Package ‘SCnorm’

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Title  Normalization of single cell RNA-seq data
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Description  This package implements SCnorm — a method to normalize single-cell RNA-seq data.
Depends  R (>= 3.4.0),
Imports  SingleCellExperiment, SummarizedExperiment, stats, methods, graphics, grDevices, parallel, quantreg, cluster, moments, data.table, BiocParallel, S4Vectors, ggplot2, forcats, BiocGenerics
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BugReports  https://github.com/rhondabacher/SCnorm/issues
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**Description**

Perform the correction within each sample (See loess normalization in original publication Risso et al., 2011 (BMC Bioinformatics)). Similar to function in EDAseq v2.8.0.

**Usage**

```r
correctWithin(y, correctFactor)
```

**Arguments**

- `y` gene to perform the regression on.
- `correctFactor` list of data needed for the regression.

**Details**

Performs within sample normalization.

**Value**

within-cell normalized expression estimates
**Description**

Median quantile regression is fit for each gene using the normalized gene expression values. A slope near zero indicate the sequencing depth effect has been successfully removed. Genes are divided into ten equally sized groups based on their non-zero median expression. Slope densities are plot for each group and estimated modes are calculated. If any of the ten group modes is larger than .1, the K is not sufficient to normalize the data.

**Usage**

```r
evaluateK(
  Data,
  SeqDepth,
  OrigData,
  Slopes,
  Name,
  Tau,
  PrintProgressPlots,
  ditherCounts
)
```

**Arguments**

- **Data**
  matrix of normalized expression counts. Rows are genes and columns are samples.

- **SeqDepth**
  vector of sequencing depths estimated as columns sums of un-normalized expression matrix.

- **OrigData**
  matrix of un-normalized expression counts. Rows are genes and columns are samples.

- **Slopes**
  vector of slopes estimated in the GetSlopes() function. Only used here to obtain the names of genes considered in the normalization.

- **Name**
  plot title

- **Tau**
  value of quantile for the quantile regression used to estimate gene-specific slopes (default is median, Tau = .5 ).

- **PrintProgressPlots**
  whether to automatically produce plot as SCnorm determines the optimal number of groups (default is FALSE, highly suggest using TRUE). Plots will be printed to the current device.

- **ditherCounts**
  whether to dither/jitter the counts, may be used for data with many ties, default is FALSE.
Value

value of largest mode and a plot of the ten normalized slope densities.

Author(s)

Rhonda Bacher

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ExampleSimSCData  Example datasets for SCnorm

---

Description

Data generated as in SIM I from the manuscript with $K = 4$.

Usage

ExampleSimSCData

Format

data matrix

Examples

data(ExampleSimSCData)

---

generateEvalPlot  Internal plotting function.

---

Description

Genes are divided into $\text{NumExpressionGroups} = 10$ equally sized groups based on their non-zero median expression. Slope densities are plot for each group.

Usage

generateEvalPlot(
  MedExpr,
  SeqDepth,
  Slopes,
  Name,
  NumExpressionGroups = 10,
  BeforeNorm = TRUE
)

getCounts

Arguments

- **MedExpr**: non-zero median expression for all genes.
- **SeqDepth**: sequencing depth for each cell/sample.
- **Slopes**: per gene estimates of the count-depth relationship.
- **Name**: name for plot title.
- **NumExpressionGroups**: the number of groups to split the data into, genes are split into equally sized groups based on their non-zero median expression.
- **BeforeNorm**: whether data have already been normalized.

Value

A plot of the un-normalized slope densities.

Author(s)

Rhonda Bacher

Description

Convenient helper function to extract the normalized expression matrix from the SummarizedExperiment

Usage

getCounts(DATA)

Arguments

- **DATA**: An object of class SummarizedExperiment that contains single-cell expression and metadata

Value

A matrix which contains the count data where genes are in rows and cells are in columns

Examples

data(ExampleSimSCData)
ExampleData <- SummarizedExperiment::SummarizedExperiment(assays=list("Counts"=ExampleSimSCData))
myData <- getCounts(ExampleData)
**getDens**

**Description**

getDens

**Usage**

getDens(ExprGroups, byGroup, RETURN = c("Mode", "Height"))

**Arguments**

- **ExprGroups**
  
  expression groups already split.

- **byGroup**
  
  factor (usually slopes) to get density based on ExprGroups.

- **RETURN**
  
  whether to return Mode or Height of density.

