

Package ‘lipidr’

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Title Data Mining and Analysis of Lipidomics Datasets

Version 2.13.0

Description lipidr an easy-to-use R package implementing a complete workflow for downstream analysis of targeted and untargeted lipidomics data. lipidomics results can be imported into lipidr as a numerical matrix or a Skyline export, allowing integration into current analysis frameworks. Data mining of lipidomics datasets is enabled through integration with Metabolomics Workbench API. lipidr allows data inspection, normalization, univariate and multivariate analysis, displaying informative visualizations. lipidr also implements a novel Lipid Set Enrichment Analysis (LSEA), harnessing molecular information such as lipid class, total chain length and unsaturation.

Depends R (>= 3.6.0), SummarizedExperiment

Imports methods, stats, utils, data.table, S4Vectors, rlang, dplyr, tidy, forcats, ggplot2, limma, fgsea, ropis, imputeLCMD, magrittr

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`lipidr-package`*Analysis workflow for targeted lipidomics*

Description

`lipidr` implements a series of functions to facilitate inspection, analysis and visualization of targeted lipidomics datasets. `lipidr` takes exported Skyline CSV as input, allowing for multiple methods to be analyzed together.

Details

`lipidr` represents Skyline files as `SummarizedExperiment` objects, which can easily be integrated with a wide variety of Bioconductor packages. Sample annotations, such as sample group or other clinical information can be loaded. `lipidr` generates various plots, such as PCA score plots and box plots, for quality control of samples and measured lipids. Normalization methods with and without internal standards are also supported.

Differential analysis can be performed using any of the loaded clinical variables, which can be readily visualized as volcano plots. A novel lipid set enrichment analysis (LSEA) is implemented to detect preferential enrichment of certain lipid classes, total chain lengths or unsaturation patterns. Plots for the visualization of enrichment results are also implemented.

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`add_sample_annotation` *Add sample annotation to Skyline data frame*

Description

Add sample annotation to Skyline data frame

Usage

```
add_sample_annotation(data, annot_file)
```

Arguments

`data` LipidomicsExperiment object.
`annot_file` CSV file or a data.frame with at least 2 columns, sample names & group(s).

Value

LipidomicsExperiment with sample group information.

Examples

```
datadir <- system.file("extdata", package = "lipidr")

# all csv files
filelist <- list.files(datadir, "data.csv", full.names = TRUE)
d <- read_skyline(filelist)

# Add clinical info to existing LipidomicsExperiment object
clinical_file <- system.file("extdata", "clin.csv", package = "lipidr")
d <- add_sample_annotation(d, clinical_file)
colData(d)
d$group

# Subset samples using clinical information
# Note we are subsetting columns
d[, d$group == "QC"]

# Subset lipids using lipid annotation
# Note we are subsetting rows
d[rowData(d)$istd, ]
```

annotate_lipids

Parse molecule names to extract lipid class and chain information.

Description

Parse lipid names to return a data.frame containing lipid class, total chain length and unsaturation. Lipids should follow the pattern 'class xx:x/yy:y', with class referring to the abbreviated lipid class, xx:x as the composition of the first chain and yy:y as the second chain. Alternatively, lipids can be supplied following the pattern 'class zz:z', where zz:z indicates the combined chain length and unsaturation information.

Usage

```
annotate_lipids(molecules, no_match = c("warn", "remove", "ignore"))
```

Arguments

| | |
|-----------|--------------------------------------------------------------------------|
| molecules | A character vector containing lipid molecule names. |
| no_match | How to handle lipids that cannot be parsed? Default is to give warnings. |

Value

A data.frame with lipid annotations as columns. Input lipid names are given in a column named "Molecule".

Examples

```
lipid_list <- c(
  "Lyso PE 18:1(d7)",
  "PE(32:0)",
  "Cer(d18:0/C22:0)",
  "TG(16:0/18:1/18:1)"
)
annotate_lipids(lipid_list)
```

as_lipidomics_experiment

Convert data.frame/matrix to LipidomicsExperiment

Description

Convert data.frame/matrix to LipidomicsExperiment

Usage

```
as_lipidomics_experiment(df, logged = FALSE, normalized = FALSE)
```

Arguments

| | |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| df | A data.frame or matrix where rows are lipids and columns are samples. Lipid names should be provided in the first column or in rownames of df. Measurements should be numeric. The data is considered summarized unless at least one lipid is duplicated (has > 1 row). |
| logged | Whether the data is log-transformed |
| normalized | Whether the data is normalized |

Value

LipidomicsExperiment

data_normalized

Example dataset (normalized and log2 transformed)

Description

A dataset containing MRM mass spectrometry-based lipidomics data from murine serum samples. Mice were fed a normal or high-fat diet and had access to normal drinking water or drinking water containing the bile acid deoxycholic acid. Lipid peaks were integrated using Skyline and exported results were imported into R using `lipidr`. The dataset has been normalized and log2 transformed. Please see [normalize_pqn](#) for details on how to generate this dataset.

