Package ‘roar’

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roar-package ...................................................... 2
checkStep ...................................................... 2
combineFisherMethod ......................................... 3
computePairedPvals .......................................... 3
computePvals .................................................. 4
computeRoars .................................................. 5
cores ............................................................. 6
countPrePost ................................................... 7
countResults ................................................... 8
fpkmResults .................................................... 9
getFisher ......................................................... 10
meanAcrossAssays ........................................... 11
pvalueCorrectFilter ......................................... 11
pvalueFilter .................................................... 12
RoarDataset ................................................... 13
Identify differential APA usage from RNA-seq alignments

Description
Identify preferential usage of APA sites, comparing two biological conditions, starting from known alternative sites and alignments obtained from standard RNA-seq experiments.

Details
The code RoarDataset class exposes methods to perform the whole analysis, in order to identify genes with preferential expression of long/short isoforms in a condition with respect to another one. The needed input data are alignments deriving from RNA-seq experiments of the two conditions and a set of coordinates of APA sites for genes with an alternative APA site proximal to the one used “normally”.

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checkStep
Private/inner/helper method to check the order of the invoked analysis methods

Description
This method should not be used by package users. It gets an rds object and a required number of analysis step and, if possible, calls the requested method to reach that step. It returns the object and a logical value that tells if the analysis can go on.

Usage
checkStep(rds, neededStep)

Arguments
rds A RoarDataset object.
neededStep The analysis step where rds should be/arrive.
combineFisherMethod

Value
A list containing a logical that shows if the needed step could be reached with rds and the object at the requested step. Check step won’t repeat a step already done and the logical value will be FALSE in this case (and rds won’t be returned modified).

Description
This method should not be used by package users. Given a numerical vector of pvalues, which should be obtained from independent tests on the same null hypothesis, this will give the combined pvalue following the Fisher method.

Usage
combineFisherMethod(pvals)

Arguments
pvals A numerical vector with pvalues of independent tests on the same H0.

Value
The combined pvalue given by the Fisher method.

computePairedPvals Computes pvalues (Fisher test) on the read counts in this roar analysis

Description
This is the third step in the Roar analyses: it applies a Fisher test comparing counts falling on the PRE and POST portion in the treatment and control conditions for every gene. The paired method should be used when the experimental setup offers multiple paired samples for the two conditions: that is foreach sample of the control condition there is a naturally paired one for the treatment (i.e. cells derived from the same plate divided in two groups and treated or not). For example in the below code sample treatment sample n.1 (rd1) is paired with control n.2 (rd4) and rd2 with rd3. The pvalue resulting from Fisher test applied on the different samples pairings will be combined with the Fisher method, therefore the pairs of samples should be independent between each other.

Usage
computePairedPvals(rds, treatmentSamples, controlSamples)
computePvals

Arguments

rds
The RoarDataset or the RoarDatasetMultipleAPA which contains the counts over PRE-POST portions in the two conditions to be compared via pvalues.

treatmentSamples
Numbers that represent the indexes of the treatmentBams/GappedAlign parameter given to the RoarDataset constructor and the order in which they are paired with control samples.

controlsamples
Numbers that represent the indexes of the controlBams/GappedAlign parameter given to the RoarDataset constructor and the order in which they are paired with treatment samples.

Value

The RoarDataset or the RoarDatasetMultipleAPA object given as rds with the compute pvalues phase of the analysis done. Pvalues will be held in the RoarDataset object itself in the case of single samples, while in a separate slot otherwise, but end user normally should not analyze those directly but use totalResults or fpkmResults at the end of the analysis.

Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id))),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rd4 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3400), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(rd1,rd2), list(rd3, rd4), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePairedPvals(rds, c(1,2), c(2,1))
```

computePvals Computes pvalues (Fisher test) on the read counts in this roar analysis

Description

This is the third step in the Roar analyses: it applies a Fisher test comparing counts falling on the PRE and POST portion in the treatment and control conditions for every gene. If there are multiple samples for a condition every combinations of comparisons between the samples lists are considered.
computeRoars

Usage

computePvals(rds)

Arguments

rds

The RoarDataset or the RoarDatasetMultipleAPA which contains the counts over PRE-POST portions in the two conditions to be compared via pvalues.

Value

The RoarDataset or the RoarDatasetMultipleAPA object given as rds with the compute pvalue phase of the analysis done. Pvalues will be held in the RoarDataset object itself in the case of single samples, while in a separate slot otherwise, but end user normally should not analyze those directly but use totalResults or fpkmResults at the end of the analysis.

Examples

library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id)) ),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1,rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePvals(rds)

computeRoars

Computes m/M and roar values

Description

This is the second step in the Roar analyses: it computes the ratio of prevalence of the short and long isoforms for every gene in the treatment and control condition (m/M) and their ratio, roar, that indicates if there is a relative shortening-lengthening in a condition over the other one. A roar > 1 for a given gene means that in the treatment condition that gene has an higher ratio of short vs long isoforms with respect to the control condition (and the opposite for roar < 1). Negative or NA m/M or roar occurs in not definite situations, such as counts equal to zero for PRE or POST portions. If for one of the conditions there are more than one samples then calculations are performed on average counts.
Usage

