

STAT 432 Final Project

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Project description and summary

The World Health Organization's International Agency for Research on Cancer (IARC) released the latest global cancer data, showing that in terms of cancer distribution types, the number of new cases of breast cancer in 2020 will reach 2.26 million, surpassing lung cancer (2.2 million) for the first time, becoming "the world's number one cancer". Thus, in this project, we are going to use what we learned in this semester to analyze a breast cancer data.

In this report, we are going to analyze the data BRCA Multi-Omics (TCGA). This data can be found here. In this report, we are going to predict four variables `PR.Status`, `ER.Status`, `HER2.Final.Status` and `histological.type`. Since, we have a raw data. Thus, we need to clean the data first. First, we are going to remove all missing data. Since, most of them are not continuous data. then we will check the collinearity between each variables. Next, we must check the distribution for each response variable and decided to modified them or not. After dealing with data, we have to find the model can help us to predict the result. We will use SVM and random forest to predict `PR.Status`, and use logistic regression and K-means clustering to predict `histological.type`. Since, we are dealing with a high dimensional data, thus some model may not be able have a good performance such as K-means clustering. Hence, we change the model to Classification Tree which has better performance on high dimensional data. Next steps will be using model to train and predict result with selected 50 variables. In this section, we use random forest as our model.

Literature Review

Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes

A rigorous examination of 817 breast tumor samples confirms invasive lobular carcinoma as a molecularly separate illness with discrete genetic traits, offering critical information for patient classification that may allow for better informed clinical follow-up.

We concluded from this paper that three ILC transcriptional subtypes linked with survival differences were identified using proliferation and immune-related markers. Cases with mixed IDC/ILC were molecularly classed as ILC-like or IDC-like, with no actual hybrid traits revealed. This multimodal molecular atlas throws fresh insight on the genetic foundations of ILC and suggests potential therapeutic treatments. This research reveals several genetic mutations that distinguish ILC from IDC, proving at the molecular level that ILC is an unique breast cancer subtype and giving new insights into ILC tumor biology and treatment alternatives.

Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer

Using microarray and quantitative reverse transcriptase polymerase chain reaction data from 189 prototype samples, a 50-gene subtype predictor was constructed. Prognosis was determined using test sets from 761 individuals, and pathologic complete response (pCR) after a taxane plus anthracycline regimen was determined using test sets from 133 patients.

We noticed that this part of Sample Subtype Prediction is particularly valuable for our project. The reliability of categorization across three centroid-based prediction approaches was tested for the 50 gene set: Prediction Analysis of Microarray, a basic nearest centroid, and Classification of Nearest Centroid. The subtype categorization is always determined based on the closest of the five centroids. The final approach comprised of centroids built as stated for the PAM algorithm and distances calculated using Spearman's rank correlation due to its reliability in subtype categorization.

Tumor characteristics and patient outcomes are similar between invasive lobular and mixed invasive ductal/lobular breast cancers but differ from pure invasive ductal breast cancers

Our first relevant paper has a total of 4,336 individuals with IDC, ILC, and mixed breast tumors were detected between 1996 and 2006. The Kaplan-Meier method was used extensively in this paper, and survival curves were constructed using it. Chi-square tests and Fisher's exact tests were used to compare clinical variables. The correlations between patient and tumor variables were summarized using contingency tables and investigated using Fisher's exact test as among three histologic groups. Patients with ILC and mixed breast

cancers were more probable as IDC patients to have tumors that were estrogen receptor and progesterone receptor positive ($P < 0.001$ and $P < 0.05$, correspondingly).

After having read, we can conclude the following from the paper: first, despite being identified at lower clinical stages of infection, patients with IDC had the poorest long-term survival; second, individuals with ILC and “mixed” malignancies had a better prognosis than patients with IDC, despite having more advanced cancer. We were also motivated to utilize the log-rank test to estimate P values if necessary.

Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome

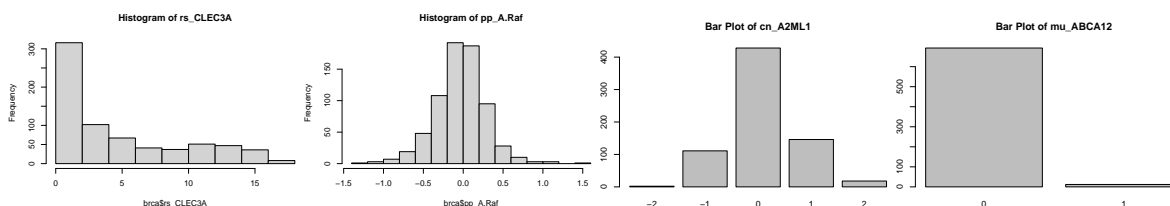
From the second paper, we summarize that these patients do not have improved clinical outcomes as IDC patients when ignoring the fact that ILC has a positive biologic pattern. Consequently, management decisions should be made based on the patient’s and tumor’s biologic characteristics, that instead of lobular histology. About statistical methods, the clinical and biologic features of lobular and ductal carcinoma were compared by contingency tables, Chi-square tests and Fisher’s exact tests, which is similar with the method using in the first paper. To see if ILC was an independent predictive predictor for recurrence and death, researchers used multivariate analysis and Cox regression models. Tumor size, number of affected nodes, age, ER status, PgR status, DNA ploidy, S-phase, and histologic type were all considered in these analyses.

The findings of this huge dataset have shown that ILC and IDC are distinct entities with distinctive clinical histories and biologic features, yet there are no clinically important variations in survival. At the present, both kinds of breast cancer should be treated identically, and histologic subtype (lobular or ductal) should not be regarded a determinant in therapeutic decision-making or an essential prognostic or predictive factor at diagnosis. Emerging technologies such as high throughput genome mapping and microchip cDNA expression arrays may help to uncover molecular distinctions between these different types of breast cancer.

Summary Statistics and Data Preprocessing

Data Overview

The dataset has 705 observations and 1941 features (1936 predictors and 5 outcomes). There are four different kinds of predictors: **rs** (gene expression), **cn** (copy number variations), **mu** (mutations), and **pp** (protein levels). Among them, **rs** and **pp** are continuous variables, and **cn** and **mu** are categorical variables.



Remove Missing Values

According to the instruction, we dropped `vital.status`, and we only considered each response variable as a binary variable. Therefore, we treated the observations that had other outcomes as missing values and removed them from our dataset.

Then the dataset `sub` had 507 observations and 1940 features.

```
dim(sub)
```

```
## [1] 507 1940
```

Deal with Multicollinearity

One of the noticeable characteristics of the data is its high dimensionality. There are 1936 predictors, almost four times as many as there are observations. Therefore, it is essential to check correlation.

Since there are four kinds of predictors, it is unlikely that two variables coming from different kinds would be highly correlated. Also, to reduce the computational cost, we split the data into four subsets: **rs**, **cn**, **mu**, and **pp**, each of which contained only one kind of predictors.

