# Package 'transcriptR'

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Type Package

**Title** An Integrative Tool for ChIP- And RNA-Seq Based Primary Transcripts Detection and Quantification

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**Description** The differences in the RNA types being sequenced have an impact on the resulting sequencing profiles. mRNA-seq data is enriched with reads derived from exons, while GRO-, nucRNA- and chrRNA-seq demonstrate a substantial broader coverage of both exonic and intronic regions. The presence of intronic reads in GRO-seq type of data makes it possible to use it to computationally identify and quantify all de novo continuous regions of transcription distributed across the genome. This type of data, however, is more challenging to interpret and less common practice compared to mRNA-seq. One of the challenges for primary transcript detection concerns the simultaneous transcription of closely spaced genes, which needs to be properly divided into individually transcribed units. The R package transcriptR combines RNA-seq data with ChIP-seq data of histone modifications that mark active Transcription Start Sites (TSSs), such as, H3K4me3 or H3K9/14Ac to overcome this challenge. The advantage of this approach over the use of, for example, gene annotations is that this approach is data driven and therefore able to deal also with novel and case specific events. Furthermore, the integration of ChIP- and RNA-seq data allows the identification all known and novel active transcription start sites within a given sample.

**Imports** BiocGenerics, caret, chipseq, e1071, GenomicAlignments, GenomicRanges, GenomicFeatures, GenomeInfoDb, ggplot2, graphics, grDevices, IRanges (>= 2.11.15), pROC, reshape2, Rsamtools, rtracklayer, S4Vectors, stats, utils

**Depends** methods, R (>= 3.3)

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Collate 'utils.R' 'TranscriptionDataSet-class.R' 'ChipDataSet-class.R' 'show.R' 'TranscriptionDataSet-generics.R' 'TranscriptionDataSet-methods.R' 'ChipDataSet-generics.R' 'ChipDataSet-methods.R'
Suggests BiocStyle, knitr, rmarkdown, TxDb.Hsapiens.UCSC.hg19.knownGene, testthat
VignetteBuilder knitr
<b>biocViews</b> ImmunoOncology, Transcription, Software, Sequencing, RNASeq, Coverage
RoxygenNote 5.0.1
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addFeature

addFeature

## Description

Add feature(s) to the ChipDataSet object.

## Usage

```
addFeature(object, feature)
## S4 method for signature 'ChipDataSet'
addFeature(object, feature)
```

## Arguments

object A ChipDataSet object.

feature List. User specified characteristics of the peaks.

## Value

The user specified feature(s) will be added to the slot features of the provided ChipDataSet object.

#### Author(s)

Armen R. Karapetyan

## See Also

constructCDS predictTssOverlap

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#### **Examples**

```
### Load ChipDataSet object
data(cds)

### As an example create some fake data
N <- length(getPeaks(cds))
fake.data <- rnorm(n = N)

addFeature(object = cds, feature = list(fake = fake.data))

### View newly added feature
getPeaks(cds)</pre>
```

annot

Reference annotation (knownGene from UCSC)

## Description

annot is an object of GRanges class, containing genomic coordinates of the genes located on human chromosome 15 (chr15:63261757-84081194).

## Usage

annot

### **Format**

**GRanges** object

#### Value

**GRanges** object

annotateTranscripts

annotate Transcripts

## **Description**

Annotate detected transcripts by the available reference annotations based on genomic overlap.

## Usage

```
annotateTranscripts(object, annot, min.overlap = 0.3)
## S4 method for signature 'TranscriptionDataSet,GRanges'
annotateTranscripts(object, annot,
    min.overlap = 0.3)
```

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#### **Arguments**

object A TranscriptionDataSet object.
annot GRanges. Reference annotations.

min.overlap Numeric. A minimal proportion of the overlap between transcript and annota-

tion. A value in the range (0, 1]. Default: 0.3.

#### **Details**

Genomic overlap between transcript and annotation is calculated as the mean of two proportions: 1) proportion of the transcript length overlapping annotation; 2) proportion of the annotation length overlapping transcript. This approach levels off differences in length between transcript and annotation and, thus better suitable for cases in which the length of either transcript or annotation is much longer than of compared element.

If there is an overlap between transcript and annotation, the ID of the associated annotation will be linked to the transcript.

#### Value

An extra column annotation.overlap will be added to the metadata portion of the GRanges object which is stored in the transcripts slot of the provided TranscriptionDataSet object.

#### Author(s)

Armen R. Karapetyan

#### See Also

detectTranscripts

```
### Load TranscriptionDataSet object
data(tds)

### Load reference annotations (knownGene from UCSC)
data(annot)

### Detect transcripts
detectTranscripts(object = tds, coverage.cutoff = 5, gap.dist = 4000,
estimate.params = TRUE, combine.by.annot = FALSE, annot = annot)

### Annotate detected transcripts
annotateTranscripts(object = tds, annot = annot)

### View detected transcripts and associated annotations
getTranscripts(tds)
```

breakTranscriptsByPeaks

breakTranscriptsByPeaks

#### **Description**

The function divides closely spaced transcripts into individually transcribed units using the detected active transcription start sites.

## Usage

```
breakTranscriptsByPeaks(tdsObj, cdsObj, estimate.params = TRUE)
## S4 method for signature 'TranscriptionDataSet,ChipDataSet'
breakTranscriptsByPeaks(tdsObj,
    cdsObj, estimate.params = TRUE)
```

## **Arguments**

tdsObj A TranscriptionDataSet object.

cdsObj A ChipDataSet object.

estimate.params

Logical. Whether to estimate expression level and coverage density of the newly detected transcripts. Default: TRUE.

### **Details**

One of the challenges for primary transcript detection concerns the simultaneous transcription of closely spaced genes, which needs to be properly divided into individually transcribed units. transcriptR combines RNA-seq data with ChIP-seq data of histone modifications that mark active Transcription Start Sites (TSSs), such as, H3K4me3 or H3K9/14Ac to overcome this challenge. The advantage of this approach over the use of, for example, gene annotations is that this approach is data driven and therefore able to deal also with novel and case specific events. Furthermore, the integration of ChIP- and RNA-seq data allows the identification all known and novel active transcription start sites within a given sample. Transcription initiation within a peak region is investigated by comparing RNA-seq read densities upstream and downstream of empirically determined TSSs. Closely spaced transcripts are divided into individually transcribed units using the detected active TSSs.

