

# Package ‘MMUPHin’

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**Type** Package

**Title** Meta-analysis Methods with Uniform Pipeline for Heterogeneity in Microbiome Studies

**Version** 1.22.0

**Author** Siyuan Ma

**Maintainer** Siyuan MA <[syma.research@gmail.com](mailto:syma.research@gmail.com)>

**Description** MMUPHin is an R package for meta-analysis tasks of microbiome cohorts. It has function interfaces for:

- a) covariate-controlled batch- and cohort effect adjustment,
- b) meta-analysis differential abundance testing,
- c) meta-analysis unsupervised discrete structure (clustering) discovery, and
- d) meta-analysis unsupervised continuous structure discovery.

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**Encoding** UTF-8

**RoxygenNote** 7.2.1

**VignetteBuilder** knitr

**SystemRequirements** glpk (>= 4.57)

**Depends** R (>= 3.6)

**Imports** Maaslin2, metafor, fpc, igraph, ggplot2, dplyr, tidyr, stringr, cowplot, utils, stats, grDevices

**Suggests** testthat, BiocStyle, knitr, rmarkdown, magrittr, vegan, phyloseq, curatedMetagenomicData, genefilter

**biocViews** Metagenomics, Microbiome, BatchEffect

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|           |                                                                                                                                |
|-----------|--------------------------------------------------------------------------------------------------------------------------------|
| adjust_EB | <i>Perform batch adjustment on standardized feature abundances, based on EB shrunk per-batch location and scale parameters</i> |
|-----------|--------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Perform batch adjustment on standardized feature abundances, based on EB shrunk per-batch location and scale parameters

**Usage**

```
adjust_EB(s_data, l_params_shrink, l_stand_feature, batchmod, n_batch,
          l_ind)
```

**Arguments**

|                 |                                                                             |
|-----------------|-----------------------------------------------------------------------------|
| s_data          | feature-by-sample matrix of standardized abundances.                        |
| l_params_shrink | list of shrunk parameters, as returned by fit_shrink.                       |
| l_stand_feature | list of per-feature standardization fits, as returned by fit_stand_feature. |
| batchmod        | design matrix for batch variables.                                          |
| n_batch         | number of batches in the data.                                              |
| l_ind           | list of indicator matrices, as returned by construct_ind.                   |

**Value**

feature-by-sample matrix of batch-adjusted feature abundances.

---

|        |                                                 |
|--------|-------------------------------------------------|
| aprior | <i>EB prior estimation for scale parameters</i> |
|--------|-------------------------------------------------|

---

**Description**

EB prior estimation for scale parameters

**Usage**

```
aprior(delta_hat, na.rm = FALSE)
```

**Arguments**

|           |                                                  |
|-----------|--------------------------------------------------|
| delta_hat | frequentist per-batch scale estimations.         |
| na.rm     | whether or not missing values should be removed. |

**Value**

shape hyper parameter

---

|     |                                                                     |
|-----|---------------------------------------------------------------------|
| AST | <i>AST transformation (modified from Maaslin2 and is different)</i> |
|-----|---------------------------------------------------------------------|

---

**Description**

AST transformation (modified from Maaslin2 and is different)

**Usage**

AST(x)

**Arguments**

x                      vector of abundance to be transformed.

**Value**

transformed vector of abundance.

---

|                    |                                                                                              |
|--------------------|----------------------------------------------------------------------------------------------|
| back_transform_abd | <i>Transform batch adjusted feature abundances back to the original scale in feature_abd</i> |
|--------------------|----------------------------------------------------------------------------------------------|

---

**Description**

Transform batch adjusted feature abundances back to the original scale in feature\_abd

**Usage**

back\_transform\_abd(adj\_data, feature\_abd, type\_feature\_abd)

**Arguments**

adj\_data              feature-by-sample matrix of batch-adjusted feature abundances with covariate effects retained.

feature\_abd          original feature-by-sample matrix of abundances (proportions or counts).

type\_feature\_abd      type of feature abundance table (counts or proportions). If counts, the final output will be rounded into counts as well.

**Value**

feature-by-sample matrix of batch-adjusted feature abundances, with covariate effects retained and scales consistent with original abundance matrix.

---

|        |                                                 |
|--------|-------------------------------------------------|
| bprior | <i>EB prior estimation for scale parameters</i> |
|--------|-------------------------------------------------|

---

**Description**

EB prior estimation for scale parameters

**Usage**

```
bprior(delta_hat, na.rm = FALSE)
```

**Arguments**

|           |                                                  |
|-----------|--------------------------------------------------|
| delta_hat | frequentist per-batch location estimations.      |
| na.rm     | whether or not missing values should be removed. |

**Value**

scale hyper parameter

---

|             |                                                    |
|-------------|----------------------------------------------------|
| catchToList | <i>Utility for catching warning/error messages</i> |
|-------------|----------------------------------------------------|

---

**Description**

Utility for catching warning/error messages

**Usage**

```
catchToList(expr)
```

**Arguments**

|      |                                                                  |
|------|------------------------------------------------------------------|
| expr | an expression to run that can generate potential errors/warnings |
|------|------------------------------------------------------------------|

**Value**

a list, capturing both the return value of the expression, as well as generated errors/warnings (NULL if no errors/warnings)

---

|             |                             |
|-------------|-----------------------------|
| check_batch | <i>Check batch variable</i> |
|-------------|-----------------------------|

---

**Description**

Check batch variable

**Usage**

```
check_batch(x, min_n_batch = 2)
```

**Arguments**

|             |                                                        |
|-------------|--------------------------------------------------------|
| x           | batch variable.                                        |
| min_n_batch | min. number of batches (for MMUPHIn functions to run). |

**Value**

if no errors then the batch variables (factorized if not already)

---

|                  |                         |
|------------------|-------------------------|
| check_covariates | <i>Check covariates</i> |
|------------------|-------------------------|

---

**Description**

Check covariates

**Usage**

```
check_covariates(data_covariates, batch)
```

**Arguments**

|                 |                           |
|-----------------|---------------------------|
| data_covariates | data frame of covariates. |
| batch           | batch variable.           |

