

# Package ‘MAST’

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

**License** GPL(>= 2)

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---

MAST-package

*MAST: Model-based Analysis of Single- cell Transcriptomics*


---

## Description

Methods for analysing single cell assay data using hurdle models.

## Details

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

## Author(s)

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Authors:

- Greg Finak <gfinak@fredhutch.org>
- Masanao Yajima <myajima@fredhutch.org>

## References

Finak, et al. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome Biology* (2015).

## See Also

Useful links:

- <https://github.com/RGLab/MAST/>
- Report bugs at <https://github.com/RGLab/MAST/issues>

---

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 | <i>Wrapper for bayesian GLM</i> |
|--------------------|---------------------------------|

---

**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?

---

|       |                                                                       |
|-------|-----------------------------------------------------------------------|
| calcZ | <i>Get Z or T statistics and P values after running gseaAfterBoot</i> |
|-------|-----------------------------------------------------------------------|

---

**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**

```
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'iguous 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
example(gseaAfterBoot)
```

---

```
colData<-,SingleCellAssay,DataFrame-method
```

*Replace colData*

---

**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**

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

**Arguments**

|       |                 |
|-------|-----------------|
| x     | SingleCellAssay |
| value | DataFrame       |

**Value**

modified SingleCellAssay

---

|                  |                                              |
|------------------|----------------------------------------------|
| collectResiduals | <i>Residual hooks and collection methods</i> |
|------------------|----------------------------------------------|

---

### 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

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

discrete_residuals_hook(x)

continuous_residuals_hook(x)

combined_residuals_hook(x)

deviance_residuals_hook(x)

fitted_phat(x)

partialScore(x, effectRegex)
```

### Arguments

|                           |                                                                                                                                         |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>            | <code>ZlmFit</code> -class                                                                                                              |
| <code>sca</code>          | <code>SingleCellAssay</code> object to which the residuals should be added                                                              |
| <code>newLayerName</code> | character name of the assay layer                                                                                                       |
| <code>effectRegex</code>  | 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, ie, difference between expected probability of expression and observed
- `continuous_residuals_hook()`: Hook to get the continuous residuals, ie, 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, ie,  $Y-E(U)*E(V)$

- `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  $p_{\text{hat}}$ , 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`

### Examples

```
data(vbetaFA)
svbeta <- subset(vbetaFA, ncells==1)
svbeta <- svbeta[freq(svbeta)>.4,]
window <- function(x1) lapply(assays(x1), function(x2) x2[1:3, 1:6])
#total residuals of the response
z1 <- zlm(~ Stim.Condition, svbeta, hook=discrete_residuals_hook)
window(collectResiduals(z1, svbeta))
z2 <- zlm(~ Stim.Condition, svbeta, hook=continuous_residuals_hook)
window(collectResiduals(z2, svbeta))
z3 <- zlm(~ Stim.Condition, svbeta, hook=combined_residuals_hook)
window(collectResiduals(z3, svbeta))
#partial residuals
colData(svbeta)$ngeneson <- colMeans(assay(svbeta)>0)
z5 <- zlm(~ 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)
```

---

```
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**

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

---

CovFromBoots

*Extract the inter-gene covariance matrices for continuous and discrete components of a MAST model for a given coefficient from bootstrap replicates*

---

**Description**

Computes the genewise covariance for a model coefficient from bootstrap replicates from ‘MAST::bootVcov1()’. If coefficients are unestimable (i.e. NA) for a gene, that row/column in the covariance matrix will be NA. Returns a list with components "C" and "D" containing the covariance matrices for the "C"ontinuous and "D"iscrete components of the MAST model.

**Usage**

```
CovFromBoots(boots = NULL, coefficient = NULL)
```

**Arguments**

|             |                                                                                                       |
|-------------|-------------------------------------------------------------------------------------------------------|
| boots       | a multidimensional array returned by ‘bootVcov1’ or ‘pbootVcov1’.                                     |
| coefficient | ‘character’ the name of the model coefficient for which to return the inter-gene covariance matrices. |

**Value**

list with components "C" and "D" containing covariance matrices for the continuous and discrete components of the model.

---

|              |                                                                            |
|--------------|----------------------------------------------------------------------------|
| defaultPrior | <i>Initialize a prior to be used a prior for BayeGLMlike/BayesGLMlike2</i> |
|--------------|----------------------------------------------------------------------------|

---

**Description**

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

**Usage**

```
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**

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

## End(Not run)
```

---

|     |                                                  |
|-----|--------------------------------------------------|
| dof | <i>Degrees of freedom of Zero inflated model</i> |
|-----|--------------------------------------------------|

---

**Description**

Degrees of freedom of Zero inflated model

**Usage**

```
dof(object)
```

**Arguments**

object                      LMlike or subclass

**Value**

vector giving the model degrees of freedom for continuous and discrete

---

|      |                                               |
|------|-----------------------------------------------|
| Drop | <i>Drop specified dimension from an array</i> |
|------|-----------------------------------------------|

---

**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 | <i>Estimate hyperparameters for hierarchical variance model for continuous component</i> |
|--------|------------------------------------------------------------------------------------------|

---

**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 mm

**Usage**

ebayes(assay\_t, ebayesControl, mm, truncate = Inf)

**Arguments**

|               |                                                                                                      |
|---------------|------------------------------------------------------------------------------------------------------|
| assay_t       | cells X genes matrix                                                                                 |
| ebayesControl | list with (optional) components 'method', 'model'. See details.                                      |
| mm            | a model matrix, 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 (v) and prior observations (df), inside a structure, with component hess, giving the Fisher Information of the hyperparameters.

