EGcode

2025-04-18

load library

1. load dataset

```
hksm <- read.csv("EG_HKSM_20E_col_03_11.csv")
e20 <- read.csv("EG_20E_col_03_11.csv")
con <- read.csv("EG_Con_col_03_11.csv")
hksm <- hksm %>% select(-Treatment)
```

2. merge data

3. Check for Common Enhancers

```
enhancer_con <- unique(con$Enhancer)
enhancer_e20 <- unique(e20$Enhancer)
enhancer_hksm <- unique(hksm$Enhancer)
common_enhancers <- Reduce(intersect, list(enhancer_con, enhancer_e20, enhancer_hksm))
length(common_enhancers)
```

```
## [1] 0
```

```
head(common_enhancers)
```

```
## character(0)
```

4. Build Machine Learning Model

4.1 Preprocess Data

```
# Check new_act_score distribution quantiles
summary(cdata$new_act_score)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   314.9   582.2   800.0   993.5  1228.1  4756.9
```

```
# Use upper quartile as threshold
threshold <- quantile(cdata$new_act_score, 0.75, na.rm = TRUE)
cdata <- cdata %>%
  filter(!is.na(new_act_score)) %>%
  mutate(new_act_binary = as.factor(ifelse(new_act_score > threshold, "High", "Low")))

# Select features
features <- setdiff(names(cdata), c("Enhancer", "Genes", "new_act_score", "new_act_binary"))
X <- cdata %>% select(all_of(features))
y <- cdata$new_act_binary
```

```r
# Impute missing values
numeric_cols <- features[sapply(X, is.numeric)]
for (col in numeric_cols) {
  if (any(is.na(X[[col]]))) {
    X[[col]][is.na(X[[col]])] <- median(X[[col]], na.rm = TRUE)
  }
}
X$treatment <- as.factor(ifelse(is.na(X$treatment), "Unknown", X$treatment))
```

4.2 Split Data

```r
set.seed(42)
trainIndex <- createDataPartition(y, p = 0.8, list = FALSE)
X_train <- X[trainIndex, ]
X_test <- X[-trainIndex, ]
y_train <- y[trainIndex]
y_test <- y[-trainIndex]
```

4.3 Train Logistic Regression Model

```r
log_model <- train(
  x = X_train,
  y = y_train,
  method = "glm",
  family = "binomial",
  trControl = trainControl(method = "cv", number = 5)
)

# Predict and evaluate
log_pred <- predict(log_model, X_test)
log_cm <- confusionMatrix(log_pred, y_test)
print(log_cm)
```

```
## Confusion Matrix and Statistics
##
##           Reference
## Prediction High  Low
##       High   12    8
##       Low   378 1164
##
##                Accuracy : 0.7529
##                  95% CI : (0.7307, 0.7741)
##     No Information Rate : 0.7503
##     P-Value [Acc > NIR] : 0.4208
##
##                   Kappa : 0.035
##
##  Mcnemar's Test P-Value : <2e-16
##
##             Sensitivity : 0.030769
##             Specificity : 0.993174
##          Pos Pred Value : 0.600000
##          Neg Pred Value : 0.754864
```

```
##              Prevalence : 0.249680
##          Detection Rate : 0.007682
##    Detection Prevalence : 0.012804
##       Balanced Accuracy : 0.511972
##
##        'Positive' Class : High
##
```

The confusion matrix of the logistic regression model showed that its accuracy on the test set was 75.29% (95% CI: 73.07%-77.41%). However, the model performed very poorly in predicting the High class (highly active enhancers), correctly predicting only 12/390 High class samples (sensitivity: 3.08%), while the specificity of the Low class was as high as 99.32% (1164/1172). The Kappa value was 0.035, indicating that the model's predictive ability was very limited compared to random guessing.

