

Package ‘glmGamPoi’

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Type Package

Title Fit a Gamma-Poisson Generalized Linear Model

Version 1.2.0

Description Fit linear models to overdispersed count data.

The package can estimate the overdispersion and fit repeated models for matrix input. It is designed to handle large input datasets as they typically occur in single cell RNA-seq experiments.

License GPL-3

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as.list.glmGamPoi	<i>Convert glmGamPoi object to a list</i>
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Description

Convert glmGamPoi object to a list

Usage

```
## S3 method for class 'glmGamPoi'
as.list(x, ...)
```

Arguments

x	an object with class glmGamPoi
...	not used

Value

The method returns a list with the following elements:

Beta a matrix with dimensions `nrow(data) x n_coefficients` where `n_coefficients` is based on the design argument. It contains the estimated coefficients for each gene.

overdispersions a vector with length `nrow(data)`. The overdispersion parameter for each gene. It describes how much more the counts vary than one would expect according to the Poisson model.

Mu a matrix with the same dimensions as `dim(data)`. If the calculation happened on disk, than Mu is a `HDF5Matrix`. It contains the estimated mean value for each gene and sample.

size_factors a vector with length `ncol(data)`. The size factors are the inferred correction factors for different sizes of each sample. They are also sometimes called the exposure factor.

model_matrix a matrix with dimensions `ncol(data) x n_coefficients`. It is build based on the design argument.

glm_gp

*Fit a Gamma-Poisson Generalized Linear Model***Description**

This function provides a simple to use interface to fit Gamma-Poisson generalized linear models. It works equally well for small scale (a single model) and large scale data (e.g. thousands of rows and columns, potentially stored on disk). The function automatically determines the appropriate size factors for each sample and efficiently finds the best overdispersion parameter for each gene.

Usage

```
glm_gp(
  data,
  design = ~1,
  col_data = NULL,
  reference_level = NULL,
  offset = 0,
  size_factors = c("normed_sum", "deconvolution", "poscounts"),
  overdispersion = TRUE,
  overdispersion_shrinkage = TRUE,
  do_cox_reid_adjustment = TRUE,
  subsample = FALSE,
  on_disk = NULL,
  verbose = FALSE
)
```

Arguments

data	any matrix-like object (e.g. matrix , DelayedArray , HDF5Matrix) or anything that can be cast to a SummarizedExperiment (e.g. MSnSet , eSet etc.) with one column per sample and row per gene.
design	a specification of the experimental design used to fit the Gamma-Poisson GLM. It can be a model.matrix() with one row for each sample and one column for each coefficient. Alternatively, design can be a formula. The entries in the formula can refer to global objects, columns in the <code>col_data</code> parameter, or the <code>colData(data)</code> of data if it is a SummarizedExperiment . The third option is that design is a vector where each element specifies to which condition a sample belongs. Default: <code>design = ~ 1</code> , which means that all samples are treated as if they belong to the same condition. Note that this is the fasted option.
col_data	a dataframe with one row for each sample in data. Default: <code>NULL</code> .
reference_level	a single string that specifies which level is used as reference when the model matrix is created. The reference level becomes the intercept and all other coefficients are calculated with respect to the <code>reference_level</code> . Default: <code>NULL</code> .
offset	Constant offset in the model in addition to <code>log(size_factors)</code> . It can either be a single number, a vector of length <code>ncol(data)</code> or a matrix with the same dimensions as <code>dim(data)</code> . Note that if data is a DelayedArray or HDF5Matrix , <code>offset</code> must be as well. Default: <code>0</code> .

