Package ‘regsplice’

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Title L1-regularization based methods for detection of differential splicing

Description Statistical methods for detection of differential splicing (differential exon usage) in RNA-seq and exon microarray data, using L1-regularization (lasso) to improve power.

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URL https://github.com/lmweber/regsplice

BugReports https://github.com/lmweber/regsplice/issues

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  'createDesignMatrix.R' 'filterLowCounts.R' 'filterZeros.R'
  'fitting_functions_multiple_genes.R'
  'fitting_functions_single_gene.R' 'helper_functions.R'
  'initializeResults.R' 'regsplice_wrapper.R'
  'runNormalization.R' 'runVoom.R' 'summaryTable.R'

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createDesignMatrix

Create design matrix.

Description
Create a model design matrix for a single gene.

Usage
createDesignMatrix(condition, n_exons)

Arguments
- condition: Experimental conditions for each sample (character or numeric vector, or factor).
- n_exons: Number of exons in the gene (integer).

Details
Creates a model design matrix for a single gene in the format required by the regsplice model fitting functions. Required inputs are the experimental conditions (groups) for each sample, and the number of exons in the gene.

The design matrix includes main effect terms for each exon and each sample, and interaction terms between the exons and conditions.

Note that the design matrix does not include main effect terms for the conditions, since these are absorbed into the main effect terms for the samples. In addition, the design matrix does not include an intercept column, since it is simpler to let the model fitting functions add an intercept term later.

The model fitting functions in subsequent steps call this function once for each gene.
filterLowCounts

Value
Returns a model design matrix for the gene, in the format required by the regsplice model fitting functions.

See Also
fitRegMultiple fitNullMultiple fitFullMultiple LRTests

Examples
condition <- rep(c(0, 1), each = 3)
 n_exons <- 10
 X <- createDesignMatrix(condition, n_exons)

filterLowCounts
Filter low-count exons.

Description
Filter low-count exons from RNA-seq read count data.

Usage
filterLowCounts(rs_data, filter_min_per_exon = 6, filter_min_per_sample = 3)

Arguments
rs_data RegspliceData object.
filter_min_per_exon
Filtering parameter: minimum number of reads per exon bin, summed across all biological samples. Default is 6.
filter_min_per_sample
Filtering parameter: minimum number of reads per biological sample; i.e. for each exon bin, at least one sample must have this number of reads. Default is 3.

Details
Filters low-count exon bins from RNA-seq read count data. Any remaining single-exon genes (after filtering) are also removed (since differential splicing requires multiple exon bins).
Input data is assumed to be in the form of a RegspliceData object. See RegspliceData for details.
The arguments filter_min_per_exon and filter_min_per_sample control the amount of filtering. Exon bins that meet the filtering conditions are kept. Default values for the arguments are provided; however, these should be adjusted depending on the total number of samples and the number of samples per condition.
After filtering low-count exon bins, any remaining genes containing only a single exon bin are also removed (since differential splicing requires multiple exon bins).
Filtering should be skipped when using exon microarray data. (When using the `regsplice` wrapper function, filtering can be disabled with the argument `filter = FALSE`).


**Value**

Returns a `RegspliceData` object.

**See Also**

`filterZeros runNormalization`

**Examples**

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
runtimeCount(rs_data)
runtimeCount(rs_data)
```

---

**filterZeros**

Filter zero-count exons.

**Description**

Filter exons with zero RNA-seq read counts in all biological samples.

**Usage**

`filterZeros(rs_data)`

**Arguments**

- `rs_data` : `RegspliceData` object.
Details

Removes exon bins with zero RNA-seq read counts in all biological samples. Any remaining single-exon genes (after filtering) are also removed (since differential splicing requires multiple exon bins).

Input data is assumed to be in the form of a RegspliceData object. See RegspliceData for details.

After filtering zero-count exon bins, any remaining genes containing only a single exon bin are also removed (since differential splicing requires multiple exon bins).

Filtering should be skipped when using exon microarray data. (When using the regsplice wrapper function, filtering can be disabled with the argument filter = FALSE).


Value

Returns a RegspliceData object.

See Also

RegspliceData filterLowCounts

Examples

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
```

