Package ‘RNAinteract’

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Type Package
Title Estimate Pairwise Interactions from multidimensional features
Version 1.22.0
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Maintainer Bernd Fischer <b.fischer@dkfz.de>
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 package 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)
Depends R (>= 2.12.0), abind, locfit, Biobase
biocViews CellBasedAssays, QualityControl, Preprocessing, Visualization
NeedsCompilation no

R topics documented:

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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
bindscreens

Main effects and pairwise interactions

- `sgisubset.sgisubsetQueryDesign`
- `bindscreens`
- `summarizeScreens`

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 <bernd.fischer@embl.de>

References


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**bindscreens**

*bind RNAinteract objects along screens*

**Description**

Bind two RNAinteract objects along screens.

**Usage**

```r
bindscreens(sgi1, sgi2)
```

**Arguments**

- **sgi1**: An object of class `RNAinteract`.
- **sgi2**: An object of class `RNAinteract`. 
computePI

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"), newscreennoname = "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.
**computePValues**

**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")

---
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**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>sgi</td>
<td>An object of class RNAinteract.</td>
</tr>
<tr>
<td>method</td>
<td>The method used to compute p-values. One of &quot;pooled.ttest&quot;, &quot;ttest&quot;, &quot;limma&quot;, &quot;HotellingT2&quot;. For &quot;ttest&quot; a Student t-test is applied for each gene pair. The variance is estimated locally for each gene pair. For &quot;pooled.ttest&quot;, 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 &quot;limma&quot; mediates between the local and the global variance estimation in a Bayesian framework. The limma-package is applied to compute the p-values. For &quot;HotellingT2&quot; 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.</td>
</tr>
<tr>
<td>mixTemplateQuery</td>
<td>If TRUE, the template query will be included in the output. If FALSE, only the p-values will be returned.</td>
</tr>
<tr>
<td>p.adjust.function</td>
<td>Function to adjust p-values.</td>
</tr>
<tr>
<td>verbose</td>
<td>Integer indicating the verbosity level.</td>
</tr>
</tbody>
</table>

---
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 (verbose).

Details
Computes p-values from a t-test, using the bioconductor package limma, or with a multidimensional Hotelling $T^2$ test.

Value
An object of class RNAinteract.

Author(s)
Bernd Fischer

References
~put references to the literature/web site here~

See Also
RNAinteract-package

Examples
```r
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 cellHTS2 object from text files

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

Usage
```r
createCellHTSFromFiles(filePlatelist = "Platelist.txt",
                        name = "anonymous",
                        path = ".", pdim = NULL)
```
createRNAinteract

Arguments

- **filePlatelist**: The platelist (See vignette("RNAinteract"))
- **name**: A (arbitrary) 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.

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

```r
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 log2 transformed.
createRNAinteractFromFiles

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`

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

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

arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>name</td>
<td>A name for the screen.</td>
</tr>
<tr>
<td>filePlatelist</td>
<td>The filename of the text file containing the plate list.</td>
</tr>
<tr>
<td>fileReagents</td>
<td>The filename of the text file containing the reagent annotation.</td>
</tr>
<tr>
<td>fileTargets</td>
<td>The filename of the text file containing the target annotation.</td>
</tr>
<tr>
<td>fileTemplateDesign</td>
<td>The filename of the text file containing the template design.</td>
</tr>
<tr>
<td>fileQueryDesign</td>
<td>The filename of the text file containing the query design.</td>
</tr>
</tbody>
</table>
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

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.

withoutgroups  Genes annotated with these groupnames are not considered for embedding.
estimateMainEffect

**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))

---

**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 very 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
**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**

```r
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`

- **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.
  - "plateffect": 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

**References**

-put references to the literature/web site here-

**See Also**

- `RNAinteract-package`

**Examples**

```r
data("sgi")
sgi <- estimateMainEffect(sgi)
getMain(sgi)
```
**getData**

- "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", do.inv.trafo = TRUE)
splots::plotScreen(Dplatelayout["1"][["nrCells"]], nx=sgi@pdim[2], ny=sgi@pdim[1], ncol=3)

Dmatrix <- getData(sgi, type="data", 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
getMain

```r
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")
```

---

### Description

Returns the main effects.

### Usage

```r
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)
```

```r
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**: Only 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)

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
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 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.
getScale

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")
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.
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 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 mainEffect 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)

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

Usage
```r
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.
normalizeMainEffectQuery

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 assigns 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.
normalizeMainEffectTemplate

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

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

---

**normalizePlateEffect**  
Normalization of plate effects

**Description**
Normalization of plate effects in the screen.

**Usage**

```r
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](https://cran.r-project.org/package=RNAinteract)

**Examples**

```r
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

plotDoublePerturbation( sgi, screen, channel, target,
withoutgroups = c("neg", "pos"), design,
main, xlab, ylab, range,
show.labels = "none", label.par, label,
avoid.overlap, col, fill,
D, MT, MQ, PV, QV, PI, ...)

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.
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).
plotHeatmap

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")

plotHeatmap

plots a heatmap for an interaction screen.

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 cannot 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 convenient 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")
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), reportDoublePerturbation(sgi, verbose = 0, path = ".", dir = "doublePerturbations", Networks, qv = 0.05, withoutgroups = c("neg", "pos"), report = NULL,
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@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"): ...

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)
sgisubset

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
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 object 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 ~~
summarizeScreens

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

Author(s)
Bernd Fischer

See Also

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

summarizeScreens summary 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

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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