The Principle of R Package GSClassifier

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Contents

Chapter 1

Prerequisites

This is a *sample* book written in **Markdown**. You can use anything that Pandoc's Markdown supports, e.g., a math equation $a^2 + b^2 = c^2$.

The **bookdown** package can be installed from CRAN or Github:

```
install.packages("bookdown")
# or the development version
# devtools::install_github("rstudio/bookdown")
```

Remember each Rmd file contains one and only one chapter, and a chapter is defined by the first-level heading `#`.

To compile this example to PDF, you need XeLaTeX. You are recommended to install TinyTeX (which includes XeLaTeX): https://yihui.org/tinytex/.

Chapter 2

Prerequisites2

You can label chapter and section titles using `{#label}` after them, e.g., we can reference Chapter 2. If you do not manually label them, there will be automatic labels anyway, e.g., Chapter 2.

Figures and tables with captions will be placed in `figure` and `table` environments, respectively.

```
par(mar = c(4, 4, .1, .1))
plot(pressure, type = 'b', pch = 19)
```

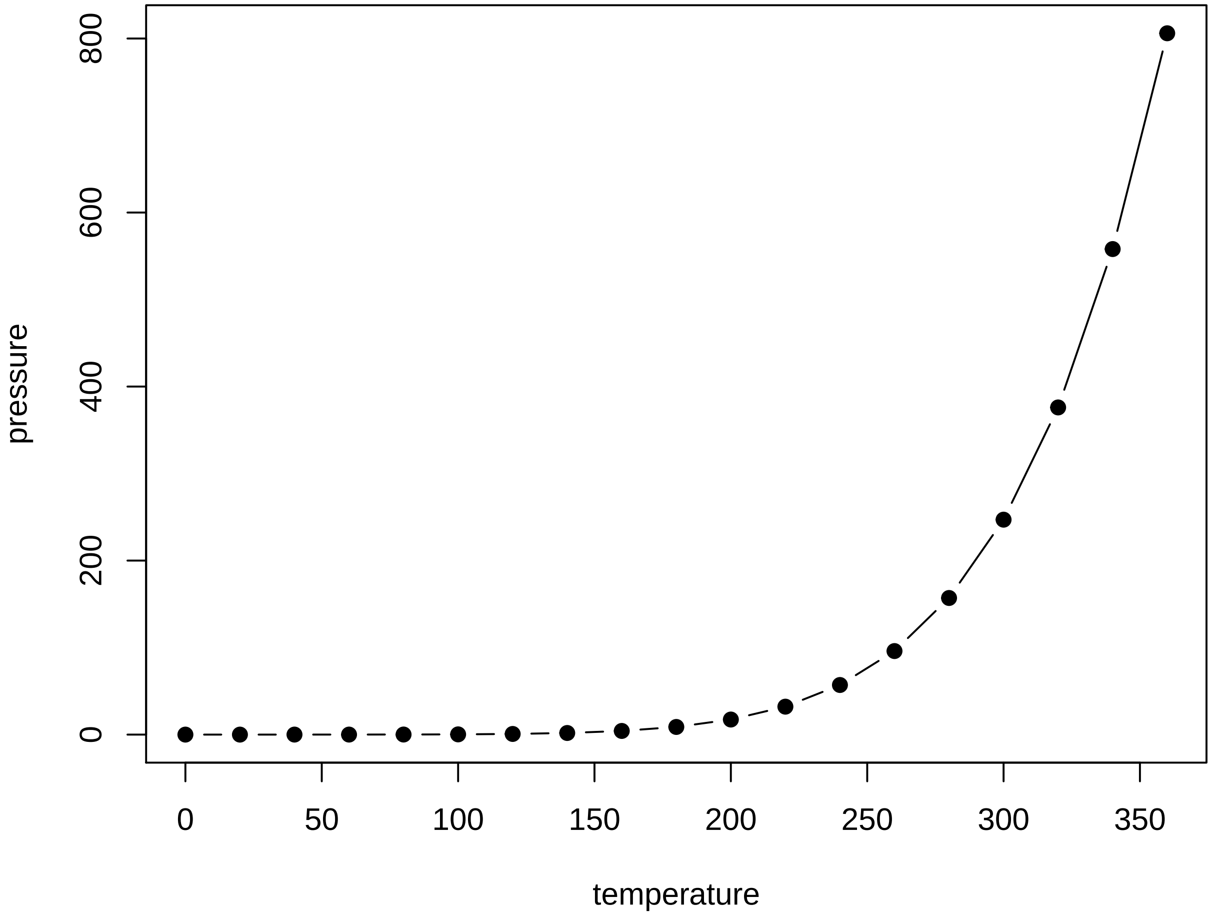


Figure 2.1: Here is a nice figure!

