check_rnaseqcomp  Sanity Check of S4 rnaseqcomp Class

Description
This function always checks if the elements are valid to create a S4 rnaseqcomp object. Specifically, check if quantData is a list of matrices, if condInfo has the correct length and levels, etc.

Usage
check_rnaseqcomp(object)

Arguments
object  A object of S4 rnaseqcomp class

Value
TRUE, or character if error happens.

plot2TX  Estimate And Plot Transcript Proportion Difference

Description
For any compared two replicates in each cell line, the proportion of one transcript for genes that only include two annotated transcripts can be different even flipped. This function estimates and plots the proportion difference stratified by detrended logsignal. Means of absolute difference will be reported for three levels of detrended logsignals. Average is used when multiple two-replicate comparisons included.

Usage
plot2TX(dat, genes, step = 0.5, thresholds = c(1, 6), plotcell = 1, ...)

Arguments
dat  A rnaseqcomp S4 class object.
genases  A vector of gene names corresponding to quantified transcripts. Note that length(genes) should equal to nrow(dat@quantData[[1]])
step  A number specifying the resolution on detrended logsignal for calculation and plotting the proportion difference. (default: 0.5)
thresholds  A vector of two numbers define cutoffs for three levels of detrended log signals, where one number summary will be generated. (default: c(1, 6))
plotcell  1 or 2 indicating which cell line will be plotted. If values other than 1 and 2, both cell lines will be plotted. This value won’t affect estimation for both cell lines. (default: 1)
...  Parameters for base function plot.
plotFC

Value

plot 2TX plots of quantification pipelines for selected cell line by plotcell.
list A list of two matrices indicating the mean and standard error of absolute proportion differences. Values are based on average of two cell lines.

Examples

data(simdata)
condInfo <- factor(simdata$samp$condition)
repInfo <- factor(simdata$samp$replicate)
evaluationFeature <- rep(TRUE, nrow(simdata$meta))
calibrationFeature <- simdata$meta$house & simdata$meta$chr == 'chr1'
unitReference <- 1
dat <- signalCalibrate(simdata$quant, condInfo, repInfo, evaluationFeature, calibrationFeature, unitReference, calibrationFeature2 = calibrationFeature)
plot2TX(dat, genes=simdata$meta$gene)

plotFC Estimate And Plot Fold Change Accuracy

Description
For each pipeline, differential expression is estimated by fold change on mean signals across replicates of cell lines. For features that are truly differential expressed, their fold changes levels are summarized based on different levels of detrended logsignals.

Usage

plotFC(dat, positive, fcsign, constant = 0.5, loessspan = 1/3, thresholds = c(1, 6), ...)

Arguments

dat A rnaseqcomp S4 class object.
positive A logical vector with length equivalent to row number of matrices in dat@quantData. TRUE means true differential and FALSE means true non-differential, while missing value NA means unknown.
fcsign A numeric vector with length equivalent to row number of matrices in dat@quantData. Only values 1, -1, 0, NA are allowed. 1 means upregulated in second cell line, -1 means downregulated in second cell line, and 0 means no change. If elements in fcsign is NA or correspond to NA in positive, these elements will be ignored in estimation.
constant A numeric constant that is added to quantifications before fold changes calculation. (default: 0.5)
loessspan A numeric number indicating span used for loess smooth. Details see loess.smooth function. (Default: 1/3)
thresholds A numeric vector defining cutoffs on fold changes as the points to make threshold averaging on ROC curves. (default: seq(12, 0, len = 300))
... Parameters for base function plot.
plotNE

Estimate And Plot Express And Non-express Features

Description

For each cell line, any compared two replicates might have a portion of transcripts that express in one replicate but not the other, depending on what cutoff is used to define non-express. This function estimate and plot the proportion of disagreement using multiple cutoffs. Average is used when multiple two-replicate comparisons included.

Usage

plotNE(dat, steps = seq(-0.5, 12, 0.5), Ks = 0:3, pchK = seq_along(Ks) - 1, plotcell = 1, ...)

Arguments

dat  
A rnaseqcomp S4 class object.

steps  
A numeric vector specifying log-scale cutoffs to be used for calculation and plotting. (default: seq(-0.5, 12, 0.5))

Ks  
A numeric vector specifying which cutoffs to be highlighted and to which the reported proportions to be corresponding.

pchK  
Plot styles of highlight points corresponding to Ks. (default: seq_along(Ks) - 1)

plotcell  
1 or 2 indicating which cell line will be plotted. If values other than 1 and 2, both cell lines will be plotted. This value won’t affect estimation for both cell lines. (default: 1)

...  
Parameters for base function plot.
Value

plot

NE plots of quantification pipelines for selected cell line by plotcell.

