The RTopper package: perform run Gene Set Enrichment across genomic platforms

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Contents

1 Overview

Gene Set Enrichment (GSE) analysis has been widely use to assist the interpretation of gene expression data. We propose here to apply GSE for the integration of genomic data obtained from distinct analytical platform.

In the present implementation of the `RTopper` GSE analysis is performed using the `geneSetTest` function from the `limma` package [6, 5, 7]. This function enables testing the hypothesis that a specific set of genes (a Functional Gene Set, FGS) is more highly ranked on a given statistics. In

particular this functions computes a p-value for each FGS by one or two-sided Wilcoxon rank-sum test. Alternative user-defined functions can also be used.

Furthermore multiple hypothesis testing correction is achieved by applying the Benjamini and Hochberg method [2] as implemented in the `multtest` R/Bioconductor package. Overall, this approach is conceptually analogous to Gene Set Enrichment Analysis (GSEA), as proposed by Mootha and colleagues [4, 8].

The integration can be achieved through two distinct approaches:

1. **GSE + INTEGRATION**: Separate GSE analysis on the individual genomic platforms followed by GSE results integration;

2. **INTEGRATION + GSE**: Integration of genomic data measurement using a logistic model followed by GSE analysis;

2   RTopper data structure

In this tutorial we demonstrate the functionality of `RTopper` package. To this end we will make use of simplified data generated within The Cancer Genome Atlas (TCGA) project, using Glioblastoma Multiforme (GBM) genomics data obtained from the same patients' cohort using distinct platforms, including Differential Gene Expression (DGE), Copy Number Variation (CNV), and Differential Methylation (DM). This data is included with the `RTopper` package as the dataset `exampleData`, which consists of genomic measurements (the list `dat`) for 500 genes (in rows) and 95 patients (in columns) from 4 distinct platforms:

1. DGE obtained using Affymetrix;

2. DGE obtained using Agilent;

3. CNV data generated ad Harvard;

4. CNV data generated ad the MSKCC;

The phenotypic class for each patient is defined in the a data.frame `pheno` consisting of 95 rows (patients, *pheno$Sample*) and 2 columns, the first being patients identifiers, and the second variable giving the group indicator (*pheno$Class*).

To load the data set type `data(exampleData)`, and to view a description of this data type `?exampleData`. The structure of the data is shown below:

```
> library(RTopper)
> data(exampleData)
> ls()

[1] "dat"    "pheno"

> class(dat)

[1] "list"

> names(dat)

[1] "dat.affy"      "dat.agilent"
[3] "dat.cnvHarvard" "dat.cnvMskcc"
```

```
> sapply(dat,class)

      dat.affy    dat.agilent dat.cnvHarvard
  "data.frame"   "data.frame"    "data.frame"
  dat.cnvMskcc
  "data.frame"

> sapply(dat,dim)

     dat.affy dat.agilent dat.cnvHarvard
[1,]      500         500            500
[2,]       95          95             95
     dat.cnvMskcc
[1,]          500
[2,]           95

> dim(pheno)

[1] 95  2

> str(pheno)

'data.frame':        95 obs. of  2 variables:
 $ Sample: chr  "TCGA.02.0003" "TCGA.02.0007" "TCGA.02.0011" "TCGA.02.0021" ...
 $ Class : int  0 0 1 1 0 0 0 0 0 0 ...
```

In summary to perform the analysis with functions from RTopper the genomic data used as input must be in the following format:

1. **Genomic measurements**: a list of data.frames, in which each list item corresponds to a genomic platform, and comprises a data.frame with rows being genes and columns patients;

2. **Phenotype data**: a data.frame with 2 columns: patients and their phenotypes;

3. The number of columns of the *Genomic measurements* data.frames must match the number of rows of the *Phenotype data*;

