

Package ‘diffHic’

October 12, 2016

Version 1.4.3

Date 2016-08-25

Title Differential Analysis of Hi-C Data

Author Aaron Lun <alun@wehi.edu.au>

Maintainer Aaron Lun <alun@wehi.edu.au>

Depends R (>= 3.3.0), GenomicRanges, InteractionSet,
SummarizedExperiment

Imports Rsamtools, Rhtslib, Biostrings, BSgenome, rhdf5, edgeR, limma,
csaw, locfit, methods, IRanges, S4Vectors, GenomeInfoDb,
BiocGenerics, grDevices, graphics, stats, utils

Suggests BSgenome.Ecoli.NCBI.20080805, Matrix

biocViews MultipleComparison, Preprocessing, Sequencing, Coverage,
Alignment, Normalization, Clustering, HiC

Description Detects differential interactions across biological
conditions in a Hi-C experiment. Methods are provided for read
alignment and data pre-processing into interaction counts.
Statistical analysis is based on edgeR and supports
normalization and filtering. Several visualization options are
also available.

License GPL-3

NeedsCompilation yes

LinkingTo Rhtslib, zlibbioc

R topics documented:

annotatePairs	2
boxPairs	4
clusterPairs	6
compartmentalize	8
connectCounts	10
consolidatePairs	12
correctedContact	14

cutGenome	17
diClusters	19
diffHicUsersGuide	20
DIList-class	21
DIList-wrappers	23
DNaseHiC	24
domainDirections	26
enrichedPairs	27
Filtering diagonals	30
Filtering methods	31
filterPeaks	34
getArea	35
getDistance	37
getPairData	38
loadData	39
marginCounts	40
mergePairs	42
neighborCounts	43
normalizeCNV	44
pairParam	47
plotDI	48
plotPlaid	51
preparePairs	53
prunePairs	56
savePairs	58
squareCounts	60
totalCounts	62
Index	64

annotatePairs	<i>Annotate bin pairs</i>
---------------	---------------------------

Description

Annotate bin pairs based on features overlapping the anchor regions.

Usage

```
annotatePairs(data.list, regions, rnames=names(regions), indices, ...)
```

Arguments

<code>data.list</code>	An InteractionSet or a list of InteractionSet objects containing bin pairs.
<code>regions</code>	A GRanges object containing coordinates for the regions of interest.
<code>rnames</code>	A character vector containing names to be used for annotation.

indices An integer vector or list of such vectors, indicating the cluster identity for each interaction in `data.list`.

... Additional arguments to pass to [findOverlaps](#).

Details

Entries in regions are identified with any overlap to anchor regions for interactions in `data.list`. The names for these entries are concatenated into a comma-separated string for easy reporting. Typically, gene symbols are used in names, but other values can be supplied depending on the type of annotation. This is done separately for the first and second anchor regions so that potential interactions between features of interest can be identified.

If `indices` is supplied, all interactions corresponding to each unique index are considered to be part of a single cluster. Overlaps with all interactions in the cluster are subsequently concatenated into a single string. Cluster indices should range from `[1, nclusters]` for any given number of clusters. This means that the annotation for a cluster corresponding to a certain index can be obtained by subsetting the output vectors with that index. Otherwise, if `indices` is not set, all interactions are assumed to be their own cluster, i.e., annotation is returned for each interaction separately.

Multiple `InteractionSet` objects can be supplied in `data.list`, e.g., if the cluster consists of bin pairs of different sizes. This means that `indices` should also be a list of vectors where each vector indicates the cluster identity of the entries in the corresponding `InteractionSet` of `data.list`.

Value

A list of two character vectors `anchor1` and `anchor2` is returned, containing comma-separated strings of names for entries in regions overlapped by the first and second anchor regions respectively. If `indices` is not specified, overlaps are identified to anchor regions of each interaction in `data.list`. Otherwise, overlaps are identified to anchor regions for any interaction in each cluster.

Author(s)

Aaron Lun

See Also

[findOverlaps](#), [clusterPairs](#)

Examples

```
# Setting up the objects.
a <- 10
b <- 20
cuts <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))
param <- pairParam(cuts)

all.combos <- combn(length(cuts), 2)
y <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,], regions=cuts, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=1))

regions <- GRanges(rep(c("chrA", "chrB"), c(3,2)), IRanges(c(1,5,8,3,3), c(1,5,8,3,4)))
```

```

names(regions) <- LETTERS[seq_along(regions)]
out <- annotatePairs(y, regions=regions)

# Again, with indices:
indices <- sample(20, length(y), replace=TRUE)
out <- annotatePairs(y, regions=regions, indices=indices)

# Again, with multiple InteractionSet objects:
out <- annotatePairs(list(y, y[1:10,]), regions=regions, indices=list(indices, indices[1:10]))

```

boxPairs

Put bin pairs into boxes

Description

Match smaller bin pairs to the larger bin pairs in which they are nested.

Usage

```
boxPairs(..., reference, minbox=FALSE, index.only=FALSE)
```

Arguments

...	One or more named InteractionSet objects produced by squareCounts , with smaller bin sizes than reference.
reference	An integer scalar specifying the reference bin size.
minbox	A logical scalar indicating whether coordinates for the minimum bounding box should be returned.
index.only	A logical scalar indicating whether only indices should be returned.

Details

Consider the bin size specified in `reference`. Pairs of these bins are referred to here as the parent bin pairs, and are described in the output `pairs` and `region`. The function accepts a number of InteractionSet objects of bin pair data in the ellipsis, referred to here as input bin pairs. The aim is to identify the parent bin pair in which each input bin pair is nested.

All input InteractionSet objects in the ellipsis must be constructed carefully. In particular, the value of width in [squareCounts](#) must be such that `reference` is an exact multiple of each width. This is necessary to ensure complete nesting. Otherwise, the behavior of the function will not be clearly defined.

In the output, one vector will be present in `indices` for each input InteractionSet in the ellipsis. In each vector, each entry represents an index for a single input bin pair in the corresponding InteractionSet. This index points to the entries in `interactions` that specify the coordinates of the parent bin pair. Thus, bin pairs with the same index are nested in the same parent.

Some users may wish to identify bin pairs in one InteractionSet that are nested within bin pairs in another InteractionSet. This can be done by supplying both InteractionSet objects in the ellipsis,

and leaving reference unspecified. The value of reference will be automatically selected as the largest width of the supplied InteractionSet objects. Nesting can be identified by [matching](#) the output indices for the smaller bin pairs to those of the larger bin pairs.

If minbox=TRUE, the coordinates in interactions represent the minimum bounding box for all nested bin pairs in each parent. This may be more precise if nesting only occurs in a portion of the interaction space of the parent bin pair.

If index.only=TRUE, only the indices are returned and coordinates are not computed. This is largely for efficiency purposes when boxPairs is called by internal functions.

Value

If index.only=FALSE, a named list is returned containing:

indices: a named list of integer vectors for every InteractionSet in the ellipsis, see [Details](#).

interactions: A ReverseStrictGInteractions object containing the coordinates of the parent bin pair or, if minbox=TRUE, the minimum bounding box.

