

Package ‘MMUPHin’

May 26, 2026

Type Package

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

Version 2.1.0

Author Siyuan Ma

Maintainer Siyuan MA <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.

License MIT + file LICENSE

Encoding UTF-8

RoxygenNote 7.3.3

VignetteBuilder knitr

SystemRequirements glpk (>= 4.57)

Depends R (>= 3.6)

Imports maaslin3, 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

git_url <https://git.bioconductor.org/packages/MMUPHin>

git_branch devel

git_last_commit 1185020

git_last_commit_date 2026-04-28

Repository Bioconductor 3.24

Date/Publication 2026-05-25

Contents

| | |
|--|----|
| add_back_covariates | 3 |
| adjust_batch | 3 |
| adjust_EB | 4 |
| aprior | 5 |
| AST | 5 |
| back_transform_abd | 6 |
| bprior | 6 |
| catchToList | 7 |
| check_batch | 7 |
| check_covariates | 8 |
| check_covariates_random | 8 |
| check_D | 9 |
| check_exposure | 9 |
| check_feature_abd | 10 |
| check_metadata | 10 |
| check_options | 11 |
| check_options_continuous | 11 |
| check_pseudo_count | 12 |
| check_rank | 12 |
| check_samples | 13 |
| check_samples_D | 13 |
| construct_design | 14 |
| construct_ind | 14 |
| continuous_discover | 15 |
| CRC_abd | 17 |
| CRC_meta | 18 |
| create_table_maaslin | 19 |
| diagnostic_adjust_batch | 19 |
| diagnostic_continuous_discover | 20 |
| diagnostic_discrete_discover | 21 |
| discrete_discover | 21 |
| fill_dimnames | 23 |
| fit_EB | 23 |
| fit_shrink | 24 |
| fit_stand_feature | 24 |
| it_sol | 25 |
| LOG | 25 |
| Maaslin3_wrapper | 26 |
| maaslin_meta | 26 |
| match_control | 28 |
| normalize_features | 29 |
| relocate_scale | 29 |
| rename_maaslin | 30 |
| rma_wrapper | 30 |
| set_pseudo | 30 |
| shorten_name | 31 |
| standardize_feature | 31 |
| transform_features | 32 |
| TSS | 32 |
| vaginal_abd | 33 |

| | |
|---|-----------|
| <i>add_back_covariates</i> | 3 |
| vaginal_meta | 34 |
| visualize_continuous_discover | 35 |
| Index | 36 |

| | |
|----------------------------|---|
| <i>add_back_covariates</i> | <i>Add back covariate effects to batch-corrected feature abundance data</i> |
|----------------------------|---|

Description

Add back covariate effects to batch-corrected feature abundance data

Usage

```
add_back_covariates(adj_data, l_stand_feature, l_ind)
```

Arguments

- adj_data feature-by-sample matrix of batch-adjusted feature abundances (but without covariate effects), as returned by `relocate_scale`.
- l_stand_feature list of per-feature standardization fits, as returned by `fit_stand_feature`.
- l_ind list of indicator matrices, as returned by `construct_ind`.

Value

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

| | |
|---------------------|---|
| <i>adjust_batch</i> | <i>Zero-inflated empirical Bayes adjustment of batch effect in compositional feature abundance data</i> |
|---------------------|---|

Description

`adjust_batch` takes as input a feature-by-sample matrix of microbial abundances, and performs batch effect adjustment given provided batch and optional covariate variables. It returns the batch-adjusted abundance matrix. Additional options and parameters can be passed through the `control` parameter as a list (see details).

Usage

```
adjust_batch(feature_abd, batch, covariates = NULL, data, control)
```

Arguments

- feature_abd feature-by-sample matrix of abundances (proportions or counts).
- 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.
- covariates name(s) of covariates to adjust for in the batch correction model.
- data data frame of metadata, columns must include batch and covariates (if specified).
- 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).

zero_inflation logical. Indicates whether or not a zero-inflated model should be run. Default to TRUE (zero-inflated model). If set to FALSE then the correction will be similar to ComBat as provided in the sva package.

pseudo_count numeric. Pseudo count to add feature_abd before the methods' log transformation. Default to NULL, in which case adjust_batch will set the pseudo count automatically to half of minimal non-zero values in feature_abd.

diagnostic_plot character. Name for the generated diagnostic figure file. Default to "adjust_batch_diagnostic.pdf". Can be set to NULL in which case no output will be generated.

conv numeric. Convergence threshold for the method's iterative algorithm for shrinking batch effect parameters. Default to 1e-4.

maxit integer. Maximum number of iterations allowed for the method's iterative algorithm. Default to 1000.

