Package 'blacksheepr'

October 15, 2023

Type Package

Title Outlier Analysis for pairwise differential comparison

Version 1.14.0

Description Blacksheep is a tool designed for outlier analysis in the context of pairwise comparisons in an effort to find distinguishing characteristics from two groups. This tool was designed to be applied for biological applications such as phosphoproteomics or transcriptomics, but it can be used for any data that can be represented by a 2D table, and has two sub populations within the table to compare.

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Encoding UTF-8

VignetteBuilder knitr

RoxygenNote 6.1.1

Imports grid, stats, grDevices, utils, circlize, viridis, RColorBrewer, ComplexHeatmap, SummarizedExperiment, pasilla

Suggests testthat (>= 2.1.0), knitr, BiocStyle, rmarkdown, curl

Depends R (>= 3.6)

biocViews Sequencing, RNASeq, GeneExpression, Transcription, DifferentialExpression, Transcriptomics

BugReports https://github.com/ruggleslab/blacksheepr/issues

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annotationlist_builder

Create the annotation object for plotting in a heatmap

Description

Create the annotation object for plotting in a heatmap

Usage

```
annotationlist_builder(metatable, customcolorlist = NULL)
```

Arguments

metatable the metatable containing information for the columns customcolorlist

DEFAULT: NULL, enter colorlist to manually set colors

Value

return the annotation object

comparison_groupings Create all of the groups based on the input metadata

Description

Create all of the groups based on the input metadata

Usage

```
comparison_groupings(comptable)
```

Arguments

comptable table where each column will have comparisons drawn from it

Value

a list with each of the groups as an entry in the list NOTE - this list will be ncol*2 long where ncol is the number comparisons

Examples

```
data("sample_annotationdata")
groupings <- comparison_groupings(sample_annotationdata)</pre>
```

count_outliers	Count up the outlier information for each of the groups you have made.
	If aggregating then you will have to turn the parameter on, but you still
	input the outliertable. Aggregate will count the total number of outliers
	AND nonoutliers in its operation, so it needs the original outlier table
	made by the <make_outlier_table> function.</make_outlier_table>

Description

Count up the outlier information for each of the groups you have made. If aggregating then you will have to turn the parameter on, but you still input the outliertable. Aggregate will count the total number of outliers AND nonoutliers in its operation, so it needs the original outlier table made by the <make_outlier_table> function.

Usage

```
count_outliers(groupings, outliertab,
    aggregate_features = FALSE, feature_delineator = "\\.")
```

Arguments

groupings	table generated by the comparison_groupings function	
outliertab	outlier table generated by make_outlier_table	
aggregate_feat	ures	
	DEFAULT: FALSE; Toggle the Aggregate feature, which will aggregate features in your table based on the given delineator. Aggregation will output counts for the TOTAL number of outliers and non- outliers across ALL sites you aggregate	
	across.	
feature_delineator		
	DEFAULT: <"\.">; What character delineates the separation between primary and secondary features. NOTE: to use proper R syntax with escape characters if necessary Ex) Protein1.Phosphosite1 uses "\." to aggregate on Protein1	

Value

the tabulated information of outliers per group

Examples

```
fractiontab <- count_outliers_out$fractiontab</pre>
```

create_heatmap Plot out a heatmap

Description

Plot out a heatmap

Usage

```
create_heatmap(counttab = counttab,
    colmetatable = NULL, colannotationlist = NULL,
    colclusterparam = FALSE, rowclusterparam = FALSE,
    nameparam)
```

deva

Arguments

counttab	table with counts, samples -x-axis, features -y-axis	
colmetatable	the metatable containing information for the columns	
colannotationli	ist	
	annotation table for columns, based off colmetatable	
colclusterparam	n	
	cluster the columns?	
rowclusterparam		
	cluster the rows?	
nameparam	the title on the heatmap	