4.4 Train Random Forest Model

```
rf_model <- train(
  x = X_train,
  y = y_train,
  method = "rf",
  trControl = trainControl(method = "cv", number = 5),
  tuneGrid = expand.grid(mtry = c(2, 4, 6))
)

# Predict and evaluate
rf_pred <- predict(rf_model, X_test)
rf_cm <- confusionMatrix(rf_pred, y_test)
print(rf_cm)
```

```
## Confusion Matrix and Statistics
##
##           Reference
## Prediction High  Low
##       High   24    8
##       Low   366 1164
##
##                Accuracy : 0.7606
##                  95% CI : (0.7386, 0.7815)
##     No Information Rate : 0.7503
##     P-Value [Acc > NIR] : 0.1827
##
##                   Kappa : 0.0789
##
##  Mcnemar's Test P-Value : <2e-16
##
##             Sensitivity : 0.06154
##             Specificity : 0.99317
##          Pos Pred Value : 0.75000
##          Neg Pred Value : 0.76078
##              Prevalence : 0.24968
##          Detection Rate : 0.01536
##    Detection Prevalence : 0.02049
##       Balanced Accuracy : 0.52736
##
```

```
##        'Positive' Class : High
##
```

```
# Feature importance
varImp(rf_model)
```

```
## rf variable importance
##
##   only 20 most important variables shown (out of 23)
##
##                 Overall
## TBS             100.000
## slp2_forkhead    39.764
## treatment        39.080
## srp_SANGER       38.328
## xrp1             33.613
## bergman_EcR_usp  29.066
## kay_Jra          26.914
## Trl              23.001
## XBP1             20.797
## CF2              18.833
## ERR              16.971
## crp              15.950
## GATA_elemento    14.618
## Rel_FFS          14.361
## h                10.536
## da                9.187
## Tgo               8.880
## bergman_Rel       8.686
## gcm               7.675
## Hnf4              2.802
```

The performance of the random forest model was slightly better than that of the logistic regression, with an accuracy of 76.06% (95% CI: 73.86%-78.15%) and a Kappa value of 0.0789, showing slightly better consistency. The confusion matrix showed that the model improved in the prediction of the High class, correctly predicting 24/390 High class samples (sensitivity: 6.15%), but still missed a large number of High class samples (366/390). The specificity of the Low class remained high (99.32%).

