

# Package ‘pairedGSEA’

July 21, 2025

**Title** Paired DGE and DGS analysis for gene set enrichment analysis

**Version** 1.8.0

**Description** pairedGSEA makes it simple to run a paired Differential Gene Expression (DGE) and Differencital Gene Splicing (DGS) analysis. The package allows you to store intermediate results for further investiation, 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, msigdb, sva, SummarizedExperiment, S4Vectors, BiocParallel, ggplot2, aggregation, stats, utils, methods, showtext

**Suggests** writexl, readxl, readr, rhdf5, plotly, testthat (>= 3.0.0), knitr, rmarkdown, BiocStyle, covr

**Config/testthat/edition** 3

**Encoding** UTF-8

**Language** en-US

**RoxygenNote** 7.3.2

**VignetteBuilder** knitr

**biocViews** DifferentialExpression, AlternativeSplicing, DifferentialSplicing, GeneExpression, ImmunoOncology, GeneSetEnrichment, Pathways, RNASeq, Software, Transcription,

**URL** <https://github.com/shdam/pairedGSEA>

**BugReports** <https://github.com/shdam/pairedGSEA/issues>

**Depends** R (>= 4.4.0)

**git\_url** <https://git.bioconductor.org/packages/pairedGSEA>

**git\_branch** RELEASE\_3\_21

**git\_last\_commit** 4931001

**git\_last\_commit\_date** 2025-04-15

**Repository** Bioconductor 3.21

**Date/Publication** 2025-07-20

**Author** Søren Helweg Dam [cre, aut] (ORCID:  
    <<https://orcid.org/0000-0002-9895-0930>>),  
Lars Rønn Olsen [aut] (ORCID: <<https://orcid.org/0000-0002-6725-7850>>),  
Kristoffer Vitting-Seerup [aut] (ORCID:  
    <<https://orcid.org/0000-0002-6450-0608>>)

**Maintainer** Søren Helweg Dam <sohdam@dtu.dk>

Contents

|                               |           |
|-------------------------------|-----------|
| example_diff_result . . . . . | 2         |
| example_gene_sets . . . . .   | 3         |
| example_ora_results . . . . . | 3         |
| example_se . . . . .          | 4         |
| paired_diff . . . . .         | 4         |
| paired_ora . . . . .          | 7         |
| plot_ora . . . . .            | 8         |
| prepare_msigdb . . . . .      | 9         |
| <b>Index</b>                  | <b>11</b> |

---

|                     |                                                     |
|---------------------|-----------------------------------------------------|
| example_diff_result | <i>Output of running paired_diff on example_se.</i> |
|---------------------|-----------------------------------------------------|

---

Description

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

Usage

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

Format

A ‘DataFrame’ with 954 rows and 7 columns.

Value

A ‘DataFrame’.

---

|                   |                                                                         |
|-------------------|-------------------------------------------------------------------------|
| example_gene_sets | <i>MSigDB gene sets from humans, category C5 with ensemble gene IDs</i> |
|-------------------|-------------------------------------------------------------------------|

---

**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 | <i>Output of running paired_ora on example_diff_result and gene sets extracted from MSigDB</i> |
|---------------------|------------------------------------------------------------------------------------------------|

---

**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 | <i>A small subset of the GEO:GSE61220 data set.</i> |
|------------|-----------------------------------------------------|

---

### 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

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61220>

---

|             |                                              |
|-------------|----------------------------------------------|
| paired_diff | <i>Run paired DESeq2 and DEXSeq analyses</i> |
|-------------|----------------------------------------------|