**Value**

vector of indicators per batch for if/which covariates can be fitted within the batches

---

|                         |                                |
|-------------------------|--------------------------------|
| check_covariates_random | <i>Check random covariates</i> |
|-------------------------|--------------------------------|

---

**Description**

Check random covariates

**Usage**

```
check_covariates_random(data_covariates, batch)
```

**Arguments**

|                 |                                  |
|-----------------|----------------------------------|
| data_covariates | data frame of random covariates. |
| batch           | batch variable.                  |

**Value**

vector of indicators per batch for if/which random covariates can be fitted within the batches

---

|         |                                   |
|---------|-----------------------------------|
| check_D | <i>Check dissimilarity object</i> |
|---------|-----------------------------------|

---

**Description**

Make sure that the input is a dissimilarity object

**Usage**

```
check_D(D)
```

**Arguments**

|   |                       |
|---|-----------------------|
| D | dissimilarity object. |
|---|-----------------------|

**Value**

returns an error if D is not a dissimilarity. Otherwise D as a matrix.

---

|                |                                |
|----------------|--------------------------------|
| check_exposure | <i>Check exposure variable</i> |
|----------------|--------------------------------|

---

**Description**

Check exposure variable

**Usage**

```
check_exposure(exposure, batch)
```

**Arguments**

|          |                    |
|----------|--------------------|
| exposure | exposure variable. |
| batch    | batch variable.    |

**Value**

vector of indicators per batch for whether or not the exposure can be fitted within the batches

---

|                   |                                      |
|-------------------|--------------------------------------|
| check_feature_abd | <i>Check feature abundance table</i> |
|-------------------|--------------------------------------|

---

**Description**

Given a feature abundance table, make sure that a) it has no missing values, b) all values are non-negative, c) it is either proportions (all no greater than 1) or counts (all integers).

**Usage**

```
check_feature_abd(feature_abd)
```

**Arguments**

|             |                                                                 |
|-------------|-----------------------------------------------------------------|
| feature_abd | feature-by-sample matrix of abundances (proportions or counts). |
|-------------|-----------------------------------------------------------------|

**Value**

returns an error if any of the check fails. Otherwise either "counts" or "proportions"

---

|                |                                                                             |
|----------------|-----------------------------------------------------------------------------|
| check_metadata | <i>Check that metadata data frame has all the variables and not missing</i> |
|----------------|-----------------------------------------------------------------------------|

---

**Description**

Check that metadata data frame has all the variables and not missing

**Usage**

```
check_metadata(data, variables, no_missing = TRUE)
```

**Arguments**

|           |                                                      |
|-----------|------------------------------------------------------|
| data      | data frame of metadata.                              |
| variables | name of variables (batch, covariates, etc.) to check |

**Value**

data reduced to include only those specified in variables

---

|               |                                     |
|---------------|-------------------------------------|
| check_options | <i>Utility for checking options</i> |
|---------------|-------------------------------------|

---

**Description**

Utility for checking options

**Usage**

```
check_options(x, x_name, options)
```

**Arguments**

|         |                             |
|---------|-----------------------------|
| x       | the specified value         |
| x_name  | name of the specified value |
| options | allowed options             |

**Value**

error if x is not in options. Otherwise returns x.

---

|                          |                                                |
|--------------------------|------------------------------------------------|
| check_options_continuous | <i>Utility for checking continuous options</i> |
|--------------------------|------------------------------------------------|

---

**Description**

Utility for checking continuous options

**Usage**

check\_options\_continuous(x, x\_name, range)

**Arguments**

|        |                             |
|--------|-----------------------------|
| x      | the specified numeric value |
| x_name | name of the specified value |
| range  | allowed range               |

**Value**

error if x is not within range (boundaries excluded). Otherwise returns x.

---

|                    |                                          |
|--------------------|------------------------------------------|
| check_pseudo_count | <i>Utility for checking pseudo count</i> |
|--------------------|------------------------------------------|

---

**Description**

Utility for checking pseudo count

**Usage**

check\_pseudo\_count(x)

**Arguments**

|   |                            |
|---|----------------------------|
| x | the specified pseudo count |
|---|----------------------------|

**Value**

error if pseudo count is smaller than zero. Otherwise returns x.

---

|            |                                              |
|------------|----------------------------------------------|
| check_rank | <i>Check if a design matrix is full rank</i> |
|------------|----------------------------------------------|

---

**Description**

Check if a design matrix is full rank

**Usage**

```
check_rank(design)
```

**Arguments**

design                  design matrix.

**Value**

TRUE/FALSE for whether or not the design matrix is full rank.

---

|               |                                                                                                    |
|---------------|----------------------------------------------------------------------------------------------------|
| check_samples | <i>Check that sample numbers and names match between a feature table and a metadata data frame</i> |
|---------------|----------------------------------------------------------------------------------------------------|

---

**Description**

Sample names (column names of the feature table, row names of the metadata data frame) must be matching exactly. Note that this dictates that they cannot be NULL because by design data (a data frame) should have non-empty row names.

**Usage**

```
check_samples(feature_abd, data)
```

**Arguments**

feature\_abd          feature-by-sample matrix of abundances (proportions or counts).  
data                  data frame of metadata.

**Value**

matched sample names

---

|                 |                                                                                                           |
|-----------------|-----------------------------------------------------------------------------------------------------------|
| check_samples_D | <i>Check that sample numbers and names match between a dissimilarity matrix and a metadata data frame</i> |
|-----------------|-----------------------------------------------------------------------------------------------------------|

---

**Description**

Sample names (row/column names of the D matrix, row names of the metadata data frame) must be matching exactly. Note that this dictates that they cannot be NULL because by design data (a data frame) should have non-empty row names.