#### Value

The slot transcripts of the provided TranscriptionDataSet object will be updated by the GRanges object, containing transcripts and, if estimated, corresponding expression levels.

## Author(s)

Armen R. Karapetyan

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#### See Also

```
predictStrand
```

## **Examples**

```
### Load TranscriptionDataSet object
data(tds)
### Load ChipDataSet object
data(cds)
### Load reference annotations (knownGene from UCSC)
data(annot)
### Detect transcripts
detectTranscripts(object = tds, coverage.cutoff = 5, gap.dist = 4000,
estimate.params = TRUE, combine.by.annot = FALSE, annot = annot)
### Classify peaks on gene associated and background
predictTssOverlap(object = cds, feature = "pileup", p = 0.75)
### Predict peak 'strand'
predictStrand(cds0bj = cds, tds0bj = tds, coverage.cutoff = 5,
quant.cutoff = 0.1, win.size = 2500)
### If `estimate.params = TRUE`, FPKM and coverage density will be re-calculated
breakTranscriptsByPeaks(tdsObj = tds, cdsObj = cds, estimate.params = TRUE)
### View detected transcripts
getTranscripts(tds)
```

cds

Example of ChipDataSet object.

#### **Description**

cds is an object of ChipDataSet class, containing H3K4me3 active histone mark ChIP-seq peaks from human chromosome 15 (chr15:63261757-84081194) profiled in prostate cancer LNCaP cells.

### Usage

cds

#### **Format**

ChipDataSet object

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#### Value

ChipDataSet object

ChipDataSet-class

ChipDataSet

#### **Description**

The ChipDataSet is a S4 class to store input values, intermediate calculations and results of ChIP-seq peaks analysis.

#### **Slots**

peaks GRanges. ChIP-seq peaks.

region GRanges. Genomic region(s) to extract peaks from.

genomicAnnotation Data.Frame. Genomic distribution of the peaks at distinct genomic features (TSSs, exons, introns, intergenic regions).

features Data. Frame. Estimated characteristics (features) of the peaks.

tssOverlapPrediction List. Prediction of the gene associated peaks. The following elements are reported:

- 'predicted.tssOverlap' predicted class (yes gene associated; no background) and probability of a ChIP-seq peak being classified as gene associated.
- 'confusionMatrix' cross-tabulation of observed and predicted classes with associated statistics.
- 'logFitSummary' statistical significance of the predictors used in the logistic regression.
- 'roc' results of the receiver operating characteristic analysis.

strandPrediction List. Prediction of the peak strandedness. The following elements are reported:

- 'predicted.strand' predicted ChIP-seq peak strand.
- 'probability.cutoff' probability cutoff for q2.
- 'results.plus' intermediate calculations for the forward DNA strand.
- 'results.minus' intermediate calculations for the reverse DNA strand.

### Author(s)

Armen R. Karapetyan

### See Also

constructCDS predictTssOverlap predictStrand

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## Description

The function constructs an object of class ChipDataSet, which is a container for holding processed sequencing data and the results of all downstream analyses. All the slots of the created object are filled during the workflow by applying specific functions to the object directly.

#### Usage

```
constructCDS(peaks, reads, region, TxDb, tssOf = c("gene", "transcript"),
  tss.region = c(-2000, 2000), reduce.peaks = FALSE, gapwidth = 1000,
  fragment.size, unique = TRUE, swap.strand = FALSE, param = NULL)
```

### **Arguments**

peaks	A path to a file with peaks. The file needs to have at least 3 columns (tabseparated): chromosome, start (peak), end (peak). The 4th column - name (peak id) is optional.
reads	A path to a BAM file with sequencing reads.
region	GRanges. Genomic region(s) to extract reads from. If not supplied, all the reads from a BAM file are extracted.
TxDb	TxDb object.
tss0f	Character. Extract Transcription Start Site (TSS) regions from either "gene" or "transcript" annotations. Default: "gene".
tss.region	A numeric vector of length two, which specifies the size of TSS region. Default: -2kb to 2kb.
reduce.peaks	Logical. Whether to merge neighboring peaks. Default: FALSE.
gapwidth	Numeric. A minimum distance (in bp) between peaks to merge. Default: 1000.
fragment.size	Numeric. Extend read length to the fragment size.
unique	Logical. Whether to remove duplicated reads (based on the genomic coordinates). Default: FALSE.
swap.strand	Logical. Whether to reverse the strand of the read. Default: FALSE.
param	ScanBamParam object influencing what fields and which records (reads) are imported from the Bam file. Default: NULL.

## **Details**

The function constructCDS initializes a ChipDataSet object, by providing the paths to the input files and information relevant to the ChIP-seq library preparation procedure. During the object construction the following steps are executed:

• The peak information is converted into the object of GRanges class.

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- The genomic distribution of the peaks is evaluated (exonic, intronic, intergenic, TSSs).
- Each peak in the data set is functionally characterized:
  - length the length of a peak (in base pairs).
  - fragments total number of fragments overlapping a peak region.
  - density number of fragments per base pair of the peak length.
  - pileup highest fragment pileup in each peak region.
  - tss0verlap overlap (binary, yes/no) of the peak with the annotated TSS region.

The estimated features are used to predict which of the peaks are gene associated in the analysis downstream.

As many peak-calling algorithms tend to divide broader peaks into the several narrower closely spaced peaks, it is advised to merge these end-to-end peaks to decrease the number of false positives and prevent unnecessary truncation of transcripts in the downstream analysis.

#### Value

An object of class ChipDataSet.

