

IOBR (Immuno-Oncology Biological Research)

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Introduction

Preface

IOBR R package workflow

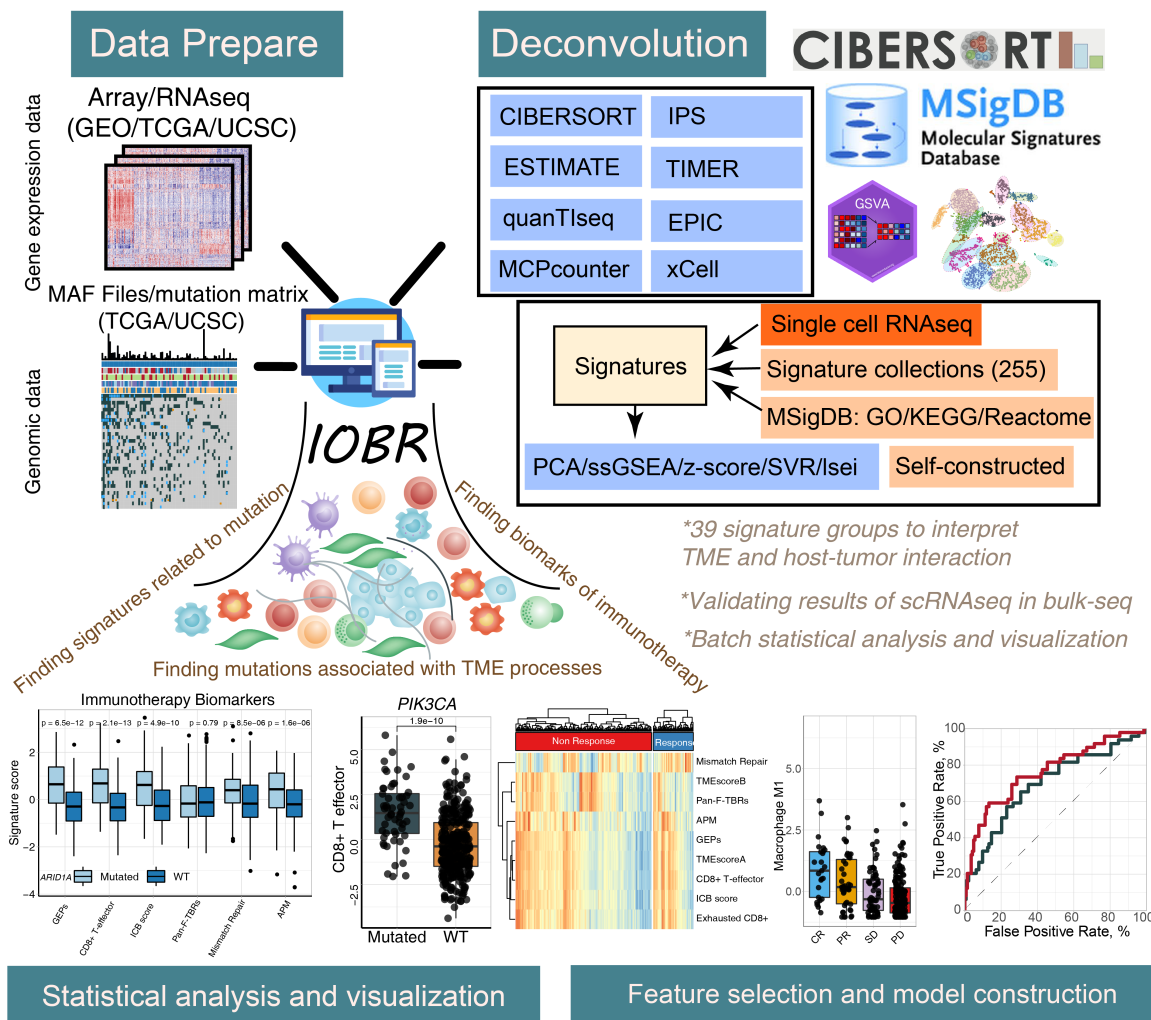


Figure 1: The workflow of IOBR

0.1 Introduction

IOBR is design for Immuno-Oncology Biological Research. Recent advance in next-generation sequencing has triggered the rapidly accumulating publicly available multi-omics data. The application of integrated omics to exploring robust signatures for clinical translation is increasingly highlighted in immuno-oncology but raises computational and biological challenges. This vignette aims to demonstrate how to utilize the package named IOBR to perform multi-omics immuno-oncology biological research to decode tumor microenvironment and signatures for clinical translation.

This R package integrates 8 published methodologies for decoding tumor microenvironment (TME) contexture: `CIBERSORT`, `TIMER`, `xCell`, `MCPcounter`, `ESITMATE`, `EPIC`, `IPS`, `quanTIseq`. Moreover, 255 published signature gene sets were collected by IOBR, involving tumor microenvironment, tumor metabolism, m6A, exosomes, microsatellite instability, and tertiary lymphoid structure. Run the function `signature_collection_citation` to obtain the source papers, and the function `signature_collection` returns the detail signature genes of all given signatures. Subsequently, IOBR adopts three computational methods to calculate the signature score, comprising `PCA`, `z-score`, and `ssGSEA`. To note, IOBR collected and employed multiple approaches for variable transition, visualization, batch survival analysis, feature selection, and statistical analysis. Batch analysis and visualization of corresponding results are supported. The details of how IOBR works are described below.

0.2 License

IOBR is released under the GPL v3.0 license. See `LICENSE` for details. The code contained in this book is simultaneously available under the GPL license; this means that you are free to use it in your packages, as long as you cite the source. The online version of this book is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

0.3 Publishment

Zeng D, Ye Z, Shen R, Yu G, Wu J, Xiong Y,..., Liao W (2021) **IOBR**: Multi-Omics Immuno-Oncology Biological Research to Decode Tumor Microenvironment and Signatures. *Frontiers in Immunology*. 12:687975. doi: 10.3389/fimmu.2021.687975

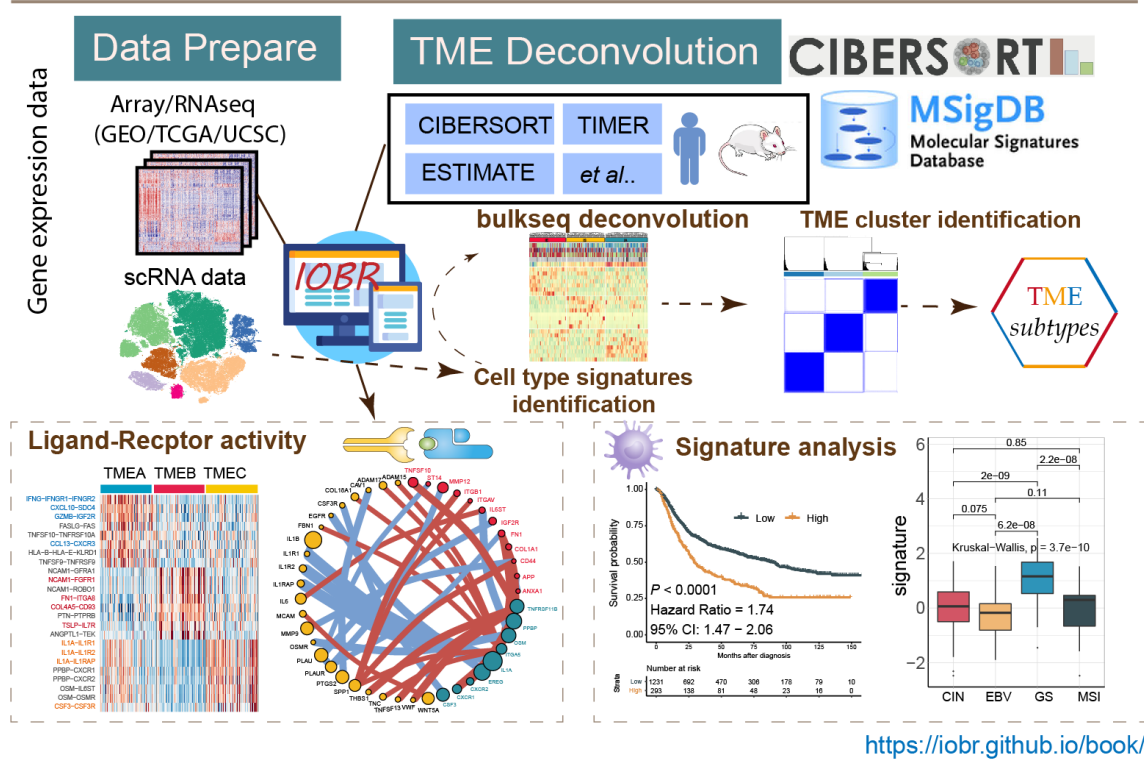


Figure 2: The workflow of IOBR

0.4 Major Updates

0.5 Reporting bugs

Please report bugs to the Github issues page

E-mail any questions to dongqiangzeng0808@gmail.com

Chapter 1

How to install IOBR

1.1 Install Dependency Packages

It is essential that you have R 3.6.3 or above already installed on your computer or server. IOBR is a pipeline that utilizes many other R packages that are currently available from CRAN, Bioconductor and GitHub.

```
if (!requireNamespace("BiocManager", quietly = TRUE)) install.packages("BiocManager")
depends<-c('tibble', 'survival', 'survminer', 'limma', "DESeq2", "devtools", 'limSolve', '
          "devtools", "tidyHeatmap", "caret", "glmnet", "ppcor", "timeROC", "pracma", "
          "FactoMineR", "WGCNA", "patchwork", 'ggplot2', "biomaRt", 'ggpubr')
for(i in 1:length(depends)){
  depen<-depends[i]
  if (!requireNamespace(depen, quietly = TRUE)) BiocManager::install(depen, update = FA
}
```

1.2 Install IOBR package

When the dependent environments are built, users are able to install IOBR from github by typing the following code into your R session:

```
if (!requireNamespace("IOBR", quietly = TRUE)) devtools::install_github("IOBR/IOBR")

library(IOBR)
```

1.3 How to update IOBR

```
detach("package:IOBR")
path<-.libPaths()
remove.packages(c('IOBR'), lib=file.path(path))
devtools::install_github("IOBR/IOBR")
```

1.4 The main pipeline of IOBR

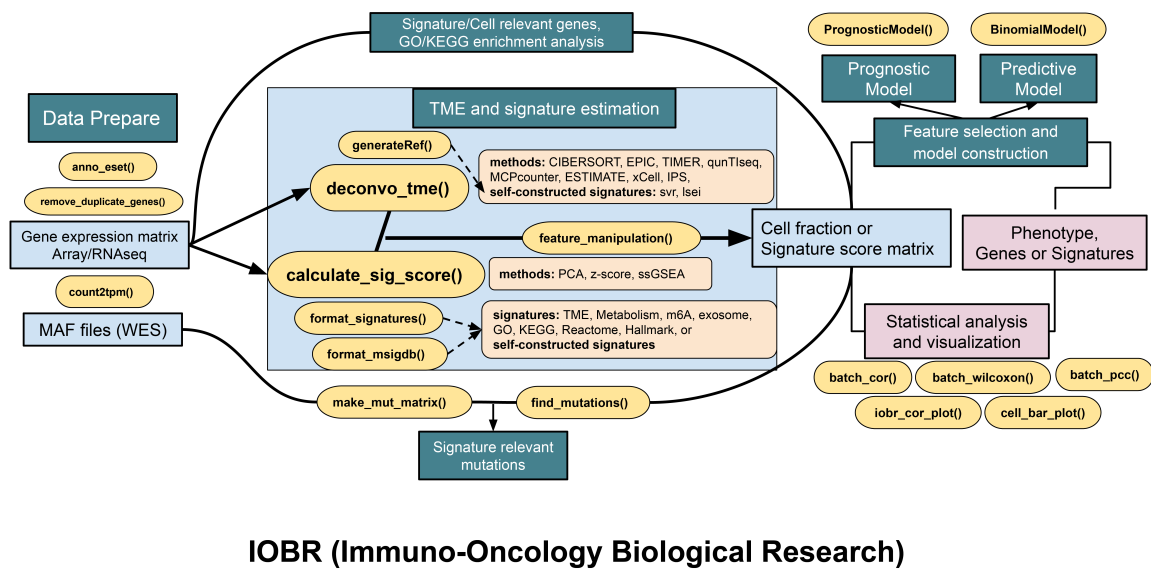


Figure 1.1: The main pipeline of IOBR

1.5 Main Functions of IOBR

- **Data Preparation: data annotation and transformation**
 - `count2tpm()`: transform count data of RNA sequencing into TPM data.
 - `anno_eset()`: annotate the normalized genes expression matrix, including RNAseq and array (Affymetrix or Illumina).
 - `remove_duplicate_genes()`: remove the genes annotated with the duplicated symbol after normalization and retain only the symbol with highest expression level.

- `mouse2human_eset()`: Converting muouse gene symbol to human gene symbol of expression set.
- `find_outlier_samples()`: Waiting for updates...
- `remove_batcheffect()`: Waiting for updates...
- **TME Deconvolution Module: integrate multiple algorithms to decode immune contexture**
 - `deconvo_tme()`: decode the TME infiltration with different deconvolution methodologies, based on bulk RNAseq, microarray or single cell RNAseq data.
 - `generateRef()`: generate a novel gene reference matrix for a specific feature such as infiltrating cell, through the SVR and lsei algorithm.
- **Signature Module: calculatint signature scores, estimate phenotype related signatures and corresponding genes, and evaluate signatures generated from single-cell RNA sequencing data**
 - `calculate_sig_score()`: estimate the interested signatures enrolled in IOBR R package, which involves TME-associated, tumor-metabolism, and tumor-intrinsic signatures.
 - `feature_manipulation()`: manipulate features including the cell fraction and signatures generated from multi-omics data for latter analysis and model construction. Remove missing values, outliers and variables without significant variance.
 - `format_signatures()`: generate the object for `calculate_sig_score()` function, by inputting a data frame with signatures as column names of corresponding gene sets, and return a list contain the signature information for calculating multiple signature scores.
 - `format_msigdb()`: transform the signature gene sets data with gmt format, which is not included in the signature collection and might be downloaded in the MS-giDB website, into the object of `calculate_sig_score()`function.
 - `sig_gsea()`: Waiting for updates...
- **Batch Analysis and Visualization: batch survival analysis and batch correlation analysis and other batch statistical analyses**
 - `batch_surv()`: batch survival analysis of multiple continuous variables including varied signature scores.
 - `subgroup_survival()`: batch survival analysis of multiple categorized variables with different number of subgroups.
 - `batch_cor()`: batch analysis of correlation between two continuous variables using Pearson correlation coefficient or Spearman's rank correlation coefficient .

- `batch_wilcoxon()`: conduct batch wilcoxon analyses of binary variables.
 - `batch_pcc()`: batch analyses of Partial Correlation coefficient(PCC) between continuous variables and minimize the interference derived from confounding factors.
 - `iobr_cor_plot()`: visualization of batch correlation analysis of signatures from ‘sig_group’. Visualize the correlation between signature or phenotype with expression of gene sets in target signature is also supported.
 - `cell_bar_plot()`: batch visualization of TME cell fraction, supporting input of deconvolution results from ‘CIBERSORT’, ‘EPIC’ and ‘quanTIseq’ methodologies to further compare the TME cell distributions within one sample or among different samples.
 - `iobr_pca()`: The `iobr_pca` function performs Principal Component Analysis (PCA), which reduces the dimensionality of data while maintaining most of the original variance, and visualizes the PCA results on a scatter plot.
 - `iobr_cor_plot()`: Integrative correlation between phenotype and features.
 - `iobr_deg()`: Waiting for updates...
 - `get_cor()`: Waiting for updates...
 - `roc_time()`: Waiting for updates...
 - `sig_box()`: Waiting for updates...
 - `sig_heatmap()`: Waiting for updates...
 - `sig_forest()`: Waiting for updates...
 - `sig_roc()`: Waiting for updates...
 - `sig_surv_plot()`: Waiting for updates...
 - `find_markers_in_bulk()`: Waiting for updates...
- **Signature Associated Mutation Module: identify and analyze mutations relevant to targeted signatures**
 - `make_mut_matrix()`: transform the mutation data with MAF format(contain the columns of gene ID and the corresponding gene alterations which including SNP, indel and frameshift) into a mutation matrix in a suitable manner for further investigating signature relevant mutations.
 - `find_mutations()`: identify mutations associated with a distinct phenotype or signature.
 - **Model Construction Module: feature selection and fast model construct to predict clinical phenotype**
 - `BinomialModel()`: select features and construct a model to predict a binary phenotype.

- `PrognosticMode()`: select features and construct a model to predict clinical survival outcome.

1.6 Current working environment

```
sessionInfo()
```

```
## R version 4.2.0 (2022-04-22 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=Chinese (Simplified)_China.utf8
## [2] LC_CTYPE=Chinese (Simplified)_China.utf8
## [3] LC_MONETARY=Chinese (Simplified)_China.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=Chinese (Simplified)_China.utf8
##
## attached base packages:
## [1] grid      stats      graphics  grDevices utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] IOBR_0.99.9          survival_3.3-1        ggpubr_0.4.0
## [4] ggplot2_3.4.2        dplyr_1.1.2           tibble_3.2.1
## [7] tidyHeatmap_1.8.1    ComplexHeatmap_2.15.4
##
## loaded via a namespace (and not attached):
## [1] readxl_1.4.0          backports_1.4.1
## [3] circlize_0.4.15       corrplot_0.92
## [5] GSEABase_1.58.0       splines_4.2.0
## [7] BiocParallel_1.30.3   GenomeInfoDb_1.34.4
## [9] digest_0.6.29         foreach_1.5.2
## [11] htmltools_0.5.2       viridis_0.6.2
## [13] fansi_1.0.3           magrittr_2.0.3
```

## [15] memoise_2.0.1	ScaledMatrix_1.4.0
## [17] cluster_2.1.3	googlesheets4_1.0.0
## [19] doParallel_1.0.17	tzdb_0.3.0
## [21] limma_3.52.1	Biostrings_2.64.0
## [23] readr_2.1.2	annotate_1.74.0
## [25] modelr_0.1.8	matrixStats_0.62.0
## [27] limSolve_1.5.6	lpSolve_5.6.15
## [29] colorspace_2.0-3	blob_1.2.3
## [31] rvest_1.0.2	haven_2.5.0
## [33] xfun_0.40	crayon_1.5.2
## [35] RCurl_1.98-1.7	jsonlite_1.8.0
## [37] graph_1.74.0	genefilter_1.78.0
## [39] zoo_1.8-10	iterators_1.0.14
## [41] glue_1.6.2	survminer_0.4.9
## [43] gtable_0.3.1	gargle_1.2.0
## [45] zlibbioc_1.42.0	XVector_0.36.0
## [47] GetoptLong_1.0.5	DelayedArray_0.22.0
## [49] BiocSingular_1.12.0	car_3.1-0
## [51] Rhdf5lib_1.18.2	SingleCellExperiment_1.18.0
## [53] shape_1.4.6	HDF5Array_1.24.1
## [55] BiocGenerics_0.42.0	abind_1.4-5
## [57] scales_1.2.0	DBI_1.1.2
## [59] rstatix_0.7.0	Rcpp_1.0.9
## [61] viridisLite_0.4.1	xtable_1.8-4
## [63] clue_0.3-61	rsvd_1.0.5
## [65] bit_4.0.4	proxy_0.4-27
## [67] preprocessCore_1.58.0	km.ci_0.5-6
## [69] GSVA_1.44.2	stats4_4.2.0
## [71] glmnet_4.1-4	httr_1.4.3
## [73] RColorBrewer_1.1-3	ellipsis_0.3.2
## [75] pkgconfig_2.0.3	XML_3.99-0.10
## [77] dbplyr_2.2.0	locfit_1.5-9.5
## [79] utf8_1.2.2	tidyselect_1.2.0
## [81] rlang_1.1.0	AnnotationDbi_1.58.0
## [83] munsell_0.5.0	cellranger_1.1.0
## [85] tools_4.2.0	cachem_1.0.6
## [87] cli_3.4.1	generics_0.1.3

```

## [89] RSQLite_2.2.14          broom_0.8.0
## [91] evaluate_0.15           stringr_1.4.0
## [93] fastmap_1.1.0           yaml_2.3.5
## [95] knitr_1.39              bit64_4.0.5
## [97] fs_1.5.2                survMisc_0.5.6
## [99] purrr_0.3.4             dendextend_1.15.2
## [101] KEGGREST_1.36.2         sparseMatrixStats_1.8.0
## [103] xml2_1.3.3              compiler_4.2.0
## [105] rstudioapi_0.13         png_0.1-7
## [107] e1071_1.7-11            ggsignif_0.6.3
## [109] reprex_2.0.1            geneplotter_1.74.0
## [111] stringi_1.7.6           forcats_0.5.1
## [113] lattice_0.20-45         Matrix_1.5-4.1
## [115] KMSurv_0.1-5            vctrs_0.6.2
## [117] rhdf5filters_1.8.0      pillar_1.9.0
## [119] lifecycle_1.0.3        GlobalOptions_0.1.2
## [121] irlba_2.3.5             data.table_1.14.2
## [123] cowplot_1.1.1           bitops_1.0-7
## [125] patchwork_1.1.1         GenomicRanges_1.48.0
## [127] R6_2.5.1                bookdown_0.35
## [129] gridExtra_2.3           IRanges_2.30.0
## [131] codetools_0.2-18        MASS_7.3-56
## [133] assertthat_0.2.1        rhdf5_2.40.0
## [135] SummarizedExperiment_1.26.1 DESeq2_1.36.0
## [137] rjson_0.2.21            withr_2.5.0
## [139] S4Vectors_0.34.0        GenomeInfoDbData_1.2.8
## [141] parallel_4.2.0          hms_1.1.1
## [143] beachmat_2.12.0         quadprog_1.5-8
## [145] tidyverse_1.3.2         tidyr_1.2.0
## [147] class_7.3-20            DelayedMatrixStats_1.18.0
## [149] rmarkdown_2.14          MatrixGenerics_1.8.0
## [151] carData_3.0-5           googledrive_2.0.0
## [153] Biobase_2.56.0          lubridate_1.8.0