```r
computeRoars(rds, qwidthTreatment=NA, qwidthControl=NA)
computeRoars(rds, qwidthTreatment, qwidthControl)
```

Arguments

- **rds**
  The `RoarDataset` or the `RoarDatasetMultipleAPA` which contains the counts over PRE-POST portions in the two conditions to be compared via roar.

- **qwidthTreatment**
  The mean length of the reads in the treatment bam files - used internally for the interaction between `RoarDataset` and `RoarDatasetMultipleAPA` objects. The default (NA) calculates this value from the bam and should not be changed.

- **qwidthControl**
  The mean length of the reads in the control bam files - used internally for the interaction between `RoarDataset` and `RoarDatasetMultipleAPA` objects. The default (NA) calculates this value from the bam and should not be changed.

Value

The `RoarDataset` or the `RoarDatasetMultipleAPA` object given as rds with the computeRoars phase of the analysis done. m/M and roars will be held in the RoarDataset object itself in the case of single samples, while in two slots otherwise, but end user normally should not analyze those directly but use `totalResults` or `fpkmResults` at the end of the analysis.

Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id))),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1,rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
```
countPrePost

Description

Right now always returns 1 as long as multi-core support has to be implemented.

Usage

cores(rds)

Arguments

rds A RoarDataset object.

Value

The number of cores used by this roar analysis.

countPrePost Counts reads falling over PRE/POST portions of the given transcripts

Description

This is the first step in the Roar analyses: it counts reads overlapping with the PRE/POST portions defined in the given gtf/GRanges annotation. See RoarDataset for details on how to define these portions. Reads of the given bam annotation files that falls over this portion are accounted for with the following rules:

1- reads that align on only one of the given features are assigned to that feature, even if the overlap is not complete 2- reads that align on both a PRE and a POST feature of the same gene (spanning reads) are assigned to the POST one, considering that they have clearly been obtained from the longest isoform

If the stranded argument is set to TRUE then strandness is considered when counting reads. When rds is a RoarDatasetMultipleAPA counts are obtained on more than two portions for each transcript in order to be able to efficiently evaluate multiple APA sites. The option stranded=TRUE is still not implemented for RoarDatasetMultipleAPA.

Usage

countPrePost(rds, stranded=FALSE)

Arguments

rds The RoarDataset or the RoarDatasetMultipleAPA which contains the alignments and annotation informations over which counts will be performed.

stranded A logical indicating if strandness should be considered when counting reads or not. Default=FALSE. WARNING: not implemented (ignored) when using RoarDatasetMultipleAPA.
The `RoarDataset` object given as `rds` with the counting reads phase of the analysis done. Counts will be held in the RoarDataset object itself in the case of single samples, while

```
library(GenomicAlignments)

gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id))),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))

rds <- RoarDataset(list(c(rd1, rd2)), list(rd3), features)
rds <- countPrePost(rds)
```

---

**countResults**

Returns a dataframe with results of the analysis for a `RoarDataset` object or a `RoarDatasetMultipleAPA` object

**Description**

The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (number of reads falling over the PRE portions).

**Usage**

```
countResults(rds)
```

**Arguments**

- **rds** The `RoarDataset` or the `RoarDatasetMultipleAPA` with all the analysis steps (countPrePost, computeRoars, computePvals) performed. If one or more steps hadn’t been performed they will be called automatically.

**Value**

The resulting dataframe will be identical to that returned by `link{totalResults}` but with two columns added: "treatmentValue" and "controlValue". These columns will contain a number that indicates the level of expression of the relative gene in the treatment (or control) condition. For `RoarDataset` this number represents the counts (averaged across samples when applicable) obtained for the PRE portion of the gene. For `RoarDatasetMultipleAPA` every possible PRE choice will have its corresponding reads counts assigned and also the length of the PRE portion (counting only exonic bases). See the vignette for more details.
Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
    seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
    strand = strand(rep("+", length(gene_id))),
    ranges = IRanges(  
        start=c(1000, 2000, 3000, 3600),  
        width=c(1000, 900, 600, 300)),
    DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1,rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePvals(rds)
dat <- countResults(rds)
```

---

**fpkmResults**

*Returns a dataframe with results of the analysis for a RoarDataset object or a RoarDatasetMultipleAPA object*

**Description**

The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM).

**Usage**

