Title  Paired DGE and DGS analysis for gene set enrichment analysis  

Description  pairedGSEA makes it simple to run a paired Differential Gene Expression (DGE) and Differential Gene Splicing (DGS) analysis. The package allows you to store intermediate results for further investigation, if desired. pairedGSEA comes with a wrapper function for running an Over-Representation Analysis (ORA) and functionalities for plotting the results.

License  MIT + file LICENSE  
Imports  DESeq2, DEXSeq, limma, fgsea, sva, SummarizedExperiment, S4Vectors, BiocParallel, ggplot2, aggregation, stats, utils, methods

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**example_diff_result**

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**R topics documented:**

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**Description**

This example result is used primarily to do package tests and for function man pages

**Usage**

```r
data("example_diff_result")
```

**Format**

A ‘DataFrame’ with 954 rows and 7 columns.

**Value**

A ‘DataFrame’.
example_gene_sets

MSigDB gene sets from humans, category C5 with ensemble gene IDs

Description
This example gene set list is used primarily to do package tests and for function man pages.

Usage
data("example_gene_sets")

Format
A list of 77 human gene sets

Value
A list of gene sets

example_ora_results

Output of running paired_ora on example_diff_result and gene sets extracted from MSigDB

Description
This example result is used primarily to do package tests and for function man pages.

Usage
data("example_ora_results")

Format
A ‘DataFrame’ with 559 rows and 18 columns.

Value
A ‘DataFrame’
example_se  A small subset of the GEO:GSE61220 data set.

Description

The subset is used in the vignettes and function man pages. The subset was created by extracting genes belonging to Telomere-related gene sets and randomly selecting 900 other genes from the original dataset.

Usage

data("example_se")

Format

A 'SummarizedExperiment'

assay  Count matrix with 5611 transcripts and 6 samples
colData  The metadata associated with the count matrix

Value

A 'SummarizedExperiment'

Source


paired_diff  Run paired DESeq2 and DEXSeq analyses

Description

With paired_diff you can run a paired differential gene expression and splicing analysis. The function expects a counts matrix or a SummarizedExperiment or DESeqDataSet object as input. A preliminary prefiltering step is performed to remove genes with a summed count lower than the provided threshold. Likewise, genes with counts in only one sample are removed. This step is mostly to speed up differential analyses, as DESeq2 will do a stricter filtering. Surrogate Variable Analysis is recommended to allow the analyses to take batch effects etc. into account. After the two differential analyses, the transcript-level p-values will be aggregated to gene-level to allow subsequent Gene-Set Enrichment Analysis. Transcript-level results can be extracted by setting store_results = TRUE.
Usage

paired_diff(
  object,
  group_col,
  sample_col,
  baseline,
  case,
  metadata = NULL,
  covariates = NULL,
  experiment_title = NULL,
  store_results = FALSE,
  run_sva = TRUE,
  use_limma = FALSE,
  prefILTER = 10,
  test = "LRT",
  quiet = FALSE,
  parallel = FALSE,
  BPPARAM = BiocParallel::bpparam(),
  expression_only = FALSE,
  custom_design = FALSE,
  ...
)

Arguments

object A data object of the types matrix, SummarizedExperiment, or DESeqDataSet. If a matrix is used, please also provide metadata.
group_col The metadata column specifying the what group each sample is associated with
sample_col The column in the metadata that specifies the sample IDs (should correspond to column names in object). Set to "rownames" if the rownames should be used.
baseline Group value of baseline samples
case Group value of case samples
metadata (Default: NULL) A metadata file or data.frame object
covariates Name of column(s) in the metadata that indicate(s) covariates. E.g., c("gender", "tissue_type")
experiment_title Title of your experiment. Your results will be stored in paste0("results/", experiment_title, ".txt")
store_results (Default: FALSE) A logical indicating if results should be stored in the folder "results/"
run_sva (Default: TRUE) A logical stating whether SVA should be run.
use_limma (Default: FALSE) A logical determining if limma+voom or DESeq2 + DEXSeq should be used for the analysis
paired_diff

prefilter (Default: 10) The prefilter threshold, where rowSums lower than the prefilter threshold will be removed from the count matrix. Set to 0 or FALSE to prevent prefiltering.

test either "Wald" or "LRT", which will then use either Wald significance tests (defined by `nbinomWaldTest`), or the likelihood ratio test on the difference in deviance between a full and reduced model formula (defined by `nbinomLRT`).

fit_type (Default: "local") Either "parametric", "local", "mean", or "glmGamPoi" for the type of fitting of dispersions to the mean intensity.

quiet (Default: FALSE) Whether to print messages.

parallel (Default: FALSE) If FALSE, no parallelization. If TRUE, parallel execution using `BiocParallel`, see next argument `BPPARAM`.

