Package ‘r3Cseq’

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Title Analysis of Chromosome Conformation Capture and Next-generation Sequencing (3C-seq)

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Depends GenomicRanges, Rsamtools, rtracklayer, VGAM, qvalue

Imports methods, GenomeInfoDb, IRanges, Biostrings, data.table, sqldf, RColorBrewer

Suggests BSgenome.Mmusculus.UCSC.mm9.masked,
BSgenome.Mmusculus.UCSC.mm10.masked,
BSgenome.Hsapiens.UCSC.hg18.masked,
BSgenome.Hsapiens.UCSC.hg19.masked,
BSgenome.Rnorvegicus.UCSC.rn5.masked

Description This package is an implementation of data analysis for the long-range interactions from 3C-seq assay.

License GPL-3

URL http://r3cseq.genereg.net

FunctionsForBatchAnalysis.R RestrictionEnzymeFunctions.R
FunctionsForNoReplicationAnalysis.R Report.R Visualize3Cseq.R
Annotation.R

biocViews Preprocessing, Sequencing

NeedsCompilation no

R topics documented:

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calculateBatchRPM

**calculate read per million (RPM) for replicates analysis**

**Description**

Normalize 3C-Seq data by transforming raw reads to read per million per each region for replication analysis

**Usage**

```r
calculateBatchRPM(object, normalized_method=c("powerlawFittedRPM", "normalRPM"))
```
**calculateRPM**

**Arguments**

- `object`: r3Cseq object
- `normalized_method`: character. method of normalization (default=powerlawFittedRPM)

**Author(s)**

S. Thongjuea

**See Also**

`calculateRPM`, `expRPM`, `contrastRPM`

**Examples**

```
#See the vignette
```
### contrInteractionRegions

#### Description

This method has been removed.

#### Usage

```r
contrInteractionRegions(object)
```

##### Arguments

- `object`  
  r3Cseq or r3CseqInBatch object

##### Value

The candidate interaction regions show in the IRange object

##### Author(s)

S. Thongjuea

##### See Also

- `expInteractionRegions`
- `getInteractions`

##### Examples

```
#See the vignette
```
contrRawData

Accessors for the 'contrRawData' slot of a r3Cseq object.

Description
The 'contrRawData' slot holds the raw aligned reads data in the GRanges object.

Usage

## S4 method for signature 'r3Cseq'
contrRawData(object)

## S4 replacement method for signature 'r3Cseq'
contrRawData(object) <- value

Arguments

object r3Cseq object
value a GRanges object of aligned reads

Author(s)

S. Thongjuea

See Also

expRawData

Examples

#See the vignette

contrReadCount

get read count per region for the control

Description
get the read count per region for the control

Usage

contrReadCount(object)

Arguments

object r3Cseq object

Author(s)

S. Thongjuea
See Also

expReadCount, getReadCountPerRestrictionFragment

Examples

#See the vignette

contrRPM(object)

Arguments

object r3Cseq or r3CseqInBatch object

Author(s)

S. Thongjuea

See Also

calculateRPM, expRPM

Examples

#See the vignette

enzymeDb

Rebase The Restriction Enzyme Database

Description

The database includes all restriction enzyme information from the REBASE database.

References

http://rebase.neb.com/rebase/rebase.html
expCoverage

This method has been removed.

expInteractionRegions

get interaction regions from the experiment

Description
get identified interaction regions from the experiment

Usage

expInteractionRegions(object)

Arguments

object r3Cseq or r3CseqInBatch object

Value

The candidate interaction regions show in the IRange object

Author(s)

S. Thongjuea

See Also

goInteractions, contrInteractionRegions

Examples

#See the vignette
**Description**

export interaction regions from RagedData to the bedGraph format, which suitable for uploading to the UCSC genome browser

**Usage**

```r
export3Cseq2bedGraph(object, datatype=c("rpm","read_count"))
```

**Arguments**

- `object`: r3Cseq object. The object might contain the interaction regions generated by function `getInteractions`
- `datatype`: `read_count` : read count per restriction fragment `rpm` : normalized read per million per restriction fragment

