Reliability Analysis of Classification of Gene Expression Data Using Efficient Gene Selection Techniques

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Abstract---**Gene expression data usually contains a large number of genes, but a small number of samples. Feature selection for gene expression data aims at finding a set of genes that best discriminate biological samples of different types. Classification of tissue samples into tumor or normal is one of the applications of microarray technology. When classifying tissue samples, gene selection plays an important role. In this paper, we propose a two-stage selection algorithm for genomic data by combining some existing statistical gene selection techniques and ROC score of SVM and k-nn classifiers. The motivation for the use of a Support Vector Machine is that DNA microarray problems can be very high dimensional and have very few training data. This type of situation is particularly well suited for an SVM approach. The proposed approach is carried out by first grouping genes with similar expression profiles into distinct clusters, calculating the cluster quality and the discriminative score for each gene by using statistical techniques, and then selecting informative genes from these clusters based on the cluster quality and discriminative score .In the second stage, the effectiveness of this technique is investigated by comparing ROC score of SVM that uses different kernel functions and k-nn classifiers. Then Leave One Out Cross Validation (LOOCV) is used to validate the techniques.**

Key Words - Fisher Criterion, Golub Signal-to-Noise, Mann-Whitney Rank Sum Statistic, Leave One Out Cross Validation (LOOCV), Support Vector Machine(SVM).

I. INTRODUCTION

The problem of cancer classification has clear implications on cancer treatment. Additionally, the advent of DNA microarrays introduces a wealth of genetic expression information for many diseases including cancer. An automated or generic approach for classification of cancer or other diseases based upon the microarray expression is an important problem.

A generic approach to classifying two types of acute leukemia was introduced in Golub et. al. [7]. They achieved good results on the problem of classifying acute myeloid leukemia (AML) versus acute lymphoblastic leukemia (ALL) using 50 gene expressions. Their approach to classification consisted of summing votes for each gene on the test data, and looking at the sign of the sum. With high-dimensional data, an alternative approach based on boosting has been proposed by Kim and Kim [13] and later used in microarray data analysis by Ma and Huang [17]. Computational cost of the boosting approach is relatively independent of the dimensionality of input variables, which makes it especially suitable for high-dimensional data. For linear models with high-dimensional input, i.e. $n \ll p$ and $g < 1$, Huang et al. [18,14] shows that the bridge can still be feature selection consistent, if the features associated with the phenotype and those not associated with the phenotype are only weakly correlated. Supervised classification and feature selection with high-dimensional input are related to the following subjects: (i) prescreening, which carries out a rough screening to remove input variables unlikely to be important[12,15,16] (ii) tuning parameter selection.

In this paper, four statistical techniques such as Fisher Criterion, Golub Signal-to-Noise, traditional t-test, and Mann-Whitney Rank Sum Statistic are studied. The objective is to investigate the impact and importance of the gene selection techniques on the tissue classification performance. The effectiveness of these techniques are investigated by comparing ROC score of SVM that uses different kernel functions i.e., the dot product, quadratic dot product, cubic dot product and the radial basis function and the *k*-nn classifiers. The LOOCV is applied to validate the techniques. Results show that a better performance of the classifiers can be achieved if genes are first selected prior to the classification task. The organization of this paper is as follows. The background on cDNA microarrays is outlined in Section 2. Gene selection methods are outlined in Section 3. The classification algorithms are briefly described in Section 4. Procedure for gene selection and classification of gene

expression data is explained in Section 5.Data set and the result evaluation method are reported in Section 6 and Section 7 respectively. Then summary of the paper is reported in Section 8.

II.   BACKGROUND ON CDNA MICROARRAYS

A *gene* consists of a segment of DNA which codes for a particular *protein*, the ultimate