Table 2.1: Here is a nice table!

Sepal.Length	Sepal.Width	Petal.Length	Petal.Width	Species
5.1	3.5	1.4	0.2	setosa
4.9	3.0	1.4	0.2	setosa
4.7	3.2	1.3	0.2	setosa
4.6	3.1	1.5	0.2	setosa
5.0	3.6	1.4	0.2	setosa
5.4	3.9	1.7	0.4	setosa
4.6	3.4	1.4	0.3	setosa
5.0	3.4	1.5	0.2	setosa
4.4	2.9	1.4	0.2	setosa
4.9	3.1	1.5	0.1	setosa
5.4	3.7	1.5	0.2	setosa
4.8	3.4	1.6	0.2	setosa
4.8	3.0	1.4	0.1	setosa
4.3	3.0	1.1	0.1	setosa
5.8	4.0	1.2	0.2	setosa
5.7	4.4	1.5	0.4	setosa
5.4	3.9	1.3	0.4	setosa
5.1	3.5	1.4	0.3	setosa
5.7	3.8	1.7	0.3	setosa
5.1	3.8	1.5	0.3	setosa

Reference a figure by its code chunk label with the `fig:` prefix, e.g., see Figure 2.1. Similarly, you can reference tables generated from `knitr::kable()`, e.g., see Table 2.1.

```r
knitr::kable(
  head(iris, 20), caption = 'Here is a nice table!',
  booktabs = TRUE
)
```

You can write citations, too. For example, we are using the **bookdown** package in this sample book, which was built on top of R Markdown and **knitr** (Xie, 2015).