Usage

```
data(data_normalized)
```

Format

An object of class `LipidomicsExperiment` with 278 rows and 56 columns.

See Also

Other lipidr datasets: [lipidDefaults](#), [lipidnames_pattern](#), [lipidr-data](#)

Examples

```
data(data_normalized)
```

 de_analysis

Differential analysis of lipids between sample groups

Description

`de_analysis` and `de_design` perform differential analysis of measured lipids that are associated with a sample group (annotation). `de_analysis` accepts a list of contrasts, while `de_design` allows users to define a design matrix, useful for complex experimental designs or for adjusting possible confounding variables.

Usage

```
de_analysis(data, ..., measure = "Area", group_col = NULL)
```

```
de_design(data, design, ..., coef = NULL, measure = "Area")
```

```
significant_molecules(de.results, p.cutoff = 0.05, logFC.cutoff = 1)
```

```
plot_results_volcano(de.results, show.labels = TRUE)
```

Arguments

| | |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>data</code> | LipidomicsExperiment object, should be normalized and log2 transformed. |
| <code>...</code> | Expressions, or character strings which can be parsed to expressions, specifying contrasts. These are passed to <code>limma::makeContrasts</code> . |
| <code>measure</code> | Which measure to use as intensity, usually Area (default). |
| <code>group_col</code> | Name of the column containing sample groups. If not provided, defaults to first sample annotation column. |
| <code>design</code> | Design matrix generated from <code>model.matrix()</code> , or a design formula. |
| <code>coef</code> | Column number or column name specifying which coefficient of the linear model is of interest. |

| | |
|--------------|----------------------------------------------------------------------------------------------------|
| de.results | Output of <code>de_analysis()</code> . |
| p.cutoff | Significance threshold. Default is 0.05. |
| logFC.cutoff | Cutoff limit for log2 fold change. Default is 1. Ignored in multi-group (ANOVA-style) comparisons. |
| show.labels | Whether labels should be displayed for significant lipids. Default is TRUE. |

Value

TopTable as returned by limma package

significant_molecules returns a character vector with names of significantly differentially changed lipids.

plot_results_volcano returns a ggplot object.

Functions

- significant_molecules: gets a list of significantly changed lipids for each contrast.
- plot_results_volcano: plots a volcano chart for differential analysis results.

Examples

```
# type ?normalize_pqn to see how to normalize and log2-transform your data
data(data_normalized)

# Specifying contrasts
de_results <- de_analysis(
  data_normalized,
  HighFat_water - NormalDiet_water,
  measure = "Area"
)

# Using formula
de_results_formula <- de_design(
  data = data_normalized,
  design = ~group,
  coef = "groupHighFat_water",
  measure = "Area"
)

# Using design matrix
design <- model.matrix(~group, data = colData(data_normalized))
de_results_design <- de_design(
  data = data_normalized,
  design = design,
  coef = "groupHighFat_water",
  measure = "Area"
)
significant_molecules(de_results)
plot_results_volcano(de_results, show.labels = FALSE)
```

| | |
|--------------|---------------------------------------------------------|
| filter_by_cv | <i>Remove molecules with CV larger than a threshold</i> |
|--------------|---------------------------------------------------------|

Description

Remove molecules with CV larger than a threshold

Usage

```
filter_by_cv(data, cv.cutoff = 20, measure = "Area")
```

Arguments

| | |
|-----------|-------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| cv.cutoff | CV threshold (numeric). Default is 20. |
| measure | Which measure used to calculate CV, usually Area (default). |

Value

LipidomicsExperiment object with molecules filtered.

Examples

```
data(data_normalized)  
filter_by_cv(data_normalized)
```

| | |
|---------------|------------------------------------------------------|
| gen_lipidsets | <i>Generate lipid sets from lipid molecule names</i> |
|---------------|------------------------------------------------------|

Description

Generate lipid sets from lipid molecule names

Usage

```
gen_lipidsets(molecules, min_size = 2)
```

Arguments

| | |
|-----------|--------------------------------------------------------------------|
| molecules | A character vector containing lipid molecule names. |
| min_size | Minimum number of molecules in a set to be included in enrichment. |

Value

List of lipid sets

Examples

```
data(data_normalized)
molecules <- rowData(data_normalized)$Molecule
gen_lipidsets(molecules)
```

| | |
|-----------|--------------------------------------------------------|
| impute_na | <i>Impute missing values in a LipidomicsExperiment</i> |
|-----------|--------------------------------------------------------|