---

|        |                            |
|--------|----------------------------|
| expavg | <i>Exponential average</i> |
|--------|----------------------------|

---

**Description**

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

**Usage**

```
expavg(x)
```

**Arguments**

|   |         |
|---|---------|
| x | numeric |
|---|---------|

**Value**

numeric

**Examples**

```
x <- 1:10
logmean(expavg(x))
```

---

```
filterLowExpressedGenes
```

*Filter low-expressing genes*

---

### Description

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

### Usage

```
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

```
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

```
fit(object, response, ...)

## S4 method for signature 'LMERlike,missing'
fit(object, response, silent = TRUE, ...)
```

### 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

---

*Summary statistics for genes in an experiment***Description**

freq returns the frequency of expression, i.e., the proportion of non-zero values in sc. NAs can be optionally removed

**Usage**

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

```
condmean(sc)
```

```
condSd(sc)
```

```
numexp(sc)
```

**Arguments**

sc                    SingleCellAssay

na.rm                should NAs be removed, or carried through?

**Value**

vector of proportions

**Functions**

- `condmean()`: Report the mean non-zero expression value for each gene. NAs are always removed.
- `condSd()`: Report standard deviation of expression, for positive et for each gene. NAs are always removed.
- `numexp()`: Report number of expressing cells ( $>0$ ) per gene. NAs are removed.

**Examples**

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

---

|            |                                                                                                       |
|------------|-------------------------------------------------------------------------------------------------------|
| FromFlatDF | <i>Construct a SingleCellAssay (or derived subclass) from a 'flat' (melted) data.frame/data.table</i> |
|------------|-------------------------------------------------------------------------------------------------------|

---

## Description

SingleCellAssay are a generic container for such data and are simple wrappers around SummarizedExperiment objects. Subclasses exist that imbue the container with additional attributes, eg [FluidigmAssay](#).

## Usage

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

## 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        | desired subclass of object. Default SingleCellAssay.                                                                                                                                                                                                                    |
| check_sanity | (default: TRUE) Set FALSE to override sanity checks that try to ensure that the default assay is log-transformed and has at least one exact zero. See <a href="#">defaultAssay</a> for details on the "default assay" which is assumed to contain log transformed data. |
| ...          | 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",
  check_sanity = TRUE,
  check_logged = check_sanity
)
```

**Arguments**

|            |                                                                                                                                           |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| exprsArray | matrix, or a list of matrices, or an array. Columns are cells, rows are genes.                                                            |
| cData      | cellData an object that can be coerced to a DataFrame, ie, data.frame, AnnotatedDataFrame. Must have as many rows as ncol(exprsArray)     |
| fData      | featureData an object that can be coerced to a DataFrame, ie, data.frame, AnnotatedDataFrame. Must have as many rows as nrow(exprsArray). |
| class      | desired subclass of object. Default SingleCellAssay.                                                                                      |

`check_sanity` (default: TRUE) Set FALSE to override sanity checks that try to ensure that the default assay is log-transformed and has at least one exact zero. See [defaultAssay](#) for details on the "default assay" which is assumed to contain log transformed data.

`check_logged` alias for `check_sanity`

**Value**

an object of class `class`

**See Also**

`defaultAssay`

**Examples**

```
ncells <- 10
ngenes <- 5
fData <- data.frame(primerid=LETTERS[1:ngenes])
cData <- data.frame(wellKey=seq_len(ncells))
mat <- matrix(rnorm(ncells*ngenes), nrow=ngenes)
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
cData$ncells <- 1
fd <- FromMatrix(mat, cData, fData)
stopifnot(inherits(fd, 'SingleCellAssay'))
```

---

`getConcordance`

*Get the concordance between two experiments*

---

**Description**

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.

**Usage**

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

```
getwss(concord, nexp)
```

```
getss(concord)
```

```
getrc(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                               |

**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 | <i>Accessor for wellKey</i> |
|------------|-----------------------------|

---

**Description**

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

**Usage**

```
getwellKey(sc)
```

**Arguments**

|    |                          |
|----|--------------------------|
| sc | An object with a wellKey |
|----|--------------------------|

**Value**

integer giving the unique id generated

**Examples**

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

---

|               |                                   |
|---------------|-----------------------------------|
| GLMlike-class | <i>Wrapper for regular glm/lm</i> |
|---------------|-----------------------------------|

---

**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(GLMlike)`: return the variance/covariance of component which

**Slots**

`weightFun` function to map expression values to probabilities of expression. Currently unused.

---

gseaAfterBoot

*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 = gsea_control(n_randomize = Inf, var_estimate = "bootall")
)

gsea_control(n_randomize = Inf, var_estimate = "bootall")
```

**Arguments**

|                           |                                                                                                                                                      |
|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>zFit</code>         | object of class <code>ZlmFit</code>                                                                                                                  |
| <code>boots</code>        | bootstraps of <code>zFit</code>                                                                                                                      |
| <code>sets</code>         | list of indices of genes                                                                                                                             |
| <code>hypothesis</code>   | a <code>Hypothesis</code> to test. Currently only one degree <code>CoefficientHypothesis</code> are supported.                                       |
| <code>control</code>      | parameters as provided by <code>gsea_control</code> . See details.                                                                                   |
| <code>n_randomize</code>  | the number of genes to sample to approximate the non-module average expression. Set to <code>Inf</code> to turn off the approximation (the default). |
| <code>var_estimate</code> | the method used to estimate the variance of the modules, one of <code>bootall</code> , <code>bootdiag</code> , or <code>modelbased</code> .          |

**Value**

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

**Functions**

- `gsea_control()`: set control parameters. See Details.

**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(~Stim.Condition, vb1)
boots = bootVcov1(zf, 5)
sets = list(A=1:5, B=3:10, C=15, D=1:5)
gsea = gseaAfterBoot(zf, boots, sets, CoefficientHypothesis('Stim.ConditionUnstim'))
## Use a model-based estimate of the variance/covariance.
gsea_mb = gseaAfterBoot(zf, boots, sets, CoefficientHypothesis('Stim.ConditionUnstim'),
control = gsea_control(var_estimate = 'modelbased'))
calcZ(gsea)
summary(gsea)
```

