

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 **GSClassifier** package 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 [2.4](#).

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

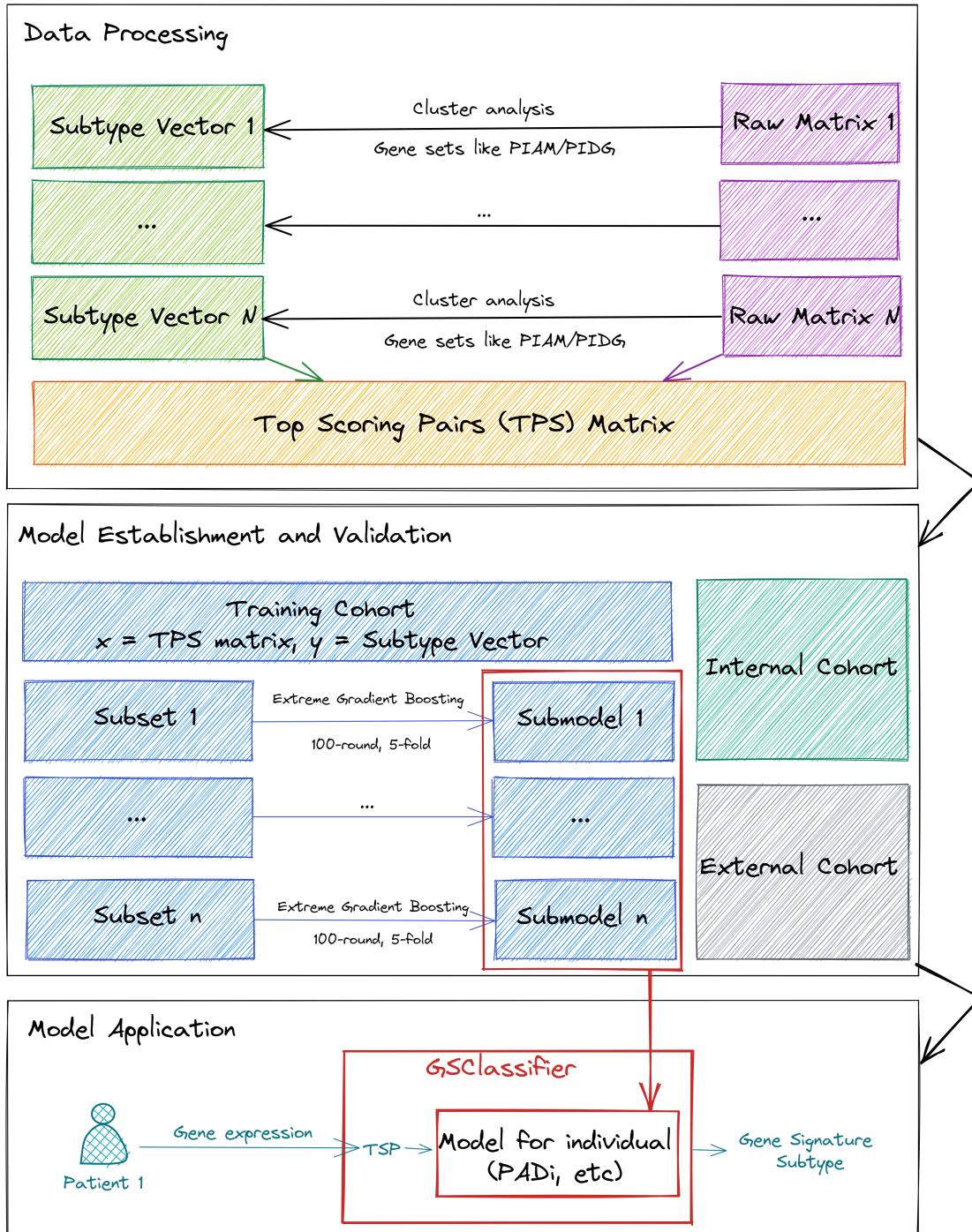


Figure 1.1: The flow chart of GSClassifier

sion 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 [1.3](#).

1.2.2 Model Establishment and Validation

The TSP matrix would be divided into training cohort and internal validation cohort. In PAD project, the rate of samples (training vs. test) is **7:3**. Next, each **Subset** (70% of the training cohort in PAD project) would be further selected randomly to build a **Submodel** via cross-validation Extreme Gradient Boosting algorithm (`xgboost::xgb.cv` function) [[Chen et al., 2015](#)]. The number of submodels is suggested over 20 (more details in [2.5](#)).

The internal validation cohort and external validation cohort (if any) would be used to test the performance of the trained model. By the way, **the data of both internal and external validation cohort would not be used during model training** so as to avoid over-fitting.

1.2.3 Model Application

In PAD project, **Model for individual**, the ensemble of submodels, is called “**PAD for individual**” (**PADI**). Supposed raw RNA expression of a sample was given. As showed in [1.1](#) and [1.2](#), **GSClassifier** would turn raw RNA expression into a TSP vector, which would be as an input to **Model for individual**. Then, **GSClassifier** would output the possibility matrix and the subtype for this sample. No extra data (RNA expression of others, follow-up data, etc) would be needed but RNA expression of the patient for subtype identification, so

we suggest **Model for individual (PADi, etc)** as personalized model.

1.3 Top scoring pairs

1.3.1 Introduction

Genes expression of an individual is normalized during the model training and the subtype identification via **Top Scoring Pairs (TSP, also called Relative Expression Orderings (REOs))** algorithm, which was previously described by Geman et al [Geman et al., 2004]. **TSP** normalization for an individual depends on its transcript data, implying that subtype calling would not be perturbed by data from other individuals or other extra information like follow-up data. **TSP** had been used in cancer research and effectively predicts cancer progression and ICIs response [Zhao et al., 2010, Youssef et al., 2011, Auslander et al., 2018].

As show in Figure 1.2, The TSP matrix in GSClassifier consists of three parts: **binned expression**, **pair difference**, and **set difference**. In this section, we would conduct some experiments to demonstrate the potential of TSP normalization for development of cross-dataset/platform GEP-based models.

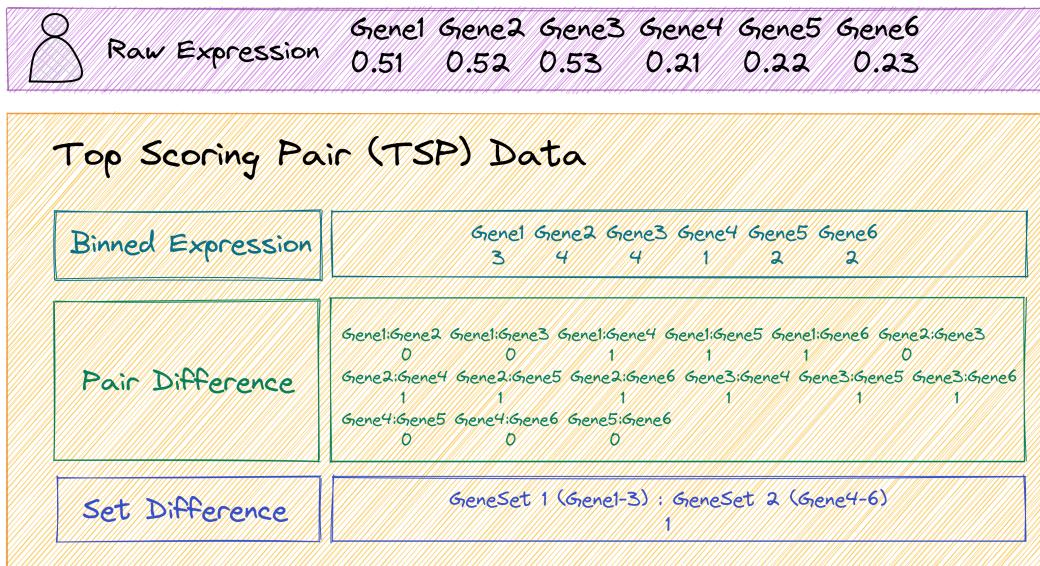


Figure 1.2: The components of TSP (2 gene sets)

1.3.2 Packages

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")

# 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",
"reshape2",
"ggplot2")

for(i in packages_needed){p_load(char=i)}

```

Here is the environment of R programming:

```

# R version 4.0.3 (2020-10-10)

# Platform: x86_64-w64-mingw32/x64 (64-bit)

# Running under: Windows 10 x64 (build 18363)

#
# Matrix products: default

#
# locale:

# [1] LC_COLLATE=Chinese (Simplified)_China.936
# [2] LC_CTYPE=Chinese (Simplified)_China.936
# [3] LC_MONETARY=Chinese (Simplified)_China.936
# [4] LC_NUMERIC=C
# [5] LC_TIME=Chinese (Simplified)_China.936
#
# attached base packages:

# [1] grid       stats      graphics  grDevices utils      datasets  methods
# [8] base
#
# other attached packages:

# [1] ggplot2_3.3.6        reshape2_1.4.4      tidyverse_1.2.0
# [4] rpart_4.1.16         GSClassifier_0.1.20  luckyBase_0.1.0
# [7] ComplexHeatmap_2.4.3  readxl_1.4.0      pacman_0.5.1
#
# loaded via a namespace (and not attached):

