

Package ‘rnaCrosslinkOO’

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Type Package

Title Analysis of RNA Crosslinking Data

Version 0.1.3

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Description Analysis of RNA crosslinking data for RNA structure prediction. The package is suitable for the analysis of RNA structure cross-linking data and chemical probing data.

License GPL-3

Encoding UTF-8

BugReports <https://github.com/JLP-BioInf/rnaCrosslinkOO/issues>

Depends seqinr, GenomicRanges, stats

Imports ggplot2, reshape2, MASS, mixtools, utils, S4Vectors, patchwork, doParallel, igraph, R4RNA, RColorBrewer, IRanges, foreach, grDevices, heatmap3, TopDom, tidyverse, RRNA, ggrepel, methods, parallel, ClassDiscovery

RoxygenNote 7.3.1

Collate 'rnaCrosslinkOO.R' 'rnaCrosslinkDataSet.R'
'clusterrnaCrosslink.R'
'clusterrnaCrosslinkMethodsAndHelpers.R'
'commonHelpersAndMethods.R' 'commonStatsAndPlots.R'
'foldrnaCrosslink.R' 'foldrnaCrosslinkMethodsAndHelpers.R'
'genericMethods.R' 'rnaCrosslinkDataSetMethodsAndHelpers.R'
'rnaCrosslinkOO-package.R' 'rnaCrosslinkQC.R'

Suggests knitr, rmarkdown, testthat (>= 3.0.0)

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`clusterGrangesList` *clusterGrangesList*

Description

Extract the cluster coordinates in granges format

Usage

```
clusterGrangesList(x)
```

Arguments

`x` A `rnaCrosslinkDataSet` object

Value

A list of Granges objects showing the positions of each cluster, one entry for each sample

Examples

```
cds = makeExemplernaCrosslinkDataSet()
clusterGrangesList(cds)
```

`clusterGrangesList<-` *clusterGrangesList<-*

Description

Set new `clusterGrangesList` slot

Usage

```
clusterGrangesList(x) <- value
```

Arguments

x A rnaCrosslinkDataSet object
value A replacement

Value

No return - Sets a new clusterGrangesList slot

Examples

```
cds = makeExemplernaCrosslinkDataSet()

newclusterGrangesList <- clusterGrangesList(cds)
clusterGrangesList(cds) <- newclusterGrangesList
```

clusterNumbers	<i>clusterNumbers</i>
----------------	-----------------------

Description

This method prints a table showing the number of clusters in each step of the analysis

Usage

```
clusterNumbers(knowClusteredCds, rna)
```

Arguments

knowClusteredCds A rnaCrosslinkDataSet object after clustering has been performed
rna The RNA ID of interest - use rna(cdsObject).

Value

A data.frame shoing the number of clusters for each sample

Examples

```
cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
                                   cores = 1,
                                   stepCount = 1,
                                   clusterCutoff = 1)
clusterNumbers(clusteredCds)
```

clusterrnaCrosslink *clusterrnaCrosslink*

Description

This method clusters the duplexes.

Usage

```
clusterrnaCrosslink(cds, cores = 3, stepCount = 2, clusterCutoff = 20)
```

Arguments

cds	rnaCrosslinkDataSet object created with rnaCrosslinkDataSet
cores	numeric - The number of cores to use
stepCount	Stringency for clustering
clusterCutoff	The minimum number of reads a cluster requires

Value

A rnaCrosslinkDataSet object

Examples

```
cds = makeExemplernaCrosslinkDataSet()

clusterrnaCrosslink(cds,
                    cores = 1,
                    stepCount = 1,
                    clusterCutoff = 0)
```

clusterTableFolded *clusterTableFolded*

Description

Extract the cluster coordinates with fold predicition in data frame format

Usage

```
clusterTableFolded(x)
```

Arguments

x	A rnaCrosslinkDataSet object
---	------------------------------

Value

A table showing the vienna structures of each cluster

Examples

```
cds = makeExemplernaCrosslinkDataSet()
```

```
clusterTableFolded(cds)
```

<code>clusterTableList</code>	<i>clusterTableList</i>
-------------------------------	-------------------------

Description

Extract the cluster coordinates in data frame format

Usage

```
clusterTableList(x)
```

Arguments

`x` A `rnaCrosslinkDataSet` object

Value

A list of tables showing the vienna structures of each cluster

Examples

```
cds = makeExemplernaCrosslinkDataSet()
```

```
clusterTableList(cds)
```

<code>clusterTableList<-</code>	<i>clusterTableList<-</i>
------------------------------------	------------------------------

Description

Set new `clusterTableList` slot

Usage

```
clusterTableList(x) <- value
```

Arguments

x A rnaCrosslinkDataSet object
 value A replacement

Value

No return - Sets a new clusterTableList slot

Examples

```
cds = makeExemplernaCrosslinkDataSet()

newclusterGrangesList <- clusterTableList(cds)
clusterTableList(cds) <- newclusterGrangesList
```

compareKnown	<i>compareKnown</i>
--------------	---------------------

Description

This method compares the current object to a know structure.run trimClusters() on the rnaCrosslinkDataSet first

