

# Package ‘SWATH2stats’

October 16, 2019

**Type** Package

**Title** Transform and Filter SWATH Data for Statistical Packages

**Version** 1.14.0

**Date** 2019-04-28

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**Description** This package is intended to transform SWATH data from the OpenSWATH software into a format readable by other statistics packages while performing filtering, annotation and FDR estimation.

**License** GPL-3

**Depends** R(>= 2.10.0)

**Imports** data.table, reshape2, grid, ggplot2, stats, grDevices, graphics, utils, biomaRt

**Suggests** testthat, knitr

**Enhances** imsbInfer, MSstats, PECA, aLFQ

**biocViews** Proteomics, Annotation, ExperimentalDesign, Preprocessing, MassSpectrometry, ImmunoOncology

**NeedsCompilation** no

**VignetteBuilder** knitr

**git\_url** <https://git.bioconductor.org/packages/SWATH2stats>

**git\_branch** RELEASE\_3\_9

**git\_last\_commit** 76f70e0

**git\_last\_commit\_date** 2019-05-02

**Date/Publication** 2019-10-15

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SWATH2stats-package      *SWATH2stats*

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## Description

This package is intended to transform SWATH data from the OpenSWATH software into a format readable by other statistics packages while performing filtering, annotation and FDR assessment.

## Details

Package: SWATH2stats  
Type: Package  
Version: 1.13.6  
Date: 2019-04-28  
License: GPLv3

## Author(s)

Peter Blattmann, Moritz Heusel and Ruedi Aebersold  
Maintainer: Peter Blattmann <blattmann@imsb.biol.ethz.ch>

## References

- Blattmann P, Heusel M, Aebersold R. SWATH2stats: An R/Bioconductor Package to Process and Convert Quantitative SWATH-MS Proteomics Data for Downstream Analysis Tools. PLoS ONE 11(4): e0153160 (2016). doi: 10.1371/journal.pone.0153160.
- Rost HL, Rosenberger G, Navarro P, Gillet L, Miladinovic SM, Schubert OT, Wolski W, Collins BC, Malmstrom J, Malmstrom L, Aebersold R. OpenSWATH enables automated, targeted analysis of data-independent acquisition MS data. Nature Biotechnology. 2014 Mar;32(3):219-23. doi: 10.1038/nbt.2841.
- Choi M, Chang CY, Clough T, Broudy D, Killeen T, MacLean B, Vitek O. MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments. Bioinformatics. 2014 Sep 1;30(17):2524-6. doi: 10.1093/bioinformatics/btu305.
- Rosenberger G, Ludwig C, Rost HL, Aebersold R, Malmstrom L. aLFQ: an R-package for estimating absolute protein quantities from label-free LC-MS/MS proteomics data. Bioinformatics. 2014 Sep 1;30(17):2511-3. doi: 10.1093/bioinformatics/btu200.
- Suomi T., Corthals G., Nevalainen O.S., and Elo L.L. (2015). Using Peptide-Level Proteomics Data for Detecting Differentially Expressed Proteins. J Proteome Res. Nov 6;14(11):4564-70. doi: 10.1021/acs.jproteome.5b00363.
- Suomi, T. and Elo L.L. (2017). Enhanced differential expression statistics for data-independent acquisition proteomics" Scientific Reports 7, Article number: 5869. doi:10.1038/s41598-017-05949-y

---

add\_genesymbol

*add\_genesymbol: Adds gene symbols to a table*

---

## Description

This function adds an additional gene or protein symbol to a table.

## Usage

```
add_genesymbol(data_table, gene.ID.table, column.name = "Protein",  
ID1 = "uniprotswissprot", ID2 = "hgnc_symbol",  
id.separator = "/", copy_nonconverted = TRUE)
```

**Arguments**

<code>data_table</code>	A data frame or file name.
<code>gene.ID.table</code>	A dictionary table containing the original and new identifiers.
<code>column.name</code>	The column name where the original protein identifiers are present.
<code>ID1</code>	The type of the original protein identifiers.
<code>ID2</code>	The type of the converted protein identifiers.
<code>id.separator</code>	Separator between protein identifiers of shared peptides.
<code>copy_nonconverted</code>	Option defining if the identifiers that cannot be converted should be copied.

**Value**

Returns the data frame with an added column of the converted protein identifiers.

**Note**

Protein identifiers from shared peptides should be separated by a forward slash.

**Author(s)**

Peter Blattmann

**Examples**

```
gene.ID.table <- data.frame(uniprotswissprot = c("Q01581", "P49327", "P60709"),
hgnc_symbol = c("HMGCS1", "FASN", "ACTB"))
data_table <- data.frame(Protein = c("Q01581", "P49327", "2/P63261/P60709"),
Abundance = c(100, 3390, 43423))
add_genesymbol(data_table, gene.ID.table)
```

---

`assess_decoy_rate`      *assess\_decoy\_rate: Assess decoy rate*

---

**Description**

This function counts the number of decoy peptides.

**Usage**

```
assess_decoy_rate(data)
```

**Arguments**

<code>data</code>	A data frame that contains at least a column named "FullPeptideName" and "decoy".
-------------------	---

**Details**

A printout is generated to indicate the number of non-decoy, decoy peptides and the rate of decoy vs non-decoy peptides. Unique peptides are counted, so a precursor with different charge states is counted as one peptide. In the column "decoy" the values need to be 1,0 or TRUE and FALSE.

**Value**

Prints the decoy rate.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data <- OpenSWATH_data
assess_decoy_rate(data)
```

---

assess_fdr_byrun	<i>Assess assay, peptide and protein level FDR by run (for each MS_injection separately) in OpenSWATH output table</i>
------------------	--

---

**Description**

This function estimates the assay, peptide and protein FDR by run in an OpenSWATH result table in dependence of a range of m\_score cutoffs. The results can be visualized and summarized by the associated method plot.fdr\_table(). It counts target and decoy assays (unique transition\_group\_id), peptides (unique FullPeptideName) and proteins (unique ProteinName) in the OpenSWATH output table in dependence of m-score cutoff, the useful m\_score cutoff range is evaluated for each dataset individually on the fly.

To arrive from decoy counts at an estimation of the false discovery rate (false positives among the targets remaining at a given mscore cutoff) the ratio of false positives to true negatives (decoys) (FFT) must be supplied. It is estimated for each run individually by pyProphet and contained in the pyProphet statistics [Injection\_name]\_full\_stat.csv. As an approximation, the FFTs of multiple runs are averaged and supplied as argument FFT. For further details see the Vignette Section 1.3 and 4.1.

To assess fdr over the entire dataset, please refer to function assess\_fdr\_overall.

FDR is calculated as  $FDR = (TN * FFT / T)$ ; TN=decoys, T=targets, FFT=see above

**Usage**

