

Package ‘doseR’

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Type Package

Title doseR

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Description doseR package is a next generation sequencing package for sex chromosome dosage compensation which can be applied broadly to detect shifts in gene expression among an arbitrary number of pre-defined groups of loci. doseR is a differential gene expression package for count data, that detects directional shifts in expression for multiple, specific subsets of genes, broad utility in systems biology research. doseR has been prepared to manage the nature of the data and the desired set of inferences. doseR uses S4 classes to store count data from sequencing experiment. It contains functions to normalize and filter count data, as well as to plot and calculate statistics of count data. It contains a framework for linear modeling of count data. The package has been tested using real and simulated data.

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dafs	<i>dafs</i>
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Description

This function filters the expression using DAFS (see ref). This is a core function invoked by the DAFS wrapper.

Usage

dafs(VEC1, PLOT)

Arguments

- VEC1 Vector 1, not intended for user interaction.
- PLOT Boolean, toggles plotting.

Details

This function filters the expression, using Data Adaptive Flag method.

Value

Returns vx[which.min(vv)] to wrapper function.

Author(s)

AJ Vaestermarck, JR Walters.

References

BMC Bioinformatics, 2014, 15:92

Examples

```
library(mclust)
data(hmel.se)
f_se <- dafsFilter(se)
```

dafsFilter

dafsFilter Function to filter expression data within an object.

Description

This function filters the expression of the supplied object, by invoking the dafsFilter (dafs) function. dafsFilter is a filtering function used to remove rows (genes) of various expression data.

Usage

```
dafsFilter(se, PLOT=TRUE)
```

Arguments

se	A SummarizedExperiment object.
PLOT	Boolean, toggles plotting.

Details

This function filters the expression of the supplied object using a Data Adaptive Flag filter. The internal function uses a vector to store Kolmogorov Smirnov distance statistics, loops through cuts of the data to determine targeted K-S statistic, selects data greater than a quantile and runs Mclust on that data to determine theoretical distribution. The wrapper uses simpleFilter to determine first left-most local minima (using the Earth library).

Value

Returns an invisible, filtered SummExp object.

Author(s)

AJ Vaestermark, JR Walters.

References

BMC Bioinformatics, 2014, 15:92

Examples

```
library(mclust)
data(hmel.se)
f_se <- dafsFilter(se)
```

generateStats	<i>generateStats Statistics function to summarize expression data of an s.e. object.</i>
---------------	--

Description

generateStats is a summary function used on various expression data.

Usage

```
generateStats(se, groupings= NULL, mode_mean=TRUE, LOG2=TRUE)
```

Arguments

se	A SummarizedExperiment object.
groupings	A grouping (annotation column), e.g. groupings="something".
mode_mean	Boolean, Calculate RowMeans or RowMedians.
LOG2	Boolean, Calculate LOG2.

Details

This function completes summary statistics of the expression of the supplied s.e. object.

Value

Returns an invisible list of summary statistics, kruskal test and raw data of an s.e. object.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
generateStats(se, groupings="annotation.ZA")
```

getLibsizes3	<i>getLibsizes3</i> Get Lib Sizes.
--------------	------------------------------------

Description

getLibsizes3 method for SummarizedExp class object, derived from getLibsizes2

Usage

```
getLibsizes3(se, subset = NULL,  
  estimationType = c("quantile", "total",  
    "edgeR"), quantile = 0.75, ...)
```

Arguments

se	A SummarizedExperiment object.
subset	Value
estimationType	e.g. quantile, total, edgeR.
quantile	A quantile, expressed as e.g. 0.75.
...	Passthrough arguments.

Value

Libsize value

Author(s)

AJ Vaestermark, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)  
getLibsizes3(se)
```

glSeq	<i>glSeq LME4 wrapper.</i>
-------	----------------------------

Description

This function is an LME4 wrapper for dosage analysis.

Usage

```
glSeq(dm, model, ...)
```

Arguments

dm	The dm data. Generally a reformatted object from se.DM.
model	The model. Expressed using standard LME4 syntax, see vignette.
...	passthrough arguments.

Details

This function is an lme4 wrapper.

Value

Returns LME4 output.

