Package 'synlet'

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Type Package

Title Hits Selection for Synthetic Lethal RNAi Screen Data

Version 2.7.0

Description Select hits from synthetic lethal RNAi screen data. For example, there are two identical celllines except one gene is knocked-down in one cellline. The interest is to find genes that lead to stronger lethal effect when they are knocked-down further by siRNA. Quality control and various visualisation tools are implemented. Four different algorithms could be used to pick up the interesting hits. This package is designed based on 384 wells plates, but may apply to other platforms with proper configuration.

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biocViews ImmunoOncology, CellBasedAssays, QualityControl, Preprocessing, Visualization, FeatureExtraction

Imports data.table, ggplot2, grDevices, magrittr, methods, patchwork, RankProd, RColorBrewer, stats, utils

Suggests BiocStyle, knitr, testthat, rmarkdown

VignetteBuilder knitr

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Description

bScore

Calculate the B-score for plates belonging to the same master plate. Positive / negative controls are removed from the calculation.

Usage

bScore(masterPlate, dta, treatment, control, outFile = FALSE)

Calculate B-score

Arguments

masterPlate	a maste plate to be normalized.
dta	synthetic lethal RNAi screen data.
treatment	the treatment experiment condition in EXPERIMENT_MODIFICATION
control	the control experiment condition in EXPERIMENT_MODIFICATION.
outFile	should calculated B-score files be written to the current folder? File names is (masterPlate).bscore.csv.

Value

A list contains B-score for each master plate, treatment plates are the first columns, followed by control plates

References

Brideau, C., Gunter, B., Pikounis, B. & Liaw, A. Improved statistical methods for hit selection in high-throughput screening. J. Biomol. Screen. 8, 634-647 (2003).

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Examples

example_dt

Synthetic lethal RNAi screen example data.

Description

A dataset containing synthetic lethal RNAi screen data to show how functions work. The variables are as follows (all are character except READOUT):

Usage

```
data(example_dt)
```

Format

A data.table with 4320 rows and 8 variables

Details

- PLATE. plate names.
- MASTER_PLATE. master plate names.
- WELL_CONTENT_NAME. siRNA targets of wells.
- EXPERIMENT_TYPE. sample, negative/positive controls.
- EXPERIMENT_MODIFICATION. experiment conditions, "treatment" or "control".
- ROW_NAME. row names of plates.
- COL_NAME. column names of plates.
- READOUT. screen results.

Value

A data.table containing RANi screen data, the READOUT value has no real biological meaning.

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madSelect

Select hits basing on median +- k*MAD

Description

Select hits basing on median +- k*MAD, by default k is three.

Usage

```
madSelect(
  masterPlate,
  dat,
  k = 3,
  treatment,
  control,
  outFile = FALSE,
  normMethod = "PLATE"
)
```

Arguments

masterPlate the master plate to analysis

dat synthetic lethal RNAi screen data

k cutoff for selecting hits, default is three

treatment the treatment condition in EXPERIMENT_MODIFICATION

control the control condition in EXPERIMENT_MODIFICATION

outFile whether or not write the median normalized results

normMethod normalization methods to be used. If "PLATE", the raw readouts are normalized by plate median, otherwise use median provided control siRNA.

Value

A data.frame contains the hits selection results.

- MASTER_PLATE: location of siRNA
- treat_cont_ratio: ratio of treatment / control
- treat_median: median value of treatment plates
- control_median: median value of control plates
- Hits: Is this siRNA a hit?

References

Chung, N. et al. Median absolute deviation to improve hits election for genome-scale RNAi screens. J. Biomol. Screen. 13, 149-158 (2008).

plateHeatmap 5

Examples

plateHeatmap

Heatmap of all plates

Description

Put all individual plates in one graph, values are the readout in experiments.

Usage

```
plateHeatmap(dta, base_size = 12, heatmap_col = NULL)
```

Arguments

dta synthetic lethal RNAi screen data

base_size basic font size used for x/y axis and title for heatmaps

heatmap_col color function generated by colorRampPalette.

Value

```
a ggplot object
```

Examples

```
data(example_dt)
plateHeatmap(example_dt)
```

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Description

Select hits by rank product methods by comparing treatment and control.

Usage

```
rankProdHits(masterPlate, dta, treatment, control, normMethod = "PLATE")
```

Arguments

masterPlate the master plate to be analyzed dta synthetic lethal RNAi screen data

treatment the treatment condition in EXPERIMENT_MODIFICATION control the control condition in EXPERIMENT_MODIFICATION

normMethod normalization methods to be used. If "PLATE", the raw readouts are normalized

by plate median, otherwise use provided control siRNA

Value

A list contains results by the rank product method for each master plate.

- MASTER_PLATE: location of siRNA
- pvalue_treat_lowerthan_cont: p-value for the hypothesis that treatment has lower normalized readout compared to control
- FDR_treat_lowerthan_cont: FDR value
- treat_cont_log2FC: log2 fold change of treatment / control

References

Breitling, R., Armengaud, P., Amtmann, A. & Herzyk, P. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Lett 573, 83-92 (2004). Hong, F. et al. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. Bioinformatics 22, 2825-2827 (2006).

Examples

rsaHits 7

rsaHits Select hits by RSA

Description

Selected hits by redundant siRNA activity method. Here is a wrapper function of RSA 1.8 by Yingyao Zhou.

