

# Package ‘DEP’

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**Title** Differential Enrichment analysis of Proteomics data

**Version** 1.19.0

**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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**Author** Arne Smits [cre, aut],  
 Wolfgang Huber [aut]  
**Maintainer** Arne Smits <smits.arne@gmail.com>

## R topics documented:

add_rejections . . . . .	3
analyze_dep . . . . .	4
DEP . . . . .	5
DiUbi . . . . .	7
DiUbi_ExpDesign . . . . .	8
filter_missval . . . . .	9
filter_proteins . . . . .	10
get_df_long . . . . .	11
get_df_wide . . . . .	12
get_prefix . . . . .	13
get_results . . . . .	13
get_suffix . . . . .	14
import_IsobarQuant . . . . .	15
import_MaxQuant . . . . .	16
impute . . . . .	17
LFQ . . . . .	18
make_se . . . . .	19
make_se_parse . . . . .	20
make_unique . . . . .	21
manual_impute . . . . .	22
meanSdPlot . . . . .	23
normalize_vsn . . . . .	24
plot_all . . . . .	24
plot_cond . . . . .	25
plot_cond_freq . . . . .	26
plot_cond_overlap . . . . .	27
plot_cor . . . . .	28
plot_coverage . . . . .	30
plot_detect . . . . .	30
plot_dist . . . . .	31
plot_frequency . . . . .	32
plot_gsea . . . . .	33
plot_heatmap . . . . .	34
plot_imputation . . . . .	36
plot_missval . . . . .	37
plot_normalization . . . . .	37

<code>add_rejections</code>	3
<code>plot_numbers</code>	38
<code>plot_pca</code>	39
<code>plot_p_hist</code>	40
<code>plot_single</code>	41
<code>plot_volcano</code>	42
<code>process</code>	44
<code>report</code>	45
<code>run_app</code>	45
<code>se2msn</code>	46
<code>test_diff</code>	47
<code>test_gsea</code>	48
<code>theme_DEP1</code>	49
<code>theme_DEP2</code>	50
<code>TMT</code>	51
<code>UbiLength</code>	52
<code>UbiLength_ExpDesign</code>	53
<b>Index</b>	<b>54</b>

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<code>add_rejections</code>	<i>Mark significant proteins</i>
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### Description

`add_rejections` marks significant proteins based on defined cutoffs.

### Usage

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

### Arguments

<code>diff</code>	SummarizedExperiment, Proteomics dataset on which differential enrichment analysis has been performed (output from <code>test_diff()</code> ).
<code>alpha</code>	Numeric(1), Sets the threshold for the adjusted P value.
<code>lfc</code>	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 <a href="#">make_se</a> or <a href="#">make_se_parse</a> .
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.

### 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.

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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](https://doi.org/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](https://doi.org/10.1016/j.molcel.2017.01.004).

---

filter_missval	<i>Filter on missing values</i>
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---

**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 <code>make_se()</code> or <code>make_se_parse()</code> ).
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 <code>make_se()</code> or <code>make_se_parse()</code> ).
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	<i>Obtain the longest common prefix</i>
------------	---

---

**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	<i>Generate a results table</i>
-------------	---------------------------------

---

**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 Summarized-Experiment 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 <a href="#">UbiLength_ExpDesign</a> 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(isobarquant_table, exp_design)

