Package 'ideal'

November 28, 2021

Type Package

Title Interactive Differential Expression AnaLysis

Version 1.18.0

Date 2021-10-15

Description This package provides functions for an Interactive Differential Expression AnaLysis of RNA-sequencing datasets, to extract quickly and effectively information downstream the step of differential expression. A Shiny application encapsulates the whole package.

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LazyData TRUE

Depends topGO

Imports DESeq2, SummarizedExperiment, GenomicRanges, IRanges, S4Vectors, ggplot2 (>= 2.0.0), heatmaply, plotly, pheatmap, pcaExplorer, IHW, gplots, UpSetR, goseq, stringr, dplyr, limma, GOstats, GO.db, AnnotationDbi, shiny (>= 0.12.0), shinydashboard, shinyBS, DT, rentrez, rintrojs, rlang, ggrepel, knitr, rmarkdown, shinyAce, BiocParallel, grDevices, base64enc, methods

Suggests testthat, BiocStyle, airway, org.Hs.eg.db, TxDb.Hsapiens.UCSC.hg38.knownGene, DEFormats, edgeR

URL https://github.com/federicomarini/ideal, https://federicomarini.github.io/ideal/

BugReports https://github.com/federicomarini/ideal/issues

biocViews ImmunoOncology, GeneExpression, DifferentialExpression, RNASeq, Sequencing, Visualization, QualityControl, GUI, GeneSetEnrichment, ReportWriting

VignetteBuilder knitr

RoxygenNote 7.1.2

Encoding UTF-8

git_url https://git.bioconductor.org/packages/ideal

git_branch RELEASE_3_14

git_last_commit d364df4

git_last_commit_date 2021-10-26

Date/Publication 2021-11-28

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R topics documented:

`deseqresult2DEgenes` *Generate a tidy table with the DE genes from the results of DESeq*

Description

Generate a tidy table with the DE genes from the results of DESeq

Usage

```
deseqresult2DEgenes(deseqresult, FDR = 0.05)
```

Arguments

`deseqresult`	A `DESeqResults` object
`FDR`	Numeric value, the significance level for thresholding adjusted p-values

Value

A "tidy" data.frame with only genes marked as differentially expressed

Examples

```
# with simulated data...
library(DESeq2)
dds <- DESeq2::makeExampleDESeqDataSet(n = 100, m = 8, betaSD = 2)
dds <- DESeq(dds)
res <- results(dds)
deseqresult2DEgenes(res)
```

`deseqresult2tbl` *Generate a tidy table with the results of DESeq*

Description

Generate a tidy table with the results of DESeq

Usage

```
deseqresult2tbl(deseqresult)
```

Arguments

`deseqresult`	A `DESeqResults` object

Value

A "tidy" data.frame with all genes

Examples

```
# with simulated data...
library(DESeq2)
dds <- DESeq2::makeExampleDESeqDataSet(n = 100, m = 8, betaSD = 1)
dds <- DESeq2::DESeq(dds)
res <- DESeq2::results(dds)
deseqresult2tbl(res)
```

`ggplotCounts` *Plot normalized counts for a gene*

Description

Plot for normalized counts of a single gene, with jittered points superimposed on the boxplot

Usage

```
ggplotCounts(
  dds,
  gene,
  intgroup = "condition",
  annotation_obj = NULL,
  transform = TRUE,
  labels_repel = TRUE
)
```

Arguments

dds	A DESeqDataSet object.
gene	A character, specifying the name of the gene to plot
intgroup	Interesting groups: a character vector of names in colData(dds) to use for grouping
annotation_obj	A data.frame object, with row.names as gene identifiers (e.g. ENSEMBL ids) and a column, gene_name, containing e.g. HGNC-based gene symbols. Optional.
transform	Logical value, corresponding whether to have log scale y-axis or not. Defaults to TRUE.
labels_repel	Logical value. Whether to use ggrepel's functions to place labels; defaults to TRUE.

Details

Note: this function relies on the plotCounts function of DESeq2, therefore pseudocounts of 0.5 are added to each point

Value

An object created by ggplot

Examples

```
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
ggplotCounts(dds_airway,
  gene = "ENSG00000103196", # CRISPLD2 in the original publication
  intgroup = "dex"
)
```

goseqTable *Extract functional terms enriched in the DE genes, based on goseq*

Description

A wrapper for extracting functional GO terms enriched in a list of (DE) genes, based on the algorithm and the implementation in the goseq package

Usage

```
goseqTable(
  de.genes,
  assayed.genes,
  genome = "hg38",
  id = "ensGene",
  testCats = c("GO:BP", "GO:MF", "GO:CC"),
  FDR_GO_cutoff = 1,
  nTop = 200,
  orgDbPkg = "org.Hs.eg.db",
  addGeneToTerms = TRUE
)
```