```
assess_fdr_byrun(data, FFT, n.range = 20, output = "pdf_csv", plot = TRUE,
filename = "FDR_report_byrun", output_mscore_levels = c(0.01, 0.001),
mscore.col = "m_score")
```

**Arguments**

data	Annotated OpenSWATH/pyProphet output table. Refer to function sample_annotation from this package for further information.
FFT	Ratio of false positives to true negatives, q-values from [Injection_name]_full_stat.csv in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument FFT. Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target).
n.range	Option to set the number of magnitude for which the m_score threshold is decreased (e.g. n.range = 10, m-score from 0.1 until 10^-10)^.

output	Choose output type. "pdf_csv" creates the output as files in the working directory, "Rconsole" triggers delivery of the output to the console enabling further computation or custom plotting / output.
plot	Logical, whether or not to create plots from the results (using the associated method plot.fdr_cube())
filename	Optional, modifying the basename of the result files if applicable.
output_mscore_levels	Define m-score levels to plot and write the estimated FDR results.
mscore.col	Defines the column from which to retrieve the m_score. If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group m_score.

### Value

Returns an array of target/decoy identification numbers and calculated FDR values at different m-score cutoffs.

### Author(s)

Moritz Heusel

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
assess_fdr_byrun(data, FFT=0.7, output = "pdf_csv", plot = TRUE,
filename="Testoutput_assess_fdr_byrun")
```

---

assess_fdr_overall	<i>Assess overall FDR in annotated OpenSWATH/pyProphet output table in dependence of m_score cutoff</i>
--------------------	---

---

### Description

This function estimates the assay, peptide and protein FDR over a multi-run OpenSWATH/pyProphet output table. It counts target and decoy assays (unique transition\_group\_id), peptides (unique FullPeptideName) and proteins (unique ProteinName) in dependence of the m-score cutoff (1e-2 to 1e-20).

To arrive from decoy counts at an estimation of the false discovery rate (false positives among the targets remaining at a given mscore cutoff) the ratio of false positives to true negatives (decoys) (FFT) must be supplied. It is estimated for each run individually by pyProphet and contained in the pyProphet statistics [Injection\_name]\_full\_stat.csv. As an approximation, the FFTs of multiple runs are averaged and supplied as argument FFT. For further details see the Vignette Section 1.3 and 4.1.

Protein FDR control on peak group quality level is a very strict filter and should be handled with caution.

FDR is calculated as  $FDR = (TN * FFT / T)$ ; TN=decoys, T=targets, FFT=see above

**Usage**

```
assess_fdr_overall(data, FFT, n.range = 20, output = "pdf_csv", plot = TRUE,
  filename="FDR_report_overall", mscore.col = "m_score")
```

**Arguments**

<code>data</code>	Data table that is produced by the OpenSWATH/pyProphet workflow
<code>n.range</code>	Option to set the number of magnitude for which the <code>m_score</code> threshold is decreased (e.g. <code>n.range = 10</code> , <code>m-score</code> from 0.1 until $10^{-10}$ ).
<code>FFT</code>	Ratio of false positives to true negatives, q-values from [Injection_name]_full_stat.csv in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument <code>FFT</code> . Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target).
<code>output</code>	Choose output type. "pdf_csv" creates the output as files in the working directory, "Rconsole" triggers delivery of the output to the console enabling further computation or custom plotting / output.
<code>plot</code>	Logical, whether or not to create plots from the results (using the associated method <code>plot.fdr_table()</code> )
<code>filename</code>	Optional, modifying the basename of the result files if applicable.
<code>mscore.col</code>	Defines the column from which to retrieve the <code>m_score</code> . If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group <code>m_score</code> .

**Value**

Returns a list of class "fdr\_table". If output "pdf\_csv" and plot = TRUE were chosen, report files are written to the working folder.

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
assess_fdr_overall(data, FFT=0.7, output = "Rconsole", plot = TRUE,
  filename="Testoutput_assess_fdr_overall")
```

---

convert4aLFQ

*convert4aLFQ: Convert table into the format for aLFQ*

---

**Description**

This functions selects the columns necessary for the aLFQ R package.

**Usage**

```
convert4aLFQ(data, annotation = TRUE, check_transitions = TRUE)
```

**Arguments**

data	A data frame containing the SWATH data in transition-level format
annotation	Option to indicate if the data has been annotated, i.e. if the columns Condition, Replicate, Run are present. If option is set to true it will write a new run_id as a string of the combination of these three columns.
check_transitions	Option if number of transitions should be checked. As input only transition-level data should be used and therefore the number of transitions can be checked to spot any irregularities. However, this makes the function slow.

**Value**

Returns a data frame in the appropriate format for aLFQ.

**Author(s)**

Peter Blattmann

**References**

Rosenberger G, Ludwig C, Rost HL, Aebersold R, Malmstrom L. aLFQ: an R-package for estimating absolute protein quantities from label-free LC-MS/MS proteomics data. *Bioinformatics*. 2014 Sep 1;30(17):2511-3. doi: 10.1093/bioinformatics/btu200.

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
raw <- disaggregate(data.filtered.decoy)
data.aLFQ <- convert4aLFQ(raw)
```

---

convert4mapDIA

*convert4mapDIA: Convert table into the format for mapDIA*

---

**Description**

This functions selects the columns necessary for mapDIA.

**Usage**

```
convert4mapDIA(data, RT=FALSE)
```

**Arguments**

data	A data frame containing SWATH data.
RT	Option to export the retention times.

**Value**

Returns a data frame in the appropriate format for mapDIA.



**Note**

The table must not contain any technical replica, the intensity of technical replica is averaged. This function requires the package reshape2.

**Author(s)**

Peter Blattmann

**References**

Teo, G., et al. (2015). "mapDIA: Preprocessing and statistical analysis of quantitative proteomics data from data independent acquisition mass spectrometry." J Proteomics 129: 108-120.

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
raw <- disaggregate(data.filtered.decoy)
data.mapDIA <- convert4mapDIA(raw, RT=TRUE)
```

---

convert4MSstats

*convert4MSstats: Convert table into the format for MSstats*

---

**Description**

This functions selects the columns necessary for MSstats and renames them if necessary.

**Usage**

```
convert4MSstats(data, replace.values = TRUE, replace.colnames = TRUE,
replace.Unimod = TRUE)
```

**Arguments**

`data` A data frame containing SWATH data.

`replace.values` Option to indicate if negative and 0 values should be replaced with NA.

`replace.colnames` Option to indicate if column names should be renamed and columns reduced to the necessary columns for MSstats

`replace.Unimod` Option to indicate if Unimod Identifier should be replaced from ":" to "\_".

**Details**

The necessary columns are selected and three columns renamed: FullPeptideName -> PeptideSequence Charge -> PrecursorCharge filename -> File

**Value**

Returns a data frame in the appropriate format for MSstats.

**Author(s)**

Peter Blattmann

**References**

Choi M, Chang CY, Clough T, Broudy D, Killeen T, MacLean B, Vitek O. MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments. *Bioinformatics*. 2014 Sep 1;30(17):2524-6. doi: 10.1093/bioinformatics/btu305.

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
raw <- disaggregate(data.filtered.decoy)
data.mapDIA <- convert4MSstats(raw)
```

---

convert4PECA

*convert4PECA: Convert table into the format for ROPECA*

---

**Description**

This functions selects the columns necessary for ROPECA.

