Package ‘pcaExplorer’

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Type Package

Title Interactive Visualization of RNA-seq Data Using a Principal Components Approach

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Description This package provides functionality for interactive visualization of RNA-seq datasets based on Principal Components Analysis. The methods provided allow for quick information extraction and effective data exploration. A Shiny application encapsulates the whole analysis.

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LazyData TRUE

Imports DESeq2, SummarizedExperiment, GenomicRanges, IRanges, S4Vectors, genefilter, ggplot2 (>= 2.0.0), d3heatmap, scales, NMF, plyr, topGO, limma, GOstats, GO.db, AnnotationDbi, shiny (>= 0.12.0), shinydashboard, shinyBS, dt, shinyAce, threejs, biomaRt, pheatmap, knitr, rmarkdown, tidy, grDevices, methods

Suggests testthat, BiocStyle, airway, org.Hs.eg.db

URL https://github.com/federicomarini/pcaExplorer

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

biocViews Visualization, RNASEq, DimensionReduction, PrincipalComponent, QualityControl, GUI, ReportWriting

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Author Federico Marini [aut, cre]

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correlatePCs

Principal components (cor)relation with experimental covariates

Description
Computes the significance of (cor)relations between PCA scores and the sample experimental covariates, using Kruskal-Wallis test for categorial variables and the cor.test based on Spearman’s correlation for continuous variables.

Usage
correlatePCs(pcaobj, coldata, pcs = 1:4)

Arguments
- pcaobj: A prcomp object
- coldata: A data.frame object containing the experimental covariates
- pcs: A numeric vector, containing the corresponding PC number

Value
A data.frame object with computed p values for each covariate and for each principal component

Examples
library(DESeq2)
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3, betaSD_tissue = 1)
rlt <- rlogTransformation(dds)
pcaobj <- prcomp(t(assay(rlt)))
correlatePCs(pcaobj, colData(dds))
distro_expr

Description
Plot distribution of expression values

Usage
distro_expr(rld, plot_type = "density")

Arguments
- rld: A `DESeqTransform` object.
- plot_type: Character, choose one of `boxplot`, `violin` or `density`. Defaults to `density`.

Value
A plot with the distribution of the expression values.

Examples
```r
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3, betaSD_tissue = 1)
rlt <- DESeq2::rlogTransformation(dds)
distro_expr(rlt)
```

GeneProfiler

Description
Extract and plot the expression profile of genes

Usage
geneProfiler(se, genelist = NULL, intgroup = "condition", plotZ = FALSE)

Arguments
- se: A `DESeqDataSet` object, or a `DESeqTransform` object.
- genelist: An array of characters, including the names of the genes of interest of which the profile is to be plotted.
- intgroup: A factor, needs to be in the `colnames` of `colData(se)`.
- plotZ: Logical, whether to plot the scaled expression values. Defaults to FALSE.

Value
A plot of the expression profile for the genes.
Examples

dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3, betaSD_tissue = 1)
rlt <- DESeq2::rlogTransformation(dds)
geneprofiler(rlt, paste0("gene", sample(1:1000, 20)))
geneprofiler(rlt, paste0("gene", sample(1:1000, 20)), plotZ = TRUE)

Description

Computes and plots the principal components of the genes, eventually displaying the samples as in a typical biplot visualization.

Usage

genespca(x, ntop, choices = c(1, 2), arrowColors = "steelblue",
groupNames = "group", biplot = TRUE, scale = 1, pc.biplot = TRUE,
obs.scale = 1 - scale, var.scale = scale, groups = NULL,
ellipse = FALSE, ellipse.prob = 0.68, labels = NULL, labels.size = 3,
alpha = 1, var.axes = TRUE, circle = FALSE, circle.prob = 0.69,
varname.size = 4, varname.adjust = 1.5, varname.abbrev = FALSE,
returnData = FALSE, coordEqual = FALSE, scaleArrow = 1,
useRownamesAsLabels = TRUE, point_size = 2, annotation = NULL)

