

Package ‘reconsi’

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Type Package

Title Resampling Collapsed Null Distributions for Simultaneous Inference

Version 1.21.0

Description Improves simultaneous inference under dependence of tests by estimating a collapsed null distribution through resampling. Accounting for the dependence between tests increases the power while reducing the variability of the false discovery proportion. This dependence is common in genomics applications, e.g. when combining flow cytometry measurements with microbiome sequence counts.

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Imports phyloseq, ks, reshape2, ggplot2, stats, methods, graphics, grDevices, matrixStats, Matrix

Suggests knitr, rmarkdown, testthat

VignetteBuilder knitr

biocViews Metagenomics, Microbiome, MultipleComparison, FlowCytometry

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binStats

Bin the test statistic into equally sized bins

Description

Bin the test statistic into equally sized bins

Usage

```
binStats(z, nBins = 82L, binEdges = c(-4.1, 4.1))
```

Arguments

z	the matrix of permuted test statistics
nBins	an integer, the number of bins
binEdges	A vector of length 2 with the outer bin edges

Value

Matrix of binned test statistics

calcWeights	<i>Obtain weights as posterior probabilities to calculate the consensus null</i>
-------------	--

Description

Obtain weights as posterior probabilities to calculate the consensus null

Usage

```
calcWeights(logDensPerm, fdr)
```

Arguments

logDensPerm	A matrix with B rows of logged density estimates of the B permutation distributions, and p columns for the p observed test statistics
fdr	A vector of local false discovery rates for the observed tests statistics of length p

Value

A vector of weights of length B

estNormal	<i>Fast estimation of mean and standard deviation of a normal distribution, optionally with weights</i>
-----------	---

Description

Fast estimation of mean and standard deviation of a normal distribution, optionally with weights

Usage

```
estNormal(y, w = NULL, p = length(y))
```

Arguments

y	vector of observations
w	optional weight vector
p	The number of features

Value

A vector of length 2 with mean and standard deviation

estP0	<i>Estimate the fraction of true null hypotheses.</i>
-------	---

Description

Estimate the fraction of true null hypotheses.

Usage

```
estP0(statObs, fitAll, z0quantRange, smooth.df, evalVal, assumeNormal)
```

Arguments

statObs	A vector of observed z-values
fitAll	the estimated normal null
z0quantRange	a number of quantiles between 0 and 0.5
smooth.df	degrees of freedom for the spline smoother
evalVal	the value of q at which to evaluate the spline
assumeNormal	A boolean, should normality be assumed for the null distribution?

Details

A natural spline is used over a range of intervals. Based on the `qvalue::qvalue()` function and Storey and Tibshirani, 2003

Value

The estimated null fraction, the value of the spline evaluated at the first element of `z0quantRange`

getApproxCovar	<i>Obtain a null covariance matrix of binned test statistics</i>
----------------	--

Description

Obtain a null covariance matrix of binned test statistics

Usage

```
getApproxCovar(statsPerm, ...)
```

Arguments

statsPerm	The <code>pxB</code> matrix of permutation z-values in the columns
...	passed on to <code>binStats</code>

Value

The covariance matrix of binned z-values

Note

This is not the covariance matrix of the p test statistic, nor of the data! It is an approximate covariance matrix of binned test statistics for visualization and diagnostic purposes.

Examples

```
p = 200; n = 50; B = 5e1
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x), n, p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5), n, p*9/10) #Non DA
)
mat = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
corMat = getApproxCovar(fdrRes$statsPerm)
```

getC1prop	<i>Find the dependence pat C1 of the approximate covariance matrix, and extract the ratio of the first eigenvalue to the sum of all positive eigenvalues</i>
-----------	--

Description

Find the dependence pat C1 of the approximate covariance matrix, and extract the ratio of the first eigenvalue to the sum of all positive eigenvalues

Usage

```
getC1prop(statsPerm, numEig = 1, ...)
```

Arguments

statsPerm	Matrix of permuted test statistics
numEig	An integer, number of first eigenvalues
...	passed onto binStats

Value

A proportion indicating the ratio of the first eigenvalues to the sum of all eigenvalues

Examples

```
p = 200; n = 50; B = 5e1
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x), n, p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5), n, p*9/10) #Non DA
)
mat = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
getC1prop(fdrRes$statsPerm)
```

getFdr	<i>Calculate tail-area (Fdr) and local (fdr) false discovery rates, based on a certain null distribution</i>
--------	--

Description

Calculate tail-area (Fdr) and local (fdr) false discovery rates, based on a certain null distribution