**Usage**

```
check_samples_D(D, data)
```

**Arguments**

|      |                                                                     |
|------|---------------------------------------------------------------------|
| D    | sample-by-sample matrix of dissimilarities (proportions or counts). |
| data | data frame of metadata.                                             |

**Value**

matched sample names

---

|                  |                                                                                                               |
|------------------|---------------------------------------------------------------------------------------------------------------|
| construct_design | <i>Construct a design model matrix given a metadata data frame, with the option to exclude the intercept.</i> |
|------------------|---------------------------------------------------------------------------------------------------------------|

---

**Description**

Construct a design model matrix given a metadata data frame, with the option to exclude the intercept.

**Usage**

```
construct_design(data, with_intercept = TRUE)
```

**Arguments**

|                |                                                 |
|----------------|-------------------------------------------------|
| data           | metadata data frame.                            |
| with_intercept | should intercept terms be included in the model |

**Value**

design matrix.

---

|               |                                                                                                                                                                |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| construct_ind | <i>Create indicator matrices for which feature/batch/samples to adjust. This is relevant for zero_inflation is TRUE and only non-zero values are adjusted.</i> |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|

---

## Description

Create indicator matrices for which feature/batch/samples to adjust. This is relevant for zero\_inflation is TRUE and only non-zero values are adjusted.

## Usage

```
construct_ind(feature_abd, n_batch, design, zero_inflation)
```

## Arguments

|                |                                                                 |
|----------------|-----------------------------------------------------------------|
| feature_abd    | feature-by-sample matrix of abundances (proportions or counts). |
| n_batch        | number of batches in the data.                                  |
| design         | design matrix.                                                  |
| zero_inflation | zero inflation flag.                                            |

## Value

list of indicator matrices needed by fitting in adjust\_batch.

---

|                     |                                                                                                                   |
|---------------------|-------------------------------------------------------------------------------------------------------------------|
| continuous_discover | <i>Unsupervised meta-analytical discovery and validation of continuous structures in microbial abundance data</i> |
|---------------------|-------------------------------------------------------------------------------------------------------------------|

---

## Description

continuous\_discover takes as input a feature-by-sample matrix of microbial abundances. It first performs unsupervised continuous structure discovery (PCA) within each batch. Loadings of top PCs from each batch are then mapped against each other to identify "consensus" loadings that are reproducible across batches with a network community discovery approach with **igraph**. The identified consensus loadings/scores can be viewed as continuous structures in microbial profiles that are recurrent across batches and valid in a meta-analytical sense. continuous\_discover returns, among other output, the identified consensus scores for continuous structures in the provided microbial abundance profiles, as well as the consensus PC loadings which can be used to assign continuous scores to any sample with the same set of microbial features.

## Usage

```
continuous_discover(feature_abd, batch, data, control)
```

## Arguments

|                          |                                                                                                                                        |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| <code>feature_abd</code> | feature-by-sample matrix of abundances (proportions or counts).                                                                        |
| <code>batch</code>       | name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise. |
| <code>data</code>        | data frame of metadata, columns must include batch.                                                                                    |
| <code>control</code>     | a named list of additional control parameters. See details.                                                                            |

## Details

`control` should be provided as a named list of the following components (can be a subset).

**normalization** character. Similar to the normalization parameter in [Maaslin2](#) but only "TSS" and "NONE" are allowed. Default to "TSS" (total sum scaling).

**transform** character. Similar to the transform parameter in [Maaslin2](#) but only "AST" and "LOG" are allowed. Default to "AST" (arcsine square root transformation).

**pseudo\_count** numeric. Pseudo count to add `feature_abd` before the transformation. Default to NULL, in which case pseudo count will be set automatically to 0 if `transform="AST"`, and half of minimal non-zero values in `feature_abd` if `transform="LOG"`.

**var\_perc\_cutoff** numeric. A value between 0 and 1 that indicates the percentage variability explained to cut off at for selecting top PCs in each batch. Across batches, the top PCs that in total explain more than `var_perc_cutoff` of the total variability will be selected for meta-analytical continuous structure discovery. Default to 0.8 (PCs included need to explain at least 80 total variability).

**cos\_cutoff** numeric. A value between 0 and 1 that indicates cutoff for absolute cosine coefficients between PC loadings to construct the method's network with. Once the top PC loadings from each batch are selected, cosine coefficients between each loading pair are calculated which indicate their similarity. Loading pairs with absolute cosine coefficients surpassing `cos_cutoff` are then considered as associated with each other, and represented as an edge between the pair in a PC loading network. Network community discovery can then be performed on this network to identified densely connected "clusters" of PC loadings, which represent meta-analytically recurrent continuous structures.

**cluster\_function** function. `cluster_function` is used to perform community structure discovery in the constructed PC loading network. This can be any of the network cluster functions provided in **igraph**. Default to `cluster_optimal`. Note that this option can be slow for larger datasets, in which case `cluster_fast_greedy` is recommended.

**network\_plot** character. Name for the generated network figure file. Default to "clustered\_network.pdf". Can be set to NULL in which case no output will be generated.

**plot\_size\_cutoff** integer. Clusters with sizes smaller than or equal to `plot_size_cutoff` will be excluded in the visualized network. Default to 2 - visualized clusters must have at least three nodes (PC loadings).

**diagnostic\_plot** character. Name for the generated diagnostic figure file. Default to "continuous\_diagnostic.pdf". Can be set to NULL in which case no output will be generated.

**verbose** logical. Indicates whether or not verbose information will be printed.

Value

a list, with the following components:

**consensus\_scores** matrix of identified consensus continuous scores. Columns are the identified consensus scores and rows correspond to samples in feature\_abd.

**consensus\_loadings** matrix of identified consensus loadings. Columns are the identified consensus scores and rows correspond to features in feature\_abd.

**mat\_vali** matrix of validation cosine coefficients of the identified consensus loadings. Columns correspond to the identified consensus scores and rows correspond to batches.

**network, communities, mat\_cos** components for the constructed PC loading network and community discovery results. network is a **igraph** graph object for the constructed network of associated PC loadings. communities is a [communities](#) object for the identified consensus loading clusters in network (output from control\$cluster\_function). mat\_cos is the matrix of cosine coefficients between all selected top PCs from all batches.