Chapter 3

Introduction

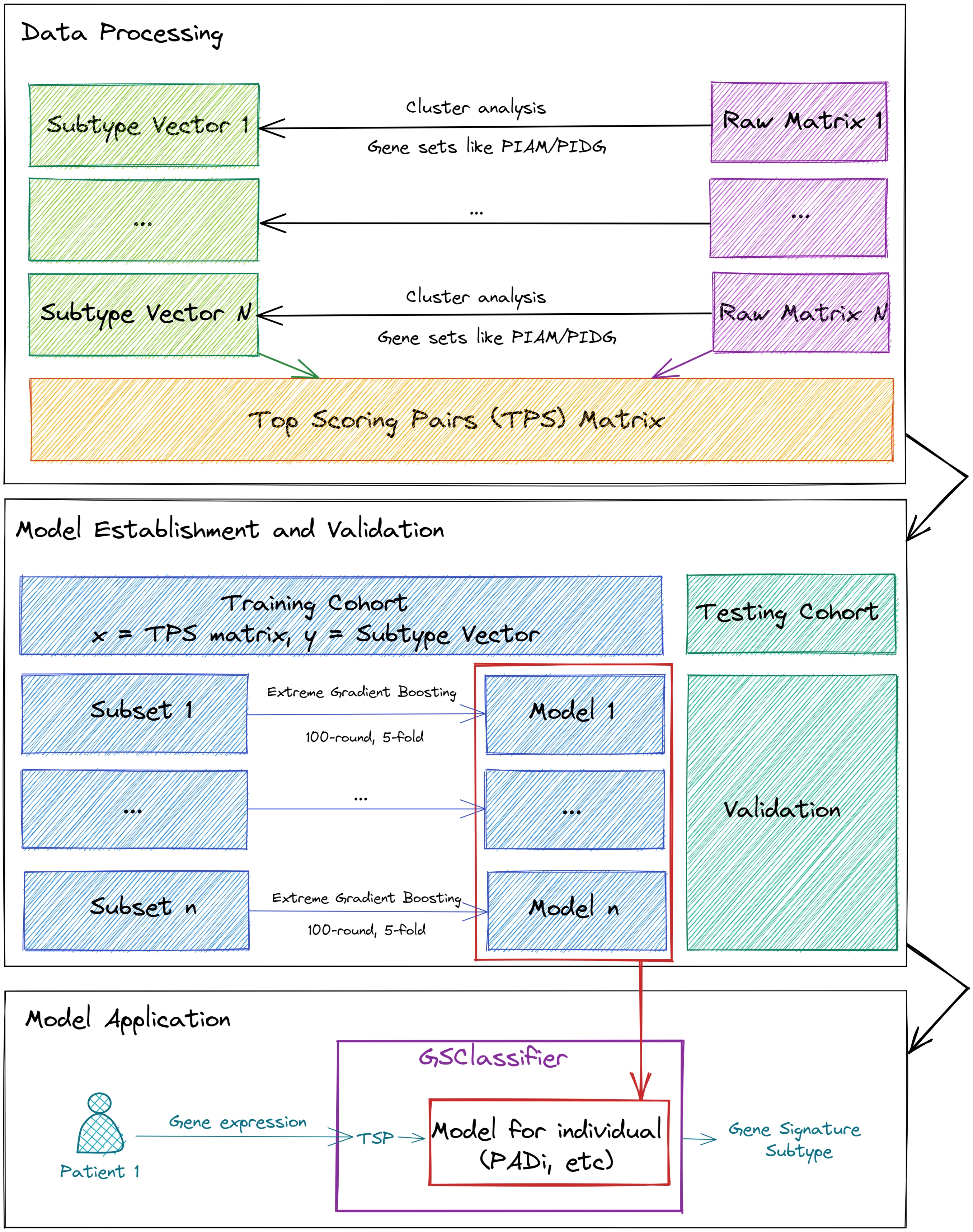
GSClassifier is an R package for modeling and identification of gene expression profiles (GEPs) subtypes. The detail of usage had been demonstrated in Github WiKi. Here, we propose to introduce the principle of GSClassifier, including flowchart, **top scoring pairs (TSP)** algorithm, and batch effect control.

Chapter 4

Flowchart

Here is the flowchart of `GSClassifier`:

```r
knitr::include_graphics(rep("./fig/flowchart.png", 1))
```


Data Processing
Subtype Vector 1
Cluster analysis
Gene sets like PIAM/PIDG
Raw Matrix 1
...
...
...
Subtype Vector N
Cluster analysis
Gene sets like PIAM/PIDG
Raw Matrix N
Top Scoring Pairs (TPS) Matrix
Model Establishment and Validation
Training Cohort
x = TPS matrix, y = Subtype Vector
Testing Cohort
Subset 1
Extreme Gradient Boosting
100-round, 5-fold
Model 1
...
...
...
Validation
Subset n
Extreme Gradient Boosting
100-round, 5-fold
Model n
Model Application
GSClassifier
Patient 1
Gene expression
TSP
Model for individual
(PADi, etc)
Gene Signature
Subtype

4.1 Data Processing

For each dataset, the RNA expression matrix would be normalized (we called `Raw Matrix` in the flowchart) internally so that the expression data of the samples in the dataset were comparable.

Next, the subtypes of the samples in each dataset would be called based on cluster analysis. Specially, we figured out PAD subtypes, which belong to `Subtype Vector` in the flowchart, via hierarchical clustering analysis.

4.2 Top scoring pairs (TSP) matrix

With `subtype vectors` and `Raw Matrix`, the TSP matrix for a specified subtypes could be calculated via function `GSClassifier::trainDataProc`:

```
trainDataProc(
  Xmat, Yvec,

  geneSet,

  subtype = 1,

  # 0.2 was Used in PAD project
  ptail = 0.2,

  # c(0, 0.25, 0.5, 0.75, 1.0) was Used in PAD project
  breakVec = c(0, 0.25, 0.5, 0.75, 1.0)
)
```

The TSP matrix consists of 3 parts: **binned expression matrix**, **top scoring of gene pairs**, and **gene set pairs**.

