

# Package ‘phenomis’

December 5, 2025

**Type** Package

**Title** Postprocessing and univariate analysis of omics data

**Version** 1.13.0

**Date** 2024-09-02

**Description** The 'phenomis' package provides methods to perform post-processing (i.e. quality control and normalization) as well as univariate statistical analysis of single and multi-omics data sets. These methods include quality control metrics, signal drift and batch effect correction, intensity transformation, univariate hypothesis testing, but also clustering (as well as annotation of metabolomics data). The data are handled in the standard Bioconductor formats (i.e. SummarizedExperiment and MultiAssayExperiment for single and multi-omics datasets, respectively; the alternative ExpressionSet and MultiDataSet formats are also supported for convenience). As a result, all methods can be readily chained as workflows. The pipeline can be further enriched by multivariate analysis and feature selection, by using the 'ropls' and 'biosigner' packages, which support the same formats. Data can be conveniently imported from and exported to text files. Although the methods were initially targeted to metabolomics data, most of the methods can be applied to other types of omics data (e.g., transcriptomics, proteomics).

**biocViews** BatchEffect, Clustering, Coverage, KEGG, MassSpectrometry, Metabolomics, Normalization, Proteomics, QualityControl, Sequencing, StatisticalMethod, Transcriptomics

**Depends** SummarizedExperiment

**Imports** Biobase, biodb, biodbChebi, data.table, futile.logger, ggplot2, ggrepel, graphics, grDevices, grid, htmlwidgets, igraph, limma, methods, MultiAssayExperiment, MultiDataSet, PMCMRplus, plotly, ranger, RColorBrewer, ropls, stats, tibble, tidyr, utils, VennDiagram

**Suggests** BiocGenerics, BiocStyle, biosigner, CLL, knitr, omicade4, rmarkdown, testthat

**VignetteBuilder** knitr

**License** CeCILL

**Encoding** UTF-8  
**LazyLoad** yes  
**URL** <https://doi.org/10.1038/s41597-021-01095-3>  
**RoxygenNote** 7.2.3  
**git\_url** <https://git.bioconductor.org/packages/phenomis>  
**git\_branch** devel  
**git\_last\_commit** 2244e33  
**git\_last\_commit\_date** 2025-10-29  
**Repository** Bioconductor 3.23  
**Date/Publication** 2025-12-04  
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Contents

|                            |           |
|----------------------------|-----------|
| phenomis-package . . . . . | 3         |
| annotating . . . . .       | 3         |
| clustering . . . . .       | 6         |
| correcting . . . . .       | 9         |
| filtering . . . . .        | 11        |
| gg_barplot . . . . .       | 14        |
| gg_boxplot . . . . .       | 16        |
| gg_pie . . . . .           | 17        |
| gg_volcanoplot . . . . .   | 18        |
| hypotesting . . . . .      | 20        |
| inspecting . . . . .       | 23        |
| normalizing . . . . .      | 26        |
| reading . . . . .          | 27        |
| reducing . . . . .         | 29        |
| transforming . . . . .     | 32        |
| vennplot . . . . .         | 33        |
| writing . . . . .          | 34        |
| <b>Index</b>               | <b>38</b> |

## Description

The 'phenomis' package provides methods to perform post-processing (i.e. quality control and normalization) as well as univariate statistical analysis of single and multi-omics data sets. These methods include quality control metrics, signal drift and batch effect correction, intensity transformation, univariate hypothesis testing, but also clustering (as well as annotation of metabolomics data). The data are handled in the standard Bioconductor formats (i.e. SummarizedExperiment and MultiAssayExperiment for single and multi-omics datasets, respectively; the alternative ExpressionSet and MultiDataSet formats are also supported for convenience). As a result, all methods can be readily chained as workflows. The pipeline can be further enriched by multivariate analysis and feature selection, by using the 'ropls' and 'biosigner' packages, which support the same formats. Data can be conveniently imported from and exported to text files. Although the methods were initially targeted to metabolomics data, most of the methods can be applied to other types of omics data (e.g., transcriptomics, proteomics).

## Author(s)

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## Examples

```
# See the package vignette
```

## Description

Annotation with chemical and biological databases by using the 'biodb' package suite. The present implementation currently enables to query the ChEBI database or a local database.

The parameters and their default values are printed for the selected database

## Usage

```
annotating(  
  x,  
  database.c = c("chebi", "local.ms")[1],  
  param.ls = list(query.type = c("mz", "chebi.id")[1], query.col = "mz", ms.mode = "pos",  
    mz.tol = 10, mz.tol.unit = "ppm", fields = c("chebi.id", "name", "formula",  
    "molecular.mass", "monoisotopic.mass"), fieldsLimit = 1, max.results = 3, local.ms.db
```

```

    = data.frame(), prefix = paste0(database.c, "."), sep = "|"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
annotating(
  x,
  database.c = c("chebi", "local.ms")[1],
  param.ls = list(query.type = c("mz", "chebi.id")[1], query.col = "mz", ms.mode = "pos",
    mz.tol = 10, mz.tol.unit = "ppm", fields = c("chebi.id", "name", "formula",
    "molecular.mass", "monoisotopic.mass"), fieldsLimit = 1, max.results = 3, local.ms.db
    = data.frame(), prefix = paste0(database.c, "."), sep = "|"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
annotating(
  x,
  database.c = c("chebi", "local.ms")[1],
  param.ls = list(query.type = c("mz", "chebi.id")[1], query.col = "mz", ms.mode = "pos",
    mz.tol = 10, mz.tol.unit = "ppm", fields = c("chebi.id", "name", "formula",
    "molecular.mass", "monoisotopic.mass"), fieldsLimit = 1, max.results = 3, local.ms.db
    = data.frame(), prefix = paste0(database.c, "."), sep = "|"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
annotating(
  x,
  database.c = c("chebi", "local.ms")[1],
  param.ls = list(query.type = c("mz", "chebi.id")[1], query.col = "mz", ms.mode = "pos",
    mz.tol = 10, mz.tol.unit = "ppm", fields = c("chebi.id", "name", "formula",
    "molecular.mass", "monoisotopic.mass"), fieldsLimit = 1, max.results = 3, local.ms.db
    = data.frame(), prefix = paste0(database.c, "."), sep = "|"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'ExpressionSet'
annotating(
  x,
  database.c = c("chebi", "local.ms")[1],
  param.ls = list(query.type = c("mz", "chebi.id")[1], query.col = "mz", ms.mode = "pos",
    mz.tol = 10, mz.tol.unit = "ppm", fields = c("chebi.id", "name", "formula",
    "molecular.mass", "monoisotopic.mass"), fieldsLimit = 1, max.results = 3, local.ms.db
    = data.frame(), prefix = paste0(database.c, "."), sep = "|"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

```

```
annotating_parameters(database.c = c("chebi", "local.ms")[1])
```

## Arguments

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>          | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <code>database.c</code> | character(1): database to be used for annotation; either the ChEBI distant database ('chebi'), or a local database ('local.ms')                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <code>param.ls</code>   | list: parameters for database query; the database can be queried by either the mass to charge ratio (mz) or the chebi ID; other query parameters include the ionization mode (ms.mode), the mz tolerance (mz.tol; e.g. 5 ppm for Orbitrap Mass Spectrometers), the fields to retrieve (fields), the maximum number of items to retrieve when a field contains more than one value (fieldsLimit), the maximum number of results to provide for each query (max.results), prefix of the new columns providing the queried information in the feature metadata (prefix), separator in case of multiple retrieved values (sep), local data base to be queried (local.ms.db); additional information is provided by the vignettes from the biodb and biodbChebi packages on Bioconductor |
| <code>report.c</code>   | character(1): File name with '.txt' extension for the printed results (call to sink()); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## Value

`SummarizedExperiment` or `MultiAssayExperiment` (or `ExpressionSet` and `MultiDataSet`) including the appended `rowData` data frame(s)

## Examples

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
# see the (default) parameters (e.g. for ChEBI query)
annotating_parameters("chebi")
# mz annotation with ChEBI

sacurine.se <- annotating(sacurine.se, database.c = "chebi",
  param.ls = list(query.type = "mz", query.col = "mass_to_charge",
    ms.mode = "neg", prefix = "chebiMZ."))