```


Chapter 2

RNA Data preprocessing

2.1 Loading packages

Load the IOBR package in your R session after the installation is complete:

```
library(IOBR)
library(tidyverse)
library(clusterProfiler)
```

2.2 Download array data using GEOquery

Obtaining data set from GEO Gastric cancer: GSE62254 using GEOquery R package.

```
if (!requireNamespace("GEOquery", quietly = TRUE)) BiocManager::install("GEOquery")
library("GEOquery")
# NOTE: This process may take a few minutes which depends on the internet connection s
eset_geo<-getGEO(GEO      = "GSE62254", getGPL = F, destdir = "./")
eset    <-eset_geo[[1]]
eset    <-exprs(eset)
eset[1:5,1:5]
```

```
##          GSM1523727 GSM1523728 GSM1523729 GSM1523744 GSM1523745
## 1007_s_at  3.2176645  3.0624323  3.0279131   2.921683   2.8456013
## 1053_at   2.4050109  2.4394879  2.2442708   2.345916   2.4328582
## 117_at    1.4933412  1.8067380  1.5959665   1.839822   1.8326058
## 121_at    2.1965561  2.2812181  2.1865556   2.258599   2.1874363
```

```
## 1255_g_at 0.8698382 0.9502466 0.8125414 1.012860 0.9441993
```

2.3 Gene Annotation

Annotation of genes in the expression matrix and removal of duplicate genes.

```
# Load the annotation file `anno_hug133plus2` in IOBR.
```

```
head(anno_hug133plus2)
```

```
## # A tibble: 6 x 2
##   probe_id symbol
##   <fct>      <fct>
## 1 1007_s_at MIR4640
## 2 1053_at   RFC2
## 3 117_at    HSPA6
## 4 121_at    PAX8
## 5 1255_g_at GUCA1A
## 6 1294_at   MIR5193
```

```
# Load the annotation file `anno_grch38` in IOBR.
```

```
head(anno_grch38)
```

```
##           id eff_length      gc entrez  symbol chr   start      end
## 1 ENSG000000000003      4536 0.3992504   7105  TSPAN6  X 100627109 100639991
## 2 ENSG000000000005      1476 0.4241192  64102   TNMD   X 100584802 100599885
## 3 ENSG000000000419      9276 0.4252911   8813   DPM1  20  50934867  50958555
## 4 ENSG000000000457      6883 0.4117391  57147  SCYL3   1 169849631 169894267
## 5 ENSG000000000460      5970 0.4298157  55732 C1orf112  1 169662007 169854080
## 6 ENSG000000000938      3382 0.5644589   2268    FGR   1  27612064  27635277
##   strand      biotype
## 1     -1 protein_coding
## 2      1 protein_coding
## 3     -1 protein_coding
## 4     -1 protein_coding
## 5      1 protein_coding
## 6     -1 protein_coding
##
## 1 tetraspanin 6 [Source:HGNC]
## 2 tenomodulin [Source:HGNC]
```

```
## 3 dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit [Source:HGNC]
## 4                                SCY1-like, kinase-like 3 [Source:HGNC]
## 5                                chromosome 1 open reading frame 112 [Source:HGNC]
## 6                                FGR proto-oncogene, Src family tyrosine kinase [Source:HGNC]
```

```
# Load the annotation file `anno_gc_vm32` in IOBR for mouse RNAseq data
head(anno_gc_vm32)
```

```
##           id eff_length      gc symbol      mgi_id      gene_type
## 1 ENSMUSG000000000001      3262 0.4350092  Gnai3  MGI:95773 protein_coding
## 2 ENSMUSG000000000003       902 0.3481153  Pbsn  MGI:1860484 protein_coding
## 3 ENSMUSG000000000028      3506 0.4962921  Cdc45  MGI:1338073 protein_coding
## 4 ENSMUSG000000000031      2625 0.5588571   H19  MGI:95891      lncRNA
## 5 ENSMUSG000000000037      6397 0.4377052  Scml2  MGI:1340042 protein_coding
## 6 ENSMUSG000000000049      1594 0.5050188  Apoh  MGI:88058 protein_coding
##      start      end transcript_id  ont
## 1 108014596 108053462          <NA> <NA>
## 2  76881507  76897229          <NA> <NA>
## 3  18599197  18630737          <NA> <NA>
## 4 142129262 142131886          <NA> <NA>
## 5 159865521 160041209          <NA> <NA>
## 6 108234180 108305222          <NA> <NA>
```

2.3.1 For Array data: HGU133PLUS-2 (Affymetrix)

```
# Conduct gene annotation using `anno_hug133plus2` file; If identical gene symbols exist
```

```
eset<-anno_eset(eset      = eset,
                annotation = anno_hug133plus2,
                symbol     = "symbol",
                probe      = "probe_id",
                method     = "mean")
eset[1:5, 1:3]
```

```
##           GSM1523727 GSM1523728 GSM1523729
## SH3KBP1      4.327974  4.316195  4.351425
## RPL41        4.246149  4.246808  4.257940
## EEF1A1       4.293762  4.291038  4.262199
## COX2         4.250288  4.283714  4.270508
```

```
## LOC101928826    4.219303    4.219670    4.213252
```

2.3.2 For RNAseq data

Download RNAseq data using UCSCXenaTools

```
if (!requireNamespace("UCSCXenaTools", quietly = TRUE)) BiocManager::install("UCSCXenaTools")
library(UCSCXenaTools)
# NOTE: This process may take a few minutes which depends on the internet connection speed
eset_stad<-XenaGenerate(subset = XenaCohorts == "GDC TCGA Stomach Cancer (STAD)") %>%
  XenaFilter(filterDatasets = "TCGA-STAD.htseq_counts.tsv") %>%
  XenaQuery() %>%
  XenaDownload() %>%
  XenaPrepare()
eset_stad[1:5, 1:3]
```

Transform gene expression matrix into TPM format, and conduct subsequent annotation.

```
# Remove the version numbers in Ensembl ID.
eset_stad$Ensembl_ID<-substring(eset_stad$Ensembl_ID, 1, 15)
eset_stad<-column_to_rownames(eset_stad, var = "Ensembl_ID")

# Revert back to original format because the data from UCSC was log2(x+1) transformed.
eset_stad<-(2^eset_stad)+1

eset_stad<-count2tpm(countMat = eset_stad, idType = "Ensembl", org="hsa", source = "local")

eset_stad[1:5,1:5]
```

2.4 Identifying outlier samples

Take ACRG microarray data for example

```
res <- find_outlier_samples(eset = eset, project = "ACRG", show_plot = TRUE)
```

```
data("pdata_acrg")
res<- iobr_pca(data = eset1,
```

```

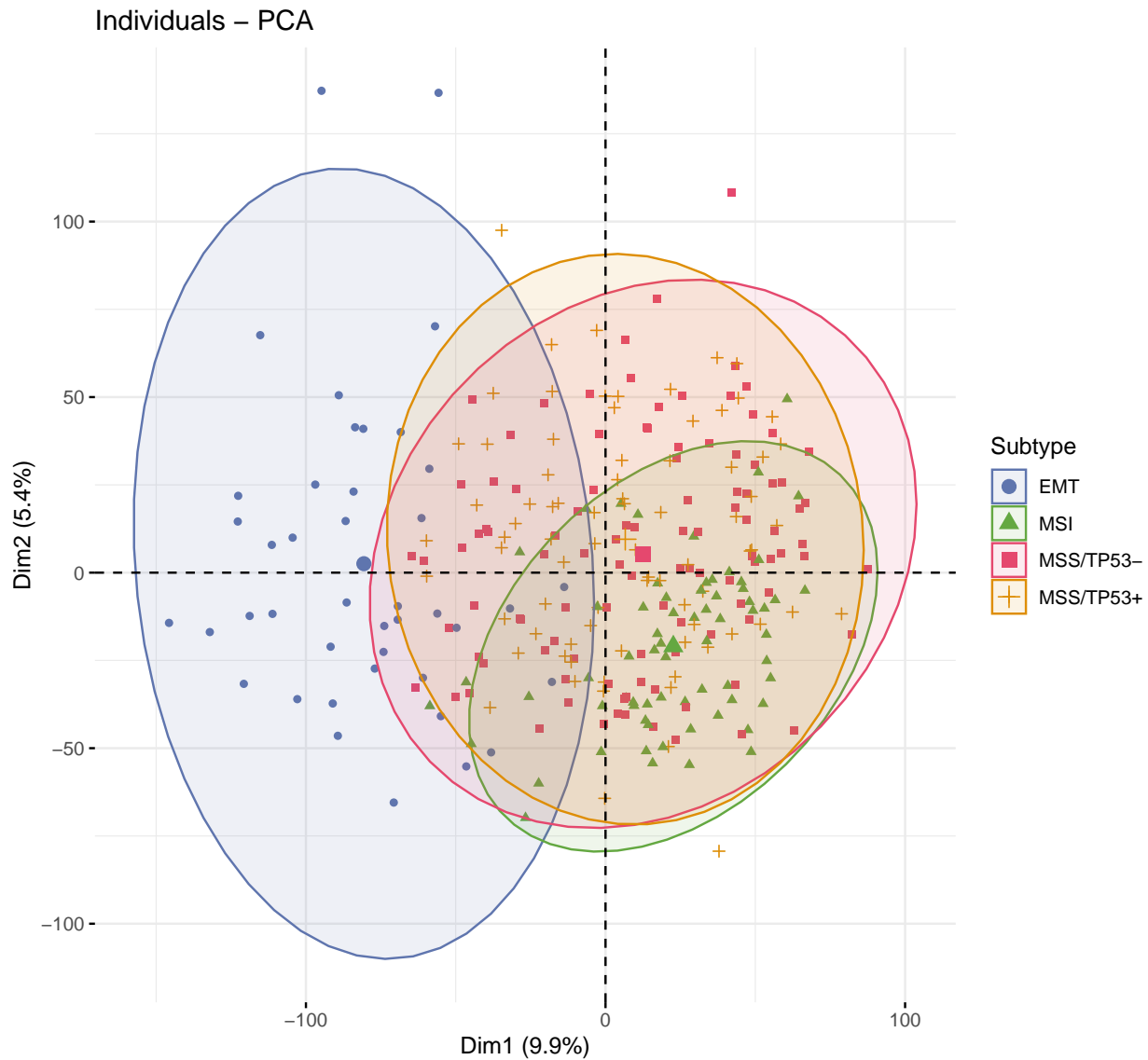
is.matrix = TRUE,
scale      = TRUE,
is.log     = FALSE,
pdata      = pdata_acrg,
id_pdata   = "ID",
group      = "Subtype",
geom.ind   = "point",
cols       = "normal",
palette    = "jama",
repel      = FALSE,
ncp        = 5,
axes       = c(1, 2),
addEllipses = TRUE)

```

```

##
##      CIN      EBV      EMT      GS      MSI MSS/TP53- MSS/TP53+
##      0        0      42      0      68      106      79
## [1] ">>-- colors for PCA: #5f75ae" ">>-- colors for PCA: #64a841"
## [3] ">>-- colors for PCA: #e5486e" ">>-- colors for PCA: #de8e06"
res

```



2.6 Batch effect correction

Obtaining another data set from GEO Gastric cancer: GSE57303 using GEOquery R package.

```
# NOTE: This process may take a few minutes which depends on the internet connection s
eset_geo<-getGEO(GEO      = "GSE57303", getGPL  = F, destdir = "./")
eset2    <-eset_geo[[1]]
eset2     <-exprs(eset2)
eset2[1:5,1:5]
```

```
##          GSM1379261 GSM1379262 GSM1379263 GSM1379264 GSM1379265
## 1007_s_at    8.34746    9.67994    8.62643    8.59301    8.63046
```

## 1053_at	5.07972	4.46377	5.29685	5.78983	4.33359
## 117_at	5.65558	4.48732	4.21615	5.47984	5.20816
## 121_at	5.95123	7.09056	6.19903	5.89872	5.91323
## 1255_g_at	1.66923	1.98758	1.73083	1.56687	1.63332

Annotation of genes in the expression matrix and removal of duplicate genes.

```

eset2<-anno_eset(eset      = eset2,
                 annotation = anno_hug133plus2,
                 symbol    = "symbol",
                 probe      = "probe_id",
                 method     = "mean")

eset2[1:5, 1:5]

```

##	GSM1379261	GSM1379262	GSM1379263	GSM1379264	GSM1379265
## ND4	13.1695	13.1804	13.0600	12.4544	13.0457
## ATP6	13.1433	13.0814	13.0502	12.4831	13.1168
## SH3KBP1	12.9390	13.1620	12.9773	12.8745	13.1169
## COX2	13.0184	13.0489	12.8621	12.7489	12.9732
## RPL41	13.0201	12.6034	12.7929	13.0153	12.9404

```

eset_com <- remove_batcheffect( eset1      = eset1,
                                eset2      = eset2,
                                eset3      = NULL,
                                id_type    = "symbol",
                                data_type   = "array",
                                cols       = "normal",
                                palette    = "jama",
                                log2       = TRUE,
                                check_eset = TRUE,
                                adjust_eset = TRUE,
                                repel     = FALSE,
                                path      = "result")

```

```

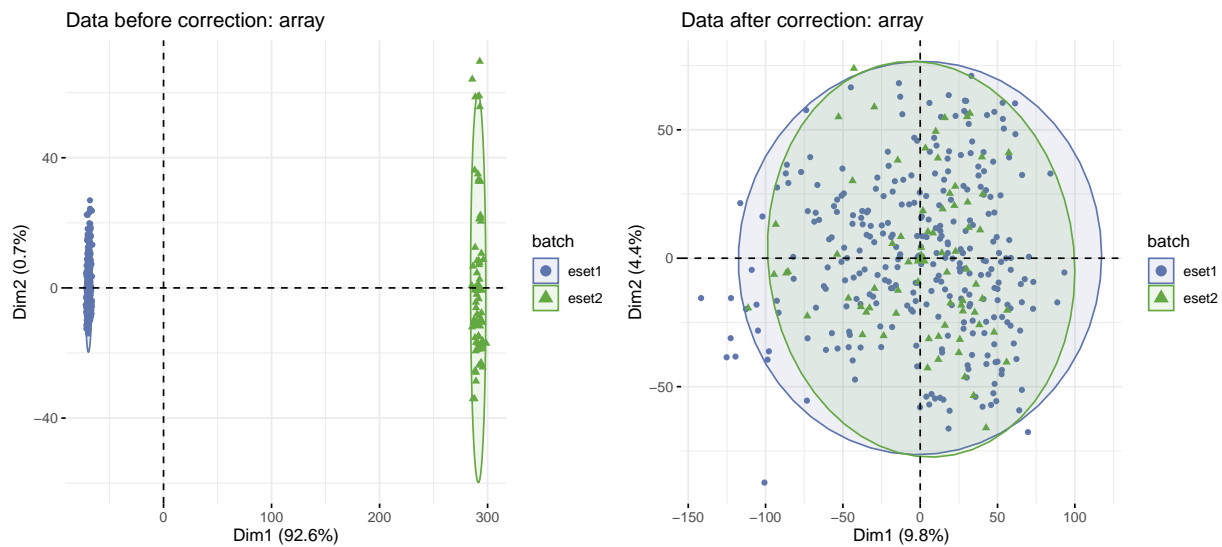
##
## eset1 eset2
##   295    70
## [1] ">>-- colors for PCA: #5f75ae" ">>-- colors for PCA: #64a841"
##
## eset1 eset2

```



```
## 295 70
```

```
## [1] ">>-- colors for PCA: #5f75ae" ">>-- colors for PCA: #64a841"
```



```
dim(eset_com)
```

```
## [1] 21752 365
```

Waiting for updates: Removing batch effect of RNAseq datasets: count, combat-seq

2.7 References

Wang et al., (2019). The UCSCXenaTools R package: a toolkit for accessing genomics data from UCSC Xena platform, from cancer multi-omics to single-cell RNA-seq. *Journal of Open Source Software*, 4(40), 1627, <https://doi.org/10.21105/joss.01627>

Yuqing Zhang and others, ComBat-seq: batch effect adjustment for RNA-seq count data, *NAR Genomics and Bioinformatics*, Volume 2, Issue 3, September 2020, lqaa078, <https://doi.org/10.1093/nargab/lqaa078>

Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E., & Storey, J. D. (2012). The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*, 28(6), 882-883.

