Package ‘ideal’

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Type Package

Title Interactive Differential Expression AnaLysis

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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, rintojs, rlang, ggrepel, knitr, rmarkdown, shinyAce, BiocParallel, grDevices, base64enc, methods

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


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

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

VignetteBuilder knitr

RoxygenNote 7.2.3

Encoding UTF-8
R topics documented:

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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)
res <- DESeq2::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)
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

```r
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

```r
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 terms
- **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

```r
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
   colData = colData(airway),
   design = ~ cell + dex
)
res_airway <- DESeq2::results(dds_airway)
res_subset <- deSeqresult2DEgenes(res_airway)[1:100, ]
myde <- res_subset$id
myassayed <- rownames(res_airway)
mygo <- goseqTable(myde, myassayed,
   testCats = "GO:BP",
   addGeneToTerms = FALSE
)
head(mygo)
```

## Not run:

```r
## 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

```r
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.

extdesign 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

```r
# 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

```r
plot_ma(
  res_obj,
  FDR = 0.05,
  point_alpha = 0.2,
  sig_color = "red",
  annotation_obj = NULL,
  draw_y0 = TRUE,
  hlines = NULL,
  title = NULL,
  xlab = "mean of normalized counts - log10 scale",
  ylim = NULL,
  add_rug = TRUE,
  intgenes = NULL,
)```
```r
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
- `draw_y0` Logical, whether to draw the horizontal line at y=0. Defaults to TRUE.
- `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 by using 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 by using the identifiers specified in the row names.

**Value**

An object created by `ggplot`

**Examples**

```r
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design = ~ cell + dex
)
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,
  labels_repel = 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
- **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 by using the identifiers specified in the row names.

Value

An object created by `ggplot`

Examples

```r
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**

```r
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**

```r
# 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**

```r
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", ";", " ")`.
sig_heatmap

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

```r
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.
sig_heatmap

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
)
wrapup_for_iSEE

## End(Not run)

### 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
```r
# with simulated data...
library(DESeq2)
dds <- DESeq2::makeExampleDESeqDataSet(n = 10000, m = 8)
dds <- DESeq(dd)
res <- results(dd)
se <- wrapup_for_iSEE(dd, 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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