# [1] colorspace_2.0-3     ggsignif_0.6.3      rjson_0.2.21

```

```
# [4] ellipsis_0.3.2           class_7.3-20          rprojroot_2.0.3
# [7] circlize_0.4.15          GlobalOptions_0.1.2  fs_1.5.2
# [10] clue_0.3-57              rstudioapi_0.13       ggpubr_0.4.0
# [13] listenv_0.8.0            remotes_2.4.2         prodlim_2019.11.13
# [16] fansi_1.0.3              lubridate_1.8.0        codetools_0.2-18
# [19] splines_4.0.3            doParallel_1.0.17     cachem_1.0.6
# [22] knitr_1.30               pkgload_1.2.4         jsonlite_1.8.0
# [25] pROC_1.18.0              caret_6.0-92          broom_1.0.0
# [28] cluster_2.1.3            png_0.1-7             compiler_4.0.3
# [31] backports_1.4.1          assertthat_0.2.1      Matrix_1.2-18
# [34] fastmap_1.1.0            cli_3.3.0             htmltools_0.5.2
# [37] prettyunits_1.1.1         tools_4.0.3            gtable_0.3.0
# [40] glue_1.6.2                dplyr_1.0.9            Rcpp_1.0.8.3
# [43] carData_3.0-5             cellranger_1.1.0      vctrs_0.4.1
# [46] nlme_3.1-149              iterators_1.0.14      timeDate_3043.102
# [49] xfun_0.33                 gower_1.0.0            stringr_1.4.0
# [52] globals_0.15.1            ps_1.4.0              testthat_3.1.0
# [55] lifecycle_1.0.1           devtools_2.4.3         rstatix_0.7.0
# [58] future_1.26.1             MASS_7.3-53            scales_1.2.0
# [61] ipred_0.9-12              parallel_4.0.3         RColorBrewer_1.1-3
# [64] yaml_2.3.5                memoise_2.0.1           stringi_1.7.6
# [67] desc_1.4.1                randomForest_4.6-14    foreach_1.5.2
# [70] hardhat_1.1.0             pkgbuild_1.3.1          lava_1.6.10
# [73] shape_1.4.6               tuneR_1.4.0            rlang_1.0.2
# [76] pkgconfig_2.0.3            evaluate_0.15          lattice_0.20-41
# [79] purrrr_0.3.4              recipes_0.2.0           processx_3.7.0
# [82] tidyselect_1.1.2           parallelly_1.32.0      plyr_1.8.7
# [85] magrittr_2.0.3             bookdown_0.21           R6_2.5.1
# [88] generics_0.1.2             DBI_1.1.3              pillar_1.7.0
# [91] withr_2.5.0               survival_3.3-1          abind_1.4-5
```

```
# [94] nnet_7.3-17           tibble_3.1.7          future.apply_1.9.0
# [97] crayon_1.5.1            car_3.1-0             xgboost_1.6.0.1
# [100] utf8_1.2.2              rmarkdown_2.14        GetoptLong_1.0.5
# [103] usethis_2.1.3            data.table_1.14.2    callr_3.7.0
# [106] ModelMetrics_1.2.2.2    digest_0.6.29       stats4_4.0.3
# [109] signal_0.7-7             munsell_0.5.0        sessioninfo_1.2.2
```

1.3.3 Simulated Dataset

We simulated a dataset to demonstrate the principle of TSP normalization in GSClassifier:

```
# 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"

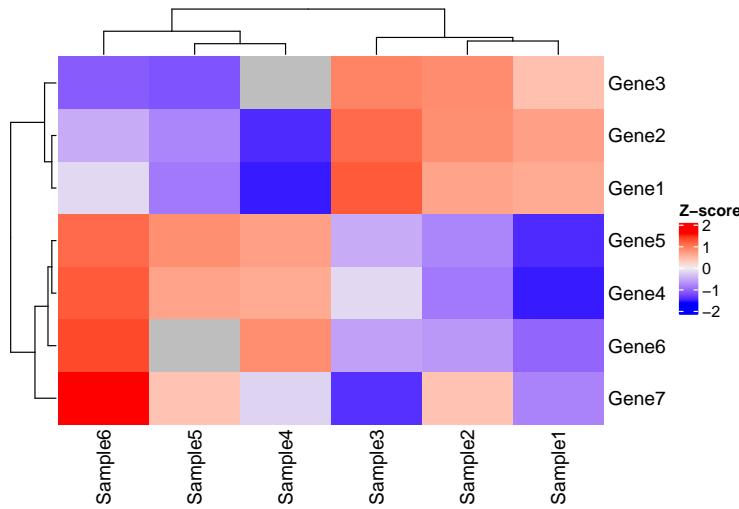
#
# RNA expression:

#       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.

1.3.4 Missing value imputation

Here, we do missing value imputation with quantile algorithm:

```
# Quantile algorithm
expr <- expr0
na.pos <- apply(expr, 2, is.one.na)
```

```

set.seed(478); seeds <- sample(1:ncol(expr)*10, sum(na.pos), replace = F)
tSample <- names(na.pos)[na.pos]
quantile_vector <- (1:1000)/1000
for(i in 1:length(tSample)){ # i=1

  sample.i <- tSample[i]
  expr.i <- expr[, sample.i]
  expr.i.max <- max(expr.i, na.rm = T)
  expr.i.min <- min(expr.i, na.rm = T)
  set.seed(seeds[i]);
  expr.i[is.na(expr.i)] <-
    expr.i.min +
    (expr.i.max-expr.i.min) * sample(quantile_vector,
                                      sum(is.na(expr.i)),
                                      replace = T)
  expr[, sample.i] <- expr.i
}

# 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

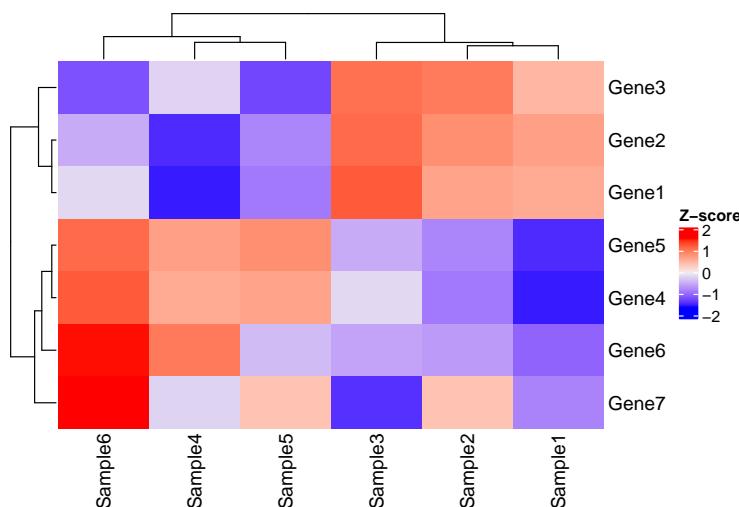
#
# RNA expression without NA value:

#           Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1      0.51   0.52   0.60  0.21000 0.30000   0.40
# Gene2      0.52   0.54   0.58  0.22000 0.31000   0.35
# Gene3      0.53   0.60   0.61  0.43256 0.29000   0.30
# Gene4      0.21   0.30   0.40  0.51000 0.52000   0.60
# Gene5      0.22   0.31   0.35  0.52000 0.54000   0.58
# Gene6      0.23   0.29   0.30  0.53000 0.32622   0.61
# Gene7      0.10   0.12   0.09  0.11000 0.12000   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.

1.3.5 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%
# 0.11000 0.21500 0.43256 0.51500 0.53000
#
# Raw expression:
# Gene1   Gene2   Gene3   Gene4   Gene5   Gene6   Gene7
# 0.21000 0.22000 0.43256 0.51000 0.52000 0.53000 0.11000
#
# 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_2^n , the removement of genes like **Gene7** before modeling could really reduce the complexity 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.

1.3.6 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 expression without NA:

#           Sample1 Sample2 Sample3 Sample4 Sample5 Sample6
# Gene1      0.51    0.52    0.60  0.21000  0.30000    0.40
# Gene2      0.52    0.54    0.58  0.22000  0.31000    0.35
# Gene3      0.53    0.60    0.61  0.43256  0.29000    0.30
# Gene4      0.21    0.30    0.40  0.51000  0.52000    0.60
# Gene5      0.22    0.31    0.35  0.52000  0.54000    0.58
# Gene6      0.23    0.29    0.30  0.53000  0.32622    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.

1.3.7 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      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      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.

Based on the structure of TSP in Figure 1.2, TSP matrix of the simulated dataset 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

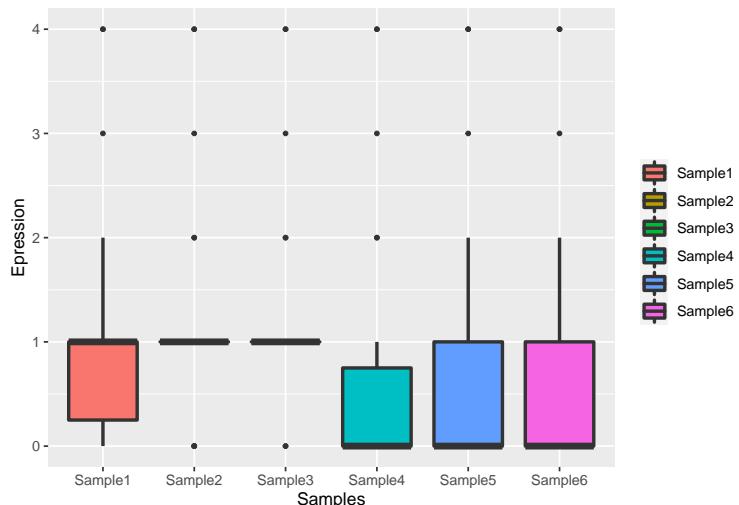
```