6. Visualize Model Evaluation

6.1 Confusion Matrix Heatmaps

```
library(ggplot2)
library(reshape2)
```

```
##
##    'reshape2'
```

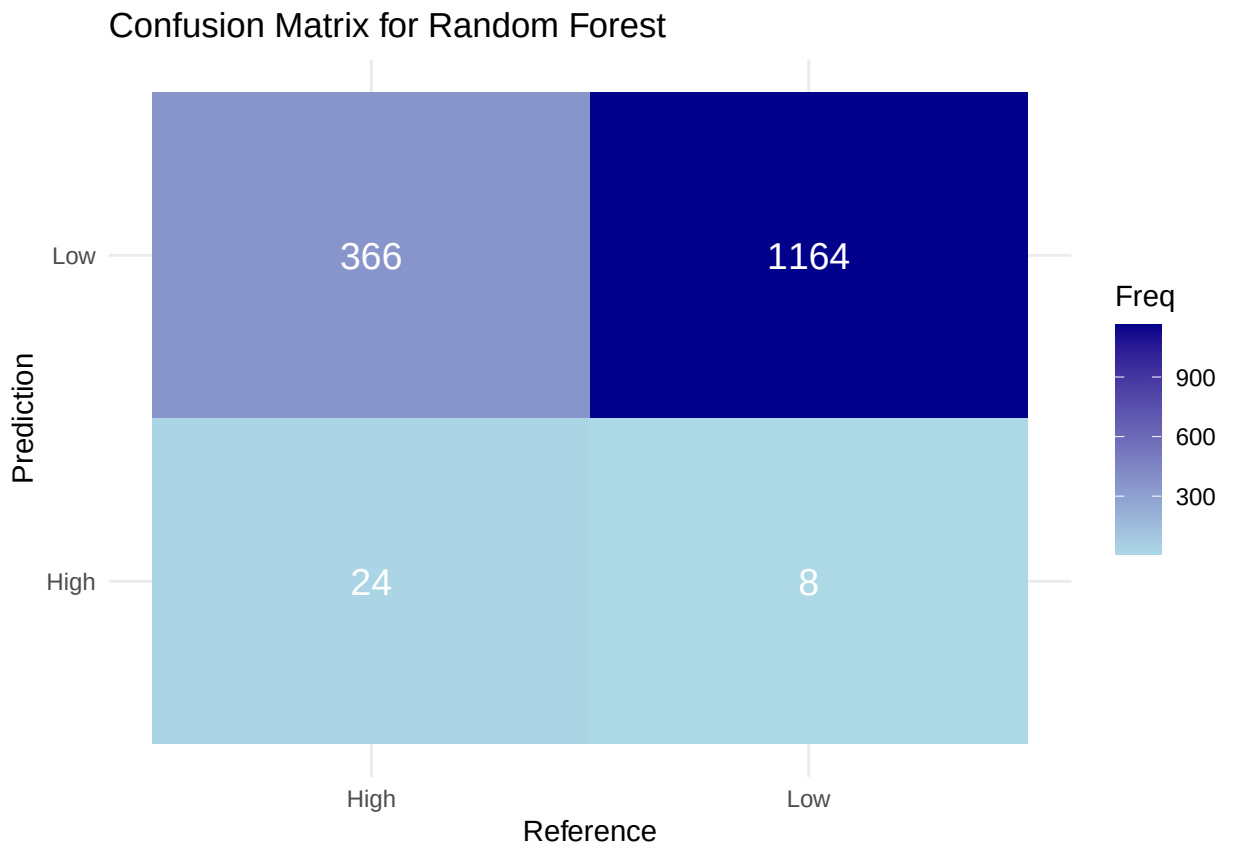
```
## The following object is masked from 'package:tidyr':
##
##     smiths
```

```
# Function to plot confusion matrix heatmap
plot_confusion_matrix <- function(cm, model_name) {
  cm_table <- as.data.frame(cm$table)
  cm_table$Prediction <- factor(cm_table$Prediction, levels = c("High", "Low"))
  cm_table$Reference <- factor(cm_table$Reference, levels = c("High", "Low"))
```

```
  ggplot(cm_table, aes(x = Reference, y = Prediction, fill = Freq)) +
    geom_tile() +
    geom_text(aes(label = Freq), color = "white", size = 5) +
    scale_fill_gradient(low = "lightblue", high = "darkblue") +
    labs(title = paste("Confusion Matrix for", model_name),
         x = "Reference", y = "Prediction") +
    theme_minimal()
}

# Plot confusion matrices
plot_confusion_matrix(log_cm, "Logistic Regression")
```


```
plot_confusion_matrix(rf_cm, "Random Forest")
```



The confusion matrix heat map clearly shows the prediction distribution of the two models. The heat map of logistic regression shows that Low class predictions (1164) dominate, and High class predictions (12+8) are very rare, reflecting the model's strong bias against the Low class. The heat map of random forest shows that the correct predictions of the High class increased to 24, but the Low class still dominated (1164). The color depth (dark blue indicates high frequency) further highlights the high frequency of Low class predictions. The ROC curve shows that random forest (red line, AUC=0.598) is slightly better than logistic regression (blue line, AUC=0.58), but both curves are close to the diagonal line, indicating limited classification ability. These visualization results highlight the shortcomings of High class predictions, suggesting that thresholds and features need to be optimized to improve the model's ability to identify highly active enhancers.

6.2 ROC Curves

```
library(pROC)
```

```
## Type 'citation("pROC")' for a citation.
```

```
##
##     'pROC'
```

```
## The following objects are masked from 'package:stats':
##
##     cov, smooth, var
```

```
# Get predicted probabilities for ROC
log_probs <- predict(log_model, X_test, type = "prob")[, "High"]
rf_probs <- predict(rf_model, X_test, type = "prob")[, "High"]

# Compute ROC curves
```