If index.only=TRUE, the indices are returned directly without computing coordinates.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [clusterPairs](#)

Examples

```
# Setting up the objects.
a <- 10
b <- 20
cuts <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))
param <- pairParam(cuts)

all.combos <- combn(length(cuts), 2) # Bin size of 1.
y <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,], regions=cuts, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=1))

a5 <- a/5
b5 <- b/5
all.combos2 <- combn(length(cuts)/5, 2) # Bin size of 5.
y2 <- InteractionSet(matrix(0, ncol(all.combos2), 1),
  GInteractions(anchor1=all.combos2[2,], anchor2=all.combos2[1,],
    regions=GRanges(rep(c("chrA", "chrB"), c(a5, b5)),
    IRanges(c((1:a5-1)*5+1, (1:b5-1)*5+1), c(1:a5*5, 1:b5*5))), mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=5))

# Clustering.
boxPairs(reference=5, larger=y2, smaller=y)
```

```

boxPairs(reference=10, larger=y2, smaller=y)
boxPairs(reference=10, larger=y2, smaller=y, minbox=TRUE)
boxPairs(larger=y2, smaller=y)

```

clusterPairs	<i>Cluster bin pairs</i>
--------------	--------------------------

Description

Aggregate bin pairs into local clusters for summarization.

Usage

```
clusterPairs(..., tol, upper=1e6, index.only=FALSE)
```

Arguments

...	One or more InteractionSet objects, optionally named.
tol	A numeric scalar specifying the maximum distance between bin pairs in base pairs.
upper	A numeric scalar specifying the maximum size of each cluster in base pairs.
index.only	A logical scalar indicating whether only indices should be returned.

Details

Clustering is performed by putting a interaction in a cluster if the smallest Chebyshev distance to any interaction already inside the cluster is less than `tol`. This is a cross between single-linkage approaches and density-based methods, especially after filtering removes low-density regions. In this manner, adjacent events in the interaction space can be clustered together. Interactions that are assigned with the same cluster ID belong to the same cluster.

The input data objects can be taken from the output of [squareCounts](#) or [connectCounts](#). For the former, inputs can consist of interactions with multiple bin sizes. It would be prudent to filter the former based on the average abundances, to reduce the density of bin pairs in the interaction space. Otherwise, clusters may be too large to be easily interpreted.

Alternatively, to avoid excessively large clusters, this function can also split each cluster into roughly-equally sized subclusters. The maximum value of any dimension of the subclusters is approximately equal to `upper`. This aims to improve the spatial interpretability of the clustering result.

There is no guarantee that each cluster forms a regular shape in the interaction space. Instead, a minimum bounding box is reported containing all bin pairs in each cluster. The coordinates of the box for each cluster is stored in each row of the output interactions. The cluster ID in each indices vector represents the row index for these coordinates.

If `index.only=TRUE`, only the indices are returned and coordinates of the bounding box are not computed. This is largely for efficiency purposes when `clusterPairs` is called by internal functions.

Value

If `index.only=FALSE`, a named list is returned containing:

indices: A named list of integer vectors where each vector contains a cluster ID for each interaction in the corresponding input `InteractionSet` object.

interactions: A `ReverseStrictGInteractions` object containing the coordinates of the sides of the bounding box for each cluster.

If `index.only=TRUE`, the indices are returned directly without computing coordinates.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [diClusters](#), [boxPairs](#)

Examples

```
# Setting up the object.
a <- 10
b <- 20
regions <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))

set.seed(3423)
all.anchor1 <- sample(length(regions), 50, replace=TRUE)
all.anchor2 <- as.integer(runif(50, 1, all.anchor1+1))
y <- InteractionSet(matrix(0, 50, 1),
  GInteractions(anchor1=all.anchor1, anchor2=all.anchor2, regions=regions, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(width=1))

# Clustering; note, small tolerances are used in this toy example.
clusterPairs(y, tol=1)
clusterPairs(y, tol=3)
clusterPairs(y, tol=5)
clusterPairs(y, tol=5, upper=5)

# Multiple bin sizes allowed.
a2 <- a/2
b2 <- b/2
rep.regions <- GRanges(rep(c("chrA", "chrB"), c(a2, b2)),
  IRanges(c(1:a2*2, 1:b2*2), c(1:a2*2, 1:b2*2)))
rep.anchor1 <- sample(length(rep.regions), 10, replace=TRUE)
rep.anchor2 <- as.integer(runif(10, 1, rep.anchor1+1))
y2 <- InteractionSet(matrix(0, 10, 1),
  GInteractions(anchor1=rep.anchor1, anchor2=rep.anchor2, regions=rep.regions, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(width=2))

clusterPairs(y, y2, tol=1)
clusterPairs(y, y2, tol=3)
clusterPairs(y, y2, tol=5)
```

```
clusterPairs(y, tol=5, upper=5)
```

compartmentalize *Identify genomic compartments*

Description

Use contact matrices to identify self-interacting genomic compartments

Usage

```
compartmentalize(data, centers=2, dist.correct=TRUE,
                 cov.correct=TRUE, robust.cov=5, ...)
```

Arguments

data	an InteractionSet object containing bin pair data, like that produced by squareCounts
centers	an integer scalar, specifying the number of clusters to form in kmeans
dist.correct	a logical scalar, indicating whether abundances should be corrected for distance biases
cov.correct	a logical scalar, indicating whether abundances should be corrected for coverage biases
robust.cov	a numeric scalar, specifying the multiple of MADs beyond which coverage outliers are removed
...	other arguments to pass to kmeans

Details

This function uses the interaction space to partition each linear chromosome into compartments. Bins in the same compartment interact more frequently with each other compared to bins in different compartments. This forms a checkerboard-like pattern in the interaction space that can be used to define the genomic intervals in each compartment. Typically, one compartment is gene-rich and is defined as “open”, while the other is gene-poor and defined as “closed”.

Compartment identification is done by setting up a ContactMatrix object, where each row/column represents a bin and each matrix entry contains the frequency of contacts between bins. Bins (i.e., rows) with similar interaction profiles (i.e., entries across columns) are clustered together with the k-means method. Those with the same ID in the output compartment vector are in the same compartment. Note that clustering is done separately for each chromosome, so bins with the same ID across different chromosomes cannot be interpreted as being in the same compartment.

If `dist.correct=TRUE`, frequencies are normalized to mitigate the effect of distance and to improve the visibility of long-range interactions. This is done by computing the residuals of the distance-dependent trend - see [filterTrended](#) for more details. If `cov.correct=TRUE`, frequencies are also normalized to eliminate coverage biases between bins. This is done by computing the average coverage of each row/column, and dividing each matrix entry by the square root averages of the relevant row and column.

Extremely low-coverage regions such as telomeres and centromeres can confound k-means clustering. To protect against this, all bins with (distance-corrected) coverages that are more than `robust.cov` MADs away from the median coverage of each chromosome are identified and removed. These bins will be marked with NA in the returned `compartment` for that chromosome. To turn off robustification, set `robust.cov` to NA.

By default, `centers` is set to 2 to model the open and closed compartments. While a larger value can be used to obtain more clusters, care is required as the interpretation of the resulting compartments becomes more difficult. If desired, users can also apply their own clustering methods on the `matrix` returned in the output.

Value

A named list of lists is returned where each internal list corresponds to a chromosome in data and contains `compartment`, an integer vector of compartment IDs for all bins in that chromosome; and `matrix`, a `ContactMatrix` object containing (normalized) contact frequencies for the intra-chromosomal space. Entries in `compartment` are named according to the matching index of `regions(data)`.

Author(s)

Aaron Lun

References

Lieberman-Aiden E et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326, 289-293.