## End(Not run)
```

---

import_MaxQuant	<i>Import from MaxQuant</i>
-----------------	-----------------------------

---

## 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 <a href="#">UbiLength_ExpDesign</a> 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	<i>Impute missing values</i>
--------	------------------------------

---

## 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 <a href="#">make_se()</a> or <a href="#">make_se_parse()</a> ). It is advised to first remove proteins with too many missing values using <a href="#">filter_missval()</a> and normalize the data using <a href="#">normalize_vsn()</a> .
fun	"bpca", "knn", "QRILC", "MLE", "MinDet", "MinProb", "man", "min", "zero", "mixed" or "nbavg", Function used for data imputation based on <a href="#">manual_impute</a> and <a href="#">impute</a> .
...	Additional arguments for imputation functions as depicted in <a href="#">manual_impute</a> and <a href="#">impute</a> .

## 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 <a href="#">manual_impute</a> and <a href="#">impute</a> .
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	<i>Data.frame to SummarizedExperiment object conversion using an experimental design</i>
---------	--

---

**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 <a href="#">make_unique()</a> ).
columns	Integer vector, Column numbers indicating the columns containing the assay data.
expdesign	Data.frame, Experimental design with 'label', 'condition' and 'replicate' information. See <a href="#">UbiLength_ExpDesign</a> 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	<i>Data.frame to SummarizedExperiment object conversion using parsing from column names</i>
---------------	---

---

**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 <code>make_unique()</code> ).
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	<i>Make unique names</i>
-------------	--------------------------

---

## 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

<code>se</code>	SummarizedExperiment, Proteomics data (output from <code>make_se()</code> or <code>make_se_parse()</code> ). It is advised to first remove proteins with too many missing values using <code>filter_missval()</code> and normalize the data using <code>normalize_vsn()</code> .
<code>scale</code>	Numeric(1), Sets the width of the distribution relative to the standard deviation of the original distribution.
<code>shift</code>	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	<i>Plot row standard deviations versus row means</i>
------------	--

---

### 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 <a href="#">stat_binhex</a> .

### 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	<i>Normalization using vsn</i>
---------------	--------------------------------

---

**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 advised 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	<i>Visualize the results in different types of plots</i>
----------	--

---

**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 <a href="#">analyze_dep</a> or the combination of <a href="#">test_diff</a> and <a href="#">add_rejections</a> .
plots	"volcano", "heatmap", "single", "freq" and/or "comparison",

**Value**

Pdfs containing 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	<i>Plot frequency of significant conditions per protein and the overlap in proteins between conditions</i>
-----------	--

---

**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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <a href="#">test_diff()</a> and <a href="#">add_rejections()</a> ).
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 <b>RColorBrewer</b> ).
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 <a href="#">Heatmap</a>

**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	<i>Plot protein coverage</i>
---------------	------------------------------

---

**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	<i>Visualize intensities of proteins with missing values</i>
-------------	--

---

**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 <a href="#">Heatmap</a>

**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 <a href="#">test_gsea()</a> ).
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", "Ctrl1")
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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <a href="#">Heatmap</a> and <a href="#">daisy</a> .
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 <a href="#">Heatmap</a>

**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	<i>Visualize imputation</i>
-----------------	-----------------------------

---

## 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 <a href="#">normalize_vsn()</a> ).
...	Other SummarizedExperiment object(s), E.g. data object after imputation (output from <a href="#">impute()</a> ).

## 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	<i>Plot a heatmap of proteins with missing values</i>
--------------	---

---

**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	<i>Visualize normalization</i>
--------------------	--------------------------------

---

**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 <code>make_se()</code> or <code>make_se_parse()</code> ).
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\_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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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\_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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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\_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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <code>test_diff()</code> and <code>add_rejections()</code> ).
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 <a href="#">import_MaxQuant</a> and <a href="#">import_IsobarQuant</a> or the generic functions <a href="#">make_se</a> and <a href="#">make_se_parse</a> .
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 <a href="#">manual_impute</a> and <a href="#">impute</a> .
...	Additional arguments for imputation functions as depicted in <a href="#">manual_impute</a> and <a href="#">impute</a> .

### 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	<i>Generate a markdown report</i>
--------	-----------------------------------

---

**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 <a href="#">LFQ</a> or <a href="#">TMT</a> 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	<i>DEP shiny apps</i>
---------	-----------------------

---

**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	<i>Differential enrichment test</i>
-----------	-------------------------------------

---

## 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 <code>make_se()</code> or <code>make_se_parse()</code> ). It is advised to first remove proteins with too many missing values using <code>filter_missval()</code> , normalize the data using <code>normalize_vsn()</code> and impute remaining missing values using <code>impute()</code> .
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 <code>test_diff()</code> and <code>add_rejections()</code> ).
databases	Character, Databases to search for gene set enrichment. See <a href="http://amp.pharm.mssm.edu/Enrichr/">http://amp.pharm.mssm.edu/Enrichr/</a> 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", "Ctrl1")
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	<i>TMT workflow</i>
-----	---------------------

---

**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 <a href="#">manual_impute</a> and <a href="#">impute</a> .
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	<i>UbiLength - Ubiquitin interactors of different linear ubiquitin lengths (UbiA-MS dataset)</i>
-----------	--

---

### 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 protein-groups 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](https://doi.org/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](https://doi.org/10.1016/j.molcel.2017.01.004).

# Index

- \* **datasets**
  - DiUbi, 7
  - DiUbi\_ExpDesign, 8
  - UbiLength, 52
  - UbiLength\_ExpDesign, 53
- add\_rejections, 3, 6, 13, 25–29, 31, 35, 39, 41–43, 48
- analyze\_dep, 4, 6, 25
- as, 46
- daisy, 35
- DEP, 5, 49, 50
- DEP-package (DEP), 5
- DiUbi, 7, 7
- DiUbi\_ExpDesign, 7, 8
- enrichr, 48
- filter\_missval, 7, 9, 17, 22, 24, 47
- filter\_proteins, 6, 10
- get\_df\_long, 7, 11
- get\_df\_wide, 7, 12
- get\_prefix, 7, 13
- get\_results, 6, 13
- get\_suffix, 7, 14
- ggplot, 26–28, 30, 31, 33, 34, 36, 38–43
- Heatmap, 29, 32, 35, 37
- import\_IsobarQuant, 6, 15, 44
- import\_MaxQuant, 6, 16, 44
- impute, 6, 17, 17, 18, 36, 44, 47, 51
- LFQ, 6, 18, 45
- make\_se, 4, 6, 9–12, 17, 19, 22, 24, 38, 39, 44, 47
- make\_se\_parse, 4, 6, 9–12, 17, 20, 22, 24, 38, 39, 44, 47
- make\_unique, 6, 19, 20, 21
- manual\_impute, 7, 17, 18, 22, 44, 51
- meanSdPlot, 23, 23
- normalize\_vsn, 6, 17, 22, 24, 36, 38, 47
- plot\_all, 6, 24
- plot\_cond, 7, 25
- plot\_cond\_freq, 7, 26
- plot\_cond\_overlap, 7, 27
- plot\_cor, 6, 28
- plot\_coverage, 6, 30
- plot\_detect, 6, 30
- plot\_dist, 31
- plot\_frequency, 6, 32
- plot\_gsea, 7, 33
- plot\_heatmap, 6, 34
- plot\_imputation, 6, 36
- plot\_missval, 6, 37
- plot\_normalization, 6, 37
- plot\_numbers, 6, 38
- plot\_p\_hist, 7, 40
- plot\_pca, 6, 39
- plot\_single, 6, 41
- plot\_volcano, 6, 42
- process, 6, 44
- report, 6, 45
- rmarkdown, 45
- run\_app, 5, 45
- se2msn, 7, 46
- stat\_binhex, 23
- test\_diff, 3, 6, 13, 25–29, 31, 35, 39, 41–43, 47, 48
- test\_gsea, 7, 33, 48
- theme\_DEP1, 49
- theme\_DEP2, 50
- TMT, 6, 45, 51

UbiLength, [7](#), [52](#)

UbiLength\_ExpDesign, [7](#), [15](#), [16](#), [19](#), [53](#)