expression of the genetic information. A *deoxyribonucleic acid* or *DNA* molecule is a double-stranded polymer composed of four basic molecular units called nucleotides. Each *nucleotide* comprises a phosphate group, a deoxy ribose sugar, and one of *four nitrogen bases*. The four different bases found in DNA are adenine (A), guanine (G), cytosine (C), and thymine (T).The two chains are held together by hydrogen bonds between nitrogen bases, with base-pairing occurring according to the following rule: G pairs with C, and A pairs with T. While a DNA molecule is built from a four-letter alphabet, proteins are sequences of twenty different types of *amino acids*. The expression of the genetic information stored in the DNA molecule occurs in two stages: (i) *transcription*, during which DNA is transcribed into *messenger ribonucleic acid* or *mRNA*, a single-stranded complementary copy of the base sequence in the DNA molecule, with the base uracil (U) replacing thymine; (ii) *translation*, during which mRNA is translated to produce a protein. The correspondence between DNA's four-letter alphabet and a protein's twenty-letter alphabet is specified by the *genetic code*, which relates nucleotide triplets to amino acids. cDNA microarrays consist of thousands of individual DNA sequences printed in a high density array on a glass microscope slide. The relative abundance of these DNA sequences in two DNA or cDNA samples may be assessed by monitoring the differential hybridization of the two samples to the sequences on the array. To this end, the two DNA samples or *targets* are labeled using different fluorescent dyes (*e.g.* a red-fluorescent dye Cy5 and a green-fluorescent dye Cy3), then mixed and hybridized with the arrayed DNA sequences or *probes*.

After this competitive hybridization, fluorescence measurements are made separately for each dye at each spot on the array. The ratio of the fluorescence intensity for each spot is indicative of the relative abundance of the corresponding DNA sequence in the two samples (see http://rana.Stanford.EDU/software/) for more information on the measurement of fluorescence intensities.

Microarrays are being applied increasingly in cancer research to study the molecular variations among tumors. This should lead to an improved classification of tumors, which in turn should result in progresses in the prevention and treatment of cancer. An important aspect of this endeavor is the ability to predict tumor types on the basis of gene expression data. We review below a number of selection and classification methods to assess their performance on the cancer dataset, described in Section 3 and 4.

III.   GENE SELECTION TECHNIQUES

A.   The Fisher Criterion

In this selection technique [9], *fisher* is a measure that indicates how much the class distributions are separated. The coefficient has the following formula:

$$fisher = \frac{(\mu_1 - \mu_2)^2}{(v_1 + v_2)}$$

where μ_i is the mean and v_i is the variance of the given gene in class *i*. There were two tissue classes in this experiment, i.e. the tumor tissue and the normal tissue. The statistic gives higher scores to genes whose means differ greatly between the two classes, relative to their variances.

B.   Golub Signal-to-Noise

This technique [7] has used a measure of correlation that emphasizes the "Signal-to-Noise" ratio, *signaltonoise*, to rank the genes.

$$signaltonoise = \frac{(\mu_1 - \mu_2)}{(\sigma_1 + \sigma_2)}$$

Where μ_i is the mean and σ_i is the standard deviation of the gene in class *i*.

C.   Traditional t-test

The traditional t-test [2] *ttest* assumes that the values of the two tissues variances are equal. The formula is as

$$ttest = \frac{(i_1 - \mu_2)}{\sqrt{\left(\frac{v_p}{n_1}\right) + \left(\frac{v_p}{n_2}\right)}}$$

where μ_i is the mean and v_p is the pooled variance,

$$v_p = \left((n_1 - 1)s_1^2 + (n_2 - 1)s_2^2\right)/(n_1 + n_2 - 2)$$

$$s_i^2 = \sum_{i=1}^{n_i} (x_i - \bar{x})^2/(n_i - 1)$$

D.   The Mann-Whitney Rank Sum Statistic

This statistic [2], *mann,* has the following formula:

$$mann = n_1 * n_2 * \frac{(n_1 + 1)}{2} - r_1$$

Where n_i is the sizes of sample *i*, and r_1 is the sum of the ranks in sample1.

These techniques are used to calculate the statistical score, S, for the genes. In these techniques, each gene is measured for correlation with the class according to some measuring criteria in the formulas. The genes are ranked according to the score, S, and the top ranked genes are selected.

IV.   TISSUE CLASSIFICATION

Two classification algorithms are proposed to evaluate the validity of the selected genes. They are the SVM [1] with different kernels and *k*- nn [6].

A.   Support Vector Machines for Tissue Classification

An SVM expects a training data set with positive and negative classes as an input.

It then creates a decision boundary between the two classes and selects the most relevant examples involved in the decision process. The construction of the linear boundary is always possible as long as the data is linearly separable. If this is not the case, SVMs can use kernels, which provide a nonlinear mapping to a higher dimensional feature space. Different kernel functions, the dot product and radial basis function are used for this experiment [4][5][8][1].