**Usage**

```
convert4PECA(data)
```

**Arguments**

data                    A data frame containing SWATH data.

**Value**

Returns a data frame in the appropriate format for ROPECA.

**Note**

The table must not contain any technical replica, the intensity of technical replica is averaged. This function requires the package reshape2.

**Author(s)**

Peter Blattmann

**References**

Suomi, T. and Elo L.L. (2017). "Enhanced differential expression statistics for data-independent acquisition proteomics" *Scientific Reports* 7, Article number: 5869. doi:10.1038/s41598-017-05949-y

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
data.PECA <- convert4PECA(data.filtered.decoy)
```

---

convert4pythonscript    *convert4bashscript: Convert data into the format for running a bash script*

---

## Description

This functions selects the columns suggested to run a python script to change the data from peptide-level to transition-level.

## Usage

```
convert4pythonscript(data, replace.Unimod = TRUE)
```

## Arguments

data                    A data frame containing SWATH data.  
replace.Unimod    Option to indicate if Unimod Identifier should be replaced form ":" to "\_".

## Details

The necessary columns are selected and the run column is renamed to filename for the script. The intensities are taken from the column `aggr_Peak_Area` and therefore the `Intensity` column is not exported.

## Value

Returns a data frame in the appropriate format to be used by a custom python script stored in the scripts folder.

## Author(s)

Peter Blattmann

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data,0.01)
data.pythonscript <- convert4pythonscript(data.filtered.decoy)
```

---

convert\_protein\_ids     *convert\_protein\_ids: Convert protein ids*

---

### Description

This function renames protein ids in a data frame or file

### Usage

```
convert_protein_ids(data_table,
                    column.name = "Protein",
                    species = "hsapiens_gene_ensembl",
                    host = "www.ensembl.org",
                    mart = "ENSEMBL_MART_ENSEMBL",
                    ID1 = "uniprotswissprot",
                    ID2 = "hgnc_symbol",
                    id.separator = "/",
                    copy_nonconverted = TRUE,
                    verbose = FALSE)
```

### Arguments

data_table	A data frame or file name.
column.name	The column name where the original protein identifiers are present. Default: "Protein".
species	The species of the protein identifiers in the term used by biomaRt (e.g. "hsapiens_gene_ensembl", "mmusculus_gene_ensembl", "drerio_gene_ensembl", etc.). Default: "hsapiens_gene_ensembl".
host	Path of the biomaRt database (e.g. "www.ensembl.org", "dec2017.archive.ensembl.org"). Default: "www.ensembl.org".
mart	The type of mart (e.g. "ENSEMBL_MART_ENSEMBL", etc.). Default: "ENSEMBL_MART_ENSEMBL"
ID1	The type of the original protein identifiers (e.g. "uniprotswissprot", "ensembl_peptide_id"). Default: "uniprotswissprot".
ID2	The type of the converted protein identifiers (e.g. "hgnc_symbol", "mgi_symbol", "external_gene_name"). Default: "hgnc_symbol".
id.separator	Separator between protein identifiers of shared peptides. Default: "/".
copy_nonconverted	Option defining if the identifiers that cannot be converted should be copied. Default: TRUE.
verbose	Option to write a file containing the version of the database used. Default: FALSE.

### Value

Returns the data frame with an added column of the converted protein identifiers.

**Note**

Protein identifiers from shared peptides should be separated by a forward slash. The host of archived ensembl databases can be introduced as well (e.g. "dec2017.archive.ensembl.org")

**Author(s)**

Peter Blattmann

**Examples**

```
data_table <- data.frame(Protein = c("Q01581", "P49327", "2/P63261/P60709"),
Abundance = c(100, 3390, 43423))
convert_protein_ids(data_table)
```

---

count\_analytes

*count\_analytes: Counts analytes in different injections*

---

**Description**

This functions counts the number of different peakgroups, peptides and proteins in different injections

**Usage**

```
count_analytes(data, column.levels = c("transition_group_id", "FullPeptideName",
"ProteinName"), column.by="run_id", rm.decoy=TRUE)
```

**Arguments**

data	A data frame containing SWATH data.
column.levels	Columns in which different identifiers should be counted.
column.by	Column for which the different identifiers should be counted for, e.g. for the different injections.
rm.decoy	Option to not remove decoy before counting.

**Value**

Returns a data frame with the count of the different identifiers per e.g. injection.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
count_analytes(data)
```

---

disaggregate	<i>disaggregate: Transforms the SWATH data from a peptide- to a transition-level table.</i>
--------------	---

---

### Description

If the SWATH data should be analyzed on transition-level the data needs to be transformed from peptide-level table to a transition-level table (one row per transition instead of one row per peptide). The columns "aggr\_Fragment\_Annotation" and "aggr\_Peak\_Area" are disaggregated into the new columns "FragmentIon" and "Intensity". The following columns are renamed if they exist: FullPeptideName -> PeptideSequence, Charge -> PrecursorCharge, Area -> Intensity, Fragment -> FragmentIon, Sequence -> NakedSequence.

### Usage

```
disaggregate(data, all.columns = FALSE)
```

### Arguments

data	A data frame containing SWATH data.
all.columns	Option that all columns are processed. Otherwise only the typical columns needed for downstream analysis are processed.

### Value

Returns a data frame containing the SWATH data in a transition-level table.

### Author(s)

Peter Blattmann

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
raw <- disaggregate(data.filtered.decoy)
```

---

filter_all_peptides	<i>Select all proteins that are supported by peptides.</i>
---------------------	--

---

### Description

This functions counts all proteins that are supported by peptides (including non proteo-typic peptides). All peptides (incl. non proteotypic peptides) are selected. For the proteins supported by proteotypic peptide the "1/" in front of the identifier is removed to facilitate further data processing.

### Usage

```
filter_all_peptides(data)
```

**Arguments**

data                    A data frame containing SWATH data.