# mz annotation with local database
msdbDF <- read.table(system.file("extdata/local_ms_db.tsv",
  package = "phenomis"),
  header = TRUE, sep = "\t", stringsAsFactors = FALSE)
sacurine.se <- annotating(sacurine.se, database.c = "local.ms",
  param.ls = list(query.type = "mz", query.col = "mass_to_charge",
    ms.mode = "neg",
    mz.tol = 5, mz.tol.unit = "ppm", local.ms.db = msdbDF, prefix = "localMS."))
rowData(sacurine.se)[!is.na(rowData(sacurine.se)[, "localMS.accession"]), ]
# annotation from ChEBI identifiers

sacurine.se <- annotating(sacurine.se, database.c = "chebi",
```

```

param.ls = list(query.type = "chebi.id", query.col = "database_identifier",
prefix = "chebiID."))
head(rowData(sacurine.se))

annotating_parameters()
annotating_parameters("chebi")

```

---

clustering

*clustering*


---

## Description

Hierarchical clustering of both samples and variables

## Usage

```

clustering(
  x,
  dissym.c = c("euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski",
    "1-cor", "1-abs(cor)")[7],
  correl.c = c("pearson", "kendall", "spearman")[1],
  agglo.c = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median",
    "centroid")[2],
  clusters.vi = c(2, 2),
  cex.vn = c(1, 1),
  palette.c = c("blueOrangeRed", "redBlackGreen")[1],
  scale_plot.l = TRUE,
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
clustering(
  x,
  dissym.c = c("euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski",
    "1-cor", "1-abs(cor)")[7],
  correl.c = c("pearson", "kendall", "spearman")[1],
  agglo.c = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median",
    "centroid")[2],
  clusters.vi = c(2, 2),
  cex.vn = c(1, 1),
  palette.c = c("blueOrangeRed", "redBlackGreen")[1],
  scale_plot.l = TRUE,
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

```

)

## S4 method for signature 'SummarizedExperiment'
clustering(
  x,
  dissym.c = c("euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski",
    "1-cor", "1-abs(cor)") [7],
  correl.c = c("pearson", "kendall", "spearman") [1],
  agglo.c = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median",
    "centroid") [2],
  clusters.vi = c(2, 2),
  cex.vn = c(1, 1),
  palette.c = c("blueOrangeRed", "redBlackGreen") [1],
  scale_plot.l = TRUE,
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf") [2],
  report.c = c("none", "interactive", "myfile.txt") [2]
)

## S4 method for signature 'MultiDataSet'
clustering(
  x,
  dissym.c = c("euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski",
    "1-cor", "1-abs(cor)") [7],
  correl.c = c("pearson", "kendall", "spearman") [1],
  agglo.c = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median",
    "centroid") [2],
  clusters.vi = c(2, 2),
  cex.vn = c(1, 1),
  palette.c = c("blueOrangeRed", "redBlackGreen") [1],
  scale_plot.l = TRUE,
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf") [2],
  report.c = c("none", "interactive", "myfile.txt") [2]
)

## S4 method for signature 'ExpressionSet'
clustering(
  x,
  dissym.c = c("euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski",
    "1-cor", "1-abs(cor)") [7],
  correl.c = c("pearson", "kendall", "spearman") [1],
  agglo.c = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median",
    "centroid") [2],
  clusters.vi = c(2, 2),
  cex.vn = c(1, 1),
  palette.c = c("blueOrangeRed", "redBlackGreen") [1],
  scale_plot.l = TRUE,

```

```

    title.c = NA,
    figure.c = c("none", "interactive", "myfile.pdf")[2],
    report.c = c("none", "interactive", "myfile.txt")[2]
  )

```

### Arguments

|                           |                                                                                                                                                                                                                                                          |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>            | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                             |
| <code>dissym.c</code>     | character(1): dissimilarity to be used in the hierarchical clustering (as provided by the <code>hclust</code> package)                                                                                                                                   |
| <code>correl.c</code>     | character(1): correlation coefficient (in case <code>'1-cor'</code> or <code>'1-abs(cor)'</code> are selected as dissimilarity)                                                                                                                          |
| <code>agglo.c</code>      | character(1): agglomeration method                                                                                                                                                                                                                       |
| <code>clusters.vi</code>  | integer(2): number of sample and variable clusters, respectively; the default values (2) are only provided as starting guess (e.g. in case of two groups of samples)                                                                                     |
| <code>cex.vn</code>       | numeric(2) [Plot parameter]: size of the sample and variable labels                                                                                                                                                                                      |
| <code>palette.c</code>    | character(1) [Plot parameter]: color palette                                                                                                                                                                                                             |
| <code>scale_plot.l</code> | logical(1) [Plot parameter]: scaling (mean-centering and unit variance scaling) to enhance contrast (for plotting only)                                                                                                                                  |
| <code>title.c</code>      | character(1) [Plot parameter]: Graphic the subtitle                                                                                                                                                                                                      |
| <code>figure.c</code>     | character(1): File name with <code>'pdf'</code> extension for the figure; if <code>'interactive'</code> (default), figures will be displayed interactively; if <code>'none'</code> , no figure will be generated                                         |
| <code>report.c</code>     | character(1): File name with <code>'txt'</code> extension for the printed results (call to <code>sink()</code> ); if <code>'interactive'</code> (default), messages will be printed on the screen; if <code>'none'</code> , no verbose will be generated |

### Value

`SummarizedExperiment` or `MultiAssayExperiment` (or `ExpressionSet` and `MultiDataSet`) including columns indicating the clusters in `rowData` and `colData` if `clusters.vi` has been specified

### Examples

```

sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se)
sacurine.se <- sacurine.se[, colData(sacurine.se)[, "sampleType"] != "pool"]
sacurine.se <- transforming(sacurine.se)
sacurine.se <- sacurine.se[, colnames(sacurine.se) != "HU_neg_096_b2"]
sacurine.se <- clustering(sacurine.se)
utils::head(rowData(sacurine.se))

# MultiAssayExperiment

prometis.mae <- reading(system.file("extdata/prometis", package="phenomis"))
prometis.mae <- clustering(prometis.mae)

```

correcting

*correcting***Description**

Signal drift and batch effect correction. The normalization strategy relies on the measurements of a pooled (or QC) sample injected periodically: for each variable, a regression model is fitted to the values of the pool and subsequently used to adjust the intensities of the samples of interest (van der Kloet et al, 2009; Dunn et al, 2011). In case the number of pool observations is below 5, the linear method is used (for all variables) and a warning is generated. In case no pool is available, the samples themselves can be used to computed the regression model (Thevenot et al., 2015). The sample metadata of each datasets (e.g. colData Data Frames) must contain 3 columns: 1) 'sampleType' (character): only the 'sample' or 'pool' values can be used to indicate the reference samples for the correction, 2) 'injectionOrder' (integer): order of injection in the instrument, and 3) 'batch' (character): batch name(s).

