Package ‘msqrob2’

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Title Robust statistical inference for quantitative LC-MS proteomics

Version 1.10.0

Description
msqrob2 provides a robust linear mixed model framework for assessing differential abundance in MS-based Quantitative proteomics experiments. Our workflows can start from raw peptide intensities or summarised protein expression values. The model parameter estimates can be stabilized by ridge regression, empirical Bayes variance estimation and robust M-estimation. msqrob2's hurdle workflow can handle missing data without having to rely on hard-to-verify imputation assumptions, and, outcompetes state-of-the-art methods with and without imputation for both high and low missingness. It builds on QFeature infrastructure for quantitative mass spectrometry data to store the model results together with the raw data and preprocessed data.

Depends R (>= 4.1), QFeatures (>= 1.1.2)

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'hypothesisTest-methods.R' 'msqrob-methods.R'
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**getContrast,StatModel-method**

*Methods for StatModel class*

**Description**

Methods for StatModel class

- `getContrast(object, L)` to calculate contrasts of the model parameters
- `varContrast(object, L)` to calculate the variance-covariance matrix of the contrasts

**Usage**

```r
## S4 method for signature 'StatModel'
getContrast(object, L)

## S4 method for signature 'StatModel'
varContrast(object, L)
```

**Arguments**

- `object` A list with elements of the class StatModel that are estimated using the `msqrob` function
- `L` contrast numeric matrix specifying one or more contrasts of the linear model coefficients to be tested equal to zero. The rownames of the matrix should be equal to the names of parameters of the model.

**Value**

A matrix with the calculated contrasts or variance-covariance matrix of contrasts

**Examples**

```r
data(pe)
# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit msqrob model
pe <- msqrob(pe, i = "protein", formula = ~condition)

# Define contrast
getCoef(rowData(pe[["protein"]]))$msqrobModels[[1]]
# Define contrast for log2 fold change between condition c and condition b:
L <- makeContrast("conditionc - conditionb=0", c("conditionb", "conditionc"))

getContrast(rowData(pe[["protein"]]))$msqrobModels[[1]], L)
varContrast(rowData(pe[["protein"]]))$msqrobModels[[1]], L)
```
Accessor functions for StatModel class

**Description**

- `getModel(object)` to get model
- `getFitMethod(object)` to get the parameter estimation method
- `getCoef(object)` to get the parameter estimates of the mean model
- `getDF(object)` to get the residual degrees of freedom of the model
- `getVar(object)` to get the residual variance of the model
- `getSigma(object)` to get the residual standard deviation of the model
- `getDfPosterior(object)` to get the degrees of freedom of the empirical Bayes variance estimator
- `getVarPosterior(object)` to get the empirical Bayes variance
- `getSigmaPosterior(object)` to get the empirical Bayes standard deviation
- `getVcovUnscaled(object)` to get the unscaled variance covariance matrix of the model parameters

**Usage**

```r
## S4 method for signature 'StatModel'
getModel(object)

## S4 method for signature 'StatModel'
getFitMethod(object)

## S4 method for signature 'StatModel'
getCoef(object)

## S4 method for signature 'StatModel'
getDfPosterior(object)

## S4 method for signature 'StatModel'
getVarPosterior(object)

## S4 method for signature 'StatModel'
getSigmaPosterior(object)

## S4 method for signature 'StatModel'
getDF(object)

## S4 method for signature 'StatModel'
getVar(object)
```
### S4 method for signature 'StatModel'

getSigma(object)

### S4 method for signature 'StatModel'

getVcovUnscaled(object)

**Arguments**

- **object** 
  StatModel object

**Value**

The requested parameter of the StatModel object

**Examples**

data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit msqrob model
pe <- msqrob(pe, i = "protein", formula = ~condition)
getCoef(rowData(pe[["protein"]])$msqrobModels[[1]])
getModel(rowData(pe[["protein"]])$msqrobModels[[1]])
getFitMethod(rowData(pe[["protein"]])$msqrobModels[[1]])

# Similar for the remaining accessors
## S4 method for signature 'SummarizedExperiment'

`hypothesisTestHurdle`

object, contrast, adjust.method = "BH", modelColumn = "msqrobHurdle", resultsColumnNamePrefix = "hurdle_", overwrite = FALSE