4. The same set of genes must be measured in each platform and gene labels must be stored as rownames;

Below are shown the first 6 rows and 4 columns of each data.frame contained in dat, which share the same genes (shown for some of the possible combinations). Similarly column names in the dat data.frames correspond to rownames of pheno.

```
> ###data structure
> lapply(dat,function(x) head(x)[,1:3])

$dat.affy
      TCGA.02.0003 TCGA.02.0007 TCGA.02.0011
AACS      7.747995     7.685409     7.535661
AARS      9.381544     9.930156    10.197194
ABI1      8.173255     8.962803     9.895811
ACHE      5.127197     4.547297     5.146552
ACTC1     6.612645     5.825879     8.067945
ACTN2     6.257383     5.330557     5.842319
```

```
$dat.agilent
      TCGA.02.0003 TCGA.02.0007 TCGA.02.0011
AACS    -1.0070000   -1.1164000     -0.913000
AARS    -1.2665000   -0.8981250      0.263500
ABI1    -0.2765000    0.3356250      1.027250
ACHE     0.4403750   -0.0222500      0.115000
ACTC1    0.3641538    0.1234615      1.046692
ACTN2    4.3348000    2.2278000      3.330600


$dat.cnvHarvard
      TCGA.02.0003 TCGA.02.0007 TCGA.02.0011
AACS   -0.08273213  -0.08917331   -0.02075644
AARS   -0.10233281  -0.20620608   -0.05157664
ABI1   -0.86886659  -0.01214599    0.59307754
ACHE    0.31560002  -1.00166150   -0.14519639
ACTC1  -1.17495078  -0.26698279   -0.95662761
ACTN2  -0.11319016  -0.09657971    0.02582138


$dat.cnvMskcc
      TCGA.02.0003 TCGA.02.0007 TCGA.02.0011
AACS    -0.0383875   -0.09140000  0.008233333
AARS     0.0075600    0.02801667  0.104850000
ABI1    -0.7006900    0.21270000  0.499472727
ACHE     0.8676000   -0.23970000  0.075000000
ACTC1   -0.9779500   -0.11625000 -0.692950000
ACTN2   -0.1258571   -0.05394444  0.010200000

> sum(rownames(dat[[1]])%in%rownames(dat[[2]]))

[1] 500

> sum(rownames(dat[[2]])%in%rownames(dat[[3]]))

[1] 500
```

2.1 Creation of Functional Gene Sets

Functional Gene Sets (FGS) are list of genes that share a specific biological function. Examples of FGS are genes that operate in the same signaling pathway (*i.e.* Notch signaling genes), or that share the same biological function (*i.e.* Cell adhesion genes). FGS can be retrieved from various database, or can be constructed *ad hoc*. A convenient source of FGS are the R-Bioconductor metaData packages, and S4 classes and methods for handling FGS are provided by the GSEABase package. Below is shown a simple way to extract FGS from the human genome metaData package org.Hs.eg.db. As a general rule the name of the metaData package, without the .db extension, can be used a function to see the content of the package, as shown below:

```
> library(org.Hs.eg.db)
> org.Hs.eg()
```

Quality control information for org.Hs.eg:

This package has the following mappings:

org.Hs.egACCNUM has 40093 mapped keys (of 63901 keys)
org.Hs.egACCNUM2EG has 805030 mapped keys (of 805030 keys)
org.Hs.egALIAS2EG has 128953 mapped keys (of 128953 keys)
org.Hs.egCHR has 63751 mapped keys (of 63901 keys)
org.Hs.egCHRLENGTHS has 595 mapped keys (of 595 keys)
org.Hs.egCHRLOC has 28297 mapped keys (of 63901 keys)
org.Hs.egCHRLOCEND has 28297 mapped keys (of 63901 keys)
org.Hs.egENSEMBL has 35374 mapped keys (of 63901 keys)
org.Hs.egENSEMBL2EG has 38475 mapped keys (of 38475 keys)
org.Hs.egENSEMBLPROT has 6872 mapped keys (of 63901 keys)
org.Hs.egENSEMBLPROT2EG has 21575 mapped keys (of 21575 keys)
org.Hs.egENSEMBLTRANS has 13044 mapped keys (of 63901 keys)
org.Hs.egENSEMBLTRANS2EG has 38736 mapped keys (of 38736 keys)
org.Hs.egENZYME has 2229 mapped keys (of 63901 keys)
org.Hs.egENZYME2EG has 975 mapped keys (of 975 keys)
org.Hs.egGENENAME has 63901 mapped keys (of 63901 keys)
org.Hs.egGENETYPE has 63901 mapped keys (of 63901 keys)
org.Hs.egGO has 20688 mapped keys (of 63901 keys)
org.Hs.egGO2ALLEGS has 22874 mapped keys (of 22874 keys)
org.Hs.egGO2EG has 18593 mapped keys (of 18593 keys)
org.Hs.egMAP has 61890 mapped keys (of 63901 keys)
org.Hs.egMAP2EG has 2002 mapped keys (of 2002 keys)
org.Hs.egOMIM has 16426 mapped keys (of 63901 keys)
org.Hs.egOMIM2EG has 22457 mapped keys (of 22457 keys)
org.Hs.egPATH has 5868 mapped keys (of 63901 keys)
org.Hs.egPATH2EG has 229 mapped keys (of 229 keys)
org.Hs.egPMID has 40970 mapped keys (of 63901 keys)
org.Hs.egPMID2EG has 710479 mapped keys (of 710479 keys)
org.Hs.egREFSEQ has 38819 mapped keys (of 63901 keys)
org.Hs.egREFSEQ2EG has 285992 mapped keys (of 285992 keys)
org.Hs.egSYMBOL has 63901 mapped keys (of 63901 keys)
org.Hs.egSYMBOL2EG has 63891 mapped keys (of 63891 keys)
org.Hs.egUCSCKG has 27333 mapped keys (of 63901 keys)
org.Hs.egUNIPROT has 19025 mapped keys (of 63901 keys)