# 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

Have a look at the distribution:

```
# Data
tsp_df <- reshape2::melt(tsp)

ggplot(tsp_df,aes(x=Var2,y=value,fill=Var2)) +
  geom_boxplot(outlier.size = 1, size = 1) +
  labs(x = 'Samples',
       y = 'Expression',
       fill = NULL)
```



1.3.8 Batch effect

TSP was widely applied to control batch effects in transcriptomic data [Zhu et al., 2022, Tong et al., 2016, Qi et al., 2016, Huang et al., 2020, Zheng et al., 2021, Kong et al., 2022, Liu et al., 2022, Zheng et al., 2022]. Still, we tested whether **TSP** is a robust method for batch effect control in real-world data. As demonstrated in Figure 1.3, the obvious batch effects across gastric cancer datasets were significantly reduced after **TSP** normalization.

In order to confirmed the association between **gene counts** in modeling and batch effect control via **TSP** normalization, we selected random genes with counts ranging 4, 8, 20, 40, and 80 for TSP matrix establishment. As shown in Figure 1.4, **TSP** normalization works greatly in different gene counts for batch effect control compared with raw expression matrix.

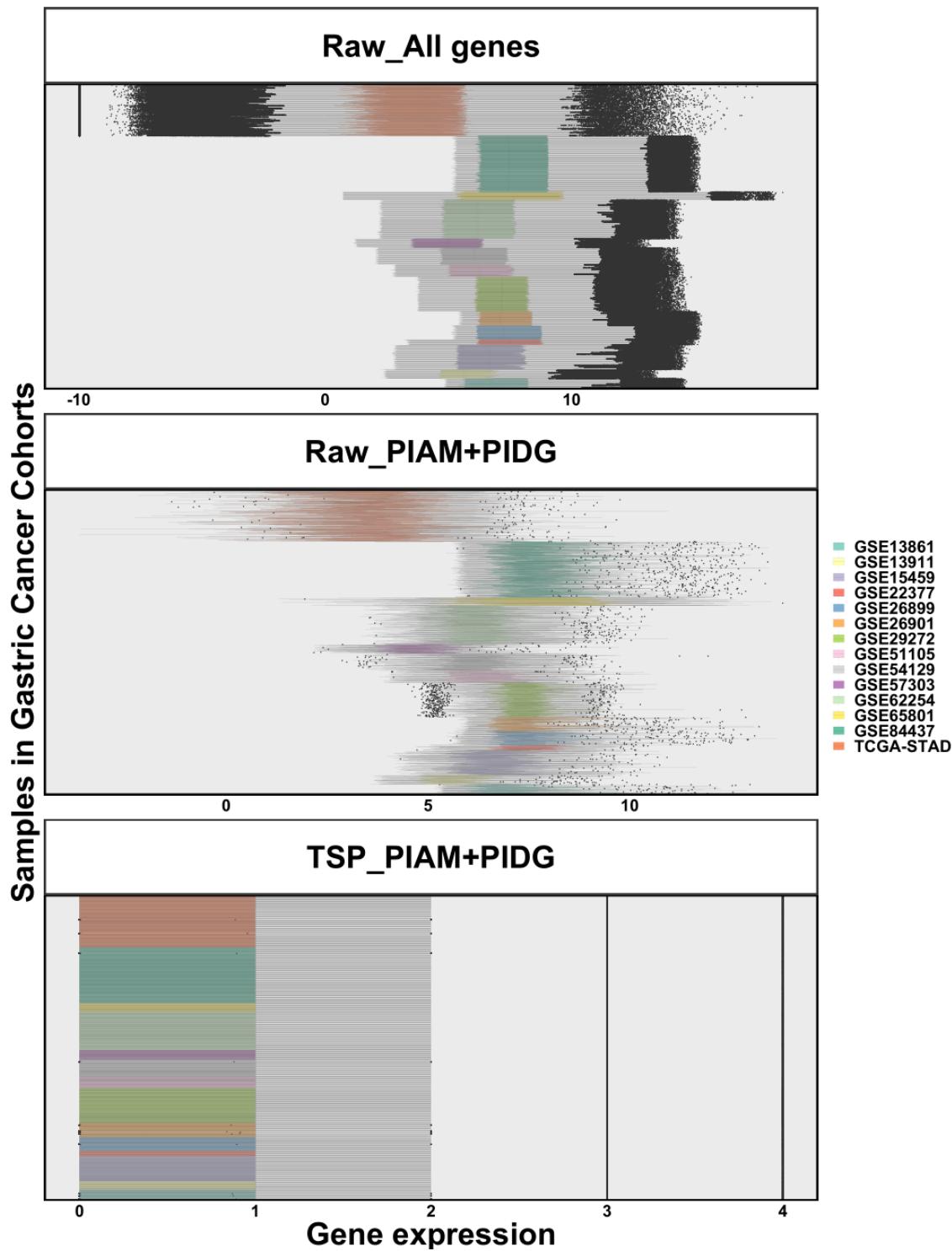


Figure 1.3: Batch effects across gastric cancer cohorts. All gene pairs were used because subtype vectors were not specified. Top: Raw expression of all genes across samples. Middle: Raw expression of PIAM and PIDG across samples. Bottom: TSP of PIAM and PIDG across samples.

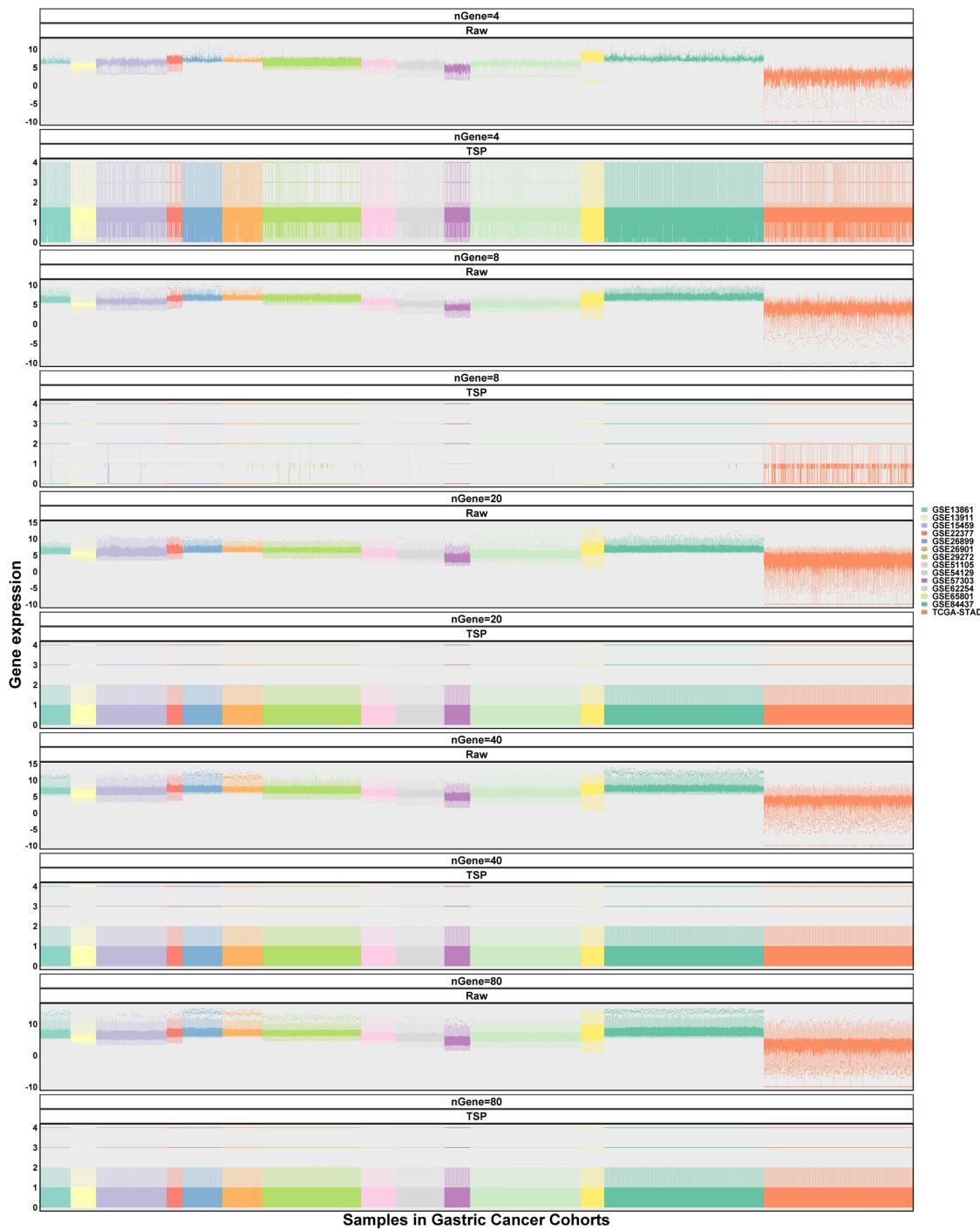


Figure 1.4: Batch effects of random genes across gastric cancer cohorts. All gene pairs were used because subtype vectors were not specified. Gene counts 4, 8, 20, 40, and 80 were detected. Data of set difference were not available because only one gene set were applied.

