Package: MSstats (via r-universe)

November 1, 2026

Title Protein Significance Analysis in DDA, SRM and DIA for Label-free or Label-based Proteomics Experiments

Version 4.20.0

Date 2025-03-05

Description A set of tools for statistical relative protein significance analysis in DDA, SRM and DIA experiments.

License Artistic-2.0

Depends R (>= 4.0)

Imports MSstatsConvert, data.table, checkmate, MASS, htmltools, limma, lme4, preprocessCore, survival, utils, Rcpp, ggplot2 (>= 3.4.0), ggrepel, gplots, plotly, marray, stats, grDevices, graphics, methods, statmod, parallel, rlang

Suggests BiocStyle, knitr, rmarkdown, tinytest, covr, markdown, mockery, kableExtra

VignetteBuilder knitr

biocViews ImmunoOncology, MassSpectrometry, Proteomics, Software, Normalization, QualityControl, TimeCourse

LazyData true

URL http://msstats.org

BugReports https://groups.google.com/forum/#!forum/msstats

RoxygenNote 7.3.3

Encoding UTF-8

NeedsCompilation no

LinkingTo Rcpp, RcppArmadillo

Config/pak/sysreqs cmake make libicu-dev libuv1-dev libssl-dev

Repository Bioconductor 3.23

Date/Publication 2026-04-28 12:38:18 UTC

RemoteUrl https://github.com/bioc/MSstats

RemoteRef RELEASE_3_23

RemoteSha 87f0523a26249daa1a0543731ba44bbb9705b508

Contents

checkRepeatedDesign . . . 3
dataProcess . . . 3
dataProcessPlots . . . 7
DDARawData . . . 10
DDARawData.Skyline . . . 11
designSampleSize . . . 13
designSampleSizePlots . . . 15
DIARawData . . . 16
example_SDRF . . . 17
extractSDRF . . . 18
getProcessed . . . 19
getSamplesInfo . . . 20
getSelectedProteins . . . 20
groupComparison . . . 21
groupComparisonPlots . . . 23
groupComparisonQCPlots . . . 27
makePeptidesDictionary . . . 29
modelBasedQCPlots . . . 29
MSstatsContrastMatrix . . . 31
MSstatsGroupComparison . . . 32
MSstatsGroupComparisonOutput . . . 33
MSstatsGroupComparisonSingleProtein . . . 34
MSstatsHandleMissing . . . 35
MSstatsMergeFractions . . . 36
MSstatsNormalize . . . 36
MSstatsPrepareForDataProcess . . . 37
MSstatsPrepareForGroupComparison . . . 38
MSstatsPrepareForSummarization . . . 39
MSstatsQualityMetricsPlot . . . 40
MSstatsSelectFeatures . . . 41
MSstatsSummarizationOutput . . . 42
MSstatsSummarizeSingleLinear . . . 43
MSstatsSummarizeSingleTMP . . . 44
MSstatsSummarizeWithMultipleCores . . . 45
MSstatsSummarizeWithSingleCore . . . 46
quantification . . . 48
savePlot . . . 50
SDRFtoAnnotation . . . 50
SRMRawData . . . 51
theme_msstats . . . 53
validateAnnotation . . . 54

Index **55**

checkRepeatedDesign *Check if data represents repeated measurements design*

Description

Check if data represents repeated measurements design

Usage

```
checkRepeatedDesign(summarization_output)
```

Arguments

summarization_output	output of the dataProcess function

Details

This extracts information required by the group comparison workflow

Value

logical, TRUE if data represent repeated measurements design

Examples

```
QuantData1 <- dataProcess(SRMRawData, use_log_file = FALSE)
checkRepeatedDesign(QuantData1)
```

dataProcess *Process MS data: clean, normalize and summarize before differential analysis*

Description

Process MS data: clean, normalize and summarize before differential analysis

Usage

```
dataProcess(
  raw,
  logTrans = 2,
  normalization = "equalizeMedians",
  nameStandards = NULL,
  featureSubset = "topN",
  remove_uninformative_feature_outlier = FALSE,
  min_feature_count = 2,
  n_top_feature = 100,
  summaryMethod = "TMP",
  equalFeatureVar = TRUE,
  censoredInt = "NA",
  MBimpute = TRUE,
  remove50missing = FALSE,
  fix_missing = NULL,
  maxQuantileforCensored = 0.999,
  use_log_file = TRUE,
  append = FALSE,
  verbose = TRUE,
  log_file_path = NULL,
  numberOfCores = 1,
  aft_iterations = 90
)
```

Arguments

raw	name of the raw (input) data set.
logTrans	base of logarithm transformation: 2 (default) or 10.
normalization	normalization to remove systematic bias between MS runs. There are three different normalizations supported: 'equalizeMedians' (default) represents constant normalization (equalizing the medians) based on reference signals is performed. 'quantile' represents quantile normalization based on reference signals 'globalStandards' represents normalization with global standards proteins. If FALSE, no normalization is performed. See MSstats vignettes for recommendations on which normalization option to use.
nameStandards	optional vector of global standard peptide names. Required only for normalization with global standard peptides.
featureSubset	"topN" (default) uses top N features which has highest average of log-intensity across runs. "top3" uses top 3 features which have highest average of log-intensity across runs. "all" uses all features that the data set has (not recommended in DIA experiments). It needs the input for n_top_feature option. "highQuality" flags uninformative feature and outliers. See MSstats vignettes for recommendations on which feature selection option to use.
remove_uninformative_feature_outlier	optional. Only required if featureSubset = "highQuality". TRUE allows to remove 1) noisy features (flagged in the column feature_quality with "Uninforma-

tive"), 2) outliers (flagged in the column, is_outlier with TRUE, before run-level summarization. FALSE (default) uses all features and intensities for run-level summarization.

`min_feature_count`
: optional. Only required if featureSubset = "highQuality". Defines a minimum number of informative features a protein needs to be considered in the feature selection algorithm.

`n_top_feature`
: Specifies the number of top features to use in summarization (100 default). Only required if featureSubset = 'topN'. Default is 100, which means to use top 100 features. Smaller numbers can be set to improve processing times. This option is by default on at a high number (100) to improve processing times without affecting differential analysis.

`summaryMethod`
: "TMP" (default) means Tukey's median polish, which is robust estimation method. "linear" uses linear mixed model. If anomaly detection algorithm is performed, "linear" must be used.

`equalFeatureVar`
: only for summaryMethod = "linear". default is TRUE. Logical variable for whether the model should account for heterogeneous variation among intensities from different features. Default is TRUE, which assume equal variance among intensities from features. FALSE means that we cannot assume equal variance among intensities from features, then we will account for heterogeneous variation from different features.

`censoredInt`
: Missing values are censored or at random. 'NA' (default) assumes that all 'NA's in 'Intensity' column are censored. '0' uses zero intensities as censored intensity. In this case, NA intensities are missing at random. The output from Skyline should use '0'. Null assumes that all NA intensites are randomly missing.

`MBimpute`
: only for summaryMethod = "TMP" and censoredInt = 'NA' or '0'. TRUE (default) imputes missing values with 'NA' or '0' (depending on censoredInt option) by Accelerated failure model. If set to FALSE, no missing values are imputed. FALSE is appropriate only when missingness is assumed to be at random. See MSstats vignettes for recommendations on which imputation option to use.

`remove50missing`
: only for summaryMethod = "TMP". TRUE removes the proteins where every run has at least 50% missing values for each peptide. FALSE is default.

`fix_missing`
: Optional, same as the 'fix_missing' parameter in MSstatsConvert::MSstatsBalancedDesign function

`maxQuantileforCensored`
: Maximum quantile for deciding censored missing values, default is 0.999

`use_log_file`
: logical. If TRUE, information about data processing will be saved to a file.

`append`
: logical. If TRUE, information about data processing will be added to an existing log file.

`verbose`
: logical. If TRUE, information about data processing wil be printed to the console.

`log_file_path`
: character. Path to a file to which information about data processing will be saved. If not provided, such a file will be created automatically. If 'append = TRUE', has to be a valid path to a file.

`numberOfCores` Number of cores for parallel processing. When > 1, a logfile named 'MSstats_dataProcess_log_progress.lo is created to track progress. Only works for Linux & Mac OS. Default is 1.

`aft_iterations` Number of iterations for AFT model fitting. Default is 90.

Value

A list containing:

FeatureLevelData A data frame with feature-level information after processing. Columns include:

- **PROTEIN** Identifier for the protein associated with the feature.
- **PEPTIDE** Identifier for the peptide sequence.
- **TRANSITION** Identifier for the transition, typically representing a specific ion pair.
- **FEATURE** Unique identifier for the feature, which could be a combination of peptide and transition.
- **LABEL** Specifies the isotopic labeling of peptides, notably for SRM-based experiments. "L" indicates light-labeled peptides while "H" denotes heavy-labeled peptides.
- **GROUP** Experimental group identifier.
- **RUN** Identifier for the specific MS run.
- **SUBJECT** Subject identifier within the experimental group.
- **FRACTION** Fraction identifier if fractionation was performed.
- **originalRUN** Original run identifier before any processing.
- **censored** Logical indicator of whether the intensity value is considered missing or below limit of detection.
- **INTENSITY** Original intensity measurement of the feature in the given run.
- **ABUNDANCE** Processed abundance or intensity value after log-transformation and normalization.
- **newABUNDANCE** The ABUNDANCE column but includes imputed missing values. It is the column that is used for protein summarization.
- **predicted** Predicted intensity values for censored data, typically derived from a statistical model.

ProteinLevelData A data frame with run-level summarized information for each protein. Columns include:

- **RUN** Identifier for the specific MS run.
- **Protein** Identifier for the protein.
- **LogIntensities** Log-transformed intensities for the protein in each run.
- **originalRUN** Original run identifier before any processing.
- **GROUP** Experimental group identifier.
- **SUBJECT** Subject identifier within the experimental group.
- **TotalGroupMeasurements** Total number of feature measurements for the protein in the given group.
- **NumMeasuredFeatures** Number of features measured for the protein in the given run.
- **MissingPercentage** Percentage of missing feature values for the protein in the given run.
- **more50missing** Logical indicator of whether more than 50 percent of the features values are missing for the protein in the given run.
- **NumImputedFeature** Number of features for which values were imputed due to missing or censored data for the protein in the given run.