#### Author(s)

Armen R. Karapetyan

#### See Also

ChipDataSet predictTssOverlap

## **Examples**

```
### Load ChipDataSet object
data(cds)
### View a short summary of the object
cds
```

constructTDS

constructTDS

## **Description**

The function constructs an object of class TranscriptionDataSet, which is a container for holding processed sequencing data and the results of all downstream analyses. All the slots of the created object are filled during the workflow by applying specific functions to the object directly.

#### Usage

```
constructTDS(file, region, fragment.size = 250, unique = FALSE,
  paired.end = FALSE, swap.strand = FALSE, param = NULL)
```

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#### **Arguments**

file A path to a BAM file with sequencing reads.

region GRanges. Genomic region(s) to extract reads from. If not supplied, all the reads

from a BAM file are extracted.

fragment.size Numeric. Extend read length to the fragment size. Default: 250.

unique Logical. Whether to remove duplicated reads (based on the genomic coordi-

nates). Default: FALSE.

paired.end Logical. Whether to treat a BAM file as paired-end. Default: FALSE. swap.strand Logical. Whether to reverse the strand of the read. Default: FALSE.

param ScanBamParam object influencing what fields and which records (reads) are im-

ported from a BAM file. Default: NULL.

#### **Details**

The slots fragments, fragmentSize, region, coveragePlus, coverageMinus are filled during the object construction. The fragments holds information about genomic coordinates of the sequenced fragments (reads extended to the fragmento size). coveragePlus and coverageMinus for each position in the genome counts the number of fragments that cover it (for the details, see coverage). region holds information about the region used for fragments extraction.

#### Value

An object of class TranscriptionDataSet.

#### Author(s)

Armen R. Karapetyan

#### **Examples**

```
### Load TranscriptionDataSet object
data(tds)
### View a short summary of the object
tds
```

detectTranscripts d

detectTranscripts

### **Description**

The function dissects transcribed regions (transcripts) genome-wide and performs expression level quantification.

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### Usage

```
detectTranscripts(object, coverage.cutoff, gap.dist, estimate.params = TRUE,
   total.reads, combine.by.annot = FALSE, annot)

## S4 method for signature 'TranscriptionDataSet'
detectTranscripts(object, coverage.cutoff,
   gap.dist, estimate.params = TRUE, total.reads, combine.by.annot = FALSE,
   annot)
```

## Arguments

object A TranscriptionDataSet object.

coverage.cutoff

Numeric. A cutoff value to discard regions with the low fragments coverage, representing expression noise. By default, the value stored in the coverageCutoff slot of the supplied TranscriptionDataSet object is used. The optimal cutoff value can be calculated by estimateBackground function call.

gap.dist

Numeric. Maximum allowed distance between transcribed regions to be merged into the one transcript. By default, the value stored in the gapDistanceTest slot of the supplied TranscriptionDataSet object is used. The optimal gap distance can be calculated by estimateGapDistance function call.

estimate.params

Logical. Whether to estimate expression level and coverage density of the detected transcripts. Default: TRUE.

total.reads

Numeric. Total number of reads used for the normalization, when calculating FPKM. By default, the total number of reads stored in the provided TranscriptionDataSet object is used.

combine.by.annot

Logical. Whether to combine transcripts overlapping the same reference annotation. Default: FALSE.

annot GRanges. Reference annotations.

### Details

The function uses two parameters to identify transcribed regions: coverage.cutoff and gap.dist as calculated by the estimateBackground and estimateGapDistance, respectively and stored in the TranscriptionDataSet object. Alternatively, the user may specify his/her own values to be passed to the function. By increasing the gap.dist, fewer transcripts of longer size will be identified, and an increase in the coverage.cutoff will result in fewer transcripts of shorter size (a typical transcript tends to have a lower fragments coverage at the 3' end, and thus, the coverage.cutoff value will have an impact on the resulting length of the detected transcript).

If estimate.params is set TRUE, the following metrics are estimated for each transcript:

- length transcript length (in base pairs).
- bases.covered the number of bases covered by the sequencing fragments.
- coverage the proportion of transcript length covered by fragments. Value in the range (0, 1].

estimateBackground 13

- fragments total number of fragments per transcript.
- fpkm Fragments Per Kilobase of transcript per Million mapped reads.

The coverage is a measure of how densely the transcript is covered by the sequencing fragments. Modestly/highly expressed transcripts will have a value close to 1, whereas lowly expressed transcripts will have a value close to 0, indicating the sparse distribution of sequencing fragments along the transcript body.

#### Value

The slot transcripts of the provided TranscriptionDataSet object will be updated by the GRanges object, containing detected transcripts and, if estimated, corresponding expression levels.

#### Author(s)

Armen R. Karapetyan

#### See Also

constructTDS

### **Examples**

```
### Load TranscriptionDataSet object
data(tds)

### Load reference annotations (knownGene from UCSC)
data(annot)

### Detect transcripts
detectTranscripts(object = tds, coverage.cutoff = 5, gap.dist = 4000,
estimate.params = TRUE, combine.by.annot = FALSE, annot = annot)

### View detected transcripts
getTranscripts(tds)
```

estimateBackground

estimateBackground

### **Description**

Gene expression is a stochastic process, which often results in substantial expression noise. To obtain a putative set of transcribed regions, it is necessary to identify those regions that are expressed significantly above the background level. Using a Poisson-based approach for estimating the noise distribution from the frequency of the transcribed regions with the low fragments coverage, estimateBackground function returns a coverage cutoff value for a specific False Discovery Rate (FDR).

estimateGapDistance

#### Usage

```
estimateBackground(object, fdr.cutoff = 0.05)
## S4 method for signature 'TranscriptionDataSet'
estimateBackground(object, fdr.cutoff = 0.05)
```

### **Arguments**

object A TranscriptionDataSet object.

fdr. cutoff Numeric. False Discovery Rate cutoff value. Default: 0.05.

#### Value

The slots coverageCutoffFdr and coverageCutoff of the provided TranscriptionDataSet object will be updated by the FDR cutoff value used in the calculations and by the corresponding estimated coverage cutoff value, respectively.

