The Principle of R Package GSClassifier

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Contents

Chapter 1

The Principle of GSClassifier

1.1 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.

1.2 Flowchart

The flowchart of **GSClassifier** is showed in Figure 1.1.

1.3 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.

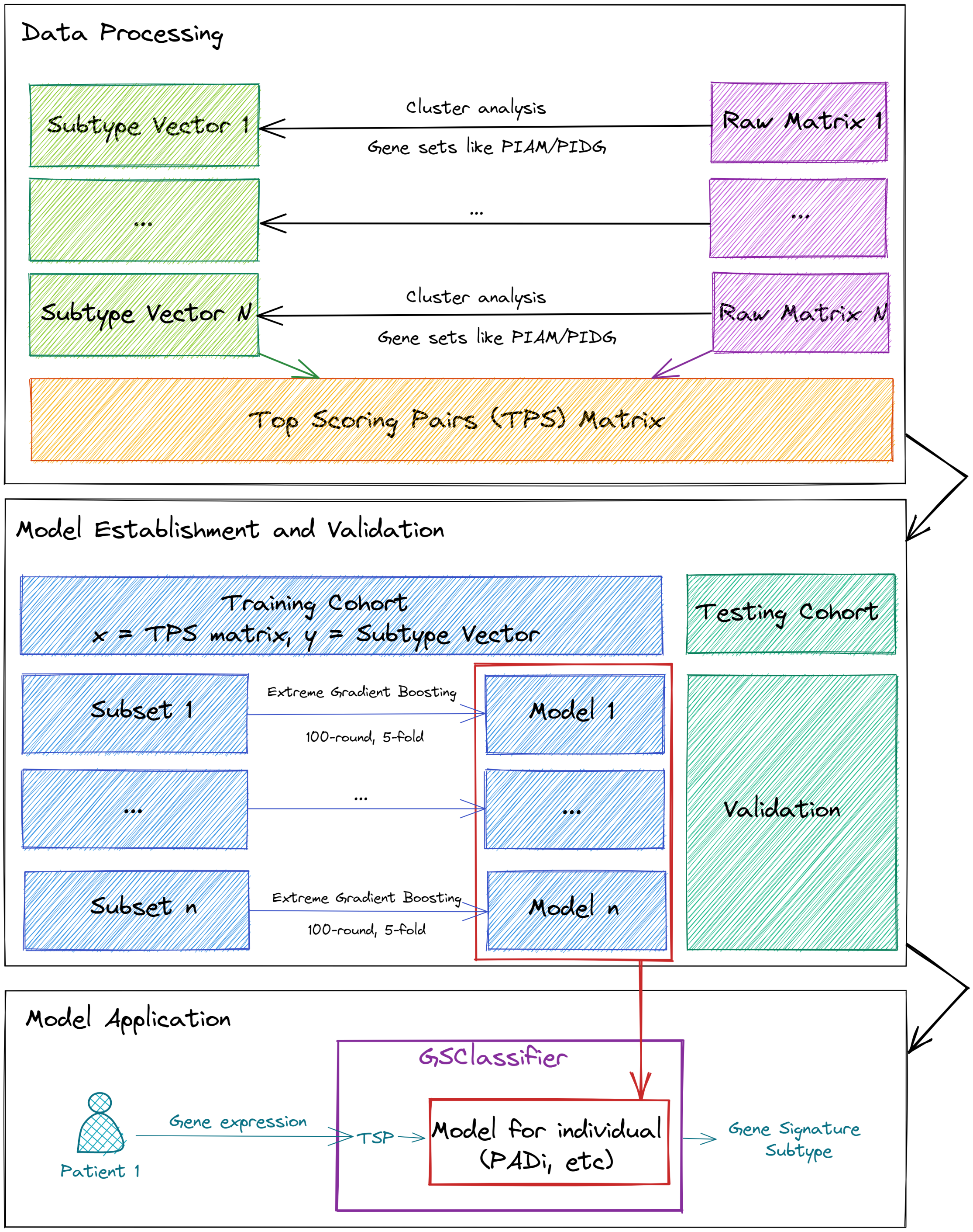


Figure 1.1: The flow chart of GSClassifier

1.4 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**.