Author(s)

AJ Vaestermark, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
f_se <- quantFilter(se, lo.bound = 0.4, hi.bound = 0.5)
dm <- se.DM(f_se)
glSeq(dm, "-1 + replicate")
```

hmel.dat	<i>Hmel data set</i>
----------	----------------------

Description

Hmel data set

Usage

```
data("hmel.data.doser")
```

Format

An object of class list of length 3.

References

To demonstrate a typical workflow using doseR, we will use data from a study in *Heliconius* butterflies. *H. melpomene* has 20 autosomes with chromosome 21 being the "Z" sex chromosome. Like all Lepidoptera, this is a female-heterogametic species, so males are diploid for the Z while females have a single Z and W chromosomes. The relevant data are included in the doseR package, which must be installed. These test data are unpublished.

iqrxFILTER	<i>iqrxFILTER Function to filter expression data within an s.e. object.</i>
------------	---

Description

This function filters the expression of the supplied se object. iqrxFILTER is a filtering function used to remove rows (genes) of various expression data.

Usage

```
iqrxFILTER(se, iqr_multi = 1.5, MEDIAN = FALSE, na.rm = TRUE)
```

Arguments

se	A SummarizedExperiment object.
iqr_multi	Numeric multiplier; removes any outliers that are iqr_multi times the mid-50 percentile distance greater or less than the 25th and 75th percentiles, by default
MEDIAN	Boolean, Calculate RowMeans or RowMedians.
na.rm	Boolean, NA removal.

Details

This function filters the expression of the supplied object, based on a selected percentage cutoff and selected interquartile range multiplier. The function iqrxFILTER will: 1) log-base two transform all RPKM values (obligatory); (2) remove any outliers that were 1.5 times the mid-50 percentile distance greater or less than the 75th and 25th percentiles (by default), respectively; and (3) uses mean values and instead of median values (by default).

Value

Returns a filtered SummarizedExperiment object.

Author(s)

AJ Vaestermark, JR Walters.

References

Jue et al. BMC Genomics 2013 14:150

Examples

```
data(hmel.se)
f_se <- iqrxFilter(se)
```

make_RPKM

make_RPKM Make RPKM.

Description

make_RPKM populates RPKM slot of SummarizedExperiment S4 object.

Usage

```
make_RPKM(se)
```

Arguments

se A SummarizedExperiment object.

Value

RPKM populated object

Author(s)

AJ Vaestermark, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
SummarizedExperiment::assays(se)$rpkm <- make_RPKM(se)
```

plotExpr	<i>plotExpr Function to generate a boxplot (expression) for a SummarizedExperiment object based on the replicate data.</i>
----------	--

Description

This function generates a boxplot of FPKM expression values from the supplied object. FPKM values are averaged across replicates and partitioned among groups of loci as specified in a selected column from the annotation slot of the provided object.

Usage

```
plotExpr(se, groupings= NULL, mode_mean=TRUE,
         treatment=levels(colData(se)$Treatment),
         LOG2=TRUE, clusterby_grouping=TRUE, ...)
```

Arguments

se	A SummarizedExp object containing FPKM values and at least one annotation column.
groupings	Specifies which column in the dataframe of the annotation slot that will be used to group loci in the boxplot. Can provide either a character value matching the column name, or a single numerical value used as an index of dataframe columns.
mode_mean	Logical. If TRUE then FPKM values are averaged by mean across replicates within treatment. If FALSE, values are averaged by median.
treatment	A character vector indicating which treatments (i.e. levels in the replicates slot vector) will be plotted. Order matters, and controls the ordering of treatments represented in the boxplot.
LOG2	Logical. If TRUE then average FPKM values are Log2 transformed.
clusterby_grouping	Logical. If TRUE then boxplots are arranged by locus annotation grouping. If FALSE they are arranged by treatment levels, as indicated in the treatment argument.
...	Additional named arguments and graphical parameters passed to the boxplot function.

