Package 'HiTC'

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Type Package

Title High Throughput Chromosome Conformation Capture analysis

Description The HiTC package was developed to explore high-throughput 'C' data such as 5C or Hi-C. Dedicated R classes as well as standard methods for quality controls, normalization, visualization, and further analysis are also provided.

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`binningC` *Windowing of high-throughput 'C' contact matrix*

Description

Binning of 'C' contact map

Usage

```
binningC(x, binsize=100000, bin.adjust=TRUE, upa=TRUE,
method=c("sum", "median","mean"), use.zero=TRUE, step=1, optimize.by = c("speed", "memory"))
```

Arguments

`x`	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.e.* window step
`optimize.by`	"speed" will use faster methods but more RAM, and "memory" will be slower, but require less RAM

Details

bin.adjust allows to work with bins of exactly the same size. Otherwise, the last bin is usually smaller than the others.
This function aims at changing the resolution of both 5C or Hi-C data. In case of 5C data (i.e. raw, not binned data), the contacts between all pairs of primers will be summarized per genomic bins (the median of all pairwise primers is usually used). In case of binned data (as Hi-C maps), the function can generate smaller resolution maps by aggregating bins. For instance, going from a 40kb resolution to a 1Mb resolution.
The method is used to combine the counts in a bin, must be 'mean', '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.*

Value

An HTCexp-class object with binned intraction data. In this case, the genomic intervals are converted into bins of fixed size. The contact 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)
## Move to a lower resolution map
E14.bin2 <- binningC(E14.bin, binsize=500000, step=1)
show(E14.bin2)
```

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, winsize=NA, dev.new=TRUE)
```

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]
winsize	used for the scat.interac.dist. If specify, the data are windowed before plotting
dev.new	if true, each graphs is plotted in a new 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)
```

---

directionalityIndex *Directionality index calculation*

Description

Calculate the directionality index as proposed by Dixon et al. 2012

Usage

```
directionalityIndex(x, winup = 2e+06, windown = 2e+06)
```

Arguments

x	HTClist object
winup	size of upstream window
windown	size of downstrean window

Details

Calculate the directionality index as proposed by Dixon et al. This index is then used to call topological domains in Hi-C/5C data.

Value

A numeric vector

Author(s)

N. Servant

See Also

HTClist-class

Examples

```
require(HiCDataHumanIMR90)
data(Dixon2012_IMR90)
hox <- extractRegion(hic_imr90_40$chr6chr6, chr="chr6", from=50e6, to=58e6)
di<-directionalityIndex(hox)
```

---

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

x	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

See Also

quantile

Examples

```
## Not run:
data(Nora_5C)

## Data binning
E14bin<-binningC(E14$chrXchrX)

## Discretize matrix
dismat<-discretize(intdata(E14bin))
mapC(dismat)

## 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, genome="mm9", per.chromosome=FALSE)
```

Arguments

x	object that inherits from class HTCexp
file	character; the prefix of the output file
genome	The genome version. This information is only used for the 'mat' export format. See details
per.chromosome	logical; export each contact maps in a different file (i.e one per chromosome pair)

Details

A tab-delimited matrix file is generated with the row and colnames defined as follow as in the my5C web tool :
REV_2|mm9|chrX:98831149-98834145

Author(s)

N. Servant

See Also

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")

## End(Not run)
```

---

exportC *Export* HTCexp *object*

Description

Export HTCexp object to tab format

Usage

```
exportC(x, file, per.chromosome=FALSE, use.names=FALSE, header=FALSE)
```

Arguments

x	object that inherits from class HTCexp
file	character; the basename of the output file
per.chromosome	logical; export each contact maps in a different files (i.e one per chromosome pair)
use.names	if TRUE, keep the original row/colnames of the contact matrix
header	if TRUE, add an header with the package version and the date

Value

Three output files will be created ; 2 BED files for each genomic intervals, and one tab file. The standard format for 5C/Hi-C data is the following :
** One list file (tab delimited) bin1 bin2 x12 bin1 bin3 x13 ... ** The BED file(s) describing the intervals ('xgi.bed' and 'ygi.bed' are usually the same for Hi-C but can be different for 5C data) chr1 1 1000000 bin1 chr1 1000001 2000000 bin2 ... Note that this format is particularly interesting for sparse data as only non null values are stored. If per.chromosome=FALSE, the data will be exported in one genome scaled file.