- `size_factors` in large scale experiments, each sample is typically of different size (for example different sequencing depths). A size factor is an internal mechanism of GLMs to correct for this effect.
`size_factors` is either a numeric vector with positive entries that has the same lengths as columns in the data that specifies the size factors that are used. Or it can be a string that species the method that is used to estimate the size factors (one of `c("normed_sum", "deconvolution", "poscounts")`). Note that "normed_sum" and "poscounts" are fairly simple methods and can lead to sub-optimal results. For the best performance, I recommend to use `size_factors = "deconvolution"` which calls `scrn::calculateSumFactors()`. However, you need to separately install the `scrn` package from Bioconductor for this method to work. Also note that `size_factors = 1` and `size_factors = FALSE` are equivalent. If only a single gene is given, no size factor is estimated (ie. `size_factors = 1`). Default: "normed_sum".
- `overdispersion` the simplest count model is the Poisson model. However, the Poisson model assumes that $variance = mean$. For many applications this is too rigid and the Gamma-Poisson allows a more flexible mean-variance relation ($variance = mean + mean^2 * overdispersion$).
`overdispersion` can either be
- a single boolean that indicates if an overdispersion is estimated for each gene.
 - a numeric vector of length `nrow(data)` fixing the overdispersion to those values.
 - the string "global" to indicate that one dispersion is fit across all genes.
- Note that `overdispersion = 0` and `overdispersion = FALSE` are equivalent and both reduce the Gamma-Poisson to the classical Poisson model. Default: TRUE.
- `overdispersion_shrinkage` the overdispersion can be difficult to estimate with few replicates. To improve the overdispersion estimates, we can share information across genes and shrink each individual overdispersion estimate towards a global overdispersion estimate. Empirical studies show however that the overdispersion varies based on the mean expression level (lower expression level => higher dispersion). If `overdispersion_shrinkage = TRUE`, a median trend of dispersion and expression level is fit and used to estimate the variances of a quasi Gamma Poisson model (Lund et al. 2012). Default: TRUE.
- `do_cox_reid_adjustment` the classical maximum likelihood estimator of the overdispersion is biased towards small values. McCarthy *et al.* (2012) showed that it is preferable to optimize the Cox-Reid adjusted profile likelihood.
`do_cox_reid_adjustment` can be either be TRUE or FALSE to indicate if the adjustment is added during the optimization of the overdispersion parameter. Default: TRUE.
- `subsample` the estimation of the overdispersion is the slowest step when fitting a Gamma-Poisson GLM. For datasets with many samples, the estimation can be considerably sped up without loosing much precision by fitting the overdispersion only on a random subset of the samples. Default: FALSE which means that the data is not subsampled. If set to TRUE, at most 1,000 samples are considered. Otherwise the parameter just specifies the number of samples that are considered for each gene to estimate the overdispersion.

on_disk	a boolean that indicates if the dataset is loaded into memory or if it is kept on disk to reduce the memory usage. Processing in memory can be significantly faster than on disk. Default: NULL which means that the data is only processed in memory if data is an in-memory data structure.
verbose	a boolean that indicates if information about the individual steps are printed while fitting the GLM. Default: FALSE.

Details

The method follows the following steps:

1. The size factors are estimated.
If `size_factors = "normed_sum"`, the column-sum for each cell is calculated and the resulting size factors are normalized so that their geometric mean is 1. If `size_factors = "poscounts"`, a slightly adapted version of the procedure proposed by Anders and Huber (2010) in equation (5) is used. To handle the large number of zeros the geometric means are calculated for $Y + 0.5$ and ignored during the calculation of the median. Columns with all zeros get a default size factor of 0.001. If `size_factors = "deconvolution"`, the method `scran::calculateSumFactors()` is called.
2. The dispersion estimates are initialized based on the moments of each row of Y .
3. The coefficients of the model are estimated.
If all samples belong to the same condition (i.e. `design = ~ 1`), the betas are estimated using a quick Newton-Raphson algorithm. This is similar to the behavior of `edgeR`. For more complex designs, the general Fisher-scoring algorithm is used. Here, the code is based on a fork of the internal function `fitBeta()` from `DESeq2`. It does however contain some modification to make the fit more robust and faster.
4. The mean for each gene and sample is calculate.
Note that this step can be very IO intensive if data is or contains a `DelayedArray`.
5. The overdispersion is estimated.
The classical method for estimating the overdispersion for each gene is to maximize the Gamma-Poisson log-likelihood by iterating over each count and summing the the corresponding log-likelihood. It is however, much more efficient for genes with many small counts to work on the contingency table of the counts. Originally, this approach had already been used by Anscombe (1950). In this package, I have implemented an extension of their method that can handle general offsets.
See also `overdispersion_mle()`.
6. The beta coefficients are estimated once more with the updated overdispersion estimates
7. The mean for each gene and sample is calculated again.

