# Package 'HiTC'

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```
binningC
```

Windowing of high-throughput 'C' interaction matrix

## Description

Windowing of 'C' interaction map

## Usage

```
binningC(x, binsize=100000, bin.adjust=TRUE, upa=TRUE,
method="median", use.zero=TRUE, step=1)
```

#### Arguments

х	object that inherits from class HTCexp
binsize	size of the bin to consider for windowing
bin.adjust	logical; adjust the size of the bin to the size of the genomic region
upa	logical; unique primer assignment. Allow one primer to belong to one or several bins
method	the method used to combine the counts. Must be 'mean', 'median' or 'sum'
use.zero	logical; use the zero values in the method calculation
step	numeric; binning step size in n coverage <i>i.e.</i> window step

## Details

bin.adjust allows to work with bin of the same size. Otherwise, the last bin will has a size different from binsize. A primer is assigned to a bin, if there is at least one base overlap between the bin and the primer region.

The method is used to combine the counts in a bin, must be 'mea', 'median' or 'sum'. The step parameter allows to choose the overlap between the bins. A step of 2 means a 50% overlap between two bins, a step of 3 means a 60% overlap between two bins, *etc*.

## CQC

## Value

An HTCexp-class object with binned intraction data. In this case, the genomic intervals are converted into bins of fixed size. The interaction matrix is symetric.

#### Author(s)

N. Servant, B. Lajoie

## See Also

HTCexp-class

## Examples

data(Nora\_5C)

```
## Data binning 100kb, with a 1/3 overlap
E14.bin <- binningC(E14$chrXchrX, binsize=100000, step=3)
show(E14.bin)
```

CQC

Quality Control for high-throughput 'C' experiment

#### Description

Quality Control for high-throughput 'C' experiment

#### Usage

```
CQC(x, cis.trans.ratio = TRUE, hist.interac=TRUE, scat.interac.dist=TRUE, hist.dist=TRUE, trim.range=0.98, dev.new=FALSE)
```

## Arguments

x	object that inherits from class HTCexp or HTClist						
cis.trans.ratio							
	logical; barplot of percentage of inter-intrachromsomal interactions						
hist.interac	logical; histogram of the interaction frequency						
scat.interac.dist							
	logical; scatter plot of interaction count versus the genomic distance between two elements						
hist.dist	logical; histogram of the distance between the 'x' and 'y' intervals						
trim.range	remove the extreme values by trimming the counts. Only use for plotting functions. [0,1]						
dev.new	logical; specifying if each plots must be in a separate graphical device						

## Details

If x is a HTClist object, all HTCexp objects are merged. The zero values are not used to compute the descriptive statistics and to display the data. If trim.range are lower than 1. The highest values (quantile probability is equal to trim.range) are discarded.

#### Value

Create quality plots and return a matrix with some simple statistics on all, cis and trans data.

#### Author(s)

N. Servant, B. Lajoie

#### See Also

HTCexp-class

## Examples

data(Nora\_5C)

## Quality Control
CQC(E14)

discretize

Transform matrix of counts data into discrete matrix

## Description

Transform matrix of counts data into discrete matrix

## Usage

discretize(x, nb.lev=4, quant=TRUE)

## Arguments

х	data matrix
nb.lev	number of discretization level
quant	logical; use quantile distribution or split data into equals 'nb.lev' levels

## Value

A discrete matrix

#### Author(s)

N. Servant

## export.my5C

## See Also

quantile

## Examples

## Not run: data(Nora\_5C)

## Data binning
E14bin<-binningC(E14\$chrXchrX)</pre>

```
## Discretize matrix
dismat<-discretize(intdata(E14bin))
mapC(dismat)</pre>
```

## End(Not run)

export.my5C

## Export HTCexp object to my5C website format

## Description

Export HTCexp object to my5C website format

#### Usage

export.my5C(x, file)

## Arguments

х	object that inherits from class HTCexp
file	character; the name of the output file

#### Value

A my5C tabbed delimited file, with : Y\_INTERVAL\_NAME/X\_INTERVAL\_NAME/INTERACTION\_COUNT

## Author(s)

N. Servant

## See Also

exportC

## *exportC*

## Examples