Lajoie BR, Dekker J, Kaplan N (2014). The hitchhiker's guide to Hi-C analysis: practical guidelines. *Methods* 72, 65-75.

See Also

[squareCounts](#), [filterTrended](#), [kmeans](#)

Examples

```
# Dummying up some data.
set.seed(3426)
npts <- 100
npairs <- 5000
nlibs <- 4
anchor1 <- sample(npts, npairs, replace=TRUE)
anchor2 <- sample(npts, npairs, replace=TRUE)
data <- InteractionSet(
  matrix(rpois(npairs*nlibs, runif(npairs, 10, 100)), nrow=npairs),
  GInteractions(anchor1=anchor1, anchor2=anchor2,
    regions=GRanges(c(rep("chrA", 80), rep("chrB", 20)),
      IRanges(c(1:80, 1:20), c(1:80, 1:20))), mode="reverse"),
  colData=DataFrame(totals=runif(nlibs, 1e6, 2e6)), metadata=List(width=1))
data <- unique(data)
```

```
# Running compartmentalization.
out <- compartmentalize(data)
head(out$chrA$compartment)
dim(out$chrA$matrix)
head(out$chrB$compartment)
dim(out$chrB$matrix)

test <- compartmentalize(data, cov.correct=FALSE)
test <- compartmentalize(data, dist.correct=FALSE)
test <- compartmentalize(data, robust.cov=NA)
```

<code>connectCounts</code>	<i>Count connecting read pairs</i>
----------------------------	------------------------------------

Description

Count the number of read pairs connecting pairs of user-specified regions

Usage

```
connectCounts(files, param, regions, filter=1L, type="any", second.regions=NULL)
```

Arguments

<code>files</code>	a character vector containing the paths to the count file for each library
<code>param</code>	a <code>pairParam</code> object containing read extraction parameters
<code>regions</code>	a <code>GRanges</code> object specifying the regions between which read pairs should be counted
<code>filter</code>	an integer scalar specifying the minimum count for each interaction
<code>type</code>	a character string specifying how restriction fragments should be assigned to regions
<code>second.regions</code>	a <code>GRanges</code> object containing the second regions of interest, or an integer scalar specifying the bin size

Details

Interactions of interest are defined as those formed by pairs of elements in regions. The number of read pairs connecting each pair of elements can then be counted in each library. This can be useful for quantifying/summarizing interactions between genomic features, e.g., promoters or gene bodies.

For a pair of intervals in regions, the interaction count is defined as the number of read pairs with one read in each interval (after rounding each interval to a fragment; see below). To avoid reporting weak interactions, pairs can be filtered to retain only those with a count sum across all libraries above `filter`. In each pair, the anchor interval is defined as that with the higher start position. Note that the end position may not be higher, due to the possibility of nested intervals in regions.

The value of `type` feeds into [findOverlaps](#) and controls the manner in which restriction fragments are assigned to each region. By default, a restriction fragment is assigned to one or more regions if said fragment overlaps with any part of those regions. This means that the boundaries of each region are expanded outwards to obtain the effective coordinates in the output region. In contrast, setting `type="within"` would contract each region inwards.

The modified regions can be extracted from the `regions` slot in the output `InteractionSet` object. These will be reordered according to the new start positions. The ordering permutation can be recovered from the `original` metadata field of the `GRanges` object. Similarly, the number of restriction fragments assigned to each interval is stored in the `nfrags` metadata field.

Counting will consider the values of `restrict`, `discard` and `cap` in `param`. See [pairParam](#) for more details.

Value

An `InteractionSet` is returned, specifying the number of read pairs in each library that are mapped between pairs of regions, or between regions and `second.regions`. Interacting regions are returned as a `ReverseStrictGInteractions` object containing the concatenated regions and `second.regions`.

Matching to a second set of regions

The `second.regions` argument allows specification of a second set of regions, where interactions are only considered between one entry in `regions` and one entry in `second.regions`. This differs from supplying all regions to `regions`, which would consider all pairwise interactions between regions regardless of whether they belong in the first or second set. If an integer scalar is supplied as `second.regions`, this value is used as a width to partition the genome into bins. These bins are then used as the set of second regions.

Specification of `second.regions` is useful for efficiently identifying interactions between two sets of regions. For example, the first set can be set to several “viewpoint” regions of interest. This is similar to the bait region in 4C-seq, or the captured regions in Capture Hi-C. Interactions between these viewpoints and the rest of the genome can then be examined by setting `second.regions` to some appropriate bin size.

The output `InteractionSet` will merge all regions and `second.regions` into a single `GRanges` object. However, those in the second set can be distinguished with the `is.second` metadata field. Each `original` index will also point towards the corresponding entry in the original `second.regions` when `is.second=TRUE`. Similarly, if `is.second=FALSE`, the index will point towards the corresponding entry in the original regions.

Note that this function does *not* guarantee that the second set of regions will be treated as the second anchor region (or the first) for each interaction. Those definitions are dependent on the sorting order of the coordinates for all regions. Users should use the `is.second` field to identify the region from the second set in each interaction.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [findOverlaps](#), [InteractionSet-class](#), [ReverseStrictGInteractions-class](#)

Examples

```

hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

# Setting up the parameters
fout <- "output"
invisible(preparePairs(hic.file, param, fout))
regions <- suppressWarnings(c(
  GRanges("chrA", IRanges(c(1, 100, 150), c(20, 140, 160))),
  GRanges("chrB", IRanges(50, 100))))

# Collating to count combinations.
con <- connectCounts(fout, param, regions=regions, filter=1L)
head(assay(con))
con <- connectCounts(fout, param, regions=regions, filter=1L, type="within")
head(assay(con))

# Still works with restriction and other parameters.
con <- connectCounts(fout, param=reform(param, restrict="chrA"),
  regions=regions, filter=1L)
head(assay(con))
con <- connectCounts(fout, param=reform(param, discard=GRanges("chrA", IRanges(1, 50))),
  regions=regions, filter=1L)
head(assay(con))
con <- connectCounts(fout, param=reform(param, cap=1), regions=regions, filter=1L)
head(assay(con))

# Specifying a second region.
regions2 <- suppressWarnings(c(
  GRanges("chrA", IRanges(c(50, 100), c(100, 200))),
  GRanges("chrB", IRanges(1, 50))))

con <- connectCounts(fout, param, regions=regions, filter=1L, second.region=regions2)
head(anchors(con, type="first"))
head(anchors(con, type="second"))
con <- connectCounts(fout, param, regions=regions, filter=1L, second.region=50)
head(anchors(con, type="first"))
head(anchors(con, type="second"))

```

consolidatePairs

Consolidate results for interactions

Description

Consolidate differential testing results for interactions from separate analyses.

Usage

```
consolidatePairs(indices, result.list, equiweight=TRUE, combine.args=list())
```

Arguments

<code>indices</code>	a list of index vectors, specifying the cluster ID to which each interaction belongs
<code>result.list</code>	a list of data frames containing the DI test results for each interaction
<code>equiweight</code>	a logical scalar indicating whether equal weighting from each bin size should be enforced
<code>combine.args</code>	a list of parameters to pass to combineTests

Details

Interactions from different analyses can be aggregated together using [boxPairs](#) or [clusterPairs](#). For example, test results can be consolidated for bin pairs of differing sizes. This usually produces a `indices` vector that can be used as an input here. Briefly, each vector in `indices` should correspond to one analysis, and each entry of that vector should correspond to an analyzed interaction. The vector itself holds cluster IDs, such that interactions within/between analyses with the same ID belong in the same cluster.