This method can handle not just in memory data, but also data stored on disk. This is essential for large scale datasets with thousands of samples, as they sometimes encountered in modern single-cell RNA-seq analysis. `glmGamPoi` relies on the `DelayedArray` and `beachmat` package to efficiently implement the access to the on-disk data.

Value

The method returns a list with the following elements:

Beta a matrix with dimensions `nrow(data) x n_coefficients` where `n_coefficients` is based on the `design` argument. It contains the estimated coefficients for each gene.

`overdispersions` a vector with length `nrow(data)`. The overdispersion parameter for each gene. It describes how much more the counts vary than one would expect according to the Poisson model.

`overdispersion_shrinkage_list` a list with additional information from the quasi-likelihood shrinkage. For details see `overdispersion_shrinkage()`.

`deviances` a vector with the deviance of the fit for each row. The deviance is a measure how well the data is fit by the model. A smaller deviance means a better fit.

`Mu` a matrix with the same dimensions as `dim(data)`. If the calculation happened on disk, than `Mu` is a `HDF5Matrix`. It contains the estimated mean value for each gene and sample.

`size_factors` a vector with length `ncol(data)`. The size factors are the inferred correction factors for different sizes of each sample. They are also sometimes called the exposure factor.

`data` a `SummarizedExperiment` that contains the input counts and the `col_data`

`model_matrix` a matrix with dimensions `ncol(data) x n_coefficients`. It is build based on the design argument.

References

- McCarthy, D. J., Chen, Y., & Smyth, G. K. (2012). Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research*, 40(10), 4288–4297. <https://doi.org/10.1093/nar/gks042>.
- Anders Simon, & Huber Wolfgang. (2010). Differential expression analysis for sequence count data. *Genome Biology*. <https://doi.org/10.1016/j.jcf.2018.05.006>.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26(1), 139–140. <https://doi.org/10.1093/bioinformatics/btp616>.
- Lun ATL, Pagès H, Smith ML (2018). “beachmat: A Bioconductor C++ API for accessing high-throughput biological data from a variety of R matrix types.” *PLoS Comput. Biol.*, 14(5), e1006135. doi: [10.1371/journal.pcbi.1006135](https://doi.org/10.1371/journal.pcbi.1006135).
- Lund, S. P., Nettleton, D., McCarthy, D. J., & Smyth, G. K. (2012). Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. *Statistical Applications in Genetics and Molecular Biology*, 11(5). <https://doi.org/10.1515/1544-6115.1826>.
- Lun ATL, Bach K and Marioni JC (2016). Pooling across cells to normalize single-cell RNA sequencing data with many zero counts. *Genome Biol.* 17:75 <https://doi.org/10.1186/s13059-016-0947-7>

See Also

`overdispersion_mle()` and `overdispersion_shrinkage()` for the internal functions that do the work. For differential expression analysis, see `test_de()`.

Examples

```
set.seed(1)
# The simplest example
y <- rnbino(n = 10, mu = 3, size = 1/2.4)
c(glm_gp(y, size_factors = FALSE))
```

```

# Fitting a whole matrix
model_matrix <- cbind(1, rnorm(5))
true_Beta <- cbind(rnorm(n = 30), rnorm(n = 30, mean = 3))
sf <- exp(rnorm(n = 5, mean = 0.7))
model_matrix
Y <- matrix(rnbinom(n = 30 * 5, mu = sf * exp(true_Beta %*% t(model_matrix)), size = 1/2.4),
            nrow = 30, ncol = 5)

fit <- glm_gp(Y, design = model_matrix, size_factors = sf, verbose = TRUE)
summary(fit)

# Fitting a model with covariates
data <- data.frame(fav_food = sample(c("apple", "banana", "cherry"), size = 50, replace = TRUE),
                  city = sample(c("heidelberg", "paris", "new york"), size = 50, replace = TRUE),
                  age = rnorm(n = 50, mean = 40, sd = 15))
Y <- matrix(rnbinom(n = 100 * 50, mu = 3, size = 1/3.1), nrow = 100, ncol = 50)
fit <- glm_gp(Y, design = ~ fav_food + city + age, col_data = data)
summary(fit)

```

loc_median_fit

Estimate local median fit

Description

This function fits y based on x through a (weighted) median using the $npoints/2$ neighborhood.

