# Package 'RNAinteract' 

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## Type Package

Title Estimate Pairwise Interactions from multidimensional features
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Description RNAinteract estimates genetic interactions from multi-dimensional read-outs like features extracted from images. The screen is assumed to be performed in multi-well plates or similar designs. Starting from a list of features (e.g. cell number, area, fluorescence intensity) per well, genetic interactions are estimated. The packages provides functions for reporting interacting gene pairs, plotting heatmaps and double RNAi plots. An HTML report can be written for quality control and analysis.

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RNAinteract-package Analysis of Pairwise Interaction Screens.

## Description

The package contains functions to organize the data from (single- and multi-parametric) genetic interaction screens. Methods to estimate main effects (single perturbation effects) and pairwise interactions. p-values are computed. Furthermore a comprehensive html-report is generated.

## Details

See vignette("RNAinteract") for details.

## Package content

Class RNAinteract (Documentation: RNAinteract-class)
Data input and creating of an object of class RNAinteract.

- createCellHTSFromFiles
- createRNAinteract, createRNAinteractFromFiles

Data access

- getData Primary data access function for multiple types of screen data.
- getMain, getMainNeg access to main effects.
- getReplicateData, getIndDesignData Comparing replicate data.
- getChannelNames, getScreenNames, getScale

Subsetting, summarizing, and binding screens

- sgisubset, sgisubsetQueryDesign
- bindscreens
- summarizeScreens

Main effects and pairwise interactions

- estimateMainEffect
- normalizeMainEffectQuery, normalizeMainEffectTemplate, normalizePlateEffect
- computePI, computePValues
- embedPCA

Plotting

- plotDoublePerturbation, plotHeatmap standard plot functions
- doublePerturbationGrob, grid.doublePerturbation, grid.sgiHeatmap specialized grid plotting functions for experts


## HTML report

- startReport, endReport starting and finalizing a report
- reportAnnotation, reportStatistics global reports
- reportDoublePerturbation, reportGeneLists, reportHeatmap, reportMainEffects, reportNetworks, reportScreenData reports specific for each screen and each channel


## Author(s)

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## References

T. Horn, T. Sandmann, B. Fischer, W. Huber, M. Boutros. Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi. Nature Methods, 2011.

## Description

Bind two RNAinteract objects along screens.

## Usage

bindscreens(sgi1, sgi2)

## Arguments

sgi1 An object of class RNAinteract.
sgi2 An object of class RNAinteract.

## Details

This function binds two double interaction screens along screens.

## Value

An object of class RNAinteract with all screens in sgi1 and sgi2.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi
sginew <- summarizeScreens(sgi, screens=c("1","2"), newscreenname = "m")
sginew
sgibind <- bindscreens(sgi, sginew)
sgibind
```

```
computePI compute pairwise interaction
```


## Description

Compute the pairwise interaction term for each single experiments.

## Usage

computePI(sgi)

## Arguments

sgi An object of class RNAinteract.

## Details

Computes the pairwise interaction term for each single experiment. Multiple values for each gene pair are not yet summarized.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi <- computePI(sgi)
PI <- getData(sgi, type="pi", format="targetMatrix")
```

```
computePValues compute p-values
```


## Description

Compute p-values for genetic interactions terms. Assess if genetic interaction term is different from zero.

## Usage

```
computePValues(sgi,
    method = "pooled.ttest",
    mixTemplateQuery = TRUE,
    p.adjust.function = function(x) { p.adjust(x, method = "BH")},
    verbose = 0)
```


## Arguments

sgi An object of class RNAinteract.
method The method used to compute p-values. One of "pooled.ttest","ttest", "limma", "HotellingT2".
For "ttest" a Student t-test is applied for each gene pair. The variance is estimated locally for each gene pair. For "pooled.ttest", a pooled variance is estimated from all gene pairs. The variance applied for each gene pair is the maximum of the pooled and the local variance estimate. This method obtains conservative p-values. For "limma" mediates between the local and the global variance estimation in a Bayesian framework. The limma-package is applied to compute the p-values. For "HotellingT2" Hotelling-T^2 statistics is computed jointly for all dimensions. It results in a single p-value summarizing all channels. For simplification the p-values are stored in a matrix of dimension genes x genes x screens $x$ channels and the $p$-values are repeated for each channel. The same holds for q -values.
mixTemplateQuery
If a gene-pair is measured twice as template-query and as query-template, a single p -value is computed by combining all measurements, if mixTemplateQuery $=$ TRUE. Else a p-value is computed independently for both cases.
p.adjust.function

A function that corrects the p-values for multiple testing. Default method is the Benjamini-Hochberg method.
verbose $\quad$ Either 0 (default, no output), 1 (minimum output), or 2 (outout)

