An effective mixed-model for screening differentially expressed genes of breast cancer based on LR-RF

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Abstract. To screen differentially expressed genes quickly and efficiently in breast cancer, two gene microarray datasets of breast cancer, GSE15852 and GSE45255, were downloaded from GEO. This paper proposed a novel method named LR-RF to select differentially expressed genes of breast cancer on microarray data by the Bonferroni test of FWER error measure. Comparing with Logistic Regression and Random Forest, our study shows that LR-FR has great facility in selecting differentially expressed genes. The average prediction accuracy of the proposed LR-RF from replicating random test ten times surprisingly reaches 93.11% with variance as low as 0.00045. In addition, through analyzing the gene interaction networks, most of the top 20 genes we selected were found to involve in the development of breast cancer. All of these results demonstrate the reliability and efficiency of LR-RF. It is anticipated that LR-RF would provide new knowledge and method for biologists, medical scientists, and cognitive computing researchers to identify disease-related genes of breast cancer.

Keywords: breast cancer, differentially expressed genes, Logistic Regression-Random Forest, Bonferroni test, gene interaction networks.

1 Introduction

Breast cancer is the most common cancer in women worldwide. Across the globe, breast cancer is the second most common type of cancer and the second leading cause of cancer death in women. According to the latest statistics, every 26 seconds, there is a woman diagnosed with breast cancer. New breast cancer worldwide each year is up to 1.2 million, with an average annual increase in 500,000[1]. For the prevention of breast cancer, early diagnosis and treatment, different ways from biomedical, bioinformatics, and so on are urgently needed in identifying the cancer-causing genes.

With the rapid development of sequencing technologies, a large amount of biological information has been stored in the gene expression data. Gene chip, also known as DNA microarray, is one of the most important technologies in the field of life science researches[2-4]. In fact, based on the extensive application of gene chip technology, the network of public databases in the growing gene chip expression data can provide an enormous powerful tool for breast cancer gene expression analysis. At the same time, the abnormal expression of polygene is the crucial biological factor of the occurrence and progression of breast cancer. The analysis of differential expression genes and their interaction networks of breast cancer is of practical significance to study the pathogenesis of breast cancer in depth, guide the individual treatment and improve the prognosis of breast cancer patients.

Actually, one of the important tasks of gene chip profiling data analysis is to screen for differentially expressed genes. For example, by comparing the differences in gene transcription and expression between normal and disease states in the study of the pathogenesis of the disease, doctors can conduct early diagnosis and treatment of the disease, and even predict the prognosis for patients.

Currently, those methods suitable for different study design and data type gene expression profiles to screen differentially expressed genes for gene expression profiles include SAM (significance analysis

of microarrays)[5], two-sample t-test[6], and so on. However, false positive of the differential expression genes by using the SAM and two sample t-test are too high. Actually, earlier researchers have tried to select differentially expressed genes by Logistic Regression (LR)[7-9], or Random Forest (RF).[10-12]Although LR is one of the classical methods and has been widely used for classification, the traditional LR model employs all (or most) variables for predicting and screening the differentially expressed genes, so requires selecting many redundant genes. On the other hand, some researchers applied RF to classify genes from microarray data and did not pre-select genes but result in overfitting due to the availability of gigantic gene data and dimensionality.

To take account of different consequences of existing methods in identifying differentially expressed genes related to breast cancer, a new method, named LR-RF method, is proposed based on microarray data in this article. Firstly, we pre-select genes by LR and get a series of differentially expressed genes based on the Bonferroni test[13] of the Family Wise Error Rate (FWER)[14] error measure. Then, best-related genes of differentially expressed of breast cancer are identified by RF method. Finally, by analyzing gene interaction networks, the LR-RF method is found to have an excellent performance for identifying differentially expressed genes.

2 Materials and methods

2.1 Materials

In this paper, we have downloaded two sets of breast cancer datasets from Gene Expression Omnibus (GEO) [15].The accession numbers were GSE15852 and GSE45255, and the chip platform was GPL96. Dataset GSE15852 included 43 paired normal persons and breast cancer patients. Dataset GSE45255 consisted of 139 breast cancer patients. By integrating data, there were 182 breast cancer patients and 43 normal cases for subsequent analysis and each of them contains 22215 genes.