**Value**

The text file in 'bedGraph' format

**Author(s)**

S. Thongjuea

**See Also**

`exportInteractions2text`

**Examples**

```r
#See the vignette
```

---

**Description**

export the interaction signal from the raw reads to the 'bedGraph' format

**Usage**

```r
export3CseqRawReads2bedGraph(object)
```
exportBatchInteractions2text

Arguments

object r3Cseq object

Value

The text file in 'bedGraph' format

Author(s)

S. Thongjuea

See Also

exportInteractions2text, export3Cseq2bedGraph,

Examples

#See the vignette

exportBatchInteractions2text

export identified interaction regions to the tab separated format for replicates analysis

Description

export interaction regions from RagedData to the tab separated format for replicates analysis

Usage

exportBatchInteractions2text(object)

Arguments

object r3CseqInBatch object

Value

The text file in the tab separated format

Author(s)

S. Thongjuea

See Also

export3Cseq2bedGraph, exportInteractions2text

Examples

#See the vignette
exportInteractions2text

export identified interaction regions to the tab separated format

Description
export interaction regions from RagedData to the tab separated format

Usage
exportInteractions2text(object)

Arguments
object r3Cseq object

Value
The text file in the tab separated format

Author(s)
S. Thongjuea

See Also
export3Cseq2bedGraph

Examples
#See the vignette

expRawData

Accessors for the 'expRawData' slot of a r3Cseq object.

Description
The 'expRawData' slot of hold the raw aligned reads data in the GRanges object.

Usage
## S4 method for signature 'r3Cseq'
expRawData(object)
## S4 replacement method for signature 'r3Cseq'
expRawData(object) <- value

Arguments
object r3Cseq object
value a GRanges object of aligned reads
Description

get the read count per region for the experiment

Usage

expReadCount(object)

Arguments

object r3Cseq

Author(s)

S. Thongjuea

See Also

contrReadCount, getReadCountPerRestrictionFragment

Examples

#See the vignette
expRPM

get read per million (RPM) for the experiment

Description
get the normalized 3C-seq data (RPM) for the experiment

Usage
expRPM(object)

Arguments
object r3Cseq or r3CseqInBatch

Author(s)
S. Thongjuea

See Also
calculateRPM, contrRPM

Examples

#See the vignette

generate3CseqReport

generate reports for analysis results from r3Cseq

Description
generate reports for analysis results from r3Cseq, the report contains all plots in one pdf file and a text separated output file.

Usage
generate3CseqReport(obj)

Arguments
obj r3Cseq or r3CseqInBatch object

Value
The text file in the tab separated format and the pdf file of all plots

Author(s)
S. Thongjuea
getBatchInteractions

See Also
exportInteractions2text plotOverviewInteractions, plotInteractionsPerChromosome, plotInteractionsNearViewpoint

Examples

#See the vignette

getBatchInteractions calculate z-score, assign p-value and q-value for each interaction region for replicates data sets

Description
Calculate z-score, assign p-value and q-value to each interaction regions for replicates data sets

Usage
getBatchInteractions(object, method=c("union", "intersection"), smoothing.parameter=0.1, fdr=0.05)

Arguments
object r3Cseq object
method character. The method for combining biological replicates for 3C-Seq analysis (default = "union")
smoothing.parameter A level at which cubic smoothing spline for the spar (see vsmooth.spline) input parameter. Must be in (0.06, 0.4] (default=0.1)
fdr A level at which to control the FDR. Must be in (0,1] (default=0.05)

Value
The interaction regions show in the RangedData

Author(s)
S. Thongjuea

See Also
getInteractions vsmooth.spline

Examples

#See the vignette
### getBatchReads

*Get aligned reads from the replicates BAM files*

**Description**

Reading in the input BAM files from the 3C-Seq replicates analysis and then save files as the local GRanged object .rData files

**Usage**

```r
getBatchReads(object)
```

**Arguments**

- `object`  
  r3CseqInBatch object

**Value**

The GRangedData represents the aligned reads from the BAM file

**Author(s)**

S. Thongjuea

**See Also**

- `getRawReads`

**Examples**

```r
# See the vignette
```

### getBatchReadCountPerRestrictionFragment

*count reads for replicates analysis*

**Description**

Counts the number of reads from 3C-Seq data per each restriction fragment for replicates analysis

**Usage**

```r
g rehabtReadCountPerRestrictionFragment(object, getReadsMethod = c("wholeReads", "adjacentFragmentEndsReads"), nFragmentExcludedReadsNearViewpoint=2)
```
getBatchReadCountPerWindow