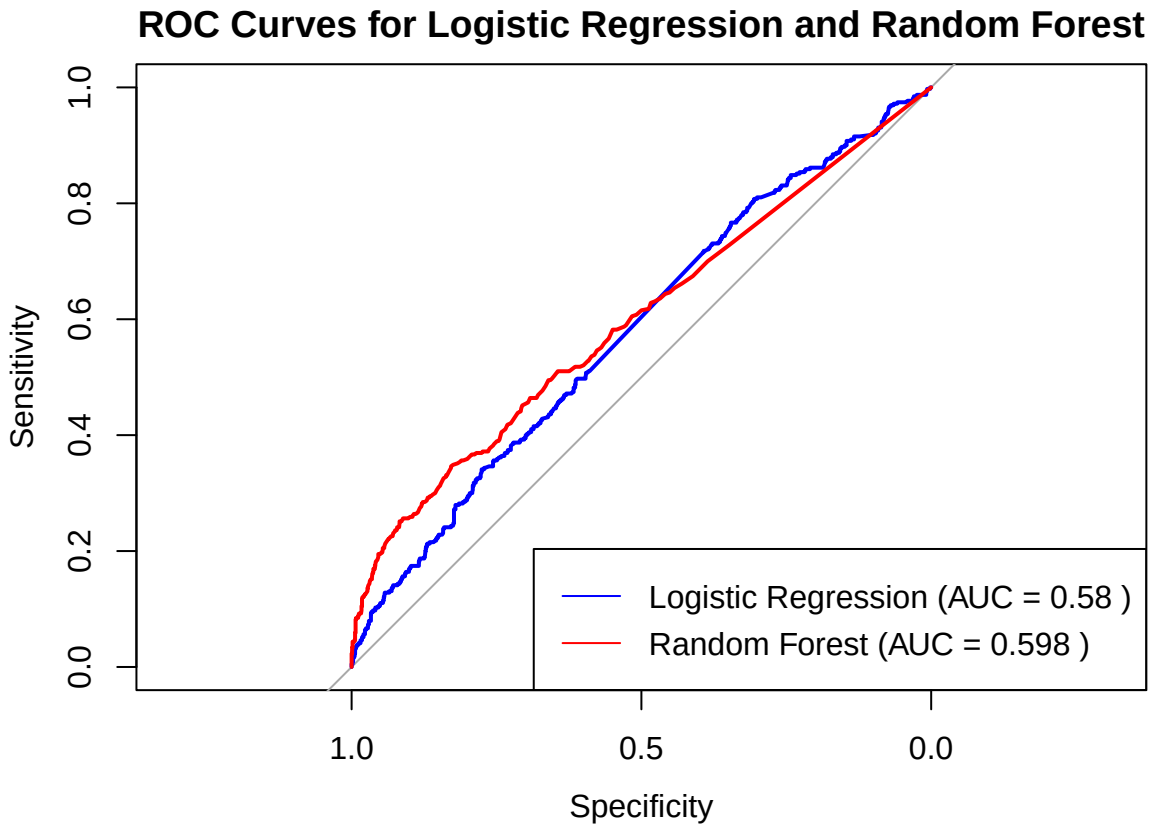
```r
log_roc <- roc(y_test, log_probs, levels = c("Low", "High"))
```

```
## Setting direction: controls < cases
```

```r
rf_roc <- roc(y_test, rf_probs, levels = c("Low", "High"))
```

```
## Setting direction: controls < cases
```

```r
# Plot ROC curves
plot(log_roc, col = "blue", main = "ROC Curves for Logistic Regression and Random Forest")
plot(rf_roc, col = "red", add = TRUE)
legend("bottomright", legend = c(paste("Logistic Regression (AUC =", round(auc(log_roc), 3), ")"),
                                 paste("Random Forest (AUC =", round(auc(rf_roc), 3), ")")),
       col = c("blue", "red"), lty = 1)
```



The ROC curve of the logistic regression model shows an AUC of 0.58, reflecting the weak overall discrimination ability of the model. In general, logistic regression may not be able to capture complex patterns in the data due to the assumption of a linear relationship between features and targets, resulting in failure to predict the High class, and the accuracy is mainly driven by the correct prediction of the Low class.

The AUC of the ROC curve of the random forest model is 0.598, which is slightly higher than that of logistic regression, indicating that random forest can capture some nonlinear relationships. Feature importance analysis shows that TBS (importance 100), slp2_forkhead, and treatment are the main contributing features. However, the low sensitivity of the High class prediction indicates that the model is still biased towards the Low class, which may be limited by data imbalance or threshold selection.