# Package 'scp'

April 12, 2022

```
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Description Utility functions for manipulating, processing, and analyzing mass
     spectrometry-based single-cell proteomics (SCP) data. The package is an
     extension to the 'QFeatures' package designed for SCP applications.
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Title Mass Spectrometry-Based Single-Cell Proteomics Data Analysis

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aggregateFeaturesOverAssays

Aggregate features over multiple assays

# Description

This function is a wrapper function around QFeatures::aggregateFeatures. It allows the user to provide multiple assays for which aggregateFeatures will be applied sequentially.

## Usage

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```
aggregateFeaturesOverAssays(object, i, fcol, name, fun, ...)
```

# Arguments

object	A QFeatures object
i	A numeric(1) or character(1) indicating which assay to transfer the colData to.
fcol	The feature variables for each assays i defining how to summarise the QFeatures. If fcol has length 1, the variable name is assumed to be the same for all assays
name	A character() naming the new assay. name must have the same length as i. Note that the function will fail if of the names in name is already present.
fun	A function used for quantitative feature aggregation.
	Additional parameters passed the fun.

# Value

A QFeatures object

#### See Also

QFeatures::aggregateFeatures

# **Examples**

computeMedianCV\_SCoPE2

(Deprecated) Compute the median coefficient of variation (CV) per cell

# Description

This function is deprecated and should no longer be used. To reproduce the SCoPE2 script, you can now use medianCVperCell with the following arguments:

## Usage

```
computeMedianCV_SCoPE2(object, i, peptideCol, proteinCol, batchCol)
```

# Arguments

```
object NULL
i NULL
peptideCol NULL
proteinCol NULL
batchCol NULL
```

# **Details**

```
• norm = "SCoPE2"
```

• nobs = 6

Make sure to provide the peptide data from separate assays so that the normalization factors are computed per batch.

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computeSCR

Compute the sample over carrier ratio (SCR)

#### **Description**

The function computes the ratio of the intensities of sample channels over the intentisty of the carrier channel for each feature. The ratios are averaged within the assay.

# Usage

```
computeSCR(
  object,
  i,
  colvar,
  samplePattern,
  sampleFUN = "mean",
  carrierPattern,
  carrierFUN = sampleFUN,
  rowDataName = "SCR"
)
```

#### **Arguments**

object A QFeatures object.

i A character() or integer() indicating for which assay(s) the SCR needs to

be computed.

colvar A character(1) indicating the variable to take from colData(object) that

gives the sample annotation.

samplePattern A character(1) pattern that matches the sample encoding in colvar.

sampleFUN A character(1) or function that provides the summarization function to use

(eg mean, sum, media, max, ...). Only used when the pattern matches multiple samples. Default is mean. Note for custom function, na.rm = TRUE is passed to sampleFUN to ignore missing values, make sure to provide a function that

accepts this argument.

carrierPattern A character(1) pattern that matches the carrier encoding in colvar. Only one

match per assay is allowed, otherwise only the first match is taken

carrierFUN A character(1) or function that provides the summarization function to use

(eg mean, sum, media, max, ...). Only used when the pattern matches multiple carriers. Default is the same function as sampleFUN. Note for custom function, na.rm = TRUE is passed to carrierFUN to ignore missing values, make sure to

provide a function that accepts this argument.

rowDataName A character(1) giving the name of the new variable in the rowData where the

computed SCR will be stored. The name cannot already exist in any of the assay

rowData.

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## Value

A QFeatures object for which the rowData of the given assay(s) is augmented with the mean SCR.

#### **Examples**

divideByReference

Divide assay columns by a reference column

# **Description**

The function divides the sample columns by a reference column. The sample and reference columns are defined based on the provided colvar variable and on regular expression matching.