Chapter 2

Discussion

2.1 Model complexity

Linear growth/exponential growth

2.2 Model extensibility

How could

2.3 Missing value

Talk about strategy in subtypes modeling and calling

2.4 Subtype vector calling

2.5 The number of submodels

Talk about the number of submodels we should train.

Chapter 3

Quick start

3.1 About

- Although with bright prospects in Pan-disease analysis, `GSClassifier` was primarily developed for clinic-friendly immune subtypes of gastric cancer (GC). Currently, only PAD subtypes and PADI for GC were supported. We would try to support more cancer types in the future as possible. More details in [Plans in the future](#) section.
- Gibbs' PanCancer immune subtypes based on five gene signatures (485 genes) could also be called in `GSClassifier`, with a pre-trained model from the [ImmuneSubtype-Classifier](#) package. If you use their jobs, please cite: [references](#).
- Particularly, all normal tissues should be eliminated before subtypes calling for cancer research.

3.2 Data

To lower the learning cost of `GSClassifier`, we provides some test data:

```
library(GSClassifier)
#      luckyBase
testData <- readRDS(system.file("extdata", "testData.rds", package = "GSClassifier"))
```

Explore the `testData`:

```
names(testData)
# [1] "Kim2018_3"                      "PanSTAD_phenotype_part" "PanSTAD_expr_part"
```

3.3 PAD

3.3.1 Preparation of the test data

load phenotype data:

```
design <- testData$PanSTAD_phenotype_part
table(design$Dataset)

#
#   GSE13861  GSE13911  GSE15459  GSE22377  GSE26899  GSE26901  GSE29272  GSE51105
#       65        39       192        43        96       108       134        94
#   GSE54129  GSE57303  GSE62254  GSE65801  GSE84437 TCGA-STAD
#      111        70       300        32       433       372
```

load target sample IDs in GSE54129 cohort:

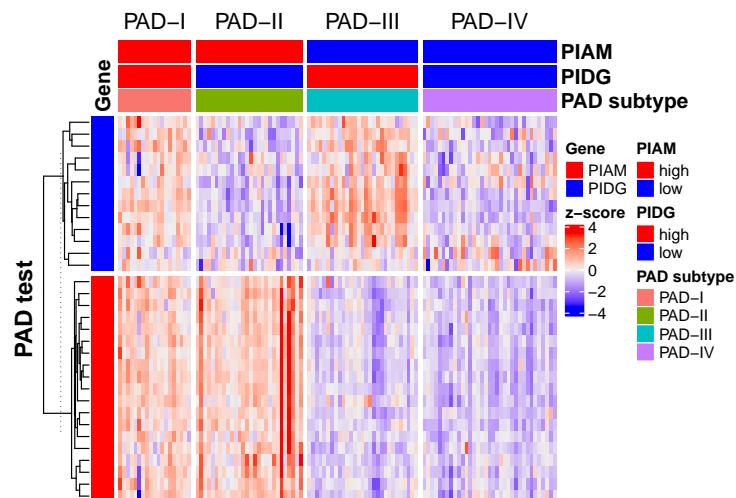
```
target_ID <- design$ID[design$Dataset %in% 'GSE54129']
expr <- testData$PanSTAD_expr_part[,target_ID]
head(expr[,1:10])

#
#           GSM1308413  GSM1308414  GSM1308415  GSM1308416  GSM1308417
# ENSG00000122122  7.888349   7.623663   6.873493   6.961102   7.150572
# ENSG00000117091  7.051760   6.217445   5.651839   5.830996   5.908532
# ENSG00000163219  6.056472   5.681844   5.411533   5.652684   5.555147
# ENSG00000136167  9.322191   8.765794   8.502315   8.838166   8.845952
# ENSG00000005844  7.119594   6.023631   5.400999   6.172863   6.059838
# ENSG00000123338  7.204051   6.925328   6.259809   6.610681   6.595882
#           GSM1308418  GSM1308419  GSM1308420  GSM1308421  GSM1308422
# ENSG00000122122  7.871423   6.953329   8.334037   6.764335   6.522554
```

# ENSG00000117091	6.526917	5.646446	6.617520	5.637693	5.742848
# ENSG00000163219	5.962885	5.361763	5.975842	5.330428	5.172705
# ENSG00000136167	9.366074	8.675718	9.118517	8.614068	8.114096
# ENSG00000005844	6.523530	6.129181	7.331588	5.547059	5.867118
# ENSG00000123338	6.699790	6.935390	7.050288	6.536710	6.200269

3.3.2 Unsupervised clustering

```
res_pad <- PAD(
  expr = expr,
  cluster.method = "ward.D2",
  extra.annot = NULL,
  plot.title = 'PAD test',
  subtype = "PAD.train_20200110",
  verbose = T
)
```



```
# Use default PIAM...
# Use default PIDG...
# Gene match: 100%.
# Done!
```

3.3.3 Of note

- It's strongly recommended that the gene type of `expr` should be alway the same, such as ENSEMBL genes (ENSG00000111640 for GAPDH, for examples).
- PAD function is only for datasets with lots of samples for its classification depends on population-based unsupervised clustering. PAD is population-dependent and non-personalized.
- Beta characteristics: You could try random forest classification based on the `randomForest` package or methods in `stats::hclust`.

3.4 PADI

- In `GSClassifier`, PADI is a pre-trained out-of-the-box model for GC personalized PAD subtypes calling.
- During the subtype calling, the gene rank relations based on individuals instead of the relative values across samples would be used. Thus, **you don't have to do batch normalization** even though the data (the `X` input) come from multiple cohorts or platform.
- More limitations were discussed in our paper that you had better know.

In this section, we would showed how to use PADI series: `PADI`, `callEnsemble`, and `parCallEnsemble` functions.

3.4.1 Preparation of the test data

```
X <- testData$Kim2018_3
head(X)
#
#           PB-16-002   PB-16-003   PB-16-004
# ENSG00000121410  0.07075272 -2.08976724 -1.43569557
# ENSG00000148584 -1.49631022 -0.23917056  0.94827471
```

```
# ENSG00000175899 -0.77315329 0.52163146 0.91264015
# ENSG00000166535 -0.28860715 -0.45964255 -0.38401295
# ENSG00000256069 -0.25034243 0.06863867 0.14429081
# ENSG00000184389 0.08215945 -0.05966481 0.04937924
```

3.4.2 Use a specific function called PADi

Very simple, just:

Check the result:

```
head(res_padi)
#   SampleIDs BestCall BestCall_Max      1      2      3      4
# 1 PB-16-002        4 0.023372779 0.02631794 0.04864784 0.3336484
# 2 PB-16-003        4 0.007271698 0.08650742 0.01974812 0.9530730
# 3 PB-16-004        4 0.011559768 0.02922151 0.09018894 0.8649045
```

Actually, PADi is exactly based on a general function called callEnsemble.

3.4.3 Use the callEnsemble function

Also simple, just:

Check the result:

```
head(res_padi)
#   SampleIDs BestCall BestCall_Max      1      2      3      4
# 1 PB-16-002        4 0.023372779 0.02631794 0.04864784 0.3336484
# 2 PB-16-003        4 0.007271698 0.08650742 0.01974812 0.9530730
# 3 PB-16-004        4 0.011559768 0.02922151 0.09018894 0.8649045
```

3.4.4 Parallel strategy for PADi

- Sometimes, the number of patients for subtype callings could be huge (hundreds or even tens of thousands). Thus, the parallel computing (Windows or Linux pass; not tested in Mac or other OS) was also developed in the current version of **GSClassifier** package.
- The parameter **numCores** was used to control the No. of CPU for computing (which depends on your CPU capacity).

```
# No run for the tiny test data. With errors.
```

```
# Method 1:
```

```
res_padi <- PADi(X = X, verbose = F, numCores = 4)
```

```
# Method 2:
```

```
res_padi <- parCallEnsemble(
  X = X,
  ens = NULL,
  geneAnnotation = NULL,
  geneSet = NULL,
  scaller = NULL,
  geneids = 'ensembl',
  subtype = 'PAD.train_20200110',
  verbose = T,
  numCores = 4)
```

3.4.5 Single sample subtype calling

In clinical practice, the single sample subtype calling might be one of the most common scenarios and is also supported by functions of **PADi** series.