#### Author(s)

Armen R. Karapetyan

#### See Also

constructTDS peakCutoff

#### **Examples**

```
### Load TranscriptionDataSet object
data(tds)

### Estimate coverage cutoff at different FDR levels
estimateBackground(object = tds, fdr = 0.01)
```

estimateGapDistance

*estimateGapDistance* 

## **Description**

The ultimate goal of transcriptR is to identify continuous regions of transcription. However, in some areas of the genome it is not possible to detect transcription, because of the presence of the low mappability regions and (high copy number) repeats. Sequencing reads can not be uniquely mapped to these positions, leading to the formation of gaps in otherwise continuous coverage profiles and segmentation of transcribed regions into multiple smaller fragments. The gap distance describes the maximum allowed distance between adjacent fragments to be merged into one transcript. To choose the optimal value for the gap distance, the detected transcripts should largely be in agreement with available reference annotations. To accomplish this, the function is build on the methodology proposed by Hah et al. (Cell, 2011). In brief, the two types of erros are defined:

estimateGapDistance 15

- dissected error the ratio of annotations that is segmented into two or more fragments.
- merged error the ratio of non-overlapping annotations that merged by mistake in the experimental data.

There is an interdependence between two types of errors. Increasing the gap distance decreases the dissected error, by detecting fewer, but longer transcripts, while the merged error will increase as more detected transcripts will span multiple annotations. The gap distance with the lowest sum of two error types is chosen as the optimal value.

## Usage

```
estimateGapDistance(object, annot, coverage.cutoff, filter.annot = TRUE,
  fpkm.quantile = 0.25, gap.dist.range = seq(from = 0, to = 10000, by =
  100))

## S4 method for signature 'TranscriptionDataSet,GRanges'
estimateGapDistance(object, annot,
  coverage.cutoff, filter.annot = TRUE, fpkm.quantile = 0.25,
  gap.dist.range = seq(from = 0, to = 10000, by = 100))
```

#### **Arguments**

object A TranscriptionDataSet object.
annot GRanges. Reference annotations.

coverage.cutoff

Numeric. A cutoff value to discard regions with the low fragments coverage, representing expression noise. By default, the value stored in the coverageCutoff slot of the supplied TranscriptionDataSet object is used. The optimal cutoff value can be calculated by estimateBackground function call.

filter annot Logical. Whether to filter out lowly expressed annotations, before estimating

error rates. Default: TRUE.

fpkm.quantile Numeric. A number in a range (0, 1). A cutoff value used for filtering lowly

expressed annotations. The value corresponds to the FPKM quantile estimated

for the supplied annotations. Default: 0.25.

gap.dist.range A numeric vector specifying a range of gap distances to test. By default, the

range is from 0 to 10000 with a step of 100.

### Value

The slot gapDistanceTest of the provided TranscriptionDataSet object will be updated by the data.frame, containing estimated error rates for each tested gap distance (see getTestedGapDistances, for the details).

#### Author(s)

Armen R. Karapetyan

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#### References

Hah N, Danko CG, Core L, Waterfall JJ, Siepel A, Lis JT, Kraus WL. A rapid, extensive, and transcriptional response to estrogen signaling in breast cancer cells. Cell. 2011.

#### See Also

constructTDS plotErrorRate getTestedGapDistances

## **Examples**

```
### Load TranscriptionDataSet object
data(tds)

### Load reference annotations (knownGene from UCSC)
data(annot)

### Estimate gap distance minimazing error rate
### Define the range of gap distances to test
gdr <- seq(from = 0, to = 10000, by = 1000)

estimateGapDistance(object = tds, annot = annot, coverage.cutoff = 5,
filter.annot = FALSE, gap.dist.range = gdr)

### View estimated gap distance
tds</pre>
```

exportCoverage

exportCoverage

#### **Description**

RNA-seq coverage profiles for both forward and reverse DNA strand can be visualized separately in the UCSC genome browser using exportCoverage. This function can generate tracks in BigWig and bedGraph formats, which can be uploaded to the genome browser as custom tracks.

### Usage

```
exportCoverage(object, file, type, strand, color,
  filter.by.coverage.cutoff = FALSE, coverage.cutoff = NULL, rpm = FALSE,
  total.reads)

## S4 method for signature 'TranscriptionDataSet'
exportCoverage(object, file, type, strand,
  color, filter.by.coverage.cutoff = FALSE, coverage.cutoff = NULL,
  rpm = FALSE, total.reads)
```

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#### **Arguments**

object A TranscriptionDataSet object.

file Character. A file name.

type Character. Track type, either "bigWig" or "bedGraph". Default: "bedGraph".

strand Character. The strand to create a track for. One of ["+", "-"].

color Object of class "integer" representing the track color (as from col2rgb). Only

works with tracks of type "bedGraph". Default: c(0L, 0L, 255L).

filter.by.coverage.cutoff

Logical. Whether to discard regions with low fragment coverage, representing

expression noise from the resulting track. Default: FALSE.

coverage.cutoff

Numeric. A cutoff value to discard regions with the low fragment coverage, representing expression noise. By default, the value stored in the coverageCutoff slot of the supplied object is used. The optimal cutoff value can be calculated

by estimateBackground function call. Default: NULL.

rpm Logical. Whether to perform normalization ('Reads Per Million'). Default:

FALSE.

number of reads stored in the provided TranscriptionDataSet object is used.

#### **Details**

There is an option to filter coverage profiles by the coverage cutoff value, either estimated for a specific FDR via estimateBackground or a user specified value. By default, the coverage cutoff value stored in the TranscriptionDataSet object is used. In order to make an informed decision about a proper FDR level, it is useful to explore the output at different FDR levels and determine the optimal cutoff value.

#### Value

A file in either BigWig or bedGraph format.