Usage

```
compareKnown(trimmedClusters, knownMat, type)
```

Arguments

trimmedClusters a rnaCrosslinkDataSet object, run trimClusters() on the rnaCrosslinkDataSet first

knownMat Matrix - A marix(ncol = lengthRNA,nrow = lengthRNA) where a value in matrix[x,y] would indicate a known interation between nucleotide x and nucleotide y

type string - the Analysis stage of clusters you would like to compare you can find available types by just running the objects name

Value

Returns a rnaCrosslinkClusteredDataSet object

The 3 attributes matrixList, clusterTableList and clusterGrangesList will gain the types "known" and "novel" and "knownAndNovel"


```
trimmedClusters = trimClusters(clusteredCds = clusteredCds, trimFactor = 1, clusterCutoff = 1)

fasta = paste(c(rep('A',25),
                rep('T',25),
                rep('A',10),
                rep('T',23)), collapse = "")

header = '>transcript1'

fastaFile = tempfile()
writeLines(paste(header,fasta,sep = "\n"),con = fastaFile)

rnaRefs = list()
rnaRefs[[rnas(cds)]] = read.fasta(fastaFile)
rnaRefs

foldedCds = foldrnaCrosslink(trimmedClusters,
                             rnaRefs = rnaRefs,
                             start = 1,
                             end = 83,
                             shape = 0,
                             ensembl = 5,
                             constraintNumber = 1,
                             evCutoff = 1)

# make an example table of "know" interactions
file = data.frame(V1 = c(6),
                  V2 = c(80))
compareKnownStructures(foldedCds,file)

## End(Not run)
```

featureInfo

featureInfo

Description

Produces a list list of 2 elements 'transcript' and 'family' Each element contains a table with the counts for each RNA in each sample that interact with the target RNA

Usage

```
featureInfo(cds)
```

Arguments

cds a rnaCrosslinkDataSet object

Value

A list - Feature level and transcript level counts for each sample

Examples

```
cds = makeExemplernaCrosslinkDataSet()
featureInfo(cds)
```

```
findBasePairsRNAcoFold2
```

```
findBasePairsRNAcoFold2
```

Description

Folds the clusters using Vienna RNAfold

Usage

```
findBasePairsRNAcoFold2(
  startPos1,
  endPos1,
  seq1,
  startPos2,
  endPos2,
  seq2,
  fasta,
  shape
)
```

Arguments

startPos1	Start of the cluster side x
endPos1	End of the cluster side x
seq1	Sequence of x
startPos2	Start of the cluster side y
endPos2	End of the cluster side y
seq2	Sequence of y
fasta	rnaRefs
shape	shape reactivities

Value

A table of clusters and coordinates with folds

findBasePairsRNAfold *findBasePairsRNAfold*

Description

Folds the clusters using Vienna RNA duplex

Usage

```
findBasePairsRNAfold(startPos, endPos, seqs, fasta, shape)
```

Arguments

startPos	Start of the cluster side x
endPos	End of the cluster side x
seqs	Sequence of x
fasta	rnaRefs
shape	shape reactivities

Value

A table of clusters and coordinates with folds

findBasePairsRNAfold2 *findBasePairsRNAfold2*

Description

Folds the clusters using Vienna RNA duplex

Usage

```
findBasePairsRNAfold2(startPos, endPos, seqs, fasta)
```

Arguments

startPos	Start of the cluster side x
endPos	End of the cluster side x
seqs	Sequence of x
fasta	rnaRefs

Value

A table of clusters and coordinates with folds

foldrnaCrosslink *foldrnaCrosslink*

Description

This methods folds an ensembl of structures for the whole RNA or chosen region of the RNA. See rnaCrosslinkDataSet for slot information.

Usage

```
foldrnaCrosslink(
  cdsObject,
  rnaRefs,
  start,
  end,
  evCutoff = 1,
  ensembl = 50,
  constraintNumber = 20,
  shape = 0
)
```

Arguments

cdsObject	rnaCrosslinkDataSet object created with rnaCrosslinkDataSet
rnaRefs	named List - a list with named elements that correspond to the .RNA of interest. The element of the list must be a fasta file that has been read with seqinr::read.fasta()
start	Start of segment to fold
end	End of segment to fold
evCutoff	Minimum number of read support for constraint to be included in folding
ensembl	Number of structures to make
constraintNumber	Number of constraints to add to each final fold
shape	shape reactivities (0 for no constraints)

Value

a rnaCrosslinkDataSet object

Examples

```
## Not run:
cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
  cores = 1,
```

```
        stepCount = 1,
        clusterCutoff = 0)

trimmedClusters = trimClusters(clusteredCds = clusteredCds,
                               trimFactor = 1,
                               clusterCutoff = 0)

fasta = paste(c(rep('A',25),
                rep('T',25),
                rep('A',10),
                rep('T',23)),collapse = "")

header = '>transcript1'

fastaFile = tempfile()
writeLines(paste(header,fasta,sep = "\n"),con = fastaFile)

rnaRefs = list()
rnaRefs[[rnas(cds)]] = read.fasta(fastaFile)
rnaRefs

foldedCds = foldrnaCrosslink(trimmedClusters,
                             rnaRefs = rnaRefs,
                             start = 1,
                             end = 83,
                             shape = 0,
                             ensembl = 5,
                             constraintNumber = 1,
                             evCutoff = 1)

foldedCds

## End(Not run)
```

getAdjacencyMat

getAdjacencyMat

Description

Makes and adjacency matrix list (for clustering)

Usage

```
getAdjacencyMat(InputGranges, nucleotideOrPerc, cutoff)
```

Arguments

InputGranges list created with InputToGRanges (but just the gap section of the list)
 nucleotideOrPerc measure difference by percentage or nucleotides
 cutoff The maximum difference before giving these two gaps 0

Details

Makes and adjacency matrix list (for clustering)

Value

A list of Adjacency matrices

`getClusterClusterShortRangeWhole`
getClusterClusterShortRangeWhole

Description

Decides if a cluster is long or short range then either grabs the whole sequence or the sequence of the two sides of the interaction separately.