**Usage**

```
correcting(
  x,
  method.vc = c("loess", "serrf")[1],
  reference.vc = c("pool", "sample")[1],
  loess_span.vn = 1,
  serrf_corvar.vi = 10,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
correcting(
  x,
  method.vc = c("loess", "serrf")[1],
  reference.vc = c("pool", "sample")[1],
  loess_span.vn = 1,
  serrf_corvar.vi = 10,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
correcting(
  x,
  method.vc = c("loess", "serrf")[1],
```

```

reference.vc = c("pool", "sample")[1],
loess_span.vn = 1,
serrf_corvar.vi = 10,
sample_intensity.c = c("median", "mean", "sum")[2],
title.c = NA,
figure.c = c("none", "interactive", "myfile.pdf")[2],
report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
correcting(
  x,
  method.vc = c("loess", "serrf")[1],
  reference.vc = c("pool", "sample")[1],
  loess_span.vn = 1,
  serrf_corvar.vi = 10,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'ExpressionSet'
correcting(
  x,
  method.vc = c("loess", "serrf")[1],
  reference.vc = c("pool", "sample")[1],
  loess_span.vn = 1,
  serrf_corvar.vi = 10,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

## Arguments

|                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>            | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                                                                                                                                                                                       |
| <code>method.vc</code>    | character of length 1 or the total number of datasets: method(s) to be used for each dataset (either 'serrf' or 'loess'); for the 'serrf' approach, the seed is internally set to 123 for reproducibility; in case the parameter is of length 1 and <code>x</code> contains multiple datasets, the same method will be used for all datasets                                                                       |
| <code>reference.vc</code> | character of length 1 or the total number of datasets: sample type to be used as reference for the correction (as indicated in the 'sampleType' column from the <code>colData(x)</code> ; e.g. 'pool' [default]); should be set to 'pool' for the 'serrf' method; in case the parameter is of length 1 and <code>x</code> contains multiple datasets, the same reference sample type will be used for all datasets |

|                                 |                                                                                                                                                                                                                                                                    |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>loess_span.vn</code>      | character of length 1 or the total number of datasets: smoothing parameter for the loess regression; between 0 and 1; (default set to 1); in case the parameter is of length 1 and x contains multiple datasets, the same span value will be used for all datasets |
| <code>serrf_corvar.vi</code>    | character of length 1 or the total number of datasets: number of correlated features for the random forest regression; (default set to 10); in case the parameter is of length 1 and x contains multiple datasets, the same value will be used for all datasets    |
| <code>sample_intensity.c</code> | character(1): metric to be used when displaying the sample intensities                                                                                                                                                                                             |
| <code>title.c</code>            | character(1): Graphic title: if NA [default] the 'title' slot from the experiment-Data will be used (metadata)                                                                                                                                                     |
| <code>figure.c</code>           | character(1): File name with '.pdf' extension for the figure; if 'interactive' (default), figures will be displayed interactively; if 'none', no figure will be generated                                                                                          |
| <code>report.c</code>           | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                  |

**Value**

SummarizedExperiment or MultiAssayExperiment (or ExpressionSet and MultiDataSet) including the corrected intensities in the assay matrix (matrices)

**Examples**

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se)

# MultiDataSet (to be done)
```

---

|                        |                                                                                                   |
|------------------------|---------------------------------------------------------------------------------------------------|
| <code>filtering</code> | <i>Filtering of the features (and/or samples) with a high proportion of NAs or a low variance</i> |
|------------------------|---------------------------------------------------------------------------------------------------|

---

**Description**

Filtering of the features (and/or samples) with a high proportion of NAs or a low variance

**Usage**

```
filtering(
  x,
  class.c = "",
  max_na_prop.n = 0.2,
  min_variance.n = .Machine$double.eps,
```

```

    dims.vc = c("features", "samples"),
    report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
filtering(
  x,
  class.c = "",
  max_na_prop.n = 0.2,
  min_variance.n = .Machine$double.eps,
  dims.vc = c("features", "samples"),
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
filtering(
  x,
  class.c = "",
  max_na_prop.n = 0.2,
  min_variance.n = .Machine$double.eps,
  dims.vc = c("features", "samples"),
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
filtering(
  x,
  class.c = "",
  max_na_prop.n = 0.2,
  min_variance.n = .Machine$double.eps,
  dims.vc = c("features", "samples"),
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'ExpressionSet'
filtering(
  x,
  class.c = "",
  max_na_prop.n = 0.2,
  min_variance.n = .Machine$double.eps,
  dims.vc = c("features", "samples"),
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

## Arguments

**x** An S4 object of class SummarizedExperiment or MultiAssayExperiment (ExpressionSet and MultiDataSet are still supported)

|                             |                                                                                                                                                                                                                                                                                      |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>class.c</code>        | character(1): name of the column of the sample metadata giving the classification groups: the filtering will be applied on each class (default: "" meaning that there are no specific classes to consider)                                                                           |
| <code>max_na_prop.n</code>  | numeric(1): maximum proportion of NAs for a feature (or sample) to be kept (e.g. the default 20 values); in case 'class.c' is provided, the maximum proportion of NAs for a feature must be achieved in at least one sample class)                                                   |
| <code>min_variance.n</code> | numeric(1): minimum variance for a feature (or sample) to be kept (e.g. the default 0 value to discard constant features (or samples); in case 'class.c' is provided, the minimum variance for a feature must be achieved in all sample classes)                                     |
| <code>dims.vc</code>        | Vector of one or two characters: dimension(s) to which the filtering should be applied; either 'features', 'samples', c('features', 'samples'), or c('samples', 'features'); in the two latter cases, the dimensions indicated in the <code>dims.vc</code> are filtered sequentially |
| <code>report.c</code>       | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                                    |

## Value

SummarizedExperiment or MultiAssayExperiment (or ExpressionSet and MultiDataSet) including the filtered data and metadata

## Examples

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
assay.mn <- assay(sacurine.se)
ropls::view(assay.mn)
filtering(sacurine.se)
assay.mn[assay.mn < 1e5] <- NA
ropls::view(assay.mn)
assay(sacurine.se) <- assay.mn
filtering(sacurine.se)
filtering(sacurine.se, class.c = "gender")
filtering(sacurine.se, class.c = "sampleType")

# MultiAssayExperiment

prometis.mae <- reading(system.file("extdata/prometis", package="phenomis"))
filtering(prometis.mae)
for (set.c in names(prometis.mae)) {
  set.se <- prometis.mae[[set.c]]
  assay.mn <- assay(set.se)
  assay.mn[assay.mn < quantile(c(assay.mn), 0.2)] <- NA
  assay(set.se) <- assay.mn
  prometis.mae[[set.c]] <- set.se
}
filtering(prometis.mae)

# MultiDataSet
```

```

prometis.mset <- reading(system.file("extdata/prometis", package="phenomis"),
                          output.c = "set")
filtering(prometis.mset)
for (set.c in names(prometis.mset)) {
  eset <- prometis.mset[[set.c]]
  exprs.mn <- Biobase::exprs(eset)
  exprs.mn[exprs.mn < quantile(c(exprs.mn), 0.2)] <- NA
  Biobase::exprs(eset) <- exprs.mn
  prometis.mset <- MultiDataSet::add_eset(prometis.mset, eset,
                                          dataset.type = set.c,
                                          GRanges = NA, overwrite = TRUE,
                                          warnings = FALSE)
}
filtering(prometis.mset)

```

---

gg\_barplot

*Barplot with ggplot2*


---

## Description

Barplot with ggplot2

## Usage

```

gg_barplot(
  data.mn,
  log10.l = FALSE,
  ylim.vn = c(NA, NA),
  title.c = "",
  xlab.c = "",
  ylab.c = "",
  row_levels.vc = NA,
  col_levels.vc = NA,
  palette.vc = "Set1",
  theme.c = c("default", "bw", "classic", "dark", "gray", "linedraw", "light", "minimal",
              "void")[3],
  flip.l = FALSE,
  legend_position.c = c("none", "bottom", "left", "top", "right")[2],
  cex_axis.i = 18,
  cex_bar.i = 10,
  cex_title.i = 28,
  bar_just.n = 0.9,
  figure.c = c("interactive", "my_barplot.pdf", "none")[1]
)

```

**Arguments**

|                                |                                                                                                                                                                |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>data.mn</code>           | Matrix of numerics: values to be barplotted                                                                                                                    |
| <code>log10.l</code>           | logical(1): should the intensities be log10 transformed?                                                                                                       |
| <code>ylim.vn</code>           | numeric(2): minimum and maximum values for the bars                                                                                                            |
| <code>title.c</code>           | Character: plot title                                                                                                                                          |
| <code>xlab.c</code>            | Character: x label                                                                                                                                             |
| <code>ylab.c</code>            | Character: y label                                                                                                                                             |
| <code>row_levels.vc</code>     | Vector of characters: levels of rownames (default: NA: alphabetical order will be used)                                                                        |
| <code>col_levels.vc</code>     | Vector of characters: levels of colnames (default: NA: alphabetical order will be used)                                                                        |
| <code>palette.vc</code>        | Character: either the name of an RColorBrewer palette (default: 'Set1'; 'Paired' can be useful for parallel plotting) or a vector manually defining the colors |
| <code>theme.c</code>           | character(1): name of the ggplot theme                                                                                                                         |
| <code>flip.l</code>            | logical(1): should the barplot be flipped (default: FALSE)                                                                                                     |
| <code>legend_position.c</code> | character(1): position of the legend: either "none", "bottom" (default), "left", "top", "right"                                                                |
| <code>cex_axis.i</code>        | Integer: size of axis text (default: 18)                                                                                                                       |
| <code>cex_bar.i</code>         | Integer: size of bar value text (default: 10)                                                                                                                  |
| <code>cex_title.i</code>       | Integer: size of title text (default: 28)                                                                                                                      |
| <code>bar_just.n</code>        | Numeric: adjustment of bar value text (default : 0.9)                                                                                                          |
| <code>figure.c</code>          | Character: either 'interactive' for interactive display, 'my_barplot.pdf' for figure saving (only the extension matters), or 'none' to prevent plotting        |