#### Usage

```
divideByReference(object, i, colvar, samplePattern = ".", refPattern)
```

### **Arguments**

object	A QFeatures object
i	A numeric() or character() vector indicating from which assays the rowData should be taken.
colvar	A character(1) indicating the variable to take from colData(object) that gives the sample annotation.
samplePattern	A character(1) pattern that matches the sample encoding in colvar. By default all samples are devided (using the regex wildcard .).
refPattern	A character(1) pattern that matches the carrier encoding in colvar. Only one match per assay is allowed, otherwise only the first match is taken

## **Details**

The supplied assay(s) are replaced with the values computed after reference division.

# Value

A QFeatures object

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## **Examples**

medianCVperCell

 $Compute \ the \ median \ coefficient \ of \ variation \ (CV) \ per \ cell$ 

# Description

The function computes for each cell the median CV and stores them accordingly in the colData of the QFeatures object. The CVs in each cell are computed from a group of features. The grouping is defined by a variable in the rowData. The function can be applied to one or more assays, as long as the samples (column names) are not duplicated. Also, the user can supply a minimal number of observations required to compute a CV to avoid that CVs computed on too few observations influence the distribution within a cell. The quantification matrix can be optionally normalized before computing the CVs. Multiple normalizations are possible.

#### Usage

```
medianCVperCell(
  object,
  i,
  groupBy,
  nobs = 5,
  na.rm = TRUE,
  colDataName = "MedianCV",
  norm = "none",
  ...
)
```

# Arguments

object	A QFeatures object
i	A numeric() or character() vector indicating from which assays the rowData should be taken.
groupBy	A character(1) indicating the variable name in the rowData that contains the feature grouping.
nobs	An integer(1) indicating how many observations (features) should at least be considered for computing the CV. Since no CV can be computed for less than 2 observations, nobs should at least be 2.

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na.rm A logical(1) indicating whether missing data should be removed before com-

putation.

colDataName A character(1) giving the name of the new variable in the colData where the

computed CVs will be stored. The name cannot already exist in the colData.

norm A character() of normalization methods that will be sequentially applied to

each feature (row) in each assay. Available methods and additional information about normalization can be found in MsCoreUtils::normalizeMethods. You can also specify norm = "SCoPE2" to reproduce the normalization performed before computing the CVs as suggested by Specht et al. norm = "none" will not nor-

malize the data (default)

... Additional arguments that are passed to the normalization method.

## **Details**

A new column is added to the colData of the object. The samples (columns) that are not present in the selection i will get assigned an NA.

#### Value

A QFeatures object.

# References

Specht, Harrison, Edward Emmott, Aleksandra A. Petelski, R. Gray Huffman, David H. Perlman, Marco Serra, Peter Kharchenko, Antonius Koller, and Nikolai Slavov. 2021. "Single-Cell Proteomic and Transcriptomic Analysis of Macrophage Heterogeneity Using SCoPE2." Genome Biology 22 (1): 50.

# Examples

mqScpData

Example MaxQuant/SCoPE2 output

#### **Description**

A data.frame with 1088 observations and 139 variables, as produced by reading a MaxQuant output file with read.delim().