```r
fpkmResults(rds)
```

**Arguments**

- **rds**
  
  The RoarDataset or the RoarDatasetMultipleAPA with all the analysis steps (*countPrePost, computeRoars, computePvals*) performed. If one or more steps hadn’t been performed they will be called automatically.

**Value**

The resulting dataframe will be identical to that returned by `totalResults` but with two columns added: "treatmentValue" and "controlValue". These columns will contain a number that indicates the level of expression of the relative gene in the treatment (or control) condition. For RoarDataset this number derives from the counts (averages across samples when applicable) obtained for the PRE portion of the gene and is similar to the RPKM standard measure of expression used in RNAseq experiment. Specifically we correct the counts on the PRE portions dividing them by portion length and total nume of reads aligned on all PRE portions and the multiply the results for 1000000000. See the vignette for more details.
For `RoarDatasetMultipleAPA` the same procedure is applied to all the possible PRE choices for genes. Note that summing all the counts for every PRE portion assigned to a gene could lead to count some reads multiple times when summing all the PRE portions counts therefore this measure is not completely comparable with the one obtained with the single APA analysis. The length column added in this case contains the length of the PRE portions (counting only exonic bases).

Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id))),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3000),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1,rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePvals(rds)
dat <- fpkmResults(rds)
```

---

**getFisher**

*Private/inner/helper method to perform Fisher test*

**Description**

This method **should not** be used by package users. Given a numerical vector of length 4 it will perform a Fisher test and return the p-value for the two-sided test. Non-integer values will be rounded.

**Usage**

```r
getFisher(counts)
```

**Arguments**

- `counts` A numerical vector of length 4.

**Value**

The p-value for the two-sided Fisher test.
meanAcrossAssays

Private/inner/helper method to get average counts across samples

Description

This method should not be used by package users. It gets average counts for "pre" or "post" portions (depending on the wantedColumns argument) given the list of assays for one of the two conditions.

Usage

meanAcrossAssays(assays, wantedColumns)

Arguments

assays A list of matrixes/dataframes.

wantedColumns The name of the columns ("pre" or "post") whose means should be computed. Average will be calculated on the corresponding rows of the list of matrices/dataframe, working on the given column.

Value

The pvalue for the two.sided Fisher test.

pvalueCorrectFilter

Returns a dataframe with results of the analysis for a RoarDataset object or a RoarDatasetMultipleAPA object

Description

The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM). Only the genes with an expression estimate bigger than a given cutoff will be considered. Also pvalues, corrected considering multiple testing, will be considered for filtering.

Usage

pvalueCorrectFilter(rds, fpkmCutoff, pvalCutoff, method)

Arguments

rds The RoarDataset or the RoarDatasetMultipleAPA with all the analysis steps (countPrePost, computeRoars, computePvals) performed. If one or more steps hadn’t been performed they will be called automatically.

fpkmCutoff The cutoff that will be used to determine if a gene is expressed or not.

pvalCutoff The cutoff that will be used to determine if a pvalue is significative or not.

method The multiple test correction method that has to be used (used only for multiple paired samples or single samples, not used for multiple unpaired samples.)
pvalueFilter

Returns a dataframe with results of the analysis for a RoarDataset object or a RoarDatasetMultipleAPA object

Description

The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM). Only the genes with an expression estimate bigger than a given cutoff will be considered. Also pvalues will be considered for filtering.

Usage

pvalueFilter(rds, fpkmCutoff, pvalCutoff)
Arguments

- **rds**: The `RoarDataset` or the `RoarDatasetMultipleAPA` with all the analysis steps `countPrePost`, `computeRoars`, `computePvals`) performed. If one or more steps hadn’t been performed they will be called automatically.
- **fpkmCutoff**: The cutoff that will be used to determine if a gene is expressed or not.
- **pvalCutoff**: The cutoff that will be used to determine if a pvalue is significant or not.

Value

For `RoarDataset`:

The resulting dataframe will be identical to that returned by `standardFilter` but after gene expression and m/M values filtering another step will be performed: for single samples comparisons only genes with a nominal p-value smaller than the given cutoff will be considered, while for multiple samples a column (nUnderCutoff) will be added to the dataframe. This column will contain an integer number representing the number of comparisons between the samples of the two conditions that results in a nominal p-value lower than the given cutoff (pvalCutoff). For multiple samples with a paired design (i.e. if `computePairedPvals` was used) the pvalues of the requested pairings will be listed together with the combined pvalued obtained with the Fisher method and the filtering will be done on this pvalue.

For `RoarDatasetMultipleAPA`: for each gene we select the APA choice that is associated with the smallest p-value then proceed exactly as above.