For all bin pairs in a cluster, the associated p-values are combined in [combineTests](#) using a weighted version of Simes' method. This yields a single combined p-value, representing the evidence against the global null. When `equiweight=TRUE`, the weight of a p-value of each bin pair is inversely proportional to the number of bin pairs of the same size in that parent bin pair. This ensures that the results are not dominated by numerous smaller bin pairs.

Value

A data frame is returned containing the combined DB results for each cluster.

Author(s)

Aaron Lun

See Also

[combineTests](#), [boxPairs](#), [clusterPairs](#)

Examples

```
# Setting up the objects.
a <- 10
b <- 20
cuts <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))
param <- pairParam(cuts)

all.combos <- combn(length(cuts), 2) # Bin size of 1.
y <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,], regions=cuts, mode="reverse"),
```

```

colData=DataFrame(lib.size=1000), metadata=List(param=param, width=1))

a5 <- a/5
b5 <- b/5
all.combos2 <- combn(length(cuts)/5, 2) # Bin size of 5.
y2 <- InteractionSet(matrix(0, ncol(all.combos2), 1),
  GInteractions(anchor1=all.combos2[2,], anchor2=all.combos2[1,],
  regions=GRanges(rep(c("chrA", "chrB"), c(a5, b5)),
  IRanges(c((1:a5-1)*5+1, (1:b5-1)*5+1), c(1:a5*5, 1:b5*5))), mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=5))

result1 <- data.frame(logFC=rnorm(nrow(y)), PValue=runif(nrow(y)), logCPM=0)
result2 <- data.frame(logFC=rnorm(nrow(y2)), PValue=runif(nrow(y2)), logCPM=0)

# Consolidating.
boxed <- boxPairs(y, y2)
out <- consolidatePairs(boxed$indices, list(result1, result2))
head(out)
out <- consolidatePairs(boxed$indices, list(result1, result2), equiweight=FALSE)
head(out)

# Repeating with three sizes.
a10 <- a/10
b10 <- b/10
all.combos3 <- combn(length(cuts)/10, 2) # Bin size of 10.
y3 <- InteractionSet(matrix(0, ncol(all.combos3), 1),
  GInteractions(anchor1=all.combos3[2,], anchor2=all.combos3[1,],
  regions=GRanges(rep(c("chrA", "chrB"), c(a10, b10)),
  IRanges(c((1:a10-1)*10+1, (1:b10-1)*10+1), c(1:a10*10, 1:b10*10))),
  mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=10))
result3 <- data.frame(logFC=rnorm(nrow(y3)), PValue=runif(nrow(y3)), logCPM=0)

boxed <- boxPairs(y, y2, y3)
out <- consolidatePairs(boxed$indices, list(result1, result2, result3))
head(out)

```

correctedContact

Iterative correction of Hi-C counts

Description

Perform iterative correction on counts for Hi-C interactions to correct for biases between fragments.

Usage

```

correctedContact(data, iterations=50, exclude.local=1, ignore.low=0.02,
  winsor.high=0.02, average=TRUE, dist.correct=FALSE)

```

Arguments

<code>data</code>	an <code>InteractionSet</code> object produced by <code>squareCounts</code>
<code>iterations</code>	an integer scalar specifying the number of correction iterations
<code>exclude.local</code>	an integer scalar, indicating the distance off the diagonal under which bin pairs are excluded
<code>ignore.low</code>	a numeric scalar, indicating the proportion of low-abundance bins to ignore
<code>winsor.high</code>	a numeric scalar indicating the proportion of high-abundance bin pairs to winsorize
<code>average</code>	a logical scalar specifying whether counts should be averaged across libraries
<code>dist.correct</code>	a logical scalar indicating whether to correct for distance effects

Details

This function implements the iterative correction procedure described by Imakaev *et al.* in their 2012 paper. Briefly, this aims to factorize the count for each bin pair into the biases for each of the two anchor bins and the true interaction probability. The bias represents the ease of sequencing/mapping/other for the genome sequence in each bin.

The data argument should be generated by taking the output of `squareCounts` after setting `filter=1`. Filtering should be avoided as counts in low-abundance bin pairs may be informative upon summation for each bin. For example, a large count sum for a bin may be formed from many bin pairs with low counts. Removal of those bin pairs would result in loss of information.

For `average=TRUE`, if multiple libraries are used to generate data, an average count will be computed for each bin pairs across all libraries using `mg1mOneGroup`. The average count will then be used for correction. Otherwise, correction will be performed on the counts for each library separately.

The maximum step size in the output can be used as a measure of convergence. Ideally, the step size should approach 1 as iterations pass. This indicates that the correction procedure is converging to a single solution, as the maximum change to the computed biases is decreasing.

Value

A list with several components.

`truth`: a numeric vector containing the true interaction probabilities for each bin pair

`bias`: a numeric vector of biases for all bins

`max`: a numeric vector containing the maximum fold-change change in biases at each iteration

`trend`: a numeric vector specifying the fitted value for the distance-dependent trend, if `dist.correct=TRUE`

If `average=FALSE`, each component is a numeric matrix instead. Each column of the matrix contains the specified information for each library in data.

Additional parameter settings

Some robustness is provided by winsorizing out strong interactions with `winsor.high` to ensure that they do not overly influence the computed biases. This is useful for removing spikes around repeat regions or due to PCR duplication. Low-abundance bins can also be removed with `ignore.low` to avoid instability during correction, though this will result in NA values in the output.

Local bin pairs can be excluded as these are typically irrelevant to long-range interactions. They are also typically very high-abundance and may have excessive weight during correction, if not removed. This can be done by removing all bin pairs where the difference between the first and second anchor indices is less than `exclude.local`. Setting `exclude.local=NA` will only use inter-chromosomal bin pairs for correction.

If `dist.correct=TRUE`, abundances will be adjusted for distance-dependent effects. This is done by computing residuals from the fitted distance-abundance trend, using the `filterTrended` function. These residuals are then used for iterative correction, such that local interactions will not always have higher contact probabilities.

Ideally, the probability sums to unity across all bin pairs for a given bin (ignoring NA entries). This is complicated by winsorizing of high-abundance interactions and removal of local interactions. These interactions are not involved in correction, but are still reported in the output `truth`. As a result, the sum may not equal unity, i.e., values are not strictly interpretable as probabilities.

Author(s)

Aaron Lun

References

Imakaev M et al. (2012). Iterative correction of Hi-C data reveals hallmarks of chromosome organization. *Nat. Methods* 9, 999-1003.

