660_at	7	Ribosomal Protein L18a Homolog
U73191_at	6	Inward rectifier potassium channel (Kir1.3)
X61072_at	6	"T cell receptor, clone IGRA17"
X66362_at	6	PCTK3 PCTAIRE protein kinase 3
Z79581_at	6	"GB DEF = LAZ3/BCL6 gene, first non coding exon"
AB002318_at	5	"KIAA0320 gene, partial cds"
D00760_at	5	PSMA3 Proteasome component C3
D14694_at	5	PHOSPHATIDYLSERINE SYNTHASE I
D86971_at	5	"KIAA0217 gene, partial cds"
X17042_at	5	"PRG1 Proteoglycan 1, secretory granule"
U26424_at	5	Stress responsive serine/threonine protein kinase Krs-1 mRNA
L33801_at	4	Protein kinase mRNA
M91592_at	4	ZNF76 Zinc finger protein 76
X96783_rna1_at	4	Syt V gene (genomic and cDNA sequence)
M19508_xpt3_s_at	4	"MPO from Human myeloperoxidase gene, exons 1-4./ntype=DNA /annot=exon"
M64676_at	3	GB DEF = K+ channel subunit gene

M92439_at	3	130 KD LEUCINE-RICH PROTEIN
M94893_at	3	"TSPY Testis specific protein, Y-linked"
J03779_at	2	"MME Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)"
X66403_at	2	"CHRNE Cholinergic receptor, nicotinic, epsilon polypeptide"
X99802_at	2	ZYG homologue
Y09306_at	2	"GB DEF = Protein kinase, Dyrk6, partial"
J03634_at	2	"INHBA Inhibin, beta A (activin A, activin AB alpha polypeptide)"
D43772_at	1	Squamous cell carcinama of esophagus mRNA for GRB-7 SH2 domain protein
D63813_at	1	Rod photoreceptor protein
HG908-HT908_at	1	Mg61 Protein (Gb:L08239)
M20530_at	1	"SPINK1 Serine protease inhibitor, Kazal type 1"
M24439_at	1	"ALPL Alkaline phosphatase, liver/bone/kidney"
M68840_at	1	MAOA Monoamine oxidase A
U38980_at	1	"PMS8 mRNA (yeast mismatch repair gene PMS1 homologue), partial cds (C-terminal region)"
U70735_at	1	GB DEF = 34 kDa mov34 isologue mRNA
U79301_at	1	Clone 23842 mRNA sequence
X54162_at	1	64 KD AUTOANTIGEN D1
X57830_at	1	Serotonin 5-HT2 receptor mRNA
X58723_at	1	GB DEF = MDR1 (multidrug resistance) gene for P-glycoprotein
X79536_at	1	HNRPA1 Heterogeneous nuclear ribonucleoprotein A1
X89894_at	1	Nuclear receptor
X90828_at	1	"Transcription factor, Lbx1"
X93036_at	1	MAT8 protein
Z11697_at	1	CD83 ANTIGEN PRECURSOR
D28791_at	1	"PIGA Phosphatidylinositol glycan, class A (paroxysmal nocturnal hemoglobinuria)"
L43576_at	1	(clone EST02946) mRNA
HG2271-HT2367_s_at	1	Profilaggrin
M16707_rna1_at	1	"Histone H4 gene, clone FO108"
M87507_s_at	1	"IL1BC Interleukin 1, beta, convertase"
X06700_s_at	1	COL3A1 Alpha-1 type 3 collagen

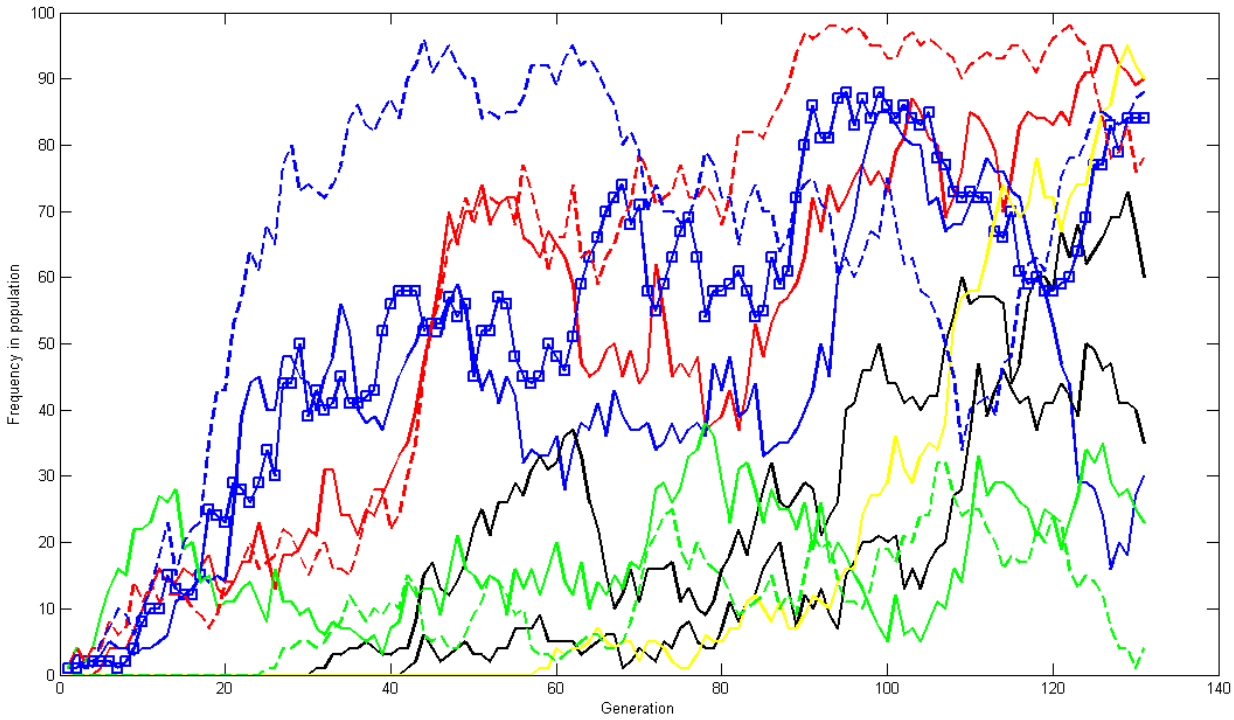
Appendix 5

Red represents high expression blue low expression. Standardized across samples



Appendix 6

Feature frequencies over generations



Appendix 7

Population Variance