Package ‘ALDEx2’

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Type Package

Title Analysis Of Differential Abundance Taking Sample and Scale Variation Into Account

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Description A differential abundance analysis for the comparison of two or more conditions. Useful for analyzing data from standard RNA-seq or meta-RNA-seq assays as well as selected and unselected values from in-vitro sequence selections.

Uses a Dirichlet-multinomial model to infer abundance from counts, optimized for three or more experimental replicates. The method infers biological and sampling variation to calculate the expected false discovery rate, given the variation, based on a Wilcoxon Rank Sum test and Welch's t-test (via aldex.ttest), a Kruskal-Wallis test (via aldex.kw), a generalized linear model (via aldex.glm), or a correlation test (via aldex.corr). All tests report predicted p-values and posterior Benjamini-Hochberg corrected p-values. ALDEx2 also calculates expected standardized effect sizes for paired or unpaired study designs. ALDEx2 can now be used to estimate the effect of scale on the results and report on the scale-dependent robustness of results.

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Description

A differential abundance analysis for the comparison of two or more conditions. For example, single-organism and meta-RNA-seq high-throughput sequencing assays, or of selected and unselected values from in-vitro sequence selections. Uses a Dirichlet-multinomial model to infer abundance from counts, that has been optimized for three or more experimental replicates. Infers sampling variation and calculates the expected false discovery rate given the biological and sampling variation using the Wilcoxon rank test or Welches t-test (aldex.ttest) or the glm and Kruskal Wallis tests (aldex.glm). Reports both P and fdr values calculated by the Benjamini Hochberg correction.

References

Please use the citation given by citation(package="ALDEx").

See Also

aldex.clr, aldex.ttest, aldex.glm, aldex.effect, selex

Examples

# see examples for the aldex.clr, aldex.ttest, aldex.effect, aldex.glm functions

aldex

Compute an aldex Object

Description

Welcome to the ALDEx2 package!

The aldex function is a wrapper that performs log-ratio transformation and statistical testing in a single line of code. Specifically, this function: (a) generates Monte Carlo samples of the Dirichlet distribution for each sample, (b) converts each instance using a log-ratio transform, then (c) returns test results for two sample (Welch’s t, Wilcoxon) or multi-sample (glm, Kruskal-Wallis) tests. This function also estimates effect size for two sample analyses.
Usage

```r
aldex(
  reads,
  conditions,
  mc.samples = 128,
  test = "t",
  effect = TRUE,
  CI = FALSE,
  include.sample.summary = FALSE,
  verbose = FALSE,
  paired.test = FALSE,
  denom = "all",
  iterate = FALSE,
  gamma = NULL,
  ...
)
```

Arguments

- **reads**: A non-negative, integer-only data.frame or matrix with unique names for all rows and columns. Rows should contain genes and columns should contain sequencing read counts (i.e., sample vectors). Rows with 0 reads in each sample are deleted prior to analysis.

- **conditions**: A character vector. A description of the data structure used for testing. Typically, a vector of group labels. For `aldex.glm`, use a `model.matrix`.

- **mc.samples**: An integer. The number of Monte Carlo samples to use when estimating the underlying distributions. Since we are estimating central tendencies, 128 is usually sufficient.

- **test**: A character string. Indicates which tests to perform. "t" runs Welch's t and Wilcoxon tests. "kw" runs Kruskal-Wallace and glm tests. "glm" runs a generalized linear model using a `model.matrix`. "corr" runs a correlation test using `cor.test`.

- **effect**: A boolean. Toggles whether to calculate abundances and effect sizes.

- **CI**: A boolean. Toggles whether to calculate effect size confidence intervals. Applies to `test = "t"` and `test = "iterative"`.

- **include.sample.summary**: A boolean. Toggles whether to include median clr values for each sample. Applies to `effect = TRUE`.

- **verbose**: A boolean. Toggles whether to print diagnostic information while running. Useful for debugging errors on large datasets. Applies to `effect = TRUE`.

- **paired.test**: A boolean. Toggles whether to do paired-sample tests. Applies to `effect = TRUE` and `test = "t"`.

- **denom**: A character string. Indicates which features to retain as the denominator for the Geometric Mean calculation. Using "iqlr" accounts for data with systematic variation and centers the features on the set features that have variance that is between the lower and upper quartile of variance. Using "zero" is a more extreme...
case where there are many non-zero features in one condition but many zeros in another. In this case the geometric mean of each group is calculated using the set of per-group non-zero features.

iterate  A boolean. Toggles whether to iteratively perform a test. For example, this will use the results from an initial "t" routine to seed the reference (i.e., denominator of Geometric Mean calculation) for a second "t" routine.

gamma  A numeric. The standard deviation on the within sample variation.

...  Arguments to embedded method (e.g., glm or cor.test).

Details

See "Examples" below for a description of the sample input.

Value

Returns a number of values that depends on the set of options. See the return values of aldex.ttest, aldex.kw, aldex.glm, and aldex.effect for explanations and examples.

Author(s)

Greg Gloor, Andrew Fernandes, and Matt Links contributed to the original package. Thom Quinn added the "glm" test method, the "corr" test method, and the "iterate" procedure. Michelle Pistner Nixon and Justin Silverman contributed the scale and PPP routines.

References

Please use the citation given by citation(package="ALDE2").

