

RNAither

April 20, 2011

BScore *BScore normalization*

Description

Normalization with BScores (see References).

Usage

```
BScore(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none">- a character string specifying the column whose values will be used for normalization- a flag specifying whether controls should be excluded for the computation of the median polish (1) or not (0) |

Value

A list containing:

| | |
|---------|---|
| header | The new header (with an added entry about the normalization procedure in the comments) |
| dataset | The new dataset with normalized values. The old values are saved in an extra column of the dataset with the suffix ".old" |

References

C. Brideau, B. Gunter, B. Pikounis, and A. Liaw. Improved statistical methods for hit selection in high-throughput screening. *J Biomol Screen*, 8:634-647, 2003

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")
normres <- BScore(header, dataset, list("SigIntensity", 0))
newheader <- normres[[1]]
newdataset <- normres[[2]]
```

channelPlot

*Plot signal channels against each other***Description**

Generates plots allowing pairwise comparison of signal channels. Fits a lowess regression curve into the plots.

Usage

```
channelPlot(header, dataset, vecOfChannels, flag, plotTitle, showPlot, smSpan=2/
```

Arguments

| | |
|---------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| vecOfChannels | A vector containing the names of the signal channels to be compared, e.g. "Sig-Intensity" |
| flag | 0, 1, or 2. 0 uses the data from the complete dataset, 1 makes comparisons for each experiment, 2 makes comparisons for each plate. |
| plotTitle | The plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |
| smSpan | The smoother span of the lowess curve. This gives the proportion of points in the plot which influence the smooth at each value. Larger values give more smoothness. Optional, defaults to 2/3 |

Value

Saves the plots in pdf and png files named after the experiment name specified in the header concatenated with the `plotTitle`, the number of the comparison, and if applicable the experiment number and/or the plate number.

When `flag == 0`, returns the plot name (`plotName`).

When `flag == 1`, returns a list containing:

| | |
|--------------|------------------------------------|
| plotName | The plot name |
| minOfScreens | The number of the first experiment |
| numOfScreens | The number of the last experiment |

When `flag == 2`, returns a list containing: the plot name, a vector with the number of the first experiment and of the last experiment, and a vector with the number of the first plate and the number of the last plate.

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")
plotname <- channelPlot(header, dataset, c("SigIntensity", "NbCells"), 0, "Channel compar
```

| | |
|---------------|---|
| closestToZero | <i>Return the replicate value closest to zero</i> |
|---------------|---|

Description

Out of a set of replicate values, returns the one closest to zero.

Usage

```
closestToZero(Ivec, na.rm = T)
```

Arguments

| | |
|-------|--|
| Ivec | All channel values for a specific siRNA/gene |
| na.rm | Removes NA values |

Value

A double giving the value closest to zero out of the given replicate values.

See Also

[rms](#), [trim](#), [furthestFromZero](#), [summarizeReps](#), [summarizeRepsNoFiltering](#)

Examples

```
data(exampleDataset, package="RNAiR")
Indexes <- findReplicates(dataset, "GeneName", "CPSF1")
replicateclosest <- closestToZero(dataset$SigIntensity[Indexes])
```

| | |
|-------------|--|
| compareHits | <i>Searching for common hits between different scoring methods</i> |
|-------------|--|

Description

Searches for common hits between different scoring methods.

Usage

```
compareHits(hitVec1, hitVec2, namesHitVec1, namesHitVec2)
```

Arguments

hitVec1, hitVec2
the two binary hit vectors to be compared

namesHitVec1, namesHitVec2
the names of the siRNAs corresponding to the hit vectors

Value

Returns a character vector indicating which siRNAs are identified as hits in two different hit scoring schemes.

See Also

[vennDiag](#), [Ttest](#), [MannWhitney](#)

Examples

```
data(scoredDataset1, package="RNAither")
data(pValVec1, package="RNAither")

data(scoredDataset2, package="RNAither")
data(pValVec2, package="RNAither")

##for details on the generation of pValVec and scoredDataset,
##see the examples of the functions Ttest and MannWhitney linked above.

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "Hits1", 0.05,
"GeneName", "pvalue_testfile1.txt")
scoredHits2 <- hitselectionPval(scoredDataset2, pValVec2, "SigIntensity", "Hits2", 0.05,
"GeneName", "pvalue_testfile2.txt")

hitVector1 <- scoredHits1[[2]]
hitVector2 <- scoredHits2[[2]]

common_hits <- compareHits(hitVector1, hitVector2, names(hitVector1), names(hitVector2))
```

compareReplicaPlates

Compare replica plates

Description

Generates plots comparing the same plates in different experiments pairwise.

Usage

```
compareReplicaPlates(header, dataset, plotTitle, col4val, showPlot)
```

Arguments

| | |
|-----------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| plotTitle | the plot title |
| col4val | a character string specifying the column whose values will be used for the plot |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

For each plate, plots of pairwise comparisons between replicate intensities are generated and saved as a pdf file named after the experiment name specified in the header concatenated with the `plotTitle`.

See Also

[compareReplicates](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

compareReplicaPlates(header, dataset, "Comparison of replica plate", "SigIntensity", 1)
```

```
compareReplicateSDPerScreen
```

Plot the standard deviation of replicates for each experiment

Description

In the same fashion as [spatialDistrib](#), generates plots of the standard deviation of replicate values for each experiment.

Usage

```
compareReplicateSDPerScreen(header, dataset, plotTitle, colname4SD, col4anno, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| plotTitle | the plot title |
| colname4SD | a character string specifying the column whose values will be used for the computation of the replicate standard deviation |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

Generates plots of the standard deviation of replicate values for each experiment. The plots are saved as png files named after the experiment name specified in the header concatenated with the `plotTitle` and the number of the experiment.

Wells showing positive controls sd are marked with a "P", wells showing negative controls sd with an "N".

The plots will also be saved as html files containing mouse-overs with the siRNA name for each well.

The function returns a list of length 3 containing:

```
basicPlotName      the plot name
minOfScreens       the number of the first experiment
numOfScreens       the number of the last experiment
```

See Also

[spatialDistrib](#), [compareReplicateSD](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

compareReplicateSDPerScreen(header, dataset, "Replicate standard intensity deviation",
"SigIntensity", "GeneName", 1)
```

`compareReplicateSD` *Plot the standard deviation of replicates*

Description

In the same fashion as [spatialDistrib](#), generates a plot of the standard deviation of replicate values.

Usage

```
compareReplicateSD(header, dataset, plotTitle, colname4SD, col4anno, showPlot)
```

Arguments

| | |
|-------------------------|---|
| <code>header</code> | the header of a dataset file generated with generateDatasetFile |
| <code>dataset</code> | an R data frame generated with generateDatasetFile |
| <code>plotTitle</code> | the plot title |
| <code>colname4SD</code> | a character string specifying the column whose values will be used for the computation of the replicate standard deviation |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| <code>showPlot</code> | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

Generates a plot of the standard deviation of replicate values of all experiments. The plot is saved as a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

Wells showing positive controls sd are marked with a "P", wells showing negative controls sd with an "N".

The plot will also be saved as an html file containing mouse-overs with the siRNA name for each well.

The function returns the plotname.

See Also

[spatialDistrib](#), [compareReplicateSDPerScreen](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

compareReplicateSD(header, dataset, "Replicate standard intensity deviation",
"SigIntensity", "GeneName", 1)
```

`compareReplicates` *Compare replicate values*

Description

Plots replicate intensities pairwise for each experiment.

Usage

```
compareReplicates(header, dataset, plotTitle, col4val, col4anno, plotDesign, showPlot)
```

Arguments

| | |
|-------------------------|---|
| <code>header</code> | the header of a dataset file generated with generateDatasetFile |
| <code>dataset</code> | an R data frame generated with generateDatasetFile |
| <code>plotTitle</code> | the plot title |
| <code>col4val</code> | a character string specifying the column whose values will be used for the plot |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| <code>plotDesign</code> | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots |
| <code>showPlot</code> | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

For each experiment, plots of pairwise comparisons between replicate intensities are generated and saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`, and the number of the experiment.

The function returns a list containing:

| | |
|--------------------------------|-------------------------------------|
| <code>plotName</code> | the plot name |
| <code>minOfScreens</code> | the number of the first experiment |
| <code>numOfScreens</code> | the number of the last experiment |
| <code>maxCombinationNum</code> | the number of replicates to compare |

See Also

[compareReplicaPlates](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

compareReplicates(header, dataset, "Comparison of Replicates", "SigIntensity", "GeneName")
```

controlDensityPerPlate

Plotting the control density per plate

Description

Plots the density of positive and negative controls (if applicable) for each plate.

Usage

```
controlDensityPerPlate(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|-------------------------|--|
| <code>header</code> | the header of a dataset file generated with generateDatasetFile |
| <code>dataset</code> | an R data frame generated with generateDatasetFile |
| <code>channel</code> | a character string specifying the name of the column containing the values for computing the density, e.g. "SigIntensity" |
| <code>plotTitle</code> | the plot title |
| <code>plotDesign</code> | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots |
| <code>showPlot</code> | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |
| <code>supHisto</code> | 0 or 1. 1 will additionally superimpose a colour histogram of the values for the positive and negative controls. Otherwise choose 0. |

Value

Generates a series of plots for each experiment and each plate, showing the density of positive and negative controls (if applicable). The plots are saved as pdf and png files named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list of length 3 containing:

```
plotName      the plot name
c(minOfScreens, numOfScreens)
                a vector with the number of the first experiment and of the last experiment
c(minOfPlates, numOfPlates)
                a vector with the number of the first plate and the number of the last plate
```

See Also

[controlDensity](#), [controlDensityPerScreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

controlDensityPerPlate(header, dataset, "SigIntensity", "Control density", 1, 1, 1)
```

```
controlDensityPerScreen
```

Plotting the control density per experiment

Description

Plots the density of positive and negative controls (if applicable) for each experiment.

Usage

```
controlDensityPerScreen(header, dataset, channel, plotTitle, showPlot, supHisto)
```

Arguments

| | |
|------------------------|--|
| <code>header</code> | the header of a dataset file generated with generateDatasetFile |
| <code>dataset</code> | an R data frame generated with generateDatasetFile |
| <code>channel</code> | a character string specifying the name of the column containing the values for computing the density, e.g. "SigIntensity" |
| <code>plotTitle</code> | the plot title |
| <code>showPlot</code> | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |
| <code>supHisto</code> | 0 or 1. 1 will additionally superimpose a colour histogram of the values for the positive and negative controls. Otherwise choose 0. |

Value

Generates a series of plots for each experiment, showing the density of positive and negative controls (if applicable). The plots are saved as pdf and png files named after the experiment name specified in the header concatenated with the `plotTitle` and the number of the experiment.

The function returns a list of length 3 containing:

```
plotName      the plotname
minOfScreens  the number of the first experiment
numOfScreens  the number of the last experiment
```

See Also

[controlDensity](#), [controlDensityPerPlate](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

controlDensityPerScreen(header, dataset, "SigIntensity", "Control density", 1, 1)
```

`controlDensity` *Plotting the control density*

Description

Plots the density of positive and negative controls (if applicable) for all controls contained in the dataset.

Usage

```
controlDensity(header, dataset, channel, plotTitle, showPlot, supHisto)
```

Arguments

```
header      the header of a dataset file generated with generateDatasetFile
dataset     an R data frame generated with generateDatasetFile
channel     a character string specifying the name of the column containing the values for
            computing the density, e.g. "SigIntensity"
plotTitle   the plot title
showPlot    0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save
            the plot(s) without opening windows
supHisto    0 or 1. 1 will additionally superimpose a colour histogram of the values for the
            positive and negative controls. Otherwise choose 0.
```

Value

Plots the density of positive and negative controls (if applicable) for all controls contained in the dataset. The plot is saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[controlDensityPerScreen](#), [controlDensityPerPlate](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

controlDensity(header, dataset, "SigIntensity", "Control density", 1, 1)
```

| | |
|-------------|----------------------------------|
| controlNorm | <i>Normalization on controls</i> |
|-------------|----------------------------------|

Description

Performs a normalization on either positive or negative controls.