## Details

Computes p-values from a t-test, using the bioconductor package limma, or with a multidimensional Hotelling $\mathrm{T}^{\wedge} 2$ test.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi <- computePValues(sgi, method = "HotellingT2")
# Hotelling T^2 test will provide one p-value for all channels, PV will be the same
# for all channels in this case
PV <- getData(sgi, type="p.value", format="targetMatrix", channel="nrCells")
```

```
createCellHTSFromFiles
```

    create cellHTS 2 object from text files
    
## Description

A cellHTS2 object is created from a set of text files.

## Usage

createCellHTSFromFiles(filePlatelist = "Platelist.txt", name = "anonymous", path $=$ ".", pdim $=$ NULL)

## Arguments

filePlatelist The platelist (See vignette("RNAinteract"))
name A (arbtrary) string providing the name for the screen
path The path were the data files are located
pdim Giving the plate dimensions, e.g. pdim $=c(8,12)$ or $\operatorname{pdim}=c(16,24)$.

## Details

See vignette("RNAinteract") for an example how to create an RNAinteract object.

## Value

An object of class cellHTS2.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

See Also
RNAinteract-package, createRNAinteractFromFiles, createRNAinteract

```
    createRNAinteract create a RNAinteract object
```


## Description

Creates a RNAinteract object given data matrices, annotation, query and template design.

## Usage

createRNAinteract(data, well, plate, pdim, Reagents, Targets, TemplateDesign, QueryDesign, Transformation = NULL)

## Arguments

data An array with dimensions features x screens x channels.
well A vector of length \#features with well names.
plate A vector of length \#features with plate numbers.
pdim A vector of length 2 with plate dimensions (e.g. pdim=c(12,8)).
Reagents A data.frame describing the reagents.
Targets A data.frame describing the targets.
TemplateDesign A data.frame with the layout of the template plates.
QueryDesign A data.frame with the layout of the query plates.
Transformation A Transformation that is applied to the data. If NULL the data is $\log 2$ transformed.

## Details

See vignette("RNAinteract") for an example how to create an RNAinteract object.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package, createRNAinteractFromFiles, createCellHTSFromFiles

```
createRNAinteractFromFiles
                    create an RNAinteract object from text files
```


## Description

Reads text files with annotation, query and template design, and data. Creates a RNAinteract object.

## Usage

createRNAinteractFromFiles(name = "anonymous", filePlatelist = "Platelist.txt", fileReagents = "Reagents.txt", fileTargets = "Targets.txt", fileTemplateDesign = "TemplateDesign.txt", fileQueryDesign = "QueryDesign.txt", path $=$ ".", pdim = NULL, Transformation = "log2")

## Arguments

name A name for the screen.
filePlatelist The filename of the text file containing the plate list.
fileReagents The filename of the text file containing the reagent annotation.
fileTargets The filename of the text file containing the target annotation.
fileTemplateDesign
The filename of the text file containing the template design.
fileQueryDesign
The filename of the text file containing the query design.
path The system directory were the textfiles filePlatelist, fileReagents, fileTargets, fileTemplateDesign, and fileQueryDesign are located.
pdim The dimensions of the multi-well plates (e.g. pdim $=c(n r o w=24, n c o l=16)$ ). if pdim =NULL (default), the plate dimension will be estimated from the input data.

Transformation The transformation that is applied to the data. All calculations are done on additive scale.

## Details

See vignette("RNAinteract") for an example how to create an RNAinteract object.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package, createRNAinteract, createCellHTSFromFiles

## Description

A principal component analysis is performed for a pairwise interaction matrix. The low-dimensional embedding is returned.

## Usage

embedPCA(sgi, screen, channel, dim $=4$, embed $=$ "template", withoutgroups $=c())$

## Arguments

sgi An object of class RNAinteract
screen The screen name whose interaction matrix will be embedded.
channel The channel name whose interaction matrix will be embedded.
dim The embedding dimension.
embed Either "template" (default) or "query" denotes if the embedding is done for rows or columns.
wi thoutgroups Genes annotated with these groupnames are not considered for embedding.

## Value

Returns a matrix with dimensions genes x dim.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
X <- embedPCA(sgi, screen="1", channel="nrCells", dim=2)
plot(X[,1], X[,2], pch=20, cex=0.01)
text(X[,1], X[,2], row.names(X))
```

estimateMainEffect estimate main effect

## Description

estimates the main effects in an additive model.

## Usage

estimateMainEffect(sgi, use.query = NULL)

## Arguments

sgi An object of class RNAinteract.
use.query A list of reagent identifiers as annotated in the RID field of the reagent list. For the estimation of the template main effects only these queries are used.