Description

Impute missing values in a LipidomicsExperiment

Usage

```
impute_na(
  data,
  measure = "Area",
  method = c("knn", "svd", "mle", "QRILC", "minDet", "minProb", "zero"),
  ...
)
```

Arguments

| | |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to use as intensity, usually Area, Area Normalized or Height. Default is Area. |
| method | The imputation method to use. All methods are wrappers for imputeLCMD package. These include <ul style="list-style-type: none"> • knn Wraps <code>imputeLCMD::impute.wrapper.KNN()</code>. Default. This requires an additional argument K (Number of neighbors used to infer the missing data). • svd Wraps <code>imputeLCMD::impute.wrapper.SVD()</code>. This requires an additional argument K (Number of principal components to use). • mle Wraps <code>imputeLCMD::impute.wrapper.MLE()</code>, • minDet Wraps <code>imputeLCMD::impute.MinDet()</code>, • minProb Wraps <code>imputeLCMD::impute.MinProb()</code>, • zero Wraps <code>imputeLCMD::impute.ZERO()</code>, |
| ... | Other arguments passed to the imputation method. |

Value

LipidomicsExperiment object with missing values imputed.

Examples

```
data(data_normalized)

# Replace with values calculated using K-nearest neighbors
impute_na(data_normalized, "Area", "knn", 10)

# Replace with values calculated from the first K principal components
impute_na(data_normalized, "Area", "svd", 3)

# Replace with Maximum likelihood estimates
impute_na(data_normalized, "Area", "mle")

# Replace with randomly drawn values from a truncated distribution
impute_na(data_normalized, "Area", "QRILC")

# Replace with a minimal value
impute_na(data_normalized, "Area", "minDet")

# Replace with randomly drawn values from a Gaussian distribution
# centered around a minimal value
impute_na(data_normalized, "Area", "minProb")

# Replace with zero (not recommended)
impute_na(data_normalized, "Area", "zero")
```

is_logged

Functions to get and set attributes of LipidomicsExperiment objects

Description

Functions to get and set attributes of LipidomicsExperiment objects

Usage

```
is_logged(data, measure)

set_logged(data, measure, val)

is_normalized(data, measure)

set_normalized(data, measure, val)

is_summarized(data)

set_summarized(data, val)
```

Arguments

| | |
|---------|-------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to get / set attributes of. |
| val | Value to be assigned to the attribute. |

Value

Modified LipidomicsExperiment.

Examples

```
data(data_normalized)
is_logged(data_normalized, "Area")
is_summarized(data_normalized)
```

| | |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| lipidDefaults | <i>Default values for lipidr internal functions A set of default mappings and annotation used internally to correctly parse lipid molecule names.</i> |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|

Description

Default values for lipidr internal functions A set of default mappings and annotation used internally to correctly parse lipid molecule names.

Usage

```
data(lipidDefaults)
```

Format

An object of class list of length 2.

See Also

Other lipidr datasets: [data_normalized](#), [lipidnames_pattern](#), [lipidr-data](#)

Examples

```
data(lipidDefaults)
```

lipidnames_pattern *Patterns used in parsing lipid names*

Description

A collection of patterns to extract lipid class and chain information from lipid names. Used internally by the package.

Usage

```
data(lipidnames_pattern)
```

Format

An object of class `list` of length 8.

See Also

Other lipidr datasets: [data_normalized](#), [lipidDefaults](#), [lipidr-data](#)

Examples

```
data(lipidnames_pattern)
```

LipidomicsExperiment *Constructor for Lipidomics experiment from list of assays*

Description

Constructor for Lipidomics experiment from list of assays

Usage

```
LipidomicsExperiment(assay_list, metadata, colData = NULL, rowData = NULL)
```

Arguments

| | |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| assay_list | A list or SimpleList of matrix-like elements, or a matrix-like object. Passed to SummarizedExperiment() . |
| metadata | A list containing arbitrary information about the experiment. It should at least contain 2 elements: <ul style="list-style-type: none"> • dimnames 2-element character vector with dimension names • summarized Has transitions been summarized? |
| colData | An optional DataFrame describing the samples (contains clinical information). Row names, if present, become the column names of the LipidomicsExperiment. |

rowData A DataFrame object describing the rows (contains generated lipid annotations). Row names, if present, become the row names of the SummarizedExperiment object. The number of rows of the DataFrame must be equal to the number of rows of the matrices in assays.

Value

LipidomicsExperiment object

LipidomicsExperiment-class

LipidomicsExperiment object

Description

LipidomicsExperiment object

lipidr-data

Description of lipidr datasets

Description

lipidr-package has 3 datasets:

- data_normalized Example lipidomics dataset, normalized & log2-transformed.
- lipidDefaults A list of default mappings and annotations for lipids.
- lipidnames_pattern A list of patterns used in parsing lipid names.