Usage

```

loc_median_fit(
  x,
  y,
  fraction = 0.1,
  npoints = max(1, round(length(x) * fraction)),
  weighted = TRUE,
  ignore_zeros = FALSE
)

```

Arguments

<code>x, y</code>	the x and y coordinates of the points.
<code>fraction, npoints</code>	the fraction / number of the points that are considered for each fit. <code>npoints</code> is the argument that is used in the end it is at least one. Default: <code>fraction = 0.1</code> and <code>npoints = length(x) * fraction</code> .
<code>weighted</code>	a boolean that indicates if a weighted median is calculated.
<code>ignore_zeros</code>	should the zeros be excluded from the fit

Details

This function is low-level and should usually not be called by the user.

See Also

locfit: a package dedicated to local regression.

Examples

```
x <- runif(n = 1000, max = 4)
y <- rpois(n = 1000, lambda = x * 10)

plot(x, y)
fit <- loc_median_fit(x, y, fraction = 0.1)
points(x, fit, col = "red")
```

overdispersion_mle *Estimate the Overdispersion for a Vector of Counts*

Description

Estimate the Overdispersion for a Vector of Counts

Usage

```
overdispersion_mle(
  y,
  mean,
  model_matrix = NULL,
  do_cox_reid_adjustment = !is.null(model_matrix),
  global_estimate = FALSE,
  subsample = FALSE,
  max_iter = 200,
  verbose = FALSE
)
```

Arguments

y	a numeric or integer vector or matrix with the counts for which the overdispersion is estimated
mean	a numeric vector of either length 1 or length(y) or if y is a matrix, a matrix with the same dimensions. Contains the predicted value for that sample. If missing: mean(y) / rowMeans(y)
model_matrix	a numeric matrix that specifies the experimental design. It can be produced using stats::model.matrix(). Default: NULL

<code>do_cox_reid_adjustment</code>	the classical maximum likelihood estimator of the overdispersion is biased towards small values. McCarthy <i>et al.</i> (2012) showed that it is preferable to optimize the Cox-Reid adjusted profile likelihood. <code>do_cox_reid_adjustment</code> can be either be TRUE or FALSE to indicate if the adjustment is added during the optimization of the overdispersion parameter. Default: TRUE if a model matrix is provided, otherwise FALSE
<code>global_estimate</code>	flag to decide if a single overdispersion for a whole matrix is calculated instead of one estimate per row. This parameter has no affect if <code>y</code> is a vector. Default: FALSE
<code>subsample</code>	the estimation of the overdispersion is the slowest step when fitting a Gamma-Poisson GLM. For datasets with many samples, the estimation can be considerably sped up without loosing much precision by fitting the overdispersion only on a random subset of the samples. Default: FALSE which means that the data is not subsampled. If set to TRUE, at most 1,000 samples are considered. Otherwise the parameter just specifies the number of samples that are considered for each gene to estimate the overdispersion.
<code>max_iter</code>	the maximum number of iterations for each gene
<code>verbose</code>	a boolean that indicates if information about the individual steps are printed while fitting the GLM. Default: FALSE.

Details

The function is optimized to be fast on many small counts. To achieve this, the frequency table of the counts is calculated and used to avoid repetitive calculations. If there are probably many unique counts the optimization is skipped. Currently the heuristic is to skip if more than half of the counts are expected to be unique. The estimation is based on the largest observed count in `y`.

An earlier version of this package (< 1.1.1) used a separate set of functions for the case of many small counts based on a paper by Bandara *et al.* (2019). However, this didn't bring a sufficient performance increase and meant an additional maintenance burden.

Value

The function returns a list with the following elements:

`estimate` the numerical estimate of the overdispersion.

`iterations` the number of iterations it took to calculate the result.

`message` additional information about the fitting process.