---

|                 |                                                   |
|-----------------|---------------------------------------------------|
| GSEATests-class | <i>An S4 class for Gene Set Enrichment output</i> |
|-----------------|---------------------------------------------------|

---

**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 | <i>Selectively muffle warnings based on output</i> |
|-------------|----------------------------------------------------|

---

**Description**

Selectively muffle warnings based on output

**Usage**

```
hushWarning(expr, regexp)
```

**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 | <i>Describe a linear model hypothesis to be tested</i> |
|------------|--------------------------------------------------------|

---

### 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

|                         |                                                                                                                                                                                                                                  |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>hypothesis</code> | a character vector specifying a hypothesis, following <code>makeContrasts</code> , or a character vector naming coefficients to be dropped.                                                                                      |
| <code>terms</code>      | an optional character vector giving the terms (column names from the <code>model.matrix</code> ) 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` `waldTest` `lrTest`

### Examples

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

---

|                     |                                                          |
|---------------------|----------------------------------------------------------|
| <code>impute</code> | <i>impute missing continuous expression for plotting</i> |
|---------------------|----------------------------------------------------------|

---

### 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**

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

---

|                    |                                  |
|--------------------|----------------------------------|
| influence.bayesglm | <i>Influence bayesglm object</i> |
|--------------------|----------------------------------|

---

**Description**

The influence function

**Usage**

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

**Arguments**

|         |                                   |
|---------|-----------------------------------|
| model   | bayesglm                          |
| do.coef | see <a href="#">influence.glm</a> |
| ...     | ignored                           |

**Value**

see [influence.glm](#)

---

|          |                                           |
|----------|-------------------------------------------|
| invlogit | <i>Inverse of logistic transformation</i> |
|----------|-------------------------------------------|

---

**Description**

Inverse of logistic transformation

**Usage**

```
invlogit(x)
```

**Arguments**

|   |         |
|---|---------|
| x | numeric |
|---|---------|

**Value**

numeric

**Examples**

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

---

|                |                               |
|----------------|-------------------------------|
| LMERlike-class | <i>Wrapper for lmer/glmer</i> |
|----------------|-------------------------------|

---

**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, keepDefaultCoef = FALSE, ...)

## 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)
```

**Arguments**

|                 |                                                                                                            |
|-----------------|------------------------------------------------------------------------------------------------------------|
| object          | LMERlike                                                                                                   |
| formula.        | formula                                                                                                    |
| design          | something coercible to a data.frame                                                                        |
| keepDefaultCoef |                                                                                                            |
|                 | logical. Should the coefficient names be preserved from object or updated if the model matrix has changed? |
| ...             | 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(LMERlike)`: update the formula or design matrix
- `vcov(LMERlike)`: return the variance/covariance of component which
- `coef(LMERlike)`: return the coefficients. The horrendous hack is attempted to be undone.
- `logLik(LMERlike)`: return the log-likelihood

**Slots**

`pseudoMM` part of this horrendous hack.

`strictConvergence` logical (default: TRUE) 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, keepDefaultCoef = FALSE, ...)

## 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                                                                        |
| keepDefaultCoef | logical. Should the coefficient names be preserved from object or updated if the model matrix has changed? |
| ...             | passed to model.matrix                                                                                     |
| hypothesis      | one of a CoefficientHypothesis, Hypothesis or contrast matrix.                                             |

**Value**

see section "Methods (by generic)"

**Methods (by generic)**

- `summary(LMlike)`: Print a summary of the coefficients in each component.
- `update(LMlike)`: update the formula or design from which the `model.matrix` is constructed

- `waldTest(object = LMlike, hypothesis = CoefficientHypothesis)`: Wald test dropping single term specified by `CoefficientHypothesis` hypothesis
- `waldTest(object = LMlike, hypothesis = matrix)`: Wald test of contrast specified by contrast matrix hypothesis
- `lrTest(object = LMlike, hypothesis = character)`: Likelihood ratio test dropping entire term specified by character hypothesis naming a term in the symbolic formula.
- `lrTest(object = LMlike, hypothesis = CoefficientHypothesis)`: Likelihood ratio test dropping single term specified by `CoefficientHypothesis` hypothesis
- `lrTest(object = LMlike, hypothesis = Hypothesis)`: Likelihood ratio test dropping single term specified by `Hypothesis` hypothesis
- `lrTest(object = LMlike, hypothesis = matrix)`: Likelihood ratio test dropping single term specified by contrast matrix hypothesis
- `logLik(GLMlike)`: 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

**fitted** A logical with components "C" and "D", TRUE if the respective component has converged

**formula** A formula for the regression

**fitArgsC**

**fitArgsD** Both lists giving arguments that will be passed to the fitter (such as convergence criteria or case weights)

## See Also

`coef`

`lrTest`

`waldTest`

`vcov`

`logLik`

logFC

*Calculate log-fold changes from hurdle model components***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**

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

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

**Arguments**

|                        |                                                                                                                                                       |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>zlmfit</code>    | ZlmFit output                                                                                                                                         |
| <code>contrast0</code> | vector of coefficients giving baseline contrast, or a <a href="#">Hypothesis</a> . If missing, then the '(Intercept)' is used as baseline.            |
| <code>contrast1</code> | matrix of coefficients giving comparison contrasts, or a <a href="#">Hypothesis</a> . 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`, ie,  $u(x) = \text{crossprod}(x, \text{coefC})$ . Likewise, Let  $v(x) = 1/(1+\exp(-\text{crossprod}(\text{coefD}, x)))$  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`.

**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`

**Caveats**

1. When method='bayesglm' (the default), it's no longer necessarily true that the log fold change from condition A to B will be the inverse of the log fold change from B to A if the models are fit separately. This is due to the shrinkage in bayesglm.
2. The log fold change can be small, but the Hurdle p-value small and significant when the sign of the discrete and continuous model components are discordant so that the marginal log fold change cancels out. The large sample sizes present in many single cell experiments also means that there is substantial power to detect even small changes.
3. When there is no expression in a gene for a coefficient that is non-zero in either condition0 or condition1 we return NA because there is not any information to estimate the continuous component. Technically we might return plus or minus infinity, but there is not a straightforward way to estimate a confidence interval in any case. See <https://support.bioconductor.org/p/99244/> for details

**See Also**

[Hypothesis](#)

[summary.ZlmFit-method](#)

**Examples**