Usage

```
rsaHits(
   dta,
   treatment,
   control,
   normMethod = "PLATE",
   LB,
   UB,
   revHits = FALSE,
   Bonferroni = FALSE,
   outputFile = "RSAhits.csv",
   scoreFile = "RSA_score.csv"
)
```

Arguments

dta synthetic lethal RNAi screen data

treatment the treatment condition in EXPERIMENT_MODIFICATION

control the control condition in EXPERIMENT_MODIFICATION

normMethod normalization methods. If "PLATE", then values are normalized by plate me-

dian, otherwise use the provided control siRNA

LB Low bound
UB up bound

revHits reverse hit picking, default the lower the score the better

Bonferroni conceptually useful when there are different number of siRNAs per gene, default

FALSE

outputFile output file name

scoreFile name of the score file to be written under the current folder

Value

A result file written to the current folder.

- Gene_ID,Well_ID,Score: columns from input spreadsheet
- LogP: OPI p-value in log10, i.e., -2 means 0.01

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- OPI_Hit: whether the well is a hit, 1 means yes, 0 means no
- #hitWell: number of hit wells for the gene
- #totalWell: total number of wells for the gene. If gene A has three wells w1, w2 and w3, and w1 and w2 are hits, #totalWell should be 3, #hitWell should be 2, w1 and w2 should have OPI Hit set as 1 and w3 should have OPI Hit set as 0.
- OPI_Rank: ranking column to sort all wells for hit picking
- Cutoff_Rank: ranking column to sort all wells based on Score in the simple activity-based method

Note: a rank value of 999999 means the well is not a hit

References

Koenig, R. et al. A probability-based approach for the analysis of large-scale RNAi screens. Nat Methods 4, 847-849 (2007).

Examples

scatterPlot

Scatter plot of RNAi screen results

Description

Produce a single plot for readous of each plate, with the option of highlighting specific signals, like positive/negative controls.

Usage

```
scatterPlot(
  dta,
  scatter_colour = rainbow(10),
  controlOnly = FALSE,
  control_name = NULL
)
```

Arguments

dta synthetic lethal RNAi screen data
scatter_colour colour for different signals
controlOnly whether or not to plot control wells only
control_name names of control siRNAs.

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Value

```
a ggplot object
```

Examples

```
data(example_dt)
scatterPlot(example_dt, control_name = c("PLK1 si1", "scrambled control si1", "lipid only"))
```

siRNAPlot

Plot siRNA data and quality metrics.

Description

Plot the normalized RNAi screen data, row data, control signals and Z' factor.

Usage

```
siRNAPlot(
   gene,
   dta,
   controlsiRNA,
   FILEPATH = ".",
   colour = rainbow(10),
   zPrimeMed,
   zPrimeMean,
   treatment,
   control,
   normMethod = c("PLATE"),
   save_plot = FALSE,
   width = 15,
   height = 14
)
```

Arguments

gene gene symbol, case sensitive
dta synthetic lethal RNAi screen data

controlsiRNA could be a vector of several siRNA, including postive/negative

control

FILEPATH path to store the figure colour colour used in graphs

zPrimeMed zPrime factor basing on median zPrimeMean zPrime factor basing on mean

treatment the treatment condition in EXPERIMENT_MODIFICATION

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control the control condition in EXPERIMENT_MODIFICATION

normMethod could be a PLATE and negative controls

save_plot whether save a png file in the working directory.

width width of the plot height height of the plot

Value

Return the ggplot2 objects in a list, which could be plotted individually.

Examples

tTest

student's t-test on B-score

Description

Select hits by student's t-test using B-score from treatment and control plates.

Usage

```
tTest(mtx, n_treat, n_cont)
```

Arguments

mtx b-score matrix.

n_treatn_contnumber of treatment platesn_contnumber of control plates

Value

A list containing student's t-test for each master plate

- pvalue: p-value of the t-test
- Treat_Cont: difference in bscore: treatment control
- p_adj: BH adjusted p-value

zFactor 11

References

Birmingham, A. et al. Statistical methods for analysis of high-throughput RNA interference screens. Nat Methods 6, 569-575 (2009).

Examples

```
data(example_dt)
bscore_res <- sapply(unique(example_dt$MASTER_PLATE), bScore,
   example_dt, control = "control", treatment = "treatment", simplify = FALSE)
tTest(bscore_res$P001, 3, 3)</pre>
```

zFactor

Calcualte the Z and Z' factor

Description

calcualte the Z and Z' factor for each plate.

Usage

```
zFactor(dta, negativeCon, positiveCon, useMean = TRUE)
```

Arguments

dta synthetic lethal RNAi screen data.

negativeCon the negative control used in the WELL_CONTENT_NAME.

positiveCon the positive control used in the WELL_CONTENT_NAME.

use Mean use mean to calcualate z factor and z' factor by default; otherwise use median.

Value

A data.frame contains z factor and z' factor

References

Zhang J.H., Chung T.D. & Oldenburg K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J. Biomol. Screen. B, 4 67-73 (1999). Birmingham, A. et al. (2009) Statistical methods for analysis of high-throughput RNA interference screens. Nat Methods, 6, 569-575.

Examples

```
data(example_dt)
res <- zFactor(example_dt, negativeCon = "scrambled control si1", positiveCon = "PLK1 si1")</pre>
```

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