See Also

aldex, aldex.clr, aldex.ttest, aldex.kw, aldex.glm, aldex.effect, aldex.corr, selex

Examples

# The 'reads' data.frame should have row # and column names that are unique, and # looks like the following:
#
#   T1a T1b T2 T3 N1 N2 Nx
# Gene_00001  0  0  2  0  0  1  0
# Gene_00002  2  8 12  5 19 26 14
# Gene_00003  3  0  2  0  0  0  1
# Gene_00004  7  5  8  7 241 149 257 188
# Gene_00005  1  3 16  4  0  4 10 10
# Gene_00006  1  2 12  6 451 223 243 149 209
# ... many more rows ...

data(selex)
selex <- selex[1201:1600,] # subset for efficiency
conds <- c(rep("NS", 7), rep("S", 7))
aldex.clr-class

The aldex.clr class

Description

The aldex.clr S4 class is a class which stores the data generated by the aldex.clr method.

Details

An aldex.clr object contains the centre-log ratio transformed Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data. It is created by the aldex.clr.function, which is invoked by the aldex.clr method. It consists of eight slots: the reads, the condition information, the number of instances, the denominator, whether it was invoked as verbose, and if multi-cores was used, the Dirichlet Monte-Carlo probabilities, and the centre-log ratio transformed Monte Carlo probabilities. These can be accessed along with information about the length of some attributes.

Value

The aldex.clr object contains the raw data, the estimated probabilities drawn from a Dirichlet distribution, and the clr transformed values for each Monte-Carlo instance. These can be accessed through getters outlined below.

Methods

In the code below, x is an aldex.clr object, and i, is a positive integer. There are N samples, D features, and M Monte-Carlo instances.

gMonteCarloInstances(x) Returns the clr transformed Monte Carlo Dirichlet instances as a list where each list entry is a single sample containing a D x M matrix.

gSampleIDs(x) Returns the names of the samples. These can be used to access the original reads for a given sample, as in x@reads\$sampleID (if the reads are a data frame).

gFeatureNames(x) Returns the names of the keys. These can be used to subset the data rows.

gFeatures(x) Returns the clr transformed values for the features in the first sample.
	numFeatures(x) Returns the number of features that were non-0 in at least one sample.
	numMCInstances(x) Returns the number of Monte-Carlo instances.

gReads(x) Returns the input data as used by the method.
	numConditions(x) Returns the number of samples in the conditions analysis.

gMonteCarloReplicate(x, i) Returns the D x M matrix containing the Monte-Carlo instances for one sample.

gMonteCarloSample(x, i) Returns the N x D matrix containing Monte-Carlo instance i for for all samples.
Author(s)

Greg Gloor, Ruth Grace Wong, Andrew Fernandes, Jia Rong Wu and Matt Links contributed to this code.

References

Please use the citation given by citation(package="ALDEx").

See Also

aldex.clr.function

Examples

# The 'reads' data.frame or
# SummarizedExperiment object should have
# row and column names that are unique,
# and looks like the following:
#
#   T1a T1b T2 T3 N1 N2 Nx
# Gene_00001 0 0 2 0 0 1 0
# Gene_00002 20 8 12 5 19 26 14
# Gene_00003 3 0 2 0 0 0 1
# Gene_00004 75 84 241 149 271 257 188
# Gene_00005 10 16 4 0 4 10 10
# Gene_00006 129 126 451 223 243 149 289
# ... many more rows ...

data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
i=1

# x is an object of type aldex.clr
x <- aldex.clr(selex, conds, mc.samples = 2, denom="all", verbose = FALSE)

# get reads plus uniform prior
reads <- getReads(x)

# get a list containing all of the clr transformed instances
monteCarloInstances <- getMonteCarloInstances(x)

# get a list containing all of the Monte-Carlo Dirichlet instances
monteCarloDirInstances <- getDirichletInstances(x)

# retrieve the clr transformed instances for sample i.
monteCarloInstance <- getMonteCarloReplicate(x,i)

# retrieve the Monte-Carlo Dirichlet instances for sample i.
monteCarloDirInstance <- getDirichletReplicate(x,i)
# retrieve the clr transformed instance i for all samples
monteCarloSample <- getMonteCarloSample(x, i)

# retrieve the Monte-Carlo Dirichlet instance i for all samples
monteCarloDirSample <- getDirichletSample(x, i)

# get sample names
sampleIDs <- getSampleIDs(x)

# get features
features <- getFeatures(x)

# get number of features with at least one count
numFeatures <- numFeatures(x)

# get number of Monte Carlo instances
numInstances <- numMCInstances(x)

# get names of features
featureNames <- getFeatureNames(x)

# get number of conditions
conditions <- numConditions(x)

# get the offset of the features in the log-ratio denominator
denom <- getDenom(x)

###

**aldex.clr.function**

*Compute an aldex.clr Object Generate Monte Carlo samples of the Dirichlet distribution for each sample. Convert each instance using a centered log-ratio transform. This is the input for all further analyses.*

**Description**

Compute an aldex.clr Object Generate Monte Carlo samples of the Dirichlet distribution for each sample. Convert each instance using a centered log-ratio transform. This is the input for all further analyses.