**control** list of additional control parameters used in the function call.

Author(s)

Siyuan Ma, <siyuanma@g.harvard.edu>

Examples

```
data("CRC_abd", "CRC_meta")
fit_continuous <- continuous_discover(feature_abd = CRC_abd,
                                     batch = "studyID",
                                     data = CRC_meta)
```

---

|         |                                                                        |
|---------|------------------------------------------------------------------------|
| CRC_abd | <i>Species level feature abundance data of five public CRC studies</i> |
|---------|------------------------------------------------------------------------|

---

Description

Species level relative abundance profiles of CRC and control patients in the five public studies used in Thomas et al. (2019). These were accessed through [curatedMetagenomicData](#).

Usage

```
data(CRC_abd)
```

Format

A feature-by-sample matrix of species-level profiles

Source

[curatedMetagenomicData](#)

## References

Thomas, Andrew Maltez, Paolo Manghi, Francesco Asnicar, Edoardo Pasolli, Federica Armanini, Moreno Zolfo, Francesco Beghini et al. "Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation." *Nature medicine* 25, no. 4 (2019): 667.

## Examples

```
data(CRC_abd)
# features included
rownames(CRC_abd)
# These are relative abundances
apply(CRC_abd, 2, sum)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# library(genefilter)
# datasets <- curatedMetagenomicData(
#   c("FengQ_2015.metaphlan_bugs_list.stool" ,
#     "HanniganGD_2017.metaphlan_bugs_list.stool",
#     "VogtmannE_2016.metaphlan_bugs_list.stool",
#     "YuJ_2015.metaphlan_bugs_list.stool",
#     "ZellerG_2014.metaphlan_bugs_list.stool"),
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset samples to only CRC and controls
#   subset_samples(study_condition %in% c("CRC", "control")) %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
#   # Filter features to be of at least 1e-5 relative abundance in five
#   # samples
#   filter_taxa(kOverA(5, 1e-5), prune = TRUE)
# CRC_abd <- otu_table(physeq)@.Data
```

---

CRC\_meta

*Sample metadata of five public CRC studies*

---

## Description

Metadata information of CRC and control patients in the five public studies used in Thomas et al. (2019). These were accessed through [curatedMetagenomicData](#).

**Usage**

```
data(CRC_meta)
```

**Format**

A data.frame of per-sample metadata information

**Source**

[curatedMetagenomicData](#)

**References**

Thomas, Andrew Maltez, Paolo Manghi, Francesco Asnicar, Edoardo Pasolli, Federica Armanini, Moreno Zolfo, Francesco Beghini et al. "Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation." *Nature medicine* 25, no. 4 (2019): 667.

**Examples**

```
data(CRC_meta)
# has CRC and control samples across five studies
table(CRC_meta$studyID, CRC_meta$study_condition)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# library(genefilter)
# datasets <- curatedMetagenomicData(
#   c("FengQ_2015.metaphlan_bugs_list.stool" ,
#     "HanniganGD_2017.metaphlan_bugs_list.stool",
#     "VogtmannE_2016.metaphlan_bugs_list.stool",
#     "YuJ_2015.metaphlan_bugs_list.stool",
#     "ZellerG_2014.metaphlan_bugs_list.stool"),
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset samples to only CRC and controls
#   subset_samples(study_condition %in% c("CRC", "control")) %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
#   # Filter features to be of at least 1e-5 relative abundance in five
#   # samples
#   filter_taxa(kOverA(5, 1e-5), prune = TRUE)
# CRC_meta <- data.frame(sample_data(physeq))
# CRC_meta$studyID <- factor(CRC_meta$studyID)
```

---

`create_table_maaslin`    *Utility for generating empty Maaslin2 results table*

---

### Description

Utility for generating empty Maaslin2 results table

### Usage

```
create_table_maaslin(features, exposure, lvl_exposure)
```

### Arguments

|                           |                                               |
|---------------------------|-----------------------------------------------|
| <code>features</code>     | name of the features fitted to Maaslin2.      |
| <code>exposure</code>     | the exposure variable.                        |
| <code>lvl_exposure</code> | levels of the exposure variable, if a factor. |

### Value

a table for each feature-exposure value pair; reference level of exposure, if a factor, is taken out because is absorbed into the intercept term in Maaslin2 regression

---

`diagnostic_adjust_batch`  
*Diagnostic visualization for adj\_batch function*

---

### Description

Diagnostic visualization for adj\_batch function

### Usage

```
diagnostic_adjust_batch(feature_abd, feature_abd_adj, var_batch, gamma_hat,  
  gamma_star, output)
```

### Arguments

|                              |                                                                                                                                                      |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>feature_abd</code>     | original feature-by-sample matrix of abundances (proportions or counts).                                                                             |
| <code>feature_abd_adj</code> | feature-by-sample matrix of batch-adjusted feature abundances, with covariate effects retained and scales consistent with original abundance matrix. |
| <code>var_batch</code>       | the batch variable (should be a factor).                                                                                                             |
| <code>gamma_hat</code>       | estimated per feature-batch gamma parameters.                                                                                                        |
| <code>gamma_star</code>      | shrunk per feature-batch gamma parameters                                                                                                            |
| <code>output</code>          | output file name                                                                                                                                     |

**Value**

(invisibly) the ggplot2 plot object

---

diagnostic\_continuous\_discover

*Diagnostic visualization for continuous.discover function*

---

**Description**

Diagnostic visualization for continuous.discover function

**Usage**

```
diagnostic_continuous_discover(mat_vali, lvl_batch, cos_cutoff, output)
```

**Arguments**

|            |                                                                                                                    |
|------------|--------------------------------------------------------------------------------------------------------------------|
| mat_vali   | matrix of maximum correlations between the cluster-specific consensus loadings and top PC loadings from each batch |
| lvl_batch  | unique batches in the data                                                                                         |
| cos_cutoff | the specified cosine coefficient cutoff                                                                            |
| output     | output file name                                                                                                   |