Value

prints a pdf heatmap out to the designated outpath

Examples

```
data("sample_phosphodata")
counttab <- sample_phosphodata
nameparam <- "testplot"
create_heatmap(counttab = counttab,
    colmetatable = NULL,
    colannotationlist = NULL,colclusterparam = FALSE,
    rowclusterparam = FALSE, nameparam)</pre>
```

deva

Run the entire blacksheep Function from Start to finish

Description

Run the entire blacksheep Function from Start to finish

Usage

```
deva(se, analyze_negative_outliers = FALSE,
    aggregate_features = FALSE, feature_delineator = "\\.",
    fraction_samples_cutoff = 0.3, fdrcutoffvalue = 0.1)
```

Arguments se

The SummarizedExperiment object containing the countdata and the associated annotation data with comparisons in the colData object.

analyze_negative_outliers

DEFAULT: FALSE; Toggle the analysis of outliers in the negative direction as well. Will lead to the output of the outlier table containing "-1" values, in addition to negative outputs for boundaries and aggregate tables (if applicable)

aggregate_features

DEFAULT: FALSE; Toggle the Aggregate feature, which will aggregate features in your table based on the given delineator. Aggregation will output an aggregate table that counts the number of outliers per feature, and also a fraction table that show the number of outliers / number of candidates (which excludes missing values)

feature_delineator

DEFAULT: <"\."> What character delineates the separation between primary and secondary features. NOTE: to use proper R syntax with escape characters if necessary Ex) Protein1.Phosphosite1 uses "\." to aggregate on Protein1

fraction_samples_cutoff

DEFAULT: 0.3; Input a fractional cut off for the of samples that need to have an outlier for feature to be considered. ex) 10 samples in ingroup - 3 need to have an outlier for feature to be considered significant

fdrcutoffvalue DEFAULT: 0.1; The FDR value for significance

Value

outputs the full output of deva, including the analysis tables, the heatmaps for the analyses, the fraction table showing the fraction of outliers per sample, and the median and boundary values that together comprise the outlier boundary

Examples

```
suppressPackageStartupMessages(library(SummarizedExperiment))
data("sample_phosphodata")
data("sample_annotationdata")
se <- SummarizedExperiment(
    assays = list(counts = as.matrix(sample_phosphodata[1:1000,])),
    colData = DataFrame(sample_annotationdata))
deva(se = se,
    analyze_negative_outliers = FALSE, aggregate_features = FALSE,
    feature_delineator = "-", fraction_samples_cutoff = 0.3,
    fdrcutoffvalue = 0.1)</pre>
```

deva_normalization	Normalization of data to prepare for deva.	Uses a Median of Ratio
method followed by a log2 transformation.		

Description

Normalization of data to prepare for deva. Uses a Median of Ratio method followed by a log2 transformation.

deva_results

Usage

deva_normalization(intable, method = "MoR-log")

Arguments

intable table with samples along the columns and features along the rows.

method DEFAULT: "MoR-log"; Method by which to normalize data in preparation for deva. Options are <"MoR-log", "MoR", "log">. Where "MoR" refers to the Median of ratio's. The "log" transformation is necessary to compress heavily skewed data and allow for proper detection. "MoR-log" as the default will perform MoR followed by a log2 transform.

Value

A normalized table for input into deva

Examples

```
library(pasilla)
pasCts <- system.file("extdata",
    "pasilla_gene_counts.tsv", package="pasilla")
cts <- as.matrix(read.csv(pasCts,sep="\t",row.names="gene_id"))
norm_cts <- deva_normalization(cts, method = "MoR-log")</pre>
```

deva_results Utility function that allows easier grabbing of data

Description

Utility function that allows easier grabbing of data

Usage

```
deva_results(deva_out, ID = NULL, type = NULL)
```

Arguments

deva_out	output from the deva function
ID	The keyword to search through analyses and grab desired output
type	<"table" "heatmap" "fraction_table" "median" "boundary"> to return the desirted analysis type

