GSEA.plot

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This vignette gives an introduction to the GSEAplot package. This package builds on the Gene Set Enrichment Analysis (GSEA), a computational method published by the Broad Institute. This package uses the enrichment data from the Broad Institute's analysis to create plots that are easier to understand and modify, and expands the amount of gene sets analyzed, thus increasing the amount of plots output by the analysis. In addition to improved plotting functionality, this package also introduces the capability to create your own geneset databases. The package includes all geneset databases published by the Broad Institute as well as 6 different transcription factor databases and two databases containing ligands and receptor pairs.

Three data files are needed in order to perform the gene set enrichment analysis. The GSEAplots function executes the analysis producing a summary results file for each phenotype in the working directory. Within the current working directory a folder will be created with the name assigned to doc.string in the GSEAplots function call. The results for each gene set within the database will be saved into this folder. Plot.ES saves a pdf of all generated enrichment graphs into the working directory.

Table of Contents:

Load the Package

```r
# remotes::install_github("kelsiereinaltt/GSEAplot")
library(devtools)
library(GSEAplot)
```

Geneset Database Options

To improve on the GSEA code published by the Broad Institute, additional geneset database options were included within this package.

Existing Databases

Calling the key included within the package shows which databases are currently saved in the package.

```
data(key)
head(key)
```

```
##                File                             Description
## 1              C1.gs                            positional all
## 2          C2.cgp.gs curated chemical and genetic perturbations
## 3 C2.cp.biocarta.gs        curated canonical pathways biocarta
## 4           C2.cp.gs                 curated canonical pathways
## 5      C2.cp.kegg.gs            curated canonical pathways kegg
## 6 C2.cp.reactome.gs        curated canonical pathways reactome
##                                           Source
## 1 http://software.broadinstitute.org/gsea/msigdb
## 2 http://software.broadinstitute.org/gsea/msigdb
## 3 http://software.broadinstitute.org/gsea/msigdb
## 4 http://software.broadinstitute.org/gsea/msigdb
## 5 http://software.broadinstitute.org/gsea/msigdb
## 6 http://software.broadinstitute.org/gsea/msigdb
```

The key has three columns: file, description, and source. The descripton correspoinding to a file makes the databases more accessible by detailing what a database contains. Users can identify a dataset of interest by reading its description then loading the databae usuing its file name.

The function **database_key** returns the file name of a database given the description of a database.

```
GO_mf_filename=database_key("GO molecular function")
GO_mf_filename
```

```
## [1] "C5.mf.gs"
```

database_key may also return the descriptons for all databases when the function input is "all". Given the descriptions of all databases, the user may re-use the function with the decription of interest to find the file of interest.

```
descriptive_names=database_key("all")
head(descriptive_names)
```

```
## [1] positional all
## [2] curated chemical and genetic perturbations
## [3] curated canonical pathways biocarta
## [4] curated canonical pathways
## [5] curated canonical pathways kegg
## [6] curated canonical pathways reactome
## 68 Levels: computational all ... TRRUST transcription factors
```

Once the file name is identified, the database of interest may be loaded intoo the workspace.

```
data(hallmark.gs)
data(C1.gs)
```

To check if a geneset database includes data of interest, all sets within a database can be viewed using the **get_genesets** function.

```r
sets=get_genesets(hallmark.gs)
head(sets)
```

```
## [1] "HALLMARK_TNFA_SIGNALING_VIA_NFKB"    "HALLMARK_HYPOXIA"
## [3] "HALLMARK_CHOLESTEROL_HOMEOSTASIS"    "HALLMARK_MITOTIC_SPINDLE"
## [5] "HALLMARK_WNT_BETA_CATENIN_SIGNALING" "HALLMARK_TGF_BETA_SIGNALING"
```

“non-existing Databases (change title later)”

~~explain what this could be (from another source, created/modified dataset)~~. The instructions for how to load a “non-existing” database will be detailed in other sections of the manuscript.

Setting up Data for be used with GSEAplot

Gene Set Enrichment Analysis requires the user to provide three dataframes: an expression file, a phenotype file, and a geneset database. The user may use dataframes provided in this package, or create his/her own dataframes. To load data included in the package, call the desired R dataset within the **data()** function. If you have not saved data to the package, simply assign your data to the corresponding function parameter making sure that it follows the correct data formatting. Instructions for how to prepare each file are included on the Broad Institute page for their GSEA analysis function.