---

**fitRegMultiple**  
*Fit models.*

**Description**

Model fitting functions for regsplice package.
Usage

```r
fitRegMultiple(
  rs_results,
  rs_data,
  alpha = 1,
  lambda_choice = c("lambda.min", "lambda.1se"),
  seed = NULL,
  ...
)
```

```r
fitNullMultiple(rs_results, rs_data, seed = NULL, ...)
```

```r
fitFullMultiple(rs_results, rs_data, seed = NULL, ...)
```

Arguments

- **rs_results** `RegspliceResults` object, which will be used to store results. Initialized using the constructor function `RegspliceResults()`. See `RegspliceResults` for details.

- **rs_data** `RegspliceData` object. In the case of RNA-seq read count data, this has been pre-transformed with `runVoom`. Contains counts and weights data matrices, and vector of experimental conditions for each biological sample stored in `colData`. See `RegspliceData` for details.

- **alpha** Elastic net parameter `alpha` for glmnet model fitting functions. Must be between 0 (ridge regression) and 1 (lasso). Default is 1 (lasso). See `glmnet` documentation for more details.

- **lambda_choice** Parameter to select which optimal `lambda` value to choose from the `cv.glmnet` cross validation fit. Choices are "lambda.min" (model with minimum cross-validated error) and "lambda.1se" (most regularized model with cross-validated error within one standard error of minimum). Default is "lambda.min". See `glmnet` documentation for more details.

- **seed** Random seed (integer). Default is NULL. Provide an integer value to set the random seed for reproducible results.

- **...** Other arguments to pass to `cv.glmnet`, `glmnet`, or `glm`.

Details

There are three model fitting functions:

- `fitRegMultiple` fits regularized (lasso) models containing an optimal subset of exon:condition interaction terms for each gene. The model fitting procedure penalizes the interaction terms only, so that the main effect terms for exons and samples are always included. This ensures that the null model is nested, allowing likelihood ratio tests to be calculated.

- `fitNullMultiple` fits the null models, which do not contain any interaction terms.

- `fitFullMultiple` fits full models, which contain all exon:condition interaction terms for each gene.

See `createDesignMatrix` for more details about the terms in each model.
The fitting functions fit models for all genes in the data set.
A random seed can be provided with the seed argument, to generate reproducible results.
If the rs_data object does not contain a weights matrix, all exon bins are weighted equally.
Previous step: Initialize RegspliceResults object with initializeResults. Next step: Calculate likelihood ratio tests with LRTests.

Value

Returns a RegspliceResults object containing deviance and degrees of freedom of the fitted models. See RegspliceResults for details.

See Also

createDesignMatrix RegspliceResults initializeResults LRTests
glmnet cv.glmnet glm

Examples

define file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl.exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene.IDs <- names(tbl.exons)
n_exons <- unname(tbl.exons)
condition <- rep(c("untreated", "treated"), each = 3)
rs_data <- RegspliceData(counts, gene.IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
rs_data <- filterLowCounts(rs_data)
rs_data <- runNormalization(rs_data)
rs_data <- runVoom(rs_data)
rs_results <- initializeResults(rs_data)
rs_results <- fitRegMultiple(rs_results, rs_data)
rs_results <- fitNullMultiple(rs_results, rs_data)
rs_results <- fitFullMultiple(rs_results, rs_data)
Usage

initializeResults(rs_data)

Arguments

rs_data  RegspliceData object. This should contain gene IDs in a column named gene_IDs in the row meta-data, which can be accessed with the accessor function rowData.

