

# Package ‘RNAseqQC’

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**Title** Quality Control for RNA-Seq Data

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**Author** Frederik Ziebell [aut, cre] (<<https://orcid.org/0000-0003-3673-1721>>), GlaxoSmithKline Research & Development Limited [cph] (GlaxoSmithKline Research & Development Limited; registered address: 980 Great West Road, Brentford, Middlesex TW8 9GS, United Kingdom)

**Maintainer** Frederik Ziebell <[frederik.x.ziebell@gsk.com](mailto:frederik.x.ziebell@gsk.com)>

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

---

## 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  <pre>library(AnnotationHub) mcols(AnnotationHub()) %&gt;%   as_tibble(rownames="ah_record") %&gt;%   filter(rdataclass=="EnsDb")</pre>

## 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 DESeq2::results() or DESeq2::lfcShrink()
dds	The DESeqDataSet that was used to build the 'de_res' 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**

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