Loading Data

The function parameter input.ds.name requires *differential expression* data. The following is an example of how gene expression data included this package can be loaded:

```r
data(aagmex_expr)
expr.input=aagmex_expr
expr.input[1:4,1:6]
```

```
##        GSM2520983 GSM2520984 GSM2520985 GSM2520986 GSM2520991 GSM2520992
## 7A5      6.416175   6.303225   6.292974   6.288139   6.354787   6.365153
## A1BG     6.442692   6.474838   6.538247   6.453630   6.498097   6.404469
## A1CF     6.429408   6.354601   6.380667   6.369513   6.386864   6.357330
## A26C3    6.431504   6.370737   6.325280   6.377389   6.376220   6.388600
```

The parameter input.cls.name takes the *phenotype* data, which maps the differential expression data to one of two phenotypes. The following is an example of how phenotype data included this package can be loaded:

```r
data(aagmex_pheno)
pheno.input=aagmex_pheno
pheno.input$phen
```

```
## [1] "Female" "Male"
```

```r
head(pheno.input$class.v)
```

```
## [1] 0 0 0 0 0 0
```

The parameter input.gs.name takes a *geneset* or a geneset database (collection of genesets). The following is an example of how geneset data included this package can be loaded:

```
data(hallmark.gs)
gene.set.input=hallmark.gs
```

GSEA Analysis

Function and Parameters

In addition to the three data frames, the GSEA analysis requires a number of other parameters.

Other Analysis Paramaters:

- **doc.string** - name of the folder where individual geneset results are saved to. The folder is created if it does not exist.

- **nperm** - determines the number of permutations. Permutations are used to analyze the significance of the association between gene expression and phenotype, and determine whether the associations are more significant than random ones.

- **fdr.q.val.threshold** - sets a limit on false discovery rate. Set at a default of 0.25, we would expect that 3 out of 4 times the result is valid. The fdr value is computed as a ratio of the enrichment score to the enrichment scores for all permutations of the gene set over the ratio of the enrichment score to the enrichment scores of the actual gene set.

- **abs.val** - When set to true (T), genes are ranked according to the absolute value of their signal to noise ratio. This vaulue is set to false (F) by default.

- **DELETEgs.size.threshold.max** - maximum number of gene symbols in expression data that match a given gene set. A geneset with more matching gene symbols than this will be excluded from the GSEA analysis.

Plotting Paramaters:

- *bar_percent*: the size of the enrichment tick mark relative to the size of the enrichment score plot.

- *gap_percent*: the size of the gaph between the running enrichment score graph and the tick marks relative to the size of the enrichment score plot.

- *under_percent*: the size of the space below the tick marks relative to the size of the enrichment score plot.

- *upper_percent*: the size of the space above the enrichment plot relative to the size of the enrichment score plot.

- *color_line*: color of the line in the enrichment score plot.

- *color_tick*: color of the tick marks that indicate a gene symbol in the expression data that is within the given geneset.

The parameters gs.size.threshold.min and gs.size.threshold.max determines how many gene symbols from the gene set and the expression data can match. Their default values are 2 and 9999999999 respectively, so as to allow genesets of all sizes to be analyzed (except for genesets less than the size of 2). Gs.size.threshold.max can be changed within the GSEAplots() parameters.

```
 pp= GSEAplots(input.ds.name=expr.input,
               input.cls.name=pheno.input, gene.set.input=gene.set.input,
               doc.string="GSEA_plots", nperm=1000,
               fdr.q.val.threshold = 0.25,abs.val=F, bar_percent=0.1, gap_percent=0.1,
               under_percent=0.1,upper_percent=0.1,color_line="black",
               color_tick="black")
```