Details

Creates a RegspliceResults object containing gene names only. This object will subsequently be populated using the functions fitRegMultiple, fitNullMultiple, fitFullMultiple, and LRTests.

Previous step: Calculate limma-voom transformation and weights with runVoom. Next step: Fit models with fitRegMultiple, fitNullMultiple, and fitFullMultiple.

Value

Returns a RegspliceResults object containing gene IDs only.

See Also

RegspliceData RegspliceResults fitRegMultiple fitNullMultiple fitFullMultiple LRTests summaryTable

Examples

file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)

rs_data <- filterZeros(rs_data)
rs_data <- filterLowCounts(rs_data)
rs_data <- runNormalization(rs_data)
rs_data <- runVoom(rs_data)

rs_results <- initializeResults(rs_data)
LRTests

*Calculate likelihood ratio tests.*

**Description**

Calculate likelihood ratio tests between fitted models and null models.

**Usage**

```r
LRTests(rs_results, when_null_selected = c("ones", "full", "NA"))
```

**Arguments**

- `rs_results` : `RegspliceResults` object containing results generated by `fitRegMultiple`, `fitNullMultiple`, and `fitFullMultiple`. If `when_null_selected = "ones"` or "NA", the "full" models are not required. See `RegspliceResults` for details.

- `when_null_selected` : Which option to use for genes where the lasso model selects zero interaction terms, i.e. identical to the null model. Options are "ones", "full", and "NA". Default is "ones". See below for details.

**Details**

The regularized (lasso) fitted models contain an optimal subset of exon:condition interaction terms for each gene, and the "full" fitted models contain all exon:condition interaction terms. The null models contain zero interaction terms, so they are nested within the fitted models.

The likelihood ratio (LR) tests compare the fitted models against the nested null models.

If the regularized (lasso) model contains at least one exon:condition interaction term, the LR test compares the lasso model against the null model. However, if the lasso model contains zero interaction terms, then the lasso and null models are identical, so the LR test cannot be calculated. The `when_null_selected` argument lets the user choose what to do in these cases: either set p-values equal to 1 (`when_null_selected = "ones"`); or calculate a LR test using the "full" model containing all exon:condition interaction terms (`when_null_selected = "full"`), which reduces power due to the larger number of terms, but allows the evidence for differential exon usage among these genes to be distinguished. You can also return NAs for these genes (`when_null_selected = "NA"`).

The default option is `when_null_selected = "ones"`. This simply calls all these genes non-significant, which in most cases is sufficient since we are more interested in genes with strong evidence for differential exon usage. However, if it is important to rank the low-evidence genes in your data set, use the `when_null_selected = "full"` option.

If `when_null_selected = "ones"` or `when_null_selected = "NA"`, the "full" fitted models are not required.

Previous step: Fit models with `fitRegMultiple`, `fitNullMultiple`, and `fitFullMultiple`. Next step: Generate summary table of results with `summaryTable`. 

Value

Returns a `RegspliceResults` object containing results of the LR tests. The results consist of the following entries for each gene:

- `p_vals`: raw p-values
- `p_adj`: multiple testing adjusted p-values (Benjamini-Hochberg false discovery rates, FDR)
- `LR_stats`: likelihood ratio test statistics
- `df_tests`: degrees of freedom of likelihood ratio tests

See Also

`RegspliceResults initializeResults fitRegMultiple fitNullMultiple fitFullMultiple summaryTable`