See Also

[glm_gp\(\)](#)

Examples

```
set.seed(1)
# true overdispersion = 2.4
y <- rnbino(n = 10, mu = 3, size = 1/2.4)
# estimate = 1.7
overdispersion_mle(y)
```

```

# true overdispersion = 0
y <- rpois(n = 10, lambda = 3)
# estimate = 0
overdispersion_mle(y)
# with different mu, overdispersion estimate changes
overdispersion_mle(y, mean = 15)
# Cox-Reid adjustment changes the result
overdispersion_mle(y, mean = 15, do_cox_reid_adjustment = FALSE)

# Many very small counts, true overdispersion = 50
y <- rnbino(m(n = 1000, mu = 0.01, size = 1/50)
summary(y)
# estimate = 31
overdispersion_mle(y, do_cox_reid_adjustment = TRUE)

# Function can also handle matrix input
Y <- matrix(rnbino(m(n = 10 * 3, mu = 4, size = 1/2.2), nrow = 10, ncol = 3)
Y
as.data.frame(overdispersion_mle(Y))

```

overdispersion_shrinkage

Shrink the overdispersion estimates

Description

Low-level function to shrink a set of overdispersion estimates following the quasi-likelihood and Empirical Bayesian framework.

Usage

```

overdispersion_shrinkage(
  disp_est,
  gene_means,
  df,
  disp_trend = TRUE,
  ql_disp_trend = NULL,
  ...,
  verbose = FALSE
)

```

Arguments

disp_est	vector of overdispersion estimates
gene_means	vector of average gene expression values. Used to fit disp_trend if that is NULL.
df	degrees of freedom for estimating the Empirical Bayesian variance prior. Can be length 1 or same length as disp_est and gene_means.
disp_trend	vector with the dispersion trend. If NULL or TRUE the dispersion trend is fitted using a (weighted) local median fit. Default: TRUE.

ql_disp_trend	a logical to indicate if a second abundance trend using splines is fitted for the quasi-likelihood dispersions. Default: NULL which means that the extra fit is only done if enough observations are present.
...	additional parameters for the <code>loc_median_fit()</code> function
verbose	a boolean that indicates if information about the individual steps are printed while fitting the GLM. Default: FALSE.

Details

The function goes through the following steps

1. Fit trend between overdispersion MLE's and the average gene expression. Per default it uses the `loc_median_fit()` function.
2. Convert the overdispersion MLE's to quasi-likelihood dispersion estimates by fixing the trended dispersion as the "true" dispersion value: $disp_{ql} = (1 + mu * disp_{mle}) / (1 + mu * disp_{trend})$
3. Shrink the quasi-likelihood dispersion estimates using Empirical Bayesian variance shrinkage (see Smyth 2004).

Value

the function returns a list with the following elements

- dispersion_trend** the dispersion trend provided by `disp_trend` or the local median fit.
- ql_disp_estimate** the quasi-likelihood dispersion estimates based on the dispersion trend, `disp_est`, and `gene_means`
- ql_disp_trend** the `ql_disp_estimate` still might show a trend with respect to `gene_means`. If `ql_disp_trend = TRUE` a spline is used to remove this secondary trend. If `ql_disp_trend = TRUE` it corresponds directly to the dispersion prior
- ql_disp_shrunken** the shrunken quasi-likelihood dispersion estimates. They are shrunken towards `ql_disp_trend`.
- ql_df0** the degrees of freedom of the empirical Bayesian shrinkage. They correspond to spread of the `ql_disp_estimate`'s

References

- Lund, S. P., Nettleton, D., McCarthy, D. J., & Smyth, G. K. (2012). Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. *Statistical Applications in Genetics and Molecular Biology*, 11(5). <https://doi.org/10.1515/1544-6115.1826>.
- Smyth, G. K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology*, 3(1). <https://doi.org/10.2202/1544-6115.1027>

See Also

`limma::squeezeVar()`

Examples

```

Y <- matrix(rnbinom(n = 300 * 4, mu = 6, size = 1/4.2), nrow = 30, ncol = 4)
disps <- sapply(seq_len(nrow(Y)), function(idx){
  overdispersion_mle(Y[idx, ])$estimate
})
shrink_list <- overdispersion_shrinkage(disps, rowMeans(Y), df = ncol(Y) - 1,
  disp_trend = FALSE, ql_disp_trend = FALSE)

plot(rowMeans(Y), shrink_list$ql_disp_estimate)
lines(sort(rowMeans(Y)), shrink_list$ql_disp_trend[order(rowMeans(Y))], col = "red")
points(rowMeans(Y), shrink_list$ql_disp_shrunken, col = "blue", pch = 16, cex = 0.5)

```

```
print.glmGamPoi      Pretty print the result from glm_gp()
```

Description

Pretty print the result from `glm_gp()`

Usage

```

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

## S3 method for class 'glmGamPoi'
format(x, ...)

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

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

## S3 method for class 'summary.glmGamPoi'
format(x, ...)

