

Package ‘DEqMS’

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Title a tool to perform statistical analysis of differential protein expression for quantitative proteomics data.

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Depends R(>= 3.5),graphics,stats,ggplot2,matrixStats,limma(>= 3.34)

Suggests BiocStyle,knitr,rmarkdown,markdown,plyr,reshape2,utils,ggrepel,ExperimentHub,LSD

LazyLoad yes

Description DEqMS is developed on top of Limma. However, Limma assumes same prior variance for all genes. In proteomics, the accuracy of protein abundance estimates varies by the number of peptides/PSMs quantified in both label-free and labelled data. Proteins quantification by multiple peptides or PSMs are more accurate. DEqMS package is able to estimate different prior variances for proteins quantified by different number of PSMs/peptides, therefore achieving better accuracy. The package can be applied to analyze both label-free and labelled proteomics data.

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biocViews ImmunoOncology, Proteomics, MassSpectrometry, Preprocessing, DifferentialExpression, MultipleComparison,Normalization,Bayesian,ExperimentHubSoftware

VignetteBuilder knitr

BugReports <https://github.com/yafeng/DEqMS/issues>

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Contents

| | |
|------------------------------------|---|
| equalMedianNormalization | 2 |
| medianSummary | 3 |

| | |
|-------------------------------|-----------|
| medianSweeping | 3 |
| medpolishSummary | 4 |
| outputResult | 5 |
| peptideProfilePlot | 6 |
| Residualplot | 7 |
| spectraCounteBayes | 8 |
| VarianceBoxplot | 10 |
| VarianceScatterplot | 11 |
| Index | 13 |

equalMedianNormalization

normalize to have equal medians in all samples

Description

This function is to normaliza out the differences of protein medians in different samples

Usage

```
equalMedianNormalization(dat)
```

Arguments

`dat` an numeric data frame or matrix containing protein relative abundance in log2 scale

Value

a data frame or matrix with normalized protein relative abundance

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])
# use the 3 ctrl samples as reference channels to calculate log2 ratio
dat.gene = medianSummary(dat.psm.log,group_col = 2,ref_col =c(3,7,10))
dat.gene.nm = equalMedianNormalization(dat.gene)
```

| | |
|---------------|--|
| medianSummary | <i>summarize peptide/PSM intensity into protein level relative abundance estimate by taking the median</i> |
|---------------|--|

Description

This function is to calculate proteins' relative abundance by median method

Usage

```
medianSummary(dat, group_col=2, ref_col)
```

Arguments

| | |
|-----------|--|
| dat | an data frame with peptide/psm intensities in log2 scale |
| group_col | the column by which peptides/psm intensity are grouped. Usually the gene/protein id column. Default is 2 |
| ref_col | an integer vector indicating the column(s) used as denominator to calculate relative peptide ratio. |

Value

a data frame containing protein relative abundance estimate in log2 scale

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])
# use the 3 ctrl samples as reference channels to calculate log2 ratio
dat.gene = medianSummary(dat.psm.log, group_col = 2, ref_col = c(3,7,10))
```

| | |
|----------------|---|
| medianSweeping | <i>summarize peptide/PSM intensity into protein level relative abundance estimate by median sweeping method</i> |
|----------------|---|

Description

This function is to calculate proteins' relative abundance by median sweeping method

Usage

```
medianSweeping(dat, group_col=2)
```

Arguments

`dat` an data frame with peptide/PSM intensities in log2 scale
`group_col` the column by which peptides/PSM intensity are grouped. Usually the gene/protein id column. Default is 2

Value

a data frame with protein relative abundance estimate in log2 scale

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)
```

| | |
|------------------|---|
| medpolishSummary | <i>summarize peptide/PSM intensity into protein level relative abundance estimate by Turkey median polish procedure</i> |
|------------------|---|

Description

This function is to calculate proteins' relative abundance by Turkey median polish

Usage

```
medpolishSummary(dat,group_col=2)
```

Arguments

`dat` an data frame containing peptide/psm intensities in log2 scale
`group_col` the column by which peptides/psm intensity are grouped. Usually the gene/protein column. Default is 2

Value

a data frame containing protein relative abundance estimate in log2 scale

Author(s)

Yafeng Zhu

Examples

```

library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene = medpolishSummary(dat.psm.log,group_col=2)

```

| | |
|--------------|--|
| outputResult | <i>output the DEqMS analysis results in a data frame</i> |
|--------------|--|

Description

This function is to generate DEqMS outputs in a data frame.

Usage

```
outputResult(fit, coef_col=1)
```

Arguments

| | |
|----------|--|
| fit | an list object produced by spectraCounteBayes function |
| coef_col | is an integer indicating the column of fit\$coefficients for which corresponding t-statistics and p-values are extracted in the output |

Value

a data frame object with the last three columns being: sca.t - Peptide or Spectra Count Adjusted posterior t-value sca.P.Value - Adjusted posterior p-value sca.adj - sca.P.Value adjusted by BH method

Author(s)

Yafeng Zhu

Examples

