

Package ‘MetaboDynamics’

July 26, 2025

Title Bayesian analysis of longitudinal metabolomics data

Version 1.1.2

URL <https://github.com/KatjaDanielzik/MetaboDynamics>

BugReports <https://github.com/KatjaDanielzik/MetaboDynamics/issues>

Description MetaboDynamics is an R-package that provides a framework of probabilistic models to analyze longitudinal metabolomics data. It enables robust estimation of mean concentrations despite varying spread between timepoints and reports differences between timepoints as well as metabolite specific dynamics profiles that can be used for identifying ``dynamics clusters" of metabolites of similar dynamics. Provides probabilistic over-representation analysis of KEGG functional modules and pathways as well as comparison between clusters of different experimental conditions.

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Encoding UTF-8

Roxygen list(markdown = TRUE)

RoxygenNote 7.3.2

Suggests knitr, rmarkdown, BiocStyle, testthat (>= 3.0.0)

Config/testthat/edition 3

Imports dplyr, ggplot2, KEGGREST, methods, Rcpp (>= 0.12.0),
RcppParallel (>= 5.0.1), rstan (>= 2.18.1), rstantools (>= 2.4.0), S4Vectors, stringr, SummarizedExperiment, tidyr,
dynamicTreeCut, dendextend, rlang

VignetteBuilder knitr

Depends R (>= 4.4.0)

LazyData false

LinkingTo BH (>= 1.66.0), Rcpp (>= 0.12.0), RcppEigen (>= 0.3.3.3.0),
RcppParallel (>= 5.0.1), rstan (>= 2.18.1), StanHeaders (>= 2.18.0)

SystemRequirements GNU make

biocViews

Software, Metabolomics, Bayesian, FunctionalPrediction, MultipleComparison, KEGG, Pathways, TimeCourse

Biarch true

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

git_branch devel
git_last_commit 98d5e8e
git_last_commit_date 2025-07-23
Repository Bioconductor 3.22
Date/Publication 2025-07-25
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Description

MetaboDynamics is an R-package that provides a framework of probabilistic models to analyze longitudinal metabolomics data. It enables robust estimation of mean concentrations despite varying spread between timepoints and reports differences between timepoints as well as metabolite specific dynamics profiles that can be used for identifying "dynamics clusters" of metabolites of similar dynamics. Provides probabilistic over-representation analysis of KEGG functional modules and pathways as well as comparison between clusters of different experimental conditions.

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See Also

Useful links:

- <https://github.com/KatjaDanielzik/MetaboDynamics>
- Report bugs at <https://github.com/KatjaDanielzik/MetaboDynamics/issues>

`.calculate_distances    compare_dynamics()`

Description

`compare_dynamics()`

Usage

```
.calculate_distances(group_a, group_b, dynamics)
```

Arguments

<code>group_a</code>	dataframe of one cluster of one condition
<code>group_b</code>	dataframe of one cluster of a different condition than <code>group_a</code>
<code>dynamics</code>	character vector specifying the columns that hold dynamic estimates in data

Value

matrix of pairwise euclidean distances between two groups of vectors

.calculate_jaccard	<i>Function to calculate Jaccard index on two character vectors of metabolite names</i>
--------------------	---

Description

Function to calculate Jaccard index on two character vectors of metabolite names

Usage

```
.calculate_jaccard(group_a, group_b)
```

Arguments

group_a	group of clusters of metabolites
group_b	group of clusters of metabolites

Value

the Jaccard index

.eu	<i>euclidean distance compare_dynamics()</i>
-----	--

Description

euclidean distance compare_dynamics()

Usage

```
.eu(a, b)
```

Arguments

a	a numeric vector
b	a numeric vector of same length as a

Value

euclidean distance between vectors

.similarity	<i>Jaccard Index: intersection/union compare_metabolites()</i>
-------------	--

Description

Jaccard Index: intersection/union compare_metabolites()

Usage

```
.similarity(a, b)
```

Arguments

a	a vector
b	a vector

Value

Jaccard Index of a and b

cluster_dynamics	<i>cluster dynamics profiles of metabolites</i>
------------------	---

Description

convenient wrapper function for clustering of metabolite dynamics employing the "hybrid" method of the [dynamicTreeCut](#) package for clustering and [hclust](#) for computing of distance matrix and hierarchical clustering needed as input for dynamicTreeCut

Usage

```
cluster_dynamics(
  data,
  distance = "euclidean",
  agglomeration = "ward.D2",
  minClusterSize = 1,
  deepSplit = 2
)
```

Arguments

data	result of estimates_dynamics() (list of dataframes or SummarizedExperiment object) or a list of dataframes (one dataframe per condition, list elements must be named by condition) with columns which are named "metabolite", "mu_mean" (mean metabolite abundance log-transformed and standarized to a mean of zero and standard deviation of one per experimental condition and metabolite) and "time.ID" (numerical, specifying the experimental time point)
distance	distance method to be used as input for hierarchical clustering dist can be "euclidean", "maximum", "manhattan", "canberra", "binary" or "minkowski"

agglomeration agglomerative method to be used for hierarchical clustering [hclust](#) can be "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"

minClusterSize minimum number of metabolites per of cluster [cutreeDynamic](#)

deepSplit rough control over sensitivity of cluster analysis. Possible values are 0:4, the higher the value, the more and smaller clusters will be produced by [cutreeDynamic](#)

Value

a list with dataframes named by experimental condition or if data is a [SummarizedExperiment](#) object clustering results are stored in metadata under "cluster"