Additional Information about this package:

DB schema: HUMAN_DB
DB schema version: 2.1
Organism: Homo sapiens
Date for NCBI data: 2021-Sep13
Date for GO data: 2021-09-01

```
Date for KEGG data: 2011-Mar15
Date for Golden Path data: 2021-Jul20
Date for Ensembl data: 2021-Apr13
```

For instance the `org.Hs.egGO2ALLEGS` environment contains the mapping of all ENTREZ Gene identifiers to the **Gene Ontology Terms** [1], while `org.Hs.egPATH2EG` maps the identifiers to **KEGG** pathways [3]. The corresponding lists of FGS can be retrieve from the corresponding environments using the the R command `as.list()`, as shown below for KEGG and GO:

```
> kegg <- as.list(org.Hs.egPATH2EG)
> go <- as.list(org.Hs.egGO2ALLEGS)
> length(kegg)

[1] 229

> length(go)

[1] 22874

> str(kegg[1:5])

List of 5
 $ 04610: chr [1:69] "2" "462" "623" "624" ...
 $ 00232: chr [1:7] "9" "10" "1544" "1548" ...
 $ 00983: chr [1:52] "9" "10" "978" "1066" ...
 $ 01100: chr [1:1130] "9" "10" "15" "18" ...
 $ 00380: chr [1:42] "15" "26" "38" "39" ...

> names(kegg)[1:5]

[1] "04610" "00232" "00983" "01100" "00380"

> str(go[1:5])

List of 5
 $ GO:0000002: Named chr [1:22] "142" "291" "1763" "1890" ...
  ..- attr(*, "names")= chr [1:22] "IMP" "TAS" "IDA" "IMP" ...
 $ GO:0000003: Named chr [1:1872] "18" "49" "49" "49" ...
  ..- attr(*, "names")= chr [1:1872] "IEA" "IBA" "IEA" "IMP" ...
 $ GO:0000012: Named chr [1:17] "142" "1161" "2074" "3981" ...
  ..- attr(*, "names")= chr [1:17] "IGI" "IDA" "IDA" "IDA" ...
 $ GO:0000017: Named chr [1:4] "6523" "6523" "6523" "6524"
  ..- attr(*, "names")= chr [1:4] "IDA" "IMP" "ISS" "IDA"
 $ GO:0000018: Named chr [1:132] "142" "604" "641" "940" ...
  ..- attr(*, "names")= chr [1:132] "IDA" "IEA" "IMP" "IEA" ...

> names(go)[1:5]

[1] "GO:0000002" "GO:0000003" "GO:0000012"
[4] "GO:0000017" "GO:0000018"
```

In the `KEGG` list genes are identified by their ENTREZ Gene identifiers, while in the `dat` genes are identified by their Gene Symbol. Below is an example of the code that can be used to perform the identifiers conversion, using only a subset of KEGG and GO FGS:

```
> kegg <- lapply(kegg[sample(1:length(kegg),5)],function(x) unique(unlist(mget(x,org.Hs.egSYMBO
> go <- lapply(go[sample(1:length(go),5)],function(x) unique(unlist(mget(x,org.Hs.egSYMBOL))))
> str(kegg)

List of 5
 $ 05323: chr [1:91] "ACP5" "ANGPT1" "ATP6V1A" "ATP6V1B1" ...
 $ 04960: chr [1:42] "ATP1A1" "ATP1A2" "ATP1A3" "ATP1A4" ...
 $ 05222: chr [1:85] "AKT1" "AKT2" "APAF1" "BIRC2" ...
 $ 04020: chr [1:177] "ADCY1" "ADCY2" "ADCY3" "ADCY7" ...
 $ 00740: chr [1:11] "ACP1" "ACP2" "ACP5" "ACP3" ...

> str(go)

List of 5
 $ GO:0099533: chr [1:3] "CACNB2" "CDK5" "CNR1"
 $ GO:0006027: chr [1:26] "CD44" "FGF2" "FUCA1" "GLB1" ...
 $ GO:0000332: chr "TERC"
 $ GO:2000816: chr [1:43] "APC" "ATM" "BUB1" "BUB1B" ...
 $ GO:0046532: chr [1:7] "CNTF" "DLX1" "DLX2" "NOTCH1" ...
```

Finally, it is also possible to annotate FGS, mapping pathways identifiers to pathway names, as shown below for KEGG, using the KEGGREST.

```
> library(KEGGREST)
> names(kegg) <- sapply(keggGet(paste0("hsa", names(kegg)[1:5])), "[[", "NAME")
```

