# Package 'RNAinteract'

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Type Package

Title Estimate Pairwise Interactions from multidimensional features

**Version** 1.52.0

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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LazyLoad yes

**Imports** RColorBrewer, ICS, ICSNP, cellHTS2, geneplotter, gplots, grid, hwriter, lattice, latticeExtra, limma, methods, splots (>= 1.13.12), abind, locfit, Biobase

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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.

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## 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)

# Bernd Fischer

Maintainer: Bernd Fischer <br/> bernd.fischer@embl.de>

### 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.

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bindscreens

bind RNAinteract objects along screens

# 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

~put references to the literature/web site here ~

## See Also

RNAinteract-package

## **Examples**

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

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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

~put references to the literature/web site here ~

# See Also

```
RNAinteract-package
```

# **Examples**

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

6 computePValues

computePValues

compute p-values

# **Description**

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

#### Usage

#### **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

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 T^2 test.

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#### Value

An object of class RNAinteract.

#### Author(s)

Bernd Fischer

#### References

~put references to the literature/web site here ~

#### 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")</pre>
```

createCellHTSFromFiles

create cellHTS2 object from text files

# Description

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

#### Usage

## **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 pdim=c(16,24).

# **Details**

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

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#### Value

An object of class cellHTS2.

#### Author(s)

Bernd Fischer

#### References

~put references to the literature/web site here ~

#### 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**

#### **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 log2 trans-

formed.

#### **Details**

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

#### Value

An object of class RNAinteract.

#### Author(s)

Bernd Fischer

#### References

~put references to the literature/web site here ~

#### 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.

file Template Design

The filename of the text file containing the template design.

fileQueryDesign

The filename of the text file containing the query design.

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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(nrow=24, ncol=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

~put references to the literature/web site here ~

## See Also

RNAinteract-package, createRNAinteract, createCellHTSFromFiles

embedPCA	
----------	--

Computes a PCA for a pairwise interaction matrix.

# Description

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

# Usage

estimateMainEffect 11

## **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.

without groups Genes annotated with these group names 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))</pre>
```

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.

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#### **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

~put references to the literature/web site here ~

#### See Also

RNAinteract-package

#### **Examples**

```
data("sgi")
sgi <- estimateMainEffect(sgi)
getMain(sgi)</pre>
```

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)
```

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#### **Arguments**

sgi An object of class RNAinteract

type 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 sym-

metrized.

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.

without groups 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 di-

mension 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

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## 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)</pre>
Dplatelayout <- getData(sgi, type="data",</pre>
     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",</pre>
     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",</pre>
     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")</pre>
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")</pre>
```

getMain

get main effects

## Description

Returns the main effects.

#### **Usage**

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```
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 re-

turned.

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

without groups 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)
```

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getReplicateData	Extract replicates measurements from the screen.

#### **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

## **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 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

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# **Examples**

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

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")
```

getScreenNames

get names of screens and channels

## **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.

grid.doublePerturbation

#### 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)
```

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#### **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 The range of the plot. Equals the xlim, ylim arguments of plot.

main An overall title of the plot.

xlab 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.

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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 param-

eter 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 helust 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)
```

```
{\tt normalize Main Effect Query}
```

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 is a vector if integers with length equal to the number of queries. It as-

signs each query to a batch. Within each batch a linear regression is estimated 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

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)</pre>
```

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.

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#### **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)</pre>
```

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 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

## **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.

plotDoublePerturbation 25

design The Either "template" (default	or "query". The single perturbation effects
---------------------------------------	---------------------------------------------

are either the template main effects or the query main effects.

main An overall title of the plot.

xlab A title of the x-axis. ylab A title of the y-axis.

range A numeric vector of length two. range equals the xlim, ylim argument in plot.

show.labels Automatically select text labels for the points. 'all' shows a text label for all

genes, "q.value" and "p.value" show a text label for all genes with a q.value (p.value) larger than label.par, "none" does not show any text label. This

argument has no effect, if label is specified.

label.par Cut-off value for q.value or p.value for displaying text labels (See show. labels).

label Either a character vector with gene names, or a named vector of text labels. The

names of the vector represent the gene names.

avoid.overlap If TRUE (default), text is moved such that text labels are not overlapping.

col A named vector with colors. The names of col define which points are colored

(See also fill).

fill A list up to four values. colors defines a set of colors from which a colorramp is

created. If colramp is specified, colors has no effect. colramp directly specifies the colorramp. values define the values that are color coded. If values is not specified, the pairwise interaction term is used instead. at is a numeric vector defining the breakpoints along the values. If not specified, breakpoints are selected to range three times the standard deviation of the values around

zero. fill has no effect, if col is specified.

D, MT, MQ, PV, QV, PI

Internal usage.

... Further argument passed to grid.doublePerturbation or doublePerturbationGrob.

#### **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")
```

26 plotHeatmap

plotHeatmap plots a heatmap for an interaction screen.
--------------------------------------------------------

# Description

A heatmap of an interaction screen is plotted.

# Usage

# 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

reportAnnotation 27

#### **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

```
(sgi, verbose = 0, path = ".", dir = "annotation",
reportAnnotation
                        prefix = "annotation", report = NULL)
                        (sgi, verbose = 0, path = ".", dir = "stats",
reportStatistics
prefix = "stat", report = NULL)
                        (sgi, verbose = 0, path = ".", dir = "hitlist",
reportGeneLists
prefix = "hitlist", report = NULL)
reportNetworks
                        (sgi, verbose = 0, path = ".", dir = "networks",
prefix = "networks", Networks, qv = 0.05,
withoutgroups = c("pos", "neg"), report = NULL)
                        (sgi, type = "data", design = "template",
reportScreenData
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), ...)
                        (sgi, verbose = 0, path = ".", dir = "maineffects",
reportMainEffects
prefix = "maineffects",
png.args = list(width = 500, height = 500),
pdf.args = list(width = 7, height = 7),
plot.args = list(), report = NULL)
                        (sgi, verbose = 0, path = ".", dir = "heatmap",
reportHeatmap
prefix = "heatmap",
png.args = list(width = 1000, height = 1000),
pdf.args = list(width = 15, height = 15),
report = NULL, withoutgroups = c("neg", "pos"))
```

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#### **Arguments**

sgi An object of class RNAinteract.

verbose Either 0 (default, no output), 1 (minimum output), or 2 (outout)

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.

without groups 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 gen-

erated png images.

pdf.args A list with entries width and height specifying the width and height of the gen-

erated 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

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#### **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.

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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@F x sgi@S x 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"): ...

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#### 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 x 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</pre>
```

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.
```

startReport 33

## 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**

```
~~ details ~~
```

## 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

34 summarizeScreens

## **Examples**

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

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</pre>
```

swaptree 35

swaptree	Swaps a branch of a helust 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 helust object.

# Author(s)

Bernd Fischer

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