**Value**

Returns a data frame with the data from both proteotypic and non-proteotypic peptides.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
data.all <- filter_all_peptides(data.filtered.decoy)
```

---

filter_mscore	<i>filter_mscore: Filter openSWATH output table according to mscore</i>
---------------	---

---

**Description**

This function filters the SWATH data according to the m\_score value, as well as to the number of occurrence in the data (requant) and within a condition (condition)

**Usage**

```
filter_mscore(data, mscore, rm.decoy=TRUE, mscore.col = "m_score")
filter_mscore_freqobs(data, mscore, percentage=NULL,
  rm.decoy = TRUE, mscore.col = "m_score")
filter_mscore_condition(data, mscore, n.replica, rm.decoy = TRUE,
  mscore.col = "m_score")
```

**Arguments**

data                    A data frame containing SWATH data.

mscore                  Value that defines the mscore threshold according to which the data will be filtered.

n.replica                Number of measurements within at least one condition that have to pass the mscore threshold for this transition.

percentage               Percentage in which replicas the transition has to reach the mscore threshold

rm.decoy                Option to remove the decoys during filtering.

mscore.col               Defines the column from which to retrieve the m\_score. If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition\_group m\_score.

**Value**

Returns a data frame with the filtered data.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered <- filter_mscore(data, 0.01)
data.filtered <- filter_mscore_freqobs(data, 0.01, 0.8)
data.filtered <- filter_mscore_condition(data, 0.01, 3)
```

---

filter_mscore_fdr	<i>Filter annotated OpenSWATH/pyProphet output table to achieve a high FDR quality data matrix with controlled overall protein FDR and quantitative values for all peptides mapping to these high-confidence proteins (up to a desired overall peptide level FDR quality).</i>
-------------------	--

---

**Description**

This function controls the protein FDR over a multi-run OpenSWATH/pyProphet output table and filters all quantitative values to a desired overall/global peptide FDR level.

It first finds a suitable m-score cutoff to minimally achieve a desired global FDR quality on a protein master list based on the function `mscore4protfdr`. It then finds a suitable m-score cutoff to minimally achieve a desired global FDR quality on peptide level based on the function `mscore4pepfdr`. Finally, it reports all the peptide quantities derived based on the peptide level cutoff for only those peptides mapping to the protein master list. It further summarizes the protein and peptide numbers remaining after the filtering. It further evaluates the individual run FDR qualities of the peptides (and quantitation events) selected.

**Usage**

```
filter_mscore_fdr(data, FFT = 1, overall_protein_fdr_target = 0.02,
  upper_overall_peptide_fdr_limit = 0.05, rm.decoy = TRUE, mscore.col = "m_score")
```

**Arguments**

<code>data</code>	Annotated OpenSWATH/pyProphet data table
<code>FFT</code>	Ratio of false positives to true negatives, q-values from [Injection_name]_full_stat.csv in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument FFT. Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target). For further details see the Vignette Section 1.3 and 4.1.
<code>overall_protein_fdr_target</code>	FDR target for the protein master list for which quantitative values down to the less strict peptide_fdr criterion will be kept/reported. Defaults to 0.02.
<code>upper_overall_peptide_fdr_limit</code>	FDR target for the quantitative values kept/reported for all peptides mapping to the high-confidence protein master list. Defaults to 0.05. If all values up to m_score 0.01 shall be kept, set = 1.



rm.decoy	Logical T/F, whether decoy entries should be removed after the analysis. Defaults to TRUE. Can be useful to disable to track the influence on decoy fraction by further filtering steps such as requiring 2 peptides per protein.
mscore.col	Defines the column from which to retrieve the m_score. If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group m_score.

**Value**

data.filtered the filtered data frame

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.fdr.filtered<-filter_mscore_fdr(data, FFT=0.7, overall_protein_fdr_target=0.02,
upper_overall_peptide_fdr_limit=0.1)
```

---

filter\_on\_max\_peptides

*Filter only for the highest intense peptides*

---

**Description**

In order to reduce the data, the data is filtered only for the proteins with the highest intensity peptides.

**Usage**

```
filter_on_max_peptides(data, n_peptides, rm.decoy = TRUE)
```

**Arguments**

data	A data frame containing SWATH data with the column names: ProteinNames, PeptideSequence, PrecursorCharge, Intensity.
n_peptides	Maximum number of highest intense peptides to filter the data on.
rm.decoy	Option to remove the decoys during filtering.

**Value**

Returns a data frame of the filtered data

**Author(s)**

Peter Blattmann

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered <- filter_mscore_freqobs(data, 0.01,0.8)
data.max <- filter_on_max_peptides(data.filtered, 5)
```

---

filter\_on\_min\_peptides

*Filter openSWATH output for proteins that are identified by a minimum of n independent peptides*

---

## Description

This function removes entries mapping to proteins that are identified by less than n\_peptides.

Removing single-hit proteins from an analysis can significantly increase the sensitivity under strict protein fdr criteria, as evaluated by e.g. assess\_fdr\_overall.

## Usage

```
filter_on_min_peptides(data, n_peptides, rm.decoy = TRUE)
```

## Arguments

data	Data table that is produced by the openSWATH/iPortal workflow.
n_peptides	Number of minimal number of peptide IDs associated with a protein ID in order to be kept in the dataset.
rm.decoy	Option to remove the decoys during filtering.

## Value

Returns the filtered data frame with only peptides that map to proteins with  $\geq$  n\_peptides peptides.

## Author(s)

Moritz Heusel

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered <- filter_mscore_freqobs(data, 0.01,0.8)
data.max <- filter_on_max_peptides(data.filtered, 5)
data.min.max <- filter_on_min_peptides(data.max, 3)
```

---

`filter_proteotypic_peptides`*Filter for proteins that are supported by proteotypic peptides.*

---

**Description**

Peptides can match to several proteins. With this function proteotypic peptides, peptides that are only contained in one protein are selected. Additionally the number of proteins are counted and printed.

**Usage**

```
filter_proteotypic_peptides(data, rm.decoy = TRUE)
```

**Arguments**

<code>data</code>	A data frame containing SWATH data.
<code>rm.decoy</code>	Option to remove the decoys during filtering.

**Value**

Returns a data frame with only the data supported by proteotypic peptides.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
data.all <- filter_proteotypic_peptides(data.filtered.decoy)
```

---

`import_data`*import\_data: Transforms the column names from a data frame to the required format.*

---

**Description**

This functions transforms the column names from a data frame from another format to a data frame with column names used by the OpenSWATH output and required for these functions. During executing of the function the corresponding columns for each column in the data need to be selected. For columns that do not correspond to a certain column 'not applicable' needs to be selected and the column names are not changed.

**Usage**

```
import_data(data)
```

**Arguments**

`data` A data frame containing the SWATH-MS data (one line per peptide precursor quantified) but with different column names.

**Value**

Returns the data frame in the appropriate format.

**Note**

List of column names of the OpenSWATH data:

`ProteinName`: Unique identifier for protein or proteingroup that the peptide maps to. Proteotypic peptides should be indicated by 1/ in order to be recognized as such by the function `filter_proteotypic_peptides`.