**Usage**

```r
aldex.clr.function(
  reads, 
  conds, 
  mc.samples = 128, 
  denom = "all", 
  verbose = FALSE, 
  useMC = FALSE, 
  summarizedExperiment = NULL, 
)```

gamma = NULL
)

Arguments

reads A data.frame or RangedSummarizedExperiment object containing non-negative integers only and with unique names for all rows and columns, where each row is a different gene and each column represents a sequencing read-count sample. Rows with 0 reads in each sample are deleted prior to analysis.

conds A vector containing a descriptor for the samples, allowing them to be grouped and compared.

mc.samples The number of Monte Carlo instances to use to estimate the underlying distributions; since we are estimating central tendencies, 128 is usually sufficient, but larger numbers may be needed with small sample sizes.

denom An any variable (all, iqr, zero, lvha, median, user) indicating features to use as the denominator for the Geometric Mean calculation The default "all" uses the geometric mean abundance of all features. Using "median" returns the median abundance of all features. Using "iqr" uses the features that are between the first and third quartile of the variance of the clr values across all samples. Using "zero" uses the non-zero features in each grop as the denominator. This approach is an extreme case where there are many nonzero features in one condition but many zeros in another. Using "lvha" uses features that have low variance (bottom quartile) and high relative abundance (top quartile in every sample). It is also possible to supply a vector of row indices to use as the denominator. Here, the experimentalist is determining a-priori which rows are thought to be invariant. In the case of RNA-seq, this could include ribosomal protein genes and other house-keeping genes. This should be used with caution because the offsets may be different in the original data and in the data used by the function because features that are 0 in all samples are removed by aldex.clr.

verbose Print diagnostic information while running. Useful only for debugging if fails on large datasets.

useMC Use multicore by default (FALSE). Multi core processing will be attempted with the BiocParallel package. Serial processing will be used if this is not possible. In practice serial and multicore are nearly the same speed because of overhead in setting up the parallel processes.

summarizedExperiment must be set to TRUE if input data are in this format.

gamma Use scale simulation if not NULL. If a matrix is supplied, scale simulation will be used assuming that matrix denotes the scale samples. If a numeric is supplied, scale simulation will be applied by relaxing the geometric mean assumption with the numeric representing the standard deviation of the scale distribution.

Value

The object produced by the clr function contains the log-ratio transformed values for each Monte-Carlo Dirichlet instance, which can be accessed through getMonteCarloInstances(x), where x is the clr function output. Each list element is named by the sample ID. getFeatures(x) returns
aldex.corr

Description

aldex.corr calculates the expected values for the correlation between each feature and a continuous variable, using data returned by aldex.clr and a vector of the continuous variable. Returns results of Pearson, Spearman and Kendall tests.

Usage

aldex.corr(clr, cont.var)

Arguments

clr An ALDEx2 object. The output of aldex.clr.
cont.var A continuous numeric vector

Value

Returns a data.frame of the average Pearson, Spearman and Kendall coefficients and their p-values for each feature, with FDR appended as a BH column.

Author(s)

Arianne Albert, Greg Gloor, Thom Quinn

References

Please use the citation given by citation(package="ALDEx2").

See Also

aldex, aldex.clr, aldex.ttest, aldex.kw, aldex.glm, aldex.effect, aldex.corr, selex
Examples

data(selex)
#subset for efficiency
selex <- selex[1:50,]
conds <- c(rep("N", 7), rep("S",7))
cont.var <- c(rep(1,7), rep(2,7))
x <- aldex.clr(selex, conds, mc.samples=16)
corr.test <- aldex.corr(x, cont.var)

aldex.effectiveffects and differences between conditions

Description

Determines the median clr abundance of the feature in all samples and in groups. Determines the median difference between the two groups. Determines the median variation within each two group. Determines the effect size, which is the median of the ratio of the between-group difference and the larger of the variance within groups.

Usage

aldex.effect(
    clr,
    verbose = TRUE,
    include.sample.summary = FALSE,
    useMC = FALSE,
    CI = FALSE,
    glm.conds = NULL,
    paired.test = FALSE
)

Arguments

clr
verbose
include.sample.summary
useMC
CI
glm.conds
paired.test
clr is the data output of aldex.clr.
Print diagnostic information while running. Useful only for debugging if fails on large datasets.
Include median clr values for each sample, defaults to FALSE.
Use multicore by default (FALSE).
Give effect 95% confidence intervals, defaults to FALSE.
Give effect for glm contrasts, note: saved as list.
Calculate effect size for paired samples, defaults to FALSE.

Details

An explicit example for two conditions is shown in the ‘Examples’ below.
Value

Returns a dataframe with the following information:

rab.all       a vector containing the median clr value for each feature.
rab.win.conditionA  a vector containing the median clr value for each feature in condition A.
rab.win.conditionB  a vector containing the median clr value for each feature in condition B.
diff.btw     a vector containing the per-feature median difference between condition A and B.
diff.win     a vector containing the per-feature maximum median difference between Dirichlet instances within conditions.
effect       a vector containing the per-feature effect size.
overlap      a vector containing the per-feature proportion of effect size that is 0 or less.

Author(s)

Greg Gloor, Andrew Fernandes, Matt Links

References

Please use the citation given by citation(package="ALDEx").

See Also

aldex.clr, aldex.ttest, aldex.glm, aldex.glm.effect, selex

Examples

# x is the output of the \code{x <- clr(data, mc.samples}) function
# conditions is a description of the data
# for the selex dataset, conditions <- c(rep("N", 7), rep("S", 7))
data(selex)
# subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples=2, denom="all")
effect.test <- aldex.effect(x)
aldex.expectedDistance

Calculate the expected values of distances between samples, given an aldex Object

Description

Calculates the expected value of distances between samples, given an aldex Object, using the median value of distances derived from n Monte-Carlo replicates.

Usage

aldex.expectedDistance(clrData)

Arguments

clrData an object of class aldex produced by the aldex function

Value

Returns a dist Object.

References

Please use the citation given by citation(package="ALDEx").

See Also

aldex, aldex.clr, dist

Examples

data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples = 128, denom = "all", verbose = FALSE)
x.dist <- aldex.expectedDistance(x)
plot(hclust(x.dist))
aldex.glm  

*Calculate glm test statistics using a model.matrix*

**Description**

`aldex.glm` calculates the expected values for each coefficient of a glm model on the data returned by `aldex.clr`. This function requires the user to define a model with `model.matrix`.

**Usage**