Usage

```
controlNorm(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column whose values will be used for normalization - 1 or 2, 1 meaning a normalization per experiment, 2 meaning a normalization per plate - 0 or 1, 0 meaning a normalization on the median of negative controls, 1 meaning a normalization on the median of positive controls. Can also be the GeneName of a specific control siRNA - 1 or 2, 1 meaning the signal values are divided by the median, 2 meaning the median is subtracted from the signal values |

Value

Returns a list containing:

| | |
|---------|---|
| header | the new header (with an added entry about the normalization procedure in the comments). |
| dataset | the new dataset with normalized values. The old values are saved in an extra column with the suffix ".old". |

Examples

```

data(exampleHeader, package="RNAiether")
data(exampleDataset, package="RNAiether")

normres <- controlNorm(header, dataset, list(2, 0, "SigIntensity", 1))

newheader <- normres[[1]]
newdataset <- normres[[2]]

```

createSubset

Creating a subset of a dataset according to a certain column value

Description

Creates a subset of a dataset containing all wells/lines having a certain value in a specified column.

Usage

```
createSubset(dataset, listIDs, equalTo)
```

Arguments

| | |
|---------|---|
| dataset | an R data frame generated with generateDatasetFile |
| listIDs | a character string and one of the following: Spotnumber, Internal_GeneID, GeneName, SpotType, SigIntensity, SDSIntensity, Background, LabtekNb, RowNb, ColNb, ScreenNb, NbCells, PercCells, ... |
| equalTo | A value or character string specifying the value in the chosen column, e.g. all wells on plate 2 |

Value

A subset of the dataset containing only the wells/lines having a certain value in a specified column.

See Also

[indexSubset](#)

Examples

```

data(exampleDataset, package="RNAiether")

subset <- createSubset(dataset, dataset$LabtekNb, 2)

```

datasetDrosophila *Genome-wide RNAi screen of cell viability in Drosophila Kc167 cells by Boutros et al.*

Description

M. Boutros et al., Genome-wide RNAi analysis of growth and viability in Drosophila cells, Science, 303(5659):832-835, 2004. 3, 18

Usage

```
datasetDrosophila
```

Format

see [generateDatasetFile](#) for details

dataset *a typical example RNAi dataset*

Description

See [generateDatasetFile](#) for details

Usage

```
dataset
```

Format

See [generateDatasetFile](#)

discardLabtek *Remove a complete plate from the analysis*

Description

Removes a plate/LabTek from the analysis by setting its spot type in the dataset to -1.

Usage

```
discardLabtek(data, screenNr, labtekNr)
```

Arguments

| | |
|----------|--|
| data | an R data frame generated with generateDatasetFile |
| screenNr | the number of the experiment that contains the plate to discard |
| labtekNr | the number of the plate to discard |

Value

A new dataset that still contains the specified plate/LabTek, but excludes it from the further analysis by setting its `SpotTypes` to -1.

See Also

[discardWells](#)

Examples

```
data(exampleDataset, package="RNAiR")  
  
newdataset <- discardLabtek(dataset, 2, 2)
```

| | |
|--------------|---------------------------------------|
| discardWells | <i>Remove wells from the analysis</i> |
|--------------|---------------------------------------|

Description

Removes wells from the analysis by setting their spot type in the dataset to -1.

Usage

```
discardWells(data, screenNr, labtekNr, vecPositions)
```

Arguments

| | |
|---------------------------|--|
| <code>data</code> | an R data frame generated with generateDatasetFile |
| <code>screenNr</code> | the number of the experiment that contains the plate to discard |
| <code>labtekNr</code> | the number of the plate to discard |
| <code>vecPositions</code> | a vector specifying the numbers of the wells to discard |

Value

A new dataset that does not contain the specified wells. A new dataset that still contains the specified wells/spots, but excludes them from the further analysis by setting their `SpotTypes` to -1.

See Also

[discardLabtek](#)

Examples

```
data(exampleDataset, package="RNAiR")  
  
newdataset <- discardWells(dataset, 2, 1, c(1, 10, 15))
```

| | |
|----------------|------------------------------|
| divideChannels | <i>Divide channel values</i> |
|----------------|------------------------------|

Description

Replace two channels by their ratio.

Usage

```
divideChannels(ch1, ch2)
```

Arguments

| | |
|-----|---|
| ch1 | a vector giving all values from channel 1 |
| ch2 | a vector giving all values from channel 2 |

Value

A vector of the ratio of channel 1 and channel 2.

See Also

[sumChannels](#)

Examples

```
data(exampleDataset, package="RNAiR")  
newch <- divideChannels(dataset$SigIntensity, dataset$NbCells)
```

| | |
|---------|--|
| divNorm | <i>Mean, median, ... , normalization</i> |
|---------|--|

Description

Normalization with the mean, median, or any other function.

Usage

```
divNorm(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with <code>generateDatasetFile</code> |
| dataset | an R data frame generated with <code>generateDatasetFile</code> |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column whose values will be used for normalization - a function to be used for the normalization, e.g. <code>mean</code>, <code>median</code>, ... - 1 or 2, 1 meaning a normalization per experiment, 2 meaning a normalization per plate - 1 or 2, 1 meaning the normalization is achieved by a division of the intensity values by the outcome of <code>funname</code>, 2, meaning by a subtraction - a flag specifying whether controls should be excluded for the computation of the result of the function specified in the first element (1) or not (0). |

Value

Returns a list containing:

| | |
|---------|--|
| header | the new header (with an added entry about the normalization procedure in the comments) |
| dataset | the new dataset with normalized values. The old values are saved in an extra column with the suffix ".old" |

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- divNorm(header, dataset, list(median, 2, 1, "SigIntensity", 1))

newheader <- normres[[1]]
newdataset <- normres[[2]]
```

Description

Computes the dynamic range per plate for a complete dataset file and plots the results.

Usage

```
DRQualControl(header, data, nbLinesHeader, channel, plotTitle, showPlot)
```


Arguments

| | |
|---------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| data | an R data frame generated with generateDatasetFile |
| nbLinesHeader | typically 3 |
| channel | A character string specifying the name of the column containing the values for computing the dynamic range, e.g. "SigIntensity" |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

Returns the dynamic range for each plate in the shell and saves them in a text file. The name of the text file will be the concatenation of the experiment name specified in the header and the character string "DR.txt".

Shows a plot of the dynamic range values and saves it as a pdf file under the experiment name specified in the header concatenated with the function argument `plotTitle`.

References

M. Boutros, L. Bras, and W. Huber. Analysis of cell-based RNAi screens. *Genome Biol*, 7(7): R66, 2006.

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

DRQualControl(header, dataset, 3, "SigIntensity", "DR per plate", 1)
```

eraseDataSetColumn *Remove columns from dataset*

Description

Removes a specified column from a dataset.

Usage

```
eraseDataSetColumn(dataset, colname)
```

Arguments

| | |
|---------|--|
| dataset | an R data frame generated with generateDatasetFile |
| colname | a character string specifying the name of the column to be removed |

Value

An R data frame with the specified column removed.

Examples

```
data(exampleDataset, package="RNAiR")
newdataset <- eraseDataSetColumn(dataset, "SDSIntensity")
```

```
findReplicates      Find all replicates of a certain siRNA/gene in a dataset
```

Description

Returns which lines in the dataset correspond to a given siRNA/gene ID.

Usage

```
findReplicates(dataset, whichCol, replicateID)
```

Arguments

`dataset` an R data frame generated with [generateDatasetFile](#)
`whichCol` a character string specifying the name of the column containing the ID, either `Internal_GeneID` or `GeneName`
`replicateID` the siRNA/gene ID of interest

Value

An integer vector containing the indexes in the main dataset of all wells corresponding to a given siRNA/gene ID

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

Indexes <- findReplicates(dataset, "GeneName", "CPSF1")
```

```
furthestFromZero  Return the replicate value furthest from zero
```

Description

Out of a set of replicate values, returns the one furthest from zero.

Usage

```
furthestFromZero(Ivec, na.rm = T)
```

Arguments

`Ivec` All channel values for a specific siRNA/gene
`na.rm` Removes NA values

Value

A double giving the value furthest from zero out of the given replicate values.

See Also

[rms](#), [trim](#), [closestToZero](#), [summarizeReps](#), [summarizeRepsNoFiltering](#)

Examples

```
data(exampleDataset, package="RNAiR")

Indexes <- findReplicates(dataset, "GeneName", "CPSF1")
replicateClosest <- furthestFromZero(dataset$SigIntensity[Indexes])
```

```
generateDatasetFile
      Generate Dataset File
```

Description

Generates a text file containing all experimental data. Needed for all subsequent analysis functions.

Usage

```
generateDatasetFile(externalExperimentName, typeOfData, comments, outputFile,
  plateLayoutInternal, plateLayoutNCBI, nbRowsPerPlate, nbColsPerPlate, screenNb_pre,
  emptyWells, poorWells, controlCoordsOutput, backgroundValOutput, meanSignalOutput,
  SDmeanSignal, objNumOutput, cellNumOutput)
```

Arguments

| | |
|------------------------|---|
| externalExperimentName | A character string specifying the experiment name, e.g. "Johns Experiment Nb. 1" |
| typeOfData | A character string specifying the type of data, e.g. "364 well plate data for virus screens" |
| comments | A character string specifying comments. NA if not available. |
| outputFile | A character string specifying the name of the text file containing the dataset. |
| plateLayoutInternal | A matrix of internal siRNA IDs specifying their position on the plate (row-wise). Each column of the matrix stands for one plate. |
| plateLayoutNCBI | A matrix of gene names specifying their position on the plate (row-wise). Each column of the matrix stands for one plate. |
| nbRowsPerPlate | The number of rows per plate |
| nbColsPerPlate | The number of columns per plate |
| screenNb_pre | The screen/experiment number |

| | |
|---------------------|---|
| emptyWells | A list containing, for each plate, an integer vector of the positions of empty wells. NA if there are no empty wells on the plate. |
| poorWells | A list containing, for each plate, an integer vector of the positions of wells that, for a certain reason, should not be taken into account during the analysis. NA if there are no such wells on the plate. |
| controlCoordsOutput | A list containing, for each plate, a list of integer vectors specifying the positions of positive (first element in sublist) and negative (second element in sublist) controls. NA if there are no positive/negative controls on the plate. |
| backgroundValOutput | A list containing, for each plate, a vector of background values per well |
| meanSignalOutput | A list containing, for each plate, a vector of intensity values for each well |
| SDmeanSignal | A list containing, for each plate, a vector of standard deviations of intensity values for each well |
| objNumOutput | A list containing, for each plate, a vector of the number of identified objects for each well |
| cellNumOutput | A list containing, for each plate, a vector of intensity values for each well, e.g. a vector of the number of identified cells for each well. |

Details

Positions on plates are specified with one integer only. For example, the position of the well in row 2 and column 5 is $(\text{RowNo}-1) * (\text{Number of columns on plate}) + \text{ColNo}$.

Value

The function generates a text file consisting of a header and a 'dataset'. The header contains the experiment description (`ExternalExperimentName`, `TypeOfData` and `Comments`). The dataset is an R data frame, each row corresponding to one well, with the following columns:

| | |
|-----------------|--|
| Spotnumber | The position of the well on the plate |
| Internal_GeneID | The ID of the siRNA |
| GeneName | The gene name |
| SpotType | Can be -1, 0, 1 or 2. Type -1 wells (e.g. empty wells, wells with poor quality) are not considered in subsequent analyses but are kept in the data set for the sake of completeness. Type 0 wells correspond to negative controls, type 1 wells to positive controls. Type 2 wells correspond to the standard data wells. |
| SigIntensity | The signal intensity (channel 1) |
| SDSIntensity | The standard deviation of the signal intensity, if available |
| Background | The background per well, if available |
| LabtekNb | The plate number |
| RowNb | The row number |
| ColNb | The column number |
| ScreenNb | The screen number |
| NbCells | E.g. the number of cells identified in the well (channel 2) |
| PercCells | The ratio (number of identified cells)/(number of identified objects) |

See Also

[joinDatasetFiles](#), [joinDatasets](#)

Examples