Chapter 3

Tumor ecosystem analysis

3.1 Loading packages

```
library(IOBR)
```

3.2 Downloading data for example

Obtaining data set from GEO Gastric cancer: GSE62254 using GEOquery R package.

```
if (!requireNamespace("GEOquery", quietly = TRUE)) BiocManager::install("GEOquery")
library("GEOquery")
# NOTE: This process may take a few minutes which depends on the internet connection speed
eset_geo<-getGEO(GEO = "GSE62254", getGPL = F, destdir = "./")
eset    <-eset_geo[[1]]
eset    <-exprs(eset)
eset[1:5,1:5]
```

##		GSM1523727	GSM1523728	GSM1523729	GSM1523744	GSM1523745
##	1007_s_at	3.2176645	3.0624323	3.0279131	2.921683	2.8456013
##	1053_at	2.4050109	2.4394879	2.2442708	2.345916	2.4328582
##	117_at	1.4933412	1.8067380	1.5959665	1.839822	1.8326058
##	121_at	2.1965561	2.2812181	2.1865556	2.258599	2.1874363
##	1255_g_at	0.8698382	0.9502466	0.8125414	1.012860	0.9441993

3.3 Gene Annotation: HGU133PLUS-2 (Affymetrix)

Conduct gene annotation using `anno_hug133plus2` file; If identical gene symbols exist

```
eset<-anno_eset(eset      = eset,
                annotation = anno_hug133plus2,
                symbol     = "symbol",
                probe      = "probe_id",
                method     = "mean")
eset[1:5, 1:3]
```

```
##          GSM1523727 GSM1523728 GSM1523729
## SH3KBP1      4.327974  4.316195  4.351425
## RPL41        4.246149  4.246808  4.257940
## EEF1A1       4.293762  4.291038  4.262199
## COX2         4.250288  4.283714  4.270508
## LOC101928826 4.219303  4.219670  4.213252
```

3.4 Determine TME subtype of gastric cancer using TMEclassifier R package

```
library(TMEclassifier)
tme <- tme_classifier(eset = eset, scale = TRUE)
```

```
## Step-1: Expression data preprocessing...
## Step-2: TME deconvolution...
## Step-3: Predicting TME phenotypes...
## [12:31:47] WARNING: amalgamation/./src/learner.cc:1040:
##   If you are loading a serialized model (like pickle in Python, RDS in R) generated by
##   older XGBoost, please export the model by calling `Booster.save_model` from that version
##   first, then load it back in current version. See:
##
##   https://xgboost.readthedocs.io/en/latest/tutorials/saving_model.html
##
##   for more details about differences between saving model and serializing.
##
## [12:31:47] WARNING: amalgamation/./src/learner.cc:749: Found JSON model saved before
```

```
## >>>--- DONE!
```

```
table(tme$TMEcluster)
```

```
##
```

```
##  IA  IE  IS
```

```
## 107  96  97
```

```
head(tme)
```

```
##           ID           IE           IS           IA TMEcluster
## 1 GSM1523727 0.204623557 0.11212681 0.68324962          IA
## 2 GSM1523728 0.009599504 0.11179146 0.87860903          IA
## 3 GSM1523729 0.852615046 0.11369089 0.03369407          IE
## 4 GSM1523744 0.053842233 0.06994632 0.87621145          IA
## 5 GSM1523745 0.055973019 0.80839488 0.13563209          IS
## 6 GSM1523746 0.545343299 0.37437568 0.08028102          IE
```

```
table(tme$TMEcluster)
```

```
##
```

```
##  IA  IE  IS
```

```
## 107  96  97
```

```
head(tme)
```

```
##           ID           IE           IS           IA TMEcluster
## 1 GSM1523727 0.204623557 0.11212681 0.68324962          IA
## 2 GSM1523728 0.009599504 0.11179146 0.87860903          IA
## 3 GSM1523729 0.852615046 0.11369089 0.03369407          IE
## 4 GSM1523744 0.053842233 0.06994632 0.87621145          IA
## 5 GSM1523745 0.055973019 0.80839488 0.13563209          IS
## 6 GSM1523746 0.545343299 0.37437568 0.08028102          IE
```

3.5 DEG analysis: method1

Differential analysis of selected immune-activated and immune-expelled gastric cancers

```
pdata <- tme[!tme$TMEcluster=="IS", ]
deg <- iobr_deg(eset           = eset,
                annoation      = NULL,
                pdata          = pdata,
```

```

group_id      = "TMEcluster",
pdata_id      = "ID",
array         = TRUE,
method        = "limma",
contrast      = c("deg_group", "IA", "IE"),
path          = NULL,
padj_cutoff   = 0.01,
logfc_cutoff  = 0.5)

##
## Attaching package: 'limma'

## The following object is masked from 'package:BiocGenerics':
##
##      plotMA

## group1 = IA
## group2 = IE

## # A tibble: 6 x 11
##   symbol log2FoldChange AveExpr      t    pvalue      padj      B sigORnot label
##   <chr>          <dbl>   <dbl> <dbl>    <dbl>    <dbl> <dbl> <chr>    <chr>
## 1 TMEM100      -0.774    1.84 -13.9 2.47e-31 5.37e-27 60.4 Down_regul~ Both
## 2 ABCA8        -0.933    1.90 -12.9 3.11e-28 3.38e-24 53.4 Down_regul~ Both
## 3 HHIP         -0.613    1.73 -12.1 7.62e-26 4.46e-22 48.0 Down_regul~ Both
## 4 LMNB2         0.287    2.25  12.1 9.28e-26 4.46e-22 47.8 NOT          Sign~
## 5 MCM6          0.211    3.02  12.1 1.02e-25 4.46e-22 47.7 NOT          Sign~
## 6 ADH1B        -0.907    1.86 -12.0 2.27e-25 7.04e-22 47.0 Down_regul~ Both
## # i 2 more variables: IA <dbl>, IE <dbl>

```

3.6 GSEA analysis based on differential express gene analysis results

Select the gene set list in IOBR's signature collection.

```

head(deg)

## # A tibble: 6 x 11
##   symbol log2FoldChange AveExpr      t    pvalue      padj      B sigORnot label

```

3.6. GSEA ANALYSIS BASED ON DIFFERENTIAL EXPRESS GENE ANALYSIS RESULTS31

```
##      <chr>          <dbl>  <dbl> <dbl>    <dbl>    <dbl> <dbl> <chr>      <chr>
## 1 TMEM100        -0.774    1.84 -13.9 2.47e-31 5.37e-27 60.4 Down_regul~ Both
## 2 ABCA8          -0.933    1.90 -12.9 3.11e-28 3.38e-24 53.4 Down_regul~ Both
## 3 HHIP           -0.613    1.73 -12.1 7.62e-26 4.46e-22 48.0 Down_regul~ Both
## 4 LMNB2           0.287    2.25  12.1 9.28e-26 4.46e-22 47.8 NOT          Sign~
## 5 MCM6            0.211    3.02  12.1 1.02e-25 4.46e-22 47.7 NOT          Sign~
## 6 ADH1B          -0.907    1.86 -12.0 2.27e-25 7.04e-22 47.0 Down_regul~ Both
## # i 2 more variables: IA <dbl>, IE <dbl>
```

```
sig_list <- signature_collection[c("TMEscoreB_CIR", "TMEscoreA_CIR", "DNA_replication",
                                   "Pan_F_TBRs", "TGFb.myCAF", "Ferroptosis", "TLS_Natur
sig_list
```

```
## $TMEscoreB_CIR
```

```
##      [1] "DCN"          "SEPP1"          "ACTA2"          "SPARCL1"        "BEX3"
##      [6] "MYLK"          "AKR1C1"          "TIMP2"          "MXRA7"          "C11orf96"
##     [11] "CAV1"          "PDGFRA"          "FHL1"          "MGP"            "EID1"
##     [16] "LOC101930400" "DST"            "GREM1"          "FERMT2"          "TNC"
##     [21] "CYBRD1"        "LTBP1"          "ACTG2"          "TMEM47"          "SERPINE2"
##     [26] "ANTXR2"        "GNG11"          "TAGLN"          "GSTA4"          "PKIG"
##     [31] "MAOA"          "PTRF"           "FAM3B"          "PBX1"           "WLS"
##     [36] "SELM"          "SVIL"           "MYH11"          "AGT"            "SPON1"
##     [41] "TGFB1I1"       "PDLIM3"          "PDK4"           "SYNP02"          "MSRB3"
##     [46] "PROS1"         "EDNRA"          "AKAP12"          "PSD3"           "TNS1"
##     [51] "JAM3"          "PDZRN3"          "DDR2"           "HMGCS2"          "SGCE"
##     [56] "MRVI1"         "WFDC1"          "FBLN1"          "FM05"           "MAOB"
##     [61] "AMOTL1"        "AKT3"           "CNRIP1"          "CPE"            "MAP1B"
##     [66] "RBP1"          "GNAI1"          "FOXF2"          "SORBS2"          "ZCCHC24"
##     [71] "ZNF704"        "ARMCX1"          "DIXDC1"          "SSTR1"           "THRB"
##     [76] "C3orf70"       "PKIB"           "CNN1"           "SYTL5"           "DACT1"
##     [81] "SYNP0"         "GAS1"           "DPYSL3"          "CCDC80"          "TSPYL5"
##     [86] "DCHS1"         "SOBP"           "AOC3"           "NDN"            "FGF7P3"
##     [91] "SMAD9"         "MCC"            "CLMP"           "MYL9"           "RBP4"
##     [96] "PLN"           "SPOCK1"          "COL14A1"         "CRYAB"          "SRPX"
##    [101] "EML1"          "RERG"           "PPP1R3C"         "LOC100506718"   "CH25H"
##    [106] "HSPB8"         "PID1"           "TTC28"           "STON1"          "ABCG2"
##    [111] "ZSCAN18"       "SCIN"           "C14orf132"       "TMEM55A"        "WASF3"
##    [116] "PAPLN"         "COLEC12"        "ACKR1"           "TMEM150C"       "RAI2"
```

```

## [121] "TSPAN7"      "MRGPRF"      "ABCA8"      "CHIC1"      "NBEA"
## [126] "FAM13C"      "SETBP1"      "LDOC1"      "TMEM100"    "LOC101930349"
## [131] "PRICKLE2"    "TSPAN18"     "FABP4"      "ARHGEF26"   "ERICH5"
## [136] "MYOCD"       "BEX2"        "PPP1R14A"   "FGF13"      "RUNX1T1"
## [141] "MAGI2-AS3"   "LINC01279"   "REEP1"      "PLAC9"      "MYEF2"
## [146] "PRKD1"       "RGN"         "CLDN11"     "ANK2"       "ESRRG"
## [151] "SYNC"        "ZNF667-AS1"  "FGF7"       "SFRP1"      "HMCN1"
## [156] "TCEAL7"      "OGN"         "MAGI2"      "MIR100HG"   "FILIP1"
## [161] "LOC100507334" "ANKRD6"      "PLEKHH2"    "ZNF542P"    "ARMCX4"
## [166] "NOV"         "DCLK1"       "ARHGAP28"   "C2orf40"    "TRHDE"
## [171] "EPHA7"       "SCRG1"       "ZNF677"     "ZFPM2"      "PEG3"
## [176] "SERP2"       "ZNF415"      "MAMDC2"     "RBM24"      "MEOX2"
##
## $TMEscoreA_CIR
## [1] "HLA-DPB1"      "UBD"          "LOC100509457"  "WARS"
## [5] "TAP1"          "HLA-DMA"      "TRIM22"        "PSAT1"
## [9] "CXCL10"        "SOCS3"        "CXCL9"         "PBK"
## [13] "CCL4"          "CCL5"         "BCL2A1"        "TRBC1"
## [17] "IDO1"          "NFE2L3"       "CCL3L3"        "DTL"
## [21] "MMP9"          "SLC2A3"       "ZNF367"        "RCC1"
## [25] "STIL"          "TRAC"         "HELLS"         "GZMB"
## [29] "RTEL1-TNFRSF6B" "CXCL11"       "GBP5"          "CD2"
## [33] "CDCA2"         "CDT1"         "TNFAIP2"       "TYMP"
## [37] "MICB"          "SLC2A14"      "GZMK"          "CD8A"
## [41] "CENPH"         "MND1"         "BATF2"         "BRIP1"
## [45] "E2F7"          "KIF18A"       "AIM2"          "ETV7"
## [49] "ITK"           "GNLY"         "GPR171"        "WDHD1"
## [53] "GBP4"          "MB21D1"       "NLRP3"         "MCEMP1"
## [57] "POLR3G"        "NLRC3"        "KLRC2"         "CLEC5A"
## [61] "ARHGAP11A"     "GPR84"        "IFNG"          "ZBED2"
##
## $DNA_replication
## [1] "RNASEH2A" "POLD3" "DNA2" "FEN1" "POLA2" "RNASEH1"
## [7] "RPA4"      "LIG1"  "MCM2" "MCM3" "MCM4"  "MCM5"
## [13] "MCM6"      "MCM7"  "PCNA" "POLE3" "POLA1" "POLD1"
## [19] "POLD2"     "POLE"  "POLE2" "PRIM1" "PRIM2" "POLE4"
## [25] "POLD4"     "RFC1"  "RFC2" "RFC3" "RFC4"  "RFC5"

```


3.6. GSEA ANALYSIS BASED ON DIFFERENTIAL EXPRESS GENE ANALYSIS RESULTS33

```
## [31] "RPA1"      "RPA2"      "RPA3"      "SSBP1"      "RNASEH2B" "RNASEH2C"
##
## $Base_excision_repair
## [1] "PARP2" "PARP3" "POLD3" "PARP1" "PARP4" "FEN1" "SMUG1" "NEIL2" "APEX2"
## [10] "POLL" "HMGB1" "APEX1" "LIG1" "LIG3" "MPG" "MUTYH" "NTHL1" "OGG1"
## [19] "PCNA" "POLE3" "POLB" "POLD1" "POLD2" "POLE" "POLE2" "NEIL3" "POLE4"
## [28] "POLD4" "UNG" "XRCC1" "NEIL1" "MBD4"
##
## $Pan_F_TBRs
## [1] "ACTA2" "ACTG2" "ADAM12" "ADAM19" "CNN1" "COL4A1"
## [7] "CTGF" "CTPS1" "FAM101B" "FSTL3" "HSPB1" "IGFBP3"
## [13] "PXDC1" "SEMA7A" "SH3PXD2A" "TAGLN" "TGFB1" "TNS1"
## [19] "TPM1"
##
## $TGFB.myCAF
## [1] "CST1" "LAMP5" "LOXL1" "EDNRA" "TGFB1" "TGFB3" "TNN"
## [8] "CST2" "HES4" "COL10A1" "ELN" "THBS4" "NKD2" "OLFM2"
## [15] "COL6A3" "LRRC17" "COL3A1" "THY1" "HTRA3" "TMEM204" "11-Sep"
## [22] "COMP" "TNFAIP6" "ID4" "GGT5" "INAFM1" "CILP" "OLFML2B"
##
## $Ferroptosis
## [1] "ACSL4" "AKR1C1-3" "ALOXs" "ATP5G3" "CARS"
## [6] "CBS" "CD44v" "CHAC1" "CISD1" "CS"
## [11] "DPP4" "FANCD2" "GCLC/GCLM" "GLS2" "GPX4"
## [16] "GSS" "HMGCR" "HSPB1/5" "KOD" "LPCAT3"
## [21] "MT1G" "NCOA4" "NFE2L2" "PTGS2" "RPL8"
## [26] "SAT1" "SLC7A11" "SQS" "TFRC" "TP53"
## [31] "TTC35/EMC2" "MESH1"
##
## $TLS_Nature
## [1] "CD79B" "CD1D" "CCR6" "LAT" "SKAP1" "CETP" "EIF1AY" "RBP5"
## [9] "PTGDS"
##
## $Glycolysis
## [1] "ACSS1" "ACSS2" "ADH1A" "ADH1B" "ADH1C" "ADH4" "ADH5"
## [8] "ADH6" "ADH7" "ADPGK" "AKR1A1" "ALDH1A3" "ALDH1B1" "ALDH2"
## [15] "ALDH3A1" "ALDH3A2" "ALDH3B1" "ALDH3B2" "ALDH7A1" "ALDH9A1" "ALDOA"
```

```
## [22] "ALDOB"    "ALDOC"    "BPGM"     "DLAT"     "DLD"      "EN01"     "EN02"
## [29] "EN03"     "FBP1"     "FBP2"     "G6PC"     "G6PC2"    "GALM"     "GAPDH"
## [36] "GAPDHS"   "GCK"      "GPI"      "HK1"      "HK2"      "HK3"      "HKDC1"
## [43] "LDHA"     "LDHAL6A"  "LDHAL6B"  "LDHB"     "LDHC"     "PANK1"    "PCK1"
## [50] "PCK2"     "PDHA1"    "PDHA2"    "PDHB"     "PFKFB1"   "PFKFB2"   "PFKFB3"
## [57] "PFKFB4"   "PFKL"     "PFKM"     "PFKP"     "PGAM1"    "PGAM2"    "PGAM4"
## [64] "PGK1"     "PGK2"     "PGM1"     "PGM2"     "PKLR"     "PKM"      "SLC2A2"
## [71] "TPI1"
```

```
gsea<- sig_gsea(deg,
  genesets      = sig_list,
  path          = "GSEA",
  gene_symbol    = "symbol",
  logfc         = "log2FoldChange",
  org           = "hsa",
  show_plot     = TRUE,
  msigdb        = TRUE,
  category      = "H",
  subcategory   = NULL,
  palette_bar    = "set2")
```

Hallmark gene signatures

```
gsea<- sig_gsea(deg,
  genesets      = NULL,
  path          = "GSEA",
  gene_symbol    = "symbol",
  logfc         = "log2FoldChange",
  org           = "hsa",
  show_plot     = TRUE,
  msigdb        = TRUE,
  category      = "H",
  subcategory   = NULL,
  palette_bar    = "aaas",
  show_bar      = 5,
  show_gsea     = 6)
```