See Also

[fit_dynamics_model\(\)](#), [estimates_dynamics\(\)](#), [plot_cluster\(\)](#)

Examples

```
data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite %in% c("ATP", "L-Alanine", "GDP")]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
data <- estimates_dynamics(
  data = data, iter = 2000,
  chains = 1, condition = "condition"
)
data <- cluster_dynamics(data)
S4Vectors::metadata(data)[["cluster"]][["A"]][["data"]]
```

compare_dynamics	<i>Comparison of metabolite dynamics clusters under different experimental conditions</i>
------------------	---

Description

Employs a Bayesian model that assumes a normal distribution of Euclidean distances between dynamics vectors (metabolite abundances at different time points) of two clusters that come from different experimental conditions to estimate the mean distance between clusters.

Usage

```
compare_dynamics(
  data,
  dynamics = metadata(data)[["cluster"]][[1]]$dynamics,
  cores = 4
)
```

Arguments

data	a dataframe or containing a column specifying the metabolite names to be compared and cluster IDs (column named "cluster") of clusters of similar dynamics, as well as a column "condition" specifying the experimental conditions. to be compared or a SummarizedExperiment storing the same information in metadata(data) under "cluster"
dynamics	vector specifying the column names of dataframe clusters that hold the dynamics information
cores	how many cores should be used for model fitting; this parallelizes the model fitting and therefore speeds it up; default=4

Value

a list holding a 1) the model fit 2) dataframe of estimates of the mean distance between #' clusters of different experimental conditions ("mean") and the standard deviation ("sigma"). If data input was a SummarizedExperiment results are stored in metadata(data) under "comparison_dynamics"

See Also

Visualization of estimates [heatmap_dynamics\(\)](#)/ compare metabolite composition of clusters [compare_metabolites\(\)](#)

Examples

```
data("longitudinalMetabolomics")
longitudinalMetabolomics <- compare_dynamics(
  data = longitudinalMetabolomics,
  dynamics = c("1", "2", "3", "4"),
  cores = 1
)
S4Vectors::metadata(longitudinalMetabolomics)[["comparison_dynamics"]]
```

compare_metabolites	<i>Comparison of metabolite sets between dynamics clusters of different experimental conditions</i>
---------------------	---

Description

Uses the Jaccard Index to compare metabolite names between dynamics clusters of different experimental conditions

Usage

```
compare_metabolites(data, metabolite = "metabolite")
```

Arguments

data	a dataframe or containing a column specifying the metabolite names to be compared and cluster IDs (column named "cluster") of clusters of similar dynamics, as well as a column "condition" specifying the experimental conditions. to be compared or a SummarizedExperiment storing the same information in metadata(data) under "cluster"
metabolite	column in "data" that specifies either metabolite name or KEGG ID or some other identifier

Value

a dataframe of Jaccard indices between data or if data input was a SummarizedExperiment results are stored in metadata(data) under "comparison_metabolites"

See Also

Visualization of metabolite similarity [heatmap_metabolites\(\)](#)/ compare dynamics of clusters [compare_dynamics\(\)](#)

Examples

```
data("longitudinalMetabolomics")
longitudinalMetabolomics <- compare_metabolites(
  data = longitudinalMetabolomics
)
S4Vectors::metadata(longitudinalMetabolomics)[["comparison_metabolites"]]
```

diagnostics_dynamics	<i>Extracts diagnostic criteria from numeric fit of Bayesian model of dynamics</i>
----------------------	--

Description

gathers number of divergences, rhat values, number of effective samples (n_eff) and provides plots for diagnostics criteria as well as posterior predictive checks. Output dataframe "model_diagnostics" contains information about experimental condition, number of divergent transitions and rhat and neff values for all timepoints.

Usage

```
diagnostics_dynamics(
  data,
  assay = "scaled_log",
  iter = 2000,
  warmup = iter/4,
  chains = 4,
  fits = metadata(data)[["dynamic_fits"]]
)
```

Arguments

data	dataframe or a SummarizedExperiment used to fit dynamics model column of "time" that contains time must be numeric, has to contain columns specifying the metabolite named "metabolite", and column specifying the time point named "time", a column named "condition" must specify the experimental condition.
assay	of the SummarizedExperiment object that was used to fit the dynamics model
iter	number of iterations used for model fit
warmup	number of warmup iterations used for model fit
chains	number of chains used for model fit
fits	list of model fits for which diagnostics should be extracted, is the object that gets returned by fit_dynamics_model(), or if a summarizedExperiment object the results of fit_dynamics_model() are stored in metadata(data) under "dynamic_fits"

Value

a list which contains diagnostics criteria of all conditions in a dataframe (named "model_diagnostics") and one dataframe per condition that contains necessary information for Posterior predictive check (named "PPC_condition"). If data is a summarizedExperiment object the diagnostics are stored in metadata(data) "diagnostics_dynamics"

See Also

[estimates_dynamics\(\)](#) parent function [fit_dynamics_model\(\)](#) visualization functions: [plot_diagnostics\(\)](#)/[plot_](#)

Examples

```
data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite == "ATP"]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
data <- diagnostics_dynamics(
  data = data, assay = "scaled_log",
  iter = 2000, chains = 1,
  fits = metadata(data)[["dynamic_fits"]]
)
S4Vectors::metadata(data)[["diagnostics_dynamics"]][["model_diagnostics"]]
S4Vectors::metadata(data)[["diagnostics_dynamics"]][["posterior_A"]]
```

estimates_dynamics	<i>Extracts parameter estimates from numeric fit of Bayesian model of dynamics</i>
--------------------	--

Description

Extracts the mean concentrations (μ) at every time point from the dynamics model fit, the 95% highest density interval (HDI), the estimated standard deviation of metabolite concentrations at every time point (σ), and the pooled standard deviation of every metabolite over all timepoints (λ). Additionally samples from the posterior of μ can be drawn. This can be helpful if p.e. one wants to estimate the clustering precision. λ can be used for clustering algorithms such as VSCLust that also take the variance into account.