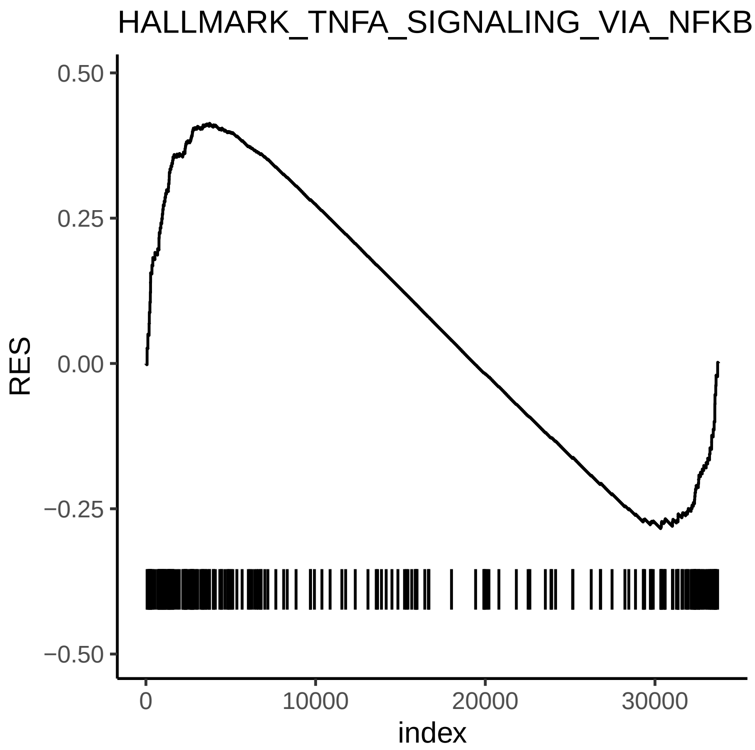
Function outputs

Outputs of GSEA include plots, variables gene.set.reference.matrix and gene.set.leading, reports and enrichment scores. All outputs will be saved to the working directory.

Plots

The following is an example of an erichment plot from the first geneset:

```
pp$plots[[1]]
```



Use the function **plot.ES** to save a pdf of enrichment plots for all significant sets in the gene set database. The pdf will save to the working directory.

```
plot.ES(list.of.plots=pp$plots,plotname="GSEA_plots")
```

gene.set.reference.matrix and gene.set.leading

gene.set.reference.matrix is a table of the gene sets being studied.

```
names(pp$gene.set.reference.matrix)[[1]]
```

```
## [1] "HALLMARK_TNFA_SIGNALING_VIA_NFKB"
```

```
head(pp$gene.set.reference.matrix[[1]])
```

```
## [1] "JUNB"    "CXCL2"   "ATF3"    "NFKBIA"  "TNFAIP3" "PTGS2"
```

gene.set.leading is a list containing the leading edge set (set of genes whose differential expression is most related to a change in phenotype) for each gene set. If differential expression is more strongly related to a change in the first phenotype, then the maximum absolute value of the runnning enrichment score (RES) will be positive. In this case the leading edge set is all of the enriched genes (have a tick mark) that are to the left of and including the max if it is enriched. If different expression is more related to the second phenotype then the largest running enrichment score is negative. In this case the leading edge set is all of the enriched genes that are to the right of and including the max if it is enriched.

The following example displays the leading gene symbols for the first gene set:

```
names(pp$gene.set.reference.matrix)[[1]]
```

```
## [1] "HALLMARK_TNFA_SIGNALING_VIA_NFKB"
```

```
head(pp$gene.set.leading[[1]])
```

```
## [1] "CCND1"  "PMEPA1" "ID2"    "MSC"    "PLEK"   "TUBB2A"
```

Reports

A report is generated for each phenotype (output tables include **report1** and **report2**. The table shows all of the information available in the report file. An example of the data is provided below. In this case, the first row contains the data for the geneset HALLMARK_PANCREAS_BETA_CELLS. The third column is the source which is ommitted for easy viewing.

```
pp$report1[1,-3]
```

```
##                              GS SIZE      ES    NES NOM p-val FDR q-val
## 1 HALLMARK_PANCREAS_BETA_CELLS   38 0.47384 1.7093  0.003884  0.013888
##   FWER p-val Tag % Gene % Signal FDR (median) glob.p.val
## 1      0.015 0.263  0.161  0.221            0      0.007
```

Enrichment Scores

The list **ES** provides access to the enrichment score data used to generate the enrichment plots along with its corresponding gene symbol.