Arguments

x A DESeqTransform object, with data in assay(x), produced for example by either rlog or varianceStabilizingTransformation
ntop Number of top genes to use for principal components, selected by highest row variance
choices Vector of two numeric values, to select on which principal components to plot
arrowColors Vector of character, either as long as the number of the samples, or one single value
groupNames Factor containing the groupings for the input data. Is efficiently chosen as the (interaction of more) factors in the colData for the object provided
biplot Logical, whether to additionally draw the samples labels as in a biplot representation
scale Covariance biplot (scale = 1), form biplot (scale = 0). When scale = 1, the inner product between the variables approximates the covariance and the distance between the points approximates the Mahalanobis distance.
pc.biplot Logical, for compatibility with biplot.princomp()
obs.scale Scale factor to apply to observations
var.scale Scale factor to apply to variables
groups Optional factor variable indicating the groups that the observations belong to. If provided the points will be colored according to groups
ellipse Logical, draw a normal data ellipse for each group
genespca

**ellipse.prob**  Size of the ellipse in Normal probability

**labels**  optional Vector of labels for the observations

**labels.size**  Size of the text used for the labels

**alpha**  Alpha transparency value for the points (0 = transparent, 1 = opaque)

**var.axes**  Logical, draw arrows for the variables?

**circle**  Logical, draw a correlation circle? (only applies when prcomp was called with scale = TRUE and when var.scale = 1)

**circle.prob**  Size of the correlation circle in Normal probability

**varname.size**  Size of the text for variable names

**varname.adjust**  Adjustment factor the placement of the variable names, >= 1 means farther from the arrow

**varname.abbrev**  Logical, whether or not to abbreviate the variable names

**returnData**  Logical, if TRUE returns a data.frame for further use, containing the selected principal components for custom plotting

**coordEqual**  Logical, default FALSE, for allowing brushing. If TRUE, plot using equal scale cartesian coordinates

**scaleArrow**  Multiplicative factor, usually >=1, only for visualization purposes, to allow for distinguishing where the variables are plotted

**useRownamesAsLabels**  Logical, if TRUE uses the row names as labels for plotting

**point.size**  Size of the points to be plotted for the observations (genes)

**annotation**  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

### Details

The implementation of this function is based on the beautiful ggbiplot package developed by Vince Vu, available at https://github.com/vqv/ggbiplot. The adaptation and additional parameters are tailored to display typical genomics data such as the transformed counts of RNA-seq experiments.

### Value

An object created by ggplot, which can be assigned and further customized.

### Examples

```r
library(DESeq2)
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3,betaSD_tissue = 1)
rlt <- rlogTransformation(dds)
groups <- colData(dds)$condition
groups <- factor(groups,levels=unique(groups))
cols <- scales::hue_pal()(2)[groups]
genespca(rlt,ntop=100,arrowColors=cols,groupName=groups)

groups_multi <- interaction(as.data.frame(colData(rlt)[,c("condition","tissue")]))
groups_multi <- factor(groups_multi,levels=unique(groups_multi))
cols_multi <- scales::hue_pal()(length(levels(groups_multi)))[factor(groups_multi)]
genespca(rlt,ntop=100,arrowColors=cols_multi,groupName=groups_multi)
```
get.annotation

Get an annotation data frame from biomaRt

Description

Get an annotation data frame from biomaRt

Usage

get.annotation(dds, biomart_dataset, idtype)

Arguments

dds A DESeqDataSet object
biomart_dataset A biomaRt dataset to use. To see the list, type mart = useMart('ensembl'), followed by listDatasets(mart).
idtype Character, the ID type of the genes as in the row names of dds, to be used for the call to getBM

Value

A data frame for ready use in pcaExplorer, retrieved from biomaRt.

