Package: DEP (via r-universe)

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Title Differential Enrichment analysis of Proteomics data

Version 4.0.0

Description This package provides an integrated analysis workflow for robust and reproducible analysis of mass spectrometry proteomics data for differential protein expression or differential enrichment. It requires tabular input (e.g. txt files) as generated by quantitative analysis softwares of raw mass spectrometry data, such as MaxQuant or IsobarQuant. Functions are provided for data preparation, filtering, variance normalization and imputation of missing values, as well as statistical testing of differentially enriched / expressed proteins. It also includes tools to check intermediate steps in the workflow, such as normalization and missing values imputation. Finally, visualization tools are provided to explore the results, including heatmap, volcano plot and barplot representations. For scientists with limited experience in R, the package also contains wrapper functions that entail the complete analysis workflow and generate a report. Even easier to use are the interactive Shiny apps that are provided by the package.

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`add_rejections` *Mark significant proteins*

Description

`add_rejections` marks significant proteins based on defined cutoffs.

Usage

```
add_rejections(diff, alpha = 0.05, lfc = 1)
```

Arguments

`diff`	SummarizedExperiment, Proteomics dataset on which differential enrichment analysis has been performed (output from `test_diff()`).
`alpha`	Numeric(1), Sets the threshold for the adjusted P value.
`lfc`	Numeric(1), Sets the threshold for the log2 fold change.

Value

A SummarizedExperiment object annotated with logical columns indicating significant proteins.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)
```

---

analyze_dep *Differential expression analysis*

Description

analyze_dep tests for differential expression of proteins based on protein-wise linear models and empirical Bayes statistics using **limma**.

Usage

```
analyze_dep(
  se,
  type = c("all", "control", "manual"),
  control = NULL,
  alpha = 0.05,
  lfc = 1,
  test = NULL,
  design_formula = formula(~0 + condition)
)
```

Arguments

se	SummarizedExperiment, Proteomics data with unique names and identifiers annotated in 'name' and 'ID' columns. Additionally, the colData should contain sample annotation including 'label', 'condition' and 'replicate' columns. The appropriate columns and objects can be generated using make_se or make_se_parse.
type	"all", "control" or "manual", The type of contrasts that will be tested. This can be all possible pairwise comparisons ("all"), limited to the comparisons versus the control ("control"), or manually defined contrasts ("manual").

control	Character(1), The condition to which contrasts are generated (a control condition would be most appropriate).
alpha	Numeric(1), Sets the threshold for the adjusted P value.
lfc	Numeric(1), Sets the threshold for the log2 fold change.
test	Character, The contrasts that will be tested if type = "manual". These should be formatted as "SampleA_vs_SampleB" or c("SampleA_vs_SampleC", "SampleB_vs_SampleC").
design_formula	Formula, Used to create the design matrix.

Value

A SummarizedExperiment object containing FDR estimates of differential expression and logical columns indicating significant proteins.

Examples

```
# Load datasets
data <- UbiLength
exp_design <- UbiLength_ExpDesign

# Import and process data
se <- import_MaxQuant(data, exp_design)
processed <- process(se)

# Differential protein expression analysis
dep <- analyze_dep(processed, "control", "Ctrl")
dep <- analyze_dep(processed, "control", "Ctrl",
    alpha = 0.01, lfc = log2(1.5))
dep <- analyze_dep(processed, "manual", test = c("Ubi6_vs_Ubi4"))
```

DEP *DEP: A package for Differential Enrichment analysis of Proteomics data.*

Description

This package provides an integrated analysis workflow for robust and reproducible analysis of mass spectrometry proteomics data for differential protein expression or differential enrichment. It requires tabular input (e.g. txt files) as generated by quantitative analysis softwares of raw mass spectrometry data, such as MaxQuant or IsobarQuant. Functions are provided for data preparation, filtering, variance normalization and imputation of missing values, as well as statistical testing of differentially enriched / expressed proteins. It also includes tools to check intermediate steps in the workflow, such as normalization and missing values imputation. Finally, visualization tools are provided to explore the results, including heatmap, volcano plot and barplot representations. For scientists with limited experience in R, the package also entails wrapper functions that entail the complete analysis workflow and generate a report. Even easier to use are the interactive Shiny apps that are provided by the package.

Value

None

Shiny apps

- run_app: Shiny apps for interactive analysis.

Workflow functions

- LFQ: Label-free quantification (LFQ) workflow wrapper.
- TMT: Tandem-mass-tags (TMT) workflow wrapper.
- report: Create a rmarkdown report wrapper.

Wrapper functions

- import_MaxQuant: Import data from MaxQuant into a SummarizedExperiment object.
- import_IsobarQuant: Import data from IsobarQuant into a SummarizedExperiment object.
- process: Perform filtering, normalization and imputation on protein data.
- analyze_dep: Differential protein expression analysis.
- plot_all: Visualize the results in different types of plots.

Main functions

- make_unique: Generate unique names.
- make_se_parse: Turn data.frame into SummarizedExperiment by parsing column names.
- make_se: Turn data.frame into SummarizedExperiment using an experimental design.
- filter_proteins: Filter proteins based on missing values.
- normalize_vsn: Normalize data using vsn.
- impute: Impute missing values.
- test_diff: Differential enrichment analysis.
- add_rejections: Mark significant proteins.
- get_results: Generate a results table.

Visualization functions

- plot_single: Barplot for a protein of interest.
- plot_volcano: Volcano plot for a specified contrast.
- plot_heatmap: Heatmap of all significant proteins.
- plot_normalization: Boxplots to inspect normalization.
- plot_detect: Density and CumSum plots of proteins with and without missing values.
- plot_imputation: Density plots to inspect imputation.
- plot_missval: Heatmap to inspect missing values.
- plot_numbers: Barplot of proteins identified.

- plot_frequency: Barplot of protein identification overlap between conditions.
- plot_coverage: Barplot of the protein coverage in conditions.
- plot_pca: PCA plot of top variable proteins.
- plot_cor: Plot correlation matrix.
- plot_cor: Plot Gower's distance matrix.
- plot_p_hist: P value histogram.
- plot_cond_freq: Barplot of the number of significant conditions per protein.
- plot_cond_overlap: Barplot of the number of proteins for overlapping conditions.
- plot_cond: Barplot of the frequency of significant conditions per protein and the overlap in proteins between conditions.

Gene Set Enrichment Analysis functions

- test_gsea: Gene Set Enrichment Analysis using enrichR.
- plot_gsea: Barplot of enriched gene sets.

Additional functions

- get_df_wide: Generate a wide data.frame from a SummarizedExperiment.
- get_df_long: Generate a long data.frame from a SummarizedExperiment.
- se2msn: SummarizedExperiment object to MSnSet object conversion.
- filter_missval: Filter on missing values.
- manual_impute: Imputation by random draws from a manually defined distribution.
- get_prefix: Obtain the longest common prefix.
- get_suffix: Obtain the longest common suffix.

Example data

- UbiLength: Ubiquitin interactors of different linear ubiquitin lengths (UbIA-MS dataset) (Zhang, Smits, van Tilburg et al. Mol. Cell 2017).
- UbiLength_ExpDesign: Experimental design of the UbiLength dataset.
- DiUbi: Ubiquitin interactors for different diubiquitin-linkages (UbIA-MS dataset) (Zhang, Smits, van Tilburg et al. Mol. Cell 2017).
- DiUbi_ExpDesign: Experimental design of the DiUbi dataset.

DiUbi *DiUbi - Ubiquitin interactors for different diubiquitin-linkages (UbIA-MS dataset)*

Description

The DiUbi dataset contains label free quantification (LFQ) and intensity-based absolute quantification (iBAQ) data for ubiquitin interactors of different diubiquitin-linkages, generated by Zhang et al 2017. The dataset contains the proteingroups output file from MaxQuant.

Usage