• Sequence: a character vector

• Length: a numeric vector

• Modifications: a character vector

• Modified.sequence: a character vector

• Deamidation..N..Probabilities: a character vector

• Oxidation..M..Probabilities: a character vector

• Deamidation..N..Score.Diffs: a character vector

• Oxidation..M..Score.Diffs: a character vector

• Acetyl..Protein.N.term.: a numeric vector

• Deamidation..N.: a numeric vector

• Oxidation..M.: a numeric vector

· Missed.cleavages: a numeric vector

• Proteins: a character vector

• Leading.proteins: a character vector

• protein: a character vector

• Gene.names: a character vector

• Protein.names: a character vector

• Type: a character vector

• Set: a character vector

• MS.MS.m.z: a numeric vector

• Charge: a numeric vector

• m.z: a numeric vector

• Mass: a numeric vector

• Resolution: a numeric vector

• Uncalibrated...Calibrated.m.z..ppm.: a numeric vector

• Uncalibrated...Calibrated.m.z..Da.: a numeric vector

• Mass.error..ppm.: a numeric vector

• Mass.error..Da.: a numeric vector

• Uncalibrated.mass.error..ppm.: a numeric vector

• Uncalibrated.mass.error..Da.: a numeric vector

- Max.intensity.m.z.0: a numeric vector
- Retention.time: a numeric vector
- Retention.length: a numeric vector
- Calibrated.retention.time: a numeric vector
- Calibrated.retention.time.start: a numeric vector
- Calibrated.retention.time.finish: a numeric vector
- Retention.time.calibration: a numeric vector
- Match.time.difference: a logical vector
- Match.m.z.difference: a logical vector
- Match.q.value: a logical vector
- Match.score: a logical vector
- Number.of.data.points: a numeric vector
- Number.of.scans: a numeric vector
- Number.of.isotopic.peaks: a numeric vector
- PIF: a numeric vector
- Fraction.of.total.spectrum: a numeric vector
- Base.peak.fraction: a numeric vector
- PEP: a numeric vector
- MS.MS.count: a numeric vector
- MS.MS.scan.number: a numeric vector
- Score: a numeric vector
- Delta.score: a numeric vector
- Combinatorics: a numeric vector
- Intensity: a numeric vector
- Reporter.intensity.corrected.0: a numeric vector
- Reporter.intensity.corrected.1: a numeric vector
- Reporter.intensity.corrected.2: a numeric vector
- Reporter.intensity.corrected.3: a numeric vector
- Reporter.intensity.corrected.4: a numeric vector
- Reporter.intensity.corrected.5: a numeric vector
- Reporter.intensity.corrected.6: a numeric vector
- Reporter.intensity.corrected.7: a numeric vector
- Reporter.intensity.corrected.8: a numeric vector
- Reporter.intensity.corrected.9: a numeric vector
- Reporter.intensity.corrected.10: a numeric vector
- RI1: a numeric vector
- RI2: a numeric vector

- RI3: a numeric vector
- RI4: a numeric vector
- RI5: a numeric vector
- RI6: a numeric vector
- RI7: a numeric vector
- RI8: a numeric vector
- RI9: a numeric vector
- RI10: a numeric vector
- RI11: a numeric vector
- Reporter.intensity.count.0: a numeric vector
- Reporter.intensity.count.1: a numeric vector
- Reporter.intensity.count.2: a numeric vector
- Reporter.intensity.count.3: a numeric vector
- Reporter.intensity.count.4: a numeric vector
- Reporter.intensity.count.5: a numeric vector
- Reporter.intensity.count.6: a numeric vector
- Reporter.intensity.count.7: a numeric vector
- Reporter.intensity.count.8: a numeric vector
- Reporter.intensity.count.9: a numeric vector
- Reporter.intensity.count.10: a numeric vector
- Reporter.PIF: a logical vector
- Reporter.fraction: a logical vector
- Reverse: a character vector
- Potential.contaminant: a logical vector
- id: a numeric vector
- Protein.group.IDs: a character vector
- Peptide.ID: a numeric vector
- Mod..peptide.ID: a numeric vector
- MS.MS.IDs: a character vector
- Best.MS.MS: a numeric vector
- AIF.MS.MS.IDs: a logical vector
- Deamidation..N..site.IDs: a numeric vector
- Oxidation..M..site.IDs: a logical vector
- remove: a logical vector
- dart\_PEP: a numeric vector
- dart\_qval: a numeric vector
- razor\_protein\_fdr: a numeric vector