Examples

```r
library("GenomicAlignments")
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id))),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1, rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePvals(rds)
dat <- pvalueFilter(rds, 1, 0.05)
```

---

**RoarDataset**

*Creates a RoarDataset object*

---

**Description**

This function creates an `RoarDataset` object from two lists of `GAlignments` and a `GRanges` containing a suitable annotation of alternative APA sites.
Usage

RoarDataset(treatmentGappedAlign, controlGappedAlign, gtfGRanges)

Arguments

treatmentGappedAlign
A list of `GAlignments` representing alignment of samples for the treatment condition (by convention it is considered the “treated” condition: this simply means that the package will compute roar values (ratios of the m/M) using this condition as the numerator) to be considered.

controlGappedAlign
A list of `GAlignments` representing alignment of samples for the control condition to be considered.

gtfGRanges
A `GRanges` object with coordinates for the portions of transcripts that has to be considered pertaining to the short (or long) isoform. This `GRanges` object must have a character metadata column called “gene_id” that ends with "_PRE" or "_POST" to address respectively the short and the long isoform. An element in the annotation is considered "PRE" (i.e. common to the short and long isoform of the transcript) if its gene_id ends with "_PRE". If it ends with "_POST" it is considered the portion present only in the long isoform. The prefix of gene_id should be a unique identifier for the gene and each identifier has to be associated with only one "_PRE" and one "_POST", leading to two genomic region associated to each gene_id. The `GRanges` object can also contain a numeric metadata column that represents the lengths of PRE and POST portions on the transcriptome. If this is omitted the lengths on the genome are used instead. Note that right now every gtf entry (or none of them) should have it.

Value

A `RoarDataset` object ready to be analyzed via the other methods.

See Also

`RoarDatasetFromFiles`

Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
    seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
    strand = strand(rep("+", length(gene_id)) ),
    ranges = IRanges(
        start=c(1000, 2000, 3000, 3600),
        width=c(1000, 900, 600, 300)),
    DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1, rd2)), list(rd3), features)
```
RoarDataset-class

Description

RoarDataset - a class to perform 3’UTR shortening analyses

Objects from the Class

Objects of this class should be created using the functions `RoarDataset` or `RoarDatasetFromFiles`, ideally the raw `new` method should never be invoked by end users. Then to perform the analysis the user should call, in order: countPrePost, computeRoars, computePvals and one of the methods to format results.

Slots

treatmentBams: Object of class "list" - a list of GappedAlignment objects for the first condition (by convention it is considered the “treated” condition) in analysis.
controlBams: Object of class "list" - a list of GappedAlignment objects for the second condition (by convention it is considered the “control” condition) in analysis.
prePostCoords: Object of class "GRanges" - represents the APA sites coords, defining "PRE" (last exon coords up until the alternative APA, defining the shorter isoform) and "POST" (from the alternative APA to the “standard” one) regions of the genes.
p后Coords: Object of class "GRanges" - private object.
countsTreatment: Object of class "RangedSummarizedExperiment" - private object.
countsControl: Object of class "RangedSummarizedExperiment" - private object.
pVals: Object of class "RangedSummarizedExperiment" - private object.
paired: "logical" slot - private.
step: "numeric" slot - private.
cores: "numeric" slot - private.
metadata: "list" slot - private.
rowRanges: Object of class "GRangesORGRangesList" - private object.
colData: Object of class "DataFrame" - private object.
assays: Object of class "Assays" - private object.

Extends

Class "RangedSummarizedExperiment", directly.

Methods

- `countPrePost` signature(rds = "RoarDataset", stranded = "logical"): Counts reads falling over PRE/POST portions of the given transcripts.
- `computeRoars` signature(rds = "RoarDataset"): Computes m/M and roar values for this `RoarDataset` object.
- `computePvals` signature(rds = "RoarDataset"): Computes pvalues (Fisher test) for this `RoarDataset` object.
**totalResults** signature(rds = "RoarDataset"): Returns a dataframe with results of the analysis for a `RoarDataset` object.

**fpkmResults** signature(rds = "RoarDataset"): The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM).

**countResults** signature(rds = "RoarDataset"): The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (counts over PRE portions).

**standardFilter** signature(rds = "RoarDataset", fpkmCutoff = "double"): Returns a dataframe with results of the analysis for a `RoarDataset` object.

**pvalueFilter** signature(rds = "RoarDataset", fpkmCutoff = "double", pvalCutoff = "double"): ...

**cores** signature(rds = "RoarDataset"): returns the number of cores used for computation, right now always 1.

**Author(s)**

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**Examples**

```
showClass("RoarDataset")
```

---

**RoarDatasetFromFiles**  *Creates a RoarDataset object*

**Description**

This function creates a `RoarDataset` object from two lists and a gtf with a suitable annotation of alternative APA sites.

**Usage**

