Package ‘qsmooth’

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Title Smooth quantile normalization

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Description Smooth quantile normalization
is a generalization of quantile normalization,
which is average of the two types of assumptions
about the data generation process: quantile
normalization and quantile normalization between
groups.

biocViews Normalization, Preprocessing, MultipleComparison, Microarray, Sequencing, RNASeq, BatchEffect

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Contents

`qsmooth` *qsmooth*

Description

This function applies a generalization of quantile normalization called smoothed quantile normalization. This function defines the qsmooth class and constructor.

Usage

```
qsmooth(object, group_factor, batch = NULL, norm_factors = NULL, window = 0.05)
```

Arguments

`object`	an object which is a `matrix` or `data.frame` with observations (e.g. probes or genes) on the rows and samples as the columns. Alternatively, a user can provide a `SummarizedExperiment` object and the `assay(object, "counts")` will be used as input for the qsmooth normalization.
`group_factor`	a group level continuous or categorial covariate associated with each sample or column in the `object`. The order of the `group_factor` must match the order of the columns in `object`.
`batch`	(Optional) batch covariate (multiple batches are not allowed). If batch covariate is provided, `Combat()` from `sva` is used prior to qsmooth normalization to remove batch effects. See `Combat()` for more details.
`norm_factors`	optional normalization scaling factors.
`window`	window size for running median which is a fraction of the number of rows in `object`. Default is 0.05.

Details

Quantile normalization is one of the most widely used normalization tools for data analysis in genomics. Although it was originally developed for gene expression microarrays it is now used across many different high-throughput applications including RNAseq and ChIPseq. The methodology relies on the assumption that observed changes in the empirical distribution of samples are due to unwanted variability. Because the data is transformed to remove these differences it has the potential to remove interesting biologically driven global variation. Therefore, applying quantile normalization, or other global normalization methods that rely on similar assumptions, may not be an appropriate depending on the type and source of variation.

This function computes a weight at every quantile that compares the variability between groups relative to within groups. In one extreme quantile normalization is applied and in the other extreme quantile normalization within each biological condition is applied. The weight shrinks the group-level quantile normalized data towards the overall reference quantiles if variability between groups is sufficiently smaller than the variability within groups. See the vignette for more details.

Value

A object of the class `qsmooth` that contains a numeric vector of the qsmooth weights in the `qsmoothWeights` slot and a matrix of normalized values after applying smoothed quantile normalization in the `qsmoothData` slot.

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
dat_qs <- qsmooth(object = dat,
                  group_factor = rep(c(0,1), each=10))
```

`qsmooth-class` *the qsmooth class*

Description

Objects of this class store all the values needed information to work with a qsmooth object

Value

`qsmoothWeights` returns the qsmooth weights and `qsmoothData` returns the qsmooth normalized data

Slots

`qsmoothWeights` qsmooth weights

`qsmoothData` qsmooth normalized data

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
dat_qs <- qsmooth(object = dat,
                  group_factor = rep(c(0,1), each=10))
```

qsmoothData *Generic function that returns the qsmooth normalized data*

Description

Given a qsmooth object, this function returns the qsmooth normalized data

Accessors for the 'qsmoothData' slot of a qsmooth object.

Usage

```
qsmoothData(object)

## S4 method for signature 'qsmooth'
qsmoothData(object)
```

Arguments

object	an object of class qsmooth.

Value

The normalized data after applying smoothed quantile normalization.

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
dat_qs <- qsmooth(object = dat,
                  group_factor = rep(c(0,1), each=10))
qsmoothData(dat_qs)
```

qsmoothGC *qsmoothGC*

Description

This function applies smoothed quantile normalization separately for groups of features that are binned according to their GC-content.

Usage

```
qsmoothGC(object, group_factor, gc, nGroups = 50, round = TRUE, ...)
```

Arguments

`object` an object which is a `matrix` or `data.frame` with observations (e.g. probes or genes) on the rows and samples as the columns. Alternatively, a user can provide a `SummarizedExperiment` object and the `assay(object, "counts")` will be used as input for the qsmooth normalization.

`group_factor` a group level continuous or categorial covariate associated with each sample or column in the `object`. The order of the `group_factor` must match the order of the columns in `object`.

`gc` GC-content of the features, ordered according to the features in `object`.

`nGroups` The number of equally-sized bins used to group the GC-content values. Groups are created using `Hmisc::cut2`.

`round` Should normalized values be rounded to integers?

`...` (Optional) Additional arguments passed to `qsmooth`.

Value

A matrix of normalized counts.

References

Van den Berge K., Chou H., Roux de Bézieux H., Street K., Risso D., Ngai J., Dudoit S. Normalization benchmark of ATAC-seq datasets shows the importance of accounting for GC-content effects. https://www.biorxiv.org/content/10.1101/2021.01.26.428252v2

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
gc <- runif(n=100, min=0.2, max=0.9)
dat_qs <- qsmoothGC(object = dat,
                    gc = gc,
                    group_factor = rep(c(0,1), each=10))
```

`qsmoothPlotWeights` *Plot weights from* `qsmooth` *function.*

Description

This function plots a scatterplot showing the `qsmoothWeights` along the y-axis and the quantiles on the x-axis.

Usage

```
qsmoothPlotWeights(
  object,
  xLab = "quantiles",
  yLab = "weights",
  mainLab = "qsmooth weights"
)
```

Arguments

`object`	a qsmooth object from `qsmooth`
`xLab`	label for x-axis. Default is "quantiles"
`yLab`	label for y-axis. Default is "weights"
`mainLab`	title of plot. Default is "qsmooth weights"

Value

A scatterplot will be created showing the `qsmoothWeights` along the y-axis and the quantiles on the x-axis.

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
dat_qs <- qsmooth(object = dat,
                  group_factor = rep(c(0,1), each=10))
qsmoothPlotWeights(dat_qs)
```

`qsmoothWeights` *Generic function that returns the qsmooth weights*

Description

Given a qsmooth object, this function returns the qsmooth weights

Accessors for the 'qsmoothWeights' slot of a qsmooth object.

Usage

```
qsmoothWeights(object)

## S4 method for signature 'qsmooth'
qsmoothWeights(object)
```

Arguments

`object`	an object of class `qsmooth`.

Value

The weights calculated for each feature after applying smoothed quantile normalization.

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
dat_qs <- qsmooth(object = dat,
                  group_factor = rep(c(0,1), each=10))
qsmoothWeights(dat_qs)
```

`qstats` *qstats*

Description

This function is a helper function that computes quantile statistics for the function `qsmooth`.

Usage

```
qstats(object, group_factor, window = 0.05)
```

Arguments

`object` an object which is a data frame or matrix with observations (e.g. probes or genes) on the rows and samples as the columns.

`group_factor` a group level continuous or categorial covariate associated with each sample or column in the `object`. The order of the `group_factor` must match the order of the columns in `object`.

`window` window size for running median which is a fraction of the number of rows in `object`. Default is 0.05.

Value

A list of quantile statistics including

`Q` sample quantiles

`Qref` reference quantile

`Qhat` linear model fit at each quantile

`SST` total sum of squares

`SSB` between sum of squares

`SSE` within sum of squares

`roughWeights` SSE / SST

`smoothWeights` smoothed weights computed using a running median with a given `window` size.

Examples

```
dat <- cbind(matrix(rnorm(1000), nrow=100, ncol=10),
             matrix(rnorm(1000, .1, .7), nrow=100, ncol=10))
qs <- qstats(object = dat,
             group_factor = rep(c(0,1), each=10),
             window = 0.05)
```

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