

The Principle of R Package GSClassifier

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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](#).

1.2.1 Data Processing

For each dataset, RNA expression matrix would be normalized internally (**Raw Matrix**) so that the expression data of the samples in the dataset were comparable and suitable for subtype identification. As demonstrated in Figure [1.1](#), the **Subtype vector** is identified based on independent cohorts instead of a merged matrix with batch effect control technologies. More details about batch effect control are discussed in [2.3](#).

There is no standard method to figure out subtype vectors. It depends on the Gene

Expression Profiles (GEPs) used, the biological problems or ideas of researchers. For Pan-immune Activation and Dysfunction (PAD) subtypes, the GEPs, Pan-Immune Activation Module (PIAM) and Pan-Immune Dysfunction Genes (PIDG), are biologically associated and suitable for calling four subtypes ($\text{PIAM}^{\text{high}}\text{PIDG}^{\text{high}}$, $\text{PIAM}^{\text{high}}\text{PIDG}^{\text{low}}$, $\text{PIAM}^{\text{low}}\text{PIDG}^{\text{high}}$, and $\text{PIAM}^{\text{low}}\text{PIDG}^{\text{low}}$). Theoretically, we can also use a category strategy like low/medium/high, but more evidences or motivations are lacked for chasing such a complex model.

With subtype vectors and raw matrices, **Top Scoring Pairs (TSP)**, the core data format for model training and application in GSClassifier, would be calculated for the following process. The details of TSP are summarized in 1.5.

1.2.2 Model Establishment and Validation

How to training

1.2.3 Model Application

How to use

1.3 Simulated Dataset

First, load needed packages:

