Package ‘MAST’

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Type Package

Title Model-based Analysis of Single Cell Transcriptomics

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Author Andrew McDavid <Andrew_McDavid@urmc.rochester.edu>, Greg Finak <gfinak@fredhutch.org>, Masanao Yajima <myajima@fredhutch.org>

Maintainer Andrew McDavid <Andrew_McDavid@urmc.rochester.edu>

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Description Methods and models for handling zero-inflated single cell assay data.

License GPL(>= 2)


RoxygenNote 5.0.1

LazyData true

biocViews GeneExpression, DifferentialExpression, GeneSetEnrichment, RNASeq, Transcriptomics, SingleCell

BugReports https://github.com/RGLab/MAST/issues

URL https://github.com/RGLab/MAST/

NeedsCompilation no
R topics documented:

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Methods for analysing single cell assay data using hurdle models.

This package provides data structures and functions for statistical analysis of single-cell assay data such as Fluidigm single cell gene expression assays.

applyFlat

Apply a vectorized binary operation recycling over last dimension

Description

When x is an array of order K, and y is an array of order K-1, whose dimensions otherwise agree, apply FUN by recycling y as necessary over dimension K of x.

Usage

applyFlat(x, y, FUN = "-")

Arguments

x
array, order K

y
array, order K-1

FUN
vectorized binary operation

Value

array, order K equal to FUN(x,y)

Examples

##Dumb example, could be done with scale(...,scale=FALSE)
x0 = matrix(1:10, nrow=2)
y0 = rowMeans(x0)
dim(y0) = c(1, 2)
x1 = MAST:::applyFlat(x0, y0)
stopifnot(rowMeans(x1)==0)

BayesGLMlike-class

Wrapper for bayesian GLM

Description

Wrapper for bayesian GLM

Slots

prior numeric optional 3d array used to specify prior for coefficients

useContinuousBayes logical should bayesglm be used to fit the continuous component as well?
**bootVcov1**

*Bootstrap a zlmfit*

**Description**
Sample cells with replacement to find bootstrapped distribution of coefficients

**Usage**
```r
bootVcov1(zlmfit, R = 99)
```

**Arguments**
- `zlmfit`: class `ZlmFit`
- `R`: number of bootstrap replicates

**Value**
array of bootstrapped coefficients

**Examples**
```r
data(vbetaFA)
zlmVbeta <- zlm.SingleCellAssay(~ Stim.Condition, subset(vbetaFA, ncells==1)[1:5,])
#Only run 3 boot straps, which you wouldn't ever want to do in practice...
bootVcov1(zlmVbeta, R=3)
```

**calcZ**

*Get Z or T statistics and P values after running gseaAfterBoot*

**Description**
The Z or T statistics may be reported by component (discrete/continuous) when combined='no' or combined by Fisher's or Stouffer's method (combined='fisher' or combined='stouffer'). Fisher's method uses the product of the p-values, while Stouffer's method uses the sum of the Z/T scores. The "Z" score returned by Fisher is the normal quantile that would yield the observed Fisher P-value, whose sign is derived from the sign of the maximum component Z score. The "Z" score returned by Stouffer when testType='normal' is the sum of the Z scores, over sqrt(2). When testType='t' it is a weighted combination of the Z scores, with weights corresponding to the degrees of freedom in each of the t statistics. A t-approximation to this sum of t-variables is derived by matching moments. It seems to be fairly accurate in practice.

**Usage**
```r
calcZ(gseaObj, testType = "t", combined = "none")
```
Arguments

gseaObj output from gseaAfterBoot
testType either 'normal' or 't'. The 't' test adjusts for excess kurtosis due to the finite number of bootstrap replicates used to estimate the variance of the statistics. This will result in more conservative inference.
combined character one of 'none', 'fisher' or 'stouffer'

Value

3D array with dimensions set (modules) comp ('cont'inuous or 'disc'rete) and metric ('Z' stat and two sided 'P' value that P(z>|Z|)) if combined='no', otherwise just a matrix.

See Also

gseaAfterBoot

Examples

## See the examples in gseaAfterBoot
eexample(gseaAfterBoot)

cData

<table>
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description

These functions are now all deprecated and will be removed in a future release.

Usage

cData(sc)
cData(sc) <- value

## S4 method for signature 'SingleCellAssay'
cData(sc)

## S4 replacement method for signature 'SingleCellAssay'
cData(sc) <- value

## S4 method for signature 'SingleCellAssay,SingleCellAssay'
combine(x, y, ...)

## S4 method for signature 'SingleCellAssay,ANY'
combine(x, y, ...)

Deprecated cell/feature data accessors/mutators

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## S4 method for signature 'SingleCellAssay,SingleCellAssay'
combine(x, y, ...)

## S4 method for signature 'SingleCellAssay,ANY'
combine(x, y, ...)
colData<-,SingleCellAssay,DataFrame-method

Arguments

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details

cData(sc): Return the cellData data.frame.
cData(sc)<-value: Replace the cellData with value, which can be either an AnnotatedDataFrame or data.frame. The replacement is checked that it has mandatory fields defined by its class.

combine(x, y, ...): Concatenate two experiments along rows/columns

Value

DataFrame or modifies the SingleCellAssay object in place

Replacement Functions

You should transition to use the following replacements:

cData colData
fData mcols
exprs assay
combine cbind2 or rbind2

See Also

exprs

Examples

data(vbetaFA)
stopifnot(all.equal(hushWarning(cData(vbetaFA), 'deprecated'), colData(vbetaFA)))
stopifnot(all.equal(hushWarning(fData(vbetaFA), 'deprecated'), mcols(vbetaFA)))
stopifnot(all.equal(hushWarning(exprs(vbetaFA), 'deprecated'), t(assay(vbetaFA)))

Description

Replace colData with a DataFrame. Checks to make sure that row.names(value) match colnames(x), in contrast to the parent method Checks for a wellKey column, as well.
Usage

```r
## S4 replacement method for signature 'SingleCellAssay,DataFrame'
colData(x) <- value
```

Arguments

- **x**: SingleCellAssay
- **value**: DataFrame

Value

modified SingleCellAssay

---

### collectResiduals

**Residual hooks and collection methods**

Description

After each gene is fit, a hook function can optionally be run and the output saved. This allows extended computations to be done using the fitted model, without keeping it in memory. Here this is used to calculate various residuals, though in some cases they can be done using only the information contained in the `ZlmFit`-class.

Usage

```r
collectResiduals(x, sca, newLayerName = "Residuals")
```

- **x**: `ZlmFit`-class
- **sca**: SingleCellAssay object to which the residuals should be added
- **newLayerName**: character name of the assay layer

Arguments

- **effectRegex**: a regular expression naming columns of the design corresponding to \( Z_0 \). Generally these should be the treatment effects of interest.

Value

copy of sca with new layer
Functions

- `discrete_residuals_hook`: Hook to get the discrete residuals, i.e., difference between expected probability of expression and observed.
- `continuous_residuals_hook`: Hook to get the continuous residuals, i.e., residuals for conditionally positive observations. If an observation is zero, it’s residual is defined to be zero as well.
- `combined_residuals_hook`: Hook to get the combined residuals, i.e., $Y - E(U)*E(V)$.
- `deviance_residuals_hook`: Standardized deviance residuals hook. Computes the sum of the standardized deviance residuals for the discrete and continuous models (scaled to have unit variance). If the observation is zero then only the discrete component is used.
- `fitted_phat`: Hook to return $p_{hat}$, the predicted probability of expression.
- `partialScore`: Compute $Y_i - E(V_i|X_i, Z_0)E(U|X_i, Z_0)$, where $Z_0$ is a treatment effect (being left in) and $X_i$ is a nuisance effect (being regressed out).

Total residual types

Each component of the model contributes several flavors of residual, which can be combined in various fashions. The discrete residual can be on the response scale (thus subtracting the predicted probability of expression from the 0/1 expression value). Or it can be a deviance residual, revealing something about the log-likelihood.

Partial residuals

It’s also possible to consider partial residuals, in which the contribution of a particular covariate is added back into the model.