## S4 method for signature 'QFeatures'

`hypothesisTest`

object, i, contrast, adjust.method = "BH", modelColumn = "msqrobModels", resultsColumnNamePrefix = "", overwrite = FALSE

## S4 method for signature 'QFeatures'

`hypothesisTestHurdle`

object, i, contrast, adjust.method = "BH", modelColumn = "msqrobHurdle", resultsColumnNamePrefix = "hurdle_", overwrite = FALSE

### Arguments

**object** SummarizedExperiment or QFeatures instance

**contrast** numeric matrix specifying one or more contrasts of the linear model coefficients to be tested equal to zero. If multiple contrasts are given (multiple columns) then results will be returned for each contrast. The rownames of the matrix should be equal to the names of parameters of the model that are involved in the contrast. The column names of the matrix will be used to construct names to store the results in the rowData of the SummarizedExperiment or of the assay of the QFeatures object. The contrast matrix can be made using the `makeContrast` function.

**adjust.method** character specifying the method to adjust the p-values for multiple testing. Options, in increasing conservatism, include "none", "BH", "BY" and "holm". See 'p.adjust' for the complete list of options. Default is "BH" the Benjamini-
Hochberg method to control the False Discovery Rate (FDR).

**modelColumn** character to indicate the variable name that was used to store the msqrob models in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "msqrobModels" when the `hypothesisTest` function is used and "msqrobHurdle" for `hypothesisTestHurdle`.

**resultsColumnNamePrefix** character to indicate the the prefix for the variable name that will be used to store test results in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "" so that the variable name with the results will be the column name of the column in the contrast matrix L. If L is a matrix with multiple columns, multiple results columns will be made, one for each contrast. If L is a matrix with a single column which has no column names and if resultsColumnNamePrefix="" the results will be stored in the column with name msqrobResults. For `hypothesisTestHurdle` the default prefix is "hurdle_". If L is a matrix with one column and has no column names and if resultsColumnNamePrefix="hurdle_" the results will be stored in the column with name hurdleResults.

**overwrite** boolean(1) to indicate if the column in the rowData has to be overwritten if the modelColumnName already exists. Default is FALSE.

**i** character or integer to specify the element of the QFeatures that contains the log expression intensities that will be modelled.

**Value**

A SummarizedExperiment or a QFeatures instance augmented with the test results.

**Author(s)**

Lieven Clement

**Examples**

```r
# Load example data
# The data are a Feature object containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit msqrob model
pe <- msqrob(pe, i = "protein", formula = ~condition)

# Define contrast
getCoef(rowData(pe[["protein"]])$msqrobModels[[1]])
# Assess log2 fold change between condition c and condition b
L <- makeContrast(
    contrasts = c("condition b" = 1, "condition c" = -1),
    coeff = 1)
```
**makeContrast**

**Description**

Construct the contrast matrix corresponding to specified contrasts of a set of parameters.

**Usage**

```r
makeContrast(contrasts, parameterNames)
```

**Arguments**

- **contrasts** character vector specifying contrasts, i.e. the linear combination of the model-parameters that equals to zero.
- **parameterNames** character vector specifying the model parameters that are involved in the contrasts, e.g if we model data of three conditions using a factor condition with three levels a, b and c then our model will have 3 mean parameters named (Intercept), conditionb and conditionc. Hence the log2 fold change between b and a is conditionb. Under the null hypothesis the log2 fold change equals 0. Which is to be encoded as "conditionb=0". If we would like to test for log2 fold change between condition c and b we assess if the log2 fold change conditionc-conditionb equals 0, encoded as "conditionb-conditionc=0".

**Value**

A numeric contrast matrix with rownames that equal the model parameters that are involved in the contrasts

**Examples**

```r
makeContrast(c("conditionb = 0"),
             parameterNames = c(
               "(Intercept)",
               "conditionb",
               "conditionc"
             )
           )
makeContrast(c("conditionc=0"),
             parameterNames = c("conditionc")
           )
makeContrast(c(
             "conditionb=0",
             "conditionc=0",
             "conditionc-conditionb=0"
           ),
             parameterNames = c(
               "conditionb",
               "conditionc"
             )
           )
```
msqrob,SummarizedExperiment-method

Methods to fit msqrob models with ridge regression and/or random effects using lme4

Description

Parameter estimation of msqrob models for QFeatures and SummarizedExperiment instance.