Examples

```
# Consider a raw data (i.e. SRMRawData) for a label-based SRM experiment from a yeast study
# with ten time points (T1-T10) of interests and three biological replicates.
# It is a time course experiment. The goal is to detect protein abundance changes
# across time points.
head(SRMRawData)
# Log2 transformation and normalization are applied (default)
QuantData<-dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
# Log10 transformation and normalization are applied
QuantData1<-dataProcess(SRMRawData, logTrans=10, use_log_file = FALSE)
head(QuantData1$FeatureLevelData)
# Log2 transformation and no normalization are applied
QuantData2<-dataProcess(SRMRawData,normalization=FALSE, use_log_file = FALSE)
head(QuantData2$FeatureLevelData)
```

---

dataProcessPlots *Visualization for explanatory data analysis*

Description

To illustrate the quantitative data after data-preprocessing and quality control of MS runs, dataProcessPlots takes the quantitative data from function (dataProcess) as input and automatically generate three types of figures in pdf files as output : (1) profile plot (specify "ProfilePlot" in option type), to identify the potential sources of variation for each protein; (2) quality control plot (specify "QCPlot" in option type), to evaluate the systematic bias between MS runs; (3) mean plot for conditions (specify "ConditionPlot" in option type), to illustrate mean and variability of each condition per protein.

Usage

```
dataProcessPlots(
  data,
  type,
  featureName = "Transition",
  ylimUp = FALSE,
  ylimDown = FALSE,
  scale = FALSE,
  interval = "CI",
  x.axis.size = 10,
  y.axis.size = 10,
  text.size = 4,
  text.angle = 0,
  legend.size = 7,
  dot.size.profile = 2,
  dot.size.condition = 3,
  width = 800,
```

```
  height = 600,
  which.Protein = "all",
  originalPlot = TRUE,
  summaryPlot = TRUE,
  save_condition_plot_result = FALSE,
  remove_uninformative_feature_outlier = FALSE,
  address = "",
  isPlotly = FALSE
)
```

Arguments

data	name of the (output of dataProcess function) data set.
type	choice of visualization. "ProfilePlot" represents profile plot of log intensities across MS runs. "QCPlot" represents quality control plot of log intensities across MS runs. "ConditionPlot" represents mean plot of log ratios (Light/Heavy) across conditions.
featureName	for "ProfilePlot" only, "Transition" (default) means printing feature legend in transition-level; "Peptide" means printing feature legend in peptide-level; "NA" means no feature legend printing.
ylimUp	upper limit for y-axis in the log scale. FALSE(Default) for Profile Plot and QC Plot use the upper limit as rounded off maximum of log2(intensities) after normalization + 3. FALSE(Default) for Condition Plot is maximum of log ratio + SD or CI.
ylimDown	lower limit for y-axis in the log scale. FALSE(Default) for Profile Plot and QC Plot is 0. FALSE(Default) for Condition Plot is minumum of log ratio - SD or CI.
scale	for "ConditionPlot" only, FALSE(default) means each conditional level is not scaled at x-axis according to its actual value (equal space at x-axis). TRUE means each conditional level is scaled at x-axis according to its actual value (unequal space at x-axis).
interval	for "ConditionPlot" only, "CI"(default) uses confidence interval with 0.95 significant level for the width of error bar. "SD" uses standard deviation for the width of error bar.
x.axis.size	size of x-axis labeling for "Run" in Profile Plot and QC Plot, and "Condition" in Condition Plot. Default is 10.
y.axis.size	size of y-axis labels. Default is 10.
text.size	size of labels represented each condition at the top of graph in Profile Plot and QC plot. Default is 4.
text.angle	angle of labels represented each condition at the top of graph in Profile Plot and QC plot or x-axis labeling in Condition plot. Default is 0.
legend.size	size of feature legend (transition-level or peptide-level) above graph in Profile Plot. Default is 7.
dot.size.profile	size of dots in profile plot. Default is 2.

`dot.size.condition`
: size of dots in condition plot. Default is 3.

`width`
: width of the saved file in pixels. Default is 800 pixels.

`height`
: height of the saved file in pixels. Default is 600 pixels.

`which.Protein`
: Protein list to draw plots. List can be names of Proteins or order numbers of Proteins from levels(data$FeatureLevelData$PROTEIN). Default is "all", which generates all plots for each protein. For QC plot, "allonly" will generate one QC plot with all proteins.

`originalPlot`
: TRUE(default) draws original profile plots.

`summaryPlot`
: TRUE(default) draws profile plots with summarization for run levels.

`save_condition_plot_result`
: TRUE saves the table with values using condition plots. Default is FALSE.

`remove_uninformative_feature_outlier`
: It only works after users used featureSubset="highQuality" in dataProcess. TRUE allows to remove 1) the features are flagged in the column, feature_quality="Uninformative" which are features with bad quality, 2) outliers that are flagged in the column, is_outlier=TRUE in Profile plots. FALSE (default) shows all features and intensities in profile plots.

`address`
: prefix for the filename that will store the results.

`isPlotly`
: Parameter to use Plotly or ggplot2. If set to TRUE, MSstats will save Plotly plots as HTML files. If set to FALSE MSstats will save ggplot2 plots as PDF files Default folder is the current working directory. The other assigned folder has to be existed under the current working directory. An output pdf file is automatically created with the default name of "ProfilePlot.pdf" or "QCplot.pdf" or "ConditionPlot.pdf" or "ConditionPlot_value.csv". The command address can help to specify where to store the file as well as how to modify the beginning of the file name. If address=FALSE, plot will be not saved as pdf file but showed in window.

Details

- Profile Plot : identify the potential sources of variation of each protein. QuantData$FeatureLevelData is used for plots. X-axis is run. Y-axis is log-intensities of transitions. Reference/endogenous signals are in the left/right panel. Line colors indicate peptides and line types indicate transitions. In summarization plots, gray dots and lines are the same as original profile plots with QuantData$FeatureLevelData. Dark dots and lines are for summarized intensities from QuantData$ProteinLevelData.
- QC Plot : illustrate the systematic bias between MS runs. After normalization, the reference signals for all proteins should be stable across MS runs. QuantData$FeatureLevelData is used for plots. X-axis is run. Y-axis is log-intensities of transition. Reference/endogenous signals are in the left/right panel. The pdf file contains (1) QC plot for all proteins and (2) QC plots for each protein separately.
- Condition Plot : illustrate the systematic difference between conditions. Summarized intensnties from QuantData$ProteinLevelData are used for plots. X-axis is condition. Y-axis is summarized log transformed intensity. If scale is TRUE, the levels of conditions is scaled according to its actual values at x-axis. Red points indicate the mean for each condition. If

interval is "CI", blue error bars indicate the confidence interval with 0.95 significant level for each condition. If interval is "SD", blue error bars indicate the standard deviation for each condition.The interval is not related with model-based analysis.

The input of this function is the quantitative data from function dataProcess.

Examples

```
# Consider quantitative data (i.e. QuantData) from a yeast study with ten time points of interests,
# three biological replicates, and no technical replicates which is a time-course experiment.
# The goal is to provide pre-analysis visualization by automatically generate two types of figures
# in two separate pdf files.
# Protein IDHC (gene name IDP2) is differentially expressed in time point 1 and time point 7,
# whereas, Protein PMG2 (gene name GPM2) is not.

QuantData<-dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
# Profile plot
dataProcessPlots(data=QuantData,type="ProfilePlot")
# Quality control plot
dataProcessPlots(data=QuantData,type="QCPlot")
# Quantification plot for conditions
dataProcessPlots(data=QuantData,type="ConditionPlot")
```

DDARawData *Example dataset from a label-free DDA, a controlled spike-in experiment.*

Description

This is a data set obtained from a published study (Mueller, et. al, 2007). A controlled spike-in experiment, where 6 proteins, (horse myoglobin, bovine carbonic anhydrase, horse Cytochrome C, chicken lysozyme, yeast alcohol dehydrogenase, rabbit aldolase A) were spiked into a complex background in known concentrations in a latin square design. The experiment contained 6 mixtures, and each mixture was analyzed in label-free LC-MS mode with 3 technical replicates (resulting in the total of 18 runs). Each protein was represented by 7-21 peptides, and each peptide was represented by 1-5 transition.

Usage

```
DDARawData
```

Format

data.frame

Details

The raw data (input data for MSstats) is required to contain variable of ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity. The variable names should be fixed.

If the information of one or more columns is not available for the original raw data, please retain the column variables and type in fixed value. For example, the original raw data does not contain the information of PrecursorCharge and ProductCharge, we retain the column PrecursorCharge and ProductCharge and then type in NA for all transitions in RawData.

Variable Intensity is required to be original signal without any log transformation and can be specified as the peak of height or the peak of area under curve.

Value

data.frame with the required format of MSstats.

Author(s)

Meena Choi, Olga Vitek.

Maintainer: Meena Choi (<mnchoi67@gmail.com>)

References

Meena Choi, Ching-Yun Chang, Timothy Clough, Daniel Broudy, Trevor Killeen, Brendan MacLean and Olga Vitek. "MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments" *Bioinformatics*, 30(17):1514-1526, 2014.

Timothy Clough, Safia Thaminy, Susanne Ragg, Ruedi Aebersold, Olga Vitek. "Statistical protein quantification and significance analysis in label-free LC-M experiments with complex designs" *BMC Bioinformatics*, 13:S16, 2012.

Mueller, L. N., Rinner, O., Schmidt, A., Letarte, S., Bodenmiller, B., Brusniak, M., Vitek, O., Aebersold, R., and Muller, M. (2007). SuperHirn - a novel tool for high resolution LC-MS based peptide/protein profiling. Proteomics, 7, 3470-3480. 3, 34

Examples

```
head(DDARawData)
```

`DDARawData.Skyline` *Example dataset from a label-free DDA, a controlled spike-in experiment, processed by Skyline.*

Description

This is a data set obtained from a published study (Mueller, et. al, 2007). A controlled spike-in experiment, where 6 proteins, (horse myoglobin, bovine carbonic anhydrase, horse Cytochrome C, chicken lysozyme, yeast alcohol dehydrogenase, rabbit aldolase A) were spiked into a complex background in known concentrations in a latin square design. The experiment contained 6 mixtures, and each mixture was analyzed in label-free LC-MS mode with 3 technical replicates (resulting in the total of 18 runs). Each protein was represented by 7-21 peptides, and each peptide was represented by 1-5 transition. Skyline is used for processing.