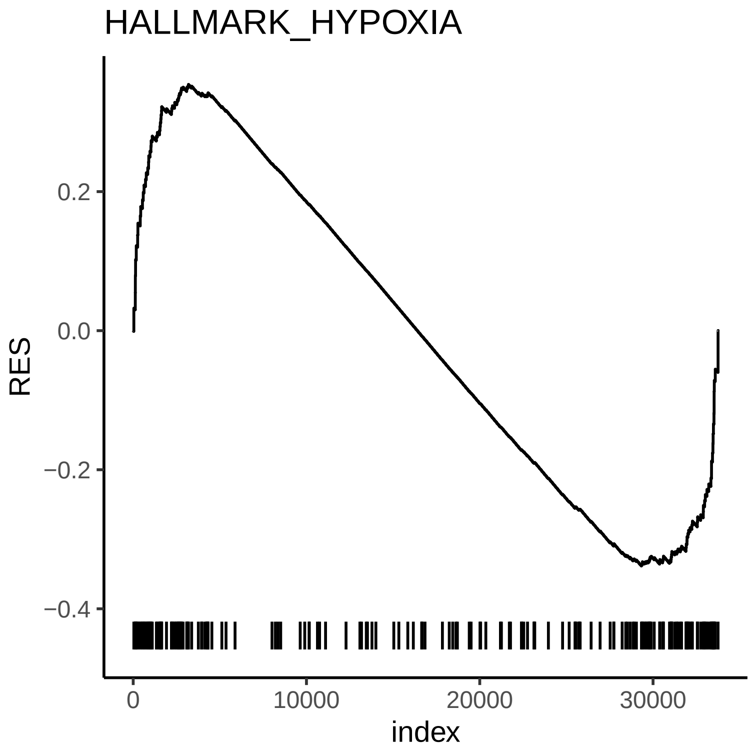
```
data1=pp$ES[[2]]
head(data1)
```

```
##   index gene.symbol           RES EStag
## 1     1        SAA2 -2.978673e-05     0
## 2     2      SEL1L2 -5.957345e-05     0
## 3     3        SAA1 -8.936018e-05     0
## 4     4       CCDC3 -1.191469e-04     0
## 5     5        SAA4 -1.489336e-04     0
## 6     6       SFRP2 -1.787204e-04     0
```

Access to enrichment score data allows for deeper analysis of the gene set enrichment. Users of the package may create their own plotting functions using the data.

The following describes the procedure for producing plots in the package as well as an example: #Left off Here Enrichment scores are plotted for each position within the gene set. A positive enrichment score indicates correlation with the first phenotype which in the case of the aagmex pheno data is Female and a negative enrichment score indicates correlation with the second phenotype, Male. The gene symbols within your gene set that are differentially expressed are indicated with a tick mark under this plot.In the enrichment score data these symbols are indicated with an EStag of 1. To specify the dimensions and positions of the tick marks a data frame is created to create line segments that have a regular height and are positioned at each position with an EStag of 1. The segments should be vertical so the vx or change in x over the segment should be 0. The y position in the data frame dictates the starting y position of the mark. It is currently set to 0.12 below the minimum of of the enrichment plot. vy dictates the end of each mark to be 0.04 higher than that starting position. In the plotting function, 0.12 and 0.04 are variables that can be modified by the bar_percent and under_percent parameters. Using ggplot, the enrichment score plot and tick marks are combined into one plot called p.

```
enrich_ind=which(data1$EStag==1)
d=data.frame(x=enrich_ind, y=matrix(min(data1$RES)-0.12,length(enrich_ind),1),
             vx=matrix(0,length(enrich_ind),1), vy=matrix(0.04,length(enrich_ind),1))
p <- ggplot(data1, aes(index,RES))+geom_line(col="black")
p <- p+geom_segment(data=d, mapping=aes(x=x, y=y, xend=x+vx, yend=y+vy),col="black")
p <- p+theme_classic()
p <- p+ggtitle(names(pp$gene.set.reference.matrix)[[2]])
print(p)
```