```r
aldex.glm(clr, verbose = FALSE, fdr.method = "holm", ...)
```

**Arguments**

- `clr`  
  An ALDEx2 object. The output of `aldex.clr`.
- `verbose`  
  A boolean. Toggles whether to print diagnostic information while running. Useful for debugging errors on large datasets. Applies to `effect = TRUE`.
- `fdr.method`  
  A string ("BH" or "holm") denoting which method to use to adjust p-values. Default is "holm"
- `...`  
  Arguments passed to `glm`.

**Value**

Returns a data.frame of the average coefficients and their p-values for each feature, with FDR appended as a `holm` column.

**Author(s)**

Thom Quinn, Michelle Pistner

**References**

Please use the citation given by `citation(package="ALDEx2")`.

**See Also**

`aldex, aldex.clr, aldex.ttest, aldex.kw, aldex.glm, aldex.effect, aldex.corr, selex`

**Examples**

```r
data(selex)  
# subset for efficiency  
selex <- selex[1201:1600,]  
covariates <- data.frame("A" = sample(0:1, 14, replace = TRUE),  
                         "B" = c(rep(0, 7), rep(1, 7)))  
mm <- model.matrix(~ A + B, covariates)  
x <- aldex.clr(selex, mm, mc.samples=4, denom="all")  
glm.test <- aldex.glm(x)
```
aldex.glm.effect

glm.eff <- aldex.glm.effect(x)
aldex.glm.plot(glm.test, eff=glm.eff, contrast='B', type='MW', post.hoc='holm')

aldex.glm.effect  
*calculate effect sizes and differences between all contrasts for the aldex.glm model matrix*

**Description**

data for this function is saved in a list with entries named by contrast determines the median clr abundance of the feature in all samples and in groups determines the median difference between the two groups determines the median variation within each two group determines the effect size, which is the median of the ratio of the between group difference and the larger of the variance within groups

**Usage**

aldex.glm.effect(clr, verbose = TRUE, include.sample.summary = FALSE, useMC=FALSE, CI=FALSE)

**Arguments**

clr  The data output of aldex.clr
verbose  Print diagnostic information while running. Useful only for debugging if fails on large datasets
include.sample.summary  include median clr values for each sample, defaults to FALSE
useMC  use multicore by default (FALSE)
CI  give effect 95% confidence intervals, defaults to FALSE

**Details**

Calculate effect sizes and differences between all contrasts for the aldex.glm model matrix
An explicit example for two conditions is shown in the ‘Examples’ below.

**Value**

A dataframe with the following information:

- `rab.all` a vector containing the median clr value for each feature
- `rab.win.conditionA` a vector containing the median clr value for each feature in condition A
- `rab.win.conditionB` a vector containing the median clr value for each feature in condition B
- `diff.btw` a vector containing the per-feature median difference between condition A and B
- `diff.win` a vector containing the per-feature maximum median difference between Dirichlet instances within conditions
- `effect` a vector containing the per-feature effect size
- `overlap` a vector containing the per-feature proportion of effect size that is 0 or less
Author(s)
Greg Gloor, Andrew Fernandes, Matt Links

References
Please use the citation given by citation(package="ALDEx").

See Also
aldex.clr, aldex.effect, aldex.ttest, aldex.glm, selex

Examples
# x is the output of the code(x <- clr(data, mc.samples)) function
# conditions is a description of the data
# for the selex dataset, conditions <- c(rep("N", 7), rep("S", 7))
data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
covariates <- data.frame("A" = sample(0:1, 14, replace = TRUE),
                        "B" = c(rep(0, 7), rep(1, 7)),
                        "Z" = sample(c(1,2,3), 14, replace=TRUE))
mm <- model.matrix(~ A + Z + B, covariates)
x <- aldex.clr(selex, mm, mc.samples=8, denom="all")
glm.effect <- aldex.glm.effect(x)
all.pch = 19,
all.cex = 0.4,
called.col = "red",
called.pch = 20,
called.cex = 0.6,
thres.line.col = "darkgrey",
thres.lwd = 1.5,
cutoff.pval = 0.05,
cutoff.effect = 1,
rare.col = "black",
rare = 0,
rare.pch = 20,
rare.cex = 0.2
)

Arguments

x an object produced by the aldex.glm function

... optional, unused arguments included for compatibility with the S3 method signature

eff an object produced by the aldex.glm.effect function

contrast the column name of the model matrix contrast to plot

test the method of calculating significance, one of "pval" or "fdr"

type which type of plot is to be produced. MA is a Bland-Altman style plot; MW is an effect plot showing the relationship of difference between and dispersion as described in: http://dx.doi.org/10.1080/10618600.2015.1131161; volcano is a volcano plot http://dx.doi.org/10.1186/gb-2003-4-4-210

xlab the x-label for the plot, as per the parent plot function

ylab the y-label for the plot, as per the parent plot function

xlim the x-limits for the plot, as per the parent plot function

ylim the y-limits for the plot, as per the parent plot function

all.col the default colour of the plotted points

all.pch the default plotting symbol

all.cex the default symbol size

called.col the colour of points with false discovery rate, q <= 0.1

called.pch the symbol of points with false discovery rate, q <= 0.1

called.cex the character expansion of points with false discovery rate, q <= 0.05

thres.line.col the colour of the threshold line where within and between group variation is equivalent

thres.lwd the width of the threshold line where within and between group variation is equivalent

cutoff.pval the fdr cutoff, default 0.05

cutoff.effect the effect size cutoff for plotting, default 1
aldex.kw

rare.col color for rare features, default black
rare relative abundance cutoff for rare features, default 0 or the mean abundance
rare.pch the default symbol of rare features
rare.cex the default symbol size of rare points

Details
Plot an aldex Object
This particular specialization of the plot function is relatively simple and provided for convenience. For more advanced control of the plot is is best to use the values returned by summary(x).

Value
None.

References
Please use the citation given by citation(package="ALDEx").

See Also
aldex, aldex.effect, aldex.ttest, aldex glm

Examples
# See the examples for 'aldex glm'

aldex.kw

Calculate the Kruskal-Wallis test and glm statistics

Description
aldex.kw calculates the expected values of the Kruskal-Wallis test and a glm on the data returned by aldex.clr.

Usage
aldex.kw(clr, useMC = FALSE, verbose = FALSE)

Arguments
clr An ALDEx2 object. The output of aldex.clr.
useMC Toggles whether to use multi-core.
verbose A boolean. Toggles whether to print diagnostic information while running. Useful for debugging errors on large datasets. Applies to effect = TRUE.
**aldex.makeScaleMatrix**

**Generate a differential scale matrix by group**

**Description**

Takes as input the conditions vector, dispersion parameter, starting scale values and the number of random instances. The ratio between the scale values is key; setting mu = c(1,1.2) will have the same effect on the analysis as a value of mu =c(0.5,0.6). The function returns a matrix of scale values of the same dimension as the number of samples and the number of mc.samples used by the aldex() or aldex.clr() function.

**Details**

use the aldex.glm function unless you really need the nonparametric KW test

**Value**

Returns a data.frame with the following information:

- **kw.ep**
  - a vector containing the expected p-value of the Kruskal-Wallis test for each feature
- **kw.eBH**
  - a vector containing the corresponding expected value of the Benjamini-Hochberg corrected p-value for each feature
- **glm.ep**
  - a vector containing the expected p-value of the glm ANOVA for each feature
- **glm.eBH**
  - a vector containing the corresponding expected value of the Benjamini-Hochberg corrected p-value for each feature. Note, you should use the aldex.glm function for better post-hoc test statistics.

**Author(s)**

Arianne Albert

**References**

Please use the citation given by citation(package="ALDEx2").

**See Also**

aldex, aldex.clr, aldex.ttest, aldex.kw, aldex.glm, aldex.effect, aldex.corr, selex

**Examples**