Usage

```
DDARawData.Skyline
```

Format

data.frame

Details

The raw data (input data for MSstats) is required to contain variable of ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity. The variable names should be fixed.

This is 'MSstats input' format from Skyline used by 'MSstats_report.skyr'. The column names, 'FileName' and 'Area', should be changed to 'Run' and 'Intensity'. There are two extra columns called 'StandardType' and 'Truncated'.'StandardType' column can be used for normalization='globalStandard' in `dataProcess`. 'Truncated' columns can be used to remove the truncated peaks with skylineReport=TRUE in `dataProcess`.

If the information of one or more columns is not available for the original raw data, please retain the column variables and type in fixed value. For example, the original raw data does not contain the information of PrecursorCharge and ProductCharge, we retain the column PrecursorCharge and ProductCharge and then type in NA for all transitions in RawData.

Variable Intensity is required to be original signal without any log transformation and can be specified as the peak of height or the peak of area under curve.

Value

data.frame with the required format of MSstats.

Author(s)

Meena Choi, Olga Vitek.

Maintainer: Meena Choi (<mnchoi67@gmail.com>)

References

Meena Choi, Ching-Yun Chang, Timothy Clough, Daniel Broudy, Trevor Killeen, Brendan MacLean and Olga Vitek. "MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments" *Bioinformatics*, 30(17):1514-1526, 2014.

Timothy Clough, Safia Thaminy, Susanne Ragg, Ruedi Aebersold, Olga Vitek. "Statistical protein quantification and significance analysis in label-free LC-M experiments with complex designs" *BMC Bioinformatics*, 13:S16, 2012.

Examples

```
head(DDARawData.Skyline)
```

`designSampleSize` *Planning future experimental designs of Selected Reaction Monitoring (SRM), Data-Dependent Acquisition (DDA or shotgun), and Data-Independent Acquisition (DIA or SWATH-MS) experiments in sample size calculation*

Description

Calculate sample size for future experiments of a Selected Reaction Monitoring (SRM), Data-Dependent Acquisition (DDA or shotgun), and Data-Independent Acquisition (DIA or SWATH-MS) experiment based on intensity-based linear model. Two options of the calculation: (1) number of biological replicates per condition, (2) power.

Usage

```
designSampleSize(
  data,
  desiredFC,
  FDR = 0.05,
  numSample = TRUE,
  power = 0.9,
  use_log_file = TRUE,
  append = FALSE,
  verbose = TRUE,
  log_file_path = NULL
)
```

Arguments

`data`	'FittedModel' in testing output from function groupComparison.
`desiredFC`	the range of a desired fold change which includes the lower and upper values of the desired fold change.
`FDR`	a pre-specified false discovery ratio (FDR) to control the overall false positive rate. Default is 0.05
`numSample`	minimal number of biological replicates per condition. TRUE represents you require to calculate the sample size for this category, else you should input the exact number of biological replicates.

power	a pre-specified statistical power which defined as the probability of detecting a true fold change. TRUE represent you require to calculate the power for this category, else you should input the average of power you expect. Default is 0.9
use_log_file	logical. If TRUE, information about data processing will be saved to a file.
append	logical. If TRUE, information about data processing will be added to an existing log file.
verbose	logical. If TRUE, information about data processing wil be printed to the console.
log_file_path	character. Path to a file to which information about data processing will be saved. If not provided, such a file will be created automatically. If 'append = TRUE', has to be a valid path to a file.

Details

The function fits the model and uses variance components to calculate sample size. The underlying model fitting with intensity-based linear model with technical MS run replication. Estimated sample size is rounded to 0 decimal. The function can only obtain either one of the categories of the sample size calculation (numSample, numPep, numTran, power) at the same time.

Value

data.frame - sample size calculation results including varibles: desiredFC, numSample, FDR, and power.

Author(s)

Meena Choi, Ching-Yun Chang, Olga Vitek.

Examples

```
# Consider quantitative data (i.e. QuantData) from yeast study.
# A time course study with ten time points of interests and three biological replicates.
QuantData <- dataProcess(SRMRawData)
head(QuantData$FeatureLevelData)
## based on multiple comparisons  (T1 vs T3; T1 vs T7; T1 vs T9)
comparison1<-matrix(c(-1,0,1,0,0,0,0,0,0,0),nrow=1)
comparison2<-matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
comparison3<-matrix(c(-1,0,0,0,0,0,0,0,1,0),nrow=1)
comparison<-rbind(comparison1,comparison2, comparison3)
row.names(comparison)<-c("T3-T1","T7-T1","T9-T1")
colnames(comparison)<-unique(QuantData$ProteinLevelData$GROUP)

testResultMultiComparisons<-groupComparison(contrast.matrix=comparison,data=QuantData)

## Calculate sample size for future experiments:
#(1) Minimal number of biological replicates per condition
designSampleSize(data=testResultMultiComparisons$FittedModel, numSample=TRUE,
                 desiredFC=c(1.25,1.75), FDR=0.05, power=0.8)
#(2) Power calculation
designSampleSize(data=testResultMultiComparisons$FittedModel, numSample=2,
```

```
desiredFC=c(1.25,1.75), FDR=0.05, power=TRUE)
```

---

designSampleSizePlots *Visualization for sample size calculation*

Description

To illustrate the relationship of desired fold change and the calculated minimal number sample size which are (1) number of biological replicates per condition, (2) number of peptides per protein, (3) number of transitions per peptide, and (4) power. The input is the result from function (designSampleSize.

Usage

```
designSampleSizePlots(data, isPlotly = FALSE)
```

Arguments

data	output from function designSampleSize.
isPlotly	Parameter to use Plotly or ggplot2. If set to TRUE, MSstats will save Plotly plots as HTML files. If set to FALSE MSstats will save ggplot2 plots as PDF files

Details

Data in the example is based on the results of sample size calculation from function designSampleSize

Value

Plot for estimated sample size with assigned variable.

Author(s)

Meena Choi, Ching-Yun Chang, Olga Vitek.

Examples

```
# Based on the results of sample size calculation from function designSampleSize,
# we generate a series of sample size plots for number of biological replicates, or peptides,
# or transitions or power plot.
QuantData<-dataProcess(SRMRawData)
head(QuantData$ProcessedData)
## based on multiple comparisons  (T1 vs T3; T1 vs T7; T1 vs T9)
comparison1<-matrix(c(-1,0,1,0,0,0,0,0,0,0),nrow=1)
comparison2<-matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
comparison3<-matrix(c(-1,0,0,0,0,0,0,0,1,0),nrow=1)
comparison<-rbind(comparison1,comparison2, comparison3)
row.names(comparison)<-c("T3-T1","T7-T1","T9-T1")
```

```
colnames(comparison)<-unique(QuantData$ProteinLevelData$GROUP)

testResultMultiComparisons<-groupComparison(contrast.matrix=comparison, data=QuantData)

# plot the calculated sample sizes for future experiments:
# (1) Minimal number of biological replicates per condition
result.sample<-designSampleSize(data=testResultMultiComparisons$FittedModel, numSample=TRUE,
                                desiredFC=c(1.25,1.75), FDR=0.05, power=0.8)
designSampleSizePlots(data=result.sample)
# (2) Power
result.power<-designSampleSize(data=testResultMultiComparisons$FittedModel, numSample=2,
                               desiredFC=c(1.25,1.75), FDR=0.05, power=TRUE)
designSampleSizePlots(data=result.power)
```

`DIARawData` *Example dataset from a label-free DIA, a group comparison study of S.Pyogenes.*

Description

This example dataset was obtained from a group comparison study of S. Pyogenes. Two conditions, S. Pyogenes with 0% and 10% of human plasma added (denoted Strep 0% and Strep 10%), were profiled in two replicates, in the label-free mode, with a SWATH-MS-enabled AB SCIEX TripleTOF 5600 System. The identification and quantification of spectral peaks was assisted by a spectral library, and was performed using OpenSWATH software (http: //proteomics.ethz.ch/openswath.html). For reasons of space, the example dataset only contains two proteins from this study. Protein FabG shows strong evidence of differential abundance, while protein Probable RNA helicase exp9 only shows moderate evidence of dif- ferential abundance between conditions.

Usage

```
DIARawData
```

Format

data.frame

Details

The raw data (input data for MSstats) is required to contain variable of ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity. The variable names should be fixed.

If the information of one or more columns is not available for the original raw data, please retain the column variables and type in fixed value. For example, the original raw data does not contain the information of PrecursorCharge and ProductCharge, we retain the column PrecursorCharge and ProductCharge and then type in NA for all transitions in RawData.

Variable Intensity is required to be original signal without any log transformation and can be specified as the peak of height or the peak of area under curve.

Value

data.frame with the required format of MSstats.

Author(s)

Meena Choi, Olga Vitek.

Maintainer: Meena Choi (<mnchoi67@gmail.com>)

Examples

```
head(DIARawData)
```

---

example_SDRF *Example SDRF.*

Description

An example SDRF file which is used to store metadata for MS-based protemics experiments.

Usage

```
example_SDRF
```

Format

data.frame

Details

An example SDRF file which is used to store metadata for MS-based protemics experiments.

Value

data.frame example of an SDRF file.

Author(s)

Mateusz Staniak, Devon Kohler, Olga Vitek.

Examples

```
head(example_SDRF)
```

extractSDRF *Extract experimental design from MSstats format into SDRF format*

Description

Extract experimental design from MSstats format into SDRF format

Usage

```
extractSDRF(
  data,
  run_name = "comment[data file]",
  condition_name = "characteristics[disease]",
  biological_replicate = "characteristics[biological replicate]",
  fraction = NULL,
  meta_data = NULL
)
```

Arguments

data	MSstats formatted data that is the output of a dedicated converter, such as 'MaxQtoMSstatsFormat', 'SkylinetoMSstatsFormat', ect.
run_name	Run column name in SDRF data
condition_name	Condition column name in SDRF data
biological_replicate	Biological replicate column name in SDRF data
fraction	Fraction column name in SDRF data (if applicable). Default is 'NULL'. If there are no fractions keep 'NULL'.
meta_data	A data.frame including any additional meta data for the SDRF file that is not included in MSstats. This meta data will be added into the final SDRF file. Please ensure the run names in the meta data matches the run names in the MSstats data.