Usage

`getClusterClusterShortRangeWhole(cluster, seq)`

Arguments

cluster cluster positions
 seq sequence of transcript

Value

The same table with an extra column

getData *getData*

Description

Get data is more generic method for retrieving data from the object and returns a list, the number of entries in the list is number of samples in the dataset and the list contain entries of the data type and analysis stage you select.

Usage

```
getData(x, data, type)
```

Arguments

x	A rnaCrosslinkDataSet object
data	The data type to return <InputFiles matrixList clusterGrangesList clusterTableList>
type	The analysis stage <original noHost originalClusters trimmedClusters>

Value

A list of the chosen data type - one entry for each sample

Examples

```
cds = makeExemplernaCrosslinkDataSet()
getData(cds, 'matrixList', 'original')
```

getInteractions *getInteractions*

Description

This method returns a table of interactions of an RNA (interactor) on the RNA of interest.

Usage

```
getInteractions(cds, interactors)
```

Arguments

cds	a rnaCrosslinkDataSet object
interactors	A vector containing the names of RNAs to show interactions with

Value

A table showing the read coverage of the specified interacting RNAs

Examples

```
cds = makeExemplernaCrosslinkDataSet()
getInteractions(cds, c("transcript1", "transcript2"))
```

getMatrices

getMatrices

Description

Make a matrix of contact interactions

Usage

```
getMatrices(InputList, rna, size)
```

Arguments

InputList	the original InputList created with readInputFiles or subsetInputList
rna	the RNA of interest that you want to subset
size	The size of the RNA

Details

Function used to create a list of matrices for plotting with plotMatrixList or plotMatrixListFull, the output list will be same as the input except for an extra list layer for the specific RNA

Value

A list of matrices

`getReverseInteractions`
getReverseInteractions

Description

This method prints interactions of the RNA of interest on another RNA transcript.

Usage

```
getReverseInteractions(cds, interactor)
```

Arguments

`cds` a `rnaCrosslinkDataSet` object
`interactor` The rna to show interactions with

Value

A long format table shoing the read coverage of chosen RNA

Examples

```
cds = makeExemplernaCrosslinkDataSet()  
getReverseInteractions(cds, 'transcript2')
```

`group` *group*

Description

Extract the indeces for each group for the instance

Usage

```
group(x)
```

Arguments

`x` A `rnaCrosslinkDataSet` object

Value

A list - The indices of the sample in the control and sample groups

Examples

```

cds = makeExemplernaCrosslinkDataSet()

group(cds)

```

InputFiles

InputFiles

Description

Extract the data in original format

Usage

```
InputFiles(x)
```

Arguments

x A rnaCrosslinkDataSet object

Value

A list of tables in the original input format, one entry for each sample

Examples

```

cds = makeExemplernaCrosslinkDataSet()

InputFiles(cds)

```

InputToGRanges

InputToGRanges

Description

This function is useful to turn a list of Input data into lists of GRanges It creates a list for each sample one for the left side one for the right side and one for the gap in the middle.

Usage

```
InputToGRanges(InputList, rna)
```

Arguments

InputList the original InputList created with readInputFiles or subsetInputList
rna The rna of interest

Value

A list of GRanges data in Input format

`makeExemplernaCrosslinkDataSet`
makeExemplernaCrosslinkDataSet

Description

Creat a minimal example `rnaCrosslinkDataSetObject`

Usage

`makeExemplernaCrosslinkDataSet()`

Value

An example `rnaCrosslinkDataSet` object

Examples

`cds = makeExemplernaCrosslinkDataSet()`

`matrixList` *matrixList*

Description

Extract the contact matrices

Usage

`matrixList(x)`

Arguments

`x` A `rnaCrosslinkDataSet` object

Value

A list of contract matrices, one entry for each sample

Examples

`cds = makeExemplernaCrosslinkDataSet()`

`matrixList(cds)`

```
matrixList<-          matrixList
```

Description

Set new matrixList slot

Usage

```
matrixList(x) <- value
```

Arguments

x A rnaCrosslinkDataSet object
value A replacement

Value

No return - Sets a new matrixList slot

Examples

```
cds = makeExemplernaCrosslinkDataSet()

newMatrixList <- matrixList(cds)
matrixList(cds) <- newMatrixList
```

```
plotClusterAgreement Plot a heatmap that plots the agreements between replicates after clus-  
terrnaCrosslink has been performed
```

Description

Plot a heatmap that plots the agreements between replicates after clusterrnaCrosslink has been performed

Usage

```
plotClusterAgreement(cds, analysisStage = "originalClusters")
```

Arguments

cds A rnaCrosslinkDataSet object
analysisStage The stage of the analysis to plot

Value

A heatmap of the agreement between replicates in the analysis stage chosen

Examples

```
cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
  cores = 1,
  stepCount = 1,
  clusterCutoff = 0)

plotClusterAgreement(cds)
```

plotClusterAgreementHeat

Plot a heatmap that plots the agreements between replicates after clusterrnaCrosslink has been performed

