

Package ‘combi’

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Type Package

Title Compositional omics model based visual integration

Version 1.22.0

Description This explorative ordination method combines quasi-likelihood estimation, compositional regression models and latent variable models for integrative visualization of several omics datasets. Both unconstrained and constrained integration are available. The results are shown as interpretable, compositional multiplots.

License GPL-2

Encoding UTF-8

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<i>addLink</i>	<i>Add a link on a compositional plot</i>
----------------	---

Description

Add a link on a compositional plot

Usage

```
addLink(  
  DIplot,  
  links,  
  Views,  
  samples,  
  variable = NULL,  
  Dims = c(1, 2),  
  addLabel = FALSE,  
  labPos = NULL,  
  projColour = "grey",  
  latentSize = 0.25  
)
```

Arguments

DIplot	A list with ggplot object where the links are to be added, and data frames with coordinates (obtained by setting plot(..., returnCoords = TRUE))
links	A matrix containing either feature names (two column matrix) or approximate coordinates (four column matrix)
Views	Indices or names of the views for which the links should be added
samples	Sample names or approximate sample coordinates
variable	Name of variable in environmental gradient for which link should be plotted
Dims	vector of length 2 referring to the model dimensions
addLabel	A boolean, should arrow with label be plotted?
labPos	The position of the label, as a numeric vector of length 2
projColour	The colour of the projection, as character string
latentSize	Size of the line from the origin to the latent variable dot

Value

A ggplot object with the links added

Examples

```
data(Zhang)
## Not run:
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)

## End(Not run)
load(system.file("extdata", "zhangFits.RData", package = "combi"))
Plot = plot(microMetaboInt, samDf = zhangMetavars, samCol = "ABX",
  returnCoords = TRUE)
addLink(Plot, links = cbind("OTU0565b3", "OTUa14fb5"), Views = 1,
  samples = c(1,1))
```

arrayMult

*Array multiplication***Description**

Array multiplication

Usage

```
arrayMult(centralMat, outerMat, ncols = ncol(outerMat))
```

Arguments

centralMat	an nxp matrix
outerMat	an nxd matrix
ncols	an integer, the number of columns of outerMat

Value

an nxpxd matrix, the stacked matrices of centralMat multiplied to every column of outermat

buildCentMat	<i>A function to build a centering matrix based on a dataframe</i>
--------------	--

Description

A function to build a centering matrix based on a dataframe

Usage

```
buildCentMat(object)
```

Arguments

object	an modelDI object or dataframe
--------	--------------------------------

Value

a centering matrix consisting of ones and zeroes, or a list with components

centMat	a centering matrix consisting of ones and zeroes
datFrame	The dataframe with factors with one level removed

buildCompMat	<i>Build the composition matrix for a certain dimension m dimensions</i>
--------------	--

Description

Build the composition matrix for a certain dimension m dimensions

Usage

```
buildCompMat(
  colMat,
  paramEsts,
  latentVar,
  m,
  norm = TRUE,
  id = seq_len(m),
  subtractMax = TRUE
)
```

Arguments

colMat	The nxp independence model composition matrix
paramEsts	The matrix of feature parameter estimates
latentVar	The matrix of latent variables
m	the required dimension
norm	a boolean, should the composition matrix be normalized?
id	The vector of dimensions to consider
subtractMax	A boolean, should the maximum be subtracted from every composition prior to exponentiation? Recommended for numerical stability

Value

A matrix with compositions in the rows

buildConfMat	<i>Build confounder design matrices with and without intercepts</i>
--------------	---

Description

Build confounder design matrices with and without intercepts

Usage

```
buildConfMat(confounders)
```

Arguments

confounders	A dataframe of confounding variables #' For the preliminary trimming, we do not include an intercept, but we do include all the levels of the factors using contrasts=FALSE: we want to do the trimming in every subgroup, so no hidden reference levels For the filtering we just use a model with an intercept and treatment coding, here the interest is only in adjusting the offset
-------------	--

Value

a list with components

confModelMatTrim	A confounder matrix without intercept, with all levels of factors present. This will be used to trim out taxa that have zero abundances in any subgroup defined by confounders
confModelMat	A confounder matrix with intercept, and with reference levels for factors absent. This will be used to fit the model to modify the independence model, and may include continuous variables

buildCovMat	<i>A function to build the covariate matrix of the constraints</i>
-------------	--

Description

A function to build the covariate matrix of the constraints

Usage

```
buildCovMat(datFrame)
```

Arguments

datFrame	the dataframe with which the covariate matrix is to be built In this case we will 1) Include dummy's for every level of the categorical variable, and force them to sum to zero. This is needed for plotting and required for reference level independent normalization. 2) Exclude an intercept. The density function f() will provide this already.
----------	--

Value

a list with components	
covModelMat	The model matrix
datFrame	The dataframe used to construct the model matrix

buildEmptyJac	<i>Prepare an empty Jacobian matrix, with useful entries prefilled. In case of distribution "gaussian", it returns the lhs matrix of the linear system for finding the feature paramters</i>
---------------	--

Description

Prepare an empty Jacobian matrix, with useful entries prefilled. In case of distribution "gaussian", it returns the lhs matrix of the linear system for finding the feature paramters

Usage

```
buildEmptyJac(
  n,
  m,
  lower,
  distribution = "quasi",
  normal = FALSE,
  nLambda1s = 1,
  centMat = NULL,
  weights = 1
)
```

Arguments

n	the number of parameters
m	the dimension
lower	the current parameter estimates
distribution	A character string, the distributional assumption for the data
normal	a boolean, are normalization restrictions in place?
nLambda1s	The number of centering restrictions
centMat	The centering matrix
weights	Vector of feature weights

Value

an empty jacobian matrix, or the lhs of the system of estimating equations

buildMarginalOffset	<i>Build an offset matrix from an marginal model object</i>
---------------------	---

Description

Build an offset matrix from an marginal model object

Usage

```
buildMarginalOffset(indepModel, invLink)
```

Arguments

indepModel	The fitted marginal model, a list
invLink	The inverse link function

Value

an offset matrix of the size of the data

buildMu	<i>A function to build the mu matrix</i>
---------	--

Description

A function to build the mu matrix

Usage

```
buildMu(offSet, latentVar, paramEsts, distribution, paramMatrix = FALSE)
```

Arguments

offSet	the offset matrix
latentVar, paramEsts, distribution	Latent variables, parameter estimates and distribution type
paramMatrix	A boolean, are feature parameters provided as matrix

Value

The mean matrix

buildMuMargins	<i>Build the marginal mu matrix</i>
----------------	-------------------------------------

Description

Build the marginal mu matrix

Usage

```
buildMuMargins(x, otherMargin, col)
```

Arguments

x	The marginal parameters begin estimated
otherMargin	The parameters of the other margin
col	A logical, are the column parameters being estimated?