Then, we created the correlation matrix for each subset, and extracted variables that are highly-correlated with at least one other variable. Take **rs** as an example. The dataframe **idx** stores all matrix indices of highly-correlated variables and the corresponding correlation coefficients. If the *i*-th variable is highly-correlated with the *j*-th variable, then we only need one of them. Thus, we removed all variables with indices *i*. For **rs**, 94 predictors were removed. We applied the same process to the other three subsets. In total, 882 predictors were removed. There are 1059 predictors remained.

```
names(idx) = c("i", "j", "corr")
idx[1:3,]

##   i  j corr
## 2 3  4 0.94
## 3 5 56 0.84
## 4 9 10 0.95

# remove highly-correlated variables
rmv = unique(idx[,1])
length(rmv)

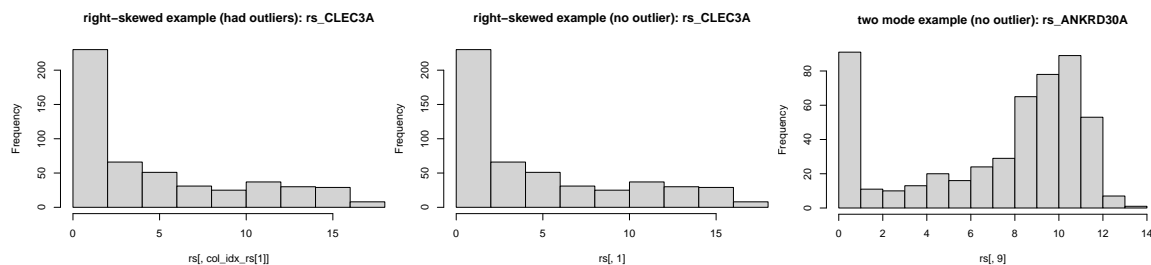
## [1] 94

rs = rs[, -rmv]
```

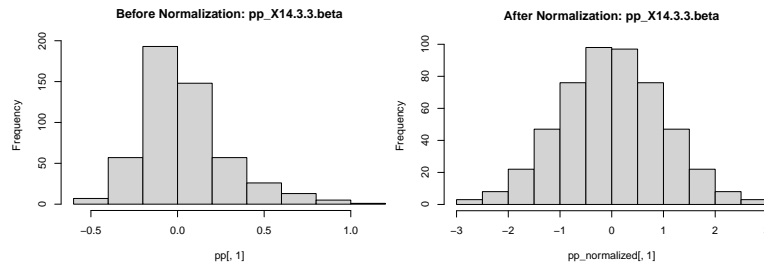
Continuous Variables

As mentioned before, **rs** and **pp** are continuous variables, so we should examine if there are any outliers. We first normalized the variable, and stored row and column indices if the data point was three standard deviations away from the mean. For the two subsets, **rs** had 100 outliers, and **pp** had no outlier.

We further looked into **rs** predictors that included outliers, and we found the vast majority of them had a long tail, mostly right and some left. In addition, a number of **rs** predictors that did not contain outliers also had a non-standard distribution. As a result, a log transformation of **rs** predictors would be beneficial.



Unlike **rs**, **pp** variables were distributed quite normally. However, many of the variables would contain outliers without normalization. Therefore, we normalized **pp** variables.



Categorical Variables

All four outcomes were more or less imbalanced, among which `histological.type` was the most imbalanced. Only 10% of the responses were ILC. In some cases, such imbalance would be problematic since models would learn nothing from the minority class. If our data also suffer from problems like this, we should resolve the imbalance by techniques like undersampling. However, if our models perform well enough on current data, no further action needs to be taken.

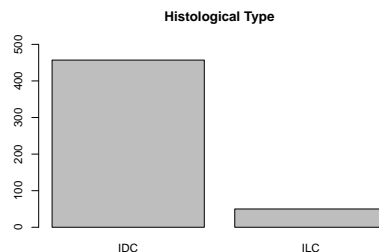
Fortunately, we trained our models first, and found the models obtained high enough accuracy and AUC scores. Therefore, we decided to not address imbalanced outcomes.

```
##
## Negative Positive
## 0.34714 0.65286

##
## Negative Positive
## 0.2445759 0.7554241

##
## infiltrating ductal carcinoma infiltrating lobular carcinoma
## 0.90138067 0.09861933

##
## Negative Positive
## 0.8382643 0.1617357
```



Modeling PR Status

Before modeling, we split the train and test datasets. We used 25% of the samples (126) for testing and 75% of the samples (381) for training.

Support Vector Machine (SVM)

The goal of the project was to make classifications. Plus, we needed to alleviate “the curse of dimensionality”. Therefore, we should choose classification models that perform well on high-dimensional data. Support vector

machines are famous for its capability in high-dimensional spaces, so we first fitted a basic linear SVM, with the default `cost = 1` to see how it worked.