Usage

```
getFdr(
  statObs,
  fitAll,
  fdr,
  p,
  p0,
  zValsDensObs,
  smoothObs,
  assumeNormal,
  fitObs,
  ...
)
```

Arguments

statObs	Vector of observed z-values
fitAll	The parameters of the estimated random null
fdr	local false discovery rate, already estimated
p	the number of hypotheses
p0	The estimated fraction of null hypotheses
zValsDensObs	estimated densities of observed test statistics
smoothObs	A boolean, should estimated observed densities of the test statistics be used in estimating the Fdr
assumeNormal	A boolean, should normality be assumed for the null distribution?
fitObs	The kernel density estimate object of all test statistics
...	more arguments, ignored

Value

A list with components

Fdr	Tail are false discovery rate
fdr	Local false discovery rate

getG0	<i>Obtain the consensus null</i>
-------	----------------------------------

Description

Obtain the consensus null

Usage

```
getG0(
  statObs,
  statsPerm,
  z0Quant,
  gridsize,
  maxIter,
  tol,
  estP0args,
  testPargs,
  B,
  p,
  pi0,
  assumeNormal,
  resamAssumeNormal
)
```

Arguments

statObs	A vector of length p with observed test statistics
statsPerm	A pxB matrix with permutation z-values
z0Quant	a vector of length of quantiles defining the central part R of the distribution. If a single number is supplied, then (z0quant, 1-z0quant) will be used
gridsize	An integer, the gridsize for the density estimation
maxIter	An integer, the maximum number of iterations in determining R
tol	The convergence tolerance.
estP0args	A list of arguments passed on to the estP0args() function
testPargs	A list of arguments passed on to quantileFun
B	an integer, the number of permutations
p	an integer, the number of hypotheses
pi0	A known fraction of true null hypotheses
assumeNormal	A boolean, should normality be assumed for the null distribution?
resamAssumeNormal	A boolean, should normality be assumed for resampling dists

Value

A list with following entries

PermDensFits	The permutation density fits
zSeq	The support of the kernel for density estimation
zValsDensObs	The estimated densities of the observed z-values
convergence	A boolean, has the algorithm converged?
weights	Vector of length B with weights for the permutation distributions
fdr	Estimated local false discovery rate along the support of the kernel
p0	The estimated fraction of true null hypotheses
iter	The number of iterations
fitAll	The consensus null fit

getTestStats	<i>A function to calculate observed and permutation z-statistics on a n-by-p matrix of observations</i>
--------------	---

Description

A function to calculate observed and permutation z-statistics on a n-by-p matrix of observations

Usage

```
getTestStats(
  Y,
  center,
  test = "wilcox.test",
  x,
  B,
  argList,
  tieBreakRan,
  replace,
  scale
)
```

Arguments

Y	The nxp data matrix
center	a boolean, should data be centered prior to permutation
test	A function name, possibly user defined. See details.
x	A vector defining the groups. Will be coerced to factor.
B	an integer, the number of permutations
argList	A list of further arguments passed on to the test function
tieBreakRan	A boolean, should ties of permutation test statistics be broken randomly? If not, midranks are used
replace	A boolean. If FALSE, samples are permuted (resampled without replacement), if TRUE the samples are bootstrapped (resampled with replacement)
scale	a boolean, should data be scaled prior to resampling

Details

For test "wilcox.test" and "t.test", fast custom implementations are used. Other functions can be supplied but must accept a y outcome variable, a x as grouping variable, and possibly a list of other arguments. It must return all arguments needed to evaluate its quantile function if z-values are to be used.

Value

A list with components

statObs	A vector of length p of observed test statistics
statsPerm	A p-by-B matrix of permutation test statistics
resamDesign	The resampling design

getTstat	<i>A function to obtain a t-test statistic efficiently. For internal use only</i>
----------	---

Description

A function to obtain a t-test statistic efficiently. For internal use only

Usage

```
getTstat(y1, y2, mm, nn)
```

Arguments

y1, y2	vectors of observed values in the two groups
mm, nn	number of observations in the corresponding groups

Value

A list with items

tstat	The t-test statistic
df	The degrees of freedom (Welch approximation)

plotApproxCovar	<i>Plot an approximation of the correlation structure of the test statistics</i>
-----------------	--

Description

Plot an approximation of the correlation structure of the test statistics

Usage

```
plotApproxCovar(
  reconsiFit,
  col = colorRampPalette(c("yellow", "blue"))(12),
  x = seq(-4.2, 4.2, 0.1),
  y = seq(-4.2, 4.2, 0.1),
  xlab = "Z-values",
  ylab = "Z-values",
  nBins = 82L,
  binEdges = c(-4.1, 4.1),
  ...
)
```

Arguments

`reconsiFit` The reconsi fit

`col, x, y, xlab, ylab, ...`
 A list of arguments for the `image()` function.