Arguments

de.genes	A vector of (differentially expressed) genes
assayed.genes	A vector of background genes, e.g. all (expressed) genes in the assays
genome	A string identifying the genome that genes refer to, as in the goseq function
id	A string identifying the gene identifier used by genes, as in the goseq function
testCats	A vector specifying which categories to test for over representation amongst DE genes - can be any combination of "GO:CC", "GO:BP", "GO:MF" & "KEGG"
FDR_GO_cutoff	Numeric value for subsetting the results
nTop	Number of categories to extract, and optionally process for adding genes to the respective
orgDbPkg	Character string, named as the org.XX.eg.db package which should be available in Bioconductor
addGeneToTerms	Logical, whether to add a column with all genes annotated to each GO term

Details

Note: the feature length retrieval is based on the goseq function, and requires that the corresponding TxDb packages are installed and available

Value

A table containing the computed GO Terms and related enrichment scores

Examples

```
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)

res_subset <- deseqresult2DEgenes(res_airway)[1:100, ]
myde <- res_subset$id
myassayed <- rownames(res_airway)
## Not run:
mygo <- goseqTable(myde,
  myassayed,
  testCats = "GO:BP",
  addGeneToTerms = FALSE
)
head(mygo)

## End(Not run)
```

`ideal` *ideal: Interactive Differential Expression Analysis*

Description

ideal makes differential expression analysis interactive, easy and reproducible. This function launches the main application included in the package.

Usage

```
ideal(
  dds_obj = NULL,
  res_obj = NULL,
  annotation_obj = NULL,
  countmatrix = NULL,
  expdesign = NULL,
  gene_signatures = NULL
)
```

Arguments

`dds_obj`	A `DESeqDataSet` object. If not provided, then a `countmatrix` and a `expdesign` need to be provided. If none of the above is provided, it is possible to upload the data during the execution of the Shiny App

res_obj — A DESeqResults object. If not provided, it can be computed during the execution of the application

annotation_obj — A data.frame object, with row.names as gene identifiers (e.g. ENSEMBL ids) and a column, gene_name, containing e.g. HGNC-based gene symbols. If not provided, it can be constructed during the execution via the org.eg.XX.db packages - these need to be installed

countmatrix — A count matrix, with genes as rows and samples as columns. If not provided, it is possible to upload the data during the execution of the Shiny App

expdesign — A data.frame containing the info on the covariates of each sample. If not provided, it is possible to upload the data during the execution of the Shiny App

gene_signatures — A list of vectors, one for each pathway/signature. This is for example the output of the read_gmt function. The provided object can also be replaced during runtime in the dedicated upload widget.

Value

A Shiny App is launched for interactive data exploration and differential expression analysis

Examples

```
# with simulated data...
library(DESeq2)
dds <- DESeq2::makeExampleDESeqDataSet(n = 100, m = 8)
cm <- counts(dds)
cd <- colData(dds)

# with the well known airway package...
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
## Not run:

ideal()
ideal(dds)
ideal(dds_airway)

dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)
ideal(dds_airway, res_airway)

## End(Not run)
```

ideal-pkg *ideal: Interactive Differential Expression Analysis*

Description

ideal makes differential expression analysis interactive, easy and reproducible. The analysis of RNA-seq datasets is guided by the Shiny app as main component of the package, which also provides a wide set of functions to efficiently extract information from the existing data. The app can be also deployed on a Shiny server, to allow its usage without any installation on the user's side.

Details

ideal makes differential expression analysis interactive, easy and reproducible. The analysis of RNA-seq datasets is guided by the Shiny app as main component of the package, which also provides a wide set of functions to efficiently extract information from the existing data. The app can be also deployed on a Shiny server, to allow its usage without any installation on the user's side.

Author(s)

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plot_ma *MA-plot from base means and log fold changes*

Description

MA-plot from base means and log fold changes, in the ggplot2 framework, with additional support to annotate genes if provided.