Here, we would use some simulated data to introduce how TSP matrix calculated.

4.2.1 Dataset

Load packages:

```
# Install "devtools" package
if (!requireNamespace("devtools", quietly = TRUE))
    install.packages("devtools")

# Install dependencies
if (!requireNamespace("luckyBase", quietly = TRUE))
    devtools::install_github("huangwb8/luckyBase")

# Install the "GSClassifier" package
if (!requireNamespace("GSClassifier", quietly = TRUE))
    devtools::install_github("huangwb8/GSClassifier")

# Install CRAN packages
if (!requireNamespace("pacman", quietly = TRUE)){
  install.packages("pacman")
  library(pacman)
} else {
  library(pacman)
}
packages_needed <- c("readxl","ComplexHeatmap","GSClassifier")
for(i in packages_needed){p_load(char=i)}
```

We simulated a dataset:

```
# Geneset
geneSet <- list(
  Set1 = paste('Gene',1:3,sep = ''),
  Set2 = paste('Gene',4:6,sep = '')
)


# RNA expression
x <- read_xlsx('./data/simulated-data.xlsx', sheet = 'RNA'); expr <- as.matrix(x[,-1]); rownames(expr) <- as.char
```

```
## New names:
```

```
# Parameters
breakVec = c(0, 0.25, 0.5, 0.75, 1.0)
subtype_vector = c(1,1,1,2,2,2)
Ybin = ifelse(subtype_vector == 1, yes = 1, no=0)

# Report
cat(c('\n', 'Gene sets:', '\n'))
```

```
##
##  Gene sets:
```

```
print(geneSet)
```

```
## $Set1
## [1] "Gene1" "Gene2" "Gene3"
##
## $Set2
## [1] "Gene4" "Gene5" "Gene6"
```

```
cat('RNA expression:', '\n')
```

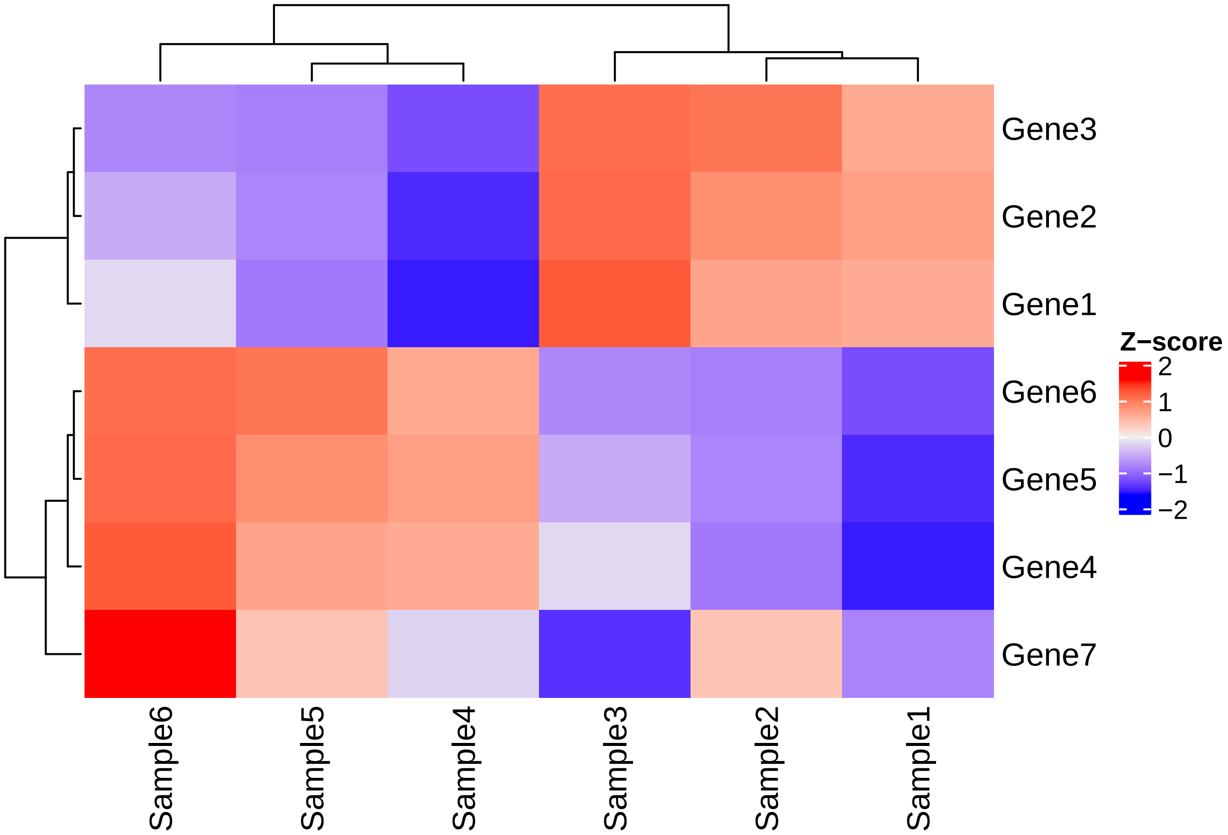
```
## RNA expression:
```

```
print(expr)
```

```
##       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
## Gene1    0.51    0.52    0.60    0.21    0.30    0.40
## Gene2    0.52    0.54    0.58    0.22    0.31    0.35
## Gene3    0.53    0.60    0.61    0.23    0.29    0.30
## Gene4    0.21    0.30    0.40    0.51    0.52    0.60
## Gene5    0.22    0.31    0.35    0.52    0.54    0.58
## Gene6    0.23    0.29    0.30    0.53    0.60    0.61
## Gene7    0.10    0.12    0.09    0.11    0.12    0.14
```