Examples

library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
colData = colData(airway),
design=~dex+cell)
## Not run:
get.annotation(dds_airway,"hsapiens_gene_ensembl","ensembl_gene_id")
## End(Not run)

get.annotation.orgdb

Get an annotation data frame from org db packages

Description

Get an annotation data frame from org db packages

Usage

get.annotation.orgdb(dds, orgdb_species, idtype)
**Arguments**

- **dds** A `DESeqDataSet` object
- **orgdb_species** Character string, named as the `org.XX.eg.db` package which should be available in Bioconductor
- **idtype** Character, the ID type of the genes as in the row names of `dds`, to be used for the call to `mapIds`

**Value**

A data frame for ready use in `pcaExplorer`, retrieved from the org db packages

**Examples**

```r
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
colData = colData(airway),
design=~dex+cell)
## Not run:
get_annotation_orgdb(dds_airway,"org.Hs.eg.db","ENSEMBL")
## End(Not run)
```

---

**Description**

Extract genes with highest loadings

**Usage**

```r
hi_loadings(pcaobj, whichpc = 1, topN = 10, exprTable = NULL,
annotation = NULL, title = "Top/bottom loadings - ")
```

**Arguments**

- **pcaobj** A `prcomp` object
- **whichpc** An integer number, corresponding to the principal component of interest
- **topN** Integer, number of genes with top and bottom loadings
- **exprTable** A matrix object, e.g. the counts of a `DESeqDataSet`. If not NULL, returns the counts matrix for the selected genes
- **annotation** 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
- **title** The title of the plot

**Value**

A base plot object, or a matrix, if `exprTable` is not null
Examples

```r
dds <- makeExampleDESeqDataSet_multifac(betaSD = 3, betaSD_tissue = 1)
rlt <- DESeq2::rlogTransformation(dds)
pcaobj <- prcomp(t(SummarizedExperiment::assay(rlt)))
hi_loadings(pcaobj,topN = 20)
hi_loadings(pcaobj,topN = 10,exprTable=dds)
hi_loadings(pcaobj,topN = 10,exprTable=counts(dds))
```

limmaquickpca2go

Functional interpretation of the principal components, based on simple overrepresentation analysis

Description

Extracts the genes with the highest loadings for each principal component, and performs functional enrichment analysis on them using the simple and quick routine provided by the limma package

Usage

```r
limmaquickpca2go(se, pca_ngenes = 10000, inputType = "ENSEMBL",
organism = "Mm", loadings_ngenes = 500, background_genes = NULL,
scale = FALSE, ...)
```

Arguments

- **se**: A DESeqTransform object, with data in assay(se), produced for example by either `rlog` or `varianceStabilizingTransformation`
- **pca_ngenes**: Number of genes to use for the PCA
- **inputType**: Input format type of the gene identifiers. Defaults to ENSEMBL, that then will be converted to ENTREZ ids. Can assume values such as ENTREZID, GENENAME or SYMBOL, like it is normally used with the select function of AnnotationDbi
- **organism**: Character abbreviation for the species, using org.XX.eg.db for annotation
- **loadings_ngenes**: Number of genes to extract the loadings (in each direction)
- **background_genes**: Which genes to consider as background.
- **scale**: Logical, defaults to FALSE, scale values for the PCA
- ... Further parameters to be passed to the topGO routine

Value

A nested list object containing for each principal component the terms enriched in each direction. This object is to be thought in combination with the displaying feature of the main `pcaExplorer` function.
Examples

library(airway)
library(DESeq2)
library(limma)
data(airway)
airway
dds_airway <- DESeqDataSet(airway, design= ~ cell + dex)
## Not run:
  rld_airway <- rlogTransformation(dds_airway)
goquick_airway <- limmaquickpca2go(rld_airway,
    pca_ngenes = 10000,
    inputType = "ENSEMBL",
    organism = "Hs")
## End(Not run)

makeExampleDESeqDataSet_multifac

Make a simulated DESeqDataSet for two or more experimental factors

Description

Constructs a simulated dataset of Negative Binomial data from different conditions. The fold changes between the conditions can be adjusted with the betaSD_condition and the betaSD_tissue arguments.