The dot product has the following formula:

$$K(x, y) = (x.y + 1)^d$$

where x and y are the vectors of the gene expression data. The parameter d is an integer which decides a rough shape of a separator. In the case where d equals to 1, a linear classifier is generated, and in the case where d is equal to or more than 2, a nonlinear classifier is generated. In this experiment, when d is equals to 1, it is called the SVM dot product, when d is equals to 2, it is called the SVM quadratic dot product and when d is equals to 3, it is called the SVM cubic dot product.

The radial basis kernel has the following formula:

$$K(x, y) = exp\left(\frac{-|x - y|}{2\sigma^2}\right)$$

where σ is the median of the Euclidean distances between the members and nonmembers of the class.

The main advantages of SVMs are that they are robust to outliers, converge quickly, and find the optimal decision boundary if the data is separable. Another advantage is that the input space can be mapped into an arbitrary high dimensional working space where the linear decision boundary can be drawn. This mapping allows for higher order interactions between the samples and can also find correlations between samples. SVMs are also very flexible as they allow for a big variety of kernel functions.

B. *k*-nearest neighbor for Tissue Classification

The *k*-nn classifier is a simple classifier based on a distance metric between the testing samples and the training samples [6]. The main idea of the method is, given a testing sample *s*, and a set of training tuples *T* containing pairs in the form of (*ti*, *ci*) where *ti's* are the expression values of gene *i* and *ci* is the class label of gene *i*. Find *k* training sample with the most similar expression value between *t* and *s*, according to a distance measure. The class label with the highest votes among the *k* training sample is assigned to *s*. The main advantage of *k*-nn is it has the ability to model very complex target functions by a collection of less complex approximations. It is easy to program and understand. It is robust to noisy training data.

V.   PROCEDURE FOR GENE SELECTION AND CLASSIFICATION OF GENE EXPRESSION DATA

The procedure for this experiment is shown:
i.   Get the input data.
ii. Select the number of genes, T based on the statistical score S of the genes using the statistical selection method and then apply the classification technique. In this experiment, the top ranked genes are selected.
iii. Apply LOOCV technique for validation and evaluation purpose.
iv. Calculate the ROC score based on the predicted class.
v.   The process is repeated for all the combinations of another gene selection technique with the classifiers for another set of genes having high statistical score.

INPUT: Gene expression data matrix, X= {x$_{11}$,.......,x$_{np}$} and the class label for each column, $y \in \{-1,1\}$, where n is the number of genes and p is the number of tissue samples.

1. Get the data with p tissues (samples).
2. Pre-set the combination: the gene selection technique, the classifier and number of genes to be selected, T.

LEAVE ONE OUT CROSS VALIDATION:

3.   For i = 1 to p
     3.1. Leave i^{th} sample out.
   GENE SELECTION:
     3.2 Calculate the discriminative score, S, for each gene of the remaining p- 1samples,   and rank the genes based on the score.
     3.3 Select top T genes based on the ranked score, S.
   CLASSIFICATION:
     3.4 Train the classifier using the selected genes, T , of p-1 samples.
     3.5 Test the trained classifier by using the left out i^{th} sample.
     3.6 Record the predicted class from 3.5, and put back the ith sample.

ROC CALCULATION:

4. Calculate the ROC score based on the predicted class and save the ROC score.
5. Go to step 2 to select another set, T, of genes for another combination of   gene selection technique and classifiers .

OUTPUT: ROC score for each set of genes to be selected, *T* and the gene selection technique.

VI . DATA SET

The colon data set were applied. It is a collection of expression levels from colon biopsy samples reported by Alon *et al.* [10]. The data set consisted of 62 tissue samples of colon epithelial cells. These samples were collected from colon-tumor patients. The tumor biopsies were collected from tumors, and the normal biopsies were collected from healthy parts of the colons of the same patients. Each sample contains more than 6,500 human genes measured using high density oligonucleotide arrays. 2000 genes with the highest minimal intensity across the 62 samples were chosen for the analysis.

VII.   RESULT EVALUATION METHOD

ROC score is used to analyze the results for the experiment. ROC score is also the area under the ROC curve. ROC score is a common way for evaluating classification performance because it takes into account both false negative and false positive errors and it reflects

the robustness of the classification. A random classification has an expected ROC score close to 0.5 while a perfect classification with no error has a ROC score of 1.