Author(s)

N. Servant

See Also

export.my5C, importC

Examples

```
## Not run:
data(Nora_5C)

## Data binning
E14.bin<-binningC(E14$chrXchrX)

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

## End(Not run)
```

---

extractRegion *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)
```

`getAnnotatedRestrictionSites`

Annotation of restriction sites

Description

Performs the annotation of all restriction sites of a given genome (i.e. GC content, mappability, effective fragment length)

Usage

```
getAnnotatedRestrictionSites(resSite="AAGCTT", overhangs5=1,
chromosomes=NULL, genomePack="BSgenome.Mmusculus.UCSC.mm9", mappability=NULL, wingc=200, winmap=5
```

Arguments

`resSite`	the sequence of the restriction site to look for. Default is HindIII restriction site
`overhangs5`	5' overhangs on the DNA resulted from the cutting
`chromosomes`	vector of chromosome number to focus on. Default all the chromosomes for the specified genome.
`genomePack`	name of the genome package to access the DNA sequence
`wingc`	size of the window upstream and downstream the restriction site used to calculate the GC content
`mappability`	a `GRanges` with a 'score' describing the mappability of the genome
`winmap`	size of the window upstream and downstream the restriction site used to calculate the mappability

Details

This function automatically annotate all the restriction sites of a given chromosome. The mappability is optional but strongly advice for Hi-C contact map normalization. This information can be easily download from public ressources like ftp://hgdownload.cse.ucsc.edu/gbdb/mm9/bbi/.

Value

Returns a `GRanges` object annotation data upstream (U) and downstream (D) the restriction sites.

Author(s)

N. Servant

See Also

normLGF, setGenomicFeatures

Examples

```
## Not run:
## Mappability data From http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeMapability/
map_hg18<- import("wgEncodeCrgMapabilityAlign100mer.bw",format="BigWig")

## 1- Example of restriction sites annnotation
cutSites <- getAnnotatedRestrictionSites(resSite="AAGCTT", overhangs5=1, chromosomes="chr1", genomePack="BSg

## End(Not run)
```

---

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, method=c("mean","loess"), asList=FALSE, ...)
```

Arguments

x	object that inherits from class HTCexp
method	method used to estimate the expected counts based on the genomic distance. See details
asList	return the results as a list. Otherwise, return an object of class HTCexp
...	arguments for mean or loess method, see below

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 two different methods, mean or loess.

The first method (default) is simply based on the mean counts of each diagonal. If logbin is false, the expected counts will be estimated for each bin of the contact maps. If logbin is true, the binsize will change according to the distance to the diagonal. Short (resp. long) distance will be estimated with smaller (larger) bins. This method works for all resolutions.

The second method is based on a Lowess regression model and works usually fine with low resolution data (250Kb to 1Mb). At higher resolution, the lowess regression might be difficult to fit. 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

For 'mean' method

logbin if true logarithm based bins are used. In practice, it means that the bin size will change as we move away from the diagonal

step multiplicative factor between each bin. Use if logbin is true

filter.low fraction of low counts to filter before normalizing. default: 0.05.

For 'loess' method

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

Author(s)

N. Servant, B. Lajoie

See Also

HTCexp-class,normPerExpected, normPerExpected, lowess

Examples

```
data(Nora_5C)

## Estimate expected interaction from distance between intervals
E14.exp<-getExpectedCounts(E14$chrXchrX, method="loess", stdev=TRUE, plot=FALSE)
mapC(E14.exp)
```

---

getPearsonMap *Pearson correlation map*

Description

Generate pearson correlation map, usually used to call chromosomal compartments

Usage

```
getPearsonMap(x, normPerExpected=TRUE, center=TRUE, ...)
```

Arguments

x	object that inherits from class HTCexp
normPerExpected	normalized by expected interaction using the loess calculation of distance dependency. see normPerExpected
center	default=true. center the observed/expected map before calculating the Pearson correlation
...	additional parameters passed to normPerExpected

Details

The function returns an HTCexp object with Pearson correlation map. This is usually the first step of the Principal Component Analysis (see pca.hic). Centering the rows of the observed/expected matrix allows to avoid bias to due to ranges of interaction counts. If true, the correlation of small values should be as valuable as correlation of large values

Value

A HTCexp object

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)