#### Author(s)

Armen R. Karapetyan

#### See Also

estimateBackground UCSC genome browser BigWig

```
### Load TranscriptionDataSet object
data(tds)

### Look at the coverage profile of the regions expressed above the background level
# exportCoverage(object = tds, file = "plus.bg", type = "bedGraph", strand = "+",
```

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```
# filter.by.coverage.cutoff = TRUE, coverage.cutoff = 3, rpm = FALSE)
### Or check the raw coverage (all expressed regions)
# exportCoverage(object = tds, file = "plus_raw.bg", type = "bedGraph",
# strand = "+", filter.by.coverage.cutoff = FALSE, rpm = FALSE)
```

 ${\tt getConfusionMatrix}$ 

getConfusionMatrix

## Description

Retrieve a cross-tabulation of observed and predicted classes (prediction of gene associated peaks) with associated statistics.

## Usage

```
getConfusionMatrix(object)
## S4 method for signature 'ChipDataSet'
getConfusionMatrix(object)
```

## Arguments

object

A ChipDataSet object.

## Value

An object of Confusion Matrix class. For the details see caret package.

#### Author(s)

Armen R. Karapetyan

### See Also

predictTssOverlap confusionMatrix

```
### Load ChipDataSet object
data(cds)
getConfusionMatrix(cds)
```

getGenomicAnnot 19

getGenomicAnnot

getGenomicAnnot

#### **Description**

Retrieve genomic distribution of ChIP-seq peaks at distinct genomic features (exons, introns, TSSs, intergenic regions)

## Usage

```
getGenomicAnnot(object)
## S4 method for signature 'ChipDataSet'
getGenomicAnnot(object)
```

### Arguments

object

A ChipDataSet object.

#### **Details**

A simple quality check of the supplied ChIP-seq peaks can be performed by investigating their genomic distribution. Ideally, these peaks should demonstrate substantial enrichment at TSS regions. Enrichment of the peaks at a given genomic feature (e.g. TSS) is defined as the ratio between the observed and expected number of peaks. The expected number of peaks is calculated from the proportion of the genome covered by the given genomic feature.

## Value

A four column Data. Frame, storing information about observed and expected number of peaks at distinct genomic features.

### Author(s)

Armen R. Karapetyan

#### See Also

constructCDS

```
### Load ChipDataSet object
data(cds)
getGenomicAnnot(cds)
```

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getPeaks

getPeaks

## Description

Retrieve ChIP-seq peak information from the ChipDataSet object.

## Usage

```
getPeaks(object)
## S4 method for signature 'ChipDataSet'
getPeaks(object)
```

## Arguments

object

A ChipDataSet object.

## Value

A GRanges object.

## Author(s)

Armen R. Karapetyan

## See Also

constructCDS predictTssOverlap predictStrand

```
### Load ChipDataSet object
data(cds)
getPeaks(cds)
```

```
{\it getPredictorSignificance} \\ {\it getPredictorSignificance}
```

## Description

Retrieve significance of each predictor used in the classification model fit (prediction of gene associated peaks).

## Usage

```
getPredictorSignificance(object)
## S4 method for signature 'ChipDataSet'
getPredictorSignificance(object)
```

## Arguments

object A ChipDataSet object.

### Value

A vector of p-values.

## Author(s)

Armen R. Karapetyan

### See Also

```
predictTssOverlap
```

```
### Load ChipDataSet object
data(cds)
getPredictorSignificance(cds)
```

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 ${\tt getProbTreshold}$ 

getProbTreshold

## Description

Retrieve estimated P(q2) threshold, used to select peaks with a putative transcription initiation event.

## Usage

```
getProbTreshold(object)
## S4 method for signature 'ChipDataSet'
getProbTreshold(object)
```

## Arguments

object

A ChipDataSet object.

## Value

Estimated P(q2) threshold.

## Author(s)

Armen R. Karapetyan

## See Also

predictStrand

```
### Load ChipDataSet object
data(cds)
getProbTreshold(cds)
```

getQuadProb 23

### Description

Retrieve all internal calculations performed by predictStrand function.

## Usage

```
getQuadProb(object, strand)
## S4 method for signature 'ChipDataSet'
getQuadProb(object, strand)
```

## **Arguments**

object A ChipDataSet object.

strand Character. The strand to extract calculations for. One of ["+", "-"].

#### Value

A nine column Data.Frame, where each row corresponds to a ChIP-seq peak and each column keeps one of the intermediate calculations:

- max.cov maximum coverage of the RNA-seq fragments inside the peak region.
- pass.cov.treshold whether the max.cov exceeds the coverage.cutoff, either user defined or estimated from RNA-seq data by estimateBackground function call and stored in TranscriptionDataSet object.
- q1q2.sepline.coord genomic coordinate corresponding to the transcription start position inside the peak region.
- q1. coord genomic coordinates of q1.
- q2. coord genomic coordinates of q2.
- q1. count total number of fragments in q1.
- q2. count total number of fragments in q2.
- q1. prob probability of a fragment being sampled from the q1.
- q2.prob probability of a fragment being sampled from the q2.

## Author(s)

Armen R. Karapetyan

#### See Also

```
predictStrand
```

#### **Examples**

```
### Load ChipDataSet object
data(cds)

### Load TranscriptionDataSet object
data(tds)

head(getQuadProb(cds, strand = "+"))
head(getQuadProb(cds, strand = "-"))
```

 ${\tt getTestedGapDistances} \ \ \textit{getTestedGapDistances}$ 

## Description

Retrieve a data. frame, containing a range of tested gap distances and estimated error rates.

## Usage

```
getTestedGapDistances(object)
## S4 method for signature 'TranscriptionDataSet'
getTestedGapDistances(object)
```

### Arguments

object

A TranscriptionDataSet object.

### Value

A data.frame containing estimated error rates (dissected, merged and sum of two errors) and corresponding gap distances.

#### Author(s)

Armen R. Karapetyan

### See Also

estimateGapDistance

```
### Load TranscriptionDataSet object
data(tds)
head(getTestedGapDistances(tds))
```

getTranscripts 25

getTranscripts getTranscripts
-------------------------------

#### **Description**

Retrieve transcripts information from the TranscriptionDataSet object.

## Usage