Value

desired subset of analysis from deva

Examples

```
suppressPackageStartupMessages(library(SummarizedExperiment))
data("sample_phosphodata")
data("sample_annotationdata")
se = SummarizedExperiment(
    assays = list(counts = as.matrix(sample_phosphodata[1:1000,])),
    colData = DataFrame(sample_annotationdata))
deva_out = deva(se = se,
    analyze_negative_outliers = FALSE, aggregate_features = TRUE,
    feature_delineator = "-", fraction_samples_cutoff = 0.3,
    fdrcutoffvalue = 0.1)
deva_results(deva_out, ID = "outlieranalysis", type = "table")
```

make_comparison_columns

Utility function that will take in columns with several subcategories, and output several columns each with binary classifications. ex) col1: A,B,C » colA: A,notA,notA; colB: notB,B,notB; colC: notC,notC,C

Description

Utility function that will take in columns with several subcategories, and output several columns each with binary classifications. ex) col1: A,B,C » colA: A,notA,notA; colB: notB,B,notB; colC: notC,notC,C

Usage

```
make_comparison_columns(intable)
```

Arguments

intable table where each column has more than one subcategory, can be multiple columns

Value

an expanded table with each of the columns as a binary labeling of each subcategory.

Examples

```
data("sample_annotationdata")
new_comparisons <- make_comparison_columns(
    sample_annotationdata[,1,drop=FALSE])</pre>
```

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make_outlier_table Separate out the "i"th gene, take the bounds, and then create a column that says whether or not this gene is high, low, or none in a sample with regards to the other samples in the dataset. Repeat this for every gene to create a reference table.

Description

Separate out the "i"th gene, take the bounds, and then create a column that says whether or not this gene is high, low, or none in a sample with regards to the other samples in the dataset. Repeat this for every gene to create a reference table.

Usage

```
make_outlier_table(intable, analyze_negative_outliers = FALSE)
```

Arguments

intable table with all of the inputted information, samples along the x-axis, features along the y-axis

analyze_negative_outliers

DEFAULT: FALSE; Toggle the analysis of outliers in the negative direction. Will lead to the output of the outlier table containing "-1" values, in addition to negative outputs for boundaries and aggregate tables (if applicable)

Value

a list with varied sections depending on parameters: \$outliertab - table converted to outlier form with 0s, 1s, and -1s, \$upperboundtab - list of upper boundaries for outliers \$lowerboundtab - list of lower boundaries of outliers \$sampmedtab - list of median value per feature

outlier_analysis

With the grouptablist generated by count_outliers - run through and run a fisher exact test to get the p.value for the difference in outlier count for each feature in each of your comparisons

Description

With the grouptablist generated by count_outliers - run through and run a fisher exact test to get the p.value for the difference in outlier count for each feature in each of your comparisons

Usage

```
outlier_analysis(grouptablist, fraction_table = NULL,
    fraction_samples_cutoff = 0.3,
    write_out_tables = FALSE, outfilepath = tempdir())
```

Arguments

grouptablist	table generated by the count_outliers function. NOTE that the inputted groupt- ablist will be deciphered to determine its content. This means that user decides to input the outliertab or aggregate tab, and the output will analyze according to what positive and negative information is contained within the table
fraction_table	DEFAULT: NULL; Input a fraction table to filter to only include features that have x an outlier.
<pre>fraction_sample</pre>	s_cutoff
	DEFAULT: 0.3; Input a fractional cut off for the of samples that need to have an outlier for feature to be considered. ex) 10 samples in ingroup - 3 need to have an outlier for feature to be considered significant
write_out_table	S
	DEFAULT: FALSE; utility in function to write out each of the analyses to a separate table to whereever <outfilepath> is specfied.</outfilepath>
outfilepath	the full string path to where the file should output to, DEFAULT is a tempdir()

Value

the analysis table with p.value, fdr, and raw data per comparison

```
data("sample_phosphodata")
reftable_function_out <- make_outlier_table(sample_phosphodata[1:1000,])
outliertab <- reftable_function_out$outliertab
data("sample_annotationdata")
groupings <- comparison_groupings(sample_annotationdata)
count_outliers_out <- count_outliers(groupings, outliertab,</pre>
```

```
aggregate_features = FALSE)
grouptablist <- count_outliers_out$grouptablist
fractiontab <- count_outliers_out$fractiontab
outlier_analysis_out <- outlier_analysis(grouptablist,
    fraction_table = fractiontab)</pre>
```

outlier_heatmap	With the grouptablist generated by count_outliers - run through and
	run a fisher exact test to get the p.value for the difference in outlier
	count for each feature in each of your comparisons

