Package ‘msmsTests’

December 21, 2016

Type Package

Title LC-MS/MS Differential Expression Tests

Version 1.12.0

Date 2013-10-02

Author Josep Gregori, Alex Sanchez, and Josep Villanueva

Maintainer Josep Gregori i Font <josep.gregori@gmail.com>

Depends R (>= 3.0.1), MSnbase, msmsEDA

Imports edgeR, qvalue

Description Statistical tests for label-free LC-MS/MS data by spectral counts, to discover differentially expressed proteins between two biological conditions. Three tests are available: Poisson GLM regression, quasi-likelihood GLM regression, and the negative binomial of the edgeR package. The three models admit blocking factors to control for nuisance variables. To assure a good level of reproducibility a post-test filter is available, where we may set the minimum effect size considered biologically relevant, and the minimum expression of the most abundant condition.

License GPL-2

biocViews Software, MassSpectrometry, Proteomics

NeedsCompilation no

R topics documented:

msmsTests-package .................................................. 2
msms.edgeR .......................................................... 2
msms(glm.pois) ......................................................... 4
msms(glm.qlll) ........................................................ 6
msms.spk .............................................................. 7
pval.by.fc .............................................................. 8
res.volcanoplot ........................................................ 9
test.results ........................................................... 11

Index 14
**msmsTests-package**  
**LC-MS/MS Differential Expression Tests**

**Description**

Statistical tests for label-free LC-MS/MS data by spectral counts, to discover differentially expressed proteins between two biological conditions. Three tests are available: Poisson GLM regression, quasi-likelihood GLM regression, and the negative binomial of the edgeR package. The three models admit blocking factors to control for nuisance variables. To assure a good level of reproducibility a post-test filter is available, where we may set the minimum effect size considered biologically relevant, and the minimum expression of the most abundant condition.

**Details**

- **Package**: msmsTests
- **Type**: Package
- **Version**: 0.99.1
- **Date**: 2013-07-26
- **License**: GPL-2

- **msms.glm.pois**: Poisson based GLM regression
- **msms.glm.qlll**: Quasi-likelihood GLM regression
- **msms.edgeR**: The binomial negative of edgeR
- **pval.by.fc**: Table of cumulative frequencies of features by p-values in bins of log fold change
- **test.results**: Multitest p-value adjustment and post-test filter
- **res.volcanoplot**: Volcanoplot of the results

**Author(s)**

Josep Gregori, Alex Sanchez, and Josep Villanueva  
Maintainer: Josep Gregori <josep.gregori@gmail.com>

**References**

Josep Gregori, Laura Villareal, Alex Sanchez, Jose Baselga, Josep Villanueva (2013). An Effect Size Filter Improves the Reproducibility in Spectral Counting-based Comparative Proteomics. Journal of Proteomics, DOI [http://dx.doi.org/10.1016/j.jprot.2013.05.030](http://dx.doi.org/10.1016/j.jprot.2013.05.030)
**msms.edgeR**

**Description**

Given a null and an alternative model, with a two level treatment factor as the two conditions to compare, executes the negative binomial test by edgeR functions to discover differentially expressed proteins between the two conditions. The null and alternative models may include blocking factors. The reference level of the main factor is considered to be the control condition.

**Usage**

```r
msms.edgeR(msnset, form1, form0, facs=NULL, div=NULL, fnm=NULL)
```

**Arguments**

- `msnset`: A MSnSet object with spectral counts in the expression matrix.
- `form1`: The alternative hypothesis model as a standard R formula, with the treatment factor of interest, and eventual blocking factors.
- `form0`: The null hypothesis model as an standard R formula. It may be the standard null model (y~.) or contain one or multiple blocking factors.
- `facs`: NULL or a data frame with the factors in its columns.
- `div`: NULL or a vector with the divisors used to compute the offsets.
- `fnm`: NULL or a character string with the treatment factor name, as used in the column names of the factors data frame, and in the formula.