`nBins, binEdges` passed on to the `getApproxCovar` function

Details

By default, yellow indicates negative correlaton between bin counts, blue positive correlation

Value

`invisible()`

Note

This is not the covariance matrix of the p test statistic, nor of the data! It is an approximate covariance matrix of binned test statistics for visualization purposes. See [plotCovar](#) for the full covariance matrix.

See Also

[plotCovar](#), [getApproxCovar](#)

Examples

```

p = 200; n = 50; B = 5e1
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x), n, p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5), n, p*9/10) #Non DA
)
mat = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
plotApproxCovar(fdrRes)

```

plotCovar	<i>Plot an the covariance matrix of the test statistics estimated through permutations</i>
-----------	--

Description

Plot an the covariance matrix of the test statistics estimated through permutations

Usage

```

plotCovar(
  reconsiFit,
  col = colorRampPalette(c("yellow", "blue"))(12),
  xlab = "Test statistic index",
  ylab = xlab,
  ...
)

```

Arguments

reconsiFit The reconsi fit
 col, xlab, ylab, ... A list of arguments for the image() function.

Details

By default, yellow indicates negative correlaton between test statistics, blue positive correlation

Value

invisible()

Note

Note the difference with the plotApproxCovar function, where the covariances between binned test statistics are shown to get an idea between covariances between tail and center values of the univariate null distribution. Here the covariance matrix between all test statistics is shown

See Also

[plotApproxCovar](#)

Examples

```

p = 200; n = 50; B = 5e1
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x),n,p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5),n,p*9/10) #Non DA
)
mat = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
plotCovar(fdrRes)

```

plotNull

Plot the obtained null distribution along with a histogram of observed test statistics

Description

Plot the obtained null distribution along with a histogram of observed test statistics

Usage

```

plotNull(
  fit,
  lowColor = "yellow",
  highColor = "blue",
  idNull = NULL,
  nResampleCurves = length(fit$Weights),
  hSize = 0.5
)

```

Arguments

fit	an object returned by the reconsi() (or testDAA()) function
lowColor, highColor	The low and high ends of the colour scale
idNull	indices of known null taxa
nResampleCurves	The number of resampling null distributions to plot
hSize	A double, the size of the line of the collapsed null estimate

Value

a ggplot2 plot object

Examples

```

p = 180; n = 50; B = 1e2
#Low number of resamples keeps computation time down
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x),n,p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5),n,p*9/10) #Non DA
)

```

```

)
#Provide just the matrix and grouping factor, and test using the random null
fdrRes = reconsi(mat, x, B = B)
plotNull(fdrRes)

```

ptEdit	<i>A custom function to calculate the distribution function of the t-test statistic. For internal use only</i>
--------	--

Description

A custom function to calculate the distribution function of the t-test statistic. For internal use only

Usage

```
ptEdit(q)
```

Arguments

q a vector with t-statistic and degrees of freedom

Value

A value between 0 and 1, the evaluation of the cdf

qtEdit	<i>A custom function to calculate the quantile function of the t-test statistic. For internal use only</i>
--------	--

Description

A custom function to calculate the quantile function of the t-test statistic. For internal use only

Usage

```
qtEdit(p)
```

Arguments

p a vector with quantile and degrees of freedom

Value

the corresponding quantile

quantCorrect	<i>Correct quantiles by not returning 0 or 1</i>
--------------	--

Description

Correct quantiles by not returning 0 or 1

Usage

```
quantCorrect(quant)
```

Arguments

quant	A vector of quantiles
-------	-----------------------

Value

The same vector of quantiles but without 0 or 1 values

reconsi	<i>Perform simultaneous inference through collapsed resampling null distributions</i>
---------	---

Description

Perform simultaneous inference through collapsed resampling null distributions

Usage

```
reconsi(
  Y,
  x = NULL,
  B = 1000L,
  test = "wilcox.test",
  argList = list(),
  distFun = "pnorm",
  zValues = TRUE,
  testPargs = list(),
  z0Quant = 0.25,
  gridsize = 801L,
  maxIter = 100L,
  tol = 1e-06,
  zVals = NULL,
  center = FALSE,
  replace = is.null(x),
  assumeNormal = TRUE,
  estP0args = list(z0quantRange = seq(0.05, 0.45, 0.0125), smooth.df = 3, evalVal = 0.05),
  resamZvals = FALSE,
  smoothObs = TRUE,
  scale = FALSE,
```

```

    tieBreakRan = FALSE,
    pi0 = NULL,
    resamAssumeNormal = TRUE
  )