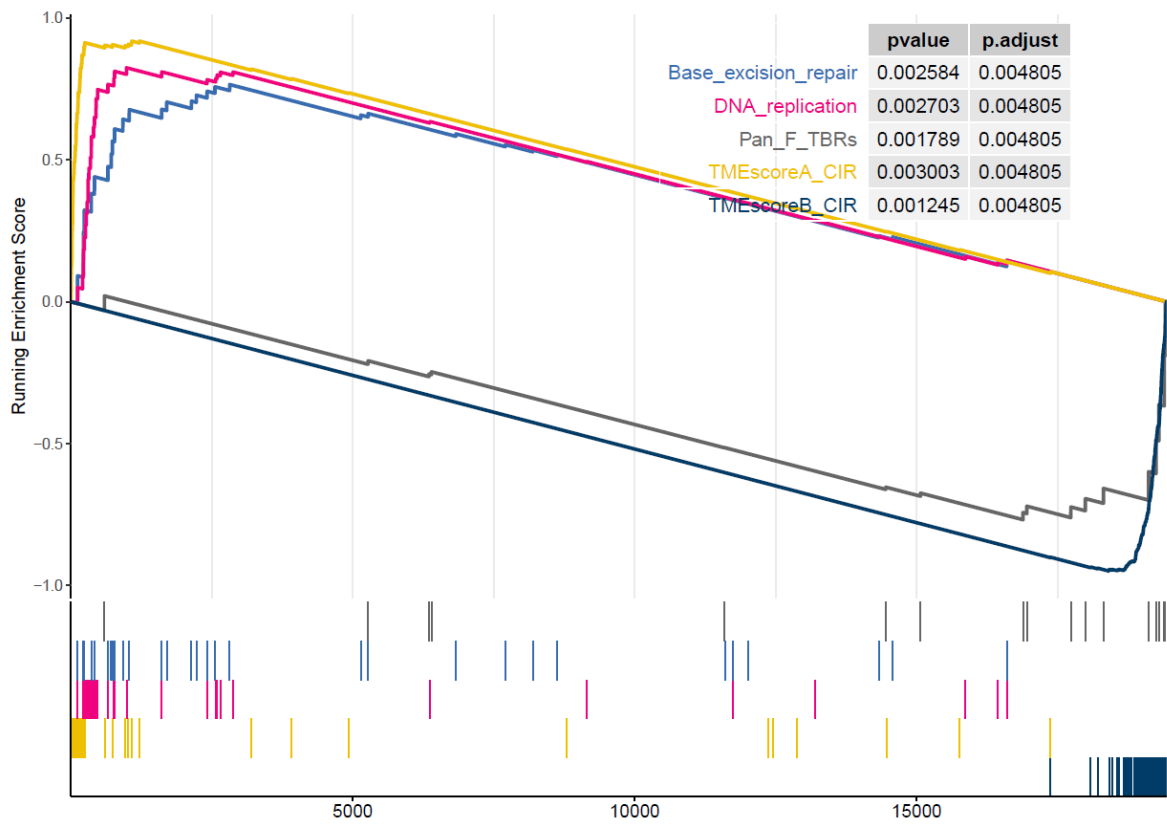


Figure 3.1: GSEA of TME gent sets

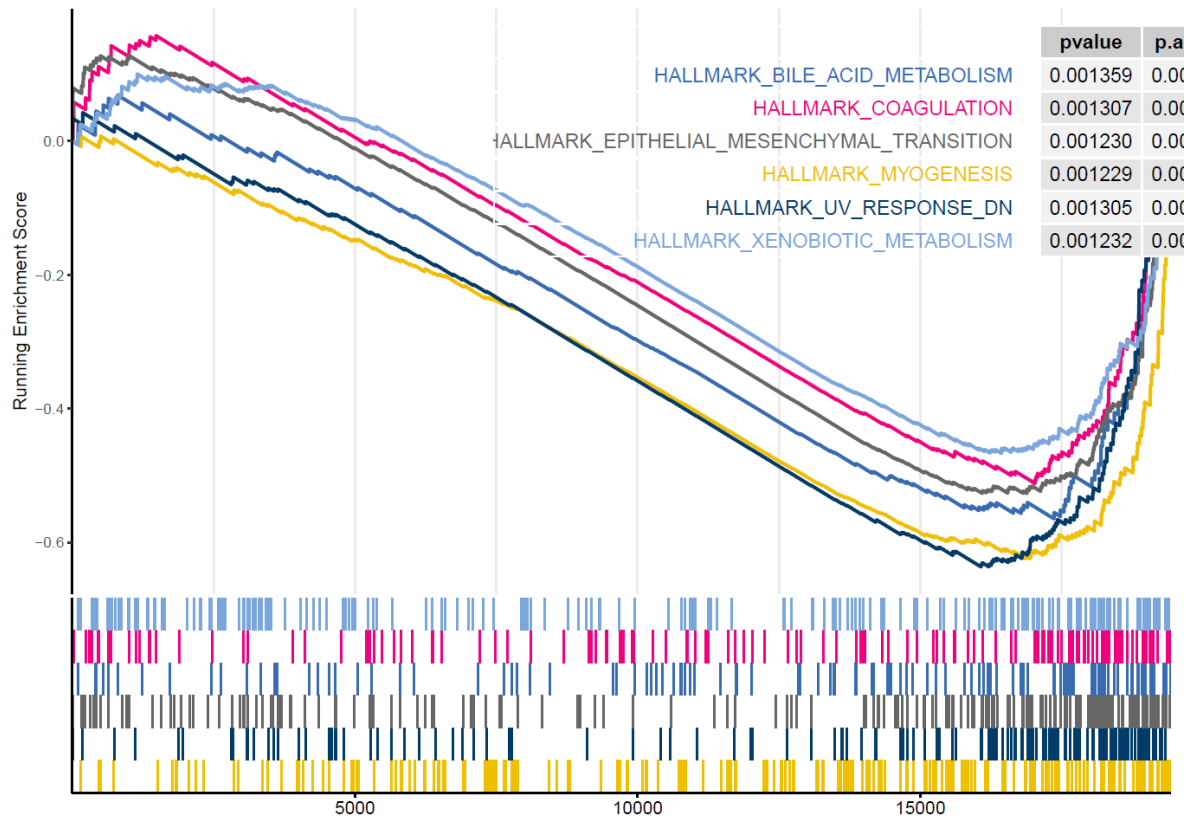


Figure 3.2: GSEA of Hallmark gent sets

3.7 DEG analysis: method2

Identifying TME subtype-related differential genes using `find_markers_in_bulk`

```
library(Seurat)
```

```
res <- find_markers_in_bulk(pdata      = tme,
                           eset       = eset,
                           group      = "TMEcluster",
                           nfeatures  = 2000,
                           top_n      = 20,
                           thresh.use = 0.15,
                           only.pos   = TRUE,
                           min.pct    = 0.10)
```

```
##
##  IA  IE  IS
## 107 96 97
## # A tibble: 56 x 7
## # Groups:   cluster [3]
##      p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
##      <dbl>      <dbl> <dbl> <dbl>      <dbl> <fct>   <chr>
##  1 3.29e-20      0.218     1     1 7.15e-16 IA      IFNG
##  2 1.81e-18      0.172     1     1 3.93e-14 IA      CXCL10
##  3 1.01e-16      0.183     1     1 2.20e-12 IA      GZMB
##  4 2.82e-16      0.251     1     1 6.12e-12 IA      CXCL11
##  5 9.68e-16      0.170     1     1 2.10e-11 IA      CXCL9
##  6 3.11e-15      0.221     1     1 6.77e-11 IA      IDO1
##  7 9.90e-15      0.156     1     1 2.15e-10 IA      POLR3G
##  8 3.02e-14      0.184     1     1 6.57e-10 IA      GBP4
##  9 9.23e-14      0.152     1     1 2.01e- 9 IA      ZBED2
## 10 9.79e-12      0.155     1     1 2.13e- 7 IA      GNLY
## # i 46 more rows
```

```
top15 <- res$top_markers %>% dplyr:: group_by(cluster) %>% dplyr::top_n(15, avg_log2F
top15$gene
```

```
## [1] "IFNG"          "CXCL10"        "GZMB"          "CXCL11"
## [5] "CXCL9"         "IDO1"          "POLR3G"        "GBP4"
## [9] "GNLY"         "PLEKHS1"       "KLRC2"         "VSNL1"
```

```
## [13] "AIM2"          "SLC01B3"      "COL11A1"      "TMEM100"
## [17] "ADH1B"         "ABCA8"        "MAMDC2"       "C1QTNF7"
## [21] "SCN7A"         "C7"           "C2orf40"      "LIPF"
## [25] "PGA4"          "SCRG1"        "OGN"          "GKN1"
## [29] "GKN2"          "GIF"          "IL1A"         "EREG"
## [33] "PPBP"          "IL11"         "CXCL6"        "PI15"
## [37] "PROK2"         "HCAR3"        "CLEC5A"       "MAGEA10-MAGEA5"
## [41] "MAGEA4"        "MAGEA12"      "MAGEA6"       "MAGEA2B"
## [45] "REG1B"
```

Heatmap visualisation using Seurat's DoHeatmap

```
#
cols <- c('#2692a4', '#fc0d3a', '#ffbe0b')
p1 <- DoHeatmap(res$sce, top15$gene, group.colors = cols )+
  scale_fill_gradientn(colours = rev(colorRampPalette(RColorBrewer::brewer.pal(11, "RdBu"))
```

Extracting variables from the expression matrix to merge with TME subtype

```
input <- combine_pd_eset(eset = eset, pdata = tme, feas = top15$gene, scale = T)
p2 <- sig_box(input, variable = "TMEcluster", signature = "IFNG", jitter = TRUE,
  cols = cols, show_pvalue = TRUE, size_of_pvalue = 4)
```

```
## # A tibble: 3 x 8
##   .y.      group1 group2      p    p.adj p.format p.signif method
##   <chr>    <chr>  <chr>    <dbl>  <dbl> <chr>    <chr>    <chr>
## 1 signature IA    IE    4.09e-17 1.20e-16 < 2e-16 ****    Wilcoxon
## 2 signature IA    IS    1.44e-13 2.90e-13 1.4e-13 ****    Wilcoxon
## 3 signature IE    IS    8.35e- 2 8.4 e- 2 0.084    ns      Wilcoxon
```

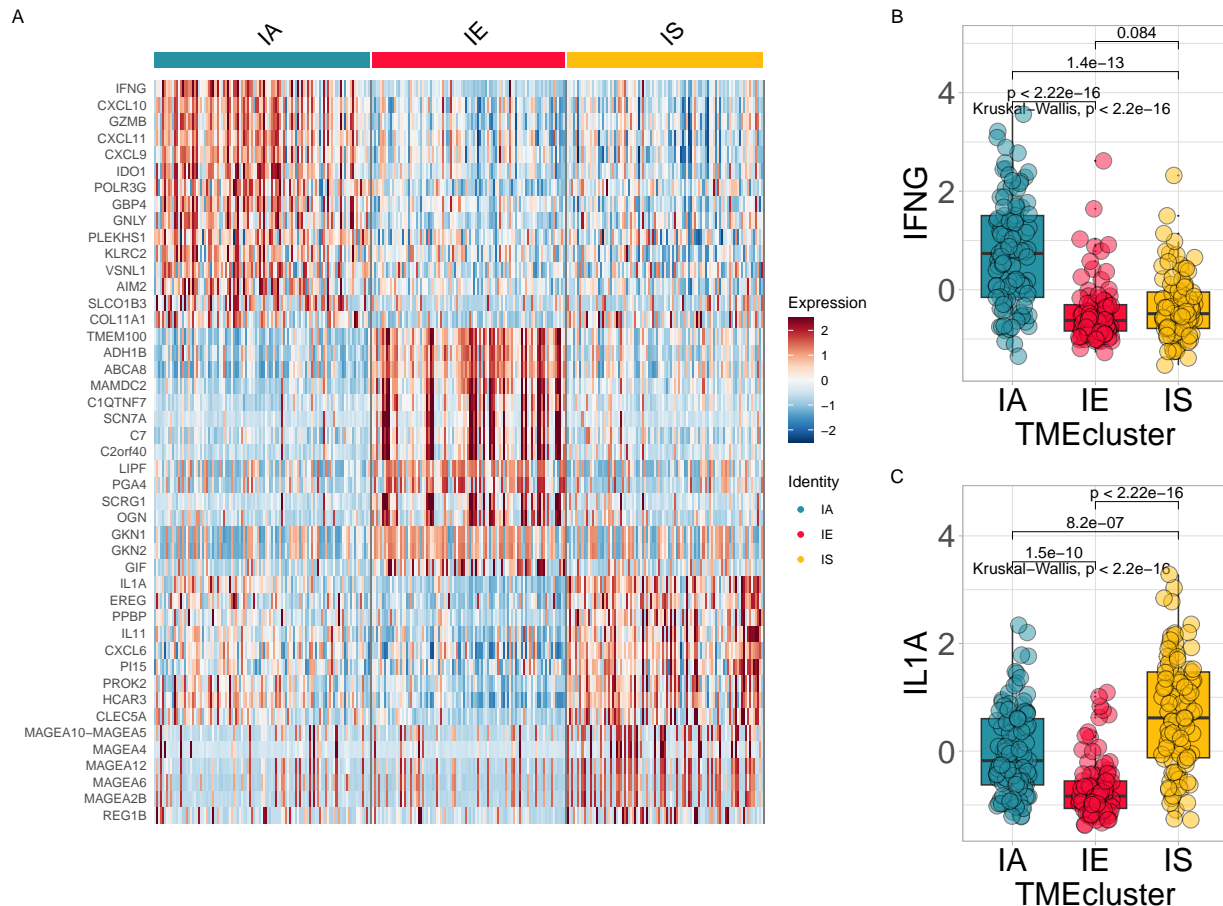
```
p3 <- sig_box(input, variable = "TMEcluster", signature = "IL1A",
  jitter = TRUE, cols = cols, show_pvalue = TRUE, size_of_pvalue = 4)
```

```
## # A tibble: 3 x 8
##   .y.      group1 group2      p    p.adj p.format p.signif method
##   <chr>    <chr>  <chr>    <dbl>  <dbl> <chr>    <chr>    <chr>
## 1 signature IA    IE    1.46e-10 2.90e-10 1.5e-10 ****    Wilcoxon
## 2 signature IA    IS    8.22e- 7 8.2 e- 7 8.2e-07 ****    Wilcoxon
## 3 signature IE    IS    4.90e-20 1.5 e-19 < 2e-16 ****    Wilcoxon
```

```

if (!requireNamespace("patchwork", quietly = TRUE)) install.packages("patchwork")
library(patchwork)
p <- (p1|p2/p3) + plot_layout(widths = c(2.3,1))
p + plot_annotation(tag_levels = 'A')

```



3.8 Identifying signatures associated with TME clusters

Calculate TME associated signatures-(through PCA method).

```

sig_tme <- calculate_sig_score(pdata = NULL,
                               eset = eset,
                               signature = signature_collection,
                               method = "pca",
                               mini_gene_count = 2)
sig_tme <- t(column_to_rownames(sig_tme, var = "ID"))

```

```
sig_tme[1:5, 1:3]
```

```
##          GSM1523727 GSM1523728 GSM1523729
## CD_8_T_effector -2.5513794  0.7789141 -2.1770675
## DDR             -0.8747614  0.7425162 -1.3272054
## APM              1.1098368  2.1988688 -0.9516419
## Immune_Checkpoint -2.3701787  0.9455120 -1.4844104
## CellCycle_Reg     0.1063358  0.7583302 -0.3649795
```

```
TMEcluster
```

```
res <- find_markers_in_bulk(pdata = tme, eset = sig_tme, group = "TMEcluster", nfeatures
```

```
##
##  IA  IE  IS
## 107 96 97
## # A tibble: 58 x 7
## # Groups:   cluster [3]
##      p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
##      <dbl>      <dbl> <dbl> <dbl>      <dbl> <fct>  <chr>
##  1 6.21e-19      4.07 0.701 0.316  1.59e-16 IA     IFNG-signature-Ayers-et-al
##  2 1.82e-17      5.18 0.813 0.399  4.66e-15 IA     Th2-cells-Bindea-et-al
##  3 2.60e-16      4.65 0.813 0.383  6.65e-14 IA     Folate-One-Carbon-Metaboli~
##  4 1.52e-15      5.12 0.804 0.352  3.89e-13 IA     Homologous-recombination
##  5 4.95e-15      4.84 0.673 0.275  1.27e-12 IA     CD-8-T-effector
##  6 7.70e-15      3.16 0.71  0.332  1.97e-12 IA     Th1-cells-Bindea-et-al
##  7 9.52e-14      3.16 0.822 0.399  2.44e-11 IA     Purine-Biosynthesis
##  8 1.42e-13      2.83 0.664 0.352  3.63e-11 IA     ADP-Ribosylation
##  9 5.96e-13      2.96 0.785 0.42  1.53e-10 IA     TIP-Release-of-cancer-cell~
## 10 3.00e-12      2.71 0.776 0.409  7.68e-10 IA     Glycine--Serine-and-Threon~
## # i 48 more rows
```

```
top15 <- res$top_markers %>% dplyr:: group_by(cluster) %>% dplyr::top_n(15, avg_log2FC
```

```
p1 <- DoHeatmap(res$sce, top15$gene, group.colors = cols)+
  scale_fill_gradientn(colours = rev(colorRampPalette(RColorBrewer::brewer.pal(11, "RdBu
```



```

top15$gene <- gsub(top15$gene, pattern = "\\-", replacement = "\\_")
input <- combine_pd_eset(eset = sig_tme, pdata = tme, feas = top15$gene, scale = T)

p2 <- sig_box(input, variable = "TMEcluster", signature = "IFNG_signature_Ayers_et_al",
              cols = cols, show_pvalue = TRUE, size_of_pvalue = 4, size_of_font = 6)

```

```

## # A tibble: 3 x 8
##   .y.      group1 group2      p    p.adj p.format p.signif method
##   <chr>    <chr> <chr>    <dbl>  <dbl> <chr>    <chr>    <chr>
## 1 signature IA    IE    2.98e-13 6 e-13 3.0e-13 ****    Wilcoxon
## 2 signature IA    IS    1.85e-15 5.6e-15 1.9e-15 ****    Wilcoxon
## 3 signature IE    IS    2.68e- 1 2.7e- 1 0.27     ns      Wilcoxon

```

```

p3 <- sig_box(input, variable = "TMEcluster", signature = "Neutrophils_Bindea_et_al",
              jitter = TRUE, cols = cols, show_pvalue = TRUE, size_of_pvalue = 4, size_of_font = 6)

```

```

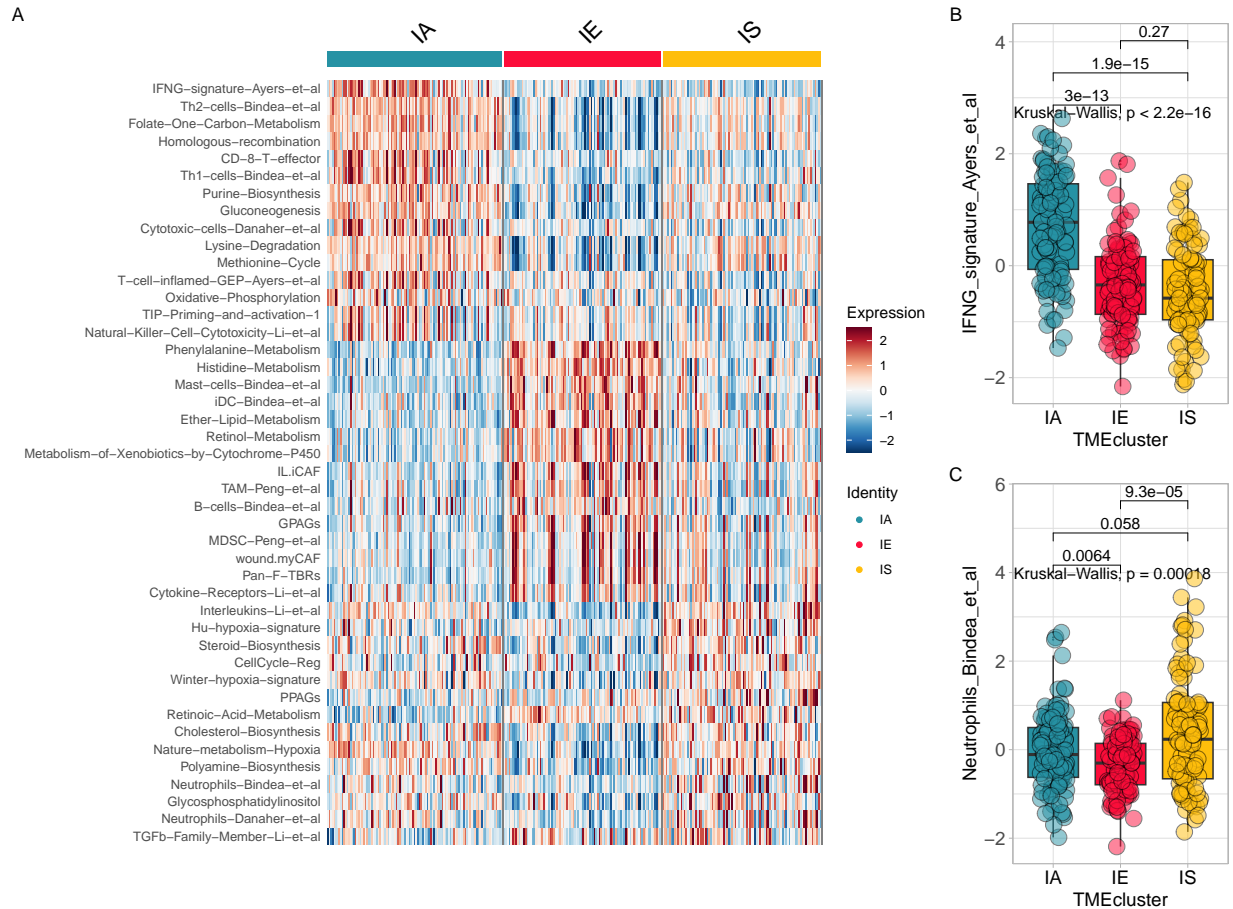
## # A tibble: 3 x 8
##   .y.      group1 group2      p    p.adj p.format p.signif method
##   <chr>    <chr> <chr>    <dbl>  <dbl> <chr>    <chr>    <chr>
## 1 signature IA    IE    0.00639  0.013  0.0064  **      Wilcoxon
## 2 signature IA    IS    0.0584   0.058  0.0584  ns      Wilcoxon
## 3 signature IE    IS    0.0000929 0.00028 9.3e-05 ****    Wilcoxon

```

```

p <- (p1|p2/p3) + plot_layout(widths = c(2.3,1))
p + plot_annotation(tag_levels = 'A')

```



```
library(survminer)
```

```
##
```

```
## Attaching package: 'survminer'
```

```
## The following object is masked from 'package:survival':
```

```
##
```

```
## myeloma
```

```
data(pdata_acrg, package = "IOBR")
```

```
input <- merge(pdata_acrg, input, by = "ID")
```

```
p1<-surv_group(input_pdata      = input,
                target_group     = "TMEcluster",
                ID               = "ID",
                reference_group   = "High",
                project           = "ACRG",
                cols              = cols,
                time              = "OS_time",
```