Running GSEA on a New Database

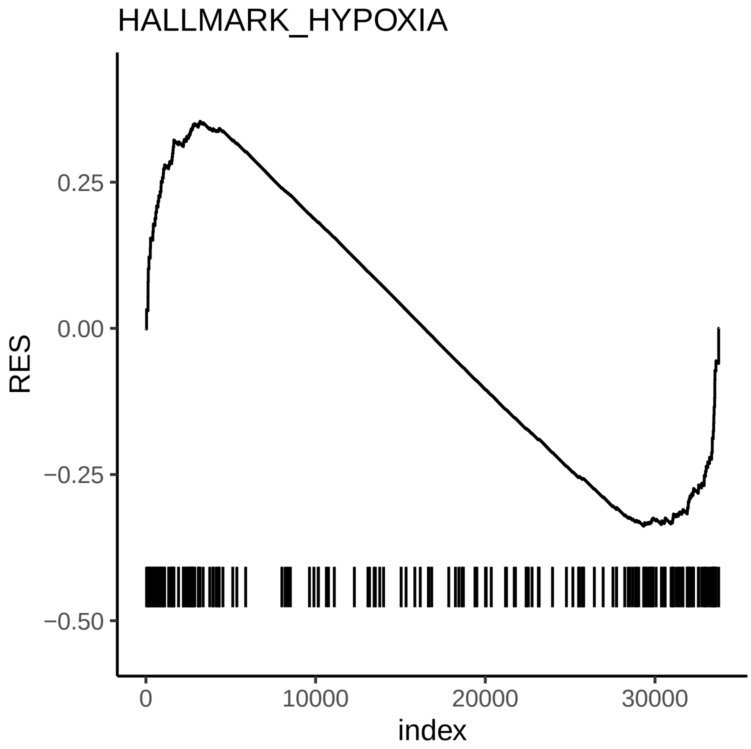
This package introduces the ability to create your own database. To create your own database you need to create a list. The names of the list must be the names of the genesets. The items in each element of the list are the description/source and gene symbols. For example the names of a potential list would be the transcription factors and the elements would be the genes affected by that specific transcription factor. The create_geneset_db() function then converts this list into a format that can go into the GSEAplots function.

This chunk creates a geneset database that may be used in the GSEAplots function which only contains the first three genesets in the Hallmark database.

```
sets=get_genesets(hallmark.gs)
symbols=get_genesymbols(hallmark.gs)
entry1=c("source_1",symbols$HALLMARK_TNFA_SIGNALING_VIA_NFKB)
entry2=c("source_2",symbols$HALLMARK_HYPOXIA)
entry3=c("source_3",symbols$HALLMARK_CHOLESTEROL_HOMEOSTASIS)
hall_13.db=list(entry1,entry2,entry3)
names(hall_13.db)=c(sets[1],sets[2],sets[3])
gene.set.input=create_geneset_db(hall_13.db)
```

```
hall_13_results= GSEAplots(input.ds.name=expr.input,
                input.cls.name=pheno.input, gene.set.input=gene.set.input,
                doc.string="Aagmex_hall13", nperm=1000,
                fdr.q.val.threshold = 0.25, bar_percent=0.1, gap_percent=0.1,
                under_percent=0.1,upper_percent=0.1,color_line="black",
                color_tick="black",abs.val=F)
```

```
hall_13_results$plots[[2]]
```



Introduction of Transcription Factor Databases

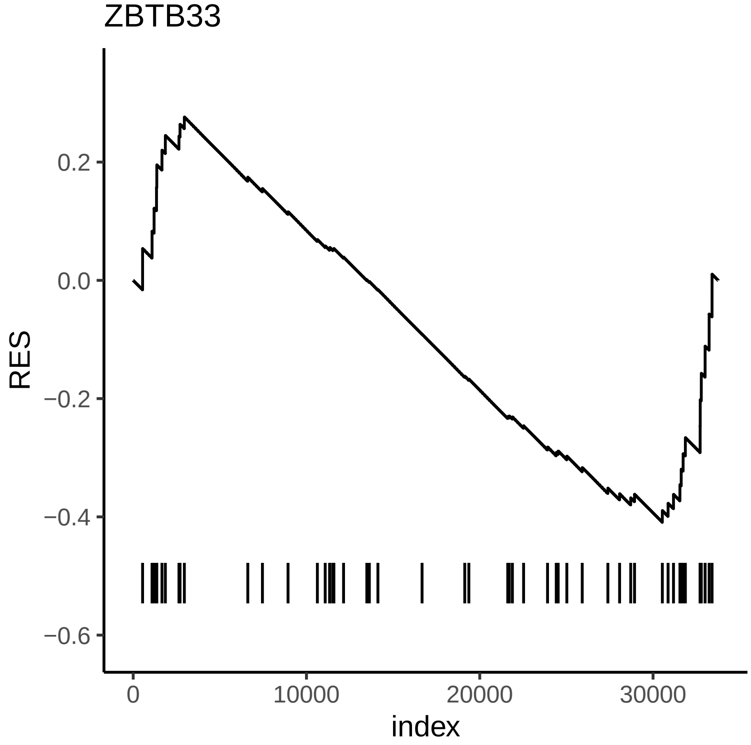
The following transcription factor databases were introduced to the Broad Institute gene set databases: Neph2012, ENCODE, ITFP, Marbach2016, TRRUST, and TRED. These databases were downloaded from the tfttargets package from https://github.com/slowkow/tftargets and reformatted for use in the GSEA function. The sets for these include the transcription factor name and then enrichment is obtained for the gene symbols affected by that transcription factor. This allows for expanded use to show which transcription factors and genes regulated by those correspond to expression data.

```
data(ENCODE.db)
gene.set.input=ENCODE.db

enrichment_TF= GSEAplots(input.ds.name=expr.input,
               input.cls.name=pheno.input, gene.set.input=gene.set.input,
               doc.string="Aagmex_TF", nperm=1000, fdr.q.val.threshold = 0.25,
               bar_percent=0.1, gap_percent=0.1, under_percent=0.1,upper_percent=0.1,color_line="black"
               color_tick="black",abs.val=F)
```

```
 enrichment_TF$plots[1]
```

```
## [[1]]
```