```
DiUbi
```

Format

A data.frame with 4071 observations and 102 variables:

Protein.IDs Uniprot IDs

Majority.protein.IDs Uniprot IDs of major protein(s) in the protein group

Protein.names Full protein names

Gene.names Gene name

Fasta.headers Header as present in the Uniprot fasta file

Peptides Number of peptides identified for this protein group

Razor...unique.peptides Number of peptides used for the quantification of this protein group

Unique.peptides Number of peptides identified which are unique for this protein group

Intensity columns (30) Raw mass spectrometry intensity, A.U.

iBAQ columns (30) iBAQ normalized mass spectrometry intensity, A.U.

LFQ.intensity columns (30) LFQ normalized mass spectrometry intensity, A.U.

Only.identified.by.site The protein is only identified by a modification site if marked ('+')

Reverse The protein is identified in the decoy database if marked ('+')

Potential.contaminant The protein is a known contaminant if marked ('+')

id The protein group ID

Value

A data.frame.

Source

Zhang, Smits, van Tilburg, et al (2017). An interaction landscape of ubiquitin signaling. Molecular Cell 65(5): 941-955. doi: 10.1016/j.molcel.2017.01.004.

DiUbi_ExpDesign *Experimental design of the DiUbi dataset*

Description

The DiUbi_ExpDesign object annotates 30 different samples of the DiUbi dataset in 10 conditions and 3 replicates.

Usage

```
DiUbi_ExpDesign
```

Format

A data.frame with 30 observations and 3 variables:

label Label names

condition Experimental conditions

replicate Replicate number

Value

A data.frame.

Source

Zhang, Smits, van Tilburg, et al (2017). An interaction landscape of ubiquitin signaling. Molecular Cell 65(5): 941-955. doi: 10.1016/j.molcel.2017.01.004.

filter_missval *Filter on missing values*

Description

`filter_missval` filters a proteomics dataset based on missing values. The dataset is filtered for proteins that have a maximum of 'thr' missing values in at least one condition.

Usage

```
filter_missval(se, thr = 0)
```

Arguments

`se`	SummarizedExperiment, Proteomics data (output from `make_se()` or `make_se_parse()`).
`thr`	Integer(1), Sets the threshold for the allowed number of missing values in at least one condition.

Value

A filtered SummarizedExperiment object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter
stringent_filter <- filter_missval(se, thr = 0)
less_stringent_filter <- filter_missval(se, thr = 1)
```

`filter_proteins` *Filter proteins based on missing values*

Description

`filter_proteins` filters a proteomic dataset based on missing values. Different types of filtering can be applied, which range from only keeping proteins without missing values to keeping proteins with a certain percent valid values in all samples or keeping proteins that are complete in at least one condition.

Usage

```
filter_proteins(
  se,
  type = c("complete", "condition", "fraction"),
  thr = NULL,
  min = NULL
)
```

Arguments

`se`	SummarizedExperiment, Proteomics data (output from `make_se()` or `make_se_parse()`).
`type`	"complete", "condition" or "fraction", Sets the type of filtering applied. "complete" will only keep proteins with valid values in all samples. "condition" will keep proteins that have a maximum of 'thr' missing values in at least one condition. "fraction" will keep proteins that have a certain fraction of valid values in all samples.
`thr`	Integer(1), Sets the threshold for the allowed number of missing values in at least one condition if type = "condition".

min Numeric(1), Sets the threshold for the minimum fraction of valid values allowed for any protein if type = "fraction".

Value

A filtered SummarizedExperiment object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter
stringent_filter <- filter_proteins(se, type = "complete")
less_stringent_filter <- filter_proteins(se, type = "condition", thr = 0)
```

---

get_df_long *Generate a long data.frame from a SummarizedExperiment*

Description

get_df_long generate a wide data.frame from a SummarizedExperiment.

Usage

```
get_df_long(se)
```

Arguments

se SummarizedExperiment, Proteomics data (output from make_se() or make_se_parse()).

Value

A data.frame object containing all data in a wide format, where each row represents a single measurement.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Get a long data.frame
long <- get_df_long(dep)
colnames(long)
```

---

get_df_wide *Generate a wide data.frame from a SummarizedExperiment*

Description

`get_df_wide` generate a wide data.frame from a SummarizedExperiment.

Usage

```
get_df_wide(se)
```

Arguments

se	SummarizedExperiment, Proteomics data (output from `make_se()` or `make_se_parse()`).

