

Package ‘broadSeq’

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Title broadSeq : for streamlined exploration of RNA-seq data

Version 1.3.3

Description This package helps user to do easily RNA-seq data analysis with multiple methods (usually which needs many different input formats). Here the user will provide the expression data as a SummarizedExperiment object and will get results from different methods. It will help user to quickly evaluate different methods.

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URL <https://github.com/dasroy/broadSeq>

BugReports <https://github.com/dasroy/broadSeq/issues>

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broadSeq-package	<i>broadSeq : for streamlined exploration of RNA-seq data</i>
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Description

This package helps user to do easily RNA-seq data analysis with multiple methods (usually which needs many different input formats). Here the user will provide the expression data as a SummarizedExperiment object and will get results from different methods. It will help user to quickly evaluate different methods.

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See Also

Useful links:

- <https://github.com/dasroy/broadSeq>
- Report bugs at <https://github.com/dasroy/broadSeq/issues>

combinedEnrichment	<i>Provides GO gene set enrichment and over-representation analysis</i>
--------------------	---

Description

This wrapper function combines `clusterProfiler::gseGO` and `clusterProfiler::enrichGO`. The input type of these two methods are different; order ranked geneList and a vector of entrez gene id. Here `combinedEnrichment` function internally generates these two data types from user defined `DEG_table` (differentially expressed genes).

Usage

```
combinedEnrichment(
  DEG_table,
  geneCol = "ID",
  logCol = "logFoldChange",
  OrgDB = "org.Hs.eg.db",
  keyType,
  universe,
  ont = "BP",
  logfoldCut = 1,
  pvalueCutoff = 0.05,
  qvalueCutoff = 0.05
)
```

Arguments

<code>DEG_table</code>	A data.frame atleast with two columns.
<code>geneCol</code>	The column name of <code>DEG_table</code> which provides gene ids and should be compatible with <code>keytype</code> parameter.
<code>logCol</code>	The column name of <code>DEG_table</code> which provides logfold(numeric) values to create a order ranked geneList for <code>gseGO</code> function.
<code>OrgDB</code>	OrgDb; passed to <code>clusterProfiler</code> functions
<code>keyType</code>	keytype of input gene(<code>geneCol</code>). One of the <code>keytypes(OrgDB)</code> ; passed to <code>clusterProfiler</code> functions
<code>universe</code>	background genes; passed to <code>clusterProfiler::enrichGO</code> .
<code>ont</code>	one of "BP", "MF", and "CC" subontologies, or "ALL" for all three.; passed to <code>clusterProfiler</code> functions
<code>logfoldCut</code>	to filter genes based on parameter <code>logCol</code>
<code>pvalueCutoff</code>	; passed to <code>clusterProfiler</code> functions
<code>qvalueCutoff</code>	; passed to <code>clusterProfiler</code> functions

Value

a named list of three data.frames which are output of `gseGO("gseResult")` and `enrichGO("oraUP" and "oraDOWN")`.

genes_plot	<i>Expression of multiple genes/features from a single assay as boxplot (or added dotplot)</i>
------------	--

Description

Expression of multiple genes/features from a single assay as boxplot (or added dotplot)

Boxplot of a single gene/feature from multiple assays

Usage

```
genes_plot(se, features, assayName = "counts", facet.by = "feature", x, ...)
```

```
assay_plot(se, feature, assayNames = c("counts"), x, ...)
```

Arguments

se	Object of SummarizedExperiment class
features	a character vector of rownames or named list of character vectors where name is one of the colnames of rowData.
assayName	One of the values from <code>SummarizedExperiment::assayNames(se)</code> ; default is "counts" assay
facet.by	must be one of the column names of <code>rowData(se)</code> . default "feature" which is equivalent to rownames of rowData
x	a column name of colData which will be used in x-axis
...	other arguments to be passed to <code>ggpubr::ggboxplot</code>
feature	a character vector of rownames or named list of character vectors where name is one of the column of rowData.
assayNames	names from <code>SummarizedExperiment::assayNames(se)</code> ; default value is "counts"

Value

ggplot object

return an object of class `ggarrange`, which is a `ggplot` or a list of `ggplot`.