**Details**

get density of slopes in different expression groups

**Value**

list, length is equal to NumGroups

---

**getSlopes**

**Description**

This is the gene-specific fitting function, where a median (Tau = .5) quantile regression is fit for each gene. Only genes having at least 10 non-zero expression values are considered.

**Usage**

getSlopes(
  Data,
  SeqDepth = 0,
  Tau = 0.5,
  FilterCellNum = 10,
  ditherCounts = FALSE
)

*Estimate gene specific count-depth relationships*
**GetTD**

**Arguments**

- **Data**
  - Data matrix of un-normalized expression counts. Rows are genes and columns are samples.
- **SeqDepth**
  - SeqDepth vector of sequencing depths estimated as columns sums of un-normalized expression matrix.
- **Tau**
  - Tau value of quantile for the quantile regression used to estimate gene-specific slopes (default is median, Tau = .5 ).
- **FilterCellNum**
  - FilterCellNum the number of non-zero expression estimate required to include the genes into the SCnorm fitting (default = 10). The initial
- **ditherCounts**
  - Whether to dither/jitter the counts, may be used for data with many ties, default is FALSE.

**Value**

- Value vector of estimated slopes.

**Author(s)**

- Rhonda Bacher

**Examples**

```r
data(ExampleSimSCData)
myslopes <- getSlopes(ExampleSimSCData)
```

**Description**

- GetTD Fit group regression for specific quantile and degree

**Usage**

```r
GetTD(x, InputData)
```

**Arguments**

- **x**
  - Specifies a column of the grid matrix of tau and d.
- **InputData**
  - InputData contains the expression values, sequencing depths to fit the group regression, and the quantile used in the individual gene regression for grouping.

**Value**

- Value estimated count-depth relationship of predicted values for one value of tau and degree.
normWrapper

Iteratively fit group regression and evaluate to choose optimal K

Description
This function iteratively normalizes using K groups and then evaluates whether K is sufficient. If the maximum mode received from the GetK() function is larger than .1, K is increased to K + 1. Uses params sent from SCnorm.

Usage

```r	normWrapper(
    Data,
    SeqDepth = NULL,
    Slopes = NULL,
    CondNum = NULL,
    PrintProgressPlots,
    PropToUse,
    Tau,
    Thresh,
    ditherCounts
)
```

Arguments

- **Data** can be a matrix of single-cell expression with cells where rows are genes and columns are samples. Gene names should not be a column in this matrix, but should be assigned to rownames(Data). Data can also be an object of class SummarizedExperiment that contains the single-cell expression matrix and other metadata. The assays slot contains the expression matrix and is named "Counts". This matrix should have one row for each gene and one sample for each column. The colData slot should contain a data.frame with one row per sample and columns that contain metadata for each sample. This data.frame should contain a variable that represents biological condition in the same order as the columns of NormCounts). Additional information about the experiment can be contained in the metadata slot as a list.
- **SeqDepth** sequencing depth for each cell/sample.
- **Slopes** per gene estimates of the count-depth relationship.
- **CondNum** name of group being normalized, just for printing messages.
- **PrintProgressPlots** whether to automatically produce plot as SCnorm determines the optimal number of groups (default is FALSE, highly suggest using TRUE). Plots will be printed to the current device.
**plotCountDepth**

**PropToUse**  proportion of genes closest to the slope mode used for the group fitting, default is set at .25. This number mainly affects speed.

**Tau**  value of quantile for the quantile regression used to estimate gene-specific slopes (default is median, Tau = .5).

**Thresh**  threshold to use in evaluating the sufficiency of K, default is .1.

**ditherCounts**  whether to dither/jitter the counts, may be used for data with many ties, default is FALSE.

**Value**

matrix of normalized and scaled expression values for all conditions and the evaluation plots are output for each attempted value of K.

**Author(s)**

Rhonda Bacher

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**plotCountDepth**  
*Evaluate the count-depth relationship before (or after) normalizing the data.*

**Description**

Quantile regression is used to estimate the dependence of read counts on sequencing depth for every gene. If multiple conditions are provided, a separate plot is provided for each and the filters are applied within each condition separately. The plot can be used to evaluate the extent of the count-depth relationship in the dataset or can be used to evaluate data normalized by alternative methods.