See Also

[squareCounts](#), [mg1mOneGroup](#)

Examples

```
# Dummying up some data.
set.seed(3423746)
npts <- 100
npairs <- 5000
nlibs <- 4
anchor1 <- sample(npts, npairs, replace=TRUE)
anchor2 <- sample(npts, npairs, replace=TRUE)
data <- InteractionSet(
  matrix(rpois(npairs*nlibs, runif(npairs, 10, 100)), nrow=npairs),
  GInteractions(anchor1=anchor1, anchor2=anchor2,
    regions=GRanges("chrA", IRanges(1:npts, 1:npts)), mode="reverse"),
  colData=DataFrame(totals=runif(nlibs, 1e6, 2e6)))

# Correcting.
stuff <- correctedContact(data)
```



```

head(stuff$truth)
head(stuff$bias)
plot(stuff$max)

# Different behavior with average=FALSE.
stuff <- correctedContact(data, average=FALSE)
head(stuff$truth)
head(stuff$bias)
head(stuff$max)

# Creating an offset matrix, for use in glmFit.
anchor1.bias <- stuff$bias[anchors(data, type="first", id=TRUE),]
anchor2.bias <- stuff$bias[anchors(data, type="second", id=TRUE),]
offsets <- log(anchor1.bias * anchor2.bias)

# Adjusting for distance, and computing offsets with trend correction.
stuff <- correctedContact(data, average=FALSE, dist.correct=TRUE)
head(stuff$truth)
head(stuff$trend)
offsets <- log(stuff$bias[anchors(data, type="first", id=TRUE),] +
  log(stuff$bias[anchors(data, type="second", id=TRUE),])

```

cutGenome

Cut up the genome

Description

Perform an in silico restriction digest of a target genome.

Usage

```
cutGenome(bs, pattern, overhang=4L)
```

Arguments

bs	a BSgenome object or a character string pointing to a FASTA file
pattern	character string describing the recognition site
overhang	integer scalar specifying the length of the 5' overhang

Details

This function simulates a restriction digestion of a specified genome, given the recognition site and 5' overhang of the cutter. The total sequence spanned by each fragment is recorded, including the two sticky ends. No support is currently provided for searching the reverse strand, so the recognition site should be an inverse palindrome.

The genome should be specified as a BSgenome object. However, a character string can also be provided, specifying a FASTA file containing all the reference sequences in a genome. The latter may be necessary to synchronise the fragments with the genome used for alignment.

Note that some of the reported fragments may be physically impossible to form, e.g., for overlapping sites or consecutive sites when `overhang==nchar(pattern)`. Nonetheless, they are still reported to maintain the correspondence between fragments and cut sites. Cleavage sites on the forward strand can be obtained as the `start` locations of all fragments (excepting the first fragment on each chromosome).

Value

A GRanges object containing the boundaries of each restriction fragment in the genome.

Warning

If `bs` is a FASTQ file, the chromosome names in the FASTQ headers will be loaded faithfully by `cutGenome`. However, many mapping pipelines will drop the rest of the name past the first whitespace when constructing the alignment index. To be safe, users should ensure that the chromosome names in the FASTQ headers consist of one word. Otherwise, there will be a discrepancy between the chromosome names in the output GRanges, and those in the BAM files after alignment.

Author(s)

Aaron Lun

See Also

[matchPattern](#)

Examples

```
require(BSgenome.Ecoli.NCBI.20080805)

cutGenome(Ecoli, "AAGCTT", overhang=4L) # HindIII
cutGenome(Ecoli, "CCGCGG", overhang=2L) # SacII
cutGenome(Ecoli, "AGCT", overhang=0L) # AluI

# Trying with FastA files.
x <- system.file("extdata", "fastaEx.fa", package="Biostrings")
cutGenome(x, "AGCT", overhang=2)
cutGenome(x, "AGCT", overhang=4)
```

diClusters	<i>Cluster significant bin pairs to DIs</i>
------------	---

Description

Cluster significant bin pairs to DIs with post-hoc cluster-level FDR control.

Usage

```
diClusters(data.list, result.list, target, equiweight=TRUE, cluster.args=list(),
           pval.col="PValue", fc.col=NA, grid.param=NULL)
```

Arguments

<code>data.list</code>	an <code>InteractionSet</code> or a list of <code>InteractionSet</code> objects containing bin pairs.
<code>result.list</code>	a data frame or a list of data frames containing the DI test results for each bin pair.
<code>target</code>	a numeric scalar specifying the desired cluster-level FDR.
<code>equiweight</code>	a logical scalar indicating whether equal weighting from each input object should be enforced
<code>cluster.args</code>	a list of parameters to supply to <code>clusterPairs</code> .
<code>pval.col</code>	a character string or integer scalar specifying the column of p-values for elements in <code>result.list</code> .
<code>fc.col</code>	a character string or integer scalar specifying the column of log-fold changes for elements in <code>result.list</code> .
<code>grid.param</code>	a list of parameters to supply to <code>controlClusterFDR</code> .

Details

Bin pairs are identified as being significant based on the adjusted p-values in the corresponding data frame of `result.list`. Only these significant bin pairs are clustered together via `clusterPairs`. This identifies DIs consisting only of significant bin pairs. By default, the `tol` parameter in `clusterPairs` is set to 1 bp, i.e., all adjacent bin pairs are clustered together. If `fc.col` is specified, all clusters consist of bin pairs that are changing in the same direction.

The aim is to avoid very large clusters from blind clustering in areas of the interaction space that have high interaction intensity. This includes interactions within structural domains, or in data sets where interactions are difficult to define due to high levels of noise. Post-hoc control of the cluster-level FDR is performed using the `controlClusterFDR` function. This is necessary as clustering is not blind to the test results. By default, the cluster-level FDR is controlled at 0.05 if `target` is not specified.

Some effort is required to equalize the contribution of the results from each element of `result.list`. This is done by setting `equiweight=TRUE`, where the weight of each bin pair is inversely proportional to the number of bin pairs from that analysis. These weights are used as frequency weights for bin pair-level FDR control, when identifying significant bin pairs prior to clustering. Otherwise, the final results would be dominated by large number of small bin pairs.

Value

A list of cluster indices and minimum bounding boxes is returned as described in [clusterPairs](#). An additional FDR field is also present, containing the estimate of the cluster-level FDR.

Author(s)

Aaron Lun

See Also

[clusterPairs](#), [controlClusterFDR](#)

Examples

```
# Setting up the objects.
a <- 10
b <- 20
cuts <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))
param <- pairParam(cuts)

all.combos <- combn(length(cuts), 2) # Bin size of 1.
y1 <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,], regions=cuts, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=1))

set.seed(1000)
result1 <- data.frame(logFC=rnorm(nrow(y1)), PValue=runif(nrow(y1)), logCPM=0)
result1$PValue[sample(nrow(result1), 50)] <- 0

# Consolidating with post-hoc control.
out <- diClusters(y1, result1, target=0.05, cluster.args=list(tol=1))
out
```

diffHicUsersGuide

View diffHic user's guide

Description

Finds the location of the user's guide and opens it for viewing.

Usage

```
diffHicUsersGuide(view=TRUE)
```

Arguments

view logical scalar specifying whether the document should be opened

Details

The diffHic package is designed for the detection of differential interactions from Hi-C data. It provides methods for read pair counting, normalization, filtering and statistical analysis via edgeR. As the name suggests, the diffHic user's guide for can be obtained by running this function.

For non-Windows operating systems, the PDF viewer is taken from `Sys.getenv("R_PDFVIEWER")`. This can be changed to `x` by using `Sys.putenv(R_PDFVIEWER=x)`. For Windows, the default viewer will be selected to open the file.

Note that this guide is not a true vignette as it is not generated using [Sweave](#) when the package is built. This is due to the time-consuming nature of the code when run on realistic case studies.

Value

A character string giving the file location. If `view=TRUE`, the system's default PDF document reader is started and the user's guide is opened.

Author(s)

Aaron Lun

See Also

[system](#)

Examples

```
# To get the location:
diffHicUsersGuide(view=FALSE)
# To open in pdf viewer:
## Not run: diffHicUsersGuide()
```

DIList-class

DIList class and methods

Description

Using the DIList class to store pairwise genomic interactions.