See below for detailed description of each dataset.

See Also

Other lipidr datasets: [data_normalized](#), [lipidDefaults](#), [lipidnames_pattern](#)

Examples

```
data(data_normalized)
```

| | |
|-----------------|-------------------------------------------|
| list_mw_studies | <i>Metabolomics Workbench integration</i> |
|-----------------|-------------------------------------------|

Description

These functions use Metabolomics Workbench REST API to support data mining of publicly available lipidomics datasets.

Usage

```
list_mw_studies(keyword = "lipid")
```

```
fetch_mw_study(study_id)
```

```
read_mwTab(mwTab)
```

```
read_mw_datamatrix(file)
```

Arguments

| | |
|----------|------------------------------------------------------------|
| keyword | A keyword to search for in Metabolomics Workbench studies. |
| study_id | The Metabolomics Workbench study ID. |
| mwTab | File path or url for a mwTab file. |
| file | File path or url for the file containing the data matrix. |

Value

list_mw_studies returns a data frame with studies matching the keyword. Study ID, title, author and details are retrieved.

All other functions return a LipidomicsExperiment object containing clinical and lipid intensity data.

Functions

- list_mw_studies: retrieves a list of lipidomics studies from Metabolomics Workbench.
- fetch_mw_study: downloads and parse full data for a study from Metabolomics Workbench using a study ID. The function returns a LipidomicsExperiment where users can directly apply lipidr analysis workflow.
- read_mwTab: parses mwTab file into a LipidomicsExperiment.
- read_mw_datamatrix: parses a Metabolomics Workbench data matrix into a LipidomicsExperiment. Data matrix downloaded from Metabolomics Workbench are parsed into a LipidomicsExperiment object to enable lipidr workflow analysis.

Examples

```
## Not run:
list_mw_studies()

## End(Not run)
## Not run:
fetch_mw_study("ST001111")

## End(Not run)
```

lsea

Lipid set enrichment analysis (LSEA)

Description

Lipid set enrichment analysis (LSEA)

Usage

```
lsea(
  de.results,
  rank.by = c("logFC", "P.Value", "adj.P.Val"),
  min_size = 2,
  ...
)

significant_lipidsets(enrich.results, p.cutoff = 0.05, size.cutoff = 2)

plot_class_enrichment(de.results, significant.sets, measure = "logFC")

plot_enrichment(
  de.results,
  significant.sets,
  annotation = c("class", "length", "unsat"),
  measure = "logFC"
)
```

Arguments

| | |
|----------------|--------------------------------------------------------------------|
| de.results | Output of de_analysis() . |
| rank.by | Statistic used to rank the lipid list. Default is logFC. |
| min_size | Minimum number of molecules in a set to be included in enrichment. |
| ... | Extra parameters passed to fgsea::fgsea() . |
| enrich.results | Output of lsea() . |
| p.cutoff | Significance threshold. Default is 0.05. |

| | |
|-------------------------------|---------------------------------------------------------------------------------------------|
| <code>size.cutoff</code> | Minimum number of lipids in a set tested for enrichment. Default is 2. |
| <code>significant.sets</code> | List of significantly changed lipid sets (output of <code>significant_lipidsets()</code>). |
| <code>measure</code> | Which measure to plot the distribution of: logFC, P.Value, Adj.P.Val. Default is logFC. |
| <code>annotation</code> | Which lipid set collection to plot. |

Value

`lsea` returns enrichment results (data.frame) as returned from `fgsea::fgsea()`. The results also contain the following attributes:

- `de.results` Original `de.results` input.
- `rank.by` Measure used to rank lipid molecules.
- `sets` Lipid sets tested, with their member molecules.

`significant_lipidsets` returns a list of character vectors of significantly enriched sets for each contrast.

`plot_enrichment` returns a ggplot object.

Functions

- `significant_lipidsets`: gets a list of significantly changed lipid sets
- `plot_enrichment`: is usually used to look at log2 fold change distribution of lipids in each class, chain length or unsaturation, marking significantly enriched sets. It can also be used to plot P.Value or Adj.P.Val.

Examples

```
data(data_normalized)
de_results <- de_analysis(
  data_normalized,
  HighFat_water - NormalDiet_water,
  measure = "Area"
)
enrich_results <- lsea(
  de_results,
  rank.by = "logFC", min_size = 4, nperm = 1000
)
sig_lipidsets <- significant_lipidsets(enrich_results)
plot_enrichment(de_results, sig_lipidsets, annotation="class")
plot_enrichment(de_results, sig_lipidsets, annotation="length")
```


Description

mva performs multivariate analysis using several possible methods. The available methods are PCA, PCoA, OPLS and OPLS-DA. The OPLS method requires a numeric y-variable, whilst OPLS-DA requires two groups for comparison. By default, for OPLS and OPLS-DA the number of predictive and orthogonal components are set to 1. Blank samples are automatically detected (using TIC) and excluded. Missing data are imputed using average lipid intensity across all samples.