Usage

makeExampleDESeqDataSet_multifac(n = 1000, m = 12, betaSD_condition = 1,
    betaSD_tissue = 3, interceptMean = 4, interceptSD = 2,
    dispMeanRel = function(x) 4/x + 0.1, sizeFactors = rep(1, m))

Arguments

n number of rows (genes)
m number of columns (samples)
betaSD_condition the standard deviation for condition betas, i.e. beta ~ N(0,betaSD)
betaSD_tissue the standard deviation for tissue betas, i.e. beta ~ N(0,betaSD)
interceptMean the mean of the intercept betas (log2 scale)
interceptSD the standard deviation of the intercept betas (log2 scale)
dispMeanRel a function specifying the relationship of the dispersions on 2^trueIntercept
sizeFactors multiplicative factors for each sample

Details

This function is designed and inspired following the proposal of makeExampleDESeqDataSet from the DESeq2 package. Credits are given to Mike Love for the nice initial implementation.
pair_corr

Value

A DESeqDataSet with true dispersion, intercept for two factors (condition and tissue) and beta values in the metadata columns. Note that the true betas are provided on the log2 scale.

Examples

```r
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3, betaSD_tissue = 1)
dds
dds2 <- makeExampleDESeqDataSet_multifac(betaSD_condition = 1, betaSD_tissue = 4)
dds2
```

---

pair_corr  
*Pairwise scatter and correlation plot of counts*

Description

Pairwise scatter and correlation plot of counts

Usage

```r
pair_corr(df, method = "pearson")
```

Arguments

df  
A data frame, containing the (raw/normalized-transformed) counts

method  
Character string, one of `pearson` (default), `kendall`, or `spearman` as in `cor`

Value

A plot with pairwise scatter plots and correlation coefficients

Examples

```r
library(airway)
data(airway)
airway
dds_airway <- DESeq2::DESeqDataSetFromMatrix(assay(airway),
  colData = colData(airway),
  design=~dex+cell)
pair_corr(counts(dds_airway)[1:100,]) # use just a subset for the example
```
Functional interpretation of the principal components

Description

Extracts the genes with the highest loadings for each principal component, and performs functional enrichment analysis on them using routines and algorithms from the topGO package.

Usage

pca2go(se, pca_ngenes = 10000, annotation = NULL, inputType = "geneSymbol", organism = "Mm", ensToGeneSymbol = FALSE, loadings_ngenes = 500, background_genes = NULL, scale = FALSE, ...)

Arguments

- `se`: A DESeqTransform object, with data in assay(se), produced for example by either `rlog` or `varianceStabilizingTransformation`.
- `pca_ngenes`: Number of genes to use for the PCA.
- `annotation`: 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.
- `inputType`: Input format type of the gene identifiers. Will be used by the routines of topGO.
- `organism`: Character abbreviation for the species, using org.XX.eg.db for annotation.
- `ensToGeneSymbol`: Logical, whether to expect ENSEMBL gene identifiers, to convert to gene symbols with the annotation provided.
- `loadings_ngenes`: Number of genes to extract the loadings (in each direction).
- `background_genes`: Which genes to consider as background.
- `scale`: Logical, defaults to FALSE, scale values for the PCA.
- `...`: Further parameters to be passed to the topGO routine.

Value

A nested list object containing for each principal component the terms enriched in each direction. This object is to be thought in combination with the displaying feature of the main pcaExplorer function.