As the confusion matrix showed, the in-sample accuracy was 1.0, which implied that we might prefer the linear kernel to the radial kernel.

```
##          actual
## predicted Negative Positive
## Negative      133         0
## Positive       0        248
```

Then we constructed two grids of tuning parameters for both linear and radial kernels, and we used 5-fold cross-validation to tune the parameters and to determine which kernel was better.

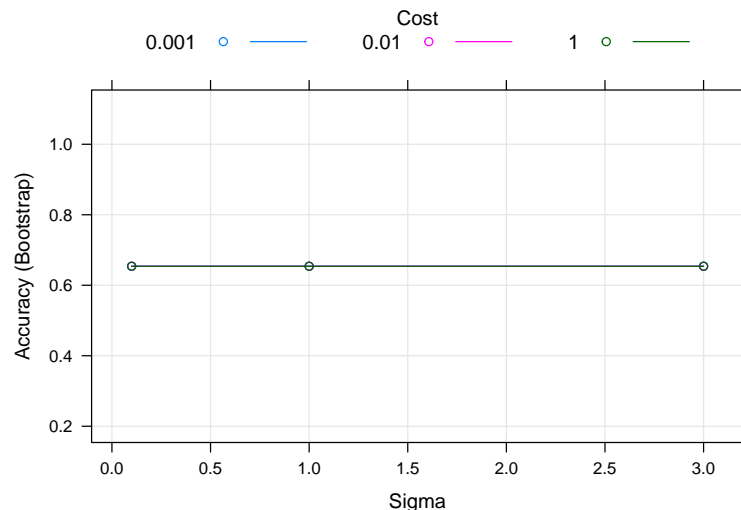
For the linear kernel, the best `C` was 0.001 with an in-sample accuracy of 0.8324.

```
##          C
## 1 0.001

##          C Accuracy      Kappa AccuracySD      KappaSD
## 1 0.001 0.8336029 0.6058770 0.02730027 0.06541415
## 2 0.010 0.7999388 0.5464954 0.02526173 0.06257166
## 3 0.050 0.8014261 0.5503720 0.02442031 0.06150767
## 4 0.100 0.8014261 0.5503720 0.02442031 0.06150767
## 5 0.500 0.8014261 0.5503720 0.02442031 0.06150767
## 6 1.000 0.8014261 0.5503720 0.02442031 0.06150767
```

For the radial kernel, the best `C` was 0.001 and the best `sigma` was 3. However, the figure showed that the radial SVM fitted poorly, since the accuracies remained the same and were merely 0.6677. The fact verified our hypothesis that a linear kernel would work better. Thus, we picked the linear SVM with `C` equals 0.001 to make classifications.

```
## sigma C
## 3      3 0.001
```



We made predictions for the test data, and printed the confusion table below. The accuracy was 0.9048. Thus, the linear SVM performed quite well.

```
##          actual
## predicted Negative Positive
## Negative      33         2
## Positive      10        81
```

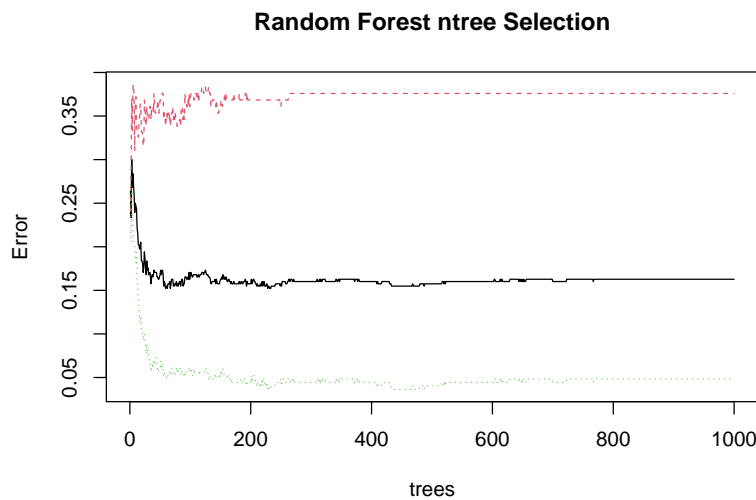
```
## [1] 0.9047619
```

Random Forest

Random forests are another classification model that is less vulnerable to “the curse of dimensionality”. Since there were many parameters that needed to be tuned, we again utilized `caret` package to cross validate the model.