Usage

```
estimates_dynamics(
  data,
  assay = "scaled_log",
  kegg = "KEGG",
  condition = "condition",
  time = "time",
  metabolite = "metabolite",
  fits = metadata(data)[["dynamic_fits"]],
  iter = 2000,
```

```

    warmup = iter/4,
    chains = 4,
    samples = 1
  )

```

Arguments

data	data frame or colData of a SummarizedExperiment used used to fit dynamics model, must contain a column specifying KEGG IDs, column named "condition" specifying the experimental condition and a column named "time" specifying the timepoints. If it is a SummarizedExperiment object the dynamic fits must be stores in metadata(data) under "dynamic_fits"
assay	of the SummarizedExperiment object that was used to fit the dynamics model
kegg	column in "data" that contains the KEGG IDs or other identifier of metabolites
condition	name of column in dataframe data that specifies the experimental condition
time	column in "data" that contains the time point identifiers
metabolite	column of "data" that contains the metabolite names or IDs
fits	list of model fits for which estimates should be extracted
iter	how many iterations were used to fit the dynamics model
warmup	how many warm-up iterations were used to fit the dynamics model
chains	how many chains were used to fit the dynamics model
samples	how many posterior samples should be drawn (p.e. for check of clustering precision)

Value

a list of dataframes (one per experimental condition) that contains the estimates at the timepoints and samples from the posterior (number as specified in samples), delta_mu specifies the difference between time point specified in column "time.ID" and subsequent time point (delta_mu in row time.ID=1: mu(time point 2)- mu(time point 1)) if number of time points in dataset is >1 If data is a summarizedExperiment object the estimates are stored in metadata(data) under "estimates_dynamics"

See Also

Fit the dynamic model [fit_dynamics_model\(\)](#). Diagnostics of the dynamic model [diagnostics_dynamics\(\)](#) Visualization of estimates with [plot_estimates\(\)](#)

Examples

```

data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite == "ATP"]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
data <- estimates_dynamics(
  data = data, iter = 2000,
  chains = 1, condition = "condition"
)

```

fit_dynamics_model	<i>Fits dynamics model</i>
--------------------	----------------------------

Description

Employs a hierarchical model that assumes a normal distribution of standardized (mean=0, sd=1) log(cpc) (cpc = normalized metabolite abundance) values for robust estimation of mean concentrations over time of single metabolites at single experimental conditions.

Usage

```
fit_dynamics_model(
  data,
  metabolite = "metabolite",
  time = "time",
  condition = "condition",
  scaled_measurement = "m_scaled",
  assay = "scaled_log",
  chains = 4,
  cores = 4,
  adapt_delta = 0.95,
  max_treedepth = 10,
  iter = 2000,
  warmup = iter/4
)
```

Arguments

data	concentration table with at least three replicate measurements per metabolite containing the columns "metabolite", "condition", and "m_scaled" by default or colData of a SummarizedExperiment object
metabolite	column of "data" that contains the metabolite names or IDs
time	column of "time" that contains time as numeric, make sure your time column is ordered from lowest to highest for the model to work
condition	column of "data" that contains the experimental conditions
scaled_measurement	column of "data" that contains the concentrations per cell, centered and normalized per metabolite and experimental condition (mean=0, sd=1)
assay	if input is a summarizedExperiment specify the assay that should be used for input, colData has to hold the columns, "condition" and "metabolite", rowData the timepoint specifications
chains	how many Markov-Chains should be used for model fitting, use at least two, default=4
cores	how many cores should be used for model fitting; this parallelizes the model fitting and therefore speeds it up; default=4
adapt_delta	target average acceptance probability, can be adapted if divergent transitions are reported, default is 0.95

max_treedepth	can be adapted if model throws warnings about hitting max_treedepth, warnings are mostly efficiency not validity concerns and treedepth can be raised, default=10
iter	how many iterations are run, increasing might help with effective sample size being to low, default=2000
warmup	how many iterations the model warms up for, increasing might facilitate efficiency, must be at least 25% of ITER, default=iter/4

Value

returns a list of model fits. One model fit named "condition" per experimental condition. If input is a summarizedExperiment object the dynamic fits are stored metadata(data) under "dynamic_fits"

See Also

Example data set [longitudinalMetabolomics](#). Get model diagnostics [diagnostics_dynamics\(\)](#) Get model estimates [estimates_dynamics\(\)](#)

Examples

```
data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite == "ATP"]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
S4Vectors::metadata(data)[["dynamic_fits"]]
```

heatmap_dynamics	<i>plot bubble heatmap from the numerical fit of compare_dynamics()</i>
------------------	---

Description

plot bubble heatmap from the numerical fit of compare_dynamics()

Usage

```
heatmap_dynamics(
  estimates = metadata(data)[["comparison_dynamics"]][["estimates"]],
  data
)
```

Arguments

estimates	dataframe of estimates of the mean distance between clusters of different experimental conditions ("mean") and the standard deviation ("sigma") obtain by function compare_dynamics()
-----------	---

data a dataframe or containing a column specifying the metabolite names to be compared and cluster IDs (column named "cluster") of clusters of similar dynamics, as well as a column "condition" specifying the experimental conditions. to be compared or a [SummarizedExperiment](#) storing the same information in meta-data(data) under "cluster"

Value

a bubble heat map where the color of the bubble represents the similarity of two clusters in regards to their dynamics in the color and the size the uncertainty of the similarity. Big bright bubbles mean high similarity with low uncertainty.