```r
data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("A", 4), rep("B", 3), rep("C", 7))
x <- aldex.clr(selex, conds, mc.samples=1, denom="all")
kw.test <- aldex.kw(x)
```

---

**aldex.makeScaleMatrix**

Generate a differential scale matrix by group
Usage

```
aldex.makeScaleMatrix(gamma, mu, conditions, log = TRUE, mc.samples = 128)
```

Arguments

- **gamma**: the base gamma value for the sdlog parameter of rlnorm
- **mu**: pair of values, or a vector of values one for each sample
- **conditions**: the conditions vector for the dataset
- **log**: scale ratio in log2 (TRUE) or as simple ratio (FALSE)
- **mc.samples**: the number of Monte-Carlo instances used by aldex()

Value

returns a matrix of gamma values that are used as an estimate of the scale for the aldex.clr() function. This allows different scale and gamma values to be applied to each group and can move the centre of mass of the data if required. The example dataset has very extreme differences in scale. Most often these are likely in the range of 10-15

Author(s)

Greg Gloor, Michelle Pistner Nixon

References

Please use the citation given by `citation(package="ALDEx")`.

See Also

`aldex.clr, aldex`

Examples

```r
# conditions is a vector describing the data
data(selex)
# subset for efficiency
conds <- c(rep("NS", 7), rep("S", 7))
mu.in <- c(1,50) # 50-fold difference in scale between groups
mu.vec <- aldex.makeScaleMatrix(1, mu.in, conds, log=TRUE, mc.samples=128)
```
aldex.plot

Plot an aldex Object

Description

Create MW- or MA-type plots from the given aldex object.

Usage

aldex.plot(
  x,
  ...,
  type = c("MW", "MA", "volcano", "volcano.var"),
  xlab = NULL,
  ylab = NULL,
  xlim = NULL,
  ylim = NULL,
  all.col = rgb(0, 0, 0, 0.2),
  all.pch = 19,
  all.cex = 0.4,
  called.col = "red",
  called.pch = 20,
  called.cex = 0.6,
  thres.line.col = "darkgrey",
  thres.lwd = 1.5,
  test = "welch",
  cutoff.pval = 0.05,
  cutoff.effect = 1,
  rare.col = "black",
  rare = 0,
  rare.pch = 20,
  rare.cex = 0.2,
  main = NULL
)

Arguments

x an object of class aldex produced by the aldex function

... optional, unused arguments included for compatibility with the S3 method signature

type which type of plot is to be produced. MA is a Bland-Altman style plot; MW is a difference between to a variance within plot as described in: http://dx.doi.org/10.1080/10618600.2015.1131161
volcano is a volcano plot of either the difference or variance type: http://dx.doi.org/10.1186/gb-2003-4-4-210

xlab the x-label for the plot, as per the parent plot function

ylab the y-label for the plot, as per the parent plot function
xlim the x-limits for the plot, as per the parent plot function
ylim the y-limits for the plot, as per the parent plot function
all.col the default colour of the plotted points
all.pch the default plotting symbol
all.cex the default symbol size
called.col the colour of points with false discovery rate, q <= 0.1
called.pch the symbol of points with false discovery rate, q <= 0.1
called.cex the character expansion of points with false discovery rate, q <= 0.05
thres.line.col the colour of the threshold line where within and between group variation is equivalent
thres.lwd the width of the threshold line where within and between group variation is equivalent
test the method of calculating significance, one of: welch = welch's t test - here a posterior predictive p-value; wilcox = wilcox rank test; effect = effect size
cutoff.pval the Benjamini-Hochberg fdr cutoff, default 0.05
cutoff.effect the effect size cutoff for plotting, default 1
rare.col color for rare features, default black
rare relative abundance cutoff for rare features, default 0 or the mean abundance
rare.pch the default symbol of rare features
rare.cex the default symbol size of rare points
main the main label for the plot

Details
Plot an aldex Object
This particular specialization of the plot function is relatively simple and provided for convenience.
For more advanced control of the plot is is best to use the values returned by summary(x).

Value
None.

References
Please use the citation given by citation(package="ALDEEx").

See Also
aldex, aldex.effect, aldex.ttest, aldex.glm

Examples
# See the examples for 'aldex'
aldex.plotFeature  Show dispersion of the expected values returned by aldex.effect

Description

aldex.plotFeature generates density plots showing the dispersion of the expected values given in the output from aldex.effect. The expected values are shown in the plots. This is a diagnostic visualization to help determine if the expected values are trustworthy.

Usage

aldex.plotFeature(
  clrData,
  featureName,
  pooledOnly = FALSE,
  densityOnly = FALSE,
  reset.par = FALSE
)

Arguments

clrData  the output object from aldex.clr
featureName  the name of the feature from the input data
pooledOnly  show only the pooled plots, default FALSE, shows all plots
densityOnly  show only the density plots, default FALSE includes expected values
reset.par  reset the plotting parameter to par(c(1,1)), default FALSE

Author(s)

Brandon Lieng, Greg Gloor

References

Please use the citation given by citation(package="ALDEx2").

See Also

aldex.clr, aldex.effect, selex

Examples

data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples=4, denom="all")
aldex.plotFeature(x, "S:D:A:D")
Sensitivity analysis using scale simulation

Description

Performs scale simulation over a range of values for gamma. Dirichlet samples are reused for computational convenience.

Usage