Before applying cross-validation, we should first determine `num.trees`, because it could not be included in the grid of parameters. Therefore, we first fitted a random forest using the `randomForest()` method with `ntree = 1000`, and plotted the error against trees.

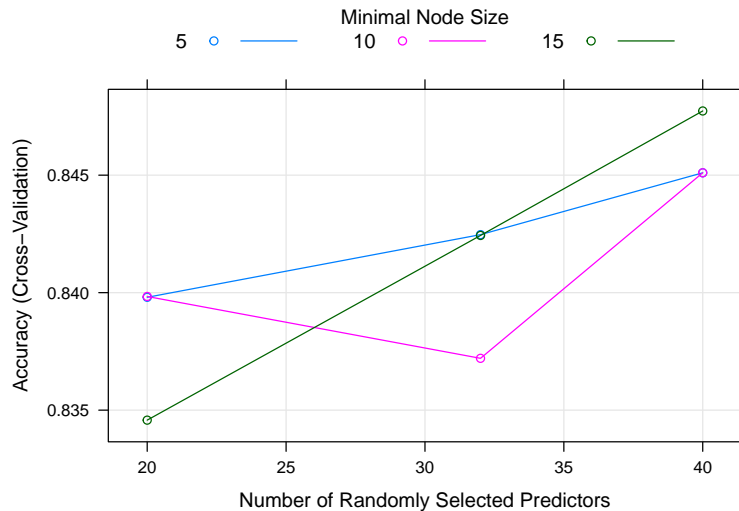
As the plot demonstrated, the error stopped decreasing after the number of trees reached around 500. Thus, `ntree = 500` should be sufficient for training.



In a 5-fold cross-validation, we tuned `mtry` and `min.node.size`. The output showed that the best parameters were `mtry = 40` and `min.node.size = 15` when `num.trees = 500` according to the test accuracy.

```
##   mtry splitrule min.node.size
## 9   40      gini             15

##   mtry splitrule min.node.size Accuracy      Kappa AccuracySD      KappaSD
## 1    20      gini             5 0.8398027 0.6199996 0.01816714 0.04744967
## 2    20      gini            10 0.8398378 0.6191721 0.01181501 0.02631224
## 3    20      gini            15 0.8345746 0.6097325 0.01575637 0.03413845
## 4    32      gini             5 0.8424694 0.6272972 0.01375275 0.03444365
## 5    32      gini            10 0.8372062 0.6150392 0.01262152 0.02909492
## 6    32      gini            15 0.8424343 0.6268844 0.01948896 0.05058240
## 7    40      gini             5 0.8451009 0.6341821 0.01487847 0.03698468
## 8    40      gini            10 0.8451009 0.6356210 0.02177987 0.05139481
## 9    40      gini            15 0.8477325 0.6408985 0.01796807 0.04555969
```



The confusion matrix and the highest test accuracy, 0.9048, were shown here.

```
##          actual
## predicted Negative Positive
## Negative      33      2
## Positive      10     81
## [1] 0.9047619
```

Modeling Histological Type

To establish the Modeling for histological Type, We observe that the response variable **Histological Type** has only two levels “infiltrating lobular carcinoma” and “infiltrating ductal carcinoma”. Thus, Logistic model become a good choice. To Peform a logistic Model, we first split the data into train and test data. Test data will be random selected 25% of the sample size.