`FullPeptideName`: Unique identifier for the peptide.

`Charge`: Charge of the peptide precursor ion quantified.

`Sequence`: Naked peptide sequence without modifications.

`aggr_Fragment_Annotation`: aggregated annotation for the different Fragments quantified for this peptide. In the OpenSWATH results the different annotation in OpenSWATH are concatenated by a semicolon.

`aggr_Peak_Area`: aggregated Intensity values for the different Fragments quantified for this peptide. In the OpenSWATH results the aggregated Peak Area intensities are concatenated by a semicolon.

`transition_group_id`: A unique identifier for each transition group used.

`decoy`: Indicating with 1 or 0 if this transition group is a decoy.

`m_score`: Column containing the score that is used to estimate FDR or filter. M-score values of identified peak groups are equivalent to a q-value and thus typically are smaller than 0.01, depending on the confidence of identification (the lower the m-score, the higher the confidence).

Column containing the score that is used to estimate FDR or filter.

`RT`: Column containing the retention time of the quantified peak.

`filename`: Column containing the filename or a unique identifier for each injection.

`Intensity`: column containing the intensity value for each quantified peptide.

Columns needed for FDR estimation and filtering functions: `ProteinName`, `FullPeptideName`, `transition_group_id`, `decoy`, `m_score`

Columns needed for conversion to transition-level format (needed for `MSSstats` and `mapDIA` input): `aggr_Fragment_Annotation`, `aggr_Peak_Area`

**Author(s)**

Peter Blattmann

**Examples**

```
data('Spyogenes', package = 'SWATH2stats')
head(data)
str(data)
```

---

`JPP_update`*Select alternate m\_score column in JPP data and avert user*

---

**Description**

The output from JPP (Rosenberger, Bludau et al. 2017) has not anymore the `m_score` column but an `ProteinName_m_score` and `transition_group_id_m_score`. To make the users aware this function tests if the `m_score` column still exists and selects as default the `transition_group_id_m_score` column.

**Usage**

```
JPP_update(data, mscore.col)
```

**Arguments**

<code>data</code>	Data table that is produced by the OpenSWATH/pyProphet workflow
<code>mscore.col</code>	Defines the column from which to retrieve the <code>m_score</code> . If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between <code>Protein</code> and <code>transition_group m_score</code> .

**Value**

Returns the `mscore.col` that might have been changed to `transition_group_id_m_score` and gives a message to the user.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
JPP_update(OpenSWATH_data, "m_score")
```

---

`load_mart`*load\_mart: Load a biomart database*

---

**Description**

This function loads a biomart database

**Usage**

```
load_mart(species, ensembl.path, mart, verbose = FALSE)
```

**Arguments**

species	The species of the protein identifiers in the term used by biomaRt (e.g. "hsapiens_gene_ensembl", "mmusculus_gene_ensembl", "drerio_gene_ensembl", etc.)
ensembl.path	Path of the biomaRt database.
mart	The species of the protein identifiers in the term used by biomaRt (e.g. "hsapiens_gene_ensembl", "mmusculus_gene_ensembl", "drerio_gene_ensembl", etc.)
verbose	Option to write a file containing the version of the database used.

**Value**

Returns the mart from biomaRt.

**Note**

Protein identifiers from shared peptides should be separated by a forward slash. The host of archived ensembl databases can be introduced as well (e.g. "dec2017.archive.ensembl.org")

**Author(s)**

Peter Blattmann

**Examples**

```
organism.mart <- load_mart("hsapiens_gene_ensembl", "www.ensembl.org", "ENSEMBL_MART_ENSEMBL")
class(organism.mart)
```

---

mscore4assayfdr	<i>Find m_score cutoff to reach a desired FDR on assay level (over the entire OpenSWATH/pyProphet output table)</i>
-----------------	---

---

**Description**

This function estimates the `m_score` cutoff required in a dataset to reach a given overall assay level FDR. It counts target and decoy assays at high resolution across the `m_score` cutoffs and reports a useful `m_score` cutoff - assay FDR pair close to the supplied `fdr_target` level over the entire dataset. The `m_score` cutoff is returned by the function and can be used in the context of the filtering functions, e.g.:

```
data.assayFDR1pc<-filter_mscore(data, mscore4assayfdr(data, fdr_target=0.01))
```

To arrive from decoy counts at an estimation of the false discovery rate (false positives among the targets remaining at a given `mscore` cutoff) the ratio of false positives to true negatives (decoys) (FFT) must be supplied. It is estimated for each run individually by pyProphet and contained in the pyProphet statistics [Injection\_name]\_full\_stat.csv. As an approximation, the FFTs of multiple runs are averaged and supplied as argument FFT. For further details see the Vignette Section 1.3 and 4.1.

For FDR evaluations on peptide and protein level, please refer to functions `mscore4pepfdr` `mscore4protfdr`

**Usage**

```
mscore4assayfdr(data, FFT = 1, fdr_target = 0.01, mscore.col = "m_score")
```

**Arguments**

data	Annotated OpenSWATH/pyProphet data table. See function sample_annotation from this package.
FFT	Ratio of false positives to true negatives, q-values from [Injection_name]_full_stat.csv in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument FFT. Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target).
fdr_target	Assay FDR target, numeric, defaults to 0.01. An m_score cutoff achieving an $FDR < fdr\_target$ will be selected. Calculated as $FDR = (TN * FFT / T)$ ; $TN = decoys$ , $T = targets$ , $FFT = \text{see above}$ .
mscore.col	Defines the column from which to retrieve the m_score. If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group m_score.

**Value**

Returns the m\_score cutoff selected to arrive at the desired FDR

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
mscore4assayfdr(data, FFT=0.7, fdr_target=0.01)
```

---

mscore4pepfd	<i>Find m_score cutoff to reach a desired FDR on peptide level (over the entire OpenSWATH/pyProphet output table)</i>
--------------	---

---

**Description**

This function estimates the m\_score cutoff required in a dataset to reach a given overall peptide level FDR. It counts target and decoy peptides (unique FullPeptideName) at high resolution across the m\_score cutoffs and reports a useful m\_score cutoff - peptide FDR pair close to the supplied fdr\_target level over the entire dataset. The m\_score cutoff is returned by the function and can be used in the context of the filtering functions, e.g.:

```
data.pepFDR2pc <- filter_mscore(data, mscore4pepfd(data, fdr_target=0.02))
```

To arrive from decoy counts at an estimation of the false discovery rate (false positives among the targets remaining at a given m\_score cutoff) the ratio of false positives to true negatives (decoys) (FFT) must be supplied. It is estimated for each run individually by pyProphet and contained in the pyProphet statistics [Injection\_name]\_full\_stat.csv. As an approximation, the FFTs of multiple runs are averaged and supplied as argument FFT. For further details see the Vignette Section 1.3 and 4.1.