Usage

## S4 method for signature 'SummarizedExperiment'
msqrob(
  object,
  formula,
  modelColumnName = "msqrobModels",
  overwrite = FALSE,
  robust = TRUE,
  ridge = FALSE,
  maxitRob = 1,
  tol = 1e-06,
  doQR = TRUE,
  lmerArgs = list(control = lmerControl(calc.derivs = FALSE))
)

## S4 method for signature 'QFeatures'
msqrob(
  object,
  i,
  formula,
  modelColumnName = "msqrobModels",
  overwrite = FALSE,
  robust = TRUE,
  ridge = FALSE,
  maxitRob = 1,
  tol = 1e-06,
  doQR = TRUE,
  lmerArgs = list(control = lmerControl(calc.derivs = FALSE))
)

Arguments

object SummarizedExperiment or QFeatures instance
formula Model formula. The model is built based on the covariates in the data object.
modelColumnLabel character to indicate the variable name that is used to store the msqrob models in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "msqrobModels".

overwrite boolean(1) to indicate if the column in the rowData has to be overwritten if the modelColumnLabel already exists. Default is FALSE.

robust boolean(1) to indicate if robust regression is performed to account for outliers. Default is TRUE. If FALSE an OLS fit is performed.

ridge boolean(1) to indicate if ridge regression is performed. Default is FALSE. If TRUE the fixed effects are estimated via penalized regression and shrunked to zero.

maxitRob numeric(1) indicating the maximum iterations in the IRWLS algorithm used in the M-estimation step of the robust regression.

tol numeric(1) indicating the tolerance for declaring convergence of the M-estimation loop.

doQR boolean(1) to indicate if QR decomposition is used when adopting ridge regression. Default is TRUE. If FALSE the predictors of the fixed effects are not transformed, and the degree of shrinkage can depend on the encoding.

lmerArgs a list (of correct class, resulting from 'lmerControl()') containing control parameters, including the nonlinear optimizer to be used and parameters to be passed through to the nonlinear optimizer, see the 'lmerControl' documentation of the lme4 package for more details. Default is list(control = lmerControl(calc.derivs = FALSE))

i character or integer to specify the element of the QFeatures that contains the log expression intensities that will be modelled.

Value
A SummarizedExperiment or a QFeatures instance with the models.

Author(s)
Lieven Clement

Examples
# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit MSqrob model using robust linear regression upon summarization of
# peptide intensities into protein expression values.
# For summarized SummarizedExperiment
se <- pe["protein"]
se
colData(se) <- colData(pe)
se <- msqrob(se, formula = ~condition, modelColumnName = "rlm")
getCoef(rowData(se)$rlm[[1]])

# For features object
pe <- msqrob(pe, i = "protein", formula = ~condition, modelColumnName = "rlm")
# with ridge regression (slower)
pe <- msqrob(pe, i = "protein", formula = ~condition, ridge = TRUE, modelColumnName = "ridge")

# compare for human protein (no DE)==> large shrinkage to zero
cbind(getCoef(rowData(pe["protein"])$rlm[[1]]), getCoef(rowData(pe["protein"])$ridge[[1]]))

# compare for ecoli protein (DE)==> almost no shrinkage to zero
cbind(
    getCoef(rowData(pe["protein"])$rlm["P00956"]),
    getCoef(rowData(pe["protein"])$ridge["P00956"])
)

---

msqrobAggregate, QFeatures-method

Method to fit msqrob models with robust regression and/or ridge regression and/or random effects. It models multiple features simultaneously, e.g. multiple peptides from the same protein.

Description

Parameter estimation of msqrob models for QFeatures instance. The method aggregates features within the model e.g. from peptides to proteins. It provides fold change estimates and their associated uncertainty at the aggregated level (e.g. protein level) while correcting for the peptide species that are observed in each sample. It also addresses the correlation in the data, e.g. the peptide data for the same protein in a sample are correlate because they originate from the same protein pool. The method however does not return aggregated expression values for each sample. For visualisation purposes aggregated expression values are provide by the aggregateFeatures function from the QFeatures Package.