## Details

The main effect is the single RNAi knockdown effect. When use.query is not specified, the main effect is estimated by minimizing the L1 distance from the non-interacting model to the double RNAi measurements. The implemented non-interacting model is the additive model (sum of single main effects). If the screen does not contain a lot of query genes with no or ery small main effect, it is recommended to estimate the template main effects only by using selected query genes. This can be obtained by specifying use.query. To estimate main effects in a multiplicative model define Transformation="log2" when creating the RNAinteract object (See createRNAinteractFromFiles), which is already the default.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## References

$\sim$ put references to the literature/web site here $\sim$

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi <- estimateMainEffect(sgi)
getMain(sgi)
```

getData Primary access function for all screen data.

## Description

This function is the primary access function for a wide range of data from the screen. It does perform normalization, transformation, and reshaping if specified.

```
Usage
getData(sgi, type = "data", format = "plain",
design = "template", mixTemplateQuery = TRUE,
screen = NULL, channel = NULL,
do.trafo = TRUE, do.inv.trafo = FALSE,
normalized = FALSE, withoutgroups = c(),
drop = TRUE)
```


## Arguments

sgi An object of class RNAinteract

Specifies which data is returned. Possible values are:

- "p.value", "q.value": returns the p-value or q-value as computed by computePValues.
- "data": returns the input data.
- "pi": returns the pairwise interaction score.
- "plateeffect": returns the plate effect estimated by normalizePlateEffect.
- "ni.model" returns the non-interacting model as estimated by estimateMainEffect.
- "main": returns the main effects.
- "mainsderr": returns the std error of the main effects.
- "mainsd": returns the std deviation of the main effects.
- "maintime": returns the estimated time effect as estimated by normalizeMainEffectQuery
- "mainspatial": returns the estimated spatial effect as estimated by normalizePlateEffect
format The output format. Possible values:
- "plain": The data can be returned as a plain vector
- "platelist": a list of plate matrices that can be passed to plotScreen
- "reagentMatrix": All values for the same reagent pair are summarized in a matrix of dimension reagents x reagents
- "targetMatrix": All values for the same gene pair are summarized in a matrix of dimension genes $x$ genes
design If type is one of the main effect types, the design can be specified to state if the "template" or "query" main effect is returned.
mixTemplateQuery
If TRUE, The template-query and query-template entries in the matrix are symmetrized.
screen The screen names of which data should be returned.
channel The channel names of which data should be returned.
do.trafo Only effective, if type is "data". If TRUE, the data is transformed.
do.inv.trafo Not effective if type is "data", "p.value", or "q.value". If TRUE, the values are back-transformed to the original scale.
normalized If TRUE, the normalization data is returned.
withoutgroups The genes from the specified groups are not returned in the data.
drop If FALSE, the returned array is reduced in dimensions, whenever there is a dimension 1 .


## Value

An array containing the specified values is returned. In the case, the format is chosen to be "platelist", a list of matrices is returned.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
# get the original data, as plain file, reshaped in plate layout,
# reshaped and summarized as target matrix
D <- getData(sgi, type="data", do.inv.trafo = TRUE)
Dplatelayout <- getData(sgi, type="data",
        format="platelist", do.inv.trafo = TRUE)
splots::plotScreen(Dplatelayout[["1"]][["nrCells"]],
        nx=sgi@pdim[2], ny=sgi@pdim[1], ncol=3)
Dmatrix <- getData(sgi, type="data",
        format="targetMatrix", do.inv.trafo = TRUE)
# get main effects as plate layout with specified transformation
# (usually log-transformed)
Mplatelayout <- getData(sgi, type="main", design="template",
    screen="1", channel="nrCells", format="platelist")
splots::plotScreen(Mplatelayout, nx=sgi@pdim[2], ny=sgi@pdim[1],
    ncol=3)
# get non-interacting model and pairwise interaction scores as matrix
NImatrix <- getData(sgi, type="ni.model", format="targetMatrix")
PImatrix <- getData(sgi, type="pi", format="targetMatrix")
PIplatelayout <- getData(sgi, type="main", design="query",
    screen="1", channel="nrCells", format="platelist")
splots::plotScreen(PIplatelayout, nx=sgi@pdim[2], ny=sgi@pdim[1],
    ncol=3)
# get p-values and q-values
PVmatrix <- getData(sgi, type="p.value", format="targetMatrix")
QVmatrix <- getData(sgi, type="q.value", format="targetMatrix")
```

```
getMain get main effects
```


## Description

Returns the main effects.

