

# Package ‘Macarron’

July 21, 2025

**Version** 1.13.2

**Title** Prioritization of potentially bioactive metabolic features from epidemiological and environmental metabolomics datasets

**Depends** R (>= 4.2.0), SummarizedExperiment

**Imports** BiocParallel, DelayedArray, WGCNA, ff, data.table, dynamicTreeCut, Maaslin2, plyr, stats, psych, xml2, httr, RJSONIO, logging, methods, utils

**Suggests** knitr, BiocStyle, optparse, testthat (>= 2.1.0), rmarkdown, markdown

**Description** Macarron is a workflow for the prioritization of potentially bioactive metabolites from metabolomics experiments. Prioritization integrates strengths of evidences of bioactivity such as covariation with a known metabolite, abundance relative to a known metabolite and association with an environmental or phenotypic indicator of bioactivity. Broadly, the workflow consists of stratified clustering of metabolic spectral features which co-vary in abundance in a condition, transfer of functional annotations, estimation of relative abundance and differential abundance analysis to identify associations between features and phenotype/condition.

**VignetteBuilder** knitr

**License** MIT + file LICENSE

**URL** <http://huttenhower.sph.harvard.edu/macarron>

**Encoding** UTF-8

**biocViews** Sequencing, Metabolomics, Coverage, FunctionalPrediction, Clustering

**BugReports** <https://forum.biobakery.org/c/microbial-community-profiling/macarron>

**RoxygenNote** 7.3.2

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

**git\_branch** devel

**git\_last\_commit** 143bd9d

**git\_last\_commit\_date** 2025-06-20

**Repository** Bioconductor 3.22

**Date/Publication** 2025-07-20

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|        |                                                                       |
|--------|-----------------------------------------------------------------------|
| calAVA | <i>Calculate abundance versus anchor (AVA) of metabolic features.</i> |
|--------|-----------------------------------------------------------------------|

---

## Description

AVA of a feature is the ratio of its abundance and the most abundant metabolite in the same module i.e. the "anchor". Anchor is an annotated/known feature if available or just the most abundant metabolic feature. For every feature, mean abundance in each phenotype or condition is calculated and the maximum is considered for AVA calculation. Singletons are assigned an AVA of 1.

## Usage

```
calAVA(se, mod.assn, metadata_variable = 1, anchor_annotation = 2)
```

## Arguments

|                   |                                                                                                                                                                                                                                                          |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| se                | SummarizedExperiment object created using Macarron::prepInput().                                                                                                                                                                                         |
| mod.assn          | the output of Macarron::findMacMod().                                                                                                                                                                                                                    |
| metadata_variable | name or index of metadata column identifying phenotypes/conditions to be used for evaluating AVA. Default: Column 1 of metadata dataframe. Note: metadata_variable must be consistent across distance matrix, ava, q-value and effect-size calculations. |
| anchor_annotation | name or index of column containing common names of the annotated metabolite. Default: Column 2 of annotation dataframe.                                                                                                                                  |

## Value

mac.ava abundance versus anchor values of metabolic features

## Examples

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                          input_annotations = annotations_df,
                          input_metadata = metadata_df)
w <- Macarron::makeDisMat(se = mbx)
modules.assign <- Macarron::findMacMod(se = mbx,
                                       w = w,
                                       input_taxonomy = taxonomy_df)
mets.ava <- Macarron::calAVA(se = mbx,
                             mod.assign = modules.assign)
```

---

calES

---

*Calculate effect size of differential abundance of metabolic features.*


---

## Description

Effect size of a metabolic feature is the difference in mean log2 transformed abundances in test and control (reference) samples. For the specified metadata variable, effect size is calculated for all test categories against the reference category.

## Usage

```
calES(se, mac.qval)
```

## Arguments

|          |                                                                  |
|----------|------------------------------------------------------------------|
| se       | SummarizedExperiment object created using Macarron::prepInput(). |
| mac.qval | the output of Macarron::calQval().                               |

## Value

mac.es effect sizes of metabolic features in phenotypes of interest.

## Examples

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
```

```

        input_annotations = annotations_df,
        input_metadata = metadata_df)
w <- Macarron::makeDisMat(se = mbx)
modules.assn <- Macarron::findMacMod(se = mbx,
                                   w = w,
                                   input_taxonomy = taxonomy_df)
mets.qval <- Macarron::calQval(se = mbx,
                             mod.assn = modules.assn)
mets.es <- Macarron::calES(se = mbx,
                          mac.qval = mets.qval)

```

calQval

*Calculate q-value of differential abundance of metabolic features.*

### Description

This function uses the MaAsLin2 package for estimating q-value of differential abundance. Multiple fixed and random effects can be specified for fitting the multiple regression model. Default analysis method is "LM". Can be run on multiple cores. `metadata_variable` and `ref` (reference group) should be the same as the one specified for effect size calculation.

