Package 'nebula'

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Type Package

Title Negative Binomial Mixed Models Using Large-Sample Approximation for Differential Expression Analysis of ScRNA-Seq Data

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Description
A fast negative binomial mixed model for conducting association analysis of multi-subject single-cell data. It can be used for identifying marker genes, differential expression and co-expression analyses. The model includes subject-level random effects to account for the hierarchical structure in multi-subject single-cell data. See He et al. (2021) <doi:10.1038/s42003-021-02146-6>.

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LinkingTo Rcpp, RcppEigen

Depends R (>= 4.4.0)

URL https://github.com/lhe17/nebula

BugReports https://github.com/lhe17/nebula/issues

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`nebula-package` *Negative Binomial Mixed Models Using Large-Sample Approximation for Differential Expression Analysis of ScRNA-Seq Data*

Description

A fast negative binomial mixed model for conducting association analysis of multi-subject single-cell data. It can be used for identifying marker genes, differential expression and co-expression analyses. The model includes subject-level random effects to account for the hierarchical structure in multi-subject single-cell data. See He et al. (2021) <doi:10.1038/s42003-021-02146-6>.

Details

nebula is an R package for performing association analysis using a fast negative binomial mixed model for multi-subject single-cell data.

Author(s)

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References

He, L., Davila-Velderrain, J., Sumida, T. S., Hafler, D. A., Kellis, M., & Kulminski, A. M. (2021). NEBULA is a fast negative binomial mixed model for differential or co-expression analysis of large-scale multi-subject single-cell data. Communications biology, 4(1), 1-17.

Examples

```
library(nebula)
data(sample_data)
pred = model.matrix(~X1+X2+cc,data=sample_data$pred)
re = nebula(count=sample_data$count,id=sample_data$sid,pred=pred)
```

group_cell *Group cells according to subject IDs*

Description

Reorders the count matrix, subject IDs, predictors, and offset factors so that cells are grouped by subject ID. If cells are already grouped, returns NULL.

Usage

```
group_cell(count, id, pred = NULL, offset = NULL)
```

Arguments

count	A raw count matrix of the single-cell data. The rows are the genes, and the columns are the cells. The matrix can be a matrix object or a sparse dgCMatrix object.
id	A vector of subject IDs. The length should be the same as the number of columns of the count matrix.
pred	A design matrix of the predictors. The rows are the cells and the columns are the predictors. If not specified, an intercept column will be generated by default.
offset	A vector of the scaling factor. The values must be strictly positive. If not specified, a vector of all ones will be generated by default.

Details

Group cells according to subject IDs

Value

count: A reordered count matrix. If the cells are already grouped, the function returns NULL.

id: A reordered subject ID vector.

pred: A reordered design matrix of the predictors.

offset: A reordered vector of the scaling factor.

Examples

```
library(nebula)
data(sample_data)
pred = model.matrix(~X1+X2+cc,data=sample_data$pred)
df_order = group_cell(count=sample_data$count,id=sample_data$sid,pred=pred)
```

load_sample_seurat *Load sample_seurat example data*

Description

Loads the sample_seurat example dataset for testing the scToNeb function. This dataset contains a subset (1000 genes and 1000 cells) of the eight-pancreas scRNA-seq datasets.

Usage

```
load_sample_seurat()
```

Details

Load sample_seurat data

This function requires the Seurat package to be installed. If Seurat is not available, the function will throw an error with installation instructions.

Value

A Seurat object containing example single-cell data

See Also

scToNeb

Examples

```
## Not run:
library(nebula)
sample_seurat <- load_sample_seurat()
re <- scToNeb(obj = sample_seurat, assay = "RNA", id = "replicate",
              pred = c("celltype", "tech"))

## End(Not run)
```

nbresidual *Extract Pearson residuals from the results of NEBULA*

Description

Calculates and returns Pearson residuals from NEBULA analysis results. Can compute either marginal or conditional residuals.

Usage

```
nbresidual(nebula, count, id, pred = NULL, offset = NULL, conditional = FALSE)
```

Arguments

nebula	An object of the result obtained from running the function nebula.
count	A raw count matrix of the single-cell data. The rows are the genes, and the columns are the cells. The matrix can be a matrix object or a sparse dgCMatrix object.
id	A vector of subject IDs. The length should be the same as the number of columns of the count matrix.
pred	A design matrix of the predictors. The rows are the cells and the columns are the predictors. If not specified, an intercept column will be generated by default.
offset	A vector of the scaling factor. The values must be strictly positive. If not specified, a vector of all ones will be generated by default.
conditional	A logical value. By default (FALSE), the function returns marginal Pearson residuals. If TRUE, the function will return conditional Pearson residuals.

Details

Extract Pearson residuals from the results of NEBULA

Value

residuals: A matrix of Pearson residuals. The number of columns is the number of cells in the count matrix. The rows correspond to gene IDs reported in the result from nebula.

gene: Gene names corresponding to the row names of the count matrix.

Examples

```
library(nebula)
data(sample_data)
pred = model.matrix(~X1+X2+cc,data=sample_data$pred)
re = nebula(count=sample_data$count,id=sample_data$sid,pred=pred)
resid = nbresidual(re,count=sample_data$count,id=sample_data$sid,pred=pred)
```

---

nebula *Association analysis of a multi-subject single-cell data set using a fast negative binomial mixed model*

Description

Performs differential expression analysis using a fast negative binomial mixed model (NEBULA) for multi-subject single-cell data. Includes subject-level random effects to account for hierarchical structure.