## Usage

getMain(sgi, type = "main", design = "template", summary = "none",
QueryNr = NULL, TemplatePlate = NULL,
do.inv.trafo = FALSE, format = "plain", withoutgroups = c(),
screen $=$ NULL, channel $=$ NULL, normalized $=$ TRUE, drop $=$ TRUE)

```
getMainNeg(sgi, type = "all", do.inv.trafo = FALSE,
screen = NULL, channel = NULL, drop = TRUE)
```


## Arguments

| sgi | An object of class RNAinteract |
| :---: | :---: |
| type | always "main" |
| design | Either "template" or "query" defining if template or query main effects are returned. |
| summary | If summary is "targets" the main effects are summarized per target gene. |
| QueryNr, TemplatePlate |  |
|  | Onle main effects of one query nr or one template plate are returned. |
| format | targetmatrix |
| withoutgroups | The genes within this group are not shown in the heatmap. It is convenient to hide screen controls. |
| do.inv.trafo | If TRUE, the data will be back-transformed for original scale of data. In the case of log-transformed data, the main effects are returned as factors, otherwise the main effects are returned as $\log$ values. |
| screen | The screen from which the main effects should be returned. |
| channel | The channel from which the main effects should be returned. |
| drop | Does return a drop array dimensions, even if only one screen or one channel is selected. |
| normalized | If true the normalized main effects are returned. |

## Value

An array containing the main effects.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
getMain(sgi)
getMainNeg(sgi)
```


## Description

A genetic interaction screen can contain within screen replicates, if some reagent pairs are measured at least twice. Usually this appears when measuring reagent pairs once as template-query and once as query-template. getReplicateData returns a list of these technical replicates.
If multiple reagents are used to target the same gene, different reagent pairs that target the same gene pair are extracted from the screen. These pairs are returned by getIndDesignData.

## Usage

```
getReplicateData(sgi, screen, channel,
    type = "data", design = "template",
    do.trafo = TRUE, do.inv.trafo = FALSE,
    normalized = FALSE)
getIndDesignData(sgi, screen, channel,
    type = "data", design = "template",
    do.trafo = TRUE, do.inv.trafo = FALSE,
    normalized = FALSE)
```


## Arguments

sgi An object of class RNAinteract.
screen The screen name from which the replicates will be extracted.
channel The channel name from which the replicates will be extracted.
type $\quad$ The type of data that is extracted. It is the type argument of the getData function.
design, do.trafo, do.inv.trafo, normalized
See the getData documentation for details.

## Value

Returns a data.frame with columns x and y .

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
res <- getIndDesignData(sgi, screen="1", channel="nrCells", type = "data")
plot(res$x, res$y)
```

    getScale get scale information for a channel.
    
## Description

Returns a character string with the scale of each channel.

## Usage

getScale(sgi, channel)

## Arguments

sgi A RNAinteract
object.
channel A channel name.

## Value

Returns a character string with scale information for each channel.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
getScale(sgi, channel="nrCells")
```


## Description

Returns the names of all screens or all channels.

## Usage

getScreenNames(sgi)
getChannelNames(sgi)

## Arguments

sgi RNAinteract

## Value

A vector of screen or channel names.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
getScreenNames(sgi)
getChannelNames(sgi)
```

```
grid.doublePerturbation
```

    Double Perturbation Plot Grob
    
## Description

These functions create a double perturbation grob for interaction screens. All interactions of one gene are displayed in one panel. The double perturbation readout level is plotted against the single perturbation level.

## Usage

```
doublePerturbationGrob( mainEffect, dpEffect, mainEffectTarget,
                    range=NULL, main=NULL, xlab=NULL, ylab=NULL,
                        text=NULL, avoid.overlap=TRUE,
                        axisOnOrigin = FALSE,
                            drawBox = TRUE,
        pch = 21, size=unit(1, "char"), fill = NULL,
                            gpMain = gpar(lty="dashed", lwd=3, col="cyan"),
                            gpNI = gpar(lty="dashed", lwd=3, col="orange"),
                            gpPoints = gpar(pch=21),
                            gpText = NULL,
                    gpAxis = NULL,
                    gpWTLines=NULL,
                            name=NULL, gp=NULL, vp=NULL )
grid.doublePerturbation(..., draw = TRUE)
# a helper function for doublePerturbationGrob:
postDrawDetails.doublePerturbation(x, recording)
```


## Arguments

mainEffect A numeric vector of main effects.
dpEffect A numeric vector of double perturbation effects.
mainEffectTarget
The main effect of the target gene (A single numeric value).
range $\quad$ The range of the plot. Equals the xlim, ylim arguments of plot.
main An overall title of the plot.
$x l a b \quad$ A title of the $x$-axis.
ylab A title of the $y$-axis.
text A character vector of text. Has to have the same length as mainEffect.
avoid.overlap If TRUE (default) the text labels are moved such that the text is not overlapping.
axisOnOrigin If TRUE, the $x$ - and $y$-axis are draw on the origin of the data. If FALSE (default), the axis are drawn on the left and on the bottom.
drawBox If TRUE (default), a box is drawn around the plot.
pch Either an integer specifying a symbol or a single character to be used in plotting points. See points for possible values.
size A unit object specifying the size of the plotting symbols.
fill A list containing (some of) the following elements: col defines a fill color for the points. Either a single value or a vector of the same length as mainEffect. If col is defined, all other elements of fill have no effect. values is a numeric vector of the same length as mainEffcet that contains values that are mapped to colors. at is a numeric vector indicating breakpoints along the values. If not specified will be equally spaced on the range of the values. colors defines a set of colors from which a colorramp is created. colramp defines a colorramp directly. colramp has no effect, if colors is defined.