**Details**

The right hand site of the formulas is expected to be "y~", with the combination of factors after the tilde. If `facs` is NULL the factors are taken as default from `pData(msnset)`. If `div` is NULL all divisors are taken equal to one. If `fnm` is NULL it is taken to be the first factor in `facs`.

**Value**

A data frame with column names 'LogFC', 'LR', 'p.value', with the estimated log fold changes, likelihood ratio statistic and corresponding p-value as obtained from a call to `glmLRT()` from the edgeR package.

**Author(s)**

Josep Gregori i Font

**References**


Josep Gregori, Laura Villareal, Alex Sanchez, Jose Baselga, Josep Villanueva (2013). An Effect Size Filter Improves the Reproducibility in Spectral Counting-based Comparative Proteomics. Journal of Proteomics, DOI [http://dx.doi.org/10.1016/j.jprot.2013.05.030](http://dx.doi.org/10.1016/j.jprot.2013.05.030)
See Also

MSnSet, edgeR, glmLRT, msmsEDA

Examples

```r
## Example
library(msmsTests)
data(msms.dataset)
e <- pp.msms.data(msms.dataset)
e
null.f <- "y~batch"
alt.f <- "y~treat+batch"
div <- apply(exprs(e),2,sum)
res <- msms.edgeR(e,alt.f,null.f,div=div,fnm="treat")
str(res)
head(res)
```

---

**msms.glm.pois**  
*Spectral counts differential expression by Poisson GLM*

**Description**

Given a null and an alternative model, with a two level treatment factor as the two conditions to compare, executes a Poisson based GLM regression to discover differentially expressed proteins between the two conditions. The null and alternative models may include blocking factors. The reference level of the main factor is considered to be the control condition.

**Usage**

```r
msms.glm.pois(msnset,form1,form0,facs=NULL,div=NULL)
```

**Arguments**

- **msnset**: A MSnSet object with spectral counts in the expression matrix.
- **form1**: The alternative hypothesis model as an standard R formula, with the treatment factor of interest, and eventual blocking factors.
- **form0**: The null hypothesis model as an standard R formula. It may be the standard null model (y~.) or contain one or multiple blocking factors.
- **facs**: NULL or a data frame with the factors in its columns.
- **div**: NULL or a vector with the divisors used to compute the offsets.

**Details**

The right hand site of the formulas is expected to be "y~", with the combination of factors after the tilde. If `facs` is NULL the factors are taken as default from `pData(msnset)`. If `div` is NULL all divisors are taken equal to one.
Value

A data frame with the following columns:

- **LogFC**: Log fold change estimated from the model parameters.
- **D**: Residual deviance as statistic of the test.
- **p.value**: The p-values obtained from the test.

Author(s)

Josep Gregori i Font

References


Josep Gregori, Laura Villareal, Alex Sanchez, Jose Baselga, Josep Villanueva (2013). An Effect Size Filter Improves the Reproducibility in Spectral Counting-based Comparative Proteomics. Journal of Proteomics, DOI [http://dx.doi.org/10.1016/j.jprot.2013.05.030](http://dx.doi.org/10.1016/j.jprot.2013.05.030)

See Also

MSnSet, glm

Examples

```
library(msmsTests)
data(msms.dataset)
# Pre-process expression matrix
e <- pp.msms.data(msms.dataset)
# Factors
pData(e)
# Control condition
levels(pData(e)$treat)[1]
# Treatment condition
levels(pData(e)$treat)[2]

# Models and normalizing condition
null.f <- "y~batch"
alt.f <- "y~treat+batch"
div <- apply(exprs(e),2,sum)

#Test
res <- msms.glm.pois(e,alt.f,null.f,div=div)
str(res)
head(res)
```
msms.glm.qlll  
*Spectral counts differential expression by quasi-likelihood GLM*

**Description**

Given a null and an alternative model, with a two level treatment factor as the two conditions to compare, executes a quasi-likelihood based GLM regression to discover differentially expressed proteins between the two conditions. The null and alternative models may include blocking factors. The reference level of the main factor is considered to be the control condition.