```
##gene names
plateLayout1 <- c("test1", "empty", "test3", "test4", "test5",
"test6", "test7", "empty", "test9", "test10", "test11", "test12")

plateLayout2 <- c("test1", "test2", "test3", "test4", "test5",
"test6", "test7", "test8", "test9", "test10", "test11", "test12")

plateLayout <- cbind(plateLayout1, plateLayout2)

emptyWells <- list(c(2, 8), NA_integer_)
##the first plate has two empty wells at position 2 and 8,
##the second plate does not have any empty wells

poorWells <- NA_integer_
##no wells of poor quality

controlCoordsOutput <- list(list(NA_integer_, NA_integer_), list(NA_integer_, c(9,10)))
##the first plate does not have any control siRNAs,
##the second plate has two negative controls at position 9 and 10

backgroundValOutput<-NA_integer_
##no background signal intensities available

sigPlate1<-c(2578, NA_integer_, 3784, 3784, 2578, 5555, 5555, NA_integer_, 8154, 2578, 3784, 3784)
sigPlate2<-c(8154, 3784, 5555, 3784, 11969, 2578, 1196, 5555, 17568, 2578, 5555, 2578)
##the signal intensities on the plates

meanSignalOutput<-list(sigPlate1, sigPlate2)

SDmeansignal<-NA_integer_
##no standard deviation available

objnumOutput<-NA_integer_
##no cell count available

cellnumOutput<-NA_integer_

generateDatasetFile("First test screen", "RNAi in virus-infected cells",
NA_character_, "testscreen_output.txt", plateLayout, plateLayout, 3, 4,
1, emptyWells, poorWells, controlCoordsOutput, backgroundValOutput,
meanSignalOutput, SDmeansignal, objnumOutput, cellnumOutput)

##load the dataset into R:
header<-readLines("testscreen_output.txt",3)
dataset<-read.table("testscreen_output.txt", skip=3, colClasses=c(NA, NA, NA, NA,
"factor", NA, NA, NA, NA, NA, NA, NA, NA, NA), stringsAsFactors=FALSE)
```

`generateReplicateMat`*Generate a matrix of replicates*

Description

Generates a matrix out of a dataset, each row corresponding to an siRNA/gene ID, each column to a channel value or its index in the dataset.

Usage

```
generateReplicateMat(data, minNbReps, IndexOrInt, col4val, col4anno)
```

Arguments

| | |
|-------------------------|---|
| <code>data</code> | an R data frame generated with generateDatasetFile |
| <code>minNbReps</code> | set to 2 if you want to exclude replicates occurring only once in the dataset, otherwise 1. |
| <code>IndexOrInt</code> | a character string - either "Index" or "Intensities" - specifying which values are to be contained in the output matrix. |
| <code>col4val</code> | a character string specifying the name of the dataset column to be used for the values of the output matrix (if <code>IndexOrIntensities</code> is set to "Intensities"), for example "SigIntensity" or "NbCells" |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used for the output matrix' rows, for example "GeneName" or "Internal_GeneID". |

Details

The function will omit values or indexes of lines/wells whose value in the column specified by `colname4val` is set to NA, (which is the case if the spot type is set to -1). If you do not want to omit those, use [generateRepMatNoFilter](#).

Value

A matrix with each row corresponding to an siRNA/gene ID (as reflected in rownames), each column to a channel value or its index in the dataset. Missing values (in case of different number of replicates occurring for different siRNAs/genes) are set to NA.

See Also

[generateRepMatNoFilter](#)

Examples

```
data(exampleDataset, package="RNAiR")
```

```
replicatematrix <- generateReplicateMat(dataset, 2, "Index", "SigIntensity", "GeneName")
```

`generateRepMatNoFilter`*Generate a matrix of replicates (II)*

Description

Generates a matrix out of a dataset, each row corresponding to an siRNA/gene ID, each column to a channel value or its index in the dataset.

Usage

```
generateRepMatNoFilter(data, minNbReps, IndexOrInt, col4val, col4anno)
```

Arguments

| | |
|-------------------------|---|
| <code>data</code> | an R data frame generated with generateDatasetFile |
| <code>minNbReps</code> | set to 2 if you want to exclude replicates occurring only once in the dataset, otherwise 1. |
| <code>IndexOrInt</code> | a character string - either "Index" or "Intensities" - specifying which values are to be contained in the output matrix. |
| <code>col4val</code> | a character string specifying the name of the dataset column to be used for the values of the output matrix (if <code>IndexOrIntensities</code> is set to "Intensities"), for example "SigIntensity" or "NbCells" |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used for the output matrix' rows, for example "GeneName" or "Internal_GeneID". |

Details

The function will not omit values or indexes of lines/wells with spot type -1. If you want to omit those, use `generateReplicateMatrix`.

Value

A matrix with each row corresponding to an siRNA/gene ID (as reflected in rownames), each column to a channel value or its index in the dataset. Missing values (in case of different number of replicates occurring for different siRNAs/genes) are set to NA.

See Also

[generateReplicateMat](#)

Examples

```
data(exampleDataset, package="RNAiR")  
replicatematrix <- generateRepMatNoFilter(dataset, 2, "Index", "SigIntensity", "GeneName")
```

gseaAnalysis *Perform a GSEA analysis of a list of genes*

Description

Performs a GSEA analysis of a list of genes using the package `topGO` (see References).

Usage

```
gseaAnalysis(hitVector, whichOnto)
```

Arguments

`hitVector` a named hit vector as generated by [hitselectionZscore](#) or [hitselectionPval](#)
`whichOnto` One of the three GO ontologies: `"biological_process"`, `"molecular_function"`
or `"cellular_component"`

Value

A table containing the enriched GO terms and their significance.

References

A. Alexa, J. Rahnenfuhrer and T. Lengauer. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics*, 22(13):1600-1607, 2006

Adrian Alexa and Jorg Rahnenfuhrer (2006). `topGO`: Enrichment analysis for Gene Ontology. R package version 1.4.0.

See Also

[Ttest](#)

Examples

```
data(scoredDataset1, package="RNAiather")
data(pValVec1, package="RNAiather")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "Hits1", 0.1,
"GeneName", "pvalue_testfile1.txt")
hitVector1 <- scoredHits1[[2]]
gseaTable <- gseaAnalysis(hitVector1, "biological_process")
```

headerDrosophila *the header of the genome-wide RNAi screen of cell viability in Drosophila Kc167 cells by Boutros et al.*

Description

M. Boutros et al., Genome-wide RNAi analysis of growth and viability in Drosophila cells, Science, 303(5659):832-835, 2004. 3, 18

Usage

headerDrosophila

Format

See [generateDatasetFile](#)

header *a typical header of an example RNAi dataset*

Description

See [generateDatasetFile](#) for details

Usage

header

Format

See [generateDatasetFile](#)

hitselectionPval *Selecting hits according to p-values*

Description

Selects significant genes according to their p-value.

Usage

hitselectionPval(dataset, pValVec, col4val, col4sel, thresh, col4anno, file4hits)

Arguments

| | |
|-----------|--|
| dataset | an R data frame generated with generateDatasetFile |
| pValVec | a vector of p-values, as generated by one of the test functions Ttest , MannWhitney or RankProduct |
| col4val | a character vector specifying a column of intensity values |
| col4sel | a character vector specifying the name of the new dataset column where hits will be stored |
| thresh | the threshold for the p-values, typically 0.05 |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| file4hits | the name of the file to store the results in |

Details

If there are no p-values under the defined threshold `thresh`, the threshold is increased to `min(pvalvec)`.

Value

A list containing:

| | |
|---------------|--|
| dataset | the dataset with an added column defining the hits in the form of a binary vector |
| hitVector | the binary vector itself |
| replicaMatrix | a matrix of replicates with corresponding values (as generated by generateReplicateMat) |
| thresh | the threshold for the p-values |

P-values and the intensity values for each siRNA are stored in a text output file.

See Also

[hitselectionZscore](#), [hitselectionZscorePval](#), [Ttest](#)

Examples

```
data(scoredDataset1, package="RNAither")
data(pValVec1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "Pval_hits", 0.
"GeneName", "pvalue_testfile1.txt")

newdataset <- scoredHits1[[1]]
hitvector <- scoredHits1[[2]]
```

hitselectionZscorePval

Selecting hits according to ZScores and p-values

Description

Selects significant genes according to their ZScore (summarized with the gene median) and p-values.

Usage

```
hitselectionZscorePval(dataset, pValVec, col4zscore, col4sel, thresh, thresh2,
flag2, col4anno, sumFunc, file4hits)
```

Arguments

| | |
|------------|---|
| dataset | an R data frame generated with generateDatasetFile |
| pValVec | a vector of p-values, as generated by one of the test functions Ttest , MannWhitney or RankProduct |
| col4zscore | a character vector specifying the name of the column containing the ZScores, usually "SigIntensity" |
| col4sel | a character vector specifying the name of the new dataset column where hits will be stored |
| thresh | the threshold for the ZScores. The interpretation depends on the choice of the parameter <code>flag2</code> . |
| thresh2 | the threshold for the p-values |
| flag2 | 2 or -2. If 2 is chosen, all Zscores greater than or equal to <code>thresh</code> are chosen. If -2 is chosen, all Zscores smaller than or equal to <code>thresh</code> are chosen. |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, for example "GeneName" or "Internal_GeneID". |
| sumFunc | the function used to summarize ZScore values, e.g. <code>mean</code> or <code>median</code> . |
| file4hits | the name of the file to store the results in |

Details

If there are no p-values under the defined threshold `thresh2`, it is increased to `min(pvalvec)`.

If `flag2 == -2` and there are no ZScores under the defined threshold `thresh`, it is increased to `min(ZScores)`.

If `flag2 == 2` and there are no ZScores over the defined threshold `thresh`, it is increased to `max(ZScores)`.

If there are not hits for the combined threshold of p-values and ZScores, the ZScore threshold is changed until there is a hit.

Value

A list containing:

| | |
|-----------|---|
| dataset | the dataset with an added column defining the hits in the form of a binary vector |
| hitVector | the binary vector itself |
| thresh | the threshold for the ZScores |
| thresh2 | the threshold for the p-values |

ZScores and p-values are stored in a text output file.

See Also

[hitselectionPval](#), [hitselectionZscore](#), [Ttest](#)

Examples

```
data(scoredDataset1, package="RNAither")
data(pValVec1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionZscorePval(scoredDataset1, pValVec1, "SigIntensity",
  "Zscore_pval_hits", -1.5, 0.05, -2, "GeneName", median, "Zscores_pvals_testfile1.txt")

newdataset <- scoredHits1[[1]]
hitvector <- scoredHits1[[2]]
```

hitselectionZscore *Selecting hits according to ZScores*

Description

Selects significant genes according to their ZScore.

Usage