See Also

zlm.SingleCellAssay

Examples

data(vbetaFA)
svbeta <- subset(vbetaFA, ncells==1)
svbeta <- svbeta[freq(svbeta)>0,]
window <- function(x1) lapply(assays(x1), function(x2) x2[1:3, 1:6])
# total residuals of the response
z1 <- zlm.SingleCellAssay(~ Stim.Condition, svbeta, hook=discrete_residuals_hook)
window(collectResiduals(z1, svbeta))
z2 <- zlm.SingleCellAssay(~ Stim.Condition, svbeta, hook=continuous_residuals_hook)
window(collectResiduals(z2, svbeta))
z3 <- zlm.SingleCellAssay(~ Stim.Condition, svbeta, hook=combined_residuals_hook)
window(collectResiduals(z3, svbeta))
# total deviance residuals
z4 <- zlm.SingleCellAssay(~ Stim.Condition, svbeta, hook=deviance_residuals_hook)
window(collectResiduals(z4, svbeta))
# partial residuals
colData(svbeta)$ngeneson <- colMeans(assay(svbeta)>0)
z5 <- zlm.SingleCellAssay(~ Stim.Condition + ngeneson, svbeta)
partialScore(z5, 'Stim.Condition')
computeEtFromCt  
Compute the Et from the Ct

Description
Computes the Et value from the Ct value in an existing data frame and returns a new data frame with the Et column appended.

Usage
computeEtFromCt(df, column = "Ct", Cmax = 40)

Arguments
- df: a data.frame
- column: The name of the Ct column. A character, 'Ct' by default.
- Cmax: the maximum number of cycles performed. 40 by default.

Value
A copy of df with the 'Et' column appended

Author(s)
Greg Finak

Examples
data(vbeta)
vbeta <- computeEtFromCt(vbeta)

condmean  
Report the mean et value for each gene

Description
NAs are always removed

Usage
condmean(sc)

Arguments
- sc: SingleCellAssay

Value
vector of means
Examples

```
data(vbetaFA)
condmean(vbetaFA)
```

---

**condSd**

*Report standard deviation of et, for positive et for each gene*

**Description**

NAs are always removed

**Usage**

```
condSd(sc)
```

**Arguments**

- `sc`  
  SingleCellAssay

**Value**

vector of standard deviations

---

**convertMASTClassicToSingleCellAssay**

*Convert a MASTClassic SingleCellAssay*

**Description**

Convert a SingleCellAssay object created with the MASTClassic package to an object recognized by the new MAST package

**Usage**

```
convertMASTClassicToSingleCellAssay(object = NULL)
```

**Arguments**

- `object`  
  of class SingleCellAssay created by MASTClassic

**Details**

The function will extract the relevant information from the attributes of the old object and construct a new SingleCellAssay that is recognized by MAST. This function checks that the object is a MASTClassic SingleCellAssay object. It will stop if it is not a SingleCellAssay, return a converted SingleCellAssay if object was created by MASTClassic, and return the original object if the object is already compatible.

**Value**

A MAST SingleCellAssay object.
Note
Type checking for old object is not performed.

Examples

```r
data(vbetaFA)
convertMASTClassicToSingleCellAssay(vbetaFA)
```

---

**defaultPrior**

*Initialize a prior to be used a prior for BayeGLMlike/BayesGLMlike2*

**Description**
Initialize a prior to be used a prior for BayeGLMlike/BayesGLMlike2

**Usage**

```r
defaultPrior(names)
```

**Arguments**

- `names`
  character vector of coefficients. The ‘(Intercept)’ will be ignored.

**Value**
3d array, with leading dimension giving the prior ‘loc’ation, ‘scale’ and degrees of freedom (df), second dimension giving the component (‘C’ontinuous or ‘D’iscrete) and trailing dimension giving the coefficient to which the prior applies. The location is initialized to be 0, the scale to 2, and degrees of freedom of 1, following the default of bayesglm.

**Examples**

```r
dp <- defaultPrior('Stim.ConditionUnstim')
## Not run:
data(vbetaFA)
zlmVbeta <- zlm.SingleCellAssay(~ Stim.Condition, vbeta.sc, method='bayesglm', coefPrior=dp)
## End(Not run)
```

---

**dof**

*Degrees of freedom of Zero inflated model*

**Description**
Degrees of freedom of Zero inflated model

**Usage**

```r
dof(object)
```
**Drop**

**Arguments**
- object: LMlike or subclass

**Value**
- vector giving the model degrees of freedom for continuous and discrete

**Description**
Like drop(x) but only dropping specified dimensions. There is no testing that the specified dimensions are actually singletons.

**Usage**
Drop(x, d)

**Arguments**
- x: array of at least d dimensions
- d: dimension(s) to drop

**Value**
- array x

**Examples**
```
x = array(1:4, dim=c(1, 2, 1, 2))
dx = MAST:::Drop(x, 1)
stopifnot(all(dim(dx)==c(2,1,2)))
```

---

**ebayes**

**Description**
ebayesControl is a named list with (optional) components 'method' (one of 'MOM' or 'MLE') and 'model' (one of 'H0' or 'H1') method MOM uses a method-of-moments estimator, while MLE using the marginal likelihood. H0 model estimates the precisions using the intercept alone in each gene, while H1 fits the full model specified by formula

**Usage**
ebayes(sca, ebayesControl, Formula, truncate = Inf)
Arguments

sca SingleCellAssay
ebayesControl list with (optional) components 'method', 'model'. See details.

Formula a formula (using variables in colData(sca) used when model='H1'.

truncate Genes with sample precisions exceeding this value are discarded when estimating the hyper parameters

Value

numeric of length two, giving the hyperparameters in terms of a variance (ν) and prior observations (df), inside a structure, with component hess, giving the Fisher Information of the hyperparameters.

Description

Puts log transformed values onto natural scale and takes mean of vector. Calculates mean(2^x - 1)

Usage

expavg(x)

Arguments

x numeric

Value

numeric

Examples

x <- 1:10
logmean(expavg(x))
#### fData

**Description**

Accessor for featureData data.frame.

**Arguments**

- `object`: An object with featureData.

**Details**

Returns the featureData data.frame.

**Value**

data.frame

---

#### featureData

**Description**

Returns the featureData.

**Arguments**

- `object`: An object with featureData.

**Value**

AnnotatedDataFrame

---

#### filter

**Description**

Remove, or flag wells that are outliers in discrete or continuous space.

**Usage**

```r
filter(sc, groups = NULL, filt_control = NULL, apply_filter = TRUE)
```

```r
burdenOfFiltering(sc, groups, byGroup = FALSE, filt_control = NULL)
```
Arguments

sc  The SingleCellAssay object

groups  An optional character naming the grouping variable

filt_control  The list with configuration parameters for the filter.

apply_filter  logical should the filter be applied, or should a matrix of booleans giving if a well would be subject to a filtering criteria be returned?

byGroup  in the case of burdenOfFiltering should the filter be stratified by groups, or only the plotting.

Details

The function filters wells that don’t pass filtering criteria described in filt_control. filt_control is a list with named elements nOutlier (minimum number of outlier cells for a cell to be filtered [default = 2]; sigmaContinuous (the z-score outlier threshold for the continuous part of the signal) [default = 7] and sigmaProportion (the z-score outlier threshold for the discrete part of the signal) [default = 7].

If groups is provided, the filtering is calculated within each level of the group, then combined again as output.

Value

A filtered result

Functions

• burdenOfFiltering: plot the proportions of wells are filtered due to different criteria

Author(s)

Andrew McDavid

See Also

burdenOfFiltering

Examples

data(vbetaFA)
## Split by 'ncells', apply to each component, then recombine
vbeta.filtered <- filter(vbetaFA, groups='ncells')
## Returned as boolean matrix
was.filtered <- filter(vbetaFA, apply_filter=FALSE)
## Wells filtered for being discrete outliers
head(subset(was.filtered, pctout))
burdenOfFiltering(vbetaFA, groups='ncells', byGroup=TRUE)
burdenOfFiltering(vbetaFA, groups='ncells')
### filterLowExpressedGenes

*Filter low-expressing genes*

**Description**
Filter out genes that have less than some percent threshold expression across all libraries.

**Usage**
```r
filterLowExpressedGenes(assay, threshold = 0.1)
```

**Arguments**
- `assay` a `SingleCellAssay` object
- `threshold` a numeric between 0, and 1, specifying the threshold frequency below which genes will be filtered out

**Value**
`SingleCellAssay`

**Examples**
```r
data(vbetaFA)
filterLowExpressedGenes(vbetaFA)
```

### fit

*fit a zero-inflated regression*

**Description**
Given a design and formula, fit the zero inflated regression, storing the fits in slots `fitC` and `fitD`.