Similarly GO Terms can be retrieved from the GO.db (please refer to the vignettes of the corresponding packages for details).

```
> library(GO.db)
> GO()

Quality control information for GO:


This package has the following mappings:

GOBPANCESTOR has 28503 mapped keys (of 28503 keys)
GOBPCHILDREN has 16438 mapped keys (of 28503 keys)
GOBPOFFSPRING has 16438 mapped keys (of 28503 keys)
GOBPPARENTS has 28503 mapped keys (of 28503 keys)
GOCCANCESTOR has 4179 mapped keys (of 4179 keys)
GOCCCHILDREN has 1385 mapped keys (of 4179 keys)
GOCCOFFSPRING has 1385 mapped keys (of 4179 keys)
GOCCPARENTS has 4179 mapped keys (of 4179 keys)
GOMFANCESTOR has 11168 mapped keys (of 11168 keys)
GOMFCHILDREN has 2014 mapped keys (of 11168 keys)
GOMFOFFSPRING has 2014 mapped keys (of 11168 keys)
GOMFPARENTS has 11168 mapped keys (of 11168 keys)
GOOBSOLETE has 3499 mapped keys (of 3499 keys)
GOTERM has 43851 mapped keys (of 43851 keys)
```

```
Additional Information about this package:

DB schema: GO_DB
DB schema version: 2.1
Date for GO data: 2021-09-01

> names(go) <- paste(names(go),Term(names(go)),sep=".")
> names(go)

[1] "GO:0099533.positive regulation of presynaptic cytosolic calcium concentration"
[2] "GO:0006027.glycosaminoglycan catabolic process"
[3] "GO:0000332.template for synthesis of G-rich strand of telomere DNA activity"
[4] "GO:2000816.negative regulation of mitotic sister chromatid separation"
[5] "GO:0046532.regulation of photoreceptor cell differentiation"
```

Finally we can be combine the two FGS collections into a named list for further used in GSE analysis (see below).

```
> fgsList <- list(go=go, kegg=kegg)
> fgsList$go

$`GO:0099533.positive regulation of presynaptic cytosolic calcium concentration`
[1] "CACNB2" "CDK5"   "CNR1"


$`GO:0006027.glycosaminoglycan catabolic process`
 [1] "CD44"    "FGF2"    "FUCA1"    "GLB1"
 [5] "GNS"     "GUSB"    "HYAL1"    "IDS"
 [9] "IDUA"    "NAGLU"   "SGSH"     "SPAM1"
[13] "TGFB1"   "HYAL3"   "HYAL2"    "PGLYRP1"
[17] "LYVE1"   "HYAL4"   "CEMIP2"   "PGLYRP4"
[21] "CEMIP"   "HPSE2"   "PGLYRP2"  "PGLYRP3"
[25] "LYG1"    "LYG2"


$`GO:0000332.template for synthesis of G-rich strand of telomere DNA activity`
[1] "TERC"


$`GO:2000816.negative regulation of mitotic sister chromatid separation`
 [1] "APC"      "ATM"       "BUB1"      "BUB1B"
 [5] "CCNB1"    "CENPF"     "DUSP1"     "IK"
 [9] "MAD2L1"   "PLK1"      "RAD21"     "TPR"
[13] "TTK"      "XRCC3"     "ZNF207"    "MAD1L1"
[17] "ZW10"     "BUB3"      "AURKB"     "PTTG1"
[21] "TRIP13"   "MAD2L1BP"  "KNTC1"     "NDC80"
[25] "MAD2L2"   "PTTG2"     "ZWINT"     "ANAPC15"
[29] "PTTG3P"   "FBXO5"     "DYNC1LI1"  "LCMT1"
[33] "SPDL1"    "ZWILCH"    "CDK5RAP2"  "PCID2"
[37] "TEX14"    "PSMG2"     "CDT1"      "USP44"
[41] "KLHL22"   "PRAP1"     "GEN1"
```

```
$`GO:0046532.regulation of photoreceptor cell differentiation`
[1] "CNTF"   "DLX1"   "DLX2"   "NOTCH1" "SOX9"
[6] "SOX8"   "TUG1"
```

3   Data analysis with RTopper

To compute gene-to-phenotype association scores the first step required is the conversion of the data into a list, where each list item corresponds to a gene, and comprises a data.frame with the rows being patients, and columns being measurements for each data type, along with the class phenotype (*the response*). Importantly each element of the list with the data should have the same genes and patients.