For FDR evaluations on assay and protein level, please refer to functions mscore4assayfdr mscore4protfdr

**Usage**

```
mscore4pepfdr(data, FFT = 1, fdr_target = 0.01, mscore.col = "m_score")
```

**Arguments**

<code>data</code>	Annotated OpenSWATH/pyProphet data table. See function <code>sample_annotation</code> from this package.
<code>FFT</code>	Ratio of false positives to true negatives, q-values from <code>[Injection_name]_full_stat.csv</code> in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument <code>FFT</code> . Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target).
<code>fdr_target</code>	FDR target, numeric, defaults to 0.01. An <code>m_score</code> cutoff achieving an $FDR < fdr\_target$ will be selected. Calculated as $FDR = (TN * FFT / T)$ ; <code>TN</code> =decoys, <code>T</code> =targets, <code>FFT</code> =see above.
<code>mscore.col</code>	Defines the column from which to retrieve the <code>m_score</code> . If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group <code>m_score</code> .

**Value**

Returns the `m_score` cutoff selected to arrive at the desired FDR

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
mscore4pepfdr(data, FFT=0.7, fdr_target=0.01)
```

---

<code>mscore4protfdr</code>	<i>Find m_score cutoff to reach a desired FDR on protein level (over the entire OpenSWATH/pyProphet output table)</i>
-----------------------------	---

---

**Description**

This function estimates the `m_score` cutoff required in a dataset to reach a given overall protein level FDR. This filter is to be used with caution as the resulting quantitative matrix is relatively sparse. It can be filled with quantitative values at a lower FDR quality level. It counts target and decoy peptides (unique ProteinName) at high resolution across the `m_score` cutoffs and reports a useful `m_score` cutoff - peptide FDR pair close to the supplied `fdr_target` level over the entire dataset. The `m_score` cutoff is returned by the function and can be used in the context of the filtering functions, e.g.:

```
data.protFDR5pc<-filter_mscore(data, mscore4protfdr(data, fdr_target=0.02))
```

To arrive from decoy counts at an estimation of the false discovery rate (false positives among the targets remaining at a given `m_score` cutoff) the ratio of false positives to true negatives (decoys) (`FFT`) must be supplied. It is estimated for each run individually by pyProphet and contained in



the pyProphet statistics [Injection\_name]\_full\_stat.csv. As an approximation, the FFTs of multiple runs are averaged and supplied as argument FFT. For further details see the Vignette Section 1.3 and 4.1.

For FDR evaluations on assay and peptide level, please refer to functions `mscore4assayfdr` `mscore4pepfdr`

### Usage

```
mscore4protfdr(data, FFT, fdr_target, mscore.col = "m_score")
```

### Arguments

<code>data</code>	Annotated OpenSWATH/pyProphet data table. See function <code>sample_annotation</code> from this package.
<code>FFT</code>	Ratio of false positives to true negatives, q-values from [Injection_name]_full_stat.csv in pyProphet stats output. As an approximation, the q-values of multiple runs are averaged and supplied as argument FFT. Numeric from 0 to 1. Defaults to 1, the most conservative value (1 Decoy indicates 1 False target).
<code>fdr_target</code>	FDR target, numeric, defaults to 0.01. An <code>m_score</code> cutoff achieving an $FDR < fdr\_target$ will be selected. Calculated as $FDR = (TN * FFT / T)$ ; $TN = decoys$ , $T = targets$ , $FFT =$ see above.
<code>mscore.col</code>	Defines the column from which to retrieve the <code>m_score</code> . If you use JPP (Rosenberger, Bludau et al. 2017) this can be used to select between Protein and transition_group <code>m_score</code> .

### Value

Returns the `m_score` cutoff selected to arrive at the desired FDR quality

### Author(s)

Moritz Heusel

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
mscore4protfdr(data, FFT=0.7, fdr_target=0.01)
```

---

MSstats\_data

*Testing dataset in MSstats format*

---

### Description

A small table with the column names corresponding to the MSstats format. This data is intended only to test functions.

### Author(s)

Peter Blattmann

---

OpenSWATH_data	<i>Testing dataset from OpenSWATH</i>
----------------	---------------------------------------

---

**Description**

A small selection of the data obtained from the iPortal pipeline for an experiment with perturbations relating to cholesterol regulation. Protein and Peptides have been anonymized as the data is unpublished. The FDR version of the test data contains modified (lowered) decoy peak group m\_scores to simulate FDR behaviour of a large dataset.

**Author(s)**

Peter Blattmann

---

plot.fdr_cube	<i>Plot functionality for FDR assessment result arrays as produced by e.g. the function assess_fdr_byrun()</i>
---------------	--

---

**Description**

This function creates standard plots from result arrays as produced by e.g. the function assess\_fdr\_byrun(), visualizing assay, peptide and protein level FDR for each run at m-score cutoffs 1e-2 and 1e-3. Furthermore, Target and Decoy ID numbers are visualized.

**Usage**

```
## S3 method for class 'fdr_cube'
plot(x, output = "Rconsole", filename = "FDR_report_byrun",
     plot_mscore_levels = c(0.01, 0.001), ...)
```

**Arguments**

x	Array of by-run FDR assessment results as produced e.g. by the function assess_fdr_byrun() from this package.
output	Choose output type. "pdf_csv" creates the output as files in the working directory, "Rconsole" triggers delivery of the output to the console enabling further computation and/or custom plotting / output.
filename	Basename for output files to be created (if output = "pdf_csv" has been selected).
plot_mscore_levels	Define m-score levels to plot the estimated FDR results.
...	further arguments passed to method.

**Value**

Plots in Rconsole or report files.

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
x <- assess_fdr_byrun(data, FFT=0.7, output = "Rconsole", plot = FALSE)
plot.fdr_cube(x, output = "pdf_csv", filename = "Assess_fdr_byrun_testplot",
plot_mscore_levels=0.01)
```

---

plot.fdr_table	<i>Plot functionality for results of class "fdr_table" as produced by e.g. the function assess_fdr_overall()</i>
----------------	--

---

**Description**

This function created standard plots from results of class "fdr\_table" as produced by e.g. the function assess\_fdr\_overall() visualizing ID numbers in dependence of estimated FDR and also estimated FDR in dependence of m\_score cutoff.

**Usage**

```
## S3 method for class 'fdr_table'
plot(x, output = "Rconsole", filename = "FDR_report_overall", ...)
```

**Arguments**

x	List of class "fdr_table" as produced e.g. by the function assess_fdr_overall() from this package.
output	Choose output type. "pdf_csv" creates the output as files in the working directory, "Rconsole" triggers delivery of the output to the console enabling further computation or custom plotting / output.
filename	Basename for output files to be created (if output = "pdf_csv" has been selected).
...	further arguments passed to method.

**Value**

Plots in Rconsole or report files.

**Author(s)**

Moritz Heusel

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
x <- assess_fdr_overall(data, FFT=0.7, output = "Rconsole", plot = FALSE)
plot.fdr_table(x, output = "pdf_csv", filename = "Assess_fdr_overall_testplot")
```

---

`plot_correlation_between_samples`*Plots the correlation between injections.*

---

### Description

This function plots the Pearson's and Spearman correlation between samples. If decoys are present these are removed before plotting.

