

Package ‘r3Cseq’

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

Author Supat Thongjuea, Bergen Center for Computational Science, Norway <supat.thongjuea@bccs.uib.no>

Maintainer Supat Thongjuea <supat.thongjuea@bccs.uib.no>

Depends R (>= 2.14.0), IRanges, BSgenome, ShortRead, rtracklayer, RColorBrewer, methods

Suggests BSgenome.Mmusculus.UCSC.mm9

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

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biocViews Preprocessing, Sequencing, HighThroughputSequencing,

Collate AllClasses.R AllGenerics.R RestrictionEnzymeFunctions.R Functions.R Export.R Report.R Visualize3Cseq.R

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calculateRPM *calculate read per million (RPM)*

Description

Normalize 3C-seq data by transforming raw reads to read per million per each restriction fragment

Usage

```
calculateRPM(object)
```

Arguments

object *r3Cseq* object. The object might contain the raw read generated by function [getReadCountPerRestrictionFragment](#)

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [contrRPM](#), [expRPM](#)

Examples

```
####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
```

contrCoverage *Get the coverage for 3C-seq data from the control*

Description

Counts the number of times a position is represented in a set of input reads from 3C-seq data in the control

Usage

```
contrCoverage(object)
```

Arguments

object r3Cseq object, The initialize input parameters are required

Value

For most methods, an [Rle](#) object representing the coverage of x. For [RangesList](#) and [RangedData](#) objects, a [SimpleRleList](#) object representing a list of coverage vectors.

An integer value called the "coverage" can be associated to each position in x, indicating how many times this position is covered by the elements contained in x. For example, if x is a [Views](#) object, the coverage of a given position in subject (x) is the number of views it belongs to.

Author(s)

S. Thongjuea

See Also

[IRanges-class](#), [Views-class](#), [Rle-class](#), [MaskCollection-class](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
contr.coverage<-contrCoverage(my.data)
```

contrInteractionRegions

get interaction regions from the control

Description

get all candidate interaction regions from the control

Usage

```
contrInteractionRegions(object)
```

Arguments

object r3Cseq object. The object might contain the interaction regions generated by function [getInteractions](#)

Value

The candidate interaction regions show in the [IRange](#) object

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [calculateRPM](#), [getInteractions](#)

Examples

```
####Create the r3Cseq object#####  
library(BSgenome.Mmusculus.UCSC.mm9)  
  load(system.file("data", "example.data.rda", package="r3Cseq"))  
calculateRPM(my.data)  
getInteractions(my.data)  
contr.interactions<-contrInteractionRegions(my.data)
```

contrRPM

get read per million (RPM) for the control

Description

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

Usage

```
contrRPM(object)
```

Arguments

object r3Cseq object. The object might contain the raw read generated by function [getReadCountPerRestrictionFragment](#)

Author(s)

S. Thongjuea

See Also

[calculateRPM](#), [expRPM](#)

Examples

```
####Create the r3Cseq object#####  
library(BSgenome.Mmusculus.UCSC.mm9)  
  load(system.file("data", "example.data.rda", package="r3Cseq"))  
calculateRPM(my.data)  
control.rpm<-contrRPM(my.data)
```

`enzyme.db`*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`*Get the coverage for 3C-seq data from the experiment*

Description

Counts the number of times a position is represented in a set of input reads from 3C-seq data in the experiment

Usage

```
expCoverage(object)
```

Arguments

`object` `r3Cseq` object, The initialize input parameters are required

Value

For most methods, an [Rle](#) object representing the coverage of `x`. For [RangesList](#) and [RangedData](#) objects, a [SimpleRleList](#) object representing a list of coverage vectors.

An integer value called the "coverage" can be associated to each position in `x`, indicating how many times this position is covered by the elements contained in `x`. For example, if `x` is a [Views](#) object, the coverage of a given position in `subject(x)` is the number of views it belongs to.

Author(s)

S. Thongjuea

See Also

[IRanges-class](#), [Views-class](#), [Rle-class](#), [MaskCollection-class](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
exp.coverage<-expCoverage(my.data)
```

expInteractionRegions *get interaction regions from the experiment*

Description

get all candidate interaction regions from the experiment

Usage

```
expInteractionRegions(object)
```

Arguments

object r3Cseq object. The object might contain the interaction regions generated by function [getInteractions](#)

Value

The candidate interaction regions show in the IRange object

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [calculateRPM](#), [getInteractions](#), [contrInteractionRegions](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)
exp.interactions<-expInteractionRegions(my.data)
```

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", "raw_read"))
```

Arguments

object	r3Cseq object, The object might contain the interaction regions generated by function getInteractions
datatype	raw_read : read count per restriction fragment rpm : read per million per restriction fragment

Value

The text file in 'bedGraph' format

Author(s)

S. Thongjuea

See Also

[getInteractions](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)
export3Cseq2bedGraph(my.data, datatype="rpm")
```

exportInteractions2text

export 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, The object might contain the interaction regions generated by function getInteractions
--------	---

Value

The text file in the tab separated format

Author(s)

S. Thongjuea

See Also

[getInteractions](#)

Examples