Value

a matrix of means

buildOffsetModel	<i>Build a marginal offset matrix given a model</i>
------------------	---

Description

Build a marginal offset matrix given a model

Usage

```
buildOffsetModel(modelObj, View, distributions, compositional)
```

Arguments

modelObj	a modelDI object
View	The view for which to build the offset
distributions, compositional	belong to the view

Value

The offset matrix

checkAlias	<i>Check for alias structures in a dataframe, and throw an error when one is found</i>
------------	--

Description

Check for alias structures in a dataframe, and throw an error when one is found

Usage

```
checkAlias(datFrame, covariatesNames)
```

Arguments

datFrame	the data frame to be checked for alias structure
covariatesNames	The names of the variables to be considered

Value

Throws an error when an alias structure is detected, returns invisible otherwise

checkMeanVarTrend	<i>Quickly check if the mean variance trend provides a good fit</i>
-------------------	---

Description

Quickly check if the mean variance trend provides a good fit

Usage

```
checkMeanVarTrend(data, meanVarFit = "spline", returnTrend = FALSE, ...)
```

Arguments

data	Data in any acceptable format (see details ?combi)
meanVarFit	The type of mean variance fit, either "cubic" or "spline"
returnTrend	A boolean, should the estimated trend be returned (TRUE) or only plotted (FALSE)?
...	passed on to the estMeanVarTrend() function

Value

A plot object

Examples

```
data(Zhang)
par(mfrow = c(1,2))
lapply(list("microbiome" = zhangMicrobio, "metabolome" = zhangMetabo),
  checkMeanVarTrend)
par(mfrow = c(1,1))
```

checkMonotonicity	<i>Check for monotonicity in compositional datasets fro given dimensions</i>
-------------------	--

Description

Check for monotonicity in compositional datasets fro given dimensions

Usage

```
checkMonotonicity(modelObj, Dim)
```

Arguments

modelObj	The combi fit
Dim	The dimensions considered

Value

A boolean matrix indicating monotonicity for every feature

combi	<i>Perform model-based data integration</i>
-------	---

Description

Perform model-based data integration

Usage

```
combi(
  data,
  M = 2L,
  covariates = NULL,
  distributions,
  compositional,
  maxIt = 300L,
  tol = 0.001,
  verbose = FALSE,
  prevCutoff = 0.95,
  minFraction = 0.1,
  logTransformGaussian = TRUE,
  confounders = NULL,
  compositionalConf = rep(FALSE, length(data)),
  nleq.control = list(maxit = 1000L, cndtol = 1e-16),
  record = TRUE,
  weights = NULL,
  fTol = 1e-05,
  meanVarFit = "spline",
  maxFeats = 2000,
```

```

    dispFreq = 10L,
    allowMissingness = FALSE,
    biasReduction = TRUE,
    maxItFeat = 20L,
    initPower = 1
)

```

Arguments

<code>data</code>	A list of data objects with the same number of samples. See details.
<code>M</code>	the required dimension of the fit, a non-negative integer
<code>covariates</code>	a dataframe of n samples with sample-specific variables.
<code>distributions</code>	a character vector describing which distributional assumption should be used. See details.
<code>compositional</code>	A logical vector with the same length as "data", indicating if the datasets should be treated as compositional
<code>maxIt</code>	an integer, the maximum number of iterations
<code>tol</code>	A small scalar, the convergence tolerance
<code>verbose</code>	Logical. Should verbose output be printed to the console?
<code>prevCutOff</code>	a scalar, the prevalence cutoff for the trimming.
<code>minFraction</code>	a scalar, each taxon's total abundance should equal at least the number of samples n times <code>minFraction</code> , otherwise it is trimmed.
<code>logTransformGaussian</code>	A boolean, should the gaussian data be logtransformed, i.e. are they log-normal?
<code>confounders</code>	A dataframe or a list of dataframes with the same length as data. In the former case the same dataframe is used for conditioning, In the latter case each view has its own conditioning variables (or NULL).
<code>compositionalConf</code>	A logical vector with the same length as "data", indicating if the datasets should be treated as compositional when correcting for confounders. Numerical problems may occur when set to TRUE
<code>nleq.control</code>	A list of arguments to the <code>nleqslv</code> function
<code>record</code>	A boolean, should intermediate estimates be stored? Can be useful to check convergence
<code>weights</code>	A character string, either 'marginal' or 'uniform', indicating rhow the feature parameters should be weighted in the normalization
<code>fTol</code>	The tolerance for solving the estimating equations
<code>meanVarFit</code>	The type of mean variance fit, see details
<code>maxFeats</code>	The maximal number of features for a Newton-Raphson procedure to be feasible
<code>dispFreq</code>	An integer, the period after which the variances should be reestimated
<code>allowMissingness</code>	A boolean, should NA values be allowed?
<code>biasReduction</code>	A boolean, should bias reduction be applied to allow for confounder correction in groups with all zeroes? Not guaranteed to work
<code>maxItFeat</code>	Integers, the maximum allowed number of iterations in the estimation of the feature parameters
<code>initPower</code>	The power to be applied to the residual matrix used to calculate the starting value. Must be positive; can be tweaked in case of numerical problems (i.e. infinite values returned by <code>nleqslv</code>)