**Usage**
```r
fit(object, response, ...)
```

**Arguments**
- `object` inheriting from `LMlike`
- `response` a vector, same length as the design, or if missing then use the current response
- `...` currently ignored
- `silent` mute some warnings emitted from the underlying modeling functions

**Value**
`LMlike` or subclass
**freq**  
*Report the proportion of expression for each gene*

**Description**

NAs can be optionally removed

**Usage**

```r
freq(sc, na.rm = TRUE)
```

**Arguments**

- `sc` SingleCellAssay
- `na.rm` should NAs be removed, or carried through?

**Value**

vector of proportions

**Examples**

```r
data(vbetaFA)
freq(vbetaFA)
```

---

**FromFlatDF**  
*Construct a SingleCellAssay (or derived subclass) from a ‘flat’ (melted) data.frame/data.table*

**Description**

SingleCellAssay are a generic container for such data and are simple wrappers around SummarizedExperiment objects. Subclasses exist that embue the container with additional attributes, eg FluidigmAssay.

**Usage**

```r
FromFlatDF(dataframe, idvars, primerid, measurement, id = numeric(0),
cellvars = NULL, featurevars = NULL, phenovars = NULL,
class = "SingleCellAssay", ...)
FluidigmAssay(...)
```
Arguments

dataframe  A ‘flattened’ data.frame or data.table containing columns giving cell and feature identifiers and a measurement column

idvars    character vector naming columns that uniquely identify a cell

primerid  character vector of length 1 that names the column that identifies what feature (i.e. gene) was measured

measurement character vector of length 1 that names the column containing the measurement

id       An identifier (eg. experiment name) for the resulting object

cellvars  Character vector naming columns containing additional cellular metadata

featurevars Character vector naming columns containing additional feature metadata

phenovars Character vector naming columns containing additional phenotype metadata

class   character providing desired subclass to construct.

... additional arguments are ignored

Value

SingleCellAssay, or derived, object

Examples

```
data(vbeta)
colnames(vbeta)
vbeta <- computeEtFromCt(vbeta)
vbeta.fa <- FromFlatDF(vbeta, idvars=c("Subject.ID", "Chip.Number", "Well"),
primerid="Gene", measurement="Et", ncells="Number.of.Cells",
geneid="Gene", cellvars=c('Number.of.Cells', 'Population'),
phenovars=c('Stim.Condition', 'Time'), id='vbeta all', class='FluidigmAssay')
show(vbeta.fa)
nrow(vbeta.fa)
ncol(vbeta.fa)
head(mcols(vbeta.fa)$primerid)
table(colData(vbeta.fa)$Subject.ID)
vbeta.sub <- subset(vbeta.fa, Subject.ID=='Sub01')
show(vbeta.sub)
```

---

**FromMatrix**

*Construct a SingleCellAssay from a matrix or array of expression*

**Description**

If the gene expression measurements are already in a rectangular form, then this function allows an easy way to construct a SingleCellAssay object while still doing some sanity checking of inputs.

**Usage**

```
FromMatrix(exprsArray, cData, fData, class = "SingleCellAssay")
```
Arguments

- `exprsArray`: matrix or array, columns are cells, rows are genes
- `cData`: cellData an object that can be coerced to a DataFrame, i.e., data.frame, AnnotatedDataFrame. Must have as many rows as `ncol(exprsArray)`
- `fData`: featureData an object that can be coerced to a DataFrame, i.e., data.frame, AnnotatedDataFrame. Must have as many rows as `nrow(exprsArray)`

Value

an object of class `class`

Examples

```r
ncells <- 10
genomes <- 5
fData <- data.frame(primerid=LETTERS[1:genomes])
cData <- data.frame(wellKey=seq_len(ncells))
mat <- matrix(rnorm(ncells*genomes), nrow=genomes)
sca <- FromMatrix(mat, cData, fData)
stopifnot(inherits(sca, 'SingleCellAssay'))
stopifnot(inherits(sca, 'SummarizedExperiment'))
## If there are mandatory keywords expected by a class, you'll have to manually set them yourself

fData$nunmbers <- 1
fd <- FromMatrix(mat, cData, fData)
stopifnot(inherits(fd, 'SingleCellAssay'))
```

Description

Get the concordance between two

Usage

```r
getConcordance(singleCellRef, singleCellcomp, groups = NULL,
    fun.natural = expavg, fun.cycle = logmean)

gtwss(concord, nexp)

gtss(concord)

gtrec(concord)
```

Arguments

- `singleCellRef`: "reference" `SingleCellAssay`
- `singleCellcomp`: "comparison" `SingleCellAssay`
- `groups`: character vector giving variable(s) on which the comparison is conditioned
fun.natural  function to transform the SingleCellAssays to a mRNA proportional level
fun.cycle  inverse function of fun.natural
concord  data.frame returned by getConcordance
nexp  number of expressed cells per row in concord

Details
Return the concordance between two assays (i.e. single cell and hundred cell) The "average" of singleCellRef (after adjusting for the number of cells) and singleCellComp are taken per gene, per groups. A data.frame with one row per gene-groups is returned with some additional columns.

Value
concordance between two assays

Functions
• getwss: getrc the sum of squares, weighted by nexp
• getss: return the sum of squares
• getrc: Return Lin’s (1989) concordance correlation coefficient

Author(s)
Andrew McDavid

See Also
plotSCAConcordance

Examples
data(vbetaFA)
sca1 <- subset(vbetaFA, ncells==1)
sca100 <- subset(vbetaFA, ncells==100)
concord <- getConcordance(sca1, sca100)
getss(concord)
getrc(concord)

getwellKey  Accessor for wellKey

Description
This returns the wellKey, which is a unique identifier generated by idvars in the mapping

Usage
getwellKey(sc)
GLMlike-class

Arguments

sc 

An object with a wellKey

Value

integer giving the unique id generated

Examples

data(vbetaFA)
getwellKey(vbetaFA)
colData(vbetaFA)$wellKey

GLMlike-class 

Wrapper for regular glm/lm

Description

Wrapper for regular glm/lm

Usage

## S4 method for signature 'GLMlike'
vcov(object, which, ...)

Arguments

object 

GLMlike

which 

character, one of 'C', 'D'.

... 

ignored

Value

covariance matrix

Methods (by generic)

• vcov: return the variance/covariance of component which

Slots

weightFun function to map expression values to probabilities of expression. Currently unused.
Gene set analysis for hurdle model

Description

Modules defined in sets are tested for average differences in expression from the "average" gene. By using bootstraps, the between-gene covariance of terms in the hurdle model is found, and is used to adjust for coexpression between genes. We drop genes if the coefficient we are testing was not estimable in original model fit in zFit or in any of the bootstrap replicates (evidenced an NA in the bootstrap array). This might yield overly conservative inference. Since bootstrapping is a randomized procedure, the degrees of freedom of a module (and its variance parameters) might differ from run-to-run. You might try setting var_estimate='modelbased' to relax this requirement by assuming independence between genes and then using the asymptotic covariance estimates, which are deterministic, but may result in overly-generous inference.

Usage

gseaAfterBoot(zFit, boots, sets, hypothesis, control = list(n_randomize = Inf, var_estimate = "bootall"))

Arguments

zFit object of class ZlmFit
boots bootstraps of zFit
sets list of indices of genes
hypothesis a Hypothesis to test. Currently only one degree CoefficientHypothesis are supported.
control list of control parameters. See details.

Value

Object of class GSEATests, containing slots tests, 4D array and bootR, the number of bootstrap replicates.

control

control is a list with elements:

• n_randomize, giving the number of genes to sample to approximate the non-module average expression. Set to Inf to turn off the approximation (the default).
• var_estimate, giving the method used to estimate the variance of the modules. bootall uses the bootstrapped covariance matrices. bootdiag uses only the diagonal of the bootstrapped covariance matrix (so assuming independence across genes). modelbased assumes independence across genes and uses the variance estimated from the model.