Examples

```
se <- readRDS(system.file("extdata", "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))
# The normalized values are added with the assay name "logCPM"
se <- broadSeq::normalizeEdgerCPM(se ,method = "none",cpm.log = TRUE )

broadSeq::genes_plot(se,
  features = list(mouse_gene_id = c("ENSMUSG00000022510" ,
    "ENSMUSG00000027985")),
  facet.by = "symbol", # column of rowData
  x = "stage", fill="stage")

broadSeq::genes_plot(se,
  features = list(symbol=c("Shh","Edar") ),
```

```

facet.by = "symbol", # column of rowData
x = "stage", fill="stage")

broadSeq::assay_plot(se, feature = c("Shh"),
  assays = c("counts", "logCPM"),
  x = "stage", fill="stage", add="dotplot", palette = "npg")

```

normalizeEdgerCPM	<i>Use of edgeR package to normalize count data</i>
-------------------	---

Description

Use of edgeR package to normalize count data

Usage

```
normalizeEdgerCPM(se, method = "TMM", cpm.log = TRUE, ...)
```

Arguments

se	Object of SummarizedExperiment class
method	value for edgeR:: normLibSizes function. default "TMM"
cpm.log	value for edgeR:: cpm function. default TRUE
...	passed to normLibSizes function

Value

Object of [SummarizedExperiment](#) class where a new assay is added to the input object.

Examples

```

se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

se <- broadSeq::normalizeEdgerCPM(se, method = "TMM", cpm.log = FALSE )
# The normalized values are added with the assay name "TMM"
SummarizedExperiment::assayNames(se)

```

plotHeatmapCluster	<i>Plot clustered heatmaps</i>
--------------------	--------------------------------

Description

Plot clustered heatmaps from SummarizedExperiment with pheatmap and return object as ggplot

Usage

```
plotHeatmapCluster(
  se,
  scaledAssay = "vst",
  ntop = 500L,
  features = NULL,
  show_geneAs = NULL,
  annotation_col = NA,
  annotation_row = NA,
  ...
)
```

Arguments

<code>se</code>	Object of SummarizedExperiment class
<code>scaledAssay</code>	an scaled assay name from <code>SummarizedExperiment::assayNames(se)</code>
<code>ntop</code>	number of most-variable genes to select. Igored if "features" is specified.
<code>features</code>	character vector features/genes to be used to measure distance between the samples
<code>show_geneAs</code>	a character vector of colnames of <code>rowData(se)</code>
<code>annotation_col</code>	a character vector of colnames of <code>colData(se)</code>
<code>annotation_row</code>	a list of character vector of colnames of <code>rowData(se)</code>
<code>...</code>	other arguments like color or shape whose values should be similar to <code>colData</code> columns names passed to pheatmap

Value

ggplot object

Examples

```
se <- readRDS(system.file("extdata", "rat_vole_mouseSE_salmon.rds", package = "broadSeq"))

se <- broadSeq::normalizeEdgerCPM(se ,method = "none", cpm.log = TRUE )

broadSeq::plotHeatmapCluster(
  se,
  scaledAssay = "logCPM",
  annotation_col = c("species", "stage"),
  annotation_row = c("Class", "gene_biotype"),
  ntop = 30, show_geneAs = "symbol",
  cluster_cols = TRUE, cluster_rows = FALSE,
  show_rownames = TRUE, show_colnames = FALSE,
  main = "Top 30 variable gene vst"
)
```

plot_MDS	<i>Classical multidimensional scaling</i>
----------	---

Description

Classical multidimensional scaling is based on measuring the distance between the samples.

Usage

```
plot_MDS(se, scaledAssay = "vst", ntop = 500L, features = NULL, ...)
```

Arguments

se	Object of SummarizedExperiment class
scaledAssay	an scaled assay name from <code>SummarizedExperiment::assayNames(se)</code>
ntop	number of most-variable genes to select. Igored if "features" is specified.
features	character vector features/genes to be used to measure distance between the samples
...	other arguments like color or shape whose values should be similar to <code>colData</code> columns names passed to <code>ggpubr::ggscatter</code>

Value

ggplot object

Examples

```
se <- readRDS(system.file("extdata", "rat_vole_mouseSE_salmon.rds", package = "broadSeq"))

se <- broadSeq::transformDESeq2(se, method = "vst" )

broadSeq::plot_MDS(se, scaledAssay = "vst", ntop=500,
  color = "species", shape = "stage",
  ellipse=TRUE, legend = "bottom")
```

prcompTidy	<i>Perform Principal Components Analysis</i>
------------	--

Description

This function returns the results of `stats::prcomp` in a tidy list format. This is more flexible for further custom PCA , biplot and exploring gene(factor) loading of the PCA.