In general, each gene has a different expression level, and gene expression value doesn't have a unified norm due to differences in the experiments on the microarray data. Thus, we normalize the microarray data by MAPMINMAX function in the MATLAB. The MAPMINMAX processes matrices by normalizing the minimum and maximum values of each row to $[[y_{\min}, y_{\max}]$. The formula is in the following

$$y = (y_{\max} - y_{\min}) \times \frac{x - x_{\min}}{x_{\max} - x_{\min}} + y_{\min} \tag{1}$$

In this study, we set $y_{\min} = 0$, $y_{\max} = 1$ and standardize the data to the internal [0, 1].

2.2 Methods

In this section, the Bonferroni test of FWER measure is briefly reviewed firstly. Further, we introduce the typical LR model and RF model. Lastly, a novel method LR-RF is proposed to screen differentially expressed genes. For all of the microarray data, we pre-select genes by LR method to reduce the dataset dimensions and then use a RF classifier to identify cancer-causing genes.

2.2.1 Bonferroni test of FWER error measure

Regression analysis for a single hypothesis usually has a straightway interpretation of the testing

result, but this may become much complicated for multiple hypotheses in a regression model based analysis. While each test of the multiple hypotheses has its type I and type II errors, and then it becomes unclear how to measure the overall error rate. FWER, which is the probability of making one or more type I errors among all the hypotheses, is the first measure being suggested for dealing with this issue. In this paper, the Bonferroni test based on the FWER measure was used to screen the differentially expressed genes.

The Bonferroni inequality[16] is often used when conducting multiple tests of significance to set an upper bound on the overall significance level α. If $T_1,\cdots,T_n$ is a set of n statistics with corresponding p-values $P_1,\cdots,P_n$ for testing hypothesis $H_1,\cdots,H_n$, the classical Bonferroni multiple test procedure is usually performed by rejecting $H_0=\{H_1,\cdots,H_n\}$ if any p-values is less than α/n. Furthermore the specific hypothesis H_i is rejected for each $P_i \le \alpha/n$ ($i=1,\cdots,n$).The Bonferroni inequality is in the following.

$$\begin{aligned} FWER &= \Pr(V \ge 1) = \Pr\left(\bigcup_i \left\{H_i = 0, P_i < \frac{\alpha}{n}\right\}\right) \\ &\le \sum_{i=1}^{n} \Pr\left(P_i < \frac{\alpha}{n} \mid H_i = 0\right) \Pr(H_i = 0) \le n \cdot \frac{\alpha}{n} = \alpha \end{aligned} \tag{2}$$

It is to ensure that the probability of rejecting at least one hypothesis when all true is no greater than α.

2.2.2 Logistic Regression model

LR[17] models are the most widely used models in the generalized linear models family. A LR model is used when the response Y is a binary variable taking only two possible values, such as the binary classification between normal and tumor considered in this paper.

Because the dependent variable Y only takes two discrete values 0, 1, it is not suitable for the regression model as a dependent variable. The basic idea of LR is that it does not regress to Y directly, but rather defines a probability function:

$$\pi = \Pr(Y=1 \mid X_1 = x_1, X_2 = x_2, \cdots, X_i = x_i) \tag{4}$$

where there requires $0 \le \pi \le 1$. Directly seeking the expression of π is a very difficult, we consider

$$\frac{1-\pi}{\pi} = \frac{P(Y \ne 1)}{P(Y=1)} = k \ , \tag{5}$$

where $0 < k < +\infty$. Then, let

$$\pi = \Pr(Y=1 \mid X_1 = x_1, X_2 = x_2, \cdots, X_i = x_i) = \frac{1}{1 + a \cdot e^{-b_1 X_1 - \cdots - b_n X_i}} . \tag{6}$$

$$(a > 0, b_n \ge 0)$$

π is a Logistic type of function. Then, we deform it and get a new function as follow:

$$\lg\left(\frac{1-\pi}{\pi}\right) = b_0 - b_1 x_1 - \cdots - b_n x_i \ . \tag{7}$$

The form of the logistic function *lg* is

$$f(x) = \frac{e^x}{1+e^x} \tag{3}$$

where $f(x)$ is a continuous curve limited to the [0, 1] interval.

In this article, the regression is relatively column by column, so the probability equation is:

$$\pi = \Pr(Y = 1 \mid X_1 = x_i) = \frac{1}{1 + a \cdot e^{-b_1 X_1}} \ (a > 0, b_1 \geq 0) \quad , \tag{8}$$

where X_i is the *i-th* gene value information, and π is the probability of being sick.