```
hitselectionZscore(dataset, col4zscore, col4sel, thresh, flag, flag2, col4anno,
  sumFunc, file4hits)
```

Arguments

| | |
|------------|---|
| dataset | an R data frame generated with generateDatasetFile |
| col4zscore | a character vector specifying the name of the column containing the ZScores, usually SigIntensity |
| col4sel | a character vector specifying the name of the new dataset column where hits will be stored |
| thresh | the threshold for the ZScores. The interpretation depends on the choice of the parameter flag2. |
| flag | 1 or 2. 1 means the ZScores are kept per well, 2 that they are summarized according to the parameter sumFunc. |

| | |
|-----------|---|
| flag2 | 1, 2 or -2. If 1 is chosen and <code>thresh == n</code> , then the <code>n</code> greatest Zscores are chosen as hits. If 1 is chosen and <code>thresh == -n</code> , then the <code>n</code> smallest Zscores are chosen. If 1 is chosen and <code>thresh == 0</code> , all ZScores are chosen and written to the output file. If 2 is chosen, all Zscores greater than or equal to <code>thresh</code> are chosen. If -2 is chosen, all Zscores smaller than or equal to <code>thresh</code> are chosen. |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, for example <code>"GeneName"</code> or <code>"Internal_GeneID"</code> |
| sumFunc | the function used to summarize ZScore values, e.g. <code>mean</code> or <code>median</code> . |
| file4hits | the name of the file to store the results in |

Details

If `flag2 == -2`, and there are no ZScores under the defined threshold `thresh`, the threshold is increased to `min(ZScores)`.

If `flag2 == 2`, and there are no ZScores over the defined threshold `thresh`, the threshold is increased to `max(ZScores)`.

Value

A list containing:

| | |
|-----------|---|
| dataset | the dataset with an added column defining the hits in the form of a binary vector |
| hitVector | the binary vector itself |
| thresh | the threshold for the ZScores |

ZScores are stored in a text output file.

References

N. Malo et al. Statistical practice in high-throughput screening data analysis. Nature Biotech, 24(2): 167-175, 2006.

See Also

[hitselectionPval](#), [hitselectionZscorePval](#), [Ttest](#)

Examples

```
data(scoredDataset1, package="RNAither")
data(pValVec1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionZscore(scoredDataset1, "SigIntensity", "Zscore_hits", -10,
2, 1, "GeneName", median, "Zscores_testfile1.txt")

newdataset <- scoredHits1[[1]]
hitvector <- scoredHits1[[2]]
```

incorporatepValVec *Incorporate a vector of p-values into a dataset*

Description

Incorporates a vector of p-values into a dataset. Also works with a dataset containing values per well (non summarized), or with a hit vector.

Usage

```
incorporatepValVec(dataset, pValVec, replicaMatrix, col4anno, colname4pval)
```

Arguments

| | |
|---------------|--|
| dataset | an R data frame generated with generateDatasetFile |
| pValVec | a vector of p-values |
| replicaMatrix | a matrix of replicate values, as generated by generateReplicateMat |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, for example "GeneName" or "Internal_GeneID" |
| colname4pval | a character string specifying the name of the dataset column the p-values will be stored in |

Value

Returns the dataset with an added column of p-values.

See Also

[multTestAdjust](#), [Ttest](#)

Examples

```
data(exampleDataset, package="RNAither")

data(scoredDataset1, package="RNAither")
##scoredDataset1 already contains the p-value column
data(pValVec1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

temp <- generateReplicateMat(dataset, 1, "Intensities", "SigIntensity", "GeneName")
replicamatrix <- temp[[1]]
newdataset <- incorporatepValVec(dataset, pValVec1, replicamatrix, "GeneName", "pvals")
##newdataset and scoredDataset1 are now equivalent
```

indexSubset *Saving the indexes of a subset in the main dataset*

Description

Used together with `createSubset`, returns the indexes in the main dataset of the wells chosen as a subset by the previous call of `createSubset`.

Usage

```
indexSubset(listIDs, equalTo)
```

Arguments

| | |
|---------|---|
| listIDs | a character string and one of the following: Spotnumber, Internal_GeneID, GeneName, SpotType, SigIntensity, SDSIntensity, Background, LabtekNb, RowNb, ColNb, ScreenNb, NbCells, PercCells, ... |
| equalTo | A value or character string specifying the value in the chosen column, e.g. all wells on plate 2 |

Value

An integer vector containing the indexes in the main dataset of the wells chosen as a subset by the previous call of `createSubset`.

See Also

`createSubset`

Examples

```
data(exampleDataset, package="RNAiR")

subset <- createSubset(dataset, dataset$LabtekNb, 2)
indexOfSubsetInDataset <- indexSubset(dataset$LabtekNb, 2)
```

joinDatasetFiles *Join dataset files*

Description

Merges two or more dataset files into one, with one common header.

Usage

```
joinDatasetFiles(listOfFiles, nbOfLinesInHeader, newHead, outputFile)
```

Arguments

`listOfFiles` a list of the names of the files to join
`nbOfLinesInHeader`
 typically 3
`newHead` the new header
`outputFile` the name of the file to save the header and concatenated dataset in

See Also

[generateDatasetFile](#), [joinDatasets](#)

Examples

```

data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")
saveDataset(header, dataset, "save_testfile1.txt")

header[[1]] <- "external_experiment_name,Test screen"
header[[2]] <- "comments,contains twice Screen Nb 1"

joinDatasetFiles(list("save_testfile1.txt", "save_testfile1.txt"), 3, header,
"concatenated_testfile.txt")

```

| | |
|---------------------------|----------------------|
| <code>joinDatasets</code> | <i>Join datasets</i> |
|---------------------------|----------------------|

Description

Merges two or more datasets into one.

Usage

```
joinDatasets(listOfDatasets)
```

Arguments

`listOfDatasets`
 a list of the datasets to join

Value

The joined datasets.

See Also

[generateDatasetFile](#), [joinDatasetFiles](#)

Examples

```

data(exampleDataset, package="RNAither")
doubledataset <- joinDatasets(list(dataset, dataset))

```

`LiWongRank`*Li Wong rank / invariant probeset normalization*

Description

Performs a Li Wong rank / invariant probeset normalization (see References).

Usage

```
LiWongRank(header, dataset, listOfArgs)
```

Arguments

| | |
|-------------------------|---|
| <code>header</code> | the header of a dataset file generated with <code>generateDatasetFile</code> |
| <code>dataset</code> | an R data frame generated with <code>generateDatasetFile</code> |
| <code>listOfArgs</code> | a list containing: <ul style="list-style-type: none">- a character string specifying the column whose values will be used for normalization- a character string specifying the name of the dataset column to be used for the computation of the siRNA/gene ranks |

Details

For each plate type/layout in each experiment, generates a ranked list of siRNAs according to their intensity values. Only siRNAs occurring only once on the plate are allowed in the list. The normalization is performed only if all plate types have a maximum of 20

For each "unique" siRNA on a plate type, the variance of its ranks across plates is computed. A histogram of variances is plotted and allows the user to choose a threshold. A list of siRNAs with rank variances under the given threshold is then returned for each plate type so that the user can choose an siRNA to normalize the plate with.

Value

Returns a list containing:

| | |
|----------------------|--|
| <code>header</code> | the new header (with an added entry about the normalization procedure in the comments) |
| <code>dataset</code> | the new dataset with normalized values. The old values are saved in an extra column with the suffix ".old" |

References

C. Li and WH Wong. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. *Genome Biol*, 2(8):research0032.1-0032.11, 2001.

E. Schadt, C. Li, B. Ellis, and WH Wong. Feature Extraction and Normalization Algorithms for High-Density Oligonucleotide Gene Expression Array Data. *J Cell Biochem Suppl*, 37:120-125, 2001.

Examples

```

data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- LiWongRank(header, dataset, list("SigIntensity", "GeneName"))
newheader=normres[[1]]
newdataset=normres[[2]]

```

lowessNorm

Lowess normalization

Description

Performs a plate-wise lowess normalization of the data.

Usage

```
lowessNorm(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column used as channel 1 (colname4ch1) - a character string specifying the column used as channel 2 (colname4ch2) - optionally: the smoother span (smSpan) of the lowess function. This gives the proportion of points which influence the smooth at each value. Larger values give more smoothness. Defaults to 2/3. |

Value

Corrects intensity values in case the values of ch2 decrease with the increase of ch1 values.

Returns a list containing:

| | |
|---------|--|
| header | the new header (with an added entry about the normalization procedure in the comments) |
| dataset | the new dataset with normalized values. The old values are saved in an extra column with the suffix ".old" |

Examples

```

data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- lowessNorm(header, dataset, list("NbCells", "SigIntensity"))
newheader <- normres[[1]]
newdataset <- normres[[2]]

```

| | |
|--------------|---|
| mainAnalysis | <i>Wrapper function for full automated analysis</i> |
|--------------|---|

Description

Performs a standard analysis of the data (quality and statistics) from a dataset file.

Usage

```
mainAnalysis(header, dataset, flagForSameExp, listOfNormalizations, listOfArgs4norm,
listOfStatTests, listOfArgs4stat, multTestAdj, hitScoringVec1, hitScoringVec2,
posNegFlag, flag4Gsea, vecOfChannels, whichOnto)
```

Arguments

| | |
|----------------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| flagForSameExp | either 0 or 1. If 1, all experiments defined in the column <code>ScreenNb</code> in the dataset file must have the same design (same type and same number of replicates - exact plate layout is irrelevant) so that Spearman's correlation coefficient can be computed between experiments (each with summarized replicates) |
| listOfNormalizations | a list of the normalization function to apply. Can be LiWongRank , varAdjust , divNorm , quantileNormalization , BScore , ZScore , ZScorePerScreen , subtractBackground , lowessNorm , controlNorm |
| listOfArgs4norm | a list containing, for each element of <code>listofnormalizations</code> , the arguments to be passed on |
| listOfStatTests | a list of the statistical tests to perform. Can be Ttest , MannWhitney , RankProduct |
| listOfArgs4stat | a list containing, for each element of <code>listofstattests</code> , the arguments to be passed on |
| multTestAdj | indicates the p-value correction for multiple testing - one of "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", or "none" (Type ?p.adjust for details) |
| hitScoringVec1 | a vector of length 3 indicating (in that order): - scoring according to p-value (0: no, 1: yes) - scoring according to ZScore with ZScore < threshold (0: no, 1: yes), or according to ZScore < threshold and p-value < hitScoringVec2[1] (2) - scoring according to ZScore with ZScore > threshold (0: no, 1: yes), or according to ZScore > threshold and p-value < hitScoringVec2[1] (2). If hitScoringVec1[2] or hitScoringVec1[3] are equal to 2, hitScoringVec1[1] must be equal to one, otherwise p-values will not be computed. |
| hitScoringVec2 | a vector of length 3 indicating the thresholds for hitscoringvec1 |

posNegFlag either 0 (no controls available) or 1 (controls available)

flag4Gsea Can be:
 - either 0: No GSEA analysis is performed
 - or 1: A GSEA analysis is performed for each hit scoring method
 - or a binary vector that allows to choose which hit scoring method(s) will be used for a GSEA analysis. Hit scoring methods are sorted as follows: first, hits are scored according to the p-values of each test specified in `listOfStatTests`. Then, if the option of scoring hits according to p-values and Intensities is chosen (see `hitScoringVec1`, for each test, a hit vector is generated. Finally, if the option of scoring hits according to Intensities only is chosen, hit vectors are generated for this option.

vecOfChannels a character vector containing the names of the channels to be used for quality plots, for example "SigIntensity" or "NbCells"

whichOnto one of the three GO hierarchies: "biological_process", "molecular_function" or "cellular_component" - used for the GSEA analysis

Value

Generates the html output files `index.html` and `indexnorm.html` containing the quality analysis of raw and normalized data, respectively, and `stats.html`, containing the statistical analysis. If several normalization methods are applied, an `indexnorm` file is generated after each.