Description

With the grouptablist generated by count_outliers - run through and run a fisher exact test to get the p.value for the difference in outlier count for each feature in each of your comparisons

Usage

Arguments

outrier	_analysis_	OUL
00.0110.		

-	the full outlier_analysis data objet
analysis_num	DEFAULT: NULL; if you only want to plot the heatmap for a particular analysis, enter number of that analysis
counttab	the raw data before outlier analysis
metatable	the complete metatable that was used to generate the comparisons, will be used for annotation of the heatmap
fdrcutoffvalue	DEFAULT: 0.1; The FDR value for significance

Value

outputs a pdf with the heatmap in the current working directory

```
grouptablist <- count_outliers_out$grouptablist
fractiontab <- count_outliers_out$fractiontab
outlier_analysis_out <- outlier_analysis(grouptablist,
    fraction_table = fractiontab)
metatable <- sample_annotationdata
counttab <- sample_phosphodata
hm1 <- outlier_heatmap(outlier_analysis_out, analysis_num = NULL,
    fractiontab, metatable, fdrcutoffvalue = 0.1)</pre>
```

sample_annotationdata sample_annotationdata

Description

Example annotation data for Outlier analysis. This example data is a subset of the data used in the CPTAC3 Breast Cancer exploration study: (doi: 10.1038/nature18003). Each row corresponds to a sample and each column is an binary annotation for that sample.

Usage

sample_annotationdata

Format

A data frame with 76 rows and 6 variables:

PAM50_Her2 The binary PAM50 Her2 classification for each sample

PAM50_Basal The binary PAM50 Basal classification for each sample

PAM50_LumA The binary PAM50 LumA classification for each sample

PAM50_LumB The binary PAM50 LumB classification for each sample

ER_Status The ER Status classification for each sample

PR_Status The PR Status classification for each sample ...

Source

https://cptac-data-portal.georgetown.edu/cptac/s/S029

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Description

Example phosphoprotein data for Outlier analysis This example data is a subset of the data used in the CPTAC3 Breast Cancer exploration study: (doi: 10.1038/nature18003). Each row corresponds to a phosphoprotein site, and each column is a sample. The values within the table are normalized massspec phosphoprotein values.

Usage

sample_phosphodata

Format

A data frame with 15532 rows and 76 variables:

TCGA-A2-A0CM phosphoprotein levels for each gene TCGA-A2-A0D2 phosphoprotein levels for each gene **TCGA-A2-A0EQ** phosphoprotein levels for each gene TCGA-A2-A0EV phosphoprotein levels for each gene TCGA-A2-A0EX phosphoprotein levels for each gene TCGA-A2-A0EY phosphoprotein levels for each gene TCGA-A2-A0SW phosphoprotein levels for each gene TCGA-A2-A0SX phosphoprotein levels for each gene TCGA-A2-A0T3 phosphoprotein levels for each gene TCGA-A2-A0T6 phosphoprotein levels for each gene TCGA-A2-A0YC phosphoprotein levels for each gene TCGA-A2-A0YD phosphoprotein levels for each gene TCGA-A2-A0YF phosphoprotein levels for each gene TCGA-A2-A0YG phosphoprotein levels for each gene TCGA-A2-A0YM phosphoprotein levels for each gene TCGA-A7-A0CE phosphoprotein levels for each gene TCGA-A7-A0CJ phosphoprotein levels for each gene TCGA-A7-A13F phosphoprotein levels for each gene TCGA-A8-A06N phosphoprotein levels for each gene TCGA-A8-A06Z phosphoprotein levels for each gene TCGA-A8-A076 phosphoprotein levels for each gene TCGA-A8-A079 phosphoprotein levels for each gene