2.2.3 Random Forest model

RF is an algorithm for classification developed by Leo Breiman [18] that uses an ensemble of classification trees. Each of the classification trees is built using a bootstrap sample of the data, and each split the candidate set of variables is a random subset of the variables. Thus, RF uses both bagging (bootstrap aggregation), a successful approach for combining unstable learners, and random variable selection for tree building. Each tree is unpruned (grown fully) to obtain low-bias trees. At the same time, bagging and random variable selection result in the low correlation of the individual trees. The algorithm yields an ensemble that can achieve both low bias and low variance.

2.2.4 Logistic Regression-Random Forest model

In this section, the LR-RF model is proposed. The model can be divided in two steps.

The first step: we pre-select genes by LR, based on the Bonferroni test of FWER error measure, and we get a series of differentially expressed genes roughly. The second step: we use the RF algorithm for the second screening and get the top potential genes related to breast cancer. As a matter of fact, RF can identify which genes were important in building a forest of trees and get the genes' importance score ranking to determine whether it is used in the model. So setting a threshold and determining the importance, we delete any genes with an importance below the threshold. Through the two steps, the differentially expressed genes in breast cancer can be obtained.

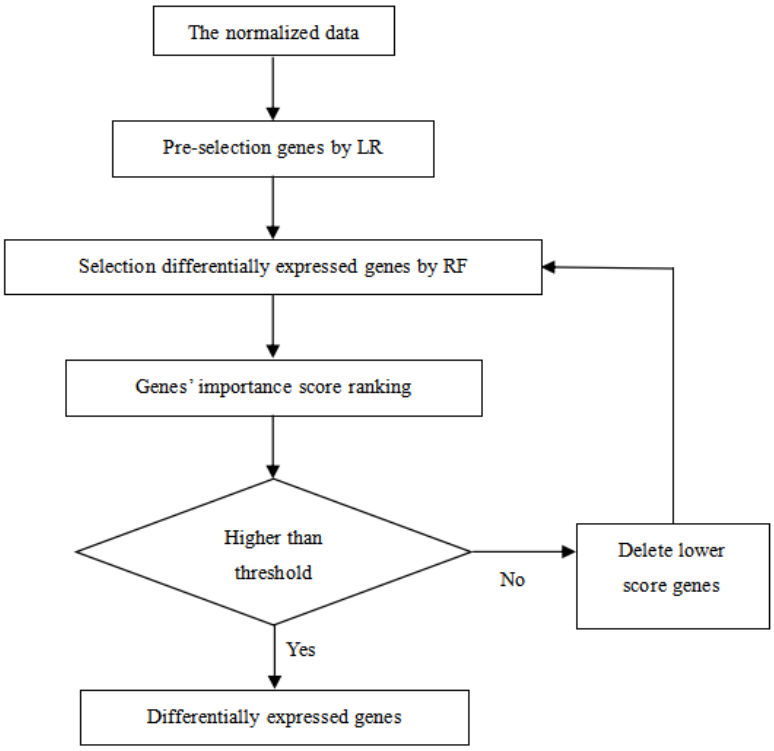

Fig.1. the process for LR-RF model

3 Results and analysis

3.1 Differentially expressed genes

For these three methods, we conducted 10 random tests and each test took 80% of the data as a training set, and the remaining 20% data as a testing set.

By screening, each method yielded a different number of differentially expressed genes, as showed in Table 1. In this study, we set 0.1 as the threshold when use the RF method to select genes.

Table 1. The number of differentially expressed genes selected by the three methods

Ten random tests / Method	1	2	3	4	5	6	7	8	9	10
LR	102	240	372	143	371	629	197	257	233	288
RF	22	33	19	24	27	14	17	25	23	20
LR-RF	102	188	158	133	143	107	174	142	163	137

The number of the differentially expressed genes selected by the three methods is listed in Table 1. A large number of genes selected by LR method are redundant genes. Although the RF method can screen out few differentially expressed genes, model may cause over fitting and then loss quite important genes due to the high dimensionality of the microarray data. In contrast, the LR-RF method can select important genes from the cancer-causing genes that have been pre-selected by the LR method, and guarantee the assurance of the veracity of identifying differential expression genes

according to RF model.