| gp | An object of class gpar, typically the output from a call to the function gpar. This is basically a list of graphical parameter settings. Overall settings for the plot are set in gp. |
| :---: | :---: |
| gpMain, gpNI | An object of class gpar (See gp). gpMain and gpNI indicate the graphics parameter for the main effect lines and the non-interacting line. |
| gpPoints, gpText, gpAxis, gpWTLines |  |
|  | An object of class gpar (See gp). These arguments define graphical parameters for single compartments of the plot. |
| name | A character identifier. |
| vp | A Grid viewport object (or NULL). |
| draw | If TRUE the grob is drawn on the current device. |
|  | Further arguments passed to doublePerturbationGrob. |
| x, recording | Internal usage only. |

## Details

This function creates a grob for a double perturbation plot. It is probably more convenient to use the function plotDoublePerturbation.

## Value

A grob is returned.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package, plotDoublePerturbation, reportDoublePerturbation

```
grid.sgiHeatmap A heatmap grob
```


## Description

A grob is created and printed for a matrix PI which is intended to represent pairwise interaction scores.

## Usage

grid.sgiHeatmap(PI, pi.max = NULL, main = expression(paste(pi, "-score")), hc. row $=$ NULL, hc.col $=$ NULL)

## Arguments

PI A matrix of pairwise interactions.
pi.max The interaction score at the top end of the colorbar. pairwise interaction score larger than this value can not be distinguished anymore.
main A title for the plot.
hc. row An hierarchical clustering as produced by hclust of the rows.
hc.col Clustering of the columns.

## Details

A heatmap is plotted with positive interaction represented in yellow and negative interactions represented in blue. A colorbar is plotted on the left and dendrograms are added. This function can be used to integrate the plot in other grid objects. It is recommended to use the function plotHeatmap to plot heatmaps of an RNAinteract object.

## Value

A grob is returned.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
PI = getData(sgi, type="pi", format="targetMatrix", screen="1", channel="nrCells")
grid.sgiHeatmap(PI)
```

```
normalizeMainEffectQuery
```

normalize query main effect

## Description

Normalize for a time effect of the query genes.

## Usage

normalizeMainEffectQuery (sgi, batch = NULL, time = NULL)

## Arguments

| sgi | An object of class RNAinteract. |
| :--- | :--- |
| batch | batch is a vector if integers with length equal to the number of queries. It as- <br> signs each query to a batch. Within each batch a linear regression is estimated <br> assuming a linear effect between the order of queries and the main effects. |
| time | batch is a vector of numbers. A linear regression is estimated fitting the main <br> effect as a function of the time. |

## Details

Normalizing the query main effect does not influence the estimation of the pairwise interaction term.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi <- normalizeMainEffectQuery(sgi)
```

```
normalizeMainEffectTemplate
```

normalize template main effect

## Description

Normalize for a spatial main effect of the template genes.

## Usage

normalizeMainEffectTemplate(sgi, screen $=$ NULL, channel = NULL)

## Arguments

sgi An object of class RNAinteract.
screen The name of the screen in which the normalization should be applied. If screen = NULL, the normalization is applied on all screens.
channel The name of the channel in which the normalization should be applied. If channel = NULL, the normalization is applied on all channels.

## Details

Normalizing the query main effect does not influence the estimation of the pairwise interaction term.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi <- normalizeMainEffectTemplate(sgi)
```

    normalizePlateEffect Normalization of plate effects
    
## Description

Normalization of plate effects in the screen.

## Usage

normalizePlateEffect(sgi, type = "Bscore", maxit = 20, verbose = 0)

## Arguments

sgi An object of class RNAinteract
type If type is "Bscore" (default) a Bscore-normalization is performed. If type is "spatial", a locfit regression is estimated that accounts for spatial effects.
maxit Maximum number of iterations for locfit.
verbose $\quad$ Either 0 (default, no output), 1 (minimum output), or 2 (outout).

## Details

The Bscore normalization estimates row and column effects for each plate. It returns the residuals to the sum of row and column effects. The spatial normalization estimates a non-linear 2D regression for each plate and returns the residuals.

## Value

An object of class RNAinteract. The returned object contains the normalization information.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
```

normalizePlateEffect(sgi)

```
plotDoublePerturbation
```


## Double Perturbation Plot

## Description

These function draws a double perturbation plot for interaction screens. All interactions of one gene are displayed in one panel. The double perturbation readout level is plotted against the single perturbation level.