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

mainAnalysis(header, dataset, 0, list(controlNorm), list(list(1, 0, "SigIntensity", 1)),
list(Ttest, MannWhitney), list(list("1", 1, "SigIntensity", "GeneName"),
list("1", 1, "SigIntensity", "GeneName")), "none", c(1, 0, 0), c(0.05, 0, 0), 1,
1, c("SigIntensity", "NbCells"), "biological_process")
```

```
makeBoxplot4PlateType
```

Generate a boxplot of the data per plate

Description

Generates a boxplot comparing the same plates in different experiments.

Usage

```
makeBoxplot4PlateType(header, dataset, channel, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the column whose values will be used for the box-plot |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

For each plate type, a boxplot of intensity values per experiment will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle` and the number of the plate.

The function returns a list containing:

| | |
|-------------|------------------------------------|
| plotName | the plotname |
| minOfPlates | the number of the first experiment |
| numOfPlates | the number of the last experiment |

See Also

[makeBoxplotControls](#), [makeBoxplotControlsPerScreen](#), [makeBoxplotControlsPerPlate](#), [makeBoxplotPerPlate](#), [makeBoxplotPerScreen](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

makeBoxplot4PlateType(header, dataset, "SigIntensity", "Data vs. Controls", 1)
```

```
makeBoxplotControlsPerPlate
```

Generate a boxplot of the data vs. the controls for each plate

Description

Generates a boxplot of intensity values of negative controls, positive controls and experimental data for each plate of each experiment available in the dataset.

Usage

```
makeBoxplotControlsPerPlate(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the column whose values will be used for the box-plot |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

For each experiment, a series of boxplots of intensity values of negative controls, positive controls and experimental data will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle` and the number of the experiment.

The function returns a list containing:

| | |
|-------------------------------|--|
| plotName | the plotname |
| c(minOfScreens, numOfScreens) | a vector with the number of the first experiment and of the last experiment |
| c(minOfPlates, numOfPlates) | a vector with the number of the first plate and the number of the last plate |

See Also

[makeBoxplotControls](#), [makeBoxplotControlsPerScreen](#), [makeBoxplotPerPlate](#), [makeBoxplotPerScreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

makeBoxplotControlsPerPlate(header, dataset, "SigIntensity", "Data vs. Controls", 1, 1)
```

```
makeBoxplotControlsPerScreen
```

Generate a boxplot of the data vs. the controls for each experiment

Description

Generates a boxplot of intensity values of negative controls, positive controls and experimental data for each experiment available in the dataset.

Usage

```
makeBoxplotControlsPerScreen(header, dataset, channel, plotTitle, plotDesign, sh
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the column whose values will be used for the box-plot |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

A series of boxplots of intensity values of negative controls, positive controls and experimental data will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

| | |
|--------------|------------------------------------|
| plotName | the plotname |
| minOfScreens | the number of the first experiment |
| numOfScreens | the number of the last experiment |

See Also

[makeBoxplotControls](#), [makeBoxplotControlsPerPlate](#), [makeBoxplotPerPlate](#), [makeBoxplotPerScreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

makeBoxplotControlsPerScreen(header, dataset, "SigIntensity", "Data vs. Controls", 1, 1)
```

```
makeBoxplotControls
```

Generate a boxplot of the data vs. the controls

Description

Generates a boxplot of intensity values of negative controls, positive controls and experimental data.

Usage

```
makeBoxplotControls(header, dataset, channel, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the column whose values will be used for the box-plot |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

A boxplot of intensity values of negative controls, positive controls and experimental data will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[makeBoxplotControlsPerScreen](#), [makeBoxplotControlsPerPlate](#), [makeBoxplotPerPlate](#), [makeBoxplotPerScreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

makeBoxplotControls(header, dataset, "SigIntensity", "Data vs. Controls", 1)
```

```
makeBoxplotPerPlate
Generate a boxplot of the data per plate
```

Description

Generates a boxplot of intensity values per plate for each experiment available in the dataset.

Usage

```
makeBoxplotPerPlate(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the column whose values will be used for the box-plot |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

For each experiment, a boxplot of intensity values per plate will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

```
plotName      the plotname
minOfScreens  the number of the first experiment
numOfScreens  the number of the last experiment
```

See Also

[makeBoxplotControls](#), [makeBoxplotControlsPerPlate](#), [makeBoxplotControlsPerScreen](#), [makeBoxplotPerScreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

makeBoxplotPerPlate(header, dataset, "SigIntensity", "Data vs. Controls", 1, 1)
```

```
makeBoxplotPerScreen
```

Generate a boxplot of the data per experiment

Description

Generates a boxplot of intensity values per experiment.

Usage

```
makeBoxplotPerScreen(header, dataset, channel, plotTitle, showPlot)
```

Arguments

```
header      the header of a dataset file generated with generateDatasetFile
dataset     an R data frame generated with generateDatasetFile
channel     a character string specifying the column whose values will be used for the box-
            plot
plotTitle   the plot title
showPlot    0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save
            the plot(s) without opening windows.
```

Value

A boxplot of intensity values per experiment will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

See Also

[makeBoxplotControls](#), [makeBoxplotControlsPerPlate](#), [makeBoxplotControlsPerScreen](#), [makeBoxplotPerPlate](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

makeBoxplotPerScreen(header, dataset, "SigIntensity", "Data vs. Controls", 1)
```

| | |
|-------------|------------------------------------|
| MannWhitney | <i>Perform a Mann-Whitney test</i> |
|-------------|------------------------------------|

Description

Performs the non-parametric Mann-Whitney test on the intensity data.

Usage

```
MannWhitney(dataset, listofargs)
```

Arguments

| | |
|------------|--|
| dataset | an R data frame generated with generateDatasetFile |
| listofargs | a list containing: <ul style="list-style-type: none"> - "g" (greater) for significant increase, "l" (lower) for significant decrease, or "two.sided" for both - either a number indicating the true value of the mean, or a character string indicating the name of the gene to compare with - a character string specifying the column whose values will be used for the test - a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |

Value

Returns a list containing:

| | |
|--|---|
| pValVec | a named vector of p-values |
| dataset | the dataset with an added column "p.value.mannwhitney" |
| paste("pValue.mannwhitney", testType, sep="_") | the character string "p.value.mannwhitney" concatenated with the testType (first element of listofargs) |
| "Mann-Whitney test" | the character string "Mann-Whitney test" |

See Also

[Ttest](#), [RankProduct](#)

Examples

```

data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

pvals1 <- MannWhitney(dataset, list("l", median(dataset$SigIntensity, na.rm=TRUE),
"SigIntensity", "GeneName"))
pValVec1 <- pvals1[[1]]
scoredDataset1 <- pvals1[[2]]

```

multTestAdjust *Adjust p-values for multiple testing*

Description

Adjusts p-values for multiple testing.

Usage

```
multTestAdjust(pValVec, adjustMethod)
```

Arguments

pValVec a vector of p-values

adjustMethod one of the following: "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". For details type ?p.adjust

Value

Returns a vector of corrected p-values. Can be integrated into a dataframe with the function [incorporatepValVec](#).

See Also

[incorporatepValVec](#), [Ttest](#)

Examples

```

data(pValVec1, package="RNAiR")

##for details on the generation of pValVec1, see the example of the Ttest function linked

newpvalvec <- multTestAdjust(pValVec1,"fdr")

```

numCellQualControl *Quality control of the number of cells*

Description

Plots a histogram of the cell number per well and allows the user to set an upper and a lower threshold so as to exclude wells from the analysis.

Usage

```
numCellQualControl(DataSetFile, nbLinesHeader, plotTitle)
```

Arguments

`DataSetFile` a dataset file generated with [generateDatasetFile](#)
`nbLinesHeader` typically 3
`plotTitle` the plot title

Value

Prints out the list of wells under and over the predefined thresholds in the shell.

Saves a list of discarded siRNA values (if applicable) in a text file named after the experiment name specified in the header concatenated with either "numCellQualControl_discarded_higher.txt" or "numCellQualControl_discarded_lower.txt".

Saves the histogram with the applied thresholds in a pdf file named after the experiment name specified in the header concatenated with the `plotTitle`.

Overwrites the given `DataSetFile` with the new dataset.

See Also

[percCellQualControl](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")
saveDataset(header, dataset, "save_testfile1.txt")

numCellQualControl("save_testfile1.txt", 3, "Histogram of the number of cells")
```

| | |
|--------------|------------------------|
| orderGeneIDs | <i>Order a dataset</i> |
|--------------|------------------------|

Description

Orders dataset according to one of its columns.

Usage

```
orderGeneIDs(dataset, ID1)
```

Arguments

| | |
|---------|--|
| dataset | an R data frame generated with generateDatasetFile |
| ID1 | a character string specifying the name of the column according to which the dataset will be sorted |

Value

An R data frame ('dataset') ordered according to its values in the specified column.

See Also

[order](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

orderedDataset=orderGeneIDs(dataset, "SigIntensity")
```

| | |
|---------------------|---|
| percCellQualControl | <i>Quality control of the percentage of cells</i> |
|---------------------|---|

Description

Plots a histogram of the percentage of cells per well (ratio of the number of identified cells and the number of identified objects) and allows the user to set an upper and a lower threshold so as to exclude wells from the analysis.

Usage

```
percCellQualControl(DataSetFile, nbLinesHeader, plotTitle)
```

Arguments

DataSetFile a dataset file generated with [generateDatasetFile](#)
 nbLinesHeader typically 3
 plotTitle the plot title

Value

Prints out the list of wells under and over the predefined thresholds in the shell.

Saves a list of discarded siRNA values (if applicable) in a text file named after the experiment name specified in the header concatenated with either "percCellQualControl_discarded_higher.txt" or "percCellQualControl_discarded_lower.txt".

Saves the histogram with the applied thresholds in a pdf file named after the experiment name specified in the header concatenated with the `plotTitle`.

Overwrites the given `DataSetFile` with the new dataset.

See Also

[numCellQualControl](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")
saveDataset(header, dataset, "save_testfile1.txt")

percCellQualControl("save_testfile1.txt", 3, "Histogram of the number of cells")
```

plotBar

Plot signal intensities per well

Description

Plots signal intensity values for each well, a blue line showing the median, two green lines showing one median absolute deviation, two red lines showing two median absolute deviations.

Usage

```
plotBar(header, dataset, col4val, flag, plotTitle, showPlot)
```

Arguments

header the header of a dataset file generated with [generateDatasetFile](#)
 dataset an R data frame generated with [generateDatasetFile](#)
 col4val a character string specifying the column whose intensity values will be used for the plot
 flag 0, 1, or 2. 0 uses the data from the complete dataset, 1 generates one plot for each experiment, 2 generates one plot for each plate.
 plotTitle the plot title
 showPlot 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows.

Value

Saves the plots in pdf and png files named after the experiment name specified in the header concatenated with the `plotTitle` and if applicable the experiment number and/or the plate number.

When `flag == 0`, returns the plot name (`plotName`).

When `flag == 1`, returns a list containing:

```
plotName      The plot name
minOfScreens  The number of the first experiment
numOfScreens  The number of the last experiment
```

When `flag == 2`, returns a list containing: the plot name, a vector with the number of the first experiment and of the last experiment, and a vector with the number of the first plate and the number of the last plate.

See Also

[ZScorePlot](#), [ZScorePlotTwo](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotname <- plotBar(header, dataset, "SigIntensity", 0, "Data per well", 1)
```

```
plotControlHistoPerplate
```

Plot a histogram of the data values and controls per plate

Description

Plots and saves a histogram of data values per experiment and per plate and shows the controls, if available, in color.

Usage

```
plotControlHistoPerplate(header, dataset, channel, plotTitle, plotDesign, showPl
```

Arguments

```
header      the header of a dataset file generated with generateDatasetFile
dataset     an R data frame generated with generateDatasetFile
channel     a character string specifying the name of the column containing the values to be
            plotted, e.g. "SigIntensity"
plotTitle   the plot title
plotDesign  1 or 2. 1 will generate one window containing all plots, 2 will generate a series
            of plots.
showPlot    0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save
            the plot(s) without opening windows.
```

Value

Saves the histograms in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

Positive controls are plotted in green, negative controls in red.