Examples

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
rs_data <- filterLowCounts(rs_data)
rs_data <- runNormalization(rs_data)
rs_data <- runVoom(rs_data)

rs_results <- initializeResults(rs_data)
rs_results <- fitRegMultiple(rs_results, rs_data)
rs_results <- fitNullMultiple(rs_results, rs_data)
rs_results <- fitFullMultiple(rs_results, rs_data)
rs_results <- LRTests(rs_results)
```

---

**regsplice**

Wrapper function to run regsplice.

**Description**

Wrapper function to run a regsplice analysis with a single command.
Usage

regsplice(
    rs_data,
    filter_zeros = TRUE,
    filter_low_counts = TRUE,
    filter_min_per_exon = 6,
    filter_min_per_sample = 3,
    normalize = TRUE,
    norm_method = "TMM",
    voom = TRUE,
    alpha = 1,
    lambda_choice = c("lambda.min", "lambda.1se"),
    when_null_selected = c("ones", "full", "NA"),
    seed = NULL,
    ...
)

Arguments

rs_data RegspliceData object containing input data. See RegspliceData for details.
filter_zeros Whether to filter zero-count exon bins, using filterZeros. Default is TRUE. Set to FALSE for exon microarray data.
filter_low_counts Whether to filter low-count exon bins, using filterLowCounts. Default is TRUE. Set to FALSE for exon microarray data.
filter_min_per_exon Filtering parameter for low-count exon bins: minimum number of reads per exon bin, summed across all biological samples. Default is 6. See filterLowCounts for details.
filter_min_per_sample Filtering parameter for low-count exon bins: minimum number of reads per biological sample; i.e. for each exon bin, at least one sample must have this number of reads. Default is 3. See filterLowCounts for details.
normalize Whether to calculate normalization factors, using runNormalization. Default is TRUE. If FALSE, non-normalized library sizes will be used. Set to FALSE for exon microarray data.
norm_method Normalization method to use. Options are "TMM", "RLE", "upperquartile", and "none". Default is "TMM". See runNormalization for details.
voom Whether to calculate limma-voom transformation and weights, using runVoom. Default is TRUE. If FALSE, model fitting functions will use the raw input data (not recommended for count data) with exon bins weighted equally. Set to FALSE for exon microarray data.
alpha Elastic net parameter alpha for glmnet model fitting functions. Must be between 0 (ridge regression) and 1 (lasso). Default is 1 (lasso). See glmnet documentation for more details.
**Details**

This wrapper function runs the `regsplice` analysis pipeline with a single command. The required input format is a `RegspliceData` object, which is created with the `RegspliceData` constructor function.

The wrapper function calls each of the individual functions in the analysis pipeline in sequence. You can also run the individual functions directly, which provides additional flexibility and insight into the statistical methodology. See the vignette for a description of the individual functions and an example workflow.

After running the analysis pipeline, a summary table of the results can be displayed with `summaryTable`.

Note that when using exon microarray data, the filtering, normalization, and voom steps should be disabled with the respective arguments.

See `RegspliceData` for details on constructing the input data object; `filterZeros` and `filterLowCounts` for details about filtering; `runNormalization` and `runVoom` for details about calculation of normalization factors and voom transformation and weights; `createDesignMatrix` for details about the model design matrices; `fitRegMultiple`, `fitNullMultiple`, or `fitFullMultiple` for details about the model fitting functions; and `LRTests` for details about the likelihood ratio tests.

**Value**

Returns a `RegspliceResults` object containing fitted model results and likelihood ratio (LR) test results. The LR test results consist of the following entries for each gene:

- **p_vals**: raw p-values
- **p_adj**: multiple testing adjusted p-values (Benjamini-Hochberg false discovery rates, FDR)
- **LR_stats**: likelihood ratio test statistics
- **df_tests**: degrees of freedom of likelihood ratio tests

See Also

`RegspliceData`, `RegspliceResults`, `initializeResults`, `filterZeros`, `filterLowCounts`, `runNormalization`, `runVoom`, `createDesignMatrix`, `fitRegMultiple`, `fitNullMultiple`, `fitFullMultiple`, `LRTests`, `summaryTable`
Examples

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_results <- regsplice(rs_data)
summaryTable(rs_results)
```

RegspliceData-class

RegspliceData objects.

Description

RegspliceData objects contain data in the format required by functions in the regsplice analysis pipeline.

