

# Package ‘qPLEXanalyzer’

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**Type** Package

**Title** Tools for qPLEX-RIME data analysis

**Version** 1.6.0

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**Description** Tools for quantitative proteomics data analysis generated from qPLEX-RIME method.

**License** GPL-2

**Imports** tidy, preprocessCore, limma, ggplot2, RColorBrewer, stats,  
utils, Biostrings, GenomicRanges, IRanges, graphics,  
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dplyr, magrittr, assertthat

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**BugReports** <https://github.com/crukci-bioinformatics/qPLEXanalyzer/issues>

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qPLEXanalyzer-package *Tools for qPLEX-RIME data analysis*

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## Description

Tools for quantitative proteomics data analysis generated from qPLEX-RIME method

## Details

Package:	qPLEXanalyzer
Type:	Package
Version:	1.0.0
Date:	2018-04-23
License:	GPL
Depends:	methods

The package offers the following functionalities

Data processing, normalization & analysis:

- `convertToMSnset`: Converts quantitative data to a MSnSet
- `summarizeIntensities`: Summarizes multiple peptide measurements for a protein
- `normalizeQuantiles`: Performs quantile normalization on the peptides/proteins intensities
- `normalizeScaling`: Performs scaling normalization on the peptides/proteins intensities (mean, median or sum)
- `groupScaling`: Performs scaling normalization on the peptides/proteins intensities within group (median or mean)

- rowScaling: Normalization by scaling peptide/protein intensity across all samples
- regressIntensity: Performs linear regression on protein intensities based on selected protein
- computeDiffStats: Compute differential statistics for the given contrasts
- getContrastResults: Get differential statistics results for given contrast

#### Visualization:

- assignColours: Assigns colours to samples in groups
- intensityPlot: Intensity distribution plot of all the samples
- intensityBoxplot: Intensity distribution boxplot of all the samples
- peptideIntensityPlot: Peptide intensity distribution plot of specific protein
- pcaPlot: PCA plot of all the samples
- maVolPlot: MA or Volcano plot of differential analysis results
- corrPlot: Correlation plot of all the samples
- rliPlot: Relative intensity plot of all the samples
- hierarchicalPlot: Hierarchical clustering plot of all the samples
- plotMeanVar: Computes and plots mean-variance for samples in MSnSet
- coveragePlot: Computes and display protein sequence coverage of selected protein in proteomics experiment

#### Author(s)

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<ads2202cu@gmail.com>

---

assignColours

*Assigns colours to samples in groups*

---

#### Description

Assigns colours to samples in groups for plotting

#### Usage

```
assignColours(MSnSetObj, colourBy="SampleGroup")
```

#### Arguments

MSnSetObj	MSnSet; an object of class MSnSet
colourBy	character: column name from pData(MSnSetObj) to use for coloring samples

#### Value

A character vector of colors for samples.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1,
metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
sampleColours <- assignColours(MSnSet_data)
```

---

<code>computeDiffStats</code>	<i>Compute differential statistics</i>
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---

**Description**

Compute differential statistics on the given contrasts, based on [limma](#) functions.

**Usage**

```
computeDiffStats(MSnSetObj, batchEffect = NULL, transform = TRUE, contrasts, trend = TRUE, robust =
```

**Arguments**

<code>MSnSetObj</code>	MSnSet; An object of class MSnSet
<code>batchEffect</code>	character; vector of variable(s) to correct for batch effect, Default : "Sample-Group"
<code>transform</code>	logical; apply log2 transformation to the raw intensities
<code>contrasts</code>	character; named character vector of contrasts for differential statistics
<code>trend</code>	logical; TRUE or FALSE
<code>robust</code>	logical; TRUE or FALSE

**Details**

A statistical analysis for the identification of differentially regulated or bound proteins is carried out using limma based analysis. It uses linear models to assess differential expression in the context of multifactor designed experiments. Firstly, a linear model is fitted for each protein where the model includes variables for each group and MS run. Then, log2 fold changes between comparisons are estimated. Multiple testing correction of p-values are applied using the Benjamini-Hochberg method to control the false discovery rate (FDR).

In order to correct for batch effect, variable(s) can be defined. It should corresponds to a column name in `pData(MSnSetObj)`. The default variable is "SampleGroup" that distinguish between two groups. If more variables are defined they are added to default.

**Value**

A list object containing three components: `MSnSetObj` (RMSnSet object), `fittedLM` (fitted linear model) and `fittedContrasts`. This object should be input into `getContrastResults` function to get differential results. See [eBayes](#) function of [limma](#) for more details on differential statistics.

**Examples**