Details

This function generates boxplots to visualize the distribution of FPKM expression values provided in an object, arranged by selected treatments and locus annotations. FPKM values are averaged (mean or median) within selected treatments, to provide a single expression value per locus per treatment. Loci are partitioned into groupings based on a specified column in the dataframe of annotations slot of the object. Thus a box is drawn for each grouping of loci for each treatment indicated. Desired treatments and their ordering are specified by the treatment argument. Groupings are arranged by sort order of the annotation column indicated, and can thus be controlled by providing a factor with a pre-specified level order. By default (`clusterby_grouping = TRUE`), boxes are arranged by annotation group first, and then by treatment, but setting this option to FALSE arranges boxes by treatment and then annotation group. This function uses the base graphics boxplot function to generate the plot, so can accept all relevant graphical arguments for customizing the figure; see `boxplot` for details.

Value

Returns an invisible data frame containing values and labels used to generate the figure.

Author(s)

AJ Vaestermark, JR Walters.

References

The 'doseR' package, 2018 (in press).

Examples

```
data(hmel.se)
plotExpr(se, groupings = "annotation.ZA", treatment = 'Male' )
```

plotMA.se	<i>plotMA.se Function to make MA plot.</i>
-----------	--

Description

This function generates MA plot.

Usage

```
plotMA.se(se, samplesA, samplesB, scale = NULL,
          xlab = 'A', ylab = 'M', ...)
```

Arguments

se	A SummarizedExperiment object.
samplesA	Either a character vector, identifying sample set A by either replicate name or sample name, or a numerical vector giving the columns of data in the object that forms sample set A.
samplesB	Either a character vector, identifying sample set B by either replicate name or sample name, or a numerical vector giving the columns of data in the object that forms sample set B.
scale	If given, defines the scale on which the log-ratios will be plotted.
xlab	Label for the X-axis. Defaults to 'A'.
ylab	Label for the Y-axis. Defaults to 'M'.
...	Any other parameters to be passed to the plot function.

Details

This function makes MA plot from SummExperiment object.

Value

Returns MA plot.

Author(s)

AJ Vaestermarck, JR Walters.

References

The 'doseR' package, 2018 (in press).

Examples

```
data(hmel.se)
plotMA.se(se, samplesA = 'Male', samplesB = 'Female')
```

plotRatioBoxes	<i>plotRatioBoxes Function to boxplot density of ratios between two treatments (using groupings from an annotation column) within a SummarizedExperiment object.</i>
----------------	--

Description

This function plots the expression of the supplied object, representing ratios between a pair of selected treatments as a boxplot for each group in the selected annotation column.

Usage

```
plotRatioBoxes(se, groupings= NULL, treatment1=NULL, treatment2=NULL,
mode_mean=TRUE, LOG2=TRUE, ...)
```

Arguments

se	A SummarizedExperiment object.
groupings	A grouping (annotation column), e.g. groupings="something".
treatment1	Symbol, treatment 1.
treatment2	Symbol, treatment 2.
mode_mean	Boolean, Calculate RowMeans or RowMedians.
LOG2	Boolean, Calculate LOG2.
...	Passthrough arguments to boxplot (additional arguments affecting the summary produced).

Details

This function boxplots expression of the supplied object using ratios of treatment1/treatment2.

Value

Returns an invisible data frame containing the values.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
plotRatioBoxes(se, groupings='annotation.ZA', treatment1 = 'Male',
treatment2 = 'Female')
```

plotRatioDensity	<i>plotRatioDensity Function to plot density of ratios between two treatments (using groupings from an annotation column) within an object.</i>
------------------	---

Description

This function plots the expression of the supplied object, using ratios between a pair of selected treatments.

Usage

```
plotRatioDensity(se, groupings= NULL, treatment1=NULL,
treatment2=NULL, mode_mean=TRUE, LOG2=TRUE,...)
```

Arguments

se	A SummarizedExperiment object.
groupings	A grouping (annotation); groupings="annotation.ZA"
treatment1	Symbol, treatment 1.
treatment2	Symbol, treatment 2.
mode_mean	Boolean, Calculate RowMeans or RowMedians.
LOG2	Boolean, Calculate LOG2.
...	Passthrough arguments to boxplot (additional arguments affecting the summary produced).

Details

This function plots expression of the supplied object using ratios of treatment1/treatment2.

Value

Returns an invisible data frame containing the x-values and corresponding density for each applicable annotation column entry.

Author(s)

AJ Vaestermark, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
plotRatioDensity(se, groupings='annotation.ZA', treatment1 = 'Male',
  treatment2 = 'Female', lty=1, type="l")
```

quantFilter	<i>quantFilter Function to filter expression data of a SummarizedExperiment object.</i>
-------------	---

Description

This function filters the expression of the supplied object; quantFilter is a filtering function used to remove rows (genes) of various expression data.