```

Arguments

<code>Y</code>	the matrix of sequencing counts
<code>x</code>	a grouping factor. If provided, this grouping factor is permuted. Otherwise a bootstrap procedure is performed
<code>B</code>	the number of resampling instances
<code>test</code>	Character string, giving the name of the function to test for differential absolute abundance. Must accept the formula interface. Features with tests resulting in observed NA test statistics will be discarded
<code>argList</code>	A list of arguments, passed on to the testing function
<code>distFun</code>	the distribution function of the test statistic, or its name. Must at least accept an argument named 'q', 'p' and 'x' respectively.
<code>zValues</code>	A boolean, should test statistics be converted to z-values. See details
<code>testPargs</code>	A list of arguments passed on to <code>distFun</code>
<code>z0Quant</code>	A vector of length 2 of quantiles of the null distribution, in between which only null values are expected
<code>gridsize</code>	The number of bins for the kernel density estimates
<code>maxIter</code>	An integer, the maximum number of iterations in the estimation of the null distribution
<code>tol</code>	The tolerance for the infinity norm of the central borders in the iterative procedure
<code>zVals</code>	An optional list of observed (<code>statObs</code>) and resampling (<code>statsPerm</code>) z-values. If supplied, the calculation of the observed and resampling test statistics is skipped and the algorithm proceeds with calculation of the consensus null distribution
<code>center</code>	A boolean, should observations be centered in each group prior to permutations? See details.
<code>replace</code>	A boolean. Should resampling occur with replacement (bootstrap) or without replacement (permutation)
<code>assumeNormal</code>	A boolean, should normality be assumed for the null distribution?
<code>estP0args</code>	A list of arguments passed on to the <code>estP0</code> function
<code>resamZvals</code>	A boolean, should resampling rather than theoretical null distributions be used?
<code>smoothObs</code>	A boolean, should the fitted rather than estimated observed distribution be used in the Fdr calculation?
<code>scale</code>	a boolean, should data be scaled prior to resampling
<code>tieBreakRan</code>	A boolean, should ties of resampling test statistics be broken randomly? If not, midranks are used
<code>pi0</code>	A known fraction of true null hypotheses. If provided, the fraction of true null hypotheses will not be estimated. Mainly for oracle purposes.
<code>resamAssumeNormal</code>	A boolean, should normality be assumed for resampling dists

Details

Efron (2007) centers the observations in each group prior to permutation. As permutations will remove any genuine group differences anyway, we skip this step by default. If `zValues = FALSE`, the density is fitted on the original test statistics rather than converted to z-values. This unlocks the procedure for test statistics with unknown distributions, but may be numerically less stable.

Value

A list with entries

<code>statsPerm</code>	Resampling Z-values
<code>statObs</code>	Observed Z-values
<code>distFun</code>	Density, distribution and quantile function as given
<code>testPargs</code>	Same as given
<code>zValues</code>	A boolean, were z-values used?
<code>resamZvals</code>	A boolean, were the resampling null distribution used?
<code>cdfValObs</code>	Cumulative distribution function evaluation of observed test statistics
<code>p0estimated</code>	A boolean, was the fraction of true null hypotheses estimated from the data?
<code>Fdr, fdr</code>	Estimates of tail-area and local false discovery rates
<code>p0</code>	Estimated or supplied fraction of true null hypotheses
<code>iter</code>	Number of iterations executed
<code>fitAll</code>	Mean and standard deviation estimated collapsed null
<code>PermDensFits</code>	Mean and standard deviations of resamples
<code>convergence</code>	A boolean, did the iterative algorithm converge?
<code>zSeq</code>	Basis for the evaluation of the densities
<code>weights</code>	weights of the resampling distributions
<code>zValsDensObs</code>	Estimated overall densities, evaluated in <code>zSeq</code>

Note

Ideally, it would be better to only use unique resamples, to avoid unnecessary replicated calculations of the same test statistics. Yet this issue is almost always ignored in practice; as the sample size grows it also becomes irrelevant. Notice also that this would require to place weights in case of the bootstrap, as some bootstrap samples are more likely than others.