```
####Create the r3Cseq object#####  
library(BSgenome.Mmusculus.UCSC.mm9)  
  load(system.file("data", "example.data.rda", package="r3Cseq"))  
calculateRPM(my.data)  
getInteractions(my.data)  
exportInteractions2text(my.data)
```

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 object. The object might contain the raw read generated by function [getReadCountPerRestrictionFragment](#)

Author(s)

S. Thongjuea

See Also

[calculateRPM](#), [contrRPM](#)

Examples

```
####Create the r3Cseq object#####  
library(BSgenome.Mmusculus.UCSC.mm9)  
  load(system.file("data", "example.data.rda", package="r3Cseq"))  
calculateRPM(my.data)  
experiment.rpm <-expRPM(my.data)
```

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 out put file.

Usage

```
generate3CseqReport(object)
```

Arguments

object r3Cseq object, The object might contain the interaction regions generated by function [getInteractions](#)

Value

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

Author(s)

S. Thongjuea

See Also

[getInteractions](#), [exportInteractions2text](#) [plotOverviewInteractions](#), [plotInteractionsPerChromosome](#), [plotInteractionsNearViewpoint](#), [plot3Cecdf](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)
generate3CseqReport(my.data)
```

getCoverage *Coverage for 3C-seq data*

Description

Counts the number of times a position is represented in a set of input reads from 3C-seq data

Usage

```
getCoverage(object)
```

Arguments

object r3Cseq object, The initialize input parameters are required

Value

For most methods, an [Rle](#) object representing the coverage of x. For [RangesList](#) and [RangedData](#) objects, a [SimpleRleList](#) object representing a list of coverage vectors.

An integer value called the "coverage" can be associated to each position in x, indicating how many times this position is covered by the elements contained in x. For example, if x is a [Views](#) object, the coverage of a given position in `subject(x)` is the number of views it belongs to.

Author(s)

S. Thongjuea

See Also

[IRanges-class](#), [Views-class](#), [Rle-class](#), [MaskCollection-class](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
expFile<-system.file("extdata","alignedReads.fetal.liver.subset.bam",package="r3Cseq")
contrFile<-system.file("extdata","alignedReads.fetal.brain.subset.bam",package="r3Cseq")

my.data<-new("r3Cseq",organismName='mm9',alignedReadsBamExpFile=expFile,
alignedReadsBamContrFile=contrFile,isControlInvolved=TRUE,isBamInputFile=TRUE,
expLabel="fetal_liver",contrLabel="fetal_brain",
restrictionEnzyme='HindIII')
getCoverage(my.data)
```

getInteractions

assign p-value and fold change to candidate interaction regions

Description

Assign p-value and fold change to each candidate interaction regions by using empirical distribution function

Usage

```
getInteractions(object)
```

Arguments

object r3Cseq object. The object might contain the RPM generated by function [getReadCountPerRestrictionFragment](#) following by [calculateRPM](#)

Value

The candidate interaction regions show in the [RangedData](#)

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [calculateRPM](#)

Examples

```
####Create the r3Cseq object#####  
library(BSgenome.Mmusculus.UCSC.mm9)  
load(system.file("data", "example.data.rda", package="r3Cseq"))  
calculateRPM(my.data)  
getInteractions(my.data)
```

`getReadCountPerRestrictionFragment`

get read count per restriction fragment for 3C-seq data

Description

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

Usage

```
getReadCountPerRestrictionFragment(object)
```

Arguments

object r3Cseq object, The initialize input parameters are required

Value

The RangedData represents the number of reads per each restriction fragment

Author(s)

S. Thongjuea

See Also

[getCoverage](#),

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
  expFile<-system.file("extdata","alignedReads.fetal.liver.subset.bam",package="r3Cseq")
  contrFile<-system.file("extdata","alignedReads.fetal.brain.subset.bam",package="r3Cseq")

my.data<-new("r3Cseq",organismName='mm9',alignedReadsBamExpFile=expFile,
alignedReadsBamContrFile=contrFile,isControlInvolved=TRUE,isBamInputFile=TRUE,
expLabel="fetal_liver",contrLabel="fetal_brain",
restrictionEnzyme='HindIII')

getReadCountPerRestrictionFragment(my.data)
```

getViewpoint	<i>get the viewpoint of 3C-seq data</i>
--------------	---

Description

The viewpoint is the region of interest, which can be a promoter region of an interested gene, an enhancer, and a transcription factor binding region. The genomic region, which show the highest number of reads per restriction fragment, represents the viewpoint.

Usage

```
getViewpoint(object)
```

Arguments

object r3Cseq object, the object is the container of interaction regions produced by [getInteractions](#) function.