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

Value

a list, with the following components:

feature_abd_adj feature-by-sample matrix of batch-adjusted abundances, normalized to the same per-sample total abundance as feature_abd.

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

Author(s)

Siyuan Ma, <syman.research@gmail.com>

Examples

```
data("CRC_abd", "CRC_meta")
CRC_abd_adj <- adjust_batch(feature_abd = CRC_abd,
                           batch = "studyID",
                           covariates = "study_condition",
                           data = CRC_meta)$feature_abd_adj
```

adjust_EB

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

Description

Perform batch adjustment on standardized feature abundances, based on EB shrunked 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

Capture values, warnings, and errors in a list

Usage

```
catchToList(expr)
```

```
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 MMUPH in functions to run).

Value

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

| | |
|------------------|--|
| check_covariates | <i>Check fixed-effect covariates for variance within batches</i> |
|------------------|--|

Description

Check fixed-effect covariates for variance within batches

Usage

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

Arguments

| | |
|-----------------|---|
| data_covariates | Data frame containing only covariate columns. |
| batch | Factor vector of batch IDs. |

Value

A logical matrix (batch x covariate) where TRUE indicates the covariate can be fitted in that batch.

| | |
|-------------------------|---|
| check_covariates_random | <i>Check random-effect covariates for clustering within batches</i> |
|-------------------------|---|

Description

Check random-effect covariates for clustering within batches

Usage

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

Arguments

| | |
|-----------------|--|
| data_covariates | Data frame of random effect variables. |
| batch | Factor vector of batch IDs. |

Value

A logical matrix indicating if random effects have clustered observations within each batch.

| | |
|---------|-----------------------------------|
| 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 consistency across batches</i> |
|----------------|---|

Description

Check exposure variable consistency across batches

Usage

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

Arguments

exposure Vector of the exposure variable.
batch Factor vector indicating batch/study membership.

Value

A logical vector indicating if the exposure has variance (>1 level) within each batch.

| | |
|-------------------|--------------------------------------|
| 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

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

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, <syma.research@gmail.com>

Examples

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

`CRC_abd`*Species level feature abundance data of five public CRC studies*

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 *Generate dummy grid for Maaslin results table*

Description

Generate dummy grid for Maaslin results table

Usage

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

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

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

Unsupervised meta-analytical discovery and validation of discrete clustering structures in microbial abundance data

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 [dist](#) 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, <syma.research@gmail.com>

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

| | |
|------------|--|
| x | matrix or data frame |
| row_prefix | prefix for the artificial row names |
| col_prefix | 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.

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

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.

| | |
|------------------|---|
| Maaslin3_wrapper | <i>Internal wrapper for Maaslin 3 to ensure name parity and handle errors</i> |
|------------------|---|

Description

Internal wrapper for Maaslin 3 to ensure name parity and handle errors

Usage

```
Maaslin3_wrapper(
  feature_abd,
  data,
  exposure,
  covariates = NULL,
  covariates_random = NULL,
  output = tempdir(),
  control
)
```

Arguments

| | |
|-------------------|---------------------------------------|
| feature_abd | Feature-by-sample matrix. |
| data | Metadata data frame. |
| exposure | Character; primary exposure variable. |
| covariates | Character vector; fixed effects. |
| covariates_random | Character vector; random effects. |
| control | List of maaslin3 parameters. |

Value

The standard Maaslin 3 fit object with restored original names.

| | |
|--------------|--|
| maaslin_meta | <i>maaslin3-based meta-analytical differential abundance testing</i> |
|--------------|--|

Description

maaslin_meta runs differential abundance models on microbial profiles within individual studies/batches using maaslin3, 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 metadata data frame which should include the batch indicator variable, the main exposure variable for differential abundance testing, and optional covariates and random covariates. The function first runs `maaslin3` models on the exposure in each batch. The per-batch effect sizes for both abundance (linear) and prevalence (logistic) components are then aggregated with `rma.uni` and reported as output. Additional parameters for both packages can be provided through `control` (see details).

Usage

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

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 maaslin3 differential abundance testing models. |
| <code>covariates_random</code> | names of random effects grouping covariates to adjust for in maaslin3 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 maaslin3. See [maaslin3](#) for details and allowed values. Default to "TSS" (total sum scaling).

transform character. transform parameter for maaslin3. See [maaslin3](#) for details and allowed values. Default to "LOG" (log transformation).

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 maaslin3 output and optional visualizations. Default to "maaslin_meta_output".

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_abundance data frame of per-feature meta-analytical results for the abundance component, including columns for effect sizes, p-values and q-values, heterogeneity statistics such as τ^2 and I^2 .

meta_fits_prevalence data frame of per-feature meta-analytical results for the prevalence component (logistic modeling).

maaslin_fits list of lists, each one corresponding to the fitted results of maaslin3 in an individual batch.

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

Author(s)

Siyuan Ma, <syma.research@gmail.com>

Examples

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

| | |
|---------------|---|
| 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>Temporary renaming of samples/features for formula safety</i> |
|----------------|--|

Description

Temporary renaming of samples/features for formula safety

Usage