Examples

```
mq_ev = data.table::fread(system.file("tinytest/raw_data/MaxQuant/mq_ev.csv",
                                      package = "MSstatsConvert"))
mq_pg = data.table::fread(system.file("tinytest/raw_data/MaxQuant/mq_pg.csv",
                                      package = "MSstatsConvert"))
annot = data.table::fread(system.file("tinytest/raw_data/MaxQuant/annotation.csv",
                                      package = "MSstatsConvert"))
maxq_imported = MaxQtoMSstatsFormat(mq_ev, annot, mq_pg, use_log_file = FALSE)
head(maxq_imported)

SDRF_file = extractSDRF(maxq_imported)
```

getProcessed *Get feature-level data to be used in the MSstatsSummarizationOutput function*

Description

Get feature-level data to be used in the MSstatsSummarizationOutput function

Usage

```
getProcessed(input)
```

Arguments

input	data.table processed by dataProcess subfunctions

Value

data.table processed by dataProcess subfunctions

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
input_all = MSstatsSelectFeatures(input, "all") # all features
input_5 = MSstatsSelectFeatures(data.table::copy(input),
"topN", top_n = 5) # top 5 features

proc1 = getProcessed(input_all)
proc2 = getProcessed(input_5)

proc1
proc2
```

getSamplesInfo *Get information about number of measurements for each group*

Description

Get information about number of measurements for each group

Usage

```
getSamplesInfo(summarization_output)
```

Arguments

summarization_output	output of the dataProcess function

Details

This function extracts information required to compute percentages of missing and imputed values in group comparison.

Value

data.table

Examples

```
QuantData <- dataProcess(DDARawData, use_log_file = FALSE)
samples_info <- getSamplesInfo(QuantData)
samples_info
```

getSelectedProteins *Get proteins based on names or integer IDs*

Description

Get proteins based on names or integer IDs

Usage

```
getSelectedProteins(chosen_proteins, all_proteins)
```

Arguments

chosen_proteins	protein names or integers IDs
all_proteins	all unique proteins

Value

character

`groupComparison` *Whole plot testing*

Description

Whole plot testing

Usage

```
groupComparison(
  contrast.matrix,
  data,
  save_fitted_models = TRUE,
  log_base = 2,
  use_log_file = TRUE,
  append = FALSE,
  verbose = TRUE,
  log_file_path = NULL,
  numberOfCores = 1
)
```

Arguments

`contrast.matrix`	comparison between conditions of interests.
`data`	name of the (output of dataProcess function) data set.
`save_fitted_models`	logical, if TRUE, fitted models will be added to the output.
`log_base`	base of the logarithm used in dataProcess.
`use_log_file`	logical. If TRUE, information about data processing will be saved to a file.
`append`	logical. If TRUE, information about data processing will be added to an existing log file.
`verbose`	logical. If TRUE, information about data processing wil be printed to the console.
`log_file_path`	character. Path to a file to which information about data processing will be saved. If not provided, such a file will be created automatically. If 'append = TRUE', has to be a valid path to a file.
`numberOfCores`	Number of cores for parallel processing. When > 1, a logfile named 'MSstats_groupComparison_log_prog is created to track progress. Only works for Linux & Mac OS. Default is 1.

Details

contrast.matrix : comparison of interest. Based on the levels of conditions, specify 1 or -1 to the conditions of interests and 0 otherwise. The levels of conditions are sorted alphabetically. Command levels(QuantData$FeatureLevelData$GROUP_ORIGINAL) can illustrate the actual order of the levels of conditions. The underlying model fitting functions are lm and lmer for the fixed effects model and mixed effects model, respectively. The input of this function is the quantitative data from function (dataProcess).

Value

A list with the following components:

ComparisonResult A 'data.frame' containing the results of the statistical testing for each protein. The columns include:

Protein The name of the protein for which the comparison is made.

Label The label of the comparison, typically derived from the 'contrast.matrix'.

log2FC The log2 fold change between the conditions being compared. The base of the logarithm is specified by the 'log_base' parameter.

- 'log2FC = Inf' or '-Inf': This occurs when one condition has entirely missing measurements for a protein, resulting in an undefined ratio.
- 'log2FC' is a numeric value but all other columns are 'NA': This occurs when there is only one sample per condition. Fold change can be estimated, but variance cannot be estimated, so no statistical testing is possible.

SE The standard error of the log2 fold change estimate. May be 'NA' when variance cannot be estimated (e.g., when only one sample per group).

Tvalue The t-statistic value for the comparison. May be 'NA' when variance cannot be estimated (e.g., when only one sample per group).

DF The degrees of freedom associated with the t-statistic. A value of 0 indicates that, although variance could be estimated, the total number of observations is too small to support hypothesis testing.

pvalue The p-value for the statistical test of the comparison. Applicable if degrees of freedom is greater than 0

adj.pvalue The adjusted p-value using the Benjamini-Hochberg method for controlling the false discovery rate.

issue Any issues encountered during the comparison. NA indicates no issues. "oneConditionMissing" occurs when data for one of the conditions being compared is entirely missing for a particular protein.

MissingPercentage The percentage of missing features for a given protein across all runs. This column is included only if missing values were imputed.

ImputationPercentage The percentage of features that were imputed for a given protein across all runs. This column is included only if missing values were imputed.

ModelQC A 'data.frame' containing quality control data used to fit models for group comparison. The columns include:

RUN Identifier for the specific MS run.

Protein Identifier for the protein.

ABUNDANCE Summarized intensity for the protein in a given run.

originalRUN Original run identifier before any processing.

GROUP Experimental group identifier.

SUBJECT Subject identifier within the experimental group.

TotalGroupMeasurements Total number of feature measurements for the protein in the given group.

NumMeasuredFeatures Number of features measured for the protein in the given run.

MissingPercentage Percentage of missing feature values for the protein in the given run.

more50missing Logical indicator of whether more than 50 percent of the features values are missing for the protein in the given run.

NumImputedFeature Number of features for which values were imputed due to missing or censored data for the protein in the given run.

residuals Contains the differences between the observed values and the values predicted by the fitted model.

fitted The predicted values obtained from the model for a protein measurement for a given run in the dataset.

FittedModel A list of fitted models for each protein. This is included only if 'save_fitted_models' is set to TRUE. Each element of the list corresponds to a protein and contains the fitted model object.

Examples

```
# Consider quantitative data (i.e. QuantData) from yeast study with ten time points of interests,
# three biological replicates, and no technical replicates.
# It is a time-course experiment and we attempt to compare differential abundance
# between time 1 and 7 in a set of targeted proteins.
# In this label-based SRM experiment, MSstats uses the fitted model with expanded scope of
# Biological replication.
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
levels(QuantData$ProteinLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
groups = levels(QuantData$ProteinLevelData$GROUP)
colnames(comparison) <- groups[order(as.numeric(groups))]
# Tests for differentially abundant proteins with models:
# label-based SRM experiment with expanded scope of biological replication.
testResultOneComparison <- groupComparison(contrast.matrix=comparison, data=QuantData,
                                           use_log_file = FALSE)
# table for result
testResultOneComparison$ComparisonResult
```

`groupComparisonPlots` *Visualization for model-based analysis and summarizing differentially abundant proteins*

Description

To summarize the results of log-fold changes and adjusted p-values for differentially abundant proteins, groupComparisonPlots takes testing results from function (groupComparison) as input and automatically generate three types of figures in pdf files as output : (1) volcano plot (specify "VolcanoPlot" in option type) for each comparison separately; (2) heatmap (specify "Heatmap" in option type) for multiple comparisons ; (3) comparison plot (specify "ComparisonPlot" in option type) for multiple comparisons per protein.

Usage