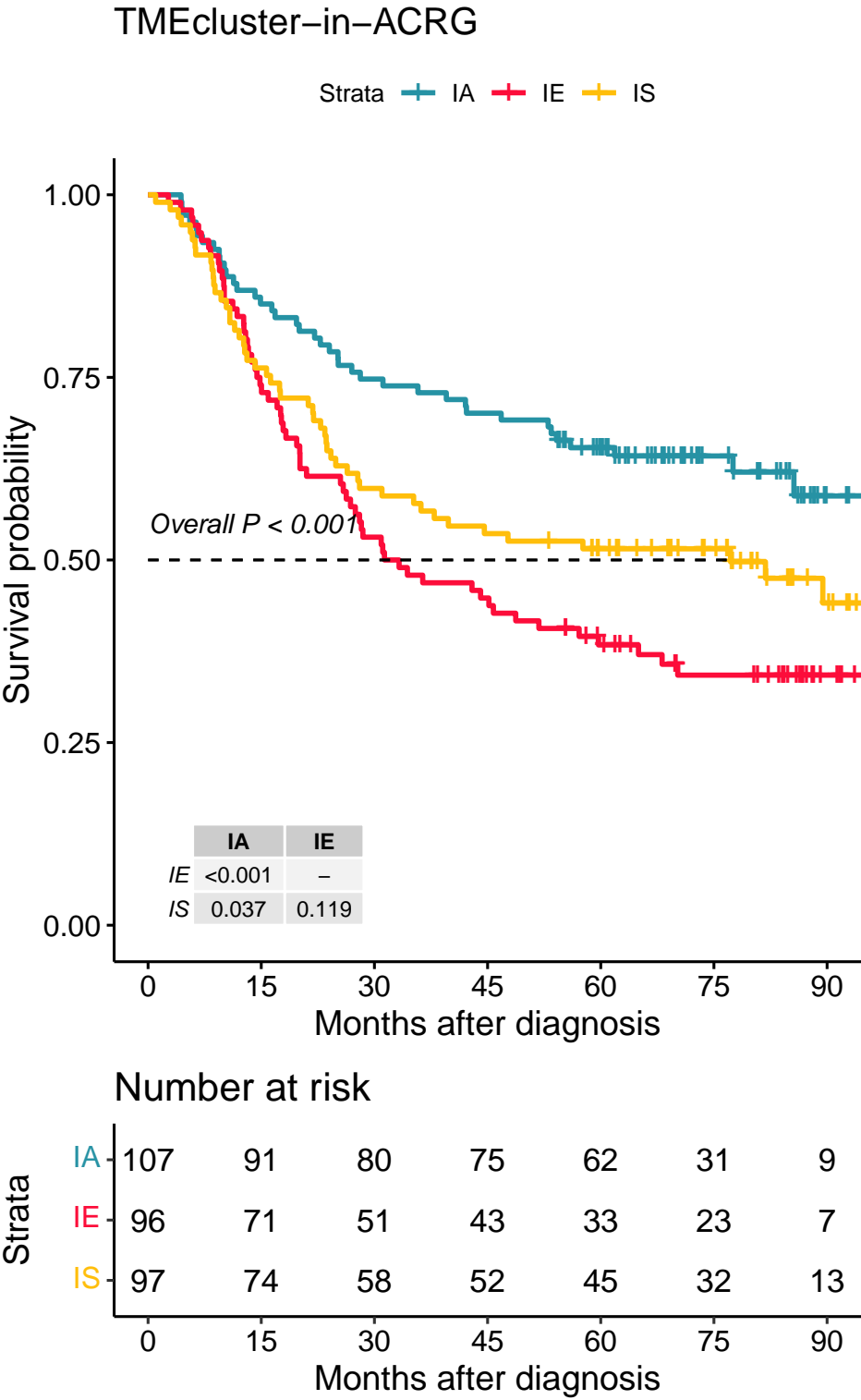
```
status      = "OS_status",  
time_type   = "month",  
save_path   = "result")
```

```
## >>> Dataset's survival follow up time is range between 1 to 105.7 months
```

```
##  IA  IE  IS  
## 107 96 97
```

```
## 1079697
```

```
## Maximum of follow up time is 105.7 months; and will be divided into 6 sections;  
p1
```



```
p1<- percent_bar_plot(input, x = "TMEcluster" , y = "Subtype", palette = "jama")

## # A tibble: 12 x 5
## # Groups:   TMEcluster [3]
```

```
##      TMEcluster Subtype      Freq  Prop count
##      <chr>      <fct>      <dbl> <dbl> <dbl>
##  1 IA          EMT          7  0.07  107
##  2 IA          MSI          49  0.46  107
##  3 IA          MSS/TP53-    27  0.25  107
##  4 IA          MSS/TP53+    24  0.22  107
##  5 IE          EMT          24  0.25   96
##  6 IE          MSI          3  0.03   96
##  7 IE          MSS/TP53-    40  0.42   96
##  8 IE          MSS/TP53+    29  0.3   96
##  9 IS          EMT          15  0.15   97
## 10 IS          MSI          16  0.16   97
## 11 IS          MSS/TP53-    40  0.41   97
## 12 IS          MSS/TP53+    26  0.27   97
## [1] "'#374E55FF', '#DF8F44FF', '#00A1D5FF', '#B24745FF', '#79AF97FF', '#6A6599FF', '#
```

```
p2<- percent_bar_plot(input, x = "TMEcluster" , y = "Lauren", palette = "jama")
```

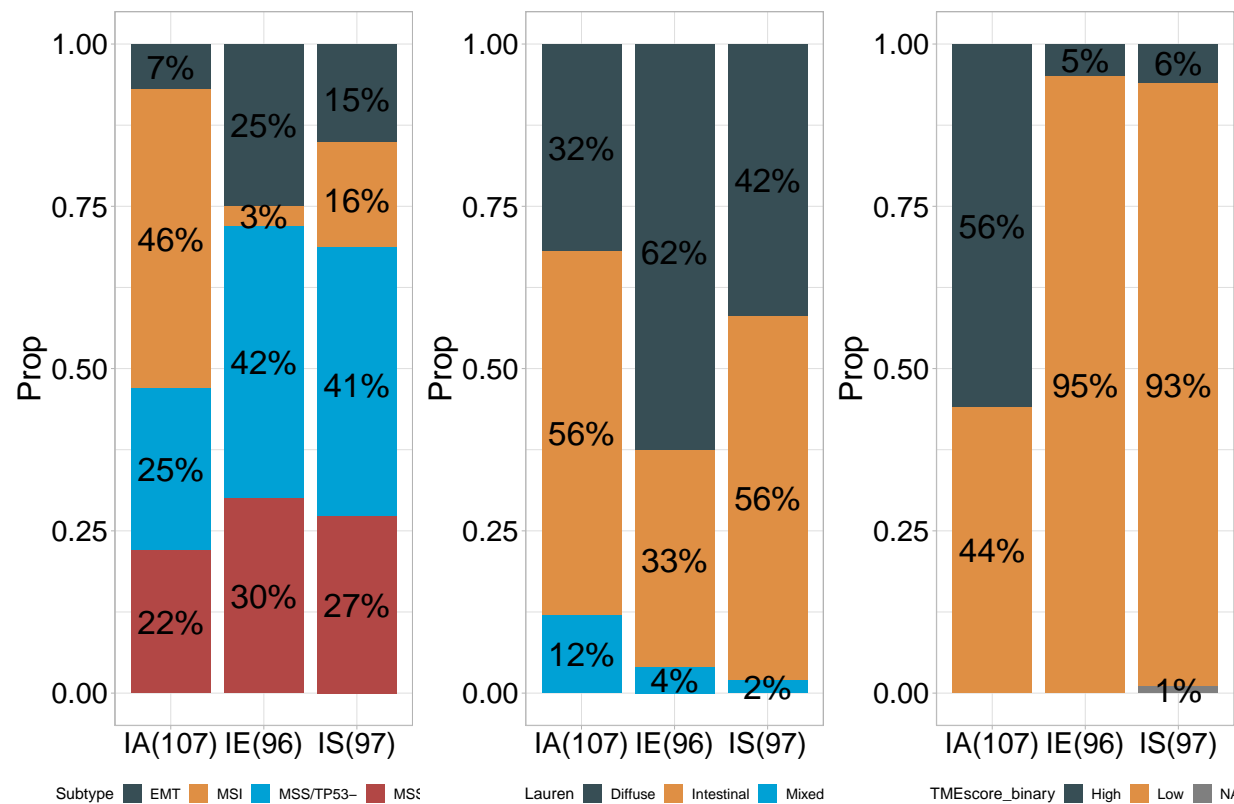
```
## # A tibble: 9 x 5
## # Groups:   TMEcluster [3]
##      TMEcluster Lauren      Freq  Prop count
##      <chr>      <fct>      <dbl> <dbl> <dbl>
##  1 IA          Diffuse      34  0.32  107
##  2 IA          Intestinal    60  0.56  107
##  3 IA          Mixed        13  0.12  107
##  4 IE          Diffuse      60  0.62   96
##  5 IE          Intestinal    32  0.33   96
##  6 IE          Mixed         4  0.04   96
##  7 IS          Diffuse      41  0.42   97
##  8 IS          Intestinal    54  0.56   97
##  9 IS          Mixed         2  0.02   97
## [1] "'#374E55FF', '#DF8F44FF', '#00A1D5FF', '#B24745FF', '#79AF97FF', '#6A6599FF', '#
```

```
p3<- percent_bar_plot(input, x = "TMEcluster" , y = "TMEscore_binary", palette = "jama")
```

```
## # A tibble: 7 x 5
## # Groups:   TMEcluster [3]
##      TMEcluster TMEscore_binary Freq  Prop count
##      <chr>      <fct>      <dbl> <dbl> <dbl>
```

##	1	IA	High	60	0.56	107
##	2	IA	Low	47	0.44	107
##	3	IE	High	5	0.05	96
##	4	IE	Low	91	0.95	96
##	5	IS	High	6	0.06	97
##	6	IS	Low	90	0.93	97
##	7	IS	<NA>	1	0.01	97
##	[1]	"'#374E55FF', '#DF8F44FF', '#00A1D5FF', '#B24745FF', '#79AF97FF', '#6A6599FF', '#				

p1|p2|p3



Chapter 4

Signature and relevant phenotypes

4.1 Loading packages

Load the IOBR package in your R session after the installation is complete:

```
library(IOBR)
library(survminer)
library(tidyverse)
```

4.2 Downloading data for example

Obtaining data set from GEO Gastric cancer: GSE62254 using GEOquery R package.

```
if (!requireNamespace("GEOquery", quietly = TRUE)) BiocManager::install("GEOquery")
library("GEOquery")
# NOTE: This process may take a few minutes which depends on the internet connection s
eset_geo <- getGEO(GEO = "GSE62254", getGPL = F, destdir = "./")
eset <- eset_geo[[1]]
eset <- exprs(eset)
eset[1:5,1:5]
```

```
##          GSM1523727 GSM1523728 GSM1523729 GSM1523744 GSM1523745
## 1007_s_at  3.2176645  3.0624323  3.0279131   2.921683   2.8456013
## 1053_at   2.4050109  2.4394879  2.2442708   2.345916   2.4328582
## 117_at    1.4933412  1.8067380  1.5959665   1.839822   1.8326058
## 121_at    2.1965561  2.2812181  2.1865556   2.258599   2.1874363
```

```
## 1255_g_at 0.8698382 0.9502466 0.8125414 1.012860 0.9441993
```

Annotation of genes in the expression matrix and removal of duplicate genes.

```
# Load the annotation file `anno_hug133plus2` in IOBR.
```

```
head(anno_hug133plus2)
```

```
## # A tibble: 6 x 2
```

```
##   probe_id symbol
```

```
##   <fct>      <fct>
```

```
## 1 1007_s_at MIR4640
```

```
## 2 1053_at   RFC2
```

```
## 3 117_at    HSPA6
```

```
## 4 121_at    PAX8
```

```
## 5 1255_g_at GUCA1A
```

```
## 6 1294_at   MIR5193
```

```
# Conduct gene annotation using `anno_hug133plus2` file; If identical gene symbols exist
```

```
eset<-anno_eset(eset      = eset,
                 annotation = anno_hug133plus2,
                 symbol     = "symbol",
                 probe      = "probe_id",
                 method     = "mean")
```

```
eset[1:5, 1:3]
```

```
##           GSM1523727 GSM1523728 GSM1523729
```

```
## SH3KBP1      4.327974  4.316195  4.351425
```

```
## RPL41        4.246149  4.246808  4.257940
```

```
## EEF1A1       4.293762  4.291038  4.262199
```

```
## COX2         4.250288  4.283714  4.270508
```

```
## LOC101928826 4.219303  4.219670  4.213252
```

4.3 Signature score estimation

4.3.1 Signature collection of IOBR

```
# Return available parameter options of signature estimation.
```

```
signature_score_calculation_methods
```



```
##          PCA          ssGSEA          z-score  Integration
##          "pca"         "ssgsea"        "zscore"  "integration"
```

```
#TME associated signatures
```

```
names(signature_tme)[1:20]
```

```
## [1] "CD_8_T_effector"      "DDR"
## [3] "APM"                  "Immune_Checkpoint"
## [5] "CellCycle_Reg"        "Pan_F_TBRs"
## [7] "Histones"             "EMT1"
## [9] "EMT2"                 "EMT3"
## [11] "WNT_target"           "FGFR3_related"
## [13] "Cell_cycle"           "Mismatch_Repair"
## [15] "Homologous_recombination" "Nucleotide_excision_repair"
## [17] "DNA_replication"      "Base_excision_repair"
## [19] "TMEscoreA_CIR"        "TMEscoreB_CIR"
```

```
#Metabolism related signatures
```

```
names(signature_metabolism)[1:20]
```

```
## [1] "Cardiolipin_Metabolism"
## [2] "Cardiolipin_Biosynthesis"
## [3] "Cholesterol_Biosynthesis"
## [4] "Citric_Acid_Cycle"
## [5] "Cyclooxygenase_Arachidonic_Acid_Metabolism"
## [6] "Prostaglandin_Biosynthesis"
## [7] "Purine_Biosynthesis"
## [8] "Pyrimidine_Biosynthesis"
## [9] "Dopamine_Biosynthesis"
## [10] "Epinephrine_Biosynthesis"
## [11] "Norepinephrine_Biosynthesis"
## [12] "Fatty_Acid_Degradation"
## [13] "Fatty_Acid_Elongation"
## [14] "Fatty_Acid_Biosynthesis"
## [15] "Folate_One_Carbon_Metabolism"
## [16] "Folate_biosynthesis"
## [17] "Gluconeogenesis"
## [18] "Glycolysis"
## [19] "Glycogen_Biosynthesis"
```

```
## [20] "Glycogen_Degradation"
```

```
#Signatures associated with biomedical basic research: such as m6A and exosomes
names(signature_tumor)
```

```
## [1] "Nature_metabolism_Hypoxia"
## [2] "Winter_hypoxia_signature"
## [3] "Hu_hypoxia_signature"
## [4] "Molecular_Cancer_m6A"
## [5] "MT_exosome"
## [6] "SR_exosome"
## [7] "Positive_regulation_of_exosomal_secretion"
## [8] "Negative_regulation_of_exosomal_secretion"
## [9] "Exosomal_secretion"
## [10] "Exosome_assembly"
## [11] "Extracellular_vesicle_biogenesis"
## [12] "MC_Review_Exosome1"
## [13] "MC_Review_Exosome2"
## [14] "CMLS_Review_Exosome"
## [15] "Ferroptosis"
## [16] "EV_Cell_2020"
```

```
#signature collection including all aforementioned signatures
names(signature_collection)[1:20]
```

```
## [1] "CD_8_T_effector"      "DDR"
## [3] "APM"                  "Immune_Checkpoint"
## [5] "CellCycle_Reg"        "Pan_F_TBRs"
## [7] "Histones"             "EMT1"
## [9] "EMT2"                 "EMT3"
## [11] "WNT_target"           "FGFR3_related"
## [13] "Cell_cycle"           "Mismatch_Repair"
## [15] "Homologous_recombination" "Nucleotide_excision_repair"
## [17] "DNA_replication"      "Base_excision_repair"
## [19] "TMEscoreA_CIR"        "TMEscoreB_CIR"
```

```
#citation of signatures
signature_collection_citation[1:20, ]
```

```
## # A tibble: 20 x 6
```

##	Signatures	Published	year	Journal	Title	PMID	DOI
##	<chr>		<dbl>	<chr>	<chr>	<chr>	<chr>
##	1 CD_8_T_effector		2018	Nature	TGF ~	2944~	10.1~
##	2 DDR		2018	Nature	TGF ~	2944~	10.1~
##	3 APM		2018	Nature	TGF ~	2944~	10.1~
##	4 Immune_Checkpoint		2018	Nature	TGF ~	2944~	10.1~
##	5 CellCycle_Reg		2018	Nature	TGF ~	2944~	10.1~
##	6 Pan_F_TBRs		2018	Nature	TGF ~	2944~	10.1~
##	7 Histones		2018	Nature	TGF ~	2944~	10.1~
##	8 EMT1		2018	Nature	TGF ~	2944~	10.1~
##	9 EMT2		2018	Nature	TGF ~	2944~	10.1~
##	10 EMT3		2018	Nature	TGF ~	2944~	10.1~
##	11 WNT_target		2018	Nature	TGF ~	2944~	10.1~
##	12 FGFR3_related		2018	Nature	TGF ~	2944~	10.1~
##	13 Cell_cycle		2018	Nature	TGF ~	2944~	10.1~
##	14 Mismatch_Repair		2018	Nature	TGF ~	2944~	10.1~
##	15 Homologous_recombination		2018	Nature	TGF ~	2944~	10.1~
##	16 Nucleotide_excision_repair		2018	Nature	TGF ~	2944~	10.1~
##	17 DNA_replication		2018	Nature	TGF ~	2944~	10.1~
##	18 Base_excision_repair		2018	Nature	TGF ~	2944~	10.1~
##	19 TMEscoreA_CIR		2019	Cancer Immunol~	Tumo~	3084~	10.1~
##	20 TMEscoreB_CIR		2019	Cancer Immunol~	Tumo~	3084~	10.1~

Three methodologies were adopted in the process of signature score evaluation, comprising Single-sample Gene Set Enrichment Analysis (ssGSEA), Principal component analysis (PCA), and Z-score.

4.3.2 Estimated by PCA method

```
sig_tme<-calculate_sig_score(pdata      = NULL,
                             eset       = eset,
                             signature  = signature_collection,
                             method     = "pca",
                             mini_gene_count = 2)

sig_tme <- t(column_to_rownames(sig_tme, var = "ID"))
sig_tme[1:5, 1:3]
```

```
## GSM1523727 GSM1523728 GSM1523729
## CD_8_T_effector -2.5513794 0.7789141 -2.1770675
## DDR -0.8747614 0.7425162 -1.3272054
## APM 1.1098368 2.1988688 -0.9516419
## Immune_Checkpoint -2.3701787 0.9455120 -1.4844104
## CellCycle_Reg 0.1063358 0.7583302 -0.3649795
```

4.3.3 Estimated by ssGSEA methodology

This method is suitable for gene sets with a large number of genes, such as those of GO, KEGG, REACTOME gene sets.