```
## Not run:
data(Nora_5C)
## Data binning
E14.bin<-binningC(E14$chrXchrX)
## Export the new intervals definition
export.my5C(E14.bin, file="E14my5C.csv")
```

## End(Not run)

exportC Export HTCexp object

## Description

Export HTCexp object to csv format

## Usage

exportC(x, file)

## Arguments

Х	object that inherits from class HTCexp
file	character; the basename of the output file

## Value

Three output files will be created ; 2 BED files for each genomic intervals, and one matrix file

## Author(s)

N. Servant

## See Also

export.my5C, importC

## Examples

## Not run: data(Nora\_5C)

## Data binning
E14.bin<-binningC(E14\$chrXchrX)</pre>

## Export the new intervals definition

## extractRegion

exportC(E14.bin, file="E14")

## End(Not run)

extractRegion Extract a s

## Extract a subset of the HTCexp object

## Description

Extract a subset of the HTCexp object based on genomic ranges

## Usage

extractRegion(x, MARGIN, chr, from, to, exact=FALSE)

#### Arguments

x	object that inherits from class HTCexp
MARGIN	a vector giving the subscripts which the function will be applied over as in 'apply' function. E.g., '1' for the 'x' intervals, and '2' for the 'y' intervals, ' $c(1, 2)$ ' indicates 'x' and 'y' intervals.
chr	character; the chromosome of the genomic region
from	numeric; start of the genomic region
to	numeric; end of the genomic region
exact	logical; exact genomic region

## Details

By default, only the intervals fully included in the genomic ranges are returned. If exact is true, the overlapping intervals are also used, and forced to start/end at the specified position. If no intervals are overlapping, an interval with NA values is added.

#### Value

A HTCexp object

#### Author(s)

N. Servant

#### See Also

GRanges-class

## Examples

data(Nora\_5C)

```
## Focus on the genomic region chrX:98000000-100000000
E14sub<-extractRegion(E14$chrXchrX, c(1,2), chr="chrX", from=98000000, to=100000000)
show(E14sub)
```

getExpectedCounts	Estimate expected interaction counts of a High-Throughput C intra-
	chromsomal map based on the genomic distance between two loci

#### Description

The expected interaction is defined as the linear relationship between the interaction counts and the distance between two loci. See details for additional informations.

#### Usage

getExpectedCounts(x, span=0.01, bin=0.005, stdev=FALSE, plot=FALSE)

#### Arguments

х	object that inherits from class HTCexp
span	fraction of the data used for smoothing at each x point.
bin	interpolation parameter
stdev	logical, calculate the variance
plot	logical, display lowess smoothing and variance estimation points

#### Details

The expected value is the interaction frequency between two loci that one would expect based on a sole dependency on the genomic proximity of these fragments in the linear genome. This can be estimated using a Lowess regression model. The lowess smoothing has two parameters : span and bin. The span corresponds to the fraction of the data used for smoothing. Instead of computing the local polynomial fitting at each data point, a window of size delta (bin parameter) is applied on the data and a linear interpolation is used to fill in the fitted values within the window. The default is 1% of the range of x. If delta=0 all but identical x values are estimated independently. The variance is then estimated using the same span and bin parameter, at each interpolation points. The points inside a window are weighted so that nearby points get the most weight (tricube weight function).

## Value

A list with the expected interaction map and the estimated variance

#### Author(s)

N. Servant, B. Lajoie

#### HTCexp-class

#### See Also

HTCexp-class,normPerExpected, normPerExpected, lowess

#### Examples

data(Nora\_5C)