- Deamidation..NQ..Probabilities: a logical vector
- Deamidation..NQ..Score.Diffs: a logical vector
- Deamidation..NQ.: a logical vector
- Reporter.intensity.corrected.11: a logical vector
- Reporter.intensity.corrected.12: a logical vector
- Reporter.intensity.corrected.13: a logical vector
- Reporter.intensity.corrected.14: a logical vector
- Reporter.intensity.corrected.15: a logical vector
- Reporter.intensity.corrected.16: a logical vector
- RI12: a logical vector
- RI13: a logical vector
- RI14: a logical vector
- RI15: a logical vector
- RI16: a logical vector
- Reporter.intensity.count.11: a logical vector
- Reporter.intensity.count.12: a logical vector
- Reporter.intensity.count.13: a logical vector
- Reporter.intensity.count.14: a logical vector
- Reporter.intensity.count.15: a logical vector
- Reporter.intensity.count.16: a logical vector
- Deamidation..NQ..site.IDs: a logical vector
- input\_id: a logical vector
- rt\_minus: a logical vector
- rt\_plus: a logical vector
- mu: a logical vector
- muij: a logical vector
- sigmaij: a logical vector
- pep\_new: a logical vector
- exp\_id: a logical vector
- peptide\_id: a logical vector
- stan\_peptide\_id: a logical vector
- exclude: a logical vector
- residual: a logical vector
- participated: a logical vector
- peptide: a character vector

### Usage

data("mqScpData")

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#### **Format**

An object of class data. frame with 1361 rows and 149 columns.

#### **Details**

The dataset is a subset of the SCoPE2 dataset (version 2, Specht et al. 2019, BioRXiv). The input file evidence\_unfiltered.csv was downloaded from a Google Drive repository. The MaxQuant evidence file was loaded and the data was cleaned (renaming columns, removing duplicate fields,...). MS runs that were selected in the scp1 dataset (see ?scp1) were kept along with a blank run. The data is stored as a data.frame.

#### See Also

readSCP() for an example on how mqScpData is parsed into a QFeatures object.

normalizeSCP	Normalize single-cell proteomics (SCP) data	
normalizeder	Normanze single cen proteomies (501) and	

# **Description**

This function normalises an assay in a QFeatures according to the supplied method (see Details). The normalized data is added as a new assay

# Usage

```
normalizeSCP(object, i, name = "normAssay", method, ...)
```

#### **Arguments**

object	An object of class QFeatures.
i	A numeric vector or a character vector giving the index or the name, respectively, of the assay(s) to be processed.
name	A character(1) naming the new assay name. Defaults is are normAssay.
method	character(1) defining the normalisation method to apply. See Details.
	Additional parameters passed to MsCoreUtils::normalizeMethods().

#### **Details**

The method parameter in normalize can be one of "sum", "max", "center.mean", "center.median", "div.median", "diff.meda", "quantiles", "quantiles.robust" or "vsn". The MsCoreUtils::normalizeMethod function returns a vector of available normalisation methods.

• For "sum" and "max", each feature's intensity is divided by the maximum or the sum of the feature respectively. These two methods are applied along the features (rows).

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• "center.mean" and "center.median" center the respective sample (column) intensities by subtracting the respective column means or medians. "div.mean" and "div.median" divide by the column means or medians. These are equivalent to sweeping the column means (medians) along MARGIN = 2 with FUN = "-" (for "center.\*") or FUN = "/" (for "div.\*").

- "diff.median" centers all samples (columns) so that they all match the grand median by subtracting the respective columns medians differences to the grand median.
- Using "quantiles" or "quantiles.robust" applies (robust) quantile normalisation, as implemented in preprocessCore::normalize.quantiles() and preprocessCore::normalize.quantiles.robust() "vsn" uses the vsn::vsn2() function. Note that the latter also glog-transforms the intensities. See respective manuals for more details and function arguments.

For further details and examples about normalisation, see MsCoreUtils::normalize\_matrix().

#### Value

A QFeatures object with an additional assay containing the normalized data.

#### See Also

**OFeatures::normalize** for more details about normalize

|--|

## **Description**

This function computes q-values from the posterior error probabilities (PEPs). The functions takes the PEPs from the given assay's rowData and adds a new variable to it that contains the computed q-values.