```r
aldex.senAnalysis(
  aldex_clr,
  gamma,
  test = "t",
  effect = TRUE,
  include.sample.summary = FALSE,
  verbose = FALSE,
  iterate = FALSE,
...
)
```

Arguments

- **aldex_clr**: An ‘aldex.clr’ object
- **gamma**: A vector of positive numeric components. Used as the standard deviation of the scale simulation model.
- **test**: A character string. Indicates which tests to perform. "t" runs Welch’s t and Wilcoxon tests. "kw" runs Kruskal-Wallace and glm tests. "glm" runs a generalized linear model using a `model.matrix`. "corr" runs a correlation test using `cor.test`.
- **effect**: A boolean. Toggles whether to calculate abundances and effect sizes.
- **include.sample.summary**: A boolean. Toggles whether to include median clr values for each sample. Applies to `effect = TRUE`.
- **verbose**: A boolean. Toggles whether to print diagnostic information while running. Useful for debugging errors on large datasets. Applies to `effect = TRUE`.
- **iterate**: A boolean. Toggles whether to iteratively perform a test. For example, this will use the results from an initial "t" routine to seed the reference (i.e., denominator of Geometric Mean calculation) for a second "t" routine.
- **...**: Arguments to embedded method (e.g., `glm` or `cor.test`).

Value

A list of results. Each element corresponds to a single result for a given value of gamma.
**aldex.set.mode**

**identify set of denominator features for log-ratio calculation**

**Description**

calculate the features that are to be used as the denominator for the Geometric Mean calculation in clr_function.R

**Usage**

`aldex.set.mode(reads, conds, denom="all")`

**Arguments**

- **reads**
  A data frame containing the samples and features per sample.
- **conds**
  A vector describing which samples belong to what condition.
- **denom**
  Character argument specifying which indicies to return. 'all' returns all features in both conditions. 'zero' returns the nonzero count features per condition. 'iqlr' returns the features whose variance falls within the inter-quantile range of the CLR-transformed data. In cases of malformed or null queries, input defaults to 'all'. Additionally, the input can be a numeric vector, which contains a set of row indicies to center the data against. Only for advanced users who can pre-determine the invariant set of features within their data. Check that the same number of features are in the input and output datasets.

**Details**

Identify set of denominator features for log-ratio calculation

An explicit example for two conditions is shown in the 'Examples' below.

**Value**

Outputs a vector containing indices per condition, or a single vector in some cases.

**Author(s)**

Jia Rong Wu

**References**

Please use the citation given by `citation(package="ALDEx")`.

**See Also**

`aldex.clr, aldex.ttest, aldex.effect, selex`
Examples

# x is the output of the \code{x <- clr(data, mc.samples)} function
# conditions is a description of the data
# for the selex dataset, conditions <- c(rep("N", 7), rep("S", 7))
# input can be "all", "iqlr", "zero" or numeric for advanced users
data(selex)
# subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples=2, denom="all")

aldex.ttest

\textit{Calculate Wilcoxon Rank Sum test and Welch's t-test statistics}

Description

\texttt{aldex.ttest} calculates the expected values of the Wilcoxon Rank Sum test and the posterior predictive value of Welch's t-test on the data returned by \texttt{aldex.clr}.

Usage

\texttt{aldex.ttest(clr, paired.test = FALSE, hist.plot = FALSE, verbose = FALSE)}

Arguments

\begin{itemize}
  \item \texttt{clr} \hspace{2cm} An \texttt{ALDEx2} object. The output of \texttt{aldex.clr}.
  \item \texttt{paired.test} \hspace{2cm} Toggles whether to calculate paired tests.
  \item \texttt{hist.plot} \hspace{2cm} Toggles whether to plot a histogram of p-values for the first Dirichlet Monte Carlo instance.
  \item \texttt{verbose} \hspace{2cm} A boolean. Toggles whether to print diagnostic information while running. Useful for debugging errors on large datasets. Applies to \texttt{effect = TRUE}.
\end{itemize}

Value

Returns a \texttt{data.frame} with the following information:

\begin{itemize}
  \item \texttt{we.ep} \hspace{2cm} a vector containing the posterior predictive p-value of Welch's t-test for each feature
  \item \texttt{we.eBH} \hspace{2cm} a vector containing the corresponding expected value of the Benjamini-Hochberg corrected p-value for each feature
  \item \texttt{wi.ep} \hspace{2cm} a vector containing the expected p-value of the Wilcoxon Rank Sum test for each feature
  \item \texttt{wi.eBH} \hspace{2cm} a vector containing the corresponding expected value of the Benjamini-Hochberg corrected p-value for each feature
\end{itemize}
getConditions

Author(s)
Greg Gloor, Michelle Pistner

References
Please use the citation given by citation(package="ALDEx2").

See Also
aldex, aldex.clr, aldex.ttest, aldex.kw, aldex.glm, aldex.effect, aldex.corr, selex

Examples

data(selex)
#subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples=2, denom="all")
ttest.test <- aldex.ttest(x)

Description
Returns the conditions, for aldex.clr object.

Usage
getConditions(.object)

Arguments
.object A aldex.clr object containing the Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

Details
Returns the conditions used.

Value
A matrix representing the conditions used.

See Also
aldex.clr
getDenom

Examples

data(selex)
  # subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  scale.samps <- getConditions(x)

getDenom

Description

Returns the offset of the features used as the denominator as the basis for the log-ratio, for an aldex.clr object.

Usage

getDenom(.object)

Arguments

.object A aldex.clr object.

Details

Returns the offset of the features used as the denominator as the basis for the log-ratio. A vector of numbers is the offset of the non-0 features used in the denominator.

Value

A vector of integer row offsets.

See Also

aldex.clr

Examples

data(selex)
  # subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "iqlr", verbose = FALSE)
  Denom <- getDenom(x)

  # to find the names of housekeeping genes used
  getFeatureNames(x)[getDenom(x)]
getDirichletInstances

Description

Returns a list of the Monte Carlo Dirichlet instances created by the \texttt{aldex.clr} function.

Usage

getDirichletInstances(.object)

Arguments

.object A \texttt{aldex.clr} object containing the Monte Carlo Dirichlet instances derived from estimating.

Details

Returns a list of the raw Monte Carlo Dirichlet instances created by the \texttt{aldex.clr} function. These are probability estimates.

Value

A list of data frames.

See Also

\texttt{aldex.clr}

Examples

data(selex)
    #subset for efficiency