**Usage**

```r
goingline(plotCountDepth(  
  Data,  
  NormalizedData = NULL,  
  Conditions = NULL,  
  Tau = 0.5,  
  FilterCellProportion = 0.1,  
  FilterExpression = 0,  
  NumExpressionGroups = 10,  
  NCores = NULL,  
  ditherCounts = FALSE
))```
Arguments

Data can be a matrix of single-cell expression with cells where rows are genes and columns are samples. Gene names should not be a column in this matrix, but should be assigned to rownames(Data). Data can also be an object of class SummarizedExperiment that contains the single-cell expression matrix and other metadata. The assays slot contains the expression matrix and is named "Counts". This matrix should have one row for each gene and one sample for each column. The colData slot should contain a data.frame with one row per sample and columns that contain metadata for each sample. This data.frame should contain a variable that represents biological condition in the same order as the columns of NormCounts). Additional information about the experiment can be contained in the metadata slot as a list.

NormalizedData matrix of normalized expression counts. Rows are genes and columns are samples. Only input this if evaluating already normalized data.

Conditions vector of condition labels, this should correspond to the columns of the un-normalized expression matrix. If not provided data is assumed to come from same condition/batch.

Tau value of quantile for the quantile regression used to estimate gene-specific slopes (default is Tau = .5 (median)).

FilterCellProportion the proportion of non-zero expression estimates required to include the genes into the evaluation. Default is .10, and will not go below a proportion which uses less than 10 total cells/samples.

FilterExpression exclude genes having median of non-zero expression below this threshold from count-depth plots (default = 0).

NumExpressionGroups the number of groups to split the data into, genes are split into equally sized groups based on their non-zero median expression.

NCores number of cores to use, default is detectCores() - 1. This will be used to set up a parallel environment using either MulticoreParam (Linux, Mac) or SnowParam (Windows) with NCores using the package BiocParallel.

ditherCounts whether to dither/jitter the counts, may be used for data with many ties, default is FALSE.

Value

returns a data.frame containing each gene’s slope (count-depth relationship) and its associated expression group. A plot will be output.

Author(s)

Rhonda Bacher
Examples

data(ExampleSimSCData)
Conditions = rep(c(1,2), each= 90)
#plotCountDepth(Data = ExampleSimSCData, Conditions = Conditions,
#FilterCellProportion = .1)

plotWithinFactor

Evaluate gene-specific factors in the the data.

Description

This function can be used to evaluate the extent of gene-specific biases in the data. If a bias exists, the plots provided here will identify whether it affects cells equally or not. Correction for such features may be considered especially if the bias is different between conditions (see SCnorm vignette for details).

Usage

plotWithinFactor(
  Data,
  withinSample = NULL,
  Conditions = NULL,
  FilterExpression = 0,
  NumExpressionGroups = 4
)

Arguments

Data can be a matrix of single-cell expression with cells where rows are genes and columns are samples. Gene names should not be a column in this matrix, but should be assigned to rownames(Data). Data can also be an object of class SummarizedExperiment that contains the single-cell expression matrix and other metadata. The assays slot contains the expression matrix and is named "Counts". This matrix should have one row for each gene and one sample for each column. The colData slot should contain a data.frame with one row per sample and columns that contain metadata for each sample. This data.frame should contain a variable that represents biological condition in the same order as the columns of NormCounts. Additional information about the experiment can be contained in the metadata slot as a list.

withinSample a vector of gene-specific features.

Conditions vector of condition labels, this should correspond to the columns of the un-normalized expression matrix. If provided the cells will be colored by Condition instead of individually.

FilterExpression exclude genes having median of non-zero expression below this threshold.

NumExpressionGroups the number of groups to split the within sample factor into, e.g genes will be split into equally sized groups based on their GC content/Gene length/etc.
