# Package 'r3Cseq'

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

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**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.

**License** GPL-3

**biocViews** Preprocessing, Sequencing, HighThroughputSequencing,

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

## R topics documented:

---

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](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](getReadCountPerRestrictionFragment)

## Author(s)

S. Thongjuea

## See Also

[calculateRPM](calculateRPM), [contrRPM](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 [ge-tReadCountPerRestrictionFragment](#) 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                    *get the viewpoint of 3C-seq data*

---

## Description

The viewpoint is the region of interest, which can be a promoter region of an interested gene, an enhancer, and a transcrition 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 (de-
                    fault=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, expInteraction-Regions, 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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