Usage

```r
RegspliceData(counts, gene_IDs = NULL, n_exons = NULL, condition = NULL)

## S4 method for signature 'RegspliceData'
assays(x, withDimnames, ..., value)

countsData(x)

## S4 method for signature 'RegspliceData'
countsData(x)

weightsData(x)

## S4 method for signature 'RegspliceData'
weightsData(x)

## S4 method for signature 'RegspliceData'
rowData(x)

## S4 method for signature 'RegspliceData'
colData(x, ..., value)
```
## S4 method for signature 'RegspliceData,ANY,ANY,ANY'
\(x[i, j]\)

### Arguments

- **counts**: RNA-seq read counts or exon microarray intensities (matrix or data frame). Rows are exons, and columns are biological samples. Alternatively, `counts` also accepts a `SummarizedExperiment` input object containing all required input data, which may be useful when running `regsplice` as part of a pipeline with other packages.
- **gene_IDs**: Vector of gene IDs (character vector). Length is equal to the number of genes.
- **n_exons**: Vector of exon lengths (numeric vector of integers), i.e. the number of exon bins per gene. Length is equal to the number of genes.
- **condition**: Experimental condition for each biological sample (character or numeric vector, or factor).
- **x**: `RegspliceData` object (for accessor or subsetting functions).
- **withDimnames**: See `SummarizedExperiment::assays()`.
- **...**: Additional arguments for replacement with `\[<-\]`.
- **value**: Value for replacement with `\[<-\]`.
- **i**: Gene names (character vector) or row numbers (numeric vector) for subsetting genes or exons. Note that when subsetting whole genes, gene names (character vector) should be provided instead of row numbers, to avoid possible errors due to selecting incorrect row numbers. Row numbers may be provided to subset individual exons.
- **j**: Column numbers (numeric vector) for subsetting biological samples.

### Details

The `RegspliceData` format is based on the `SummarizedExperiment` container. Initially, objects contain raw data along with meta-data for rows (genes and exons) and columns (biological samples). During subsequent steps in the `regsplice` analysis pipeline, the data values are modified, and additional data and meta-data are added to the object. Final results are stored in a `RegspliceResults` object.

`RegspliceData` objects are created with the constructor function `RegspliceData()`. Required inputs for the constructor function are `counts` (matrix or data frame of RNA-seq read counts or exon microarray intensities), `gene_IDs` (vector of gene IDs), `n_exons` (vector of exon lengths, i.e. number of exon bins per gene), and `condition` (vector of experimental conditions for each biological sample).

Alternatively, the inputs can be provided as a `SummarizedExperiment` object, which will be parsed to extract each of these components. This may be useful when running `regsplice` as part of a pipeline together with other packages.

See the vignette for an example showing how to construct `gene_IDs` and `n_exons` from a column of gene:exon IDs.
Exon microarray intensities should be log2-transformed, which is usually done during pre-processing of microarray data. (RNA-seq counts will be transformed automatically during the regsplice analysis pipeline; see runVoom.)

After creating a RegspliceData object, the wrapper function regsplice can be used to run the analysis pipeline with a single command. Alternatively, you can run the individual functions for each step in the pipeline, beginning with filterZeros (see vignette for details).

Value

Returns a RegspliceData object.

Fields

- **counts** Matrix of RNA-seq read counts or exon microarray intensities. Rows are exons, and columns are biological samples.
- **weights** (Optional) Matrix of observation-level weights. Rows are exons, and columns are biological samples. Created by the runVoom function.
- **rowData** DataFrame of row meta-data. This should contain two columns: gene_IDs and exon_IDs, which are created by the RegspliceData constructor function.
- **colData** DataFrame of column meta-data. This contains the experimental condition and (optionally) normalization factors for each biological sample. Normalization factors are created by the runVoom function.