Usage

```r
## S4 method for signature 'QFeatures'
msqrobAggregate(
  object,
  formula,
  i,
  fcol,
  name = "msqrobAggregate",
  aggregateFun = MsCoreUtils::robustSummary,
```
msqrobAggregate,QFeatures-method

    modelColumnNames = "msqrobModels",
    robust = TRUE,
    ridge = FALSE,
    maxitRob = 1,
    tol = 1e-06,
    doQR = TRUE,
    lmerArgs = list(control = lmerControl(calc.derivs = FALSE))
  )

Arguments

object QFeatures instance
formula Model formula. The model is built based on the covariates in the data object.
i character or integer to specify the element of the QFeatures that contains the log expression intensities that will be modelled.
fcol The feature variable of assay ‘i’ defining how to summerise the features.
name A ‘character(1)’ naming the new assay. Default is ‘newAssay’. Note that the function will fail if there’s already an assay with ‘name’.
aggregateFun A function used for quantitative feature aggregation. Details can be found in the documentation of the aggregateFeatures of the QFeatures package.
modelColumnNames character to indicate the variable name that is used to store the msqrob models in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "msqrobModels".
robust boolean(1) to indicate if robust regression is performed to account for outliers. Default is TRUE. If FALSE an OLS fit is performed.
ridge boolean(1) to indicate if ridge regression is performed. Default is FALSE. If TRUE the fixed effects are estimated via penalized regression and shrunken to zero.
maxitRob numeric(1) indicating the maximum iterations in the IRWLS algorithm used in the M-estimation step of the robust regression.
tol numeric(1) indicating the tolerance for declaring convergence of the M-estimation loop.
doQR boolean(1) to indicate if QR decomposition is used when adopting ridge regression. Default is TRUE. If FALSE the predictors of the fixed effects are not transformed, and the degree of shrinkage can depend on the encoding.
lmerArgs a list (of correct class, resulting from ‘lmerControl()’ containing control parameters, including the nonlinear optimizer to be used and parameters to be passed through to the nonlinear optimizer, see the ‘lmerControl’ documentation of the lme4 package for more details. Default is list(control = lmerControl(calc.derivs = FALSE))

Value

A ‘QFeatures’ object with an additional assay.
Examples

```r
# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

# Fit MSqrob model using robust ridge regression starting from peptide intensities
# The fold changes are calculated at the protein level while correcting for
# the different peptide species in each sample and the correlation between
# peptide intensities of peptides of the same protein in the same sample.
colData(pe)$samples <- rownames(colData(pe))
pe <- msqrobAggregate(pe, i = "peptide", fcol = "Proteins",
    formula = ~condition + (1|samples) + (1|Sequence),
    ridge = TRUE)
getCoef(rowData(pe[["msqrobAggregate"]])$msqrobModels[["P00956"])
```

Description

Function to fit msqrob models to peptide counts using glm

Usage

```r
msqrobGlm(y, npep, formula, data, priorCount = 0.1, binomialBound = TRUE)
```

Arguments

- **y**: A matrix with the peptide counts. The features are along the rows and samples along the columns.
- **npep**: A vector with number of peptides per protein. It has as length the number of rows of y. The counts are equal or larger than the largest peptide count in y.
- **formula**: Model formula. The model is built based on the covariates in the data object.
- **data**: A DataFrame with information on the design. It has the same number of rows as the number of columns (samples) of y.
- **priorCount**: A `numeric(1)`, which is a prior count to be added to the observations to shrink the estimated log-fold-changes towards zero.
- **binomialBound**: logical, if ‘TRUE’ then the quasibinomial variance estimator will be never smaller than 1 (no underdispersion).
Value

A list of objects of the StatModel class.