Value

A data.frame object containing all data in a wide format, where each row represents a protein.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")
```

```
# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Get a wide data.frame
wide <- get_df_wide(dep)
colnames(wide)
```

`get_prefix` *Obtain the longest common prefix*

Description

`get_prefix` returns the longest common prefix of the supplied words.

Usage

```
get_prefix(words)
```

Arguments

`words`	Character vector, A list of words.

Value

A character vector containing the prefix.

Examples

```
# Load example
data <- UbiLength
columns <- grep("LFQ.", colnames(data))

# Get prefix
names <- colnames(data[, columns])
get_prefix(names)
```

`get_results` *Generate a results table*

Description

`get_results` generates a results table from a proteomics dataset on which differential enrichment analysis was performed.

Usage

```
get_results(dep)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from `test_diff()` and `add_rejections()`).

Value

A data.frame object containing all results variables from the performed analysis.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Get results
results <- get_results(dep)
colnames(results)

significant_proteins <- results[results$significant,]
nrow(significant_proteins)
head(significant_proteins)
```

get_suffix *Obtain the longest common suffix*

Description

get_suffix returns the longest common suffix of the supplied words.

Usage

```
get_suffix(words)
```

Arguments

words	Character vector, A list of words.

Value

A character vector containing the suffix

Examples

```
# Get suffix
names <- c("xyz_rep", "abc_rep")
get_suffix(names)
```

import_IsobarQuant *Import from IsobarQuant*

Description

import_IsobarQuant imports a protein table from IsobarQuant and converts it into a SummarizedExperiment object.

Usage

```
import_IsobarQuant(
  proteins,
  expdesign,
  intensities = "signal_sum",
  names = "gene_name",
  ids = "protein_id",
  delim = "[|]"
)
```

Arguments

proteins	Data.frame, Protein table for which unique names will be created.
expdesign	Data.frame, Experimental design with 'label', 'condition' and 'replicate' information. See UbiLength_ExpDesign for an example experimental design.
intensities	Character(1), Prefix of the columns containing sample intensities.
names	Character(1), Name of the column containing feature names.
ids	Character(1), Name of the column containing feature IDs.
delim	Character(1), Sets the delimiter separating the feature names within on protein group.

Value

A SummarizedExperiment object with log2-transformed values and "name" and "ID" columns containing unique names and identifiers.

Examples

```
## Not run:
# Load data
isobarquant_table <- read.csv("testfile.txt", header = TRUE,
                              stringsAsFactors = FALSE, sep = "\t")
exp_design <- read.csv("test_experimental_design.txt", header = TRUE,
                              stringsAsFactors = FALSE, sep = "\t")
# Import data
se <- import_IsobarQuant(isabarquant_table, exp_design)


## End(Not run)
```

---

import_MaxQuant *Import from MaxQuant*

Description

import_MaxQuant imports a protein table from MaxQuant and converts it into a SummarizedExperiment object.

Usage

```
import_MaxQuant(
  proteins,
  expdesign,
  filter = c("Reverse", "Potential.contaminant"),
  intensities = "LFQ",
  names = "Gene.names",
  ids = "Protein.IDs",
  delim = ";"
)
```

Arguments

proteins	Data.frame, Protein table originating from MaxQuant.
expdesign	Data.frame, Experimental design with 'label', 'condition' and 'replicate' information. See UbiLength_ExpDesign for an example experimental design.
filter	Character, Name of the column(s) containing features to be filtered on.
intensities	Character(1), Prefix of the columns containing sample intensities.
names	Character(1), Name of the column containing feature names.
ids	Character(1), Name of the column containing feature IDs.
delim	Character(1), Sets the delimiter separating the feature names within on protein group.

Value

A SummarizedExperiment object with log2-transformed values and "name" and "ID" columns containing unique names and identifiers.

Examples

```
# Load example data and experimental design
data <- UbiLength
exp_design <- UbiLength_ExpDesign

# Import data
se <- import_MaxQuant(data, exp_design)
```

---

impute *Impute missing values*

Description

impute imputes missing values in a proteomics dataset.

Usage

```
impute(
  se,
  fun = c("bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "man", "min", "zero",
    "mixed", "nbavg"),
  ...
)
```

Arguments

se	SummarizedExperiment, Proteomics data (output from make_se() or make_se_parse()). It is adviced to first remove proteins with too many missing values using filter_missval() and normalize the data using normalize_vsn().
fun	"bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "man", "min", "zero", "mixed" or "nbavg", Function used for data imputation based on manual_impute and impute.
...	Additional arguments for imputation functions as depicted in manual_impute and impute.

Value

An imputed SummarizedExperiment object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and normalize
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)

# Impute missing values using different functions
imputed_MinProb <- impute(norm, fun = "MinProb", q = 0.05)
imputed_QRILC <- impute(norm, fun = "QRILC")

imputed_knn <- impute(norm, fun = "knn", k = 10, rowmax = 0.9)
imputed_MLE <- impute(norm, fun = "MLE")

imputed_manual <- impute(norm, fun = "man", shift = 1.8, scale = 0.3)
```

LFQ *LFQ workflow*

Description

LFQ is a wrapper function running the entire differential enrichment/expression analysis workflow for label free quantification (LFQ)-based proteomics data. The protein table from MaxQuant is used as direct input.

Usage

```
LFQ(
  proteins,
  expdesign,
  fun = c("man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero",
    "mixed", "nbavg"),
  type = c("all", "control", "manual"),
  control = NULL,
  test = NULL,
  filter = c("Reverse", "Potential.contaminant"),
  name = "Gene.names",
  ids = "Protein.IDs",
  alpha = 0.05,
  lfc = 1
)
```

Arguments

proteins	Data.frame, The data object.
expdesign	Data.frame, The experimental design object.
fun	"man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero", "mixed" or "nbavg", Function used for data imputation based on manual_impute and impute.
type	'all', 'control' or 'manual', The type of contrasts that will be generated.
control	Character(1), The sample name to which the contrasts are generated (the control sample would be most appropriate).
test	Character, The contrasts that will be tested if type = "manual". These should be formatted as "SampleA_vs_SampleB" or c("SampleA_vs_SampleC", "SampleB_vs_SampleC").
filter	Character, Name(s) of the column(s) to be filtered on.
name	Character(1), Name of the column representing gene names.
ids	'Character(1), Name of the column representing protein IDs.
alpha	Numeric(1), sets the false discovery rate threshold.
lfc	Numeric(1), sets the log fold change threshold.

