Package ‘r3Cseq’

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

Author Supat Thongjuea, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK

<supat.thongjuea@ndcls.ox.ac.uk>

Maintainer Supat Thongjuea <supat.thongjuea@ndcls.ox.ac.uk>

Depends GenomicRanges, Rsamtools, rtracklayer, VGAM, qvalue

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


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

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URL http://r3cseq.genereg.net


biocViews Preprocessing, Sequencing

NeedsCompilation no

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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

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

**Arguments**

- `object`: r3CseqInBatch object
- `normalized_method`: character. method of normalization (default=powerlawFittedRPM)

**Author(s)**

S. Thongjuea

**See Also**

`calculateRPM`, `expRPM`, `contrRPM`

**Examples**

```r
#See the vignette
```

---

**Description**

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

**Usage**

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

**Arguments**

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

**Author(s)**

S. Thongjuea

**See Also**

`contrRPM`, `expRPM`, `calculateBatchRPM`

**Examples**

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

Description

This method has been removed.

contrInteractionRegions

get interaction regions from the control

Description

get all identified interaction regions from the control

Usage

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**

**Description**

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

**Usage**

```r
## 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**

```r
contrReadCount
```

#See the vignette

---

**contrReadCount**

**Description**

get read count per region for the control

**Usage**

```r
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**

```r
expInteractionRegions(object)
```

**Arguments**

- `object`: r3Cseq or r3CseqInBatch object

**Value**

The candidate interaction regions show in the IRange object

**Author(s)**

S. Thongjuea

**See Also**

- `getInteractions`
- `contrInteractionRegions`

**Examples**

```r
#See the vignette
```
export3Cseq2bedGraph  export interaction regions to the 'bedGraph' format

Description

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

Usage

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

#See the vignette

export3CseqRawReads2bedGraph  export the interaction signal from the raw reads to the 'bedGraph' format

Description

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

Usage

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

---

**Description**

Export identified interaction regions 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**

```r
# See the vignette
```

---

expRawData

---

**Description**

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

**Usage**

```r
## S4 method for signature 'r3Cseq'
expRawData(object)
## S4 method for signature 'r3Cseq'
expRawData(object) <- value
```

**Arguments**

- `object`: r3Cseq object
- `value`: a GRanges object of aligned reads
**expReadCount**

Author(s)

S. Thongjuea

See Also

expRawData

Examples

#See the vignette

---

**expReadCount**

*get read count per region for the experiment*

**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
generateInteractions, plotOverviewInteractions, plotInteractionsPerChromosome, plotInteractionsNearViewpoint

Examples

#See the vignette

generateInteractions 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

generateInteractions(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

generateInteractions vsmooth.spline

Examples

#See the vignette
getBatchRawReads

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

getBatchRawReads(object)

Arguments

object r3CseqInBatch object

Value

The GRangedData represents the aligned reads from the BAM file

Author(s)

S. Thongjuea

See Also

getRawReads,

Examples

#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

getBatchReadCountPerRestrictionFragment(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

```r
#See the vignette
```

```
getBatchReadCountPerWindow

  count reads per window size for replicates analysis
```

Description

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

Usage

```r
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
Author(s)
S. Thongjuea

See Also
getReadCountPerRestrictionFragment, getBatchReadCountPerRestrictionFragment, getReadCountPerWindow.

Examples
# See the vignette

getContrInteractionsInRefseq
identified significant interaction regions for RefSeq genes

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

Usage
getContrInteractionsInRefseq(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
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**

```r
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**

```r
# See the vignette
```
getInteractions

**Description**

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

**Usage**

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**

getchInteractions vsmooth.spline

**Examples**

#See the vignette

getRawReads

**Description**

Get aligned reads from the BAM file.

**Usage**

getRawReads(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 each 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

getReadCountPerRestrictionFragment

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

getViewpoint(obj)
**hg18refGene**

**Arguments**

obj         r3Cseq or r3CseqInBatch object

**Value**

The viewpoint shows in the IRanges

**Author(s)**

S. Thongjuea

**Examples**

#See the vignette

---

**hg18refGene**  *hg18's refGenes*

**Description**

The human (hg18) reference genes from UCSC

---

**hg19refGene**  *hg19's refGenes*

**Description**

The human (hg19) reference genes from UCSC

---

**mm10refGene**  *mm10's refGenes*

**Description**

The mouse (mm10) reference genes from UCSC

---

**mm9refGene**  *mm9's refGenes*

**Description**

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

**Description**
The example aligned reads generated by 3C-Seq protocol from fetal brain. The promoter region of the Myb’s gene was selected as the viewpoint. This data was transformed from aligned reads shown in the BAM file to GRanged object by using Rsamtools.

### Myb_prom_FL

**Description**
The example aligned reads generated by 3C-Seq protocol from fetal liver. The promoter region of the Myb’s gene was selected as the viewpoint. This data was transformed from aligned reads shown in the BAM file to GRanged object by using Rsamtools.

### plot3Cecdf

**Description**
This method has been removed.

### plotDomainogramNearViewpoint

**Plot domainogram of interaction regions near the viewpoint**

**Description**
Plot domainogram of interaction regions near the viewpoint

**Usage**
```
plotDomainogramNearViewpoint(object, smoothing.parameter=0.1, distance=5e5, maximum_window=25e3, view=c("experiment","control","both"))
```

**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")
**plotInteractionsNearViewpoint**

**Value**

Plots of domainogram for interaction regions close to the viewpoint

**Author(s)**

S. Thongjuea

**See Also**

plotOverviewInteractions, plotInteractionsPerChromosome, plotInteractionsNearViewpoint

**Examples**

```r
# See the vignette
```
plotInteractionsPerChromosome

Plot interaction regions per each chromosome of interest

Description
Plot the distribution of interaction regions per each chromosome

Usage
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

# See the vignette

plotOverviewInteractions

Plot overview of identified interaction regions for genome-wide

Description
Plot the distribution of identified interaction regions across genome

Usage
plotOverviewInteractions(obj, cutoff.qvalue=0.05)

Arguments
obj object 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 per million in control
- **expInteractionRegions**: Object of class "RangedData" the identified interaction regions in experiment
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
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

---

rn5refGene  

rn5's refGenes

Description
The rat (rn5) reference genes from UCSC
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