**Value**

invisible ggplot2 object

**Examples**

```

prometis.mae <- reading(system.file("extdata/prometis", package = "phenomis"))
dims.mn <- vapply(names(prometis.mae),
                  function(set.c) { dim(prometis.mae[[set.c]])},
                  FUN.VALUE = integer(2))
dims.mn <- t(dims.mn)
colnames(dims.mn) <- c("features", "samples")
gg_barplot(dims.mn, title.c = "ProMetIS data",
            row_levels = c("proteo", "metabo"),
            col_levels = c("samples", "features"),
            ylim.vn = c(NA, 110),
            bar_just = -0.25,
            cex_bar.i = 6,
            cex_title.i = 15)

```

gg\_boxplot

*Boxplot with ggplot2***Description**

Boxplot with ggplot2

**Usage**

```
gg_boxplot(
  data.tb,
  x.c = "",
  y.c = "",
  color.c = "",
  title.c = NA,
  xlab.c = NA,
  ylab.c = "",
  label.vc = "",
  palette.vc = "Set1",
  theme.c = c("default", "bw", "classic", "dark", "gray", "linedraw", "light", "minimal",
    "void")[3],
  size.ls = list(dot.n = 0.7, lab.i = 20, tick.i = 20, title.i = 20),
  figure.c = c("interactive", "my_boxplot.pdf")[1]
)
```

**Arguments**

|            |                                                                                                                                                                                                     |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data.tb    | Data frame (or tibble) containing the information                                                                                                                                                   |
| x.c        | Character: name of the column with qualitative levels                                                                                                                                               |
| y.c        | Character: name of the column with quantitative values                                                                                                                                              |
| color.c    | Character: optional name of the column for color information                                                                                                                                        |
| title.c    | Character: plot title                                                                                                                                                                               |
| xlab.c     | Character: x label                                                                                                                                                                                  |
| ylab.c     | Character: y label                                                                                                                                                                                  |
| label.vc   | Character (vector): either the name of a character column from the data or a character vector of the same length as the row number of the data, containing the feature labeling for outlier display |
| palette.vc | Character: either the name of an RColorBrewer palette (default: 'Set1'; 'Paired' can be useful for parallel plotting) or a vector manually defining the colors                                      |
| theme.c    | character(1): name of the ggplot theme                                                                                                                                                              |
| size.ls    | List of sizes for dots (default is 0.7), labels (default is 16), ticks (14) and title (20)                                                                                                          |
| figure.c   | Character: either 'interactive' for interactive display or 'my_barplot.pdf' for figure saving (only the extension matters)                                                                          |

**Value**

character vector of outlier labels (same dimension as the number of rows from data.tb)

**Examples**

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine_pda.df <- as.data.frame(colData(sacurine.se))
sacurine_pda.df <- sacurine_pda.df[!grepl("QC", rownames(sacurine_pda.df)), ]
gg_boxplot(sacurine_pda.df, y.c = "age")
gg_boxplot(sacurine_pda.df, x.c = "gender", y.c = "bmi", color.c = "gender")
gg_boxplot(sacurine_pda.df, x.c = "gender", y.c = "bmi", color.c = "gender",
  label.vc = rownames(sacurine_pda.df))
```

gg\_pie

*Pie with ggplot2***Description**

Pie with ggplot2

**Usage**

```
gg_pie(
  data.tb,
  y.c = "",
  color.c = "",
  title.c = "",
  palette.vc = "Set1",
  label.c = c("none", "value", "percent")[1],
  geom_text.ls = list(lab.i = 7, legend_title.i = 16, legend_text.i = 14, title.i = 16),
  figure.c = c("interactive", "my_pie.pdf", "none")[1]
)
```

**Arguments**

|            |                                                                                                                                                                                                                            |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| data.tb    | Tibble (or data frame) containing the information                                                                                                                                                                          |
| y.c        | Character: name of the column with the factor to be displayed; alternatively, name of the column with the counts (in this case set the name of the column with the names of the factor levels with the 'color.c' argument) |
| color.c    | Character: optional name of the column with the names of the factor levels                                                                                                                                                 |
| title.c    | Character: plot title                                                                                                                                                                                                      |
| palette.vc | Character: either the name of an RColorBrewer palette (default: 'Set1'; 'Paired' can be useful for parallel plotting) or a vector manually defining the colors                                                             |
| label.c    | Character: (relative) counts to be displayed on the pie; either 'none' (default), 'value' or 'percent'                                                                                                                     |

`geom_text.ls` List of sizes for `lab.i` (default 7), `legend_title.i` (16), `legend_text.i` (14), and `title.i` (16)

`figure.c` Character: either 'interactive' for interactive display, 'my\_pie.pdf' for figure saving (only the extension matters), or 'none' to prevent plotting

## Value

invisible ggplot2 object

## Examples

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine_pda.df <- colData(sacurine.se)
sacurine_pda.df <- sacurine_pda.df[!grepl("QC", rownames(sacurine_pda.df)), ]
gg_pie(sacurine_pda.df, y.c = "gender", label.c = "value")
```

---

gg\_volcanoplot

*Volcano plot with ggplot2*

---

## Description

Volcano plot with ggplot2

## Usage

```
gg_volcanoplot(
  fold_change.vn,
  adjusted_pvalue.vn,
  adjust_method.c = "",
  adjust_thresh.n = 0.05,
  label.vc = "",
  title.c = "",
  xlab.c = "Fold Change",
  signif_palette.vc = c(yes = RColorBrewer::brewer.pal(9, "Greens")[8], no =
    RColorBrewer::brewer.pal(9, "Greys")[7]),
  signif_shape.vi = c(yes = 16, no = 1),
  class_name.vc = "",
  class_color.vc = "",
  size.ls = list(class.i = 5, lab.i = 16, point.i = 3, tick.i = 14, title.i = 20),
  figure.c = c("interactive", "interactive_plotly", "my_volcanoplot.pdf",
    "my_volcanoplot.html")[2]
)
```

**Arguments**

|                                 |                                                                                                                                                                                                                                                                                                                           |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>fold_change.vn</code>     | Numeric vector: fold changes                                                                                                                                                                                                                                                                                              |
| <code>adjusted_pvalue.vn</code> | Numeric vector: (adjusted) p-values                                                                                                                                                                                                                                                                                       |
| <code>adjust_method.c</code>    | Character: method for multiple testing correction                                                                                                                                                                                                                                                                         |
| <code>adjust_thresh.n</code>    | Numeric: significance threshold                                                                                                                                                                                                                                                                                           |
| <code>label.vc</code>           | Character (vector): either the name of a character column from the data or a character vector of the same length as the row number of the data, containing the feature labeling                                                                                                                                           |
| <code>title.c</code>            | Character: plot title                                                                                                                                                                                                                                                                                                     |
| <code>xlab.c</code>             | Character: x label (default: "Fold Change")                                                                                                                                                                                                                                                                               |
| <code>signif_palette.vc</code>  | Character vector: color palette (default 'green4' for significant features and 'gray' otherwise)                                                                                                                                                                                                                          |
| <code>signif_shape.vi</code>    | Integer vector: shapes for significant (respectively, non significant) features; default is 16 (respectively, 1)                                                                                                                                                                                                          |
| <code>class_name.vc</code>      | Character vector: names of the two compared class labels                                                                                                                                                                                                                                                                  |
| <code>class_color.vc</code>     | Character vector: colors of the two compared class labels                                                                                                                                                                                                                                                                 |
| <code>size.ls</code>            | List of sizes for classes (default: 5), xy labels (default: 16), points (default: 3), ticks (default: 14) and title (default: 20)                                                                                                                                                                                         |
| <code>figure.c</code>           | Character: either 'interactive' (respectively, 'interactive_plotly') for interactive display with ggplot2 (respectively, with plotly::ggplotly [default]), or 'my_volcanoplot.pdf' (respectively 'my_volcanoplot.html') for figure saving (only the extension matters) with ggplot2 (respectively, with plotly::ggplotly) |