Usage

```
mva(  
  data,  
  measure = "Area",  
  method = c("PCA", "PCoA", "OPLS", "OPLS-DA"),  
  group_col = NULL,  
  groups = NULL,  
  ...  
)
```

```
plot_mva(  
  mvaresults,  
  components = c(1, 2),  
  color_by = NULL,  
  ellipse = TRUE,  
  hotelling = TRUE  
)
```

```
plot_mva_loadings(  
  mvaresults,  
  components = c(1, 2),  
  color_by = NULL,  
  top.n = nrow(mvaresults$loadings)  
)
```

```
top_lipids(mvaresults, top.n = 10)
```

Arguments

| | |
|---------|---------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to use as intensity, usually Area (default). The measure should be already summarized and normalized. |
| method | Either PCA, PCoA, OPLS or OPLS-DA. Default is PCA. |

| | |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| <code>group_col</code> | Sample annotation to use as grouping column. If not provided, samples are treated independently. |
| <code>groups</code> | A numeric grouping (OPLS) or two groups to be used for supervised analysis (OPLS-DA), ignored in other methods. |
| <code>...</code> | Extra arguments to be passed to <code>opls()</code> for OPLS-DA, ignored in other methods. |
| <code>mvaresults</code> | Results obtained from <code>mva()</code> . |
| <code>components</code> | Which components to plot. Ignored for PCoA, OPLS and OPLS-DA results. Default is first 2 components. |
| <code>color_by</code> | Sample annotation (or lipid annotation in case of <code>plot_mva_loadings</code>) to use as color. Defaults to individual samples / lipids |
| <code>ellipse</code> | Whether to plot ellipses around groups |
| <code>hotelling</code> | Whether to plot Hotelling T2. |
| <code>top.n</code> | Number of top ranked features to highlight in the plot. If omitted, returns top 10 lipids. |

Value

Multivariate analysis results in `mvaresults` object. The object contains the following:

- `scores` Sample scores
- `loadings` Feature or component loadings (not for PCoA)
- `method` Multivariate method that was used
- `row_data` Lipid molecule annotations
- `col_data` Sample annotations
- `original_object` Original output object as returned by corresponding analysis methods

`plot_mva` returns a `ggplot` of the sample scores.

`plot_mva_loadings` returns a `ggplot` of the loadings.

`top_lipids` returns a dataframe of `top.n` lipids with their annotations.

Functions

- `plot_mva`: plots a multivariate scatterplot of sample scores to investigate sample clustering.
- `plot_mva_loadings`: Plot a multivariate scatterplot of feature loadings to investigate feature importance.
- `top_lipids`: extracts top lipids from OPLS-DA results

Examples

```
data(data_normalized)

# PCA
mvaresults <- mva(data_normalized, measure = "Area", method = "PCA")
plot_mva(mvaresults, color_by = "group")
```

```
# NOT RUN
# plot_mva(mvareults, color_by = "Diet", components = c(2, 3))

# PCoA
mvareults <- mva(data_normalized, measure = "Area", method = "PCoA")
# NOT RUN
# plot_mva(mvareults, color_by = "group")

# OPLS-DA
mvareults <- mva(
  data_normalized,
  method = "OPLS-DA", group_col = "Diet", groups = c("HighFat", "Normal")
)
plot_mva(mvareults, color_by = "group")
plot_mva_loadings(mvareults, color_by = "Class", top.n = 10)
top_lipids(mvareults, top.n = 10)
```

non_parsed_molecules *Get a list of molecules that couldn't be parsed by lipidr*

Description

Get a list of molecules that couldn't be parsed by lipidr

Usage

```
non_parsed_molecules(data)
```

Arguments

data LipidomicsExperiment object.

Value

A character vector of the molecule names that could not be parsed.

Examples

```
data(data_normalized)
non_parsed_molecules(data_normalized)
```

| | |
|----------------|------------------------------------------------------------------------|
| normalize_istd | <i>Normalize each class by its corresponding internal standard(s).</i> |
|----------------|------------------------------------------------------------------------|

Description

Normalize each class by its corresponding internal standard(s). Lipid classes are normalized using corresponding internal standard(s) of the same lipid class. If no corresponding internal standard is found the average of all measured internal standards is used instead.