TCGA-A8-A08Z phosphoprotein levels for each gene TCGA-A8-A09G phosphoprotein levels for each gene TCGA-AN-A04A phosphoprotein levels for each gene TCGA-AN-A0AJ phosphoprotein levels for each gene TCGA-AN-A0AL phosphoprotein levels for each gene TCGA-AN-A0AM phosphoprotein levels for each gene TCGA-AN-A0FK phosphoprotein levels for each gene TCGA-AN-A0FL phosphoprotein levels for each gene **TCGA-AO-A03O** phosphoprotein levels for each gene TCGA-AO-A0J6 phosphoprotein levels for each gene TCGA-AO-A0J9 phosphoprotein levels for each gene TCGA-AO-A0JC phosphoprotein levels for each gene **TCGA-AO-A0JE** phosphoprotein levels for each gene **TCGA-AO-A0JJ** phosphoprotein levels for each gene **TCGA-AO-A0JL** phosphoprotein levels for each gene TCGA-AO-A0JM phosphoprotein levels for each gene **TCGA-AO-A126** phosphoprotein levels for each gene TCGA-AO-A12B phosphoprotein levels for each gene TCGA-AO-A12D phosphoprotein levels for each gene TCGA-AO-A12E phosphoprotein levels for each gene TCGA-AO-A12F phosphoprotein levels for each gene TCGA-AR-A0TR phosphoprotein levels for each gene TCGA-AR-A0TT phosphoprotein levels for each gene TCGA-AR-A0TV phosphoprotein levels for each gene TCGA-AR-A0TX phosphoprotein levels for each gene TCGA-AR-A0U4 phosphoprotein levels for each gene TCGA-AR-A1AP phosphoprotein levels for each gene TCGA-AR-A1AS phosphoprotein levels for each gene TCGA-AR-A1AV phosphoprotein levels for each gene TCGA-AR-A1AW phosphoprotein levels for each gene TCGA-BH-A0AV phosphoprotein levels for each gene TCGA-BH-A0BV phosphoprotein levels for each gene **TCGA-BH-A0C1** phosphoprotein levels for each gene **TCGA-BH-A0C7** phosphoprotein levels for each gene TCGA-BH-A0DD phosphoprotein levels for each gene TCGA-BH-A0DG phosphoprotein levels for each gene TCGA-BH-A0E1 phosphoprotein levels for each gene

TCGA-BH-A0E9 phosphoprotein levels for each gene TCGA-BH-A18N phosphoprotein levels for each gene TCGA-BH-A18Q phosphoprotein levels for each gene TCGA-BH-A18U phosphoprotein levels for each gene TCGA-C8-A12L phosphoprotein levels for each gene TCGA-C8-A12T phosphoprotein levels for each gene TCGA-C8-A12U phosphoprotein levels for each gene TCGA-C8-A12V phosphoprotein levels for each gene TCGA-C8-A12Z phosphoprotein levels for each gene TCGA-C8-A130 phosphoprotein levels for each gene TCGA-C8-A131 phosphoprotein levels for each gene TCGA-C8-A134 phosphoprotein levels for each gene **TCGA-C8-A135** phosphoprotein levels for each gene TCGA-C8-A138 phosphoprotein levels for each gene TCGA-D8-A142 phosphoprotein levels for each gene TCGA-E2-A154 phosphoprotein levels for each gene TCGA-E2-A158 phosphoprotein levels for each gene

Source

https://cptac-data-portal.georgetown.edu/cptac/s/S029

sample_rnadata sample_rnadata

Description

Example RNA data for Outlier analysis This example data is a subset of the data used in the CPTAC3 Breast Cancer exploration study: (doi: 10.1038/nature18003). Each row corresponds to a gene, and each column is a sample. The values within the table are normalized transcript counts.