```
getTranscripts(object, min.length, min.fpkm, min.coverage)
## S4 method for signature 'TranscriptionDataSet'
getTranscripts(object, min.length, min.fpkm,
    min.coverage)
```

### **Arguments**

object A TranscriptionDataSet object.

min.length Numeric. A minimum length (in bp) of the reported transcripts.

min.fpkm Numeric. A minimum FPKM of the reported transcripts.

min.coverage Numeric. A minimum coverage of the reported transcripts.

#### **Details**

The coverage is a measure of how densely the transcript is covered by the sequencing fragments. Modestly/highly expressed transcripts will have a value close to 1, whereas lowly expressed transcripts will have a value close to 0, indicating the sparse distribution of sequencing fragments along the transcript body.

## Value

A GRanges object.

#### Author(s)

Armen R. Karapetyan

#### See Also

detectTranscripts

```
### Load TranscriptionDataSet object
data(tds)

### View detected transcripts
getTranscripts(tds)
```

26 peaksToBed

peaksToBed

peaksToBed

## **Description**

A convenient way to explore output of the predictions made on the ChIP peaks is to visualize them in the UCSC genome browser. The peaksToBed function returns a file in BED format, which can be uploaded directly to the genome browser. To improve the visual perception, peaks are color-coded by the predicted strand.

## Usage

```
peaksToBed(object, file, strand.pred.color = c("blue", "red", "green4",
    "black"), gene.associated.peaks = TRUE)

## S4 method for signature 'ChipDataSet'
peaksToBed(object, file, strand.pred.color = c("blue",
    "red", "green4", "black"), gene.associated.peaks = TRUE)
```

### **Arguments**

object A ChipDataSet object.

file Character. A file name.
strand.pred.color

Character. A vector of length four, specifying colors of the predicted peak strand. The order of colors corresponds to 1) plus strand, 2) minus strand, 3) bideractional, 4) strand not determined. Default: c("blue", "red", "green4", "black").

gene.associated.peaks

Logical. Whether to return gene associated peaks (based on the prediction) only. Default: TRUE

### Value

A file in the BED format.

## Author(s)

Armen R. Karapetyan

#### See Also

constructCDS predictTssOverlap predictStrand UCSC genome browser BED

plotErrorRate 27

#### **Examples**

```
### Load ChipDataSet object
data(cds)

### Load TranscriptionDataSet object
data(tds)

### Classify peaks on gene associated and background
predictTssOverlap(object = cds, feature = "pileup", p = 0.75)

### Predict peak 'strandedness'
predictStrand(cdsObj = cds, tdsObj = tds, coverage.cutoff = 5,
quant.cutoff = 0.1, win.size = 2500)

# peaksToBed(object = cds, file = "peaks.bed", gene.associated.peaks = TRUE)
```

plotErrorRate

plotErrorRate

### Description

A simple helper function that plot results of estimateGapDistance function call.

#### Usage

```
plotErrorRate(object, color = c("#1B9E77", "#D95F02", "#7570B3"),
    xlab = "Gap distance (kb)", ylab = "Error rate", ...)

## S4 method for signature 'TranscriptionDataSet'
plotErrorRate(object, color = c("#1B9E77",
    "#D95F02", "#7570B3"), xlab = "Gap distance (kb)", ylab = "Error rate",
    ...)
```

#### **Arguments**

```
object A TranscriptionDataSet object.

color Character. Colors to be used to plot estimated errors.

xlab Character. Label of the x-axis.

ylab Character. Lable of the y-axis.

... Further arguments passed to plot.
```

### **Details**

The tested gap distances are plotted on the x-axis and corresponding error rates on the y-axis. Three curved lines depict the two error types calculated by <code>estimateGapDistance</code> and the sum of both errors. The vertical dashed line depicts the gap distance with the smallest sum of two errors.

28 plotFeatures

#### Value

plot

#### Author(s)

Armen R. Karapetyan

#### See Also

```
estimateGapDistance
```

### **Examples**

```
### Load TranscriptionDataSet object
data(tds)

### Load reference annotations (knownGene from UCSC)
data(annot)

### Estimate gap distance minimazing error rate
### Define the range of gap distances to test
gdr <- seq(from = 0, to = 10000, by = 1000)

estimateGapDistance(object = tds, annot = annot, coverage.cutoff = 5,
filter.annot = FALSE, gap.dist.range = gdr)

plotErrorRate(object = tds, lwd = 2)</pre>
```

plotFeatures

plotFeatures

## **Description**

Visualize the relations between predictors and response variable ('tssOverlap').

#### Usage

```
plotFeatures(object, plot.type = c("box", "density"), feature, ncol, xlab,
  ylab, color = c("#E41A1C", "#377EB8"), alpha = 1)

## S4 method for signature 'ChipDataSet'
plotFeatures(object, plot.type = c("box", "density"),
  feature, ncol, xlab, ylab, color = c("#E41A1C", "#377EB8"), alpha = 1)
```

plotFeatures 29

## Arguments

object	A ChipDataSet object.
plot.type	One of ["box", "density"]. Default: "box"
feature	Feature to plot. By default, all the features are plotted.
ncol	Numeric. Arrange individual plots in columns. By default, the number of columns correspond to the number of features used for plotting.
xlab	Character. Title of the x-axis
ylab	Character. Title of the y-axis.
color	A character vector of length two. Default: ["#E41A1C","#377EB8"].
alpha	Color transparency. In a range [0, 1]. Default: 1.

#### **Details**

In order to discriminate between functional or gene associated peaks and non-functional or background peaks, each peak in the data set is characterized by several features. Moreover, the user might supply her/his own list of features with the addFeature. Prior to fitting the logistic model, the relations between predictors and response variable (tssOverlap) can be explored with plotFeatures. Based on the plots, poor predictors can be excluded from the analysis to improve the model fit.

#### Value

ggplot2 object.

#### Author(s)

Armen R. Karapetyan

### See Also

constructCDS

```
### Load ChipDataSet object
data(cds)

### The data can be plotted in two ways
### As a boxplot
plotFeatures(object = cds, plot.type = "box")

### Or as a density plot
plotFeatures(object = cds, plot.type = "density")

### Additionally, only the subset of features can be shown
plotFeatures(object = cds, plot.type = "box", feature = c("pileup", "length"))