```
data(vbetaFA)
zz <- zlm( ~ Stim.Condition+Population, vbetaFA[1:5,])
##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  $\log_2(\text{mean}(x) + 1)$

Usage

```
logmean(x)
```

Arguments

x                    numeric

Value

numeric

Examples

```
x <- 1:10
expavg(logmean(x))
```

---

|     |                                                    |
|-----|----------------------------------------------------|
| LRT | <i>Likelihood Ratio Tests for SingleCellAssays</i> |
|-----|----------------------------------------------------|

---

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 ZlmFit

**Examples**

```
data(vbetaFA)
LRT(vbetaFA, 'Stim.Condition', 'Unstim')
```

---

lrTest

---

*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.       |
| ...        | optional arguments, passed to fitting functions |

**Value**

array giving test statistics

**See Also**

fit  
waldTest  
Hypothesis  
CoefficientHypothesis

**Examples**

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

---

lrTest,ZlmFit,character-method

*Likelihood ratio test*


---

## 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, ...)
```

## Arguments

|               |                                                                                                                                         |
|---------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| object        | ZlmFit                                                                                                                                  |
| hypothesis    | See Details                                                                                                                             |
| ...           | Arguments passed on to <a href="#">zlm</a>                                                                                              |
| 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 <a href="#">ebayes</a> .                                                        |
| 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).                             |
| exprs_values  | character or integer passed to 'assay' specifying which assay to use for testing                                                        |

## Value

3D array

---

|                   |                               |
|-------------------|-------------------------------|
| magic_assay_names | <i>Default assay returned</i> |
|-------------------|-------------------------------|

---

## Description

Methods in this package operate on log-transformed (multiplicative scale) expression. We attempt to check for this at construction, and then over-ride the assay method to return the "layer" containing such log-transformed data.

## Usage

```
magic_assay_names()
```

```
assay_idx(x)
```

```
## S4 method for signature 'SingleCellAssay,missing'
assay(x, i, withDimnames = TRUE, ...)
```

## Arguments

|              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| x            | SingleCellAssay                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| i            | must be missing for this method to apply                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| withDimnames | A logical(1), indicating whether the dimnames of the SummarizedExperiment object should be applied (i.e. copied) to the extracted assays. More precisely, setting withDimnames=FALSE in the <i>getter</i> returns the assays <i>as-is</i> whereas setting withDimnames=TRUE return them with possibly modified dimnames.<br>Setting withDimnames=FALSE in the <i>setter</i> (assays<-) is required when the dimnames on the supplied assays are not identical to the dimnames on the SummarizedExperiment object; it does not influence actual assignment of dimnames to assays (they're always stored as-is).<br>Note that<br><pre>assays(x, withDimnames=FALSE) &lt;- assays(x, withDimnames=TRUE)</pre> is guaranteed to always work and be a no-op. This is not the case if withDimnames=FALSE is used or if withDimnames is not specified. |
| ...          | passed to parent method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

## Details

By default we return the assay whose names, as given by assayNames(x), matches the first element in the vector c('thresh', 'et', 'Et', 'lCount', 'logTPM', 'logCounts', 'logcounts').

## Functions

- magic\_assay\_names(): list of names assumed to represent log-transformed data, in order of usage preference
- assay\_idx(): what index is returned by default by 'assay'

Examples

