Package 'ScreenR'

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Type Package

Title Package to Perform High Throughput Biological Screening

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Description ScreenR is a package suitable to perform hit identification in loss of function High Throughput Biological Screenings performed using barcoded shRNA-based libraries. ScreenR combines the computing power of software such as edgeR with the simplicity of use of the Tidyverse metapackage. ScreenR executes a pipeline able to find candidate hits from barcode counts, and integrates a wide range of visualization modes for each step of the analysis.

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Contents

`ScreenR-package` *Tools for analyzing shRNAs screening data*

Description

ScreenR is an easy and effective package to perform hits identification in loss of function High Throughput Biological Screening performed with shRNAs library. ScreenR combines the power of software like edgeR with the simplicity of the Tidyverse metapackage. ScreenR executes a pipeline able to find candidate hits from barcode counts data and integrates a wide range of visualization for each step of the analysis.

Details

ScreenR takes the a count table as input and create the screenr_object to perform the analysis. Throught the pipeline **ScreenR** enable the user to perform quality control, visual inspection, dimensionality reduction of the data. Using three statistical methods:

- ROAST
- CAMERA
- Z-score

it is able to find new candidate hits. Moreover in order to improve the quality of the hit found it is also possible to further filter the list of hit using other filter like the variance and the slope filters.

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annotation_table *Table for the annotation of Barcode*

Description

Table for the annotation of Barcode

Usage

```
data(annotation_table)
```

Format

A data frame with 5320 rows and 2 columns obtained from a loss-of-function genetic screening. This table is used to store information about the shRNAs:

Gene It Contains the gene name

Barcode It contains an ID that identify each barcode (it is an unique identifier for an shRNA). I t can be use to merge the annotation table with t he count table

Gene_ID It Contains a unique Gene ID

Sequence It contains the cDNA sequence of the shRNA associated to the barcode

Library It contains the library from which the shRNA come from. In this case is a pooled from https://cellecta.com/cellecta

barcode_lost *Count number of barcode lost*

Description

This function counts the number of barcodes lost during the sequencing. A barcode is lost if its associated shRNA has zero mapped read in a sample.

Usage

```
barcode_lost(screenR_Object)
```

Arguments

`screenR_Object` The ScreenR object obtained using the `create_screenr_object`

Value

Return a tibble containing the number of barcode lost for each sample

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

# In order to count the number of barcodes lost just the ScreenR object is
# needed
head(barcode_lost(object))
```

compute_camera *Compute Camera*

Description

This internal function computes the actual hits using the camera method.

Usage

```
compute_camera(
  xglm,
  lrt,
  DGEList,
  matrix_model,
  contrast,
  number_barcode = 3,
  thresh = 1e-04,
  lfc = 1
)
```

Arguments

xglm	object created with estimateDisp
lrt	object created with glmFit
DGEList	edgeR object
...	Arguments passed on to find_camera_hit
	matrix_model The matrix that will be used to perform the linear model analysis created using model.matrix
	thresh The threshold for the False Discovery Rate (FDR) that has to be used to select the statistically significant hits.
	lfc The Log2FC threshold.
	number_barcode Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.

Value

The list of hits found by the camera method

compute_data_table *Compute data Table*

Description

This function computes the data table that will be used for the analysis. The data_table is a tidy and normalized version of the original count_table and will be used throughout the analysis.

Usage

```
compute_data_table(screenR_Object)
```

Arguments

`screenR_Object` The ScreenR object obtained using the `create_screenr_object`

Value

ScreenR_Object with the data_table filed containing the table.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
object <- compute_data_table(object)
head(slot(object, "data_table"))
```

compute_explained_variance *Compute explained variance*

Description

This is an internal function used to compute the explained variance by each of the Principal Components.

Usage

```
compute_explained_variance(screenR_Object)
```

Arguments

`screenR_Object` The Object of the package

Value

A data.frame containing all the information of the variance expressed by the components

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

compute_explained_variance(object)
```

compute_metrics *Compute Metrics*

Description

This function computes the metrics that will be then used to compute the z-score using the function find_zscore_hit starting from the screenr object for a given treatment in a given day. More information about the z-score and other metrics used in genetic screening can be found at this paper z-score

Usage

```
compute_metrics(screenR_Object, control, treatment, day)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
control	A string specifying the sample that as to be used as control in the analysis. This string has to be equal to the interested sample in the Treatment column of the data_table slot
treatment	A string specifying the sample that as to be used as treatment in the analysis. This string has to be equal to the interested sample in the Treatment column of the data_table slot.
day	A string containing the day (time point) to consider in the metrics computation. This string has to be equal to the interested sample in the Day column of the data_table slot.