**Value**

invisible ggplot2 object

**Examples**

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se, figure.c = "none")
sacurine.se <- sacurine.se[, colData(sacurine.se)[, "sampleType"] != "pool"]
sacurine.se <- transforming(sacurine.se)
sacurine.se <- hypotesting(sacurine.se, test.c = "wilcoxon",
                           factor_names.vc = "gender",
                           figure.c = "none", report.c = "none")
fold.vn <- rowData(sacurine.se)[, "wilcoxon_gender_Female.Male_diff"]
fdr.vn <- rowData(sacurine.se)[, "wilcoxon_gender_Female.Male_BH"]
feat.vc <- rownames(sacurine.se)
gg_volcanoplot(fold.vn,
               fdr.vn,
```

```

        label.vc = make.names(feats.vc),
        adjust_method.c = "BH")
feats_signif.vc <- vapply(seq_along(feats.vc),
                        function(feats.i)
                          ifelse(fdr.vn[feats.i] <= 0.05, feats.vc[feats.i], ""),
                          FUN.VALUE = character(1))
gg_volcanoplot(fold.vn,
              fdr.vn,
              label.vc = make.names(feats_signif.vc),
              adjust_method.c = "BH",
              figure.c = "interactive")

```

---

hypotesting

*Univariate hypothesis testing*


---

## Description

The hypotesting method is a wrapper of the main R functions for hypothesis testing and corrections for multiple testing. The list of available tests includes two sample tests (t-test and Wilcoxon rank test, but also the limma test), analysis of variance (for one and two factors) and Kruskal-Wallis rank test, and correlation tests (by using either the pearson or the spearman correlation).

## Usage

```

hypotesting(
  x,
  test.c = c("ttest", "limma", "wilcoxon", "anova", "kruskal", "pearson", "spearman",
            "limma2ways", "limma2waysInter", "anova2ways", "anova2waysInter")[2],
  factor_names.vc,
  factor_levels.ls = list(factor1.vc = "default", factor2.vc = "default"),
  adjust.c = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")[5],
  adjust_thresh.n = 0.05,
  signif_maxprint.i = NA,
  title.c = NA,
  display_signif.l = FALSE,
  prefix.c = "",
  figure.c = c("none", "interactive", "interactive_plotly", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

```
## S4 method for signature 'MultiAssayExperiment'
```

```

hypotesting(
  x,
  test.c = c("ttest", "limma", "wilcoxon", "anova", "kruskal", "pearson", "spearman",
            "limma2ways", "limma2waysInter", "anova2ways", "anova2waysInter")[2],
  factor_names.vc,
  factor_levels.ls = list(factor1.vc = "default", factor2.vc = "default"),
  adjust.c = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")[5],

```

```

    adjust_thresh.n = 0.05,
    signif_maxprint.i = NA,
    title.c = NA,
    display_signif.l = FALSE,
    prefix.c = "",
    figure.c = c("none", "interactive", "interactive_plotly", "myfile.pdf")[2],
    report.c = c("none", "interactive", "myfile.txt")[2]
  )

## S4 method for signature 'SummarizedExperiment'
hypotesting(
  x,
  test.c = c("ttest", "limma", "wilcoxon", "anova", "kruskal", "pearson", "spearman",
    "limma2ways", "limma2waysInter", "anova2ways", "anova2waysInter")[2],
  factor_names.vc,
  factor_levels.ls = list(factor1.vc = "default", factor2.vc = "default"),
  adjust.c = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")[5],
  adjust_thresh.n = 0.05,
  signif_maxprint.i = NA,
  title.c = NA,
  display_signif.l = FALSE,
  prefix.c = "",
  figure.c = c("none", "interactive", "interactive_plotly", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
hypotesting(
  x,
  test.c = c("ttest", "limma", "wilcoxon", "anova", "kruskal", "pearson", "spearman",
    "limma2ways", "limma2waysInter", "anova2ways", "anova2waysInter")[2],
  factor_names.vc,
  factor_levels.ls = list(factor1.vc = "default", factor2.vc = "default"),
  adjust.c = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")[5],
  adjust_thresh.n = 0.05,
  signif_maxprint.i = NA,
  title.c = NA,
  display_signif.l = FALSE,
  prefix.c = "",
  figure.c = c("none", "interactive", "interactive_plotly", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'ExpressionSet'
hypotesting(
  x,
  test.c = c("ttest", "limma", "wilcoxon", "anova", "kruskal", "pearson", "spearman",
    "limma2ways", "limma2waysInter", "anova2ways", "anova2waysInter")[2],

```

```

factor_names.vc,
factor_levels.ls = list(factor1.vc = "default", factor2.vc = "default"),
adjust.c = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")[5],
adjust_thresh.n = 0.05,
signif_maxprint.i = NA,
title.c = NA,
display_signif.l = FALSE,
prefix.c = "",
figure.c = c("none", "interactive", "interactive_plotly", "myfile.pdf")[2],
report.c = c("none", "interactive", "myfile.txt")[2]
)

```

## Arguments

|                                |                                                                                                                                                                                                                                                                                                                                               |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>                 | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                                                                                                                  |
| <code>test.c</code>            | character(1): One of the 9 available hypothesis tests can be selected (either 'ttest', 'limma', 'wilcoxon', 'anova', 'kruskal', 'pearson', 'spearman', 'limma2ways', 'limma2waysInter', 'anova2ways', 'anova2waysInter')                                                                                                                      |
| <code>factor_names.vc</code>   | (Vector of) character(s): Factor(s) of interest (up to two), i.e. name(s) of a column from the <code>pData(x)</code>                                                                                                                                                                                                                          |
| <code>factor_levels.ls</code>  | List: for each factor of interest (up to two), the levels of the factor can be specified (i.e. re-ordered) by including a character vector with those levels in the list; by default (no specification), the two vectors are set to "default".                                                                                                |
| <code>adjust.c</code>          | character(1): Name of the method for correction of multiple testing (the <code>p.adjust</code> function is used)                                                                                                                                                                                                                              |
| <code>adjust_thresh.n</code>   | numeric(1): Threshold for (corrected) p-values                                                                                                                                                                                                                                                                                                |
| <code>signif_maxprint.i</code> | integer(1): Maximum number of significant feature to display on the screen (by default, 'NA', all significant features are displayed)                                                                                                                                                                                                         |
| <code>title.c</code>           | character(1): Title of the graphics                                                                                                                                                                                                                                                                                                           |
| <code>display_signif.l</code>  | logical(1): In case of two sample tests (or correlation test), should individual boxplots (or scatterplots) of significant features be shown?                                                                                                                                                                                                 |
| <code>prefix.c</code>          | character(1): prefix to be added to the supplementary columns from the <code>variableMetadata</code> to prevent overwriting of pre-existing columns with identical names [default: ""]                                                                                                                                                        |
| <code>figure.c</code>          | character(1): File name with '.pdf' extension for the figure (for venn diagrams, e.g. in the 'anova2ways' test, the extension will be internally changed to '.tiff' for compatibility with the <code>VennDiagram</code> package); if 'interactive' (default), figures will be displayed interactively; if 'none', no figure will be generated |
| <code>report.c</code>          | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                                                                                             |

**Value**

SummarizedExperiment or MultiAssayExperiment (or ExpressionSet and MultiDataSet) including the difference in means/medians or correlations and the adjusted p-values in feature meta-data

**Examples**

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se, figure.c = 'none')
sacurine.se <- sacurine.se[, colData(sacurine.se)[, "sampleType"] != "pool"]
sacurine.se <- transforming(sacurine.se)
sacurine.se <- sacurine.se[, colnames(sacurine.se) != "HU_neg_096_b2"]
# Student's T test
sacurine.se <- hypotesting(sacurine.se, "ttest", "gender")
# Pearson correlation test
sacurine.se <- hypotesting(sacurine.se, "pearson", "age")
# ANOVA
colData(sacurine.se)[, "ageGroup"] <- vapply(colData(sacurine.se)[, "age"],
      function(x) {
        if (x < 35) {
          return("thirty")
        } else if (x < 50) {
          return("fourty")
        } else {
          return("fifty")}},
      FUN.VALUE = character(1))
sacurine.se <- hypotesting(sacurine.se, "anova", "ageGroup")