---

### 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",
  fit_type = "local",
  quiet = FALSE,
  parallel = FALSE,
  BPPARAM = BiocParallel::bpparam(),
  expression_only = FALSE,
  custom_design = FALSE,
  ...
)
```

**Arguments**

|                  |                                                                                                                                                                |
|------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object           | A data object of the types matrix, <a href="#">SummarizedExperiment</a> , or <a href="#">DESeqDataSet</a> . 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, "_pairedGSEA.RDS").                                              |
| 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                                                        |

|                              |                                                                                                                                                                                                                                                                      |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>prefilter</code>       | (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                                                                                             |
| <code>test</code>            | either "Wald" or "LRT", which will then use either Wald significance tests (defined by <a href="#">nbinomWaldTest</a> ), or the likelihood ratio test on the difference in deviance between a full and reduced model formula (defined by <a href="#">nbinomLRT</a> ) |
| <code>fit_type</code>        | (Default: "local") Either "parametric", "local", "mean", or "glmGamPoi" for the type of fitting of dispersions to the mean intensity.                                                                                                                                |
| <code>quiet</code>           | (Default: FALSE) Whether to print messages                                                                                                                                                                                                                           |
| <code>parallel</code>        | (Default: FALSE) If FALSE, no parallelization. If TRUE, parallel execution using <a href="#">BiocParallel</a> , see next argument BPPARAM.                                                                                                                           |
| <code>BPPARAM</code>         | (Default: <a href="#">bpparam()</a> ) An optional parameter object passed internally to <a href="#">bplapply</a> when <code>parallel = TRUE</code> . If not specified, the parameters last registered with <code>register</code> will be used.                       |
| <code>expression_only</code> | (Default: FALSE) A logical that indicates whether to only run <a href="#">DESeq2</a> analysis. Not generally recommended. The setting was implemented to make the SVA impact analysis easier                                                                         |
| <code>custom_design</code>   | (Default: FALSE) A logical or formula. Can be used to apply a custom design formula for the analysis. Generally not recommended, as <code>pairedGSEA</code> will make its own design formula from the group and covariate columns                                    |
| <code>...</code>             | Additional parameters passed to <a href="#">DESeq()</a>                                                                                                                                                                                                              |

**Value**

A DFrame of aggregated pvalues

**See Also**

Other paired: [paired\\_orc\(\)](#)

**Examples**

```
# 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\_or*Paired Over-Representation Analysis*

---

## Description

paired\_or 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_or(  
  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 <a href="#">paired_diff</a>                                                                                 |
| 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 <code>paste0("results/", experiment_title, "_fora.RDS")</code> . |
| expression_only    | (Default: FALSE) A logical that indicates whether to only run <a href="#">DESeq2</a> 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

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

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

---

|          |                                                             |
|----------|-------------------------------------------------------------|
| plot_ora | <i>Scatter plot of Over-Representation Analysis results</i> |
|----------|-------------------------------------------------------------|

---

Description

Scatter plot of Over-Representation Analysis results

Usage

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

Arguments

|         |                                                                                                                      |
|---------|----------------------------------------------------------------------------------------------------------------------|
| ora     | Output of <a href="#">paired_ora</a>                                                                                 |
| 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 <a href="#">plotly</a> 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



**Note**

suggestion: `importFrom plotly ggplotly`

**Examples**

```
data(example_ora_results)

plot_ora(example_ora_results)
```

---

|                |                                                           |
|----------------|-----------------------------------------------------------|
| prepare_msigdb | <i>Load MSigDB and convert to names list of gene sets</i> |
|----------------|-----------------------------------------------------------|

---

**Description**

This function is wrapper around `msigdb()`. 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",
  db_species = c("HS", "MM"),
  collection = "C5",
  subcollection = NULL,
  category = NULL,
  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 for output genes, such as "Homo sapiens" or "Mus musculus". Use <code>msigdb_species()</code> for available options. |
| db_species    | Species abbreviation for the human or mouse databases ("HS" or "MM").                                                             |
| collection    | Collection abbreviation, such as "H" or "C1". Use <code>msigdb_collections()</code> for the available options.                    |
| subcollection | Sub-collection abbreviation, such as "CGP" or "BP". Use <code>msigdb_collections()</code> for the available options.              |
| category      | <b>[Deprecated]</b> use the collection argument                                                                                   |
| subcategory   | <b>[Deprecated]</b> use the subcollection argument                                                                                |

**Value**

A list of gene sets

**Examples**

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

# Index

- \* **datasets**
  - example\_diff\_result, [2](#)
  - example\_gene\_sets, [3](#)
  - example\_ora\_results, [3](#)
  - example\_se, [4](#)
- \* **paired**
  - paired\_diff, [4](#)
  - paired\_ora, [7](#)
- \* **plotting**
  - plot\_ora, [8](#)
- BiocParallel, [6](#)
- bplapply, [6](#)
- bpparam(), [6](#)
- DESeq(), [6](#)
- DESeq2, [4](#), [6](#), [7](#)
- DESeqDataSet, [4](#), [5](#)
- DEXSeq, [7](#)
- example\_diff\_result, [2](#)
- example\_gene\_sets, [3](#)
- example\_ora\_results, [3](#)
- example\_se, [4](#)
- fora, [7](#)
- msigdb(), [9](#)
- nbinomLRT, [6](#)
- nbinomWaldTest, [6](#)
- paired\_diff, [4](#), [7](#)
- paired\_ora, [6](#), [7](#), [8](#)
- paired\_ora(), [9](#)
- plot\_ora, [8](#)
- plotly, [8](#)
- prepare\_msigdb, [7](#), [9](#)
- SummarizedExperiment, [4](#), [5](#)