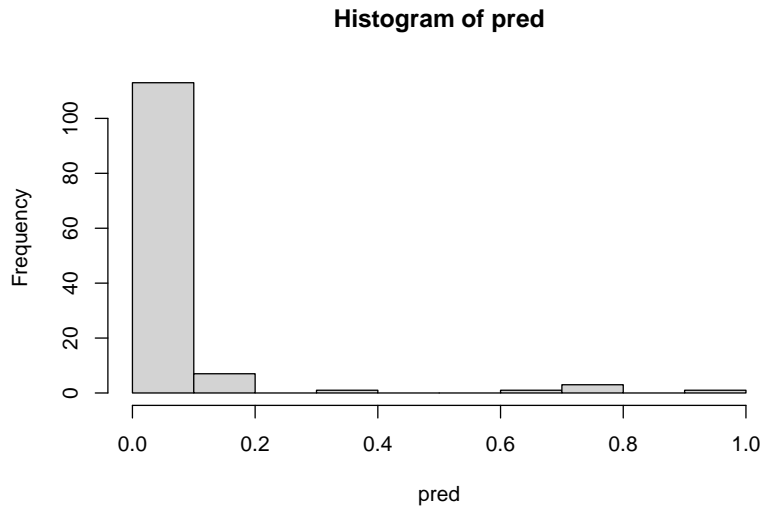
Logistic Regression

Once, we attempted to fit logistic model with our data, the algorithm throw a warning said “algorithm did not converge”. In other words, we are experience a perfect sepration. As the confusion matrix shows below, our model has 100% accuracy and AUC with 1.

```
## Warning: glm.fit: algorithm did not converge
## AUC = 1
##
##          0    1
## FALSE 457    0
## TRUE   0    50
```

Logistic Regression with ridge penalty and cross validation.

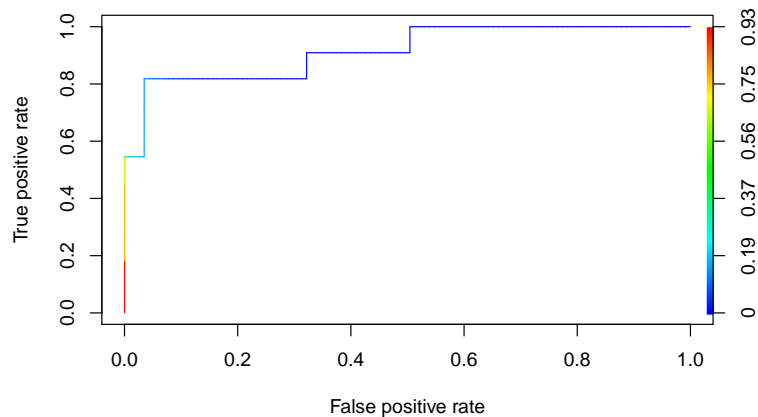
The above model is obviously not correct. Thus, we decided to use penalized regression and cross validation. Thus, we are going to perform a logistic model with ridge penalty and 10-fold cross validation. Since, the Logistic Regression Model will not return value with 0 and 1 Thus, we decided to choose the cut-off value based on the distribution of the predict result. Thus, we plot the histogram of the prediction result.



Based on the graph shows above, we decided to choose cut-off value as 0.5. The the prediction result will give 121 FALSE and 5 TRUE.

```
##
## FALSE  TRUE
##   121    5
```

Then, we are going to plot the ROC and calculate AUC.