Details

Data can be provided as raw matrices with features in the columns, or as phyloseq, Summarized-Experiment or ExpressionSet objects. Estimation of independence model and view wise parameters can be parametrized. See `?BiocParallel::bplapply` and `?BiocParallel::register`. `meanVarFit = "spline"` yields a cubic spline fit for the abundance-variance trend, `"cubic"` gives a third degree polynomial. Both converge to the diagonal line with slope 1 for small means. Distribution can be either `"quasi"` for quasi likelihood or `"gaussian"` for Gaussian data

Value

An object of the `"combi"` class, containing all information on the data integration and fitting procedure

Examples

```
data(Zhang)
#The method works on several datasets at once, and simply is not very fast.
#Hence the "Not run" statement
## Not run:
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)
#Constrained
microMetaboIntConstr = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, covariates = zhangMetavars, verbose = TRUE)

## End(Not run)
```

convPlot

Plot the convergence of the different parameter estimates in a line plot

Description

Plot the convergence of the different parameter estimates in a line plot

Usage

```
convPlot(
  model,
  latent = is.null(View),
  nVars = Inf,
  Dim = 1L,
  View = NULL,
  size = 0.125
)
```

Arguments

model	A fitted modelDI object
latent	A boolean, should latent variable trajectory be plotted
nVars	An integer, the number of variables to plot. By default all are plotted
Dim	An integer, the dimension to be plotted
View	An integer or character string, indicating the view to be plotted (if latent = FALSE)
size	The line size (see ?geom_path)

Value

A ggplot object containing the convergence plot

Examples

```
## Not run:
data(Zhang)
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)
## End(Not run)
load(system.file("extdata", "zhangFits.RData", package = "combi"))
convPlot(microMetaboInt)
convPlot(microMetaboInt, Dim = 2)
convPlot(microMetaboInt, View = "microbiome")
```

deriv2LagrangianFeatures

The score function to estimate the latent variables

Description

The score function to estimate the latent variables

Usage

```
deriv2LagrangianFeatures(
  x,
  data,
  distribution,
  offSet,
  latentVars,
  numVar,
  paramEstsLower,
  mm,
  Jac,
  meanVarTrend,
  weights,
```

```

    compositional,
    indepModel,
    ...
)

```

Arguments

x	parameter estimates
data	A list of data matrices
distribution, compositional, meanVarTrend, offSet, numVar	Characteristics of the view
latentVars	A vector of latent variables
paramEstsLower	lower dimension estimates
mm	the current dimension
Jac	a prefab jacobian
weights	The normalization weights
indepModel	the independence model
...	Additional arguments passed on to the score and jacobian functions

Value

A vector of length n, the evaluation of the score functions of the latent variables

deriv2LagrangianLatentVars

The jacobian function to estimate the latent variables

Description

The jacobian function to estimate the latent variables

Usage

```

deriv2LagrangianLatentVars(
  x,
  data,
  distributions,
  offsets,
  paramEsts,
  paramMats,
  numVars,
  latentVarsLower,
  n,
  m,
  Jac,
  numSets,
  meanVarTrends,
  links,
  varPosts,

```

```

    indepModels,
    compositional,
    ...
)

```

Arguments

<code>x</code>	The current estimates of the latent variables
<code>distributions, links, compositional, data, meanVarTrends, offsets, numVars, numSets, paramMats, paramEsts, varPosts, indepModels</code>	Characteristics of the views
<code>latentVarsLower</code>	The parameter estimates of the lower dimensions
<code>n, m</code>	integers, number of samples and dimensions
<code>Jac</code>	an empty jacobian matrix
<code>...</code>	arguments to the jacobian function, currently ignored

Value

A vector of length `n`, the evaluation of the score functions of the latent variables

deriv2LagrangianLatentVarsConstr

The score function to estimate the latent variables

Description

The score function to estimate the latent variables

Usage

```

deriv2LagrangianLatentVarsConstr(
  x,
  data,
  distributions,
  offsets,
  paramEsts,
  paramMats,
  numVars,
  latentVarsLower,
  nn,
  m,
  Jac,
  numSets,
  meanVarTrends,
  links,
  numCov,
  covMat,
  nLambda1s,
  varPosts,

```



```

    compositional,
    indepModels,
    ...
)

```

Arguments

x	The current estimates of the latent variables
distributions, data, links, compositional, meanVarTrends, offsets, numVars, paramMats, paramEsts	
latentVarsLower	Characteristics of the view
nn	The parameter estimates of the lower dimensions
m, numSets, varPosts, indepModels	number of samples
	other arguments
Jac	an empty jacobian matrix
numCov	The number of covariates
covMat	the covariates matrix
nLambda1s	The number of centering restrictions
...	arguments to the jacobian function, currently ignored

Value

A vector of length nn, the evaluation of the score functions of the latent variables

derivLagrangianFeatures

The score function to estimate the feature parameters

Description

The score function to estimate the feature parameters

Usage

```

derivLagrangianFeatures(
  x,
  data,
  distribution,
  offSet,
  latentVars,
  numVar,
  paramEstsLower,
  mm,
  indepModel,
  meanVarTrend,
  weights,
  compositional,
  ...
)

```

Arguments

x	current parameter estimates
data	A list of data matrices
distribution, compositional, meanVarTrend, offSet, numVar, indepModel, paramEstsLower	Characteristics of the view
latentVars	A vector of latent variables
mm	the current dimension
weights	The normalization weights
...	arguments to the jacobian function, currently ignored

Value

A vector with the evaluation of the score functions of the feature parameters

derivLagrangianLatentVars

The score function to estimate the latent variables

Description

The score function to estimate the latent variables

Usage

```
derivLagrangianLatentVars(
  x,
  data,
  distributions,
  offsets,
  paramEsts,
  paramMats,
  numVars,
  n,
  m,
  numSets,
  meanVarTrends,
  links,
  varPosts,
  latentVarsLower,
  compositional,
  indepModels,
  ...
)
```

Arguments

x	The current estimates of the latent variables
n	The number of samples
m	The dimensions
numSets	The number of views
latentVarsLower	The parameter estimates of the lower dimensions
compositional, links, indepModels, meanVarTrends, numVars, distributions, data, offsets, varPosts, paramMats, paramEsts	Lists of information on all the views
...	arguments to the jacobian function, currently ignored