Value

The viewpoint shows in the IRanges

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [calculateRPM](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
  load(system.file("data","example.data.rda",package="r3Cseq"))
calculateRPM(my.data)
viewpoint<-getViewpoint(my.data)
```

viewpoint

my.data

a simple example of r3Cseq object

Description

The example r3Cseq object which is used for the examples in the package help pages.

plot3Cecdf

Plot the empirical distribution of interaction regions

Description

Plot the empirical distribution of interaction regions

Usage

```
plot3Cecdf(object)
```

Arguments

object r3Cseq object. The object is the container of interaction regions produced by [getInteractions](#) function.

Value

Plots the empirical distribution of interaction regions on the active graphical device.

Author(s)

S. Thongjuea

See Also

[plotOverviewInteractions](#), [plotInteractionsPerChromosome](#), [plotInteractionsNearViewpoint](#)

Examples

```
####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
  load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)

####Plot

plot3Cecdf(my.data)
```

plotInteractionsNearViewpoint

Plot candidate interaction regions near the viewpoint

Description

Plot candidate interaction regions near the viewpoint by zooming at 10Mb, 1Mb, 500Kb, and 100Kb respectively

Usage

```
plotInteractionsNearViewpoint(object)
```

Arguments

object r3Cseq object. The object is the container of interaction regions produced by [getInteractions](#) function.

Value

Plots interaction regions close to the viewpoint on the active graphical device.

Author(s)

S. Thongjuea

See Also

[plotOverviewInteractions](#), [plotInteractionsPerChromosome](#), [plot3Cecdf](#)

Examples

```
####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
  load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)

####Plot

plotInteractionsNearViewpoint(my.data)
```

`plotInteractionsPerChromosome`*Plot interaction regions per each chromosome of interest*

Description

Plot the distribution of interaction regions per each interested input chromosome

Usage

```
plotInteractionsPerChromosome(object, chromosomeName)
```

Arguments

`object` `r3Cseq` object. The object is the container of interaction regions produced by [getInteractions](#) function.

`chromosomeName` Character. The input chromosome name (e.g. "chr1")

Value

Plots interaction regions per chromosome on the active graphical device.

Author(s)

S. Thongjuea

See Also

[plotInteractionsNearViewpoint](#), [plotOverviewInteractions](#), [plot3Cecdf](#)

Examples

```
#####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)

####Plot

plotInteractionsPerChromosome(my.data, "chr10")
```

`plotOverviewInteractions`*Plot the overview of interaction regions for the genome-wide*

Description

Plot the distribution of interaction regions across genome with the level of interaction signal

Usage

```
plotOverviewInteractions(object, cutoff.p_value=0.05,cutoff.fold_change=2)
```

Arguments

`object` r3Cseq object. The object is the container of interaction regions produced by [getInteractions](#) function.

`cutoff.p_value` Numeric. The cutoff p-value from empirical distribution function (default=0.05)

`cutoff.fold_change` Numeric. The cutoff fold change compare between experiment and control (default=2)

Value

Plots interaction regions genome-wide on the active graphical device.

Author(s)

S. Thongjuea

See Also

[plotInteractionsNearViewpoint](#), [plotInteractionsPerChromosome](#), [plot3Cecdf](#)

Examples

```
####Create the r3Cseq object#####
library(BSgenome.Mmusculus.UCSC.mm9)
load(system.file("data", "example.data.rda", package="r3Cseq"))
calculateRPM(my.data)
getInteractions(my.data)

####Plot

plotOverviewInteractions(my.data)
```

r3Cseq-class

r3Cseq objects

Description

The r3Cseq class is a general container for storing and manipulating a set of input parameters, RangeData of interactions regions from r3Cseq analysis , and Rle coverage vector of the genome-wide interaction signal.

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" the restriction enzyme name using in cutting the 3C interaction fragments

`alignedReadsExpFile` Object of class "character" the file name of the experiment

`alignedReadsContrFile` Object of class "character" the file name of the control

`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

`alignedReadsType` Object of class "character" the file type of aligned read (see [readAligned](#))

`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

`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 read read per million in experiment

`contrRPM` Object of class "RangedData" the read read per million in control

`expInteractionRegions` Object of class "RangedData" the interaction regions in experiment

`contrInteractionRegions` Object of class "RangedData" the interaction regions in control

`expCoverage` Object of class "RleList" the Rle coverage vector in experiment

`contrCoverage` Object of class "RleList" the Rle coverage vector in control

`isControlInvolved` Object of class "logical" the logical to ask whether the control is involved in the analysis or not

`isBamInputFile` Object of class "logical" the logical to ask whether the BAM file format is the input file or not

Methods

`expInteractionRegions(x)`: The interaction regions in experiment x.

`contrInteractionRegions(x)`: The interaction regions in control x.

Author(s)

S. Thongjuea

See Also

[getCoverage](#), [getReadCountPerRestrictionFragment](#), [calculateRPM](#), [getInteractions](#), [expInteractionRegions](#), [contrInteractionRegions](#)

Examples

```
library(BSgenome.Mmusculus.UCSC.mm9)
expFile<-system.file("extdata","alignedReads.fetal.liver.subset.bam",package="r3Cseq")
contrFile<-system.file("extdata","alignedReads.fetal.brain.subset.bam",package="r3Cseq")

my.data<-new("r3Cseq",organismName='mm9',alignedReadsBamExpFile=expFile,
alignedReadsBamContrFile=contrFile,isControlInvolved=TRUE,isBamInputFile=TRUE,
expLabel="fetal_liver",contrLabel="fetal_brain",
restrictionEnzyme='HindIII')
my.data
```

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