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

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

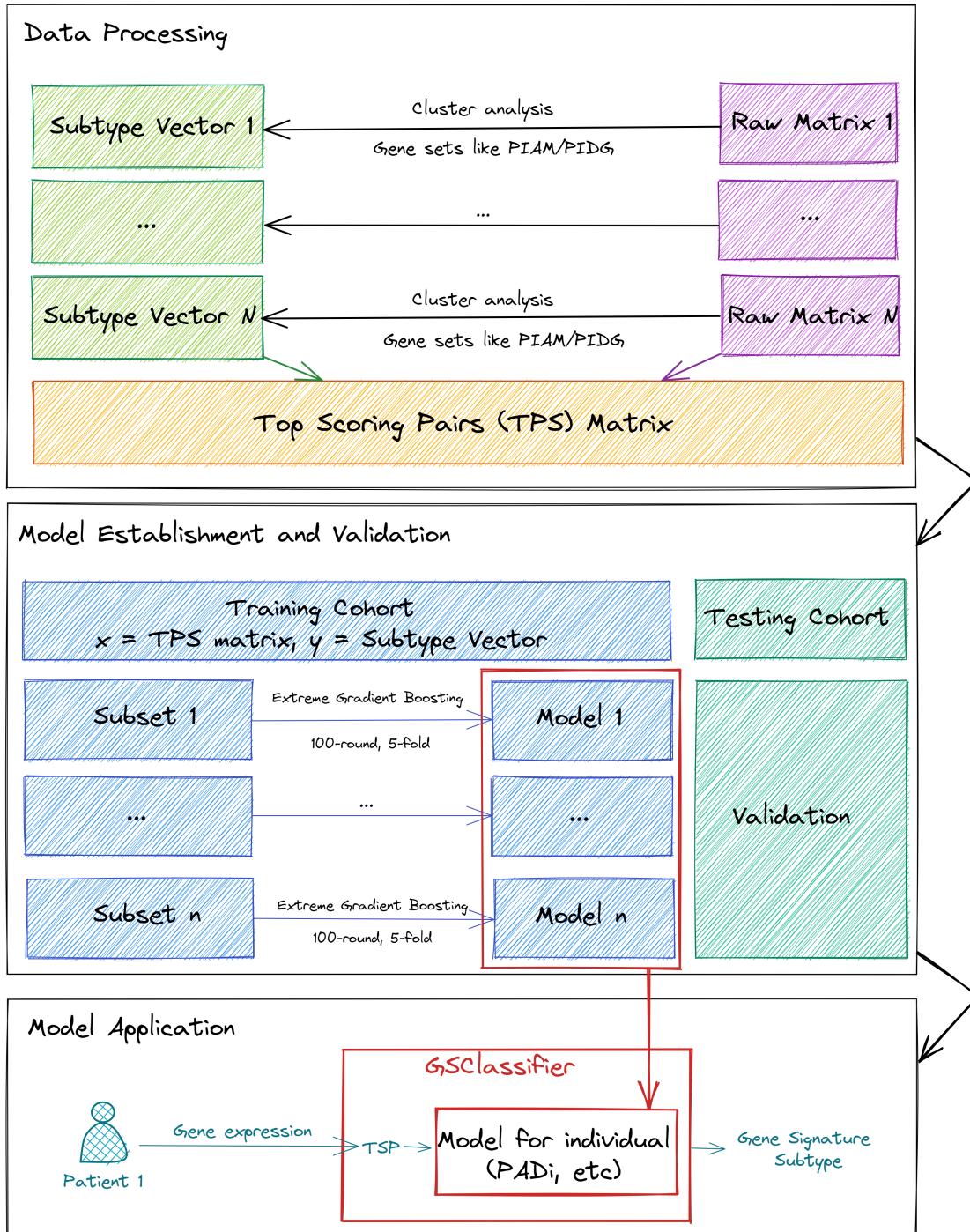


Figure 1.1: The flow chart of GSClassifier

```

# Install the "GSClassifier" package

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

# Install the "pacman" package

if (!requireNamespace("pacman", quietly = TRUE)){
  install.packages("pacman")
  library(pacman)
} else {
  library(pacman)
}

# Load needed packages

packages_needed <- c(
  "readxl",
  "ComplexHeatmap",
  "GSClassifier",
  "rpart",
  "tidyverse")

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')

```

```

expr0 <- as.matrix(x[,-1])
rownames(expr0) <- as.character(as.matrix(x[,1])); rm(x)

# Subtype information
# It depends on the application scenarios of GEPs
subtype_vector <- c(1, 1, 1, 2, 2, 2)
# Binned data for subtype 1
Ybin <- ifelse(subtype_vector == 1, 1, 0)

# Parameters
breakVec = c(0, 0.25, 0.5, 0.75, 1.0)

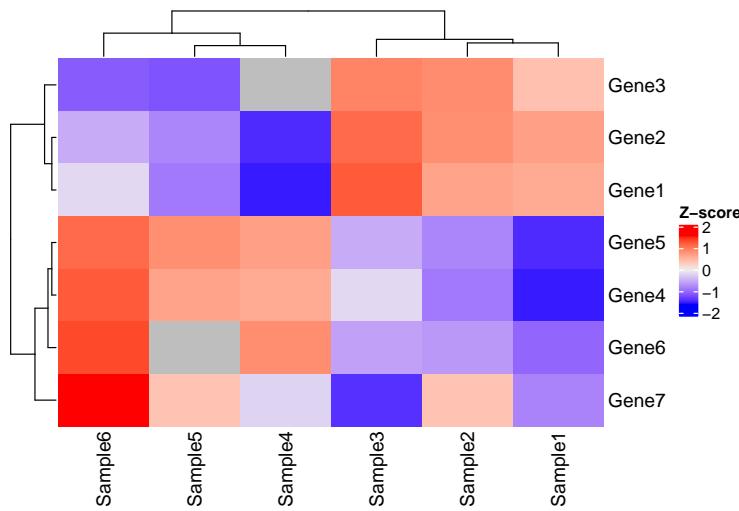
# Report
cat(c('\n', 'Gene sets:', '\n'))
print(geneSet)
cat('RNA expression:', '\n')
print(expr0)
#
# Gene sets:
# $Set1
# [1] "Gene1" "Gene2" "Gene3"
#
# $Set2
# [1] "Gene4" "Gene5" "Gene6"
#
# RNA expression:
# Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1 0.51 0.52 0.60 0.21 0.30 0.40
# Gene2 0.52 0.54 0.58 0.22 0.31 0.35

```

# Gene3	0.53	0.60	0.61	NA	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	NA	0.61
# Gene7	0.10	0.12	0.09	0.11	0.12	0.14

Look at the matrix via heatmap:

```
Heatmap(t(scale(t(expr0))), name = "Z-score")
```



This is an interesting dataset with features as following:

- **Distinguished gene sets:** The expression profile between **Gene 1-3** and **Gene 4-6** is obviously different across samples. Thus, these gene sets might represent different biology meaning.
- **Stable gene:** The expression level and rank of **Gene 7** seemed to be similar across samples. Thus, **Gene 7** might not be a robust marker for subtype modeling. Thus, it could help us to understand how filtering of **GSClassifier** works.
- **Expression heterogeneity & rank homogeneity:** Take **Sample1** and **Sample3** as examples. The expression of **Gene 1-6** in **Sample3** seemed to be higher than those of **Sample1**. However, the expression of **Gene 1-3** is higher than **Gene 4-6** in both **Sample1** and **Sample3**, indicating similar bioprocess in these samples exists so that

they should be classified as the same subtype.