```
groupComparisonPlots(
  data,
  type,
  sig = 0.05,
  FCcutoff = FALSE,
  logBase.pvalue = 10,
  ylimUp = FALSE,
  ylimDown = FALSE,
  xlimUp = FALSE,
  x.axis.size = 10,
  y.axis.size = 10,
  dot.size = 3,
  text.size = 4,
  text.angle = 0,
  legend.size = 13,
  ProteinName = TRUE,
  colorkey = TRUE,
  numProtein = 100,
  clustering = "both",
  width = 800,
  height = 600,
  which.Comparison = "all",
  which.Protein = "all",
  address = "",
  isPlotly = FALSE
)
```

Arguments

data	'ComparisonResult' in testing output from function groupComparison.
type	choice of visualization. "VolcanoPlot" represents volcano plot of log fold changes and adjusted p-values for each comparison separately. "Heatmap" represents heatmap of adjusted p-values for multiple comparisons. "ComparisonPlot" represents comparison plot of log fold changes for multiple comparisons per protein.
sig	FDR cutoff for the adjusted p-values in heatmap and volcano plot. level of significance for comparison plot. 100(1-sig)% confidence interval will be drawn. sig=0.05 is default.

FCcutoff	for volcano plot or heatmap, whether involve fold change cutoff or not. FALSE (default) means no fold change cutoff is applied for significance analysis. FC-cutoff = specific value means specific fold change cutoff is applied.
logBase.pvalue	for volcano plot or heatmap, (-) logarithm transformation of adjusted p-value with base 2 or 10(default).
ylimUp	for all three plots, upper limit for y-axis. FALSE (default) for volcano plot/heatmap use maximum of -log2 (adjusted p-value) or -log10 (adjusted p-value). FALSE (default) for comparison plot uses maximum of log-fold change + CI.
ylimDown	for all three plots, lower limit for y-axis. FALSE (default) for volcano plot/heatmap use minimum of -log2 (adjusted p-value) or -log10 (adjusted p-value). FALSE (default) for comparison plot uses minimum of log-fold change - CI.
xlimUp	for Volcano plot, the limit for x-axis. FALSE (default) for use maximum for absolute value of log-fold change or 3 as default if maximum for absolute value of log-fold change is less than 3.
x.axis.size	size of axes labels, e.g. name of the comparisons in heatmap, and in comparison plot. Default is 10.
y.axis.size	size of axes labels, e.g. name of targeted proteins in heatmap. Default is 10.
dot.size	size of dots in volcano plot and comparison plot. Default is 3.
text.size	size of ProteinName label in the graph for Volcano Plot. Default is 4.
text.angle	angle of x-axis labels represented each comparison at the bottom of graph in comparison plot. Default is 0.
legend.size	size of legend for color at the bottom of volcano plot. Default is 7.
ProteinName	for volcano plot only, whether display protein names or not. TRUE (default) means protein names, which are significant, are displayed next to the points. FALSE means no protein names are displayed.
colorkey	TRUE(default) shows colorkey.
numProtein	For ggplot2: The number of proteins which will be presented in each heatmap. Default is 100. Maximum possible number of protein for one heatmap is 180. For Plotly: use this parameter to adjust the number of proteins to be displayed on the heatmap
clustering	Determines how to order proteins and comparisons. Hierarchical cluster analysis with Ward method(minimum variance) is performed. 'protein' means that protein dendrogram is computed and reordered based on protein means (the order of row is changed). 'comparison' means comparison dendrogram is computed and reordered based on comparison means (the order of comparison is changed). 'both' means to reorder both protein and comparison. Default is 'protein'.
width	width of the saved file in pixels. Default is 800.
height	height of the saved file in pixels. Default is 600.
which.Comparison	list of comparisons to draw plots. List can be labels of comparisons or order numbers of comparisons from levels(data$Label), such as levels(testResultMultiComparisons$Compariso Default is "all", which generates all plots for each protein.

`which.Protein` Protein list to draw comparison plots. List can be names of Proteins or order numbers of Proteins from levels(testResultMultiComparisons$ComparisonResult$Protein). Default is "all", which generates all comparison plots for each protein.

`address` the name of folder that will store the results. Default folder is the current working directory. The other assigned folder has to be existed under the current working directory. An output pdf file is automatically created with the default name of "VolcanoPlot.pdf" or "Heatmap.pdf" or "ComparisonPlot.pdf". The command address can help to specify where to store the file as well as how to modify the beginning of the file name. If address=FALSE, plot will be not saved as pdf file but showed in window.

`isPlotly` This parameter is for MSstatsShiny application for plotly render, this cannot be used for saving PDF files as plotly do not have suppprt for PDFs currently. address and isPlotly cannot be set as TRUE at the same time.

Details

- Volcano plot : illustrate actual log-fold changes and adjusted p-values for each comparison separately with all proteins. The x-axis is the log fold change. The base of logarithm transformation is the same as specified in "logTrans" from `dataProcess`. The y-axis is the negative log2 or log10 adjusted p-values. The horizontal dashed line represents the FDR cutoff. The points below the FDR cutoff line are non-significantly abundant proteins (colored in black). The points above the FDR cutoff line are significantly abundant proteins (colored in red/blue for up-/down-regulated). If fold change cutoff is specified (FCcutoff = specific value), the points above the FDR cutoff line but within the FC cutoff line are non-significantly abundant proteins (colored in black)/
- Heatmap : illustrate up-/down-regulated proteins for multiple comparisons with all proteins. Each column represents each comparison of interest. Each row represents each protein. Color red/blue represents proteins in that specific comparison are significantly up-regulated/down-regulated proteins with FDR cutoff and/or FC cutoff. The color scheme shows the evidences of significance. The darker color it is, the stronger evidence of significance it has. Color gold represents proteins are not significantly different in abundance.
- Comparison plot : illustrate log-fold change and its variation of multiple comparisons for single protein. X-axis is comparison of interest. Y-axis is the log fold change. The red points are the estimated log fold change from the model. The blue error bars are the confidence interval with 0.95 significant level for log fold change. This interval is only based on the standard error, which is estimated from the model.

Examples

```
QuantData<-dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
## based on multiple comparisons  (T1 vs T3; T1 vs T7; T1 vs T9)
comparison1<-matrix(c(-1,0,1,0,0,0,0,0,0,0),nrow=1)
comparison2<-matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
comparison3<-matrix(c(-1,0,0,0,0,0,0,0,1,0),nrow=1)
comparison<-rbind(comparison1,comparison2, comparison3)
row.names(comparison)<-c("T3-T1","T7-T1","T9-T1")
groups = levels(QuantData$ProteinLevelData$GROUP)
colnames(comparison) <- groups[order(as.numeric(groups))]
```

```
testResultMultiComparisons<-groupComparison(contrast.matrix=comparison,
data=QuantData,
use_log_file = FALSE)
testResultMultiComparisons$ComparisonResult
# Volcano plot with FDR cutoff = 0.05 and no FC cutoff
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="VolcanoPlot",
logBase.pvalue=2, address="Ex1_")
# Volcano plot with FDR cutoff = 0.05, FC cutoff = 70, upper y-axis limit = 100,
# and no protein name displayed
# FCcutoff=70 is for demonstration purpose
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="VolcanoPlot",
FCcutoff=70, logBase.pvalue=2, ylimUp=100, ProteinName=FALSE,address="Ex2_")
# Heatmap with FDR cutoff = 0.05
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="Heatmap",
logBase.pvalue=2, address="Ex1_")
# Heatmap with FDR cutoff = 0.05 and FC cutoff = 70
# FCcutoff=70 is for demonstration purpose
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="Heatmap",
FCcutoff=70, logBase.pvalue=2, address="Ex2_")
# Comparison Plot
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="ComparisonPlot",
address="Ex1_")
# Comparison Plot
groupComparisonPlots(data=testResultMultiComparisons$ComparisonResult, type="ComparisonPlot",
ylimUp=8, ylimDown=-1, address="Ex2_")
```

`groupComparisonQCPlots` *Visualization for model-based quality control in fitting model*

Description

To check the assumption of linear model for whole plot inference, groupComparisonQCPlots takes the results after fitting models from function (`groupComparison`) as input and automatically generate two types of figures in pdf files as output: (1) normal quantile-quantile plot (specify "QQPlot" in option type) for checking normally distributed errors.; (2) residual plot (specify "ResidualPlot" in option type).

Usage

```
groupComparisonQCPlots(
  data,
  type,
  axis.size = 10,
  dot.size = 3,
  width = 10,
  height = 10,
  which.Protein = "all",
```

```
  address = ""
)
```

Arguments

data	output from function groupComparison.
type	choice of visualization. "QQPlots" represents normal quantile-quantile plot for each protein after fitting models. "ResidualPlots" represents a plot of residuals versus fitted values for each protein in the dataset.
axis.size	size of axes labels. Default is 10.
dot.size	size of points in the graph for residual plots and QQ plots. Default is 3.
width	width of the saved file. Default is 10.
height	height of the saved file. Default is 10.
which.Protein	Protein list to draw plots. List can be names of Proteins or order numbers of Proteins from levels(testResultOneComparison$ComparisonResult$Protein). Default is "all", which generates all plots for each protein.
address	name that will serve as a prefix to the name of output file.

Details

Results based on statistical models for whole plot level inference are accurate as long as the assumptions of the model are met. The model assumes that the measurement errors are normally distributed with mean 0 and constant variance. The assumption of a constant variance can be checked by examining the residuals from the model.

- QQPlots : a normal quantile-quantile plot for each protein is generated in order to check whether the errors are well approximated by a normal distribution. If points fall approximately along a straight line, then the assumption is appropriate for that protein. Only large deviations from the line are problematic.
- ResidualPlots : The plots of residuals against predicted(fitted) values. If it shows a random scatter, then the assumption is appropriate.

Value

produce a pdf file

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
levels(QuantData$FeatureLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
colnames(comparison) <- unique(QuantData$ProteinLevelData$GROUP)
# Tests for differentially abundant proteins with models:
# label-based SRM experiment with expanded scope of biological replication.
testResultOneComparison <- groupComparison(contrast.matrix=comparison, data=QuantData,
use_log_file = FALSE)
```

```
# normal quantile-quantile plots
groupComparisonQCPlots(data=testResultOneComparison, type="QQPlots", address="")
# residual plots
groupComparisonQCPlots(data=testResultOneComparison, type="ResidualPlots", address="")
```

`makePeptidesDictionary` *Prepare a peptides dictionary for global standards normalization*

Description

Prepare a peptides dictionary for global standards normalization

Usage

```
makePeptidesDictionary(input, normalization)
```

Arguments

`input`	'data.table' in MSstats standard format
`normalization`	normalization method

Details

This function extracts information required to perform normalization with global standards. It is useful for running the summarization workflow outside of the dataProcess function.

Examples

```
input = data.table::as.data.table(DDARawData)
peptides_dict = makePeptidesDictionary(input, "GLOBALSTANDARDS")
head(peptides_dict) # ready to be passed to the MSstatsNormalize function
```

`modelBasedQCPlots` *Visualization for model-based quality control in fitting model*

Description

To check the assumption of linear model for whole plot inference, modelBasedQCPlots takes the results after fitting models from function (`groupComparison`) as input and automatically generate two types of figures in pdf files as output: (1) normal quantile-quantile plot (specify "QQPlot" in option type) for checking normally distributed errors.; (2) residual plot (specify "ResidualPlot" in option type).

Usage

```
modelBasedQCPlots(
  data,
  type,
  axis.size = 10,
  dot.size = 3,
  width = 10,
  height = 10,
  which.Protein = "all",
  address = "",
  displayDeprecationMessage = TRUE
)
```

Arguments

data	output from function groupComparison.
type	choice of visualization. "QQPlots" represents normal quantile-quantile plot for each protein after fitting models. "ResidualPlots" represents a plot of residuals versus fitted values for each protein in the dataset.
axis.size	size of axes labels. Default is 10.
dot.size	size of points in the graph for residual plots and QQ plots. Default is 3.
width	width of the saved file. Default is 10.
height	height of the saved file. Default is 10.
which.Protein	Protein list to draw plots. List can be names of Proteins or order numbers of Proteins from levels(testResultOneComparison$ComparisonResult$Protein). Default is "all", which generates all plots for each protein.
address	name that will serve as a prefix to the name of output file.