Author(s)

Lieven Clement

Examples

# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit MSqrob model using robust regression with the MASS rlm function
models <- msqrobGlm(
  aggcounts(pe[,"protein"]),
  rowData(pe[,"protein"])[["n"]],
  ~condition,
  colData(pe)
)
gGetCoef(models[[1]])

---

msqrobHurdle, SummarizedExperiment-method

Function to fit msqrob hurdle models

Description

Fitting a hurdle msqrob model with an intensity component for assessing differential abundance and an count component that is modeling peptide counts using quasibinomial glm for differential detection of the number of features that were not missing and used for aggregation

Usage

## S4 method for signature 'SummarizedExperiment'
msqrobHurdle(
  object,
  formula,
  modelColumnName = "msqrobHurdle",
  overwrite = FALSE,
robust = TRUE,
ridge = FALSE,
maxitRob = 1,
tol = 1e-06,
doQR = TRUE,
lmerArgs = list(control = lmerControl(calc.derivs = FALSE)),
priorCount = 0.1,
binomialBound = TRUE
)

## S4 method for signature 'QFeatures'
msqrobHurdle(
  object,
  i,
  formula,
  modelColumnName = "msqrobHurdle",
  overwrite = FALSE,
  robust = TRUE,
  ridge = FALSE,
  maxitRob = 1,
  tol = 1e-06,
  doQR = TRUE,
  lmerArgs = list(control = lmerControl(calc.derivs = FALSE)),
  priorCount = 0.1,
  binomialBound = TRUE
)

Arguments

**object** SummarizedExperiment or QFeatures instance with an assay that is generated with the aggregateFeatures function from the QFeatures package

**formula** Model formula. Both model components are built based on the covariates in the data object.

**modelColumnName** character to indicate the variable name that is used to store the msqrob models in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "msqrobHurdle".

**overwrite** boolean(1) to indicate if the column in the rowData has to be overwritten if the modelColumnName already exists. Default is FALSE.

**robust** boolean(1) to indicate if robust regression is performed to account for outliers when fitting the intensity component of the hurdle model. Default is TRUE. If FALSE an OLS fit is performed.

**ridge** boolean(1) to indicate if ridge regression is performed. Default is FALSE. If TRUE the fixed effects of the intensity component of the hurdle model are estimated via penalized regression and shrunken to zero.

**maxitRob** numeric(1) indicating the maximum iterations in the IRWLS algorithm used in the M-estimation step of the robust regression for fitting the intensity component of the hurdle model.
tol numeric(1) indicating the tolerance for declaring convergence of the M-estimation loop of the intensity component of the hurdle model.

doQR boolean(1) to indicate if QR decomposition is used when adopting ridge regression for the intensity component of the model. Default is TRUE. If FALSE the predictors of the fixed effects are not transformed, and the degree of shrinkage can depend on the encoding.

lmerArgs a list (of correct class, resulting from `lmerControl()’) containing control parameters, including the nonlinear optimizer to be used and parameters to be passed through to the nonlinear optimizer, see the ‘lmerControl’ documentation of the lme4 package for more details. Default is `list(control = lmerControl(calc.derivs = FALSE))`

priorCount A 'numeric(1)', which is a prior count to be added to the observations to shrink the estimated odds ratios of the count component towards zero. Default is 0.1.

binomialBound logical, if 'TRUE' then the quasibinomial variance estimator will be never smaller than 1 (no underdispersion). Default is TRUE.

i character or integer to specify the element of the QFeatures that contains the log expression intensities that will be modelled.

Value
SummarizedExperiment or QFeatures instance

Author(s)
Lieven Clement

Examples

# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

# Aggregate peptide intensities to protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit Hurdle MSqrob model
# For summarized SummarizedExperiment
se <- pe[["protein"]]
se$colData(se) <- colData(pe)
se <- msqrobHurdle(se, formula = ~condition)
getCoef(rowData(se)$msqrobHurdleIntensity[[1]])
getCoef(rowData(se)$msqrobHurdleCount[[1]])

# For features object
msqrobLm Function to fit msqrob models using lm and rlm

Description

Low-level function for parameter estimation with msqrob using the ordinary least squares or robust regression base on the MASS::rlm function.