1.4 Missing values

Here, we fill missing value with Recursive Partitioning and Regression Trees (RPART) algorithm:

```
# RPART

expr <- GSClassifier:::na_fill(expr0, method="anova", na.action = na.rpart)

# Report
cat('RNA expression:', '\n')
print(expr0)
cat('\n')
cat('RNA expression without NA value:', '\n')
print(expr)

# RNA expression:

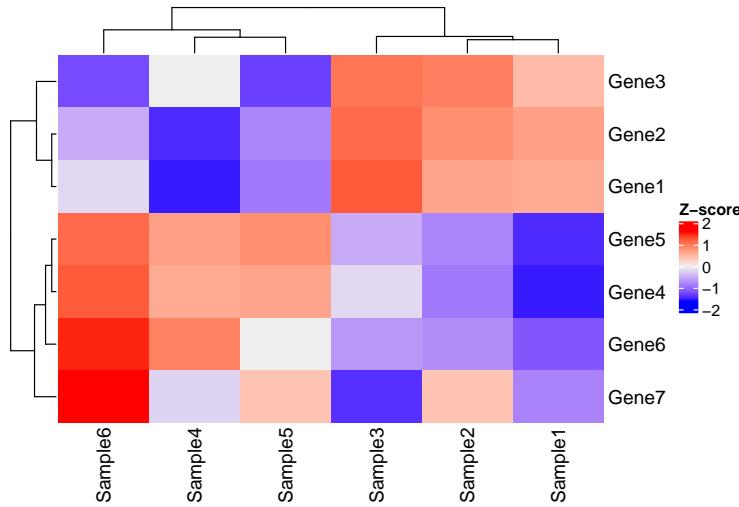
#       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1    0.51    0.52    0.60    0.21    0.30    0.40
# Gene2    0.52    0.54    0.58    0.22    0.31    0.35
# Gene3    0.53    0.60    0.61     NA    0.29    0.30
# Gene4    0.21    0.30    0.40    0.51    0.52    0.60
# Gene5    0.22    0.31    0.35    0.52    0.54    0.58
# Gene6    0.23    0.29    0.30    0.53     NA    0.61
# Gene7    0.10    0.12    0.09    0.11    0.12    0.14
#
# RNA expression without NA value:

#       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1    0.51    0.52    0.60    0.210   0.300    0.40
# Gene2    0.52    0.54    0.58    0.220   0.310    0.35
# Gene3    0.53    0.60    0.61    0.466   0.290    0.30
```

# Gene4	0.21	0.30	0.40	0.510	0.520	0.60
# Gene5	0.22	0.31	0.35	0.520	0.540	0.58
# Gene6	0.23	0.29	0.30	0.530	0.392	0.61
# Gene7	0.10	0.12	0.09	0.110	0.120	0.14

Look at the new matrix via heatmap, where the clustering result is not obviously disturbed by **NA** filling:

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



Although RPART algorithm is proved to be powerful dealing with NA value, we should try to use markers with less NA as possible. During PAD subtype establishment, only genes occurring in over 80% of datasets were retained so as to minimize the impact from missing value.

1.5 Top scoring pairs (TSP)

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)