    selex <- selex[1201:1600,]
    conds <- c(rep("NS", 7), rep("S", 7))
    x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
    monteCarloDirInstances <- getDirichletInstances(x)
getDirichletReplicate

Description

Returns the raw per-sample Monte Carlo Dirichlet replicates generated from analysis, for an aldex.clr object.

Usage

getDirichletReplicate(.object,i)

Arguments

.object A aldex.clr object containing the Monte Carlo Dirichlet replicates derived from estimating the technical variance of the raw read count data, along with sample and feature information.
i The numeric index of the desired sample replicate.

Details

Returns the raw per-sample Monte Carlo Dirichlet replicates. These are estimated probabilities.

Value

A numeric matrix.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  DirichletReplicate <- getDirichletReplicate(x,1)
getDirichletSample

Description

Returns a single Monte Carlo Dirichlet instance for all samples for an aldex.clr object.

Usage

getDirichletSample(.object,i)

Arguments

.object A aldex.clr object containing the raw Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

i The numeric index of the desired Monte-Carlo instance.

Details

Returns the designated Monte Carlo Dirichlet instance for all samples generated from analysis.

Value

A matrix representing the designated Monte Carlo Dirichlet instance for all samples.

See Also

aldex.clr

Examples

data(selex)

# subset for efficiency
selex <- selex[1201:1600,]
conds <- c(rep("NS", 7), rep("S", 7))
x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
DirichletSample <- getDirichletSample(x,i)
Description

Returns the names of the features as a vector, for an aldex.clr object.

Usage

getFeatureNames(.object)

Arguments

.object A aldex.clr object.

Details

Returns the names of the keys that can be used to subset the data rows. The keys values are the rsid's.

Value

A vector of feature names.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom="all", verbose = FALSE)
  featureNames <- getFeatureNames(x)
getFeatures

Description
Returns the features as a vector, for an aldex.clr object.

Usage
getFeatures(.object)

Arguments
.object A aldex.clr object.

Details
Returns the features from the first sample and first Monte-Carlo replicate as a vector, for an aldex.clr object. Used only for troubleshooting purposes.

Value
A vector of features.

See Also
aldex.clr

Examples
data(selex)
  # subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom="all", verbose = FALSE)
  features <- getFeatures(x)
getMonteCarloInstances

Description

Returns a list of the log-ratio transformed Monte Carlo Dirichlet instances created by the aldex.clr function.

Usage

getMonteCarloInstances(.object)

Arguments

.object

A aldex.clr object containing the clr-transformed Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

Details

Returns a list of the log-ratio transformed Monte Carlo Dirichlet instances created by the aldex.clr function.

Value

A list of data frames.

See Also

aldex.clr

Examples

data(selex)
   #subset for efficiency
   selex <- selex[1201:1600,]
   conds <- c(rep("NS", 7), rep("S", 7))
   x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
   monteCarloInstances <- getMonteCarloInstances(x)
getMonteCarloReplicate

Description

Returns the log-ratio transformed per-sample Monte Carlo Dirichlet replicates generated from analysis, for an aldex.clr object.

Usage

getMonteCarloReplicate(.object, i)

Arguments

.object  A aldex.clr object containing the Monte Carlo Dirichlet replicates derived from estimating the technical variance of the raw read count data, along with sample and feature information.

i        The numeric index of the desired sample replicate.

Details

Returns the log-ratio transformed per-sample Monte Carlo Dirichlet replicates.

Value

A numeric matrix.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  monteCarloReplicate <- getMonteCarloReplicate(x, 1)
getMonteCarloSample

Description

Returns a single Monte Carlo Dirichlet instance for all samples for an aldex.clr object.

Usage

getMonteCarloSample(.object,i)

Arguments

.object A aldex.clr object containing the log-ratio transformed Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

i The numeric index of the desired Monte-Carlo instance.

Details

Returns the designated Monte Carlo Dirichlet instance for all samples generated from analysis.

Value

A matrix representing the designated log-ratio transformed Monte Carlo Dirichlet instance for all samples.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  monteCarloSample <- getMonteCarloSample(x,1)
getReads

Description

Returns the count table used as input for analysis, for aldex.clr object. Note this count table has features that are 0 in all samples removed, and a uniform prior of 0.5 is applied.

Usage

getReads(.object)

Arguments

.object A aldex.clr object containing the Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

Details

Returns the count table. Note this count table has features that are 0 in all samples removed, and a uniform prior of 0.5 is applied.

Value

A data frame representing the count table used as input for analysis.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  reads <- getReads(x)
getSampleIDs

Description

Returns the names of the samples for an aldex.clr object. These can be used to access the original reads, as in \texttt{reads}\$\texttt{sampleID} (if the reads are a data frame).

Usage

\texttt{getSampleIDs(.)\texttt{object}}

Arguments

\texttt{.object} \hspace{1cm} A \texttt{aldex.clr} object.

Details

Returns the names of the samples. These can be used to access the original reads, as in \texttt{reads}\$\texttt{sampleID} (if the reads are a data frame).

Value

A vector of sample names.

See Also

\texttt{aldex.clr}

Examples

\begin{verbatim}
data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  sampleIDs <- getSampleIDs(x)
\end{verbatim}
getScaleSamples

Description

Returns the log2 scale samples if scale simulation is used, for aldex.clr object.

Usage

getScaleSamples(.object)

Arguments

.object A aldex.clr object containing the Monte Carlo Dirichlet instances derived from estimating the technical variance of the raw read count data, along with sample and feature information.

Details

Returns NULL if scale simulation was not used or a matrix of log2 scale samples if scale simulation was used.

Value

A matrix representing the log2 scale samples if scale simulation was used.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  scale.samps <- getScaleSamples(x)
Interpret the scale model implied by a certain level of gamma or scale model

**Description**

Interpret the scale model implied by a certain level of gamma or scale model

**Usage**