Value

A list of 9 objects:

data	data.frame containing the original data
se	SummarizedExperiment object containing the original data
filt	SummarizedExperiment object containing the filtered data
norm	SummarizedExperiment object containing the normalized data
imputed	SummarizedExperiment object containing the imputed data

diff	SummarizedExperiment object containing FDR estimates of differential expression
dep	SummarizedExperiment object annotated with logical columns indicating significant proteins
results	data.frame containing containing all results variables from the performed analysis
param	data.frame containing the test parameters

Examples

```
data <- UbiLength
expdesign <- UbiLength_ExpDesign
results <- LFQ(data, expdesign, 'MinProb', 'control', 'Ctrl')
```

---

make_se *Data.frame to SummarizedExperiment object conversion using an experimental design*

Description

make_se creates a SummarizedExperiment object based on two data.frames: the protein table and experimental design.

Usage

```
make_se(proteins_unique, columns, expdesign)
```

Arguments

proteins_unique	Data.frame, Protein table with unique names annotated in the 'name' column (output from make_unique()).
columns	Integer vector, Column numbers indicating the columns containing the assay data.
expdesign	Data.frame, Experimental design with 'label', 'condition' and 'replicate' information. See UbiLength_ExpDesign for an example experimental design.

Value

A SummarizedExperiment object with log2-transformed values.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)
```

`make_se_parse` *Data.frame to SummarizedExperiment object conversion using parsing from column names*

Description

`make_se_parse` creates a SummarizedExperiment object based on a single data.frame.

Usage

```
make_se_parse(
  proteins_unique,
  columns,
  mode = c("char", "delim"),
  chars = 1,
  sep = "_"
)
```

Arguments

`proteins_unique`	Data.frame, Protein table with unique names annotated in the 'name' column (output from `make_unique()`).
`columns`	Integer vector, Column numbers indicating the columns containing the assay data.
`mode`	"char" or "delim", The mode of parsing the column headers. "char" will parse the last number of characters as replicate number and requires the 'chars' parameter. "delim" will parse on the separator and requires the 'sep' parameter.
`chars`	Numeric(1), The number of characters to take at the end of the column headers as replicate number (only for mode == "char").
`sep`	Character(1), The separator used to parse the column header (only for mode == "delim").

Value

A SummarizedExperiment object with log2-transformed values.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
se <- make_se_parse(data_unique, columns, mode = "char", chars = 1)
se <- make_se_parse(data_unique, columns, mode = "delim", sep = "_")
```

`make_unique` *Make unique names*

Description

`make_unique` generates unique identifiers for a proteomics dataset based on "name" and "id" columns.

Usage

```
make_unique(proteins, names, ids, delim = ";")
```

Arguments

`proteins`	Data.frame, Protein table for which unique names will be created.
`names`	Character(1), Name of the column containing feature names.
`ids`	Character(1), Name of the column containing feature IDs.
`delim`	Character(1), Sets the delimiter separating the feature names within one protein group.

Value

A data.frame with the additional variables "name" and "ID" containing unique names and identifiers, respectively.

Examples

```
# Load example
data <- UbiLength

# Check colnames and pick the appropriate columns
colnames(data)
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")
```

manual_impute *Imputation by random draws from a manually defined distribution*

Description

manual_impute imputes missing values in a proteomics dataset by random draws from a manually defined distribution.

Usage

```
manual_impute(se, scale = 0.3, shift = 1.8)
```

Arguments

se	SummarizedExperiment, Proteomics data (output from make_se() or make_se_parse()). It is adviced to first remove proteins with too many missing values using filter_missval() and normalize the data using normalize_vsn().
scale	Numeric(1), Sets the width of the distribution relative to the standard deviation of the original distribution.
shift	Numeric(1), Sets the left-shift of the distribution (in standard deviations) from the median of the original distribution.

Value

An imputed SummarizedExperiment object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and normalize
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)

# Impute missing values manually
imputed_manual <- impute(norm, fun = "man", shift = 1.8, scale = 0.3)
```

meanSdPlot *Plot row standard deviations versus row means*

Description

meanSdPlot generates a hexagonal heatmap of the row standard deviations versus row means from SummarizedExperiment objects. See meanSdPlot.

Usage

```
meanSdPlot(
  x,
  ranks = TRUE,
  xlab = ifelse(ranks, "rank(mean)", "mean"),
  ylab = "sd",
  pch,
  plot = TRUE,
  bins = 50,
  ...
)
```

Arguments

x	SummarizedExperiment, Data object.
ranks	Logical, Whether or not to plot the row means on the rank scale.
xlab	Character, x-axis label.
ylab	Character, y-axis label.
pch	Ignored - exists for backward compatibility.
plot	Logical, Whether or not to produce the plot.
bins	Numeric vector, Data object before normalization.
...	Other arguments, Passed to stat_binhex.

Value

A scatter plot of row standard deviations versus row means(generated by stat_binhex)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
```

```
se <- make_se(data_unique, columns, exp_design)

# Filter and normalize
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)

# Plot meanSdPlot
meanSdPlot(norm)
```

`normalize_vsn` *Normalization using vsn*

Description

`normalize_vsn` performs variance stabilizing transformation using the `vsn-package`.

Usage

```
normalize_vsn(se)
```

Arguments

`se`	SummarizedExperiment, Proteomics data (output from `make_se()` or `make_se_parse()`). It is adviced to first remove proteins with too many missing values using `filter_missval()`.

Value

A normalized SummarizedExperiment object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and normalize
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
```

plot_all *Visualize the results in different types of plots*

Description

plot_all visualizes the results of the differential protein expression analysis in different types of plots. These are (1) volcano plots, (2) heatmaps, (3) single protein plots, (4) frequency plots and/or (5) comparison plots.

Usage

```
plot_all(dep, plots = c("volcano", "heatmap", "single", "freq", "comparison"))
```

Arguments

dep	SummarizedExperiment, Data object which has been generated by analyze_dep or the combination of test_diff and add_rejections.
plots	"volcano", "heatmap", "single", "freq" and/or "comparison",

Value

Pdfs containg the desired plots.

Examples

```
# Load datasets
data <- UbiLength
exp_design <- UbiLength_ExpDesign

# Import and process data
se <- import_MaxQuant(data, exp_design)
processed <- process(se)

# Differential protein expression analysis
dep <- analyze_dep(processed, "control", "Ctrl")

## Not run:
# Plot all plots
plot_all(dep)

## End(Not run)
```

plot_cond *Plot frequency of significant conditions per protein and the overlap in proteins between conditions*

Description

plot_cond generates a histogram of the number of proteins per condition and stacks for overlapping conditions.

Usage

```
plot_cond(dep, plot = TRUE)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
plot	Logical(1), If TRUE (default) the barplot is produced. Otherwise (if FALSE), the data which the barplot is based on are returned.