Value

A vector of length n, the evaluation of the score functions of the latent variables

derivLagrangianLatentVarsConstr

The score function to estimate the latent variables

Description

The score function to estimate the latent variables

Usage

```
derivLagrangianLatentVarsConstr(
  x,
  data,
  distributions,
  offsets,
  paramEsts,
  numVars,
  latentVarsLower,
  n,
  m,
  numSets,
  meanVarTrends,
  links,
  covMat,
  numCov,
  centMat,
  nLambdas,
  varPosts,
  compositional,
  indepModels,
  paramMats,
  ...
)
```

Arguments

x	The current estimates of the latent variables
latentVarsLower	The parameter estimates of the lower dimensions
n	The number of samples
m	The dimensions
numSets	The number of views
covMat	The covariance matrix
numCov	The number of covariates
centMat	A centering matrix
nLambdas	The number of dummy variables
compositional, links, indepModels, meanVarTrends, numVars, distributions, data, offsets, varPosts, paramMats, paramEsts	Lists of information on all the views
...	arguments to the jacobian function, currently ignored

Value

A vector of length n, the evaluation of the score functions of the latent variables

estFeatureParameters *Estimate the feature parameters*

Description

Estimate the feature parameters

Usage

```
estFeatureParameters(
  paramEsts,
  lambdasParams,
  seqSets,
  data,
  distributions,
  offsets,
  nCores,
  m,
  JacFeatures,
  meanVarTrends,
  latentVars,
  numVars,
  control,
  weights,
  compositional,
  indepModels,
  fTol,
  allowMissingness,
  maxItFeat,
  ...
)
```

Arguments

paramEsts	Current list of parameter estimates for the different views
lambdasParams	The lagrange multipliers
seqSets	A vector with view indices
data	A list of data matrices
distributions	A character vector describing the distributions
offsets	A list of offset matrices
nCores	The number of cores to use in multithreading
m	The dimension
JacFeatures	An empty Jacobian matrix
meanVarTrends	The mean-variance trends of the different views
latentVars	A vector of latent variables
numVars	The number of variables
control	A list of control arguments for the nleqslv function
weights	The normalization weights
compositional	A list of booleans indicating compositionality
indepModels	A list of independence model
fTol	A convergence tolerance
allowMissingness	A boolean indicating whether missing values are allowed
maxItFeat	An integer, the maximum number of iterations
...	Additional arguments passed on to the score and jacobian functions

Value

A vector with estimates of the feature parameters

estIndepModel	<i>Estimate the independence model belonging to one view</i>
---------------	--

Description

Estimate the independence model belonging to one view

Usage

```
estIndepModel(
  data,
  distribution,
  compositional,
  maxIt,
  tol,
  link,
  invLink,
  meanVarFit,
  newtonRaphson,
  dispFreq,
  ...
)
```

Arguments

data	a list of data matrices with the same number of samples n in the rows. Also phyloseq objects are acceptable
distribution	a character string describing which distributional assumption should be used.
compositional	A logical indicating if the dataset should be treated as compositional
maxIt	an integer, the maximum number of iterations
tol	A small scalar, the convergence tolerance
link, invLink	link and inverse link function
meanVarFit	mean variance model
newtonRaphson	a boolean, should newton-raphson be used
dispFreq	An integer, frequency of dispersion estimation
...	passed on to the estOff() function

Value

A list with elements

rowOff	The row offsets
colOff	The column offsets
converged	A logical flag, indicating whether the fit converged
iter	An integer, the number of iterations

estLatentVars	<i>Estimate the latent variables</i>
---------------	--------------------------------------

Description

Estimate the latent variables

Usage

```
estLatentVars(latentVars, lambdasLatent, constrained, fTol, ...)
```

Arguments

latentVars	A vector of latent variables
lambdasLatent	A vector of Lagrange multipliers
constrained	A boolean, is the ordination constrained?
fTol	The convergence tolerance
...	additional arguments passed on to score and jacobian functions

Value

A vector of length n, the estimates of the latent variables

estMeanVarTrend	<i>Estimate a column-wise mean-variance trend</i>
-----------------	---

Description

Estimate a column-wise mean-variance trend

Usage

```
estMeanVarTrend(  
  data,  
  meanMat,  
  baseAbundances,  
  libSizes,  
  plot = FALSE,  
  meanVarFit,  
  degree = 2L,  
  constraint = "none",  
  ...  
)
```

Arguments

data	the data matrix with n rows
meanMat	the estimated mean matrix
baseAbundances	The baseline abundances
libSizes	Library sizes
plot	A boolean, should the trend be plotted?
meanVarFit	A character string describing the type of trend to be fitted: either "spline" or "cubic"
degree	The degree of the spline
constraint	Constraint to the spline
...	additional arguments passed on to the plot() function

Value

A list with components

meanVarTrend	An smoothed trend function, that can map a mean on a variance
meanVarTrendDeriv	A derivative function of this

estOff	<i>Estimate the row/column parameters of the independence model</i>
--------	---

Description

Estimate the row/column parameters of the independence model

Usage

```
estOff(
  data,
  distribution,
  rowOff,
  colOff,
  meanVarTrend,
  col,
  newtonRaphson,
  libSizes,
  ...
)
```

Arguments

data	a list of data matrices with the same number of samples <i>n</i> in the rows. Also phyloseq objects are acceptable
distribution	a character string describing which distributional assumption should be used.
rowOff, colOff	current row and column offset estimates
meanVarTrend	The estimated mean-variance trend
col	A logical, should column offsets be estimated
newtonRaphson	A boolean, should Newton-Raphson be used to solve the estimating equations
libSizes	The library sizes, used to evaluate the mean-variance trend
...	passed onto <code>nleqslv</code>

Value

The estimated marginal parameters

extractCoords	<i>Extract coordinates from fitted object</i>
---------------	---

Description

Extract coordinates from fitted object

Usage

```
extractCoords(modelObj, Dim)
```


Arguments

modelObj	The fitted model
Dim	the required dimensions

Value

A list with components (matrices with two columns)

latentData	The latent variables
featureData	The feature parameters
varData	The variables

