

# Package ‘Macarron’

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**Version** 1.8.0

**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** 7.1.2

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## Contents

calAVA	2
calES	3
calQval	4
decorateID	6
findMacMod	6
Macarron	8
makeDisMat	10
prepInput	11
prioritize	12
showBest	13
<b>Index</b>	<b>15</b>

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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 log<sub>2</sub> 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.assign <- Macarron::findMacMod(se = mbx,
                                       w = w,
                                       input_taxonomy = taxonomy_df)

mets.qval <- Macarron::calQval(se = mbx,
                              mod.assign = modules.assign)

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.assign,
  metadata_variable = 1,
  fixed_effects = NULL,
  random_effects = NULL,
  reference = NULL,
  output_folder = NULL,
  cores = 1,
  plot_heatmap = TRUE,
  plot_scatter = FALSE,
  heatmap_first_n = 50
)
```



```
mets.qval <- Macarron::calQval(se = mbx,  
                             mod.assn = modules.assn)
```

---

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                    *Cluster metabolic features based on covarying abundances into modules*

---

### 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

*Macarron*

---

## 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 = TRUE,  
  plot_scatter = FALSE,  
  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.



<code>input_metadata</code>	a comma-delimited file or dataframe (samples x metadata) containing sample metadata.
<code>input_taxonomy</code>	a comma-delimited file or dataframe containing the chemical class and subclass information of annotated features.
<code>output</code>	name of the folder where Macarron output files will be written. Default: "Macarron_output".
<code>metadata_variable</code>	Name or index of the column that identifies the phenotypes/conditions in the study. Default: Column 1 of metadata dataframe.
<code>min_prevalence</code>	prevalence threshold (percentage). Default = 0.7.
<code>execution_mode</code>	BiocParallel execution mode. Options: "serial" or "multi" Default = "serial".
<code>standard_identifier</code>	Name or index of column containing HMDB or PubChem IDs. Default: Column 1 in annotation dataframe.
<code>anchor_annotation</code>	Name or index of column containing common names of the annotated metabolite. Default: Column 2 of annotation dataframe.
<code>min_module_size</code>	Integer that defines the size of the smallest covariance module. Default: Cube root of number of prevalent metabolic features.
<code>fixed_effects</code>	Covariates for linear modeling with MaAsLin2. Default: All columns of metadata dataframe.
<code>random_effects</code>	Random effects for linear modeling with MaAsLin2. Default: NULL.
<code>reference</code>	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.
<code>cores</code>	MaAsLin2 option-The number of R processes to be run in parallel.
<code>plot_heatmap</code>	MaAslin2 option-Generate a heatmap for the significant associations. Default: TRUE
<code>plot_scatter</code>	MaAslin2 option-Generate scatter plots for the significant associations. Default: FALSE
<code>heatmap_first_n</code>	MaAslin2 option-Generate heatmap for top n significant associations. Default = 50
<code>show_best</code>	write 1000 or fewer highly prioritized metabolic features into a separate file. Default: TRUE
<code>priority_threshold</code>	cut-off of priority score for showing highly prioritized features. Default = 0.9
<code>per_module</code>	show first n highly prioritized features in a module. Default = 10
<code>per_phenotype</code>	show highly prioritized n features per phenotype/condition. Default = 1000
<code>only_characterizable</code>	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**

se	SummarizedExperiment object created using Macarron::prepInput().
metadata_variable	metadata column identifying phenotypes/conditions to be used to evaluate prevalence of features. Default = Column 1 of metadata dataframe.
min_prevalence	prevalence threshold (percentage). Default = 0.7.
execution_mode	"serial" or "multi" processing with BiocParallel. Default: "serial" (recommended for laptops). "multi" may be used when running Macarron on a cluster.
optimize_for	runtime or memory. 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 `WGCNA::bicor()`.

### 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)
```

---

prioritize

*Rank metabolic features and prioritize based on predicted bioactivity.*

---

**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.assn <- Macarron::findMacMod(se = mbx,
                                   w = w,
                                   input_taxonomy = taxonomy_df)

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

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

```

# Index

calAVA, [2](#)  
calES, [3](#)  
calQval, [4](#)  
  
decorateID, [6](#)  
  
findMacMod, [6](#)  
  
Macarron, [8](#)  
makeDisMat, [10](#)  
  
prepInput, [11](#)  
prioritize, [12](#)  
  
showBest, [13](#)