```
data(vbetaFA)
assay(vbetaFA)[1:3,1:3]
assay(vbetaFA, 'thresh', withDimnames = FALSE) = assay(vbetaFA)*0 - 9
assay(vbetaFA)[1:3, 1:3]
```

---

|       |                               |
|-------|-------------------------------|
| maits | <i>MAITs data set, RNASeq</i> |
|-------|-------------------------------|

---

Description

MAITs data set, RNASeq

Format

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

See Also

[FromMatrix](#)

---

|              |                                            |
|--------------|--------------------------------------------|
| MAST-defunct | <i>Defunct functions in package ‘MAST’</i> |
|--------------|--------------------------------------------|

---

Description

These functions are defunct or have been renamed.

Functions (and replacements, if available)

- filter** `mast_filter`
- `cData` [colData](#)
- `fData` [mcols](#)
- `exprs` [assay](#)
- `zlm.SingleCellAssay` [zlm](#)
- `combine` [cbind](#) or [rbind](#)
- `deviance_residuals_hook` No replacement available, underlying API changed

---

|             |                                 |
|-------------|---------------------------------|
| mast_filter | <i>Filter a SingleCellAssay</i> |
|-------------|---------------------------------|

---

**Description**

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

**Usage**

```
mast_filter(sc, groups = NULL, filt_control = NULL, apply_filter = TRUE)
```

```
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 <- mast_filter(vbetaFA, groups='ncells')
## Returned as boolean matrix
was.filtered <- mast_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')
```

---

|                |                                                                                          |
|----------------|------------------------------------------------------------------------------------------|
| meld_list_left | <i>Combine lists, preferentially taking elements from x if there are duplicate names</i> |
|----------------|------------------------------------------------------------------------------------------|

---

**Description**

Combine lists, preferentially taking elements from x if there are duplicate names

**Usage**

```
meld_list_left(x, y)
```

**Arguments**

- x                    list
- y                    list

**Examples**

```
MAST:::meld_list_left(list(A=1, B=2), list(A = 0))
```

---

|                      |                                        |
|----------------------|----------------------------------------|
| melt.SingleCellAssay | <i>"Melt" a SingleCellAssay matrix</i> |
|----------------------|----------------------------------------|

---

**Description**

Return a molten (flat) representation, taking the cross-product of the expression values, the colData (column meta data), and the feature data (mcols).

**Usage**

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

**Arguments**

|            |                                           |
|------------|-------------------------------------------|
| data       | SingleCellAssay                           |
| ...        | ignored                                   |
| na.rm      | ignored                                   |
| value.name | name of 'values' column in returned value |

**Value**

A data.table, with the cartesian product of the row and column attributes and the expression values

**Examples**

```
data(vbetaFA)
melt.SingleCellAssay(vbetaFA[1:10,])
as(vbetaFA[1:10,], 'data.table')
```

---

|              |                              |
|--------------|------------------------------|
| model.matrix | <i>Model matrix accessor</i> |
|--------------|------------------------------|

---

**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)**

- `model.matrix(LMlike)`: return the `model.matrix`

---

|                                |                             |
|--------------------------------|-----------------------------|
| <code>model.matrix&lt;-</code> | <i>Replace model matrix</i> |
|--------------------------------|-----------------------------|

---

**Description**

Replace model matrix

**Usage**

```
model.matrix(object) <- value
```

**Arguments**

|        |                    |
|--------|--------------------|
| object | LMlike or subclass |
| value  | matrix             |

**Value**

modify object

---

|                       |                            |
|-----------------------|----------------------------|
| <code>myBiplot</code> | <i>Makes a nice BiPlot</i> |
|-----------------------|----------------------------|

---

**Description**

Creates a custom BiPlot for visualizing the results of PCA

**Usage**

```
myBiplot(pc, colorfactor, scaling = 100, nudge = 1.2, N = 10, dims = 1:2, ...)
```



**Arguments**

|             |                                                                          |
|-------------|--------------------------------------------------------------------------|
| pc          | output of 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

---

new\_with\_repaired\_slots

*Instantiate a class, but warn rather than error for badly named slots*

---

**Description**

Instantiate a class, but warn rather than error for badly named slots

**Usage**

```
new_with_repaired_slots(classname, ..., extra)
```

**Arguments**

|           |                                              |
|-----------|----------------------------------------------|
| classname | ‘character’ naming a class                   |
| ...       | slots in ‘classname’                         |
| extra     | named list giving other slots in ‘classname’ |

**Value**

‘new(classname)’

**Examples**

```
MAST::new_with_repaired_slots("SimpleList", listData = list(x = LETTERS),
extra = list(elementType = 'character', food = "tasty", beer = "cold"))
```

---

|            |                           |
|------------|---------------------------|
| pbootVcov1 | <i>Bootstrap a zlmfit</i> |
|------------|---------------------------|

---

## Description

Sample cells with replacement to find bootstrapped distribution of coefficients

## Usage

```
pbootVcov1(cl, zlmfit, R = 99)

bootVcov1(zlmfit, R = 99, boot_index = NULL)
```

## Arguments

|            |                                                                          |
|------------|--------------------------------------------------------------------------|
| cl         | a cluster object created by makeCluster                                  |
| zlmfit     | class ZlmFit                                                             |
| R          | number of bootstrap replicates                                           |
| boot_index | list of indices to resample. Only one of R or boot_index can be offered. |

## Value

array of bootstrapped coefficients  
 array of bootstrapped coefficients

## Functions

- pbootVcov1(): parallel version of bootstrapping

## Examples

```
data(vbetaFA)
zlmVbeta <- zlm(~ 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)
```

---

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                                                                                                                                                                                                                                          |

### Value

displays plots

### Examples