### Usage

```
plot_correlation_between_samples(data, column.values = "Intensity",  
Comparison = transition_group_id ~ Condition + BioReplicate,  
fun.aggregate = NULL, label=TRUE, ...)
```

### Arguments

<code>data</code>	Data frame that is produced by the OpenSWATH/pyProphet workflow
<code>column.values</code>	Indicates the columns for which the correlation is assessed. This can be the Intensity or Signal, but also the retention time.
<code>Comparison</code>	The comparison for assessing the variability. Default is to assess the variability per <code>transition_group_id</code> over the different Condition and Replicates. Comparison is performed using the <code>dcast()</code> function of the <code>reshape2</code> package.
<code>fun.aggregate</code>	If for the comparison values have to be aggregated one needs to provide the function here.
<code>label</code>	Option to print correlation value in the plot.
<code>...</code>	further arguments passed to method.

### Value

Plots in Rconsole a correlation heatmap and returns the data frame used to do the plotting.

### Author(s)

Peter Blattmann

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")  
data("Study_design", package="SWATH2stats")  
data <- sample_annotation(OpenSWATH_data, Study_design)  
plot_correlation_between_samples(data)
```

---

plot_variation	<i>Plots the coefficient of variation for different replicates</i>
----------------	--

---

### Description

This function plots the coefficient of variation within replicates for a given value. If decoys are present these are removed before plotting.

### Usage

```
plot_variation(data, column.values = "Intensity",  
Comparison = transition_group_id + Condition ~ BioReplicate,  
fun.aggregate = NULL, label=FALSE, title = "cv across conditions",  
boxplot = TRUE, ...)
```

### Arguments

data	Data frame that is produced by the OpenSWATH/pyProphet workflow
column.values	Indicates the columns for which the variation is assessed. This can be the Intensity or Signal, but also the retention time.
Comparison	The comparison for assessing the variability. Default is to assess the variability per transition_group_id and Condition over the different Replicates. Comparison is performed using the dcast() function of the reshape2 package.
fun.aggregate	If for the comparison values have to be aggregated one needs to provide the function here.
label	Option to print value of median cv.
title	Option to define title of plot.
boxplot	Option to add boxplot.
...	further arguments passed to method.

### Value

Returns a list with the data and calculated cv and a table that summarizes the mean, median and mode cv per Condition (if Condition is contained in the comparison). In addition it plots in Rconsole a violin plot with the observed coefficient of variations.

### Author(s)

Peter Blattmann

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")  
data("Study_design", package="SWATH2stats")  
data <- sample_annotation(OpenSWATH_data, Study_design)  
plot_variation(data)
```

---

`plot_variation_vs_total`*Plots the total variation versus variation within replicates*

---

**Description**

This function plots the total variation and the variation within replicates for a given value. If decoys are present these are removed before plotting.

**Usage**

```
plot_variation_vs_total(data, column.values = "Intensity",
  Comparison1 = transition_group_id ~ BioReplicate + Condition,
  Comparison2 = transition_group_id + Condition ~ BioReplicate,
  fun.aggregate = NULL, label=FALSE,
  title = "coefficient of variation - total versus within replicates",
  boxplot = TRUE,...)
```

**Arguments**

<code>data</code>	Data table that is produced by the OpenSWATH/pyProphet workflow
<code>column.values</code>	Indicates the columns for which the variation is assessed. This can be the Intensity or Signal, but also the retention time.
<code>Comparison1</code>	The comparison for assessing the total variability. Default is to assess the variability per <code>transition_group_id</code> over the combination of Replicates and different Conditions.
<code>Comparison2</code>	The comparison for assessing the variability within the replicates. Default is to assess the variability per <code>transition_group_id</code> and Condition over the different Replicates.
<code>fun.aggregate</code>	If depending on the comparison values have to be aggregated one needs to provide the function here.
<code>label</code>	Option to print value of median cv.
<code>title</code>	Option to define title of plot.
<code>boxplot</code>	Option to add boxplot.
<code>...</code>	further arguments passed to method.

**Value**

Plots in Rconsole a violin plot comparing the total variation with the variation within replicates. In addition it returns the data frame from which the plotting is done and a table with the calculated mean, median and mode of the cv for the total or replicate data.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
plot_variation_vs_total(data)
```

---

```
reduce_OpenSWATH_output
```

*Reduce columns of OpenSWATH data*

---

**Description**

This function selects the columns from the standard OpenSWATH output to column needed for MSstats, aLFQ and mapDIA.

**Usage**

```
reduce_OpenSWATH_output(data, column.names=NULL)
```

**Arguments**

`data` A data frame containing SWATH data.  
`column.names` A vector of column names that can be selected.

**Value**

Returns a data frame with the selected columns.

**Note**

A basic set of columns are defined in the function and are used if no column names are indicated

**Note**

The `column.names` can be omitted and then the following columns are selected that are needed for MSstats and mapDIA analysis: ProteinName, FullPeptideName, Sequence, Charge, aggr\_Fragment\_Annotation, aggr\_Peak\_Area, filename, m\_score, decoy, Intensity, RT. This function should be omitted if the data is analyzed afterwards with the aLFQ or imsbInfer package that needs further columns.

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered <- reduce_OpenSWATH_output(data)
```

---

removeDecoyProteins     *Removes decoy proteins from the protein group label*

---

### Description

There exist peptides annotated as protein groups with 2/ProteinA/DECOY\_ProteinB. However these are in principal proteotypic peptides and should be annotated 1/ProteinA. This function changes these labels accordingly. The subfunction rmDecoyProt removes the Decoy protein, calling removeDecoyProteins also changes the number before the protein group accordingly.

### Usage

```
removeDecoyProteins(data)
```

### Arguments

data                    A data frame containing SWATH data.

### Value

Returns a data frame with changed protein labels.