Usage

```
nebula(
  count,
  id,
  pred = NULL,
  offset = NULL,
  min = c(1e-04, 1e-04),
  max = c(10, 1000),
  model = "NBGMM",
  method = "LN",
  cutoff_cell = 20,
  kappa = 800,
  opt = "lbfgs",
  verbose = TRUE,
  cpc = 0.005,
  mincp = 5,
  covariance = FALSE,
  output_re = FALSE,
  reml = 0,
  ncore = 2,
  fmaxsize = Inf
)
```

Arguments

count	A raw count matrix of the single-cell data. The rows are the genes, and the columns are the cells. The matrix can be a matrix object or a sparse dgCMatrix object.
id	A vector of subject IDs. The length should be the same as the number of columns of the count matrix.
pred	A design matrix of the predictors. The rows are the cells and the columns are the predictors. If not specified, an intercept column will be generated by default.
offset	A vector of the scaling factor. The values must be strictly positive. If not specified, a vector of all ones will be generated by default.
min	Minimum values for the overdispersions parameters σ^2 and ϕ. Must be positive. The default is c(1e-4,1e-4).
max	Maximum values for the overdispersions parameters σ^2 and ϕ. Must be positive. The default is c(10,1000).
model	'NBGMM', 'PMM' or 'NBLMM'. 'NBGMM' is for fitting a negative binomial gamma mixed model. 'PMM' is for fitting a Poisson gamma mixed model. 'NGLMM' is for fitting a negative binomial lognormal mixed model (the same model as that in the lme4 package). The default is 'NBGMM'.
method	'LN' or 'HL'. 'LN' is to use NEBULA-LN and 'HL' is to use NEBULA-HL. The default is 'LN'.
cutoff_cell	The data will be refit using NEBULA-HL to estimate both overdispersions if the product of the cells per subject and the estimated cell-level overdispersion parameter ϕ is smaller than cutoff_cell. The default is 20.

kappa	Please see the vignettes for more details. The default is 800.
opt	'lbfgs' or 'trust'. Specifying the optimization algorithm used in NEBULA-LN. The default is 'lbfgs'. If it is 'trust', a trust region algorithm based on the Hessian matrix will be used for optimization.
verbose	An optional logical scalar indicating whether to print additional messages. Default is FALSE.
cpc	A non-negative threshold for filtering low-expression genes. Genes with counts per cell smaller than the specified value will not be analyzed.
mincp	A positive integer threshold for filtering low-expression genes. A gene will not be analyzed if its number of cells that have a non-zero count is smaller than the specified value .
covariance	If TRUE, nebula will output the covariance matrix for the estimated log(FC), which can be used for testing contrasts.
output_re	If TRUE, nebula will output the subject-level random effects. Only effective for model='NBGMM' or 'NBLMM'.
reml	Either 0 (default) or 1. If it is one, REML will be used to estimate the overdispersions.
ncore	The number of cores used for parallel computing.
fmaxsize	The maximum allowed total size (in bytes) of global variables (future.globals.maxSize) when using parallel computing.

Details

Association analysis of a multi-subject single-cell data set using a fast negative binomial mixed model

Value

summary: The estimated coefficient (logFC_), standard error (se_) and p-value (p_) for each predictor.

overdispersion: The estimated cell-level and subject-level overdispersions σ^2 and ϕ^{-1}.

convergence: More information about the convergence of the algorithm for each gene. A value of -20 or lower indicates a potential failure of the convergence. A value of one indicates that the convergence is reached due to a sufficiently small improvement of the function value. A value of -10 indicates that the convergence is reached because the gradients are close to zero (i.e., the critical point) and no improvement of the function value can be found.

algorithm: The algorithm used for analyzing the gene. More information can be found in the vignettes.

covariance: The covariance matrix for the estimated log(FC).

random_effect: The subject-level random effects.

Examples

```
library(nebula)
data(sample_data)
pred = model.matrix(~X1+X2+cc,data=sample_data$pred)
re = nebula(count=sample_data$count,id=sample_data$sid,pred=pred)
```

`sample_data` *An example data set for testing nebula*

Description

A dataset containing a count matrix, subject IDs, a data frame of predictors and scaling factors.

Format

A list of four objects:

count A raw count matrix

sid A vector of subject IDs

pred A data frame of three predictors

offset A vector of scaling factors

Details

An example data set for testing nebula

`scToNeb` *Retrieve data from Seurat or SingleCellExperiment object to prepare for use in nebula*

Description

Extracts count matrix and metadata from Seurat or SingleCellExperiment objects and formats them for use with the nebula function. Handles predictors, sample IDs, and offset factors.

Usage

```
scToNeb(
  obj,
  assay = NULL,
  id = NULL,
  pred = NULL,
  offset = NULL,
  verbose = TRUE
)
```

Arguments

obj	Seurat or SingleCellExperiment object to create data set for Nebula.
assay	Assay to retrieve counts from the corresponding Seurat count matrix.
id	Sample ID to use metadata object i.e. obj$id.
pred	Character vector of predictors from metadata in Seurat or SingleCellExperiment objects.
offset	Metadata column corresponding to per-cell scaling factor e.g. TMM.
verbose	Indicating whether to print additional messages.

Details

Retrieve data from Seurat or SingleCellExperiment object to prepare for use in nebula

Value

data_neb: A list usable for nebula.

Examples

```
## Not run:
library(Seurat)
library(nebula)

sample_seurat <- load_sample_seurat()
re <- scToNeb(obj = sample_seurat, assay = "RNA", id = "replicate", pred = c("celltype", "tech"))

## End(Not run)
```

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