Usage

msqrobLm(y, formula, data, robust = TRUE, maxitRob = 5)

Arguments

y A matrix with the quantified feature intensities. The features are along the rows and samples along the columns.

formula Model formula. The model is built based on the covariates in the data object.

data A DataFrame with information on the design. It has the same number of rows as the number of columns (samples) of y.

robust boolean(1) to indicate if robust regression is performed to account for outliers. Default is TRUE. If FALSE an OLS fit is performed.

maxitRob numeric(1) indicating the maximum iterations in the IRWLS algorithm used in the M-estimation step of the robust regression.

Value

A list of objects of the StatModel class.

Author(s)

Lieven Clement, Oliver M. Crook

Examples

# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

pe <- msqrobHurdle(pe, i = "protein", formula = ~condition)
gtc = getCoef(rowData(pe["protein"])$msqrobHurdleIntensity[[1]])
gtc = getCoef(rowData(pe["protein"])$msqrobHurdleCount[[1]])
msqrobLmer

pe

# Fit MSqrob model using robust regression with the MASS rlm function
models <- msqrobLm(assay(pe["protein"]), ~condition, colData(pe))
#' getCoef(models[[1]])

msqrobLmer Function to fit msqrob models with ridge regression and/or random
effects using lme4

Description

Low-level function for parameter estimation with msqrob using the robust ridge regression. The
models can be fitted for each feature (e.g. summarised protein expression values) or multiple fea-
tures belonging to the same accession can be modelled simultaneously e.g. peptide-based models
where all peptide intensities for the same protein are modelled simultaneously. The fold changes
and uncertainty estimates are then calculated at the protein level while correcting for peptide species
and within sample correlation.

Usage

msqrobLmer(
  y, formula, data,
  rowdata = NULL, tol = 1e-06,
  robust = TRUE, ridge = FALSE,
  maxitRob = 1,
  doQR = TRUE, featureGroups = NULL,
  lmerArgs = list(control = lmerControl(calc.derivs = FALSE))
)

Arguments

y A matrix with the quantified feature intensities. The features are along the rows
and samples along the columns.

formula Model formula. The model is built based on the covariates in the data object.

data A DataFrame with information on the design. It has the same number of rows
as the number of columns (samples) of y.

rowdata A DataFrame with the rowData information of the SummarizedExperiment. It
has the same number of rows as the number of rows (features) of y.

tol numeric(1) indicating the tolerance for declaring convergence of the M-estimation
loop.
msqrobLmer

robust boolean(1) to indicate if robust regression is performed to account for outliers. Default is TRUE. If FALSE an OLS fit is performed.

ridge boolean(1) to indicate if ridge regression is performed. Default is FALSE. If TRUE the fixed effects are estimated via penalized regression and shrunken to zero.

maxitRob numeric(1) indicating the maximum iterations in the IRWLS algorithm used in the M-estimation step of the robust regression.

doQR boolean(1) to indicate if QR decomposition is used when adopting ridge regression. Default is TRUE. If FALSE the predictors of the fixed effects are not transformed, and the degree of shrinkage can depend on the encoding.

featureGroups vector of type character or vector of type factor indicating how to aggregate the features. Is only used when multiple features are used to build the model, e.g. when starting from peptide data and modelling the fold change at the protein level. The default is NULL

lmerArgs a list (of correct class, resulting from ‘lmerControl()’ containing control parameters, including the nonlinear optimizer to be used and parameters to be passed through to the nonlinear optimizer, see the ‘lmerControl’ documentation of the lme4 package for more details. Default is list(control = lmerControl(calc.derivs = FALSE))

Value
A list of objects of the StatModel class.

Author(s)
Lieven Clement, Oliver M. Crook

Examples
# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit MSqrob model using robust ridge regression upon summarization of
# peptide intensities into protein expression values
modelsRidge <- msqrobLmer(assay(pe["protein"])), ~condition, data = colData(pe),
ridge = TRUE)

calc.derivs = FALSE))