### Usage

```

calQval(
  se,
  mod.assn,
  metadata_variable = 1,
  fixed_effects = NULL,
  random_effects = NULL,
  reference = NULL,
  output_folder = NULL,
  cores = 1,
  plot_heatmap = FALSE,
  plot_scatter = FALSE,
  heatmap_first_n = 50
)

```

### Arguments

|                                |                                                                                                                                                                                                                                                                                                                |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>se</code>                | SummarizedExperiment object created using <code>Macarron::prepInput()</code> .                                                                                                                                                                                                                                 |
| <code>mod.assn</code>          | the output of <code>Macarron::findMacMod()</code> .                                                                                                                                                                                                                                                            |
| <code>metadata_variable</code> | name or index of metadata column identifying phenotypes/conditions to be used for differential abundance testing. Default: Column 1 of metadata dataframe<br>Note: <code>metadata_variable</code> must be consistent across <code>ava</code> , <code>q-value</code> and <code>effect-size</code> calculations. |
| <code>fixed_effects</code>     | fixed effects, comma delimited e.g. <code>c("metadata1","metadata2")</code> . Default: all columns in metadata.                                                                                                                                                                                                |
| <code>random_effects</code>    | random effects, comma delimited. Default: <code>NULL</code> .                                                                                                                                                                                                                                                  |

|                 |                                                                                                                                                                                                                                                                                                                      |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| reference       | a reference level/group in each metadata column with more than 3 levels, semi-colon delimited for multiple variables e.g. c("metadata1,ref1";"metadata2,ref2"). Default: alphabetically first phenotype/condition will be used as reference. Note: Reference must be specified for metadata with more than 2 levels. |
| output_folder   | the name of the output folder where all MaAsLin2 results will be written. Default: maaslin2_output                                                                                                                                                                                                                   |
| cores           | the number of R processes to run in parallel.                                                                                                                                                                                                                                                                        |
| plot_heatmap    | Maaslin2 option-Generate a heatmap for the significant associations. Default: TRUE                                                                                                                                                                                                                                   |
| plot_scatter    | Maaslin2 option-Generate scatter plots for the significant associations. Default: FALSE                                                                                                                                                                                                                              |
| heatmap_first_n | Maaslin2 option-Generate heatmap for top n significant associations. Default: 50                                                                                                                                                                                                                                     |

### Value

mac.qval q-value of metabolic features in phenotypes of interest.

### Examples

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                          input_annotations = annotations_df,
                          input_metadata = metadata_df)
w <- Macarron::makeDisMat(se = mbx)
modules.assign <- Macarron::findMacMod(se = mbx,
                                       w = w,
                                       input_taxonomy = taxonomy_df)
mets.qval <- Macarron::calQval(se = mbx,
                              mod.assign = modules.assign)
```

---

decorateID

*Create a chemical taxonomy table for annotated metabolic features.*

---

### Description

Create a chemical taxonomy table for annotated metabolic features.

### Usage

```
decorateID(input_annotations)
```

**Arguments**

input\_annotations

a dataframe (features x annotations) containing the available feature annotations.  
 ^^Column 1 must contain standard annotations such as HMDB ID or PubChem CID for the subset of identified/annotated metabolic features.

**Value**

tax\_df input\_taxonomy-dataframe containing ID (HMDB or PubChem), chemical sub class and chemical class of annotated metabolic features.

**Examples**

```
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
input_taxonomy <- decorateID(annotations_df)
```

---

|            |                                                                              |
|------------|------------------------------------------------------------------------------|
| findMacMod | <i>Cluster metabolic features based on covarying abundances into modules</i> |
|------------|------------------------------------------------------------------------------|

---

**Description**

Cluster metabolic features based on covarying abundances into modules

**Usage**