**Usage**

`msms.glm.qlll(msnset, form1, form0, facs=NULL, div=NULL)`

**Arguments**

- `msnset`: A MSnSet object with spectral counts in the expression matrix.
- `form1`: The alternative hypothesis model as an standard R formula, with the treatment factor of interest, and eventual blocking factors.
- `form0`: The null hypothesis model as an standard R formula. It may be the standard null model (`y~.`) or contain one or multiple blocking factors.
- `facs`: NULL or a data frame with the factors in its columns.
- `div`: NULL or a vector with the divisors used to compute the offsets.

**Details**

The right hand site of the formulas is expected to be "y~", with the combination of factors after the tilde. If `facs` is NULL the factors are taken as default from `pData(msnset)`. If `div` is NULL all divisors are taken equal to one.

**Value**

A data frame with the following columns:

- `LogFC`: Log fold change estimated from the model parameters.
- `D`: Residual deviance as statistic of the test.
- `p.value`: The p-values obtained from the test.

**Author(s)**

Josep Gregori i Font

**References**


See Also
MSnSet, glm

Examples
library(msmsTests)
data(msms.dataset)
# Pre-process expression matrix
e <- pp.msms.data(msms.dataset)
# Factors
pData(e)
# Control condition
levels(pData(e)$treat)[1]
# Treatment condition
levels(pData(e)$treat)[2]

# Models and normalizing condition
null.f <- "y~batch"
alt.f <- "y~treat+batch"
div <- apply(exprs(e),2,sum)

#Test
res <- msms.glm.qlll(e,alt.f,null.f,div=div)
str(res)
head(res)

msms.spk
Yeast lisate samples spiked with human proteins

Description
A MSnSet with a spectral counts in the expression matrix and a treatment factor in the phenoData slot.
The spectral counts matrix has samples in the columns, and proteins in the rows. Each sample consists in 500ng of standard yeast lisate spiked with 100, 200, 400 and 600fm of a mix of 48 equimolar human proteins (UPS1, Sigma-Aldrich). The dataset contains a different number of technical replicates of each sample.

Usage
data(msms.spk)
Format
A MSnSet

References
Josep Gregori, Laura Villareal, Alex Sanchez, Jose Baselga, Josep Villanueva (2013). An Effect
Size Filter Improves the Reproducibility in Spectral Counting-based Comparative Proteomics. Jour-
nal of Proteomics, DOI http://dx.doi.org/10.1016/j.jprot.2013.05.030

Laurent Gatto and Kathryn S. Lilley, MSnbase - an R/Bioconductor package for isobaric tagged
mass spectrometry data visualization, processing and quantitation, Bioinformatics 28(2), 288-289
(2012).

See Also
See MSnSet for detail on the class, and the exprs and pData accessors.

Examples
data(msms.spk)
msms.spk
dim(msms.spk)
table(pData(msms.spk))
head(exprs(msms.spk))

pval.by.fc Table of cumulative frequencies of p-values by log fold change bins

Description
Given the set of p-values and log fold changes that result from a test, computes a table of cumulative
frequencies of features by p-values in bins of log fold changes.

Usage
pval.by.fc(pvals,lfc)

Arguments
lfc The log fold changes estimated from the tests.
pvals The p-values, adjusted or not, obtained from the tests.

Value
A matrix of cumulated frequencies with descriptive row and column names.