```
RoarDatasetFromFiles(treatmentBams, controlBams, gtf)
```

**Arguments**

- `treatmentBams`: A list of filenames of bam alignments with data for the treatment condition (by convention it is considered the “treated” condition: this simply means that the package will compute roar values (ratios of the m/M) using this condition as the numerator) to be considered.
- `controlBams`: A list of filenames of bam alignments with data for the control condition to be considered.
- `gtf`: A filename of a gtf with coordinates for the portions of transcripts that has to be considered pertaining to the short (or long) isoform. This gtf must have an attribute called "gene_id" that ends with ".PRE" or ".POST" to address respectively the short and the long isoform. A ready-to-go gtf, with coordinates
RoarDatasetMultipleAPA

derived from the PolyADB on the human genome (version hg19), is available in
the "examples" package directory. An element in the annotation is considered
"PRE" (i.e. common to the short and long isoform of the transcript) if its gene_id
feature in the gtf ends with "_PRE". If it ends with "_POST" it is considered
the portion present only in the long isoform. The prefix of gene_id should be
an identifier for the gene and each identifier has to be associated with only one
"_PRE" and one "_POST", leading to two genomic region associated to each
gene_id. The gtf can also contain an attribute that represents the lengths of PRE
and POST portions on the transcriptome. If this is omitted the lengths on the
genome are used instead. Note that right now every gtf entry (or none of them)
should have it.

Value

A RoarDataset object ready to be analyzed via the other methods.

See Also

RoarDataset

Examples

# rds <- RoarDatasetFromFiles(treatmentBams, controlBams, gtf)

---

RoarDatasetMultipleAPA

Creates a RoarDatasetMultipleAPA object

Description

This function creates a RoarDatasetMultipleAPA object from two lists of of GAlignments and a
GRanges containing a suitable annotation of alternative APA sites and gene exon structure. A Mul-
tipleAPA analysis computes several roar values and p-values for each gene: one for every possible
combination of APA-canonical end of a gene (i.e. the end of its last exon). This is more efficient
than performing several different “standard” roar analyses choosing the PRE and POST portions
corresponding to different APAs because reads overlaps are computed only once.

Usage

RoarDatasetMultipleAPA(treatmentBamsGenomicAlignments, controlBamsGenomicAlignments, gtfGRanges)

Arguments

treatmentBamsGenomicAlignments

A list of GAlignments representing alignment of samples for the treatment condi-
tion (by convention it is considered the “treated” condition: this simply means
that the package will compute roar values (ratios of the m/M) using this condi-
tion as the numerator) to be considered.
controlBamsGenomicAlignments
A list of `GAlignments` representing alignment of samples for the control condition to be considered.

gtfGRanges
A `GRanges` containing a suitable annotation of alternative APA sites and gene exonic structure. Minimal requirements are: metadata columns called "gene", "apa" and "type." APA should be single bases falling over one of the given genes and need to have the metadata column "type" equal to "apa" and the "apa" column composed of unambiguous id and the corresponding gene id pasted together with an underscore. The "gene" metadata columns for these entries should not be initialized. All the studied gene exons need to be reported, in this case the metadata column "gene" should contain the gene id (the same one reported for each gene APAs) while "type" should be set to "gene" and "apa" to NA. All apa entries assigned to a gene should have coordinates that falls inside it and every gene that appears should contain at least one APA.

Value
A `RoarDatasetMultipleAPA` object ready to be analyzed via the other methods.

See Also
`RoarDatasetMultipleAPAFromFiles`

Examples
```r
library(GenomicAlignments)
gene <- c("A", "B", NA, NA)
type <- c("gene","gene","apa", "apa")
apa <- c(NA, NA, "apa1_A", "apa2_B")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr2", "chr1", "chr2")),
  strand = strand(rep("+", length(gene))),
  ranges = IRanges(
    start=c(1000, 2000, 1300, 2050),
    width=c(500, 900, 1, 1)),
  DataFrame(gene, apa, type)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("-"))
rds <- RoarDatasetMultipleAPA(list(c(rd1,rd1)), list(c(rd1,rd1)), features)
```
Objects from the Class

Objects from this class should be created using the functions RoarDatasetMultipleAPA or RoarDatasetMultipleAPAFromFiles; ideally the raw new method should never be invoked by end users. Then to perform the analysis the user should call, in order: countPrePost, computeRoars, computePvals and one of the methods to format results. This class is used to allow efficient analyses that allow to study more than one APA site for each gene: internally it uses a RoarDataset object that stores PRE/POST counts for all possible alternative APA choices for each gene.

Slots

treatmentBams: Object of class "list" - a list of GappedAlignment objects for the first condition (by convention it is considered the “treated” condition) in analysis.

treatmentBams: Object of class "list" - a list of GappedAlignment objects for the second condition (by convention it is considered the “control” condition) in analysis.
geneCoords: Object of class "GRangesList" - private object that represents the exon structures of genes in study.

apaCoords: Object of class "GRangesList" - private object that represents the APA falling on genes in study.

fragments: Object of class "GRangesList" - private object used to efficiently count reads falling on short and long isoforms.

prePostDef: Object of class "list" - private object representing all possible short and long isoforms.

roars: Object of class "list" - private object with a list of RoarDataset objects, each one representing all possible PRE/POST choices for a single gene.

corrTreatment: "numeric" slot - private, integer representing the mean length of reads for the treatment samples.

corrControl: "numeric" slot - private, integer representing the mean length of reads for the control samples.
paired: "logical" slot - private.

step: "numeric" slot - private.

cores: "numeric" slot - private.

Methods

countPrePost signature(rds = "RoarDatasetMultipleAPA", stranded = "logical"): Counts reads falling over all the possible PRE/POST portions of the given transcripts. WARNING: stranded = TRUE is still unsupported and could give unpredictable results.

computeRoars signature(rds = "RoarDatasetMultipleAPA"): Computes m/M and roar values for this RoarDatasetMultipleAPA object.

computePvals signature(rds = "RoarDatasetMultipleAPA"): Computes pvalues (Fisher test) for this RoarDatasetMultipleAPA object.

totalResults signature(rds = "RoarDatasetMultipleAPA"): Returns a dataframe with results of the analysis for a RoarDatasetMultipleAPA object.

fpkmResults signature(rds = "RoarDatasetMultipleAPA"): The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM).
RoarDatasetMultipleAPAFromFiles

Description

This function creates an RoarDatasetMultipleAPA object from two lists and a gtf with a suitable annotation of alternative APA sites and exonic structures of genes. A MultipleAPA analysis computes several roar values and p-values for each gene: one for every possible combination of APA-canonical end of a gene (i.e. the end of its last exon). This is more efficient than performing several different “standard” roar analyses choosing the PRE and POST portions corresponding to different APAs because reads overlaps are computed only once.

Usage

RoarDatasetMultipleAPAFromFiles(treatmentBams, controlBams, gtf)

Arguments

treatmentBams A list of filenames of bam alignments with data for the treatment condition (by convention it is considered the “treated” condition: this simply means that the package will compute roar values (ratios of the m/M) using this condition as the numerator) to be considered.

controlBams A list of filenames of bam alignments with data for the control condition to be considered.

gtf A filename of a gtf with coordinates for alternative APA sites and gene exonic structure. This gtf must have three attributes called "gene", "apa" and "type" to distinguish different features. APA should be single bases falling over one of the given genes and need to have the attribute "type" equal to "apa" and the "apa" attribute composed of unambiguous id and the corresponding gene id pasted.
together with an underscore. The "gene" attributes for these entries should not be initialized. All the studied gene exons need to be reported, in this case the attribute "gene" should contain the gene id (the same one reported for each gene APAs) while "type" should be set to "gene" and "apa" to NA. All apa entries assigned to a gene should have coordinates that falls inside it and every gene that appears should contain at least one APA. A ready-to-go gtf, with coordinates derived from the PolyADB on the human genome (version hg19), is available in the "examples" package directory.

Value

A RoarDatasetMultipleAPA object ready to be analyzed via the other methods.

See Also

RoarDatasetMultipleAPA

Examples