Value

Return a tibble with all the measure computed.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
metrics <- compute_metrics(object,
    control = "TRT",
    treatment = "Time3", day = "Time3"
)
head(metrics)
```

compute_slope *Compute Slope of a Gene*

Description

This function is used to compute the slope of the gene passed as input

Usage

```
compute_slope(screenR_Object, genes, group_var)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
genes	The genes for which the slope as to be computed. Those genes are the result of the three statistical methods selection
group_var	The variable to use as independent variable (x) for the linear model

Value

A tibble containing in each row the gene and the corresponding Slope

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

compute_slope(object,
    genes = c("Gene_42", "Gene_24"),
    group_var = c("T1", "T2", "TRT")
)
```

---

compute_trend *Compute trend*

Description

This is an internal function used to computes the trend of a gene

Usage

```
compute_trend(screenR_Object, genes, group_var)
```

Arguments

screenR_Object	object created with estimateDisp
genes	a list of genes
group_var	the variable that as to be used as grouping variable

Value

A table with the trend of the genes passed as input

`count_mapped_reads` *Count the number of mapped read*

Description

This function counts the number of reads for each barcode in each sample. It is a quality control function (QC) to see if the biological protocol went as planned. If a sample has very low mapped compared to the other means that is has a lower quality.

Usage

```
count_mapped_reads(screenR_Object)
```

Arguments

`screenR_Object` The ScreenR object obtained using the `create_screenr_object`

Value

Return a tibble containing the number of mapped read for sample

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
head(count_mapped_reads(object))
```

`count_table` *Table of the count table*

Description

Table of the count table

Usage

```
data(count_table)
```

Format

A data frame with 5323 rows and 15 variables obtained from barcode alignment to the reference library. It is generated from a [Cellecta](https://cellecta.com/) protocol. The samples generated are then sequenced using an RNA-seq protocol. Due to the fact that different shRNAs are sequenced for a gene each barcode has its associated reads. This reads were aligned to the reference library using [bowtie2](http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) and then sorted with [samtools](https://github.com/samtools/samtools). Since this dataset comes from a Chemical Synthetic Lethality experiments the samples treated and combined with the shRNAs knockdown should present a decreased number of reads compared to the controls.

Barcode It contains an ID that identify each barcode. It can be use to marge the annotation table with the count table. A Barcode is a unique identifier of an shRNA. In a genetic screening multiple slightly different shRNAs perform a knockout of a gene each with its efficacy. For this reason it is important to keep track of each shRNA using a unique barcode.

Time_1 It contains the counts at time zero. This is the first time point at which cells are not treated and not infected.

Time_2 It contains the counts after the cell were washed. At this time point the cells are infected and following the Cellecta protocol are washed with the puromycin.

Time_3_TRT_rep1 It contains the counts for the first replicate of the treated at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_3_TRT_rep2 It contains the counts for the second replicate of the treated at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_3_TRT_rep3 It contains the counts for the third replicate of the treated at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_3_rep1 It contains the counts for the first replicate of the control at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_3_rep2 It contains the counts for the second replicate of the control at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_3_rep3 It contains the counts for the third replicate of the control at the first time point. Usually the first time point is 7 day after the puromycin wash.

Time_4_TRT_rep1 It contains the counts for the first replicate of the treated at the second time point. Usually the first time point is 14 day after the puromycin wash.

Time_4_TRT_rep2 It contains the counts for the second replicate of the treated at the second time point. Usually the first time point is 14 day after the puromycin wash.

Time_4_TRT_rep3 It contains the counts for the third replicate of the treated at the second time point. Usually the first time point is 14 day after the puromycin wash.

Time_4_rep1 It contains the counts for the first replicate of the control at the second time point. Usually the first time point is 14 day after the puromycin wash.

Time_4_rep2 It contains the counts for the second replicate of the control at the second time point. Usually the first time point is 14 day after the puromycin wash.

Time_4_rep3 It contains the counts for the third replicate of the control at the second time point. Usually the first time point is 14 day after the puromycin wash.

`create_edger_obj` *Create edgeR Object*

Description

Utility function that using the screenr-class object create the corresponding edgeR object. This function and other utility function enables the user to not worry abut the implementation and just focus on the analysis. The ScreenR package will take care of the rest.