Usage

```
normalize_istd(data, measure = "Area", exclude = "blank", log = TRUE)
```

Arguments

| | |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to use as intensity, usually Area, Area Normalized or Height. Default is Area. |
| exclude | Samples to exclude, can be either: "blank" - automatically detected blank samples and exclude them logical vector with the same length as samples. Default. |
| log | whether the normalized values should be log ₂ transformed. Default is TRUE. |

Value

A LipidomicsExperiment object with normalized values. Each molecule is normalized against the internal standard from the same class.

Examples

```
datadir <- system.file("extdata", package = "lipidr")
filelist <- list.files(datadir, "data.csv", full.names = TRUE)
d <- read_skyline(filelist)
clinical_file <- system.file("extdata", "clin.csv", package = "lipidr")
d <- add_sample_annotation(d, clinical_file)
d_summarized <- summarize_transitions(d, method = "average")

# Normalize data that have been summarized (single value per molecule).
data_norm_istd <- normalize_istd(
  d_summarized,
  measure = "Area", exclude = "blank", log = TRUE
)
```

| | |
|---------------|----------------------------------------------------------------------|
| normalize_pqn | <i>Perform Probabilistic Quotient Normalization for intensities.</i> |
|---------------|----------------------------------------------------------------------|

Description

Perform Probabilistic Quotient Normalization (PQN) for sample intensities. The PQN method determines a dilution factor for each sample by comparing the distribution of quotients between samples and a reference spectrum, followed by sample normalization using this dilution factor. The reference spectrum in this method is the average lipid abundance of all samples (excluding blanks).

Usage

```
normalize_pqn(data, measure = "Area", exclude = "blank", log = TRUE)
```

Arguments

| | |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to use as intensity, usually Area, Area Normalized or Height. Default is Area. |
| exclude | Samples to exclude, can be either: "blank" - automatically detected blank samples and exclude them logical vector with the same length as samples. Default. |
| log | Whether the normalized values should be log2 transformed. Default is TRUE. |

Value

A LipidomicsExperiment object with normalized values

References

Dieterle, F., Ross, A., Schlotterbeck, G., & Senn, H. (2006). Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. *Analytical chemistry*, 78(13), 4281-4290.

Examples

```
datadir <- system.file("extdata", package = "lipidr")
filelist <- list.files(datadir, "data.csv", full.names = TRUE)
d <- read_skyline(filelist)
clinical_file <- system.file("extdata", "clin.csv", package = "lipidr")
d <- add_sample_annotation(d, clinical_file)
d_summarized <- summarize_transitions(d, method = "average")

# Normalize data that have been summarized (single value per molecule).
data_normalized <- normalize_pqn(
  d_summarized,
  measure = "Area", exclude = "blank", log = TRUE
)
```

`plot_chain_distribution`*Plot logFC of lipids per class showing chain information*

Description

Plot a chart of (log2) fold changes of lipids per class showing chain lengths and unsaturations. If multiple molecules with the same total chain length and unsaturation are present in the dataset, the measure is averaged, and the number of molecules is indicated on the plot.

Usage

```
plot_chain_distribution(de_results, contrast = NULL, measure = "logFC")
```

Arguments

| | |
|-------------------------|----------------------------------------------------------------------------------------|
| <code>de_results</code> | Output of <code>de_analysis()</code> . |
| <code>contrast</code> | Which comparison to plot. if not provided, defaults to the the first comparison. |
| <code>measure</code> | Which measure to plot the distribution of: logFC, P.Value, Adj.P.Val. Default is logFC |

Value

A ggplot object.

Examples

```
data(data_normalized)
de_results <- de_analysis(
  data_normalized,
  HighFat_water - NormalDiet_water,
  measure = "Area"
)
plot_chain_distribution(de_results)
```

`plot_heatmap`*Plot an annotated heatmap Plots a hierarchically clustered heatmap showing selected sample and lipid molecule annotations.*

Description

Plot an annotated heatmap Plots a hierarchically clustered heatmap showing selected sample and lipid molecule annotations.

Usage

```
plot_heatmap(  
  data,  
  measure = "Area",  
  molecule_annotation = "Class",  
  sample_annotation = "all",  
  cluster_cols = "hclust",  
  cluster_rows = "hclust",  
  scale = "rows",  
  ...  
)
```

Arguments

| | |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| measure | Which measure to plot the distribution of: usually Area, Area Normalized, Height or Retention Time. Default is Area. |
| molecule_annotation | The column name for lipid annotation, default to Class. |
| sample_annotation | The column name for sample annotation, default to all. |
| cluster_cols | "none", "hclust", or "k-means" for no clustering, hierarchical clustering, and k-means clustering of rows respectively. Default is "hclust". |
| cluster_rows | "none", "hclust", or "k-means" for no clustering, hierarchical clustering, and k-means clustering of rows respectively. Default is "hclust". |
| scale | character indicating if the values should be centered and scaled in either the row direction or the column direction, or none. Corresponding values are "row", "cols" and "none". |
| ... | Additional parameters passed to <code>iheatmapr::iheatmap()</code> . |