**Value**

the invisible ggplot2 plot object

---

diagnostic\_discrete\_discover

*Diagnostic visualization for discrete.discover function*

---

**Description**

Diagnostic visualization for discrete.discover function

**Usage**

```
diagnostic_discrete_discover(stats_internal, stats_external, lvl_batch,  
output)
```

**Arguments**

|                |                                                |
|----------------|------------------------------------------------|
| stats_internal | list of internal evaluation summary statistics |
| stats_external | list of external validation summary statistics |
| lvl_batch      | unique batches in the data                     |
| output         |                                                |

**Value**

the invisible ggplot2 plot object

---

|                   |                                                                                                                            |
|-------------------|----------------------------------------------------------------------------------------------------------------------------|
| discrete_discover | <i>Unsupervised meta-analytical discovery and validation of discrete clustering structures in microbial abundance data</i> |
|-------------------|----------------------------------------------------------------------------------------------------------------------------|

---

**Description**

discrete\_discover takes as input sample-by-sample dissimilarity measurements (generated from microbial abundance profiles), and performs unsupervised clustering within each batch across a range of cluster numbers. It then evaluates the support for each cluster number with both internal (i.e., samples within the batch) and external (i.e., samples in other batches) data. Internal evaluation is realized with [prediction.strength](#) and external evaluation is based on a generalized version of the same method. discrete\_discover generates as output the evaluation statistics for each cluster number. A cluster number with good support from both internal and external evaluations provides meta-analytical evidence for discrete structures in the microbial abundance profiles.

**Usage**

```
discrete_discover(D, batch, data, control)
```

**Arguments**

|         |                                                                                                                                        |
|---------|----------------------------------------------------------------------------------------------------------------------------------------|
| D       | sample-by-sample dissimilarity measurements. Should be provided as a <a href="#">dist</a> object.                                      |
| batch   | name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise. |
| data    | data frame of metadata, columns must include batch.                                                                                    |
| control | a named list of additional control parameters. See details.                                                                            |

**Details**

control should be provided as a named list of the following components (can be a subset).

**k\_max** integer. Maximum number of clusters to evaluate. discrete\_discover will evaluate clustering structures corresponding to cluster numbers ranging from 2 to k\_max. Default to 10.

**cluster\_function** an interface function. This function will be used for unsupervised clustering for discrete structure evaluation. This corresponds to the clustermethod parameter in [prediction.strength](#), and similarly, should also follow the specifications as detailed in [clusterboot](#). Default to [claraCBI](#)

**classify\_method** character. Classification method used to assign observations in the method's internal and external evaluation stage. Corresponds to the classification parameter in [prediction.strength](#), and can only be either "centroid" or "knn". Default to "centroid".

**M** integer. Number of random iterations to partition the batch during method's internal evaluation. Corresponds to the M parameter in [prediction.strength](#). Default to 30.

- nnk** integer. Number of nearest neighbors if `classify_method="knn"`. Corresponds to the `nnk` parameter in `prediction.strength`. Default to 1.
- diagnostic\_plot** character. Name for the generated diagnostic figure file. Default to "discrete\_diagnostic.pdf". Can be set to NULL in which case no output will be generated.
- verbose** logical. Indicates whether or not verbose information will be printed.

## Value

a list, with the following components:

- internal\_mean, internal\_se** matrices of internal clustering structure evaluation measurements (prediction strengths). Columns and rows corresponds to different batches and different numbers of clusters, respectively. `internal_mean` and `internal_se`, as the names suggest, are the mean and standard error of prediction strengths for each batch/cluster number.
- external\_mean, external\_se** same structure as `internal_mean` and `internal_se`, but records external clustering structure evaluation measurements (generalized prediction strength).
- control** list of additional control parameters used in the function call.

## Author(s)

Siyuan Ma, <siyuanma@g.harvard.edu>

## Examples

```
data("CRC_abd", "CRC_meta")
# Calculate Bray-Curtis dissimilarity between the samples
library(vegan)
D <- vegdist(t(CRC_abd))
fit_discrete <- discrete_discover(D = D,
                                  batch = "studyID",
                                  data = CRC_meta)
```

---

|               |                                                                                           |
|---------------|-------------------------------------------------------------------------------------------|
| fill_dimnames | <i>Fill in artificial row/column names to a matrix or data frame, if they are missing</i> |
|---------------|-------------------------------------------------------------------------------------------|

---

## Description

Fill in artificial row/column names to a matrix or data frame, if they are missing

## Usage

```
fill_dimnames(x, row_prefix, col_prefix)
```

## Arguments

- |                         |                                        |
|-------------------------|----------------------------------------|
| <code>x</code>          | matrix or data frame                   |
| <code>row_prefix</code> | prefix for the artificial row names    |
| <code>col_prefix</code> | prefix for the artificial column names |

**Value**

x but with the missing dimension names filled in

---

|        |                                                                                                                         |
|--------|-------------------------------------------------------------------------------------------------------------------------|
| fit_EB | <i>Parametric estimation of per-batch location and scale parameters, and Empirical Bayes estimation of their priors</i> |
|--------|-------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Parametric estimation of per-batch location and scale parameters, and Empirical Bayes estimation of their priors

**Usage**

```
fit_EB(s_data, l_stand_feature, batchmod, n_batch, l_ind)
```

**Arguments**

|                 |                                                                             |
|-----------------|-----------------------------------------------------------------------------|
| s_data          | feature-by-sample matrix of standardized abundances.                        |
| l_stand_feature | list of per-feature standardization fits, as returned by fit_stand_feature. |
| batchmod        | design matrix for batch variables.                                          |
| n_batch         | number of batches in the data.                                              |
| l_ind           | list of indicator matrices, as returned by construct_ind.                   |

**Value**

list of parameter estimations.