Examples

```r
library(airway)
library(DESeq2)
data(airway)
airway

## Not run:
dds_airway <- DESeqDataSet(airway, design= ~ cell + dex)

rld_airway <- rlogTransformation(dds_airway)
```
# constructing the annotation object
anno_df <- data.frame(gene_id = rownames(dds_airway),
  stringsAsFactors=FALSE)
library("AnnotationDbi")
library("org.Hs.eg.db")
anno_df$gene_name <- mapIds(org.Hs.eg.db,
  keys=anno_df$gene_id,
  column="SYMBOL",
  keytype="ENSEMBL",
  multiVals="first")
rownames(anno_df) <- anno_df$gene_id
bg_ids <- rownames(dds_airway)[rowSums(counts(dds_airway)) > 0]
library(topGO)
pca2go_airway <- pca2go(rld_airway,
  annotation = anno_df,
  organism = "Hs",
  ensToGeneSymbol = TRUE,
  background_genes = bg_ids)
## End(Not run)

---

**Description**

pcaExplorer provides functionality for interactive visualization of RNA-seq datasets based on Principal Components Analysis. The methods provided allow for quick information extraction and effective data exploration. A Shiny application encapsulates the whole analysis.

Launch a Shiny App for interactive exploration of a dataset from the perspective of Principal Components Analysis

**Usage**

```r
pcaExplorer(dds = NULL, rlt = NULL, countmatrix = NULL, coldata = NULL,
  pca2go = NULL, annotation = NULL)
```

**Arguments**

- **dds**: A DESeqDataSet object. If not provided, then a countmatrix and a coldata 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.
- **rlt**: A DESeqTransform object. Can be computed from the dds object if left NULL. If none is provided, then a countmatrix and a coldata 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.
- **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.
**Description**

Plots the results of PCA on a 2-dimensional space
pcaplot3d

Sample PCA plot for transformed data

Usage

pcaplot3d(x, intgroup = "condition", ntop = 500, returnData = FALSE, title = NULL, pcX = 1, pcY = 2, pcZ = 3, text_labels = TRUE, point_size = 3)

Description

Plots the results of PCA on a 3-dimensional space, interactively

Usage

pcaplot3d(x, intgroup = "condition", ntop = 500, returnData = FALSE, title = NULL, pcX = 1, pcY = 2, pcZ = 3, text_labels = TRUE, point_size = 3)
Arguments

- **x**: A `DESeqTransform` object, with data in `assay(x)`, produced for example by either `rlog` or `varianceStabilizingTransformation`
- **intgroup**: Interesting groups: a character vector of names in `colData(x)` to use for grouping
- **ntop**: Number of top genes to use for principal components, selected by highest row variance
- **returnData**: logical, if TRUE returns a data.frame for further use, containing the selected principal components and intgroup covariates for custom plotting
- **title**: The plot title
- **pcX**: The principal component to display on the x axis
- **pcY**: The principal component to display on the y axis
- **pcZ**: The principal component to display on the z axis
- **text_labels**: Logical, whether to display the labels with the sample identifiers
- **point_size**: Integer, the size of the points for the samples

Value

A html-based visualization of the 3d PCA plot

Examples

```r
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3,betaSD_tissue = 1)
rlt <- DESeq2::rlogTransformation(dds)
pcaplot3d(rlt, ntop=200)
```

### pcascree

**Scree plot of the PCA on the samples**

Description

Produces a scree plot for investigating the proportion of explained variance, or alternatively the cumulative value

Usage

```r
pcascree(obj, type = c("pev", "cev"), pc_nr = NULL, title = NULL)
```

Arguments

- **obj**: A `prcomp` object
- **type**: Display absolute proportions or cumulative proportion. Possible values: "pev" or "cev"
- **pc_nr**: How many principal components to display max
- **title**: Title of the plot

Value

An object created by `ggplot`, which can be assigned and further customized.
Examples

```r
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3,betaSD_tissue = 1)
rlt <- DESeq2:::rlogTransformation(dds)
pcaobj <- prcomp(t(SummarizedExperiment:::assay(rlt)))
pcascree(pcaobj,type="pev")
pcascree(pcaobj,type="cev",title="Cumulative explained proportion of variance - Test dataset")
```

---

**plotPCcorrs**

`plotPCcorrs`  
---

**Plot significance of (cor)relations of covariates VS principal components**

**Description**

Plots the significance of the (cor)relation of each covariate vs a principal component