```
## See thresholdSCRNACountMatrix
example(thresholdSCRNACountMatrix)
```

---

plotlrt

*Plot a likelihood ratio test object*


---

### Description

Constructs a forest-like plot of signed log<sub>10</sub> p-values, possibly adjusted for multiple comparisons adjust can be one of "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none".

**Usage**

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

**Arguments**

|        |                                                                                                                            |
|--------|----------------------------------------------------------------------------------------------------------------------------|
| lr     | output from lrttest, with returnall=FALSE                                                                                  |
| adjust | character, passed along to p.adjust, see below                                                                             |
| thres  | numeric genes with adjusted pvalues 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 lrttest. Plots done separately for each group. |

**Value**

Constructs a dotplot

**Author(s)**

andrew

---

|                    |                                                              |
|--------------------|--------------------------------------------------------------|
| plotSCAConcordance | <i>Concordance plots of filtered single vs n-cell assays</i> |
|--------------------|--------------------------------------------------------------|

---

**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 mcols column ncells 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 | <i>Return predictions from a ZlmFit object.</i> |
|----------------|-------------------------------------------------|

---

**Description**

Return predictions from a ZlmFit object.

**Usage**

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

**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 (on the link scale) and standard errors.

**Examples**

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

---

|               |                             |
|---------------|-----------------------------|
| predicted_sig | <i>Predicted signatures</i> |
|---------------|-----------------------------|

---

### Description

Predicted signatures

### Format

A data frame of predicted gene expression signatures for stimulated and unstimulated cells.

---

|               |                                                                 |
|---------------|-----------------------------------------------------------------|
| primerAverage | <i>Average expression values for duplicated/redundant genes</i> |
|---------------|-----------------------------------------------------------------|

---

### Description

Takes an average, potentially on a different scale given by `fun.natural` of some genes. The average is then transformed with `fun.cycle`.

### Usage

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

### Arguments

|                          |                                                                       |
|--------------------------|-----------------------------------------------------------------------|
| <code>fd</code>          | SingleCellAssay or subclass                                           |
| <code>geneGroups</code>  | character naming a column in the featureData that keys the duplicates |
| <code>fun.natural</code> | transformation to be used to collapse the duplicate expression values |
| <code>fun.cycle</code>   | transformation to be used after collapsing                            |

### Value

averaged version of `fd`.

### Note

This code needs to be tested more extensively after a refactoring. Caveat calculator.

---

```
print.summaryZlmFit
```

*Print summary of a ZlmFit*

---

## Description

Shows the top ‘n’ genes by z score on ‘by’

## Usage

```
## 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
```

*Reads a Fluidigm Biomark (c. 2011) raw data file (or set of files)*

---

## Description

This function reads a raw Fluidigm Biomark data file or set of files and constructs a SingleCellAssay (or FluidigmAssay) object. This was written c. 2011 and has not been tested lately. The Biomark format may have changed.

**Usage**

```
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",
  ncells = "SampleRConc"
)
```

**Arguments**

|                                 |                                                                                                                                                                                                                                                                                   |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>files</code>              | A character vector of files to read.                                                                                                                                                                                                                                              |
| <code>metadata</code>           | A character path and filename of a CSV file containing additional metadata about the samples                                                                                                                                                                                      |
| <code>header.size</code>        | A numeric indicating the number of lines in the header (default 2)                                                                                                                                                                                                                |
| <code>skip</code>               | numeric how many lines to skip before reading (default 8)                                                                                                                                                                                                                         |
| <code>cycle.threshold</code>    | The maximum number of PCR cycles performed (default 40) numeric                                                                                                                                                                                                                   |
| <code>metadataColClasses</code> | Optional character vector giving the column classes of the metadata file. See <a href="#">read.table</a> .                                                                                                                                                                        |
| <code>meta.key</code>           | Optional character vector that identifies the key column between the metadata and the fluidigm data                                                                                                                                                                               |
| <code>idvars</code>             | Optional character vector that defines the set of columns uniquely identifying a well (unique cell, gene, and condition).                                                                                                                                                         |
| <code>splitby</code>            | Optional character that defines the column / variable used to split the resulting data into a list of SingleCellAssay, such that unique levels of <code>splitby</code> each fall into their own SingleCellAssay. Usually the experimental unit subjected to different treatments. |
| <code>unique.well.id</code>     | The column that uniquely identifies a sample well in the data. Default is "Chamber.ID".                                                                                                                                                                                           |
| <code>raw</code>                | 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.                                          |

**Value**

list of SingleCellAssay holding the data.

**Author(s)**

Greg Finak

---

|                |                                                            |
|----------------|------------------------------------------------------------|
| removeResponse | <i>Remove the left hand side (response) from a formula</i> |
|----------------|------------------------------------------------------------|

---

**Description**

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

**Usage**

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

**Arguments**

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

**Value**

formula

**Author(s)**

Andrew

---

|                                 |                                        |
|---------------------------------|----------------------------------------|
| <code>rstandard.bayesglm</code> | <i>rstandard for bayesglm objects.</i> |
|---------------------------------|----------------------------------------|

---

### Description

`rstandard` bayesglm object S3 method

### Usage

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

### Arguments

|                    |                               |
|--------------------|-------------------------------|
| <code>model</code> | bayesglm                      |
| <code>infl</code>  | see <a href="#">rstandard</a> |
| <code>type</code>  | see <a href="#">rstandard</a> |
| <code>...</code>   | ignored                       |

### Value

numeric residuals

---

|                                   |                                                                    |
|-----------------------------------|--------------------------------------------------------------------|
| <code>SceToSingleCellAssay</code> | <i>Coerce a SingleCellExperiment to some class defined in MAST</i> |
|-----------------------------------|--------------------------------------------------------------------|

---

### Description

Coerce a SingleCellExperiment to some class defined in MAST

### Usage