---

|            |                                                                                            |
|------------|--------------------------------------------------------------------------------------------|
| fit_shrink | <i>A posteriori shrink per-batch location and scale parameters towards their EB priors</i> |
|------------|--------------------------------------------------------------------------------------------|

---

**Description**

A posteriori shrink per-batch location and scale parameters towards their EB priors

**Usage**

```
fit_shrink(s_data, l_params, batchmod, n_batch, l_ind, control)
```

**Arguments**

|          |                                                           |
|----------|-----------------------------------------------------------|
| s_data   | feature-by-sample matrix of standardized abundances.      |
| l_params | list of parameter fits, as returned by fit_EB.            |
| batchmod | design matrix for batch variables.                        |
| n_batch  | number of batches in the data.                            |
| l_ind    | list of indicator matrices, as returned by construct_ind. |
| control  | list of control parameters (passed on to it_sol)          |

**Value**

list of shrunked per-batch location and scale parameters.

---

|                   |                                            |
|-------------------|--------------------------------------------|
| fit_stand_feature | <i>Fit lm and standardize all features</i> |
|-------------------|--------------------------------------------|

---

**Description**

Fit lm and standardize all features

**Usage**

```
fit_stand_feature(s_data, design, l_ind)
```

**Arguments**

|        |                                                                 |
|--------|-----------------------------------------------------------------|
| s_data | feature-by-sample matrix of abundances (proportions or counts). |
| design | design matrix.                                                  |
| l_ind  | list of indicator matrices, as returned by construct_ind.       |

**Value**

list of two componet: the standardized feature abundance matrix, and a list of per-feature standardization fits.

---

|        |                                                                                   |
|--------|-----------------------------------------------------------------------------------|
| it_sol | <i>Iteratively solve for one feature's shrunked location and scale parameters</i> |
|--------|-----------------------------------------------------------------------------------|

---

### Description

Iteratively solve for one feature's shrunked location and scale parameters

### Usage

```
it_sol(s_data, g_hat, d_hat, g_bar, t2, a, b, control)
```

### Arguments

|         |                                                           |
|---------|-----------------------------------------------------------|
| s_data  | the feature's standardized abundances.                    |
| g_hat   | the feature's location parameter frequentist estimations. |
| d_hat   | the feature's scale parameter frequentist estimations.    |
| g_bar   | EB estimation of location hyper parameters.               |
| t2      | EB estimation of location hyper parameters.               |
| a       | EB estimation of scale hyper parameters.                  |
| b       | EB estimation of scale hyper parameters.                  |
| control | list of control parameters                                |

### Value

matrix of shrunked location and scale parameters.

---

|         |                                                                          |
|---------|--------------------------------------------------------------------------|
| lm_meta | <i>Covariate adjusted meta-analytical differential abundance testing</i> |
|---------|--------------------------------------------------------------------------|

---

### Description

lm\_meta runs differential abundance models on microbial profiles within individual studies/batches, and aggregates per-batch effect sizes with a meta-analysis fixed/random effects model. It takes as input a feature-by-sample microbial abundance table and the accompanying meta data data frame which should includes the batch indicator variable, the main exposure variable for differential abundance testing, and optional covariates and random covariates. The function first runs [Maaslin2](#) models on the exposure with optional covariates/random covariates in each batch. The per-batch effect sizes are then aggregated with [rma.uni](#) and reported as output. Additional parameters, including those for both [Maaslin2](#) and [rma.uni](#) can be provided through control (see details).

**Usage**

```
lm_meta(
  feature_abd,
  batch,
  exposure,
  covariates = NULL,
  covariates_random = NULL,
  data,
  control
)
```

**Arguments**

|                                |                                                                                                                                        |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| <code>feature_abd</code>       | feature-by-sample matrix of abundances (proportions or counts).                                                                        |
| <code>batch</code>             | name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise. |
| <code>exposure</code>          | name of the exposure variable for differential abundance testing.                                                                      |
| <code>covariates</code>        | names of covariates to adjust for in Maaslin2 differential abundance testing models.                                                   |
| <code>covariates_random</code> | names of random effects grouping covariates to adjust for in Maaslin2 differential abundance testing models.                           |
| <code>data</code>              | data frame of metadata, columns must include exposure, batch, and covariates and covariates_random (if specified).                     |
| <code>control</code>           | a named list of additional control parameters. See details.                                                                            |

**Details**

`control` should be provided as a named list of the following components (can be a subset).

**normalization** character. normalization parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "TSS" (total sum scaling).

**transform** character. transform parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "AST" (arcsine square root transformation).

**analysis\_method** character. analysis\_method parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "LM" (linear modeling).

**rma\_method** character. method parameter for rma.uni. See [rma.uni](#) for details and allowed values. Default to "REML" (restricted maximum-likelihood estimator).

**output** character. Output directory for intermediate Maaslin2 output and the optional forest plots. Default to "MMUPHin\_lm\_meta".

**forest\_plot** character. Suffix in the name for the generated forest plots visualizing significant meta-analytical differential abundance effects. Default to "forest.pdf". Can be set to NULL in which case no output will be generated.

**rma\_conv** numeric. Convergence threshold for rma.uni (corresponds to `control$threshold`). See [rma.uni](#) for details. Default to 1e-4.

**rma\_maxit** integer. Maximum number of iterations allowed for `rma.uni` (corresponds to `control$maxiter`. See [rma.uni](#) for details. Default to 1000.

**verbose** logical. Indicates whether or not verbose information will be printed.

### Value

a list, with the following components:

**meta\_fits** data frame of per-feature meta-analytical differential abundance results, including columns for effect sizes, p-values and q-values, heterogeneity statistics such as  $\tau^2$  and  $I^2$ , as well as weights for individual batches. Many of these statistics are explained in detail in [rma.uni](#).

**maaslin\_fits** list of data frames, each one corresponding to the fitted results of Maaslin2 in a individual batch. See [Maaslin2](#) on details of these output.