# Fit MSqrob model using robust ridge regression starting from peptide intensities
# The fold changes are calculated at the protein level while correcting for
# the different peptide species in each sample and the correlation between
# peptide intensities of peptides of the same protein in the same sample.
# Add the samples variable to colData
colData(pe)$samples <- rownames(colData(pe))
modelsPepBased <- msqrobLmer(assay(pe["peptide"]),
    formula = ~condition + (1|samples) + (1|Sequence), data = colData(pe),
    rowdata = rowData(pe["peptide"])), featureGroups = rowData(pe["peptide"])$Proteins,
    ridge = TRUE)
getCoef(modelsPepBased[[1]])

msqrobQB,SummarizedExperiment-method

Function to fit msqrob models to peptide counts using glm

Description

Low-level function for parameter estimation with msqrob by modeling peptide counts using quasi-binomial glm

Usage

## S4 method for signature 'SummarizedExperiment'
msqrobQB(
    object,
    formula,
    modelColumnName = "msqrobQbModels",
    overwrite = FALSE,
    priorCount = 0.1,
    binomialBound = TRUE
)

## S4 method for signature 'QFeatures'
msqrobQB(
    object,
    i,
    formula,
    modelColumnName = "msqrobQbModels",
    overwrite = FALSE,
    priorCount = 0.1,
    binomialBound = TRUE
)

Arguments

  object SummarizedExperiment or QFeatures instance
  formula Model formula. The model is built based on the covariates in the data object.
modelColumnName

class to indicate the variable name that is used to store the msqrob models in the rowData of the SummarizedExperiment instance or of the assay of the QFeatures instance. Default is "msqrobModels".

overwrite

boolean(1) to indicate if the column in the rowData has to be overwritten if the modelColumnName already exists. Default is FALSE.

priorCount

A 'numeric(1)', which is a prior count to be added to the observations to shrink the estimated log-fold-changes towards zero. Default is 0.1.

binomialBound

logical, if 'TRUE' then the quasibinomial variance estimator will be never smaller than 1 (no underdispersion). Default is TRUE.

i

character or integer to specify the element of the QFeatures that contains the log expression intensities that will be modelled.

Value

SummarizedExperiment or QFeatures instance

Author(s)

Lieven Clement

Examples

# Load example data
# The data are a Feature object with containing
# a SummarizedExperiment named "peptide" with MaxQuant peptide intensities
# The data are a subset of spike-in the human-ecoli study
# The variable condition in the colData of the Feature object
# contains information on the spike in condition a-e (from low to high)
data(pe)

data(pe)

# Aggregate by counting how many peptide we observe for each protein
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit MSqrob model to peptide counts using a quasi-binomial model
# For summarized SummarizedExperiment
se <- pe[["protein"]]
se

# For features object
pe <- msqrobQB(pe, i = "protein", formula = ~condition)
getCoef(rowData(se)$msqrobQbModels[[1]])
Example data for 100 proteins

Description

Subset of peptides from 100 proteins from a quantitative mass spectrometry based proteomics dataset (PRIDE identifier: PXD003881 Shen et al. (2018)). E. Coli lysates were spiked at five different concentrations (3%, 4.5%, 6%, 7.5% and 9%wt/wt) in a stable human background (4 repl. per treatment). The twenty resulting samples were run on an Orbitrap Fusion mass spectrometer. Raw data files were processed with MaxQuant (version 1.6.1.0, Cox and Mann (2008)) using default search settings unless otherwise noted. Spectra were searched against the UniProtKB/SwissProt human and E. Coli reference proteome databases (07/06/2018), concatenated with the default MaxQuant contaminant database. Carbamidomethylation of Cystein was set as a fixed modification, and oxidation of Methionine and acetylation of the protein amino-terminus were allowed as variable modifications. In silico cleavage was set to use trypsin/P, allowing two miscleavages. Match between runs was also enabled using default settings. The resulting peptide-to-spectrum matches (PSMs) were filtered by MaxQuant at 1% FDR.

Usage

data(pe)

Format

Feature set with an instance "peptide":

- **assay**: contains the raw peptide intensities

- **rowData**: contains a variable "Proteins" with the protein accession and an variable ecoli to indicate if the protein is a spikin.

- **colData**: contains a factor condition indicating the spike-in condition

Examples

data(pe)
head(colData(pe))
head(rowData(pe))
head(assay(pe))
smallestUniqueGroups  Smallest unique protein groups

Description

For a given vector of protein group names, outputs the names of those protein groups for which none of its member proteins is present in a smaller protein group.