Return Value

A 4D array is returned, with dimensions "set" (each module), "comp" (‘disc’rete or ‘cont’inuous), "metric" (‘stat’ gives the average of the coefficient, ‘var’ gives the variance of that average, ‘dof’ gives the number of genes that were actually tested in the set), "group" (‘test’ for the genes in test-set, "null" for all genes outside the test-set).
See Also

calcZ
summary,GSEATests-method

Examples

data(vbetaFA)
vb1 = subset(vbetaFA, ncells==1)
vb1 = vb1[,freq(vb1)>.1][1:15,]
zf = zlm.SingleCellAssay(~Stim.Condition, vb1)
boots = bootVcov(zf, 5)
sets=list(A=1:5, B=3:10, C=15, D=1:5)
gsea=gseaAfterBoot(zf, boots, sets, CoefficientHypothesis('Stim.ConditionUnstim'))
calcZ(gsea)
summary(gsea)

GSEATests-class
An S4 class for Gene Set Enrichment output

Description
This holds output from a call to gseaAfterBoot. It primarily provides a summary method.

Slots

tests array: gene sets X discrete,continuous X stat, variance, degrees of freedom, avg correlation X test, null
bootR number of bootstrap replicates

See Also

gseaAfterBoot
calcZ
summary,GSEATests-method

hushWarning
Selectively muffle warnings based on output

Description
Selectively muffle warnings based on output

Usage
hushWarning(expr, regexp)
Hypothesis

Arguments

expr an expression
regexp a regexp to be matched (with str_detect)

Value

the result of expr

Examples

hushWarning(warning('Beware the rabbit'), 'rabbit')
hushWarning(warning('Beware the rabbit'), 'hedgehog')

Hypothesis

Describe a linear model hypothesis to be tested

Description

A Hypothesis can be any linear combination of coefficients, compared to zero. Specify it as a character vector that can be parsed to yield the desired equalities ala makeContrasts. A CoefficientHypothesis is a hypothesis for which terms are singly or jointly tested to be zero (generally the case in a t-test or F-test), by dropping coefficients from the model.

Usage

Hypothesis(hypothesis, terms)

Arguments

hypothesis a character vector specifying a hypothesis, following makeContrasts, or a character vector naming coefficients to be dropped.
terms an optional character vector giving the terms (column names from the model.matrix) out of which the contrasts will be contrasted. If missing then most functions will attempt to fill this in for you at run time.

Value

a Hypothesis with a "transformed" component

See Also

zlm.SingleCellAssay waldTest lrTest

Examples

h <- Hypothesis('Stim.ConditionUnstim', c('(Intercept)', 'Stim.ConditionUnstim'))
h@contrastMatrix
impute  

**impute missing continuous expression for plotting**

**Description**

If there are no positive observations for a contrast, it is generally not estimable. However, for the purposes of testing we can replace it with the least favorable value with respect to the contrasts that are defined.

**Usage**

impute(object, groupby)

**Arguments**

- **object**: Output of predict
- **groupby**: Variables (column names in predict) to group by for imputation (facets of the plot)

**Value**

data.table

**Examples**

```r
##See stat_ell
example(stat_ell)
```

---

**influence.bayesglm**  

**Influence bayesglm object**

**Description**

The influence function

**Usage**

```r
## S3 method for class 'bayesglm'
influence(model, do.coef = TRUE, ...)
```

**Arguments**

- **model**: bayesglm
- **do.coef**: see influence.glm
- **...**: ignored

**Value**

see influence.glm
invlogit

Inverse of logistic transformation

Description
Inverse of logistic transformation

Usage
invlogit(x)

Arguments
x numeric

Value
numeric

Examples
x <- 1:5
invlogit(log(x/(1-x)))

LMERlike-class
Wrapper for lmer/glmer

Description
A horrendous hack is employed in order to do arbitrary likelihood ratio tests: the model matrix is built, the names possibly mangled, then fed in as a symbolic formula to glmer/lmer. This is necessary because there is no (easy) way to specify an arbitrary fixed-effect model matrix in glmer.

Usage

## S4 method for signature 'LMERlike'
update(object, formula., design, ...)

## S4 method for signature 'LMERlike'
vcov(object, which, ...)

## S4 method for signature 'LMERlike'
coef(object, which, singular = TRUE, ...)

## S4 method for signature 'LMERlike'
logLik(object)
LMlike-class

Arguments

object
  LMERlike
formula.
  formula
design
  something coercible to a data.frame
...
  In the case of vcov, ignored. In the case of update, passed to model.matrix.
which
  character, one of 'C', 'D'.
singular
  logical. Should NA coefficients be returned?

Value

see the section "Methods (by generic)"

Methods (by generic)

• update: update the formula or design matrix
• vcov: return the variance/covariance of component which
• coef: return the coefficients. The horrendous hack is attempted to be undone.
• logLik: return the log-likelihood

Slots

pseudoMM part of this horrendous hack.
strictConvergence logical return results even when the optimizer or *lmer complains about convergence
optimMsg character record warnings from lme. NA_character_ means no warnings.

LMLike-class

Linear Model-like Class

Description

Wrapper around modeling function to make them behave enough alike that Wald tests and Likelihood ratio are easy to do. To implement a new type of zero-inflated model, extend this class. Depending on how different the method is, you will definitely need to override the fit method, and possibly the model.matrix, model.matrix<-, update, coef, vcov, and logLik methods.

Usage

## S4 method for signature 'LMLike'
summary(object)

## S4 method for signature 'LMLike'
update(object, formula., design, ...)

## S4 method for signature 'LMLike,CoefficientHypothesis'
waldTest(object, hypothesis)

## S4 method for signature 'LMLike,matrix'
waldTest(object, hypothesis)
## S4 method for signature 'LMlike,character'
lrTest(object, hypothesis)
## S4 method for signature 'LMlike,CoefficientHypothesis'
lrTest(object, hypothesis)
## S4 method for signature 'LMlike,Hypothesis'
lrTest(object, hypothesis)
## S4 method for signature 'LMlike,matrix'
lrTest(object, hypothesis)
## S4 method for signature 'GLMlike'
logLik(object)

Arguments

object LMlike
formula. formula
design something coercible to a data.frame
... passed to model.matrix
hypothesis one of a CoefficientHypothesis, Hypothesis or contrast matrix.

Value
see section "Methods (by generic)"

Methods (by generic)

- summary: Print a summary of the coefficients in each component.
- update: update the formula or design from which the model.matrix is constructed
- waldTest: Wald test dropping single term specified by CoefficientHypothesis hypothesis
- waldTest: Wald test of contrast specified by contrast matrix hypothesis
- lrTest: Likelihood ratio test dropping entire term specified by character hypothesis naming a term in the symbolic formula.
- lrTest: Likelihood ratio test dropping single term specified by CoefficientHypothesis hypothesis
- lrTest: Likelihood ratio test dropping single term specified by Hypothesis hypothesis
- lrTest: Likelihood ratio test dropping single term specified by contrast matrix hypothesis
- logLik: return the log-likelihood of a fitted model

Slots

design a data.frame from which variables are taken for the right hand side of the regression
fitC The continuous fit
fitD The discrete fit
response The left hand side of the regression
**Description**

Using the delta method, estimate the log-fold change from a state given by a vector contrast0 and the state(s) given by contrast1.

**Usage**

```r
logFC(zlmfit, contrast0, contrast1)
getLogFC(zlmfit, contrast0, contrast1)
```

**Arguments**

- `zlmfit`: ZlmFit output
- `contrast0`: vector of coefficients giving baseline contrast, or a `Hypothesis`. If missing, then the `(Intercept)` is used as baseline.
- `contrast1`: matrix of coefficients giving comparison contrasts, or a `Hypothesis`. If missing, then all non-(Intercept) coefficients are compared.

