

Package ‘RNAseqQC’

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Title Quality Control for RNA-Seq Data

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filter_genes	<i>Filter genes with low counts</i>
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Description

Filter genes with low counts

Usage

```
filter_genes(dds, min_count = 5, min_rep = 3)
```

Arguments

dds	A DESeqDataSet
min_count, min_rep	keep genes with at least min_count counts in at least min_rep replicates

Value

A DESeq2::DESeqDataSet object with only those genes that meet the filter criteria.

Examples

```
library("DESeq2")
dds <- makeExampleDESeqDataSet()
filter_genes(dds)
```

make_dds	<i>Make DESeqDataSet from counts matrix and metadata</i>
----------	--

Description

Make DESeqDataSet from counts matrix and metadata

Usage

```
make_dds(counts, metadata, ah_record, design = ~1)
```

Arguments

counts	The genes x samples counts matrix. The row names must be ENSEMBL gene IDs.
metadata	data.frame of sample information. Order of rows corresponds to the order of columns in the counts matrix.
ah_record	ID of AnnotationHub record used to retrieve an EnsDb object.
design	The design formula specified in DESeqDataSet() To view all valid record IDs, run library(AnnotationHub) mcols(AnnotationHub()) %>% as_tibble(rownames="ah_record") %>% filter(rdataclass=="EnsDb")

Value

A DESeq2::DESeqDataSet object containing the counts matrix and metadata.

Examples

```
library("DESeq2")  
count_mat <- counts(T47D)  
meta <- data.frame(colData(T47D))  
dds <- make_dds(counts = count_mat, metadata = meta, ah_record = "AH89426")
```

mean_sd_plot	<i>Create a mean-sd plot Make a scatterplot that shows for each gene its standard deviation versus mean.</i>
--------------	--

Description

Create a mean-sd plot Make a scatterplot that shows for each gene its standard deviation versus mean.

Usage

```
mean_sd_plot(vsd)
```

Arguments

vsd A DESeqTransform object

Value

A ggplot object of the ggplot2 package that contains the mean-sd plot.

Examples

```
library("DESeq2")
dds <- makeExampleDESeqDataSet(interceptMean=10, n=5000)
vsd <- vst(dds)
mean_sd_plot(vsd)
```

plot_biotypes	<i>Plot number of counts per sample and biotype</i>
---------------	---

Description

Plot the total number of counts for each sample and the major classes of ENSEMBL gene biotypes (protein coding, lncRNA, etc.)

Usage

```
plot_biotypes(dds)
```

Arguments

dds A DESeqDataSet

Value

A ggplot object of the ggplot2 package.

Examples

```
plot_biotypes(T47D)
```

plot_chromosome	<i>Plot gene expression along a chromosome</i>
-----------------	--

Description

Plot gene expression along a chromosome

Usage

```
plot_chromosome(vsd, chr, scale = FALSE, trunc_val = NULL)
```

Arguments

vsd	An object generated by DESeq2::vst()
chr	A string denoting a chromosome as annotated by ENSEMBL, e.g. '1', '2', 'X', 'Y', 'MT'
scale	Whether to scale the columns of the heatmap
trunc_val	Truncate the expression matrix to this value prior to plotting. This is useful if some very high expression values dominate the heatmap. By default, the heatmap is truncated to expression values at most 3 standard deviations from the mean.

Value

A Heatmap-class object of the ComplexHeatmap package that contains the heatmap of expression values.

Examples

```
library("DESeq2")
chr1 <- T47D[which(mcols(T47D)$chromosome=="1"),]
vsd <- vst(chr1)
plot_chromosome(vsd, chr="1")
```

plot_gene

Plot a gene

Description

Plot a gene

Usage

```
plot_gene(
  gene,
  dds,
  x_var = NULL,
  color_by = NULL,
  point_alpha = 0.7,
  point_rel_size = 2,
  show_plot = TRUE
)
```

Arguments

gene	A gene ID or gene name, i.e. an element of <code>rownames(dds)</code> or of <code>rowData(dds)\$gene_name</code>
dds	a <code>DESeqDataSet</code>
x_var	Variable to plot on the x-axis. If <code>NULL</code> , then each sample is plotted separately.
color_by	Variable (column in <code>colData(dds)</code>) to color points by.
point_alpha	alpha value of <code>geom_point()</code>
point_rel_size	relative size of <code>geom_point()</code>
show_plot	Whether to show the plot or not