Examples

```
data(Zhang)
## Not run:
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)

## End(Not run)
#Load the fits
load(system.file("extdata", "zhangFits.RData", package = "combi"))
extractCoords(microMetaboInt, Dim = c(1,2))
```

extractData	<i>Helper function to extract data matrix from phyloseq, expressionset objects etc. Also filters out all zero rows</i>
-------------	--

Description

Helper function to extract data matrix from phyloseq, expressionset objects etc. Also filters out all zero rows

Usage

```
extractData(data, logTransformGaussian = TRUE)
```

Arguments

data	The list of data objects, either matrix, phyloseq or ExpressionSet objects
logTransformGaussian	A boolean, should array data be logtransformed

Value

the raw data matrices, samples in the rows

Examples

```
data(Zhang)
matrixList = extractData(list("microbiome" = zhangMicrobio,
"metabolome" = zhangMetabo))
```

extractMat	<i>A function to extract a data matrix from a number of objects</i>
------------	---

Description

A function to extract a data matrix from a number of objects

Usage

```
extractMat(Y, ...)

## S4 method for signature 'ExpressionSet'
extractMat(Y, logTransformGaussian, ...)

## S4 method for signature 'SummarizedExperiment'
extractMat(Y, ...)

## S4 method for signature 'matrix'
extractMat(Y, ...)
```

Arguments

Y	a phyloseq or eSet object, or another object, or a raw data matrix
...	additional arguments for the extractor function
logTransformGaussian	A boolean, should array data be logtransformed

Value

A data matrix with samples in the rows and features in the columns

filterConfounders	<i>Filter out the effect of known confounders</i>
-------------------	---

Description

Filter out the effect of known confounders

Usage

```

filterConfounders(
  confMat,
  data,
  distribution,
  link,
  invLink,
  compositional,
  control,
  meanVarTrend,
  offSet,
  numVar,
  marginModel,
  biasReduction,
  allowMissingness
)

```

Arguments

confMat	A confounder design matrix
data	data matrix
distribution, link, invLink, compositional, meanVarTrend, offSet, numVar, marginModel	Characteristics of the view
control	A list of control elements to the nleqslv function
biasReduction	A boolean, should bias reduction be applied
allowMissingness	A boolean, are missing values allowed?

Value

Parameter estimates accounting for the effects of the confounders

getInflLatentVar	<i>Extract the influence on the estimation of the latent variable</i>
------------------	---

Description

Extract the influence on the estimation of the latent variable

Usage

```
getInflLatentVar(score, InvJac, i)
```

Arguments

score	The score matrix
InvJac	The inverse Jacobian
i	the sample index

Value

The influence of all observations on the i-th latent variable

gramSchmidtOrth	<i>Gram schmidt orhtogonalize a with respect to b, and normalize</i>
-----------------	--

Description

Gram schimdt orhtogonalize a with respect to b, and normalize

Usage

```
gramSchmidtOrth(a, b, weights = 1, norm = TRUE)
```

Arguments

a	the vector to be orthogonalized
b	the vector to be orthogonalized to
weights	weights vector
norm	a boolean, should the result be normalized?

Value

The orthogonalized vector

indentPlot	<i>Functions to indent the plot to include the entire labels</i>
------------	--

Description

Functions to indent the plot to include the entire labels

Usage

```
indentPlot(plt, xInd = 0, yInd = 0)
```

Arguments

plt	a ggplot object
xInd	a scalar or a vector of length 2, specifying the indentation left and right of the plot to allow for the labels to be printed entirely
yInd	a a scalar or a vector of length 2, specifying the indentation top and bottom of the plot to allow for the labels to be printed entirely

Value

a ggplot object, squared

inflPlot

*A ggplot line plot showing the influences***Description**

A ggplot line plot showing the influences

Usage

```
inflPlot(
  modelObj,
  plotType = ifelse(length(modelObj$data) <= 2, "pointplot", "boxplot"),
  pointFun = "sum",
  lineSize = 0.07,
  Dim = 1,
  samples = seq_len(nrow(if (is.null(modelObj$covariates)) modelObj$latentVars else
    modelObj$alphas)),
  ...
)
```

Arguments

modelObj	The fitted data integration
plotType	The type of plot requested, see details
pointFun	The function to calculate the summary measure to be plotted
lineSize	The line size
Dim	The dimension required
samples	index vector of which samples to be plotted
...	additional arguments passed on to the influence() function

Details

The options for plotType are: "pointPlot": Dot plot of total influence per view and sample, "boxplot": plot boxplot of influence of all observations per view and sample, "boxplotSingle": boxplot of log absolute total influence per view, "lineplot": line plot of total influence per view and sample. In the pointplot, dots crosses represent parameter estimates

Value

A ggplot object

Examples

```
data(Zhang)
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)
#Constrained
```

```

microMetaboIntConstr = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, covariates = zhangMetavars, verbose = TRUE)
load(system.file("extdata", "zhangFits.RData", package = "combi"))
inflPlot(microMetaboInt)
#Constrained
inflPlot(microMetaboIntConstr)

```

influence.combi	<i>Evaluate the influence function</i>
-----------------	--

Description

Evaluate the influence function

Usage

```

## S3 method for class 'combi'
influence(modelObj, samples = is.null(View), Dim = 1, View = NULL)

```

Arguments

modelObj	The model object
samples	A boolean, should we look at sample variables? Throws an error otherwise
Dim, View	Integers, the dimension and views required

Details

Especially the influence of the different views on the latent variable or gradient estimation may be of interest. The influence values are not all calculated. Rather, the score values and inverse jacobian are returned so they can easily be calculated

Value

A list with components

score	The evaluation of the score function
InvJac	The inverted jacobian matrix

jacConfounders

Jacobian when estimating confounder variables

Description

Jacobian when estimating confounder variables

Usage

```
jacConfounders(
  confMat,
  data,
  distribution,
  x,
  meanVarTrend,
  offSet,
  CompMat,
  libSizes,
  allowMissingness
)
```