```

data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1,
metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- groupScaling(MSnSet_data, scalingFunction=median)
MSnset_Pnorm <- summarizeIntensities(MSnset_norm, sum, human_anno)
contrasts <- c(tam.24h_vs_vehicle = "tam.24h - vehicle", tam.6h_vs_vehicle = "tam.6h - vehicle")
diffstats <- computeDiffStats(MSnSetObj=MSnset_Pnorm, contrasts=contrasts)

```

---

convertToMSnset	<i>Converts proteomics TMT intensity data to MSnSet</i>
-----------------	---------------------------------------------------------

---

**Description**

Converts processed TMT peptide intensities to MSnSet

**Usage**

```
convertToMSnset(ExpObj, metadata, indExpData, Sequences, Accessions, type="peptide", rmMissing=TRUE)
```

**Arguments**

ExpObj	data.frame; a data.frame consisting of quantitative peptide intensities and peptide annotation
metadata	data.frame; a data.frame describing the samples
indExpData	numeric; a numeric vector indicating the column indexes of intensities in ExpObj
Sequences	numeric; a numeric value indicating the index of column consisting of peptide sequence in ExpObj
Accessions	numeric; a numeric value indicating the index of column consisting of protein accession in ExpObj
type	character; what type of data set to create, either 'peptide' or 'protein'
rmMissing	logical; TRUE or FALSE to indicate whether to remove missing data or not

**Details**

This function builds an object of class MSnSet from a dataframe consisting of quantitative proteomics intensities data and a meta-data describing the samples information. This function creates an MSnSet object from the intensities and metadata file. The metadata must contain "Sample-Name", "SampleGroup", "BioRep" and "TechRep" columns. The function can be used for either peptide intensities or data that has already been summarized to protein level. The type argument should be set to 'protein' for the latter.

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
```

---

corrPlot

*Correlation plot*


---

**Description**

Computes and display correlation plot for samples within MSnSet

**Usage**

```
corrPlot(MSnSetObj, addValues=TRUE, title="", low_cor_colour = "#FFFFFF",
high_cor_colour = "#B90505")
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
addValues	logical: adds correlation values to the plot
title	character; title of the plot
low_cor_colour	colour; colour for lowest correlation in scale
high_cor_colour	colour; colour for highest correlation in scale

**Value**

(Invisibly) returns a reordered correlation matrix plot.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1,
  metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16),
  Sequences=2,
  Accessions=6)
corrPlot(MSnSet_data, addValues=TRUE, title="Correlation plot")

# change colours
corrPlot(MSnSet_data, addValues=TRUE, title="Correlation plot",
  low_cor_colour="yellow", high_cor_colour="green")
```

---

coveragePlot	<i>Plot peptide sequence coverage</i>
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---

### Description

Computes and displays peptide sequence coverage in proteomics experiment

### Usage

```
coveragePlot(MSnSetObj, ProteinID, ProteinName, fastaFile, myCol="brown")
```

### Arguments

MSnSetObj	MSnSet: an object of class MSnSet
ProteinID	character: Uniprot ID of the protein
ProteinName	character: name of the protein
fastaFile	character: fasta file of protein sequence
myCol	character: colour for plotting

### Details

In the qPLEX-RIME experiment it is imperative for bait protein to have good sequence coverage. This function plots the protein sequence coverage of the bait protein in the qPLEX-RIME experiment. It requires the fasta sequence file of bait protein as input to generate the plot.