```r
treatmentBams <- readBams(treatmentBams)
controlBams <- readBams(controlBams)
gtf <- readGtf(gtf)

rds <- RoarDatasetMultipleAPAFromFiles(treatmentBams, controlBams, gtf)

standardFilter(rds, fpkmCutoff)
```

Description

The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values, pvalues and estimates of expression (a measure recalling FPKM). Only the genes with an expression estimate bigger than a given cutoff will be considered.

Usage

```r
standardFilter(rds, fpkmCutoff)
```

Arguments

- **rds**
  The RoarDataset or the RoarDatasetMultipleAPA with all the analysis steps (countPrePost, computeRoars, computePvals) performed. If one or more steps hadn’t been performed they will be called automatically.

- **fpkmCutoff**
  The cutoff that will be used to determine if a gene is expressed or not.

Value

For RoarDataset and RoarDatasetMultipleAPA:

The resulting dataframe will be identical to that returned by fpkmResults but it will contains rows relative only with genes with an expression estimate (treatment or controlValue) bigger than the given fpkmCutoff in both the conditions and with sensitive m/M and roar values (it removes negative or NA m/M values/roar - these values arise when there aren’t enough information to draw a conclusion about the shortening/lengthening of the gene).
Examples

```r
classicRoar <- function(...)
{
  library(GenomicAlignments)
  gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
  features <- GRanges(
    seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
    strand = strand(rep("+", length(gene_id))),
    ranges = IRanges(
      start = c(1000, 2000, 3000, 3600),
      width = c(1000, 900, 600, 300)),
    DataFrame(gene_id)
  )
  rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
  rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
  rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
  rds <- RoarDataset(list(c(rd1, rd2)), list(rd3), features)
  rds <- countPrePost(rds, FALSE)
  rds <- computeRoars(rds)
  rds <- computePvals(rds)
  dat <- standardFilter(rds, 1)
}

totalResults <- function(rds)
{
  Returns a dataframe with results of the analysis for a RoarDataset or a RoarDatasetMultipleAPA object

  Description

  The last step of a classical Roar analyses: it returns a dataframe containing m/M values, roar values and pvalues.