BPPARAM (Default: `bpparam()`) An optional parameter object passed internally to `bplapply` when parallel = TRUE. If not specified, the parameters last registered with register will be used.

expression_only (Default: FALSE) A logical that indicates whether to only run `DESeq2` analysis. Not generally recommended. The setting was implemented to make the SVA impact analysis easier.

custom_design (Default: FALSE) A logical or formula. Can be used to apply a custom design formula for the analysis. Generally not recommended, as `pairedGSEA` will make its own design formula from the group and covariate columns.

Value

A DFrame of aggregated pvalues

See Also

Other paired: `paired_ora()`

Examples

```r
# Run analysis on included example data
data("example_se")
diff_results <- paired_diff(
  object = example_se[1:15, ],
  group_col = "group_nr",
  sample_col = "id",
  baseline = 1,
  case = 2,
  experiment_title = "Example",
  store_results = FALSE
)
```
paired_ora

Paired Over-Representation Analysis

Description

paired_ora uses fora to run the over-representation analysis. First the aggregated pvalues are adjusted using the Benjamini & Hochberg method. The analysis is run on all significant genes found by DESeq2 and DEXSeq individually. I.e., two runs of fora are executed and subsequently joined into a single object. You can use prepare_msigdb to create a list of gene_sets.

Usage

paired_ora(
  paired_diff_result,
  gene_sets,
  cutoff = 0.05,
  min_size = 25,
  experiment_title = NULL,
  expression_only = FALSE,
  quiet = FALSE
)

Arguments

paired_diff_result  The output of paired_diff
gene_sets         List of gene sets to analyse
cutoff            (Default: 0.05) Adjusted p-value cutoff for selecting significant genes
min_size          (Default: 25) Minimal size of a gene set to test. All pathways below the threshold are excluded.
experiment_title  Title of your experiment. Your results will be stored in paste0("results/", experiment_title, ".fora.RDS").
expression_only   (Default: FALSE) A logical that indicates whether to only run DESeq2 analysis. Not generally recommended.
quiet             (Default: FALSE) Whether to print messages

Value

A data.table of merged ORA results

See Also

Other paired: paired_diff()
Examples

```r
data("example_diff_result")
data("example_gene_sets")

ora <- paired_ora(
  example_diff_result,
  example_gene_sets)
```

Description

Scatter plot of Over-Representation Analysis results

Usage

```r
plot_ora(
  ora,
  pattern = NULL,
  paired = TRUE,
  plotly = FALSE,
  cutoff = 0.05,
  lines = TRUE,
  colors = c("darkgray", "purple", "lightblue", "maroon")
)
```

Arguments

- **ora**: Output of `paired_ora`
- **pattern**: Highlight pathways containing a specific regex pattern
- **paired**: (Default: TRUE) New plotting mode for paired ora analysis
- **plotly**: (Default: FALSE) Logical on whether to return plot as an interactive `plotly` plot or a simple `ggplot`.
- **cutoff**: (Default: 0.2) Adjusted p-value cutoff for pathways to include
- **lines**: (Default: TRUE) Whether to show dashed lines
- **colors**: (Default: c("darkgray", "purple", "navy")) Colors to use in plot. The colors are ordered as "Both", "DGS", and "DGE"

Value

A `ggplot`
prepare_msigdb

Note
Suggested: importFrom plotly ggplotly

Examples

data(example_ora_results)
plot_ora(example_ora_results, pattern = "Telomer")

gene_sets <- prepare_msigdb(species = "Homo sapiens")

Description
This function is wrapper around msigdbr(). Please see their manual for details on its use. The function extracts the gene set name and a user-defined gene id type (Default: "ensembl_gene"). Please make sure the gene IDs match those from your DE analysis. This function will format the gene sets such that they can be directly used with paired_ora().

Usage
prepare_msigdb(
  gene_id_type = "ensembl_gene",
  species = "Homo sapiens",
  category = "C5",
  subcategory = NULL
)

Arguments

gene_id_type (Default: "ensembl_gene") The gene ID type to extract. The IDs should match the gene IDs from your DE analysis.

species Species name, such as Homo sapiens or Mus musculus.

category MSigDB collection abbreviation, such as H or C1.

subcategory MSigDB sub-collection abbreviation, such as CGP or BP.

Value
A list of gene sets

Note
Suggested: importFrom msigdbr msigdbr

Examples

gene_sets <- prepare_msigdb(species = "Homo sapiens")
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