**control** list of additional control parameters used in the function call.

### Author(s)

Siyuan Ma, <siyuanma@g.harvard.edu>

### Examples

```
data("CRC_abd", "CRC_meta")
fit_meta <- lm_meta(feature_abd = CRC_abd,
                    exposure = "study_condition",
                    batch = "studyID",
                    covariates = c("gender", "age"),
                    data = CRC_meta)$meta_fits
```

---

LOG

*LOG transformation (modified from Maaslin2 and is different)*

---

### Description

LOG transformation (modified from Maaslin2 and is different)

### Usage

`LOG(x)`

### Arguments

**x** vector of abundance to be transformed.

### Value

transformed vector of abundance.

---

|                  |                                      |
|------------------|--------------------------------------|
| Maaslin2_wrapper | <i>Wrapper function for Maaslin2</i> |
|------------------|--------------------------------------|

---

**Description**

Wrapper function for Maaslin2

**Usage**

```
Maaslin2_wrapper(feature_abd, data, exposure, covariates = NULL,
  covariates_random = NULL, output = tempdir(),
  normalization = "TSS", transform = "AST", analysis_method = "LM")
```

**Arguments**

|                   |                                             |
|-------------------|---------------------------------------------|
| feature_abd       | feature*sample matrix of feature abundance. |
| data              | data frame of metadata.                     |
| exposure          | name of exposure variable.                  |
| covariates        | name of covariates.                         |
| covariates_random | name of random covariates.                  |
| output            | directory for Maaslin2.                     |
| normalization     | normalization parameter for Maaslin2.       |
| transform         | transformation parameter for Maaslin2.      |
| analysis_method   | analysis method parameter for Maaslin2.     |

**Value**

a data frame recording per-feature coefficients, p-values, etc. from running Maaslin2.

---

|               |                                                                                   |
|---------------|-----------------------------------------------------------------------------------|
| match_control | <i>Match user-specified control parameters with default, and modify if needed</i> |
|---------------|-----------------------------------------------------------------------------------|

---

**Description**

Match user-specified control parameters with default, and modify if needed

**Usage**

```
match_control(default, control)
```

**Arguments**

|         |                                          |
|---------|------------------------------------------|
| default | list of default control parameters       |
| control | list of user-provided control parameters |

**Value**

list of control parameters, set to user provided values if specified and default other wise

---

|                    |                                                                   |
|--------------------|-------------------------------------------------------------------|
| normalize_features | <i>Normalize feature abundance table (modified from Maaslin2)</i> |
|--------------------|-------------------------------------------------------------------|

---

**Description**

Normalize feature abundance table (modified from Maaslin2)

**Usage**

```
normalize_features(features, normalization = "NONE", pseudo_count = 0)
```

**Arguments**

|               |                                                                 |
|---------------|-----------------------------------------------------------------|
| features      | feature-by-sample matrix of abundances (proportions or counts). |
| normalization | normalization method.                                           |
| pseudo_count  | pseudo count to be added to feature_abd.                        |

**Value**

normalized abundance table.

---

|                |                                                                                                                                   |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------|
| relocate_scale | <i>Relocate and scale feature abundances to correct for batch effects, given shrinked per-batch location and scale parameters</i> |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Relocate and scale feature abundances to correct for batch effects, given shrinked per-batch location and scale parameters

**Usage**

```
relocate_scale(s_data, l_params_shrink, batchmod, n_batch, l_ind)
```

**Arguments**

|                 |                                                           |
|-----------------|-----------------------------------------------------------|
| s_data          | feature-by-sample matrix of standardized abundances.      |
| l_params_shrink | list of shrinked parameters, as returned by fit_shrink.   |
| batchmod        | design matrix for batch variables.                        |
| n_batch         | number of batches in the data.                            |
| l_ind           | list of indicator matrices, as returned by construct_ind. |

**Value**

feature-by-sample matrix of batch-adjusted feature abundances (but without covariate effects).

---

|                |                                                                                                                                                    |
|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| rename_maaslin | <i>Utility for temporarily renaming samples/features for Maaslin2 run to bypass the rare cases where unconventional names can cause exceptions</i> |
|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Utility for temporarily renaming samples/features for Maaslin2 run to bypass the rare cases where unconventional names can cause exceptions

**Usage**

```
rename_maaslin(old_names, prefix)
```

**Arguments**

|           |                                                  |
|-----------|--------------------------------------------------|
| old_names | vector of names.                                 |
| prefix    | prefix for the replacement (new numbered names). |

**Value**

vector of new names - numbered vector with same length as old names and with the specified prefix

---

|             |                                                                                   |
|-------------|-----------------------------------------------------------------------------------|
| rma_wrapper | <i>Wrapper for fitting fixed/random effects meta-analysis model using metafor</i> |
|-------------|-----------------------------------------------------------------------------------|

---

### Description

Wrapper for fitting fixed/random effects meta-analysis model using metafor

### Usage

```
rma_wrapper(maaslin_fits, method = "REML", output = tempdir(),
  forest_plot = NULL, rma_conv = 1e-06, rma_maxit = 1000,
  verbose = TRUE)
```

### Arguments

|              |                                                                             |
|--------------|-----------------------------------------------------------------------------|
| maaslin_fits | list of Maaslin2 result data frames, outputted from Maaslin2_wrapper.       |
| method       | meta-analysis model to run, options provided in metafor::rma.               |
| output       | directory for the output forest plots.                                      |
| forest_plot  | logical. should forest plots be generated for the significant associations. |
| rma_conv     | rma threshold control.                                                      |
| rma_maxit    | rma maximum iteration control.                                              |
| verbose      | should verbose information be printed.                                      |

### Value

a data frame recording per-feature meta-analysis association results. (coefficients, p-values, etc.)