NOTE: this class has been deprecated in favour of the InteractionSet class from the InteractionSet package. Users should use InteractionSet objects for future work. Existing DIList objects can be converted into InteractionSet objects using the DI2IS function.

Details

Each DIList stores counts for pairwise genomic interactions. Slots are defined as:

`counts`: an integer matrix containing the number of read pairs for an interaction in each library

`colData`: a `DataFrame` object containing library-specific information in each row, e.g., the total number of read pairs

exptData: a List object containing data or parameters pertinent to counting
anchors: an integer vector specifying the index of the first interacting region
targets: an integer vector specifying the index of the second interacting region
regions: a GRanges object containing the coordinates of all interacting regions

Each row of counts corresponds to an interaction, while each column corresponds to a library. Each interaction is parameterized by an anchors and targets index, pointing to the anchor and target intervals in regions, respectively. The anchor interval is defined as that with the higher genomic start coordinate, compared to that of the target interval. This means that anchors is never less than targets to avoid redundant permutations.

Constructor

`DIList(counts, totals=colSums(counts), anchors, targets, regions, exptData=List(), ...)`:
 Returns a DIList object where each entry is used to fill the corresponding slots. Type coercion is performed as required. Arguments in ... are used as columns in colData.

Accessors

In the code snippets below, x is a DIList object.

anchors(x, id=FALSE): Get the GRanges corresponding to the anchor intervals for each interaction. If id=TRUE, indices to the corresponding intervals in regions are returned instead.
targets(x, id=FALSE): Get the GRanges corresponding to the target intervals for each interaction. If id=TRUE, indices to the corresponding intervals in regions are returned instead.
regions(x): Get the GRanges for all interacting regions.
counts(x): Get the matrix of counts for all interactions in all libraries.
colData(x): Get the DataFrame of library-specific information.
exptData(x): Get the List of experiment-specific information.
x\$name: Get the value of colData(x)\$name.
dim(x): Get the dimensions, i.e., number of interactions (rows) against number of libraries (columns).
dimnames(x): Get the dimension names. This returns a list of length 2, where each element is NULL or a character vector.

Modifiers

In the code snippets below, x is a DIList object.

x\$name <- value: Assign value to the name field of the colData in x. Primarily intended for modification of the library sizes in totals, but can also be used to store additional library-specific information.
exptData(x) <- value: Assign a SimpleList object named value to the exptData slot of x. Note that this also works with list accessors, e.g., by assigning to exptData(x)\$name.

Subsetting and combining

In the code snippets below, `x` is a `DIList` object.

`x[i, j]`: Get count and coordinate data for all interactions `i` in libraries `j`. Either `i` or `j` can be missing, in which case all interactions or libraries are returned, respectively.

`c(x, ..., add.totals=TRUE)`: Merge `x` with other `DIList` objects in `...`, by concatenating the count matrices along with the anchor and target indices. By default, the totals will be added across all objects to be merged. This can be turned off by setting `add.totals=FALSE`, whereby only the totals of `x` are used (a warning will be generated if totals are not identical between objects). In all cases, objects to be merged should have the same value in the regions slot.

Other methods

In the code snippets below, `x` is a `DIList` object.

`show(x)`: Shows a summary of the information in `x`. Specifically, a string is printed that reports the number of libraries, number of interactions and total number of regions in `x`.

`as.matrix(x, first=NULL, second=first, fill=NULL, ...)`: Converts `x` into a contact matrix where rows and columns represent regions on the `first` and `second` chromosomes, respectively. Entries of the matrix correspond to an interaction of `x`. Each entry is extracted from `fill`, where `fill` is a numeric vector with one value per interaction in `x`. By default, the average abundance of each interaction in `x` is used as the entry. For intra-chromosomal spaces, both sides of the diagonal are filled. Multiple chromosomes can also be specified in `first` or `second` (all chromosomes are used by default). Each row and column is named according to the matching index of `regions(x)`.

Author(s)

Aaron Lun

DIList-wrappers

Statistical wrappers for DIList objects

Description

Convenience wrappers for statistical routines operating on `DIList` objects.

NOTE: These methods are all deprecated, along with the `DIList` class itself. Users can apply the same methods to `InteractionSet` objects.

Usage

```
## S4 method for signature 'DIList'
normOffsets(object, lib.sizes, ...)
## S4 method for signature 'DIList'
normalize(object, lib.sizes, ...) # deprecated, use normOffsets
## S4 method for signature 'DIList'
asDGEList(object, lib.sizes, ...)
```

Arguments

object	a DIList object, like that produced by squareCounts
lib.sizes	an (optional) integer vector of library sizes
...	other arguments to be passed to the function being wrapped

Details

Counts are extracted from the matrix in the DIList object. If not specified in lib.sizes, library sizes are taken from the totals field in the column data of object. Warnings will be generated if this field is not present.

In the normOffsets method, the extracted counts and library sizes are supplied to [normOffsets, matrix-method](#). Similarly, the asDGEList method wraps the [DGEList](#) constructor. In both cases, any arguments in ... are also passed to the wrapped functions.

Value

For normOffsets, either a numeric matrix or vector is returned; see [normOffsets, matrix-method](#).

For asDGEList, a DGEList object is returned.

Author(s)

Aaron Lun

DNaseHiC

Methods for processing DNase Hi-C data

Description

Processing of BAM files for DNase Hi-C into index files

Usage

```
segmentGenome(bs, size=500)
prepPseudoPairs(bam, param, file, dedup=TRUE, ichim=TRUE,
  chim.span=1000, minq=NA, output.dir=NULL)
```

Arguments

bs	a BSgenome object, or a character string pointing to a FASTA file, or a named integer vector of chromosome lengths
size	an integer scalar indicating the size of the pseudo-fragments
bam	a character string containing the path to a name-sorted BAM file
param	a pairParam object containing read extraction parameters
file	a character string specifying the path to an output index file

<code>dedup</code>	a logical scalar indicating whether marked duplicate reads should be removed
<code>ichim</code>	a logical scalar indicating whether invalid chimeras should be counted
<code>chim.span</code>	an integer scalar specifying the maximum span between a chimeric 3' end and a mate read
<code>minq</code>	an integer scalar specifying the minimum mapping quality for each read
<code>output.dir</code>	a character string specifying a directory for temporary files

Details

DNase Hi-C involves random fragmentation with DNase instead of restriction enzymes. This is accommodated in `diffHiC` by partitioning the genome into small pseudo-fragments, using `segmentGenome`. Reads are then assigned into these pseudo-fragments using `prepPseudoPairs`. The rest of the analysis pipeline can then be used in the same manner as that for standard Hi-C.

The behavior of `prepPseudoPairs` is almost identical to that for `preparePairs`, if the latter were asked to assign reads into pseudo-fragments. However, for `prepPseudoPairs`, no reporting or removal of self-circles or dangling ends is performed, as these have no meaning for artificial fragments. Also, invalidity of chimeras is determined by checking whether the 3' end is more than `chim.span` away from the mate read, rather than checking for localization in different fragments.

The size of the pseudo-fragments is determined by, well, `size` in `segmentGenome`. Smaller sizes provide better resolution but increase computational work. Needless to say, the `param$fragments` field should contain the output from `segmentGenome`, rather than from `cutGenome`. Also see `cutGenome` documentation for a warning about the chromosome names.