Supposed that there is a GC patient, its information should be:

```
X_ind <- X[,1]; names(X_ind) <- rownames(X)
head(X_ind)

# ENSG00000121410 ENSG00000148584 ENSG00000175899 ENSG00000166535 ENSG00000256069
#      0.07075272     -1.49631022     -0.77315329     -0.28860715     -0.25034243
# ENSG00000184389
#      0.08215945
```

Or it can also be another format:

```
X_ind <- as.matrix(X[,1]); rownames(X_ind) <- rownames(X)
head(X_ind)

#                  [,1]
# ENSG00000121410 0.07075272
# ENSG00000148584 -1.49631022
# ENSG00000175899 -0.77315329
# ENSG00000166535 -0.28860715
# ENSG00000256069 -0.25034243
# ENSG00000184389 0.08215945
```

Similar to multiples sample calling, just:

check the result:

```
head(res_padi)

#   SampleIDs BestCall BestCall_Max      1      2      3      4
# 1    target        4  0.02337278 0.02631794 0.04864784 0.3336484
```

Similarly, there is alternative choice:

Check the result:

```
head(res_padi)

#   SampleIDs BestCall BestCall_Max      1      2      3      4
# 1    target        4  0.02337278 0.02631794 0.04864784 0.3336484
```

3.4.6 Of note

- In the results of PADi, two types of subtypes (`BestCall` and `BestCall_Max`) were integrated. `BestCall` was predicted based on a xgboost-trained model based on prior knowlege of PAD subtypes and the possibility matrix (columns 4 to 7 of four-subtype calling, for example), while `BestCall_Max` was predicted via maximum strategy. You should use THE SAME ONE in a specific practice no matter which one you use.
- PADi is individual-dependent and personalized, which means that the result of subtype calling would not be influenced by the data of others.

3.5 Use external models from luckyModel package

In the future, there might be lots of models available as a resource of `GSClassifier`, such as `luckyModel`. Here we show how `luckyModel` support `GSClassifier`.

First, intall and load `luckyModel`:

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

Check projects supported in current `luckyModel`:

```
list_project()
# [1] "GSClassifier"
```

Check available models in the project:

```
list_model(project='GSClassifier')
# Available models in GSClassifier:
#   *Gibbs_PanCancerImmuneSubtype_v20190731
#   *HWB_PAD_v20200110
```

Here, `HWB_PAD_v20200110` is a standard name of PADi. They are exactly the same.

Taking PAdi as an example, we here show how to use an external model from luckyModel.

First, load a model:

```
model <- lucky_model(project = 'GSClassifier',
                      developer='HWB',
                      model = 'PAD',
                      version = 'v20200110')
```

Then, check the gene id type:

```
model$geneSet

# $PIAM

# [1] "ENSG00000122122" "ENSG00000117091" "ENSG00000163219" "ENSG00000136167"
# [5] "ENSG0000005844" "ENSG00000123338" "ENSG00000102879" "ENSG00000010671"
# [9] "ENSG00000185862" "ENSG00000104814" "ENSG00000134516" "ENSG00000100055"
# [13] "ENSG00000082074" "ENSG00000113263" "ENSG00000153283" "ENSG00000198821"
# [17] "ENSG00000185811" "ENSG00000117090" "ENSG00000171608"

#
# $PIDG

# [1] "ENSG00000116667" "ENSG00000107771" "ENSG00000196782" "ENSG00000271447"
# [5] "ENSG00000173517" "ENSG00000134686" "ENSG00000100614" "ENSG00000134247"
# [9] "ENSG00000109686" "ENSG00000197321" "ENSG00000179981" "ENSG00000187189"
# [13] "ENSG00000140836"
```

The model should use `ensembl` as the value of `geneid` parameter in `callEnsemble` series.

Next, you can use the model like:

Or just:

They are exactly the same.

Finally, check the result:

```
head(res_padi)

#   SampleIDs BestCall BestCall_Max      1      2      3      4
# 1 PB-16-002        4  4 0.023372779 0.02631794 0.04864784 0.3336484
# 2 PB-16-003        4  4 0.007271698 0.08650742 0.01974812 0.9530730
# 3 PB-16-004        4  4 0.011559768 0.02922151 0.09018894 0.8649045
```

3.6 PanCancer immune subtypes

`GSClassifier` could also call the PanCancer immune subtypes of Gibbs'.

First, see data available in current `GSClassifier`:

```
GSClassifier_Data()

# Available data:
# Usage example:
#   ImmuneSubtype.rds
#   PAD.train_20200110.rds
#   PAD.train_20220916.rds
#   PAD <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClassi
#   ImmuneSubtype <- readRDS(system.file("extdata", "ImmuneSubtype.rds", package = "GS
```

Let's use our test data to do this:

```
X <- testData$Kim2018_3
symbol <- convert(rownames(X))
rownames(X) <- symbol
X <- X[!is.na(symbol),]
dim(X)
# [1] 19118      3
```

Have a check

```
head(X)

#          PB-16-002   PB-16-003   PB-16-004
# A1BG      0.07075272 -2.08976724 -1.43569557
# A1CF     -1.49631022 -0.23917056  0.94827471
# A2M      -0.77315329  0.52163146  0.91264015
# A2ML1    -0.28860715 -0.45964255 -0.38401295
# RP11-118B22.6 -0.25034243  0.06863867  0.14429081
# A3GALT2     0.08215945 -0.05966481  0.04937924
```

PanCan Immune Subtype callings:

Check the result:

```
head(res_pis)

#   SampleIDs BestCall BestCall_Max           1           2           3
# 1 PB-16-002      2      2 9.677974e-04 0.561300665 0.0000031733
# 2 PB-16-003      4      4 4.963801e-07 0.018167170 0.0001958085
# 3 PB-16-004      3      3 5.107389e-06 0.001126488 0.2720691785
#               4           5           6
# 1 0.2133894339 0.007000939 0.005137485
# 2 0.3924977928 0.002049010 0.002650601
# 3 0.0001744295 0.006241082 0.004441003
```

Also, you can try to use luckyModel:

```
pci <- lucky_model(
  project = "GSClassifier",
  model = "PanCancerImmuneSubtype",
  developer = "Gibbs",
  version = "v20190731"
)
```

PanCan Immune Subtype callings:

Finally, we take a look at the PanCancer immune subtypes model:

```
ImmuneSubtype <- readRDS(system.file("extdata", "ImmuneSubtype.rds", package = "GSClassi
names(ImmuneSubtype)
# [1] "ens"           "scaller"        "geneAnnotation" "geneSet"
```

Its gene annotation:

```
head(ImmuneSubtype$geneAnnotation)
```

	<i>SYMBOL</i>	<i>ENTREZID</i>	<i>ENSEMBL</i>
# 235	<i>ACTL6A</i>	86	<i>ENSG00000136518</i>
# 294	<i>ADAM9</i>	8754	<i>ENSG00000168615</i>
# 305	<i>ADAMTS1</i>	9510	<i>ENSG00000154734</i>
# 322	<i>ADAR</i>	103	<i>ENSG00000160710</i>
# 340	<i>ADCY7</i>	113	<i>ENSG00000121281</i>
# 479	<i>AIMP2</i>	7965	<i>ENSG00000106305</i>