Usage

```
prcompTidy(se, scaledAssay = "vst", ntop = 500L, features = NULL, ...)

plotAnyPC(computedPCA, x = 1, y = 2, ...)

biplotAnyPC(computedPCA, x = 1, y = 2, genes = NULL, genesLabel = NULL, ...)

getFeatureLoadRanking(computedPCA, pcs = seq_len(5), topN = 10, keep)
```

Arguments

<code>se</code>	Object of <code>SummarizedExperiment</code> class
<code>scaledAssay</code>	an scaled assay name from <code>SummarizedExperiment::assayNames(se)</code>
<code>ntop</code>	number of most-variable genes to select. Igored if "features" is specified.
<code>features</code>	character vector features/genes to be used for PCA
<code>...</code>	other arguments like color or shape whose values should be similar to colData columns names passed to <code>ggpubr::ggscatter</code>
<code>computedPCA</code>	a list of data.frame returned by <code>prcompTidy</code>
<code>x</code>	PC number for x-axis default 1
<code>y</code>	PC number for y-axis default 2
<code>genes</code>	if genes is NULL then top max and min loaded genes of each PCs are plotted
<code>genesLabel</code>	one of rowData column names
<code>pcs</code>	The numbers of PCs
<code>topN</code>	Number of features per PC
<code>keep</code>	the column names of rowData to keep the corresponding information

Details

Reused code

Value

a list with four data.frame objects: `pc_scores`, `eigen_values`, `loadings` (eigen vectors) and the original data.
 ggplot object
 ggplot object
 a data.frame

Examples

```
se <- readRDS(system.file("extdata", "rat_vole_mouseSE_salmon.rds", package = "broadSeq"))

se <- broadSeq::normalizeEdgerCPM(se ,method = "none",cpm.log = TRUE )
computedPCA_logCPM <- broadSeq::prcompTidy(se, scaledAssay = "logCPM", ntop = 500)

plotAnyPC(computedPCA = computedPCA_logCPM, x = 1, y = 2, color = "species",
           shape = "stage", legend = "bottom")
plotAnyPC(computedPCA = computedPCA_logCPM, x = 2, y = 3, color = "species",
           shape = "stage", legend = "bottom")

computedPCA_logCPM$eigen_values %>%
  dplyr::filter(var_exp >= 0.5) %>% # Selecting PC explaining more than 1% variance
  ggbarplot(x="PC",y="var_exp", label = TRUE, label.pos = "out")
```

round_df	<i>Applies round function only on numeric columns of a data.frame.</i>
----------	--

Description

Applies round function only on numeric columns of a data.frame.

Usage

```
round_df(df, digits)
```

Arguments

df	data.frame object
digits	passed to round

Value

data.frame object

Examples

```
data("iris")
iris %>% round_df(digits = 0) %>% head()
```

sampleAssay_plot	<i>Useful to visualize distribution of assay values for each sample. Plots 'boxplot' of any assay for each sample. Aesthetic can be added from colData.</i>
------------------	---

Description

Useful to visualize distribution of assay values for each sample. Plots 'boxplot' of any assay for each sample. Aesthetic can be added from colData.

Usage

```
sampleAssay_plot(se, assayName = "counts", ...)
```

Arguments

se	Object of SummarizedExperiment class
assayName	One of the values from <code>SummarizedExperiment::assayNames(se)</code>
...	other arguments to be passed to <code>ggpubr::ggboxplot</code>

Value

ggplot object

Examples

```
se <- readRDS(system.file("extdata", "rat_vole_mouseSE_salmon.rds", package = "broadSeq"))

sampleAssay_plot(se, assayName = "counts",
  fill="stage", # stage is a column name of colData(se)
  yscale="log2")

se <- broadSeq::normalizeEdgerCPM(se, method = "none", cpm.log = TRUE )

sampleAssay_plot(se, assayName = "logCPM", fill="stage")
```

transformDESeq2

Transform SummarizedExperiment with DESeq2 package

Description

To use SummarizedExperiment with DESeq2, this function makes sure that 'counts' assay should be the first in assays list and the mode is integer.