# MultiAssayExperiment

prometis.mae <- reading(system.file("extdata/prometis", package="phenomis"))
prometis.mae <- hypotesting(prometis.mae, "limma", "gene")

# MultiDataSet

prometis.mset <- reading(system.file("extdata/prometis", package="phenomis"),
      output.c = "set")
prometis.mset <- hypotesting(prometis.mset, "limma", "gene")
```

---

inspecting

*Inspecting*


---

**Description**

Provides numerical metrics and graphical overview of SummarizedExperiment, MultiAssayExperiment, ExpressionSet, or MultiDataSet instance

**Usage**

```

inspecting(
  x,
  pool_as_pool1.l = FALSE,
  pool_cv.n = 0.3,
  loess_span.n = 1,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  plot_dims.l = TRUE,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
inspecting(
  x,
  pool_as_pool1.l = FALSE,
  pool_cv.n = 0.3,
  loess_span.n = 1,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  plot_dims.l = TRUE,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
inspecting(
  x,
  pool_as_pool1.l = FALSE,
  pool_cv.n = 0.3,
  loess_span.n = 1,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  plot_dims.l = TRUE,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
inspecting(
  x,
  pool_as_pool1.l = FALSE,
  pool_cv.n = 0.3,
  loess_span.n = 1,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  plot_dims.l = TRUE,

```

```

    figure.c = c("none", "interactive", "myfile.pdf")[2],
    report.c = c("none", "interactive", "myfile.txt")[2]
  )

## S4 method for signature 'ExpressionSet'
inspecting(
  x,
  pool_as_pool1.l = FALSE,
  pool_cv.n = 0.3,
  loess_span.n = 1,
  sample_intensity.c = c("median", "mean", "sum")[2],
  title.c = NA,
  plot_dims.l = TRUE,
  figure.c = c("none", "interactive", "myfile.pdf")[2],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

### Arguments

|                                 |                                                                                                                                                                                                                                                                                                             |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>                  | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                                                                                |
| <code>pool_as_pool1.l</code>    | logical(1): should pool be included (as <code>pool1</code> ) in the correlation with the dilution factor? [default = <code>FALSE</code> ]                                                                                                                                                                   |
| <code>pool_cv.n</code>          | numeric(1): threshold for the coefficient of variation of the pools; the default value (30%) is often used in metabolomics                                                                                                                                                                                  |
| <code>loess_span.n</code>       | numeric(1): span parameter used in the loess trend estimation; the default value is set to 1 to prevent overfitting                                                                                                                                                                                         |
| <code>sample_intensity.c</code> | Character: function to be used to display the global sample intensity; default: 'mean'                                                                                                                                                                                                                      |
| <code>title.c</code>            | character(1): <code>MultiAssayExperiment</code> : title of the barplot showing the number of samples and variables in each dataset; <code>ExpressionSet</code> : title of the multipanel graphic displaying the metrics (if <code>NA</code> -default- the title slot from the experiment-Data will be used) |
| <code>plot_dims.l</code>        | ( <code>MultiAssayExperiment</code> ) logical(1): should an overview of the number of samples and variables in all datasets be barplotted?                                                                                                                                                                  |
| <code>figure.c</code>           | character(1): File name with '.pdf' extension for the figure; if 'interactive' (default), figures will be displayed interactively; if 'none', no figure will be generated                                                                                                                                   |
| <code>report.c</code>           | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                                                           |

### Value

`SummarizedExperiment` or `MultiAssayExperiment` (or `ExpressionSet` and `MultiDataSet`) including the computed sample and variable metrics in the `rowData` and `colData` metadata.

## Examples

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- inspecting(sacurine.se)
sacurine.se <- correcting(sacurine.se)
sacurine.se <- inspecting(sacurine.se)
sacurine.se <- transforming(sacurine.se)
sacurine.se <- inspecting(sacurine.se)

# MultiAssayExperiment
prometis.mae <- reading(system.file("extdata/prometis",
                                   package = "phenomis"))
prometis.mae <- inspecting(prometis.mae)
```

---

normalizing

*Normalization of the data matrix intensities*


---

## Description

The matrix intensities may be normalized by using the Probabilistic Quotient Normalization to scale the spectra to the same virtual overall concentration

## Usage

```
normalizing(
  x,
  method.vc = "pqn",
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
normalizing(
  x,
  method.vc = "pqn",
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
normalizing(
  x,
  method.vc = "pqn",
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
normalizing(
  x,
  method.vc = "pqn",
```

```

    report.c = c("none", "interactive", "myfile.txt")[2]
  )

  ## S4 method for signature 'ExpressionSet'
  normalizing(
    x,
    method.vc = "pqn",
    report.c = c("none", "interactive", "myfile.txt")[2]
  )

```

### Arguments

|                        |                                                                                                                                                                                                                                                    |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>         | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported)                                                                       |
| <code>method.vc</code> | character of length 1 or the total number of datasets: method(s) to be used for each dataset (default is 'pqn'); in case the parameter is of length 1 and <code>x</code> contains multiple datasets, the same method will be used for all datasets |
| <code>report.c</code>  | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                  |

### Value

`SummarizedExperiment` or `MultiAssayExperiment` (or `ExpressionSet` and `MultiDataSet`) including the (list of) matrix with normalized intensities

### Examples

```

sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- sacurine.se[, colnames(sacurine.se) != 'HU_neg_096_b2']
sacurine.se <- transforming(sacurine.se, method.vc = "log10")
norm.se <- normalizing(sacurine.se, method.vc = "pqn")

# MultiDataSet

```

---

reading

*reading*

---

### Description

Reading dataset(s) in the 3 tables 'dataMatrix' (or 'DM'), sampleMetadata' (or 'SM') and variableMetadata' (or 'VM') tabular format. In case of a single dataset (3 tables in the specified directory), a `SummarizedExperiment` instance is returned. In case of a multiple dataset (several subfolders containing 3 tables), a `MultiAssayExperiment` instance is created.

**Usage**

```
reading(
  dir.c,
  files.ls = NULL,
  subsets.vc = NA,
  output.c = c("exp", "set")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)
```

**Arguments**

|                         |                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>dir.c</code>      | character(1): directory containing the 3 tabular files (single dataset), or containing several subdirectories with 3 tabular files (multiple datasets)                                                                                                                                                                                                                                  |
| <code>files.ls</code>   | list: if <code>dir.c</code> is set to NA, the full names of the individual files can be provided; in case of a <code>SummarizedExperiment</code> , the names of the list must be 'dataMatrix', 'sampleMetadata', and 'variableMetadata' with the corresponding file full names; in case of a <code>MultiAssayExperiment</code> , the list must consists of one such sublist per dataset |
| <code>subsets.vc</code> | character(): specifying a subset of the subdirectories to be included in the <code>MultiAssayExperiment</code> (by default, all subdirectories containing the 3 tables will be considered as datasets)                                                                                                                                                                                  |
| <code>output.c</code>   | character(1): Either 'exp' for <code>SummarizedExperiment</code> (or <code>MultiAssayExperiment</code> ), or 'set' for <code>ExpressionSet</code> (or <code>MultiDataSet</code> ) output formats (the latter are supported for convenience)                                                                                                                                             |
| <code>report.c</code>   | character(1): File name for the printed results (call to 'sink()'); if NA (default), messages will be printed on the screen; if NULL, no verbose will be generated                                                                                                                                                                                                                      |

**Value**

`SummarizedExperiment` (one dataset) or `MultiAssayExperiment` (multiple datasets) instance containing the dataset(s)

**Examples**

```
data_dir.c <- system.file("extdata", package = "phenomis")
## 1) Single set
sacurine_dir.c <- file.path(data_dir.c, "sacurine")
sacurine.se <- reading(sacurine_dir.c)
# or
sacurine.se <- reading(NA,
  files.ls = list(dataMatrix = file.path(sacurine_dir.c,
    "Galaxy1_dataMatrix.tabular"),
    sampleMetadata = file.path(sacurine_dir.c,
    "Galaxy2_sampleMetadata.tabular"),
    variableMetadata = file.path(sacurine_dir.c,
    "Galaxy3_variableMetadata.tabular")))
## 2) Multiple sets
prometis_dir.c <- file.path(data_dir.c, "prometis")
prometis.mae <- reading(prometis_dir.c)
```