3.2 Stability analysis of the three methods

Using the differential expression genes screened to predict whether the testing set samples are breast cancer patients. In this paper, the Rand index[19] is applied to calculate the prediction accuracy rate.

a：The patient is predicted to be a patient.

b：The patient is predicted to be normal.

c：The normal person is predicted to be a patient.

d：The normal person is predicted to be normal.

$$\text{The prediction accuracy}=\frac{a+d}{a+b+c+d}.$$

Through validating and comparing the models, we can get the prediction accuracy rate of the three methods and evaluate the stability of the method by using the variance of accuracy. The smaller the variance is, the more stable the method is.

Table 2. The prediction accuracy rate and stability of the three methods

Ten random tests / Method	1	2	3	4	5	6	7	8	9	10	average	variance
LR	90.2	91.9	89.2	93.0	96.3	85.4	96.2	96.0	93.3	92.2	92.37	0.0011
RF	84.4	82.2	91.1	88.9	88.9	82.2	91.1	86.7	84.4	80.0	85.99	0.0014
LR-RF	95.6	93.3	91.1	91.1	93.3	95.6	93.3	93.3	95.6	88.9	93.11	0.00045

(**Note:** the unit of 2 to 12 columns is %.)

Seen from Table 2, the average prediction accuracy rate of the LR-RF method is 93.11%, which is higher than LR and RF methods. The variance of the LR-RF method is 0.00045, and the variances of the LR and RF are 0.0011, 0.0014, respectively. LR-RF method's variance is smaller than the other methods' variances. Obviously, comparing with the other two methods, the method LR-RF we proposed is more stable on the premise that selecting differentially expressed genes effectively.

Remark: the average prediction accuracy rate of the RF method is 85.99%, it is because the differentially expressed genes selected by the RF method are too few including true disease-related genes have been deleted, and ones selected by the LR method too many genes. The average prediction accuracy rate doesn't vary much between LR method (92.37%) and LR-RF method (93.11%), but the number of the genes selected by the LR-RF method is smaller than the LR method. Compared with the LR method, the differentially expressed genes system built by the LR-RF is simpler.

3.3 Hierarchical clustering analysis

We took the union of the differentially expressed genes selected by LR-RF methods ten times, and the clustering analysis was performed by using R software. The hierarchical clustering chart was as follows:

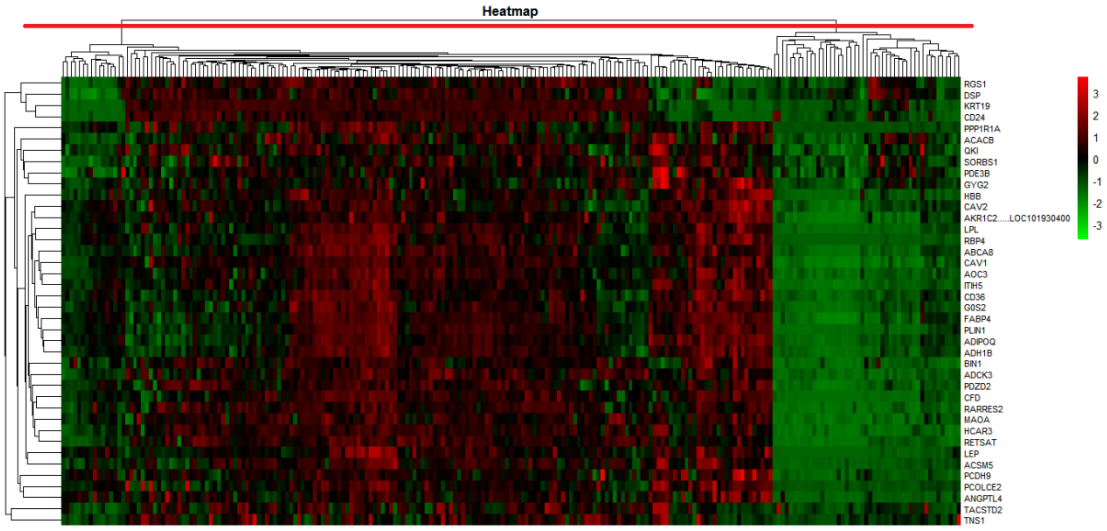

Fig.2. The heat map of cluster analysis of differentially expressed genes of 139 breast cancer patients and 43 normal specimens.