See Also

Do calculations for comparison of dynamics between clusters [compare_dynamics\(\)](#)

Examples

```
data("longitudinalMetabolomics")
longitudinalMetabolomics <- compare_dynamics(
  data = longitudinalMetabolomics,
  dynamics = c("1", "2", "3", "4"),
  cores = 1
)
heatmap_dynamics(data = longitudinalMetabolomics)
```

heatmap_metabolites	<i>plot heatmap from comparison of metabolite composition compare_metabolites()</i>
---------------------	---

Description

plot heatmap from comparison of metabolite composition [compare_metabolites\(\)](#)

Usage

```
heatmap_metabolites(
  distances = metadata(data)[["comparison_metabolites"]],
  data
)
```

Arguments

distances dataframe of Jaccard indices between clusters obtained by function [compare_metabolites\(\)](#). If [compare_metabolites\(\)](#) was executed on as [SummarizedExperiment](#) or a [SummarizedExperiment](#) than this is stored in metadata(data) under "comparison_metabolites"

data a dataframe containing the columns "metabolite" specifying the metabolite names to be compared and cluster IDs(column named "cluster") of clusters of similar dynamics, as well as a column "condition" specifying the experimental conditions to be compared

Value

a heatmap where the color of the tile represents the similarity of two clusters in regards to their metabolite composition. The brighter the color the more similar the metabolite compositions.

See Also

Do calculations for comparison of metabolites between clusters `compare_metabolites()`

Examples

```
data("longitudinalMetabolomics")
longitudinalMetabolomics <- compare_metabolites(
  data = longitudinalMetabolomics
)
heatmap_metabolites(data = longitudinalMetabolomics)
```

longitudinalMetabolomics

A simulated data set of longitudinal concentration tables of metabolites.

Description

A simulated data set of 98 metabolites. 3 replicate measurements of 4 time points and at 3 experimental conditions. Metabolites are in 8 dynamics groups per experimental condition. 4 groups have varying dynamics between conditions. Is represented as a SummarizedExperiment object where concentration tables of each experimental condition are stored in assays (raw concentrations in "concentrations", log-transformed transformations in "log_con" and scaled log-transformed concentrations in "scaled_log") and metabolite names, KEGG IDs, experimental conditions and clustering solutions per experimental condition are stored in colData and timepoint specifications in rowData. ([SummarizedExperiment](#)).

Usage

```
data("longitudinalMetabolomics")
```

Format

A SummarizedExperiment object with concentration tables in assays. RowData contains the time point specification. ColData as specified below.

condition experimental condition

metabolite metabolite name

KEGG KEGG ID of metabolites

replicate column that specifies the measurement replicate

cluster cluster ID that is condition specific for every metabolite

Source**Script used to create simulated data**

```

# load KEGG database for assignment of metabolite names: data("metabolite_modules")
# metabolite_db <- metabolite_modules # Group <- middle_hierarchy
library(dplyr) library(SummarizedExperiment) # Parameters (as before)
n_features <- 98
n_groups <- 8 # Number of groups (randomly choose between 6-8)
n_time_points <- 4 # Number of time points
n_replicates <- 3 # Number of replicates for all features and time points
n_conditions <- 3 # Number of experimental conditions
x_varying_groups <- 4 # Number of groups with varying dynamics across conditions
condition_names <- c("A","B","C")

# Probability matrix for assigning metabolites from different database groups to dynamic groups #
# For simplicity, we assume equal probability; customize as needed
group_probabilities <- matrix(c(0.8,rep(0.01,7), #amino acid metabolism rep(0.01,7),0.8, #nucleotide
metabolism 0.1,0.8,0.8,rep(0.1,5), # energy and carbohydrate metabolism runif(5 * length(unique(metabolite_modules$middle_hierarchy)))
nrow = n_groups, ncol = length(unique(metabolite_modules$middle_hierarchy)))

# Generate group dynamics (base trends over time) for each condition
group_dynamics <- list()

# Define the base group dynamics for condition 1
group_dynamics[[1]] <- lapply(1:n_groups, function(g) trend <- rnorm(n_time_points, mean = g *
2, sd = 0.5) return(trend) )

# Define varying dynamics for selected groups across other conditions
varying_groups <- sample(1:n_groups, x_varying_groups, replace = FALSE)
for (cond in 2:n_conditions) group_dynamics[[cond]] <- group_dynamics[[1]] for (g in varying_groups)
group_dynamics[[cond]][[g]] <- rnorm(n_time_points, mean = g * 2, sd = 1)

# Assign each feature to a group
feature_to_group <- sample(1:n_groups, n_features, replace = TRUE)

# Initialize a list to store the simulated data
simulated_data <- list()

# Assign metabolite names to features
available_metabolites <- metabolite_modules # Copy of metabolite database to keep track of unused
names

# Simulate data for each feature across all conditions
for (feature in 1:n_features)
# Get the group for this feature
group <- feature_to_group[feature]

# Determine probability of each metabolite database group for this dynamic group
group_probs <- group_probabilities[group, ]

# Subset the metabolite database for selection based on group probabilities