Usage

```
create_edger_obj(screenR_Object)
```

Arguments

screenR_Object The ScreenR object obtained using the create_screenr_object

Value

The edgeR object will all the needed information for the analysis.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
create_edger_obj(object)
```

---

create_screenr_object *Create the ScreenR Object*

Description

Initial function to create the Screen Object.

Usage

```
create_screenr_object(
  table = NULL,
  annotation = NULL,
  groups = NULL,
  replicates = c("")
)
```

Arguments

table	The count table obtained from the read alignment that contains the Barcodes as rows and samples as columns.
annotation	The annotation table containing the information for each Barcode and the association to the corresponding Gene
groups	A factor containing the experimental design label
replicates	A vector containing the replicates label

Value

An object containing all the needed information for the analysis.

Examples

```
count_table <-
    data.frame(
        Barcode = c("Code_1", "Code_2", "Code_3", "Code_3"),
        Time_3_rep1 = c("3520", "3020", "1507", "1400"),
        Time_3_rep2 = c("3500", "3000", "1457", "1490"),
        Time_3_TRT_rep1 = c("1200", "1100", "1300", "1350"),
        Time_3_TRT_rep2 = c("1250", "1000", "1400", "1375")
    )
```

```
annotation_table <-
    data.frame(
        Gene = c("Gene_1", "Gene_1", "Code_2", "Code_2"),
        Barcode = c("Code_1", "Code_2", "Code_3", "Code_3"),
        Gene_ID = rep(NA, 4), Sequence = rep(NA, 4),
        Library = rep(NA, 4)
    )

groups <- factor(c("Control", "Control", "Treated", "Treated"))
obj <- create_screenr_object(
    table = count_table,
    annotation = annotation_table,
    groups = groups, replicates = c("")
)
obj
```

`filter_by_slope` *Filter using the slope filter*

Description

This function is used to improve the quality of the hits found. It computes a regression line in the different samples ad uses the slope of this line to see the trend

Usage

```
filter_by_slope(
  screenR_Object,
  genes,
  group_var_treatment,
  group_var_control,
  slope_control,
  slope_treatment
)
```

Arguments

`screenR_Object`	The ScreenR object obtained using the `create_screenr_object`
`genes`	The genes for which the slope as to be computed. Those genes are the result of the three statistical methods selection
`group_var_treatment`	The variable to use as independent variable (x) for the linear model of the treatment
`group_var_control`	The variable to use as independent variable (x) for the linear model of the the control
`slope_control`	A value used as threshold for the control slope
`slope_treatment`	A value used as threshold for the treatment slope

Value

A data frame with the slope for the treatment and the control for each gene

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

filter_by_slope(
    screenR_Object = object, genes = c("Gene_1", "Gene_2"),
    group_var_treatment = c("T1", "T2", "TRT"),
    group_var_control = c("T1", "T2", "Time3", "Time4"),
    slope_control = 0.5, slope_treatment = 1
)
```

`filter_by_variance` *Filter using the variance filter*

Description

This function is used to improve the quality of the hits. It compute the variance among the hits and filter the one with a value greater than the threshold set

Usage

```
filter_by_variance(
  screenR_Object,
  genes,
  matrix_model,
  variance = 0.5,
  contrast
)
```

Arguments

`screenR_Object`	The ScreenR object obtained using the `create_screenr_object`
`genes`	The genes for which the variance as to be computed. Those genes are the result of the three statistical methods selection
`matrix_model`	a matrix created using `model.matrix`
`variance`	The maximum value of variance accepted
`contrast`	The variable to use as X for the linear model for the Treatment

Value

A data frame with the variance for the treatment and the control for each gene

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
matrix_model <- model.matrix(~ slot(object, "groups"))
colnames(matrix_model) <- c("Control", "T1_T2", "Treated")
contrast <- limma::makeContrasts(Treated - Control, levels = matrix_model)

data <- filter_by_variance(
    screenR_Object = object, genes = c("Gene_42"),
```

```
    matrix_model = matrix_model, contrast = contrast
)
head(data)
```

`find_camera_hit` *Find Camera Hit*

Description

This function implements the method by proposed by Wu and Smyth (2012). The original `camera` method is a gene set test, here is applied in the contest of a genetic screening and so it erforms a competitive barcode set test. The paper can be found here CAMERA

Usage

```
find_camera_hit(
  screenR_Object,
  matrix_model,
  contrast,
  number_barcode = 3,
  thresh = 1e-04,
  lfc = 1,
  direction = "Down"
)
```

Arguments

`screenR_Object`	The ScreenR object obtained using the `create_screenr_object`
`matrix_model`	The matrix that will be used to perform the linear model analysis created using `model.matrix`
`contrast`	A vector or a single value indicating the index or the name of the column the model_matrix with which perform the analysis
`number_barcode`	Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.
`thresh`	The threshold for the False Discovery Rate (FDR) that has to be used to select the statistically significant hits.
`lfc`	The Log2FC threshold.
`direction`	String containing the direction of the variation, "Down" for the down regulation "Up" for the up regulation.

Value

The data frame containing the hit found using the camera method

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

matrix <- model.matrix(~ slot(object, "groups"))
colnames(matrix) <- c("Control", "T1/T2", "Treated")

result <- find_camera_hit(
    screenR_Object = object,
    matrix_model = matrix, contrast = "Treated"
)
head(result)
```