```
interpretGamma(clr)
```

**Arguments**

- `clr` A aldex.clr object

**Value**

A table. For each variable, an estimate of theta^perp that is implied by the scale model is returned. The average and 95

---

Returns the number of conditions compared for analysis, for an aldex.clr object.

**Usage**

```
numConditions(.object)
```

**Arguments**

- `.object` A aldex.clr object.

**Details**

Returns the number of samples compared.

**Value**

A numeric representing the number of samples compared.

**See Also**

aldex.clr
Examples

data(selex)
    #subset for efficiency
    selex <- selex[1201:1600,]
    conds <- c(rep("NS", 7), rep("S", 7))
    x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
    conditions <- numConditions(x)

numFeatures <- numFeatures(x)

Description

Returns the number of non-0 features associated with the data, for an aldex.clr object.

Usage

numFeatures(.object)

Arguments

.object A aldex.clr object.

Details

Returns the number of features associated with the data that are not 0 in all samples.

Value

A numeric representing the number of non-0 features associated with the data.

See Also

aldex.clr

Examples

data(selex)
    #subset for efficiency
    selex <- selex[1201:1600,]
    conds <- c(rep("NS", 7), rep("S", 7))
    x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
    numFeatures <- numFeatures(x)
numMCInstances

Description

Returns the number of Monte Carlo Dirichlet instances generated for analysis, for an aldex.clr object.

Usage

numMCInstances(.object)

Arguments

.object A aldex.clr object.

Details

Returns the number of Monte Carlo Dirichlet instances generated for analysis.

Value

A numeric representing the number of Monte Carlo Dirichlet instances generated for analysis.

See Also

aldex.clr

Examples

data(selex)
  #subset for efficiency
  selex <- selex[1201:1600,]
  conds <- c(rep("NS", 7), rep("S", 7))
  x <- aldex.clr(selex, conds, mc.samples = 2, denom = "all", verbose = FALSE)
  numInstances <- numMCInstances(x)
plotGamma

Create gamma diagram for scale simulation sensitivity result

Description

Create gamma diagram for scale simulation sensitivity result

Usage

plotGamma(
  sen_results,
  test = "t",
  thresh = 0.05,
  taxa_to_label = 10,
  glmVar = NULL,
  blackWhite = FALSE,
  cex = 1
)

Arguments

sen_results  A list return by aldex.senAnalysis()

  test        A character string. What test was used to calculate the results

  thresh      A numeric between 0 and 1. What threshold should be used for significance?

  taxa_to_label  A positive integer. How many taxa should be labeled in the plot?

  glmVar       If 'test = "glm"'; what variable do you want plotted?

  blackWhite   boolean. If TRUE, returns the plot in black and white.

  cex          Default == 1. Controls the size of the axis and text labels in the plots.

Value

A plot object

selex

Selection-based differential sequence variant abundance dataset

Description

This data set gives the differential abundance of 1600 enzyme variants grown under selective (NS) and selective (S) conditions

Usage

data(selex)
Format
A dataframe of 1600 features and 14 samples. The first 7 samples are non-selected, the last 7 are selected.

Source

References

| synth2 | Synthetic asymmetric dataset |

Description
This synthetic dataset contains 2 percent sparsity as 0 values asymmetrically distributed. It is used as a test dataset.

Usage
data(synth2)

Format
A dataframe of 1000 features and 16 samples. The first 8 samples contain 20 features set to 0, the last 8 samples contain counts.

Source
Gloor et al (2017) notes
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