A list of two matrices. The first matrix gives the proportion of disagreement and
the second matrix gives the proportion of both replicates under (non-express)
corresponding cutoff Ks. Values are based on average of two cell lines.

Examples

data(simdata)
condInfo <- factor(simdata$samp$condition)
repInfo <- factor(simdata$samp$replicate)
evaluationFeature <- rep(TRUE, nrow(simdata$meta))
calibrationFeature <- simdata$meta$house & simdata$meta$chr == 'chr1'
unitReference <- 1
dat <- signalCalibrate(simdata$quant, condInfo, repInfo, evaluationFeature,
calibrationFeature, unitReference, calibrationFeature2 = calibrationFeature)
plotNE(dat)

plotROC Estimate And Plot Differential Expression

Description

For each pipeline, differential expression is first estimated by fold change on 1 vs. 1 comparison
between cell lines. ROC curves then are made by comparing fold changes with predefined true
differentials. Then, ROC curves from multiple 1 vs. 1 comparisons are averaged using threshold
averaging strategy. Standardized partial area under the curve (pAUC) is reported for each pipeline.

Usage

plotROC(dat, positive, fcsign, cut = 1, constant = 0.5,
thresholds = seq(12, 0, len = 300), arrow = FALSE, ...)

Arguments

dat A rnaseqcomp S4 class object.
positive A logical vector with length equivalent to row number of matrices in dat@quantData.
TRUE means true differential and FALSE means true non-differential, while missing
value NA means unknown.
fcsign A numeric vector with length equivalent to row number of matrices in dat@quantData.
Only values 1, -1, 0 are allowed. 1 means upregulated in second cell line, -1
means downregulated in second cell line, and 0 means no change. If elements
in fcsign correspond to NA in positive, these elements will be ignored in esti-
mation.
cut A numeric cutoff used to decide if fold change should be estimated. For a 1 vs
1 comparison, if features have signals less than cut in both samples, their fold
changes will be set to 0. (default: 1)
constant A numeric constant that is added to quantifications before fold changes calcula-
tion. (default: 0.5)
plotSD

Thresholds

A numeric vector defining cutoffs on fold changes as the points to make threshold averaging on ROC curves. (default: seq(12, 0, len = 300))

Arrow

A logical indicating if error bars should be added to the averaged ROC curves. (default: FALSE)

...

Parameters for base function plot.

Value

plot

ROC plots for all the quantification pipelines.

pAUC

A numeric vector indicating pipeline accuracy. This is standardized partial AUC based on ranges chosen on false positive rate.

Examples

```r
data(simdata)
condInfo <- factor(simdata$samp$condition)
repInfo <- factor(simdata$samp$replicate)
evaluationFeature <- rep(TRUE, nrow(simdata$meta))
calibrationFeature <- simdata$meta$house & simdata$meta$chr == "chr1"
unitReference <- 1
dat <- signalCalibrate(simdata$quant, condInfo, repInfo, evaluationFeature, calibrationFeature, unitReference, calibrationFeature2 = calibrationFeature)
plotROC(dat, simdata$meta$positive, simdata$meta$fcsign)
```

plotSD

Estimate And Plot Median Standard Deviation

Description

For each cell line in each pipeline, the standard deviation of detrend log signals are calculated for individual features. Then, loess smooth on standard deviation are plotted stratified by detrended log signals for select cell line. The median of standard deviation at three different levels of detrended log signals are reported.

Usage

```r
plotSD(dat, constant = 0.5, loessspan = 1/3, thresholds = c(1, 6), plotcell = 1, ...)
```

Arguments

dat

A rnaseqcomp S4 class object.

constant

A numeric pseudo-constant to be added on all the signals before transferred to log scale. (default: 0.5)

loessspan

A numeric number indicating span used for loess smooth. Details see loess.smooth function. (Default: 1/3)

thresholds

A vector of two numbers define cutoffs for three levels of detrended log signals. (default: c(1, 6))

plotcell

1 or 2 indicating which cell line will be plotted. If values other than 1 and 2, both cell lines will be plotted. This value won’t affect estimation for both cell lines. (default: 1)

...

Parameters for base function plot.
**Value**

<table>
<thead>
<tr>
<th>plot</th>
<th>SD plots of quantification pipelines for selected cell line by plotcell.</th>
</tr>
</thead>
<tbody>
<tr>
<td>list</td>
<td>A list of two matrices of median and standard error of standard deviations.</td>
</tr>
</tbody>
</table>

**Examples**

data(simdata)
condInfo <- factor(simdata$samp$condition)
repInfo <- factor(simdata$samp$replicate)
evaluationFeature <- rep(TRUE, nrow(simdata$meta))
calibrationFeature <- simdata$meta$house & simdata$meta$chr == 'chr1'
unitReference <- 1
dat <- signalCalibrate(simdata$quant, condInfo, repInfo, evaluationFeature, calibrationFeature, unitReference, calibrationFeature2 = calibrationFeature)
plotSD(dat)