`find_common_hit` *Find common hit*

Description

This method find the hit in common between the three methods

Usage

```
find_common_hit(hit_zscore, hit_camera, hit_roast, common_in = 3)
```

Arguments

`hit_zscore`	The matrix obtained by the `find_zscore_hit` method
`hit_camera`	The matrix obtained by the `find_camera_hit` method
`hit_roast`	The matrix obtained by the `find_roast_hit` method
`common_in`	Number of methods in which the hit has to be in common in order to be considered a candidate hit. The default value is 3, which means that has to be present in the result of all the three methods.

Value

A vector containing the common hit

Examples

```
hit_zscore <- data.frame(Gene = c("A", "B", "C", "D", "E"))
hit_camera <- data.frame(Gene = c("A", "B", "C", "F", "H", "G"))
hit_roast <- data.frame(Gene = c("A", "L", "N"))

# common among all the three methods
find_common_hit(hit_zscore, hit_camera, hit_roast)

# common among at least two of the three methods
find_common_hit(hit_zscore, hit_camera, hit_roast, common_in = 2)
```

`find_roast_hit` *Find Roast Hit*

Description

Find the hit using the roast method. Roast is a competitive gene set test which uses rotation instead of permutation. Here is applied in a contest of a genetic screening so it perform a barcode competitive test testing for barcode which are differentially expressed within a gene. More information can be found in Roast

Usage

```
find_roast_hit(
  screenR_Object,
  matrix_model,
  contrast,
  nrot = 9999,
  number_barcode = 3,
  direction = "Down",
  p_val = 0.05
)
```

Arguments

`screenR_Object`	The ScreenR object obtained using the `create_screenr_object`
`matrix_model`	The matrix that will be used to perform the linear model analysis. Created using `model.matrix`
`contrast`	A vector or a single value indicating the index or the name of the column the model_matrix to which perform the analysis
`nrot`	Number of rotation to perform the test. Higher number of rotation leads to more statistically significant result.
`number_barcode`	Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.
`direction`	Direction of variation
`p_val`	The value that as to be used as p-value cut off

Value

The hits found by ROAST method

Examples

```
set.seed(42)
object <- get0("object", envir = asNamespace("ScreenR"))
matrix_model <- model.matrix(~ slot(object, "groups"))
colnames(matrix_model) <- c("Control", "T1_T2", "Treated")

result <- find_roast_hit(object,
    matrix_model = matrix_model,
```

```
    contrast = "Treated", nrot = 100
)
head(result)
```

`find_robust_zscore_hit` *Title Find robust Z-score Hit*

Description

Title Find robust Z-score Hit

Usage

```
find_robust_zscore_hit(table_treate_vs_control, number_barcode)
```

Arguments

`table_treate_vs_control`	A table computed with the function `compute_data_table`. It contain for each barcode the associated Gene the counts in the treated and control and the value for the Log2FC, Zscore, ZscoreRobust in each day.
`number_barcode`	Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.

Value

Return a tibble containing the hit for the robust Z-score

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
table <- compute_metrics(object,
    control = "TRT", treatment = "Time3",
    day = "Time3"
)
result <- find_robust_zscore_hit(table, number_barcode = 6)
head(result)
```

`find_zscore_hit` *Title Find Z-score Hit*

Description

Title Find Z-score Hit

Usage

```
find_zscore_hit(table_treate_vs_control, number_barcode = 6, metric = "median")
```

Arguments

table_treate_vs_control	table computed with the function compute_data_table
number_barcode	Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.
metric	A string containing the metric to use. The value allowed are "median" or "mean".

Value

Return a tibble containing the hit for the Z-score

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
table <- compute_metrics(object,
    control = "TRT", treatment = "Time3",
    day = "Time3"
)

# For the the median
result <- find_zscore_hit(table, number_barcode = 6)
head(result)

# For the mean
result <- find_zscore_hit(table, number_barcode = 6, metric = "mean")
head(result)
```