Arguments

data, confMat, meanVarTrend
 Characteristics of the views

distribution, offSet
 distribution and offset of the view

x
 the parameter estimates

libSizes, CompMat
 Library sizes and relative abundance

allowMissingness
 a boolean, should missing values be allowed

Value

the jacobian matrix

jacConfoundersComp

Jacobian for conditioning under compositionality

Description

Jacobian for conditioning under compositionality

Usage

```
jacConfoundersComp(
  x,
  confMat,
  data,
  meanVarTrend,
  marginModel,
  allowMissingness,
  biasReduction,
  subtractMax = TRUE
)
```

Arguments

`x` the parameter estimates
`confMat`, `data`, `meanVarTrend` arguments belonging to views
`marginModel`, `biasReduction`, `subtractMax` The marginal mode, and booleans indicating bias reduction and maximum subtraction
`allowMissingness` a boolean, should missing values be allowed

Value

the jacobian matrix

<code>jacFeatures</code>	<i>Evaluate the jacobian for estimating the feature parameters for one view</i>
--------------------------	---

Description

Evaluate the jacobian for estimating the feature parameters for one view

Usage

```
jacFeatures(
  latentVars,
  data,
  distribution,
  paramEsts,
  meanVarTrend,
  offSet,
  compositional,
  indepModel,
  m,
  paramEstsLower,
  allowMissingness,
  ...
)
```


Arguments

latentVars	A vector of latent variables
data	A list of data matrices
distribution, compositional, meanVarTrend, offSet, paramEsts, paramEstsLower, indepModel	Characteristics of each view
m	dimension
allowMissingness	a boolean, are missing values allowed?
...	Additional arguments passed on to the score and jacobian functions

Value

The jacobian matrix

jacLatentVars	<i>Evaluate the jacobian for estimating the latent variable for one view</i>
---------------	--

Description

Evaluate the jacobian for estimating the latent variable for one view

Usage

```
jacLatentVars(
  latentVar,
  data,
  distribution,
  paramEsts,
  paramMats,
  offSet,
  meanVarTrend,
  n,
  varPosts,
  mm,
  indepModel,
  latentVarsLower,
  compositional,
  allowMissingness,
  ...
)
```

Arguments

latentVar	the latent variable estimates
distribution, data, varPosts, compositional, meanVarTrend, offSet, paramEsts, paramMats, indepModel	Characteristics of each view
n	the number of samples

mm	the current dimension
latentVarsLower	the lower dimensional latent variables
allowMissingness	a boolean, should missing values be allowed
...	additional arguments passed on to score and jacobian functions

Value

The diagonal of the jacobian matrix

jacLatentVarsConstr	<i>Evaluate the jacobian for estimating the latent variable for one view for constrained ordination</i>
---------------------	---

Description

Evaluate the jacobian for estimating the latent variable for one view for constrained ordination

Usage

```
jacLatentVarsConstr(
  latentVar,
  data,
  distribution,
  paramEsts,
  offSet,
  meanVarTrend,
  numCov,
  covMat,
  varPosts,
  compositional,
  mm,
  indepModel,
  latentVarsLower,
  ...
)
```

Arguments

latentVar	current latent variable estimates
distribution, compositional, meanVarTrend, offSet, paramEsts, indepModel, varPosts, data	Characteristics of each view
numCov	the number of covariates
covMat	the covariates matrix
mm	the dimension
latentVarsLower	latent variable estimates of lower dimensions
...	additional arguments passed on to score and jacobian functions

Value

The jacobian matrix

plot.combi	<i>Make multiplots of the data integration object</i>
------------	---

Description

Make multiplots of the data integration object

Usage

```
## S3 method for class 'combi'
plot(
  x,
  ...,
  Dim = c(1, 2),
  samDf = NULL,
  samShape = NULL,
  samCol = NULL,
  featurePlot = "threshold",
  featNum = 15L,
  samColValues = NULL,
  manExpFactorTaxa = 0.975,
  featSize = switch(featurePlot, threshold = 2.5, points = samSize * 0.7, density = 0.35),
  crossSize = 4,
  manExpFactorVar = 0.975,
  varNum = nrow(x$alphas),
  varSize = 2.5,
  samSize = 1.75,
  featCols = c("darkblue", "darkgreen", "grey10", "turquoise4", "blue", "green", "grey",
    "cornflowerblue", "lightgreen", "grey75"),
  strokeSize = 0.05,
  warnMonotonicity = FALSE,
  returnCoords = FALSE,
  squarePlot = TRUE,
  featAlpha = 0.5,
  featShape = 8,
  xInd = 0,
  yInd = 0,
  checkOverlap = FALSE,
  shapeValues = (21:(21 + length(unique(samDf[[samShape]]))))
)
```

Arguments

x	the fit
...	additional arguments, currently ignored
Dim	the dimensions to be plotted
samDf	a dataframe of sample variables

samShape	A variable name from samDf used to shape the samples
samCol	A variable name from samDf used to colour the samples
featurePlot	A character string, either "threshold", "points" or "density". See details
featNum, varNum	The number of features and variables to plot
samColValues	Colours for the samples
manExpFactorTaxa, manExpFactorVar	Expansion factors for taxa and variables, normally calculated natively
featSize, crossSize, varSize, samSize, strokeSize	Size parameters for the features (text, dots or density contour lines), central cross, variable labels, sample dots, sample strokes and feature contour lines
featCols	Colours for the features
warnMonotonicity	A boolean, should a warning be thrown when the feature proportions of compositional views do not all vary monotonically with all latent variables?
returnCoords	A boolean, should coordinates be returned, e.g. for use in third party software
squarePlot	A boolean, should the axes be square? Strongly recommended
featAlpha	Controls the transparency of the features
featShape	Shape of feature dots when featurePlot = "points"
xInd, yInd	x and y indentations
checkOverlap	A boolean, should overlapping labels be omitted?
shapeValues	the shapes, as numeric values

Details

It is usually impossible to plot all features with their labels. Therefore, the default option of the 'featurePlot' parameter is "threshold", whereby only the 'featNum' features furthest away from the origin are shown. Alternatively, the "points" or "density" options are available to plot all features as a point or density cloud, but without labels.