Usage

```
transformDESeq2(se, method = "vst", ...)
```

Arguments

se	Object of SummarizedExperiment class
method	"vst", "normTransform" or "rlog" to choose either of DESeq2:: varianceStabilizingTransformation DESeq2:: normTransform and DESeq2:: rlog function. default is "vst"
...	arguments passed to varianceStabilizingTransformation normTransform and rlog

Value

Object of [SummarizedExperiment](#) class where a new assay is added to the input object.

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

se <- broadSeq::transformDESeq2(se, method = "vst" )
# The transformed values are added with the assay name "vst"
SummarizedExperiment::assayNames(se)
```

use_DELocal	<i>To use SummarizedExperiment with package DELocal</i>
-------------	---

Description

A wrapper function of DELocal where input is an object of [SummarizedExperiment](#)

Usage

```
use_DELocal(se, colData_id, control, treatment, rank = FALSE, ...)
```

Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an additional column named "rank" and the results are ranked on "relative.logFC"
...	other arguments to be passed to main function DELocal::DELocal.

Value

a data.frame from DELocal

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

result <-
  use_DELocal(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE)
```

use_deseq2	<i>To use SummarizedExperiment with package DESeq2</i>
------------	--

Description

A wrapper function of DESeq2 where input is an object of [SummarizedExperiment](#)

Usage

```
use_deseq2(se, colData_id, control, treatment, rank = FALSE, ...)
```

Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an additional column named "rank" and the results are ranked on "padj"
...	other arguments to be passed to main function DESeq2::results.

Value

a data.frame converted from DESeq2::DESeqResults

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

result <-
  use_deseq2(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE)
```

use_EBSeq

To use SummarizedExperiment with package EBSeq

Description

A wrapper function of EBSeq where input is an object of [SummarizedExperiment](#)

Usage

```
use_EBSeq(se, colData_id, control, treatment, rank = FALSE, ...)
```

Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an additional column named "rank" and the results are ranked on "PPDE"
...	other arguments to be passed to main function EBSeq::GetDEResults.

Value

a data.frame object converted from the output of `EBSeq::GetDEResults`.

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

result <-
  use_EBSeq(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE)
```

use_edgeR_GLM

To use SummarizedExperiment with package edgeR

Description

A wrapper function of DESeq2 where input is an object of [SummarizedExperiment](#)

Usage

```
use_edgeR_GLM(se, colData_id, control, treatment, rank = FALSE, ...)

use_edgeR_exact(se, colData_id, control, treatment, rank = FALSE, ...)

use_edgeR(
  se,
  colData_id,
  control,
  treatment,
  rank = FALSE,
  edgeR.n = Inf,
  edgeR.adjust.method = "BH",
  edgeR.sort.by = "PValue",
  option = "GLM",
  ...
)
```

Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an additional column named "rank"

```

...          other arguments to be passed to edgeR::glmLRT or edgeR::exactTest
edgeR.n      argument for edgeR::topTags
edgeR.adjust.method
              argument for edgeR::topTags
edgeR.sort.by argument for edgeR::topTags
option       "GLM" or "exact" to indicate to use either edgeR::glmLRT or edgeR::exactTest

```

Value

a data.frame of output from edgeR::topTags

Examples

```

se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

result <-
  use_edgeR(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE)

```

use_limma_trend	<i>To use SummarizedExperiment with package limma</i>
-----------------	---

Description

A wrapper function of limma where input is an object of [SummarizedExperiment](#)

Usage

```

use_limma_trend(se, colData_id, control, treatment, rank = FALSE, ...)

use_limma_voom(se, colData_id, control, treatment, rank = FALSE, ...)

use_limma(
  se,
  colData_id,
  control,
  treatment,
  rank = FALSE,
  useVoom = TRUE,
  showPlot = FALSE,
  limma.adjust = "BH",
  limma.sort.by = "p",
  limma.number = Inf,
  ...
)

```

Arguments

se	Object of <code>SummarizedExperiment</code> class
colData_id	One of the columns of <code>colData(se)</code> . It should be factors of more than one value.
control	Base level and one of the factor values of <code>colData(se)[[colData_id]]</code>
treatment	one of the factor values of <code>colData(se)[[colData_id]]</code>
rank	Logical value default FALSE. If true the result will have an additional column named "rank"
...	other arguments to be passed to main function <code>edgeR::calcNormFactors</code> .
useVoom	whether to use <code>limma::voom</code> or <code>edgeR::cpm</code>
showPlot	whether to use <code>limma::plotSA</code> ; default FALSE
limma.adjust	argument for <code>limma::topTable</code>
limma.sort.by	argument for <code>limma::topTable</code>
limma.number	argument for <code>limma::topTable</code>