```

Arguments

<code>x</code>	the <code>glmGamPoi</code> object
<code>...</code>	additional parameters, currently ignored
<code>object</code>	the <code>glmGamPoi</code> object that is summarized

Value

The `print()` methods return the object `x`. The `format()` method returns a string. The `summary()` method returns an object of class `summary.glmGamPoi`.

residuals.glmGamPoi *Extract Residuals of Gamma Poisson Model*

Description

Extract Residuals of Gamma Poisson Model

Usage

```
## S3 method for class 'glmGamPoi'
residuals(
  object,
  type = c("deviance", "pearson", "randomized_quantile", "working", "response"),
  ...
)
```

Arguments

object a fit of type glmGamPoi. It is usually produced with a call to `glm_gp()`.

type the type of residual that is calculated. See details for more information. Default: "deviance".

... currently ignored.

Details

This method can calculate a range of different residuals:

deviance The deviance for the Gamma-Poisson model is

$$dev = 2 * (1 / theta * log((1 + m * theta) / (1 + y * theta)) - y * log((m + y * theta) / (y + y * m * theta)))$$

and the residual accordingly is

$$res = sign(y - m) * sqrt(dev).$$

pearson The Pearson residual is $res = (y - m) / sqrt(m + m^2 * theta)$

randomized_quantile The randomized quantile residual was originally developed by Dunn & Smyth, 1995. Please see that publication or `statmod::qresiduals()` for more information.

working The working residuals are $res = (y - m) / m$.

response The response residuals are $res = y - m$

Value

a matrix with the same size as `fit$data`. If `fit$data` contains a `DelayedArray` then the result will be a `DelayedArray` as well.

See Also

`glm_gp()` and `'stats::residuals.glm()`

test_de

*Test for Differential Expression***Description**

Conduct a quasi-likelihood ratio test for a Gamma-Poisson fit.

Usage

```
test_de(
  fit,
  contrast,
  reduced_design = NULL,
  full_design = fit$model_matrix,
  subset_to = NULL,
  pseudobulk_by = NULL,
  pval_adjust_method = "BH",
  sort_by = NULL,
  decreasing = FALSE,
  n_max = Inf,
  verbose = FALSE
)
```

Arguments

fit	object of class <code>glmGamPoi</code> . Usually the result of calling <code>glm_gp(data, ...)</code>
contrast	The contrast to test. Can be a single column name (quoted or as a string) that is removed from the full model matrix of <code>fit</code> . Or a complex contrast comparing two or more columns: e.g. <code>A - B</code> , <code>"A - 3 * B"</code> , <code>(A + B) / 2 - C</code> etc. Only one of <code>contrast</code> or <code>reduced_design</code> must be specified.
reduced_design	a specification of the reduced design used as a comparison to see what how much better <code>fit</code> describes the data. Analogous to the <code>design</code> parameter in <code>glm_gp()</code> , it can be either a formula, a <code>model.matrix()</code> , or a vector. Only one of <code>contrast</code> or <code>reduced_design</code> must be specified.
full_design	option to specify an alternative <code>full_design</code> that can differ from <code>fit\$model_matrix</code> . Can be a formula or a matrix. Default: <code>fit\$model_matrix</code>
subset_to	a vector with the same length as <code>ncol(fit\$data)</code> or an expression that evaluates to such a vector. The expression can reference columns from <code>colData(fit\$data)</code> . A typical use case in single cell analysis would be to subset to a specific cell type (e.g. <code>subset_to = cell_type == "T-cells"</code>). Note that if this argument is set a new the model for the <code>full_design</code> is re-fit. Default: <code>NULL</code> which means that the data is not subset.
pseudobulk_by	a vector with the same length as <code>ncol(fit\$data)</code> that is used to split the columns into different groups (calls <code>split()</code>). <code>pseudobulk_by</code> can also be an expression that evaluates to a vector. The expression can reference columns from <code>colData(fit\$data)</code> . The counts are summed across the groups to create "pseudobulk" samples. This is typically used in single cell analysis if the cells come from different samples to get a proper estimate of the differences. This is particularly powerful in combination with the <code>subset_to</code> parameter to analyze differences between samples