Accessor functions

- **countsData()**: Accesses the counts data matrix.
- **weightsData()**: Accesses the (optional) weights data matrix.
- **rowData()**: Accesses the DataFrame of row meta-data. This should contain two columns: gene_IDs and exon_IDs.
- **colData()**: Accesses the DataFrame of column meta-data. This contains the experimental condition and (optionally) normalization factors for each biological sample.

Subsetting

Subsetting of RegspliceData objects is performed with square brackets, `x[i, j]`, where `x` is the name of the object. The subsetting operations are designed to keep data and meta-data in sync.

For subsetting by rows, there are two possibilities:

- **Subsetting genes**: To subset whole genes, provide a character vector of gene names to the argument `i`. The returned object will contain all rows corresponding to these genes. Row numbers should not be used when subsetting whole genes, since this risks potential errors due to selecting incorrect rows.
- **Subsetting exons**: To subset individual exons, provide the corresponding row numbers to the argument `i`.

For subsetting by columns (biological samples), provide the corresponding column numbers to the argument `j`. 
See Also

regsplice filterZeros

Examples

# Example 1

counts <- matrix(sample(100:200, 14 * 6, replace = TRUE), nrow = 14, ncol = 6)
gene_IDs <- paste0("gene", 1:5)
n_exons <- c(3, 2, 3, 1, 5)
condition <- rep(c(0, 1), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)

rs_data
countsData(rs_data)
rowData(rs_data)
colData(rs_data)

rs_data[1, ]
rs_data[1, 1:3]

rs_data["gene1", ]
rs_data["gene1", 1:3]

# Vignette

file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)

head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, "\:"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)

rs_data
head(countsData(rs_data))
rowData(rs_data)
colData(rs_data)

rs_data[1, ]
rs_data[1, 1:3]
**Description**

RegspliceResults objects contain the results of a regsplice analysis.

**Usage**

RegspliceResults(gene_IDs)

gene_IDs(x)

## S4 method for signature 'RegspliceResults'
gene_IDs(x)

p_vals(x)

## S4 method for signature 'RegspliceResults'
p_vals(x)

p_adj(x)

## S4 method for signature 'RegspliceResults'
p_adj(x)

LR_stats(x)

## S4 method for signature 'RegspliceResults'
LR_stats(x)

df_tests(x)

## S4 method for signature 'RegspliceResults'
df_tests(x)

**Arguments**

gene_IDs Gene identifiers or names (character vector).
x RegspliceResults object (for accessor functions).
Details

RegspliceResults objects are created with the constructor function RegspliceResults(), which requires the gene IDs as an argument.

Once created, RegspliceResults objects are then populated using the functions fitRegMultiple, fitNullMultiple, fitFullMultiple, and LRTests.

The function summaryTable can be used to display a summary table of the results.

Value

Returns an empty RegspliceResults object.

Fields

gene_IDs  Gene identifiers or names (character vector).
fit_reg_dev  Deviance of fitted regularized (lasso) models from fitRegMultiple.
fit_reg_df  Degrees of freedom of fitted regularized (lasso) models from fitRegMultiple.
fit_null_dev  Deviance of fitted null models from fitNullMultiple.
fit_null_df  Degrees of freedom of fitted null models from fitNullMultiple.
fit_full_dev  Deviance of fitted full models from fitFullMultiple.
fit_full_df  Degrees of freedom of fitted full models from fitFullMultiple.
p_vals  Raw p-values (numeric vector).
p_adj  Multiple testing adjusted p-values (Benjamini-Hochberg false discovery rates, FDR).
LR_stats  Likelihood ratio test statistics.
df_tests  Degrees of freedom of likelihood ratio tests.

Accessor functions

- gene_IDs(): Accesses gene identifiers or names.
- p_vals(): Accesses raw p-values.
- p_adj(): Accesses multiple testing adjusted p-values (Benjamini-Hochberg false discovery rates, FDR).
- LR_stats(): Accesses likelihood ratio test statistics.
- df_tests(): Accesses degrees of freedom of likelihood ratio tests.