### The position of the graphs on the plot, can be adjusted by 'ncol' argument
plotFeatures(object = cds, plot.type = "box", ncol = 2)
```

30 plotGenomicAnnot

plotGenomicAnnot plotGenomicAnnot

## Description

Visualize genomic distribution of ChIP-seq peaks.

## Usage

```
plotGenomicAnnot(object, plot.type = c("distrib", "enrich"), xlab, ylab,
    color)

## S4 method for signature 'ChipDataSet'
plotGenomicAnnot(object, plot.type = c("distrib",
    "enrich"), xlab, ylab, color)
```

## **Arguments**

object	A ChipDataSet object.
plot.type	Character. One of ("distrib", "enrich"). Default: "enrich".
xlab	Character. Title of the x-axis.
ylab	Character. Title of the y-axis.
color	A character vector of length four, specifying colors for distinct genomic features (TSSs, exons, introns, intergenic regions).

### **Details**

Genomic distribution of the peaks can be visualized in two ways, either by observing the total number of peaks overlapping given genomic feature or by looking at the enrichment levels.

## Value

ggplot2 object.

#### Author(s)

Armen R. Karapetyan

### See Also

constructCDS

plotROC 31

#### **Examples**

```
### Load ChipDataSet object
data(cds)

### Plot the total number of peaks overlapping distinct genomic features
plotGenomicAnnot(object = cds, plot.type = "distrib")

### Plot enrichment of the peaks at a given genomic feature (e.g. TSS)
plotGenomicAnnot(object = cds, plot.type = "enrich")
```

plotROC

plotROC

### **Description**

Visualize the performance of the classification model fit (prediction of the gene associated peaks).

#### Usage

```
plotROC(object, ...)
## S4 method for signature 'ChipDataSet'
plotROC(object, ...)
```

## Arguments

object A ChipDataSet object.

... Further arguments passed to plot.

#### **Details**

The plotROC is a simple wrapper for the plot function implemented in pROC package.

The ROC curve is created by plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity) at various threshold settings. The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test. The area under the curve (AUC) is a measure of accuracy.

### Value

ROC plot.

### Author(s)

Armen R. Karapetyan

32 predictStrand

### See Also

```
predictTssOverlap
```

#### **Examples**

```
### Load ChipDataSet object
data(cds)

### Classify peaks on gene associated and background
predictTssOverlap(object = cds, feature = "pileup", p = 0.75)

### Visualize the performance of the model fit
plotROC(object = cds, col = "red3", grid = TRUE, auc.polygon = TRUE)
```

predictStrand

predictStrand

#### **Description**

The function evaluates transcription initiation within a peak region by comparing RNA-seq read densities upstream and downstream of an empirically determined transcription start sites. Putative transcription of both forward and reverse genomic strands is tested and the results are stored with each ChIP-seq peak.

### Usage

```
predictStrand(cds0bj, tds0bj, coverage.cutoff, quant.cutoff = 0.1,
   win.size = 2500, prob.cutoff)

## S4 method for signature 'ChipDataSet,TranscriptionDataSet'
predictStrand(cds0bj, tds0bj,
   coverage.cutoff, quant.cutoff = 0.1, win.size = 2500, prob.cutoff)
```

## Arguments

cdsObj A ChipDataSet object.

tdsObj A TranscriptionDataSet object.

coverage.cutoff

Numeric. A cutoff value to discard regions with the low fragments coverage, representing expression noise. By default, the value stored in the coverageCutoff slot of the supplied TranscriptionDataSet object is used. The optimal cutoff value can be calculated by estimateBackground function call.

quant.cutoff

Numeric. A cutoff value for the cumulative distribution of the RNA-seq signal along the ChIP-seq peak region. Must be in a range (0, 1). For the details, see step 1 in the "Details" section below. Default: 0.1.

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win.size Numeric. The size of the q1 and q2 regions flanking transcription start position

at the 5' and 3', respectively. For the details, see step 2 in the "Details" section

below. Default: 2500.

 $\label{eq:prob:cutoff} \textbf{Numeric. A cutoff value for the probability of reads to be sampled from the } q2$ 

flanking region. If not supplied, the value estimated from the data will be used. Must be in a range (0, 1). For the details, see step 6 in the "Details" section

below.

#### **Details**

RNA-seq data is incorporated to find direct evidence of active transcription from every putatively gene associated peak. In order to do this, we determine the 'strandedness' of the ChIP-seq peaks, using strand specific RNA-seq data. The following assumptions are made in order to retrieve the peak 'strandedness':

- The putatively gene associated ChIP-seq peaks are commonly associated with transcription initiation.
- This transcription initiation occurs within the ChIP peak region.
- When a ChIP peak is associated with a transcription initiation event, we expect to see a strandspecific increase in RNA-seq fragment count downstream the transcription initiation site.

Each peak in the data set is tested for association with transcription initiation on both strands of DNA. Steps 1-5 are performed for both forward and reverse DNA strand separately and step 6 combines the data from both strands. If the peak is identified as associated with the transcription on both strands, than it is considered to be a bidirectional.

ChIP peak 'strandedness' prediction steps:

- Identify a location within the ChIP-seq peak near the transcription start site. This is accomplished by calculating the cumulative distribution of RNA-seq fragments within a peak region. The position is determined where 100% 'quant.cutoff' \* 100% of RNA-seq fragments are located downstream. This approach performs well on both gene-poor and gene-dense regions where transcripts may overlap.
- 2. Two equally sized regions are defined (q1 and q2), flanking the position identified in (1) on both sides. RNA-seq fragments are counted in each region.
- ChIP peaks with an RNA-seq fragment coverage below an estimated threshold are discarded from the analysis.
- 4. The probability is calculated for RNA-seq fragments to be sampled from either q1 or q2. Based on the assumptions we stated above, a ChIP peak that is associated with transcription initiation should have more reads in q2 (downstream of the transcription start position) compared to q1, and subsequently, the probability of a fragment being sampled from q2 would be higher.
- 5. ChIP-seq peaks are divided into gene associated and background based on the prediction.
- 6. Iteratively, the optimal P(q2) threshold is identified, which balances out the False Discovery Rate (FDR) and False Negative Rate (FNR). Peaks with the P(q2) exceeding the estimated threshold are considered to be associated with the transcription initiation event.

34 predictTssOverlap

#### Value

The slot strandPrediction of the provided ChipDataSet object will be updated by the the following elements: 'predicted.strand', 'probability.cutoff', 'results.plus' and 'results.minus'.

#### Author(s)