Description

Plot a heatmap that plots the agreements between replicates after clusterrnaCrosslink has been performed

Usage

```
plotClusterAgreementHeat(cds, analysisStage = "originalClusters")
```

Arguments

cds A rnaCrosslinkDataSet object
analysisStage The stage of the analysis to plot

Value

A heatmap of the agreement between replicates in the analysis stage chosen

Examples

```
cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
  cores = 1,
  stepCount = 1,
  clusterCutoff = 0)

plotClusterAgreementHeat(cds)
```

plotCombinedMatrix	<i>Plots a contact map of two chosen samples for chosen stages in the analysis, with each chosen sample on separate halves of the contact map</i>
--------------------	---

Description

Plots a contact map of two chosen samples for chosen stages in the analysis, with each chosen sample on separate halves of the contact map

Usage

```
plotCombinedMatrix(  
  cds,  
  type1 = "original",  
  type2 = "original",  
  sample1 = 1,  
  sample2 = 1,  
  directory = 0,  
  a = 1,  
  b = 50,  
  c = 1,  
  d = 50,  
  h = 3,  
  returnData = FALSE  
)
```

Arguments

cds	A rnaCrosslinkDataSet object
type1	The analysis stage to plot on the upper half of the heatmap

type2	The analysis stage to plot on the lower half of the heatmap
sample1	The sample number to plot on the upper half of the heatmap
sample2	The sample number to plot on the upper half of the heatmap
directory	An output directory for the heatmap (use 0 for no output)
a	To make a subsetting plot (left value on x)
b	To make a subsetting plot (right value on x)
c	To make a subsetting plot (left value on y)
d	To make a subsetting plot (right value on y)
h	Height of image (inches) (only useful if plotting)
returnData	if TRUE matrix is returned instead of plotting

Value

A heatmap of the reads of the chosen sample numbers, in the analysis stages chosen, with each chosen sample on a separate half of the heatmap

Examples

```

cds = makeExampleRnaCrosslinkDataSet()

plotCombinedMatrix(cds,
  type1 = "original",
  type2 = "noHost",
  b = rnaSize(cds),
  d = rnaSize(cds))

```

plotComparisonArc *plotComparisonArc*

Description

This method plots two structures chosen from the plotEnsemblePCA method

Usage

```
plotComparisonArc(foldedCds, s1 = "s1", s2 = "s2", n1 = 1, n2 = 2)
```

Arguments

foldedCds	rnaCrosslinkDataSet after running foldrnaCrosslink
s1	sample of structure 1
s2	sample of structure 2
n1	number of structure from first sample
n2	number of structure from first sample

Value

an ark diagram of the two structures

Examples

```
## Not run:
cds = makeExemplernaCrosslinkDataSet()
clusteredCds = clusterrnaCrosslink(cds = cds,
                                  cores = 3,
                                  stepCount = 2,
                                  clusterCutoff = 1)

trimmedClusters = trimClusters(clusteredCds = clusteredCds, trimFactor = 1, clusterCutoff = 1)

fasta = paste(c(rep('A',25),
                rep('T',25),
                rep('A',10),
                rep('T',23)),collapse = "")

header = '>transcript1'

fastaFile = tempfile()
writeLines(paste(header,fasta,sep = "\n"),con = fastaFile)

rnaRefs = list()
rnaRefs[[rnas(cds)]] = read.fasta(fastaFile)
rnaRefs

foldedCds = foldrnaCrosslink(trimmedClusters,
                             rnaRefs = rnaRefs,
                             start = 1,
                             end = 83,
                             shape = 0,
                             ensembl = 5,
                             constraintNumber = 1,
                             evCutoff = 1)

plotComparisonArc(foldedCds,"s1","s1",1,3)

## End(Not run)
```

plotEnsemblePCA	<i>plotEnsemblePCA</i>
-----------------	------------------------

Description

This method plots a PCA of the ensembl

Usage

```
plotEnsemblePCA(foldedCds, labels = TRUE, split = TRUE)
```

Arguments

foldedCds	rnaCrosslinkDataSet after running foldrnaCrosslink
labels	plot with labels or not (TRUE/FALSE)
split	split the plot using facets based on the samples (TRUE/FALSE)

Value

a PCA plot of the ensembl

Examples

```
## Not run:
cds = makeExemplernaCrosslinkDataSet()
clusteredCds = clusterrnaCrosslink(cds = cds,
                                  cores = 3,
                                  stepCount = 2,
                                  clusterCutoff = 1)

trimmedClusters = trimClusters(clusteredCds = clusteredCds, trimFactor = 1, clusterCutoff = 1)

fasta = paste(c(rep('A',25),
                rep('T',25),
                rep('A',10),
                rep('T',23)),collapse = "")

header = '>transcript1'

fastaFile = tempfile()
writeLines(paste(header,fasta,sep = "\n"),con = fastaFile)

rnaRefs = list()
rnaRefs[[rnas(cds)]] = read.fasta(fastaFile)
```

```

rnaRefs

foldedCds = foldrnaCrosslink(trimmedClusters,
                             rnaRefs = rnaRefs,
                             start = 1,
                             end = 83,
                             shape = 0,
                             ensembl = 5,
                             constraintNumber = 1,
                             evCutoff = 1)

plotEnsemblePCA(foldedCds)

## End(Not run)