Some loss of spatial resolution is inevitable when reads are summarized into pseudo-fragments. This is largely irrelevant, though, as counting across the interaction space will ultimately use much larger bins (usually at least 2 kbp).

Value

For `segmentGenome`, a `GRanges` object is produced containing the coordinates of the pseudo-fragments in the specified genome.

For `prepPseudoPairs`, a HDF5-formatted index file is produced at the specified location. A list of diagnostic vectors are also returned in the same format as that from `preparePairs`, without the same `.id` entry.

Author(s)

Aaron Lun

See Also

[preparePairs](#), [cutGenome](#)

Examples

```
require(BSgenome.Ecoli.NCBI.20080805)
segmentGenome(BSgenome.Ecoli.NCBI.20080805)
segmentGenome(BSgenome.Ecoli.NCBI.20080805, size=1000)
```

```
# Pretend that this example is DNase Hi-C.
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
pseudo <- segmentGenome(seqlengths(cuts), size=50)
param <- pairParam(pseudo)

tmpf <- "gunk.h5"
prepPseudoPairs(hic.file, param, tmpf)
prepPseudoPairs(hic.file, param, tmpf, dedup=FALSE)
prepPseudoPairs(hic.file, param, tmpf, minq=50)
prepPseudoPairs(hic.file, param, tmpf, chim.span=20)
```

domainDirections	<i>Calculate domain directionality</i>
------------------	--

Description

Collect directionality statistics for domain identification with genomic bins.

Usage

```
domainDirections(files, param, width=50000, span=10)
```

Arguments

files	a character vector containing paths to the index files generated from each Hi-C library
param	a pairParam object containing read extraction parameters
width	an integer scalar specifying the width of each bin in base pairs
span	an integer scalar specifying the distance to consider for up/downstream interactions

Details

The genome is partitioned into bins of size width. For each bin, this function computes the total count for all bin pairs involving that bin and the span upstream bins. This is repeated for the span downstream bins. If multiple files are specified, the total of the averages across all libraries for each bin pair is computed instead.

The total up- and downstream counts are returned in the Up and Down fields of the output GRanges object. This can be used to compute a directionality statistic, e.g., as defined by Dixon et al, or by computing the log-fold change between fields. The latter is easier to compute and model with a Gaussian distribution. In any case, the directionalities can be used in a HMM to identify domains - see the user's guide for more details.

Value

A GRanges object with one entry for each bin in the genome. The Up and Down fields in the metadata contain the total average count for the up-/downstream interactions involving each bin.

Author(s)

Aaron Lun

References

Dixon JR et al. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485:376-380.

See Also

[squareCounts](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(fragments=cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, file=fout))

# Not really that informative; see user's guide.
out <- domainDirections(fout, param, width=10)
out
domainDirections(fout, param, width=10, span=1)

# Calculating directionality log-FC with a large prior.
dir.logFC <- log2((out$Up+10)/(out$Down+10))
dir.logFC

# Calculating directionality index with Dixon's method.
dixon.stat <- sign(out$Up-out$Down)*2*((out$Up-out$Down)/(out$Up+out$Down))^2
dixon.stat
```

enrichedPairs

Compute local enrichment for bin pairs

Description

Calculate the log-fold increase in abundance for each bin pair against its local neighborhood.

Usage

```
enrichedPairs(data, flank=5, exclude=0, prior.count=2, abundances=NULL)
```

Arguments

<code>data</code>	an InteractionSet object containing bin pair counts, generated by squareCounts
<code>flank</code>	an integer scalar, specifying the number of bins to consider as the local neighborhood
<code>exclude</code>	an integer scalar, specifying the number of bins to exclude from the neighborhood
<code>prior.count</code>	a numeric scalar indicating the prior count to use in computing the log-fold increase
<code>abundances</code>	a numeric vector of abundances for each bin pair

Value

A numeric vector containing the log-fold increase (i.e., enrichment value) for each bin pair in data.

Definition of the neighborhoods

Consider the coordinates of the interaction space in terms of bins, and focus on any particular bin pair (named here as the target bin pair). This target bin pair is characterized by four neighborhood regions, from A to D. Region A is a square with side lengths equal to $flank*2+1$, where the target bin pair is positioned in the center. Region B is a square with side lengths equal to $flank$, positioned such that the target bin pair lies at the corner furthest from the diagonal (only used for intra-chromosomal targets). Region C is a horizontal rectangle with dimensions $(1, flank*2+1)$, containing the target bin pair at the center. Region D is the vertical counterpart to C.

Obviously, the target bin pair itself is excluded in the definition of each neighborhood. If `exclude` is positive, additional bin pairs closest to the target will also be excluded. For example, region A* is constructed with `exclude` instead of `flank`, and the resulting area is excluded from region A (and so on for all other regions). This avoids problems where diffuse interactions are imperfectly captured by the target bin pair, such that genuine interactions spill over into the neighborhood. Spill-over is undesirable as it will inflate the neighborhood abundance for genuine interactions. Setting a larger `exclude` ensures that this does not occur.

The size of `flank` requires consideration, as it defines the size of each neighborhood region. If the value is too large, other peaks may be included in the background such that the neighborhood abundance is inflated. On the other hand, if `flank` is too small, there will not be enough neighborhood bin pairs to dilute the increase in abundance from spill-over. Both scenarios result in a decrease in enrichment values and loss of power to detect punctate events. The default value of 5 seems to work well, though users may wish to test several values for themselves.

Computing the enrichment values

For a target bin pair in data, the `enrichedPairs` function computes the mean abundance for each of its surrounding neighborhoods. This is defined as the mean of the counts for all constituent bin pairs in that neighborhood (average counts are used for multiple libraries). The local background for the target bin pair is defined as the maximum of the mean abundances for all neighborhoods.

The enrichment value is then defined as the the difference between the target bin pair's abundance and its local background. The idea is that bin pairs with high enrichments are likely to represent punctate interactions between clearly defined loci. Selecting for high enrichments can then select for these peak-like features in the interaction space.

The maximizing strategy is designed to mitigate the effects of structural features. Region B will capture the high interaction intensity within genomic domains like TADs, while the C and D will capture any bands in the interaction space. The abundance will be high for any neighborhood that captures a high-intensity feature, as the average counts will be large for all bin pairs within the features. This will then be chosen as the maximum during calculation of enrichment values. Otherwise, if only region A were used, the background abundance would be decreased by low-intensity bin pairs outside of the features. This results in spuriously high enrichment values for target bin pairs on the feature boundaries.

By default, nothing is done to adjust for the effect of distance on abundance for intra-chromosomal bin pairs. This is because the counts are generally too low to routinely fit a reliable trend. That said, users can still supply distance-adjusted abundances as abundances. Such values can be defined as the residuals of the fit from `filterTrended`. Obviously, no such work is required for inter-chromosomal bin pairs.

Author(s)

Aaron Lun

References

Rao S et al. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*. 159, 1665-1690.

See Also

[squareCounts](#), [filterPeaks](#)

Examples

```
# Setting up the object.
a <- 10
b <- 20
regions <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))

set.seed(23943)
all.anchor1 <- sample(length(regions), 50, replace=TRUE)
all.anchor2 <- as.integer(runif(50, 1, all.anchor1+1))
data <- InteractionSet(matrix(rnbinom(200, mu=10, size=10), 50, 4),
  GInteractions(anchor1=all.anchor1, anchor2=all.anchor2,
    regions=regions, mode="reverse"),
  colData=DataFrame(lib.size=1:4*1000), metadata=List(width=1))
data$totals <- colSums(assay(data))