Its gene sets:

```
ImmuneSubtype$geneSet
# $LIexpression_score
# [1] "CCL5"   "CD19"   "CD37"   "CD3D"   "CD3E"   "CD3G"   "CD3Z"   "CD79A"  "CD79B"
# [10] "CD8A"   "CD8B1"  "IGHG3"  "IGJ"    "IGLC1"  "CD14"   "LCK"    "LTB"    "MS4A1"
#
# $CSF1_response
# [1] "CORO1A"  "MNDA"   "CCRL2"  "SLC7A7"  "HLA-DMA" "FYB"
# [7] "RNASE6"  "TLR2"   "CTSC"   "LILRB4"  "PSCDBP"  "CTSS"
# [13] "RASSF4"  "MSN"    "CYBB"   "LAPTM5"  "DOCK2"   "FCGR1A"
# [19] "EVI2B"   "ADCY7"  "CD48"   "ARHGAP15" "ARRB2"   "SYK"
# [25] "BTK"     "TNFAIP3" "FCGR2A" "VSIG4"   "FPRL2"   "IL10RA"
# [31] "IFI16"   "ITGB2"  "IL7R"   "TBXAS1"  "FMNL1"   "FLI1"
# [37] "RASSF2"  "LYZ"    "CD163"  "CD97"   "CCL2"    "FCGR2B"
# [43] "MERTK"   "CD84"   "CD53"   "CD86"   "HMHA1"   "CTSL"
```

```

# [49] "EVI2A"      "TNFRSF1B"   "CXCR4"      "LCP1"       "SAMHD1"     "CPVL"
# [55] "HLA-DRB1"    "C13orf18"   "GIMAP4"     "SAMSN1"     "PLCG2"      "OSBPL3"
# [61] "CD8A"        "RUNX3"      "FCGR3A"     "AMPD3"      "MYO1F"      "CECR1"
# [67] "LYN"         "MPP1"       "LRMP"       "FGL2"       "NCKAP1L"    "HCLS1"
# [73] "SELL"        "CASP1"      "SELPLG"     "CD33"       "GPNMB"     "NCF2"
# [79] "FNBP1"       "IL18"       "B2M"        "SP140"      "FCER1G"    "LCP2"
# [85] "LY86"        "LAIR1"      "IFI30"      "TNFSF13B"   "LST1"      "FGR"
# [91] "NPL"         "PLEK"       "CCL5"       "PTPRC"     "GNPTAB"    "SLC1A3"
# [97] "HCK"         "NPC2"       "C3AR1"     "PIK3CG"    "DAPK1"      "ALOX5AP"
# [103] "CSF1R"      "CUGBP2"     "APOE"       "APOC1"     "CD52"      "LHFPL2"
# [109] "C1orf54"    "IKZF1"      "SH2B3"     "WIPF1"      "#"
# $Module3_IFN_score
# [1] "IFI44"       "IFI44L"     "DDX58"      "IFI6"       "IFI27"      "IFIT2"      "IFIT1"      "IFIT3"
# [9] "CXCL10"      "MX1"        "OAS1"       "OAS2"      "OAS3"      "HERC5"      "SAMD9"      "HERC6"
# [17] "DDX60"       "RTP4"       "IFIH1"      "STAT1"     "TAP1"      "OASL"       "RSAD2"      "ISG15"
# $TGFB_score_21050467
# [1] "MMP3"        "MARCKSL1"   "IGF2R"      "LAMB1"     "SPARC"     "FN1"
# [7] "ITGA4"       "SMO"        "MMP19"     "ITGB8"     "ITGA5"     "NID1"
# [13] "TIMP1"       "SEMA3F"     "RHOQ"      "CTNNB1"    "MMP2"      "SERPINE1"
# [19] "EPHB2"       "COL16A1"    "EPHA2"     "TNC"       "JUP"       "ITGA3"
# [25] "TCF7L2"      "COL3A1"     "CDH6"      "WNT2B"     "ADAM9"     "DSP"
# [31] "HSPG2"       "ARHGAP1"    "ITGB5"     "IGFBP5"    "ARHGDI"    "LRP1"
# [37] "IGFBP2"      "CTNNA1"     "LRRK17"    "MMP14"     "NEO1"      "EFNA5"
# [43] "ITGB3"       "EPHB3"      "CD44"      "IGFBP4"    "TNFRSF1A"  "RAC1"
# [49] "PXN"         "PLAT"       "COL8A1"    "WNT8B"    "IGFBP3"    "RHOA"
# [55] "EPHB4"       "MMP1"       "PAK1"      "MTA1"      "THBS2"     "CSPG2"
# [61] "MMP17"       "CD59"       "DVL3"      "RHOB"      "COL6A3"    "NOTCH2"

```

```

# [67] "BSG"      "MMP11"     "COL1A2"     "ZYX"       "RND3"      "THBS1"
# [73] "RHOG"      "ICAM1"      "LAMA4"      "DVL1"      "PAK2"      "ITGB2"
# [79] "COL6A1"    "FGD1"

#
# $CHANG_CORE_SERUM_RESPONSE_UP

# [1] "CEP78"      "LSM3"       "LRRC40"     "STK17A"    "RPN1"      "JUNB"
# [7] "NUP85"      "FLNC"       "HMGN2"      "RPP40"     "UQCR10"    "AIMP2"
# [13] "CHEK1"      "VTA1"       "EXOSC8"     "CENPO"     "PNO1"      "SLC16A1"
# [19] "WDR77"      "UBE2J1"     "NOP16"      "NUDT1"     "SMC2"      "SLC25A5"
# [25] "NUPL1"      "DLEU2"      "PDAP1"      "CCBL2"     "COX17"     "BCCIP"
# [31] "PLG"        "RGS8"       "SNRPC"      "PLK4"      "NUTF2"     "LSM4"
# [37] "SMS"        "EBNA1BP2"   "C13orf27"   "VDAC1"     "PSMD14"    "MYCBP"
# [43] "SMURF2"     "GNG11"      "F3"         "IL7R"      "BRIP1"     "HNRNPA2B1"
# [49] "DCK"        "ALKBH7"     "HN1L"       "MSN"       "TPM1"      "HYLS1"
# [55] "HAUS1"      "NUP93"      "SNRPE"      "ITGA6"     "CENPN"     "C11orf24"
# [61] "GGH"        "PFKP"       "FARSA"      "EIF2AK1"   "CENPW"     "TUBA4A"
# [67] "TRA2B"      "UMPS"       "MRT04"      "NUDT15"    "PGM2"      "DBNDD1"
# [73] "SNRPB"      "MNAT1"      "NUP35"      "TCEB1"     "HSPB11"    "C19orf48"
# [79] "ID3"        "IPO4"       "FARSB"      "EIF4G1"    "SKA1"      "MFSD11"
# [85] "PLAUR"      "MARVELD2"   "MCM3"       "DHFR"     "RNF41"     "ID2"
# [91] "H2AFZ"      "CDK2"       "NCLN"       "ZWILCH"    "DYNLT1"    "C16orf61"
# [97] "SLC25A40"   "RHOC"       "CCT5"       "PDIA4"     "SNRPA"     "RBM14"
# [103] "PDLIM7"    "PITPNC1"   "TPM3"       "CORO1C"    "ERLIN1"    "PAICS"
# [109] "TPRKB"      "SKA2"       "MYBL1"      "SH3BP5L"   "BRCA2"     "SAR1A"
# [115] "POLR3K"    "MRPS28"    "NUP107"     "TUBG1"     "PNN"       "FAM167A"
# [121] "RFC3"       "MYL6"       "MCM7"       "MAGOHB"   "FAM89B"    "TOMM40"
# [127] "CDCA4"      "MT3"        "MTHFD1"     "PSMD12"   "MYBL2"     "CKLF"
# [133] "NRIP3"      "EZR"        "C12orf24"   "GPLD1"     "SRM"       "RAB3B"
# [139] "NLN"        "MT1F"       "TNFRSF12A"  "TPI1"     "HAS2"      "APOO"

```

# [145]	"FBXO41"	"MRPL37"	"GSTCD"	"SDC1"	"WDR54"	"RNF138"
# [151]	"APITD1"	"RMND5B"	"EN01"	"MAP3K8"	"TMEM130"	"SNX17"
# [157]	"KRR1"	"TAGLN"	"PA2G4"	"RUVBL1"	"SNRPD1"	"LOXL2"
# [163]	"POLE2"	"MAPRE1"	"IMP4"	"EMP2"	"PSMD2"	"MET"
# [169]	"IFRD2"	"LMNB2"	"PLOD2"	"NCEH1"	"NME1"	"STRA13"
# [175]	"ACTL6A"	"DLEU1"	"SNRPA1"	"CBX1"	"LYAR"	"PTPLB"
# [181]	"PFN1"	"CENPJ"	"COTL1"	"SPRYD7"	"USPL1"	"MRPL12"
# [187]	"ADAMTS1"	"GLRX3"	"WSB2"	"MRPS16"	"DCLRE1B"	"MKKS"
# [193]	"C3orf26"	"CPEB4"	"SPAG17"	"MLF1IP"	"UAP1"	"COQ2"
# [199]	"WDHD1"	"DCBLD2"	"KIAA0090"	"SAR1B"	"PSMA7"	"PSMC3"
# [205]	"COPS6"	"DUT"	"PPIH"	"PHF19"	"TPM2"	"MCTS1"
# [211]	"EIF4EBP1"	"HNRNPR"				

Enjoy GSClassifier!

Chapter 4

Model establishment via GSClassifier

4.1 About

Sometimes, researchers might have their own gene signatures and know how many subtypes they want to call before (based on some knowledges). Gratifyingly, comprehensive functions were also provided in `GSClassifier`. In this section, we would show how to build a `GSClassifier` model like PADi.