Have a look at the matrix:

```
Heatmap(t(scale(t(expr))), name = "Z-score")
```



4.2.2 Binned expression

```
# Data of one sample

x <- expr[,1]

# Create quantiles
brks <- quantile(as.numeric(x), probs=breakVec, na.rm = T)

# Get interval orders
xbin <- .bincode(x = x, breaks = brks, include.lowest = T)
xbin <- as.numeric(xbin)

# Report
cat('Quantiles:', '\n'); print(brks)
```

```
## Quantiles:
```

```
##    0%   25%   50%   75%  100%
## 0.100 0.215 0.230 0.515 0.530
```

```
cat('\n')
```

```
cat('Raw expression:', '\n');print(as.numeric(x))
```

```
## Raw expression:
```

```
## [1] 0.51 0.52 0.53 0.21 0.22 0.23 0.10
```

```
cat('\n')
```

```
cat('Binned expression:', '\n'); print(xbin)
```

```
## Binned expression:
```

```
## [1] 3 4 4 1 2 2 1
```

For example, `0.10` is the minimun of the raw expression vector, so its binned expression is `1`. Similarly, the binned expression of maximum `0.53` is `4`.

We calculated binned expression via function `breakBin` in `GSClassifier`:

```
expr_binned <- apply(expr, 2,
                 GSClassifier:::breakBin,
                 breakVec)
rownames(expr_binned) <- rownames(expr)
print(expr_binned)
```

```
##       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
## Gene1       3       3       4       1       2       2
## Gene2       4       4       3       2       2       2
## Gene3       4       4       4       2       1       1
## Gene4       1       2       2       3       3       4
## Gene5       2       2       2       4       4       3
## Gene6       2       1       1       4       4       4
## Gene7       1       1       1       1       1       1
```

4.2.3 Genes with large rank differences

In this simulated dataset, `Gene7` is a gene whose expression is always the lowest across all samples. In other words, the rank of `Gene7` is stable or invariable across samples so that it's not robust for identification of differentail subtypes.

Except binned expression, we also calculated gene-pair scores later. Due to the number of gene-pair is C_n^2, the removement of genes like `Gene7` before modeling could really reduce the complexibility of the model and save computing resources. In all, genes like `Gene7` could be dropped out in the following analysis.