The function returns a list containing:

```
histoName      the plotname
c(minOfScreens, numOfScreens)
                a vector with the number of the first experiment and of the last experiment
c(minOfPlates, numOfPlates)
                a vector with the number of the first plate and the number of the last plate
```

See Also

[plotControlHisto](#), [plotControlHistoPerscreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotControlHistoPerplate(header, dataset, "SigIntensity", "Distribution of Data and Contr
```

```
plotControlHistoPerscreen
```

Plot a histogram of the data values and controls per experiment

Description

Plots and saves a histogram of data values per experiment and shows the controls, if available, in color.

Usage

```
plotControlHistoPerscreen(header, dataset, channel, plotTitle, plotDesign, showP
```

Arguments

| | |
|-------------------------|--|
| <code>header</code> | the header of a dataset file generated with generateDatasetFile |
| <code>dataset</code> | an R data frame generated with generateDatasetFile |
| <code>channel</code> | a character string specifying the name of the column containing the values to be plotted, e.g. <code>"SigIntensity"</code> |
| <code>plotTitle</code> | the plot title |
| <code>plotDesign</code> | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| <code>showPlot</code> | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the histograms in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

Positive controls are plotted in green, negative controls in red.

The function returns a list containing:

```
histoName      the plotname
minOfScreens   the number of the first experiment
numOfScreens   the number of the last experiment
```

See Also

[plotControlHisto](#), [plotControlHistoPerplate](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotControlHistoPerscreen(header, dataset, "SigIntensity", "Distribution of Data and Cont
```

`plotControlHisto` *Plot a histogram of the data values and controls*

Description

Plots and saves a histogram of data values and shows the controls, if available, in color.

Usage

```
plotControlHisto(header, dataset, channel, plotTitle, showPlot)
```

Arguments

```
header      the header of a dataset file generated with generateDatasetFile
dataset     an R data frame generated with generateDatasetFile
channel     a character string specifying the name of the column containing the values to be
            plotted, e.g. "SigIntensity"
plotTitle   the plot title
showPlot    0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save
            the plot(s) without opening windows.
```

Value

Saves the histogram in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

Positive controls are plotted in green, negative controls in red.

The function returns the plot name.

See Also

[plotControlHistoPerplate](#), [plotControlHistoPerscreen](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

plotControlHisto(header, dataset, "SigIntensity", "Distribution of Data and Controls", 1)
```

plotHistoPerplate *Plot a histogram of the data values per plate*

Description

Plots and saves a histogram of the chosen data values per experiment and per plate.

Usage

```
plotHistoPerplate(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the histograms in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

| | |
|-------------------------------|--|
| histoName | the plotname |
| c(minOfScreens, numOfScreens) | a vector with the number of the first experiment and of the last experiment |
| c(minOfPlates, numOfPlates) | a vector with the number of the first plate and the number of the last plate |

See Also

[plotHisto](#), [plotHistoPerscreen](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")
```

```
plotHistoPerplate(header, dataset, "SigIntensity", "Distribution of Data and Controls", 1
```

```
plotHistoPerscreen Plot a histogram of the data values per experiment
```

Description

Plots and saves a histogram of the chosen data values.

Usage

```
plotHistoPerscreen(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the histograms in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

| | |
|--------------|------------------------------------|
| histoName | the plotname |
| minOfScreens | the number of the first experiment |
| numOfScreens | the number of the last experiment |

See Also

[plotHisto](#), [plotHistoPerplate](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")
```

```
plotHistoPerscreen(header, dataset, "SigIntensity", "Distribution of Data and Controls", 1
```

plotHisto *Plot a histogram of the data values*

Description

Plots and saves a histogram of the chosen data values.

Usage

```
plotHisto(header, dataset, channel, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the histogram in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[plotHistoPerplate](#), [plotHistoPerscreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotHisto(header, dataset, "SigIntensity", "Distribution of Data and Controls", 1)
```

plotQQperplate *Make a QQ plot per plate*

Description

Shows and saves a QQ plot of the data for each experiment and each plate in the dataset.

Usage

```
plotQQperplate(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the QQ plots in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

| | |
|-------------------------------|--|
| histoName | the plotname |
| c(minOfScreens, numOfScreens) | a vector with the number of the first experiment and of the last experiment |
| c(minOfPlates, numOfPlates) | a vector with the number of the first plate and the number of the last plate |

See Also

[plotQQ](#), [plotQQperscreen](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotQQperplate(header, dataset, "SigIntensity", "QQplot", 1, 1)
```

plotQQperscreen *Make a QQ plot per experiment*

Description

Shows and saves a QQ plot of the data for each experiment in the dataset.

Usage

```
plotQQperscreen(header, dataset, channel, plotTitle, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the QQ plots in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns a list containing:

| | |
|--------------|------------------------------------|
| histoName | the plotname |
| minOfScreens | the number of the first experiment |
| numOfScreens | the number of the last experiment |

See Also

[plotQQ](#), [plotQQperplate](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

plotQQperscreen(header, dataset, "SigIntensity", "QQplot", 1, 1)
```

plotQQ

Make a QQ plot

Description

Shows and saves a QQ plot of the data.

Usage

```
plotQQ(header, dataset, channel, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values to be plotted, e.g. "SigIntensity" |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the QQ plot in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[plotQQperscreen](#), [plotQQperplate](#)

Examples

```
data(exampleHeader, package="RNAiether")
data(exampleDataset, package="RNAiether")

plotQQ(header, dataset, "SigIntensity", "QQplot", 1)
```

pValVec1

A vector of p-values after a median normalization and a t-test

Description

See [divNorm](#) and [Ttest](#) for details

Usage

```
pValVec1
```

Format

vector

pValVec2 *A vector of p-values after a Mann-Whitney test*

Description

See [MannWhitney](#) for details

Usage

pValVec2

Format

vector

quantileNormalization
 Quantile normalization

Description

Quantile normalization (see References)

Usage

quantileNormalization(header, dataset, listOfArgs)

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column whose values will be used for normalization - 1 or 2, 1 meaning a normalization per experiment, 2 meaning a normalization per plate |

Value

Returns a list, containing:

| | |
|---------|--|
| header | the new header (with an added entry about the normalization procedure in the comments) |
| dataset | the new dataset with normalized values. The old values are saved in an extra column with the suffix ".old" |

References

B.M. Bolstad, R.A. Irizarry, M. Astrand, and T.P. Speed. A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Variance and Bias. *Bioinformatics*, 19(2): 185-193, 2003

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- quantileNormalization(header, dataset, list(2, "SigIntensity"))
newheader <- normres[[1]]
newdataset <- normres[[2]]
```

| | |
|-------------|------------------------------------|
| RankProduct | <i>Perform a Rank Product test</i> |
|-------------|------------------------------------|

Description

Performs the non-parametric rank product test on the intensity data.

Usage

```
RankProduct(dataset, listofargs)
```

Arguments

| | |
|------------|--|
| dataset | an R data frame generated with generateDatasetFile |
| listofargs | a list containing: <ul style="list-style-type: none"> - the number of permutations to perform to compute the p-values (usually 100) - 1 or 2, depending if the search is for a significant decrease or increase - a character string specifying the column whose values will be used for the test - a character string specifying the name of the dataset column to be used to define the replicate, for example "GeneName" or "Internal_GeneID" |

Value

Returns a list containing

| | |
|--|--|
| pValVec | a named vector of p-values |
| dataset | the dataset with an added column "p.value.rankproduct" |
| paste("pValue.rankproduct", testType, sep="_") | the character string "p.value.rankproduct" |
| "Rank product test" | the character string "Rank product test" |

The p values returned are equivalent to the percentage of false prediction (pfp), which in theory is the equivalent of false discovery rate (FDR). It is possible that they are larger than 1.

See Also

[Ttest](#), [MannWhitney](#)

Examples

```
data(exampleHeader, package="RNAiether")
data(exampleDataset, package="RNAiether")

pvals1 <- RankProduct(dataset, list(100, 1, "SigIntensity", "GeneName"))
pValVec1 <- pvals1[[1]]
scoredDataset1 <- pvals1[[2]]
```

| | |
|--------------|--|
| replicatesCV | <i>Compute the correlation of variation (CV)</i> |
|--------------|--|

Description

Computes the correlation of variation as defined in Tseng et al. (see References)

Usage

```
replicatesCV(header, dataset, PlotTitle, col4val, col4anno, plotDesign, showPlot)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| PlotTitle | the plot title |
| col4val | a character string specifying the column whose values will be used to compute the correlation of variation |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| plotDesign | 1 or 2. 1 will generate one window containing all plots, 2 will generate a series of plots. |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

The correlation of variation of an siRNA is defined as the standard deviation of its values divided by their mean.

The function generates a plot of the average intensity against the CV for each experiment. The plot will be saved as a pdf and a png file named after the experiment name specified in the header concatenated with the `PlotTitle`.

The function returns a list containing:

| | |
|--------------|------------------------------------|
| histoName | the plotname |
| minOfScreens | the number of the first experiment |
| numOfScreens | the number of the last experiment |

References

G. C. Tseng et al. Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. *Nucleic Acids Res*, 29(12): 2549-2557, 2001.

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

replicatesCV(header, dataset, "Correlation of Variation versus Mean Intensity",
"SigIntensity", "GeneName", 1, 0)
```

```
replicatesSpearmanCor
```

Compute the correlation coefficient between replicates or experiments

Description

Computes Spearman's rank correlation coefficient for each replicate - either inside each experiment, or between experiments.

Usage

```
replicatesSpearmanCor(header, dataset, flag, col4val, col4anno, fileNameSuffix)
```

Arguments

| | |
|----------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| flag | 1 or 2. 1 will compute the coefficient for a maximum of 3 replicates, for each experiment available in the dataset. 2 will summarize the replicates from each experiment with their root mean square and compute the correlation coefficient between experiments. |
| col4val | a character string specifying the column whose values will be used to compute the correlation coefficient |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| fileNameSuffix | a character string that will be used to name the output file containing a table with the correlation coefficients. |

Value

For `flag==1`, the correlation coefficients are printed out to the shell and saved in a text file named after the experiment name specified in the header concatenated with the character string `filenamesuffix` and "SpearmanCor.txt".

For `flag==2`, the correlation coefficients are printed out to the shell and saved in a text file named after the experiment name specified in the header concatenated with the character string `filenamesuffix` and "SpearmanCor_AllExp.txt".

The function returns a table containing the correlation coefficients.

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

replicatesSpearmanCor(header, dataset, 1, "SigIntensity", "GeneName", "testfile1_")
```

rms

*Compute the replicate root mean square***Description**

Computes the root mean square of replicate values

Usage

```
rms(Ivec, na.rm = T)
```

Arguments

| | |
|-------|--|
| Ivec | All channel values for a specific siRNA/gene |
| na.rm | Removes NA values |

Value

A double giving the root mean square of the given replicate values.

See Also

[trim](#), [closestToZero](#), [furthestFromZero](#), [summarizeReps](#), [summarizeRepsNoFiltering](#)

Examples

```
data(exampleDataset, package="RNAither")

Indexes <- findReplicates(dataset, "GeneName", "CPSF1")
rmsval <- rms(dataset$SigIntensity[Indexes])
```

RNAither-package

*Statistical analysis of high-throughput RNAi screens***Description**

RNAither analyzes cell-based RNAi screens, and includes quality assessment, customizable normalization and statistical tests, leading to lists of significant genes and biological processes.

Details

| | |
|----------|----------------------|
| Package: | RNAither |
| Type: | Package |
| Version: | 1.0 |
| Date: | 2008-07-20 |
| License: | Artistic License 2.0 |

Author(s)

Nora Rieber and Lars Kaderali

Maintainer: Nora Rieber <Rieber Nor [at] gmx [dot] de>

| | |
|-------------|---|
| saveDataset | <i>Save the normalized dataset into a dataset text file</i> |
|-------------|---|

Description

Saves the normalized dataset and corresponding header into the specified dataset text file.