```
SceToSingleCellAssay(sce, class = "SingleCellAssay", check_sanity = TRUE)
```

### Arguments

|                           |                                                                                                                                                                                                                                                                         |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>sce</code>          | object inheriting from SingleCellExperiment                                                                                                                                                                                                                             |
| <code>class</code>        | character naming the class to be coerced to                                                                                                                                                                                                                             |
| <code>check_sanity</code> | (default: TRUE) Set FALSE to override sanity checks that try to ensure that the default assay is log-transformed and has at least one exact zero. See <a href="#">defaultAssay</a> for details on the "default assay" which is assumed to contain log transformed data. |

**Value**

object of the indicated class.

---

|         |                                           |
|---------|-------------------------------------------|
| se.coef | <i>Return coefficient standard errors</i> |
|---------|-------------------------------------------|

---

**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
example('ZlmFit-class')
```

---

|                    |             |
|--------------------|-------------|
| show,LMlike-method | <i>show</i> |
|--------------------|-------------|

---

**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

**Value**

side effect of printing to console

**Methods (by class)**

- `show(ZlmFit)`: print info on ZlmFit

---

*split, SingleCellAssay, character-method*  
*Split into list*

---

**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**

|                   |                                                              |
|-------------------|--------------------------------------------------------------|
| <code>x</code>    | <code>SingleCellAssay</code>                                 |
| <code>f</code>    | length-1 character, or atomic of length <code>ncol(x)</code> |
| <code>drop</code> | drop unused factor levels                                    |
| <code>...</code>  | 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 <code>aes</code> or <code>aes_</code> . If specified and <code>inherit.aes = TRUE</code> (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 <code>NULL</code> , the default, the data is inherited from the plot data as specified in the call to <code>ggplot</code> . A <code>data.frame</code> , or other object, will override the plot data. All objects will be fortified to produce a data frame. See <code>fortify</code> for which variables will be created. A function will be called with a single argument, the plot data. The return value must be a <code>data.frame</code> ., and will be used as the layer data. |
| 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 <code>FALSE</code> (the default), removes missing values with a warning. If <code>TRUE</code> silently removes missing values.                                                                                                                                                                                                                                                                                                                                                                                                                         |
| show.legend | logical. Should this layer be included in the legends? <code>NA</code> , the default, includes if any aesthetics are mapped. <code>FALSE</code> never includes, and <code>TRUE</code> always includes.                                                                                                                                                                                                                                                                                                                                                    |

|                          |                                                                                                                                                                                                                                               |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>inherit.aes</code> | 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. |
| <code>fill</code>        | A color or aesthetic mapping to fill color. Defaults to NA for empty ellipses.                                                                                                                                                                |
| <code>level</code>       | The confidence level at which to draw an ellipse (default is level=0.95).                                                                                                                                                                     |
| <code>lty</code>         | The linetype to use. Can map to a variable. Defaults to 2 (dashed line)                                                                                                                                                                       |
| <code>invert</code>      | 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.                                            |
| <code>alpha</code>       | transparency                                                                                                                                                                                                                                  |
| <code>...</code>         | 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**

```
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,modelmatrix=MM)
plt <- ggplot(predicted)+aes(x=invlogit(etaD),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=etaD,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**

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

Arguments

x                    SingleCellAssay  
...                  expression

Value

SingleCellAssay

Examples

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

---

|           |                                                        |
|-----------|--------------------------------------------------------|
| summarize | <i>Return programmatically useful summary of a fit</i> |
|-----------|--------------------------------------------------------|

---

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

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

## Arguments

|                     |                                 |
|---------------------|---------------------------------|
| <code>object</code> | A <code>GSEATests</code> object |
| <code>...</code>    | passed to <code>calcZ</code>    |

## Value

`data.table`

## See Also

`gseaAfterBoot`

## Examples

```
## See the examples in gseaAfterBoot
example(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

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

### Arguments

|                       |                                                                                                                                    |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------|
| <code>object</code>   | A <code>ZlmFit</code> object                                                                                                       |
| <code>logFC</code>    | If <code>TRUE</code> , calculate log-fold changes, or output from a call to <code>getLogFC</code> .                                |
| <code>doLRT</code>    | if <code>TRUE</code> , calculate <code>lrTests</code> on each coefficient, or a character vector of such coefficients to consider. |
| <code>level</code>    | what level of confidence coefficient to return. Defaults to 95 percent.                                                            |
| <code>parallel</code> | If <code>TRUE</code> and <code>option(mc.cores)&gt;1</code> then multiple cores will be used in fitting.                           |
| <code>...</code>      | 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` component to get normal print method
zs$datatable
## Can use parallel processing for LRT now
summary(z, doLRT = TRUE, parallel = TRUE)
```

---

```
summary.thresholdSCRNACountMatrix
```

*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 'thresholdSCRNACountMatrix'
summary(object, ...)

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

**Arguments**

|        |                                                                                  |
|--------|----------------------------------------------------------------------------------|
| object | a thresholdSCRNACountMatrix                                                      |
| ...    | currently ignored                                                                |
| x      | a summaryThresholdSCRNA object, ie output from summary.thresholdSCRNACountMatrix |

**Value**

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

## Functions

- `print(summaryThresholdSCRNA)`: 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 bi-modal density estimates of the binned data. These density estimates currently exclude the zeros due to complications with how the bandwidth is selected. (If the bandwidth is too small, then extra peaks/modes are found and everything goes haywire). If the diagnostic plots don't reveal any bi-modal bins, this is probably the reason, and you may not need to threshold since background in the data are exact zeros.

## Usage