Usage

smallestUniqueGroups(proteins, split = ";")

Arguments

proteins  A vector of characters or factors containing single proteins and/or protein groups (i.e. proteins separated by a separator symbol).
split  The character string that is used to separate the individual protein names in each protein group.

Value

A character vector containing the names of the protein groups for which none of its proteins is present in a smaller protein group.

Examples

data(pe)
smallestUniqueGroups(rowData(pe["peptide"])$Proteins)

StatModel-class  The StatModel class for msqrob

Description

The StatModel class contains a statistical model as applied on a feature.

Models are created by the dedicated user-level functions (msqrob(), mqrObAggregate()) or manually, using the StatModel() constructor. In the former case, each quantitative feature is assigned its statistical model and the models are stored as a variable in a DataFrame object, as illustrated in the example below.

Function for constructing a new StatModel object.
Usage

## S4 method for signature 'StatModel'
show(object)

StatModel(
    type = "fitError",
    params = list(),
    varPosterior = NA_real_,
    dfPosterior = NA_real_
)

Arguments

object StatModel object
type default set to fit-error, can be a "lm", "rlm" (robust lm with M estimation), "lmer" (when mixed models or ridge regression is adopted), "quasibinomial" (when peptide counts are fitted)
params A list containing the parameters of the fitted model
varPosterior Numeric, posterior variance, default is NA
dfPosterior Numeric, posterior degrees of freedom, default is NA

Value

A StatModel object

Slots

type character(1) defining type of the used model. Default is "fitError", i.e. a error model. Other include "lm", "rlm", ...
params A list() containing information of the used model.
varPosterior numeric() of posterior variance.
dfPosterior numeric() of posterior degrees of freedom.

Author(s)

Oliver M. Crook, Laurent Gatto, Lieven Clement

Examples

## A fully specified dummy model
myModel <- StatModel(
    type = "rlm",
    params = list(x = 3, y = 7, b = 4),
    varPosterior = c(0.1, 0.2, 0.3),
    dfPosterior = c(6, 7, 8)
)
myModel
## A collection of models stored as a variable in a DataFrame

```r
mod1 <- StatModel(type = "rlm")
mod2 <- StatModel(type = "lm")
df <- DataFrame(x = 1:2)
df$mods <- c(mod1, mod2)
df
# TODO
```

### topFeatures

**Toplist of DE proteins, peptides or features**

#### Description

Summary table of the differentially expressed Features

#### Usage

```r
topFeatures(models, contrast, adjust.method = "BH", sort = TRUE, alpha = 1)
```

#### Arguments

- `models` A list with elements of the class `StatModel` that are estimated using the `msqrob` function.
- `contrast` numeric (matrix)vector specifying one contrast of the linear model coefficients to be tested equal to zero. The (row)names of the vector should be equal to the names of parameters of the model.
- `adjust.method` character specifying the method to adjust the p-values for multiple testing. Options, in increasing conservatism, include "none", "BH", "BY" and "holm". See `p.adjust` for the complete list of options. Default is "BH" the Benjamini-Hochberg method to control the False Discovery Rate (FDR).
- `sort` boolean(1) to indicate if the features have to be sorted according to statistical significance.
- `alpha` numeric specifying the cutoff value for adjusted p-values. Only features with lower p-values are listed.

#### Value

A dataframe with log2 fold changes (logFC), standard errors (se), degrees of freedom of the test (df), t-test statistic (t), p-values (pval) and adjusted pvalues (adjPval) using the specified adjust.method in the p.adjust function of the stats package.

#### Author(s)

Lieven Clement
Examples

data(pe)

# Aggregate peptide intensities in protein expression values
pe <- aggregateFeatures(pe, i = "peptide", fcol = "Proteins", name = "protein")

# Fit msqrob model
pe <- msqrob(pe, i = "protein", formula = ~condition)

# Define contrast
getCoef(rowData(pe[["protein"]])$msqrobModels[[1]])

# Assess log2 fold change between condition c and condition b:
L <- makeContrast("conditionc - conditionb=0", c("conditionb", "conditionc"))
topDeProteins <- topFeatures(rowData(pe[["protein"]])$msqrobModels, L)
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