The 225 samples are clustered into two groups using differentially expressed genes selected by the LR-RF method. The 139 samples ahead of the figure are breast cancer patients and the remaining samples are 43 normal specimens. It can be seen that a significant difference between the two groups in Fig.2.This indicates these genes can distinguish the normal samples from and patient ones.

3.4 The analysis of genes interaction networks

Top 20 differentially expressed genes related to breast cancer selected by our method is listed in Table3. The most exciting results from the table, majority of genes had been described in accumulating papers of breast cancer.

Table 3. The top 20 differentially expressed genes in breast cancer

No.	Gene ID	Gene Symbol	Average Importance	No.	Gene ID	Gene Symbol	Average Importance
1	201650	KRT19	1.155279813	11	218168	ADCK3	0.399596245
2	209493	PDZD2	0.641707676	12	216379	CD24	0.383872561
3	209763	CHRDL1	0.57484195	13	215695	GYG2	0.37417914
4	206488	CD36	0.567017651	14	214439	BIN1	0.374115711
5	211696	HBB	0.53992312	15	43427	ACACB	0.360580478
6	207092	LEP	0.489604283	16	218723	RGCC	0.34986191
7	205478	PPP1R1A	0.471361386	17	210201	BIN1	0.342833509
8	203548	LPL	0.436124485	18	219140	RBP4	0.332710044
9	203853	GAB2	0.420885256	19	221009	ANGPTL4	0.31555432
10	209699	AKR1C2	0.408219183	20	204894	AOC3	0.297631023

Previous research has identified a highly deregulated gene Keratin 19(KRT19) in breast cancer. Furthermore, KRT19 expression was associated with breast tumor subtyping, among estrogen receptor(ER) and Luminal B, and KRT19 expression correlated with poor overall survival[20-24]. PDZ domain containing 2(PDZD2) does not possess an intrinsic enzymatic activity through a number of

direct and indirect interactions with breast tumor suppressors. It inhibits the activities of P and PDZ proteins, or enhances the activity of telomerase.[25] Other studies suggest that the CD36 gene[26] is located on the chromosome 7q11.2, and its encoded protein CD36 molecule is a transmembrane glycoprotein expressed on the surface of platelets and a variety of tumor cells. Seewaldt *et al*[27] found that inhibition of CD36 gene expression in normal breast cells can lead to a decrease in adipocyte surrounding cells and an increase in extracellular matrix collagen deposition, which is a key factor in increased mammalian gland density, and the lack of CD36 gene infection may be an important event in the early development of breast cancer.

To further demonstrate the predictive ability of LR-RF, we annotated the differentially expressed genes screened by LR-RF method to Gene MANIA database and found that most of these genes were significantly enriched in pathways related to the breast cancer, such as adipocytokine signaling pathway, neurotrophin signaling pathway. This demonstrates these genes play important roles in the cancers.