```
findMacMod(
  se,
  w,
  input_taxonomy,
  standard_identifier = 1,
  min_module_size = NULL,
  evaluateMOS = TRUE
)
```

**Arguments**

se SummarizedExperiment object created using Macarron::prepInput().

w distance matrix from function Macarron::makeDisMat().

input\_taxonomy chemical taxonomy file with 3 columns specifying annotation, subclass and class of annotated features. Can be created using the decorateID.R utility of Macarron. Annotation specified with "standard\_identifier" and annotation in the first column of the chemical taxonomy file must match.

standard\_identifier name or index of column containing HMDB or PubChem IDs. Default: Column 1 in annotation dataframe.

min\_module\_size minimum module size to be used for module identification with dynamicTreeCut::cutreeDynamic(). Default is cube root of number of prevalent features.

evaluateMOS      examine measure of success for modules identified using min\_module\_size, min\_module\_size + 5, min\_module\_size + 10, min\_module\_size - 5, min\_module\_size - 10

Value

mod.assn metabolic features clustered into "modules" based on covarying abundances and measures of success.

Examples

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                           input_annotations = annotations_df,
                           input_metadata = metadata_df)
w <- Macarron::makeDisMat(se = mbx)
modules.assn <- Macarron::findMacMod(se = mbx,
                                     w = w,
                                     input_taxonomy = taxonomy_df)
```

---

|          |                 |
|----------|-----------------|
| Macarron | <i>Macarron</i> |
|----------|-----------------|

---

Description

Macarron

Usage

```
Macarron(
  input_abundances,
  input_annotations,
  input_metadata,
  input_taxonomy,
  output = "Macarron_output",
  metadata_variable = 1,
  min_prevalence = 0.7,
  execution_mode = "serial",
  standard_identifier = 1,
  anchor_annotation = 2,
  min_module_size = NULL,
  fixed_effects = NULL,
  random_effects = NULL,
```

```

reference = NULL,
cores = 1,
plot_heatmap = FALSE,
plot_scatter = TRUE,
heatmap_first_n = 50,
show_best = TRUE,
priority_threshold = 0.9,
per_module = 10,
per_phenotype = 1000,
only_characterizable = TRUE
)

```

## Arguments

**input\_abundances** a comma-delimited file or dataframe (features x samples) containing metabolic feature intensities (abundances).

**input\_annotations** a comma-delimited file or dataframe (features x annotations) containing available feature annotations.

**input\_metadata** a comma-delimited file or dataframe (samples x metadata) containing sample metadata.

**input\_taxonomy** a comma-delimited file or dataframe containing the chemical class and subclass information of annotated features.

**output** name of the folder where Macarron output files will be written. Default: "Macarron\_output".

**metadata\_variable** Name or index of the column that identifies the phenotypes/conditions in the study. Default: Column 1 of metadata dataframe.

**min\_prevalence** prevalence threshold (percentage). Default = 0.7.

**execution\_mode** BiocParallel execution mode. Options: "serial" or "multi" Default = "serial".

**standard\_identifier** Name or index of column containing HMDB or PubChem IDs. Default: Column 1 in annotation dataframe.

**anchor\_annotation** Name or index of column containing common names of the annotated metabolite. Default: Column 2 of annotation dataframe.

**min\_module\_size** Integer that defines the size of the smallest covariance module. Default: Cube root of number of prevalent metabolic features.

**fixed\_effects** Covariates for linear modeling with MaAsLin2. Default: All columns of metadata dataframe.

**random\_effects** Random effects for linear modeling with MaAsLin2. Default: NULL.

**reference** Reference category (factor) in categorical metadata covariates containing three or more levels. Must be provided as a string of 'covariate,reference' semi-colon delimited for multiple covariates.