```
rename_maaslin(old_names, 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, stream = "abundance", control)
```

Arguments

| | |
|--------------|---|
| maaslin_fits | list of results from individual batches. |
| stream | stream to aggregate ("abundance" or "prevalence"). |
| control | control parameters including output path and plot settings. |

Value

a data frame recording per-feature meta-analysis association results.

| | |
|------------|---|
| 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

Utility to shorten names for plotting

Usage

```
shorten_name(x, cutoff = 15)
```

```
shorten_name(x, cutoff = 15)
```

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

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

Value

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

Index

* datasets

- CRC_abd, [17](#)
- CRC_meta, [18](#)
- vaginal_abd, [33](#)
- vaginal_meta, [34](#)

* internal

- add_back_covariates, [3](#)
- adjust_EB, [4](#)
- aprior, [5](#)
- AST, [5](#)
- back_transform_abd, [6](#)
- bprior, [6](#)
- catchToList, [7](#)
- check_batch, [7](#)
- check_covariates, [8](#)
- check_covariates_random, [8](#)
- check_D, [9](#)
- check_exposure, [9](#)
- check_feature_abd, [10](#)
- check_metadata, [10](#)
- check_options, [11](#)
- check_options_continuous, [11](#)
- check_pseudo_count, [12](#)
- check_rank, [12](#)
- check_samples, [13](#)
- check_samples_D, [13](#)
- construct_design, [14](#)
- construct_ind, [14](#)
- create_table_maaslin, [19](#)
- diagnostic_adjust_batch, [19](#)
- diagnostic_continuous_discover, [20](#)
- diagnostic_discrete_discover, [21](#)
- fill_dimnames, [23](#)
- fit_EB, [23](#)
- fit_shrink, [24](#)
- fit_stand_feature, [24](#)
- it_sol, [25](#)
- LOG, [25](#)
- Maaslin3_wrapper, [26](#)
- match_control, [28](#)
- normalize_features, [29](#)
- relocate_scale, [29](#)
- rename_maaslin, [30](#)

- rma_wrapper, [30](#)
- set_pseudo, [30](#)
- shorten_name, [31](#)
- standardize_feature, [31](#)
- transform_features, [32](#)
- TSS, [32](#)
- visualize_continuous_discover, [35](#)

- add_back_covariates, [3](#)
- adjust_batch, [3](#)
- adjust_EB, [4](#)
- aprior, [5](#)
- AST, [5](#)

- back_transform_abd, [6](#)
- bprior, [6](#)

- catchToList, [7](#)
- check_batch, [7](#)
- check_covariates, [8](#)
- check_covariates_random, [8](#)
- check_D, [9](#)
- check_exposure, [9](#)
- check_feature_abd, [10](#)
- check_metadata, [10](#)
- check_options, [11](#)
- check_options_continuous, [11](#)
- check_pseudo_count, [12](#)
- check_rank, [12](#)
- check_samples, [13](#)
- check_samples_D, [13](#)

- claraCBI, [22](#)
- cluster_fast_greedy, [16](#)
- cluster_optimal, [16](#)
- clusterboot, [22](#)
- communities, [16](#)
- construct_design, [14](#)
- construct_ind, [14](#)
- continuous_discover, [15](#)
- CRC_abd, [17](#)
- CRC_meta, [18](#)
- create_table_maaslin, [19](#)
- curatedMetagenomicData, [17](#), [18](#), [33](#), [34](#)
- diagnostic_adjust_batch, [19](#)

diagnostic_continuous_discover, 20
diagnostic_discrete_discover, 21
discrete_discover, 21
dist, 21

fill_dimnames, 23
fit_EB, 23
fit_shrink, 24
fit_stand_feature, 24

it_sol, 25

LOG, 25

Maaslin2, 15
maaslin3, 26, 27
Maaslin3_wrapper, 26
maaslin_meta, 26
match_control, 28

normalize_features, 29

prediction_strength, 21, 22

relocate_scale, 29
rename_maaslin, 30
rma.uni, 26, 27
rma_wrapper, 30

set_pseudo, 30
shorten_name, 31
standardize_feature, 31

transform_features, 32
TSS, 32

vaginal_abd, 33
vaginal_meta, 34
visualize_continuous_discover, 35