4.2 Data preparation

Note: The test data is only for the demonstration of the modeling

Load packages:

Load data:

```
testData <- readRDS(system.file("extdata", "testData.rds", package = "GSClassifier"))
expr <- testData$PanSTAD_expr_part
design <- testData$PanSTAD_phenotype_part
```

Select training and testing cohorts across different platforms and PAD subtypes from PAD function:

```
modelInfo <- modelData(
  design,
  id.col = "ID",
  variable = c("platform", "PAD_subtype"),
  Prop = 0.7,
  seed = 145
)
```

Check the result `modelInfo`:

```
names(modelInfo)

head(modelInfo$Data$Train)

head(modelInfo$Data$Valid)
# [1] "Repeat" "Data"
#           ID Dataset PAD_subtype PIAM_subtype PIDG_subtype platform
# GSM1606509 GSM1606509 GSE65801      PAD-I     high     high GPL14550
# GSM1606517 GSM1606517 GSE65801      PAD-I     high     high GPL14550
# GSM1606503 GSM1606503 GSE65801      PAD-I     high     high GPL14550
# GSM1606525 GSM1606525 GSE65801      PAD-I     high     high GPL14550
# GSM1606511 GSM1606511 GSE65801      PAD-I     high     high GPL14550
# GSM1606527 GSM1606527 GSE65801      PAD-I     high     high GPL14550
#           ID Dataset PAD_subtype PIAM_subtype PIDG_subtype platform
# GSM2235558 GSM2235558 GSE84437      PAD-I     high     high GPL6947
# GSM2235561 GSM2235561 GSE84437      PAD-II    high     low  GPL6947
# GSM2235562 GSM2235562 GSE84437      PAD-IV   low     low  GPL6947
# GSM2235563 GSM2235563 GSE84437      PAD-IV   low     low  GPL6947
# GSM2235564 GSM2235564 GSE84437      PAD-IV   low     low  GPL6947
# GSM2235567 GSM2235567 GSE84437      PAD-IV   low     low  GPL6947
```

Get training data Xs and Ys:

```
# Training data

Xs <- expr[,modelInfo$Data$Train$ID]

y <- modelInfo$Data$Train

y <- y[colnames(Xs),]

Ys <- ifelse(y$PAD_subtype == 'PAD-I', 1, ifelse(y$PAD_subtype == 'PAD-II', 2, ifelse(y$PAD_subtype == 'PAD-III', 3, 4)))

# Ys

#          1           2           3           4
# 0.1010169 0.2474576 0.1694915 0.4820339
```

Get the number of subtype:

```
# No. of subtypes

nSubtype <- length(unique(Ys))

print(nSubtype)

# [1] 4
```

Also, you can take a look at the validation data:

```
# Validating data

Xs_valid <- expr[,modelInfo$Data$Valid$ID]

y <- modelInfo$Data$Valid

y <- y[colnames(Xs_valid),]

Ys_valid <- ifelse(y$PAD_subtype == 'PAD-I', 1, ifelse(y$PAD_subtype == 'PAD-II', 2, ifelse(y$PAD_subtype == 'PAD-III', 3, 4)))

table(Ys_valid)/length(Ys_valid)

# Ys_valid

#          1           2           3           4
# 0.09609121 0.24592834 0.16612378 0.49185668
```

Note: When you convert your phenotype into numeric, **You CANNOT USE A ZERO VALUE**, which is not supported by the xGboost.

Other parameteres for modeling:

```
# Build 20 models
n=20

# In every model, 70% samples in the training cohort would be selected.
sampSize=0.7

# Seed for sampling
sampSeed = 2020
na.fill.seed = 2022

# A vector for approximate gene rank estimation
breakVec=c(0, 0.25, 0.5, 0.75, 1.0)

# Use 80% most variable gene & gene-pairs for modeling
ptail=0.8/2

# Automatical selection of parameters for xGboost
auto = F

if(!auto){

  # Self-defined params. Fast.
  params = list(max_depth = 10,
                eta = 0.5,
                nrounds = 100,
                nthread = 10,
                nfold=5)
  caret.seed = NULL}
```

```

# No. of CPU for parallel computing. The optimized value depends on your CPU and
numCores = 4

} else {

# caret::train strategy by GSClassifier:::cvFitOneModel2. Time consuming
params = NULL
caret.seed = 105

# Self-defined. For this example training grid, there are 2×1×1×3×2×1×2=24 grids
grid = expand.grid(
  nrounds = c(100, 200),
  colsample_bytree = 1,
  min_child_weight = 1,
  eta = c(0.01, 0.1, 0.3),
  gamma = c(0.5, 0.3),
  subsample = 0.7,
  max_depth = c(5,8)
)

# If you don't know how to set, just use the same number of your subtypes
numCores = 4
}

```

Finally, you have to provide your gene sets as a `list` object:

```
geneSet = <Your gene sets>
```

Let's take PAD as an example:

```
PAD <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClassifier"))
geneSet <- PAD$geneSet
```

```
print(geneSet)

# $PIAM

# [1] "ENSG00000122122" "ENSG00000117091" "ENSG00000163219" "ENSG00000136167"
# [5] "ENSG0000005844" "ENSG00000123338" "ENSG00000102879" "ENSG0000010671"
# [9] "ENSG00000185862" "ENSG00000104814" "ENSG00000134516" "ENSG00000100055"
# [13] "ENSG00000082074" "ENSG00000113263" "ENSG00000153283" "ENSG00000198821"
# [17] "ENSG00000185811" "ENSG00000117090" "ENSG00000171608"

#
# $PIDG

# [1] "ENSG00000116667" "ENSG00000107771" "ENSG00000196782" "ENSG00000271447"
# [5] "ENSG00000173517" "ENSG00000134686" "ENSG00000100614" "ENSG00000134247"
# [9] "ENSG00000109686" "ENSG00000197321" "ENSG00000179981" "ENSG00000187189"
# [13] "ENSG00000140836"
```

4.3 Fitting models

4.3.1 GSClassifier model training

Just fit the model like:

```
    n = n,
    sampSize = sampSize,
    sampSeed = sampSeed ,
    breakVec = breakVec,
    params = params,
    ptail = ptail,
    caret.grid = NULL,
    caret.seed = caret.seed,
    verbose = verbose,
    numCores = numCores)
)

# user    system   elapsed
# 0.08s  0.18s   92.70s

} else {

  # caret::train-defined and time-consuming
  system.time(
    res <- fitEnsembleModel(Xs,
      Ys,
      geneSet = geneSet,
      na.fill.method = c('quantile','rpart',NULL)[1],
      na.fill.seed = na.fill.seed,
      n = n,
      sampSize = sampSize,
      sampSeed = sampSeed ,
      breakVec = breakVec,
      params = NULL,  # This must be NULL
      ptail = ptail,
```

```
        caret.grid = grid,
        caret.seed = caret.seed,
        verbose = verbose,
        numCores = numCores)
    )
}

# user      system    elapsed
# 1.10s    2.60s    2311.55s

}

mymusic() # Remind me with a music when the process completed
```

You should save it for convenience:

```
saveRDS(res, '<your path>/train_ens.rds')
```

Although a `auto-parameter strategy` was provided in `GSClassifier`, it's unknown for this method to improve your model performance to what extent. You can just try. It's not a prior recommendation. In general, setting `auto=F` in this script could be more cost-effective. Empirically, the speed of `caret::train` depends on single-core performance of the CPU instead of the core number.

4.3.2 Scaller for the best call

Next, we model the `scaller` for the training cohort, which would be used for `BestCall` based on the probability matrix in `callEnsemble` series. Here, `scaller=NULL` would cause an NA value of `BestCall` col. It's not a big deal, because the probability matrix is the information we need.

```
        geneSet = geneSet,
        scaller = NULL,
        geneids = "ensembl",
        subtype = NULL,
        numCores = numCores)

# xgboost via best interation
library(xgboost)
dtrain <- xgb.DMatrix(as.matrix(resTrain[4:(3 + nSubtype)]), label = Ys-1)

cvRes <- xgb.cv(data = dtrain,
                  nrounds=100,
                  nthread=10,
                  nfold=5,
                  max_depth=5,
                  eta=0.5,
                  early_stopping_rounds=100,
                  num_class = 4,
                  objective = "multi:softmax")

# xgboost via best interation
bst <- xgboost(data = dtrain,
                 max_depth=5,
                 eta=0.5,
                 nrounds = cvRes$best_iteration,
                 nthread=10,
                 num_class = 4,
                 objective = "multi:softmax")

Ys_pred <- predict(bst, as.matrix(resTrain[4:7])) + 1
mean(Ys_pred == Ys) # Prediction rates
```

```
# Ensemble results
scaller.train <- list(
  Repeat = list(
    data = dtrain,
    max_depth=5,
    eta=0.5,
    nrounds = cvRes$best_iteration,
    nthread=10,
    num_class = 4,
    objective = "multi:softmax"
  ),
  Model = bst
)
```

4.3.3 Assemble your model

For more information of `geneAnnotation`, you could see [Advanced development: Gene Annotation](#) section for assistance.

Here we give an example:

```
l.train <- list()

# bootstrap models based on the training cohort
l.train[['ens']] <- res

# Scaller model
l.train[['scaller']] <- scaller.train

# a data frame containing gene annotation for IDs conversion
l.train[['geneAnnotation']] <- <Your gene annotation>
```

```
# Your gene sets
l.train[['geneSet']] <- geneSet

Finally, save it for downstream analysis

saveRDS(l.train, '<Your path>/train.rds')
```

About model contributions, you can go Advanced development in [here](#) or [here](#) for more information.

4.3.4 Of note

You can take a look at the `PAD.train_20200110` model (PADi). You have to make your model frame similar to the `PAD.train_20200110` model.

```
l.train <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClassifier"))
names(l.train)
# [1] "ens"           "scaller"        "geneAnnotation" "geneSet"
```

The time of `GSClassifier` modeling depends on the number of individual models (controlled by `n`)/called subtypes/gene signatures, automatic parameter selection, and your CPU capacity.

4.4 Calling subtypes

Supposed that you had got a `GSClassifier` model, next you want to use it for personalized subtype calling.