Arguments

object  r3CseqInBatch object
getReadsMethod  character. To count all reads found in the particular restriction fragment uses wholeReads option. To count reads found around the edge of restriction fragment both 5'utr and 3'utr uses adjacentFragmentEndsReads option (default=wholeReads)
nFragmentExcludedReadsNearViewpoint
   Numeric. The number of excluded fragments around the viewpoint, reads found in these fragments will be removed from the analysis (default=2)

Value

The RangedData represents the number of reads per each restriction fragment

Author(s)

S. Thongjuea

See Also

getReadCountPerWindow, getReadCountPerRestrictionFragment

Examples

```
#See the vignette
```

Description

Counts the number of reads from 3C-Seq data per each window size for replicates analysis

Usage

```
getBatchReadCountPerWindow(object, windowSize=5e3, nFragmentExcludedReadsNearViewpoint=2, mode=c("non-overlapping", "overlapping"))
```

Arguments

object  r3CseqInBatch object
windowSize  Numeric. non-overlapping window size for counting reads (default=5e3)
nFragmentExcludedReadsNearViewpoint
   Numeric. The number of excluded fragments around the viewpoint, reads found in these fragments will be removed from the analysis (default=2)
mode  character. The window-based modes analysis (default="non-overlapping")

Value

The RangedData represents the number of reads per each window size
getContrInteractionsInRefseq

Author(s)
S. Thongjuea

See Also
getReadCountPerRestrictionFragment, getBatchReadCountPerRestrictionFragment, getReadCountPerWindow.

Examples
# See the vignette

getcConInteractionsInRefseq

identified significant interaction regions for RefSeq genes

Description
Get a list of genes that contain strong interaction signals in the control

Usage
getcConInteractionsInRefseq(obj,cutoff.qvalue=0.05,expanded_upstream=50e3,expanded_downstream=10e3)

Arguments

obj obj is r3Cseq or r3CseqInBatch object
cutoff.qvalue Numeric. The cutoff q-value (default=0.05)
expanded_upstream Numeric. The expanded distance from the upstream of a gene start (default=50e3)
expanded_downstream Numeric. The expanded distance from the downstream of a gene end (default =10e3)

Value
List of identified genes, which contain strong interaction signals

Author(s)
S. Thongjuea

See Also
getcConInteractionsInRefseq

Examples
# See the vignette
getCoverage

This method has been removed.

getExpInteractionsInRefseq

identified significant interaction regions for RefSeq genes

Description

Get a list of genes that contain strong interaction signals in the experiment

Usage

getExpInteractionsInRefseq(obj,cutoff.qvalue=0.05,expanded_upstream=50e3,expanded_downstream=10e3)

Arguments

obj obj is r3Cseq or r3CseqInBatch object

cutoff.qvalue Numeric. The cutoff q-value (default=0.05)

expanded_upstream Numeric. The expanded distance from the upstream of a gene start (default=50e3)

expanded_downstream Numeric. The expanded distance from the downstream of a gene end (default =10e3)

Value

List of identified genes, which contain strong interaction signals

Author(s)

S. Thongjuea

See Also

getContrInteractionsInRefseq

Examples

# See the vignette
getInteractions

**Description**

Calculate z-score, assign p-value and q-value for each interaction region

**Usage**

```r
getInteractions(object, smoothing.parameter=0.1, fdr=0.05)
```

**Arguments**

- `object`: r3Cseq object
- `smoothing.parameter`: A level at which cubic smoothing spline for the spar (see vsmooth.spline) input parameter. Must be in (0.06,0.4] (default=0.1)
- `fdr`: A level at which to control the FDR. Must be in (0,1] (default=0.05)

**Value**

The interaction regions show in the RangedData

**Author(s)**

S. Thongjuea

**See Also**

- `getBatchInteractions`
- `vsmooth.spline`

**Examples**

```r
#See the vignette
```

getRawReads

**Description**

Get aligned reads from the BAM file

**Usage**

```r
generateRawReads(object)
```

**Arguments**

- `object`: r3Cseq object
Value
The GRangedData represents the aligned reads from the BAM file

