Package 'reconsi'

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Type Package

Title Resampling Collapsed Null Distributions for Simultaneous Inference

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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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LazyData true

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Imports phyloseq, KernSmooth, reshape2, ggplot2, stats, methods, graphics, grDevices, matrixStats

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`calcWeights` *Obtain weights as posterior probabilities to calculate the consensus null*

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 — *Fast estimation of mean and standard deviation of a normal distrbution, optionally with weights*

Description

Fast estimation of mean and standard deviation of a normal distrbution, 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 — *Estimate the fraction of true null hypotheses.*

Description

Estimate the fraction of true null hypotheses.

Usage

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

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

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` *Obtain a null covariance matrix of binned test statistics*

Description

Obtain a null covariance matrix of binned test statistics

Usage

```
getApproxCovar(statsPerm, nBins = 82L, binEdges = c(-4.1, 4.1))
```

Arguments

`statsPerm`	The pxB matrix of permutation z-values in the columns
`nBins`	an integer, the number of bins
`binEdges`	A vector of length 2 with the outer bin edges

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 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 = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
corMat = getApproxCovar(fdrRes$statsPerm)
```

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

Description

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

Usage

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

Arguments

`statObs`	Vector of observed z-values
`fitAll`	The parameters of the estimated random null
`fdr`	local false discovery rate, already estimated
`zSeq`	Support of the density estimation
`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
`...`	more arguments, ignored

Value

A list with components

`Fdr`	Tail are false discovery rate
`fdr`	Local false discovery rate

`getG0` *Obtain the consensus null*

Description

Obtain the consensus null

Usage

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

Arguments

statObs	A vector of lenght p with observed test statistics
statsPerm	A pxB matrix with permuation 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.

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

Description

A function to calculate observed and permuation 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 permuation
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 permuations
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` *A function to obtain a t-test statistic efficiently. For internal use only*

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 obsereved 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` *Plot an approximatio of the correlation structure of the test statistics*

Description

Plot an approximatio 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.

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 = mat + rnorm(n, sd = 0.3) #Introduce some dependence
fdrRes = reconsi(mat, x, B = B)
plotApproxCovar(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",
  idDA = 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
idDA	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 = 175; 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
 )
fdrRes = reconsi(mat, x, B = B)
plotNull(fdrRes)
```

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

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

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 *Correct quantiles by not returning 0 or 1*

Description

Correct quantiles by not returning 0 or 1

Usage

```
quantCorrect(quants)
```

Arguments

quants	A vector of quantiles

Value

The same vector of quantiles but without 0 or 1 values

reconsi *Perform simultaneous inference through collapsed resampling null distributions*

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 = pnorm(c(-1, 1)),
  gridsize = 801L,
  maxIter = 1000L,
  tol = 1e-08,
  center = FALSE,
  replace = is.null(x),
  zVals = NULL,
  estP0args = list(z0quantRange = seq(0.05, 0.45, 0.0125), smooth.df = 3),
```

```
  resamZvals = FALSE,
  smoothObs = TRUE,
  scale = FALSE,
  tieBreakRan = FALSE,
  pi0 = NULL
)
```

Arguments

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

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

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

Note

Ideally, it would be better to only use unique resamples, to avoid unnecesarry replicated calculations of the same test statistics. Yet this issue is almost alwyas 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 = 50; 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` *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*

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 multplied by the vector

---

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

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

---

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

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, ...)
```

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.

Value

See the reconsi() function

Examples

```
#Test for phyloseq object
library(phyloseq)
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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