```
## Estimate expected interaction from distance between intervals
E14.exp<-getExpectedCounts(E14$chrXchrX, stdev=TRUE, plot=FALSE)
mapC(HTCexp(E14.exp$exp.interaction, xgi=x_intervals(E14$chrXchrX), ygi=y_intervals(E14$chrXchrX)))
```

HTCexp-class

Class 'HTCexp'

#### Description

A class for representing high throughput Chromosome Conformation Capture data from nextgeneration sequencing experiments.

#### Details

The normPerExpected method estimates the expected interactions based on a the dependency on the genomic proximity between two loci. If stdev is false, the ratio observed/expected is returned, otherwise, the zscore ((observed-expected)/stdev) is returned Look at the getExpectedCounts function for details.

The normPerTrans method is based on the assumption that all trans interactions should be the same. Thus, the cis interactions can be normalized by the interaction level of trans data. The xtrans trans map has to share its 'xgi' ranges with the cis map, and the ytrans has to share its 'ygi' ranges with the cismap. The method is used to combine the normalization factor from x and y ranges. Must be 'sum', 'mult' or 'mean'.

#### **Objects from the Class**

Objects can be created either by:

- 1. calls of the form new("HTCexp", intdata, GRanges, GRanges).
- 2. using the auxiliary function HTCexp and supplying interaction matrix with x and y intervals definition.

#### Slots

- intdata: Integer matrix, holding the interaction level between each pairs of 'x-y' intervals. The 'y' intervals must be in rows, and the 'x' in columns.
- ygi: Genomic ranges of y intervals; see class granges for details
- xgi: Genomic ranges of x intervals; see class granges for details

#### Methods

**detail(x)** signature("HTCexp"): a more detailed output of the experiment than provided by show.

- divide(x) comparison of two signature("HTCexp") objects. Perform the division of the two interaction matrices on the common 'x' and 'y' intervals. The operation is done only on the common intervals of both objects. If one of the two objects has a count to zero, the divided value will be NA
- intdata(x) return the intdata matrix counts
- export(x) Defunct. See exportC method
- **isBinned** return TRUE if the data are binned. The method tests if the 'x' and 'y' genome intervals are the same, if 90% of the bins have the same size and if the full genomic range is covered
- isIntraChrom(x) return TRUE if the current signature("HTCexp") object contains intrachromosomal interaction data
- **normPerReads**(x) normalize the interaction matrix by the total number of reads of the matrix.
- **normPerExpected**(**x**, **stdev=TRUE**) normalize the interaction matrix by the expected number of reads based on the distance between two loci. See details.
- normPerZscore(x) Defunct. See normPerExpected method
- **normPerTrans(x, xtrans, ytrans, method=''sum'')** Normalize cis interaction map based on the trans interactions. See details
- plot(x) visualization method; Display an heatmap of the interaction data. Refer to the documentation of mapC for more details of the plotting function
- **range**(**x**) return the genomic range of the signature("HTCexp") object
- seq\_name(x) Defunct. See seqlevels method
- seqlevels(x) return the sequence levels of the signature("HTCexp") object
- show(x) summarized output of the experiment, with informations about the data dimension and the
  genomic region studied
- substract(x) comparison of two signature("HTCexp") objects. Perform the substraction of the two interaction matrices on the common 'x' and 'y' intervals. The operation is done only on the common intervals of both objects. If one of the two objects has a count to zero, the divided value will be NA
- x\_intervals(x) return the xgi GRanges object defining the x intervals
- y\_intervals(x) return the ygi GRanges object defining the y intervals

xy\_intervals(x) return both xgi and ygi objects as a GRangesList object

#### Author(s)

Nicolas Servant

#### See Also

GRanges-class, GRangesList-class

#### HTClist-class

#### Examples

data(Nora\_5C)

## HTCexp descriptio
show(E14)
detail(E14)

## Is binned data ?
isBinned(E14\$chrXchrX)

## Is a inter or intrachromsomal experiment ?
isIntraChrom(E14\$chrXchrX)

## Divide by expected interaction counts
E14norm<-normPerExpected(E14\$chrXchrX)</pre>

```
## Operation on HTCexp object
E14_d_MEF<-divide(normPerReads(E14$chrXchrX), normPerReads(MEF$chrXchrX))
E14_s_MEF<-substract(normPerReads(E14$chrXchrX), normPerReads(MEF$chrXchrX))</pre>
```

## Overlap with genomic annotation
require(rtracklayer)
gene <- import(file.path(system.file("extdata", package="HiTC"),"refseq\_mm9\_chrX\_98831149\_103425150.bed"), forma
plot(E14\$chrXchrX, tracks=list(RefSeqGene=gene))</pre>