```

metabo.mae <- reading(prometis_dir.c, subsets.vc = "metabo")
# or
prometis.mae <- reading(NA,
                        files.ls = list(metabo = list(dataMatrix = file.path(prometis_dir.c,
                                                                              "metabo", "dataMatrix.tsv"),
                                                                              sampleMetadata = file.path(prometis_dir.c,
                                                                              "metabo", "sampleMetadata.tsv"),
                                                                              variableMetadata = file.path(prometis_dir.c,
                                                                              "metabo", "variableMetadata.tsv")),
                        proteo = list(dataMatrix = file.path(prometis_dir.c,
                                                             "proteo", "dataMatrix.tsv"),
                                                             sampleMetadata = file.path(prometis_dir.c,
                                                             "proteo", "sampleMetadata.tsv"),
                                                             variableMetadata = file.path(prometis_dir.c,
                                                             "proteo", "variableMetadata.tsv"))))

```

reducing

*Grouping chemically redundant MSI features*

## Description

This method groups chemically redundant features from a peak table, based on 1) correlation of sample profiles, 2) retention time window, 3) referenced m/z differences. The initial algorithm is named 'Analytic Correlation Filtration' (Monnerie et al., 2019; DOI:10.3390/metabo9110250) and is available in Perl and on the Workflow4Metabolomics platform. Here, the algorithm described in the paper was implemented in R as follows: An adjacency matrix of all pairs of features is built, containing a 1 when the features have a (Pearson) correlation above the (0.9) threshold, a retention time difference between the (6) seconds threshold, and an m/z difference belonging to referenced adducts, isotopes and fragments m/z difference, and containing a 0 otherwise. The connex components of this adjacency matrix are extracted ('igraph' package). Within each component, the features are ranked by decreasing average intensity in samples; all features except the first one are flagged as 'redundant'. Note: the algorithm relies on the 'mzdiff\_db.tsv' file referencing the known adducts, isotopes, and fragments.

## Usage

```

reducing(
  x,
  cor_method.c = "pearson",
  cor_threshold.n = 0.9,
  rt_tol.n = 6,
  rt_colname.c = "rt",
  mzdiff_tol.n = 0.005,
  mz_colname.c = "mz",
  return_adjacency.l = FALSE,
  report.c = c("none", "interactive", "myfile.txt")[2]
)

```

```
## S4 method for signature 'MultiAssayExperiment'
reducing(
  x,
  cor_method.c = "pearson",
  cor_threshold.n = 0.9,
  rt_tol.n = 6,
  rt_colname.c = "rt",
  mzdiff_tol.n = 0.005,
  mz_colname.c = "mz",
  return_adjacency.l = FALSE,
  report.c = c("none", "interactive", "myfile.txt")[2]
)
```

```
## S4 method for signature 'SummarizedExperiment'
reducing(
  x,
  cor_method.c = "pearson",
  cor_threshold.n = 0.9,
  rt_tol.n = 6,
  rt_colname.c = "rt",
  mzdiff_tol.n = 0.005,
  mz_colname.c = "mz",
  return_adjacency.l = FALSE,
  report.c = c("none", "interactive", "myfile.txt")[2]
)
```

```
## S4 method for signature 'MultiDataSet'
reducing(
  x,
  cor_method.c = "pearson",
  cor_threshold.n = 0.9,
  rt_tol.n = 6,
  rt_colname.c = "rt",
  mzdiff_tol.n = 0.005,
  mz_colname.c = "mz",
  return_adjacency.l = FALSE,
  report.c = c("none", "interactive", "myfile.txt")[2]
)
```

```
## S4 method for signature 'ExpressionSet'
reducing(
  x,
  cor_method.c = "pearson",
  cor_threshold.n = 0.9,
  rt_tol.n = 6,
  rt_colname.c = "rt",
  mzdiff_tol.n = 0.005,
  mz_colname.c = "mz",

```

```

    return_adjacency.l = FALSE,
    report.c = c("none", "interactive", "myfile.txt")[2]
  )

```

## Arguments

|                                 |                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>                  | An S4 object of class <code>SummarizedExperiment</code> or <code>MultiAssayExperiment</code> ( <code>ExpressionSet</code> and <code>MultiDataSet</code> are still supported): the dataset(s) must contain the <code>dataMatrix</code> and the <code>variableMetadata</code> (with the <code>mz</code> and <code>rt</code> columns) |
| <code>cor_method.c</code>       | character(1): correlation method (default: 'pearson')                                                                                                                                                                                                                                                                              |
| <code>cor_threshold.n</code>    | numeric(1): correlation threshold (default: 0.9)                                                                                                                                                                                                                                                                                   |
| <code>rt_tol.n</code>           | numeric(1): retention time width in seconds (default: 6 s); the time window may be increased when using hydrophilic interaction (HILIC) chromatography                                                                                                                                                                             |
| <code>rt_colname.c</code>       | character(1): column name for the retention time in the <code>rowData/fData</code> (default: 'rt')                                                                                                                                                                                                                                 |
| <code>mzdiff_tol.n</code>       | numeric(1): tolerance in Da for the matching of m/z differences and referenced adducts, isotopes, and fragments (default: 0.005 Da)                                                                                                                                                                                                |
| <code>mz_colname.c</code>       | character(1): column name for the m/z in the <code>rowData/fData</code> (default: 'mz')                                                                                                                                                                                                                                            |
| <code>return_adjacency.l</code> | logical(1): should the adjacency matrix be returned (in addition to the updated <code>SummarizedExperiment/ExpressionSet</code> )?                                                                                                                                                                                                 |
| <code>report.c</code>           | character(1): File name with '.txt' extension for the printed results (call to <code>sink()</code> ); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated                                                                                                                  |

## Value

updated `SummarizedExperiment` or `MultiAssayExperiment` (or `ExpressionSet` and `MultiDataSet`): the `SummarizedExperiment(s)` (resp. `ExpressionSet(s)`) now include(s) 5 new columns in the `rowData` (resp. `fData`): `redund_samp_mean`, `redund_is`, `redund_group`, `redund_iso_add_frag`, `redund_repres` and `redund_relative` containing, respectively, the redundant features (coded by 1; i.e. features with a relative annotation distinct from " and 'M'), the connected components, the m/z diff. chemical annotations, the representative ion of each group, and the annotations relative to this representative ion within each group

## Examples

```

metabo.se <- reading(system.file("extdata/prometis/metabo",
                                package = "phenomis"),
                    report.c = "none")
metabo.se <- reducing(metabo.se,
                    rt_tol.n = 15)
# Note: in the 'prometis' example data set from this package, the chemical
# redundancy has already been filtered out

```

transforming

*Transformation of the data matrix intensities***Description**

A logarithmic or square root transformation may be applied to the data matrix intensities in (each of) the data set (e.g. to stabilize the variance)

**Usage**

```
transforming(
  x,
  method.vc = c("log2", "log10", "sqrt", "none")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiAssayExperiment'
transforming(
  x,
  method.vc = c("log2", "log10", "sqrt", "none")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'SummarizedExperiment'
transforming(
  x,
  method.vc = c("log2", "log10", "sqrt", "none")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'MultiDataSet'
transforming(
  x,
  method.vc = c("log2", "log10", "sqrt", "none")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)

## S4 method for signature 'ExpressionSet'
transforming(
  x,
  method.vc = c("log2", "log10", "sqrt", "none")[1],
  report.c = c("none", "interactive", "myfile.txt")[2]
)
```

**Arguments**

**x** An S4 object of class SummarizedExperiment or MultiAssayExperiment (ExpressionSet and MultiDataSet are still supported)

|           |                                                                                                                                                                                                     |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| method.vc | character of length 1 or the total number of datasets: transformation to be used for each dataset (either 'log2', 'log10', 'sqrt', or 'none')                                                       |
| report.c  | character(1): File name with '.txt' extension for the printed results (call to sink()); if 'interactive' (default), messages will be printed on the screen; if 'none', no verbose will be generated |

## Value

SummarizedExperiment or MultiAssayExperiment (or ExpressionSet and MultiDataSet) including the (list of) matrix with transformed intensities

## Examples

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se)
sacurine.se <- sacurine.se[, colData(sacurine.se)[, "sampleType"] != "pool"]
sacurine.se <- transforming(sacurine.se)
# MultiAssayExperiment
prometis.mae <- reading(system.file("extdata/prometis",
                                   package = "phenomis"))
prometis.mae <- transforming(prometis.mae, method.vc = c("log2", "none"))
# Note: in the 'prometis' example data set from the package, the data are
# already log2 transformed
```