The `convertToDr` function is used to make such conversion. Below is a short description of the arguments to this function:

- `dataIntersection`: a list of data.frames containing the same set of patients(columns) and genes (rows)

- `response`: a data.frame indicating patients' phenotypic class;

- `nPlatforms`: the number of platforms;

This can be achieved as follows using our examples data:

```
> dataDr <- convertToDr(dat, pheno, 4)
> class(dataDr)

[1] "list"

> length(dataDr)

[1] 500

> names(dataDr)[1:5]

[1] "AACS"  "AARS"  "ABI1"   "ACHE"   "ACTC1"

> str(dataDr[1:2])

List of 2
 $ AACS:'data.frame':        95 obs. of  5 variables:
  ..$ dat.affy      : num [1:95] 7.75 7.69 7.54 7.3 7.01 ...
  ..$ dat.agilent   : num [1:95] -1.007 -1.116 -0.913 -1.061 -1.775 ...
  ..$ dat.cnvHarvard: num [1:95] -0.0827 -0.0892 -0.0208 -0.1811 -0.0625 ...
  ..$ dat.cnvMskcc  : num [1:95] -0.03839 -0.0914 0.00823 0.03456 0.0573 ...
  ..$ response      : int [1:95] 0 0 1 1 0 0 0 0 0 0 ...
 $ AARS:'data.frame':        95 obs. of  5 variables:
  ..$ dat.affy      : num [1:95] 9.38 9.93 10.2 9.54 9.37 ...
  ..$ dat.agilent   : num [1:95] -1.266 -0.898 0.264 -0.599 -1.437 ...
  ..$ dat.cnvHarvard: num [1:95] -0.1023 -0.2062 -0.0516 -0.0923 -0.1199 ...
  ..$ dat.cnvMskcc  : num [1:95] 0.00756 0.02802 0.10485 0.0841 0.12262 ...
  ..$ response      : int [1:95] 0 0 1 1 0 0 0 0 0 0 ...
```

It is now possible to compute gene-to-phenotype association scores, using as input the gene-centered list produced by `convertToDr`. Therefore the `computeDrStat` function assumes that each gene-centered data.frame contains a column (the last one) called 'response', as created by the `convertToDr`. Below is a short description of the arguments to this function:

- **data**: a list of data.frames, one for each gene analyzed, containing the the genomic measurements from all platforms (by column) for all the patients (by row), along with the phenotypic response;

- **columns**: a numeric vector indicating column indexes corresponding the genomic measurements to be used for computing the gene-to-phenotype association scores; the default is `columns = c(1:(ncol(data) - 1))`, assuming the phenotypic response to be the last column;

- **method**: the method used to compute the association score;

- **integrate**: logical, whether an integrated gene-to-phenotype score should be computed, or separate scores for each platform/data sets specified by `columns`;

In the current implementation of the `RTopper` there are three methods for computing gene-to-phenotype association scores:

1. **dev**: this approach computes the score as the difference of deviances (as described in Tyekucheva et al, manuscript under review [9]);

2. **aic**: this approach computes the score as the Akaike information criterion for model selection;

3. **bic**: this approach computes the score as the penalized likelihood ratio;

3.1 Integrated Gene-to-Phenotype score computation

This approach first integrates genomic data across platform, and subsequently perform GSE to identify the FGS most strongly associated with the integrated score. Below is an example of application to compute the gene-to-phenotype association scores for 4 data type simultaneously:

```
> bicStatInt <- computeDrStat(dataDr, columns = c(1:4), method="bic", integrate = TRUE)
> names(bicStatInt)

[1] "integrated"

> str(bicStatInt)

List of 1
 $ integrated: Named num [1:500] -11.43 -15.93 -8.85 -13.52 -7.26 ...
  ..- attr(*, "names")= chr [1:500] "AACS" "AARS" "ABI1" "ACHE" ...
```

3.2 Separate Gene-to-Phenotype score computation

This approach first computes computes gene-to-phenotype score separately for each platform, uses the scores to perform separate GSE analysis in each platform for identifying the FGS most strongly associated with the score, and finally integrates the results from GSE analysis, Below is an example of this approach:

```
> bicStatSep <- computeDrStat(dataDr, columns = c(1:4), method="bic", integrate = FALSE)
> names(bicStatSep)
```

```
[1] "dat.affy"       "dat.agilent"
[3] "dat.cnvHarvard" "dat.cnvMskcc"

> str(bicStatSep)

List of 4
 $ dat.affy      : Named num [1:500] 0.545 -4.269 -2.334 -4.471 -3.625 ...
  ..- attr(*, "names")= chr [1:500] "AACS" "AARS" "ABI1" "ACHE" ...
 $ dat.agilent   : Named num [1:500] -3.57 -4.5 -3.66 -4.52 -1.05 ...
  ..- attr(*, "names")= chr [1:500] "AACS" "AARS" "ABI1" "ACHE" ...
 $ dat.cnvHarvard: Named num [1:500] -4.49 -3.64 3.13 -3.26 -2.57 ...
  ..- attr(*, "names")= chr [1:500] "AACS" "AARS" "ABI1" "ACHE" ...
 $ dat.cnvMskcc  : Named num [1:500] -4.53 -4.48 2.1 -2.55 -4.25 ...
  ..- attr(*, "names")= chr [1:500] "AACS" "AARS" "ABI1" "ACHE" ...
```

3.3 Gene Set Enrichment using integrated and separate score

After the gene-to-phenotype scores have been obtained it is possible to perform a GSE analysis. To this end we will use the `runBatchGSE` function, as shown below. This function enables to perform GSE analysis over multiple collections of FGS, and over multiple ranking statistics. In the current implementation of the `runBatchGSE` the default is performing the enrichment analysis using the `geneSetTest` function from the `limma` package, and most of the arguments passed to `runBatchGSE` are indeed passed to `geneSetTest` (see the relative help for the details).