```

plotInteractions	<i>Plots a contact map of interactions of each sample of an RNA (interactor) on the RNA of interest</i>
------------------	---

Description

Plots a contact map of interactions of each sample of an RNA (interactor) on the RNA of interest

Usage

```

plotInteractions(
  cds,
  rna,
  interactor,
  directory = 0,
  a = 1,
  b = 50,
  c = 1,
  d = 50,
  h = 3
)

```

Arguments

cds	A rnaCrosslinkDataSet object
rna	The RNA of interest
interactor	The RNA to show interactions with
directory	An output directory for the heatmap (use 0 for no output)
a	To make a subsetted plot (left value on x)

- b To make a subsetting plot (right value on x) (use 'max' to plot the whole RNA strand length)
- c To make a subsetting plot (left value on y)
- d To make a subsetting plot (right value on y) (use 'max' to plot the whole RNA strand length)
- h Height of image (inches) (only useful if plotting)

Value

A heatmap of interactions of the RNA (interactor) on the RNA of interest

Examples

```
cds = makeExemplernaCrosslinkDataSet()

plotInteractions(cds,
  rna = "transcript1",
  interactor = "transcript2",
  b = "max",
  d = "max")
```

plotInteractionsAverage

Plots a contact map of interactions of all samples of an RNA (interactor) on the RNA of interest

Description

Plots a contact map of interactions of all samples of an RNA (interactor) on the RNA of interest

Usage

```
plotInteractionsAverage(
  cds,
  rna,
  interactor,
  directory = 0,
  a = 1,
  b = 50,
  c = 1,
  d = 50,
  h = 3
)
```

Arguments

cds	A rnaCrosslinkDataSet object
rna	The RNA of interest
interactor	The RNA to show interactions with
directory	An output directory for the heatmap (use 0 for no output)
a	To make a subsetting plot (left value on x)
b	To make a subsetting plot (right value on x) (use 'max' to plot the whole RNA strand length)
c	To make a subsetting plot (left value on y)
d	To make a subsetting plot (right value on y) (use 'max' to plot the whole RNA strand length)
h	Height of image (inches) (only useful if plotting)

Value

A heatmap of interactions of all samples of the RNA (interactor) on the RNA of interest

Examples

```
cds = makeExemplernaCrosslinkDataSet()

plotInteractionsAverage(cds,
  rna = "transcript1",
  interactor = "transcript2",
  b = "max",
  d = "max")
```

plotMatrices	<i>Plots a number of contact maps to file of each sample for a stage in the analysis</i>
--------------	--

Description

Plots a number of contact maps to file of each sample for a stage in the analysis

Usage

```
plotMatrices(
  cds,
  type = "original",
  directory = 0,
  a = 1,
  b = 50,
  c = 1,
  d = 50,
  h = 3
)
```

Arguments

cds	A rnaCrosslinkDataSet object
type	The analysis stage to plot
directory	An output directory for the heatmap (use 0 for no output)
a	To make a subsetted plot (left value on x)
b	To make a subsetted plot (right value on x)
c	To make a subsetted plot (left value on y)
d	To make a subsetted plot (right value on y)
h	Height of image (inches) (only useful if plotting)

Value

A heatmap of the reads in the analysis stage chosen

Examples

```
cds = makeExemplernaCrosslinkDataSet()

plotMatrices(cds,
              b = rnaSize(cds),
              d = rnaSize(cds))
```

plotMatricesAverage *plotMatricesAverage*

Description

Plots a contact map of all samples for two chosen stages in the analysis, with each chosen stage on separate halves of the contact map

Usage

```
plotMatricesAverage(
  cds,
  type1 = "original",
  type2 = "blank",
  directory = 0,
  a = 1,
  b = 50,
  c = 1,
  d = 50,
  h = 3
)
```

Arguments

<code>cds</code>	A <code>rnaCrosslinkDataSet</code> object
<code>type1</code>	The analysis stage to plot on the upper half of the heatmap (use 'blank' to leave this half blank)
<code>type2</code>	The analysis stage to plot on the lower half of the heatmap (use 'blank' to leave this half blank)
<code>directory</code>	An output directory for the heatmap (use 0 for no output)
<code>a</code>	To make a subsetted plot (left value on x)
<code>b</code>	To make a subsetted plot (right value on x)
<code>c</code>	To make a subsetted plot (left value on y)
<code>d</code>	To make a subsetted plot (right value on y)
<code>h</code>	Height of image (inches) (only useful if plotting)

Value

A heatmap of the reads in the two analysis stages chosen, with each chosen stage on a separate half of the heatmap

Examples

```
cds = makeExamplernaCrosslinkDataSet()

plotMatricesAverage(cds,
                    b = rnaSize(cds),
                    d = rnaSize(cds))
```

`plotStructure`

plotStructure

Description

This method plots a structures chosen from the `plotEnsemblePCA` method

Usage

```
plotStructure(foldedCds, rnaRefs, s = "s1", n = 1)
```

Arguments

<code>foldedCds</code>	<code>rnaCrosslinkDataSet</code> after running <code>foldrnaCrosslink</code>
<code>rnaRefs</code>	A fasta of the transcript (made with <code>seqinr::read.fasta</code>)
<code>s</code>	sample of structure
<code>n</code>	number of structure