Value

A heatmap plot

Examples

```
data(data_normalized)  
plot_heatmap(data_normalized, sample_annotation = "group")
```

plot_lipidclass *Informative plots to investigate lipid classes*

Description

lipidr supports two types of plots for to visualize at lipid classes.

sd plots a bar chart for standard deviation of a certain measure in each class. This plot type is usually used to look at standard deviations of intensity in each class, but can also be used to look at different measures such as Retention Time, to ensure all lipids are eluted within the expected range. To assess instrumental variation apply the function to technical quality control samples.

boxplot Plots a boxplot chart to examine the distribution of values per class. This plot type is usually used to look at the intensity distribution in each class, but can also be used to look at different measures, such as Retention Time or Background.

Usage

```
plot_lipidclass(data, type = c("boxplot", "sd"), measure = "Area", log = TRUE)
```

Arguments

| | |
|---------|---------------------------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| type | plot type, either boxplot or sd. Default is boxplot. |
| measure | Which measure to plot the distribution of: usually Area, Area Normalized, Height or Retention Time. Default is Area |
| log | Whether values should be log2 transformed. Default is TRUE (Set FALSE for retention time). |

Value

A ggplot object.

Examples

```
data(data_normalized)

d_qc <- data_normalized[, data_normalized$group == "QC"]
plot_lipidclass(d_qc, "sd", "Area", log = TRUE)
plot_lipidclass(d_qc, "sd", "Retention Time", log = FALSE)
plot_lipidclass(d_qc, "boxplot", "Area", log = TRUE)
plot_lipidclass(d_qc, "boxplot", "Retention Time", log = FALSE)
```

| | |
|----------------|--------------------------------------------------------------------|
| plot_molecules | <i>Informative plots to investigate individual lipid molecules</i> |
|----------------|--------------------------------------------------------------------|

Description

lipidr supports three types of plots for to visualize at lipid molecules.

cv plots a bar chart for coefficient of variation of lipid molecules. This plot type is usually used to investigate the CV in lipid intensity or retention time, in QC samples.

sd plots a bar chart for standard deviations of a certain measure in each lipid. This plot type is usually used to look at standard deviation of intensity for each lipid, but can also be used to look at different measures such as Retention Time, to ensure all lipids elute within expected range.

boxplot plots a boxplot chart to examine the distribution of values per lipid. This plot type is usually used to look at intensity distribution for each lipid, but can also be used to look at different measures, such as Retention Time or Background.

Usage

```
plot_molecules(  
  data,  
  type = c("cv", "sd", "boxplot"),  
  measure = "Area",  
  log = TRUE,  
  color = "Class"  
)
```

Arguments

| | |
|---------|-----------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| type | plot type, either cv, sd or boxplot. Default is cv. |
| measure | Which measure to plot the distribution of: usually Area, Area Normalized or Height. Default is Area |
| log | Whether values should be log2 transformed (Set FALSE for retention time). Default is TRUE |
| color | The column name of a row annotation to be used as color |

Value

A ggplot object.

Examples

```

data(data_normalized)
d_qc <- data_normalized[, data_normalized$group == "QC"]

# plot the variation in intensity and retention time of all measured
# lipids in QC samples
plot_molecules(d_qc, "cv", "Area")
plot_molecules(d_qc, "cv", "Retention Time", log = FALSE)

# plot the variation in intensity, RT of ISTD (internal standards)
# in QC samples
d_istd_qc <- data_normalized[
  rowData(data_normalized)$istd,
  data_normalized$group == "QC"
]
plot_molecules(d_istd_qc, "sd", "Area")
plot_molecules(d_istd_qc, "sd", "Retention Time", log = FALSE)

plot_molecules(d_istd_qc, "boxplot")
plot_molecules(d_istd_qc, "boxplot", "Retention Time", log = FALSE)

```

plot_samples

Informative plots to investigate samples

Description

lipidr supports two types of plots for sample quality checking.

tic plots a bar chart for total sample intensity.

boxplot plots a boxplot chart to examine the distribution of values per sample.

Usage

```

plot_samples(
  data,
  type = c("tic", "boxplot"),
  measure = "Area",
  log = TRUE,
  color = NULL
)

```

Arguments

| | |
|---------|---------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| type | plot type, either tic or boxplot. Default is tic. |
| measure | Which measure to use as intensity, usually Area, Area Normalized or Height. Default is Area |

log Whether values should be log2 transformed. Default is TRUE
 color The column name of a sample annotation to be used as color

Value

A ggplot object.