---

get_annotation_table *Get ScreenR annotation table*

Description

Get function for the annotation table of the ScreenR object

Usage

```
get_annotation_table(object)

## S4 method for signature 'screenr_object'
get_annotation_table(object)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object

Value

The annotation table of the ScreenR object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
annotation_table <- get_annotation_table(object)
head(annotation_table)
```

`get_count_table` *Get ScreenR count table*

Description

Get function for the count table of the ScreenR object

Usage

```
get_count_table(object)

## S4 method for signature 'screenr_object'
get_count_table(object)
```

Arguments

`object`	The ScreenR object obtained using the `create_screenr_object`

Value

The count table of the ScreenR object

Slots

`count_table` It is used to store the count table to perform the analysis

`annotation_table` It is used to store the annotation of the shRNA

`groups` It is used to store the vector of treated and untreated

`replicates` It is used to store information about the replicates

`normalized_count_table` It is used to store a normalized version of the count table

`data_table` It is used to store a tidy format of the count table

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
count_table <- get_count_table(object)
head(count_table)
data("count_table", package = "ScreenR")
data("annotation_table", package = "ScreenR")

groups <- factor(c(
    "T1/T2", "T1/T2", "Treated", "Treated", "Treated",
    "Control", "Control", "Control", "Treated", "Treated",
    "Treated", "Control", "Control", "Control"
))

obj <- create_screenr_object(
```

```
    table = count_table,
    annotation = annotation_table,
    groups = groups,
    replicates = c("")
)
```

`get_data_table` *Get ScreenR data_table*

Description

Get function for the data_table of the ScreenR object

Usage

```
get_data_table(object)

## S4 method for signature 'screenr_object'
get_data_table(object)
```

Arguments

`object`	The ScreenR object obtained using the `create_screenr_object`

Value

The data_table of the ScreenR object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
data_table <- get_data_table(object)
data_table
```

`get_groups` *Get ScreenR groups*

Description

Get function for the groups of the ScreenR object

Usage

```
get_groups(object)

## S4 method for signature 'screenr_object'
get_groups(object)
```

Arguments

`object`	The ScreenR object obtained using the `create_screenr_object`

Value

The groups of the ScreenR object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
groups <- get_groups(object)
groups
```

---

get_normalized_count_table *Get ScreenR normalized_count_table*

Description

Get function for the normalized_count_table of the ScreenR object

Usage

```
get_normalized_count_table(object)

## S4 method for signature 'screenr_object'
get_normalized_count_table(object)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object

Value

The normalized_count_table of the ScreenR object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
normalized_count_table <- get_normalized_count_table(object)
normalized_count_table
```

---

get_replicates *Get ScreenR replicates*

Description

Get function for the replicates of the ScreenR object

Usage

```
get_replicates(object)

## S4 method for signature 'screenr_object'
get_replicates(object)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object

Value

The replicates of the ScreenR object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
replicates <- get_replicates(object)
replicates
```

---

mapped_reads *Mapped Reads*

Description

This function returns the number of mapped reads inside the ScreenR object

Usage

```
mapped_reads(screenR_Object)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object

Value

Return a tibble containing the number of mapped read for sample

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
mapped_reads(object)
```

---

normalize_data *Normalize data*

Description

This function perform a normalization on the data considering the fact that each shRNA has a defined length so this will not influence the data. Basically is computed the sum for each row and then multiply by 1e6. At the end the data obtained will be Count Per Million.

Usage

```
normalize_data(screenR_Object)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object

Value

Return the ScreenR object with the normalize data

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
object <- normalize_data(object)

slot(object, "normalized_count_table")
```

---

plot_barcode_hit *Plot barcode hit*

Description

Create a barcode plot for a hit. A barcode plot displays if the hit is differentially up or down regulated. If most of the vertical line are on the left side the gene associated to the barcodes is down regulated otherwise is up regulated.

Usage

```
plot_barcode_hit(
  screenR_Object,
  matrix_model,
  contrast,
  number_barcode = 3,
  gene,
  quantile = c(-0.5, 0.5),
  labels = c("Negative logFC", "Positive logFC")
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
matrix_model	The matrix that will be used to perform the linear model analysis. It is created using model.matrix.
contrast	An object created with makeContrasts function.
number_barcode	Number of barcode that as to be differentially expressed (DE) in order to consider the associated gene DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.
gene	The name of the gene that has to be plot
quantile	Quantile to display on the plot
labels	The label to be displayed on the quantile side

Value

The barcode plot

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
matrix_model <- model.matrix(~ slot(object, "groups"))
colnames(matrix_model) <- c("Control", "T1_T2", "Treated")
contrast <- limma::makeContrasts(Treated - Control, levels = matrix_model)

plot_barcode_hit(object, matrix_model,
    contrast = contrast,
    gene = "Gene_300"
)
```

---

plot_barcode_lost *Plot number of barcode lost*

Description

This function plots the number of barcode lost in each sample. Usually lots of barcodes lost mean that the sample has low quality.

Usage

```
plot_barcode_lost(
  screenR_Object,
  palette = NULL,
  alpha = 1,
  legende_position = "none"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
palette	A vector of colors to be used to fill the barplot.
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
legende_position	Where to positioning the legend of the plot. Allowed values are in the "top", "bottom", "right", "left", "none".