Value

a data.frame of output from `limma::topTable`

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]
result <-
  use_limma(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE)
```

use_multDE

To identify differentially expressed genes by multiple methods

Description

To identify differentially expressed genes by multiple methods

Usage

```
use_multDE(
  deFun_list,
  return.df = FALSE,
  se,
  colData_id,
  control,
  treatment,
  ...
)
```

Arguments

deFun_list	a list of function which can perform differential expression analysis
return.df	whether to return all results aggregated form of data.frame or a list of results. Default is FALSE
se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
...	other arguments to be passed to functions listed in deFun_list

Value

a list or data.frame

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

# First define a named list of functions
funs <- list(limma_trend = use_limma_trend, limma_voom = use_limma_voom,
  edgeR_exact = use_edgeR_exact, edgeR_glm = use_edgeR_GLM,
  deseq2 = use_deseq2,
  DELocal = use_DELocal, noiseq = use_NOIseq,
  EBSseq = use_EBSseq)

multi_result <- broadSeq::use_multDE(
  se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"],
  deFun_list = funs, return.df = TRUE,
  colData_id = "stage", control = "Bud", treatment = "Cap",
  rank = TRUE)
```

use_NOIseq

Differential expression method for NOIseq

Description

This is a wrapper function of NOIseq::[noiseqbio](#) whose input class is 'eSet' and output class is Output which are not widely used. We can use as(se, "ExpressionSet") to get an eSet easily but then it will be hard to refer the treatment and control. The order of factors influence the log fold change sign. To keep it comparable to other methods the NOIseq::readData() is used internally.

Usage

```
use_NOIseq(se, colData_id, control, treatment, rank = FALSE, ...)
```


Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an additional column named "rank" which is ordered by 'prob' values returned by function NOISeq::noiseqbio .
...	other arguments to be passed to main function NOISeq::noiseqbio . The 'input' and 'factor' argument should not be used.

Value

A data.frame object from the results of [NOISeq::noiseqbio\(\)](#). For details check the documentation of 'NOISeq'

Examples

```
se <- readRDS(system.file("extdata",
  "rat_vole_mouseSE_salmon.rds",
  package = "broadSeq"))

# To reduce runtime
se <- se[rowData(se)$chromosome_name == 2,colData(se)$species == "Mouse"]

result_Noiseq <-
  use_NOISeq(se = se,
    colData_id = "stage", control = "Bud", treatment = "Cap",
    rank = TRUE,
    r = 10) # r is an argument of NOISeq::noiseqbio
```

use_SAMseq	<i>To use SummarizedExperiment with package samr</i>
------------	--

Description

To use SummarizedExperiment with package samr

Usage

```
use_SAMseq(se, colData_id, control, treatment, rank = FALSE, ...)
```

Arguments

se	Object of SummarizedExperiment class
colData_id	One of the columns of colData(se). It should be factors of more than one value.
control	Base level and one of the factor values of colData(se)[[colData_id]]
treatment	one of the factor values of colData(se)[[colData_id]]
rank	Logical value default FALSE. If true the result will have an
...	other arguments to be passed to samr::SAMseq

Value

a data.frame object as a result

volcanoPlot	<i>Volcano plot with formatted x and y axis label.</i>
-------------	--

Description

Volcano plot with formatted x and y axis label.

Usage

```
volcanoPlot(
  df,
  pValName,
  lFCName,
  sigThreshold = 0.05,
  logFCThreshold = 1,
  labelName = NULL,
  selectedLabel = NULL,
  palette = "nejm"
)
```

Arguments

df	a data.frame object
pValName	column name of df which provides p-values
lFCName	column name of df which provides log fold change values
sigThreshold	Threshold for p-values
logFCThreshold	Threshold for log fold change values
labelName	column name of df to label the dots
selectedLabel	which dots to highlight
palette	one of "npg", "aaas", "lancet", "jco", "ucscgb", "uchicago", "simpsons" and "nejm" or similar to viridis::cividis(3)

Value

ggplot object

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