

# Package ‘synlet’

April 6, 2026

**Type** Package

**Title** Hits Selection for Synthetic Lethal RNAi Screen Data

**Version** 2.10.0

**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.

**License** GPL-3

**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

**NeedsCompilation** no

**RoxygenNote** 7.2.3

**Encoding** UTF-8

**git\_url** <https://git.bioconductor.org/packages/synlet>

**git\_branch** RELEASE\_3\_22

**git\_last\_commit** efe839e

**git\_last\_commit\_date** 2025-10-29

**Repository** Bioconductor 3.22

**Date/Publication** 2026-04-05

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bScore	<i>Calculate B-score</i>
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### 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	<i>Synthetic lethal RNAi screen example data.</i>
------------	---

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**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	<i>Select hits basing on median +- k*MAD</i>
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**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	<i>Select hits by the rank product method</i>
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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**

```
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 readouts 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 positive/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	<i>Calculate the Z and Z' factor</i>
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**Description**

calculate 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 calculate 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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