A.   Results and Discussion

In this section, the impact and importance of gene selection to the classification performance is first studied. This is carried out by comparing the classification performance by using all genes and gene selected by statistical techniques which are mentioned above. After that, the classification performance of   each classifier is discussed. Finally, the effectiveness of each statistical technique to the best classifier is discussed.

B.   Importance of Gene Selection Technique Prior to Tissue Classification

Figure-1 shows the classification performance by using all genes and gene selected by using statistical techniques. The ROC scores recorded for the gene selection techniques in the figure are the average ROC scores for number of genes selected with high statistical score S.

From the figure, by using all genes, the best performance is obtained by using SVMs with radial basis function while *1*-nn, *2*-nn and *5*-nn have worst performance. *3*-nn and *4*-nn are comparable to each other when all genes are used. The performances of the classifiers are improved after genes are selected by gene selection techniques especially for *k*-nn classifier. This shows the importance of applying gene selection techniques to select informative genes prior to the classification task. Applying gene selection techniques in selecting genes helps in removing a large number of irrelevant genes which improves the classification performance. Since one of the advantages of SVMs is, it is robust to outliers and allows nonlinear classification to be done, gene selection techniques does not give big impact to its performance, but, a better performance still can be obtained after applying gene selection techniques, which can be seen from the figure.
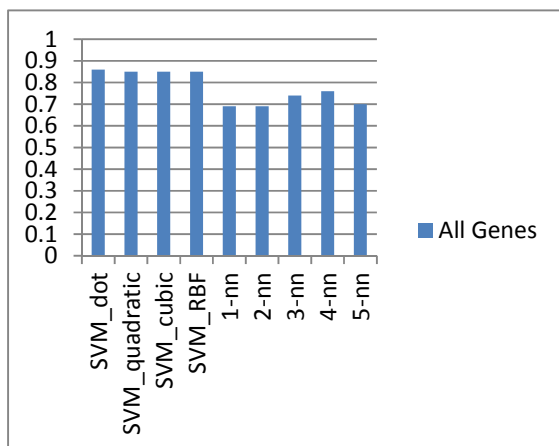

Figure 1: Classification performance of the classifiers without using selection methods
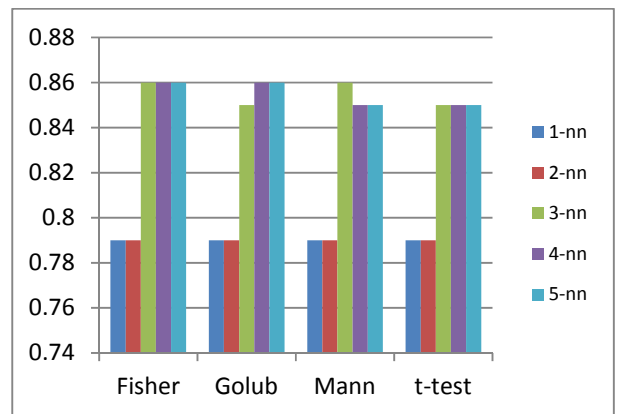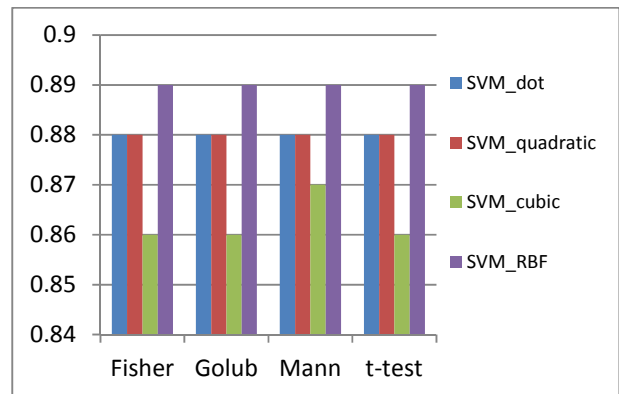

Figure 2: Classification performance using genes selected by statistical technique

Figure.2 shows that a better classification performance can be achieved if genes are first selected by the gene selection techniques. However, which combination of statistical techniques and classifier increases the performance of the classifier? Next section answers this question.

C.   Classification Performance between Different Classifiers

Table-1 summarizes the performance for each SVM classifier. The ROC scores recorded in the table are the average ROC score over all trials with the number of selected genes.