Value

A ggplot object containing the plot

Examples

```
data(Zhang)
## Not run:
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)
#Constrained
microMetaboIntConstr = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, covariates = zhangMetavars, verbose = TRUE)
## End(Not run)
#Load the fits
load(system.file("extdata", "zhangFits.RData", package = "combi"))
```

```

plot(microMetaboInt)
plot(microMetaboInt, samDf = zhangMetavars, samCol = "ABX")
#Plot all features as points or density
plot(microMetaboInt, samDf = zhangMetavars, samCol = "ABX",
     featurePlot = "points")
plot(microMetaboInt, samDf = zhangMetavars, samCol = "ABX",
     featurePlot = "density")
#Constrained
plot(microMetaboIntConstr, samDf = zhangMetavars, samCol = "ABX")

```

polyHorner	<i>Horner's method to evaluate a polynomial, copied from the polynom package. the most efficient way</i>
------------	--

Description

Horner's method to evaluate a polynomial, copied from the polynom package. the most efficient way

Usage

```
polyHorner(coefs, x)
```

Arguments

coefs	the polynomial coefficients
x	the input values for the polynomial function

Value

the evaluated polynomial

predictSpline	<i>A custom spline prediction function, extending linearly with a slope such that prediction never drops below first bisectant</i>
---------------	--

Description

A custom spline prediction function, extending linearly with a slope such that prediction never drops below first bisectant

Usage

```

predictSpline(
  fit,
  newdata,
  linX,
  coefsQuad,
  deriv = 0L,
  meanVarFit,

```

```

    minFit,
    new.knots,
    degree
  )

```

Arguments

fit	The existing spline fit
newdata	points in which the spline needs to be evaluated
linX	The x at which the fit becomes linear and intersects the diagonal line
coefsQuad	parameters of a quadratic fit
deriv	An integer. Which derivative is required?
meanVarFit	A character string, indicating which type of mean variance fit is being used
minFit	The lower bound of the cubic fit
new.knots	The knots at which the spline is to be evaluated
degree	The degree of the polynomial fit

Value

The evaluation of the spline, i.e. the predicted variance

prepareJacMat	<i>prepare the jacobian matrix</i>
---------------	------------------------------------

Description

prepare the jacobian matrix

Usage

```
prepareJacMat(mu, data, meanVarTrend, CompMat, libSizes)
```

Arguments

mu	the mean matrix
data	the count matrix
meanVarTrend	The mean variance trend
CompMat	The compisition matrix
libSizes	The library sizes

Value

the matrix which can be summed over

prepareJacMatComp	<i>prepare the jacobian for the latent variabels compostional</i>
-------------------	---

Description

prepare the jacobian for the latent variabels compostional

Usage

```
prepareJacMatComp(mu, paramEsts, CompMat0, meanVarTrend, data, libSizes)
```

Arguments

mu	the mean matrix
paramEsts	Current parameter estimates
CompMat0	The composition matrix
meanVarTrend	The mean variance trend
data	the count matrix
libSizes	The library sizesv

Value

The empty jacobian matrix with entries maximally filled out

prepareScoreMat	<i>Prepare a helper matrix for score function evaluation under quasi-likelihood</i>
-----------------	---

Description

Prepare a helper matrix for score function evaluation under quasi-likelihood

Usage

```
prepareScoreMat(data, mu, meanVarTrend, CompMat, libSizes)
```

Arguments

data	the count matrix
mu	the mean matrix
meanVarTrend	The mean variance trend
CompMat	The composition matrix
libSizes	The library sizes

Value

The helper matrix

print.combi	<i>Print an overview of a fitted combi x</i>
-------------	--

Description

Print an overview of a fitted combi x

Usage

```
## S3 method for class 'combi'
print(x, ...)
```

Arguments

x	a fitted combi x
...	Further arguments, currently ignored

Value

An overview of the number of dimensions, views and parameters, type of ordination and importance parameters

Examples

```
data(Zhang)
## Not run:
#Unconstrained
microMetaboInt = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, verbose = TRUE)
#Constrained
microMetaboIntConstr = combi(
  list("microbiome" = zhangMicrobio, "metabolomics" = zhangMetabo),
  distributions = c("quasi", "gaussian"), compositional = c(TRUE, FALSE),
  logTransformGaussian = FALSE, covariates = zhangMetavars, verbose = TRUE)
## End(Not run)
#Load the fits
load(system.file("extdata", "zhangFits.RData", package = "combi"))
print(microMetaboInt)
print(microMetaboIntConstr)
#Or simply
microMetaboInt
```

quasiJacIndep	<i>The jacobian for column offset estimation</i>
---------------	--

Description

The jacobian for column offset estimation

Usage

```
quasiJacIndep(x, data, otherMargin, meanVarTrend, col, libSizes, ...)
```

Arguments

x	the initial guess for the current margin
data	the data matrix
otherMargin	The other margin
meanVarTrend	the function describing the mean-variance trend
col	A logical, is the column being estimated?
libSizes	The library sizes
...	passed on to prepareJacMat

Value

the jacobian matrix

quasiScoreIndep	<i>Quasi score equations for column offset parameters of sequence count data</i>
-----------------	--

Description

Quasi score equations for column offset parameters of sequence count data

Usage

```
quasiScoreIndep(x, data, otherMargin, meanVarTrend, col, libSizes, ...)
```

Arguments

x	the initial guess for the current margin
data	the data matrix
otherMargin	The other margin
meanVarTrend	the function describing the mean-variance trend
col	A logical, is the column being estimated?
libSizes	The library sizes
...	passed on to prepareJacMat

Value

the evaluated estimating equation

rowMultiply	<i>A function to efficiently row multiply a matrix and a vector</i>
-------------	---

Description

A function to efficiently row multiply a matrix and a vector

Usage

```
rowMultiply(matrix, vector)
```

Arguments

matrix	a numeric matrix of dimension a-by-b
vector	a numeric vector of length b t(t(matrix)*vector) but then faster