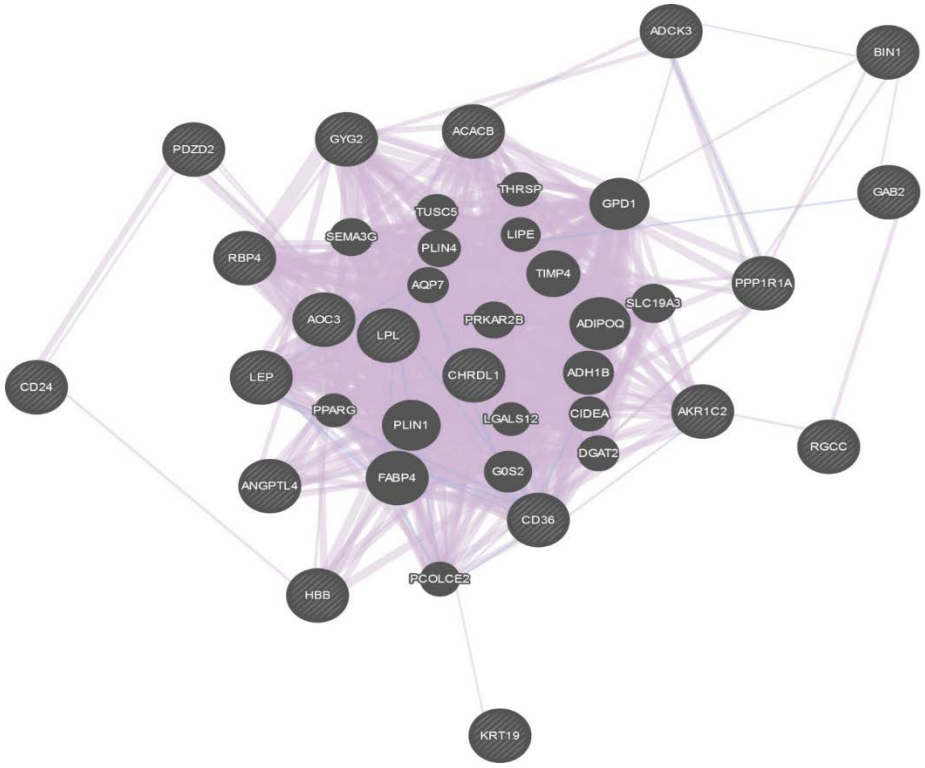

Fig.3. Network of the differentially expressed genes constructed by Gene MANIA

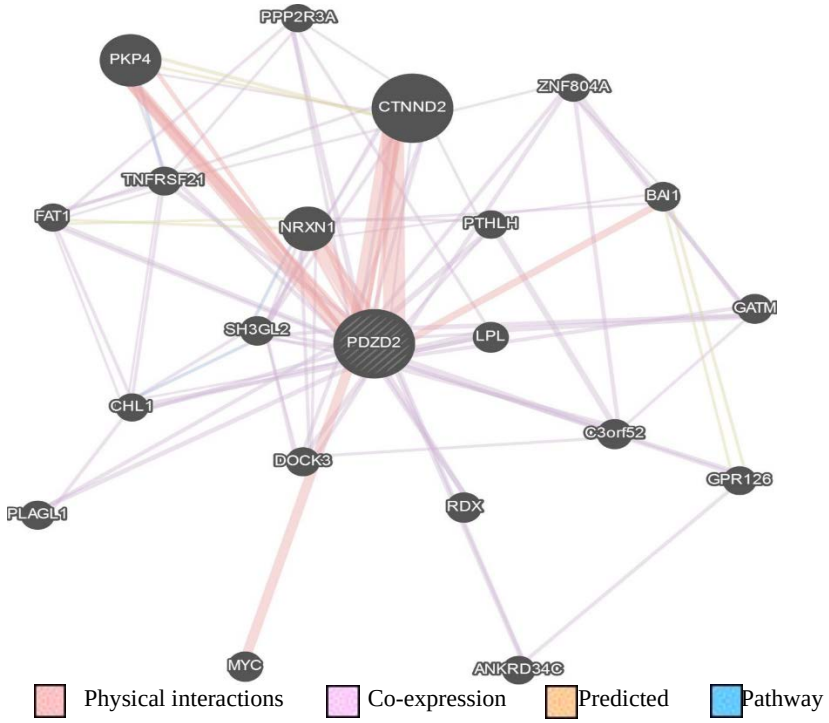

Fig.4. Network of the PDZD2 gene constructed by Gene MANIA

In Fig.4, the differentially expressed genes are involved in many known pathways and harbor many physical interactions. From the two figures we can see, these differentially expressed genes are densely connected which several, such as PDZD2, LPL and CD36 have been confirmed to be closely related to breast cancer. Fig.4 also shows that the interaction network of PDZD2 gene with other genes can be seen clearly.

4 Conclusions

It is well known that many cancer-causing genes of breast cancer are still unclear. However it is crucial to select the differentially expressed genes by bioinformatics methods from the availability of huge DNA microarray data. We have found candidate genes related to breast cancer based on microarray data and proposed a method for screening differentially expressed genes of breast cancer by combining LR and RF as a machine-learning technique. From 22215 genes of breast cancer, we pre-select a series of differentially expressed genes, and then screen breast cancer genes again. LR and RF have been trained before and after screening differentially expressed genes. The LR-RF method greatly improves not only the accuracy of the screening of cancer-causing genes but also the speed.

Via replicating a random experiment ten times, two microarray datasets related to breast cancer have been used to measure the stability of methods by using variance. The analyses show that the proposed mixed model can produce almost similar pattern of results for all considered selections, and select differentially expressed genes successfully.

Although some genes including in the results have not been identified, LR-RF is shown to have the potential to screen differentially expressed genes related to breast cancer efficiently in a short time. It is anticipated that LR-RF would provide new knowledge and method for biologists, medical scientists, and cognitive computing researchers to identify disease-related genes of breast cancer.

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