## get Pearson correlation map
pm <- getPearsonMap(hiC$chr14chr14)
mapC(HTClist(pm), maxrange=1, col.pos=c("black","red"), col.neg=c("black","blue"))
```

---

getRestrictionFragmentsPerChromosome

Get a list of DNA restriction fragments

Description

Performs the detection of restriction sites on a given genome and convert this information as a list of restriction fragments.

Usage

```
getRestrictionFragmentsPerChromosome(resSite="AAGCTT", overhangs5=1,
chromosomes=NULL, genomePack="BSgenome.Mmusculus.UCSC.mm9")
```

Arguments

resSite	the sequence of the restriction site to look for. Default is HindIII restriction site
overhangs5	5' overhangs on the DNA resulted from the cutting
chromosomes	vector of chromosome number to focus on. Default all the chromosomes for the specified genome.
genomePack	name of the genome package to access the DNA sequence

Value

Returns a GRanges object with all restriction fragments for a given genome/chromosome.

Author(s)

N. Servant

See Also

normLGF, setGenomicFeatures, getAnnotatedRestrictionSites

Examples

```
## Not run:
## Mappability data From http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeMapability/
map_hg18<- import("wgEncodeCrgMapabilityAlign100mer.bw",format="BigWig")

## 1- Get the list of restriction fragments for Human hg18 after HindIII digestion
resFrag <- getRestrictionFragmentsPerChromosome(resSite="AAGCTT", overhangs5=1, chromosomes="chrX", genomePa

## End(Not run)
```

HiTC-internal *Internal HiTC functions*

Description

Called internally by other HiTC functions. In theory, not called by the user.

Author(s)

Nicolas Servant

See Also

HTCexp-class, HTClist-class

HTCexp-class *Class 'HTCexp'*

Description

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

Details

The `normPerExpected` method estimates the expected interactions based on a the dependency on the genomic proximity between two loci. See `getExpectedCounts` function for details.

The `normPerTrans` method is based on the assumption that all trans contacts should be the same. Thus, the cis contacts 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 contact Matrix with x and y intervals definition. The forceSymmetric option can used to force intra-chromosomal contact maps to be stored as symmetrical matrix.

Slots

`intdata`**:** Dense or Sparse 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

c(x, ...) Combines 'x' and the `signature("HTCexp")` objects in '...' together. The results is an object of class `signature("HTCList")`

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 contact 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. Note that triangular matrices are always returned as symmetric matrices.

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

forceSymmetric(x) force the interaction data to 'symmetricMatrix'

forceTriangular(x) force the interaction data to triangular, ie. symmetric. Lower triangle of the matrix is set to zero, therefore reducing the size of the data in memory

isIntraChrom(x) return TRUE if the current `signature("HTCexp")` object contains intrachromosomal contact data

isSymmetric(x) return TRUE if the contact map is symmetrical, i.e inherits the `symmetricMatrix` class

normPerReads(x) normalize the contact matrix by the total number of reads of the matrix.

normPerExpected(x, ...) normalize the contact 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 contact map based on the trans interactions. See details

plot(x) visualization method; Display an heatmap of the contact 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 contact 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

summary(x) return descriptive summary statistics about the contact map

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`,`Matrix-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)

## Bin the data
E14.bin <- binningC(E14$chrXchrX, binsize=100000, step=3)

## Divide by expected interaction counts
E14norm<-normPerExpected(E14.bin)

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

## Overlap with genomic annotation
require(rtracklayer)
gene <- import(file.path(system.file("extdata", package="HiTC"),"refseq_mm9_chrX_98831149_103425150.bed"), f
plot(E14$chrXchrX, tracks=list(RefSeqGene=gene))

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

## Look at raw contact map
mapC(ENCODE$chr7chr7)

## look at normalize by trans contact 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.

Details

A `signature("HTClist")` is composed of a list of contact maps, representing the chromosomal interactions between pair of chromosomes.
The expected number of maps for a complete `signature("HTClist")` object should be equal to 'lchrs+(lchrs*(lchrs-1)/2)'. In this case, the chr1-chr2 map is stored once, but the dataset is complete.
If a `signature("HTClist")` object is composed of all pairwise interaction maps, it means that the chr1-chr2 AND the chr2-chr1 maps will be stored. This way of storing the data is less memory efficient but can ease the use of some genome-wide algorithm.
Note that the `getCombinedContact` method should be used carefully. This method merges all

single contact maps in one genome-wide map, therefore creating a very big matrix requiring memory space. However, this method is useful for many genome-wide analysis. The `normPerExpected` method applied to HTClist object is only available with the 'mean' method (see `getExpectedCounts` for details). In this case, the mean counts per distance are calculated over all intra-chromosomal maps.

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

c(x, ...) Combines a `signature("HTClist")` object 'x' with `signature("HTClist")` or `signature("HTCexp")` objects in '...'. The results is an object of class `signature("HTCList")`

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

forcePairwise(x) return a `signature("HTClist")` with all the pairwise contact maps

forceSymmetric(x) return a `signature("HTClist")` with half of the pairwise contact maps

isComplete(x) Logical; true if 'x' contains all intra and interchromosomal maps

isPairwise(x) Logical; true if 'x' contains all interchromosomal pairs, i.e. chr1chr2 and chr2chr1

isBinned(x) applies 'isBinned' to each element in 'x'

isIntraChrom(x) applies 'isIntraChrom' to each element in 'x'

getCombinedContacts(x) merge all contact maps in a single big matrix

getCombinedIntervals(x, merge=FALSE) merge all x and y intervals in single GRangesList object, or in a single GRanges object if merge=TRUE

normPerExpected(x) normalized by genomic distance all intra-chromosomal maps. See details

ranges(x) applies 'range' to each element in 'x'

range(x) return the reduce range of all elements in 'x'

reduce(x, chr, cis=TRUE, trans=TRUE, extend=FALSE) reduce a HTClist object to the list of provided chromosomes. Intra/Interchromosomal maps are returned accoring to the cis and trans args. If extend = True, all maps involving one of the chromosomes are returned

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

show(x) summarized output of the experiment, with informations about the data dimension

summary(x) return descriptive summary statistics for each interaction map

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