**Details**

The log-fold change is defined as follows. For each gene, let $u(x)$ be the expected value of the continuous component, given a covariate $x$ and the estimated coefficients `coefC`, i.e., $u(x) = \text{crossprod}(x, \text{coefC})$. Likewise, let $v(x) = 1/(1+\exp(-\text{crossprod(coefD, x)}))$ be the expected value of the discrete component. The log fold change from contrast0 to contrast1 is defined as

$$u(\text{contrast1})v(\text{contrast1}) - u(\text{contrast0})v(\text{contrast0}).$$

Note that for this to be a log-fold change, then the regression for $u$ must have been fit on the log scale. This is returned in the matrix `logFC`. An approximation of the variance of `logFC` (applying the delta method to formula defined above) is provided in `varLogFC`. 
logmean

Value
list of matrices 'logFC' and 'varLogFC', giving the log-fold-changes for each contrast (columns) and genes (rows) and the estimated sampling variance thereof

Functions
• getLogFC: Return results as a perhaps friendlier data.table

See Also
Hypothesis

Examples
data(vbetaFA)
##log-fold changes in terms of intercept (which is Stim(SEB) and CD154+VbetaResponsive)
lfcStim <- logFC(zz)
##If we want to compare against unstim, we can try the following
coefnames <- colnames(coef(zz, 'D'))
contrast0 <- setNames(rep(0, length(coefnames)), coefnames)
contrast0[c('Intercept', 'Stim.ConditionUnstim')] <- 1
contrast1 <- diag(length(coefnames))
rownames(contrast1) <- colnames(contrast1) <- coefnames
contrast1[('(Intercept)', ]<-1
lfcUnstim <- logFC(zz, contrast0, contrast1)
##log-fold change with itself is 0
stopifnot(all(lfcUnstim$logFC[,2]==0))
##inverse of log-fold change with Stim as reference
stopifnot(all(lfcStim$logFC[,1]==(-lfcUnstim$logFC[,1])))
##As a data.table:
getLogFC(zz)

logmean

Log mean

Description
Takes mean of natural scaled values and then logarithm Approximately the inverse operation of expavg Calculates log2(mean(x) + 1)

Usage
logmean(x)

Arguments
x numeric

Value
numeric
LRT

Likelihood Ratio Tests for SingleCellAssays

Description

Tests for a change in ET binomial proportion or mean of positive ET Likelihood Ratio Test for SingleCellAssay objects

Usage

LRT(sca, comparison, ...)  
## S4 method for signature 'SingleCellAssay,character'
LRT(sca, comparison, referent = NULL,  
    groups = NULL, returnall = FALSE)

Arguments

sca A SingleCellAssay class object
comparison A character specifying the factor for comparison
... ignored
referent A character specifying the reference level of comparison.
groups A optional character specifying a variable on which to stratify the test. For each level of groups, there will be a separate likelihood ratio test.
returnall A logical specifying if additional rows should be returned with information about the different components of the test.

Details

Combined Likelihood ratio test (binomial and normal) for SingleCellAssay and derived objects. This function is deprecated, please use lrTest instead.

Value

data.frame

See Also

zlm.SingleCellAssay, ZlmFit

Examples

data(vbetaFA)
LRT(vbetaFA, 'Stim.Condition', 'Unstim')
Run a likelihood-ratio test

Description

Compares the change in likelihood between the current model and one subject to contrasts tested in hypothesis. hypothesis can be one of a character giving complete factors or terms to be dropped from the model, CoefficientHypothesis giving names of coefficients to be dropped, Hypothesis giving contrasts using the symbolically, or a contrast matrix, with one row for each coefficient in the full model, and one column for each contrast being tested.

Usage

lrTest(object, hypothesis)

Arguments

object LMlike or subclass
hypothesis the hypothesis to be tested. See details.

Value

array giving test statistics

See Also

fit
waldTest
Hypothesis
CoefficientHypothesis

Examples

#see ZlmFit-class for examples
d example('ZlmFit-class')

Description

A 3D array with first dimension being the genes, next dimension giving information about the test (the degrees of freedom, Chisq statistic, and P value), and final dimension being the value of these quantities on the discrete, continuous and hurdle (combined) levels.

Usage

## S4 method for signature 'ZlmFit,character'
lrTest(object, hypothesis)

lrTest,ZlmFit,character-method

Likelihood ratio test
melt.SingleCellAssay

Arguments

- **object**: ZlmFit  
- **hypothesis**: See Details

Value

- 3D array

maits

**MAITs data set, RNASeq**

Description

MAITs data set, RNASeq

Format

A list containing an expression matrix (expressionmat), cell cdat and feature fdat.

See Also

FromMatrix

melt.SingleCellAssay

**Melt a rectangular array**

Description

Return a molten (flat) representation of a rectangular array

Usage

```r
## S3 method for class 'SingleCellAssay'
melt(data, ..., na.rm = FALSE,
     value.name = "value")
```

Arguments

- **data**: A rectangular array, with attributes attached to its rows and columns
- **...**: ignored
- **na.rm**: ignored
- **value.name**: name of 'values' column containing the measurement

Value

A data.frame typically, with the cartesian product of the row and column attributes and the values from the rectangular array

Examples

```r
data(vbetaFA)
as(vbetaFA[1:10,], 'data.table')
```
model.matrix

Model matrix accessor

Description
Model matrix accessor

Usage
model.matrix(object, ...)

## S4 method for signature 'LMlike'
model.matrix(object, ...)

Arguments
- object LMlike or subclass
- ... ignored

Value
model.matrix if present

Methods (by class)
- LMlike: return the model.matrix

model.matrix<-

Replace model matrix

Description
Replace model matrix

Usage
model.matrix(object) <- value

Arguments
- object LMlike or subclass
- value matrix

Value
modify object
myBiplot \hspace{1cm} \textit{Makes a nice BiPlot}

**Description**

Creates a custom BiPlot for visualizing the results of PCA

**Usage**

\[
\text{myBiplot}(\text{pc, colorfactor, scaling = 100, nudge = 1.2, N = 10, dims = 1:2, ...})
\]

**Arguments**

- **pc**: output of \texttt{prcomp}
- **colorfactor**: a factor the same length as nrow(pc$x) to color the points
- **scaling**: integer to scale the vectors showing loadings
- **nudge**: numeric to offset labels for loadings
- **N**: number of variables with longest dim[1] or dim[2] projections to display
- **dims**: numeric vector of length 2 indicating which PCs to plot
- **...**: passed to plot

**Value**

printed plot

---

\textbf{numexp} \hspace{1cm} \textit{Report number of expressing cells per gene}

**Description**

NAs are removed

**Usage**

\[
\text{numexp}(\text{sc})
\]

**Arguments**

- **sc**: SingleCellAssay

**Value**

numeric vector
pbootVcov1

Bootstrap a zlmfit

Description
Sample cells with replacement to find bootstrapped distribution of coefficients

Usage
pbootVcov1(cl, zlmfit, R = 99)

Arguments

cl a cluster object created by makeCluster
zlmfit class ZlmFit
R number of bootstrap replicates

Value
array of bootstrapped coefficients

plot.thresholdSCRNAcountMatrix
Plot cutpoints and densities for thresholding

Description
Plot cutpoints and densities for thresholding

Usage
## S3 method for class 'thresholdSCRNAcountMatrix'
plot(x, ask = FALSE, wait.time = 0,
     type = "bin", indices = NULL, ...)

Arguments

x output of thresholdSCRNAcountMatrix
ask if TRUE then will prompt before displaying each plot
wait.time pause (in seconds) between each plot
type one or more of the following: 'bin' (plot the genes by the binning used for
thresholding), or 'gene' (plot thresholding by gene – see next argument)
indices if type is equal to 'gene', and is a integer of length 1, then a random sample
of indices genes is taken. If it is NULL, then 10 genes are sampled. If it is a
integer vector of length > 1, then it is interpreted as giving a list of indices of
genes to be displayed.
... further arguments passed to plot
plotlrt

## Description

Constructs a forest-like plot of signed log10 p-values, possibly adjusted for multiple comparisons. Adjust can be one of "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", or "none".

## Usage

```r
plotlrt(lr, adjust = "fdr", thres = 0.1, trunc = 1e-06, groups = NULL)
```

## Arguments

- `lr`: output from lrtest, with returnall=FALSE
- `adjust`: character, passed along to `p.adjust`, see below
- `thres`: numeric, genes with adjusted p-values above this value are not depicted
- `trunc`: numeric, p values below this value are truncated at this value
- `groups`: character, grouping value. If provided, must match groups argument passed to lrtest. Plots done separately for each group.

## Value

Constructs a dotplot

## Author(s)

andrew
plotSCAConcordance

Concordance plots of filtered single vs n-cell assays

Description
Plot the average expression value of two subsets of the data. Generally these might be 1 cell and multiple-cell replicates, in which case if the ncol$s column n cells is set then the averages will be adjusted accordingly. But it could be any grouping.

Usage
plotSCAConcordance(SCellAssay, NCellAssay, filterCriteria = list(nOutlier = 2, sigmaContinuous = 9, sigmaProportion = 9), groups = NULL, ...)

Arguments
SCellAssay is a FluidigmAssay for the 1-cell per well assay
NCellAssay is a FluidigmAssay for the n-cell per well assay
filterCriteria is a list of filtering criteria to apply to the SCellAssay and NCellAssay
groups is a character vector naming the group within which to perform filtering. NULL by default.
... passed to getConcordance

Value
printed plot

See Also
getConcordance

Examples
data(vbetaFA)
sca1 <- subset(vbetaFA, ncells==1)
sca100 <- subset(vbetaFA, ncells==100)
plotSCAConcordance(sca1, sca100)

predict.ZlmFit

Return predictions from a ZlmFit object.