---

|            |                                                                                                                   |
|------------|-------------------------------------------------------------------------------------------------------------------|
| set_pseudo | <i>Set pseudo count for an abundance matrix. Pseudo count is currently set to half of minimum non-zero values</i> |
|------------|-------------------------------------------------------------------------------------------------------------------|

---

### Description

Set pseudo count for an abundance matrix. Pseudo count is currently set to half of minimum non-zero values

### Usage

```
set_pseudo(features)
```

### Arguments

|          |                                                                 |
|----------|-----------------------------------------------------------------|
| features | feature-by-sample matrix of abundances (proportions or counts). |
|----------|-----------------------------------------------------------------|

**Value**

the pseudo count

---

|              |                                                                                                               |
|--------------|---------------------------------------------------------------------------------------------------------------|
| shorten_name | <i>Utility for shorter names Useful when plotting per-feature figures where feature names could be cutoff</i> |
|--------------|---------------------------------------------------------------------------------------------------------------|

---

**Description**

Utility for shorter names Useful when plotting per-feature figures where feature names could be cutoff

**Usage**

```
shorten_name(x, cutoff = 3, replacement = "..")
```

**Arguments**

|        |                                                                     |
|--------|---------------------------------------------------------------------|
| x      | vector of names                                                     |
| cutoff | number of maximum string length before start cutting off the middle |

**Value**

vector of new names with .. replacing the middle part if name is longer than cutoff

---

|                     |                                                                                                                                         |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| standardize_feature | <i>Centralize (by design matrix) and standardize (by pooled variance across all batches) feature abundances for empirical Bayes fit</i> |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Centralize (by design matrix) and standardize (by pooled variance across all batches) feature abundances for empirical Bayes fit

**Usage**

```
standardize_feature(y, i_design, n_batch)
```

**Arguments**

|          |                                                                                             |
|----------|---------------------------------------------------------------------------------------------|
| y        | vector of non-zero abundance of a single feature (if zero-inflated is true).                |
| i_design | design matrix for the feature; samples with zeros are taken out (if zero-inflated is true). |
| n_batch  | number of batches in the data.                                                              |

**Value**

a list with component: y\_stand for vector of centralized and standardized feature abundance, and stand\_mean/varpooled for the location and scale factor (these are used later to back transform the batch-shrunked feature abundance).

---

|                    |                                                                  |
|--------------------|------------------------------------------------------------------|
| transform_features | <i>Transform feature abundnce table (modified from Maaslin2)</i> |
|--------------------|------------------------------------------------------------------|

---

**Description**

Transform feature abundnce table (modified from Maaslin2)

**Usage**

```
transform_features(features, transform = "NONE", pseudo_count = 0)
```

**Arguments**

|              |                                                                 |
|--------------|-----------------------------------------------------------------|
| features     | feature-by-sample matrix of abundances (proportions or counts). |
| transform    | transformation method.                                          |
| pseudo_count | pseudo count to be added to feature_abd..                       |

**Value**

transformed abundance table.

---

|     |                                                   |
|-----|---------------------------------------------------|
| TSS | <i>TSS normalization (modified from Maaslin2)</i> |
|-----|---------------------------------------------------|

---

**Description**

TSS normalization (modified from Maaslin2)

**Usage**

```
TSS(x)
```

**Arguments**

|   |                                       |
|---|---------------------------------------|
| x | vector of abundance to be normalized. |
|---|---------------------------------------|

**Value**

normalized vector of abundance.

---

vaginal\_abd*Species level feature abundance data of two public vaginal studies*

---

**Description**

Species level relative abundance profiles of vaginal samples in the two public studies provided in [curatedMetagenomicData](#).

**Usage**

```
data(vaginal_abd)
```

**Format**

A feature-by-sample matrix of species-level profiles

**Source**

[curatedMetagenomicData](#)

**References**

Pasolli, Edoardo, Lucas Schiffer, Paolo Manghi, Audrey Renson, Valerie Obenchain, Duy Tin Truong, Francesco Beghini et al. "Accessible, curated metagenomic data through ExperimentHub." Nature methods 14, no. 11 (2017): 1023.

**Examples**

```
data(vaginal_abd)
# features included
rownames(vaginal_abd)
# These are relative abundances
apply(vaginal_abd, 2, sum)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# datasets <- curatedMetagenomicData(
#   "*metaphlan_bugs_list.vagina*",
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
```

```
# Filter features to be of at least 1e-5 relative abundance in two samples
# filter_taxa(kOverA(2, 1e-5), prune = TRUE)
# vaginal_abd <- otu_table(physeq)@.Data
```

---

vaginal\_meta

*Sample metadata of two public vaginal studies*


---

## Description

Metadata information of vaginal samples in the two public studies provided in [curatedMetagenomicData](#).

## Usage

```
data(vaginal_meta)
```

## Format

A data.frame of per-sample metadata information

## Source

[curatedMetagenomicData](#)

## References

Pasolli, Edoardo, Lucas Schiffer, Paolo Manghi, Audrey Renson, Valerie Obenchain, Duy Tin Truong, Francesco Beghini et al. "Accessible, curated metagenomic data through ExperimentHub." Nature methods 14, no. 11 (2017): 1023.

## Examples

```
data(vaginal_meta)
# has vaginal samples across two studies
table(vaginal_meta$studyID, vaginal_meta$body_site)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# datasets <- curatedMetagenomicData(
#   "*metaphlan_bugs_list.vagina*",
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
```

```
# Filter features to be of at least 1e-5 relative abundance in two samples
# filter_taxa(kOverA(2, 1e-5), prune = TRUE)
# vaginal_meta <- data.frame(sample_data(physeq))
# vaginal_meta$studyID <- factor(vaginal_meta$studyID)
```

---

visualize\_continuous\_discover

*Visualization of the clustered network for the continuous.discover function*

---

## Description

Visualization of the clustered network for the continuous.discover function

## Usage

```
visualize_continuous_discover(graph_pc, membership_loading,
  size_communities, plot_size_cutoff, short_names, output)
```

## Arguments

|                    |                                                                               |
|--------------------|-------------------------------------------------------------------------------|
| graph_pc           | the full pc network constructed from correlated PCs                           |
| membership_loading | membership of PC loadings from community discovery                            |
| size_communities   | ordered (largest to smallest) size of the identified communities              |
| plot_size_cutoff   | cluster size cutoff (for cluster to be included in the visualized PC network) |
| short_names        | shorter names of the loadings                                                 |
| output             | output file name                                                              |

## Value

an invisible list of the subsetting network and memberships (to reproduce the plot)

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