## Usage

```
pep2qvalue(object, i, groupBy, PEP, rowDataName = "qvalue")
```

## **Arguments**

object	A QFeatures object
i	A numeric() or character() vector indicating from which assays the rowData should be taken.
groupBy	A character(1) indicating the variable name in the rowData that contains the grouping variable, for instance to compute protein FDR. When groupBy is not missing, the best feature approach is used to compute the PEP per group, meaning that the smallest PEP is taken as the PEP of the group.
PEP	A character(1) indicating the variable names in the rowData that contains the PEPs. Since, PEPs are probabilities, the variable must be contained in $(0, 1)$ .
rowDataName	A character(1) giving the name of the new variable in the rowData where the computed FDRs will be stored. The name cannot already exist in any of the assay rowData.

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#### **Details**

The q-value of a feature (PSM, peptide, protein) is the minimum FDR at which that feature will be selected upon filtering (Savitski et al.). On the other hand, the feature PEP is the probability that the feature is wrongly matched and hence can be seen as a local FDR (Kall et al.). While filtering on PEP is guaranteed to control for FDR, it is usually too conservative. Therefore, we provide this function to convert PEP to q-values.

We compute the q-value of a feature as the average of the PEPs associated to PSMs that have equal or greater identification confidence (so smaller PEP). See Kall et al. for a visual interpretation.

We also allow inference of q-values at higher level, for instance computing the protein q-values from PSM PEP. This can be performed by supplying the groupBy argument. In this case, we adopt the best feature strategy that will take the best (smallest) PEP for each group (Savitski et al.).

#### Value

A QFeatures object.

#### References

Käll, Lukas, John D. Storey, Michael J. MacCoss, and William Stafford Noble. 2008. "Posterior Error Probabilities and False Discovery Rates: Two Sides of the Same Coin." Journal of Proteome Research 7 (1): 40–44.

Savitski, Mikhail M., Mathias Wilhelm, Hannes Hahne, Bernhard Kuster, and Marcus Bantscheff. 2015. "A Scalable Approach for Protein False Discovery Rate Estimation in Large Proteomic Data Sets." Molecular & Cellular Proteomics: MCP 14 (9): 2394–2404.

#### **Examples**

readSCP

Read single-cell proteomics data as a QFeatures object from tabular data and metadata

## **Description**

Convert tabular quantitative MS data and metadata from a spreadsheet or a data.frame into a QFeatures object containing SingleCellExperiment objects.

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#### Usage

```
readSCP(
   featureData,
   colData,
   batchCol,
   channelCol,
   suffix = NULL,
   removeEmptyCols = FALSE,
   verbose = TRUE,
   ...
)
```

### **Arguments**

featureData File or object holding the quantitative data. Can be either a character(1) with

the path to a text-based spreadsheet (comma-separated values by default, but see ...) or an object that can be coerced to a data.frame. It is advised not to

encode characters as factors.

colData A data.frame or any object that can be coerced to a data.frame. colData is

expected to contains all the sample meta information. Required fields are the acquisition batch (given by batchCol) and the acquisition channel within the batch (e.g. TMT channel, given by channelCol). Additional fields (e.g. sample

type, acquisition date,...) are allowed and will be stored as sample meta data.

batchCol A numeric(1) or character(1) pointing to the column of featureData and

colData that contain the batch names. Make sure that the column name in both table are either identical and syntactically valid (if you supply a character) or have the same index (if you supply a numeric). Note that characters can be

converted to syntactically valid names using make.names

channelCol A numeric(1) or character(1) pointing to the column of colData that con-

tains the column names of the quantitative data in featureData (see Example).

suffix A character() giving the suffix of the column names in each assay. The length

of the vector should equal the number of quantification channels and should contain unique character elements. If NULL, the names of the quantification

columns in featureData are taken as suffix.

removeEmptyCols

A logical(1). If true, the function will remove in each batch the columns that

contain only missing values.

verbose A logical(1) indicating whether the progress of the data reading and format-

ting should be printed to the console. Default is TRUE.

Further arguments that can be passed on to read.csv except stringsAsFactors,

which is always FALSE.