Description
Return predictions from a ZlmFit object.

Usage
## S3 method for class 'ZlmFit'
predict(object, newdata = NULL, modelmatrix = NULL, ...)
primerAverage

Arguments

- **object**: A `ZlmFit`
- **newdata**: The data to predict from. Currently ignored, will use the data in the object.
- **modelmatrix**: The model matrix specifying the linear combination of coefficients.
- ... ignored

Value

Predictions and standard errors.

Examples

```r
## See stat_ell
example(stat_ell)
```

---

primerAverage

*Average within duplicated genes/primers*

Description

.

Usage

```r
primerAverage(fd, geneGroups, fun.natural = expavg, fun.cycle = logshift)
```

Arguments

- **fd**: `SingleCellAssay` or subclass
- **geneGroups**: character naming a column in the `featureData` that keys the duplicates
- **fun.natural**: transformation to be used to collapse the duplicate expression values
- **fun.cycle**: transformation to be used after collapsing

Value

collapsed version of `fd`. 
**print.summaryZlmFit**  
*Print summary of a ZlmFit*

**Description**

Shows the top 'n' genes by z score on 'by'

**Usage**

```r
## S3 method for class 'summaryZlmFit'
print(x, n = 2, by = "logFC", ...)
```

**Arguments**

- `x`: output from `summary(ZlmFit)`
- `n`: number of genes to show
- `by`: one of `"C"`, `"D"` or `"logFC"` for continuous, discrete and log fold change z-scores for each contrast
- `...`: ignored

**Value**

prints a pretty table and invisibly returns a `data.table` representing the table.

**See Also**

`summary.ZlmFit-method`

---

**read.fluidigm**

**read.fluidigm**

**Description**

Reads a fluidigm raw data file (or set of files)

**Usage**

```r
read.fluidigm(files = NULL, metadata = NULL, header.size = 2, skip = 8,
cycle.threshold = 40, metadataColClasses = NULL, meta.key = NULL,
idvars = NULL, splitby = NULL, unique.well.id = "Chamber.ID",
raw = TRUE, assay = NULL, geneid = "Assay.Name", sample = NULL,
well = "Well", measurement = "X40.Ct", measurement.processed = "Ct",
cells = "SampleRConc")
```
Arguments

files  A character vector of files to read.
metadata  A character path and filename of a CSV file containing additional metadata about the samples
header.size  A numeric indicating the number of lines in the header (default 2)
skip  numeric how many lines to skip before reading (default 8)
cycle.threshold  The maximum number of PCR cycles performed (default 40) numeric
metadataColClasses  Optional character vector giving the column classes of the metadata file. See read.table.
meta.key  Optional character vector that identifies the key column between the metadata and the fluidigm data
idvars  Optional character vector that defines the set of columns uniquely identifying a well (unique cell, gene, and condition).
splitby  Optional character that defines the column / variable used to split the resulting data into a list of SingleCellAssay, such that unique levels of splitby each fall into their own SingleCellAssay. Usuually the experimental unit subjected to different treatments.
unique.well.id  The column that uniquely identifies a sample well in the data. Default is "Chamber.ID".
raw  logical flag indicating this is raw data coming off the instrument. Thus we make some assumptions about the column names that are present.
assay  character name of a column that uniquely identifies an Assay (i.e. gene). Default is NULL
geneid  character names of the column that identifies a gene. Default is "Assay.Name"
sample  character name of a column that uniquely identifies a sample
well  character name of a column that uniquely identifies a well. Default "Well"
measurement  character name of the column that holds the measurement. Default "X40.Ct".
measurement.processed  character one of "Ct","40-Ct", or "et". If not "Ct", the measurement will be transformed.
ncells  The column with the number of cells in this well.

Details

This function reads a raw Fluidigm data file or set of files and constructs a SingleCellAssay (or FluigidmAssay) object.

Value

list of SingleCellAssay holding the data.

Author(s)

Greg Finak
**removeResponse**

*Remove the left hand side (response) from a formula*

**Description**

The order of terms will be rearrange to suit R’s liking for hierarchy but otherwise the function should be idempotent for

**Usage**

```r
removeResponse(Formula, warn = TRUE)
```

**Arguments**

- `Formula`:
  - formula
- `warn`:
  - Issue a warning if a response variable is found?

**Value**

`formula`

**Author(s)**

Andrew

---

**rstandard.bayesglm**

*rstandard for bayesglm objects.*

**Description**

rstandard bayesglm object S3 method

**Usage**

```r
## S3 method for class 'bayesglm'
rstandard(model, infl = influence(model, do.coef = FALSE),
  type = c("deviance", "pearson"), ...)
```

**Arguments**

- `model`:
  - bayesglm
- `infl`:
  - see `rstandard`
- `type`:
  - see `rstandard`
- `...`:
  - ignored

**Value**

numeric residuals
se.coef

Return coefficient standard errors

Description
Given a fitted model, return the standard errors of the coefficient

Usage
se.coef(object, ...)

Arguments

object a model implementing vcov

... passed to methods

Value
vector or matrix

See Also
ZlmFit-class

Examples
# see ZlmFit-class for examples
eample('ZlmFit-class')

show,LMlike-method

Description
Display info

Usage
## S4 method for signature 'LMlike'
show(object)

## S4 method for signature 'ZlmFit'
show(object)

Arguments

object an object of some type

Details
Prints information on a LMlike object
split, SingleCellAssay, character-method

Value
side effect of printing to console

Methods (by class)
• ZlmFit: print info on ZlmFit

Description
Splits a SingleCellAssay into a list by a factor (or something coercible into a factor) or a character giving a column of colData(x)

Usage
## S4 method for signature 'SingleCellAssay,character'
split(x, f, drop = FALSE, ...)

Arguments
x SingleCellAssay
f length-1 character, or atomic of length ncol(x)
drop drop unused factor levels
... ignored

Value
List

Examples
data(vbetaFA)
split(vbetaFA, 'ncells')
fa <- as.factor(colData(vbetaFA)$ncells)
split(vbetaFA, fa)
stat_ell  

Plot confidence ellipse in 2D

Description

The focus of the ellipse will be the point (x, y) and semi-major axes aligned with the coordinate axes and scaled by xse, yse and the level.

Usage

stat_ell(mapping = NULL, data = NULL, geom = "polygon", position = "identity", na.rm = FALSE, show.legend = NA, inherit.aes = TRUE, fill = NA, level = 0.95, lty = 2, invert = FALSE, alpha = 1, ...)

Arguments

mapping  Set of aesthetic mappings created by aes or aes_. If specified and inherit.aes = TRUE (the default), it is combined with the default mapping at the top level of the plot. You must supply mapping if there is no plot mapping.

data  The data to be displayed in this layer. There are three options: If NULL, the default, the data is inherited from the plot data as specified in the call to ggplot. A data.frame, or other object, will override the plot data. All objects will be fortified to produce a data frame. See fortify for which variables will be created.

geom  The geometric object to use display the data

position  Position adjustment, either as a string, or the result of a call to a position adjustment function.

na.rm  If FALSE (the default), removes missing values with a warning. If TRUE silently removes missing values.

show.legend  logical. Should this layer be included in the legends? NA, the default, includes if any aesthetics are mapped. FALSE never includes, and TRUE always includes.

inherit.aes  If FALSE, overrides the default aesthetics, rather than combining with them. This is most useful for helper functions that define both data and aesthetics and shouldn't inherit behaviour from the default plot specification, e.g. borders.

fill  A color or aesthetic mapping to fill color. Defaults to NA for empty ellipses.

level  The confidence level at which to draw an ellipse (default is level=0.95).

lty  The linetype to use. Can map to a variable. Defaults to 2 (dashed line)

invert  vector of length 1 that should either be "x", "y", or TRUE. Specifies whether to plot the estimates from the discrete component on the inverse logit scale. invert specifies which axis to invert.

alpha  transparency

...  other arguments passed on to layer. These are often aesthetics, used to set an aesthetic to a fixed value, like color = "red" or size = 3. They may also be parameters to the paired geom/stat.
**Value**