### Value

A protein coverage plot of selected protein for quantitative proteomics data.

### Examples

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
mySequenceFile <- system.file('extdata', "P03372.fasta", package="qPLEXanalyzer")
coveragePlot(MSnSet_data, ProteinID="P03372", ProteinName="ERa", fastaFile=mySequenceFile)
```

---

exp2_Xlink	<i>exp2_Xlink dataset</i>
------------	---------------------------

---

### Description

An ER qPLEX-RIME experiment was performed to compare two different methods of crosslinking. MCF7 cells were double crosslinked with DSG/formaldehyde (double) or with formaldehyde alone (single). Four biological replicates were obtained for each condition along with two IgG pooled samples from each replicate.

**Usage**

```
data(exp2_Xlink)
```

**Format**

An object of class `list` related to peptides quantification. It consists of qPLEX-RIME data of 10 samples divided into three conditions (FA, DSG.FA and IgG).

**Value**

An object of class `list` related to peptides quantification.

---

exp3_OHT_ESR1	<i>exp3_OHT_ESR1 dataset</i>
---------------	------------------------------

---

**Description**

Three ER qPLEX-RIME (10plex) experiments were performed to investigate the dynamics of the ER complex assembly upon 4-hydrotamoxifen (OHT) treatment at 2h, 6h and 24h or at 24h post-treatment with the drug-vehicle alone (ethanol). Two biological replicates of each condition were included in each experiment to finally consider a total of six replicates per time point. Additionally, MCF7 cells were treated with OHT or ethanol and cross-linked at 24h post-treatment in each experiment to be used for mock IgG pull-downs and to enable discrimination of non-specific binding in the same experiment. This is a timecourse experiment to study the effect of tamoxifen in ER interactome using qPLEX-RIME method.

**Usage**

```
data(exp3_OHT_ESR1)
```

**Format**

An object of class `list` related to peptides quantification. It consists of qPLEX-RIME data from three experimental runs. Each run contains 10 samples divided into five conditions (IgG, vehicle, tam.2h, tam.6h and tam.24h).

**Value**

An object of class `list` related to peptides quantification.



---

getContrastResults      *Get differential statistics results*

---

### Description

Get differential statistics results for given contrasts.

### Usage

```
getContrastResults(diffstats, contrast, controlGroup = NULL, transform = TRUE, writeFile= FALSE)
```

### Arguments

diffstats	list; output of computeDiffStats function
contrast	character; contrast of interest for which to retrieve differential statistics results
controlGroup	character; control group such as IgG
transform	logical; apply log2 transformation to the raw intensities
writeFile	logical; whether to write the results into a text file

### Value

A `data.frame` object and text file containing the result of the differential statistics.

### Examples

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- groupScaling(MSnSet_data, scalingFunction=median)
MSnset_Pnorm <- summarizeIntensities(MSnset_norm, sum, human_anno)
contrasts <- c(tam.24h_vs_vehicle = "tam.24h - vehicle")
diffstats <- computeDiffStats(MSnset_Pnorm, contrasts=contrasts)
diffexp <- getContrastResults(diffstats=diffstats, contrast=contrasts)
```

---

groupScaling      *Normalization by scaling within group*

---

### Description

Performs scaling normalization on the intensities within group (median or mean)

### Usage

```
groupScaling(MSnSetObj, scalingFunction, groupingColumn="SampleGroup")
```

**Arguments**

MSnSetObj        MSnSet; an object of class MSnSet  
 scalingFunction        function; median or mean  
 groupingColumn    character; the feature on which groups would be based; default="SampleGroup"

**Details**

In this normalization method the central tendencies (mean or median) of the samples within groups are aligned. The argument "groupingColumn" is used to define separate groups to normalize. The function takes one of the column of pData(data) as the variable for classifying group. The default variable is "SampleGroup". It is imperative in qPLEX-RIME experiment to define IgG as a separate group and normalize it separately from others. You could add a column into the metadata to define this classification.