Value

The function displays the plot and returns invisible the data frame of expression values and `colData` annotation for the gene.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet()
colData(dds)$type <- c("A", "A", "A", "B", "B", "B")
colData(dds)$patient <- c("1", "1", "2", "2", "3", "3")
dds <- estimateSizeFactors(dds)
plot_gene("gene1", dds)
plot_gene("gene1", dds, x_var="patient", color_by="type")
```

plot_gene_detection *Plot number of detected genes for each sample*

Description

For specified thresholds, the number of detected genes is shown for each sample.

Usage

```
plot_gene_detection(dds, thresholds = c(3, 10, 20, 50))
```

Arguments

dds	A DESeqDataSet
thresholds	Vector of thresholds for which the number of genes with counts greater or equal than the thresholds is plotted

Value

A ggplot object of the ggplot2 package that contains the gene detection plot.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet()
plot_gene_detection(dds)
```

plot_library_complexity

Plot the library complexity

Description

Plot per sample the fraction of genes, versus the fraction of total counts.

Usage

```
plot_library_complexity(dds)
```

Arguments

dds	A DESeqDataSet
-----	----------------

Value

A ggplot object of the ggplot2 package that contains the library complexity plot.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet()
plot_library_complexity(dds)
```

plot_loadings	<i>Plot loadings of a principal component</i>
---------------	---

Description

Plot loadings of a principal component

Usage

```
plot_loadings(
  pca_res,
  PC = 1,
  color_by = NULL,
  annotate_top_n = 0,
  highlight_genes = NULL,
  show_plot = TRUE
)
```

Arguments

pca_res	A result returned from plot_pca()
PC	Number of the principal component to plot
color_by	Variable (column in pca_res\$loadings) to color points by.
annotate_top_n	Annotate the top n features with positive or negative loading
highlight_genes	Vector of gene names or gene IDs to highlight on the plot (overwrites top_n annotation)
show_plot	Whether to show the plot

Value

The function displays the loadings plot and returns invisible a list of the plot, the data.frame of the PCA loadings.

Examples

```
set.seed(1)
data <- matrix(rnorm(100*6), ncol=6)
data <- t(t(data)+c(-1, -1.1, -1.2, 1, 1.1, 1.2))
pca_res <- plot_pca(data)
plot_loadings(pca_res)
```

plot_ma	<i>MA-plot of a differential testing result</i>
---------	---

Description

MA-plot of a differential testing result

Usage

```
plot_ma(de_res, dds, annotate_top_n = 5, highlight_genes = NULL)
```

Arguments

de_res	An object returned by <code>DESeq2::results()</code> or <code>DESeq2::lfcShrink()</code>
dds	The <code>DESeqDataSet</code> that was used to build the <code>'de_res'</code> object. This is needed for gene name annotation.
annotate_top_n	Annotate the top n significant genes by fold change (up- and down-regulated)
highlight_genes	Vector of gene names or gene IDs to highlight on the plot (overwrites top_n annotation)

Value

A `ggplot` object of the `ggplot2` package that contains the MA-plot. The plot shows three classes of points: Light gray points are genes with low counts that are removed from the analysis by independent filtering. Darker gray points are not significant genes that show a density map to visualize where the majority of non-significant points are located. Finally, red point show significant genes.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet(n=1500, m=6, betaSD=.3, interceptMean=6)
rowData(dds)$gene_name <- rownames(dds)
dds <- DESeq(dds)
de_res <- results(dds)
plot_ma(de_res, dds)
```

plot_pca

Plot results of a principal component analysis

Description

Plot results of a principal component analysis

Usage

```
plot_pca(
  obj,
  PC_x = 1,
  PC_y = 2,
  n_feats = 500,
  scale_feats = FALSE,
  na_frac = 0.3,
  metadata = NULL,
  color_by = NULL,
  shape_by = NULL,
  point_alpha = 0.7,
  point_rel_size = 2,
  show_plot = TRUE
)
```