Details

Results based on statistical models for whole plot level inference are accurate as long as the assumptions of the model are met. The model assumes that the measurement errors are normally distributed with mean 0 and constant variance. The assumption of a constant variance can be checked by examining the residuals from the model.

- QQPlots : a normal quantile-quantile plot for each protein is generated in order to check whether the errors are well approximated by a normal distribution. If points fall approximately along a straight line, then the assumption is appropriate for that protein. Only large deviations from the line are problematic.
- ResidualPlots : The plots of residuals against predicted(fitted) values. If it shows a random scatter, then the assumption is appropriate.

Value

produce a pdf file

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
levels(QuantData$FeatureLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
colnames(comparison) <- unique(QuantData$ProteinLevelData$GROUP)
# Tests for differentially abundant proteins with models:
# label-based SRM experiment with expanded scope of biological replication.
testResultOneComparison <- groupComparison(contrast.matrix=comparison, data=QuantData,
use_log_file = FALSE)
# normal quantile-quantile plots
modelBasedQCPlots(data=testResultOneComparison, type="QQPlots", address="")
# residual plots
modelBasedQCPlots(data=testResultOneComparison, type="ResidualPlots", address="")
```

`MSstatsContrastMatrix` *Create a contrast matrix for groupComparison function*

Description

Create a contrast matrix for groupComparison function

Usage

```
MSstatsContrastMatrix(contrasts, conditions, labels = NULL)
```

Arguments

`contrasts`	One of the following: i) list of lists. Each sub-list consists of two vectors that name conditions that will be compared. See the details section for more information ii) matrix. In this case, it's correctness will be checked iii) "pairwise". In this case, pairwise comparison matrix will be generated iv) data.frame. In this case, input will be converted to matrix
`conditions`	unique condition labels
`labels`	labels for contrasts (row.names of the contrast matrix)

`MSstatsGroupComparison` *Group comparison*

Description

Group comparison

Usage

```
MSstatsGroupComparison(
  summarized_list,
  contrast_matrix,
  save_fitted_models,
  repeated,
  samples_info,
  numberOfCores = 1
)
```

Arguments

`summarized_list`	output of MSstatsPrepareForGroupComparison
`contrast_matrix`	contrast matrix
`save_fitted_models`	if TRUE, fitted models will be included in the output
`repeated`	logical, output of checkRepeatedDesign function
`samples_info`	data.table, output of getSamplesInfo function
`numberOfCores`	Number of cores for parallel processing. When > 1, a logfile named 'MSstats_groupComparison_log_prog is created to track progress. Only works for Linux & Mac OS.

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
group_comparison_input = MSstatsPrepareForGroupComparison(QuantData)
levels(QuantData$ProteinLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
groups = levels(QuantData$ProteinLevelData$GROUP)
colnames(comparison) <- groups[order(as.numeric(groups))]
samples_info = getSamplesInfo(QuantData)
repeated = checkRepeatedDesign(QuantData)
group_comparison = MSstatsGroupComparison(group_comparison_input, comparison,
                                          FALSE, repeated, samples_info)
length(group_comparison) # list of length equal to number of proteins
group_comparison[[1]][[1]] # data used to fit linear model
```

```
group_comparison[[1]][[2]] # comparison result
group_comparison[[2]][[3]] # NULL, because we set save_fitted_models to FALSE
```

`MSstatsGroupComparisonOutput` *Create output of group comparison based on results for individual proteins*

Description

Create output of group comparison based on results for individual proteins

Usage

```
MSstatsGroupComparisonOutput(input, summarization_output, log_base = 2)
```

Arguments

`input`	output of MSstatsGroupComparison function
`summarization_output`	output of dataProcess function
`log_base`	base of the logarithm used in fold-change calculation

Value

list, same as the output of 'groupComparison'

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
group_comparison_input = MSstatsPrepareForGroupComparison(QuantData)
levels(QuantData$ProteinLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
groups = levels(QuantData$ProteinLevelData$GROUP)
colnames(comparison) <- groups[order(as.numeric(groups))]
samples_info = getSamplesInfo(QuantData)
repeated = checkRepeatedDesign(QuantData)
group_comparison = MSstatsGroupComparison(group_comparison_input, comparison,
                                          FALSE, repeated, samples_info)
group_comparison_final = MSstatsGroupComparisonOutput(group_comparison,
                                                      QuantData)
group_comparison_final[["ComparisonResult"]]
```

MSstatsGroupComparisonSingleProtein
Group comparison for a single protein

Description

Group comparison for a single protein

Usage

```
MSstatsGroupComparisonSingleProtein(
  single_protein,
  contrast_matrix,
  repeated,
  groups,
  samples_info,
  save_fitted_models,
  has_imputed
)
```

Arguments

single_protein	data.table with summarized data for a single protein
contrast_matrix	contrast matrix
repeated	if TRUE, repeated measurements will be modeled
groups	unique labels of experimental conditions
samples_info	number of runs per group
save_fitted_models	if TRUE, fitted model will be saved. If not, it will be replaced with NULL
has_imputed	TRUE if missing values have been imputed

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
group_comparison_input <- MSstatsPrepareForGroupComparison(QuantData)
levels(QuantData$ProteinLevelData$GROUP)
comparison <- matrix(c(-1,0,0,0,0,0,1,0,0,0),nrow=1)
row.names(comparison) <- "T7-T1"
groups = levels(QuantData$ProteinLevelData$GROUP)
colnames(comparison) <- groups[order(as.numeric(groups))]
samples_info <- getSamplesInfo(QuantData)
repeated <- checkRepeatedDesign(QuantData)
single_output <- MSstatsGroupComparisonSingleProtein(
  group_comparison_input[[1]], comparison, repeated, groups, samples_info,
  FALSE, TRUE)
single_output # same as a single element of MSstatsGroupComparison output
```

`MSstatsHandleMissing` *Handle censored missing values*

Description

Handle censored missing values

Usage

```
MSstatsHandleMissing(
  input,
  summary_method,
  impute,
  missing_symbol,
  censored_cutoff
)
```

Arguments

`input`	'data.table' in MSstats data format
`summary_method`	summarization method ('summaryMethod' parameter to 'dataProcess')
`impute`	if TRUE, missing values are supposed to be imputed ('MBimpute' parameter to 'dataProcess')
`missing_symbol`	'censoredInt' parameter to 'dataProcess'
`censored_cutoff`	'maxQuantileforCensored' parameter to 'dataProcess'

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
head(input)
```

MSstatsMergeFractions *Re-format the data before feature selection*

Description

Re-format the data before feature selection

Usage

```
MSstatsMergeFractions(input)
```

Arguments

input	'data.table' in MSstats format

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
head(input)
```

MSstatsNormalize *Normalize MS data*

Description

Normalize MS data

Usage

```
MSstatsNormalize(
  input,
  normalization_method,
  peptides_dict = NULL,
  standards = NULL
)
```

Arguments

input	data.table in MSstats format
normalization_method	name of a chosen normalization method: "NONE" or "FALSE" for no normalization, "EQUALIZEMEDIANS" for median normalization, "QUANTILE" normalization for quantile normalization from ‘preprocessCore‘ package, "GLOBALSTANDARDS" for normalization based on selected peptides or proteins.
peptides_dict	‘data.table‘ of names of peptides and their corresponding features.
standards	character vector with names of standards, required if "GLOBALSTANDARDS" method was selected.

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS") # median normalization
head(input)
```

MSstatsPrepareForDataProcess

Prepare data for processing by ‘dataProcess‘ function

Description

Prepare data for processing by ‘dataProcess‘ function

Usage

```
MSstatsPrepareForDataProcess(input, log_base, fix_missing)
```

Arguments

input	‘data.table‘ in MSstats format
log_base	base of the logarithm to transform intensities
fix_missing	str, optional. Defaults to NULL, which means no action. If not NULL, must be one of the options: "zero_to_na" or "na_to_zero". If "zero_to_na", Intensity values equal exactly to 0 will be converted to NA. If "na_to_zero", missing values will be replaced by zeros.

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
head(input)
```

MSstatsPrepareForGroupComparison

Prepare output for dataProcess for group comparison

Description

Prepare output for dataProcess for group comparison

Usage

```
MSstatsPrepareForGroupComparison(summarization_output)
```

Arguments

summarization_output	output of dataProcess

Value

list of run-level data for each protein in the input. This list has a "has_imputed" attribute that indicates if missing values were imputed in the input dataset.

Examples

```
QuantData <- dataProcess(SRMRawData, use_log_file = FALSE)
group_comparison_input = MSstatsPrepareForGroupComparison(QuantData)
length(group_comparison_input) # list of length equal to number of proteins
# in protein-level data of QuantData
head(group_comparison_input[[1]])
```

MSstatsPrepareForSummarization
Prepare feature-level data for protein-level summarization

Description

Prepare feature-level data for protein-level summarization

Usage

```
MSstatsPrepareForSummarization(
  input,
  method,
  impute,
  censored_symbol,
  remove_uninformative_feature_outlier
)
```

Arguments

input	feature-level data processed by dataProcess subfunctions
method	summarization method - ‘summaryMethod‘ parameter of the dataProcess function
impute	if TRUE, censored missing values will be imputed - ‘MBimpute‘ parameter of the dataProcess function
censored_symbol	censored missing value indicator - ‘censoredInt‘ parameter of the dataProcess function
remove_uninformative_feature_outlier	if TRUE, features labeled as outlier of uninformative by the MSstatsSelectFeatures function will not be used in summarization

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
head(input)
```

MSstatsQualityMetricsPlot *Plot quality metrics from converter output*

Description

Visualizes a quality metric column from the output of MSstats converter functions against run order for a single protein. Each PeptideSequence + PrecursorCharge combination is drawn as a distinct coloured line, mirroring the feature-level view in `dataProcessPlots`.

Usage