### Author(s)

Moritz Heusel

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
data.2 <- removeDecoyProteins(data.filtered.decoy)
```

---

sample\_annotation     *sample\_annotation: Annotate the SWATH data with the sample information*

---

### Description

For statistical analysis and filtering the measurements need to be annotated with Filename, Condition, BioReplicate, and Run. This functions takes this information from a txt file containing this meta-data.

### Usage

```
sample_annotation(data, sample.annotation, data.type="OpenSWATH",
column.file = "filename", change.run.id = TRUE, verbose=FALSE)
```



**Arguments**

<code>data</code>	A data frame containing SWATH data.
<code>sample.annotation</code>	A data frame containing the columns: Filename, Condition, BioReplicate, Run. The values contained in the column filename have to be present in the filename of the SWATH data.
<code>data.type</code>	Option to specify the format of the table, if the column names from an OpenSWATH output or MSstats table are used.
<code>column.file</code>	Option to specify the column name where the injection file is specified. Default is set to "filename".
<code>change.run.id</code>	Option to choose if the run\_id column shall be reassigned to a unique value combining the values of Condition, BioReplicate and Run. (Option only possible if data is of format "OpenSWATH")
<code>verbose</code>	Option to turn on reporting on which filename it is working on.

**Value**

Returns a dataframe with each row annotated for the study design

**Author(s)**

Peter Blattmann

**Examples**

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
```

---

Spyogenes

*S.pyogenes example data*

---

**Description**

A table containing SWATH-MS data from *S.pyogenes*

**Source** This table was generated from the original data deposited on PeptideAtlas (PASS00289, file "rawOpenSwathResults\_1pcnt\_only.tsv") by selecting only the column necessary for the SWATH2stats.

**References** Rost, H. L., et al. (2014). OpenSWATH enables automated, targeted analysis of data-independent acquisition MS data. *Nat Biotechnol* 32(3): 219-223.

---

Study_design	<i>Study design table</i>
--------------	---------------------------

---

**Description**

A table containing the meta-data defining the study design.

**Filename** A unique identifier corresponding to the filename in the SWATH data.

**Condition** The Condition explains the perturbation performed on this sample.

**BioReplicate** Number indicating the biological replicate of this sample.

**Run** A unique number for each MS-injection.

**Author(s)**

Peter Blattmann

**Source**

Peter Blattmann

---

transform\_MSstats\_OpenSWATH

*transform\_MSstats\_OpenSWATH: Transforms column names to OpenSWATH column names*

---

**Description**

This functions transforms the column names from a data frame in MSstats format to a data frame with column names used by the OpenSWATH output. The original table needs to contain at least the 10 columns defined by MSstats: ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity.)

**Usage**

```
transform_MSstats_OpenSWATH(data)
```

**Arguments**

data                    A data frame containing the SWATH data in the MSstats format

**Value**

Returns the data frame in the appropriate format.

**Author(s)**

Peter Blattmann

## References

Choi M, Chang CY, Clough T, Broudy D, Killeen T, MacLean B, Vitek O. MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments. *Bioinformatics*. 2014 Sep 1;30(17):2524-6. doi: 10.1093/bioinformatics/btu305.

## Examples

```
data("MSstats_data", package="SWATH2stats")
transform_MSstats_OpenSWATH(MSstats_data)
```

---

unifyProteinGroupLabels

*Unify the protein group labels*

---

## Description

Unify the protein group labels (2/ProteinA/ProteinB and 2/ProteinB/ProteinA) to one common label (e.g. 2/ProteinA/ProteinB)

## Usage

```
unifyProteinGroupLabels(data)
```

## Arguments

data                    A data frame containing SWATH data.

## Value

Returns a data frame with the unified protein labels.

## Author(s)

Moritz Heusel

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")
data("Study_design", package="SWATH2stats")
data <- sample_annotation(OpenSWATH_data, Study_design)
data.filtered.decoy <- filter_mscore(data, 0.01)
data.unified <- unifyProteinGroupLabels(data.filtered.decoy)
```

---

`write_matrix_peptides` *write\_matrix\_peptides: Writes out an overview matrix of peptides mapping to a FDR quality controlled protein master list at controlled global peptide FDR quality.*

---

## Description

Writes out an overview matrix on peptide level of a supplied (unfiltered or prefiltered) OpenSWATH results data frame. The peptide quantification is achieved by summing the areas under all 6 transitions per precursor and summing all precursors per FullPeptideName. In order to keep the peptide-to-protein association, the FullPeptideName is joined with the ProteinName.

## Usage

```
write_matrix_peptides(data, write.csv=FALSE,  
filename = "SWATH2stats_overview_matrix_peptidelevel.csv",  
rm.decoy = FALSE)
```

## Arguments

<code>data</code>	A data frame containing annotated OpenSWATH/pyProphet data.
<code>write.csv</code>	Option to determine if table should be written automatically into csv file.
<code>filename</code>	File base name of the .csv matrix written out to the working folder
<code>rm.decoy</code>	Logical whether decoys will be removed from the data matrix. Defaults to FALSE. It's sometimes useful to know how decoys behave across a dataset and how many you allow into your final table with the current filtering strategy.

## Value

No return value, output .csv matrix is written to the working folder.

## Author(s)

Moritz Heusel

## Examples

```
data("OpenSWATH_data", package="SWATH2stats")  
data("Study_design", package="SWATH2stats")  
data <- sample_annotation(OpenSWATH_data, Study_design)  
write_matrix_peptides(data)
```

---

`write_matrix_proteins` *write\_matrix\_proteins*: Writes out an overview matrix of summed signals per protein identifier (lines) over run\_id(columns).

---

### Description

Writes out an overview matrix on protein level of a supplied (unfiltered or filtered) OpenSWATH results data frame. The protein quantification is achieved by summing the areas under all 6 transitions per precursor, summing all precursors per FullPeptideName and all FullPeptideName signals per ProteinName entry.

This function does not select consistently quantified or top peptides but sums all signals available that may or may not originate from the same set of peptides across different runs. A more detailed overview can be generated using the function `write_matrix_peptides()`.

Peptide selection can be achieved upstream using e.g. the functions `filter_mscore_requant()`, `filter_on_max_peptides()` and `filter_on_min_peptides()`.

### Usage

```
write_matrix_proteins(data, write.csv = FALSE,  
filename = "SWATH2stats_overview_matrix_proteinlevel.csv",  
rm.decoy = FALSE)
```

### Arguments

<code>data</code>	A data frame containing annotated OpenSWATH/pyProphet data.
<code>write.csv</code>	Option to determine if table should be written automatically into csv file.
<code>filename</code>	File base name of the .csv matrix written out to the working folder
<code>rm.decoy</code>	Logical whether decoys will be removed from the data matrix. Defaults to FALSE. It's sometimes useful to know how decoys behave across a dataset and how many you allow into your final table with the current filtering strategy.

### Value

No return value, output .csv matrix is written to the working folder.

### Author(s)

Moritz Heusel

### Examples

```
data("OpenSWATH_data", package="SWATH2stats")  
data("Study_design", package="SWATH2stats")  
data <- sample_annotation(OpenSWATH_data, Study_design)  
write_matrix_proteins(data)
```

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