Author(s)
S. Thongjuea

See Also
getBatchRawReads,

Examples
#See the vignette

getReadCountPerRestrictionFragment

Description
Counts the number of reads from 3C-Seq data per each restriction fragment

Usage
getReadCountPerRestrictionFragment(object, getReadsMethod = c("wholeReads", "adjacentFragmentEndsReads"), nFragmentExcludedReadsNearViewpoint=2)

Arguments
object r3Cseq object
getReadsMethod character. To count all reads found in the particular restriction fragment uses wholeReads option. To count reads found around the edge of restriction fragment both 5’utr and 3’utr uses adjacentFragmentEndsReads option (default=wholeReads)
nFragmentExcludedReadsNearViewpoint Numeric. The number of excluded fragments around the viewpoint, reads found in these fragments will be removed from the analysis (default=2)

Value
The RangedData represents the number of reads per restriction fragment

Author(s)
S. Thongjuea

See Also
getReadCountPerWindow, getBatchReadCountPerRestrictionFragment

Examples
#See the vignette
Description

Counts the number of reads from 3C-Seq data per each window size

Usage

getReadCountPerWindow(object, windowSize=5e3, nFragmentExcludedReadsNearViewpoint=2, mode=c("non-overlapping", "overlapping"))

Arguments

- **object**: r3Cseq object
- **windowSize**: Numeric. non-overlapping window size for counting reads (default=5e3)
- **nFragmentExcludedReadsNearViewpoint**: Numeric. The number of excluded fragments around the viewpoint, reads found in these fragments will be removed from the analysis (default=2)
- **mode**: character. The window-based modes analysis (default="non-overlapping")

Value

The RangedData represents the number of reads per each window size

Author(s)

S. Thongjuea

See Also

generateReadCountPerRestrictionFragment,

Examples

#See the vignette

Description

The viewpoint is the bait of 3C method, which can be a promoter region of an interested gene, an enhancer, and a transcription factor binding region.

Usage

generateReadCountPerRestrictionFragment(obj)
**Arguments**

`obj` r3Cseq or r3CseqInBatch object

**Value**

The viewpoint shows in the IRanges

**Author(s)**

S. Thongjuea

**Examples**

#See the vignette

<table>
<thead>
<tr>
<th><code>hg18refGene</code></th>
<th><code>hg18’s refGenes</code></th>
</tr>
</thead>
</table>

**Description**

The human (hg18) reference genes from UCSC

<table>
<thead>
<tr>
<th><code>hg19refGene</code></th>
<th><code>hg19’s refGenes</code></th>
</tr>
</thead>
</table>

**Description**

The human (hg19) reference genes from UCSC

<table>
<thead>
<tr>
<th><code>mm10refGene</code></th>
<th><code>mm10’s refGenes</code></th>
</tr>
</thead>
</table>

**Description**

The mouse (mm10) reference genes from UCSC

<table>
<thead>
<tr>
<th><code>mm9refGene</code></th>
<th><code>mm9’s refGenes</code></th>
</tr>
</thead>
</table>

**Description**

The mouse (mm9) reference genes from UCSC
## plotDomainogramNearViewpoint

**Description**

Plot domainogram of interaction regions near the viewpoint.

**Usage**

```r
plotDomainogramNearViewpoint(object, smoothing.parameter = 0.1, distance = 5e5, maximum_window = 25e3, view = "experiment")
```

**Arguments**

- `object`: r3Cseq or r3CseqInBatch object
- `smoothing.parameter`: A level at which cubic smoothing spline for the spar (see vsmooth.spline) input parameter. Must be in (0.06, 0.4] (default = 0.1)
- `distance`: Numeric. The distance relative to the viewpoint (default = 5e5)
- `maximum_window`: Numeric. The maximum windowing (default = 25e3). We normally compute the interaction regions per window starting from 2Kb to maximum window (default = 25kb) to make the interaction matrix for visualizing the domainogram.
- `view`: character. The selected view of data (default = "experiment")
Value

Plots of domainogram for interaction regions close to the viewpoint

Author(s)

S. Thongjuea

See Also

plotOverviewInteractions, plotInteractionsPerChromosome, plotInteractionsNearViewpoint