Usage

```
saveDataset(header, data, dataSetFile)
```

Arguments

| | |
|-------------|--|
| header | the header of a dataset file generated with <code>generateDatasetFile</code> |
| data | an R data frame generated with <code>generateDatasetFile</code> |
| dataSetFile | the name of the text file the data will be saved in; can be the same as the old file (will be overwritten without prompting) |

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

normres <- divNorm(header, dataset, list(median, 2, 1, "SigIntensity", 1))
newheader <- normres[[1]]
newdataset <- normres[[2]]
saveDataset(newheader, newdataset, "save_testfile1.txt")
```

| | |
|-------------------------|---|
| saveOldIntensityColumns | <i>Save old intensity value columns</i> |
|-------------------------|---|

Description

Duplicates the specified column and adds it to the end of the dataset.

Usage

```
saveOldIntensityColumns(dataset, col4val)
```

Arguments

| | |
|---------|---|
| dataset | an R data frame generated with generateDatasetFile |
| col4val | a character string specifying the column whose values will be saved as an extra column before normalization |

Value

The values in the chosen column are saved in an extra column with the suffix ".old".

Examples

```
data(exampleDataset, package="RNAiR")
newdataset <- saveOldIntensityColumns(dataset, "SigIntensity")
```

| | |
|-------------|------------------------------|
| savepValVec | <i>Save p-values to file</i> |
|-------------|------------------------------|

Description

Saves a vector of p-values to a text file.

Usage

```
savepValVec(pValVec, filename)
```

Arguments

| | |
|----------|--|
| pValVec | a vector of p-values |
| filename | the name of the text file to save the p-values to. |

See Also

[Ttest](#)

Examples

```
data(pValVec1, package="RNAiR")

##for details on the generation of pValVec1, see the example of the Ttest function linked

savepValVec(pValVec1, "pvals_testfile1.txt")
```

| | |
|----------------|--|
| scoredDataset1 | <i>A dataset containing an additional column showing the p-values, after a median normalization and a t-test</i> |
|----------------|--|

Description

See [divNorm](#) and [Ttest](#) for details

Usage

```
scoredDataset1
```

Format

see [generateDatasetFile](#) for details

| | |
|----------------|---|
| scoredDataset2 | <i>A dataset containing an additional column showing the p-values after a Mann-Whitney test</i> |
|----------------|---|

Description

See [MannWhitney](#) for details

Usage

```
scoredDataset1
```

Format

see [generateDatasetFile](#) for details

| | |
|----------------|--------------------------|
| SNRQualControl | <i>Computing the SNR</i> |
|----------------|--------------------------|

Description

Computes the signal to noise ratio for all data, per experiment and per plate for a complete dataset file and plots histograms of the results.

Usage

```
SNRQualControl(dataSetFile, nbLinesHeader, channel, noise, plotTitle, showPlot)
```

Arguments

| | |
|---------------|---|
| dataSetFile | a dataset file generated with generateDatasetFile |
| nbLinesHeader | typically 3 |
| channel | a character string specifying the name of the column containing the values for computing the SNR, e.g. "SigIntensity" |
| noise | A character string specifying the name of the column containing the values for computing the SNR, e.g. "Background" |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

Shows histogram plots of the SNR for the whole dataset file, per experiment and per plate and saves them in a pdf file. The name of the file will be the concatenation of the experiment name specified in the header and the function argument `plotTitle`.

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")
saveDataset(header, dataset, "save_testfile1.txt")

SNRQualControl("save_testfile1.txt", 3, "SigIntensity", "Background", "SNR", 1)
```

spatialDistribHits *Plotting the spatial distribution of the hits*

Description

Plots the plates showing the spatial distribution of the hits using the `plotPlate` function of the `prada` package.

Usage

```
spatialDistribHits(header, dataset, plotTitle, col4hits, col4anno, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| plotTitle | the plot title |
| col4hits | a character vector specifying the name of the dataset column containing the binary hit vector |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

For each plate, the plot will be saved as a png file named after the experiment name specified in the header concatenated with the `plotTitle`, the number of the experiment, and the number of the plate.

Wells containing positive controls are marked with a "P", wells containing negative controls with an "N".

Each plate will also be saved as an html file containing mouse-overs with the siRNA name for each well.

The function returns a list containing:

```
histoName      the plotname
c(minOfScreens, numOfScreens)
                a vector with the number of the first experiment and of the last experiment
c(minOfPlates, numOfPlates)
                a vector with the number of the first plate and the number of the last plate
```

See Also

[Ttest](#)

Examples

```
data(exampleHeader, package="RNAither")
data(pValVec1, package="RNAither")
data(scoredDataset1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "Hits1", 0.05,
"GeneName", "pvalue_testfile1.txt")

hitDataset1 <- scoredHits1[[1]]

spatialDistribHits(header, hitDataset1, "Spatial distribution of hits", "Hits1", "GeneName")
```

spatialDistrib *Generate spatial plots of intensity values*

Description

Generate plots of plates and their intensity values.

Usage

```
spatialDistrib(header, dataset, plotTitle, col4plot, col4anno, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with <code>generateDatasetFile</code> |
| dataset | an R data frame generated with <code>generateDatasetFile</code> |
| plotTitle | the plot title |
| col4plot | a character string specifying the column whose values will be used for the plot |
| col4anno | a character string specifying the column whose values will be used for the annotation of the plot |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

For each plate, the plot will be saved as a png file named after the experiment name specified in the header concatenated with the `plotTitle`, the number of the experiment, and the number of the plate.

Wells containing positive controls are marked with a "P", wells containing negative controls with an "N".

Each plate will also be saved as an html file containing mouse-overs with the siRNA name for each well.

The function returns a list containing:

| | |
|-------------------------------|--|
| histoName | the plotname |
| c(minOfScreens, numOfScreens) | a vector with the number of the first experiment and of the last experiment |
| c(minOfPlates, numOfPlates) | a vector with the number of the first plate and the number of the last plate |

See Also

`compareReplicateSD`, `compareReplicateSDPerScreen`

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

spatialDistrib(header, dataset, "Spatial distribution of cell counts", "NbCells", "GeneNa
```

subtractBackground *Background subtraction*

Description

Subtracts a specified background value from the intensity values.

Usage

```
subtractBackground(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column whose values will be used for background subtraction - a character string specifying the column whose values will be used as background |

Value

A list containing:

| | |
|---------|---|
| header | The new header (with an added entry about the normalization procedure in the comments) |
| dataset | The new dataset with normalized values. The old values are saved in an extra column of the dataset with the suffix ".old" |

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- subtractBackground(header, dataset, list("SigIntensity", "Background"))
newheader <- normres[[1]]
newdataset <- normres[[2]]
```

| | |
|-------------|---------------------------|
| sumChannels | <i>Summarize channels</i> |
|-------------|---------------------------|

Description

Summarizes two channels, for example by computing their ratio.

Usage

```
sumChannels(header, dataset, funName, colname4ch1, colname4ch2)
```

Arguments

| | |
|-------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| funName | the function used to summarize the two channels, for example divideChannels |
| colname4ch1 | a character string specifying the name of the dataset column containing the first channel |
| colname4ch2 | a character string specifying the name of the dataset column containing the second channel |

Details

The original dataset columns are saved as extra columns with the suffix `".old"` by the function `saveOldIntensityColumns`.

Value

A list containing:

| | |
|-------------------------|--|
| <code>header</code> | the header with an entry about the channel summarization added in the comments section |
| <code>newDataset</code> | the new dataset |

See Also

`eraseDataSetColumn`, `divideChannels`, `saveOldIntensityColumns`

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

newdataset=sumChannels(header, dataset, divideChannels, "SigIntensity", "NbCells")
```

```
summarizeRepsNoFiltering
```

Generate a new dataset with summarized replicates

Description

Generates a new dataset with summarized replicates. Keeps wells/spots with `SpotType -1` in the dataset, but intensity values are replaced with NA.

Usage

```
summarizeRepsNoFiltering(data, funSum, col4val, col4anno, cols2del)
```

Arguments

| | |
|-----------------------|--|
| <code>data</code> | an R data frame generated with <code>generateDatasetFile</code> |
| <code>funSum</code> | a function used to summarize the values of a replicate, e.g. <code>mean</code> , <code>median</code> , <code>rms</code> , <code>trim</code> , <code>max</code> , <code>min</code> , <code>closestToZero</code> , <code>furthestFromZero</code> , ... |
| <code>col4val</code> | a character vector (containing for example <code>"SigIntensity"</code> , <code>Background</code> , <code>NbCells</code> , <code>PercCells</code> , ...) specifying the columns that will be summarized by <code>funSum</code> |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used to define the replicate, e.g. <code>"GeneName"</code> or <code>"Internal_GeneID"</code> |
| <code>cols2del</code> | a character vector containing the columns to delete, for example <code>"SDSIntensity"</code> |

Details

All columns containing replicate values will be summarized by `funSum`. For all columns containing positions, screen numbers, plate numbers, etc., all information for different replicates will be kept, comma-separated. All columns containing standard deviations of channels should be specified in `colnames2delete`.

Value

Returns the summarized dataset.

See Also

[summarizeReps](#), [eraseDataSetColumn](#), [generateReplicateMat](#), [generateRepMatNoFilter](#), [mean](#), [median](#), [rms](#), [trim](#), [max](#), [min](#), [closestToZero](#), [furthestFromZero](#)

Examples

```
data(exampleDataset, package="RNAither")

colname4val <- c("SigIntensity", "Background", "NbCells", "PercCells")
summarizeddataset <- summarizeRepsNoFiltering(dataset, mean, colname4val, "GeneName", "SD")
```

`summarizeReps`

Generate a new dataset with summarized replicates

Description

Generates a new dataset with summarized replicates.

Usage

```
summarizeReps(data, funSum, col4val, col4anno, cols2del)
```

Arguments

| | |
|-----------------------|--|
| <code>data</code> | an R data frame generated with generateDatasetFile |
| <code>funSum</code> | a function used to summarize the values of a replicate, e.g. <code>mean</code> , <code>median</code> , rms , trim , <code>max</code> , <code>min</code> , closestToZero , furthestFromZero , ... |
| <code>col4val</code> | a character vector (containing for example <code>"SigIntensity"</code> , <code>Background</code> , <code>NbCells</code> , <code>PercCells</code> , ...) specifying the columns that will be summarized by <code>funSum</code> |
| <code>col4anno</code> | a character string specifying the name of the dataset column to be used to define the replicate, e.g. <code>"GeneName"</code> or <code>"Internal_GeneID"</code> |
| <code>cols2del</code> | a character vector containing the columns to delete, for example <code>"SDSIntensity"</code> |

Details

All columns containing replicate values will be summarized by `funSum`. For all columns containing positions, screen numbers, plate numbers, etc., all information for different replicates will be kept, comma-separated. All columns containing standard deviations of channels should be specified in `colnames2delete`.

Value

Returns the summarized dataset.