```

```

metabolite_candidates <- available_metabolites group_by(middle_hierarchy) mutate(Probability =
group_probs[match(middle_hierarchy, unique(metabolite_modules$middle_hierarchy))]) ungroup()
filter(metabolite

# Randomly sample a metabolite based on these probabilities
metabolite_name <- sample(metabolite_candidates$metabolite, 1, prob = metabolite_candidates$Probability)
# Remove this metabolite from available pool
available_metabolites <- available_metabolites[available_metabolites$metabolite != metabolite_name,
]

# Generate a random base mean for this feature between 0.001 and 1000
base_mean <- runif(1, min = 0.001, max = 1000)
# Generate feature-specific variances for each time point
feature_variances <- runif(n_time_points, min = 0.1, max = 2)
# Store data for each condition
for (cond in 1:n_conditions) trend <- group_dynamics[[cond]][[group]] feature_means <- base_mean
* trend / max(abs(trend))

feature_data <- data.frame( metabolite = metabolite_name, # Assign metabolite name here condi-
tion = paste0(condition_names[[cond]]), time = rep(1:n_time_points, each = n_replicates), replicate
= rep(1:n_replicates, times = n_time_points) )

# Generate the actual data points with strictly positive concentrations
feature_data$measurement <- unlist(lapply(1:n_time_points, function(t) rlnorm(n_replicates, mean-
log = log(feature_means[t]), sdlog = feature_variances[t]) ))

simulated_data[[length(simulated_data) + 1]] <- feature_data

rm(base_mean,cond,feature,feature_means,feature_to_group,feature_variances, g,group,group_probs,metabolite_name,
n_time_points,trend,varying_groups,x_varying_groups,available_metabolites,feature_data,group_dynamics,
group_probabilities,metabolite_candidates)

# Combine all features and conditions into one data frame
simulated_data_df <- do.call(rbind, simulated_data)

simulated_data_df <- simulated_data_df group_by(metabolite, condition) mutate( log_m = log10(measurement),
m_scaled = (log_m - mean(log_m)) / sd(log_m) )

# add KEGG IDs name_map_HMDB_CAS <- readr::read_csv("name_map_HMDB_CAS.csv")
longitudinalMetabolomics <- dplyr::left_join(longitudinalMetabolomics,name_map_HMDB_CAS[,c("Query","KEGG")])

## concentrations temp <- longitudinalMetabolomics temp <- temp select(condition,metabolite,KEGG,time,measurement)
pivot_wider(id_cols=c(condition,metabolite,KEGG,replicate), names_from = time, values_from =
measurement) concentrations <- temp ## transform matrix so that conditions are in columns to facil-
itate access ## with colData -> se[,se$condition="A"] concentrations <- t(as.matrix(concentrations))
row.names(concentrations) <- NULL # prepare log_transformed data temp <- data temp <- temp se-
lect(condition,metabolite,KEGG,time,log_m,replicate) pivot_wider(id_cols=c(condition,metabolite,KEGG,replicate),
names_from = time, values_from = log_m) log_con <- temp log_con <- t(as.matrix(log_con))
row.names(log_con) <- NULL # prepare scaled log_transformed data temp <- data temp <- temp se-
lect(condition,metabolite,KEGG,time,m_scaled,replicate) pivot_wider(id_cols=c(condition,metabolite,KEGG,replicate),
names_from = time, values_from = m_scaled) scaled_log <- temp scaled_log <- t(as.matrix(scaled_log))
row.names(scaled_log) <- NULL

# prepare row and colData ##### row_data <- DataFrame(time_points=c("time_point_1","time_point_2","time_point_3",
"time_point_4")) col_data <- DataFrame(condition=temp$condition,metabolite=temp$metabolite,
KEGG=temp$KEGG,replicate=temp$replicate)

```

```

se <- SummarizedExperiment(assays=SimpleList(concentrations=concentrations, log_con=log_con,
scaled_log=scaled_log), rowData = row_data, colData = col_data) # set row and colnames #####
rownames(se) <- c("time_point_1", "time_point_2", "time_point_3", "time_point_4") colnames(se)
<- temp$metabolite

# add metadata ##### metadata(se)[["data origin"]] <- "Simulated data of 98 metabolites with three
concentration observations at four time points and at three different biological conditions. Script to
construct dataset can be seen with ?longitudinalMetabolomics"

# se <- fit_dynamics_model(se, scaled_measurement = "m_scaled", assay = "scaled_log", time =
"time", condition = "condition", max_treedepth = 10, adapt_delta = 0.95, # default 0.95 iter = 5000)

se <- estimates_dynamics(data = se, iter = 5000, chains = 2, condition = "condition")

se <- cluster_dynamics(data=se, distance="euclidean", agglomeration="ward.D2", minClusterSize=0,
deepSplit=4)

longitudinalMetabolomics <- se devtools::use_data(longitudinalMetabolomics)

```

longitudinalMetabolomics_df

A simulated data set of longitudinal concentration tables of metabolites. In contrast to "longitudinalMetabolomics" this dataset is in data frame format. It was simulated with a different seed compared to longitudinalMetabolomics so the results can deviate.

