

# Package ‘msmsTests’

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**Type** Package

**Title** LC-MS/MS Differential Expression Tests

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**Depends** R (>= 3.0.1), MSnbase, msmsEDA

**Imports** edgeR, qvalue

**Suggests** xtable

**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** ImmunoOncology, Software, MassSpectrometry, Proteomics

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| msmsTests-package | <i>LC-MS/MS Differential Expression Tests</i> |
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**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: Volcanplot of the results

**Author(s)**

Josep Gregori, Alex Sanchez, and Josep Villanueva  
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**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>

msms.edgeR

*Spectral counts differential expression by 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**

```
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 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.                                                                          |
| 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**

- Robinson MD, McCarthy DJ and Smyth GK (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139-140
- Robinson MD and Smyth GK (2007). Moderated statistical tests for assessing differences in tag abundance. *Bioinformatics* 23, 2881-2887
- Robinson MD and Smyth GK (2008). Small-sample estimation of negative binomial dispersion, with applications to SAGE data. *Biostatistics*, 9, 321-332

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>

### See Also

[MSnSet](#), [edgeR](#), [glmLRT](#), [msmsEDA](#)

### Examples

```
## 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

```
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 a standard R formula, with the treatment factor of interest, and eventual blocking factors.         |
| form0  | The null hypothesis model as a 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 side 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**

- Agresti, A. (2002) Categorical Data Analysis, 2nd Edition, John Wiley & Sons, Inc., Hoboken, New Jersey
- Thompson L.A. (2009) R (and S-PLUS) Manual to Accompany Agresti's Categorical Data Analysis (2002), 2nd edition <https://home.comcast.net/~lthompson221/Splusdiscrete2.pdf>
- Dobson, A.J. (2002) An Introduction to Generalized Linear Models, 2nd Edition, Chapman & Hall/CRC, New York
- Venables, W. N. and Ripley, B. D. (2002) Modern Applied Statistics with S. New York: Springer
- 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>

**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**

- Agresti, A. (2002) *Categorical Data Analysis*, 2nd Edition, John Wiley & Sons, Inc., Hoboken, New Jersey
- Thompson L.A. (2009) *R (and S-PLUS) Manual to Accompany Agresti's Categorical Data Analysis*, 2nd edition <https://home.comcast.net/~lthompson221/Splusdiscrete2.pdf>
- Dobson, A.J. (2002) *An Introduction to Generalized Linear Models*, 2nd Edition, Chapman & Hall/CRC, New York

Venables, W. N. and Ripley, B. D. (2002) *Modern Applied Statistics with S*, New York: Springer

Li, M.; Gray, W.; Zhang, H.; Chung, C. H.; Billheimer, D.; Yarbrough, W. G.; Liebler, D. C.; Shyr, Y.; Slebos, R. J. C. *Comparative shotgun proteomics using spectral count data and quasi-likelihood modeling*, J Proteome Res 2010, 9, 4295-4305.

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, <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.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. Journal 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

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>

## 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 multitest 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,
                ylbls=20)
```

**Arguments**

|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>tres</code>     | The dataframe with test results as obtained from <code>test.results()</code> . Or a data frame with, at least, the following columns: <code>LogFC</code> with log fold changes, <code>adjp</code> with multitest adjusted p-values, and <code>DEP</code> with <code>TRUE</code> or <code>FALSE</code> as post test filter results, being the <code>TRUE</code> features both statistically significant and relevant for reproducibility. |
| <code>max.pval</code> | The maximum adjusted p-value considered as statistically significant.                                                                                                                                                                                                                                                                                                                                                                    |
| <code>min.LFC</code>  | The minimum absolute log fold change considered as biologically relevant.                                                                                                                                                                                                                                                                                                                                                                |
| <code>maxx</code>     | The maximum value in abscissas (i.e. $\log_2(\text{fold change})$ ).                                                                                                                                                                                                                                                                                                                                                                     |
| <code>maxy</code>     | The maximum value in ordinates (i.e. $-\log_{10}(\text{p.val})$ )                                                                                                                                                                                                                                                                                                                                                                        |
| <code>ylbls</code>    | All features with $-\log_{10}(\text{p.val})$ 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  $-\log_{10}$  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**

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>

**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`), multitest 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, msnsset, gpf, gp1, gp2, div, alpha = 0.05,
             minSpC = 2, minLFC = 1, method = "BH")

```

## Arguments

|                      |                                                                                                                                                                                               |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>test</code>    | The dataframe obtained from either <code>msms.glm.pois()</code> , <code>msms.glm.qlll()</code> or <code>msms.edgeR()</code>                                                                   |
| <code>msnsset</code> | A MSnSet object with spectral counts in the expression matrix.                                                                                                                                |
| <code>gpf</code>     | The factor used in the tests.                                                                                                                                                                 |
| <code>gp1</code>     | The treatment level name.                                                                                                                                                                     |
| <code>gp2</code>     | The control level name. Should be the factor's reference level. See R function <code>relevel</code> .                                                                                         |
| <code>div</code>     | The weights used as divisors (offsets) in the GLM model. Usually the sum of spectral counts of each sample.                                                                                   |
| <code>alpha</code>   | The multi test adjusted p-value significance threshold.                                                                                                                                       |
| <code>minSpC</code>  | The minimum spectral counts considered as relevant in the most abundant condition. This filter aims at reaching good reproducibility.                                                         |
| <code>minLFC</code>  | 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. |
| <code>method</code>  | 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 multitest 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>

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B, 57, 289-300.

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**

```
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.ql11(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)]
```

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