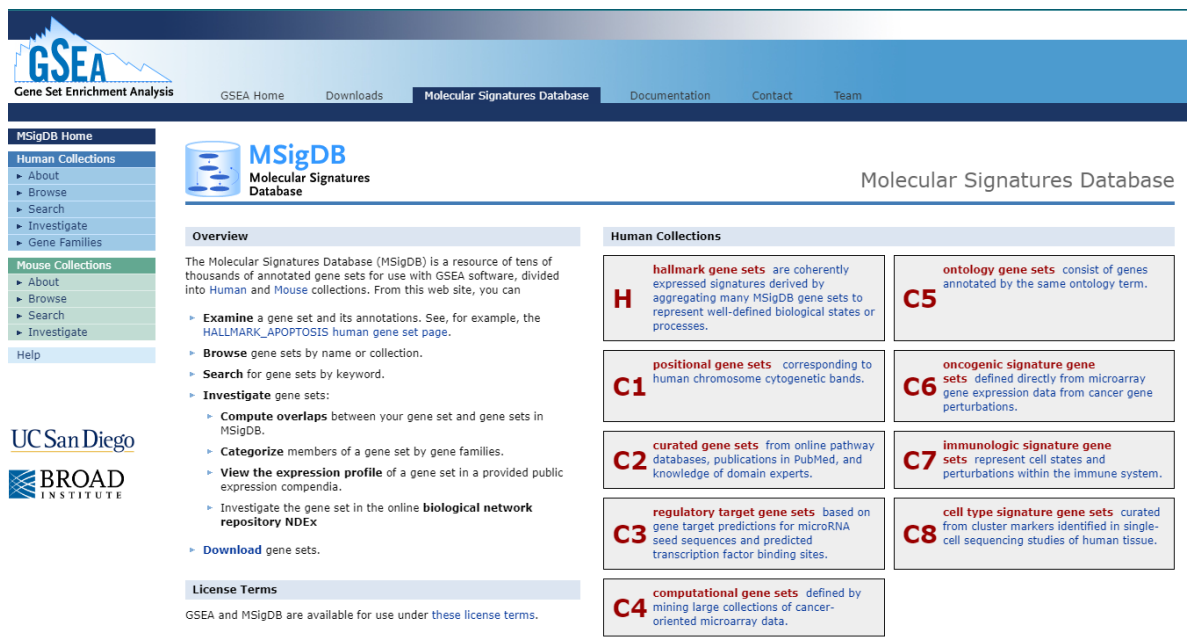


Figure 4.1: Gene sets of MSigDb

```
sig_tme<-calculate_sig_score(pdata = NULL,
                             eset   = eset,
                             signature = go_bp,
                             method  = "ssgsea",
                             mini_gene_count = 2)
```

4.3.4 Estimated by zscore function

```
sig_tme<-calculate_sig_score(pdata = NULL,
                             eset   = eset,
```

```
signature      = signature_collection,
method         = "zscore",
mini_gene_count = 2)
```

4.3.5 Reference

ssgsea: Barbie, D.A. et al (2009). Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, 462(5):108-112.

gsva: Hänzelmann, S., Castelo, R. and Guinney, J. (2013). GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14(1):7.

zscore: Lee, E. et al (2008). Inferring pathway activity toward precise disease classification. *PLoS Comp Biol*, 4(11):e1000217.

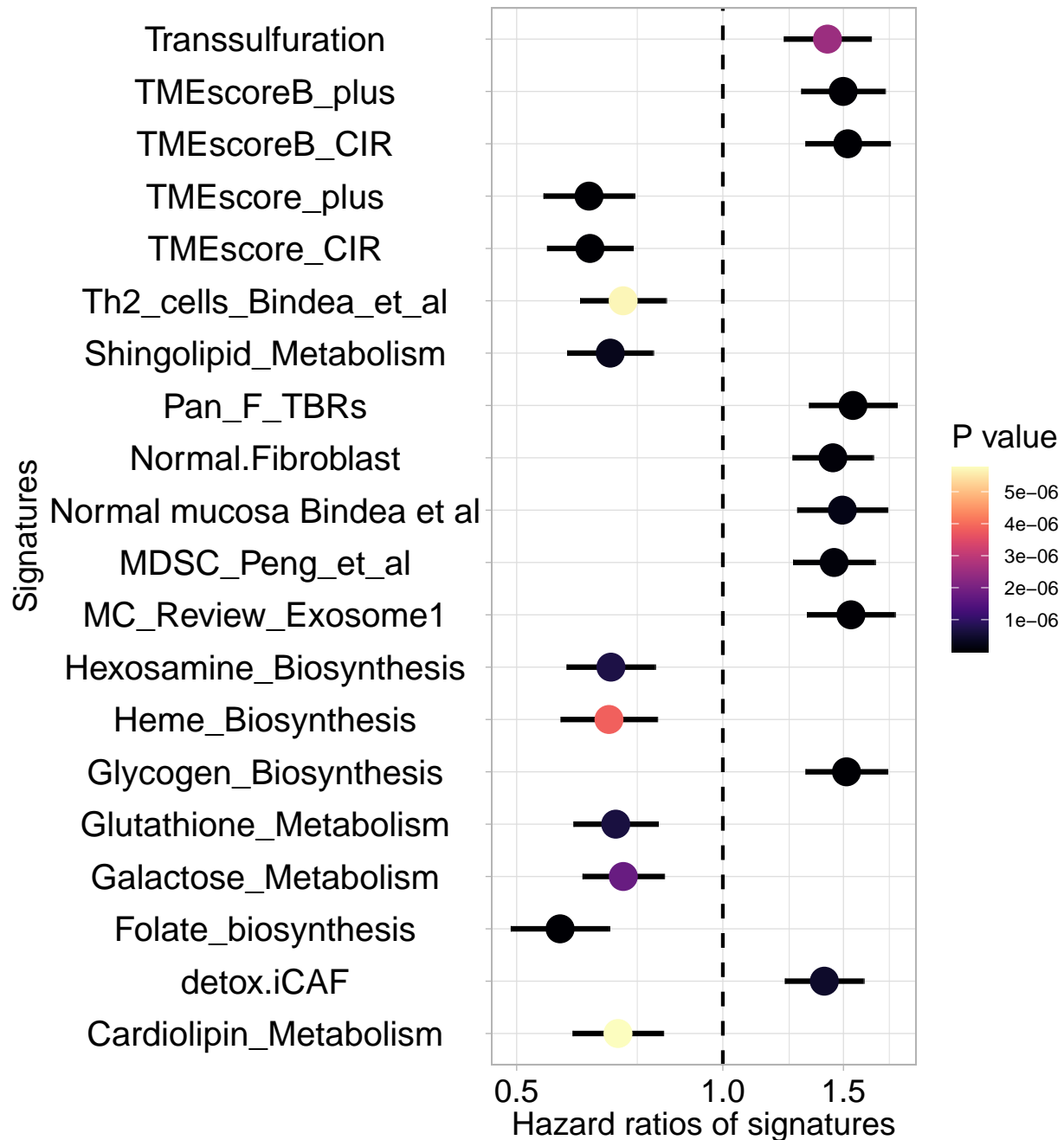
PCA method: Mariathasan S, Turley SJ, Nickles D, et al. TGF α attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018 Feb 22;554(7693):544-548.

4.4 Identifying features associated with survival

```
data("pdata_acrg")
input <- combine_pd_eset(eset = sig_tme, pdata = pdata_acrg, scale = T)
res<- batch_surv(pdata      = input,
                  time       = "OS_time",
                  status     = "OS_status",
                  variable   = colnames(input)[69:ncol(input)])
head(res)
```

```
## # A tibble: 6 x 5
##   ID                P      HR CI_low_0.95 CI_up_0.95
##   <chr>             <dbl> <dbl>      <dbl>      <dbl>
## 1 Folate_biosynthesis 1.00e-10 0.579      0.490      0.683
## 2 TMEscore_CIR        1.32e- 9 0.640      0.554      0.739
## 3 Glycogen_Biosynthesis 3.24e- 9 1.52       1.32       1.74
## 4 Pan_F_TBRs         6.33e- 9 1.55       1.34       1.80
## 5 TMEscoreB_CIR       7.17e- 9 1.52       1.32       1.75
## 6 TMEscore_plus       8.08e- 9 0.638      0.547      0.743
```

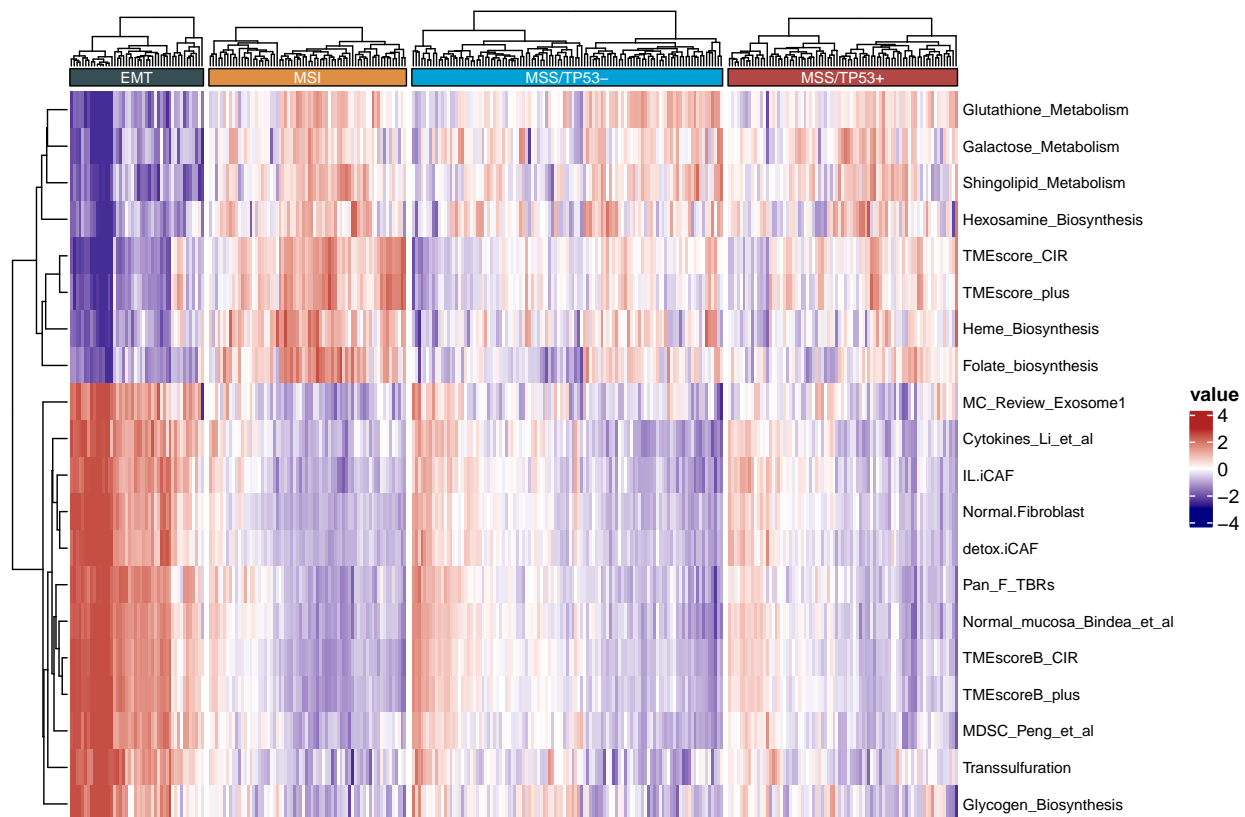
```
res<- res[nchar(res$ID)<=28, ]
p1<- sig_forest(res, signature = "ID", n = 20)
```



4.5 Visulization using heatmap

Signatures IOBR sig_heatmap

```
p2 <- sig_heatmap(input      = input,
                  features    = res$ID[1:20],
                  group       = "Subtype",
                  palette_group = "jama",
                  palette      = 6)
```



4.6 Focus on target signatures

```
p1 <- sig_box(data      = input,
              signature   = "Glycogen_Biosynthesis",
              variable    = "Subtype",
              jitter      = FALSE,
              cols        = NULL,
              palette      = "jama",
              show_pvalue = TRUE,
              size_of_pvalue = 5,
```

```
hjust      = 1,
angle_x_text = 60,
size_of_font = 8)
```

```
## # A tibble: 6 x 8
```

	.y.	group1	group2	p	p.adj	p.format	p.signif	method
	<chr>	<chr>	<chr>	<dbl>	<dbl>	<chr>	<chr>	<chr>
## 1	signature	EMT	MSI	5.39e-15	3.20e-14	5.4e-15	****	Wilcoxon
## 2	signature	EMT	MSS/TP53-	5.53e-13	2.8 e-12	5.5e-13	****	Wilcoxon
## 3	signature	EMT	MSS/TP53+	1.90e-12	7.6 e-12	1.9e-12	****	Wilcoxon
## 4	signature	MSI	MSS/TP53-	1.14e- 3	3.4 e- 3	0.0011	**	Wilcoxon
## 5	signature	MSI	MSS/TP53+	7.05e- 3	1.4 e- 2	0.0071	**	Wilcoxon
## 6	signature	MSS/TP53-	MSS/TP53+	7.16e- 1	7.2 e- 1	0.7161	ns	Wilcoxon

```
p2 <- sig_box(data      = input,
               signature  = "Pan_F_TBRs",
               variable   = "Subtype",
               jitter     = FALSE,
               cols       = NULL,
               palette     = "jama",
               show_pvalue = TRUE,
               angle_x_text = 60,
               hjust      = 1,
               size_of_pvalue = 5,
               size_of_font = 8)
```

```
## # A tibble: 6 x 8
```

	.y.	group1	group2	p	p.adj	p.format	p.signif	method
	<chr>	<chr>	<chr>	<dbl>	<dbl>	<chr>	<chr>	<chr>
## 1	signature	EMT	MSI	7.98e-17	3.20e-16	<2e-16	****	Wilcoxon
## 2	signature	EMT	MSS/TP53-	1.70e-17	1 e-16	<2e-16	****	Wilcoxon
## 3	signature	EMT	MSS/TP53+	2.57e-17	1.3 e-16	<2e-16	****	Wilcoxon
## 4	signature	MSI	MSS/TP53-	1.32e- 2	4 e- 2	0.013	*	Wilcoxon
## 5	signature	MSI	MSS/TP53+	6.99e- 2	1.4 e- 1	0.070	ns	Wilcoxon
## 6	signature	MSS/TP53-	MSS/TP53+	4.02e- 1	4 e- 1	0.402	ns	Wilcoxon

```
p3 <- sig_box(data      = input,
               signature  = "Immune_Checkpoint",
               variable   = "Subtype",
```

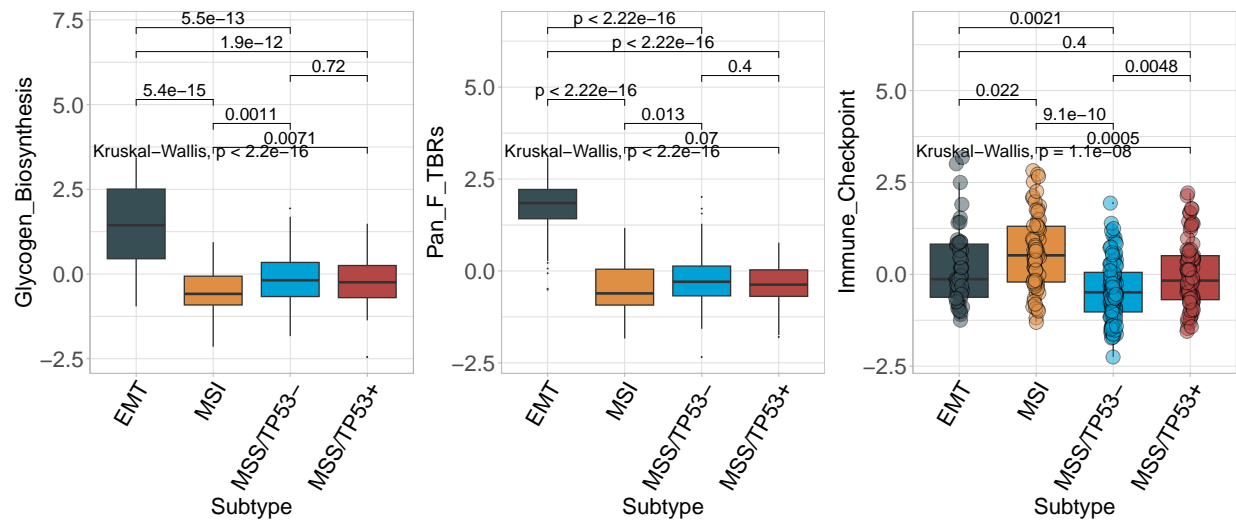


```
jitter      = TRUE,  
cols        = NULL,  
palette     = "jama",  
show_pvalue = TRUE,  
angle_x_text = 60,  
hjust       = 1,  
size_of_pvalue = 5,  
size_of_font  = 8)
```

```
## # A tibble: 6 x 8
```

##	.y.	group1	group2	p	p.adj	p.format	p.signif	method
##	<chr>	<chr>	<chr>	<dbl>	<dbl>	<chr>	<chr>	<chr>
## 1	signature	EMT	MSI	2.20e- 2	0.044	0.0220	*	Wilcoxon
## 2	signature	EMT	MSS/TP53-	2.11e- 3	0.0085	0.0021	**	Wilcoxon
## 3	signature	EMT	MSS/TP53+	4.03e- 1	0.4	0.4026	ns	Wilcoxon
## 4	signature	MSI	MSS/TP53-	9.13e-10	0.0000000055	9.1e-10	****	Wilcoxon
## 5	signature	MSI	MSS/TP53+	5.03e- 4	0.0025	0.0005	***	Wilcoxon
## 6	signature	MSS/TP53-	MSS/TP53+	4.82e- 3	0.014	0.0048	**	Wilcoxon

```
p1|p2|p3
```



4.7 Survival analysis

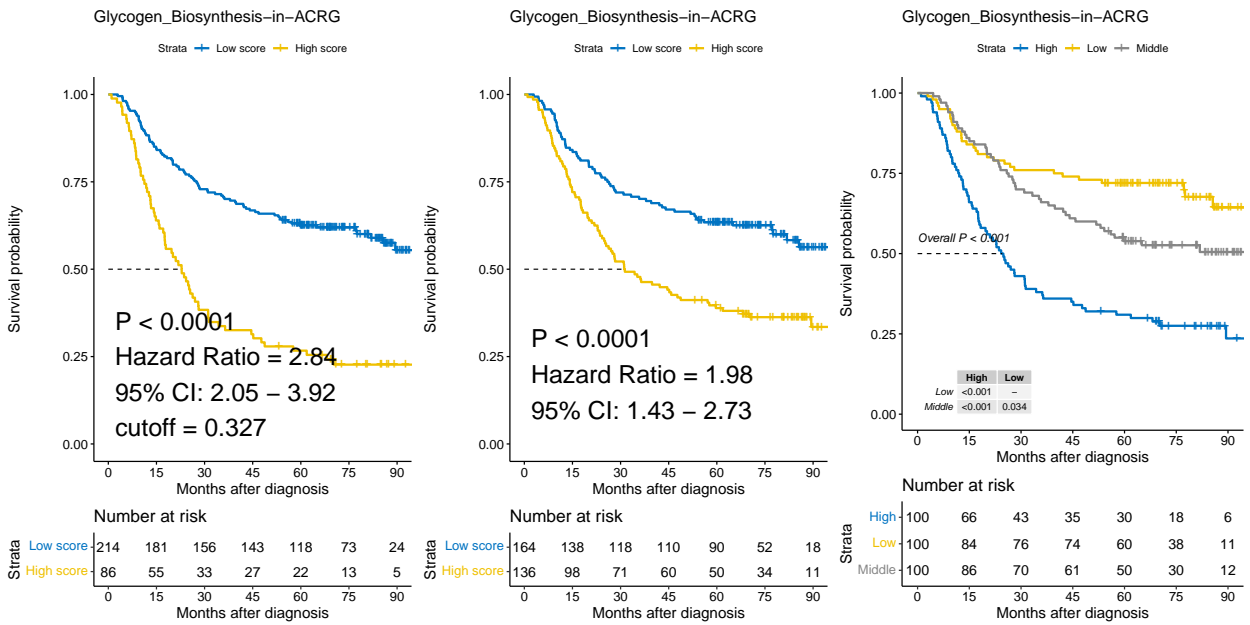
Signature

```
res <- sig_surv_plot(input_pdata = input,
                    signature = "Glycogen_Biosynthesis",
                    cols = NULL,
                    palette = "jco",
                    project = "ACRG",
                    time = "OS_time",
                    status = "OS_status",
                    time_type = "month",
                    save_path = "result")
```

```
##           ID   time status Glycogen_Biosynthesis group3 group2 bestcutoff
## 1 GSM1523727  88.73     0      -0.3612213 Middle    Low      Low
## 2 GSM1523728  88.23     0      -0.6926726 Low       Low      Low
## 3 GSM1523729  88.23     0      -0.9388531 Low       Low      Low
## 4 GSM1523744 105.70     0      -1.1825136 Low       Low      Low
## 5 GSM1523745 105.53     0      -0.3034304 Middle    Low      Low
## 6 GSM1523746  25.50     1       0.7517934 High      High     High

## [1] ">>>>>>>>>"
```

res\$plots



Signature ROC

```
p1<- roc_time(input      = input,
              vars       = "Glycogen_Biosynthesis",
              time       = "OS_time",
              status     = "OS_status",
              time_point = c(12, 24, 36),
              time_type  = "month",
              palette    = "jama",
              cols       = "normal",
              seed       = 1234,
              show_col   = FALSE,
              path       = "result",
              main       = "OS",
              index      = 1,
              fig.type   = "pdf",
              width      = 5,
              height     = 5.2)
```

```
## [1] ">>>-- Range of Time: "  
## [1] 1.0 105.7
```