Description

A simulated data set of 98 metabolites. 3 replicate measurements of 4 time points and at 3 experimental conditions. Metabolites are in 8 dynamics groups per experimental condition. 4 groups have varying dynamics between conditions. Is represented as a SummarizedExperiment object where concentration tables of each experimental condition are stored in assays (raw concentrations in "concentrations", log-transformed transformations in "log_con" and scaled log-transformed concentrations in "scaled_log") and metabolite names, KEGG IDs, experimental conditions and clustering solutions per experimental condition are stored in colData and timepoint specifications in rowData. ([SummarizedExperiment](#)).

Usage

```
data("longitudinalMetabolomics")
```

Format

A SummarizedExperiment object with concentration tables in assays. RowData contains the time point specification. ColData as specified below.

condition experimental condition

metabolite metabolite name

KEGG KEGG ID of metabolites

replicate column that specifies the measurement replicate

cluster cluster ID that is condition specific for every metabolite

Source**Script used to create simulated data**

```

# load KEGG database for assignment of metabolite names: data("metabolite_modules")
# metabolite_db <- metabolite_modules # Group <- middle_hierarchy
library(dplyr) library(SummarizedExperiment) # Parameters (as before)
n_features <- 98
n_groups <- 8 # Number of groups (randomly choose between 6-8)
n_time_points <- 4 # Number of time points
n_replicates <- 3 # Number of replicates for all features and time points
n_conditions <- 3 # Number of experimental conditions
x_varying_groups <- 4 # Number of groups with varying dynamics across conditions
condition_names <- c("A","B","C")

# Probability matrix for assigning metabolites from different database groups to dynamic groups #
# For simplicity, we assume equal probability; customize as needed
group_probabilities <- matrix(c(0.8,rep(0.01,7), #amino acid metabolism rep(0.01,7),0.8, #nucleotide
metabolism 0.1,0.8,0.8,rep(0.1,5), # energy and carbohydrate metabolism runif(5 * length(unique(metabolite_modules$middle_hierarchy)))
nrow = n_groups, ncol = length(unique(metabolite_modules$middle_hierarchy)))

# Generate group dynamics (base trends over time) for each condition
group_dynamics <- list()

# Define the base group dynamics for condition 1
group_dynamics[[1]] <- lapply(1:n_groups, function(g) trend <- rnorm(n_time_points, mean = g *
2, sd = 0.5) return(trend) )

# Define varying dynamics for selected groups across other conditions
varying_groups <- sample(1:n_groups, x_varying_groups, replace = FALSE)
for (cond in 2:n_conditions) group_dynamics[[cond]] <- group_dynamics[[1]] for (g in varying_groups)
group_dynamics[[cond]][[g]] <- rnorm(n_time_points, mean = g * 2, sd = 1)

# Assign each feature to a group
feature_to_group <- sample(1:n_groups, n_features, replace = TRUE)

# Initialize a list to store the simulated data
simulated_data <- list()

# Assign metabolite names to features
available_metabolites <- metabolite_modules # Copy of metabolite database to keep track of unused
names

# Simulate data for each feature across all conditions
for (feature in 1:n_features)
# Get the group for this feature
group <- feature_to_group[feature]

# Determine probability of each metabolite database group for this dynamic group
group_probs <- group_probabilities[group, ]

# Subset the metabolite database for selection based on group probabilities

```

```

metabolite_candidates <- available_metabolites group_by(middle_hierarchy) mutate(Probability =
group_probs[match(middle_hierarchy, unique(metabolite_modules$middle_hierarchy))]) ungroup()
filter(metabolite

# Randomly sample a metabolite based on these probabilities
metabolite_name <- sample(metabolite_candidates$metabolite, 1, prob = metabolite_candidates$Probability)
# Remove this metabolite from available pool
available_metabolites <- available_metabolites[available_metabolites$metabolite != metabolite_name,
]
# Generate a random base mean for this feature between 0.001 and 1000
base_mean <- runif(1, min = 0.001, max = 1000)
# Generate feature-specific variances for each time point
feature_variances <- runif(n_time_points, min = 0.1, max = 2)
# Store data for each condition
for (cond in 1:n_conditions) trend <- group_dynamics[[cond]][[group]] feature_means <- base_mean
* trend / max(abs(trend))

feature_data <- data.frame( metabolite = metabolite_name, # Assign metabolite name here condi-
tion = paste0(condition_names[[cond]]), time = rep(1:n_time_points, each = n_replicates), replicate
= rep(1:n_replicates, times = n_time_points) )
# Generate the actual data points with strictly positive concentrations
feature_data$measurement <- unlist(lapply(1:n_time_points, function(t) rlnorm(n_replicates, mean-
log = log(feature_means[t]), sdlog = feature_variances[t]) ))
simulated_data[[length(simulated_data) + 1]] <- feature_data

rm(base_mean,cond,feature,feature_means,feature_to_group,feature_variances, g,group,group_probs,metabolite_name,
n_time_points,trend,varying_groups,x_varying_groups,available_metabolites,feature_data,group_dynamics,
group_probabilities,metabolite_candidates)
# Combine all features and conditions into one data frame
simulated_data_df <- do.call(rbind, simulated_data)
simulated_data_df <- simulated_data_df group_by(metabolite, condition) mutate( log_m = log10(measurement),
m_scaled = (log_m - mean(log_m)) / sd(log_m) )
# add KEGG IDs name_map_HMDB_CAS <- readr::read_csv("name_map_HMDB_CAS.csv")
longitudinalMetabolomics_df <- dplyr::left_join(simulated_data_df,name_map_HMDB_CAS[,c("Query","KEGG")],join=

```

metabolite_modules	<i>KEGG Query Results of experimental metabolites</i>
--------------------	---

Description

Using the package KEGGREST (<https://www.bioconductor.org/packages/release/bioc/html/KEGGREST.html>) all experimental metabolites (see `data("intra")`) were queried with there KEGG-IDs and all functional modules recorded to which the metabolite is annotated in the KEGG-database.