See Also

fitRegMultiple fitNullMultiple fitFullMultiple LRTests summaryTable

Examples

# initialize RegspliceResults object
gene_IDs <- paste0("gene", 1:5)
RegspliceResults(gene_IDs)
runNormalization

---

runNormalization  
*Calculate normalization factors.*

Description

Calculate normalization factors to scale library sizes, using the TMM (trimmed mean of M-values) method implemented in edgeR.

Usage

```r
runNormalization(rs_data, norm_method = "TMM")
```

Arguments

- **rs_data**: `RegspliceData` object, which has already been filtered with `filterZeros` and `filterLowCounts`.
- **norm_method**: Normalization method to use. Options are "TMM", "RLE", "upperquartile", and "none". See documentation for `calcNormFactors` in the edgeR package for details. Default is "TMM".

Details

Normalization factors are used to scale the raw library sizes (total read counts per sample). We use the TMM (trimmed mean of M-values) normalization method (Robinson and Oshlack, 2010), as implemented in the edgeR package.

For more details, see the documentation for `calcNormFactors` in the edgeR package.

This step should be performed after filtering with `filterZeros` and `filterLowCounts`. The normalization factors are then used by limma-voom in the next step (`runVoom`).

The normalization factors are stored in a new column named `norm_factors` in the column metadata (colData slot) of the `RegspliceData` object. The colData can be accessed with the accessor function `colData()`.

Normalization should be skipped when using exon microarray data. (When using the `regsplice` wrapper function, normalization can be disabled with the argument `normalize = FALSE`).


Value

Returns a `RegspliceData` object. Normalization factors are stored in the column `norm_factors` in the column meta-data (colData slot), which can be accessed with the `colData()` accessor function.

See Also

- `filterLowCounts runVoom`
Examples

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, "\""), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)
rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
rs_data <- filterLowCounts(rs_data)
rs_data <- runNormalization(rs_data)
```

---

**runVoom**

*Calculate 'voom' transformation and weights.*

**Description**

Use limma-voom to transform counts and calculate exon-level weights.

**Usage**

```r
runVoom(rs_data)
```

**Arguments**

- `rs_data` *RegspliceData* object, which has been filtered with `filterZeros` and `filterLowCounts`, and (optionally) normalization factors added with `runNormalization`.

**Details**

Raw counts do not fulfill the statistical assumptions required for linear modeling. The limma-voom methodology transforms counts to log2-counts per million (logCPM), and calculates exon-level weights based on the observed mean-variance relationship. Linear modeling methods can then be applied.

For more details, see the documentation for *voom* in the limma package.

Note that voom assumes that exon bins (rows) with zero or low counts have already been removed, so this step should be done after filtering with `filterZeros` and `filterLowCounts`.

Normalization factors can be provided in a column named `norm_factors` in the column meta-data (colData slot) of the *RegspliceData* object. These will be used by voom to calculate normalized library sizes. If normalization factors are not provided, voom will use non-normalized library sizes (columnwise total counts) instead.
The experimental conditions or group labels for each biological sample are assumed to be in a column named `condition` in the column meta-data (colData slot) of the `RegspliceData` object. This column is created when the object is initialized with the `RegspliceData()` constructor function.

The transformed counts are stored in the updated `counts` matrix, which can be accessed with the `countsData` accessor function. The weights are stored in a new data matrix labeled weights, which can be accessed with the `weightsData` accessor function. In addition, the normalized library sizes (if available) are stored in a new column named `lib_sizes` in the column meta-data (colData slot).

If you are using exon microarray data, this step should be skipped, since exon microarray intensities are already on a continuous scale.

Previous step: Calculate normalization factors with `runNormalization`. Next step: Initialize `RegspliceResults` object with the constructor function `RegspliceResults()`.

**Value**

Returns a `RegspliceData` object. Transformed counts are stored in the `counts` matrix, and weights are stored in a new `weights` data matrix. The data matrices can be accessed with the accessor functions `countsData` and `weightsData`.

**See Also**

`runNormalization` `fitRegMultiple` `fitNullMultiple` `fitFullMultiple` 

**Examples**

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data’exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)

rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_data <- filterZeros(rs_data)
rs_data <- filterLowCounts(rs_data)
rs_data <- runNormalization(rs_data)
rs_data <- runVoom(rs_data)
```

**summaryTable**

Summary table.

