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

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April 11, 2014

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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

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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 analysisn 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 33077 mapped keys (of 47938 keys)
org.Hs.egACCNUM2EG has 653161 mapped keys (of 653161 keys)
org.Hs.egALIAS2EG has 103510 mapped keys (of 103510 keys)
org.Hs.egCHR has 47442 mapped keys (of 47938 keys)
org.Hs.egCHRLENGTHS has 93 mapped keys (of 93 keys)
org.Hs.egCHRLOC has 23638 mapped keys (of 47938 keys)
org.Hs.egCHRLOCEND has 23638 mapped keys (of 47938 keys)
org.Hs.egENSEMBL has 25832 mapped keys (of 47938 keys)
org.Hs.egENSEMBL2EG has 28046 mapped keys (of 28046 keys)
org.Hs.egENSEMBLPROT has 19851 mapped keys (of 47938 keys)
org.Hs.egENSEMBLPROT2EG has 102658 mapped keys (of 102658 keys)
org.Hs.egENSEMBLTRANS has 20698 mapped keys (of 47938 keys)
org.Hs.egENSEMBLTRANS2EG has 161214 mapped keys (of 161214 keys)
org.Hs.egENZYME has 2230 mapped keys (of 47938 keys)
org.Hs.egENZYME2EG has 975 mapped keys (of 975 keys)
org.Hs.egGENENAME has 47938 mapped keys (of 47938 keys)
org.Hs.egGO has 18207 mapped keys (of 47938 keys)
org.Hs.egGO2ALLEGS has 18078 mapped keys (of 18078 keys)
org.Hs.egGO2EG has 14134 mapped keys (of 14134 keys)
org.Hs.egMAP has 35492 mapped keys (of 47938 keys)
org.Hs.egMAP2EG has 2446 mapped keys (of 2446 keys)
org.Hs.egOMIM has 15762 mapped keys (of 47938 keys)
org.Hs.egOMIM2EG has 19659 mapped keys (of 19659 keys)
org.Hs.egPATH has 5870 mapped keys (of 47938 keys)
org.Hs.egPATH2EG has 229 mapped keys (of 229 keys)
org.Hs.egPMID has 32512 mapped keys (of 47938 keys)
org.Hs.egPMID2EG has 412133 mapped keys (of 412133 keys)
org.Hs.egREFSEQ has 31817 mapped keys (of 47938 keys)
org.Hs.egREFSEQ2EG has 177188 mapped keys (of 177188 keys)
org.Hs.egSYMBOL has 47938 mapped keys (of 47938 keys)
org.Hs.egSYMBOL2EG has 47912 mapped keys (of 47912 keys)
org.Hs.egUCSCKG has 22905 mapped keys (of 47938 keys)
org.Hs.egUNIGENE has 25020 mapped keys (of 47938 keys)
org.Hs.egUNIGENE2EG has 26760 mapped keys (of 26760 keys)
org.Hs.egUNIPROT has 19146 mapped keys (of 47938 keys)

Additional Information about this package:

DB schema: HUMAN_DB
DB schema version: 2.1
Organism: Homo sapiens
Date for NCBI data: 2014-Mar13

```
Date for GO data: 20140308
Date for KEGG data: 2011-Mar15
Date for Golden Path data: 2010-Mar22
Date for Ensembl data: 2014-Feb26
```

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] 18078

> 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 [1:18] "291" "1763" "1890" "3980" ...
  ..- attr(*, "names")= chr [1:18] "TAS" "IDA" "TAS" "IEA" ...
 $ GO:0000003: Named chr [1:1268] "18" "49" "49" "49" ...
  ..- attr(*, "names")= chr [1:1268] "IEA" "IEA" "IMP" "ISS" ...
 $ GO:0000012: Named chr [1:9] "3981" "7141" "7515" "23411" ...
  ..- attr(*, "names")= chr [1:9] "IDA" "IDA" "IEA" "IMP" ...
 $ GO:0000018: Named chr [1:55] "604" "641" "641" "940" ...
  ..- attr(*, "names")= chr [1:55] "IEA" "IEA" "IMP" "IEA" ...
 $ GO:0000019: Named chr [1:4] "641" "4292" "4361" "10111"
  ..- attr(*, "names")= chr [1:4] "IEA" "IEA" "TAS" "IDA"

> names(go)[1:5]