Introduction of Kadoki Ligand and Receptor Database

This package offers two new databases, the Kadoki Ligand and Receptor Databases. For both databases, gs.size.threshold.max is increased to 3000 to enable the analysis.

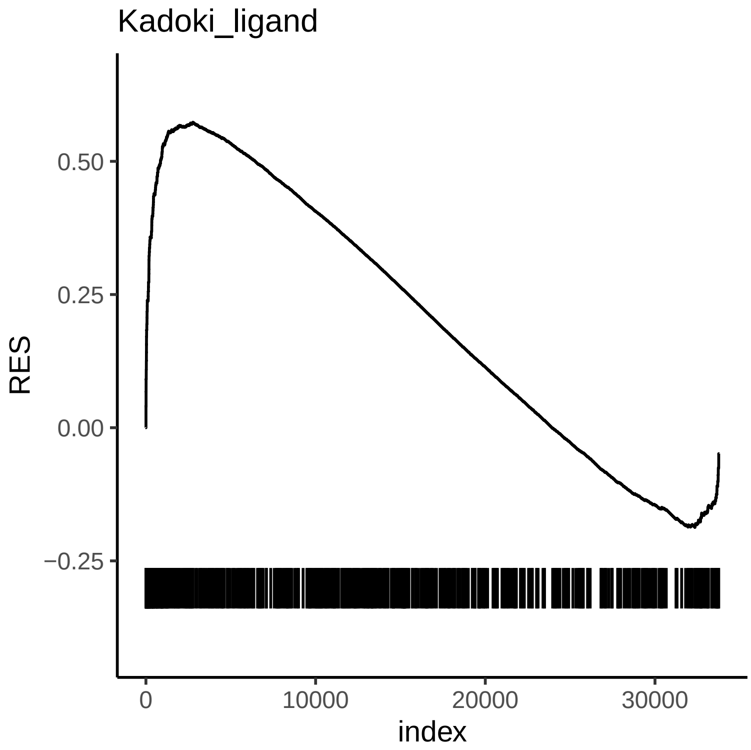
```r
data(Kadoki_ligands.db)
gene.set.input=Kadoki_ligands.db

ligand_results=GSEAplots(input.ds.name=expr.input,
               input.cls.name=pheno.input, gene.set.input=gene.set.input,
               doc.string="Aagmex_ligands", nperm=1000,fdr.q.val.threshold = 0.25,
               bar_percent=0.1, gap_percent=0.1, under_percent=0.1,upper_percent=0.1,color_line="black"
               color_tick="black",abs.val=F)
```

```
## [1] " *** Running GSEA Analysis..."
## [1] "gs.size.threshold.max: " "10000002197"
## [1] "Number of genes:" "33759"
## [1] "Number of Gene Sets:" "1"
## [1] "Number of samples:" "252"
## [1] "Original number of Gene Sets:" "1"
## [1] "Maximum gene set size:" "2198"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  1 -- 100"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  101 -- 200"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  201 -- 300"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  301 -- 400"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  401 -- 500"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  501 -- 600"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  601 -- 700"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  701 -- 800"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  801 -- 900"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  901 -- 1000"
## [1] "Computing observed enrichment for gene set: 1 Kadoki_ligand"
## [1] "Computing random permutations' enrichment for gene set: 1 Kadoki_ligand"
## [1] "Computing nominal p-values..."
## [1] "Computing rescaling normalization for each gene set null..."
## [1] "Computing FWER p-values..."
## [1] "Computing FDR q-values..."
## [1] "Producing result tables and plots..."
```

```r
 ligand_results$plots[1]
```

```
## [[1]]
```