Table-1: Summary for classification performance by using SVM with different kernels after gene selection by using statistical techniques

SVMs	Fisher	Golub	Mann	t-test
SVM_dot	0.88	0.88	0.88	0.88
SVM_quadratic	0.88	0.88	0.88	0.88
SVM_cubic	0.86	0.86	0.87	0.86
SVM_RBF	0.89	0.89	0.89	0.89

Table-1 shows that, SVM radial basis function performs the best. Of the three product kernels, dot-product and quadratic product have better ROC score than

cubic-product. These results indicate that over-fitting causes the misclassification for the cubic-product kernel. If more samples are obtained and they are not separable linearly, nonlinear classification may perform well [3]. Table-2 summarizes the performance for each *k*-nn classifier. The ROC scores recorded in the table are the average ROC score over all trials with the number of selected genes.

Table-2: Summary for classification performance by using different *k*-nn after gene selection using statistical techniques

k-nn	Fisher	Golub	Mann	t-test
1-nn	0.79	0.79	0.79	0.79
2-nn	0.79	0.79	0.79	0.79
3-nn	0.86	0.85	0.86	0.85
4-nn	0.86	0.86	0.85	0.85
5-nn	0.86	0.86	0.85	0.85

Table-2 shows that *k*-nn with *k* more than 2 outperform the *k* which is equal to 1 and 2. One of the reasons for this to happen is that in the case of mislabeled training samples, it will have much greater effect on the classification result of *1*-nn since one mislabel will result in misclassifying the test sample. *3*-nn and *4*-nn is less prone to bias in the data and more tolerable to noise since it makes use of several training samples to determine the class of a test sample.

Figure-3 shows that SVM with radial basis function as the kernel function always produced higher ROC score than *3*-nn. Generally, the results have lower ROC score with fewer genes for both classifiers. One reason for the lower scores might due to the characteristic of genes itself where genes do not act alone, but they interact with other genes for certain functions.

For example, if Gene A and Gene B are in the same function it could be that they have similar regulation and therefore similar expression profiles. If Gene A has a good discriminative score it is highly likely that Gene B will, as well. Hence the statistical techniques are likely to include both genes in a classifier, yet the pair of genes provides little additional information compared to either gene alone. If there are 5 functions in the dataset, 10 genes for each function, and if the genes in first function have the highest scores, so these 10 genes might be selected for the classification task. In this case, the genes being selected are highly redundant and thus provide little additional information.

When the number of genes increase, the ROC score for SVMs and *k*-nn becomes more stable, because the possibility to select meaningful genes increase.
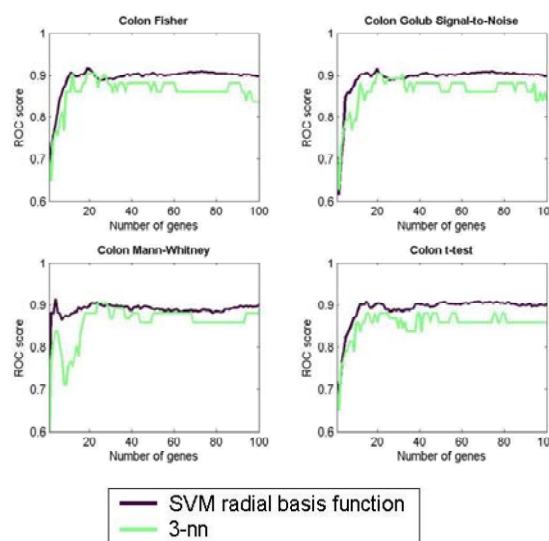

Figure-3: Classification performance between different classifiers after gene selection using statistical techniques (the best classifier is selected from SVM and *k*nn).

VIII.   SUMMARY

This paper reports the application of different statistical techniques to the colon dataset. These techniques include Fisher Criterion, Golub Signal-to-Noise, traditional t-test, and Mann-Whitney Rank Sum Statistic. By using these techniques, the data is rank based on the discriminative score and top *T* numbers of genes are selected. In conjunction with these gene selection techniques, several SVMs and *k*-nn classifiers are applied. Based on the genes selected by the gene selection techniques, ROC score of different combination of gene selection techniques and classifiers are obtained for analysis. The main objective of this experiment is to study the impact and importance of applying gene selection techniques prior to the classification task. Results show that a better classification performance is achieved by the classifiers if informative genes are first selected. However, finding a way to reduce redundant genes being selected in order to obtain a better classification performance is important.

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