[1] "GO:0000002" "GO:0000003" "GO:0000012"
[4] "GO:0000018" "GO:0000019"
```

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

List of 5
 $ 04320: chr [1:24] "EGFR" "ETS1" "ETS2" "ETV6" ...
 $ 03450: chr [1:13] "DNTT" "FEN1" "XRCC6" "LIG4" ...
 $ 05340: chr [1:35] "ADA" "AIRE" "BTK" "CD3D" ...
 $ 05320: chr [1:52] "FAS" "FASLG" "CD28" "CD80" ...
 $ 04620: chr [1:102] "AKT1" "AKT2" "CASP8" "CD14" ...

> str(go)

List of 5
 $ GO:0034113: chr [1:17] "APOA1" "BMP7" "CD1D" "CD44" ...
 $ GO:0008013: chr [1:62] "APC" "SHROOM2" "AR" "BCL9" ...
 $ GO:0016830: chr [1:49] "ALDOA" "ALDOB" "ALDOC" "AMD1" ...
 $ GO:0071409: chr [1:2] "KLF4" "KLF2"
 $ GO:0032355: chr [1:90] "ADCYAP1R1" "ANXA1" "AQP4" "ASS1" ...
```

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

```
> library(KEGG.db)
> KEGG()

Quality control information for KEGG:


This package has the following mappings:

KEGGENZYMEID2GO has 4012 mapped keys (of 4012 keys)
KEGGEXTID2PATHID has 75100 mapped keys (of 75100 keys)
KEGGGO2ENZYMEID has 4137 mapped keys (of 4137 keys)
KEGGPATHID2EXTID has 3152 mapped keys (of 3152 keys)
KEGGPATHID2NAME has 390 mapped keys (of 390 keys)
KEGGPATHNAME2ID has 390 mapped keys (of 390 keys)


Additional Information about this package:

DB schema: KEGG_DB
DB schema version: 2.1
Date for KEGG data: 2011-Mar15

> names(kegg) <- paste(names(kegg),unlist(mget(names(kegg),KEGGPATHID2NAME)),sep=".")
> names(kegg)

[1] "04320.Dorso-ventral axis formation"
[2] "03450.Non-homologous end-joining"
[3] "05340.Primary immunodeficiency"
[4] "05320.Autoimmune thyroid disease"
```

[5] "04620.Toll-like receptor signaling pathway"

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 25683 mapped keys (of 25683 keys)
GOBPCHILDREN has 14773 mapped keys (of 25683 keys)
GOBPOFFSPRING has 14773 mapped keys (of 25683 keys)
GOBPPARENTS has 25683 mapped keys (of 25683 keys)
GOCCANCESTOR has 3441 mapped keys (of 3441 keys)
GOCCCHILDREN has 1152 mapped keys (of 3441 keys)
GOCCOFFSPRING has 1152 mapped keys (of 3441 keys)
GOCCPARENTS has 3441 mapped keys (of 3441 keys)
GOMFANCESTOR has 9679 mapped keys (of 9679 keys)
GOMFCHILDREN has 1963 mapped keys (of 9679 keys)
GOMFOFFSPRING has 1963 mapped keys (of 9679 keys)
GOMFPARENTS has 9679 mapped keys (of 9679 keys)
GOOBSOLETE has 1860 mapped keys (of 1860 keys)
GOTERM has 38804 mapped keys (of 38804 keys)

Additional Information about this package:

DB schema: GO_DB
DB schema version: 2.1
Date for GO data: 20140308

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

[1] "GO:0034113.heterotypic cell-cell adhesion"
[2] "GO:0008013.beta-catenin binding"
[3] "GO:0016830.carbon-carbon lyase activity"
[4] "GO:0071409.cellular response to cycloheximide"
[5] "GO:0032355.response to estradiol"

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)
```

3 Data analysis with RTopper

To compute gene-to-phenotype association scores the first step required is the convertion 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, contining 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"
```

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```
> 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, usin 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 ot 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 assensing 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 0.777 0.464 NA 0.934
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.607 NA 0.454 0.391 0.179
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
```

```
> gseABSsim.int
```

```
$integrated
$integrated$go
    GO:0034113.heterotypic cell-cell adhesion
                                           NA
           GO:0008013.beta-catenin binding
                                    0.2797203
      GO:0016830.carbon-carbon lyase activity
                                    0.5084915
GO:0071409.cellular response to cycloheximide
                                           NA
           GO:0032355.response to estradiol
                                    0.1768232