`ggplot` layer

**Examples**

```r
data(vbetaFA)
library(ggplot2)
zlmCond <- zlm(~Stim.Condition, vbetaFA[1:10,])
MM <- model.matrix(~Stim.Condition, unique(colData(vbetaFA)[,c("Stim.Condition"), drop=FALSE]))
predicted <- predict(zlmCond, model.matrix=MM)
plt <- ggplot(predicted)+aes(x=invlogit(muD), y=muC, xse=seD, yse=seC, col=sample)+
  facet_wrap(~primerid, scales="free_y")+theme_linedraw()+
  geom_point(size=0.5)+scale_x_continuous("Proportion expression")+
  scale_y_continuous("Estimated Mean")+
  stat_ell(aes(x=muD, y=muC), level=0.95, invert=’x’)
## plot with inverse logit transformed x-axis
print(plt)
# doesn't do anything in this case because there are no inestimable coefficients
predictI <- impute(predicted, groupby=’primerid’)
```

**subset,SingleCellAssay-method**

*Subset a SingleCellAssay by cells (columns)*

**Description**

Evaluates the expression in ... in the context of `colData(x)` and returns a subsetted version of `x`

**Usage**

```r
## S4 method for signature 'SingleCellAssay'
subset(x, ...)
```

**Arguments**

- `x`: `SingleCellAssay`
- `...`: `expression`

**Value**

`SingleCellAssay`

**Examples**

```r
data(vbetaFA)
subset(vbetaFA, ncells==1)
```
summarize

Return programmatically useful summary of a fit

Description

Return programmatically useful summary of a fit

Usage

summarize(object, ...)

Arguments

object 
LMlike or subclass
...
other arguments

Value

list of parameters characterizing fit

summarize,GSEATests-method

Summarize gene set enrichment tests

Description

Returns a data.table with one row per gene set. This data.table contains columns:

set name of gene set
cond.Z Z statistic for continuous component
cont.P wald P value
cont_effect difference in continuous regression coefficients between null and test sets (ie, the numerator of the Z-statistic.)
disc.Z Z statistic for discrete
disc.P wald P value
disc_effect difference in discrete regression coefficients between null and test sets.
combined.Z combined discrete and continuous Z statistic using Stouffer’s method
combined.P combined P value
combined_adj FDR adjusted combined P value

Usage

## S4 method for signature 'GSEATests'
summary(object, ...)

---

**summarize**

Return programmatically useful summary of a fit

**Description**

Return programmatically useful summary of a fit

**Usage**

summarize(object, ...)

**Arguments**

- object: LMlike or subclass
- ...: other arguments

**Value**

list of parameters characterizing fit

**summary,GSEATests-method**

Summarize gene set enrichment tests

**Description**

Returns a data.table with one row per gene set. This data.table contains columns:

- set: name of gene set
- cond.Z: Z statistic for continuous component
- cont.P: wald P value
- cont_effect: difference in continuous regression coefficients between null and test sets (ie, the numerator of the Z-statistic.)
- disc.Z: Z statistic for discrete
- disc.P: wald P value
- disc_effect: difference in discrete regression coefficients between null and test sets.
- combined.Z: combined discrete and continuous Z statistic using Stouffer’s method
- combined.P: combined P value
- combined_adj: FDR adjusted combined P value

**Usage**

```r
## S4 method for signature 'GSEATests'
summary(object, ...)
```
Arguments

object A GSEATests object
... passed to calcZ

Value

data.table

See Also

gseaAfterBoot

Examples

## See the examples in gseaAfterBoot
eexample(gseaAfterBoot)

---

**summary.ZlmFit-method**  
Summarize model features from a ZlmFit object

Description

Returns a data.table with a special print method that shows the top 2 most significant genes by contrast. This data.table contains columns:

- **primerid**: the gene
- **component**: C=continuous, D=discrete, logFC=log fold change, S=combined using Stouffer’s method, H=combined using hurdle method
- **contrast**: the coefficient/contrast of interest
- **ci.hi**: upper bound of confidence interval
- **ci.lo**: lower bound of confidence interval
- **coef**: point estimate
- **z**: z score (coefficient divided by standard error of coefficient)
- **Pr(>Chisq)**: likelihood ratio test p-value (only if doLRT=TRUE)

Some of these columns will contain NAs if they are not applicable for a particular component or contrast.

Usage

```r
## S4 method for signature 'ZlmFit'
summary(object, logFC = TRUE, doLRT = FALSE, level = 0.95, ...)
```

Arguments

- **object**: A ZlmFit object
- **logFC**: If TRUE, calculate log-fold changes, or output from a call to getLogFC.
- **doLRT**: if TRUE, calculate lrTests on each coefficient, or a character vector of such coefficients to consider.
- **level**: what level of confidence coefficient to return. Defaults to 95 percent.
- **...**: ignored
Value

data.table

See Also

print.summaryZlmFit

Examples

data(vbetaFA)
z <- zlm(~Stim.Condition, vbetaFA[1:5,])
zs <- summary(z)
names(zs)
print(zs)
##Select 'datatable' copmonent to get normal print method
zs$datatable

summary.thresholdSCRNA

Summarize the effect of thresholding

Description

Returns the proportion of (putative) expression, the variance of expressed cells, and -log10 shapiro-wilk tests for normality on the expressed cells

Usage

## S3 method for class 'thresholdSCRNA'
summary(object, ...)

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

Arguments

object a thresholdSCRNA
... currently ignored
x a summaryThresholdSCRNA object, ie output from summary.thresholdSCRNA

Value

a list of statistics on the original data, and thresholded data

Methods (by generic)

* print: prints five-number distillation of the statistics and invisibly returns the table used to generate the summary
**thresholdSCRNACountMatrix**

Threshold a count matrix using an adaptive threshold.

---

**Description**

An adaptive threshold is calculated from the conditional mean of expression, based on 10 bins of the genes with similar expression levels. Thresholds are chosen by estimating cutpoints in the bimodal density estimates of the binned data.

**Usage**

```r
thresholdSCRNACountMatrix(data_all, conditions = NULL, cutbins = NULL,
                          nbins = 10, bin_by = "median", qt = 0.975, min_per_bin = 50,
                          absolute_min = 0, data_log = TRUE, adj = 1)
```

**Arguments**

- `data_all` matrix of (possibly log-transformed) counts or TPM. Rows are genes and columns are cells.
- `conditions` Bins are be determined per gene and per condition. Typically contrasts of interest should be specified.
- `cutbins` vector of cut points.
- `nbins` integer number of bins when cutbins is not specified.
- `bin_by` character "median", "proportion", "mean"
- `qt` when bin_by is "quantile", what quantile should be used to form the bins
- `min_per_bin` minimum number of genes within a bin
- `absolute_min` numeric giving a hard threshold below which everything is assumed to be noise
- `data_log` is `data_all` log+1 transformed? If so, it will be returned on the (log+1)-scale as well.
- `adj` bandwith adjustment, passed to `density`

**Value**

list of thresholded counts (on natural scale), thresholds, bins, densities estimated on each bin, and the original data

**Examples**

```r
data(maits,package='MAST', envir = environment())
sca <- FromMatrix(t(maits$expressionmat[,1:1000]), maits$cdat, maits$fdat[1:1000,])
tt <- thresholdSCRNACountMatrix(assay(sca))
tt <- thresholdSCRNACountMatrix(2^assay(sca)-1, data_log=FALSE)
opar <- par()
on.exit(par(opar))
par(mfrow=c(4,2))
plot(tt)
```
waldTest

Description

Run a Wald test on discrete and continuous components. Hypothesis can be one of a character giving complete factors or terms to be dropped from the model, CoefficientHypothesis giving names of coefficients to be dropped, Hypothesis giving contrasts using the symbolically, or a contrast matrix, with one row for each coefficient in the full model, and one column for each contrast being tested.

Usage

waldTest(object, hypothesis)

Arguments

object LMLike or subclass
hypothesis the hypothesis to be tested. See details.
waldTest,ZlmFit,matrix-method

Value

array giving test statistics

See Also

fit
lt
lt

Examples

#see ZlmFit-class for examples
eexample('ZlmFit-class')

Description

A 3D array with first dimension being the genes, next dimension giving information about the test
(the degrees of freedom, Chisq statistic, and P value), and final dimension being the value of these
quantities on the discrete, continuous and hurdle (combined) levels.

Usage

## S4 method for signature 'ZlmFit,matrix'
waldTest(object, hypothesis)

Arguments

object ZlmFit
hypothesis See Details

Value

3D array
xform

Make matrix of continuous expression values, orthogonal to discrete

Description
This centers each column of mat around the mean of its non-zero values.