**cores** MaAsLin2 option-The number of R processes to be run in parallel.

**plot\_heatmap** MaAslin2 option-Generate a heatmap for the significant associations. Default: TRUE



|                      |                                                                                                             |
|----------------------|-------------------------------------------------------------------------------------------------------------|
| plot_scatter         | MaAslin2 option-Generate scatter plots for the significant associations. Default: FALSE                     |
| heatmap_first_n      | MaAslin2 option-Generate heatmap for top n significant associations. Default = 50                           |
| show_best            | write 1000 or fewer highly prioritized metabolic features into a separate file. Default: TRUE               |
| priority_threshold   | cut-off of priority score for showing highly prioritized features. Default = 0.9                            |
| per_module           | show first n highly prioritized features in a module. Default = 10                                          |
| per_phenotype        | show highly prioritized n features per phenotype/condition. Default = 1000                                  |
| only_characterizable | show highly prioritized features in modules which contain at least one annotated metabolite. Default = TRUE |

### Value

mac.result dataframes containing metabolic features listed according to their priority (potential bioactivity) in a phenotype of interest.

### Examples

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
mets.prioritized <- Macarron::Macarron(input_abundances = prism_abundances,
                                       input_annotations = prism_annotations,
                                       input_metadata = prism_metadata,
                                       input_taxonomy = met_taxonomy)
```

---

|            |                                                                                 |
|------------|---------------------------------------------------------------------------------|
| makeDisMat | <i>Create a biweight midcorrelation (WGCNA::bicor()) based distance matrix.</i> |
|------------|---------------------------------------------------------------------------------|

---

### Description

Create a biweight midcorrelation (WGCNA::bicor()) based distance matrix.

### Usage

```
makeDisMat(
  se,
  metadata_variable = 1,
  min_prevalence = 0.7,
  execution_mode = "serial",
  optimize_for = c("runtime", "memory")
)
```

**Arguments**

|                                |                                                                                                                                                                                                                                                                                                                                                                                                                       |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>se</code>                | SummarizedExperiment object created using <code>Macarron::prepInput()</code> .                                                                                                                                                                                                                                                                                                                                        |
| <code>metadata_variable</code> | metadata column identifying phenotypes/conditions to be used to evaluate prevalence of features. Default = Column 1 of metadata dataframe.                                                                                                                                                                                                                                                                            |
| <code>min_prevalence</code>    | prevalence threshold (percentage). Default = 0.7.                                                                                                                                                                                                                                                                                                                                                                     |
| <code>execution_mode</code>    | "serial" or "multi" processing with BiocParallel. Default: "serial" (recommended for laptops). "multi" may be used when running Macarron on a cluster.                                                                                                                                                                                                                                                                |
| <code>optimize_for</code>      | runtime or memory.<br><br>Features present (i.e. not NA) in "min_prevalence" of samples in each category of a "metadata_variable" will be considered e.g. if min_prevalence is 0.7 and metadata_variable has 2 categories A and B, union of (i) features present in at least 70 and (ii) features present in at least 70 Correlation between feature abundances are is calculated using <code>WGCNA::bicor()</code> . |

**Value**

w distance matrix where  $\text{distance} = 1 - \text{bicor}^3$

**Examples**

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                           input_annotations = annotations_df,
                           input_metadata = metadata_df)
w <- Macarron::makeDisMat(se = mbx)
```

---

```
prepInput
```

---

*Create a SummarizedExperiment object*

---

**Description**

Create a SummarizedExperiment object

**Usage**

```
prepInput(input_abundances, input_annotations, input_metadata)
```

**Arguments**

- `input_abundances` a dataframe (features x samples) containing metabolic feature intensities (abundances).
- `input_annotations` a dataframe (features x annotations) containing the available feature annotations. ^^Column 1 must contain standard annotations such as HMDB ID or Pubchem CID for the subset of identified/annotated features. ^^Column 2 must contain metabolite name. ^^Column 3 must contain a continuous numeric chemical property such as m/z or shift/ppm.
- `input_metadata` a dataframe (samples x metadata) containing sample metadata. ^^Row names must identify samples. ^^Column 1 must identify phenotypes or conditions (categorical metadata) associated with the samples. Must not contain NA. Rows with no specified phenotype/condition will be removed.