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- groupScaling(MSnSet_data, scalingFunction=median, groupingColumn="SampleGroup")
```

---

hierarchicalPlot        *Hierarchical plot*

---

**Description**

Computes and display hierarchical clustering plot for samples in MSnSet

**Usage**

```
hierarchicalPlot(MSnSetObj, sampleColours=NULL, colourBy="SampleGroup", horizontal=TRUE,
  title="")
```

**Arguments**

MSnSetObj        MSnSet; an object of class MSnSet  
 sampleColours    character: a vector of colors for samples  
 colourBy        character: column name from pData(MSnSetObj) to use for coloring samples  
 horizontal       logical: define orientation of the dendrogram  
 title            character: the main title for the dendrogram

**Value**

A hierarchical clustering plot for quantitative proteomics data.

**Examples**

```

data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
exprs(MSnSet_data) <- exprs(MSnSet_data)+0.01
hierarchicalPlot(MSnSet_data, title="qPLEX_RIME_ER")

```

---

human_anno	<i>human_anno dataset</i>
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---

**Description**

Uniprot Human protein annotation table.

**Usage**

```
data(human_anno)
```

**Format**

An object of class [data.frame](#) consisting of uniprot human protein annotation.

---

intensityBoxplot	<i>Intensity Distribution boxplot</i>
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---

**Description**

Intensity distribution boxplot of all the samples

**Usage**

```
intensityBoxplot(MSnSetObj, title="", sampleColours=NULL, colourBy="SampleGroup")
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
title	character; title of the plot
sampleColours	character: a named character vector of colors for samples
colourBy	character: column name from pData(MSnSetObj) to use for coloring samples

**Details**

The column provided to the "colourBy" argument will be used to colour the samples. The colours will be determined using the function [assignColours](#), alternatively the user may specify a named vector of colours using the "sampleColours" argument. The names of the "sampleColours" vector should match the unique values in the "colourBy" column.

**Value**

An intensity distribution boxplot for quantitative proteomics data.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
intensityBoxplot(MSnSet_data, title = "qPLEX_RIME_ER")

# custom colours
customCols <- rainbow(length(unique(pData(MSnSet_data)$SampleGroup)))
names(customCols) <- unique(pData(MSnSet_data)$SampleGroup)
intensityBoxplot(MSnSet_data, title = "qPLEX_RIME_ER", sampleColours = customCols)
```

---

intensityPlot

*Intensity Distribution Plot*


---

**Description**

Intensity distribution plot of all the samples

**Usage**

```
intensityPlot(MSnSetObj, sampleColours=NULL, title="", colourBy="SampleGroup",
              transform=TRUE, xlab="log2(intensity)",
              trFunc=log2xplus1)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
sampleColours	character: a vector of colors for samples
title	character: title for the plot
colourBy	character: column name from pData(MSnSetObj) to use for coloring samples
transform	logical: whether to log transform intensities
xlab	character: label for x-axis
trFunc	func: internal helper function for log transformation

**Details**

The column provided to the "colourBy" argument will be used to colour the samples. The colours will be determined using the function [assignColours](#), alternatively the user may specify a named vector of colours using the "sampleColours" argument. The names of the "sampleColours" vector should match the unique values in the "colourBy" column.

**Value**

An intensity distribution plot for quantitative proteomics data.