Value

A histogram (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot histogram with overlaps
plot_cond(dep)
```

plot_cond_freq *Plot frequency of significant conditions per protein*

Description

plot_cond_freq generates a histogram of the number of significant conditions per protein.

Usage

```
plot_cond_freq(dep, plot = TRUE)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
plot	Logical(1), If TRUE (default) the histogram is produced. Otherwise (if FALSE), the data which the histogram is based on are returned.

Value

A histogram (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot frequency of significant conditions
plot_cond_freq(dep)
```

plot_cond_overlap *Plot conditions overlap*

Description

plot_cond_overlap generates a histogram of the number of proteins per condition or overlapping conditions.

Usage

```
plot_cond_overlap(dep, plot = TRUE)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
plot	Logical(1), If TRUE (default) the barplot is produced. Otherwise (if FALSE), the data which the barplot is based on are returned.

Value

A histogram (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot condition overlap
plot_cond_overlap(dep)
```

plot_cor *Plot correlation matrix*

Description

plot_cor generates a Pearson correlation matrix.

Usage

```
plot_cor(
  dep,
  significant = TRUE,
  lower = -1,
  upper = 1,
  pal = "PRGn",
  pal_rev = FALSE,
  indicate = NULL,
  font_size = 12,
  plot = TRUE,
  ...
)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
significant	Logical(1), Whether or not to filter for significant proteins.
lower	Integer(1), Sets the lower limit of the color scale.
upper	Integer(1), Sets the upper limit of the color scale.
pal	Character(1), Sets the color panel (from **RColorBrewer**).
pal_rev	Logical(1), Whether or not to invert the color palette.
indicate	Character, Sets additional annotation on the top of the heatmap based on columns from the experimental design (colData).
font_size	Integer(1), Sets the size of the labels.
plot	Logical(1), If TRUE (default) the correlation matrix plot is produced. Otherwise (if FALSE), the data which the correlation matrix plot is based on are returned.
...	Additional arguments for Heatmap function as depicted in Heatmap

Value

A heatmap plot (generated by Heatmap)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot correlation matrix
plot_cor(dep)
```

---

plot_coverage *Plot protein coverage*

Description

plot_coverage generates a barplot of the protein coverage in all samples.

Usage

```
plot_coverage(se, plot = TRUE)
```

Arguments

se	SummarizedExperiment, Data object for which to plot observation frequency.
plot	Logical(1), If TRUE (default) the barplot is produced. Otherwise (if FALSE), the data which the barplot is based on are returned.

Value

Barplot of protein coverage in samples (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and plot coverage
filt <- filter_missval(se, thr = 0)
plot_coverage(filt)
```

---

plot_detect *Visualize intensities of proteins with missing values*

Description

plot_detect generates density and CumSum plots of protein intensities with and without missing values

Usage

```
plot_detect(se)
```

Arguments

se	SummarizedExperiment, Data object with missing values.

Value

Density and CumSum plots of intensities of proteins with and without missing values (generated by ggplot).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter
```

```
filt <- filter_missval(se, thr = 0)

# Plot intensities of proteins with missing values
plot_detect(filt)
```

`plot_dist` *Plot Gower's distance matrix*

Description

`plot_dist` generates a distance matrix heatmap using the Gower's distance.

Usage

```
plot_dist(
  dep,
  significant = TRUE,
  pal = "YlOrRd",
  pal_rev = TRUE,
  indicate = NULL,
  font_size = 12,
  plot = TRUE,
  ...
)
```

Arguments

`dep`	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from `test_diff()` and `add_rejections()`).
`significant`	Logical(1), Whether or not to filter for significant proteins.
`pal`	Character(1), Sets the color panel (from **RColorBrewer**).
`pal_rev`	Logical(1), Whether or not to invert the color palette.
`indicate`	Character, Sets additional annotation on the top of the heatmap based on columns from the experimental design (colData).
`font_size`	Integer(1), Sets the size of the labels.
`plot`	Logical(1), If `TRUE` (default) the distance matrix plot is produced. Otherwise (if `FALSE`), the data which the distance matrix plot is based on are returned.
`...`	Additional arguments for Heatmap function as depicted in `Heatmap`

Value

A heatmap plot (generated by `Heatmap`)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot correlation matrix
plot_dist(dep)
```

---

plot_frequency *Plot protein overlap between samples*

Description

plot_frequency generates a barplot of the protein overlap between samples

Usage

```
plot_frequency(se, plot = TRUE)
```

Arguments

se	SummarizedExperiment, Data object for which to plot observation frequency.
plot	Logical(1), If TRUE (default) the barplot is produced. Otherwise (if FALSE), the data which the barplot is based on are returned.

Value

Barplot of overlap of protein identifications between samples (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and plot frequency
filt <- filter_missval(se, thr = 0)
plot_frequency(filt)
```

---

plot_gsea *Plot enriched Gene Sets*

Description

plot_gsea plots enriched gene sets from Gene Set Enrichment Analysis.

Usage

```
plot_gsea(
  gsea_results,
  number = 10,
  alpha = 0.05,
  contrasts = NULL,
  databases = NULL,
  nrow = 1,
  term_size = 8
)
```

Arguments

gsea_results	Data.frame, Gene Set Enrichment Analysis results object. (output from test_gsea()).
number	Numeric(1), Sets the number of enriched terms per contrast to be plotted.
alpha	Numeric(1), Sets the threshold for the adjusted P value.
contrasts	Character, Specifies the contrast(s) to plot. If 'NULL' all contrasts will be plotted.
databases	Character, Specifies the database(s) to plot. If 'NULL' all databases will be plotted.
nrow	Numeric(1), Sets the number of rows for the plot.
term_size	Numeric(1), Sets the text size of the terms.

Value

A barplot of the enriched terms (generated by ggplot).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

## Not run:

# Test enrichments
gsea_results <- test_gsea(dep)
plot_gsea(gsea_results)


## End(Not run)
```

---

plot_heatmap *Plot a heatmap*

Description

plot_heatmap generates a heatmap of all significant proteins.