```
exDir <- system.file("extdata", package="HiTC")
l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
            import.my5C)
hiC <- HTClist(l)
names(hiC)

## Methods
ranges(hiC)
range(hiC)
isComplete(hiC)
isPairwise(hiC)
isBinned(hiC)
isIntraChrom(hiC)
seqlevels(hiC)
```

`import.my5C` *Import data from my5C webtool*

Description

Import data from my5C webtool

Usage

```
import.my5C(file, allPairwise=FALSE, rm.trans=FALSE, lazyload=FALSE)
```

Arguments

`file`	input file from the my5C webtool
`allPairwise`	logical; generate all pairwise chromosomal contact maps, i.e chr1-chr2, chr2-chr1
`rm.trans`	if true, only intra-chromosomal maps are loaded
`lazyload`	logical; force the intra-chromosomal contact maps to be stored as triangular matrix

Details

This function allows data import from the the my5C webtool.
The matrix format is a tab-delimited format, corresponding to the contact map. The rownames and columnames are splitted to created the genome intervals (example : REV_2|mm9|chrX:98831149-98834145).

The `allPairwise` option is not necessary in case of symetric design. Otherwise, it will return all the pairwise contact maps.

The matrix will be stored as a matrix inheriting from `Matrix` class. If forcesymmetrical=TRUE, the intrachromosomal matrix as coerced to `symmetricMatrix` class allowing a much more efficient memory usage.

Value

A `HTClist` object

Author(s)

N. Servant

See Also

HTClist-class,HTCexp-class,importC, Matrix-class, symmetricMatrix-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)
```

---

importC *Import high-htroughput 'C' data*

Description

Import 5C or Hi-C data from list file

Usage

```
importC(con, xgi.bed, ygi.bed=NULL, allPairwise=FALSE,
rm.trans=FALSE, lazyload=FALSE)
```

Arguments

con	input csv file. See details
xgi.bed	BED file describing the 'x' Intervals (i.e. column names) of the contact map
ygi.bed	BED file describing the 'y' intervals (i.e. row names) of the contact map
allPairwise	logical; generate all pairwise chromosomal contact maps, i.e chr1-chr2, chr2-chr1
rm.trans	if true, returns only intra-chromosomal maps
lazyload	logical; see details

Details

This function import high-throughput data from a tab list file.
The standard format for 5C/Hi-C data is the following :
** One list file (tab delimited) bin1 bin2 x12 bin1 bin3 x13 ... ** The BED file(s) describing the intervals ('xgi.bed' and 'ygi.bed' are usually the same for Hi-C but can be different for 5C data) chr1 1 1000000 bin1 chr1 1000001 2000000 bin2 ...

Note that this format is particularly interesting for sparse data as only non null values are stored.