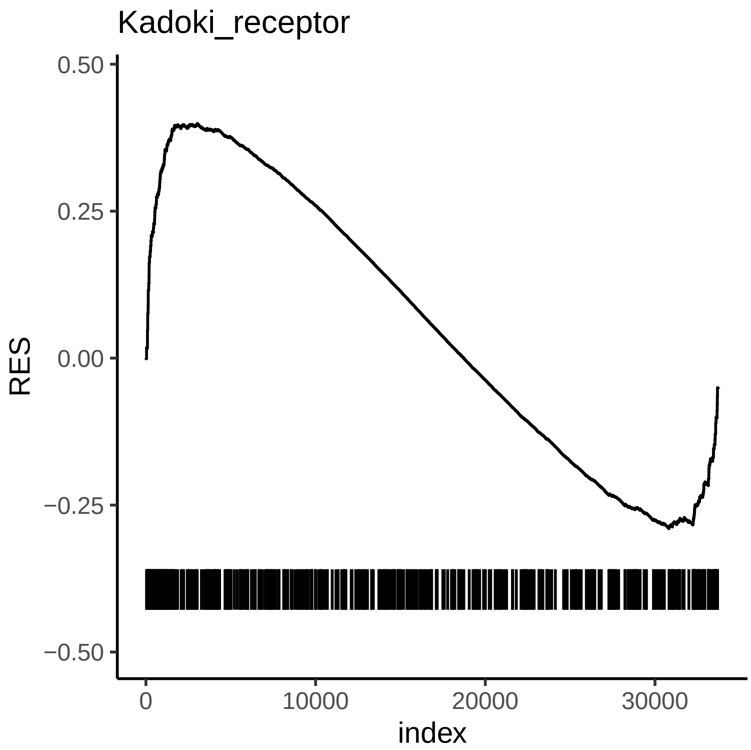
```r
data(Kadoki_receptors.db)
gene.set.input=Kadoki_receptors.db

receptor_results=GSEAplots(input.ds.name=expr.input,
               input.cls.name=pheno.input, gene.set.input=gene.set.input,
               doc.string="Aagmex_receptors", nperm=1000,fdr.q.val.threshold = 0.25,
               bar_percent=0.1, gap_percent=0.1, under_percent=0.1,upper_percent=0.1,color_line="black"
               color_tick="black",abs.val=F)
```

```
## [1] " *** Running GSEA Analysis..."
## [1] "gs.size.threshold.max: " "10000002197"
## [1] "Number of genes:" "33759"
## [1] "Number of Gene Sets:" "1"
## [1] "Number of samples:" "252"
## [1] "Original number of Gene Sets:" "1"
## [1] "Maximum gene set size:" "2198"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  1 -- 100"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  101 -- 200"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  201 -- 300"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  301 -- 400"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  401 -- 500"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  501 -- 600"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  601 -- 700"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  701 -- 800"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  801 -- 900"
## [1] "Computing ranked list for actual and permuted phenotypes.......permutations:  901 -- 1000"
## [1] "Computing observed enrichment for gene set: 1 Kadoki_receptor"
## [1] "Computing random permutations’ enrichment for gene set: 1 Kadoki_receptor"
## [1] "Computing nominal p-values..."
## [1] "Computing rescaling normalization for each gene set null..."
## [1] "Computing FWER p-values..."
## [1] "Computing FDR q-values..."
## [1] "Producing result tables and plots..."
```

```r
receptor_results$plots[1]
```

```
## [[1]]
```



Add to an Existing Gene Set Database

This new function allows you to add genesets into an exsiting database. The database input needs to be already in the tab separated format that is used in the GSEAplots function. The genesets to add have a separate line for each geneset with the geneset name, source, and gene symbols. These will be converted into the tab separated format in the add_to_database() function.