As an alternative the user can also define his own function to test for FGS enrichment, passing the selection of genes within the FGS and the ranking ranking statistics in the same way as done for `geneSetTest`. In this tutorial we apply `geneSetTest` in order to perform a Wilcoxon rank-sum test, using the absolute value of the gene-to-phenotype scores as the ranking statistics.

```
> args(runBatchGSE)

function (dataList, fgsList, ...)
NULL
```

Below a short description of the arguments that can be passed to this function:

- `dataList`: a list containing gene-to-phenotype scores to be used as ranking statistics in the GSE analysis;

- `fgsList`: a list of FGS collection, in which each element is a list of character vectors, one for each gene set;

- `...`: any other argument to be passed to lower level functions, including the lower level enrichment function to be used (like the `geneSetTest` function from the `limma` package, which is used as the default);

- `absolute`: logical specifying whether the absolute values of the ranking statistics should be used in the test (the default being TRUE);

- `gseFunc`: a function to perform GSE analysis, when not specified (the default) the `geneSetTest` from the `limma` package is used. When a function is specified, the membership of the analyzed genes to a FGS, and the ranking statistics must be defined in the same way this is done for `geneSetTest`, and the new function must return an integer (usually a p-value) (see the help for `geneSetTest` in the `limma` package)

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Below are few examples to perform Wilcoxon rank-sum test over multiple FGS collections, and over multiple ranking statistics, using the `runBatchGSE`. To this end we will use the **KEGG** and **GO** collections created above, and the separate and integrated gene-to-phenotype scores computed using the `computeDrStat`. The output of this function is a named list of lists, containing an element for each ranking statistics considered in the input. Each one of these elements, in turn, is another list, containing the GSE results for each collection sets. In the examples below we will therefore obtain a list of length one in the case of the integrated gene-to-phenotype score, and a list of length four (on element for each genomic platform) in the case of the separate scores. For all the rankings we will obtain GSE result for both the collections of FGS.

3.4 INTEGRATION + GSE

The integrated gene-to-phenotype scores we have computed can be used to perform a GSE analysis. Below are reported few examples, using the default options, as well as passing several specific arguments to `geneSetTest` (see the relative help for details).

3.4.1 One-sided Wilcoxon rank-sum test using absolute ranking statistics

This can be accomplished by calling the `runBatchGSE` with default values, or by specifying each argument, as shown below:

```
> gseABS.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList)
> gseABS.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                   absolute=TRUE, type="f", alternative="mixed")
```

3.4.2 One-sided Wilcoxon rank-sum test using signed ranking statistics

When the signed ranking statistics has a sign, it is possible to perform a one-sided test assessing both tails separately, as well as a two-sided test. This can be accomplished by passing the corresponding arguments to `runBatchGSE`, as shown below:

```
> gseUP.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                  absolute=FALSE, type="t", alternative="up")
> gseDW.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                  absolute=FALSE, type="t", alternative="down")
> gseBOTH.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                  absolute=FALSE, type="t", alternative="either")
```

3.4.3 Performing a simulation-based GSE test

It is also possible to perform an enrichment analysis comparing each FGS to randomly selected gene lists of the same size of the FGS. In this case the p-value is computed by simulation as the proportion of times the mean of the statistics in the FGS is smaller (or larger) than in the `nsim` random simulated sets of genes.

```
> gseABSsim.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                     absolute=TRUE, type="f", alternative="mixed",
+                                     ranks.only=FALSE, nsim=1000)
> gseUPsim.int <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                     absolute=FALSE, type="t", alternative="up",
+                                     ranks.only=FALSE, nsim=1000)
```

Results from this analysis are named lists of lists, as shown below:

```
> str(gseUP.int)

List of 1
 $ integrated:List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.882 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.893 NA 0.424 0.569 0.574
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone

> gseABSsim.int

$integrated
$integrated$go
GO:0099533.positive regulation of presynaptic cytosolic calcium concentration
                                                                            NA
                        GO:0006027.glycosaminoglycan catabolic process
                                                                            NA
  GO:0000332.template for synthesis of G-rich strand of telomere DNA activity
                                                                            NA
       GO:2000816.negative regulation of mitotic sister chromatid separation
                                                                     0.1628372
             GO:0046532.regulation of photoreceptor cell differentiation
                                                                            NA


$integrated$kegg
                        Rheumatoid arthritis - Homo sapiens (human)
                                                          0.0999001
Aldosterone-regulated sodium reabsorption - Homo sapiens (human)
                                                                 NA
             Small cell lung cancer - Homo sapiens (human)
                                                  0.5034965
          Calcium signaling pathway - Homo sapiens (human)
                                                  0.5394605
             Riboflavin metabolism - Homo sapiens (human)
                                                  0.4405594
```

