

Package ‘MetaLonDA’

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Type Package

Title Metagenomics Longitudinal Differential Abundance Method

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URL <https://github.com/aametwally/MetaLonDA>

BugReports <https://github.com/aametwally/MetaLonDA/issues>

Description Identify time intervals of differentially abundant metagenomic features in longitudinal studies (Metwally AA, et al., Microbiome, 2018 <doi:10.1186/s40168-018-0402-y>).

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Depends R(>= 3.5.0)

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areaPermutation	<i>Calculate Area Ratio (AR) of each feature's time interval for all permutations</i>
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Description

Fits longitudinal samples from the same group using negative binomial or LOWESS for all permutations

Usage

```
areaPermutation(perm)
```

Arguments

perm list has all the permuted models

Value

returns a list of all permutation area ratio

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```

data(metalonda_test_data)
n.sample = 5 # sample size;
n.timepoints = 10 # time point;
n.perm = 3
n.group= 2 # number of group;
Group = factor(c(rep(0,n.sample*n.timepoints), rep(1,n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
aggretage.df = data.frame(Count = metalonda_test_data[,], Time = Time, Group = Group, ID = ID)
perm = permutation(aggretage.df, n.perm = 3, method = "nbinomial", points)
areaPermutation(perm)

```

curveFitting

Fit longitudinal data

Description

Fits longitudinal samples from the same group using negative binomial smoothing splines or LOWESS

Usage

```
curveFitting(df, method = "nbinomial", points)
```

Arguments

df	dataframe has the Count, Group, ID, Time
method	fitting method (nbinomial, lowess)
points	points at which the prediction should happen

Value

returns the fitted model

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```

data(metalonda_test_data)
n.sample = 5
n.timepoints = 10
n.group = 2
Group = factor(c(rep(0, n.sample*n.timepoints), rep(1, n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))

```

```

points = seq(1, 10, length.out = 10)
aggretage.df = data.frame(Count = metalonda_test_data[,1], Time = Time, Group = Group, ID = ID)
cf = curveFitting(df = aggretage.df, method= "nbinomial", points)

```

<code>findSigInterval</code>	<i>Find significant time intervals</i>
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Description

Identify significant time intervals

Usage

```
findSigInterval(adjusted.pvalue, threshold = 0.05, sign)
```

Arguments

<code>adjusted.pvalue</code>	vector of the adjusted p-value
<code>threshold</code>	p-value cut off
<code>sign</code>	vector hold area sign of each time interval

Value

returns a list of the start and end points of all significant time intervals

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```

p = c(0.04, 0.01, 0.02, 0.04, 0.06, 0.2, 0.06, 0.04)
sign = c(1, 1, 1, 1, -1, -1, 1, 1)
findSigInterval(p, threshold = 0.05, sign)

```

intervalArea	<i>Calculate Area Ratio (AR) of each feature's time interval</i>
--------------	--

Description

Calculate Area Ratio (AR) of each feature's time interval

Usage

```
intervalArea(curve.fit.df)
```

Arguments

curve.fit.df gss data object of the fitted spline

Value

returns the area ratio for all time intervals

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```
data(metalonda_test_data)
n.sample = 5
n.timepoints = 10
n.group= 2
Group = factor(c(rep(0,n.sample*n.timepoints), rep(1,n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
aggregate.df = data.frame(Count = metalonda_test_data[1,], Time = Time, Group = Group, ID = ID)
model = curveFitting(df = aggregate.df, method= "nbinomial", points)
intervalArea(model)
```

metalonda	<i>Metagenomic Longitudinal Differential Abundance Analysis for one feature</i>
-----------	---

Description

Find significant time intervals of the one feature

Usage

```

metalonda(
  Count,
  Time,
  Group,
  ID,
  n.perm = 500,
  fit.method = "nbinomial",
  points,
  text = 0,
  parall = FALSE,
  pvalue.threshold = 0.05,
  adjust.method = "BH",
  time.unit = "days",
  ylabel = "Normalized Count",
  col = c("blue", "firebrick"),
  prefix = "Test"
)

```

Arguments

Count	matrix has the number of reads that mapped to each feature in each sample.
Time	vector of the time label of each sample.
Group	vector of the group label of each sample.
ID	vector of the subject ID label of each sample.
n.perm	number of permutations.
fit.method	fitting method (nbinomial, lowess).
points	points at which the prediction should happen.
text	Feature's name.
parall	boolean to indicate whether to use multicore.
pvalue.threshold	p-value threshold cutoff for identifying significant time intervals.
adjust.method	multiple testing correction method.
time.unit	time unit used in the Time vector (hours, days, weeks, months, etc.)
ylabel	text to be shown on the y-axis of all generated figures (default: "Normalized Count")
col	two color to be used for the two groups (eg., c("red", "blue")).
prefix	prefix to be used to create directory for the analysis results

Value

returns a list of the significant time intervals for the tested feature.