1.4.1 Simulated Dataset

Here, we would use some simulated data to introduce how TSP matrix calculated. First, load packages:

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

# Install dependencies
```

```r
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)}
```

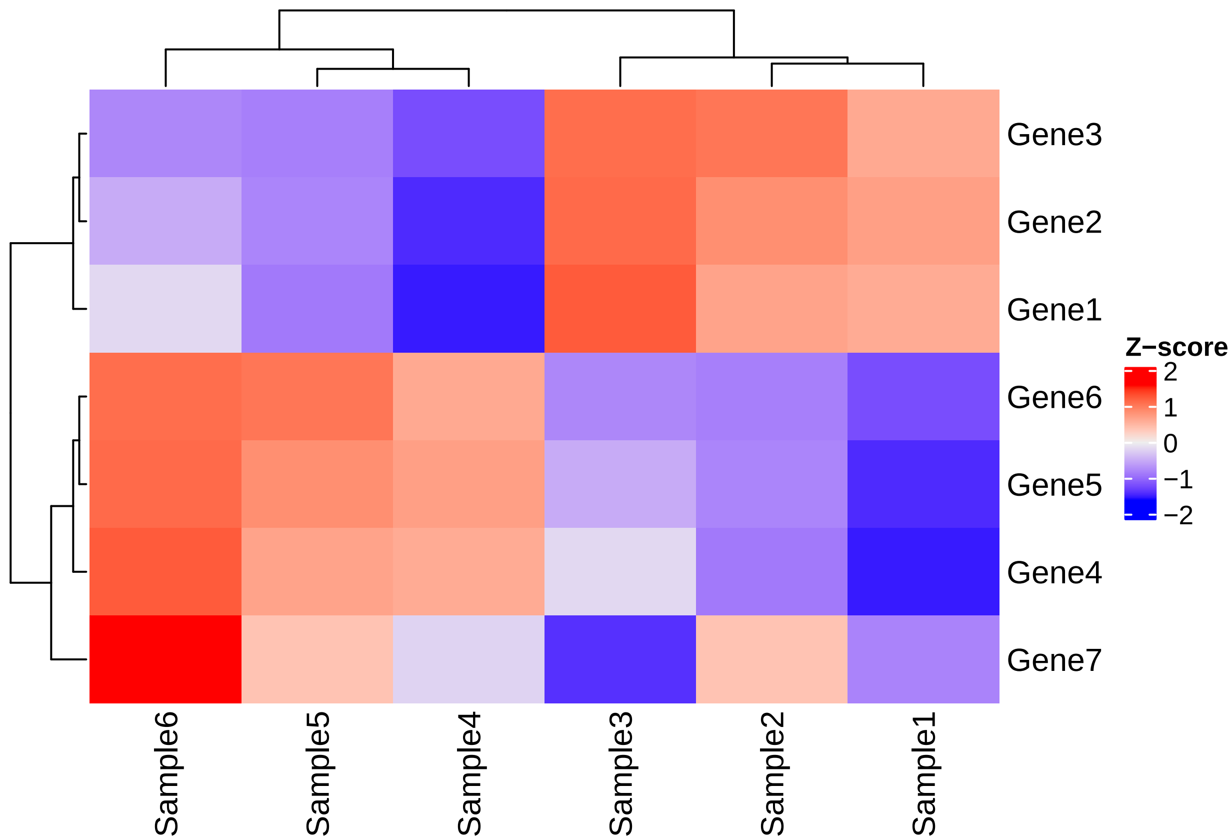
We simulated a dataset:

```r
# 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')
# New names:
expr <- as.matrix(x[,-1])
rownames(expr) <- as.character(as.matrix(x[,1])); rm(x)
```

```
# 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'))
print(geneSet)
cat('RNA expression:', '\n')
print(expr)
#
#  Gene sets:
# $Set1
# [1] "Gene1" "Gene2" "Gene3"
#
# $Set2
# [1] "Gene4" "Gene5" "Gene6"
#
# RNA expression:
#       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")
```



1.4.2 Genes with large rank differences

First, we binned genes with diffrent quantile intervals so that the distribution of rank information could be more consistent across samples.

Take data of **Sample1** as an example:

```
# Data of Sample1

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)
cat('\n')
cat('Raw expression:', '\n');print(as.numeric(x))
cat('\n')
cat('Binned expression:', '\n'); print(xbin)
# Quantiles:
#    0%   25%   50%   75%  100%
# 0.100 0.215 0.230 0.515 0.530
#
# Raw expression:
# [1] 0.51 0.52 0.53 0.21 0.22 0.23 0.10
#
# 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**.

Generally, we calculated binned expression via function **breakBin** of **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
```

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){
    GSClassifier:::testFun(
      as.numeric(expr_binned_rank[gi,]),
      Ybin)
  }
)
names(testRes) <- rownames(expr_binned_rank)
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')
cat('Target genes:','\n');print(gene_bigRank); cat('\n')
# Index of target genes:
# Gene1 Gene2 Gene3 Gene4 Gene5 Gene6
#     1     2     3     4     5     6
#
# 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.

1.4.3 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 less than or equal to 0, the pair score is 0.

1.4.4 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
```