Value

a diagram of the predicted structure

Examples

```
## Not run:
cds = makeExemplernaCrosslinkDataSet()
clusteredCds = clusterrnaCrosslink(cds = cds,
                                  cores = 3,
                                  stepCount = 2,
                                  clusterCutoff = 1)

trimmedClusters = trimClusters(clusteredCds = clusteredCds, trimFactor = 1, clusterCutoff = 1)

fasta = paste(c(rep('A',25),
                rep('T',25),
                rep('A',10),
                rep('T',23)),collapse = "")

header = '>transcript1'

fastaFile = tempfile()
writeLines(paste(header,fasta,sep = "\n"),con = fastaFile)

rnaRefs = list()
rnaRefs[[rnas(cds)]] = read.fasta(fastaFile)
rnaRefs

foldedCds = foldrnaCrosslink(trimmedClusters,
                             rnaRefs = rnaRefs,
                             start = 1,
                             end = 83,
                             shape = 0,
                             ensembl = 5,
                             constraintNumber = 1,
                             evCutoff = 1)

plotStructure(foldedCds,rnaRefs,"s1",3)

## End(Not run)
```

printClustersFast *printClustersFast*

Description

Makes a table with the coordinates of the clusters

Usage

```
printClustersFast(dir, clustering, highest_clusters, left, right)
```

Arguments

dir	the directory that contains the *Inputrids.Input files
clustering	The output from the iGraph function cluster_walktrap for the (made with adjacency matrix input)
highest_clusters	The cluster you are interested in keeping
left	list created with InputToGRanges (but just the left section of the list)
right	list created with InputToGRanges (but just the right section of the list)

Details

Does the same as printClusters but is a lot faster and does not create plots of each cluster

Value

A table of clusters and coordinates

readNumbers *readNumbers*

Description

This method prints a table showing the number of duplexes in the clusters in each step of the analysis

Usage

```
readNumbers(knowClusteredCds, rna)
```

Arguments

knowClusteredCds	A rnaCrosslinkDataSet object after clustering has been performed
rna	The RNA ID of interest - use rna(cdsObject).

Value

A data.frame showing the number of reads in clusters for each sample

Examples

```

cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
  cores = 1,
  stepCount = 1,
  clusterCutoff = 1)
readNumbers(clusteredCds)

```

rnaCrosslinkDataSet-class

rnaCrosslinkDataSet

Description

rnaCrosslinkDataSet objects are used to store the input meta-data, data and create a framework for the storage of results. Whilst creating the object, the original Input files are also filtered for the RNA of interest. Check the package vignette for more information.

Usage

```

rnaCrosslinkDataSet(
  rnas,
  rnaSize = 0,
  sampleTable,
  subset = "all",
  sample = "all"
)

```

Arguments

rnas	vector - The names of the RNA interest, these must be displayed the same way as in the input Input Files.
rnaSize	named list - The sizes (nt) of the RNAs of interest, the list elements must have same names as the rnas vector and each each contain one numeric value.
sampleTable	string - The address of the sample table, the sample table must have 4 columns, fileName (the full path and file name of the input Input file for each sample), group ("s" - sample or "c" - control), sample (1,2,3, etc), sampleName (must be unique).
subset	a vector of 4 values to subset based on structural read size. c(l-min,l-max,r-min,r-max)
sample	The number of reads to sample for each sample.

Value

A rnaCrosslinkDataSet object.

Slots

`clusterTableFolded` table - a table similar to the `clusterTableList` it contains coordinates of the clusters along with vienna format fold and RNA sequences for each cluster

`clusterTableList` List - Follows the pattern for list slots of rnaCrosslinkDataSet objects, `matrixList(cds)[[rna]][[type]]` contains a table with coordinates and information about the clusters identified

`clusterGrangesList` List - Follows the pattern for list slots of rnaCrosslinkDataSet objects, `matrixList(cds)[[rna]][[type]]` contains GRanges objects of the original duplexes with their cluster membership

`sampleTable` table - Column names; fileName, group (s or c), sample (1,2,3, etc), sampleName (must be unique)

`rnas` string - a single RNA to analyse - must be present in `rnas(cdsObject)`

`rnaSize` if set to 0 this will be calculated

`matrixList` List - Follows the pattern for list slots of rnaCrosslinkDataSet objects, `matrixList(cds)[[rna]][[type]][[sample]]` Contains a set of contact matrices, each cell contains the number of duplexes identified for position x,y.

`InputFiles` List - Follows the pattern for list slots of rnaCrosslinkDataSet objects, `InputFiles(cds)[[rna]][[type]][[sample]]` Contains a set of tables, these are the original Input files that were read in.