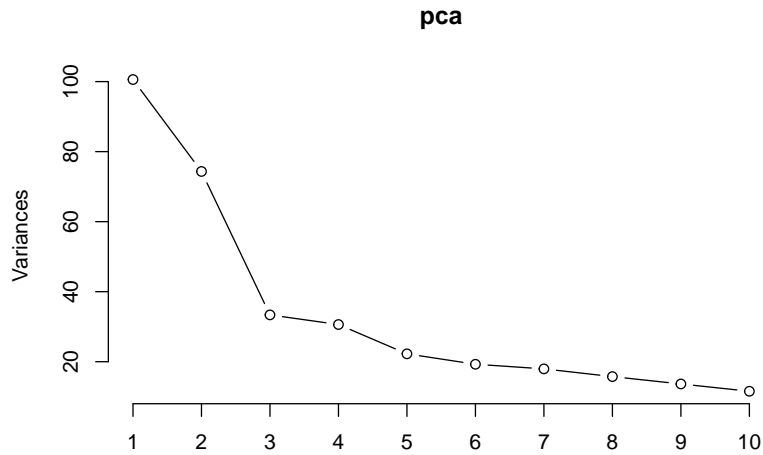
```
## AUC = 0.915415
```

```
##      ytest
##      0    1
## FALSE 115  6
##  TRUE   0  5
```

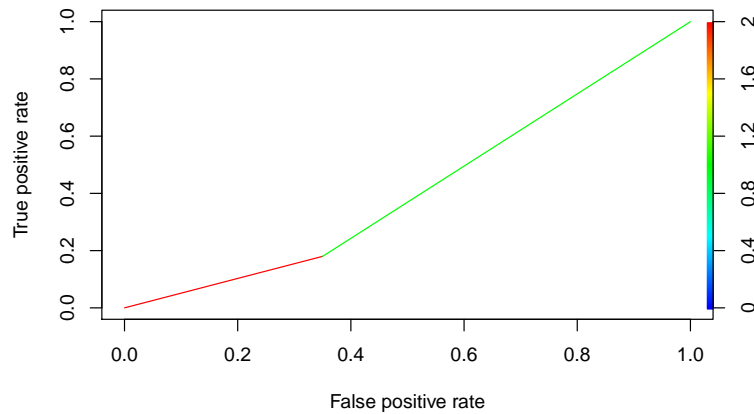
Based on the ROC and output given above, AUC is 0.915415, which is a good result

K mean Clustering with Principal Components Analysis

We decided to using kmean clustering as our second model. Since, we known that Kmean clustering does not have a good performace on high demision data .Thus, we decided to use dimensionality reduction technique such as principal components analysis.



To be able get a better result we decided to scale the data, thus, there will be no variable dominate. Then we have to choose the number of components which can represent our data. By using the rule of thumbs called “elbow” method. Based one the above graph, we are going to retain the first three components.

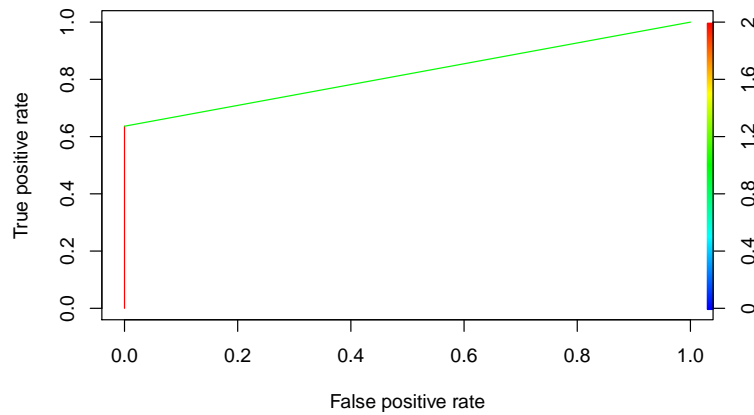


```
## [1] 0.4149453
```

However, the result of the kmeans did not gives us a good result. The value of AUC is 0.4149453. This is not a good result. Therefore, we believe that k means is not suitable for analyzing and predicting this set of data. Then, we are looking for other method. Next, we are going to perform the method called Classification Tree.

Classification Tree

The reason that we choose Classification Tree is becasue it has high accuracy on high-dimensional data. Therefore, we will choose 10-fold Classification Tree as our next model. Thus, we will set the parameters `control` as `rpart.control(xval = 10)`.



```
## [1] 0.8181818
```

Based on the result given above. The ROC seems good an the AUC is 0.8181818 which is a good result.

