Package ‘ideal’

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**Type** Package

**Title** Interactive Differential Expression AnaLysis

**Version** 1.26.0

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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.

**License** MIT + file LICENSE

**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, markdown, 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, ShinyApps

**VignetteBuilder** knitr

**RoxygenNote** 7.2.3

**Encoding** UTF-8
`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**

```r
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

```r
# 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

```r
deseqresult2tbl(deseqresult)
```

Arguments

- `deseqresult`: A `DESeqResults` object

Value

A "tidy" data.frame with all genes

Examples

```r
# 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
ggplotCounts

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.
lables_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

degenes 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 )
dds_airway <- DESeq2::DESeq(ddis_airway)
res_airway <- DESeq2::results(ddis_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**

```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

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(dd_airway)

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

## End(Not run)
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.

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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,
  draw_y0 = TRUE,
  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
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 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

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**

```r
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

\textit{Read in a GMT file}\n
\begin{description}
\item[Description] Returns a list of pathways from a GMT file.
\item[Usage] \texttt{read_gmt(gmtfile)}
\item[Arguments]
\begin{itemize}
\item \texttt{gmtfile} A character value, containing the location of the GMT formatted file. It can also be a file found online
\end{itemize}
\item[Value] A list of vectors, one for each pathway in the GMT file.
\item[Examples]\begin{verbatim}
# 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)
## End(Not run)
\end{verbatim}\end{description}

sepguesser

\textit{Make an educated guess on the separator character}\n
\begin{description}
\item[Description] This function tries to guess which separator was used in a text delimited file
\item[Usage] \texttt{sepguesser(file, sep_list = c(",", "\t", ":", " "}}
\item[Arguments]
\begin{itemize}
\item \texttt{file} The name of the file which the data are to be read from
\item \texttt{sep_list} A vector containing the candidates for being identified as separators. Defaults to \texttt{c(",", "\t", ":", " "})
\end{itemize}
\end{description}
**Value**

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

**Examples**

```r
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

**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
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
)
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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