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**rnaseqcomp-class**

**Description**

This is a S4 class to organize data ready for benchmark summarization. There are 5 S3 objects inside this class. `quantData` documents a list of data matrices ready for evaluation by functions `plotSD`, `plotNE`, `plot2TX` or `plotROC`. `condInfo` is a factor corresponding to columns of `quantData` matrices, indicating to which cell lines each sample belongs. `repInfo` is a factor corresponding to columns of `quantData` matrices indicating replicate information. `repInfo` is a legacy from previous versions, and doesn’t have too much meanings in current version. `refMed` is the median log2 signal of calibration references. `scaler` is a number that point to the median log2 signal of reference pipeline. `refMed` and `scaler` were used to calibrate and generate `quantData`.

**signalCalibrate**

**Quantification Filtering And Calibration**

**Description**

This is the function to do any pre-filtering or pre-processing analysis for downstream benchmark estimation and visualization. Pre-filtering includes row selection (e.g. protein coding genes) of quantification table; pre-processing includes calculation on a set of rows as calibration reference (e.g. house keeping genes) across different quantification pipelines, calibration of quantifications across all the pipelines based on given cutoffs from selected pipelines.

**Usage**

```r
signalCalibrate(quantData, condInfo, repInfo, evaluationFeature, calibrationFeature, unitReference, unitCutoff = 0, calibrationFeature2 = NULL, fixMedian = 4.776)
```
**Arguments**

- **quantData**: A list of quantification matrices each with rows by features (transcripts, genes, junctions or exons) and columns by samples. Names of the list should be provided. The sizes of each element should be the same. Missing data will be set to 0.
- **condInfo**: A factor documenting condition information of samples, corresponding to the columns of each element in `quantData`.
- **repInfo**: A factor documenting replicate information of samples, corresponding to the columns of each element in `quantData`.
- **evaluationFeature**: A logical vector corresponding to the rows of each element in `quantData`, providing which features should be considered for downstream evaluation, e.g. protein coding genes.
- **calibrationFeature**: A logical vector corresponding to the rows of each element in `quantData`, providing which features should be considered as calibration reference, e.g. housekeeping genes.
- **unitReference**: A numeric number specifying which pipeline will be selected as reference pipeline, i.e. the index of one element in `quantData`.
- **unitCutoff**: A numeric number for signal cutoff on reference pipeline specified by `unitReference` (default: 0). Equivalent effects of cutoffs will be applied to other pipelines accordingly.
- **calibrationFeature2**: A logical vector corresponding to the rows of each element in `quantData`, providing which features should be considered as references for calibration across different datasets. Default `NULL` means no calibration needed.
- **fixMedian**: A numeric number specifying the median of detrend logsignals for features specified by `calibrationFeature2`. When comparing across datasets, those features will be calibrated to have the same median as `fixMedian`, while other features calibrated accordingly. The default is 4.776, which was calculated based on one ENCODE dataset used in our web tool.

**Details**

In the functions `plotSD` and `plot2TX`, detrended signals with value 0 will be at the same level as value 1 for giving pipeline by `unitReference`.

**Value**

A `rnaseqcomp` S4 class object

- **quantData**: A filtered and calibrated list of quantifications for downstream analysis.
- **condInfo**: A factor documenting sample condition information.
- **repInfo**: A factor documenting sample replicate information.
- **refMed**: A list of numeric vectors giving the log scale medians of calibration features in different pipelines.
- **scaler**: A number that was used for scaling quantifications onto reference pipeline.
Examples

data(simdata)
condInfo <- factor(simdata$samp$condition)
repInfo <- factor(simdata$samp$replicate)
evaluationFeature <- rep(TRUE, nrow(simdata$meta))
calibrationFeature <- simdata$meta$house & simdata$meta$chr == 'chr1'
unitReference <- 1
dat <- signalCalibrate(simdata$quant, condInfo, repInfo, evaluationFeature, calibrationFeature, unitReference, calibrationFeature2 = calibrationFeature)

Description

This dataset include quantifications on 15776 transcripts on two cell lines each with 8 replicates. The true differential expressed transcripts were simulated. Quantifications from two pipelines (RSEM and FluxCapacitor) are included in this dataset at simdata$quant. Meta information of transcripts is included at simdata$meta, including if they belongs to house keeping genes and their true fold change status. Sample information is included at simdata$samp.

Format

A list of objects including list of two 15776*16 quantification matrices, one 15776*6 data frame with meta information and one 16*3 data frame with sample information.
Index

check_rnaseqcomp, 2
plot2TX, 2
plotFC, 3
plotNE, 4
plotROC, 5
plotSD, 6
rnaseqcomp-class, 7
signalCalibrate, 7
simdata, 9