Usage

```
quantFilter (se, lo.bound=.25, hi.bound=.75, MEDIAN = FALSE,
  na.rm = TRUE)
```

Arguments

se	A SummarizedExperiment object.
lo.bound	The lower cutoff, expressed as a percentage.
hi.bound	The upper cutoff, expressed as a percentage.
MEDIAN	Boolean, Calculate RowMeans or RowMedians.
na.rm	Boolean, NA removal.

Details

This function filters the expression of the supplied object, based on a selected percentage cutoff.

Value

Returns a filtered SummarizedExperiment object.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
f_se <- quantFilter(se, lo.bound=0.5)
```

se	<i>Hmel data set</i>
----	----------------------

Description

Hmel data set

Usage

```
data("hmel.se")
```

Format

An object of class SummarizedExperiment with 13619 rows and 6 columns.

References

To demonstrate a typical workflow using doseR, we will use data from a study in *Heliconius* butterflies. *H. melpomene* has 20 autosomes with chromosome 21 being the "Z" sex chromosome. Like all Lepidoptera, this is a female-heterogametic species, so males are diploid for the Z while females have a single Z and W chromosomes. The relevant data are included in the doseR package, which must be installed. These test data are unpublished. This is the SummarizedExperiment version of the sample data.

se.DM	<i>se.DM Function to convert SummarizedExperiment object to LME4 input.</i>
-------	---

Description

This function generates LME4 input.

Usage

```
se.DM(se, weightByLL = TRUE)
```

Arguments

se	A SummarizedExperiment object containing FPKM values and at least one annotation column.
weightByLL	Logical, weigh by log likelihood score.

Details

This function converts SummarizedExperiment object.

Value

Returns LME4 input.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)
f_se <- quantFilter(se, lo.bound = 0.4, hi.bound = 0.5)
dm <- se.DM(f_se)
```

simpleFilter

simpleFilter Function to filter expression data of an object.

Description

simpleFilter is a filtering function used to remove rows (genes) of various expression data.

Usage

```
simpleFilter(se, mean_cutoff=NULL, min_cutoff=NULL, median_cutoff=
NULL, counts=TRUE)
```

Arguments

se	A SummarizedExperiment object.
mean_cutoff	The lower cutoff, using mean.
min_cutoff	The cutoff, expressed as a minimum acceptable value.
median_cutoff	The cutoff, using a median value.
counts	Boolean, use raw counts data (default) or RPKM.

Details

This function filters the expression of the supplied obj.

Value

Returns a filtered SummarizedExperiment object.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se) ; f_se <- simpleFilter(se, mean_cutoff=0.5)
```

test_diffs	<i>test_diffs</i> Statistics function to summarize expression data of an object, using ratios between selected treatments.
------------	--

Description

generateStats is a summary function used on various expression data, using ratios between selected treatments.

Usage

```
test_diffs(se, groupings= NULL, treatment1=NULL, treatment2=NULL,  
mode_mean=TRUE, LOG2=TRUE)
```

Arguments

se	A SummarizedExperiment object.
groupings	A grouping (annotation column); groupings="annotation.ZA".
treatment1	Symbol, treatment 1.
treatment2	Symbol, treatment 2.
mode_mean	Boolean, Calculate RowMeans or RowMedians.
LOG2	Boolean, Calculate LOG2.

Details

This function completes summary statistics of the expression of the supplied SummarizedExperiment object.

Value

Returns an invisible list of summary statistics, kruskal test and raw data of an object, using ratios between selected treatments.

Author(s)

AJ Vaestermarck, JR Walters.

References

The "doseR" package, 2018 (in press).

Examples

```
data(hmel.se)  
test_diffs(se, groupings='annotation.ZA', treatment1="Male",  
treatment2="Female" )
```

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