The lazyload option allow to reduce the memory size of imported object. Therefore, only half of inter-chromosomal maps are stored. And intra-chromosomal maps are stored as sparse triangular matrix. Note that even if the contact maps are stored as triangular matrix, the indata method always returns a symmetrical map.

Value

A HTClist object

Author(s)

N. Servant

See Also

exportC,import.my5C, HTCexp-class

Examples

```
## Not run:
data(Nora_5C)

## Data binning
E14.bin<-binningC(E14$chrXchrX)

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

##Import
importC(con="E14.count", xgi="E14_xgi.bed", ygi="E14_ygi.bed")

## End(Not run)
```

---

intervalsDist *intervalsDist*

Description

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

Usage

```
intervalsDist(x, use.zero=TRUE)
```

Arguments

x	object that inherits from class HTCexp
use.zero	if FALSE, the distance for non interacting regions (zero counts) are not reported

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 contact 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
d<-intervalsDist(E14$chrXchrX)
```

---

mapC *Visualize 'C' ontact map*

Description

Visualize 'C' contact map

Details

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

By default, the trim.range value is fixed so that the 98th percentile (resp. 2th percentile) of each interaction matrix is discarded. It therefore allow to remove the extreme values from the matrix, but each map is plotted independently. If the maxrange argument is set, data higher that this threshold will be fixed to the maxrange value for all maps. In addition, color ranges are ajusted in a way that all maps are plotted within the same color range allowing visual maps comparison.

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 contact 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.zero logical; plot the zero values

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 contact counts. Must be a vectore of size 3. mid can be NA

col.neg color for (low,mid,high) negative contact 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 HTCexp plot(s)

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

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)

## Contact 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")
mapC(E14$chrXchrX, tracks=list(Refseq=gene))

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

Nora_5C *HiTC - 5C data*

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)
```

normICE *Iterative Correction of Hi-C data (ICE)*

Description

Iterative correction leverages the unique pairwise genome-wide structure of Hi-C data to decompose the data into a set of biases and a map of relative contact probabilities between any two genomic loci, achieving equal visibility across all genomic regions.

Usage

```
normICE(x, max_iter=50, eps=1e-4, sparse.filter=0.02)
```

Arguments

x	object that inherits from class HTCexp
max_iter	maximum number of iteration
eps	the relative increment in the results before declaring convergence
sparse.filter	Define which pourcentage of bins with high sparsity should be force to zero

Details

The normalization of Hi-C data is based on matrix balancing algorithm which consists of iteratively estimating the matrix bias using the l1 norm. The method implemented here is the Sinkhorn-Knopp algorithm as used in the Imakaev et al. paper. Note that the original method is applied on the genome-wide Hi-C map, but that the method could be applied on intra-chromosomal maps at high resolution.

Value

Returns a HTCexp object with a corrected contact map.

Author(s)

N. Servant, N. Varoqaux

References

Imakaev M, Fudenberg G, McCord RP, Naumova N, Goloborodko A, Lajoie BR, Dekker J, Mirny LA. Iterative correction of Hi-C data reveals hallmarks of chromosome organization.Nat Methods. 2012 Oct;9(10):999-1003.

See Also

normLGF

Examples

```
## Not run:
##Lieberman data
exDir <- system.file("extdata", package="HiTC")
l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
            import.my5C)
hiC <- HTClist(l)
hiC <- hiC[isIntraChrom(hiC)]

## Run ICE
hiC_iced <- HTClist(lapply(hiC, normICE))

## End(Not run)
```

---

normLGF *Local Genomic Feature (LGF) normalization*

Description

Parametric model to remove systematic biases in the raw contact maps

Usage

```
normLGF(x, family=c("poisson", "nb"))
```

Arguments

x	object that inherits from class HTCexp
family	parametric model to fit (poisson or nb)

Details

This function implements the HiCNorm method proposed by Hu et al. Briefly, the method uses a generalized linear model to correct the systematic biases (effective fragment length, GC content, mappability) in a Hi-C contact map.

Value

Returns a HTCexp object with a normalized contact map.

Author(s)

N. Servant, M. Hu, S. Selvaraj

References

Hu M, Deng K, Selvaraj S, Qin Z, Ren B, Liu JS. HiCNorm: removing biases in Hi-C data via Poisson regression. Bioinformatics. 2012;28(23):3131-3.