## Usage

$$
\begin{aligned}
& \text { plotDoublePerturbation( sgi, screen, channel, target, } \\
& \text { withoutgroups = c("neg", "pos"), design, } \\
& \text { main, xlab, ylab, range, } \\
& \text { show.labels = "none", label.par, label, } \\
& \text { avoid.overlap, col, fill, } \\
& \text { D , MT, MQ, PV, QV, PI, ...) }
\end{aligned}
$$

## Arguments

sgi An object of class RNAinteract.
target A character name of the target gene.
screen The character name of the screen to display. If not specified, the first screen is used. Does not have to be specified, if sgi contains only one screen.
channel The character name of the channel to display. If not specified, the first channel is used. Does not have to be specified, if sgi contains only one channel.
withoutgroups Interactions to genes from these groups (as specified in the reagent or target annotation) are excluded from the plot, e.g. positive and negative controls.
$\left.\begin{array}{ll}\text { design } & \begin{array}{l}\text { The Either "template" (default) or "query". The single perturbation effects } \\ \text { are either the template main effects or the query main effects. }\end{array} \\ \text { main } & \text { An overall title of the plot. } \\ \text { xlab } \\ \text { ylab } \\ \text { range } \\ \text { show. labels } & \begin{array}{l}\text { A title of the x-axis. }\end{array} \\ \text { A title of the y-axis. } \\ \text { A numeric vector of length two. range equals the xlim, ylim argument in plot. } \\ \text { genes, "q.value" and "p.value" show a text label for all genes with a q.value } \\ \text { (p.value) larger than label. par, "none" does not show any text label. This } \\ \text { argument has no effect, if label is specified. }\end{array}\right\}$

## Details

Plots a double perturbation plot. It shows the interaction profile for one (query) gene.

## Value

A grob is returned.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package, grid.doublePerturbation, reportDoublePerturbation

## Examples

```
data("sgi")
```

plotDoublePerturbation( sgi, screen="1", channel="nrCells", target="rl", show.labels="p.value")

## Description

A heatmap of an interaction screen is plotted.

## Usage

```
plotHeatmap(sgi, screen, channel, pi.max = NULL,
                main = expression(paste(pi, "-score")),
    hc.row = NULL, hc.col = NULL,
    withoutgroups = c("neg", "pos"))
```


## Arguments

sgi An object of class RNAinteract
screen The screen name of which the interaction matrix is plotted.
channel The channel name of which the interaction matrix is plotted.
pi.max The pairwise interaction score that is represented at the top of the color scale. All interaction scores above this value can not be distinguished any more.
main The title of the plot.
hc.row A hierarchical clustering (hclust) for the rows.
hc.col A hierarchical clustering (hclust) for the columns.
withoutgroups The genes within this group are not shown in the heatmap. It is convinient to hide screen controls in the heatmap.

## Details

A heatmap for one screen and one channel is plotted. Positive interactions are marked blue, negative ones are marked yellow. A colorbar is shown on the left hand side.

## Value

Returns a grob.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
plotHeatmap(sgi, screen="1", channel="nrCells")
```

reportAnnotation Specialized report functions

## Description

Functions that provide a html report of genetic interactions screens for specific topics.

## Usage