QuickReg produces a plot and returns the data the plot is based on.

Author(s)
Rhonda Bacher

Examples
```r
data(ExampleSimSCData)
Conditions = rep(c(1,2), each= 90)
exampleFactor = runif(dim(ExampleSimSCData)[1], 0, 1)
names(exampleFactor) = rownames(ExampleSimSCData)
#plotWithinFactor(Data = ExampleSimSCData,
    #withinSample=exampleFactor, Conditions = Conditions)
```

Description
Perform the single gene regressions using quantile regression.

Usage
quickReg(x, InputData)

Arguments
- x: gene to perform the regression on.
- InputData: list of data needed for the regression.

Details
Perform the single gene regressions using quantile regression.

Value
gene slope.
**redoBox**

**Description**

redoBox

**Usage**

`redoBox(DATA, smallc)`

**Arguments**

- **DATA**: data set to.
- **smallc**: what value to ignore, typically is zero.

**Details**

Function to log data and turn zeros to NA to mask/ignore in functions.

**Value**

the dataset has been logged with values below smallc masked.

---

**results**

**Description**

Convenient helper function to extract the results (normalized data, list of genes filtered out, or scale factors). Results data.frames/matrices are stored in the metadata slot and can also be accessed without the help of this convenience function by calling `metadata(DataNorm)`.

**Usage**

`results(DATA, type = c("NormalizedData", "ScaleFactors", "GenesFilteredOut"))`

**Arguments**

- **DATA**: An object of class SummarizedExperiment that contains normalized single-cell expression and other metadata, and the output of the SCnorm function.
- **type**: A character variable specifying which output is desired, with possible values "NormalizedData", "ScaleFactors", and "GenesFilteredOut". By default results() will return type="NormalizedData", which is the matrix of normalized counts from SCnorm. By specifying type="ScaleFactors" a matrix of scale factors (only returned if reportSF=TRUE when running SCnorm()) can be obtained. type="GenesFilteredOut" returns a list of genes that were not normalized using SCnorm, these are genes that did not pass the filter criteria.
Value
A data.frame containing output as detailed in the description of the type input parameter

Examples
```
data(ExampleSimSCData)
Conditions = rep(c(1), each = 90)
#NormData <- SCnorm(Data=ExampleSimSCData, Conditions=Conditions)
#normDataMatrix <- results(NormData)
```

Description
After conditions are independently normalized with the count-depth effect removed, conditions need to be additionally scaled prior to further analysis. Genes that were normalized in both conditions are split into quartiles based on their un-normalized non-zero medians. Genes in each quartile are scaled to the median fold change of condition specific gene means and overall gene means. This function can be used independently if SCnorm was run across different Conditions separately. However, the input must be as follow: NormData <- list(list(NormData = normalizedDataSet1), list(NormData = normalizedDataSet2)) where normalizedDataSet1 is the normalized matrix obtained using normcounts() on the output of SCnorm().

Usage
```
scaleNormMultCont(NormData, OrigData, Genes, useSpikes, useZerosToScale)
```

Arguments
```
NormData list of matrices of normalized expression counts and scale factors for each condition. Matrix rows are genes and columns are samples.
OrigData list of matrices of un-normalized expression counts. Matrix rows are genes and columns are samples. Each item in list is a different condition.
Genes vector of genes that will be used to scale conditions, only want to use genes that were normalized.
useSpikes whether to use spike-ins to perform between condition scaling (default=FALSE). Assumes spike-in names start with "ERCC-".
useZerosToScale whether to use zeros when scaling across conditions (default=FALSE).
```

Value
matrix of normalized and scaled expression values for all conditions.

Author(s)
Rhonda Bacher
**Description**

Quantile regression is used to estimate the dependence of read counts on sequencing depth for every gene. Genes with similar dependence are then grouped, and a second quantile regression is used to estimate scale factors within each group. Within-group adjustment for sequencing depth is then performed using the estimated scale factors to provide normalized estimates of expression. If multiple conditions are provided, normalization is performed within condition and then normalized estimates are scaled between conditions. If withinSample=TRUE then the method from Risso et al. 2011 will be implemented.

**Usage**