**Usage**

```r
plotPCcorrs(pccorrs, pc = 1, logp = TRUE)
```

**Arguments**

- `pccorrs`: A `data.frame` object generated by `correlatePCs`
- `pc`: An integer number, corresponding to the principal component of interest
- `logp`: Logical, defaults to `TRUE`, displays the -log10 of the pvalue instead of the p value itself

**Value**

A base plot object

**Examples**

```r
library(DESeq2)
dds <- makeExampleDESeqDataSet_multifac(betaSD_condition = 3,betaSD_tissue = 1)
rlt <- rlogTransformation(dds)
pcaobj <- prcomp(t(assay(rlt)))
res <- correlatePCs(pcaobj,colData(dds))
plotPCcorrs(res)
```
topGOtable

Extract functional terms enriched in the DE genes, based on topGO

Description
A wrapper for extracting functional GO terms enriched in the DE genes, based on the algorithm and the implementation in the topGO package

Usage
topGOtable(DEgenes, BGgenes, ontology = "BP", annot = annFUN.org, mapping = "org.Mm.eg.db", geneID = "symbol", topTablerows = 200, fullNamesInRows = TRUE, addGeneToTerms = TRUE, plotGraph = FALSE, plotNodes = 10, writeOutput = FALSE, outputFile = "")

Arguments
- DEgenes: A vector of (differentially expressed) genes
- BGgenes: A vector of background genes, e.g. all (expressed) genes in the assays
- ontology: Which Gene Ontology domain to analyze: BP (Biological Process), MF (Molecular Function), or CC (Cellular Component)
- annot: Which function to use for annotating genes to GO terms. Defaults to annFUN.org
- mapping: Which org.XX.eg.db to use for annotation - select according to the species
- geneID: Which format the genes are provided. Defaults to symbol, could also be entrez or ENSEMBL
- topTablerows: How many rows to report before any filtering
- fullNamesInRows: Logical, whether to display or not the full names for the GO terms
- addGeneToTerms: Logical, whether to add a column with all genes annotated to each GO term
- plotGraph: Logical, if TRUE additionally plots a graph on the identified GO terms
- plotNodes: Number of nodes to plot
- writeOutput: Logical, if TRUE additionally writes out the result to a file
- outputFile: Name of the file the result should be written into

Value
A table containing the computed GO Terms and related enrichment scores

Examples
library(airway)
library(DESeq2)
data(airway)
airway
dds_airway <- DESeqDataSet(airway, design= ~ cell + dex)

# Example, performing extraction of enriched functional categories in
## detected significantly expressed genes

```r
# Not run:
dds_airway <- DESeq(dds_airway)
res_airway <- results(dds_airway)
library("AnnotationDbi")
library("org.Hs.eg.db")
res_airway$symbol <- mapIds(org.Hs.eg.db,
                           keys=row.names(res_airway),
                           column="SYMBOL",
                           keytype="ENSEMBL",
                           multiVals="first")
res_airway$entrez <- mapIds(org.Hs.eg.db,
                           keys=row.names(res_airway),
                           column="ENTREZID",
                           keytype="ENSEMBL",
                           multiVals="first")
resOrdered <- as.data.frame(res_airway[order(res_airway$padj),,drop=FALSE])
de_df <- resOrdered[resOrdered$padj < .05 & !is.na(resOrdered$padj),]
de_symbols <- de_df$symbol
bg_ids <- rownames(dds_airway)[rowSums(counts(dds_airway)) > 0]
bg_symbols <- mapIds(org.Hs.eg.db,
                     keys=bg_ids,
                     column="SYMBOL",
                     keytype="ENSEMBL",
                     multiVals="first")
library(topGO)
topgoDE_airway <- topGOtable(de_symbols, bg_symbols,
                             ontology = "BP",
                             mapping = "org.Hs.eg.db",
                             geneID = "symbol")
```

## End(Not run)
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