Usage

\[
xform(mat, scale = FALSE)
\]

Arguments

- mat: matrix (such as produced by exprs)
- scale: should the columns also be scaled to have unit variance

Value

matrix

zlm

Convenience function for running a zero-inflated regression

Description

Fits a hurdle model on zero-inflated continuous data in which the zero process is modeled as a logistic regression and (conditional on the the response being >0), the continuous process is Gaussian, ie, a linear regression.

Usage

\[
zlm(formula, data, method = \text{"bayesglm"}, silent = \text{TRUE}, \ldots)\]

Arguments

- formula: model formula
- data: a data.frame, list, environment or SingleCellAssay in which formula is evaluated
- method: one of ‘glm’, ‘glmer’ or ‘bayesglm’. See MAST:::methodDict for other possibilities.
- silent: if TRUE suppress common errors from fitting continuous part
- \ldots: passed to fit, and eventually to the linear model fitting function

Value

list with "disc"rete part and "cont"inuous part

See Also

GLMlike, LMERlike, BayesGLMlike
Examples

data <- data.frame(x=rnorm(500), z=rbinom(500, 1, .3))
logit.y <- with(data, x*2 + z*2); mu.y <- with(data, 10+10*x+10*z + rnorm(500))
y <- (runif(500)<exp(logit.y)/(1+exp(logit.y)))*1
y[y>0] <- mu.y[y>0]
data$y <- y
fit <- zlm(y ~ x+z, data)
summary.glm(fit$disc)
summary.glm(fit$cont)


Description

For each gene in sca, fits the hurdle model in formula (linear for et>0), logistic for et==0 vs et>0. Return an object of class ZlmFit containing slots giving the coefficients, variance-covariance matrices, etc. After each gene, optionally run the function on the fit named by ’hook’

Usage

zlm.SingleCellAssay(formula, sca, method = "bayesglm", silent = TRUE, 
  ebayes = TRUE, ebayesControl = NULL, force = FALSE, hook = NULL, 
  parallel = TRUE, LMlike, onlyCoef = FALSE, ...)

Arguments

  formula a formula with the measurement variable on the LHS and predictors present in 
colData on the RHS
  sca SingleCellAssay object
  method character vector, either ’glm’, ’glmer’ or ’bayesglm’
  silent Silence common problems with fitting some genes
  ebayes if TRUE, regularize variance using empirical bayes method
  ebayesControl list with parameters for empirical bayes procedure. See ebayes.
  force Should we continue testing genes even after many errors have occurred?
  hook a function called on the fit after each gene.
  parallel If TRUE and option(mc.cores)>1 then multiple cores will be used in fitting.
  LMlike if provided, then the model defined in this object will be used, rather than following the formulas. This is intended for internal use.
  onlyCoef If TRUE then only an array of model coefficients will be returned (probably only useful for bootstrapping).
  ... arguments passed to the S4 model object upon construction. For example, 
  fitArgsC and fitArgsD, or coefPrior.

Value

  a object of class ZlmFit with methods to extract coefficients, etc.
Empirical Bayes variance regularization

The empirical bayes regularization of the gene variance assumes that the precision (1/variance) is drawn from a gamma distribution with unknown parameters. These parameters are estimated by considering the distribution of sample variances over all genes. The procedure used for this is determined from `ebayesControl`, a named list with components 'method' (one of 'MOM' or 'MLE') and 'model' (one of 'H0' or 'H1') method MOM uses a method-of-moments estimator, while MLE using the marginal likelihood. H0 model estimates the precisions using the intercept alone in each gene, while H1 fits the full model specified by formula

See Also

`ebayes`, `glmlike-class`, `ZlmFit-class`, `BayesGLMlike-class`

Examples

data(vbetaFA)
zlmVbeta <- zlm.SingleCellAssay(~ Stim.Condition, subset(vbetaFA, ncells==1)[1:10,])
slotNames(zlmVbeta)
  #A matrix of coefficients
  coef(zlmVbeta, 'D')[['CCL2',]
  #An array of covariance matrices
  vcov(zlmVbeta, 'D')[,,'CCL2']
  waldTest(zlmVbeta, CoefficientHypothesis('Stim.ConditionUnstim'))

ZlmFit-class

An S4 class to hold the output of a call to zlm

Description

This holds output from a call to zlm.SingleCellAssay. Many methods are defined to operate on it. See below.

Usage

````
## S4 method for signature 'ZlmFit,CoefficientHypothesis'
lrTest(object, hypothesis)
```

````
## S4 method for signature 'ZlmFit,Hypothesis'
lrTest(object, hypothesis)
```

````
## S4 method for signature 'ZlmFit,matrix'
lrTest(object, hypothesis)
```

````
## S4 method for signature 'ZlmFit,CoefficientHypothesis'
waldTest(object, hypothesis)
```

````
## S4 method for signature 'ZlmFit,Hypothesis'
waldTest(object, hypothesis)
```

````
## S4 method for signature 'ZlmFit'
coef(object, which, ...)
```
## S4 method for signature 'ZlmFit'
vcov(object, which, ...)

## S4 method for signature 'ZlmFit'
se.coef(object, which, ...)

### Arguments

- **object**: `ZlmFit`
- **hypothesis**: call to `Hypothesis` or `CoefficientHypothesis` or a matrix giving such contrasts.
- **which**: character vector, one of "C" (continuous) or "D" (discrete) specifying which component should be returned
- **...**: ignored

### Value

see "Methods (by generic)"

### Methods (by generic)

- 1rTest: Returns an array with likelihood-ratio tests on contrasts defined using `CoefficientHypothesis()`.
- 1rTest: Returns an array with likelihood-ratio tests specified by `Hypothesis`, which is a `Hypothesis`.
- 1rTest: Returns an array with likelihood-ratio tests specified by `Hypothesis`, which is a contrast matrix.
- waldTest: Returns an array with Wald Tests on contrasts defined using `CoefficientHypothesis()`.
- waldTest: Returns an array with Wald Tests on contrasts defined in `Hypothesis()`
- coef: Returns the matrix of coefficients for component which.
- vcov: Returns an array of variance/covariance matrices for component which.
- se.coef: Returns a matrix of standard error estimates for coefficients on component which.

### Slots

- `coefC`: matrix of continuous coefficients
- `coefD`: matrix of discrete coefficients
- `vcovC`: array of variance/covariance matrices for coefficients
- `vcovD`: array of variance/covariance matrices for coefficients
- `LMlike`: the LmWrapper object used
- `sca`: the SingleCellAssay object used
- `deviance`: matrix of deviances
- `loglik`: matrix of loglikelihoods
- `df.null`: matrix of null (intercept only) degrees of freedom
- `df.resid`: matrix of residual DOF
- `dispersion`: matrix of dispersions (after shrinkage)
- `dispersionNoShrink`: matrix of dispersion (before shrinkage)
- `priorDOF`: shrinkage weight in terms of number of psuedo-obs
priorVar  shrinkage target
converged  output that may optionally be set by the underlying modeling function
hookOut  a list of length ngenes containing output from a hook function, if zlm was called with one

See Also
zlml.SingleCellAssay summary,zlmFit-method

Examples

data(vbetaFA)
zlmVbeta <- zlm.SingleCellAssay(~ Stim.Condition+Population, subset(vbetaFA, ncells==1)[1:10,])
#Coefficients and standard errors
coef(zlmVbeta, 'D')
coef(zlmVbeta, 'C')
se.coef(zlmVbeta, 'C')
#Test for a Population effect by dropping the whole term (a 5 degree of freedom test)
lrTest(zlmVbeta, 'Population')
#Test only if the VbetaResponsive cells differ from the baseline group
lrTest(zlmVbeta, CoefficientHypothesis('PopulationVbetaResponsive'))
# Test if there is a difference between CD154+/Unresponsive and CD154-/Unresponsive.
# Note that because we parse the expression
# the columns must be enclosed in backquotes
# to protect the \quote{+} and \quote{-} characters.
lrTest(zlmVbeta, Hypothesis("PopulationCD154+VbetaUnresponsive" ~
  'PopulationCD154-VbetaUnresponsive'))
waldTest(zlmVbeta, Hypothesis("PopulationCD154+VbetaUnresponsive" ~
  'PopulationCD154+VbetaUnresponsive'))
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