Author(s)
Josep Gregori i Font
References


See Also
test.results

Examples

library(msmsTests)
data(msms.spk)
# Subset
treat <- pData(msms.spk)
jdx <- which(treat=="U200" | treat=="U600")
e <- msms.spk[,jdx]
pData(e)$treat <- treat[jdx,1,drop=TRUE]
# Pre-process expression matrix
e <- pp.msms.data(e)
# Models and normalizing condition
null.f <- "y~1"
alt.f <- "y~treat"
div <- apply(exprs(e),2,sum)
#Test
res <- msms.glm.pois(e,alt.f,null.f,div=div)
# Post-test filter
lst <- test.results(res,e,pData(e)$treat,"U600","U200",div,
alpha=0.05,minSpC=2,minLFC=1,
method="BH")

## On all features, with multtest adjusted p-values
pval.by.fc(lst$tres$adjp, lst$tres$LogFC)

### On all features deemed significant and biologically relevant
flags <- lst$tres$DEP
pval.by.fc(lst$tres$adjp[flags], lst$tres$LogFC[flags])

res.volcanoplot Volcanoplot

Description

Given the data frame obtained from test.results() a volcano plot is drawn. The features are colored according to significance and relevance.

Usage

res.volcanoplot(tres, max.pval=0.05, min.LFC=1, maxx=3, maxy=10, ylbs=20)
res.volcanoplot

Arguments

tres  The dataframe with test results as obtained from test.results(). Or a dataframe with, at least, the following columns: LogFC with log fold changes, adjp with multtest adjusted p-values, and DEP with TRUE or FALSE as post test filter results, being the TRUE features both statistically significant and relevant for reproducibility.

max.pval  The maximum adjusted p-value considered as statistically significant.

min.LFC  The minimum absolute log fold change considered as biologically relevant.

maxx  The maximum value in abscissas (i.e. log2(fold change)).

maxy  The maximum value in ordinates (i.e. -log10(p.val)).

ylbls  All features with -log10(p.val) above this value will be plotted with feature labels.

Details

Abscissas and ordinates may be limited giving a value other than NULL to the parameters maxx and maxy. All features deemed significant and relevant are plotted by a blue dot, all features deemed significant but not passing the post test filter are plotted by a red dot. The non-significant features are plotted as smaller black dots. All features deemed significant and relevant and with a -log10 p-value above ylbls are plotted with a label showing their row index in the test results dataframe. The borders limiting the values given by max.pval and min.LFC are plotted as dash-and-dot red lines.

Value

No return value.

Author(s)

Josep Gregori i Font

References


See Also

test.results, volcanoplot

Examples

library(msmsTests)
data(msms.dataset)
# Pre-process expression matrix
e <- pp.msms.data(msms.dataset)
# Models and normalizing condition
null.f <- "y~batch"
alt.f <- "y~treat+batch"
div <- apply(exprs(e),2,sum)
# Test
res <- msms.glm.qlll(e,alt.f,null.f,div=div)
lst <- test.results(res,e,pData(e)$treat,"U600","U200",div,
                      alpha=0.05,minSpC=2,minLFC=log2(1.8),
                      method="BH")

# Plot
res.volcanoplot(lst$tres, max.pval=0.05, min.LFC=1, maxx=3, maxy=NULL,
                 ylbls=4)

test.results

Multitest p-value adjustment and post-test filter

Description

Operates on the statistic tests results obtained from msms.glm.pois(), msms.glm.qlll() or msms.edgeR(). The following variables are computed: Raw expression mean values for each condition (control and treatment), log fold change based on these expression levels and taking into account the normalizing divisors (div), multtest adjusted p-values with FDR control, and a post test filter based on minimum spectral counts and minimum absolute log fold change as estimated by the statistic test. According to the results of this post-test filter, features are flagged as T or F depending on whether they result relevant or not, beyond their statistic significance.

Usage

test.results(test, msnset, gpf, gp1, gp2, div, alpha = 0.05,
             minSpC = 2, minLFC = 1, method = "BH")

Arguments

test The dataframe obtained from either msms.glm.pois(), msms.glm.qlll() or msms.edgeR().
msnset A MSnSet object with spectral counts in the expression matrix.
gpf The factor used in the tests.
gp1 The treatment level name.
gp2 The control level name. Should be the factor’s reference level. See R function relevel.
div The weights used as divisors (offsets) in the GLM model. Usually the sum of spectral counts of each sample.
alpha The multi test adjusted p-value significance threshold.
minSpC The minimum spectral counts considered as relevant in the most abundant condition. This filter aims at reaching good reproducibility.
minLFC The minimum absolute log fold change considered both, relevant and biologically significant. This filter aims at assuring enough biological effect size and at reaching good reproducibility.
method One among BH or qval. The p-values are FDR adjusted by the Benjamini-Hochberg method (BH) or by qvalue (qval).