**Examples**

```

data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
intensityPlot(MSnSet_data, title = "qPLEX_RIME_ER")

# custom colours
customCols <- rainbow(length(unique(pData(MSnSet_data)$SampleGroup)))
names(customCols) <- unique(pData(MSnSet_data)$SampleGroup)
intensityPlot(MSnSet_data, title = "qPLEX_RIME_ER", sampleColours = customCols)

```

---

maVolPlot

*MA or Volcano Plot*


---

**Description**

MA or Volcano plot of differential statistics results

**Usage**

```

maVolPlot(diffstats, contrast, title="", controlGroup = NULL,
           selectedGenes=NULL, fdrCutOff=0.05,
           lfcCutOff=1, controlLfcCutOff=1, plotType="MA")

```

**Arguments**

diffstats	list; output of computeDiffStats function
contrast	character; contrast of interest to plot differential statistics results
title	character: title for the plot
controlGroup	character; control group such as IgG
selectedGenes	character: a vector defining genes to plot
fdrCutOff	numeric: False Discovery Rate (adj.P.Val) cut off
lfcCutOff	numeric: Log Fold Change (log2FC) cutoff for
controlLfcCutOff	numeric: only plot genes above controlLogFoldChange cutoff
plotType	character: which type of plot to generate: "MA" or "Volcano"

**Details**

Genes determined as significant according to the Log Fold Change and False Discovery Rate cutoffs are highlighted in red.

A user specified selection of genes can be highlighted by passing a character vector of Accessions to the selectedGenes argument. The contents of this vector will be matched with the Accessions column of the diffstats object to identify rows to highlight. These will be plotted in blue and labelled with the contents of the GeneSymbol column. Note that if the GeneSymbol for a selected gene is missing, no label will be apparent.

**Value**

A "MA" or "Volcano" plot to visualize differential statistics results.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- groupScaling(MSnSet_data, scalingFunction=median)
MSnset_Pnorm <- summarizeIntensities(MSnset_norm, sum, human_anno)
contrasts <- c(tam.24h_vs_vehicle = "tam.24h - vehicle")
diffstats <- computeDiffStats(MSnset_Pnorm, contrasts=contrasts)
maVolPlot(diffstats, contrast = contrasts, plotType="MA")
maVolPlot(diffstats, contrast = contrasts, plotType="Volcano")
```

---

mergePeptides

*Merge identical peptides intensities*


---

**Description**

Merge identical peptides intensities belonging to same protein to single peptide intensity. This function is especially useful for phosphopeptide analysis.

**Usage**

```
mergePeptides(MSnSetObj, summarizationFunction, annotation)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
summarizationFunction	function; method used to aggregate the peptides. Sum, mean or median
annotation	data.frame; a data.frame of protein annotation of four columns: "Accessions", "Gene", "Description" and "GeneSymbol"

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_P <- mergePeptides(MSnSet_data, sum, human_anno)
```

---

normalizeQuantiles	<i>Quantile normalization</i>
--------------------	-------------------------------

---

**Description**

Performs quantile normalization on the intensities within columns

**Usage**

```
normalizeQuantiles(MSnSetObj)
```

**Arguments**

MSnSetObj      MSnSet; an object of class MSnSet

**Details**

The peptide intensities are roughly replaced by the order statistics on their abundance. This normalization technique has the effect of making the distributions of intensities from the different samples identical in terms of their statistical properties. It is the strongest normalization method and should be used carefully as it erases most of the difference between the samples.

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- normalizeQuantiles(MSnSet_data)
```

---

normalizeScaling	<i>Normalization by scaling</i>
------------------	---------------------------------

---

**Description**

Performs scaling normalization on the peptide/protein intensities (median or mean)

**Usage**

```
normalizeScaling(MSnSetObj, scalingFunction, ProteinId = NULL)
```

**Arguments**

MSnSetObj      MSnSet; an object of class MSnSet  
 scalingFunction      function; median or mean  
 ProteinId      character; protein Id

**Details**

In this normalization method the central tendencies (mean or median) of the samples are aligned. The central tendency for each sample is computed and log transformed. A scaling factor is determined by subtracting from each central tendency the mean of all the central tendencies. The raw intensities are then divided by the scaling factor to get normalized ones. The intensities can also be normalized based on the peptides intensities of a selected protein. For this the argument "ProteinId" allows you to define the protein that will be used for scaling the intensities.