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```
data(metalonda_test_data)
n.sample = 5
n.timepoints = 10
n.group = 2
Group = factor(c(rep(0, n.sample*n.timepoints), rep(1,n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
## Not run:
output.nbinomial = metalonda(Count = metalonda_test_data[1,], Time = Time, Group = Group,
ID = ID, fit.method = "nbinomial", n.perm = 10, points = points,
text = rownames(metalonda_test_data)[1], parall = FALSE, pvalue.threshold = 0.05,
adjust.method = "BH", time.unit = "hours", ylabel = "Normalized Count", col = c("black", "green"))

## End(Not run)
```

metalondaAll

Metagenomic Longitudinal Differential Abundance Analysis for all Features

Description

Identify significant features and their significant time interval

Usage

```
metalondaAll(
  Count,
  Time,
  Group,
  ID,
  n.perm = 500,
  fit.method = "nbinomial",
  num.intervals = 100,
  parall = FALSE,
  pvalue.threshold = 0.05,
  adjust.method = "BH",
  time.unit = "days",
  norm.method = "none",
  prefix = "Output",
  ylabel = "Normalized Count",
  col = c("blue", "firebrick")
)
```

Arguments

Count	Count matrix of all features
Time	Time label of all samples
Group	Group label of all samples
ID	individual ID label for samples
n.perm	number of permutations
fit.method	The fitting method (nbinomial, lowess)
num.intervals	The number of time intervals at which metalonda test differential abundance
parall	logic to indicate whether to use multicore
pvalue.threshold	p-value threshold cutoff
adjust.method	Multiple testing correction methods
time.unit	time unit used in the Time vector (hours, days, weeks, months, etc.)
norm.method	normalization method to be used to normalize count matrix (css, tmm, ra, log10, median_ratio)
prefix	prefix for the output figure
ylabel	text to be shown on the y-axis of all generated figures (default: "Normalized Count")
col	two color to be used for the two groups (eg., c("red", "blue")).

Value

Returns a list of the significant features a long with their significant time intervals

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```
## Not run:
data(metalonda_test_data)
n.sample = 5
n.timepoints = 10
n.group = 2
Group = factor(c(rep(0, n.sample*n.timepoints), rep(1,n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
output.nbinomial = metalondaAll(Count = metalonda_test_data, Time = Time, Group = Group,
ID = ID, n.perm = 10, fit.method = "nbinomial", num.intervals = 100,
parall = FALSE, pvalue.threshold = 0.05, adjust.method = "BH",
time.unit = "hours", norm.method = "none", prefix = "Test", time.unit = "hours",
ylabel = "Normalized Count", col = c("black", "green"))

## End(Not run)
```

metalonda_test_data	<i>Simulated data of OTU abundance for 2 phenotypes each has 5 subjects at 10 time-points</i>
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Description

The dataset is used for testing the MetaLonDA

Usage

metalonda_test_data

Format

A data frame with 2 OTUs patterns

normalize	<i>Normalize count matrix</i>
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Description

Normalize count matrix

Usage

```
normalize(count, method = "css")
```

Arguments

count	count matrix
method	normalization method

References

Ahmed Metwally (ametwall@stanford.edu)

permutation	<i>Permute group labels</i>
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Description

Permutes the group label of the samples in order to construct the AR empirical distribution

Usage

```
permutation(
  perm.dat,
  n.perm = 500,
  method = "nbinomial",
  points,
  lev,
  parall = FALSE
)
```

Arguments

perm.dat	dataframe has the Count, Group, ID, Time
n.perm	number of permutations
method	The fitting method (negative binomial, LOWESS)
points	The points at which the prediction should happen
lev	the two level's name
parall	boolean to indicate whether to use multicore.

Value

returns the fitted model for all the permutations

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```
data(metalonda_test_data)
n.sample = 5
n.timepoints = 10
n.perm = 3
n.group = 2
Group = factor(c(rep(0, n.sample*n.timepoints), rep(1, n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
aggregate.df = data.frame(Count = metalonda_test_data[,1], Time = Time, Group = Group, ID = ID)
prm = permutation(aggregate.df, n.perm = 3, method = "nbinomial", points)
```

visualizeArea	<i>Visualize significant time interval</i>
---------------	--

Description

Visualize significant time interval

Usage