```
## Not run:
## normPerTrans data normalization applied on \href{http://genome.ucsc.edu/cgi-bin/hgFileUi?db=hg19&g=wgEncodeUm
ENCODE=import.my5C("./ENM-GM12878-R1.matrix")
```

## Look at raw interaction map
mapC(ENCODE\$chr7chr7)

## look at normalize by trans interaction map mapC(normPerTrans(ENCODE\$chr7chr7, xtrans=ENCODE\$chr7chr5, ytrans=ENCODE\$chr5chr7))

## End(Not run)

## Not run: ## Export exportC(E14\$chrXchrX, con="E14.csv")

## End(Not run)

HTClist-class Class 'HTClist'

#### Description

A class for representing a list of high throughput Chromosome Conformation Capture data from next-generation sequencing experiments.

#### Constructor

The HTClist represents a list of HTCexp objects and can be created as follow :

HTClist(...) : Creates a HTClist object using HTCexp objects supplied in '...'

## Methods

isBinned(x) applies 'isBinned' to each element in 'x'
isIntraChrom(x) applies 'isIntraChrom' to each element in 'x'
ranges(x) applies 'range' to each element in 'x'
range(x) return the reduce range of all elements in 'x'
seqlevels(x) return the sequence levels of all elements in 'x'
as.list(x) coercion to simple list object
names(x) get the names of the elements
x[i] Get elements i from x. Can be the positional index or its name.

#### Author(s)

Nicolas Servant

## See Also

GRangesList-class, HTCexp-class

## Examples

## TODO on Hi-C data

import.my5C Import data from my5C webtool

#### Description

Import data from my5C webtool

#### Usage

import.my5C(my5C.datafile, xgi.bed, ygi.bed, all.pairwise=TRUE)

## import.my5C

#### Arguments

my5C.datafile	input file from the my5C webtool
xgi.bed	BED file describing the 'x' Intervals (i.e. column names) of the interaction map. Required for the my5C list format
ygi.bed	BED file describing the 'y' intervals (i.e. row names) of the interaction map. Required for the my5C list format
all.pairwise	logical; generate all pairwise chromosomal interaction maps, i.e chr1-chr2, chr2-chr1

#### Details

This function allows data import from the the my5C webtool. Two input formats can be used : - The list format is composed of three files; two BED files describing the genomic intervals (i.e. primers); and a tabbed delimited format to specify the interaction between each genomic regions, with : FORWARD\_PRIMER\_NAME/REVERSE\_PRIMER\_NAME/INTERACTION\_COUNT

- The matrix format is a tab-delimited format, corresponding to the interaction map. The rownames and columnames are splitted to created the genome intervals (example : REV\_2lmm9lchrX:98831149-98834145).

The BED format is a standard format provided by the the UCSC Genome Browser. The all.pairwise option is not necessary in case of symetric design. Otherwise, it will return all the pairwise interaction maps.

#### Value

A HTClist object(s)

#### Author(s)

N. Servant

#### See Also

import, codeHTClist-class,

#### Examples