Arguments

obj	A (features x samples) matrix or SummarizedExperiment object
PC_x	The PC to show on the x-axis.
PC_y	The PC to show on the y-axis.
n_feats	Number of top-variable features to include.
scale_feats	Whether to scale the features.
na_frac	Only consider features with the stated maximum fraction of NAs or NaNs. NA/NaNs will be mean-imputed for PCA.
metadata	A data.frame used for annotating samples. rownames(metadata) must match colnames(obj).
color_by	Variable by which to color points. Must be a column in metadata or in colData(obj).
shape_by	Variable by which to color points. Must be a column in metadata or in colData(obj).
point_alpha	alpha value of geom_point()
point_rel_size	relative size of geom_point()
show_plot	Whether to show the plot or not

Details

If the metadata or colData of obj contain a column colname, this column will be removed in the \$pca_data slot, because this column contains the colnames of the data matrix. Similarly, for the \$loadings slot, the column rowname is reserved for the rownames of the data matrix.

Value

The function displays the plot and returns invisible a list of the plot, the data.frame to make the plot, the vector of percentages of variance explained and the loadings matrix.

Examples

```
set.seed(1)
data <- matrix(rnorm(100*6), ncol=6)
data <- t(t(data)+c(-1, -1.1, -1.2, 1, 1.1, 1.2))
plot_pca(data)
```

plot_pca_scatters	<i>Plot matrix of PCA scatter plots</i>
-------------------	---

Description

Plot matrix of PCA scatter plots

Usage

```
plot_pca_scatters(
  obj,
  n_PCs = min(10, nrow(obj), ncol(obj)),
  show_var_exp = T,
  n_feats = 500,
  scale_feats = FALSE,
  na_frac = 0.3,
  metadata = NULL,
  color_by = NULL,
  shape_by = NULL,
  point_alpha = 0.7,
  point_rel_size = 2
)
```

Arguments

obj	A (features x samples) matrix or SummarizedExperiment object
n_PCs	Number of principal components to plot
show_var_exp	Whether to show a plot of the percentage of variance explained by each PC in the bottom left corner.

n_feats	Number of top-variable features to include.
scale_feats	Whether to scale the features.
na_frac	Only consider features with the stated maximum fraction of NAs or NaNs. NA/NaNs will be mean-imputed for PCA.
metadata	A data.frame used for annotating samples. rownames(metadata) must match colnames(obj).
color_by	Variable by which to color points. Must be a column in metadata or in colData(obj).
shape_by	Variable by which to color points. Must be a column in metadata or in colData(obj).
point_alpha	alpha value of geom_point()
point_rel_size	relative size of geom_point()

Value

The function displays the scatter plots of the PCs

Examples

```
set.seed(1)
data <- matrix(rnorm(100*6), ncol=6)
data <- t(t(data)+c(-1, -1.1, -1.2, 1, 1.1, 1.2))
plot_pca_scatters(data)
```

plot_sample_clustering

Plot clustering of samples in a distance heatmap

Description

Plot clustering of samples in a distance heatmap

Usage

```
plot_sample_clustering(
  se,
  n_feats = 500,
  anno_vars = NULL,
  anno_title = "group",
  distance = "euclidean",
  ...
)
```

Arguments

se	A SummarizedExperiment object.
n_feats	Number of top-variable features (genes) to consider
anno_vars	Character vector of columns in colData(se) to annotate samples
anno_title	The title of the color legend for anno_vars
distance	The type of distance metric to consider. Either 'euclidean', 'pearson' or 'spearman'
...	Other arguments passed on to ComplexHeatmap::Heatmap()

Value

A Heatmap-class object of the ComplexHeatmap package that contains the heatmap of pairwise sample distances.

Examples

```
library("DESeq2")
dds <- makeExampleDESeqDataSet(m=8, interceptMean=10)
vsd <- vst(dds)
plot_sample_clustering(vsd)
```

plot_sample_MAs *MA plots of samples*

Description

For each level of the grouping variable, the gene-wise median over all samples is computed to obtain a reference sample. Then, each sample is plotted against the reference.