$integrated$kegg
        04320.Dorso-ventral axis formation
                                 0.4725275
         03450.Non-homologous end-joining
                                        NA
          05340.Primary immunodeficiency
                                 0.6203796
         05320.Autoimmune thyroid disease
                                 0.6023976
04620.Toll-like receptor signaling pathway
                                 0.6643357
```

3.4.4 Passsing 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 0.777 0.464 NA 0.934
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.607 NA 0.454 0.391 0.179
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous

> 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 differentially 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-represtation of the a specific gene set within the genes defined as differentially 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 1 1 NA 1
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 1 NA 1 1 1
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous

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

                                         fisher
04320.Dorso-ventral axis formation            1
03450.Non-homologous end-joining             NA
05340.Primary immunodeficiency                1
05320.Autoimmune thyroid disease              1
04620.Toll-like receptor signaling pathway    1
```

```
                                           wilcoxon
04320.Dorso-ventral axis formation        0.6072531
03450.Non-homologous end-joining                 NA
05340.Primary immunodeficiency            0.4537338
05320.Autoimmune thyroid disease          0.3908406
04620.Toll-like receptor signaling pathway 0.1790416
```

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 minimun 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 0.1802 0.9193 NA 0.0396
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.0284 NA 0.7016 0.2619 0.3872
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
 $ dat.agilent   :List of 2
  ..$ go  : Named num [1:5] NA 0.0976 0.5614 NA 0.1856
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.569 NA 0.703 0.957 0.88
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
 $ dat.cnvHarvard:List of 2
  ..$ go  : Named num [1:5] NA 0.868 0.021 NA 0.227
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.84788 NA 0.00288 0.35674 0.97666
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
 $ dat.cnvMskcc  :List of 2
```

```
  ..$ go  : Named num [1:5] NA 0.827 0.227 NA 0.337
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.7607 NA 0.0442 0.5715 0.9344
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
```

```
> str(gseABS.geoMean.sep)
```

```
List of 1
 $ combinedScore:List of 2
  ..$ go  : Named num [1:5] NA 0.335 0.223 NA 0.154
  .. ..- attr(*, "names")= chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.be
  ..$ kegg: Named num [1:5] 0.319 NA 0.089 0.475 0.747
  .. ..- attr(*, "names")= chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous
```

```
> gseABS.geoMean.sep
```

```
$combinedScore
$combinedScore$go
    GO:0034113.heterotypic cell-cell adhesion
                                           NA
            GO:0008013.beta-catenin binding
                                    0.3351430
      GO:0016830.carbon-carbon lyase activity
                                    0.2228891
GO:0071409.cellular response to cycloheximide
                                           NA
            GO:0032355.response to estradiol
                                    0.1540070


$combinedScore$kegg
        04320.Dorso-ventral axis formation
                              0.31947474
          03450.Non-homologous end-joining
                                        NA
           05340.Primary immunodeficiency
                              0.08901106
          05320.Autoimmune thyroid disease
                              0.47547290
04620.Toll-like receptor signaling pathway
                              0.74665141
```

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 0.2251 0.54 NA 0.0683 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.beta-catenin bi
  .. .. ..$ : chr [1:2] "rawp" "BH"
  ..$ kegg: num [1:5, 1:2] 0.398 NA 0.554 0.614 0.825 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous end-joining"
  .. .. ..$ : chr [1:2] "rawp" "BH"

> str(gseABS.int.holm)

List of 1
 $ integrated:List of 2
  ..$ go  : num [1:5, 1:2] NA 0.2251 0.54 NA 0.0683 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "GO:0034113.heterotypic cell-cell adhesion" "GO:0008013.beta-catenin bi
  .. .. ..$ : chr [1:2] "rawp" "Holm"
  ..$ kegg: num [1:5, 1:2] 0.398 NA 0.554 0.614 0.825 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:5] "04320.Dorso-ventral axis formation" "03450.Non-homologous end-joining"
  .. .. ..$ : chr [1:2] "rawp" "Holm"
```

4 System Information

Session information:

```
> sessionInfo()

R version 3.1.0 (2014-04-10)
Platform: x86_64-unknown-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8
 [2] LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8
 [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] parallel  stats     graphics  grDevices
[5] utils     datasets  methods   base

other attached packages:
 [1] limma_3.20.0         GO.db_2.14.0
 [3] KEGG.db_2.14.0       org.Hs.eg.db_2.14.0
 [5] RSQLite_0.11.4       DBI_0.2-7
 [7] AnnotationDbi_1.26.0 GenomeInfoDb_1.0.0
 [9] RTopper_1.10.0       Biobase_2.24.0
[11] BiocGenerics_0.10.0

loaded via a namespace (and not attached):
[1] IRanges_1.21.45 MASS_7.3-31
[3] multtest_2.20.0 splines_3.1.0
[5] stats4_3.1.0    survival_2.37-7
[7] tools_3.1.0
```

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