```
reportAnnotation (sgi, verbose = 0, path = ".", dir = "annotation",
    prefix = "annotation", report = NULL)
reportStatistics (sgi, verbose = 0, path = ".", dir = "stats",
    prefix = "stat", report = NULL)
reportGeneLists (sgi, verbose = 0, path = ".", dir = "hitlist",
    prefix = "hitlist", report = NULL)
reportNetworks (sgi, verbose = 0, path = ".", dir = "networks",
    prefix = "networks", Networks, qv = 0.05,
    withoutgroups = c("pos", "neg"), report = NULL)
reportScreenData (sgi, type = "data", design = "template",
    do.trafo = TRUE, do.inv.trafo = FALSE, verbose = 0,
        path = ".", dir = "screenplots", prefix = "screenplot",
    png.args = list(width = 960, height = 960),
    pdf.args = list(width = 7, height = 7),
    plotScreen.args = list(ncol = 6L, do.legend = TRUE,
            fill = c("red", "white", "blue")),
    png.scatter.args = list(width = 400, height = 400),
    pdf.scatter.args = list(width = 7, height = 7), report = NULL)
reportDoublePerturbation(sgi, verbose = 0, path = ".", dir = "doublePerturbations",
    prefix = "doublePerturbationPlots", report = NULL,
            withoutgroups = c("neg", "pos"),
            png.args = list(width = 500, height = 500),
    pdf.args = list(width = 7, height = 7), ...)
reportMainEffects (sgi, verbose = 0, path = ".", dir = "maineffects",
    prefix = "maineffects",
    png.args = list(width = 500, height = 500),
    pdf.args = list(width = 7, height = 7),
    plot.args = list(), report = NULL)
reportHeatmap (sgi, verbose = 0, path = ".", dir = "heatmap",
    prefix = "heatmap",
    png.args = list(width = 1000, height = 1000),
    pdf.args = list(width = 15, height = 15),
    report = NULL, withoutgroups = c("neg", "pos"))
```


## Arguments

| sgi | An object of class RNAinteract. |
| :---: | :---: |
| verbose | Either 0 (default, no output), 1 (minimum output), or 2 (outout) |
| path | The main path to the HTML report directory. |
| dir | A subdirectory where the report is written to. |
| prefix | A prefix for each file written in the subdirectory. Using different prefixes, one can write multiple reports in the same directory. |
| report | A report object as generated by startReport. |
| Networks | A boolean array with edges from interaction graphs. |
| qv | A cut-off value for the q-values. |
| withoutgroups | Genes annotated with these groups are not plotted in this report. |
| type | Any "type" that can be passed to getData. |
| design | Either "template" (default) or "query" |
| do.trafo, do.inv.trafo |  |
|  | Apply the (inverse) transformation before plotting. |
| png.args | A list with entries width and height specifying the width and height of the generated png images. |
| pdf.args | A list with entries width and height specifying the width and height of the generated of files. |
| plotScreen.args |  |
|  | Arguments for the screen plots |
| png.scatter.args, pdf.scatter.args, plot.args |  |
|  | Arguments for the scatter plots |
|  | Parameters passed to the plotting functions. |

## Details

Each of these function generates a HTML report. It is added to a HTML frame.
The report object has to be created with startReport beforehand. Multiple report functions can be called afterwards. When all reports are written, the report is finalized and closed by endReport.

## Value

All functions return a report object as returned by startReport.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package, startReport, endReport

## Examples

```
    data("sgi")
    report = startReport("report")
    reportAnnotation(sgi, report = report)
    endReport(report)
    # browseURL(file.path("report","index.html"))
```

RNAinteract-class Class "RNAinteract"

## Description

A class for double perturbation experiments (genetic interaction screens, drug-drug interaction screens). There are functions for creation, analysis, and display of interaction screens.

## Objects from the Class

Objects can be created by calls of createRNAinteractFromFiles. See vignette("RNAinteract") for an example of creating an RNAinteract object.

## Slots

data: Object of class "array" with dimension sgi@F x sgi@S x sgi@c. The raw data of the screen. screenNames: Object of class "character" with length sgi@s.
channelNames: Object of class "character" with length sgi@c.
well: Object of class "character" with length sgi@F. Well name (e.g. F04) for each measurement.
plate: Object of class "integer" with length sgi@F. Number of the plate for each measurement pdim: Object of class "integer" of length 2. Plate dimensions.
NT: Object of class "integer" of length 1. Number of template reagents.
NQ: Object of class "integer" of length 1 . Number of query reagents.
C: Object of class "integer" of length 1. Number of readout channels.
S: Object of class "integer" of length 1. Number of screens.
F: Object of class "integer" of length 1. Number of measurements or single experiments per screen.
reagents: Object of class "data.frame" describing each reagents. Obligatory columns: RID and TID.
targets: Object of class "data.frame" describing each target gene. Obligatory columns: TID, Symbol, group, GID.
templateDesign: Object of class "data.frame" with sgi@NT rows describing the template design. Obligatory columns: TemplatePlate, Well, RID, QueryNr.
queryDesign: Object of class "data.frame" with sgi@NQ rows describing the query design. Obligatory columns: Plate, TemplatePlate, QueryNr, RID.
transformation: Object of class "character" of length sgi@c. The transformation applied to the input data.
mainTemplate: Object of class "array" with dimension sgi@NT x sgi@s x sgi@c. The main effect of the template reagents.
mainQuery: Object of class "array" with dimension sgi@NQ x sgi@S x sgi@c. The main effect of the query reagents.
mainSderrTemplate: Object of class "array" with dimension sgi@NT x sgi@S x sgi@C. The standard error of the main effect of the template reagents.
mainSderrQuery: Object of class "array" with dimension sgi@NQ x sgi@s x sgi@c. The standard error of the main effect of the query reagents.
mainSdTemplate: Object of class "array" with dimension sgi@NQ x sgi@S x sgi@c. The standard deviation of the main effect of the query reagents.
mainSdQuery: Object of class "array" with dimension sgi@NQ x sgi@S x sgi@c. The standard deviation of the main effect of the query reagents.
mainTimeEffect: Object of class "array" with dimension sgi@NQ x sgi@S x sgi@c. The systematic changes of the query main effects, e.g. decreasing cell number over time.
mainSpatialEffect: Object of class "array" with dimension sgi@F x sgi@S x sgi@c. The systematic spatial plate effects.
mainSpatialEffectRow: Object of class "array". Spatial effects per row (as computed by Bscore).
mainSpatialEffectCol: Object of class "array". Spatial effects per column (as computed by Bscore).
mainNeg: Object of class "array" with dimension sgi@s x sgi@c. The main effect of the negative control.
mainNegTemplate: Object of class "array" with dimension sgi@S x sgi@c. The template main effect of the negative control.
mainNegQuery: Object of class "array" with dimension sgi@S x sgi@c. The query main effect of the negative control.
data2mainTemplate: Object of class "integer" with dimension sgi@F. Mapping of single experiments to template reagents.
data2mainQuery: Object of class "integer" with dimension sgi@F. Mapping of single experiments to query reagents.
ni.model: Object of class "array" with dimension sgi@F x sgi@s x sgi@c. The expected values of the non-interacting model.
pi: Object of class "array" with dimension sgi@ $\times$ sgi@s $\times$ sgi@c. The pairwise interaction score.
plateeffect: Object of class "array".
p.value: Object of class "array" with dimension sgi@NT x sgi@NQ x sgi@s x sgi@C describing the p.values.
q.value: Object of class "array" with dimension sgi@NT x sgi@NQ x sgi@S x sgi@C describing the q.values.

## Methods

show signature(object = "RNAinteract"): ..

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
    showClass("RNAinteract")
```