`interactionTable` Table of interactions discovered in step1 of the folding

`viennaStructures` List of vienna format structures from final prediction

`dgs` List of free energies

Examples

```
# make example input
cds = makeExemplernaCrosslinkDataSet()

cds
```

rnaCrosslinkQC

rnaCrosslinkQC

Description

get a plot fo the read lengths and transcripts in the dataset The fuction will make 1 pdf and 2 text file in the directory provided

Usage

```
rnaCrosslinkQC(sampleTable, directory, topTranscripts = TRUE)
```

Arguments

sampleTable string - The address of the sample table, the sample table must have 4 columns, fileName (the full path and file name of the input Input file for each sample), group ("s" - sample or "c" - control), sample (1,2,3, etc), sampleName (must be unique).

directory A directory address to write the files

topTranscripts If FALSE a table of top transcripts will not be written to file

Value

ggplot and txt file

Examples

```

c4 = c(rep("transcript1",100),rep("transcript2",100) )
c10 = c(rep("transcript1",200) )
c1 = 1:200
c2 = rep(paste(rep("A", 40), collapse = ""),200)
c3 = rep(".",200)
c9 = rep(".",200)
c15 = rep(".",200)
c5 = rep(1,200)
c11 = rep(21,200)
c6 = rep(20,200)
c12= rep(40,200)
# short distance 50
c7 = sample(1:5, 50, replace = TRUE)
c8 = sample(20:25, 50, replace = TRUE)
c13 = sample(20:25, 50, replace = TRUE)
c14 = sample(40:45, 50, replace = TRUE)
# long distance 50
c7 = c(c7,sample(1:5, 50, replace = TRUE))
c8 = c(c8,sample(20:25, 50, replace = TRUE))
c13 = c(c13,sample(60:70, 50, replace = TRUE))
c14 = c(c14,sample(80:83, 50, replace = TRUE))
# inter RNA 100
c7 = c(c7,sample(1:5, 100, replace = TRUE))
c8 = c(c8,sample(20:25, 100, replace = TRUE))
c13 = c(c13,sample(1:5, 100, replace = TRUE))
c14 = c(c14,sample(20:25, 100, replace = TRUE))

exampleInput = data.frame(V1 = c1,
                           V2 = c2,
                           V3 = c3,
                           V4 = c4,
                           V5 = as.numeric(c5),
                           V6 = as.numeric(c6),
                           V7 = as.numeric(c7),
                           V8 = as.numeric(c8),
                           V9 = c9,
                           V10 = c10,

```

```

V11 = as.numeric(c11),
V12 = as.numeric(c12),
V13 = as.numeric(c13),
V14 = as.numeric(c14),
V15 = c15)

file = tempfile()
write.table(exampleInput,
            file = file,
            quote = FALSE,
            row.names = FALSE,
            sep = "\t", col.names = FALSE)

c4 = c(rep("transcript1",55),rep("transcript2",90) )
c10 = c(rep("transcript1",145) )
c1 = 1:145
c2 = rep(paste(rep("A", 40), collapse = ""),145)
c3 = rep(".",145)
c9 = rep(".",145)
c15 = rep(".",145)
c5 = rep(1,145)
c11 = rep(21,145)
c6 = rep(20,145)
c12= rep(40,145)
# short distance 55
c7 = sample(1:5, 55, replace = TRUE)
c8 = sample(20:25, 55, replace = TRUE)
c13 = sample(20:25, 55, replace = TRUE)
c14 = sample(40:45, 55, replace = TRUE)

# inter RNA 100
c7 = c(c7,sample(1:40, 90, replace = TRUE))
c8 = c(c8,sample(20:75, 90, replace = TRUE))
c13 = c(c13,sample(1:40, 90, replace = TRUE))
c14 = c(c14,sample(20:75, 90, replace = TRUE))

exampleInput = data.frame(V1 = c1,
                          V2 = c2,
                          V3 = c3,
                          V4 = c4,
                          V5 = as.numeric(c5),
                          V6 = as.numeric(c6),
                          V7 = as.numeric(c7),
                          V8 = as.numeric(c8),
                          V9 = c9,
                          V10 = c10,
                          V11 = as.numeric(c11),
                          V12 = as.numeric(c12),
                          V13 = as.numeric(c13),

```

```
V14 = as.numeric(c14),
V15 = c15)

file2 = tempfile()
write.table(exampleInput,
            file = file2,
            quote = FALSE,
            row.names = FALSE,
            sep = "\t",
            col.names = FALSE)

# Set up the sample table. ----
sampleTable1 = c(file, "s", "1", "s1")
sampleTable2 = c(file2, "c", "1", "c1")
# make the sample table
sampleTable2 = rbind.data.frame(sampleTable1, sampleTable2)
# add the column names
colnames(sampleTable2) = c("file", "group", "sample", "sampleName")

rnaCrosslinkQC(sampleTable2, tempdir())
```

rnas

rnas

Description

Extract the rna ID for the instance

Usage

```
rnas(x)
```

Arguments

x A `rnaCrosslinkDataSet` object

Value

A character - the ID of the RNA

Examples

```
cds = makeExemplernaCrosslinkDataSet()

rnas(cds)
```

`rnaSize`*rnaSize*

Description

Extract the size of the RNA for the instance

Usage

```
rnaSize(x)
```

Arguments

`x` A `rnaCrosslinkDataSet` object

Value

A numeric - the size of the RNA (nucleotides)

Examples

```
cds = makeExemplernaCrosslinkDataSet()
rnaSize(cds)
```

`sampleChimeras`*sampleChimeras*

Description

This function samples chimeras into smaller chunks so that clustering is quicker

Usage

```
sampleChimeras(chimeraList)
```

Arguments

`chimeraList` list of chimeras

sampleNames	<i>sampleNames</i>
-------------	--------------------

Description

Extract the sample names for the instance

Usage

```
sampleNames(x)
```

Arguments

x A rnaCrosslinkDataSet object

Value

A character vector - the sample names

Examples

```
cds = makeExemplernaCrosslinkDataSet()
sampleNames(cds)
```

sampleTable	<i>sampleTable</i>
-------------	--------------------

Description

Extract the sample table for the instance

Usage

```
sampleTable(x)
```

Arguments

x A rnaCrosslinkDataSet object

Value

A data frame - The original meta-data table

Examples

```
cds = makeExemplernaCrosslinkDataSet()
sampleTable(cds)
```

subsetInputList2	<i>subsetInputList2</i>
------------------	-------------------------