Value

Returns the plot displaying the number of barcode lost in each sample

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_barcode_lost(object)
```

plot_barcode_lost_for_gene

Plot number of barcode lost for gene

Description

This function plots the number of barcodes lost in each sample for each gene. Usually in a genetic screening each gene is is associated with multiple shRNAs and so barcodes. For this reason a reasonable number of barcodes associated with the gene has to be retrieved in order to have a robust result. Visualizing the number of genes that have lost lot's of barcode is a Quality Check procedure in order to be aware of the number of barcode for the hit identified.

Usage

```
plot_barcode_lost_for_gene(screenR_Object, facet = TRUE, samples)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
facet	A boolean to use the facet.
samples	A vector of samples that as to be visualize

Value

Return the plot displaying the number of barcode lost for each gene in each sample.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_barcode_lost_for_gene(object,
    samples = c("Time3_A", "Time3_B")
)
plot_barcode_lost_for_gene(object,
    samples = c("Time3_A", "Time3_B"),
    facet = FALSE
)
```

plot_barcode_trend

Plot the trend over time of the barcodes

Description

Plot the log2FC over time of the barcodes in the different time point. This plot is useful to check we efficacy of each shRNA. Good shRNAs should have consistent trend trend over time.

Usage

```
plot_barcode_trend(
  list_data_measure,
  genes,
  n_col = 1,
  size_line = 1,
  color = NULL
)
```

Arguments

list_data_measure	A list containing the measure table of the different time point. Generated using the compute_metrics function.
genes	The vector of genes name.
n_col	The number of column to use in the facet wrap.
size_line	The thickness of the line.
color	The vector of colors. One color for each barcode.

Value

The trend plot for the genes in input.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

metrics <- dplyr::bind_rows(
    compute_metrics(object,
        control = "TRT", treatment = "Time3",
        day = "Time3"
    ),
    compute_metrics(object,
        control = "TRT", treatment = "Time4",
        day = "Time4"
    )
)
# Multiple Genes
plot_barcode_trend(metrics,
    genes = c("Gene_1", "Gene_50"),
    n_col = 2
)
# Single Gene
plot_barcode_trend(metrics, genes = "Gene_300")
```

---

plot_boxplot *Plot Barcodes Hit*

Description

This function plots a boxplot for each sample for the genes passed as input. It can be used to see the overall trend of a gene and so to visualize if the gene is up or down regulated.

Usage

```
plot_boxplot(
  screenR_Object,
  genes,
  group_var,
  alpha = 0.5,
  nrow = 1,
  ncol = 1,
  fill_var = "Sample",
  type = "boxplot",
  scales = "free"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
genes	The vector of genes that will be displayed
group_var	The variable that as to be used to filter the data, for example the different treatment
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
nrow	The number of rows in case multiple genes are plotted
ncol	The number of columns in case multiple genes are plotted
fill_var	The variable used to fill the boxplot
type	The type of plot to use "boxplot" or "violinplot"
scales	The scales used for the facet. Possible values can be "free", "fixed" and "free_y"

Value

A boxplot

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_boxplot(object,
    genes = c("Gene_34"),
    group_var = c("T1", "T2", "TRT"), nrow = 1, ncol = 2,
    fill_var = "Day", type = "violinplot"
)
```

---

plot_common_hit *Plot common hit*

Description

This method plot the hits in common among the three methods is a wrapper for the ggvenn function.

Usage

```
plot_common_hit(
  hit_zscore,
  hit_camera,
  hit_roast,
  alpha = 0.5,
  stroke_size = 0.5,
  set_name_size = 4,
  text_color = "black",
  text_size = 4,
  show_percentage = TRUE,
  title = "",
  color = c("#1B9E77", "#D95F02", "#7570B3"),
  show_elements = TRUE
)
```

Arguments

hit_zscore	The list of hits of the find_zscore_hit
hit_camera	The list of hits of the find_camera_hit
hit_roast	The list of hits of the find_roast_hit
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
stroke_size	Stroke size for drawing circles
set_name_size	Text size for set names
text_color	Text color for intersect contents
text_size	Text size for intersect contents
show_percentage	Show percentage for each set
title	The title to display above the plot
color	The three vector color for the venn
show_elements	Show set elements instead of count/percentage.