```
exDir <- system.file("extdata", package="HiTC")
## Load my5C matrix format
hiC<-import.my5C(file.path(exDir,"HIC_gm06690_chr14_chr14_1000000_obs.txt"))
detail(hiC)</pre>
```

importC

## Description

Import 5C or Hi-C data from csv file

## Usage

importC(con, xgi.bed = NULL, ygi.bed = NULL, all.pairwise=TRUE)

## Arguments

con	input csv file. See details
xgi.bed	BED file describing the 'x' Intervals (i.e. column names) of the interaction map. Required for the my5C list format
ygi.bed	BED file describing the 'y' intervals (i.e. row names) of the interaction map. Required for the my5C list format
all.pairwise	logical; generate all pairwise chromosomal interaction maps, i.e chr1-chr2, chr2-chr1

#### Details

This function import high-throughput data from a csv file. The expected format is the following : chrA,startA,endA,nameA,strandA,chrB,startB,endB,nameB,strandB,countAB

### Value

A list of HTCexp object(s)

#### Author(s)

N. Servant

#### See Also

exportC,import.my5C, HTCexp-class

## Examples

## Not run: data(Nora\_5C)

## Data binning E14.bin<-binningC(E14\$chrXchrX)</pre>

## Export the new intervals definition
exportC(E14.bin, file="E14")

## intervalsDist

##Import
importC("E14.csv")
## End(Not run)

intervalsDist intervalsDist

## Description

Compute the distance of intrachromosomal interactions of a 'C' experiment

## Usage

intervalsDist(x)

#### Arguments

#### х

object that inherits from class HTCexp

#### Details

If A and B are the two sets of intervals and s and e, the start and end of an interval, the distance is calculated as :

$$\min(|A_e - B_s|, |A_s - B_e|)$$

Only intrachromsomal interaction maps can be use for this operation.

## Value

A matrix of distances between genomic intervals

#### Author(s)

N. Servant

#### See Also

HTCexp-class

## Examples

data(Nora\_5C)

## Calculate distances between primers/intervals
intervalsDist(E14\$chrXchrX)

### Description

Visualize 'C' interaction map

#### Details

This function implements the plot method for objects of class HTCexp and HTClist.

By default, the maxrange and minrange values are fixed as the 98th percentile (resp. 2th percentile) of the interaction matrix. These values are useful to play with the contrast and remove the extreme values from the matrix.

The HTCexp and HTClist are not represented in the same way. The heatmap view is used to display the HTClist objects in two dimension. This view is mainly useful to have an overview of the data, as Hi-C data. The triangle view is used for HTCexp only and represent the top-right part the interaction matrix. If two HTCexp objects are specified, they will be displayed in order to compare both interaction maps. The two maps have to be binned to ensure comparison between genomic ranges.

Annotation tracks can be added to both views. In case of binned data, the exact genomic positions of each features are takken into account. Otherwise, the 'C' intervals which overlap with the annotation features are colored.

#### Value

Returns NULL; this function is called for the side-effect of creating the plot.

#### For HTCexp and HTClist objects

x object that inherits from class HTCexp or HTClist

tracks List of GRanges objects of data to display as annotation track(s)

minrange the minimum range of values used to define the color palette

maxrange the maximum range of values used to define the color palette

trim.range define the maxrange and minrange values using the percentile of the interaction matrix.

show.na logical; show the NA values in gray

log.data logical; do you want to log the data before plotting the heatmap

- **col.pos** color for (low,mid,high) positive interaction counts. Must be a vectore of size 3. mid can be NA
- **col.neg** color for (low,mid,high) negative interaction counts. Must be a vectore of size 3. mid can be NA
- col.na color for NA values
- grid logical; add a grid on the heatmap
- title character; add a title to the heatmap

mapC

## mapC

value logical; display the interaction values on the matrix. Useful for small matrices

**mask.data** matrix to add to the heatmap as a mask. Must have the same dimension as the interaction matrix

#### For HTCexp objects only

y optional. object that inherits from class HTCexp.

#### For HTClist objects only

names logical; display the names of the intervals. Useful for small matrices

## Author(s)

N. Servant, B. Lajoie

## See Also

HTCexp-class, HTClist-class

#### Examples

data(Nora\_5C)

```
## Interaction map
## HTClist view
mapC(E14)
```

```
## HTCexp view
mapC(E14$chrXchrX)
```

```
## Play with contrast and color
mapC(E14$chrXchrX, maxrange=100, col.pos=c("black","red","yellow"))
```

```
## Add annotation and change view
require(rtracklayer)
exDir <- system.file("extdata", package="HiTC")
gene <- import(file.path(exDir,"refseq_mm9_chrX_98831149_103425150.bed"), format="bed", asRangedData=FALSE)
mapC(E14$chrXchrX, tracks=list(Refseq=gene))</pre>
```