Usage

```
plot_heatmap(
  dep,
  type = c("contrast", "centered"),
  kmeans = FALSE,
  k = 6,
  col_limit = 6,
  indicate = NULL,
```

```
  clustering_distance = c("euclidean", "maximum", "manhattan", "canberra", "binary",
    "minkowski", "pearson", "spearman", "kendall", "gower"),
  row_font_size = 6,
  col_font_size = 10,
  plot = TRUE,
  ...
)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
type	'contrast' or 'centered', The type of data scaling used for plotting. Either the fold change ('contrast') or the centered log2-intensity ('centered').
kmeans	Logical(1), Whether or not to perform k-means clustering.
k	Integer(1), Sets the number of k-means clusters.
col_limit	Integer(1), Sets the outer limits of the color scale.
indicate	Character, Sets additional annotation on the top of the heatmap based on columns from the experimental design (colData). Only applicable to type = 'centered'.
clustering_distance	"euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski", "pearson", "spearman", "kendall" or "gower", Function used to calculate clustering distance (for proteins and samples). Based on Heatmap and daisy.
row_font_size	Integer(1), Sets the size of row labels.
col_font_size	Integer(1), Sets the size of column labels.
plot	Logical(1), If TRUE (default) the heatmap is produced. Otherwise (if FALSE), the data which the heatmap is based on are returned.
...	Additional arguments for Heatmap function as depicted in Heatmap

Value

A heatmap (generated by Heatmap)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
```

```
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot heatmap
plot_heatmap(dep)
plot_heatmap(dep, 'centered', kmeans = TRUE, k = 6, row_font_size = 3)
plot_heatmap(dep, 'contrast', col_limit = 10, row_font_size = 3)
```

`plot_imputation` *Visualize imputation*

Description

`plot_imputation` generates density plots of all conditions for input objects, e.g. before and after imputation.

Usage

```
plot_imputation(se, ...)
```

Arguments

`se`	SummarizedExperiment, Data object, e.g. before imputation (output from `normalize_vsn()`).
`...`	Other SummarizedExperiment object(s), E.g. data object after imputation (output from `impute()`).

Value

Density plots of all conditions of all conditions for input objects, e.g. before and after imputation (generated by `ggplot`).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
```

```
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Plot imputation
plot_imputation(filt, norm, imputed)
```

`plot_missval` *Plot a heatmap of proteins with missing values*

Description

`plot_missval` generates a heatmap of proteins with missing values to discover whether values are missing by random or not.

Usage

```
plot_missval(se)
```

Arguments

`se`	SummarizedExperiment, Data object with missing values.

Value

A heatmap indicating whether values are missing (0) or not (1) (generated by `Heatmap`).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)

# Plot missing values heatmap
plot_missval(filt)
```

plot_normalization *Visualize normalization*

Description

plot_normalization generates boxplots of all conditions for input objects, e.g. before and after normalization.

Usage

```
plot_normalization(se, ...)
```

Arguments

se	SummarizedExperiment, Data object, e.g. before normalization (output from make_se() or make_se_parse()).
...	Additional SummarizedExperiment object(s), E.g. data object after normalization (output from normalize_vsn).

Value

Boxplots of all conditions for input objects, e.g. before and after normalization (generated by ggplot). Adding components and other plot adjustments can be easily done using the ggplot2 syntax (i.e. using '+')

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and normalize
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)

# Plot normalization
plot_normalization(se, filt, norm)
```

plot_numbers *Plot protein numbers*

Description

plot_numbers generates a barplot of the number of identified proteins per sample.

Usage

```
plot_numbers(se, plot = TRUE)
```

Arguments

se	SummarizedExperiment, Data object for which to plot protein numbers (output from make_se() or make_se_parse()).
plot	Logical(1), If TRUE (default) the barplot is produced. Otherwise (if FALSE), the data which the barplot is based on are returned.

Value

Barplot of the number of identified proteins per sample (generated by ggplot)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter and plot numbers
filt <- filter_missval(se, thr = 0)
plot_numbers(filt)
```

plot_p_hist *Plot a P value histogram*

Description

plot_p_hist generates a p value histogram.

Usage

```
plot_p_hist(dep, adjusted = FALSE, wrap = FALSE)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
adjusted	Logical(1), Whether or not to use adjusted p values.
wrap	Logical(1), Whether or not to display different histograms for the different contrasts.

Value

A histogram (generated by ggplot).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot p value histogram
plot_p_hist(dep)
plot_p_hist(dep, wrap = TRUE)
```

---

plot_pca *Plot PCA*

Description

plot_pca generates a PCA plot using the top variable proteins.

Usage

```
plot_pca(
  dep,
  x = 1,
  y = 2,
  indicate = c("condition", "replicate"),
  label = FALSE,
  n = 500,
  point_size = 4,
  label_size = 3,
  plot = TRUE
)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
x	Integer(1), Sets the principle component to plot on the x-axis.
y	Integer(1), Sets the principle component to plot on the y-axis.
indicate	Character, Sets the color, shape and facet_wrap of the plot based on columns from the experimental design (colData).
label	Logical, Whether or not to add sample labels.
n	Integer(1), Sets the number of top variable proteins to consider.
point_size	Integer(1), Sets the size of the points.
label_size	Integer(1), Sets the size of the labels.
plot	Logical(1), If TRUE (default) the PCA plot is produced. Otherwise (if FALSE), the data which the PCA plot is based on are returned.

Value

A scatter plot (generated by ggplot).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
```

```
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot PCA
plot_pca(dep)
plot_pca(dep, indicate = "condition")
```

`plot_single` *Plot values for a protein of interest*

Description

`plot_single` generates a barplot of a protein of interest.

Usage

```
plot_single(dep, proteins, type = c("contrast", "centered"), plot = TRUE)
```

Arguments

`dep`	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from `test_diff()` and `add_rejections()`).
`proteins`	Character, The name(s) of the protein(s) to plot.
`type`	'contrast' or 'centered', The type of data scaling used for plotting. Either the fold change ('contrast') or the centered log2-intensity ('centered').
`plot`	Logical(1), If `TRUE` (default) the barplot is produced. Otherwise (if `FALSE`), the summaries which the barplot is based on are returned.

Value

A barplot (generated by `ggplot`).