	for subgroups of cells. Note that this does a fresh fit for both the full and the reduced design. Default: NULL which means that the data is not aggregated.
pval_adjust_method	one of the p-value adjustment method from p.adjust.methods . Default: "BH".
sort_by	the name of the column or an expression used to sort the result. If sort_by is NULL the table is not sorted. Default: NULL
decreasing	boolean to decide if the result is sorted increasing or decreasing order. Default: FALSE.
n_max	the maximum number of rows to return. Default: Inf which means that all rows are returned
verbose	a boolean that indicates if information about the individual steps are printed while fitting the GLM. Default: FALSE.

Value

a data.frame with the following columns

name the rownames of the input data

pval the p-values of the quasi-likelihood ratio test

adj_pval the adjusted p-values returned from [p.adjust\(\)](#)

f_statistic the F-statistic: $F = (Dev_{full} - Dev_{red}) / (df_1 * disp_{ql} - shrunken)$

df1 the degrees of freedom of the test: $ncol(design) - ncol(reduced_design)$

df2 the degrees of freedom of the fit: $ncol(data) - ncol(design) + df_0$

lfc the log2-fold change. If the alternative model is specified by reduced_design will be NA.

References

- Lund, S. P., Nettleton, D., McCarthy, D. J., & Smyth, G. K. (2012). Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. *Statistical Applications in Genetics and Molecular Biology*, 11(5). <https://doi.org/10.1515/1544-6115.1826>.

See Also

[glm_gp\(\)](#)

Examples

```
Y <- matrix(rnbinom(n = 30 * 100, mu = 4, size = 0.3), nrow = 30, ncol = 100)
annot <- data.frame(sample = sample(LETTERS[1:6], size = 100, replace = TRUE),
                   cont1 = rnorm(100), cont2 = rnorm(100, mean = 30))
annot$condition <- ifelse(annot$sample %in% c("A", "B", "C"), "ctrl", "treated")
head(annot)
se <- SummarizedExperiment::SummarizedExperiment(Y, colData = annot)
fit <- glm_gp(se, design = ~ condition + cont1 + cont2)

# Test with reduced design
res <- test_de(fit, reduced_design = ~ condition + cont1)
head(res)

# Test with contrast argument, the results are identical
res2 <- test_de(fit, contrast = cont2)
```

```
head(res2)

# The column names of fit$Beta are valid variables in the contrast argument
colnames(fit$Beta)

# You can also have more complex contrasts:
# the following compares cont1 vs cont2:
test_de(fit, cont1 - cont2, n_max = 4)

# You can also sort the output
test_de(fit, cont1 - cont2, n_max = 4,
        sort_by = "pval")

test_de(fit, cont1 - cont2, n_max = 4,
        sort_by = - abs(f_statistic))

# If the data has multiple samples, it is a good
# idea to aggregate the cell counts by samples.
# This is called "pseudobulk".
test_de(fit, contrast = "conditiontreated", n_max = 4,
        pseudobulk_by = sample)

# You can also do the pseudobulk only on a subset of cells:
cell_types <- sample(c("Tcell", "Bcell", "Makrophages"), size = 100, replace = TRUE)
test_de(fit, contrast = "conditiontreated", n_max = 4,
        pseudobulk_by = sample,
        subset_to = cell_types == "Bcell")

# Be care full, if you included the cell type information in
# the original fit, after subsetting the design matrix would
# be degenerate. To fix this, specify the full_design in 'test_de()'
SummarizedExperiment::colData(se)$ct <- cell_types
fit_with_celltype <- glm_gp(se, design = ~ condition + cont1 + cont2 + ct)
test_de(fit_with_celltype, contrast = cont1, n_max = 4,
        full_design = ~ condition + cont1 + cont2,
        pseudobulk_by = sample,
        subset_to = ct == "Bcell")
```


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