Usage

```
data("metabolite_modules")
```

Format

A data frame with 348 observations on the following 8 variables.

... 1 row number of the dataframe
 metabolite name of the experimental metabolite
 KEGG KEGG ID of the experimental metabolite
 module_id ID of the KEGG module to which the metabolite is annotated
 module_name name of the KEGG module to which the metabolite is annotated
 upper_hierarchy name of the highest hierarchy level of module organisation
 middle_hierarchy name of the middle hierarchy = functional module, p.e. "Amino acid metabolism"
 lower_hierarchy name of the lowest level of modules, this often contain only a couple pathways
 p.e. "Arginine and proline metabolism"

Source

<https://www.genome.jp/kegg/module.html>

See Also

[modules_compounds](#)

modules_compounds

Background KEGG Query Results Of Functional Modules

Description

Using the package KEGGREST (<https://www.bioconductor.org/packages/release/bioc/html/KEGGREST.html>) a list of all KEGG-modules (KeggList("module")) including their upper, middle and lower hierarchy as given by the KEGG-database and the corresponding annotated metabolites was queried.

Usage

```
data("modules_compounds")
```

Format

A data frame with 1988 observations on the following 6 variables.

KEGG KEGG ID of a metabolite annotated to a functional module
 upper_hierarchy name of the highest hierarchy level of module organisation
 middle_hierarchy name of the middle hierarchy = functional module, p.e. "Amino acid metabolism"
 lower_hierarchy name of the lowest level of modules, this often contain only a couple pathways
 p.e. "Arginine and proline metabolism"
 module_id the ID of the KEGG functional module
 module_name name of the KEGG module

Source

<https://www.genome.jp/kegg/module.html>

See Also[metabolite_modules](#)

ORA_hypergeometric *OverRepresentationAnalysis with a hypergeometric model*

Description

Testing the hypothesis that certain KEGG modules are over-represented in clusters of metabolites. A module is considered over-represented in a cluster the number of metabolites in a cluster being annotated to a functional module (n_{obs}) is higher than the expected number of metabolites in a cluster of this size being annotated to a functional module (n_{theo}). We can calculate the OvE (Observed versus Expected = $n_{\text{obs}}/n_{\text{theo}}$) and show the probabilities of these ratios. $\log(p(\text{OvE})) > 0$ indicates an over-representation of the functional module in the cluster, $\log(p(\text{OvE})) < 0$ an under-representation.

Usage

```
ORA_hypergeometric(
  background,
  annotations,
  data,
  tested_column = "middle_hierarchy"
)
```

Arguments

background	dataframe that contains KEGG IDs of metabolites that are assigned to functional modules, is incorporated in the package modules_compounds
annotations	dataframe tha contains information to which functional modules our experimental metabolites are annotated in KEGG, can be constructed by filtering the provided KEGG background modules_compounds for the experimental metabolites
data	dataframe containing columns "KEGG" specifying the KEGG identifiers of metabolites, "cluster" specifying the cluster ID of metabolites and a column specifying the experimental condition called "condition" or if data is a SummarizedExperiment or a SummarizedExperiment clustering solution must be stored in metadata(data) under "cluster"
tested_column	column that is in background and annotations and on which the hypergeometric model will be executed

Value

a dataframe containing the ORA results or if data is SummarizedExperiment [SummarizedExperiment](#) object the output is stored in metadata(data) under "ORA_tested_column"

See Also

Function to visualize ORA results [plot_ORA\(\)](#)

Examples

```

data("longitudinalMetabolomics")
data("modules_compounds")
head(modules_compounds)
data("metabolite_modules")
head(metabolite_modules)
# middle hierarchy
longitudinalMetabolomics <- ORA_hypergeometric(
  data = longitudinalMetabolomics,
  annotations = metabolite_modules,
  background = modules_compounds,
  tested_column = "middle_hierarchy"
)
S4Vectors::metadata(longitudinalMetabolomics)[["ORA_middle_hierarchy"]]

```

plot_cluster	<i>visualize clustering solution of cluster_dynamics()</i>
--------------	--

Description

visualize clustering solution of cluster_dynamics()

Usage

```
plot_cluster(data)
```

Arguments

data result of `cluster_dynamics()` function

Value

a list of plots. Per experimental condition one dendrogram, colored by cluster and one visualization of PCA-analysis of the clustering solution. Additionally one plot visualizing the clustered dynamics over all conditions

See Also

[cluster_dynamics\(\)](#)

Examples

```

data("longitudinalMetabolomics")
plot_cluster(longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A"])

```

plot_diagnostics	<i>Plot diagnostic criteria of numerical fit of Bayesian model of dynamics</i>
------------------	--

Description

Plot diagnostic criteria of numerical fit of Bayesian model of dynamics

Usage

```
plot_diagnostics(
  data,
  assay = "scaled_log",
  diagnostics = metadata(data)[["diagnostics_dynamics"]][["model_diagnostics"]],
  divergences = TRUE,
  max_tredepth = TRUE,
  Rhat = TRUE,
  n_eff = TRUE
)
```

Arguments

data	dataframe or colData of a SummarizedExperiment used to fit dynamics model must contain column "time"
assay	of the SummarizedExperiment object that was used to fit the dynamics model
diagnostics	dataframe containing diagnostics criteria from the numerical fit of Bayesian model of dynamics obtained by function <code>diagnostics_dynamics()</code>
divergences	should number of divergent transitions be visualized?
max_tredepth	should number of exceeded maximum tredepth be visualized?
Rhat	should Rhat be visualized?
n_eff	should number of effective samples be visualized?