```
Armen R. Karapetyan
```

#### See Also

ChipDataSet constructCDS

## **Examples**

```
### Load TranscriptionDataSet object
data(tds)

### Load ChipDataSet object
data(cds)

### Classify peaks on gene associated and background
predictTssOverlap(object = cds, feature = "pileup", p = 0.75)

### Predict peak 'strand'
predictStrand(cdsObj = cds, tdsObj = tds, coverage.cutoff = 5,
quant.cutoff = 0.1, win.size = 2500)

### View a short summary of the 'strand' prediction
cds

### View 'strand' prediction
getPeaks(cds)
```

 ${\tt predictTssOverlap}$ 

predictTssOverlap

#### **Description**

The function classifies ChIP-seq peaks on gene associated and background using classification model based on a logistic regression.

## Usage

```
predictTssOverlap(object, feature, p = 0.8)
## S4 method for signature 'ChipDataSet'
predictTssOverlap(object, feature, p = 0.8)
```

predictTssOverlap 35

## **Arguments**

object A ChipDataSet object.

feature Character. Specify feature(s) that are be used as predictors in the classification

model. By default, all the features stored in the ChipDataSet object will be

used.

p Numeric. The proportion of data that is used to train the model. Default: 0.8.

#### **Details**

In order to improve the accuracy of the model the data is internally partitioned into a training and testing data sets. A repeated 10-Fold Cross-Validation is used to calculate performance measures on the training data set and to prevent over-fitting.

The model fit and validation is internally acomplised by the functions implemented in the caret package.

#### Value

The slot tssOverlapPrediction of the provided TranscriptionDataSet object will be updated by the the following elements: 'predicted.tssOverlap', 'confusionMatrix', 'logFitSummary' and 'roc'.

## Author(s)

Armen R. Karapetyan

#### See Also

ChipDataSet constructCDS caret

```
### Load ChipDataSet object
data(cds)
predictTssOverlap(object = cds, feature = "pileup", p = 0.75)
### View a short summary of the gene associated peaks prediction
cds
### View peaks and associated prediction
getPeaks(cds)
```

36 tds

show

Show method for ChipDataSet and TranscriptionDataSet objects

## Description

Show method for objects of class ChipDataSet and TranscriptionDataSet

#### Usage

```
## S4 method for signature 'TranscriptionDataSet'
show(object)

## S4 method for signature 'ChipDataSet'
show(object)
```

## Arguments

object

A TranscriptionDataSet or ChipDataSet object.

## Value

Displays an overview of the TranscriptionDataSet or ChipDataSet object.

### **Examples**

```
### Load TranscriptionDataSet object
data(tds)
### View a short summary of the object
tds
```

tds

Example of TranscriptionDataSet object.

## Description

tds is an object of TranscriptionDataSet class, containing nuclear RNA-seq data for human chromosome 15 (chr15:63261757-84081194), profiled in prostate cancer LNCaP cells.

#### Usage

tds

### Format

TranscriptionDataSet object

#### Value

TranscriptionDataSet object

TranscriptionDataSet-class

**TranscriptionDataSet** 

#### **Description**

The TranscriptionDataSet is a S4 class to store input values, intermediate calculations and results of the transcripts detection and quantification analysis.

#### **Slots**

bamFile Character. Path to a BAM file.

fragments GRanges. Sequencing reads extended to the fragment size.

fragmentSize Numeric. Fragment size in base pairs (bp).

region GRanges. Genomic region(s) to extract reads from.

coveragePlus RleList. Fragment coverage profile for the forward DNA strand.

coverageMinus RleList. Fragment coverage profile for the reverse DNA strand.

coverageCutoff Numeric. Background coverage cutoff value.

coverageCutoffFdr Numeric. False Discovery Rate (FDR) used to estimate background coverage cutoff.

gapDistanceTest Data.Frame. Tested gap distances and corresponding error rates.

gapDistanceTestCovCutoff Numeric. Coverage cutoff value used for the gap distance estimation.

transcripts GRanges. Identified transcripts.

transcriptsCovCutoff Numeric. Coverage cutoff value used for the transcripts detection.

transcriptsGapDist Numeric. Gap distance value used for the transcripts detection.

transcriptsNormalization Numeric. Total number of reads used for normalization when calculating FPKM.

#### Author(s)

Armen R. Karapetyan

## See Also

constructTDS

38 transcriptsToBed

transcriptsToBed transcriptsToBed

#### **Description**

A convenient graphical way to explore the identified transcripts is to visualize them in the UCSC genome browser. The transcriptsToBed function returns a file in BED format, which can be directly uploaded to the genome browser. To improve the visual perception, transcripts are color-coded by DNA strand orientation.

## Usage

## **Arguments**

object A GRanges object.
file Character. A file name.

strand.color A character vector of length two, specifying color for each DNA strand. Default:

c("blue", "red").

### Value

A file in the BED format.

#### Author(s)

Armen R. Karapetyan

#### See Also

estimateBackground UCSC genome browser BED

```
### Load TranscriptionDataSet object
data(tds)

### Load reference annotations (knownGene from UCSC)
data(annot)

### Detect transcripts
detectTranscripts(object = tds, coverage.cutoff = 5, gap.dist = 4000, estimate.params = TRUE, combine.by.annot = FALSE, annot = annot)
```

transcriptsToBed 39

```
### View detected transcripts
trx <- getTranscripts(tds)

### Export to BED
# transcriptsToBed(object = trx, file = "transcripts.bed",
# strand.color = c("blue", "red"))</pre>
```

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