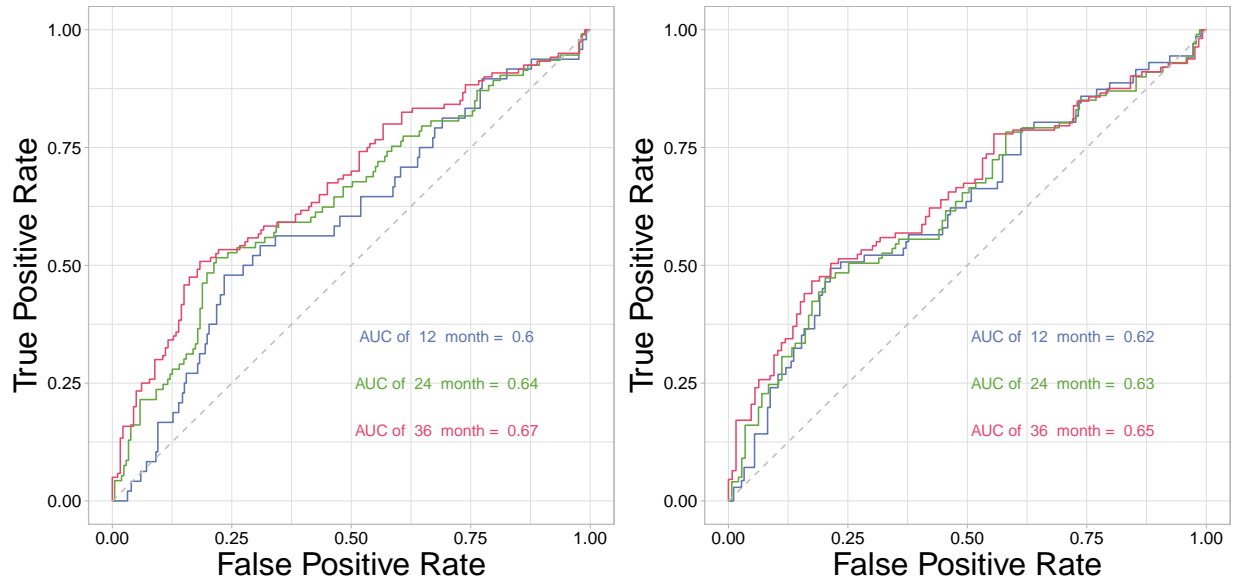
```
p2<- roc_time(input      = input,
              vars       = "Glycogen_Biosynthesis",
              time       = "RFS_time",
              status     = "RFS_status",
              time_point = c(12, 24, 36),
              time_type  = "month",
              palette    = "jama",
              cols       = "normal",
              seed       = 1234,
              show_col   = FALSE,
              path       = "result",
              main       = "OS",
              index      = 1,
              fig.type   = "pdf",
              width      = 5,
              height     = 5.2)
```

```
## [1] ">>>-- Range of Time: "
```

```
## [1] 0.10 100.87
```

```
p1|p2
```

Glycogen_Biosynthesis, OS = 12, 24, 36 m Glycogen_Biosynthesis, OS = 12, 24, 36 m



4.8 Batch correlation analysis

signature signatures

```
res <- batch_cor(data = input, target = "Glycogen_Biosynthesis", feature = colnames(input))
head(res)
```

```
## # A tibble: 6 x 6
```

##	sig_names	p.value	statistic	p.adj	log10pvalue	stars
##	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<fct>
## 1	TMEscoreB_CIR	8.89e-42	0.678	2.27e-39	41.1	****
## 2	Glycine__Serine_and_Threonine_M~	7.49e-40	-0.666	9.54e-38	39.1	****
## 3	Ether_Lipid_Metabolism	3.84e-39	0.662	3.27e-37	38.4	****
## 4	MDSC_Peng_et_al	1.13e-38	0.659	7.21e-37	37.9	****
## 5	Glycerophospholipid_Metabolism	8.72e-38	-0.653	4.44e-36	37.1	****
## 6	TIP_Release_of_cancer_cell_anti~	2.32e-37	-0.650	9.86e-36	36.6	****

```
p1<- get_cor(eset = sig_tme, pdata = pdata_acrg, var1 = "Glycogen_Biosynthesis", var2 =
```

```
##
```

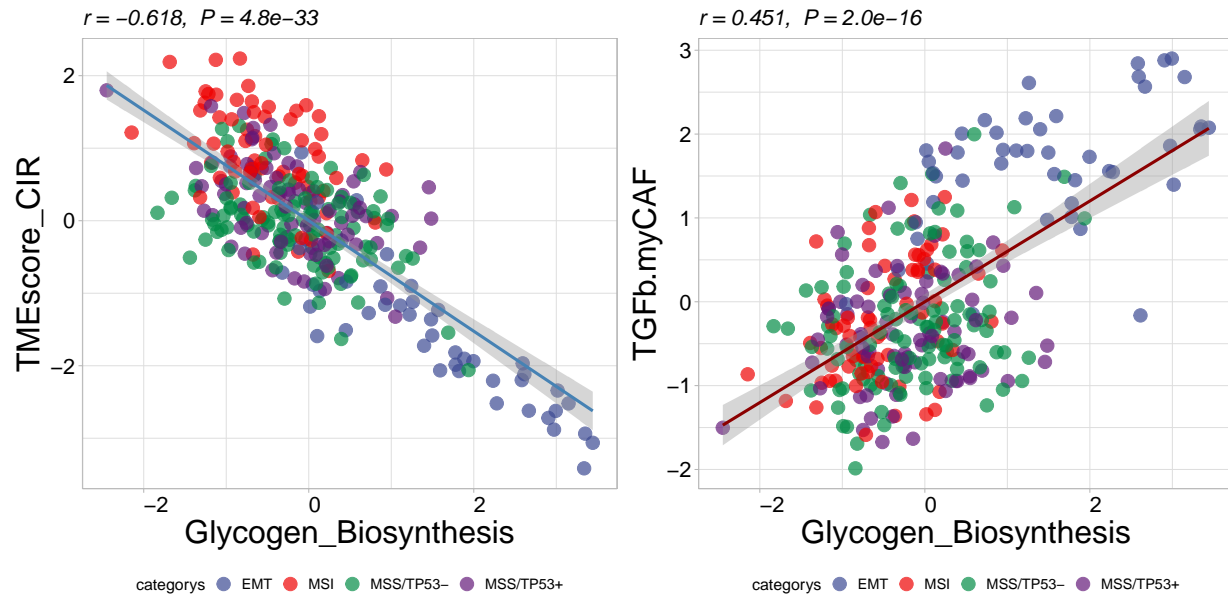
```
## Spearman's rank correlation rho
```

```
##
## data:  data[, var1] and data[, var2]
## S = 7282858, p-value < 2.2e-16
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## -0.6184309
##
## [1] ">>>--- The exact p value is: 4.78971420439895e-33"
##      EMT      MSI MSS/TP53- MSS/TP53+
##      46      68      107      79

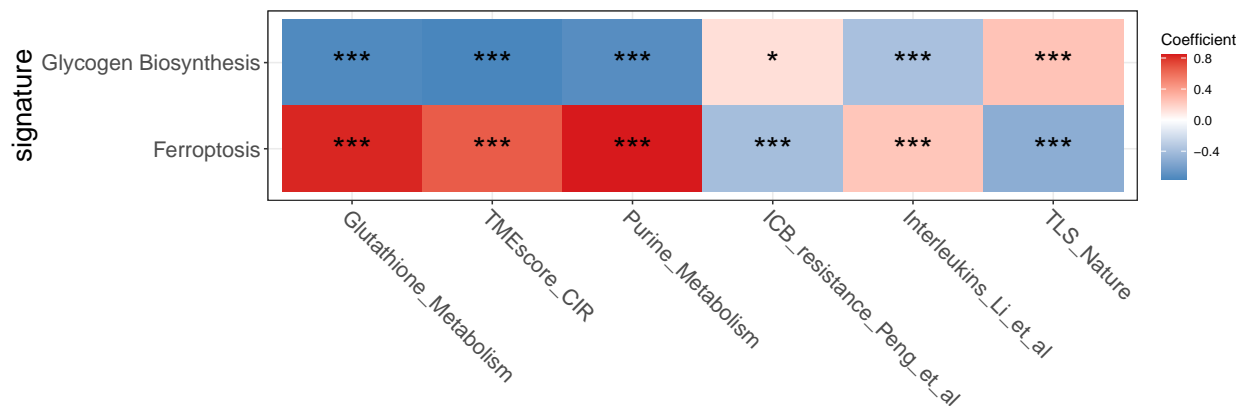
p2<- get_cor(eset = sig_tme, pdata = pdata_acrg, var1 = "Glycogen_Biosynthesis", var2 =
```

```
##
## Spearman's rank correlation rho
##
## data:  data[, var1] and data[, var2]
## S = 2471758, p-value < 2.2e-16
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## 0.4507143
##
## [1] ">>>--- The exact p value is: 2.04505761057615e-16"
##      EMT      MSI MSS/TP53- MSS/TP53+
##      46      68      107      79
```

```
p1|p2
```



```
feas1 <- c("Glycogen_Biosynthesis", "Ferroptosis")
feas2 <- c("Glutathione_Metabolism", "TMEscore_CIR", "Purine_Metabolism", "ICB_resistance")
p <- get_cor_matrix(data = input,
                     feas1 = feas2,
                     feas2 = feas1,
                     method = "pearson",
                     font.size.star = 8,
                     font.size = 15,
                     fill_by_cor = FALSE,
                     round.num = 1)
```

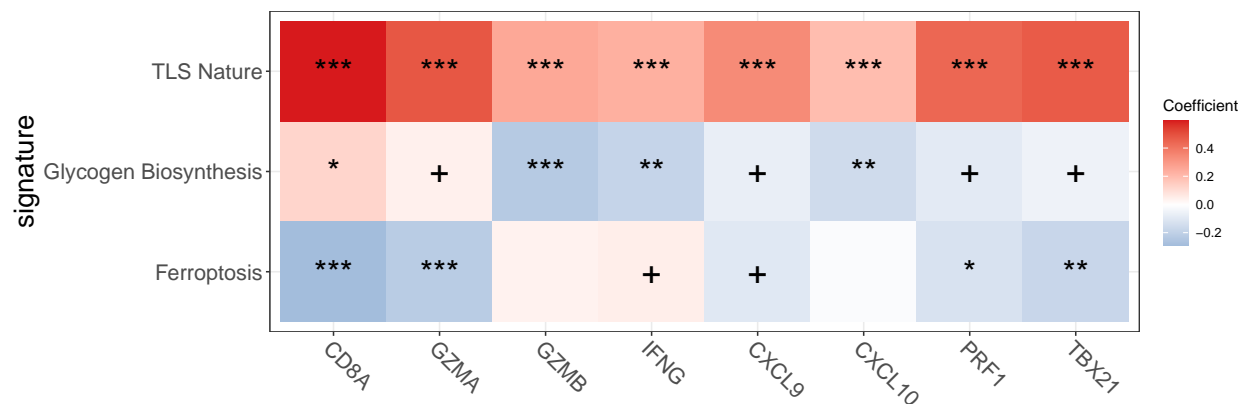


4.9 Visulization of correlations

```
input2 <- combine_pd_eset(eset = eset, pdata = input[, c("ID", "Glycogen_Biosynthesis",
feas1 <- c("Glycogen_Biosynthesis", "TLS_Nature", "Ferroptosis")
feas2 <- signature_collection$CD_8_T_effector
feas2
```

```
## [1] "CD8A" "GZMA" "GZMB" "IFNG" "CXCL9" "CXCL10" "PRF1" "TBX21"
```

```
p <- get_cor_matrix(data      = input2,
                    feas1     = feas2,
                    feas2     = feas1,
                    method    = "pearson",
                    scale     = T,
                    font.size.star = 8,
                    font.size  = 15,
                    fill_by_cor = FALSE,
                    round.num  = 1)
```



```
p <- get_cor_matrix(data      = input2,
                    feas1     = feas2,
                    feas2     = feas1,
                    method    = "pearson",
                    scale     = T,
                    font.size.star = 8,
                    font.size  = 15,
                    fill_by_cor = TRUE,
                    round.num  = 2)
```



Chapter 5

TME deconvolution

5.1 Loading packages

Load the IOBR package in your R session after the installation is complete:

```
library(IOBR)
library(survminer)
library(tidyverse)
```

5.2 Downloading data for example

Obtaining data set from GEO Gastric cancer: GSE62254 using GEOquery R package.

```
if (!requireNamespace("GEOquery", quietly = TRUE)) BiocManager::install("GEOquery")
library("GEOquery")
# NOTE: This process may take a few minutes which depends on the internet connection s
eset_geo<-getGEO(GEO      = "GSE62254", getGPL = F, destdir = "./")
eset    <-eset_geo[[1]]
eset    <-exprs(eset)
eset[1:5,1:5]
```

```
##          GSM1523727 GSM1523728 GSM1523729 GSM1523744 GSM1523745
## 1007_s_at  3.2176645  3.0624323  3.0279131   2.921683   2.8456013
## 1053_at   2.4050109  2.4394879  2.2442708   2.345916   2.4328582
## 117_at    1.4933412  1.8067380  1.5959665   1.839822   1.8326058
## 121_at    2.1965561  2.2812181  2.1865556   2.258599   2.1874363
```

```
## 1255_g_at 0.8698382 0.9502466 0.8125414 1.012860 0.9441993
```

Annotation of genes in the expression matrix and removal of duplicate genes.

```
library(IOBR)
```

```
# Load the annotation file `anno_hug133plus2` in IOBR.
```

```
head(anno_hug133plus2)
```

```
## # A tibble: 6 x 2
```

```
##   probe_id symbol
```

```
##   <fct>      <fct>
```

```
## 1 1007_s_at MIR4640
```

```
## 2 1053_at   RFC2
```

```
## 3 117_at    HSPA6
```

```
## 4 121_at    PAX8
```

```
## 5 1255_g_at GUCA1A
```

```
## 6 1294_at   MIR5193
```

```
# Conduct gene annotation using `anno_hug133plus2` file; If identical gene symbols exist
```

```
eset<-anno_eset(eset      = eset,
                 annotation = anno_hug133plus2,
                 symbol     = "symbol",
                 probe       = "probe_id",
                 method      = "mean")
```

```
eset[1:5, 1:3]
```

```
##           GSM1523727 GSM1523728 GSM1523729
```

```
## SH3KBP1      4.327974  4.316195  4.351425
```

```
## RPL41        4.246149  4.246808  4.257940
```

```
## EEF1A1       4.293762  4.291038  4.262199
```

```
## COX2         4.250288  4.283714  4.270508
```

```
## LOC101928826 4.219303  4.219670  4.213252
```

5.3 Available Methods to Decode TME Contexture

```
tme_deconvolution_methods
```

```
##           MCPcounter           EPIC           xCell           CIBERSORT
```

```
##          "mcpcounter"          "epic"          "xcell"          "cibersort"
## CIBERSORT Absolute            IPS            ESTIMATE            SVR
##          "cibersort_abs"        "ips"          "estimate"        "svr"
##                  lsei          TIMER          quanTIseq
##                  "lsei"         "timer"        "quantiseq"

# Return available parameter options of deconvolution methods
```

The input data is a matrix subseted from ESET of ACRG cohort, with genes in rows and samples in columns. The row name must be HGNC symbols and the column name must be sample names.

```
eset_acrg <- eset[, 1:50]
eset_acrg[1:5, 1:3]
```

```
##          GSM1523727 GSM1523728 GSM1523729
## SH3KBP1          4.327974   4.316195   4.351425
## RPL41            4.246149   4.246808   4.257940
## EEF1A1           4.293762   4.291038   4.262199
## COX2             4.250288   4.283714   4.270508
## LOC101928826     4.219303   4.219670   4.213252
```

Check detail parameters of the function

```
# help(deconvo_tme)
```

5.4 Method 1: CIBERSORT

```
cibersort<-deconvo_tme(eset = eset_acrg, method = "cibersort", arrays = TRUE, perm = 1000)
```

```
##
```

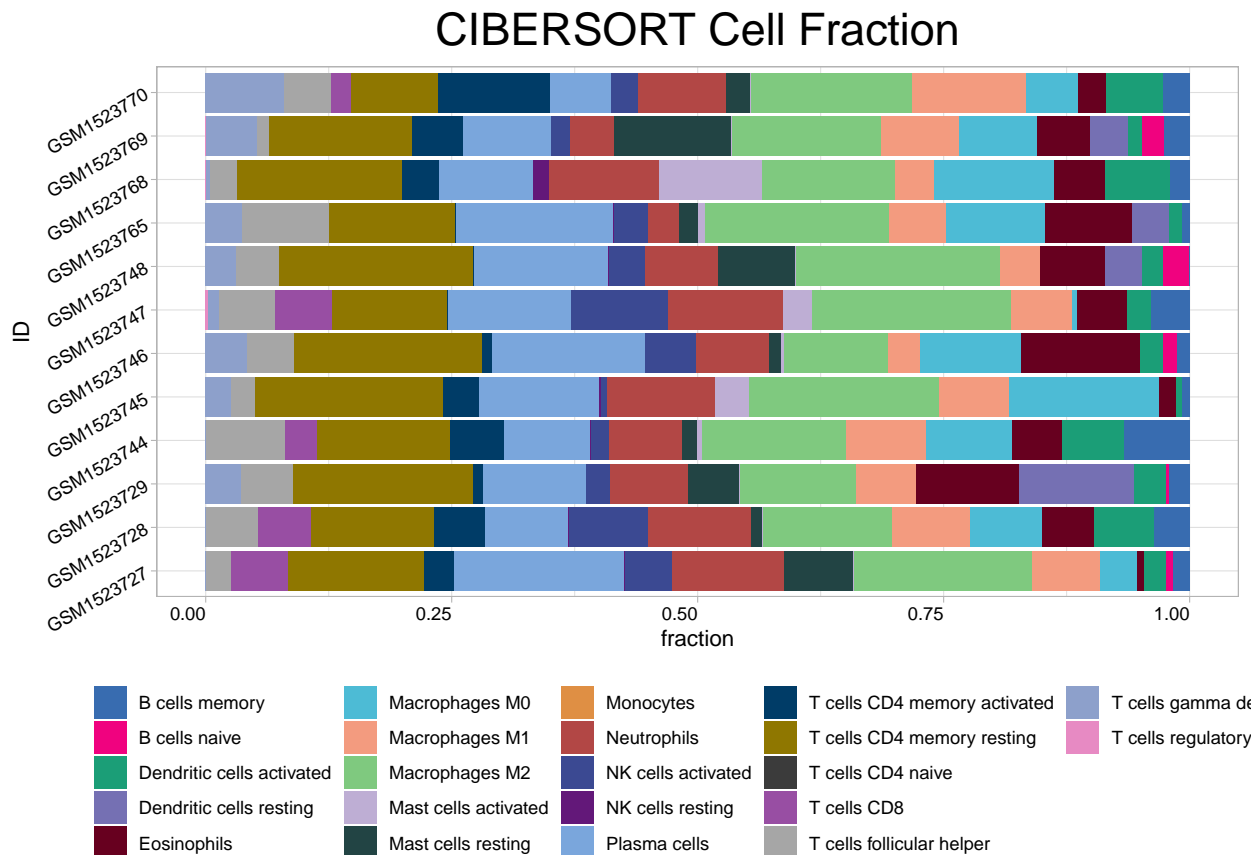
```
## >>> Running CIBERSORT
```

```
# head(cibersort)
```

```
res<-cell_bar_plot(input = cibersort[1:12,], title = "CIBERSORT Cell Fraction")
```

```
## There are seven categories you can choose: box, continue2, continue, random, heatmap,
```

```
## >>>=== Palette option for random: 1: palette1; 2: palette2; 3: palette3; 4: palette4
```