Usage

sample_rnadata

Format

A data frame with 4317 rows and 76 variables:

TCGA-A2-A0CM RNA levels for each gene TCGA-A2-A0D2 RNA levels for each gene TCGA-A2-A0EQ RNA levels for each gene TCGA-A2-A0EV RNA levels for each gene TCGA-A2-A0EX RNA levels for each gene TCGA-A2-A0EY RNA levels for each gene TCGA-A2-A0SW RNA levels for each gene TCGA-A2-A0SX RNA levels for each gene TCGA-A2-A0T3 RNA levels for each gene TCGA-A2-A0T6 RNA levels for each gene TCGA-A2-A0YC RNA levels for each gene TCGA-A2-A0YD RNA levels for each gene TCGA-A2-A0YF RNA levels for each gene TCGA-A2-A0YG RNA levels for each gene TCGA-A2-A0YM RNA levels for each gene TCGA-A7-A0CE RNA levels for each gene TCGA-A7-A0CJ RNA levels for each gene TCGA-A7-A13F RNA levels for each gene TCGA-A8-A06N RNA levels for each gene TCGA-A8-A06Z RNA levels for each gene TCGA-A8-A076 RNA levels for each gene TCGA-A8-A079 RNA levels for each gene TCGA-A8-A08Z RNA levels for each gene TCGA-A8-A09G RNA levels for each gene TCGA-AN-A04A RNA levels for each gene TCGA-AN-A0AJ RNA levels for each gene TCGA-AN-A0AL RNA levels for each gene TCGA-AN-A0AM RNA levels for each gene TCGA-AN-A0FK RNA levels for each gene TCGA-AN-A0FL RNA levels for each gene TCGA-AO-A03O RNA levels for each gene TCGA-AO-A0J6 RNA levels for each gene TCGA-AO-A0J9 RNA levels for each gene TCGA-AO-A0JC RNA levels for each gene TCGA-AO-A0JE RNA levels for each gene

TCGA-AO-A0JJ RNA levels for each gene TCGA-AO-A0JL RNA levels for each gene TCGA-AO-A0JM RNA levels for each gene TCGA-AO-A126 RNA levels for each gene TCGA-AO-A12B RNA levels for each gene TCGA-AO-A12D RNA levels for each gene TCGA-AO-A12E RNA levels for each gene TCGA-AO-A12F RNA levels for each gene TCGA-AR-A0TR RNA levels for each gene **TCGA-AR-A0TT** RNA levels for each gene TCGA-AR-A0TV RNA levels for each gene TCGA-AR-A0TX RNA levels for each gene TCGA-AR-A0U4 RNA levels for each gene TCGA-AR-A1AP RNA levels for each gene TCGA-AR-A1AS RNA levels for each gene TCGA-AR-A1AV RNA levels for each gene TCGA-AR-A1AW RNA levels for each gene TCGA-BH-A0AV RNA levels for each gene TCGA-BH-A0BV RNA levels for each gene TCGA-BH-A0C1 RNA levels for each gene TCGA-BH-A0C7 RNA levels for each gene TCGA-BH-A0DD RNA levels for each gene TCGA-BH-A0DG RNA levels for each gene TCGA-BH-A0E1 RNA levels for each gene TCGA-BH-A0E9 RNA levels for each gene TCGA-BH-A18N RNA levels for each gene TCGA-BH-A18Q RNA levels for each gene TCGA-BH-A18U RNA levels for each gene TCGA-C8-A12L RNA levels for each gene TCGA-C8-A12T RNA levels for each gene TCGA-C8-A12U RNA levels for each gene TCGA-C8-A12V RNA levels for each gene TCGA-C8-A12Z RNA levels for each gene TCGA-C8-A130 RNA levels for each gene TCGA-C8-A131 RNA levels for each gene TCGA-C8-A134 RNA levels for each gene TCGA-C8-A135 RNA levels for each gene TCGA-C8-A138 RNA levels for each gene TCGA-D8-A142 RNA levels for each gene TCGA-E2-A154 RNA levels for each gene TCGA-E2-A158 RNA levels for each gene

Source

https://cptac-data-portal.georgetown.edu/cptac/s/S029

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