**Value**

SummarizedExperiment object

**Examples**

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                           input_annotations = annotations_df,
                           input_metadata = metadata_df)
```

---

|                         |                                                                               |
|-------------------------|-------------------------------------------------------------------------------|
| <code>prioritize</code> | <i>Rank metabolic features and prioritize based on predicted bioactivity.</i> |
|-------------------------|-------------------------------------------------------------------------------|

---

**Description**

Metabolic features are ranked based on AVA, and q-value and effect size of differential abundance. The harmonic mean of these three ranks is calculated and used as the meta-rank to prioritize potentially bioactive features in a phenotype (or condition). Top-ranked features have good relative abundance, and are significantly perturbed in the specified environment/phenotype.

**Usage**

```
prioritize(se, mod.assn, mac.ava, mac.qval, mac.es)
```

**Arguments**

- `se` SummarizedExperiment object created using `Macarron::prepInput()`
- `mod.assn` the output of `Macarron::findMacMod()`
- `mac.ava` the output of `Macarron::calAVA()`
- `mac.qval` the output of `Macarron::calQval()`
- `mac.es` the output of `Macarron::calES()`

**Value**

mac.result - metabolic features listed according to priority

**Examples**

```
prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                           input_annotations = annotations_df,
                           input_metadata = metadata_df)

w <- Macarron::makeDisMat(se = mbx)
modules.assign <- Macarron::findMacMod(se = mbx,
                                       w = w,
                                       input_taxonomy = taxonomy_df)

mets.ava <- Macarron::calAVA(se = mbx,
                             mod.assign = modules.assign)
mets.qval <- Macarron::calQval(se = mbx,
                              mod.assign = modules.assign)
mets.es <- Macarron::calES(se = mbx,
                           mac.qval = mets.qval)
mets.prioritized <- Macarron::prioritize(se = mbx,
                                         mod.assign = modules.assign,
                                         mac.ava = mets.ava,
                                         mac.qval = mets.qval,
                                         mac.es = mets.es)
```

---

showBest

---

View highly prioritized bioactives grouped by modules.

---

**Description**

Modules are listed in the order of priority. Only the top-ranked *n* features in each module are shown. The priority of a module is the ratio of number of features in it that are ranked higher than the cut-off and the size of the module. This utility function makes it easier to understand default prioritization results of large datasets where a few hundred metabolic features are highly-prioritized.

**Usage**

```
showBest(
  mac.result,
  priority_threshold = 0.9,
  per_module = 10,
  per_phenotype = 1000,
  only_characterizable = TRUE
)
```

## Arguments

**mac.result**        the output of `Macarron::Macarron()` or `Macarron::prioritize()`.  
**priority\_threshold**        cut-off of priority score. Default = 0.9.  
**per\_module**        show first n highly prioritized features in a module. Default = 10  
**per\_phenotype**    show highly prioritized n features per phenotype/condition. Default = 1000  
**only\_characterizable**    show highly prioritized features in modules which contain at least one annotated metabolite. Default = TRUE

## Value

best.mets -highly-prioritized bioactives in each module in each phenotype

## Examples

```

prism_abundances = system.file("extdata", "demo_abundances.csv", package="Macarron")
abundances_df = read.csv(file = prism_abundances, row.names = 1)
prism_annotations = system.file("extdata", "demo_annotations.csv", package="Macarron")
annotations_df = read.csv(file = prism_annotations, row.names = 1)
prism_metadata = system.file("extdata", "demo_metadata.csv", package="Macarron")
metadata_df = read.csv(file = prism_metadata, row.names = 1)
met_taxonomy = system.file("extdata", "demo_taxonomy.csv", package="Macarron")
taxonomy_df = read.csv(file = met_taxonomy)
mbx <- Macarron::prepInput(input_abundances = abundances_df,
                           input_annotations = annotations_df,
                           input_metadata = metadata_df)

w <- Macarron::makeDisMat(se = mbx)
modules.assign <- Macarron::findMacMod(se = mbx,
                                       w = w,
                                       input_taxonomy = taxonomy_df)

mets.ava <- Macarron::calAVA(se = mbx,
                             mod.assign = modules.assign)
mets.qval <- Macarron::calQval(se = mbx,
                              mod.assign = modules.assign)
mets.es <- Macarron::calES(se = mbx,
                           mac.qval = mets.qval)
mets.prioritized <- Macarron::prioritize(se = mbx,
                                         mod.assign = modules.assign,
                                         mac.ava = mets.ava,
                                         mac.qval = mets.qval,
                                         mac.es = mets.es)

best.mets <- Macarron::showBest(mac.result = mets.prioritized)
  
```

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