Package 'synlet'

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Type Package

Title Hits Selection for Synthetic Lethal RNAi Screen Data

Version 2.12.0

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

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`bScore` *Calculate B-score*

Description

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)
```

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).

Examples

```
data(example_dt)
res <- sapply(unique(example_dt$MASTER_PLATE), bScore, example_dt,
              treatment = "treatment", control = "control", simplify = FALSE)
```

`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.

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

Examples

```
data(example_dt)
res <- sapply((unique(example_dt$MASTER_PLATE)),
              madSelect,
              example_dt,
              control   = "control",
              treatment = "treatment",
              simplify  = FALSE)
```

`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)
```

rankProdHits *Select hits by the rank product method*

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

```
data(example_dt)
res <- sapply(unique(example_dt$MASTER_PLATE),
              rankProdHits,
              example_dt,
              control   = "control",
              treatment = "treatment",
              simplify  = FALSE)
```

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 median, 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

- 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

```
data(example_dt)
rsaHits(example_dt, treatment = "treatment", control = "control",
        normMethod = "PLATE", LB = 0.2, UB = 0.8, revHits = FALSE,
        Bonferroni = FALSE, outputFile = "RSAhits.csv")
```

---

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.

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`	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

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

```
data(example_dt)
zF_mean <- zFactor(example_dt, negativeCon = "scrambled control si1", positiveCon = "PLK1 si1")
zF_med  <- zFactor(example_dt, negativeCon = "scrambled control si1", positiveCon = "PLK1 si1",
                   useMean = FALSE)
p01 <- siRNAPlot("AAK1", example_dt,
                 controlsiRNA = c("lipid only", "scrambled control si1"),
                 FILEPATH = ".",  zPrimeMed = zF_med, zPrimeMean = zF_mean,
                 treatment = "treatment", control = "control",
                 normMethod = c("PLATE", "lipid only", "scrambled control si1"))
```

---

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_treat	number of treatment plates
n_cont	number 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

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)
```

`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.
`useMean`	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")
```

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