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
```

```
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot single proteins
plot_single(dep, 'USP15')
plot_single(dep, 'USP15', 'centered')
plot_single(dep, c('USP15', 'CUL1'))
plot_single(dep, c('USP15', 'CUL1'), plot = FALSE)
```

`plot_volcano` *Volcano plot*

Description

`plot_volcano` generates a volcano plot for a specified contrast.

Usage

```
plot_volcano(
  dep,
  contrast,
  label_size = 3,
  add_names = TRUE,
  adjusted = FALSE,
  plot = TRUE
)
```

Arguments

`dep`	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from `test_diff()` and `add_rejections()`).
`contrast`	Character(1), Specifies the contrast to plot.
`label_size`	Integer(1), Sets the size of name labels.
`add_names`	Logical(1), Whether or not to plot names.
`adjusted`	Logical(1), Whether or not to use adjusted p values.
`plot`	Logical(1), If `TRUE` (default) the volcano plot is produced. Otherwise (if `FALSE`), the data which the volcano plot is based on are returned.

Value

A volcano plot (generated by `ggplot`)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

# Plot volcano
plot_volcano(dep, 'Ubi6_vs_Ctrl', label_size = 5, add_names = TRUE)
plot_volcano(dep, 'Ubi6_vs_Ctrl', label_size = 5,
   add_names = TRUE, adjusted = TRUE)
plot_volcano(dep, 'Ubi6_vs_Ctrl', add_names = FALSE)
plot_volcano(dep, 'Ubi4_vs_Ctrl', label_size = 5, add_names = TRUE)
```

`process` *Proteomics data processing*

Description

`process` performs data processing on a SummarizedExperiment object. It (1) filters a proteomics dataset based on missing values, (2) applies variance stabilizing normalization and (3) imputes eventual remaining missing values.

Usage

```
process(
  se,
  thr = 0,
 fun = c("man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero",
    "mixed", "nbavg"),
  ...
)
```

Arguments

se	SummarizedExperiment, Proteomics data with unique names and identifiers annotated in 'name' and 'ID' columns. The appropriate columns and objects can be generated using the wrapper import functions import_MaxQuant and import_IsobarQuant or the generic functions make_se and make_se_parse.
thr	Integer(1), Sets the threshold for the allowed number of missing values per condition.
fun	"man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero", "mixed" or "nbavg", Function used for data imputation based on manual_impute and impute.
...	Additional arguments for imputation functions as depicted in manual_impute and impute.

Value

A filtered, normalized and imputed SummarizedExperiment object.

Examples

```
# Load datasets
data <- UbiLength
exp_design <- UbiLength_ExpDesign

# Import data
se <- import_MaxQuant(data, exp_design)

# Process data
processed <- process(se)
```

---

report *Generate a markdown report*

Description

report generates a report of the analysis performed by TMT and LFQ wrapper functions. Additionally, the results table is saved as a tab-delimited file.

Usage

```
report(results)
```

Arguments

results	List of SummarizedExperiment objects obtained from the LFQ or TMT wrapper functions.

Value

A rmarkdown report is generated and saved. Additionally, the results table is saved as a tab-delimited txt file.

Examples

```
## Not run:

data <- UbiLength
expdesign <- UbiLength_ExpDesign

results <- LFQ(data, expdesign, 'MinProb', 'control', 'Ctrl')
report(results)


## End(Not run)
```

---

run_app *DEP shiny apps*

Description

run_app launches an interactive shiny app for interactive differential enrichment/expression analysis of proteomics data.

Usage

```
run_app(app)
```

Arguments

app	'LFQ' or 'TMT', The name of the app.

Value

Launches a browser with the shiny app

Examples

```
## Not run:
# Run the app
run_app('LFQ')

run_app('TMT')


## End(Not run)
```

se2msn *Deprecated Function to coerce SummarizedExperiment to MSnSet object*

Description

Use as instead.

Usage

```
se2msn(se)
```

Arguments

se	SummarizedExperiment, Object which will be turned into a MSnSet object.

Value

A MSnSet object.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Convert to MSnSet
data_msn <- as(se, "MSnSet")
# Convert back to SE
se_back <- as(data_msn, "SummarizedExperiment")
```

test_diff *Differential enrichment test*

Description

test_diff performs a differential enrichment test based on protein-wise linear models and empirical Bayes statistics using **limma**. False Discovery Rates are estimated using **fdrtool**.

Usage

```
test_diff(
  se,
  type = c("control", "all", "manual"),
  control = NULL,
  test = NULL,
  design_formula = formula(~0 + condition)
)
```

Arguments

se	SummarizedExperiment, Proteomics data (output from make_se() or make_se_parse()). It is adviced to first remove proteins with too many missing values using filter_missval(), normalize the data using normalize_vsn() and impute remaining missing values using impute().
type	"control", "all" or "manual", The type of contrasts that will be tested. This can be all possible pairwise comparisons ("all"), limited to the comparisons versus the control ("control"), or manually defined contrasts ("manual").
control	Character(1), The condition to which contrasts are generated if type = "control" (a control condition would be most appropriate).
test	Character, The contrasts that will be tested if type = "manual". These should be formatted as "SampleA_vs_SampleB" or c("SampleA_vs_SampleC", "SampleB_vs_SampleC").
design_formula	Formula, Used to create the design matrix.

Value

A SummarizedExperiment object containing fdr estimates of differential expression.

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- test_diff(imputed, "control", "Ctrl")
diff <- test_diff(imputed, "manual",
```

```
    test = c("Ubi4_vs_Ctrl", "Ubi6_vs_Ctrl"))

# Test for differentially expressed proteins with a custom design formula
diff <- test_diff(imputed, "control", "Ctrl",
    design_formula = formula(~ 0 + condition + replicate))
```

---

test_gsea *Gene Set Enrichment Analysis*

Description

test_gsea tests for enriched gene sets in the differentially enriched proteins. This can be done independently for the different contrasts.

Usage

```
test_gsea(
  dep,
  databases = c("GO_Molecular_Function_2017b", "GO_Cellular_Component_2017b",
    "GO_Biological_Process_2017b"),
  contrasts = TRUE
)
```

Arguments

dep	SummarizedExperiment, Data object for which differentially enriched proteins are annotated (output from test_diff() and add_rejections()).
databases	Character, Databases to search for gene set enrichment. See http://amp.pharm.mssm.edu/Enrichr/ for available databases.
contrasts	Logical(1), Whether or not to perform the gene set enrichment analysis independently for the different contrasts.

