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.Mmusc1us.UCSC.mm9.masked, BSgenome.Mmusc1us.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


biocViews Preprocessing, Sequencing

NeedsCompilation no

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**calculateBatchRPM**

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

Author(s)

S. Thongjuea

See Also

`calculateRPM`, `expRPM`, `contrRPM`

Examples

```
#See the vignette
```

---

**Description**

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

**Usage**

```
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

```
#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**

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

**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**

#See the vignette

**contrReadCount**

get read count per region for the control

**Description**

get the 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 <- 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 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

getInteractions, contrInteractionRegions

Examples

#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**

```r
eexport3Cseq2bedGraph(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
```

---

**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**

```r
eexport3CseqRawReads2bedGraph(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**

```r
## 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
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
- exportInteractions2text
- plotOverviewInteractions, plotInteractionsPerChromosome
- plotInteractionsNearViewpoint

Examples

#See the vignette

getBatchInteractions  

**Description**

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

generateBatchReadCountPerRestrictionFragment

Description

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

Usage

generateBatchReadCountPerRestrictionFragment(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

gBatchReadCountPerWindow

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

gBatchReadCountPerWindow(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

---

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

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
getRawReads(object)
```

**Arguments**

- `object`: r3Cseq object
getReadCountPerRestrictionFragment

Value

The GRangedData represents the aligned reads from the BAM file

Author(s)

S. Thongjuea

See Also

getBatchRawReads,

Examples

#See the vignette

getReadCountPerRestrictionFragment

count reads per restriction fragment

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)
**Arguments**

obj rCseq or rCseqInBatch 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
plotDomainogramNearViewpoint

Myb_prom_FB  Myb_prom_FB a data set for the example of r3Cseq analysis

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  Myb_prom_FL a data set for the example of r3Cseq analysis

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  This method has been removed.

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

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>object</td>
<td>r3Cseq or r3CseqInBatch object</td>
</tr>
<tr>
<td>smoothing.parameter</td>
<td>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)</td>
</tr>
<tr>
<td>distance</td>
<td>Numeric. The distance relative to the viewpoint (default=5e5)</td>
</tr>
<tr>
<td>maximum_window</td>
<td>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.</td>
</tr>
<tr>
<td>view</td>
<td>character. The selected view of data (default=&quot;experiment&quot;)</td>
</tr>
</tbody>
</table>
Value

Plots of domainogram for interaction regions close to the viewpoint

Author(s)

S. Thongjuea

See Also

plotOverviewInteractions, plotInteractionsPerChromosome, plotInteractionNearViewpoint

Examples

# See the vignette

plotInteractionNearViewpoint

Plot identified interaction regions near the viewpoint

Description

Plot identified interaction regions near the viewpoint

Usage

plotInteractionNearViewpoint(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
plotOverviewInteractions

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

```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
`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 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

---

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
corrReadCount Object of class "RangedData" the read count in control

expRPM Object of class "RangedData" the normalized read per million in experiment
corrRPM Object of class "RangedData" the normalized read per million in control

expInteractionRegions Object of class "RangedData" the identified interaction regions in experiment
corrInteractionRegions 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

r3CseqInBatch-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.
The rat (rn5) reference genes from UCSC
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