Usage

```
plot_ma(
  res_obj,
  FDR = 0.05,
  point_alpha = 0.2,
  sig_color = "red",
  annotation_obj = NULL,
  hlines = NULL,
  title = NULL,
  xlab = "mean of normalized counts - log10 scale",
  ylim = NULL,
  add_rug = TRUE,
  intgenes = NULL,
  intgenes_color = "steelblue",
```

```
  labels_intgenes = TRUE,
  labels_repel = TRUE
)
```

Arguments

res_obj	A DESeqResults object
FDR	Numeric value, the significance level for thresholding adjusted p-values
point_alpha	Alpha transparency value for the points (0 = transparent, 1 = opaque)
sig_color	Color to use to mark differentially expressed genes. Defaults to red
annotation_obj	A data.frame object, with row.names as gene identifiers (e.g. ENSEMBL ids) and a column, gene_name, containing e.g. HGNC-based gene symbols. Optional
hlines	The y coordinate (in absolute value) where to draw horizontal lines, optional
title	A title for the plot, optional
xlab	X axis label, defaults to "mean of normalized counts - log10 scale"
ylim	Vector of two numeric values, Y axis limits to restrict the view
add_rug	Logical, whether to add rug plots in the margins
intgenes	Vector of genes of interest. Gene symbols if a symbol column is provided in res_obj, or else the identifiers specified in the row names
intgenes_color	The color to use to mark the genes on the main plot.
labels_intgenes	Logical, whether to add the gene identifiers/names close to the marked plots
labels_repel	Logical, whether to use geom_text_repel for placing the labels on the features to mark

Details

The genes of interest are to be provided as gene symbols if a symbol column is provided in res_obj, or else b< using the identifiers specified in the row names

Value

An object created by ggplot

Examples

```
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
# subsetting for quicker run, ignore the next two commands if regularly using the function
gene_subset <- c(
```

```
  "ENSG00000103196", # CRISPLD2
  "ENSG00000120129", # DUSP1
  "ENSG00000163884", # KLF15
  "ENSG00000179094", # PER1
  rownames(dds_airway)[rep(c(rep(FALSE, 99), TRUE), length.out = nrow(dds_airway))]
) # 1% of ids
dds_airway <- dds_airway[gene_subset, ]

dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)

plot_ma(res_airway, FDR = 0.05, hlines = 1)

plot_ma(res_airway,
  FDR = 0.1,
  intgenes = c(
    "ENSG00000103196", # CRISPLD2
    "ENSG00000120129", # DUSP1
    "ENSG00000163884", # KLF15
    "ENSG00000179094"
  ) # PER1
)
```

`plot_volcano` *Volcano plot for log fold changes and log p-values*

Description

Volcano plot for log fold changes and log p-values in the ggplot2 framework, with additional support to annotate genes if provided.

Usage

```
plot_volcano(
  res_obj,
  FDR = 0.05,
  ylim_up = NULL,
  vlines = NULL,
  title = NULL,
  intgenes = NULL,
  intgenes_color = "steelblue",
  labels_intgenes = TRUE
)
```

Arguments

`res_obj`	A `DESeqResults` object
`FDR`	Numeric value, the significance level for thresholding adjusted p-values

ylim_up	Numeric value, Y axis upper limits to restrict the view
vlines	The x coordinate (in absolute value) where to draw vertical lines, optional
title	A title for the plot, optional
intgenes	Vector of genes of interest. Gene symbols if a symbol column is provided in res_obj, or else the identifiers specified in the row names
intgenes_color	The color to use to mark the genes on the main plot.
labels_intgenes	Logical, whether to add the gene identifiers/names close to the marked plots

Details

The genes of interest are to be provided as gene symbols if a symbol column is provided in res_obj, or else b< using the identifiers specified in the row names

Value

An object created by ggplot

Examples

```
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)

# subsetting for quicker run, ignore the next two commands if regularly using the function
gene_subset <- c(
  "ENSG00000103196", # CRISPLD2
  "ENSG00000120129", # DUSP1
  "ENSG00000163884", # KLF15
  "ENSG00000179094", # PER1
  rownames(dds_airway)[rep(c(rep(FALSE, 99), TRUE), length.out = nrow(dds_airway))]
) # 1% of ids
dds_airway <- dds_airway[gene_subset, ]

dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)

plot_volcano(res_airway)
```

read_gmt *Read in a GMT file*

Description

Returns a list of pathways from a GMT file.

Usage

```
read_gmt(gmtfile)
```

Arguments

gmtfile	A character value, containing the location of the GMT formatted file. It can also be a file found online

Value

A list of vectors, one for each pathway in the GMT file.