Examples

# See the vignette

```r
plotInteractionsNearViewpoint
```

Description

Plot identified interaction regions near the viewpoint

Usage

```r
plotInteractionsNearViewpoint(obj, distance=5e5, log2fc_cutoff=1, yLim=0)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>obj</td>
<td>obj is r3Cseq or r3CseqInBatch object</td>
</tr>
<tr>
<td>distance</td>
<td>Numeric. The distance relative to the viewpoint (default=5e5)</td>
</tr>
<tr>
<td>log2fc_cutoff</td>
<td>Numeric. The log2 cutoff ratio between the experiment and control (default=1)</td>
</tr>
<tr>
<td>yLim</td>
<td>Numeric. The limited height of y-axis (default=0)</td>
</tr>
</tbody>
</table>

Value

Plots of identified interaction regions close to the viewpoint

Author(s)

S. Thongjuea

See Also

plotOverviewInteractions, plotInteractionsPerChromosome, plotDomainogramNearViewpoint

Examples

# See the vignette
plotInteractionsPerChromosome

*Plot interaction regions per each chromosome of interest*

**Description**

Plot the distribution of interaction regions per each chromosome

**Usage**

```r
plotInteractionsPerChromosome(obj, chromosomeName)
```

**Arguments**

- `obj` obj is `r3Cseq` or `r3CseqInBatch` object.
- `chromosomeName` Character. The input chromosome name (e.g. "chr1")

**Value**

Plots of interaction regions per chromosome.

**Author(s)**

S. Thongjuea

**See Also**

`plotInteractionsNearViewpoint`, `plotOverviewInteractions`, `plotDomainogramNearViewpoint`

**Examples**

```r
# See the vignette
```

---

plotOverviewInteractions

*Plot overview of identified interaction regions for genome-wide*

**Description**

Plot the distribution of identified interaction regions across genome

**Usage**