Details

Memory intensive but that does not matter with given matrix sizes

Value

a matrix, row multiplied by the vector

scaleCoords	<i>A helper function to rescale coordinates</i>
-------------	---

Description

A helper function to rescale coordinates

Usage

```
scaleCoords(featsCoords, latentData, manExpFactorTaxa, featNum = NULL)
```

Arguments

featsCoords	the feature coordinates to be rescaled
latentData	latent variables
manExpFactorTaxa	an expansion factor
featNum	the number of features to retain

Value

The rescaled feature coordinates

scoreConfounders	<i>Score functions for confounder variables</i>
------------------	---

Description

Score functions for confounder variables

Usage

```
scoreConfounders(  
  x,  
  data,  
  distribution,  
  offSet,  
  confMat,  
  meanVarTrend,  
  allowMissingness,  
  libSizes,  
  CompMat  
)
```

Arguments

x the parameter estimates
data, distribution, offSet, confMat, meanVarTrend
 Characteristics of the views
allowMissingness
 a boolean, should missing values be allowed
libSizes, CompMat
 Library sizes and relative abundance

Value

The evaluation of the estimating equations

scoreConfoundersComp	<i>Score equations for conditioning under compositionality</i>
----------------------	--

Description

Score equations for conditioning under compositionality

Usage

```
scoreConfoundersComp(
  x,
  confMat,
  data,
  meanVarTrend,
  marginModel,
  biasReduction,
  allowMissingness,
  subtractMax = TRUE
)
```

Arguments

x	Confounder parameter estimates
confMat	confounder matrix
data	data
meanVarTrend	mean variance trend
marginModel	marginal models
biasReduction	A boolean, should a bias reduced estimation be applied?
allowMissingness	A boolean, are missing values allowed
subtractMax	A boolean, should the maximum be subtracted before softmax transformation? Recommended for numerical stability

Value

The evaluation of the estimating equations

scoreFeatureParams	<i>Evaluate the score functions for the estimation of the feature parameters for a single dataset</i>
--------------------	---

Description

Evaluate the score functions for the estimation of the feature parameters for a single dataset

Usage

```
scoreFeatureParams(
  x,
  data,
  distribution,
  offSet,
  latentVar,
  meanVarTrend,
  mm,
  indepModel,
  compositional,
```

```

    paramEstsLower,
    allowMissingness,
    ...
)

```

Arguments

x	the parameter estimates
data, distribution, offSet, meanVarTrend, indepModel, compositional, paramEstsLower	Characteristics of the views
latentVar	the latent variables
mm	the dimension
allowMissingness	a boolean, should missing values be allowed
...	Additional arguments passed on to the score and jacobian functions

Value

A vector with evaluated score function

scoreLatentVars	<i>Evaluate the score functions for the estimation of the latent variables for a single dataset</i>
-----------------	---

Description

Evaluate the score functions for the estimation of the latent variables for a single dataset

Usage

```

scoreLatentVars(
  data,
  distribution,
  paramEsts,
  paramMats,
  offSet,
  latentVar,
  meanVarTrend,
  constrained = FALSE,
  covMat = NULL,
  varPosts,
  compositional,
  indepModel,
  mm,
  latentVarsLower,
  allowMissingness,
  ...
)

```

Arguments

data, distribution, offSet, meanVarTrend, indepModel, varPosts,
 paramEsts, paramMats, compositional
 Characteristics of the views

latentVar the latent variable estimates

constrained a boolean, is this a constrained analysis

covMat a matrix of constraining covariates

mm the current dimension

latentVarsLower
 the lower dimensional latent variables

allowMissingness
 a boolean, should missing values be allowed

... additional arguments passed on to score and jacobian functions

Value

A vector of length n, with evaluated score function

 seqM

A small auxiliary function for the indices of the lagrange multipliers

Description

A small auxiliary function for the indices of the lagrange multipliers

Usage

```
seqM(y, normal = TRUE, nLambda1s = 1)
```

Arguments

y an integer, the current dimension

normal a logical, is there a normalization restriction?

nLambda1s the number of centering restrictions

Value

a vector containing the ranks of the current lagrangian multipliers

trimOnConfounders	<i>Trim based on confounders to avoid taxa with only zero counts</i>
-------------------	--

Description

Trim based on confounders to avoid taxa with only zero counts

Usage

```
trimOnConfounders(confounders, data, prevCutOff, minFraction, n)
```

Arguments

confounders	a n x t confounder matrix
data	the data matrix
prevCutOff	a scalar between 0 and 1, the prevalence cut off
minFraction	a scalar between 0 and 1, each taxon's total abundance should equal at least the number of samples n times minFraction, otherwise it is trimmed
n	the number of samples

Should be called prior to fitting the independence model

Value

A trimmed data matrix n x p'

zhangMetabo	<i>Metabolomes of mice that underwent Pulsed Antibiotic Treatment (PAT) and controls</i>
-------------	--

Description

Metabolome of mice that underwent Pulsed Antibiotic Treatment (PAT) and controls

Usage

```
data(Zhang)
```

Format

SummarizedExperiment with metabolome data

zhangMetabo The metabolome data as a SummarizedExperiment object

Source

<https://www.ibdmdb.org/>

zhangMetavars	<i>Baseline sample variables of PAT and control mice</i>
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Description

Baseline covariates of PAT mice and healthy controls

Usage

`data(Zhang)`

Format

A dataframe with baseline sample variables

zhangMetavars The metadata on the mice

Source

<https://www.ibdmdb.org/>

zhangMicrobio	<i>Microbiomes of mice that underwent Pulsed Antibiotic Treatment (PAT) and controls</i>
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Description

Microbiome of mice that underwent Pulsed Antibiotic Treatment (PAT) and controls. The data were extracted from the source <https://www.ibdmdb.org/>, and then only the samples matching between microbiome and metabolome were retained.

Usage

`data(Zhang)`

Format

A phyloseq object containing microbiome data

zhangMicrobio The microbiome dataset pruned for matches with the metabolome object

Source

<https://www.ibdmdb.org/>

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