To add the geneset HALLMARK_KLF14 create a row vector with the name of the set then the source and then the gene symbols.

```r
data(transf)
data(transm)
tx = t(c("HALLMARK_KLF14","http://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_KLF14",transf[,1], 
```

The add_to_database function adds this set to the hallmark sets and formats it for use in the function. An example of it being used is in the section below.

```r
gene.set.input=add_to_database(database=hallmark.gs,addition=tx)
head(gene.set.input)
```

```
## [1] "HALLMARK_TNFA_SIGNALING_VIA_NFKB\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_TNFA_
## [2] "HALLMARK_HYPOXIA\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_HYPOXIA\tPGK1\tPDK1\
## [3] "HALLMARK_CHOLESTEROL_HOMEOSTASIS\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_CHOL
## [4] "HALLMARK_MITOTIC_SPINDLE\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_MITOTIC_SPIN
## [5] "HALLMARK_WNT_BETA_CATENIN_SIGNALING\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_W
## [6] "HALLMARK_TGF_BETA_SIGNALING\thttp://www.broadinstitute.org/gsea/msigdb/cards/HALLMARK_TGF_BETA_
```

Create a Phenotype Input File

The pheno.input parameter for GSEA.plot requires a list with a phen element and a class.v element.

```r
data(aagmex_pheno)
aagmex_pheno$phen
```

```
## [1] "Female" "Male"
```

```r
head(aagmex_pheno$class.v)
```

```
## [1] 0 0 0 0 0 0
```

The create_phenoinput file takes a character column with labels for the phenotype of each sample and makes it into a working pheno.input for the GSEA.plot function.

```r
data(gtex_ann)
head(gtex_ann)
```

```
## [1] "Female" "Female" "Female" "Female" "Female" "Female"
```

```r
pheno.input=create_phenoinput(gtex_ann)
pheno.input$phen
```

```
## [1] "Female" "Male"
```

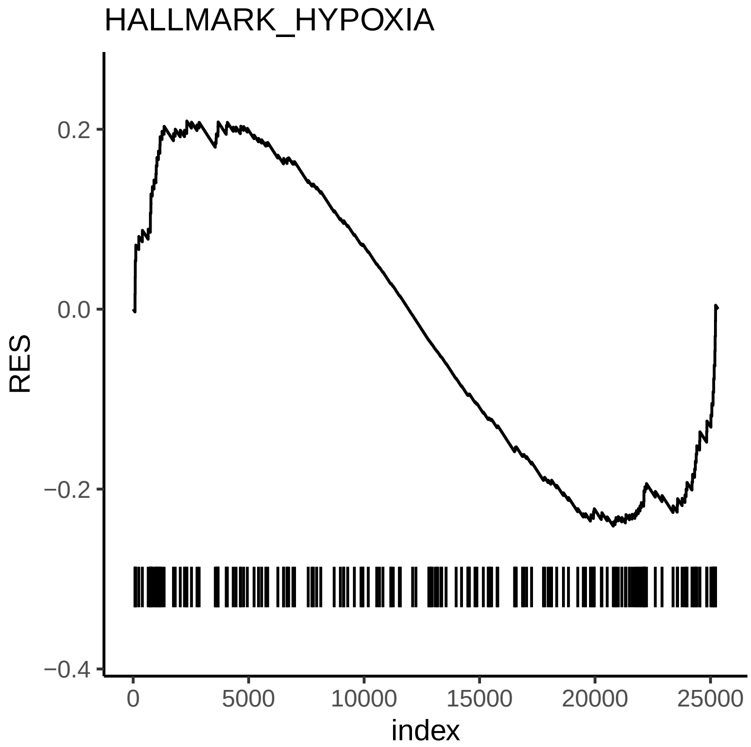
```r
head(pheno.input$class.v)
```

```
## [1] 0 0 0 0 0 0
```

```r
data(gtex_expr)
expr.input=gtex_expr
```

```r
gtex_example= GSEAplots(input.ds.name=expr.input,
               input.cls.name=pheno.input, gene.set.input=gene.set.input,
               doc.string="Gtex_hall", nperm=1000,
               fdr.q.val.threshold = 0.25,abs.val=F,bar_percent=0.1, gap_percent=0.1,
               under_percent=0.1,upper_percent=0.1,color_line="black",
               color_tick="black")
```

```
gtex_example$plots[[2]]
```



References

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