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16), Sequences=2, Accessions=6)
MSnSet_norm <- normalizeScaling(MSnSet_data, scalingFunction=median)
```

---

pcaPlot

*PCA plot*

---

**Description**

PCA plots of the samples within MSnset

**Usage**

```
pcaPlot(MSnSetObj, omitIgG=FALSE, sampleColours=NULL, transFunc=log2xplus1, transform=TRUE,
  colourBy="SampleGroup", title="", labelColumn="BioRep", labelsize=4, pointsize=4,
  x.nudge=4, x.PC=1)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
omitIgG	Logical: whether to remove IgG from the PCA plot
sampleColours	character: A named vector of colours for samples
transFunc	func: internal helper function for log transformation
transform	logical: whether to log transform intensities
colourBy	character: column name to use for colouring samples from pData(MSnSetObj)
title	character: title for the plot
labelColumn	character: column name from pData(MSnSetObj) to use for labelling points on the plot
labelsize	numeric: size of the labels
pointsize	numeric: size of plotting points
x.nudge	numeric: distance to move labels along the x-axis away from the plotting points
x.PC	numeric: The principle component to plot on the x-axis; the following PC will be plotted on the y-axis



**Details**

The column provided to the "colourBy" argument will be used to colour the samples. The colours will be determined using the function `assignColours`, alternatively the user may specify a named vector of colours using the "sampleColours" argument. The names of the "sampleColours" vector should match the unique values in the "colourBy" column.

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16), Sequences=2, Accessions=6)
exprs(MSnSet_data) <- exprs(MSnSet_data)+0.01
pcaPlot(MSnSet_data, omitIgG = TRUE, labelColumn = "BioRep")

# custom colours and PC2 v PC3
customCols <- rainbow(length(unique(pData(MSnSet_data)$SampleGroup)))
names(customCols) <- unique(pData(MSnSet_data)$SampleGroup)
pcaPlot(MSnSet_data, omitIgG = TRUE, labelColumn = "BioRep", sampleColours = customCols, x.PC=2)
```

---

peptideIntensityPlot *Plot peptide intensities*

---

**Description**

Plots all the peptide intensities for the selected protein

**Usage**

```
peptideIntensityPlot(MSnSetObj, ProteinID, ProteinName,
  combinedIntensities=NULL, selectedSequence=NULL,
  selectedModifications=NULL)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet containing peptide level intensities
ProteinID	character: Uniprot ID of the protein
ProteinName	character: name of the protein
combinedIntensities	MSnSet; an object of class MSnSet containing protein level intensities
selectedSequence	character: sequence present in the "Sequences" column in fData(MSnSetObj)
selectedModifications	character: modification present in the "Modifications" column in fData(MSnSetObj)

**Details**

Providing a summarised protein level MSnSet object to the 'combinedIntensities' argument will add a summed protein intensity trace to the plot along with the peptide intensities.

**Value**

An intensity plot for selected protein and associated peptides.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_P <- summarizeIntensities(MSnSet_data, sum, human_anno)
peptideIntensityPlot(MSnSet_data, combinedIntensities=MSnset_P, ProteinID="P03372", ProteinName= "ESR1")
```

---

plotMeanVar

*Mean variance plot*


---

**Description**

Computes and plots mean-variance for samples in MSnset

**Usage**

```
plotMeanVar(MSnSetObj, title="")
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
title	character: title for the plot

**Value**

Plot of mean variance.

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
plotMeanVar(MSnSet_data, title="Mean_Variance")
```

---

regressIntensity	<i>Regression based analysis</i>
------------------	----------------------------------

---

### Description

Performs linear regression on protein intensities based on selected protein (qPLEX-RIME bait)

### Usage