Examples

```
data(data_normalized)

plot_samples(data_normalized, type = "tic", "Area", log = TRUE)
plot_samples(data_normalized, type = "tic", "Background", log = FALSE)
plot_samples(
  data_normalized[, data_normalized$group == "QC"],
  type = "boxplot",
  measure = "Retention Time", log = FALSE
)
```

 plot_trend

Plot a regulation trend line between logFC and chain annotation

Description

Fit and plot a regression line of (log2) fold changes and total chain lengths or unsaturations. If multiple comparisons are included, one regression is plotted for each.

Usage

```
plot_trend(de_results, annotation = c("length", "unsat"))
```

Arguments

de_results Output of `de_analysis`.
 annotation Whether to fit trend line against chain length or unsat.

Value

A ggplot object.

Examples

```
data(data_normalized)
de_results <- de_analysis(
  data_normalized,
  HighFat_water - NormalDiet_water,
  NormalDiet_DCA - NormalDiet_water,
  measure = "Area"
)
plot_trend(de_results, "length")
```

| | |
|--------------|------------------------------------|
| read_skyline | <i>Read Skyline exported files</i> |
|--------------|------------------------------------|

Description

Read Skyline exported files

Usage

```
read_skyline(files)
```

Arguments

files Character vector with filepaths to Skyline exported files in CSV format.

Value

LipidomicsExperiment object.

Examples

```
datadir <- system.file("extdata", package = "lipidr")  
  
# all csv files  
filelist <- list.files(datadir, "data.csv", full.names = TRUE)  
d <- read_skyline(filelist)  
  
# View automatically generated lipid annotations  
rowData(d)
```

| | |
|-----------------------------|----------------------------------------------------------------------------|
| remove_non_parsed_molecules | <i>Remove molecules that couldn't be parsed by lipidr from the dataset</i> |
|-----------------------------|----------------------------------------------------------------------------|

Description

Remove molecules that couldn't be parsed by lipidr from the dataset

Usage

```
remove_non_parsed_molecules(data)
```

Arguments

data LipidomicsExperiment object.

Value

A filtered LipidomicsExperiment object.

Examples

```
data(data_normalized)
remove_non_parsed_molecules(data_normalized)
```

summarize_transitions *Summarize transitions*

Description

Calculate a single intensity for molecules with multiple transitions, by determining the average or maximum intensity.

Usage

```
summarize_transitions(data, method = c("max", "average"))
```

Arguments

| | |
|--------|-----------------------------------------------------------------------------------------------------|
| data | LipidomicsExperiment object. |
| method | Choose to summarize multiple transitions by taking the average or maximum intensity. Default is max |

Value

A LipidomicsExperiment object with single intensities per lipid molecule

Examples

```
datadir <- system.file("extdata", package = "lipidr")
filelist <- list.files(datadir, "data.csv", full.names = TRUE)
d <- read_skyline(filelist)
clinical_file <- system.file("extdata", "clin.csv", package = "lipidr")
d <- add_sample_annotation(d, clinical_file)
d_summarized <- summarize_transitions(d, method = "average")
```

update_molecule_names *Rename molecules in a dataset.*

Description

This function enables users to rename selected molecules in the dataset, so that they can be parsed correctly by `lipidr` or modify the lipid class. `lipidr` automatically updates the annotation for the renamed molecules.

Usage

```
update_molecule_names(data, old, new)
```

Arguments

| | |
|------|---------------------------------------------------------|
| data | LipidomicsExperiment object. |
| old | A character vector of the molecule names to be renamed. |
| new | A character vector of the new molecule names. |

Value

A LipidomicsExperiment object with molecules name and annotation updated.

Examples

```
data(data_normalized)
old_names <- rowData(data_normalized)$Molecule
# replace PCO with plasmeynLPC
new_names <- sub("^LPE", "LysoPE", old_names)
update_molecule_names(data_normalized, old_names, new_names)
```

use_interactive_graphics
Activate interactive graphics

Description

Use this function to turn on/off interactive graphics plotting. Interactive plots require `plotly` to be installed. Interactive graphics are disabled by default.

Usage

```
use_interactive_graphics(interactive = TRUE)
```

Arguments

`interactive` Should interactive plots be displayed? Default is TRUE.

Value

None

Examples

```
data(data_normalized)
use_interactive_graphics()

# plot the variation in intensity and retention time of all measured
# lipids in QC samples
d_qc <- data_normalized[, data_normalized$group == "QC"]
# plot_molecules(d_qc, "cv", "Area")

# turn off interactivity
use_interactive_graphics(interactive = FALSE)
```

%>%

Pipe operator

Description

See [magrittr::%>%](#) for details.

Usage

```
lhs %>% rhs
```

Value

Result of `rhs(lhs, ...)`.

Examples

```
data(data_normalized)
data_normalized %>% filter_by_cv()
```

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