Description

Subset a list of Input files

Usage

```
subsetInputList2(InputList, min, max, length)
```

Arguments

InputList	the original InputList created with readInputFiles
min	the rna of interest that you want to subset
max	The number of randomly subsetted chimeric reads you need
length	The number of randomly subsetted chimeric reads you need

Details

Function used to subset a list of Input data created by readInputFiles This function produces the same size list as before but it returns ONLY the rna of interest and also Choose duplexes where the nt difference in position between the one side and other side of an interaction is between min and max

Value

A list of subsetted Input files

swapInputs	<i>swapInputs</i>
------------	-------------------

Description

Swap the table to ensure that 3 prime most duplex side is on he left of the table used to make one sides heatmaps and other reasons where having the left of the table coming after the right side is a problem. Different from swapInputs as it ensure that BOTH duplex sides originate from the RNA of interest.

Usage

```
swapInputs(InputList, rna)
```


Arguments

InputList	the original InputList created with readInputFiles or subsetInputList
rna	The rna of interest

Value

A list of "swapped" Input datas

swapInputs2	<i>swapInputs2</i>
-------------	--------------------

Description

Swap the table to ensure that 3 prime most duplex side is on the left of the table used to make one sides heatmaps and other reasons where having the left of the table coming after the right side is a problem. Different from swapInputs as it ensure that BOTH duplex sides originate from the RNA of interest.

Usage

```
swapInputs2(InputList, rna)
```

Arguments

InputList	the original InputList created with readInputFiles or subsetInputList
rna	The rna of interest

Value

A list of "swapped" Input data

swapInputs3	<i>swapInputs3</i>
-------------	--------------------

Description

Swap the table to ensure that 3 prime most duplex side is ont he left of the table used to make one sides heatmaps and other reasons where having the left of the table coming after the right side is a problem. Different from swapInputs as it ensure that BOTH duplex sides originate from the RNA of interest.

Usage

```
swapInputs3(InputList, rna)
```

Arguments

InputList the original InputList created with readInputFiles or subsetInputList
 rna The rna of interest

Value

A list of "swapped" Input datas

topInteractors	<i>topInteractors</i>
----------------	-----------------------

Description

This method prints the top transcripts that have the most duplexes assigned that interact with the transcript of interest

Usage

```
topInteractors(cds, ntop = 10, sds = TRUE)
```

Arguments

cds a rnaCrosslinkDataSet object
 ntop the number of entries to display
 sds known bug, doesn't work for small data sets fix incoming

Value

A table, the number of counts per sample per interacting transcript

Examples

```
cds = makeExemplernaCrosslinkDataSet()
topInteractors(cds, sds = TRUE)
```

topInteractions	<i>topInteractions</i>
-----------------	------------------------

Description

This method prints the top transcript interactions that have the most duplexes assigned

Usage

```
topInteractions(cds, ntop = 10)
```

Arguments

cds	a rnaCrosslinkDataSet object
ntop	the number of entries to display

Value

A table, the number of counts per sample per interaction

Examples

```
cds = makeExemplernaCrosslinkDataSet()  
topInteractions(cds)
```

topTranscripts	<i>topTranscripts</i>
----------------	-----------------------

Description

This method prints the top transcripts that have the most duplexes assigned

Usage

```
topTranscripts(cds, ntop = 10)
```

Arguments

cds	a rnaCrosslinkDataSet object
ntop	the number of entries to display

Value

A table, the number of counts per sample per transcript

Examples

```

cds = makeExemplernaCrosslinkDataSet()
topTranscripts(cds)

```

trimClusters	<i>trimClusters</i>
--------------	---------------------

Description

Trimming of the clusters removes redundant information derived from random fragmentation of the reads during library preparation. This method takes a `rnaCrosslinkDataSet` object where clustering has been performed with the `clusterrnaCrosslink` method and trims the clusters according to the `trimFactor` argument.

Usage

```
trimClusters(clusteredCds, trimFactor = 2.5, clusterCutoff = 1)
```

Arguments

<code>clusteredCds</code>	a <code>rnaCrosslinkDataSet</code> object
<code>trimFactor</code>	a positive value that defines how much the clusters will
<code>clusterCutoff</code>	Minimum number of reads before discarding cluster be trimmed = $\text{mean} + (\text{sd} * \text{trimFactor})$

Details

The 3 attributes; `matrixList`, `clusterTableList` and `clusterGrangesList` will gain the types "superClusters" and "trimmedClusters"

Value

Returns a `rnaCrosslinkDataSet` object

Examples

```

cds = makeExemplernaCrosslinkDataSet()

clusteredCds = clusterrnaCrosslink(cds,
  cores = 1,
  stepCount = 1,
  clusterCutoff = 0)

trimClusters(clusteredCds = clusteredCds,
  trimFactor = 1,
  clusterCutoff = 0)

```

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