```
regressIntensity(MSnSetObj, ProteinId, controlInd=NULL, plot=TRUE)
```

### Arguments

MSnSetObj	MSnSet; an object of class MSnSet
controlInd	numeric; index of IgG within MSnSet
ProteinId	character; Uniprot protein ID
plot	character; Whether or not to plot the QC histograms

### Details

This function performs regression based analysis upon protein intensities based on a selected protein. In qPLEX RIME this method could be used to regress out the effect of target protein on other interactors. This function corrects this dependency of many proteins on the target protein levels by linear regression. It sets the target protein levels as the independent variable (x) and each of the other proteins as the dependent variable (y). The resulting residuals of the linear regressions  $y=ax+b$  are the protein levels corrected for target protein dependency.

### Value

An instance of class "MSnSet". This consists of corrected protein levels. In addition the function can also plot histograms of correlation of target protein with all other proteins before and after this correction.

### Examples

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_P <- summarizeIntensities(MSnSet_data, sum, human_anno)
IgG_ind <- which(pData(MSnset_P)$SampleGroup == "IgG")
MSnset_reg <- regressIntensity(MSnset_P, controlInd=IgG_ind, ProteinId="P03372")
```

rliPlot

*RLI plot***Description**

Relative log intensity (RLI) plots of the samples within MSnset

**Usage**

```
rliPlot(MSnSetObj, title="", sampleColours=NULL, colourBy="SampleGroup", omitIgG=TRUE)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
title	character: title for the plot
sampleColours	character: a named vector of colours for samples
colourBy	character: column name to use for colouring samples from pData(MSnSetObj)
omitIgG	logical: whether to remove IgG from the RLI plot

**Details**

An RLI-plot is a boxplot that can be used to visualise unwanted variation in a data set. It is similar to the relative log expression plot developed for microarray analysis - see Gandolfo and Speed (2018). Rather than examining gene expression, the RLI plot uses the MS intensities for each peptide or the summarised protein intensities.

The column provided to the "colourBy" argument will be used to colour the samples. The colours will be determined using the function [assignColours](#), alternatively the user may specify a named vector of colours using the "sampleColours" argument. The names of the "sampleColours" vector should match the unique values in the colourBy column.

**Value**

An instance of class "MSnSet".

**References**

Gandolfo LC, Speed TP (2018) RLE plots: Visualizing unwanted variation in high dimensional data. PLoS ONE 13(2): e0191629. <https://doi.org/10.1371/journal.pone.0191629>

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
rliPlot(MSnSet_data, title = "qPLEX_RIME_ER")

# custom colours
customCols <- rainbow(length(unique(pData(MSnSet_data)$SampleGroup)))
names(customCols) <- unique(pData(MSnSet_data)$SampleGroup)
rliPlot(MSnSet_data, title = "qPLEX_RIME_ER", sampleColours = customCols)
```

---

rowScaling	<i>Normalization by scaling peptide/protein intensity across all samples</i>
------------	------------------------------------------------------------------------------

---

**Description**

Divide each peptide/protein by the row mean/median and transform to log2

**Usage**

```
rowScaling(MSnSetObj, scalingFunction)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
scalingFunction	function; median or mean

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1,
  metadata=exp3_OHT_ESR1$metadata_qPLEX1,
  indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_norm <- rowScaling(MSnSet_data, scalingFunction=median)
```

---

summarizeIntensities	<i>Summarizes peptides intensities to proteins</i>
----------------------	----------------------------------------------------

---

**Description**

Summarizes multiple peptides intensities measurements to protein level.

**Usage**

```
summarizeIntensities(MSnSetObj, summarizationFunction, annotation)
```

**Arguments**

MSnSetObj	MSnSet; an object of class MSnSet
summarizationFunction	function; method used to aggregate the peptides into proteins. Sum, mean or median
annotation	data.frame; a data.frame of protein annotation of four columns: "Accessions", "Gene", "Description" and "GeneSymbol"

**Value**

An instance of class "MSnSet".

**Examples**

```
data(human_anno)
data(exp3_OHT_ESR1)
MSnSet_data <- convertToMSnset(exp3_OHT_ESR1$intensities_qPLEX1, metadata=exp3_OHT_ESR1$metadata_qPLEX1,
indExpData=c(7:16), Sequences=2, Accessions=6)
MSnset_P <- summarizeIntensities(MSnSet_data, sum, human_anno)
```

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