Usage

```
plot_sample_MAs(vsd, group, y_lim = 3)
```

Arguments

vsd	An object generated by DESeq2::vst()
group	A grouping variable, must be a column of colData(vsd)
y_lim	Y-axis limits, the axis will run from -y_lim to y_lim

Value

A list of ggplot objects of the ggplot2 package, with each element corresponding to one MA-plot.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet(n=1000, m=4, interceptMean=10)
colData(dds)$type <- c("A", "A", "B", "B")
vsd <- vst(dds)
plot_sample_MAs(vsd, group="type")
```

plot_total_counts	<i>Plot total counts per sample</i>
-------------------	-------------------------------------

Description

Plot the distribution of the total number of counts per sample as histogram.

Usage

```
plot_total_counts(dds, n_bins = 50)
```

Arguments

dds	A DESeqDataSet
n_bins	Number of histogram bins

Value

A ggplot object of the ggplot2 package that contains the histogram of total counts per sample.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet(m=30)
plot_total_counts(dds)
```

`plot_within_level_sample_MAs`*Plot correlations of samples within a level of a group*

Description

For the given level, the gene-wise median over all samples is computed to obtain a reference sample. Then, each sample is plotted against the reference as MA-plot.

Usage

```
plot_within_level_sample_MAs(vsd, group, level, y_lim = 4)
```

Arguments

<code>vsd</code>	An object generated by <code>DESeq2::vst()</code>
<code>group</code>	A grouping variable, must be a column of <code>colData(vsd)</code>
<code>level</code>	A level of the grouping variable
<code>y_lim</code>	Y-axis limits, the axis will run from <code>-y_lim</code> to <code>y_lim</code>

Value

A list of `ggplot` objects of the `ggplot2` package that contains for each sample of the specified level the the sample vs reference MA-plot.

Examples

```
library("DESeq2")
set.seed(1)
dds <- makeExampleDESeqDataSet(n=1000, m=4, interceptMean=10)
colData(dds)$type <- c("A", "A", "B", "B")
vsd <- vst(dds)
plot_within_level_sample_MAs(vsd, group="type", level="A")
```

`save_plots_to_pdf`*Save list of plots to PDF*

Description

This function takes a list of plots as input and makes a pdf with `ncol` x `nrow` plots per page.

Usage

```
save_plots_to_pdf(
  plots,
  file = "plots.pdf",
  ncol,
  nrow,
  subfig_width = subfig_height * 16/9,
  subfig_height = 2.5,
  legend_position = "original"
)
```

Arguments

plots	List of plots that is passed to the plotlist argument of cowplot::plot_grid
file	file where the plots are saved
ncol	number of columns per page for the grid of plots
nrow	number of rows per page for the grid of plots
subfig_width	width of a plot of the grid in inches
subfig_height	height of a plot of the grid in inches
legend_position	either 'original' if the original legend of each sub-plot is shown, 'none', if no legend should be shown in any of the sub-plots, 'bottom', if no legend should be shown in the sub plots and one shared legend at the bottom or 'right', which is same as 'bottom', but shown on the right

Value

The function returns nothing but is called for it's side effect, which is to save a pdf of plots to the filesystem.

Examples

```
library("ggplot2")
manuf <- unique(mpg$manufacturer)
plots <- lapply(manuf, function(x){
  df <- mpg[mpg$manufacturer==x,]
  ggplot(df, aes(cty, hwy)) +
    geom_point() +
    labs(title=x)
})
save_plots_to_pdf(plots, ncol=3, nrow=2)
```

T47D

The T47D cell line data of RNA-seq experiment GSE89888

Description

The dataset contains the read counts of experiment GSE89888 in which T47D cells with different mutation statuses were treated with E2 (estradiol) or vehicle.

Usage

T47D

Format

A DESeqDataSet with 43576 rows (of genes) and 24 columns (of samples).

Source

[doi:10.1101/2021.05.21.445138](https://doi.org/10.1101/2021.05.21.445138)

T47D_diff_testing

Differential expression results corresponding to the T47D data set.

Description

Differential expression results corresponding to the T47D data set.

Usage

T47D_diff_testing

Format

A DESeqResults object with 36562 rows and 3 columns.

Source

See the 'data' vignette on how to reproduce this object.

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