Value

A data.frame with enrichment terms (generated by enrichr)

Examples

```
# Load example
data <- UbiLength
data <- data[data$Reverse != "+" & data$Potential.contaminant != "+",]
data_unique <- make_unique(data, "Gene.names", "Protein.IDs", delim = ";")

# Make SummarizedExperiment
columns <- grep("LFQ.", colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)
```

```
# Filter, normalize and impute missing values
filt <- filter_missval(se, thr = 0)
norm <- normalize_vsn(filt)
imputed <- impute(norm, fun = "MinProb", q = 0.01)

# Test for differentially expressed proteins
diff <- diff <- test_diff(imputed, "control", "Ctrl")
dep <- add_rejections(diff, alpha = 0.05, lfc = 1)

## Not run:

# Test enrichments
gsea_results_per_contrast <- test_gsea(dep)
gsea_results <- test_gsea(dep, contrasts = FALSE)

gsea_kegg <- test_gsea(dep, databases = "KEGG_2016")


## End(Not run)
```

---

theme_DEP1 *DEP ggplot theme 1*

Description

theme_DEP1 is the default ggplot theme used for plotting in DEP with horizontal x-axis labels.

Usage

```
theme_DEP1()
```

Value

ggplot theme

Examples

```
data <- UbiLength
data <- data[data$Reverse != '+' & data$Potential.contaminant != '+',]
data_unique <- make_unique(data, 'Gene.names', 'Protein.IDs', delim = ';')

columns <- grep('LFQ.', colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

filt <- filter_missval(se, thr = 0)
plot_frequency(filt) # uses theme_DEP1() style
```

theme_DEP2 *DEP ggplot theme 2*

Description

theme_DEP2 is the ggplot theme used for plotting in DEP with vertical x-axis labels.

Usage

```
theme_DEP2()
```

Value

ggplot theme

Examples

```
data <- UbiLength
data <- data[data$Reverse != '+' & data$Potential.contaminant != '+',]
data_unique <- make_unique(data, 'Gene.names', 'Protein.IDs', delim = ';')

columns <- grep('LFQ.', colnames(data_unique))
exp_design <- UbiLength_ExpDesign
se <- make_se(data_unique, columns, exp_design)

filt <- filter_missval(se, thr = 0)
plot_numbers(filt) # uses theme_DEP2() style
```

TMT *TMT workflow*

Description

TMT is a wrapper function running the entire differential enrichment/expression analysis workflow for TMT-based proteomics data. The protein table from IsobarQuant is used as direct input.

Usage

```
TMT(
  proteins,
  expdesign,
  fun = c("man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero",
    "mixed", "nbavg"),
  type = c("all", "control", "manual"),
  control = NULL,
```

```
  test = NULL,
  name = "gene_name",
  ids = "protein_id",
  alpha = 0.05,
  lfc = 1
)
```

Arguments

proteins	Data.frame, The data object.
expdesign	Data.frame, The experimental design object.
fun	"man", "bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "min", "zero", "mixed" or "nbavg", Function used for data imputation based on manual_impute and impute.
type	'all', 'control' or 'manual', The type of contrasts that will be generated.
control	Character(1), The sample name to which the contrasts are generated (the control sample would be most appropriate).
test	Character, The contrasts that will be tested if type = "manual". These should be formatted as "SampleA_vs_SampleB" or c("SampleA_vs_SampleC", "SampleB_vs_SampleC").
name	Character(1), Name of the column representing gene names.
ids	'Character(1), Name of the column representing protein IDs.
alpha	Numeric(1), sets the false discovery rate threshold.
lfc	Numeric(1), sets the log fold change threshold.

Value

A list of 8 objects:

se	SummarizedExperiment object containing the original data
filt	SummarizedExperiment object containing the filtered data
norm	SummarizedExperiment object containing the normalized data
imputed	SummarizedExperiment object containing the imputed data
diff	SummarizedExperiment object containing FDR estimates of differential expression
dep	SummarizedExperiment object annotated with logical columns indicating significant proteins
results	data.frame containing containing all results variables from the performed analysis
param	data.frame containing the test parameters

Examples

```
## Not run:

TMT_res <- TMT()


## End(Not run)
```

`UbiLength` *UbiLength - Ubiquitin interactors of different linear ubiquitin lengths (UbIA-MS dataset)*

Description

The UbiLength dataset contains label free quantification (LFQ) data for ubiquitin interactors of different linear ubiquitin lengths, generated by Zhang et al 2017. The dataset contains the proteinGroups output file from MaxQuant.

Usage

```
UbiLength
```

Format

A data.frame with 3006 observations and 35 variables:

Protein.IDs Uniprot IDs

Majority.protein.IDs Uniprot IDs of major protein(s) in the protein group

Protein.names Full protein names

Gene.names Gene name

Fasta.headers Header as present in the Uniprot fasta file

Peptides Number of peptides identified for this protein group

Razor...unique.peptides Number of peptides used for the quantification of this protein group

Unique.peptides Number of peptides identified which are unique for this protein group

Intensity columns (12) Raw mass spectrometry intensity, A.U.

LFQ.intensity columns (12) LFQ normalized mass spectrometry intensity, A.U.

Only.identified.by.site The protein is only identified by a modification site if marked ('+')

Reverse The protein is identified in the decoy database if marked ('+')

Potential.contaminant The protein is a known contaminant if marked ('+')

Value

A data.frame.

Source

Zhang, Smits, van Tilburg, et al (2017). An interaction landscape of ubiquitin signaling. Molecular Cell 65(5): 941-955. doi: 10.1016/j.molcel.2017.01.004.

`UbiLength_ExpDesign` *Experimental design of the UbiLength dataset*

Description

The UbiLength_ExpDesign object annotates 12 different samples of the UbiLength dataset in 4 conditions and 3 replicates.

Usage

```
UbiLength_ExpDesign
```

Format

A data.frame with 12 observations and 3 variables:

label Label names

condition Experimental conditions

replicate Replicate number

Value

A data.frame.

Source

Zhang, Smits, van Tilburg, et al (2017). An interaction landscape of ubiquitin signaling. Molecular Cell 65(5): 941-955. doi: 10.1016/j.molcel.2017.01.004.

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