)

```

As shown in Figure 1.2, The TSP matrix consists of 3 parts: **binned expression**, **pair difference**, and **set difference**. Next, we would use a simulated dataset to introduce how TSP matrix calculated in GSClassifier.

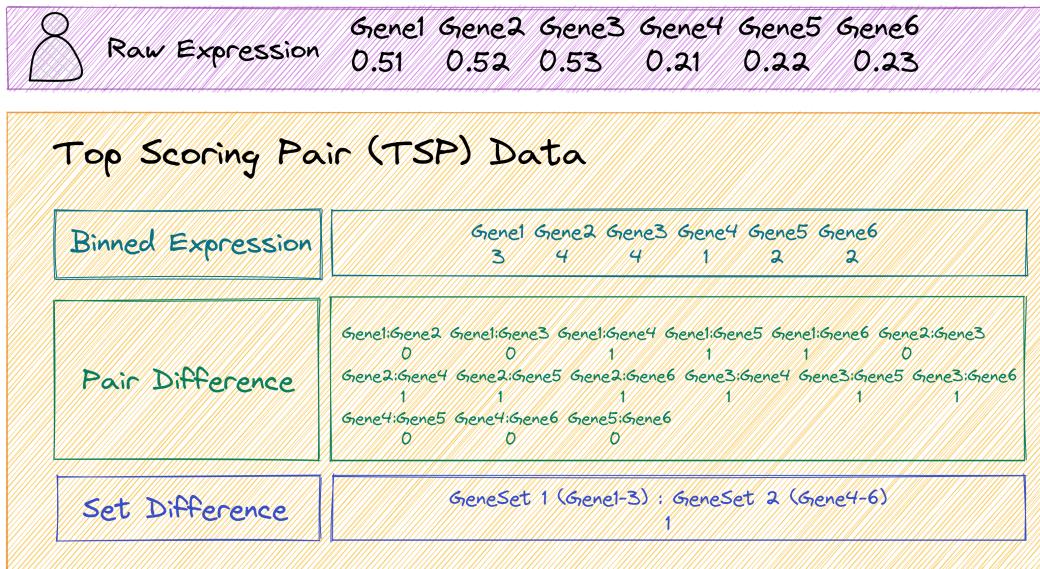


Figure 1.2: The components of TSP (2 gene sets)

1.5.1 Binned expression

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

Take **Sample4** as an example:

```
# Data of Sample4

x <- expr[,4]

# 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)
names(xbin) <- names(x)

# Report
cat('Quantiles:', '\n'); print(brks)
cat('\n')
cat('Raw expression:', '\n'); print(x)
cat('\n')
cat('Binned expression:', '\n'); print(xbin)
# Quantiles:
#      0%    25%    50%    75%   100%
# 0.110 0.215 0.466 0.515 0.530
#
# Raw expression:
# Gene1 Gene2 Gene3 Gene4 Gene5 Gene6 Gene7
```

```
# 0.210 0.220 0.466 0.510 0.520 0.530 0.110
#
# Binned expression:
# Gene1 Gene2 Gene3 Gene4 Gene5 Gene6 Gene7
#   1   2   2   3   4   4   1
```

For example, **0.110** is the minimum of the raw expression vector, so its binned expression is **1**. Similarly, the binned expression of maximum **0.530** is **4**.

Generally, we calculate 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      4      4
# Gene5      2      2      2      4      4      3
# Gene6      2      1      1      4      3      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 pair difference 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 and save computing resources. In all, genes with low rank difference should

be dropped out in some extent in **GSClassifier**.

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    6.5    6.5
# Gene5    3.5    3.5    3.5    6.5    6.5    5.0
# Gene6    3.5    1.5    1.5    6.5    5.0    6.5
# Gene7    1.5    1.5    1.5    1.5    1.5    1.5
```

Then, get weighted average rank difference of each gene based on specified subtype distribution (**Ybin**):

```
testRes <- sapply(
  1:nrow(expr_binned_rank),
  function(gi){

    # Rank vector of each gene
    rankg = expr_binned_rank[gi,];

    # Weighted average rank difference of a gene for specified subtype
    # Here is subtype 1 vs. others
    (sum(rankg[Ybin == 0], na.rm = T) / sum(Ybin == 0, na.rm = T)) -
    (sum(rankg[Ybin == 1], na.rm = T) / sum(Ybin == 1, na.rm = T))
  }
)
```

```

    }
)

names(testRes) <- rownames(expr_binned_rank)
print(testRes)

#      Gene1      Gene2      Gene3      Gene4      Gene5      Gene6      Gene7
# -2.666667 -2.500000 -4.333333  3.166667  2.500000  3.833333  0.000000

```

Gene7 is the one with the lowest absolute value (0) of rank difference. By the way, **Gene 1-3** have the same direction (<0), so do **Gene 4-6** (>0), which indicates the nature of clustering based on these two gene sets.

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

```

# ptail is a number 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)

# 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. By the way, both **ptail** and **breakVec** are hyperparameters in GSClassifier modeling.

1.5.2 Pair difference

In GSClassifier, we use a ensemble function **featureSelection** to select data for pair difference scoring.