**Description**

Display summary table of results from a `regsplice` analysis.
Usage

```r
summaryTable(
  rs_results,
  n = 20,
  threshold = 0.05,
  rank_by = c("FDR", "p-value", "none")
)
```

Arguments

- **rs_results**: `RegspliceResults` object containing results of a regsplice analysis, generated with wrapper function `regsplice` (or individual functions up to `LRTests`). See `RegspliceResults` for details.
- **n**: Number of genes to display in summary table. Default is 20. If the total number of significant genes up to the significance threshold is less than `n`, only the significant genes are shown. Set to `Inf` to display all significant genes; or set both `n = Inf` and `threshold = 1` to display all genes in the data set.
- **threshold**: Significance threshold (for either FDR or raw p-values, depending on choice of argument `rank_by`). Default is 0.05. Set to 1 to display all `n` genes; or set both `n = Inf` and `threshold = 1` to display all genes in the data set.
- **rank_by**: Whether to rank genes by false discovery rate (FDR), raw p-values, or no ranking. Choices are "FDR", "p-value", and "none". Default is "FDR".

Details

The results of a regsplice analysis consist of a set of multiple testing adjusted p-values (Benjamini-Hochberg false discovery rates, FDR) quantifying the statistical evidence for differential exon usage (DEU) for each gene. Typically, the adjusted p-values are used to rank the genes in the data set according to their evidence for DEU, and an appropriate significance threshold (e.g. FDR < 0.05) can be used to generate a list of genes with statistically significant evidence for DEU.

The main regsplice functions return results in the form of a `RegspliceResults` object, which contains slots for gene names, fitted model results, raw p-values, multiple testing adjusted p-values (Benjamini-Hochberg FDR), likelihood ratio (LR) test statistics, and degrees of freedom of the LR tests. See `RegspliceResults` and the main regsplice wrapper function `regsplice` for details.

This function generates a summary table of the results. The results are displayed as a data frame of the top `n` most highly significant genes, ranked according to either FDR or raw p-values, up to a specified significance threshold (e.g. FDR < 0.05).

The argument `rank_by` controls whether to rank by FDR or raw p-values. The default is to rank by FDR.

To display results for all genes up to the significance threshold, set the argument `n = Inf`. To display results for all genes in the data set, set both `n = Inf` and `threshold = 1`.

Previous step: Run regsplice pipeline with the `regsplice` wrapper function (or individual functions up to `LRTests`).
**summaryTable**

**Value**

Returns a data frame containing results for the top \( n \) most highly significant genes, up to the specified significance threshold for the FDR or raw p-values.

**See Also**

*RegspliceResults* regsplice

**Examples**

```r
file_counts <- system.file("extdata/vignette_counts.txt", package = "regsplice")
data <- read.table(file_counts, header = TRUE, sep = "\t", stringsAsFactors = FALSE)
head(data)

counts <- data[, 2:7]
tbl_exons <- table(sapply(strsplit(data$exon, ":"), function(s) s[[1]]))
gene_IDs <- names(tbl_exons)
n_exons <- unname(tbl_exons)
condition <- rep(c("untreated", "treated"), each = 3)
rs_data <- RegspliceData(counts, gene_IDs, n_exons, condition)
rs_results <- regsplice(rs_data)

summaryTable(rs_results)
summaryTable(rs_results, n = Inf, threshold = 1)
```
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