See Also

[summarizeRepsNoFiltering](#), [eraseDataSetColumn](#), [generateReplicateMat](#), [generateRepMatNoFiltering](#), [mean](#), [median](#), [rms](#), [trim](#), [max](#), [min](#), [closestToZero](#), [furthestFromZero](#)

Examples

```
data(exampleDataset, package="RNAiR")

colname4val <- c("SigIntensity", "Background", "NbCells", "PercCells")
summarizeddataset <- summarizeReps(dataset, mean, colname4val, "GeneName", "SDSIntensity")
```

trim

Compute the replicate mean with trimmed values

Description

Computes the mean of replicate values, omitting the highest and the lowest 5

Usage

```
trim(Ivec, na.rm = T)
```

Arguments

| | |
|-------|--|
| Ivec | All channel values for a specific siRNA/gene |
| na.rm | Removes NA values |

Value

A double giving the trimmed mean of the given replicate values, i.e. omitting the highest and the lowest 5

See Also

[rms](#), [closestToZero](#), [furthestFromZero](#), [summarizeReps](#), [summarizeRepsNoFiltering](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

Indexes <- findReplicates(dataset, "GeneName", "CPSF1")
replicatemean <- trim(dataset$SigIntensity[Indexes])
```

Ttest

Perform a Student's t-test

Description

Performs a Student's t-test on the intensity data.

Usage

```
Ttest(dataset, listofargs)
```

Arguments

| | |
|------------|--|
| dataset | an R data frame generated with generateDatasetFile |
| listofargs | a list containing: <ul style="list-style-type: none"> - "g" (greater) for significant increase, "l" (lower) for significant decrease, or "two.sided" for both - either a number indicating the true value of the mean, or a character string indicating the name of the gene to compare with - a character string specifying the column whose values will be used for the test - a character string specifying the name of the dataset column to be used to define the replicate, e.g. "GeneName" or "Internal_GeneID" |

Value

Returns a list containing:

| | |
|--|--|
| pValVec | a named vector of p-values |
| dataset | the dataset with an added column "p.value.mannwhitney" |
| paste("pValue.ttest", testType, sep="_") | the character string "pValue.ttest" concatenated with the testType (first element of listofargs) |
| "t test" | the character string "t test" |

See Also

[MannWhitney](#), [RankProduct](#)

Examples

```
data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

pvals1 <- Ttest(dataset, list("l", median(dataset$SigIntensity, na.rm=TRUE), "SigIntensit
pValVec1 <- pvals1[[1]]
scoredDataset1 <- pvals1[[2]]
```

| | |
|-----------|----------------------------|
| varAdjust | <i>Variance adjustment</i> |
|-----------|----------------------------|

Description

Divides the intensity values by their median absolute deviation (of the experiment or of the plate)

Usage

```
varAdjust(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none">- a character string specifying the column whose values will be used for normalization- 1 or 2, 1 meaning a normalization per screen, 2 a normalization per plate- a flag specifying whether controls should be excluded for the computation of the median absolute deviation (1) or not (0). |

Value

Divides the intensity values by their median absolute deviation (of the experiment or of the plate).

Returns a list containing:

| | |
|---------|---|
| header | The new header (with an added entry about the normalization procedure in the comments) |
| dataset | The new dataset with normalized values. The old values are saved in an extra column of the dataset with the suffix ".old" |

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- varAdjust(header, dataset, list(1, "SigIntensity", 1))
newheader <- normres[[1]]
newdataset <- normres[[2]]
```

 vennDiag

Plotting a Venn Diagram to compare hits

Description

Plots a Venn Diagram of up to three binary hit vectors.

Usage

```
vennDiag(header, listOfCols, listOfNames, plotTitle, showPlot)
```

Arguments

| | |
|-------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| listOfCols | a list of binary hit vectors to compare |
| listOfNames | a list of character strings for the annotation of the Venn Diagram |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

The plot is saved in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[Ttest](#), [MannWhitney](#)

Examples

```
data(exampleHeader, package="RNAither")

data(pValVec1, package="RNAither")
data(pValVec2, package="RNAither")
data(scoredDataset1, package="RNAither")
data(scoredDataset2, package="RNAither")

##for details on the generation of pValVec and scoredDataset,
##see the examples of the functions Ttest and MannWhitney linked above.

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "pValue.ttest_1",
"GeneName", "pvalue_testfile1.txt")

scoredHits2 <- hitselectionPval(scoredDataset2, pValVec2, "SigIntensity", "pValue.mannwhi",
"GeneName", "pvalue_testfile2.txt")

hitvector1 <- scoredHits1[[2]]
hitvector2 <- scoredHits2[[2]]

plot_name <- vennDiag(header, list(hitvector1, hitvector2), list("t test", "Mann-Whitney",
"Venn diagram", 1)
```

volcanoPlot *Making a volcano plot*

Description

Makes a volcano plot of the data.

Usage

```
volcanoPlot(header, dataset, col4plotx, col4ploty, col4anno, plotTitle, sigLevel,
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| col4plotx | a character vector specifying the name of the column containing the intensity values, usually <code>SigIntensity</code> |
| col4ploty | a character vector specifying the name of the dataset column containing the corresponding p-values |
| col4anno | a character string specifying the name of the dataset column to be used to define the replicate, e.g. <code>"GeneName"</code> or <code>"Internal_GeneID"</code> . |
| plotTitle | the plot title |
| sigLevel | the significance level for the p-value, indicating where a horizontal green line will be drawn |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows |

Value

Plots the intensity values against the negative decadic logarithm of the p-values. A green horizontal line is drawn at the specified significance level.

The plot is saved in a pdf and a png file named after the experiment name specified in the header concatenated with the `plotTitle`.

The function returns the plot name.

See Also

[Ttest](#)

Examples

```
data(exampleHeader, package="RNAither")
data(pValVec1, package="RNAither")
data(scoredDataset1, package="RNAither")

##for details on the generation of pValVec1 and scoredDataset1, see the example of the Tt

scoredHits1 <- hitselectionPval(scoredDataset1, pValVec1, "SigIntensity", "pValue.ttest_1
"GeneName", "pvalue_testfile1.txt")
```

```
hitDataset1 <- scoredHits1[[1]]
hitvector1 <- scoredHits1[[2]]

volcano_name <- volcanoPlot(header, hitDataset1, "SigIntensity", "pValue.ttest_1", "GeneN
"Volcano Plot", 0.05, 1)
```

ZPRIMEQualControl *Computing the Z' factor*

Description

Computes the Z' factor per plate for a complete dataset file and plots the results.

Usage

```
ZPRIMEQualControl(header, data, channel, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| data | an R data frame generated with generateDatasetFile |
| channel | a character string specifying the name of the column containing the values for computing the Z' factor, e.g. "SigIntensity" |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Returns the Z' values in the shell for each plate and saves them in a text file. The name of the text file will be the concatenation of the experiment name specified in the header and the character string "Z'Scores.txt".

Shows a plot of the Z' factor values and saves it as a png and a pdf file under the experiment name specified in the header concatenated with the function argument `plotTitle`.

The function returns a list containing:

| | |
|---------------|--------------------------------|
| plotName | the plot name |
| ZPrimeTabelle | table containing the Z' values |

References

J. Zhang, T. Chung, and K. Oldenburg. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen*, 4:67-73, 1999.

Examples

```

data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

res <- ZPRIMEQualControl(header, dataset, "SigIntensity", "Z' factors per plate", 1)
zprime_plot <- res[[1]]
zprime_table <- res[[2]]

```

ZScorePerScreen *ZScore normalization per experiment*

Description

ZScore normalization not per plate, but per experiment (see Value and References)

Usage

```
ZScorePerScreen(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|--|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none"> - a character string specifying the column whose values will be used for normalization - a flag specifying whether controls should be excluded for the computation of the median and median absolute deviation (1) or not (0). |

Value

The ZScore is defined as the quotient of the difference between an intensity value and the median of the experiment, and of the median absolute deviation.

Returns a list containing:

| | |
|---------|---|
| header | The new header (with an added entry about the normalization procedure in the comments) |
| dataset | The new dataset with normalized values. The old values are saved in an extra column of the dataset with the suffix ".old" |

References

N. Malo et al. Statistical practice in high-throughput screening data analysis. Nature Biotech, 24(2): 167-175, 2006.

See Also

[ZScore](#), [BScore](#)

Examples

```

data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- ZScorePerScreen(header, dataset, list("SigIntensity", 1))
newheader <- normres[[1]]
newdataset <- normres[[2]]

```

ZScorePlot

*Plot normalized intensity values per well***Description**

Plots the normalized intensity values for each well, together with a black line showing the mean, two green lines showing the standard deviation, and two red lines showing 2 standard deviations.

Usage

```
ZScorePlot(header, dataset, flag, col4plot, col4anno, plotTitle, showPlot)
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| flag | either 1 or 2. 1 if the dataset contains values per well, 2 if the dataset contains summarized values for each siRNA (e.g. a dataset summarized with summarizeReps). |
| col4plot | a character string specifying the column whose values will be used for the plot |
| col4anno | a character string specifying the column that will be used for the plot annotation |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Plots the normalized intensity values for each well, together with a black line showing the mean, and two red lines showing 2 standard deviations. Clicking on the points shows the gene/siRNA name.

The plot is saved as a pdf and a png file named after the experiment name specified in the header concatenated with the plotTitle.

The function returns the plot name.

See Also

[plotBar](#), [ZScorePlotTwo](#)

Examples

```

data(exampleHeader, package="RNAither")
data(exampleDataset, package="RNAither")

normedvals <- ZScore(header, dataset, list("SigIntensity", 1))
ZScorePlot(normedvals[[1]], normedvals[[2]], 1, "SigIntensity", "GeneName",
"Normed intensity values per well", 1)

```

ZScorePlotTwo *Plot signal intensities per well (II)*

Description

Plots signal intensity values for each well, a black line showing the median, two green lines showing one median absolute deviation, two red lines showing two median absolute deviations.

Usage

```
ZScorePlotTwo(header, dataset, flag, flag2, col4plot, col4anno, plotTitle, showP
```

Arguments

| | |
|-----------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| flag | 0, 1, or 2. 0 uses the data from the complete dataset, 1 generates one plot for each experiment, 2 generates one plot for each plate. |
| flag2 | 0 draws lines using mean and sd, 1 draws lines using median and mad. |
| col4plot | a character string specifying the column whose intensity values will be used for the plot |
| col4anno | in case showPlot == 1, a character string specifying the column used for identifying points |
| plotTitle | the plot title |
| showPlot | 0 or 1. 1 will open one or several plot windows in the R GUI, 0 will only save the plot(s) without opening windows. |

Value

Saves the plots in pdf and png files named after the experiment name specified in the header concatenated with the `plotTitle` and if applicable the experiment number and/or the plate number.

When `flag == 0`, returns the plot name (`plotName`).

When `flag == 1`, returns a list containing:

| | |
|--------------|------------------------------------|
| plotName | The plot name |
| minOfScreens | The number of the first experiment |
| numOfScreens | The number of the last experiment |

When `flag == 2`, returns a list containing: the plot name, a vector with the number of the first experiment and of the last experiment, and a vector with the number of the first plate and the number of the last plate.

See Also

[plotBar](#), [ZScorePlot](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

plotname <- ZScorePlotTwo(header, dataset, 0, 1, "SigIntensity", "GeneName", "Data per we
```

ZScore

ZScore normalization

Description

ZScore normalization (see Value and References)

Usage

```
ZScore(header, dataset, listOfArgs)
```

Arguments

| | |
|------------|---|
| header | the header of a dataset file generated with generateDatasetFile |
| dataset | an R data frame generated with generateDatasetFile |
| listOfArgs | a list containing: <ul style="list-style-type: none">- a character string specifying the column whose values will be used for normalization- a flag specifying whether controls should be excluded for the computation of the median and median absolute deviation (1) or not (0). |

Value

The ZScore is defined as the quotient of the difference between an intensity value and the median of the plate, and of the median absolute deviation.

Returns a list containing:

| | |
|---------|---|
| header | The new header (with an added entry about the normalization procedure in the comments) |
| dataset | The new dataset with normalized values. The old values are saved in an extra column of the dataset with the suffix ".old" |

References

N. Malo et al. Statistical practice in high-throughput screening data analysis. Nature Biotech, 24(2): 167-175, 2006.

See Also

[ZScorePerScreen](#), [BScore](#)

Examples

```
data(exampleHeader, package="RNAiR")
data(exampleDataset, package="RNAiR")

normres <- ZScore(header, dataset, list("SigIntensity", 1))
newheader <- normres[[1]]
newdataset <- normres[[2]]
```


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