Examples

```
# this example reads in the freely available pathways from wikipathways
## Not run:
mysigs <- read_gmt(
  "http://data.wikipathways.org/20180910/gmt/wikipathways-20180910-gmt-Homo_sapiens.gmt"
)
head(mysigs)
# see how the gene identifiers are encoded as ENTREZ id

## End(Not run)
```

sepguesser *Make an educated guess on the separator character*

Description

This function tries to guess which separator was used in a text delimited file

Usage

```
sepguesser(file, sep_list = c(",", "\t", ";", " "))
```

Arguments

file	The name of the file which the data are to be read from
sep_list	A vector containing the candidates for being identified as separators. Defaults to c(",","\t",";"," ")

Value

A character value, corresponding to the guessed separator. One of "," (comma), "\t" (tab), ";" (semicolon)," " (whitespace)

Examples

```
sepguesser(system.file("extdata/design_commas.txt", package = "ideal"))
sepguesser(system.file("extdata/design_semicolons.txt", package = "ideal"))
sepguesser(system.file("extdata/design_spaces.txt", package = "ideal"))
mysep <- sepguesser(system.file("extdata/design_tabs.txt", package = "ideal"))

# to be used for reading in the same file, without having to specify the sep
```

---

sig_heatmap *Plot a heatmap of the gene signature on the data*

Description

Plot a heatmap for the selected gene signature on the provided data, with the possibility to compactly display also DE only genes

Usage

```
sig_heatmap(
  vst_data,
  my_signature,
  res_data = NULL,
  FDR = 0.05,
  de_only = FALSE,
  annovec,
  title = "",
  cluster_rows = TRUE,
  cluster_cols = FALSE,
  anno_colData = NULL,
  center_mean = TRUE,
  scale_row = FALSE
)
```

Arguments

vst_data	A DESeqTransform object - usually the variance stabilized transformed data, which will be used to extract the expression values
my_signature	A character vector, usually named, containing the genes which compose the gene signature
res_data	A DESeqResults object. If not provided, it can be computed during the execution of the application

FDR	Numeric value between 0 and 1, the False Discovery Rate
de_only	Logical, whether to display only DE genes belonging to the pathway - defaults to FALSE
annovec	A named character vector, with the corresponding annotation across IDs
title	Character, title for the heatmap
cluster_rows	Logical, whether to cluster rows - defaults to TRUE
cluster_cols	Logical, whether to cluster column - defaults to FALSE. Recommended to be set to TRUE if de_only is also set to TRUE
anno_colData	Character vector, specifying the elements of the colData information to be displayed as a decoration of the heatmap. Can be a vector of any length, as long as these names are included as colData. Defaults to NULL, which would plot no annotation on the samples.
center_mean	Logical, whether to perform mean centering on the expression values. Defaults to TRUE, as it improves the general readability of the heatmap
scale_row	Logical, whether to perform row-based standardization of the expression values

Value

A plot based on the pheatmap function

Examples

```
# with the well known airway package...
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
## Not run:
dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)
vst_airway <- DESeq2::vst(dds_airway)
library(org.Hs.eg.db)
annovec <- mapIds(org.Hs.eg.db, rownames(dds_airway), "ENTREZID", "ENSEMBL")
mysignatures <- read_gmt(
  "http://data.wikipathways.org/20190210/gmt/wikipathways-20190210-gmt-Homo_sapiens.gmt"
)
mysignature_name <- "Lung fibrosis%WikiPathways_20190210%WP3624%Homo sapiens"
library(pheatmap)
sig_heatmap(vst_airway,
  mysignatures[[mysignature_name]],
  res_data = res_airway,
  de_only = TRUE,
  annovec = annovec,
  title = mysignature_name,
  cluster_cols = TRUE
)
```

```
## End(Not run)
```

---

wrapup_for_iSEE *wrapup_for_iSEE*

Description

Combine data from a typical DESeq2 run

Usage

```
wrapup_for_iSEE(dds, res)
```

Arguments

dds	A DESeqDataSet object.
res	A DESeqResults object.

Details

Combines the DESeqDataSet input and DESeqResults into a SummarizedExperiment object, which can be readily explored with iSEE.

A typical usage would be after running the DESeq2 pipeline as specified in one of the workflows which include this package, e.g. in the context of the ideal package.

Value

A SummarizedExperiment object, with raw counts, normalized counts, and variance-stabilizing transformed counts in the assay slots; and with colData and rowData extracted from the corresponding input parameters

Examples

```
# with simulated data...
library(DESeq2)
dds <- DESeq2::makeExampleDESeqDataSet(n = 10000, m = 8)
dds <- DESeq(dds)
res <- results(dds)
se <- wrapup_for_iSEE(dds, res)
# library(iSEE)
# iSEE(se)
## Not run:
# or with the well known airway package...
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
```

```
  colData = colData(airway),
  design = ~ cell + dex
)
dds_airway <- DESeq2::DESeq(dds_airway)
res_airway <- DESeq2::results(dds_airway)
se_airway <- wrapup_for_iSEE(dds_airway, res_airway)
# iSEE(se_airway)

## End(Not run)
```

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