  Usage

  totalResults(rds)

  Arguments

  rds  The RoarDataset or RoarDatasetMultipleAPA with all the analysis steps (countPrePost, computeRoars, computePvals) performed.

  Value

  The RoarDataset or the RoarDatasetMultipleAPA object given as rds with all the analysis steps performed. If one or more steps hadn’t been performed they will be called automatically. The resulting dataframe will have the "gene_id" of the initial annotation as row names (without the trailing ".PRE"/".POST") and as columns the m/M ratio for the treatment and control conditions, the roar value and the Fisher test pvalue (respectively: mM_treatment, mM_control, roar, pval). If more than one sample has been given for a condition the "pval" column will contain the product of all the comparisons pvalue and there will be other columns containing the pvalues resulting from all the pairwise treatment vs control contrasts, with names "pvalue_X_Y" where X represent the position of the sample in the treatment list of bam files (or GappedAlignment) and Y the position for the control list. When using RoarDatasetMultipleAPA this dataframe will report multiple results for each gene_id with the _PRE"/".POST" suffix removed.
}
gene that corresponds to the pairings between every APA associated with that gene in the gtf and the
gene’s end - rownames in this case will be in the form geneid_apaid. **WARNING:** this method does
not filter in any way the results, therefore there will be negative m/M values/ROAR and also NA - in
these cases there aren’t enough information to draw a conclusion about the shortening/lengthening
of the gene in the given samples and thus the pvalues should not be kept in consideration. Furthermore
there isn’t any filter on the expression level of the genes. See `fpkmResults`, `standardFilter`
and `pvalueFilter` about results filtering possibilities.

Examples

```r
library(GenomicAlignments)
gene_id <- c("A_PRE", "A_POST", "B_PRE", "B_POST")
features <- GRanges(
  seqnames = Rle(c("chr1", "chr1", "chr2", "chr2")),
  strand = strand(rep("+", length(gene_id)) ),
  ranges = IRanges(
    start=c(1000, 2000, 3000, 3600),
    width=c(1000, 900, 600, 300)),
  DataFrame(gene_id)
)
rd1 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(1000), cigar = "300M", strand = strand("+"))
rd2 <- GAlignments("a", seqnames = Rle("chr1"), pos = as.integer(2000), cigar = "300M", strand = strand("+"))
rd3 <- GAlignments("a", seqnames = Rle("chr2"), pos = as.integer(3000), cigar = "300M", strand = strand("+"))
rds <- RoarDataset(list(c(rd1, rd2)), list(rd3), features)
rds <- countPrePost(rds, FALSE)
rds <- computeRoars(rds)
rds <- computePvals(rds)
dat <- totalResults(rds)
```
Index

*Topic RoarDatasetFromFiles
  RoarDatasetFromFiles, 16
  RoarDatasetMultipleAPAFromFiles, 20
*Topic RoarDataset
  RoarDataset, 13
  RoarDatasetMultipleAPA, 17
*Topic checkStep
  checkStep, 2
  cores, 6
*Topic classes
  RoarDataset-class, 15
  RoarDatasetMultipleAPA-class, 18
*Topic combineFisherMethod
  combineFisherMethod, 3
*Topic computePairedPvals
  computePairedPvals, RoarDataset, numeric, numeric-method (RoarDataset-class), 3
  computePairedPvals, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 3
  computePairedPvals, RoarDataset, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
  computePairedPvals, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
*Topic computePvals
  computePvals, RoarDataset, numeric, numeric-method (RoarDataset-class), 4
  computePvals, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
  computePvals, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
*Topic computeRoars
  computeRoars, RoarDataset, numeric, numeric-method (RoarDataset-class), 5
  computeRoars, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
  computeRoars, RoarDatasetMultipleAPA, numeric, numeric-method (RoarDatasetMultipleAPA-class), 18
*Topic countPrePost
  countPrePost, logical, 7
  countPrePost, logical-method (RoarDataset-class), 15
  countPrePost, RoarDataset, logical-method (RoarDataset-class), 15
*Topic countResults
  countResults, 8
*Topic fpkmResults
  fpkmResults, 9
*Topic getFisher
  getFisher, 10
*Topic meanAcrossAssays
  meanAcrossAssays, 11
*Topic package
  roar-package, 2
*Topic pvalueCorrectFilter
  pvalueCorrectFilter, 11
*Topic pvalueFilter
  pvalueFilter, 12
*Topic standardFilter
  standardFilter, 21
*Topic totalResults
  totalResults, 22