Value

plots of diagnostic criteria of numerical fit of Bayesian model of dynamics

See Also

parent function [diagnostics_dynamics\(\)](#) visualization function for posterior predictive check [plot_PPC\(\)](#)

Examples

```
data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite %in% c("ATP", "ADP")]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_tredepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
```

```
data <- diagnostics_dynamics(
  data = data, assay = "scaled_log",
  iter = 2000, chains = 1,
  fits = metadata(data)[["dynamic_fits"]]
)
plot_diagnostics(data = data, assay = "scaled_log")
```

plot_estimates	<i>Visualization of parameter estimates from numeric fit of Bayesian model of dynamics</i>
----------------	--

Description

Visualization of parameter estimates from numeric fit of Bayesian model of dynamics

Usage

```
plot_estimates(
  data,
  estimates = metadata(data)[["estimates_dynamics"]],
  assay = "scaled_log",
  time = "time",
  delta_t = TRUE,
  dynamics = TRUE
)
```

Arguments

data	dataframe or SummarizedExperiment used used to fit dynamics model and extract the estimates
estimates	a list of dataframes (one per experimental condition) that contains the estimates at the timepoints and samples from the posterior generated by estimates_dynamics() or if data is a SummarizedExperiment estimates must be stored in metadata(data) under "estimates_dynamics"
assay	of the SummarizedExperiment object that was used to fit the dynamics model
time	column in "data" that contains the time point identifiers
delta_t	should differences between timepoints be plotted?
dynamics	should dynamics be plotted?

Value

Visualization of differences between timepoints(delta_t) and dynamics profiles of single metabolites

See Also

parent function [estimates_dynamics\(\)](#)

Examples

```

data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite == "ATP"]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)
data <- estimates_dynamics(
  data = data, iter = 2000,
  chains = 1, condition = "condition"
)
plot_estimates(data = data, delta_t = FALSE)
plot_estimates(data = data, dynamics = FALSE)

```

plot_ORA	<i>Plot results of over-representation analysis with ORA_hypergeometric()</i>
----------	---

Description

Plot results of over-representation analysis with ORA_hypergeometric()

Usage

```
plot_ORA(data, tested_column = "middle_hierarchy")
```

Arguments

data	result dataframe from ORA_hypergeometric() or SummarizedExperiment object where the ORA_hypergeometric() results are stored in metadata(data) under "ORA_tested_column"
tested_column	KEGG module hierarchy level on which ORA was executed

Value

a plot of the over-representation analysis

See Also

do over-representation analysis of KEGG functional modules [ORA_hypergeometric\(\)](#)

Examples

```

data("longitudinalMetabolomics")
data("modules_compounds")
head(modules_compounds)
data("metabolite_modules")
head(metabolite_modules)
# middle hierarchy
longitudinalMetabolomics <- ORA_hypergeometric(
  data = longitudinalMetabolomics,

```

```

    annotations = metabolite_modules,
    background = modules_compounds,
    tested_column = "middle_hierarchy"
  )
plot_ORA(longitudinalMetabolomics)

```

plot_PPC	<i>Plots posterior predictive check of numerical fit of Bayesian dynamics model</i>
----------	---

Description

Plots posterior predictive check of numerical fit of Bayesian dynamics model

Usage

```

plot_PPC(
  posterior = metadata(data)[["diagnostics_dynamics"]],
  data,
  assay = "scaled_log",
  scaled_measurement = "scaled_measurement"
)

```

Arguments

posterior	a list of one dataframe per condition that contains necessary information for Posterior predictive check obtained by function <code>diagnostics_dynamics()</code> (named "PPC_condition")
data	dataframe or colData of a SummarizedExperiment used to fit dynamics model
assay	of the SummarizedExperiment object that was used to fit the dynamics model
scaled_measurement	column name of concentration values used to model fit, should be normalized by experimental condition and metabolite to mean of zero and standard deviation of one

Value

a list of visual posterior predictive check, one per experimental condition

See Also

parent function [diagnostics_dynamics\(\)](#) visualization function for diagnostics [plot_diagnostics\(\)](#)

Examples

```

data("longitudinalMetabolomics")
data <- longitudinalMetabolomics[, longitudinalMetabolomics$condition == "A" &
  longitudinalMetabolomics$metabolite %in% c("ATP", "ADP")]
data <- fit_dynamics_model(
  data = data,
  scaled_measurement = "m_scaled", assay = "scaled_log",
  max_treedepth = 14, adapt_delta = 0.95, iter = 2000, cores = 1, chains = 1
)

```

```
)  
data <- diagnostics_dynamics(  
  data = data, assay = "scaled_log",  
  iter = 2000, chains = 1,  
  fits = metadata(data)[["dynamic_fits"]]  
)  
plot_PPC(  
  data = data, assay = "scaled_log"  
)
```

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