Value

A vector containing the common hit

Examples

```
hit_zscore <- data.frame(Gene = c("A", "B", "C", "D", "E"))
hit_camera <- data.frame(Gene = c("A", "B", "C", "F", "H", "G"))
hit_roast <- data.frame(Gene = c("A", "L", "N"))
plot_common_hit(hit_zscore, hit_camera, hit_roast)
```

plot_explained_variance *Plot the explained variance by the PC*

Description

This function plot the explained variance by the Principal Component analysis.

Usage

```
plot_explained_variance(
  screenR_Object,
  cumulative = FALSE,
  color = "steelblue"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
cumulative	A boolean value which indicates whether or not to plot the cumulative variance. The default value is FALSE.
color	The color to fill the barplot the default value is steelblue

Value

The explained variance plot

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_explained_variance(object)

# For the cumulative plot
plot_explained_variance(object, cumulative = TRUE)
```

plot_mapped_reads *Plot mapped reads*

Description

This function plots the number of reads mapped for each sample. It internally call the count_mapped_reads function, to compute the number of mapped reads.

Usage

```
plot_mapped_reads(
  screenR_Object,
  palette = NULL,
  alpha = 1,
  legende_position = "none"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
palette	A vector of color that as to be used to fill the barplot.
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
legende_position	Where to positioning the legend of the plot ("none", "left", "right", "bottom", "top")

Value

return a ggplot object

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_mapped_reads(object)
```

---

plot_mapped_reads_distribution

Plot the distribution of the mapped reads

Description

This function creates a boxplot or a densityplot to show the distribution of the mapped reads in different samples. This function can be used to assess the quality of the samples. Samples which show roughly the same distribution have good quality.

Usage

```
plot_mapped_reads_distribution(
  screenR_Object,
  palette = NULL,
  alpha = 1,
  type = "boxplot"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object.
palette	The color vector that as to be used for the plot.
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
type	The type of plot. The default is "boxplot" the other option is "density."

Value

Return a tibble containing the number of mapped read for each sample

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

# Boxplot
plot_mapped_reads_distribution(object)

# Density
plot_mapped_reads_distribution(object, type = "density")

plot_mapped_reads_distribution(object, type = "density", alpha = 0.2)
```

`plot_mds` *Multidimensional Scaling Plot*

Description

Plot samples on a two-dimensional scatterplot so that distances on the plot approximate the typical log2 fold changes between the samples.

Usage

```
plot_mds(
  screenR_Object,
  groups = NULL,
  alpha = 0.8,
  size = 2.5,
  color = "black"
)
```

Arguments

`screenR_Object`	The Object of the package `create_screenr_object`
`groups`	The vector that has to be used to fill the plot if NULL the function will use the default groups slot in the object passed as input.
`alpha`	The opacity of the labels. Possible value are in a range from 0 to 1.
`size`	The dimension of the labels. The default value is 2.5
`color`	The color of the labels. The default value is black

Value

The MDS Plot

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_mds(object)
```

plot_trend *Plot the trend hit gene*

Description

This function plot the trend of a gene resulted as hit

Usage

```
plot_trend(
  screenR_Object,
  genes,
  group_var,
  alpha = 0.5,
  se = FALSE,
  point_size = 1,
  line_size = 1,
  nrow = 1,
  ncol = 1,
  scales = "free"
)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object
genes	The vector of genes to use
group_var	The variable that as to be used to filter the data, for example the different treatment
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1
se	A boolean to indicate where or not to plot the standard error
point_size	The dimension of each dot
line_size	The dimension of the line
nrow	The number of rows in case multiple genes are plotted
ncol	The number of columns in case multiple genes are plotted
scales	The scales to be used in the facette

Value

The plot of the trend over time for a specific treatment.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

plot_trend(object, genes = "Gene_42", group_var = c("T1", "T2", "TRT"))

plot_trend(object,
    genes = c("Gene_42", "Gene_100"),
    group_var = c("T1", "T2", "TRT"),
    nrow = 2
)
```

plot_zscore_distribution *Plot distribution Z-score*

Description

This function plots the Log2FC Z-score distribution of the treated vs control in the different time points.

Usage

```
plot_zscore_distribution(time_point_measure, alpha = 1)
```

Arguments

time_point_measure	A list containing the table for each time point. Each table contains for each barcode the counts for the treated and control the Log2FC, Zscore, ZscoreRobust, Day.
alpha	A value for the opacity of the plot. Allowed values are in the range 0 to 1

Value

return the density plot of the distribution of the Z-score

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))

table1 <- compute_metrics(object,
    control = "TRT", treatment = "Time3",
    day = "Time3"
)

table2 <- compute_metrics(object,
    control = "TRT", treatment = "Time4",
    day = "Time4"
)

plot_zscore_distribution(list(table1, table2), alpha = 0.5)
```

remove_all_zero_row *Remove rows that have zero count in all samples*

Description

This function removes the rows that have zero count in all samples. It takes care of updating both count_table and annotation_table. Very_Important: It has to be performed before the data normalization.