checkStep, 2
combineFisherMethod, 3
computePairedPvals, 3
computePairedPvals, RoarDataset
  (computePairedPvals), 3
computePairedPvals,
  RoarDatasetMultipleAPA
  (computePairedPvals), 3
computePairedPvals, RoarDataset, numeric, numeric-method
  (RoarDataset-class), 15
computePairedPvals, RoarDatasetMultipleAPA, numeric, numeric-method
  (RoarDatasetMultipleAPA-class), 18
computePairedPvals, RoarDatasetMultipleAPA, numeric, numeric-method
  (RoarDatasetMultipleAPA-class), 18
computePvals, RoarDataset
  (computePvals), 4
computePvals, RoarDatasetMultipleAPA
  (computePvals), 4
computePvals, RoarDataset-method
  (RoarDataset-class), 15
computePvals, RoarDatasetMultipleAPA-method
  (RoarDatasetMultipleAPA-class), 18
computePvals, RoarDatasetMultipleAPA-method
  (RoarDatasetMultipleAPA-class), 18
computeRoars, RoarDataset, numeric, numeric-method
  (computeRoars), 5
computeRoars, RoarDatasetMultipleAPA, numeric, numeric-method
  (computeRoars), 5
computeRoars, RoarDataset-method
  (RoarDataset-class), 15
computeRoars, RoarDatasetMultipleAPA-method
  (RoarDatasetMultipleAPA-class), 18
coreS, RoarDataset-method
  (RoarDataset-class), 15
cores, RoarDatasetMultipleAPA-method
  (RoarDatasetMultipleAPA-class), 18
countPrePost, 7, 8, 9, 11, 13, 15, 19, 21, 22
countPrePost, RoarDataset, logical-method
  (countPrePost), 7
countPrePost, RoarDataset, logical-method
  (RoarDataset-class), 15
countPrePost, RoarDataset-method
(RoarDataset-class), 15
pvalueFilter, RoarDatasetMultipleAPA
(pvalueFilter), 12
pvalueFilter, RoarDataset, numeric, numeric-method
(RoarDataset-class), 15

RangedSummarizedExperiment, 15
roar (roar-package), 2
RoarDataset, 2, 4–9, 11–13, 13, 14–17, 19,
21, 22
RoarDataset-class, 15
RoarDatasetFromFiles, 14, 15, 16
RoarDatasetMultipleAPA, 4–13, 17, 17,
18–22
RoarDatasetMultipleAPA-class, 18
RoarDatasetMultipleAPAFromFiles, 18, 19, 20
standardFilter, J2, 13, 16, 20, 21, 23
standardFilter, RoarDataset
(stdandardFilter), 21
standardFilter,
RoarDatasetMultipleAPA
(stdandardFilter), 21
standardFilter, RoarDataset, numeric-method
(RoarDataset-class), 15
standardFilter, RoarDatasetMultipleAPA, numeric-method
(RoarDatasetMultipleAPA-class), 18

totalResults, 4–6, 9, 22
totalResults, RoarDataset
(totalResults), 22
totalResults, RoarDatasetMultipleAPA
(totalResults), 22
totalResults, RoarDataset-method
(RoarDataset-class), 15
totalResults, RoarDatasetMultipleAPA-method
(RoarDatasetMultipleAPA-class), 18

fpkmResults, 4–6, 9, 16, 19, 21, 23
fpkmResults, RoarDataset (fpkmResults), 9
fpkmResults, RoarDatasetMultipleAPA
(fpkmResults), 9
fpkmResults, RoarDataset-method
(RoarDataset-class), 15
fpkmResults, RoarDatasetMultipleAPA-method
(RoarDatasetMultipleAPA-class), 18

GAlignments, 13, 14, 17, 18
getFisher, 10
GRanges, 13, 14, 17, 18

meanAcrossAssays, 11
new, 15, 19

pvalueCorrectFilter, 11
pvalueCorrectFilter, RoarDataset
(pvalueCorrectFilter), 11
pvalueCorrectFilter,
RoarDatasetMultipleAPA
(pvalueCorrectFilter), 11
pvalueCorrectFilter, RoarDataset, numeric, numeric, character-method
(RoarDataset-class), 15
pvalueCorrectFilter, RoarDatasetMultipleAPA, numeric, numeric, character-method
(RoarDatasetMultipleAPA-class), 18
pvalueFilter, 12, 16, 20, 23
pvalueFilter, RoarDataset
(pvalueFilter), 12