```r
SCnorm(
  Data = NULL,
  Conditions = NULL,
  PrintProgressPlots = FALSE,
  reportSF = FALSE,
  FilterCellNum = 10,
  FilterExpression = 0,
  Thresh = 0.1,
  K = NULL,
  NCores = NULL,
  ditherCounts = FALSE,
  PropToUse = 0.25,
  Tau = 0.5,
  withinSample = NULL,
  useSpikes = FALSE,
  useZerosToScale = FALSE
)
```

**Arguments**

- `Data` can be a matrix of single-cell expression with cells where rows are genes and columns are samples. Gene names should not be a column in this matrix, but should be assigned to rownames(Data). Data can also be an object of class SummarizedExperiment that contains the single-cell expression matrix and other metadata. The assays slot contains the expression matrix and is named "Counts". This matrix should have one row for each gene and one sample for each column. The colData slot should contain a data.frame with one row per sample and columns that contain metadata for each sample. This data.frame should contain a variable that represents biological condition in the same order as the columns of NormCounts). Additional information about the experiment can be contained in the metadata slot as a list.
Conditions  vector of condition labels, this should correspond to the columns of the expression matrix.

PrintProgressPlots  whether to automatically produce plot as SCnorm determines the optimal number of groups (default is FALSE, highly suggest using TRUE). Plots will be printed to the current device.

reportSF  whether to provide a matrix of scaling counts in the output (default = FALSE).

FilterCellNum  the number of non-zero expression estimate required to include the genes into the SCnorm fitting (default = 10). The initial grouping fits a quantile regression to each gene, making this value too low gives unstable fits.

FilterExpression  exclude genes having median of non-zero expression from the normalization.

Thresh  threshold to use in evaluating the sufficiency of K, default is .1.

K  the number of groups for normalizing. If left unspecified, an evaluation procedure will determine the optimal value of K (recommended).

NCores  number of cores to use, default is detectCores() - 1. This will be used to set up a parallel environment using either MulticoreParam (Linux, Mac) or SnowParam (Windows) with NCores using the package BiocParallel.

ditherCounts  whether to dither/jitter the counts, may be used for data with many ties, default is FALSE.

PropToUse  proportion of genes closest to the slope mode used for the group fitting, default is set at .25. This number # mainly affects speed.

Tau  value of quantile for the quantile regression used to estimate gene-specific slopes (default is median, Tau = .5 ).

withinSample  a vector of gene-specific features to correct counts within a sample prior to SCnorm. If NULL(default) then no correction will be performed. Examples of gene-specific features are GC content or gene length.

useSpikes  whether to use spike-ins to perform across condition scaling (default=FALSE). Spike-ins must be stored in the SingleCellExperiment object using altExp() function from SingleCellExperiment. See vignette for example.

useZerosToScale  whether to use zeros when scaling across conditions (default=FALSE).

Value

List containing matrix of normalized expression (and optionally a matrix of size factors if reportSF = TRUE ).

Author(s)

Rhonda Bacher
Examples

data(ExampleSimSCData)
Conditions = rep(c(1,2), each= 45)
#DataNorm <- SCnorm(ExampleSimSCData, Conditions,
#FilterCellNum = 10)
#str(DataNorm)

SCnormFit

Fit group quantile regression for K groups

Description

For each group K, a quantile regression is fit over all genes (PropToUse) for a grid of possible
degree’s d and quantile’s tau. For each value of tau and d, the predicted expression values are
obtained and regressed against the original sequencing depths. The optimal tau and d combination
is chosen as that closest to the mode of the gene slopes.

Usage

SCnormFit(Data, SeqDepth, Slopes, K, PropToUse = 0.25, Tau = 0.5, ditherCounts)

Arguments

Data can be a matrix of single-cell expression with cells where rows are genes and
columns are samples. Gene names should not be a column in this matrix, but
should be assigned to rownames(Data). Data can also be an object of class
SummarizedExperiment that contains the single-cell expression matrix and other
metadata. The assays slot contains the expression matrix and is named "Counts".
This matrix should have one row for each gene and one sample for each column.
The colData slot should contain a data.frame with one row per sample and
columns that contain metadata for each sample. This data.frame should contain
a variable that represents biological condition in the same order as the columns
of NormCounts). Additional information about the experiment can be contained
in the metadata slot as a list.

SeqDepth sequencing depth for each cell/sample.

Slopes per gene estimates of the count-depth relationship.

K the number of groups for normalizing. If left unspecified, an evaluation proce-
dure will determine the optimal value of K (recommended).

PropToUse proportion of genes closest to the slope mode used for the group fitting, default
is set at .25. This number # mainly affects speed.

Tau value of quantile for the quantile regression used to estimate gene-specific slopes
(default is median, Tau = .5 ).

ditherCounts whether to dither/jitter the counts, may be used for data with many ties, default
is FALSE.
splitGroups

Value

normalized expression matrix and matrix of scaling factors.

Author(s)

Rhonda Bacher

splitGroups

Description

splitGroups

Usage

splitGroups(DATA, NumGroups = 10)

Arguments

<table>
<thead>
<tr>
<th>DATA</th>
<th>vector to be splot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NumGroups</td>
<td>number of groups</td>
</tr>
</tbody>
</table>

Details

helper function to get split a vector into a specified number of groups

Value

list, length is equal to NumGroups
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