```
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

|                          |                                                                                                      |
|--------------------------|------------------------------------------------------------------------------------------------------|
| <code>data_all</code>    | matrix of (possibly log-transformed) counts or TPM. Rows are genes and columns are cells.            |
| <code>conditions</code>  | Bins are determined per gene and per condition. Typically contrasts of interest should be specified. |
| <code>cutbins</code>     | vector of cut points.                                                                                |
| <code>nbins</code>       | integer number of bins when <code>cutbins</code> is not specified.                                   |
| <code>bin_by</code>      | character "median", "proportion", "mean"                                                             |
| <code>qt</code>          | when <code>bin_by</code> is "quantile", what quantile should be used to form the bins                |
| <code>min_per_bin</code> | 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                 bandwidth adjustment, passed to density

**Value**

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

**Examples**

```
data(maits,package='MAST', envir = environment())
sca <- FromMatrix(t(maits$expressionmat[,1:1000]), maits$scdat, maits$fdat[1:1000,])
tt <- thresholdSCRNACountMatrix(assay(sca))
tt <- thresholdSCRNACountMatrix(2^assay(sca)-1, data_log=FALSE)
opar <- par(no.readonly = TRUE)
on.exit(par(opar))
par(mfrow=c(4,2))
plot(tt)
```

---

|       |                       |
|-------|-----------------------|
| vbeta | <i>Vbeta Data Set</i> |
|-------|-----------------------|

---

**Description**

Vbeta Data Set

**Format**

a data frame with 11 columns. Column Ct contains the cycle threshold, with NA denoting that the threshold was never crossed. So it is inversely proportional to the log2 mRNA, and should be negated (and NAs set to zero) if it is used as a expression measurement for a FluidigmAssay.

---

|         |                                      |
|---------|--------------------------------------|
| vbetaFA | <i>Vbeta Data Set, FluidigmAssay</i> |
|---------|--------------------------------------|

---

**Description**

Vbeta Data Set, FluidigmAssay

**Format**

a FluidigmAssay of the vbeta data set.

**See Also**

[vbeta](#), [FromFlatDF](#)

---

|          |                        |
|----------|------------------------|
| waldTest | <i>Run a Wald test</i> |
|----------|------------------------|

---

**Description**

Run a Wald tests 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.

**Value**

array giving test statistics

**See Also**

fit  
lrTest  
lht

**Examples**

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

---

|                               |
|-------------------------------|
| waldTest,ZlmFit,matrix-method |
| <i>Wald test</i>              |

---

**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 | <i>Make matrix of continuous expression values, orthogonal to discrete</i> |
|-------|----------------------------------------------------------------------------|

---

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 | <i>Zero-inflated regression for SingleCellAssay</i> |
|-----|-----------------------------------------------------|

---

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(
  formula,
  sca,
  method = "bayesglm",
  silent = TRUE,
  ebayes = TRUE,
  ebayesControl = NULL,
  force = FALSE,
  hook = NULL,
  parallel = TRUE,
  LMlike,
  onlyCoef = FALSE,
  exprs_values = assay_idx(sca)$aidx,
  ...
)
```

## 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 <a href="#">ebayes</a> .                                                        |
| 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).                             |
| exprs_values  | character or integer passed to 'assay' specifying which assay to use for testing                                                        |
| ...           | 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. OR, if data is a data.frame just a list of the discrete and continuous fits.

### Empirical Bayes variance regularization

The empirical bayes regularization of the gene variance assumes that the precision ( $1/\text{variance}$ ) 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

ZlmFit-class, ebayes, GLMlike-class, BayesGLMlike-class

### Examples

```
data(vbetaFA)
zlmVbeta <- zlm(~ 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'))

## Can also provide just a \code{data.frame} instead
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)
```

---

ZlmFit-class

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

---

### Description

This holds output from a call to `zlm`. 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 <a href="#">Hypothesis</a> or <a href="#">CoefficientHypothesis</a> or a matrix giving such contrasts. |
| ...        | ignored                                                                                                        |
| which      | character vector, one of "C" (continuous) or "D" (discrete) specifying which component should be returned      |

### Value

see "Methods (by generic)"

### Methods (by generic)

- `lrTest(object = ZlmFit, hypothesis = CoefficientHypothesis)`: Returns an array with likelihood-ratio tests on contrasts defined using `CoefficientHypothesis()`.
- `lrTest(object = ZlmFit, hypothesis = Hypothesis)`: Returns an array with likelihood-ratio tests specified by `Hypothesis`, which is a [Hypothesis](#).
- `lrTest(object = ZlmFit, hypothesis = matrix)`: Returns an array with likelihood-ratio tests specified by `Hypothesis`, which is a contrast matrix.
- `waldTest(object = ZlmFit, hypothesis = CoefficientHypothesis)`: Returns an array with Wald Tests on contrasts defined using `CoefficientHypothesis()`.
- `waldTest(object = ZlmFit, hypothesis = Hypothesis)`: Returns an array with Wald Tests on contrasts defined in `Hypothesis()`
- `coef(ZlmFit)`: Returns the matrix of coefficients for component `which`.
- `vcov(ZlmFit)`: Returns an array of variance/covariance matrices for component `which`.
- `se.coef(ZlmFit)`: 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  
 exprs\_values 'character' or 'integer' with the 'assay' used.

**See Also**

zlm summary, ZlmFit-method

**Examples**

```

data(vbetaFA)
zlmVbeta <- zlm(~ 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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