---

vennplot

*Venn diagram with VennDiagram*

---

## Description

Venn diagram with VennDiagram

## Usage

```
vennplot(
  input.ls,
  palette.vc = RColorBrewer::brewer.pal(9, "Set1")[seq_len(5)],
  title.c = NA,
  sub.c = "",
  cat_pos.vi = NA,
  label_col.c = "black",
  lwd.i = 2,
  inverted.l = FALSE,
  figure.c = "none"
)
```

**Arguments**

|                          |                                                                                                                                                                                                               |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>input.ls</code>    | Named list of vectors to be compared                                                                                                                                                                          |
| <code>palette.vc</code>  | Character vector: Color palette                                                                                                                                                                               |
| <code>title.c</code>     | Character: Plot title                                                                                                                                                                                         |
| <code>sub.c</code>       | Character: Plot subtitle                                                                                                                                                                                      |
| <code>cat_pos.vi</code>  | Integer vector giving the position (in degrees) of each category name along the circle, with 0 at 12 o'clock; if NA, (-50, 50), (-40, 40, 180), (-15, 15, 0, 0), and (0, 287.5, 215, 145, 70) values are used |
| <code>label_col.c</code> | Character: Label color                                                                                                                                                                                        |
| <code>lwd.i</code>       | Integer: Width of the circle's circumference                                                                                                                                                                  |
| <code>inverted.l</code>  | Logical: Should the Venn diagram be flipped along its vertical axis (pairwise venn only)                                                                                                                      |
| <code>figure.c</code>    | Character: Filename for image output (with either .tiff, .png, or .svg extensions); if 'none' (default) the grid object is displayed interactively                                                            |

**Value**

invisible grid object

**Examples**

```
sacurine.se <- reading(system.file("extdata/sacurine", package = "phenomis"))
sacurine.se <- correcting(sacurine.se, figure.c = 'none')
sacurine.se <- sacurine.se[, colData(sacurine.se)[, "sampleType"] != "pool"]
sacurine.se <- transforming(sacurine.se)
sacurine.se <- sacurine.se[, colnames(sacurine.se) != "HU_neg_096_b2"]
# Student's T test
sacurine.se <- hypotesting(sacurine.se, "ttest", "gender")
# Wilcoxon T test
sacurine.se <- hypotesting(sacurine.se, "wilcoxon", "gender")
signif.ls <- list(ttest = which(rowData(sacurine.se)[, "ttest_gender_Female.Male_signif"] > 0),
wilcoxon = which(rowData(sacurine.se)[, "wilcoxon_gender_Female.Male_signif"] > 0))
vennplot(signif.ls, label_col.c = "black",
title.c = "Signif. features\nwith Student or Wilcoxon tests")
```

---

writing

*Exporting a SummarizedExperiment (or MultiAssayExperiment) instance into (subfolders with) the 3 tabulated files 'dataMatrix.tsv', 'sampleMetadata.tsv', 'variableMetadata.tsv'*

---

**Description**

Note that the `dataMatrix` is transposed before export (e.g., the samples are written column wise in the `'dataMatrix.tsv'` exported file).

**Usage**

```
writing(  
  x,  
  dir.c,  
  prefix.c = "",  
  files.ls = NULL,  
  overwrite.l = FALSE,  
  metadata.l = FALSE,  
  report.c = c("none", "interactive", "myfile.txt")[2]  
)
```

```
## S4 method for signature 'MultiAssayExperiment'
```

```
writing(  
  x,  
  dir.c,  
  prefix.c = "",  
  files.ls = NULL,  
  overwrite.l = FALSE,  
  metadata.l = FALSE,  
  report.c = c("none", "interactive", "myfile.txt")[2]  
)
```

```
## S4 method for signature 'SummarizedExperiment'
```

```
writing(  
  x,  
  dir.c,  
  prefix.c = "",  
  files.ls = NULL,  
  overwrite.l = FALSE,  
  metadata.l = FALSE,  
  report.c = c("none", "interactive", "myfile.txt")[2]  
)
```

```
## S4 method for signature 'MultiDataSet'
```

```
writing(  
  x,  
  dir.c,  
  prefix.c = "",  
  files.ls = NULL,  
  overwrite.l = FALSE,  
  metadata.l = FALSE,  
  report.c = c("none", "interactive", "myfile.txt")[2]  
)
```

```
## S4 method for signature 'ExpressionSet'
```

```
writing(  
  x,  
  dir.c,
```



```
"pro_sampleMetadata.tsv"),  
variableMetadata = file.path(getwd(),  
"pro_variableMetadata.tsv"))))
```

# Index

**\* package**  
phenomis-package, 3

annotating, 3  
annotating, ExpressionSet-method  
(annotating), 3  
annotating, MultiAssayExperiment-method  
(annotating), 3  
annotating, MultiDataSet-method  
(annotating), 3  
annotating, SummarizedExperiment-method  
(annotating), 3  
annotating\_parameters (annotating), 3

clustering, 6  
clustering, ExpressionSet-method  
(clustering), 6  
clustering, MultiAssayExperiment-method  
(clustering), 6  
clustering, MultiDataSet-method  
(clustering), 6  
clustering, SummarizedExperiment-method  
(clustering), 6

correcting, 9  
correcting, ExpressionSet-method  
(correcting), 9  
correcting, MultiAssayExperiment-method  
(correcting), 9  
correcting, MultiDataSet-method  
(correcting), 9  
correcting, SummarizedExperiment-method  
(correcting), 9

filtering, 11  
filtering, ExpressionSet-method  
(filtering), 11  
filtering, MultiAssayExperiment-method  
(filtering), 11  
filtering, MultiDataSet-method  
(filtering), 11

filtering, SummarizedExperiment-method  
(filtering), 11

gg\_barplot, 14  
gg\_boxplot, 16  
gg\_pie, 17  
gg\_volcanoplot, 18

hypotesting, 20  
hypotesting, ExpressionSet-method  
(hypotesting), 20  
hypotesting, MultiAssayExperiment-method  
(hypotesting), 20  
hypotesting, MultiDataSet-method  
(hypotesting), 20  
hypotesting, SummarizedExperiment-method  
(hypotesting), 20

inspecting, 23  
inspecting, ExpressionSet-method  
(inspecting), 23  
inspecting, MultiAssayExperiment-method  
(inspecting), 23  
inspecting, MultiDataSet-method  
(inspecting), 23  
inspecting, SummarizedExperiment-method  
(inspecting), 23

normalizing, 26  
normalizing, ExpressionSet-method  
(normalizing), 26  
normalizing, MultiAssayExperiment-method  
(normalizing), 26  
normalizing, MultiDataSet-method  
(normalizing), 26  
normalizing, SummarizedExperiment-method  
(normalizing), 26

phenomis (phenomis-package), 3  
phenomis-package, 3

reading, [27](#)  
reducing, [29](#)  
reducing, ExpressionSet-method  
    (reducing), [29](#)  
reducing, MultiAssayExperiment-method  
    (reducing), [29](#)  
reducing, MultiDataSet-method  
    (reducing), [29](#)  
reducing, SummarizedExperiment-method  
    (reducing), [29](#)  
  
transforming, [32](#)  
transforming, ExpressionSet-method  
    (transforming), [32](#)  
transforming, MultiAssayExperiment-method  
    (transforming), [32](#)  
transforming, MultiDataSet-method  
    (transforming), [32](#)  
transforming, SummarizedExperiment-method  
    (transforming), [32](#)  
  
vennplot, [33](#)  
  
writing, [34](#)  
writing, ExpressionSet-method (writing),  
    [34](#)  
writing, MultiAssayExperiment-method  
    (writing), [34](#)  
writing, MultiDataSet-method (writing),  
    [34](#)  
writing, SummarizedExperiment-method  
    (writing), [34](#)