    sgi
        Dataset of class 'RNAinteract'
    
## Description

Sample object of class RNAinteract. The data are real but anonymized. The object contains two replicate screens with three readout channels.

## Usage

data(sgi)

## Format

The data contains two screens with three readout channels. The screen is performed on multiwell plates with $8 \times 12$ wells.

## Examples

```
data(sgi)
sgi
```

    sgisubset subset of an RNAinteract object.
    
## Description

A new object of class RNAinteract is created that contains a subset of screens and channels.

## Usage

sgisubset(sgi, screen = getScreenNames(sgi), channel = getChannelNames(sgi))

## Arguments

| sgi | An object of class RNAinteract. |
| :--- | :--- |
| screen | Names of the selected screens. |
| channel | Names of the selected channels. |

## Details

This function returns a RNAinteract object that only contains the selected screens and channels.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

```
data("sgi")
sgi
sgi1 <- sgisubset(sgi, screen = "1")
sgi1
sgi2 <- sgisubset(sgi, channel = "nrCells")
sgi2
```

sgisubsetQueryDesign Subsetting query genes in a RNAinteract object.

## Description

A RNAinteract object with a subset of query genes is returned.

## Usage

sgisubsetQueryDesign(sgi, query.targets = NULL, query.reagents = NULL)

## Arguments

## sgi

An RNAinteract object.
query.targets
The query target ids to be selected.
query. reagents The query reagent ids to be selected.

## Value

Returns an oject of class RNAinteract.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

```
startReport start and end a RNAinteract report
```


## Description

startReport will open a html page and starts writing an html report for a RNAinteract screen. endReport finishes the report and closes the html-file.

## Usage

startReport(outputpath)
endReport(report)

## Arguments

outputpath the path to the output directory were the report is written to.
report An report object as returned by startReport or any report... function.

## Details

$\sim \sim$ details $\sim \sim$

## Value

startReport returns an report object. It is handed over to each report-function.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package, reportAnnotation, reportStatistics, reportGeneLists, reportNetworks, reportScreenData, reportDoublePerturbation, reportMainEffects

## Examples

```
    data("sgi")
    report <- startReport("report")
    reportAnnotation(sgi, report = report)
    endReport(report)
    # browseURL(file.path("report","index.html"))
```

    summarizeScreens summarize screens
    
## Description

Creates a new object of class RNAinteract with one screen. The new screen is the mean of all screens in the input object.

## Usage

summarizeScreens(sgi, screens, newscreenname = "mean")

## Arguments

sgi An object of class RNAinteract.
screens The screen names to be summarized.
newscreenname The name of the new summary screen.

## Details

If multiple screens with the same layout are stored in the same RNAinteract object, then these screens are summarized by averaging to a new screen. The returned object contains one screen.

## Value

An object of class RNAinteract.

## Author(s)

Bernd Fischer

## See Also

RNAinteract-package

## Examples

data("sgi")
sgi
sginew <- summarizeScreens(sgi, screens=c("1","2"), newscreenname = "m")
sginew
swaptree Swaps a branch of a hclust object.

## Description

Swaps the left and right branch at a specified level of a dendrogram.

## Usage

swaptree(hc, level)

## Arguments

| hc | An hierarchical clustering object as produced by hclust. |
| :--- | :--- |
| level | The level to be swapped. |

## Value

Returns an hclust object.

## Author(s)

Bernd Fischer

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