```r
plotOverviewInteractions(obj, cutoff.qvalue=0.05)
```

**Arguments**

- `obj` obj is `r3Cseq` or `r3CseqInBatch` object
- `cutoff.qvalue` Numeric. The cutoff q-value (default=0.05)
Value

Plots of identified 3C-Seq interaction regions genome-wide

Author(s)

S. Thongjuea

See Also

plotInteractionsNearViewpoint, plotInteractionsPerChromosome, plotDomainogramNearViewpoint

Examples

# See the vignette

---

r3Cseq-class  
r3Cseq objects

Description

The r3Cseq class is the extended class from r3CseqCommon class. It is a general container for storing and manipulating a set of input parameters, RangeData of interactions regions from r3Cseq analysis, and the raw reads GRanged data of the genome-wide interaction signal generated by next-generation sequencing.

Extends

Class r3CseqCommon, directly.

Slots

- **organismName**: Object of class "character" the version of particular assembly genome from UCSC (e.g. mm9, hg18, hg19). The package supports three genome assemblies consisting of mouse (mm9), and human (hg18, hg19).
- **restrictionEnzyme**: Object of class "character" this is the primary restriction enzyme name using in 3C-Seq experiment
- **viewpoint_chromosome**: Object of class "character" chromosome name of where is the viewpoint located eg. chr10, chrX etc.
- **viewpoint_primer_forward**: Object of class "character" the forward primer DNA sequences for the viewpoint amplification
- **viewpoint_primer_reverse**: Object of class "character" the reverse primer DNA sequences for the viewpoint amplification
- **expReadCount**: Object of class "RangedData" the read count in experiment
- **contrReadCount**: Object of class "RangedData" the read count in control
- **expRPM**: Object of class "RangedData" the normalized read read per million in experiment
- **contrRPM**: Object of class "RangedData" the normalized read read per million in control
- **expInteractionRegions**: Object of class "RangedData" the identified interaction regions in experiment
contrInteractionRegions  Object of class "RangedData" the identified interaction regions in control
isControlInvolved  Object of class "logical" the logical to ask whether the control is involved in the analysis or not
alignedReadsBamExpFile  Object of class "character" the file name of experiment in BAM format
alignedReadsBamContrFile  Object of class "character" the file name of control in BAM format
expLabel  Object of class "character" the experiment name
contrLabel  Object of class "character" the control name
expLibrarySize  Object of class "integer" the library size of experiment
contrLibrarySize  Object of class "integer" the library size of control
expReadLength  Object of class "integer" the read length of experiment
contrReadLength  Object of class "integer" the read length of experiment
expRawData  Object of class "GRanges" the raw reads found in experiment
contrRawData  Object of class "GRanges" the raw reads found in control

Author(s)
S. Thongjuea

See Also
r3CseqCommon, r3CseqInBatch

Examples

# See the vignette

---

r3CseqCommon-class  r3CseqCommon objects

Description
The r3CseqCommon class is a general container for storing and manipulating a set of input parameters, RangeData of interactions regions from r3Cseq analysis. It is a root class for r3Cseq and r3CseqInBatch classes.

Slots
organismName  Object of class "character" the version of particular assembly genome from UCSC (e.g. mm9, hg18, hg19). The package supports three genome assemblies consisting of mouse (mm9), and human (hg18, hg19).
restrictionEnzyme  Object of class "character" this is the primary restriction enzyme name using in 3C-Seq experiment
viewpoint_chromosome  Object of class "character" chromosome name of where is the viewpoint located eg. chr10, chrX etc.
viewpoint_primer_forward Object of class "character" the forward primer DNA sequences for the viewpoint amplification
viewpoint_primer_reverse Object of class "character" the reverse primer DNA sequences for the viewpoint amplification
expReadCount Object of class "RangedData" the read count in experiment
contrReadCount Object of class "RangedData" the read count in control
expRPM Object of class "RangedData" the normalized read read per million in experiment
contrRPM Object of class "RangedData" the normalized read per million in control
expInteractionRegions Object of class "RangedData" the identified interaction regions in experiment
contrInteractionRegions Object of class "RangedData" the identified interaction regions in control
isControlInvolved Object of class "logical" the logical to ask whether the control is involved in the analysis or not

Author(s)

S. Thongjuea

See Also

r3Cseq, r3CseqInBatch

Examples

# See the vignette

---

t3CseqInBatch-class  r3CseqInBatch objects

Description

The r3CseqInBatch class is the extended class from r3CseqCommon class. It is a general container for storing and manipulating a set of input parameters, RangeData of interactions regions from r3Cseq analysis for replicates data sets.

Extends

Class r3CseqCommon, directly.

Slots

organismName Object of class "character" the version of particular assembly genome from UCSC (e.g. mm9, hg18, hg19). The package supports three genome assemblies consisting of mouse (mm9), and human (hg18, hg19).
restrictionEnzyme Object of class "character" this is the primary restriction enzyme name using in 3C-Seq experiment
viewpoint_chromosome Object of class "character" chromosome name of where is the viewpoint located eg. chr10, chrX etc.
viewpoint_primer_forward Object of class "character" the forward primer DNA sequences for the viewpoint amplification
viewpoint_primer_reverse Object of class "character" the reverse primer DNA sequences for the viewpoint amplification
expReadCount Object of class "RangedData" the read count in experiment
contrReadCount Object of class "RangedData" the read count in control
expRPM Object of class "RangedData" the normalized read read per million in experiment
contrRPM Object of class "RangedData" the normalized read read per million in control
expInteractionRegions Object of class "RangedData" the identified interaction regions in experiment
contrInteractionRegions Object of class "RangedData" the identified interaction regions in control
isControlInvolved Object of class "logical" the logical to ask whether the control is involved in the analysis or not
bamFilesDirectory Object of class "character" the path name of directory that contains BAM files
BamExpFiles Object of class "vector" the file names of BAM files in the experiment
BamContrFiles Object of class "vector" the file names of BAM files in the control
expBatchLabel Object of class "vector" the labeled experiment names
contrBatchLabel Object of class "vector" the labeled control names
readCountTable Object of class "RangedData" the read count table
RPMsTable Object of class "RangedData" the normalized read per million table
expBatchLibrarySize Object of class "vector" the library size of each experiment
contrBatchLibrarySize Object of class "vector" the library size of each control
expBatchReadLength Object of class "vector" the read length of experiments
contrBatchReadLength Object of class "vector" the read length of controls

Author(s)
S. Thongjuea

See Also
r3CseqCommon, r3CseqInBatch

Examples
# See the vignette

<table>
<thead>
<tr>
<th>rn5refGene</th>
<th>rn5's refGenes</th>
</tr>
</thead>
</table>

Description
The rat (rn5) reference genes from UCSC
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