```
visualizeArea(  
  aggregate.df,  
  model.ss,  
  method,  
  start,  
  end,  
  text,  
  group.levels,  
  unit = "days",  
  ylabel = "Normalized Count",  
  col = c("blue", "firebrick"),  
  prefix = "Test"  
)
```

Arguments

aggregate.df	Dataframe has the Count, Group, ID, Time
model.ss	The fitted model
method	Fitting method (negative binomial or LOWESS)
start	Vector of the start points of the time intervals
end	Vector of the end points of the time intervals
text	Feature name
group.levels	Level's name
unit	time unit used in the Time vector (hours, days, weeks, months, etc.)
ylabel	text to be shown on the y-axis of all generated figures (default: "Normalized Count")
col	two color to be used for the two groups (eg., c("red", "blue")).
prefix	prefix to be used to create directory for the analysis results

References

Ahmed Metwally (ametwall@stanford.edu)

visualizeARHistogram *Visualize Area Ratio (AR) empirical distribution*

Description

Visualize Area Ratio (AR) empirical distribution for each time interval

Usage

```
visualizeARHistogram(permuted, text, method, prefix = "Test")
```

Arguments

permuted	Permutation of the permuted data
text	Feature name
method	fitting method
prefix	prefix to be used to create directory for the analysis results

References

Ahmed Metwally (ametwall@stanford.edu)

visualizeFeature *Visualize Longitudinal Feature*

Description

Visualize Longitudinal Feature

Usage

```
visualizeFeature(  
  df,  
  text,  
  group.levels,  
  unit = "days",  
  ylabel = "Normalized Count",  
  col = c("blue", "firebrick"),  
  prefix = "Test"  
)
```

Arguments

df	dataframe has the Count, Group, ID, Time
text	feature name
group.levels	The two level's name
unit	time interval unit
ylabel	text to be shown on the y-axis of all generated figures (default: "Normalized Count")
col	two color to be used for the two groups (eg., c("red", "blue")).
prefix	prefix to be used to create directory for the analysis results

References

Ahmed Metwally (ametwall@stanford.edu)

Examples

```
data(metalonda_test_data)
pfx = tempfile()
dir.create(file.path(pfx))
n.sample = 5
n.timepoints = 10
n.group = 2
Group = factor(c(rep(0, n.sample*n.timepoints), rep(1, n.sample*n.timepoints)))
Time = rep(rep(1:n.timepoints, times = n.sample), 2)
ID = factor(rep(1:(2*n.sample), each = n.timepoints))
points = seq(1, 10, length.out = 10)
aggregate.df = data.frame(Count = metalonda_test_data[1,], Time = Time, Group = Group, ID = ID)
visualizeFeature(df = aggregate.df, text = rownames(metalonda_test_data)[1],
group.levels = Group, prefix = pfx)
```

visualizeFeatureSpline

Visualize the feature trajectory with the fitted Splines

Description

Plot the longitudinal features along with the fitted splines

Usage

```
visualizeFeatureSpline(
  df,
  model,
  method,
  text,
  group.levels,
```

```
    unit = "days",  
    ylabel = "Normalized Count",  
    col = c("blue", "firebrick"),  
    prefix = "Test"  
  )
```

Arguments

df	dataframe has the Count , Group, ID, Time
model	the fitted model
method	The fitting method (negative binomial, LOWESS)
text	feature name
group.levels	The two level's name
unit	time unit used in the Time vector (hours, days, weeks, months, etc.)
ylabel	text to be shown on the y-axis of all generated figures (default: "Normalized Count")
col	two color to be used for the two groups (eg., c("red", "blue")).
prefix	prefix to be used to create directory for the analysis results

References

Ahmed Metwally (ametwall@stanford.edu)

visualizeTimeIntervals

Visualize all significant time intervals for all tested features

Description

Visualize all significant time intervals for all tested features

Usage

```
visualizeTimeIntervals(  
  interval.details,  
  prefix = "Test",  
  unit = "days",  
  col = c("blue", "firebrick")  
)
```

Arguments

interval.details	Dataframe has information about significant interval (feature name, start, end, dominant, p-value)
prefix	prefix for the output figure
unit	time unit used in the Time vector (hours, days, weeks, months, etc.)
col	two color to be used for the two groups (eg., c("red", "blue")).

References

Ahmed Metwally (ametwall@stanford.edu)

visualizeVolcanoPlot *Visualize log2 fold-change and significance of each interval as volcano plot*

Description

Visualize log2 fold-change and significance of each interval as volcano plot

Usage

```
visualizeVolcanoPlot(df, text, prefix = "Test")
```

Arguments

df	Dataframe has a detailed summary about feature's significant intervals
text	Feature name
prefix	prefix to be used to create directory for the analysis results

References

Ahmed Metwally (ametwall@stanford.edu)

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