```

library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)

psm.count.table = as.data.frame(table(dat.psm$gene)) # generate PSM count table
rownames(psm.count.table)=psm.count.table$Var1

cond = c("ctrl","miR191","miR372","miR519","ctrl",

```

```

"miR372", "miR519", "ctrl", "miR191", "miR372")

sampleTable <- data.frame(
  row.names = colnames(dat.psm)[3:12],
  cond = as.factor(cond)
)

gene.matrix = as.matrix(dat.gene.nm)
design = model.matrix(~cond, sampleTable)

fit1 <- eBayes(lmFit(gene.matrix, design))
# add PSM count for each gene
fit1$count <- psm.count.table[rownames(fit1$coefficients), 2]

fit2 = spectraCounteBayes(fit1)

DEqMS.results = outputResult(fit2, coef_col=3)

```

peptideProfilePlot *plot log₂ intensities of all peptides for one gene in different samples*

Description

This function is to plot log₂ intensities of all peptides for one gene in different samples.

Usage

```
peptideProfilePlot(dat, col=2, gene)
```

Arguments

| | |
|------|--|
| dat | a data frame with peptide/psm log ₂ intensities |
| col | an integer indicates the column number where the gene protein id is. default is 2, assuming the gene/protein is in the second column |
| gene | an character indicates the gene name/id to be plotted |

Value

return a ggplot2 object

Author(s)

Yafeng Zhu

Examples

```

library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[, 3:12] = log2(dat.psm[, 3:12])

```

```
peptideProfilePlot(dat.psm.log,col=2,gene="TGFB2")
```

| | |
|--------------|---|
| Residualplot | <i>plot the residuals against the number of quantified peptides/PSMs.</i> |
|--------------|---|

Description

This function is to plot the residuals of fit model on the vertical axis and the peptide or PSM count on the horizontal axis.

Usage

```
Residualplot(fit, xlab="log2(count)",  
ylab="Variance(fitted - observed)", main="")
```

Arguments

| | |
|------|---|
| fit | an object returned from spectraCounteBayes function |
| xlab | the title for x axis |
| ylab | the title for y axis |
| main | the title for the figure |

Value

return a plot graphic

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)  
eh = ExperimentHub(localHub=TRUE)  
query(eh, "DEqMS")  
dat.psm = eh[["EH1663"]]  
  
dat.psm.log = dat.psm  
dat.psm.log[,3:12] = log2(dat.psm[,3:12])  
  
dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)  
  
psm.count.table = as.data.frame(table(dat.psm$gene)) # generate PSM count table  
rownames(psm.count.table)=psm.count.table$Var1  
  
cond = c("ctrl","miR191","miR372","miR519","ctrl",  
"miR372","miR519","ctrl","miR191","miR372")  
  
sampleTable <- data.frame(  
row.names = colnames(dat.psm)[3:12],  
cond = as.factor(cond))
```

```

)

gene.matrix = as.matrix(dat.gene.nm)
design = model.matrix(~cond,sampleTable)

fit1 <- eBayes(lmFit(gene.matrix,design))
# add PSM count for each gene
fit1$count <- psm.count.table[rownames(fit1$coefficients),2]

fit2 = spectraCounteBayes(fit1)

Residualplot(fit2,xlab="log2(PSM count)",main="TMT data PXD004163")

```

| | |
|--------------------|---|
| spectraCounteBayes | <i>Peptide/Spectra Count Based Empirical Bayes Statistics for Differential Expression</i> |
|--------------------|---|

Description

This function is to calculate peptide/PSM count adjusted t-statistics, p-values.

Usage

```
spectraCounteBayes(fit, fit.method="loess", coef_col)
```

Arguments

| | |
|------------|--|
| fit | an list object produced by Limma eBayes function, it should have one additional attribute \$count, which stored the peptide or PSM count quantified for the gene in label-free or isobaric labelled data. |
| fit.method | the method used to fit variance against the number of peptides/PSM count quantified. Two available methods: "loess", "nls" and "spline". default "loess". "loess" uses loess and span = 0.75, "nls" uses a explicit formula $y \sim a + b/x$. "spline" uses smooth.spline and "generalized cross-validation" for smoothing parameter computation. For "nls", independent variable x is peptide/PSM count, response y is pooled variance ($fit\$sigma^2$). For "loess" and "spline" method, both x and y are log transformed before applying the two methods. In most of time, "loess" is sufficient. To quickly assess the fit model, use VarianceScatterplot and Residualplot functions. |
| coef_col | an integer vector indicating the column(s) of fit\$coefficients for which the function is to be performed. if not specified, all coefficients are used. |

Details

This function adjusts the T-statistics and p-values for quantitative MS proteomics experiment according to the number of peptides/PSMs used for quantification. The method is similar in nature to intensity-based Bayes method (Maureen A. Sartor et al BMC Bioinformatics 2006).