```
MSstatsQualityMetricsPlot(
  input,
  metric = "AnomalyScores",
  which.Protein,
  address = FALSE,
  isPlotly = FALSE
)
```

Arguments

`input`	data.frame or data.table returned by an MSstatsConvert converter function (e.g. `SpectronauttoMSstatsFormat`).
`metric`	character, name of the column to plot on the y-axis. Defaults to `"AnomalyScores"`. Must be a column of `input`.
`which.Protein`	character, name of the protein to plot. Required.
`address`	prefix for the filename used when saving the plot. If `FALSE` (default), the plot is returned without saving. When `isPlotly = FALSE` a PDF is saved; when `isPlotly = TRUE` an HTML file is saved.
`isPlotly`	logical. If `TRUE` returns an interactive `plotly` object (and saves as HTML when `address` is provided). If `FALSE` (default) returns a `ggplot` object.

Details

The x-axis order is determined by the factor levels of the `Run` column. When `runOrder` is passed to the converter the `Run` column is automatically set to an ordered factor; otherwise the runs appear in alphabetical order.

Metric values are averaged across fragment ions within each PeptideSequence + PrecursorCharge + Run combination before plotting, so each precursor contributes exactly one point per run.

Value

A `ggplot` object, or a `plotly` object when `isPlotly = TRUE`.

Examples

```
## Not run:
result <- SpectronauttoMSstatsFormat(
  input, calculateAnomalyScores = TRUE,
  anomalyModelFeatures = c("FGShapeQualityScoreMS2", "EGDeltaRT"),
  anomalyModelFeatureTemporal = c("mean_decrease", "dispersion_increase"),
  runOrder = my_run_order
)
MSstatsQualityMetricsPlot(result, which.Protein = "ProteinA")
MSstatsQualityMetricsPlot(result, metric = "EGDeltaRT",
                          which.Protein = "ProteinA", isPlotly = TRUE)

## End(Not run)
```

MSstatsSelectFeatures *Feature selection before feature-level data summarization*

Description

Feature selection before feature-level data summarization

Usage

```
MSstatsSelectFeatures(input, method, top_n = 3, min_feature_count = 2)
```

Arguments

input	data.table
method	"all" / "highQuality", "topN"
top_n	number of features to use for "topN" method
min_feature_count	number of quality features for "highQuality" method

Value

data.table

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
```

```
input_all = MSstatsSelectFeatures(input, "all") # all features
input_5 = MSstatsSelectFeatures(data.table::copy(input), "topN", top_n = 5) # top 5 features
input_informative = MSstatsSelectFeatures(input, "highQuality") # feature selection

head(input_all)
head(input_5)
head(input_informative)
```

`MSstatsSummarizationOutput` *Post-processing output from MSstats summarization*

Description

Post-processing output from MSstats summarization

Usage

```
MSstatsSummarizationOutput(
  input,
  summarized,
  processed,
  method,
  impute,
  censored_symbol
)
```

Arguments

`input`	'data.table' in MSstats format
`summarized`	output of the 'MSstatsSummarizeWithSingleCore' function
`processed`	output of MSstatsSelectFeatures
`method`	name of the summarization method ('summaryMethod' parameter to 'dataProcess')
`impute`	if TRUE, censored missing values were imputed ('MBimpute' parameter to 'dataProcess')
`censored_symbol`	censored missing value indicator ('censoredInt' parameter to 'dataProcess')

Value

A list with the following elements:

`FeatureLevelData`	Feature-level data after processing.
`ProteinLevelData`	Protein-level (summarized) data.
`SummaryMethod`	String: name of the summarization method used.

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
input = MSstatsSelectFeatures(input, "all")
processed = getProcessed(input)
input = MSstatsPrepareForSummarization(input, method, impute, cens, FALSE)
summarized = MSstatsSummarizeWithSingleCore(input, method, impute, cens, FALSE, TRUE, 100)
output = output = MSstatsSummarizationOutput(input, summarized, processed,
method, impute, cens)
```

MSstatsSummarizeSingleLinear

Linear model-based summarization for a single protein

Description

Linear model-based summarization for a single protein

Usage

```
MSstatsSummarizeSingleLinear(
  single_protein,
  impute,
  censored_symbol,
  remove50missing,
  aft_iterations = 90,
  equal_variances = TRUE
)
```

Arguments

single_protein	feature-level data for a single protein
impute	boolean for whether imputation should be performed
censored_symbol	Character string indicating how censored values are represented
remove50missing	if TRUE, proteins with more than 50% missing values in each run are removed
aft_iterations	number of iterations for AFT model fitting
equal_variances	if TRUE, observation are assumed to be homoskedastic

Value

list with protein-level data

Examples

```
raw = DDARawData
method = "linear"
cens = NULL
impute = FALSE
# currently, MSstats only supports MBimpute = FALSE for linear summarization
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
input = MSstatsSelectFeatures(input, "all")
input = MSstatsPrepareForSummarization(input, method, impute, cens, FALSE)
input_split = split(input, input$PROTEIN)
single_protein_summary = MSstatsSummarizeSingleLinear(input_split[[1]], impute, cens, TRUE, 100)
head(single_protein_summary[[1]])
```

MSstatsSummarizeSingleTMP

Tukey Median Polish summarization for a single protein

Description

Tukey Median Polish summarization for a single protein

Usage

```
MSstatsSummarizeSingleTMP(
  single_protein,
  impute,
  censored_symbol,
  remove50missing,
  aft_iterations = 90
)
```

Arguments

single_protein	feature-level data for a single protein
impute	only for summaryMethod = "TMP" and censoredInt = 'NA' or '0'. TRUE (default) imputes 'NA' or '0' (depending on censoredInt option) by Accelated failure model. FALSE uses the values assigned by cutoffCensored

censored_symbol
: Missing values are censored or at random. 'NA' (default) assumes that all 'NA's in 'Intensity' column are censored. '0' uses zero intensities as censored intensity. In this case, NA intensities are missing at random. The output from Skyline should use '0'. Null assumes that all NA intensites are randomly missing.

remove50missing
: only for summaryMethod = "TMP". TRUE removes the proteins where every run has at least 50% missing values for each peptide. FALSE is default.

aft_iterations
: number of iterations for AFT model fitting

Value

list of two data.tables: one with fitted survival model, the other with protein-level data

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
# currently, MSstats only supports MBimpute = FALSE for linear summarization
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
input = MSstatsSelectFeatures(input, "all")
input = MSstatsPrepareForSummarization(input, method, impute, cens, FALSE)
input_split = split(input, input$PROTEIN)
single_protein_summary = MSstatsSummarizeSingleTMP(input_split[[1]],
                                                   impute, cens, FALSE, 100)
head(single_protein_summary[[1]])
```

MSstatsSummarizeWithMultipleCores

Feature-level data summarization with multiple cores

Description

Feature-level data summarization with multiple cores

Usage

```
MSstatsSummarizeWithMultipleCores(
  input,
  method,
  impute,
```

```
  censored_symbol,
  remove50missing,
  equal_variance,
  numberOfCores = 1,
  aft_iterations = 90
)
```

Arguments

input	feature-level data processed by dataProcess subfunctions
method	summarization method: "linear" or "TMP"
impute	only for summaryMethod = "TMP" and censoredInt = 'NA' or '0'. TRUE (default) imputes 'NA' or '0' (depending on censoredInt option) by Accelated failure model. FALSE uses the values assigned by cutoffCensored
censored_symbol	Missing values are censored or at random. 'NA' (default) assumes that all 'NA's in 'Intensity' column are censored. '0' uses zero intensities as censored intensity. In this case, NA intensities are missing at random. The output from Skyline should use '0'. Null assumes that all NA intensites are randomly missing.
remove50missing	only for summaryMethod = "TMP". TRUE removes the proteins where every run has at least 50% missing values for each peptide. FALSE is default.
equal_variance	only for summaryMethod = "linear". Default is TRUE. Logical variable for whether the model should account for heterogeneous variation among intensities from different features. Default is TRUE, which assume equal variance among intensities from features. FALSE means that we cannot assume equal variance among intensities from features, then we will account for heterogeneous variation from different features.
numberOfCores	Number of cores for parallel processing. When > 1, a logfile named 'MSstats_dataProcess_log_progress.lo is created to track progress. Only works for Linux & Mac OS. Default is 1.
aft_iterations	Number of iterations for AFT model fitting. Default is 90.

Value

list of length one with run-level data.

`MSstatsSummarizeWithSingleCore`
Feature-level data summarization with 1 core

Description

Feature-level data summarization with 1 core

Usage

```
MSstatsSummarizeWithSingleCore(
  input,
  method,
  impute,
  censored_symbol,
  remove50missing,
  equal_variance,
  aft_iterations = 90
)
```

Arguments

input	feature-level data processed by dataProcess subfunctions
method	summarization method: "linear" or "TMP"
impute	only for summaryMethod = "TMP" and censoredInt = 'NA' or '0'. TRUE (default) imputes 'NA' or '0' (depending on censoredInt option) by Accelated failure model. FALSE uses the values assigned by cutoffCensored
censored_symbol	Missing values are censored or at random. 'NA' (default) assumes that all 'NA's in 'Intensity' column are censored. '0' uses zero intensities as censored intensity. In this case, NA intensities are missing at random. The output from Skyline should use '0'. Null assumes that all NA intensites are randomly missing.
remove50missing	only for summaryMethod = "TMP". TRUE removes the proteins where every run has at least 50% missing values for each peptide. FALSE is default.
equal_variance	only for summaryMethod = "linear". Default is TRUE. Logical variable for whether the model should account for heterogeneous variation among intensities from different features. Default is TRUE, which assume equal variance among intensities from features. FALSE means that we cannot assume equal variance among intensities from features, then we will account for heterogeneous variation from different features.
aft_iterations	Number of iterations for AFT model fitting. Default is 90.

Value

list of length one with run-level data.