See Also

getAnnotatedRestrictionSites, setGenomicFeatures

Examples

```
## Not run:
require(HiTC)
require(BSgenome.Hsapiens.UCSC.hg18)

##Lieberman data
exDir <- system.file("extdata", package="HiTC")
l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
            import.my5C)
hiC <- HTClist(l)
hiC <- hiC[isIntraChrom(hiC)]
names(hiC)

## Mappability data From http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeMapability/
map_hg18<- import("wgEncodeCrgMapabilityAlign100mer.bw", format="BigWig")

## Get the genomic feature of the chromosome 12
hiC_annot <- HTClist(lapply(hiC, setGenomicFeatures, resSite="AAGCTT", overhangs5=1, genomePack="BSgenome.Hsa
hiC_annot$chr12chr12

## Normalize the data
hiCnorm <- HTClist(lapply(hiC_annot, normLGF))


## End(Not run)
```

`pca.hic` *Perform Principle Component Analysis on Hi-C contact map*

Description

Perform Principle Component Analysis on Hi-C contact map

Usage

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

Arguments

`x`	object that inherits from class `HTCexp`
`normPerExpected`	normalized by expected interaction using the loess calculation of distance dependency. 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
`gene.gr`	object of class `GenomicRanges` describing the genes position. If used, the A/B compartments classes are defined based on gene density
`...`	additional parameters passed to `normPerExpected` function

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)

## Performed PCA
pr<-pca.hic(hiC$chr14chr14, npc=1, asGRangesList=TRUE)
```

---

removeIntervals *Remove intervals from HTCexp object*

Description

Remove primers intervals from HTCexp object

Usage

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

Arguments

x	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"))
```

---

setGenomicFeatures *Annotation of Hi-C contact map*

Description

Annotate a Hi-C contact map with the genomic local features (i.e. GC content, mappability, effective fragment length)

Usage

```
setGenomicFeatures(x, cutSites, minFragMap=.5, effFragLen=1000)
```

Arguments

x	HTCexp object to annotate
cutSites	GRangesList or GRanges object with restriction sites annotation obtained using the getAnnotatedRestrictionSites function
minFragMap	Minimum Fragment Mappbility. All fragments with a lower mappability are not used for the annotation.
effFragLen	Efective Fragment Length. Size of specific fragment ligation

Details

The function require the restriction sites annotation as provided by the getAnnotatedRestrictionSites function. The restriction sites are first filtered according to their mappability. This threshold has to be defined according to the data pre-processing. All remaining restriction sites are then intersected with the genomic bins of the contact map. All restriction sites included within a bin are averaged. The effective fragment length is defined as the size of specific ligation product. (See Yaffe and Tanay, 2011). In this paper, the authors define specific ligation as sum of distance to cutter sites (d1+d2) <= 500 bp. Such criterion implies that d1<=500 bp and d2 <= 500 bp. So for each fragment end, only reads mapped within 500 bp to cutter sites are used for downstream analysis. All defults paramters correspond to the ones used in the HiCNorm method.

Value

Returns a HTCexp object with local genomic features annotations.

Author(s)

N. Servant

See Also

normLGF, setGenomicFeatures

Examples

```
## Not run:
require(BSgenome.Hsapiens.UCSC.hg18)
require(rtracklayer)

##Lieberman data
exDir <- system.file("extdata", package="HiTC")
l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
            import.my5C)
hiC <- HTClist(l)
hiC <- hiC[isIntraChrom(hiC)]
names(hiC)

## Mappability data From http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeMapability/
map_hg18<- import("wgEncodeCrgMapabilityAlign100mer.bw", format="BigWig")

## Get the genomic feature of the HiC chr12 data
cutSites <- getAnnotatedRestrictionSites(resSite="AAGCTT", overhangs5=1, chromosomes=seqlevels(hiC), genomeP

chr12_annot <- setGenomicFeatures(hiC$chr12chr12, cutSites)

## End(Not run)
```

---

setIntervalScale *Set x and y interval of the HTCexp object*

Description

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

Usage

```
setIntervalScale(x, xgi, ygi, upa=TRUE, method=c("sum","median","mean"),
use.zero=TRUE, optimize.by = c("speed", "memory"))
```

Arguments

x	object that inherits from class HTCexp
ygi	y intervals; see class GRanges for details
xgi	x intervals; see class GRanges for details
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
optimize.by	"speed" will use faster methods but more RAM, and "memory" will be slower, but require less RAM

Details

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

This function has to be used carefully and can has important impact on the contact 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)

## 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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