```
expr_feat <- featureSelection(expr, Ybin,
                                testRes = testRes,
                                ptail = 0.4)

expr_sub <- expr_feat$Xsub
gene_bigRank <- expr_feat$Genes

# Report
cat('Raw xpression without NA:', '\n')
print(expr_sub)
cat('\n')
cat('Genes with large rank diff:', '\n')
print(gene_bigRank)

# Raw xpression without NA:
#       Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1    0.51    0.52    0.60   0.210    0.300    0.40
# Gene2    0.52    0.54    0.58   0.220    0.310    0.35
# Gene3    0.53    0.60    0.61   0.466    0.290    0.30
# Gene4    0.21    0.30    0.40   0.510    0.520    0.60
# Gene5    0.22    0.31    0.35   0.520    0.540    0.58
```

```
# Gene6      0.23     0.29     0.30    0.530    0.392    0.61
#
# Genes with large rank diff:
# [1] "Gene1" "Gene2" "Gene3" "Gene4" "Gene5" "Gene6"
```

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      1      0
# Gene5:Gene6      0      1      1      0      1      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. In addition, the difference of gene pair scoring between **Sample 1-3** and

Sample 4-6 is obvious, revealing the robustness of pair difference for subtype identification.

1.5.3 Set difference

In GSClassifier, **Set difference** is defined as a weight average of gene-geneset rank difference.

```
# No. of gene sets
nGS = 2

# Name of gene set comparision, which is like s1s2, s1s3 and so on.
featureNames <- 's1s2'

# Gene set difference across samples
resultList <- list()

for (i in 1:ncol(expr_sub)) { # i=1
  res0 <- numeric(length=length(featureNames))
  idx <- 1
  for (j1 in 1:(nGS-1)) { # j1=1
    for (j2 in (j1+1):nGS) { # j2=2
      # If j1=1 and j2=2, gene sets s1/s2 would be selected

      # Genes of different gene sets
      set1 <- geneSet[[j1]] # "Gene1" "Gene2" "Gene3"
      set2 <- geneSet[[j2]] # "Gene4" "Gene5" "Gene6"

      # RNA expression of Genes by different gene sets
      vals1 <- expr_sub[rownames(expr_sub) %in% set1,i]
      # Gene1 Gene2 Gene3
      # 0.51 0.52 0.53
```

```

vals2 <- expr_sub[rownames(expr_sub) %in% set2,i]
# Gene4 Gene5 Gene6
# 0.21 0.22 0.23

# Differences between one gene and gene sets
# Compare expression of each gene in Set1 with all genes in Set2.
# For example, 0.51>0.21/0.22/0.23, so the value of Gene1:s2 is 3.
res1 <- sapply(vals1, function(v1) sum(v1 > vals2, na.rm=T))
# Gene1:s2  Gene2:s2  Gene3:s2
# 3          3          3

# Weight average of gene-geneset rank difference
res0[idx] <- sum(res1, na.rm = T) / (length(vals1) * length(vals2))

# Next gene set pair
idx <- idx + 1
}

}
resultList[[i]] <- as.numeric(res0)
}

resMat <- do.call(cbind, resultList)
colnames(resMat) <- colnames(expr_sub)
rownames(resMat) <- featureNames

# Report
cat('Set difference across samples: ', '\n')
print(resMat)

# Set difference across samples:
#      Sample1 Sample2 Sample3 Sample4 Sample5 Sample6

```

```
# s1s2      1      1      0      0      0
```

In **GSClassifier**, we established **makeSetData** to evaluate set difference across samples:

```
# Gene set difference across samples
geneset_interaction <- GSClassifier:::makeSetData(expr_sub, geneSet)

# Report
cat('Set difference across samples: ', '\n')
print(resMat)

# Set difference across samples:
#      Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# s1s2      1      1      0      0      0
```

We have known that the subtype of **Sample 1-3** differs from that of **Sample 4-6**, which revealed the robustness of set difference for subtype identification.

As shown in Figure 1.2, TSP matrix here should be :

```
# TSP matrix
tsp <- rbind(
  # Binned expression
  expr_binned[gene_bigRank,],
  # Pair difference
  gene_bigRank_pairs,
  # Set difference
  resMat
)
```

```

# Report

cat('TSP matrix: ', '\n')
print(tsp)

# TSP matrix:

#          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      4      4
# Gene5      2      2      2      4      4      3
# Gene6      2      1      1      4      3      4
# 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      1      0
# Gene5:Gene6 0      1      1      0      1      0
# s1s2       1      1      1      0      0      0

```


Chapter 2

Discussion

2.1 Model complexity

Linear growth/exponential growth

2.2 Missing value

Talk about strategy in subtypes modeling and calling

2.3 Subtype vector calling