Examples

```
raw = DDARawData
method = "TMP"
cens = "NA"
impute = TRUE
MSstatsConvert::MSstatsLogsSettings(FALSE)
input = MSstatsPrepareForDataProcess(raw, 2, NULL)
input = MSstatsNormalize(input, "EQUALIZEMEDIANS")
input = MSstatsMergeFractions(input)
```

```
input = MSstatsHandleMissing(input, "TMP", TRUE, "NA", 0.999)
input = MSstatsSelectFeatures(input, "all")
processed = getProcessed(input)
input = MSstatsPrepareForSummarization(input, method, impute, cens, FALSE)
summarized = MSstatsSummarizeWithSingleCore(input, method, impute, cens, FALSE, TRUE, 100)
length(summarized) # list of summarization outputs for each protein
head(summarized[[1]][[1]]) # run-level summary
```

`quantification` *Protein sample quantification or group quantification*

Description

Model-based quantification for each condition or for each biological sample per protein in a targeted Selected Reaction Monitoring (SRM), Data-Dependent Acquisition (DDA or shotgun), and Data-Independent Acquisition (DIA or SWATH-MS) experiment. Quantification takes the processed data set by `dataProcess` as input and automatically generate the quantification results (data.frame) in a long or matrix format.

Usage

```
quantification(
  data,
  type = "Sample",
  format = "matrix",
  use_log_file = TRUE,
  append = FALSE,
  verbose = TRUE,
  log_file_path = NULL
)
```

Arguments

`data`	name of the (processed) data set.
`type`	choice of quantification. "Sample" or "Group" for protein sample quantification or group quantification.
`format`	choice of returned format. "long" for long format which has the columns named Protein, Condition, LogIntensities (and BioReplicate if it is subject quantification), NumFeature for number of transitions for a protein, and NumPeaks for number of observed peak intensities for a protein. "matrix" for data matrix format which has the rows for Protein and the columns, which are Groups(or Conditions) for group quantification or the combinations of BioReplicate and Condition (labeled by "BioReplicate"_"Condition") for sample quantification. Default is "matrix"
`use_log_file`	logical. If TRUE, information about data processing will be saved to a file.

append	logical. If TRUE, information about data processing will be added to an existing log file.
verbose	logical. If TRUE, information about data processing wil be printed to the console.
log_file_path	character. Path to a file to which information about data processing will be saved. If not provided, such a file will be created automatically. If 'append = TRUE', has to be a valid path to a file.

Details

- Sample quantification : individual biological sample quantification for each protein. The label of each biological sample is a combination of the corresponding group and the sample ID. If there are no technical replicates or experimental replicates per sample, sample quantification is the same as run summarization from dataProcess. If there are technical replicates or experimental replicates, sample quantification is median among run quantification corresponding MS runs.
- Group quantification : quantification for individual group or individual condition per protein. It is median among sample quantification.
- The quantification for endogenous samples is based on run summarization from subplot model, with TMP robust estimation.

Value

data.frame as described in details.

Examples

```
# Consider quantitative data (i.e. QuantData) from a yeast study with ten time points of
# interests, three biological replicates, and no technical replicates which is
# a time-course experiment.
# Sample quantification shows model-based estimation of protein abundance in each biological
# replicate within each time point.
# Group quantification shows model-based estimation of protein abundance in each time point.
QuantData<-dataProcess(SRMRawData, use_log_file = FALSE)
head(QuantData$FeatureLevelData)
# Sample quantification
sampleQuant<-quantification(QuantData, use_log_file = FALSE)
head(sampleQuant)
# Group quantification
groupQuant<-quantification(QuantData, type="Group", use_log_file = FALSE)
head(groupQuant)
```

savePlot *Save a plot to pdf file*

Description

Save a plot to pdf file

Usage

```
savePlot(name_base, file_name, width, height)
```

Arguments

name_base	path to a folder (or "" for working directory)
file_name	name of a file to save. If this file already exists, an integer will be appended to this name
width	width of a plot
height	height of a plot

SDRFtoAnnotation *Convert SDRF experimental design file into an MSstats annotation file*

Description

Takes an SDRF file and outputs an MSstats annotation file. Note the information in the SDRF file must be correctly annotated for MSstats so that MSstats can identify the experimental design. In particular the biological replicates must be correctly annotated, with group comparison experiments having a unique ID for each BioReplicate. For more information on this please see the Supplementary of the most recent MSstats paper

Usage

```
SDRFtoAnnotation(
  data,
  run_name = "comment[data file]",
  condition_name = "characteristics[disease]",
  biological_replicate = "characteristics[biological replicate]",
  fraction = NULL
)
```

Arguments

data	SDRF annotation file
run_name	Column name in SDRF file which contains the name of the MS run. The information in this column must match exactly with the run names in the PSM file
condition_name	Column name in SDRF file which contains information on the conditions in the data.
biological_replicate	Column name in SDRF file which contains the identifier for the biological replicte. Note MSstats uses this column to determine if the experiment is a repeated measure design. BioReplicte IDs should only be reused if the replicate was measured multiple times.
fraction	Column name in SDFT file which contains information on the fractionation in the data. Only required if data contains fractions. Default is 'NULL'

Examples

```
head(example_SDRF)

msstats_annotation = SDRFtoAnnotation(example_SDRF)

head(msstats_annotation)
```

SRMRawData *Example dataset from a SRM experiment with stable isotope labeled reference of a time course yeast study*

Description

This is a partial data set obtained from a published study (Picotti, et. al, 2009). The experiment targeted 45 proteins in the glycolysis/gluconeogenesis/TCA cycle/glyoxylate cycle network, which spans the range of protein abundance from less than 128 to 10E6 copies per cell. Three biological replicates were analyzed at ten time points (T1-T10), while yeasts transited through exponential growth in a glucose-rich medium (T1-T4), diauxic shift (T5-T6), post-diauxic phase (T7-T9), and stationary phase (T10). Prior to trypsinization, the samples were mixed with an equal amount of proteins from the same N15-labeled yeast sample, which was used as a reference. Each sample was profiled in a single mass spectrometry run, where each protein was represented by up to two peptides and each peptide by up to three transitions. The goal of this study is to detect significantly change in protein abundance across time points. Transcriptional activity under the same experimental conditions has been previously investigated by (DeRisi et. al., 1997). Genes coding for 29 of the proteins are differentially expressed between conditions similar to those represented by T7 and T1 and could be treated as external sources to validate the proteomics analysis. In this exampled data set, two of the targeted proteins are selected and validated with gene expression study: Protein IDHC (gene name IDP2) is differentially expressed in time point 1 and time point 7, whereas, Protein PMG2 (gene name GPM2) is not. The protein names are based on Swiss Prot Name.

Usage

```
SRMRawData
```

Format

data.frame

Details

The raw data (input data for MSstats) is required to contain variable of ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity. The variable names should be fixed.

If the information of one or more columns is not available for the original raw data, please retain the column variables and type in fixed value. For example, the original raw data does not contain the information of ProductCharge, we retain the column ProductCharge and type in NA for all transitions in RawData.

The column BioReplicate should label with unique patient ID (i.e., same patients should label with the same ID).

Variable Intensity is required to be original signal without any log transformation and can be specified as the peak of height or the peak of area under curve.

Value

data.frame with the required format of MSstats.

Author(s)

Meena Choi, Olga Vitek.

Maintainer: Meena Choi (<mnchoi67@gmail.com>)

References

Ching-Yun Chang, Paola Picotti, Ruth Huttenhain, Viola Heinzelmann-Schwarz, Marko Jovanovic, Ruedi Aebersold, Olga Vitek. Protein significance analysis in selected reaction monitoring (SRM) measurements. *Molecular & Cellular Proteomics*, 11:M111.014662, 2012.

Examples

```
head(SRMRawData)
```

`theme_msstats` *Theme for MSstats plots*

Description

Theme for MSstats plots

Usage

```
theme_msstats(
  type,
  x.axis.size = 10,
  y.axis.size = 10,
  legend_size = 13,
  strip_background = element_rect(fill = "gray95"),
  strip_text_x = element_text(colour = c("black"), size = 14),
  legend_position = "top",
  legend_box = "vertical",
  text_angle = 0,
  text_hjust = NULL,
  text_vjust = NULL,
  ...
)
```

Arguments

`type`	type of a plot
`x.axis.size`	size of text on the x axis
`y.axis.size`	size of text on the y axis
`legend_size`	size of the legend
`strip_background`	background of facet
`strip_text_x`	size of text on facets
`legend_position`	position of the legend
`legend_box`	legend.box
`text_angle`	angle of text on the x axis (for condition and comparison plots)
`text_hjust`	hjust parameter for x axis text (for condition and comparison plots)
`text_vjust`	vjust parameter for x axis text (for condition and comparison plots)
`...`	additional parameters passed on to ggplot2::theme()

`validateAnnotation` *Check if annotation matches intended experimental design*

Description

Check if annotation matches intended experimental design

Usage

```
validateAnnotation(msstats_table, design_type = "group comparison")
```

Arguments

`msstats_table`	output of a converter function
`design_type`	character, "group comparison" or "repeated measures"

Value

TRUE if annotation file is consistent with intended experimental design. Otherwise, an error is thrown

Index

checkRepeatedDesign, 3

dataProcess, 3, *7*, *10*, *12*, *26*, *48*
dataProcessPlots, 7, *40*
DDARawData, 10
DDARawData.Skyline, 11
designSampleSize, 13, *15*
designSampleSizePlots, 15
DIARawData, 16

example_SDRF, 17
extractSDRF, 18

getProcessed, 19
getSamplesInfo, 20
getSelectedProteins, 20
ggplot, *40*
groupComparison, 21, *24*, *27*, *29*
groupComparisonPlots, 23
groupComparisonQCPlots, 27

makePeptidesDictionary, 29
modelBasedQCPlots, 29
MSstatsContrastMatrix, 31
MSstatsGroupComparison, 32
MSstatsGroupComparisonOutput, 33
MSstatsGroupComparisonSingleProtein, 34
MSstatsHandleMissing, 35
MSstatsMergeFractions, 36
MSstatsNormalize, 36
MSstatsPrepareForDataProcess, 37
MSstatsPrepareForGroupComparison, 38
MSstatsPrepareForSummarization, 39
MSstatsQualityMetricsPlot, 40
MSstatsSelectFeatures, 41
MSstatsSummarizationOutput, 42
MSstatsSummarizeSingleLinear, 43
MSstatsSummarizeSingleTMP, 44
MSstatsSummarizeWithMultipleCores, 45
MSstatsSummarizeWithSingleCore, 46

plotly, *40*

quantification, 48

savePlot, 50
SDRFtoAnnotation, 50
SRMRawData, 51

theme_msstats, 53

validateAnnotation, 54