First, We use `base::rank`to return the sample ranks of the values in a vector:

```
expr_binned_rank <- apply(
  expr_binned,
  2,
  function(x)rank(x, na.last = TRUE)
)
print(expr_binned_rank)
```

```
##       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
## Gene1     5.0     5.0     6.5     1.5     3.5     3.5
## Gene2     6.5     6.5     5.0     3.5     3.5     3.5
## Gene3     6.5     6.5     6.5     3.5     1.5     1.5
## Gene4     1.5     3.5     3.5     5.0     5.0     6.5
## Gene5     3.5     3.5     3.5     6.5     6.5     5.0
## Gene6     3.5     1.5     1.5     6.5     6.5     6.5
## Gene7     1.5     1.5     1.5     1.5     1.5     1.5
```

`na.last = TRUE` means that missing values in the data are put last.

Then, get rank differences of each gene based on specified subtype distribution (`Ybin`):

```
testRes <- sapply(1:nrow(expr_binned_rank), function(gi) testFun(as.numeric(expr_binned_rank[gi,]), Ybin)); names
print(testRes)
```

```
##     Gene1     Gene2     Gene3     Gene4     Gene5     Gene6     Gene7 
## -2.666667 -2.500000 -4.333333  2.666667  2.500000  4.333333  0.000000
```

Gene7 is the one with the lowest absolute value (0) of rank diffrence.

In GSClassifier, we use ptail to select differential genes based on rank diffrences. **Less ptail is, less gene kept**. Here, we just set ptail=0.4:

```
# ptail is a numeber ranging (0,0.5].
ptail = 0.4

# Index of target genes with big rank differences
idx <- which((testRes < quantile(testRes, ptail, na.rm = T)) | (testRes > quantile(testRes, 1.0-ptail, na.rm = T)

# Target genes
gene_bigRank <- names(testRes)[idx]

# Report
cat('Index of target genes: ','\n');print(idx); cat('\n')
```

```
## Index of target genes:

## Gene1 Gene2 Gene3 Gene4 Gene5 Gene6 
##     1     2     3     4     5     6
```

```
cat('Target genes:','\n');print(gene_bigRank); cat('\n')
```

```
## Target genes:

## [1] "Gene1" "Gene2" "Gene3" "Gene4" "Gene5" "Gene6"
```

Hence, Gene7 was filtered and excluded in the following analysis. In practice, both ptail and breakVec are hyperparameters in modeling.

4.2.4 Pair scores of top genes

In GSClassifier, we use function makeGenePairs to calculate s

```
gene_bigRank_pairs <- GSClassifier:::makeGenePairs(gene_bigRank, expr[gene_bigRank,])
print(gene_bigRank_pairs)
```

```
##             Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
## Gene1:Gene2       0       0       1       0       0       1
## Gene1:Gene3       0       0       0       0       1       1
## Gene1:Gene4       1       1       1       0       0       0
## Gene1:Gene5       1       1       1       0       0       0
## Gene1:Gene6       1       1       1       0       0       0
## Gene2:Gene3       0       0       0       0       1       1
## Gene2:Gene4       1       1       1       0       0       0
## Gene2:Gene5       1       1       1       0       0       0
## Gene2:Gene6       1       1       1       0       0       0
## Gene3:Gene4       1       1       1       0       0       0
## Gene3:Gene5       1       1       1       0       0       0
## Gene3:Gene6       1       1       1       0       0       0
## Gene4:Gene5       0       0       1       0       0       1
## Gene4:Gene6       0       1       1       0       0       0
## Gene5:Gene6       0       1       1       0       0       0
```

Take `Gene1:Gene4` of `Sample1` as an example. $Expression_{Gene1} - Expression_{Gene4} = 0.51 - 0.21 = 0.3 > 0$, so the pair score is 1; if the difference is 0, the pair score is 0 instead.

4.2.5 Gene set difference score

In GSClassifier, we use function `makeSetData` to calculate gene set difference score:

```
geneset_interaction <- GSClassifier:::makeSetData(expr,geneSet)
print(geneset_interaction)
```

```
##      Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
## s1s2       1       1       1       0       0       0
```

Bibliography

Xie, Y. (2015). *Dynamic Documents with R and knitr*. Chapman and Hall/CRC, Boca Raton, Florida, 2nd edition. ISBN 978-1498716963.