## Value

An instance of class QFeatures. The expression data of each batch is stored in a separate assay as a SingleCellExperiment object.

#### Note

The SingleCellExperiment class is built on top of the RangedSummarizedExperiment class. This means that some column names are forbidden in the rowData. Avoid using the following names: seqnames, ranges, strand, start, end, width, element

## Author(s)

Laurent Gatto, Christophe Vanderaa

# **Examples**

readSingleCellExperiment

Read SingleCellExperiment from tabular data

# Description

Convert tabular data from a spreadsheet or a data.frame into a SingleCellExperiment object.

# Usage

```
readSingleCellExperiment(table, ecol, fnames, ...)
```

### **Arguments**

table

File or object holding the quantitative data. Can be either a character(1) with the path to a text-based spreadsheet (comma-separated values by default, but see ...) or an object that can be coerced to a data. frame. It is advised not to encode characters as factors.

ecol

A numeric indicating the indices of the columns to be used as assay values. Can also be a character indicating the names of the columns. Caution must be taken if the column names are composed of special characters like ( or - that will be converted to a . by the read.csv function. If ecol does not match, the error message will dislpay the column names as seen by the read.csv function.

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fnames An optional character(1) or numeric(1) indicating the column to be used as

row names.

... Further arguments that can be passed on to read.csv except stringsAsFactors,

which is always FALSE.

#### Value

An instance of class SingleCellExperiment.

#### Note

The SingleCellExperiment class is built on top of the RangedSummarizedExperiment class. This means that some column names are forbidden in the rowData. Avoid using the following names: seqnames, ranges, strand, start, end, width, element

#### Author(s)

Laurent Gatto, Christophe Vanderaa

#### See Also

The code relies on QFeatures::readSummarizedExperiment.

### **Examples**

rowDataToDF

Extract the rowData of a QFeatures object to a DataFrame

#### **Description**

This function is deprecated. You should rather use [QFeatures::rbindRowData]

# Usage

```
rowDataToDF(object, i, vars)
```

### **Arguments**

object	A QFeatures object	

i A numeric() or character() vector indicating from which assays the rowData

should be taken.

vars A character() vector indicating which variables from the rowData should be

extracted.

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#### Value

A DataFrame object with the rowData row-binded over the required assays.

sample Annotation

Single cell sample annotation

## **Description**

A data frame with 48 observations on the following 6 variables.

• Set: a character vector

· Channel: a character vector

• SampleType: a character vector

lcbatch: a character vectorsortday: a character vector

• digest: a character vector

### Usage

```
data("sampleAnnotation")
```

### **Format**

An object of class data. frame with 64 rows and 6 columns.

## **Details**

##' The dataset is a subset of the SCoPE2 dataset (version 2, Specht et al. 2019, BioRXiv). The input files batch.csv and annotation.csv were downloaded from a Google Drive repository. The two files were loaded and the columns names were adapted for consistency with mqScpData table (see ?mqScpData). The two tables were filtered to contain only sets present in "mqScpData. The tables were then merged based on the run ID, hence merging the sample annotation and the batch annotation. Finally, annotation for the blank run was added manually. The data is stored as a data.frame'.

#### See Also

readSCP() to see how this file is used.

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scp1

Single Cell QFeatures data

# Description

A small QFeatures object with SCoPE2 data. The object is composed of 5 assays, including 3 PSM-level assays, 1 peptide assay and 1 protein assay.

#### Usage

```
data("scp1")
```

#### **Format**

An object of class QFeatures of length 5.

#### **Details**

The dataset is a subset of the SCoPE2 dataset (version 2, Specht et al. 2019, BioRXiv). This dataset was converted to a QFeatures object where each assay in stored as a SingleCellExperiment object. One assay per chromatographic batch ("LCA9", "LCA10", "LCB3") was randomly sampled. For each assay, 100 proteins were randomly sampled. PSMs were then aggregated to peptides and joined in a single assay. Then peptides were aggregated to proteins.

# **Examples**

```
data("scp1")
scp1
```

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