Usage

```
remove_all_zero_row(screenR_Object)
```

Arguments

screenR_Object	The ScreenR object obtained using the create_screenr_object

Value

The ScreenR object with the count_table and the annotation_table filtered.

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
counts <- get_count_table(object)
nrow(counts)
object <- remove_all_zero_row(object)
counts <- get_count_table(object)
nrow(counts)
```

---

select_number_barcode *Select number of Barcode*

Description

Compute a unique gene symbol for gene

Usage

```
select_number_barcode(gene, gene_symbols, number_barcode)
```

Arguments

gene	The gene name
...	Arguments passed on to unique_gene_symbols
	gene_symbols The gene symbols list

Value

The barcode of the gene passed as input

set_annotation_table *Set ScreenR annotation table*

Description

Set function for the annotation table of the ScreenR object

Usage

```
set_annotation_table(object, annotation_table)

## S4 method for signature 'screenr_object'
set_annotation_table(object, annotation_table)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object
annotation_table	a table containing the annotation for each shRNA

Value

The ScreenR object with the annotation table

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
annotation <- get_annotation_table(object)
set_annotation_table(object, annotation)
```

set_count_table *Set ScreenR count table*

Description

Set function for the count table of the ScreenR object

Usage

```
set_count_table(object, count_table)

## S4 method for signature 'screenr_object'
set_count_table(object, count_table)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object
count_table	A count table containing in each row an shRNA and in each column a sample

Value

The ScreenR object with the count table

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
counts <- get_count_table(object)
set_count_table(object, counts)
```

`set_data_table` *Set ScreenR data_table*

Description

Set function for the data_table of the ScreenR object

Usage

```
set_data_table(object, data_table)

## S4 method for signature 'screenr_object'
set_data_table(object, data_table)
```

Arguments

`object`	The ScreenR object obtained using the `create_screenr_object`
`data_table`	A count table in a tidy format

Value

The ScreenR object with the set data_table

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
data_table <- get_data_table(object)
set_data_table(object, data_table)
```

`set_groups` *Set ScreenR groups*

Description

Set function for the groups of the ScreenR object

Usage

```
set_groups(object, groups)

## S4 method for signature 'screenr_object'
set_groups(object, groups)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object
groups	The treatment and control groups

Value

The ScreenR object containing the group field

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
groups <- get_groups(object)
set_groups(object, groups)
```

---

set_normalized_count_table

Set ScreenR normalized_count_table

Description

Set function for the normalized_count_table of the ScreenR object

Usage

```
set_normalized_count_table(object, normalized_count_table)

## S4 method for signature 'screenr_object'
set_normalized_count_table(object, normalized_count_table)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object
normalized_count_table	A table of the normalized count table

Value

The ScreenR object with the set normalized_count_table

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
normalized_count_table <- get_normalized_count_table(object)
normalized_count_table
set_normalized_count_table(object, normalized_count_table)
```

set_replicates *Set ScreenR replicates*

Description

Set function for the replicates of the ScreenR object

Usage

```
set_replicates(object, replicates)

## S4 method for signature 'screenr_object'
set_replicates(object, replicates)
```

Arguments

object	The ScreenR object obtained using the create_screenr_object
replicates	The vector containing the replicates name

Value

The ScreenR object with the specific replicates

Examples

```
object <- get0("object", envir = asNamespace("ScreenR"))
replicates <- get_replicates(object)
set_replicates(object, replicates)
```

unique_gene_symbols *Unique gene Symbols*

Description

Compute a unique gene symbol for gene

Usage

```
unique_gene_symbols(gene_symbols, number_barcode = 3)
```

Arguments

gene_symbols	The gene symbols list
...	Arguments passed on to find_camera_hit
number_barcode	Number of barcode that as to be differentially expressed (DE)in order to consider the gene associated DE. Example a gene is associated with 10 shRNA we consider a gene DE if it has at least number_barcode = 5 shRNA DE.

Value

A list of unique gene symbols

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