```
## Compare two samples
mapC(binningC(E14$chrXchrX), binningC(MEF$chrXchrX), tracks=list(Refseq=gene))
```

Nora\_5C

#### Description

5C data described by Nora et al. (2012)

#### Usage

data(Nora\_5C)

#### Format

Contains two HTClist objects (E14 and MEF). Each of them containing the ChrX intrachromosomal maps as a HTCexp object.

#### Details

This 5C dataset published by Nora et al (GSE35721), contains two different samples, a male undifferentiated ES cells (E14, GSM873935) and a mouse embryonic fibroblasts (MEF, GSM873924). This dataset is mainly used to describe the available functionalities of the HiTC package. The data provided with the package are count data.

## Source

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35721

#### References

Nora EP, Lajoie BR, Schulz EG, Giorgetti L et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 2012 Apr 11;485(7398):381-5. PMID: 22495304

## Examples

```
data(Nora_5C)
show(E14)
show(MEF)
```

pca.hic

## Description

Perform Principle Component Analysis on Hi-C interaction map

#### Usage

```
pca.hic(x, normPerExpected=TRUE, npc=2, asGRangesList=TRUE)
```

## Arguments

Х	object that inherits from class HTCexp	
normPerExpected		
	normalized by expected interaction using the loess calculation of distance de- pendency. see normPerExpected	
npc	numeric; number of first principal component to return	
asGRangesList	if TRUE a GRangesList object is returned where the scores represent the eigenvector	

#### Details

This method was apply by Lieberman-Aiden et al. 2009 to correlate the annotation profiles (genes, ChIP-seq, etc.) with the topological domains observed in Hi-C (see Fig3G of Lieberman-Aiden et al. 2009)

#### Value

A list with the eigen vector(s) of the npc first principal component(s), and their importance

#### Author(s)

N. Servant, B. Lajoie, R. McCord

## See Also

normPerExpected

## Examples

```
## Get Lieberman-Aiden Hi-C data
exDir <- system.file("extdata", package="HiTC")
l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
import.my5C)
hiC <- HTClist(l)</pre>
```

## Performed PCA

pr<-pca.hic(hiC\$chr14chr14, npc=1, asGRangesList=TRUE)</pre>

removeIntervals *Remove intervals from HTCexp object* 

## Description

Remove primers intervals from HTCexp object

## Usage

```
removeIntervals(x, ids)
```

## Arguments

х	object that inherits from class HTCexp
ids	character; vector of primers Ids to remove from the object

## Value

A HTCexp object without the discarded intervals

#### Author(s)

N. Servant

## See Also

GRanges-class

## Examples

data(Nora\_5C)

## Remove intervals from a HTCexp object
removeIntervals(E14\$chrXchrX, ids=c("5C\_938\_XIC-3\_REV\_2", "5C\_938\_XIC-3\_REV\_4"))

setIntervalScale Set x and y interval of the HTCexp object

## Description

Set x and y interval of the HTCexp object and update the interaction map accordingly

## Usage

```
setIntervalScale(x, xgi, ygi, upa=TRUE, method="mean", use.zero=TRUE)
```

#### Arguments

x	object that inherits from class HTCexp
ygi	y intervals; see class GRanges for details
xgi	x intervals; see class GRanges for details
ира	logical; unique primer assignment. Allow one primer to belong to one or several bins
method	the method used to combine the counts. Must be 'mean', 'median' or 'sum'
use.zero	logical; use the zero values in the method calculation

## Details

Define new interaction map based on the specified xgi and ygi intervals.

This function has to be used carefully and can has important impact on the interaction map. It is important to note that the setIntervalScale function is different from the binningC function in the way that the output is not symetrical.

#### Value

A HTCexp object

#### Author(s)

N. Servant

## See Also

HTCexp-class

## Examples

data(Nora\_5C)

E14.bin<-binningC(E14\$chrXchrX)</pre>

## I have two HTCexp samples defined with different intervals. show(E14.bin) show(MEF\$chrXchrX)

## How to compare them ?
## One idea is to force the intervals definition of one object using the
## intervals of the other.

setIntervalScale(MEF\$chrXchrX, xgi=x\_intervals(E14.bin), ygi=y\_intervals(E14.bin))

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