3.4.4 Passing alternative enrichment functions to runBatchGSE

Below is show how to define and pass alternative enrichment functions to **runBatchGSE**. We will first show how to use the `limma` **wilcoxGST** function, which is a synonym for **geneSetTest** using `ranks.only=TRUE` and `type="t"`.

```
> library(limma)
> gseUP.int.2 <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                 absolute=FALSE, gseFunc=wilcoxGST, alternative="up")
```

As shown below this approach will return the same results obtained with **geneSetTest** passing appropriate arguments.

```
> str(gseUP.int.2)
```

```
List of 1
 $ integrated:List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.882 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.893 NA 0.424 0.569 0.574
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone

> all(gseUP.int.2$go==gseUP.int$go)

[1] TRUE
```

We can finally also pass any new user-defined enrichment function, provided that the arguments
are passed in the same way as with `geneSetTest`, as shown below using the Fisher's exact test,
and a threshold for defining the list of deferentially expressed genes.

```
> gseFunc <- function (selected, statistics, threshold) {
+         diffExpGenes <- statistics > threshold
+         tab <- table(diffExpGenes, selected)
+         pVal <- fisher.test(tab)[["p.value"]]
+         }
> gseUP.int.3 <- runBatchGSE(dataList=bicStatInt, fgsList=fgsList,
+                                   absolute=FALSE, gseFunc=gseFunc, threshold=7.5)
```

As shown below this approach will test for over-representation of the a specific gene set within the
genes defined as deferentially expressed (in our example the genes showing an integrated association
score larger than 7.5). Results are somewhat comparable to what obtained using the Wilcoxon
rank-sum test.

```
> str(gseUP.int.3)

List of 1
 $ integrated:List of 2
  ..$ go  : Named num [1:5] NA NA NA 1 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 1 NA 1 1 1
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone

> data.frame(fisher=na.omit(gseUP.int.3$integrated$kegg), wilcoxon=na.omit(gseUP.int$integrated
```

```
                                                    fisher
Rheumatoid arthritis - Homo sapiens (human)            1
Small cell lung cancer - Homo sapiens (human)          1
Calcium signaling pathway - Homo sapiens (human)       1
Riboflavin metabolism - Homo sapiens (human)           1
                                                 wilcoxon
Rheumatoid arthritis - Homo sapiens (human)      0.8926682
Small cell lung cancer - Homo sapiens (human)    0.4235427
Calcium signaling pathway - Homo sapiens (human) 0.5688934
Riboflavin metabolism - Homo sapiens (human)     0.5741939
```

3.5 GSE + INTEGRATION

The individual gene-to-phenotype scores computed for each platform can be similarly used to perform separate GSE analyses for each considered genomic platform, applying the same code and functions used to perform GSE analysis in the **INTEGRATION + GSE** approach above.

```
> gseABS.sep <- runBatchGSE(dataList=bicStatSep, fgsList=fgsList)
```

This step of GSE analysis on separate platform is then followed by GSE results integration, which is achieved using the `combineGSE` function, which summarizes the individual p-values from the tests. To this end different methods are available, including the computation of the geometric or arithmetic means, the use of the median, the selection of the minimum or the maximum p-value, and the random selection (respectively `geometricMean`, `mean`, `median`, `min`, `max`, and `random`). Few examples are shown below:

```
> gseABS.geoMean.sep <- combineGSE(gseABS.sep, method="geometricMean")
> gseABS.max.sep <- combineGSE(gseABS.sep, method="max")
```