Examples

```
#Important notice: low number of resamples B necessary to keep
# computation time down, but not recommended. Pray set B at 200 or higher.
p = 60; n = 20; B = 5e1
x = rep(c(0,1), each = n/2)
mat = cbind(
  matrix(rnorm(n*p/10, mean = 5+x), n, p/10), #DA
  matrix(rnorm(n*p*9/10, mean = 5), n, p*9/10) #Non DA
)
fdrRes = reconsi(mat, x, B = B)
fdrRes$p0
#Indeed close to 0.9
estFdr = fdrRes$Fdr
```

```

#The estimated tail-area false discovery rates.

#With another type of test. Need to supply quantile function in this case
fdrResLm = reconsi(mat, x, B = B,
test = function(x, y){
fit = lm(y~x)
c(summary(fit)$coef["x","t value"], fit$df.residual)},
distFun = function(q){pt(q = q[1], df = q[2])})

#With a test statistic without known null distribution(for small samples)
fdrResKruskal = reconsi(mat, x, B = B,
test = function(x, y){
kruskal.test(y~x)$statistic}, zValues = FALSE)

#Provide an additional covariate through the 'argList' argument
z = rpois(n , lambda = 2)
fdrResLmZ = reconsi(mat, x, B = B,
test = function(x, y, z){
fit = lm(y~x+z)
c(summary(fit)$coef["x","t value"], fit$df.residual)},
distFun = function(q){pt(q = q[1], df = q[2])},
argList = list(z = z))

#When nog grouping variable is provided, a bootstrap is performed
matBoot = cbind(
matrix(rnorm(n*p/10, mean = 1), n, p/10), #DA
matrix(rnorm(n*p*9/10, mean = 0), n, p*9/10) #Non DA
)
fdrResBoot = reconsi(matBoot, B = B,
test = function(y, x){testRes = t.test(y, mu = 0, var.equal = TRUE);
c(testRes$statistic, testRes$parameter)},
distFun = function(q){pt(q = q[1], df = q[2])},
center = TRUE, replace = TRUE)

```

rowMultiply	<i>A function to efficiently row multiply a a-by-b matrix by a vector of length b. More memory intensive but that does not matter with given matrix sizes</i>
-------------	---

Description

A function to efficiently row multiply a a-by-b matrix by a vector of length b. More memory intensive but that does not matter with given matrix sizes

Usage

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

Arguments

matrix	a numeric matrix of dimension a-by-b
vector	a numeric vector of length b

Details

`t(t(matrix)*vector)` but then faster

Value

a matrix, row multiplied by the vector

<code>stabExp</code>	<i>A function to numerically stabilize an exponentiation. For internal use only</i>
----------------------	---

Description

A function to numerically stabilize an exponentiation. For internal use only

Usage

```
stabExp(exps)
```

Arguments

`exps` the vector to be exponentiated

Value

the vector with the maximum subtracted

<code>testDAA</code>	<i>A function to test for differential absolute abundance on a phyloseq object</i>
----------------------	--

Description

A function to test for differential absolute abundance on a phyloseq object

Usage

```
testDAA(Y, ...)
```

S4 method for signature 'phyloseq'

```
testDAA(Y, groupName, FCname, ...)
```

S4 method for signature 'matrix'

```
testDAA(Y, FC, x, S = rowSums(Y), tieBreakRan = TRUE, assumeNormal = TRUE, ...)
```

Arguments

Y	A phyloseq object, or a data matrix with samples in the rows and OTUs in the columns
...	passed on to the reconsi() function
groupName	A character string, the name of a variable in physeq indicating the grouping factor
FCname	A character string, the name of a variable in physeq containing the total cell count
FC	a vector of length n with total flow cytometry cell counts
x	a grouping factor of length n
S	a vector of library sizes. Will be calculated if not provided
tieBreakRan	A boolean, should ties be broken at random.
assumeNormal	A boolean, should normality be assumed for the null distribution?

Value

See the reconsi() function

Examples

```
#Test for phyloseq object
library(phyloseq)
data("VandeputteData")
VandeputtePruned = prune_samples(Vandeputte,
samples = sample_names(Vandeputte)[20:40])
testVanDePutte = testDAA(VandeputtePruned, "Health.status", "absCountFrozen",
B = 15)
#Test for matrix
testMat = testDAA(as(otu_table(VandeputtePruned), "matrix"),
get_variable(VandeputtePruned, "Health.status"),
get_variable(VandeputtePruned, "absCountFrozen"), B = 15)
```

Vandeputte

Microbiomes of Crohn's disease patients and healthy controls

Description

Microbiome sequencing data of Crohn's disease patients, and healthy controls, together with other baseline covariates. Both sequencing and flow cytometry data are available.

Usage

Vandeputte

Format

A phyloseq object with an OTU-table and sample data

otu_table Count data matrix of 234 taxa in 135 samples

sample_data Data frame of patient covariates

Source

<https://www.ncbi.nlm.nih.gov/pubmed/29143816>

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