5.5 Method 2: EPIC

```
# help(deconvo_epic)
```

```
epic<-deconvo_tme(eset = eset_acrg, method = "epic", arrays = TRUE)
```

```
##
```

```
## >>> Running EPIC
```

```
## Warning in IOBR::EPIC(bulk = eset, reference = ref, mRNA_cell = NULL, scaleExprs = TR
```

```
## GSM1523744; GSM1523746; GSM1523781; GSM1523786
```

```
## - check fit.gof for the convergeCode and convergeMessage
```

```
## Warning in IOBR::EPIC(bulk = eset, reference = ref, mRNA_cell = NULL, scaleExprs
```

```
## = TRUE): mRNA_cell value unknown for some cell types: CAFs, Endothelial - using
```

```
## the default value of 0.4 for these but this might bias the true cell proportions
```

```
## from all cell types.
```

```
head(epic)
```

```
## # A tibble: 6 x 9
##   ID      Bcells_EPIC CAFs_EPIC CD4_Tcells_EPIC CD8_Tcells_EPIC Endothelial_EPIC
##   <chr>          <dbl>    <dbl>          <dbl>          <dbl>          <dbl>
## 1 GSM152~      0.0292  0.00888        0.145          0.0756         0.0876
## 2 GSM152~      0.0293  0.0109         0.159          0.0745         0.0954
## 3 GSM152~      0.0308  0.0106         0.149          0.0732         0.0941
## 4 GSM152~      0.0273  0.0108         0.145          0.0704         0.0860
## 5 GSM152~      0.0280  0.0111         0.151          0.0707         0.0928
## 6 GSM152~      0.0320  0.00958        0.148          0.0716         0.0907
## # i 3 more variables: Macrophages_EPIC <dbl>, NKcells_EPIC <dbl>,
## #   otherCells_EPIC <dbl>
```

5.6 Method 3: MCPcounter

```
mcp<-deconvo_tme(eset = eset_acrg, method = "mcpcounter")
```

```
##
## >>> Running MCP-counter
```

```
head(mcp)
```

```
## # A tibble: 6 x 11
##   ID      T_cells_MCPcounter CD8_T_cells_MCPcounter Cytotoxic_lymphocytes_M~1
##   <chr>          <dbl>          <dbl>          <dbl>
## 1 GSM1523727      1.47          1.11          1.33
## 2 GSM1523728      1.53          1.05          1.60
## 3 GSM1523729      1.47          1.07          1.37
## 4 GSM1523744      1.46          1.02          1.44
## 5 GSM1523745      1.51          1.10          1.49
## 6 GSM1523746      1.51          0.992         1.40
## # i abbreviated name: 1: Cytotoxic_lymphocytes_MCPcounter
## # i 7 more variables: B_lineage_MCPcounter <dbl>, NK_cells_MCPcounter <dbl>,
## #   Monocytic_lineage_MCPcounter <dbl>,
## #   Myeloid_dendritic_cells_MCPcounter <dbl>, Neutrophils_MCPcounter <dbl>,
## #   Endothelial_cells_MCPcounter <dbl>, Fibroblasts_MCPcounter <dbl>
```

5.7 Method 4: xCELL

```
xcell<-deconvo_tme(eset = eset_acrg, method = "xcell", arrays = TRUE)
```

```
head(xcell)
```

```
## # A tibble: 6 x 68
##   ID          aDC_xCell Adipocytes_xCell Astrocytes_xCell `B-cells_xCell`
##   <chr>          <dbl>          <dbl>          <dbl>          <dbl>
## 1 GSM1523727  4.78e-19          0.0250          0              0
## 2 GSM1523728  9.41e- 2          0.00433         7.70e- 3       0
## 3 GSM1523729  1.02e- 1          0.0789         2.04e- 2       0
## 4 GSM1523744  7.88e- 2          0.0538         4.82e-18       0.0126
## 5 GSM1523745  9.02e- 2          0.0136         1.93e- 2       0
## 6 GSM1523746  3.40e- 2          0.0331         9.22e- 2       0
## # i 63 more variables: Basophils_xCell <dbl>,
## #   `CD4+_memory_T-cells_xCell` <dbl>, `CD4+_naive_T-cells_xCell` <dbl>,
## #   `CD4+_T-cells_xCell` <dbl>, `CD4+_Tcm_xCell` <dbl>, `CD4+_Tem_xCell` <dbl>,
## #   `CD8+_naive_T-cells_xCell` <dbl>, `CD8+_T-cells_xCell` <dbl>,
## #   `CD8+_Tcm_xCell` <dbl>, `CD8+_Tem_xCell` <dbl>, cDC_xCell <dbl>,
## #   Chondrocytes_xCell <dbl>, `Class-switched_memory_B-cells_xCell` <dbl>,
## #   CLP_xCell <dbl>, CMP_xCell <dbl>, DC_xCell <dbl>, ...
```

5.8 Method 5: ESTIMATE

```
estimate<-deconvo_tme(eset = eset_acrg, method = "estimate")
```

```
## [1] "Merged dataset includes 9940 genes (472 mismatched)."
```

```
## [1] "1 gene set: StromalSignature overlap= 136"
```

```
## [1] "2 gene set: ImmuneSignature overlap= 138"
```

```
head(estimate)
```

```
## # A tibble: 6 x 5
##   ID          StromalScore_estimate ImmuneScore_estimate ESTIMATEScore_estimate
##   <chr>          <dbl>          <dbl>          <dbl>
## 1 GSM1523727    -1250.          268.          -982.
## 2 GSM1523728     197.          1334.         1531.
## 3 GSM1523729    -111.          822.          711.
```

```
## 4 GSM1523744          -119.          662.          544.
## 5 GSM1523745           324.        1015.        1339.
## 6 GSM1523746        -594.          621.          27.0
## # i 1 more variable: TumorPurity_estimate <dbl>
```

5.9 Method 6: TIMER

```
timer<-deconvo_tme(eset = eset_acrg, method = "timer", group_list = rep("stad",dim(eset.
```

```
## [1] "Outlier genes: AGR2 B2M COL1A2 COL3A1 COX2 CYAT1 EEF1A1 EIF1 FTH1 GKN1 HUWE1 IGR
```

```
head(timer)
```

```
## # A tibble: 6 x 7
##   ID          B_cell_TIMER T_cell_CD4_TIMER T_cell_CD8_TIMER Neutrophil_TIMER
##   <chr>          <dbl>          <dbl>          <dbl>          <dbl>
## 1 GSM1523727      0.104          0.128          0.183          0.108
## 2 GSM1523728      0.103          0.130          0.192          0.118
## 3 GSM1523729      0.106          0.130          0.190          0.110
## 4 GSM1523744      0.101          0.126          0.187          0.111
## 5 GSM1523745      0.104          0.127          0.191          0.116
## 6 GSM1523746      0.105          0.129          0.192          0.111
## # i 2 more variables: Macrophage_TIMER <dbl>, DC_TIMER <dbl>
```

5.10 Method 7: quanTIseq

```
quantiseq<-deconvo_tme(eset = eset_acrg, tumor = TRUE, arrays = TRUE, scale_mrna = TRUE,
```

```
##
```

```
## Running quanTIseq deconvolution module
```

```
## Gene expression normalization and re-annotation (arrays: TRUE)
```

```
## Removing 17 genes with high expression in tumors
```

```
## Signature genes found in data set: 152/153 (99.35%)
```

```
## Mixture deconvolution (method: lsei)
```

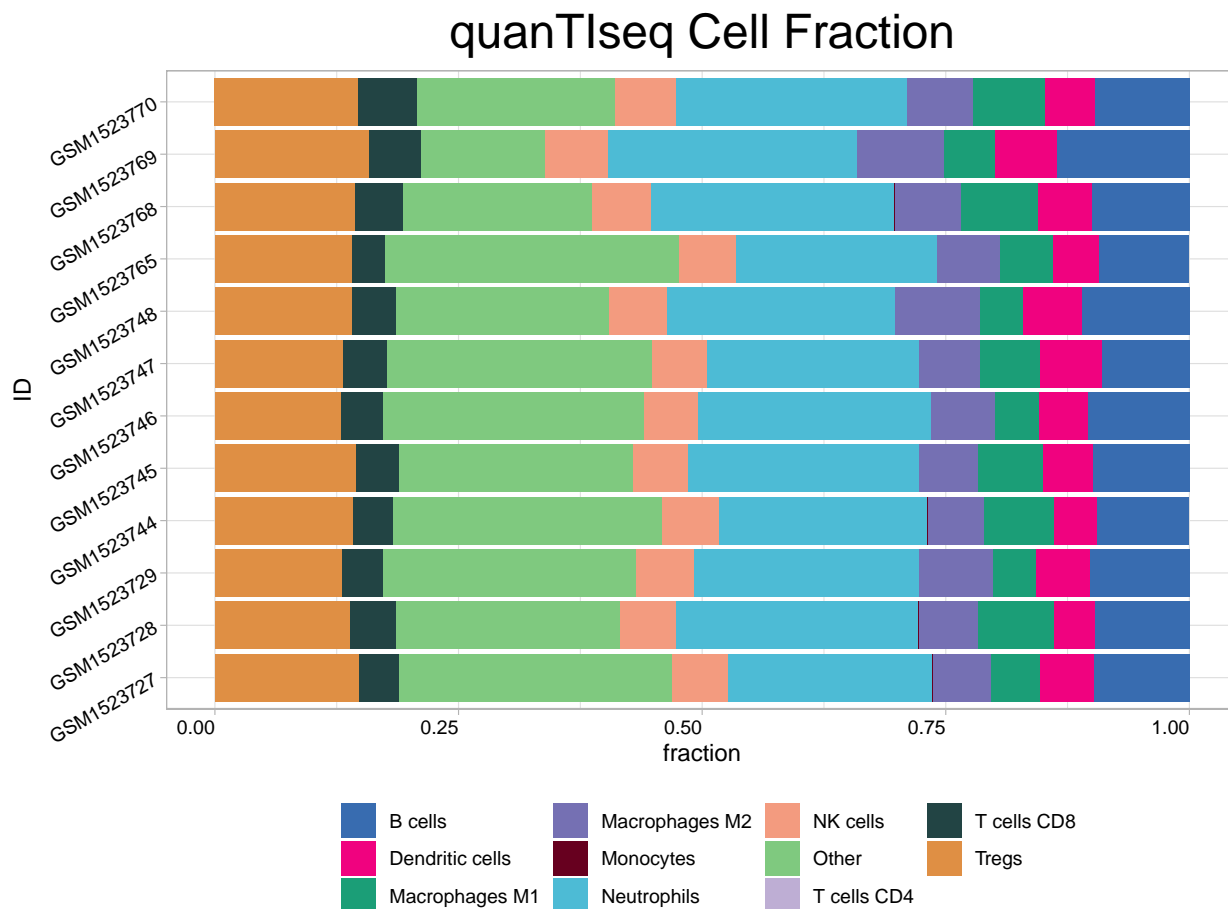
```
## Deconvolution sucessful!
```

```
head(quantiseq)
```

```
## # A tibble: 6 x 12
##   ID          B_cells_quantiseq Macrophages_M1_quantiseq Macrophages_M2_quantiseq
##   <chr>                <dbl>                <dbl>                <dbl>
## 1 GSM1523727          0.0983                0.0510                0.0598
## 2 GSM1523728          0.0967                0.0795                0.0607
## 3 GSM1523729          0.102                 0.0450                0.0758
## 4 GSM1523744          0.0954                0.0725                0.0579
## 5 GSM1523745          0.0991                0.0669                0.0613
## 6 GSM1523746          0.105                 0.0453                0.0662
## # i 8 more variables: Monocytes_quantiseq <dbl>, Neutrophils_quantiseq <dbl>,
## #   NK_cells_quantiseq <dbl>, T_cells_CD4_quantiseq <dbl>,
## #   T_cells_CD8_quantiseq <dbl>, Tregs_quantiseq <dbl>,
## #   Dendritic_cells_quantiseq <dbl>, Other_quantiseq <dbl>
res<-cell_bar_plot(input = quantiseq[1:12, ], title = "quanTIseq Cell Fraction")
```

```
## There are seven categories you can choose: box, continue2, continue, random, heatmap,
```

```
## >>>>== Palette option for random: 1: palette1; 2: palette2; 3: palette3; 4: palette
```

5.11 Method 8: IPS

```
ips<-deconvo_tme(eset = eset_acrg, method = "ips", plot= FALSE)
head(ips)
```

```
## # A tibble: 6 x 7
```

##	ID	MHC_IPS	EC_IPS	SC_IPS	CP_IPS	AZ_IPS	IPS_IPS
##	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
## 1	GSM1523727	2.25	0.404	-0.192	0.220	2.68	9
## 2	GSM1523728	2.37	0.608	-0.578	-0.234	2.17	7
## 3	GSM1523729	2.10	0.480	-0.322	0.0993	2.36	8
## 4	GSM1523744	2.12	0.535	-0.333	0.0132	2.34	8
## 5	GSM1523745	1.91	0.559	-0.479	0.0880	2.08	7
## 6	GSM1523746	1.94	0.458	-0.346	0.261	2.31	8

5.12 Combination of above deconvolution results

```
tme_combine<-cibersort %>%
  inner_join(.,mcp,by      = "ID") %>%
  inner_join(.,xcell,by    = "ID") %>%
  inner_join(.,epic,by     = "ID") %>%
  inner_join(.,estimate,by = "ID") %>%
  inner_join(.,timer,by    = "ID") %>%
  inner_join(.,quantiseq,by = "ID") %>%
  inner_join(.,ips,by      = "ID")
dim(tme_combine)
```

```
## [1] 50 138
```

If you use this package in your work, please cite both our package and the method(s) you are using.

Citation and licenses of these deconvolution methods

CIBERSORT; free for non-commercial use only; Newman, A. M., Liu, C. L., Green, M. R., Gentles, A. J., Feng, W., Xu, Y., ... Alizadeh, A. A. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods*, 12(5), 453–457. <https://doi.org/10.1038/nmeth.3337>;

ESTIMATE; free (GPL2.0); Vegesna R, Kim H, Torres-Garcia W, ..., Verhaak R. (2013). Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature Communications* 4, 2612. <http://doi.org/10.1038/ncomms3612>;

quanTIseq; free (BSD); Finotello, F., Mayer, C., Plattner, C., Laschober, G., Rieder, D., Hackl, H., ..., Sopper, S. (2019). Molecular and pharmacological modulators of the tumor immune contexture revealed by deconvolution of RNA-seq data. *Genome medicine*, 11(1), 34. <https://doi.org/10.1186/s13073-019-0638-6>;

TIMER; free (GPL 2.0); Li, B., Severson, E., Pignon, J.-C., Zhao, H., Li, T., Novak, J., ... Liu, X. S. (2016). Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biology*, 17(1), 174. <https://doi.org/10.1186/s13059-016-1028-7>;

IPS; free (BSD); P. Charoentong et al., Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Reports* 18, 248-262 (2017). <https://doi.org/10.1016/j.celrep.2016.12.019>;

MCPCounter; free (GPL 3.0); Becht, E., Giraldo, N. A., Lacroix, L., Buttard, B., Elarouci,

N., Petitprez, F., ... de Reyniès, A. (2016). Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biology*, 17(1), 218. <https://doi.org/10.1186/s13059-016-1070-5>;

xCell; free (GPL 3.0); Aran, D., Hu, Z., & Butte, A. J. (2017). xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biology*, 18(1), 220. <https://doi.org/10.1186/s13059-017-1349-1>;

EPIC; free for non-commercial use only (Academic License); Racle, J., de Jonge, K., Baumgaertner, P., Speiser, D. E., & Gfeller, D. (2017). Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data. *ELife*, 6, e26476. <https://doi.org/10.7554/eLife.26476>;

GSVA free (GPL (≥ 2)) Hänzelmann S, Castelo R, Guinney J (2013). “GSVA: gene set variation analysis for microarray and RNA-Seq data.” *BMC Bioinformatics*, 14, 7. doi: 10.1186/1471-2105-14-7, <http://www.biomedcentral.com/1471-2105/14/7>

Chapter 6

References

If IOBR R package is utilized in your published research, please cite:

Zeng D, Ye Z, Shen R, Yu G, Wu J, Xiong Y,..., Liao W (2021) **IOBR**: Multi-Omics Immuno-Oncology Biological Research to Decode Tumor Microenvironment and Signatures. *Frontiers in Immunology*. 12:687975. doi: 10.3389/fimmu.2021.687975

6.1 TME deconvolution

Please cite the following papers appropriately for TME deconvolution algorithm if used:

CIBERSORT: Newman, A. M., Liu, C. L., Green, M. R., Gentles, A. J., Feng, W., Xu, Y., ... Alizadeh, A. A. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods*, 12(5), 453–457. <https://doi.org/10.1038/nmeth.3337>

ESTIMATE: Vegesna R, Kim H, Torres-Garcia W, ..., Verhaak R.*(2013). Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature Communications* 4, 2612. <http://doi.org/10.1038/ncomms3612>

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TIMER: Li, B., Severson, E., Pignon, J.-C., Zhao, H., Li, T., Novak, J., ... Liu, X. S.* (2016). Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biology*, 17(1), 174.

IPS: P. Charoentong et al.* (2017). Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Reports* 18, 248-262 (2017). <https://doi.org/10.1016/j.celrep.2016.12.019>

MCPCounter: Becht, E., Giraldo, N. A., Lacroix, L., Buttard, B., Elarouci, N., Petitprez, F., ... de Reyniès, A*. (2016). Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biology*, 17(1), 218. <https://doi.org/10.1186/s13059-016-1070-5>

xCell: Aran, D., Hu, Z., & Butte, A. J.* (2017). xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biology*, 18(1), 220. <https://doi.org/10.1186/s13059-017-1349-1>

EPIC: Racle, J., de Jonge, K., Baumgaertner, P., Speiser, D. E., & Gfeller, D*. (2017). Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data. *ELife*, 6, e26476. <https://doi.org/10.7554/eLife.26476>

6.2 TME Signatures

For signature score estimation, please cite corresponding literature below:

ssgsea: Barbie, D.A. et al (2009). Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, 462(5):108-112.

gsva: Hänzelmann, S., Castelo, R. and Guinney, J. (2013). GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14(1):7.

zscore: Lee, E. et al (2008). Inferring pathway activity toward precise disease classification. *PLoS Comp Biol*, 4(11):e1000217.

6.3 Data sets

For the datasets enrolled in IOBR, please cite the data sources:

UCSCXena: Wang et al., et al (2019). The UCSCXenaTools R package: a toolkit for accessing genomics data from UCSC Xena platform, from cancer multi-omics to single-cell RNA-seq. *Journal of Open Source Software*, 4(40), 1627

TLScore: Helmink BA, Reddy SM, Gao J, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*. 2020 Jan;577(7791):549-555.

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HCP5: Li, Y., Jiang, T., Zhou, W. et al. Pan-cancer characterization of immune-related lncRNAs identifies potential oncogenic biomarkers. *Nat Commun* 11, 1000 (2020). **HCP5:** Sun J, Zhang Z, Bao S, et al Identification of tumor immune infiltration-associated lncRNAs for improving prognosis and immunotherapy response of patients with non-small cell lung cancer *Journal for ImmunoTherapy of Cancer* 2020;8:e000110.

LINC00657: Feng Q, Zhang H, Yao D, Chen WD, Wang YD. Emerging Role of Non-Coding RNAs in Esophageal Squamous Cell Carcinoma. *Int J Mol Sci*. 2019 Dec 30;21(1):258. doi: 10.3390/ijms21010258.

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TCGA-STAD: Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014 Sep 11;513(7517):202-9. doi: 10.1038/nature13480. TCGA.STAD MAF data: <https://api.gdc.cancer.gov/data/c06465a3-50e7-46f7-b2dd-7bd654ca206b>

6.4 Others

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2. Vegesna R, Kim H, Torres-Garcia W, ..., Verhaak R.*(2013). Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature Communications* 4, 2612.
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