Details

No feature is removed in the filter, but instead they are flagged as TRUE or FALSE depending on whether they are considered as differentially expressed or not, in the DEP column, taking into account statistic significance and reproducibility metrics.
Value

A data frame with the following columns:

- **first column**: Column named as the treatment level with the mean raw spectral counts observed for this condition.
- **second column**: Column named as the control level with the mean raw spectral counts observed for this condition.
- **lFC.Av**: Log fold change computed from the mean expression levels taking into account the given normalization factors.
- **logFC**: Log fold change estimated by fitting the given GLM model. The reference level of the main factor is taken as control.
- **D or LR**: The statistic obtained from the tests. The residual deviance $D$ for Poisson and quasi-likelihood, or the likelihood ratio $LR$ for edgeR.
- **p.val**: The unadjusted $p$-values obtained from the tests.
- **adjp**: The multtest adjusted $p$-values with FDR control.
- **DEP**: A logical flagging the features considered both as statistically significant and relevant for reproducibility.

Author(s)

Josep Gregori i Font

References

Josep Gregori, Laura Villareal, Alex Sanchez, Jose Baselga, Josep Villanueva (2013). An Effect Size Filter Improves the Reproducibility in Spectral Counting-based Comparative Proteomics. Journal of Proteomics, DOI [http://dx.doi.org/10.1016/j.jprot.2013.05.030](http://dx.doi.org/10.1016/j.jprot.2013.05.030)


Alan Dabney, John D. Storey and with assistance from Gregory R. Warnes. qvalue: Q-value estimation for false discovery rate control. R package version 1.30.0.

See Also

- [pval.by.fc](#), [p.adjust](#), [qvalue](#)

Examples

```r
library(msmsTests)
data(msms.dataset)
# Pre-process expression matrix
e <- pp.msms.data(msms.dataset)
# Factors
pData(e)
# Control condition
levels(pData(e)$treat)[1]
# Treatment condition
levels(pData(e)$treat)[2]
# Models and normalizing condition
null.f <- "y~batch"
alt.f <- "y~treat+batch"
```
div <- apply(exprs(e),2,sum)
# Test
res <- msms.glm.qlll(e,alt.f,null.f,div=div)
# Post-test filter
lst <- test.results(res,e,pData(e)$treat,"U600","U200",div,
alpha=0.05,minSpC=2,minLFC=1,
method="BH")
str(lst)
lst$cond
head(lst$tres)
rownames(lst$tres)[which(lst$tres$DEP)]
Index

*Topic datasets
  msms.spk, 7

*Topic design
  msms.edgeR, 2
  msms.glm.pois, 4
  msms.glm.qlll, 6

*Topic hplot
  msmsTests-package, 2
  res.volcanoplot, 9

*Topic htest
  msmsTests-package, 2
  pval.by.fc, 8
  res.volcanoplot, 9

*Topic models
  msms.edgeR, 2
  msms.glm.pois, 4
  msms.glm.qlll, 6

*Topic univar
  msms.edgeR, 2
  msms.glm.pois, 4
  msms.glm.qlll, 6
  pval.by.fc, 8
  res.volcanoplot, 9

designR, 4

glm, 5, 7
glmLRT, 4

msms.edgeR, 2
msms.glm.pois, 4
msms.glm.qlll, 6
msms.spk, 7
msmsEDA, 4
msmsTests (msmsTests-package), 2
msmsTests-package, 2
MSnSet, 4, 5, 7, 8

p.adjust, 12
pval.by.fc, 8, 12

qvalue, 12

res.volcanoplot, 9

test.results, 9, 10, 11
volcanoplot, 10