Value

a list object with the following components

| | |
|--------------|---|
| count | Peptide or PSM count used for quantification |
| sca.t | Spectra Count Adjusted posterior t-value |
| sca.p | Spectra Count Adjusted posterior p-value |
| sca.dfprior | Spectra Count Adjusted prior degrees of freedom |
| sca.priorvar | Spectra Count Adjusted prior variance |
| sca.postvar | Spectra Count Adjusted posterior variance |
| model | fitted model |
| fit.method | The method used to fit the model |

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)

psm.count.table = as.data.frame(table(dat.psm$gene)) # generate PSM count table
rownames(psm.count.table)=psm.count.table$Var1

cond = c("ctrl","miR191","miR372","miR519","ctrl",
"miR372","miR519","ctrl","miR191","miR372")

sampleTable <- data.frame(
row.names = colnames(dat.psm)[3:12],
cond = as.factor(cond)
)

gene.matrix = as.matrix(dat.gene.nm)
design = model.matrix(~cond,sampleTable)

fit1 <- eBayes(lmFit(gene.matrix,design))
# add PSM count for each gene
fit1$count <- psm.count.table[rownames(fit1$coefficients),2]

fit2 = spectraCounteBayes(fit1)
```

VarianceBoxplot *generate a boxplot of the variance*

Description

This function is to draw a boxplot of the variance of genes quantified by different number of peptides/PSMs. Red curve indicate DEqMS prior variance.

Usage

```
VarianceBoxplot(fit,n=20, xlab="count", ylab = "log(Variance)", main="")
```

Arguments

| | |
|------|--|
| fit | an object returned from spectraCounteBayes function |
| n | set a number to plot only the genes with count value smaller or equal to n |
| xlab | the title for x axis |
| ylab | the title for y axis |
| main | the title for the figure |

Value

return a plot graphic

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)

psm.count.table = as.data.frame(table(dat.psm$gene)) # generate PSM count table
rownames(psm.count.table)=psm.count.table$Var1

cond = c("ctrl","miR191","miR372","miR519","ctrl",
"miR372","miR519","ctrl","miR191","miR372")

sampleTable <- data.frame(
row.names = colnames(dat.psm)[3:12],
cond = as.factor(cond)
)

gene.matrix = as.matrix(dat.gene.nm)
```

```
design = model.matrix(~cond,sampleTable)

fit1 <- eBayes(lmFit(gene.matrix,design))
# add PSM count for each gene
fit1$count <- psm.count.table[rownames(fit1$coefficients),2]

fit2 = spectraCounteBayes(fit1)

VarianceBoxplot(fit2,xlab="PSM count",main="TMT data PXD004163")
```

VarianceScatterplot *generate a scatter plot of the variance*

Description

This function is to draw a scatter plot of the variance against the number of quantified peptides/PSMs. Red curve indicate DEqMS prior variance.

Usage

```
VarianceScatterplot(fit, xlab="log2(count)",
  ylab = "log(Variance)", main="")
```

Arguments

| | |
|------|---|
| fit | an object returned from spectraCounteBayes function |
| xlab | the title for x axis |
| ylab | the title for y axis |
| main | the title for the figure |

Value

return a plot graphic

Author(s)

Yafeng Zhu

Examples

```
library(ExperimentHub)
eh = ExperimentHub(localHub=TRUE)
query(eh, "DEqMS")
dat.psm = eh[["EH1663"]]

dat.psm.log = dat.psm
dat.psm.log[,3:12] = log2(dat.psm[,3:12])

dat.gene.nm = medianSweeping(dat.psm.log,group_col = 2)

psm.count.table = as.data.frame(table(dat.psm$gene)) # generate PSM count table
rownames(psm.count.table)=psm.count.table$Var1
```

```
cond = c("ctrl","miR191","miR372","miR519","ctrl",
"miR372","miR519","ctrl","miR191","miR372")

sampleTable <- data.frame(
row.names = colnames(dat.psm)[3:12],
cond = as.factor(cond)
)

gene.matrix = as.matrix(dat.gene.nm)
design = model.matrix(~cond,sampleTable)

fit1 <- eBayes(lmFit(gene.matrix,design))
# add PSM count for each gene
fit1$count <- psm.count.table[rownames(fit1$coefficients),2]

fit2 = spectraCounteBayes(fit1)

VarianceScatterplot(fit2,xlab="log2(PSM count)",main="TMT data PXD004163")
```

Index

[equalMedianNormalization](#), 2

[medianSummary](#), 3

[medianSweeping](#), 3

[medpolishSummary](#), 4

[outputResult](#), 5

[peptideProfilePlot](#), 6

[Residualplot](#), 7

[spectraCounteBayes](#), 8

[VarianceBoxplot](#), 10

[VarianceScatterplot](#), 11