Also in this case the results from the combination are named lists of lists, as shown below:

```
> names(gseABS.sep)

[1] "dat.affy"       "dat.agilent"
[3] "dat.cnvHarvard" "dat.cnvMskcc"

> str(gseABS.sep)

List of 4
 $ dat.affy       :List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.816 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.0915 NA 0.6958 0.5137 0.0587
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone
 $ dat.agilent    :List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.63 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.4542 NA 0.7954 0.0573 0.7536
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone
 $ dat.cnvHarvard:List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.119 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.0235 NA 0.3029 0.6948 0.8029
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone
 $ dat.cnvMskcc   :List of 2
  ..$ go  : Named num [1:5] NA NA NA 0.111 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.541 NA 0.283 0.581 0.669
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone

> str(gseABS.geoMean.sep)

List of 1
 $ combinedScore:List of 2
```

```
  ..$ go  : Named num [1:5] NA NA NA 0.288 NA
  .. ..- attr(*, "names")= chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic c
  ..$ kegg: Named num [1:5] 0.152 NA 0.467 0.33 0.393
  .. ..- attr(*, "names")= chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone

> gseABS.geoMean.sep

$combinedScore
$combinedScore$go
GO:0099533.positive regulation of presynaptic cytosolic calcium concentration
                                                                          NA
                          GO:0006027.glycosaminoglycan catabolic process
                                                                          NA
  GO:0000332.template for synthesis of G-rich strand of telomere DNA activity
                                                                          NA
        GO:2000816.negative regulation of mitotic sister chromatid separation
                                                                   0.2875944
              GO:0046532.regulation of photoreceptor cell differentiation
                                                                          NA


$combinedScore$kegg
                     Rheumatoid arthritis - Homo sapiens (human)
                                                       0.1516756
Aldosterone-regulated sodium reabsorption - Homo sapiens (human)
                                                              NA
               Small cell lung cancer - Homo sapiens (human)
                                                       0.4665677
           Calcium signaling pathway - Homo sapiens (human)
                                                       0.3302224
               Riboflavin metabolism - Homo sapiens (human)
                                                       0.3925857
```

3.6 Multiple testing correction

Finally the `adjustPvalGSE` enables to adjust the p-values computed by the `runBatchGSE`. This functions is an interface to the `mt.rawp2adjp` function from the `multtest` package.

```
> gseABS.int.BH <- adjustPvalGSE(gseABS.int)
> gseABS.int.holm <- adjustPvalGSE(gseABS.int, proc = "Holm")
```

Also in this case the results after the adjustment are named lists of lists, as shown below:

```
> names(gseABS.int.BH)

[1] "integrated"

> names(gseABS.int.holm)

[1] "integrated"

> str(gseABS.int.BH)
```

```
List of 1
 $ integrated:List of 2
  ..$ go  : num [1:5, 1:2] NA NA NA 0.119 NA ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic calcium concer
  .. .. ..$ : chr [1:2] "rawp" "BH"
  ..$ kegg: num [1:5, 1:2] 0.109 NA 0.582 0.441 0.431 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone-regulated so
  .. .. ..$ : chr [1:2] "rawp" "BH"

> str(gseABS.int.holm)

List of 1
 $ integrated:List of 2
  ..$ go  : num [1:5, 1:2] NA NA NA 0.119 NA ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "GO:0099533.positive regulation of presynaptic cytosolic calcium concer
  .. .. ..$ : chr [1:2] "rawp" "Holm"
  ..$ kegg: num [1:5, 1:2] 0.109 NA 0.582 0.441 0.431 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "Rheumatoid arthritis - Homo sapiens (human)" "Aldosterone-regulated so
  .. .. ..$ : chr [1:2] "rawp" "Holm"
```

4   System Information

Session information:

```
> sessionInfo()

R version 4.1.2 (2021-11-01)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 20.04.3 LTS

Matrix products: default
BLAS:   /home/biocbuild/bbs-3.14-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.14-bioc/R/lib/libRlapack.so

locale:
 [1] LC_CTYPE=en_US.UTF-8
 [2] LC_NUMERIC=C
 [3] LC_TIME=en_GB
 [4] LC_COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8
 [6] LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8
 [8] LC_NAME=C
 [9] LC_ADDRESS=C
[10] LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8
```

```
[12] LC_IDENTIFICATION=C

attached base packages:
[1] stats4     stats     graphics  grDevices
[5] utils      datasets  methods   base

other attached packages:
 [1] limma_3.50.0        GO.db_3.14.0
 [3] KEGGREST_1.34.0     org.Hs.eg.db_3.14.0
 [5] AnnotationDbi_1.56.2 IRanges_2.28.0
 [7] S4Vectors_0.32.3    RTopper_1.40.1
 [9] Biobase_2.54.0      BiocGenerics_0.40.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.8          compiler_4.1.2
 [3] GenomeInfoDb_1.30.0 XVector_0.34.0
 [5] bitops_1.0-7        tools_4.1.2
 [7] zlibbioc_1.40.0     bit_4.0.4
 [9] RSQLite_2.2.9       memoise_2.0.1
[11] lattice_0.20-45     pkgconfig_2.0.3
[13] png_0.1-7           rlang_1.0.0
[15] Matrix_1.4-0        DBI_1.1.2
[17] cli_3.1.1           curl_4.3.2
[19] fastmap_1.1.0       GenomeInfoDbData_1.2.7
[21] httr_1.4.2          Biostrings_2.62.0
[23] vctrs_0.3.8         bit64_4.0.5
[25] multtest_2.50.0     grid_4.1.2
[27] R6_2.5.1            survival_3.2-13
[29] blob_1.2.2          MASS_7.3-55
[31] splines_4.1.2       RCurl_1.98-1.5
[33] cachem_1.0.6        crayon_1.4.2
```

5 References

References

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