Variable Selection for All Outcomes

We used both literature and random forest to determine the 50 variables.

Literature

Based on “Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer”, we selected 7 variables: pp_PTEN, mu_PTEN, mu_TBX3, cn_FOXA1, mu_FOXA1, rs_FOXA1, and pp_E.Cadherin.

Fit Random Forest to Select Variables

Then, we fitted Random Forest using `randomForest()` to select the other 43 important variables and make predictions with the total 50 variables of all outcomes.

First, we fitted a random forest with best parameters chosen by cross-validation previously. Then we sorted the importances of variables and picked the most important 43 variables. Combined with the 7 variables from the paper, we constructed our input matrix.

Generate Fold ID

Using the provided code, we generate the fold ID for all 705 observations. Since we removed null values in data preprocessing, we would use only the first 507 IDs.

Predict All Four Outcomes

To perform 3-fold cross-validation, we wrote a for loop. For each $ID \in \{1, 2, 3\}$, we created test indices and split train and test datasets. We stored the three AUC scores in a vector and took its mean after the for loop ended. Below is an example for `ER.Status`. The same process was applied for the other three outcomes.

```
#fit for `ER.Status`
AUC = c()
set.seed(651978735)
for (i in 1:3) {
```

```

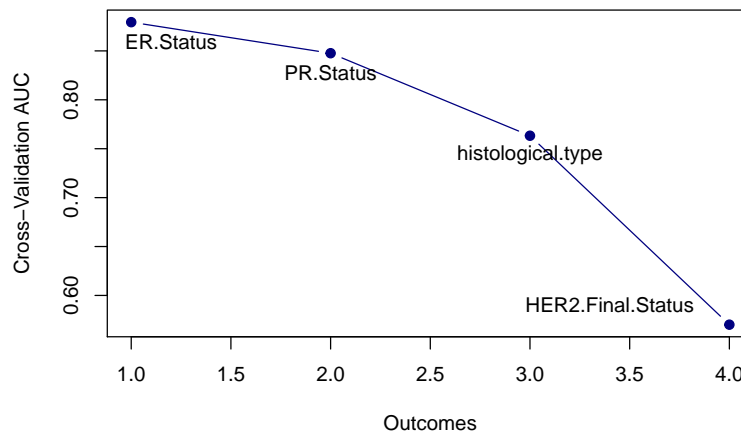
tst_idx = which(fold_id == i)
Xtrain = sub4[-tst_idx, 1:50]
Xtest = sub4[tst_idx, 1:50]
ytrain = sub4$ER.Status[-tst_idx]
ytest = sub4$ER.Status[tst_idx]
rf.fit = randomForest(Xtrain, ytrain,
                      ntree=500,
                      mtry=40,
                      nodesize=15,
                      samplesize=400,
                      importance=TRUE)

pred = predict(rf.fit, Xtest)
roc = prediction(as.numeric(pred), ytest)
auc = performance(roc, measure = "auc")@y.values[[1]]
AUC = c(AUC, auc)
}
mean_1=mean(AUC)
mean_1

```

```
## [1] 0.8793647
```

As shown in the graph below. The cross-validation AUC of ER.Status, PR.Status, histological.type were satisfactory. However, CV AUC of HER2.Final.Status was the lowest (0.5584). Since we did not model for HER2.Final.Status in previous prediction, it is possible that the model did not work well on the outcome.



```

# average the cross-validated AUC of all four outcomes
mean(c(mean_1,mean_2,mean_3,mean_4))

```

```
## [1] 0.7650967
```

At last, we took the average of the four cross-validation AUC. The final AUC value we obtained was `mean(c(mean_1,mean_2,mean_3,mean_4))`. Based on our experience and conclusion drawn from relevant papers and analysis of our upper results, a small set of biomarkers could predict four outcomes well, but the performance was not as good as the prediction with all variables.