Just:

```
# Load your model
l <- readRDS('<Your path>/train.rds')

# subtype calling
```

```
res_i = callEnsemble(  
  X,  
  ens = l$ens$Model,  
  geneAnnotation = l$geneAnnotation,  
  geneSet = l$geneSet,  
  scaller = l$scaller$Model,  
  geneid = <ID type of your training data>,  
  subtype = NULL,  
  verbose = T  
)
```

The usage of `parCallEnsemble` (for huge amount of samples) is similar.

Chapter 5

Suggestions for GSClassifier model developers

5.1 About

- The book **R packages** is a straightaway and useful reference book for R developers. The free-access website of **R packages** is <https://r-pkgs.org/>. As a developer of R, if you haven't hear about it, it's strongly recommended to just read it. Hadley Wickham, the main author of the book, is an active R developer and have led some master works like **ggplot2** and **plyr**.
- With **GSClassifier** package, it could be easy for users to build a model only with certain gene sets and transcriptomics data. If you are interesting in sharing your model, **GSClassifier** also provides a simple methodology for this vision. In this section, let's see how to achieve it!

First, load the package

```
library(GSClassifier)  
#       luckyBase
```

5.2 Available models

With `GSClassifier_Data()`, all models supported in the current `GSClassifier` package would be showed.

```
GSClassifier_Data()

# Available data:

# Usage example:

# ImmuneSubtype.rds
# PAD.train_20200110.rds
# PAD.train_20220916.rds
# PAD <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClass
# ImmuneSubtype <- readRDS(system.file("extdata", "ImmuneSubtype.rds", package = "GS
```

For more details of `GSClassifier_Data()`, just:

```
?GSClassifier_Data()
```

Set `model=F`, all .rds data would be showed:

```
GSClassifier_Data(model = F)

# Available data:

# Usage example:

# general-gene-annotation.rds
# ImmuneSubtype.rds
# PAD.train_20200110.rds
# PAD.train_20220916.rds
# testData.rds
# PAD <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClass
# ImmuneSubtype <- readRDS(system.file("extdata", "ImmuneSubtype.rds", package = "GS
```

5.3 Components of a GSClassifier model

Currently, a GSClassifier model and related product environments is designed as a `list` object. Let's take `PAD.train_20210110`(also called PADi) as an example.

```
PADi <- readRDS(system.file("extdata", "PAD.train_20200110.rds", package = "GSClassifier"))
```

This picture shows the components of PADi:

As shown, a typical `GSClassifier` model is consist of four parts (with different colors in the picture):

- 1. `ens`:
 - Repeat: productive parameters of `GSClassifier` models
 - Model: `GSClassifier` models. Here, PADi had 20 models from different subs of the training cohorts
- 2. `scaller`:
 - Repeat: productive parameters of the `scaller` model, which was used for `BestCall` calling
 - Model: the `scaller` model
- 3. `geneAnnotation`: a data frame containing gene annotation information
- 4. `geneSet`: a list contains several gene sets

Thus, you can assemble your model like:

```
model <- list()

# bootstrap models based on the training cohort
model[['ens']] <- <Your model for subtypes calling>

# Scaller model
model[['scaller']] <- <Your scaller for BestCall calling>

# a data frame containing gene annotation for IDs conversion
```

Name	Type	Value
PADi	list [4]	List of length 4
ens	list [2]	List of length 2
Repeat	list [10]	List of length 10
Xs	list [32 x 1475] (S3: data.frame)	A data.frame with 32 rows and 1475 columns
Ys	double [1475]	1 1 1 1 1 1 ...
geneSet	list [2]	List of length 2
n	double [1]	20
sampSize	double [1]	0.7
sampSeed	double [1]	2020
breakVec	double [5]	0.00 0.25 0.50 0.75 1.00
params	list [5]	List of length 5
ptail	double [1]	0.4
numCores	double [1]	20
Model	list [20]	List of length 20
[[1]]	list [4]	List of length 4
[[2]]	list [4]	List of length 4
[[3]]	list [4]	List of length 4
[[4]]	list [4]	List of length 4
[[5]]	list [4]	List of length 4
[[6]]	list [4]	List of length 4
[[7]]	list [4]	List of length 4
[[8]]	list [4]	List of length 4
[[9]]	list [4]	List of length 4
[[10]]	list [4]	List of length 4
[[11]]	list [4]	List of length 4
[[12]]	list [4]	List of length 4
[[13]]	list [4]	List of length 4
[[14]]	list [4]	List of length 4
[[15]]	list [4]	List of length 4
[[16]]	list [4]	List of length 4
[[17]]	list [4]	List of length 4
[[18]]	list [4]	List of length 4
[[19]]	list [4]	List of length 4
[[20]]	list [4]	List of length 4
scaler	list [2]	List of length 2
Repeat	list [7]	List of length 7
Model	list [9] (S3: xgb.Booster)	List of length 9
geneAnnotation	list [32 x 3] (S3: data.frame)	A data.frame with 32 rows and 3 columns
ENSEMBL	character [32]	'ENSG00000122122' 'ENSG00000117091' 'ENSG00000163219' 'ENSG00000136167' 'ENSG000 ...
SYMBOL	character [32]	'SASH3' 'CD48' 'ARHGAP25' 'LCP1' 'ITGAL' 'NCKAP1L' ...
ENTREZID	character [32]	'54440' '962' '9938' '3936' '3683' '3071' ...
geneSet	list [2]	List of length 2
PIAM	character [19]	'ENSG00000122122' 'ENSG00000117091' 'ENSG00000163219' 'ENSG00000136167' 'ENSG000 ...
PIDG	character [13]	'ENSG00000116667' 'ENSG00000107771' 'ENSG00000196782' 'ENSG00000271447' 'ENSG000 ...

Figure 5.1: Details of a GSClassifier model

```

model[['geneAnnotation']] <- <Your gene annotation>

# Your gene sets

model[['geneSet']] <- <Your gene sets>

saveRDS(model, 'your-model.rds')

```

More tutorials for model establishment, please go to [markdown tutorial](#) or [html tutorial](#).

5.4 Submit models to luckyModel package

Considering most users of `GSClassifier` might have no need for lots of models, We divided the model storage feature into an new ensembl package called `luckyModel`. Don't worry, the usage is very easy!

If you want to submit your model, you should apply for a contributor of `luckyModel` first. Then, just send the model (.rds) into the `inst/extdata/<project>` path of `luckyModel`. After audit, your branch would be accepted and available for the users.

The name of your model must be the format as following:

```

# <project>
GSClassifier

# <creator>_<model>_v<yyyymmdd>:
HWB_PAD_v20211201.rds

```

5.5 Repeatability of models

For repeatability, you had better submit a `.zip` or `.tar.gz` file that containing the information of your model. Here are some suggestions:

- `<creator>_<model>_v<yyyymmdd>.md`

- **Destinations:** Why you develop the model
- **Design:** The evidence for gene signatures, et al
- **Data sources:** The data for model training and validating, et al
- **Applications:** Where to use your model
- **Limitations:** Limitation or improvement direction of your model
- <creator>_<model>_v<yyyymmdd>.R: The code you used for model training and validating.
- Data-of-<creator>_<model>_v<yyyymmdd>.rds (Optional): Due to huge size of omics data, it's OK for you not to submit the raw data.

:cupid: Welcome your contributions!

5.6 Gene Annotation

For convenience, we provided a general gene annotation dataset for different genomics:

```
gga <- readRDS(system.file("extdata", "general-gene-annotation.rds", package = "GSClassifier"))
names(gga)
# [1] "hg38" "hg19" "mm10"
```

I believe they're enough for routine medicine studies.

Here, take a look at hg38:

```
hg38 <- gga$hg38
head(hg38)
#          ENSEMBL      SYMBOL ENTREZID
# 1 ENSG00000223972    DDX11L1 100287102
# 3 ENSG00000227232    WASH7P     <NA>
# 4 ENSG00000278267 MIR6859-1 102466751
# 5 ENSG00000243485 RP11-34P13.3     <NA>
```

```
# 6 ENSG00000284332      MIR1302-2 100302278
# 7 ENSG00000237613      FAM138A     645520
```

With this kind of data, it's simple to customize your own gene annotation (take PADi as examples):

```
tGene <- as.character(unlist(PADi$geneSet))
geneAnnotation <- hg38[hg38$ENSEMBL %in% tGene, ]
dim(geneAnnotation)
# [1] 32 3
```

Have a check:

```
head(geneAnnotation)
#           ENSEMBL SYMBOL ENTREZID
# 353 ENSG00000171608 PIK3CD      5293
# 1169 ENSG00000134686 PHC2       1912
# 2892 ENSG00000134247 PTGFRN     5738
# 3855 ENSG00000117090 SLAMF1      6504
# 3858 ENSG00000117091 CD48       962
# 4043 ENSG00000198821 CD247      919
```

This `geneAnnotation` could be the `model[['geneAnnotation']]`.

Also, we use a function called `convert` to do gene ID conversion.

```
luckyBase::convert(c('GAPDH', 'TP53'), 'SYMBOL', 'ENSEMBL', hg38)
# [1] "ENSG00000111640" "ENSG00000141510"
```

Note: the `luckyBase` package integrates lots of useful tiny functions, you could explore it sometimes.

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