# Getting peaks.
head(enrichedPairs(data))
head(enrichedPairs(data, flank=3))
```

```

head(enrichedPairs(data, flank=1))
head(enrichedPairs(data, exclude=1))

# Accounting for distance.
filtered <- suppressWarnings(filterTrended(data, prior.count=0))
adj.ab <- filtered$abundances - filtered$threshold

head(enrichedPairs(data, abundances=adj.ab))

```

Filtering diagonals *Filtering of diagonal bin pairs*

Description

Filtering to remove bin pairs on or near the diagonal of the interaction space.

Usage

```
filterDiag(data, by.dist=0, by.diag=0L, dist, ...)
```

Arguments

<code>data</code>	an <code>InteractionSet</code> object produced by squareCounts
<code>by.dist</code>	a numeric scalar indicating the base-pair distance threshold below which bins are considered local
<code>by.diag</code>	an integer scalar indicating the bin distance threshold below which bins are considered local
<code>dist</code>	a optional numeric vector containing pre-computed distances
<code>...</code>	other arguments to pass to getDistance , if <code>dist</code> is not specified

Details

Pairs of the same bin will lie on the diagonal of the interaction space. Counts for these pairs can be affected by local artifacts (e.g., self-circles, dangling ends) that may not have been completely removed during earlier quality control steps. These pairs are also less interesting, as they capture highly local structure that may be the result of non-specific compaction. In many cases, these bin pairs are either removed or, at least, normalized separately within the analysis.

This function provides a convenience wrapper in order to separate diagonal bin pairs from those in the rest of the interaction space. Users can also consider near-diagonal bin pairs, which are defined as pairs of local bins on the linear genome. Specifically, bins are treated as local if they separated by less than `by.dist` in terms of base pairs, or by less than `by.diag` in terms of bins. These can be separated with the diagonal bin pairs if they are subject to the same issues described above.

Note that if `by.dist` is specified, it should be set to a value greater than 1.5 times the average bin size. Otherwise, the distance between the midpoints of adjacent bins will always be larger than `by.dist`, such that no near-diagonal bin pairs are removed.

Users can expedite processing by supplying a pre-computed vector of distances in `dist`. This vector may already be available if it was generated elsewhere in the pipeline. However, the supplied vector should have the same number of entries as that in `data`.

Value

A logical vector indicating whether each bin pair in data is a non-diagonal (or non-near-diagonal) element.

Author(s)

Aaron Lun

See Also

[pairedist](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(fragments=cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, file=fout))

# Collating to count combinations.
y <- squareCounts(fout, param, width=50, filter=1)

summary(filterDiag(y))
summary(filterDiag(y, by.dist=100))
summary(filterDiag(y, by.diag=1))
summary(filterDiag(y, dist=pairedist(y)))
```

Filtering methods

Filtering strategies for bin pairs

Description

Implementations of the direct and trended filtering strategies for bin pair abundances.

Usage

```
filterDirect(data, prior.count=2, reference=NULL)
filterTrended(data, span=0.25, prior.count=2, reference=NULL)
```

Arguments

<code>data</code>	an <code>InteractionSet</code> object produced by <code>squareCounts</code>
<code>span</code>	a numeric scalar specifying the bandwidth for loess curve fitting
<code>prior.count</code>	a numeric scalar indicating the prior count to use for calculating the average abundance
<code>reference</code>	another <code>InteractionSet</code> object, usually containing data for larger bin pairs

Details

The `filterDirect` function implements the direct filtering strategy. The rate of non-specific ligation is estimated as the median of average abundances from inter-chromosomal bin pairs. This rate or some multiple thereof can be used as a minimum threshold for filtering, to keep only high-abundance bin pairs. When calculating the median, some finesse is required to consider empty parts of the interaction space, i.e., areas that are not represented by bin pairs.

The `filterTrended` function implements the trended filtering strategy. The rate of non-specific compaction is estimated by fitting a trend to the average abundances against the log-distance for all intra-chromosomal bin pairs. This rate can then be used as a minimum threshold for filtering. For inter-chromosomal bin pairs, the threshold is the same as that from the direct filter.

Curve fitting in `filterTrended` is done using `loessFit` with a bandwidth of `span`. Lower values may need to be used for a more accurate fit when the trend is highly non-linear. The bin size is also added to the distance prior to log-transformation, to avoid problems with undefined values when distances are equal to zero. Empty parts of the interaction space are considered by inferring the abundances and distances of the corresponding bin pairs (though this is skipped if too much of the space is empty).

If `reference` is specified, it will be used to compute filter thresholds instead of `data`. This is intended for large bin pairs that have been loaded with `filter=1`. Larger bins provide larger counts for more precise threshold estimates, while the lack of filtering ensures that estimates are not biased. All threshold estimates are adjusted to account for differences in bin sizes between `reference` and `data`. The final values can be used to directly filter on abundances in `data`; check out the user's guide for more details.

Value

A list is returned containing abundances, a numeric vector with the average abundances of all bin pairs in `data`. For `filterDirect`, the list contains a numeric scalar `threshold`, i.e., the non-specific ligation rate. For `filterTrended`, the list contains `threshold`, a numeric vector containing the threshold for each bin pair; and `log.distance`, a numeric vector with the log-distances for each bin pair.

If `reference` is specified in either function, an additional list named `ref` is also returned. This contains the filtering information for the bin pairs in `reference`, same as that reported above for each bin pair in `data`.

Author(s)

Aaron Lun

References

Lin, YC et al. (2012) Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. *Nat. Immunol.* 13. 1196-1204

See Also

[squareCounts](#), [scaledAverage](#)

Examples

```
# Setting up the object.
a <- 10
b <- 20
regions <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))

set.seed(138153)
npairs <- 500
all.anchor1 <- sample(length(regions), npairs, replace=TRUE)
all.anchor2 <- as.integer(runif(npairs, 1, all.anchor1+1))
counts <- matrix(rnbinom(npairs*4, mu=10, size=10), npairs, 4)
y <- InteractionSet(counts, GInteractions(anchor1=all.anchor1,
    anchor2=all.anchor2, regions=regions, mode="reverse"),
    colData=DataFrame(totals=colSums(counts)), metadata=List(width=1))

# Requiring at least 1.5-fold change.
direct <- filterDirect(y)
keep <- direct$abundances > direct$threshold + log2(1.5)
y[keep,]

# Requiring to be above the threshold.
trended <- filterTrended(y)
keep <- trended$abundances > trended$threshold
y[keep,]

# Running reference comparisons, using larger bin pairs.
w <- 5L
a2 <- a/w
b2 <- b/w
rep.regions <- GRanges(rep(c("chrA", "chrB"), c(a2, b2)),
    IRanges(c(1:a2, 1:b2)*w-w+1L, c(1:a2, 1:b2)*w))
npairs2 <- 20
rep.anchor1 <- sample(length(rep.regions), npairs2, replace=TRUE)
rep.anchor2 <- as.integer(runif(npairs2, 1, rep.anchor1+1))
y2 <- InteractionSet(matrix(rnbinom(npairs2*4, mu=10*w^2, size=10), npairs2, 4),
    GInteractions(anchor1=rep.anchor1, anchor2=rep.anchor2, regions=rep.regions, mode="reverse"),
    colData=DataFrame(totals=y$totals), metadata=List(width=w))

direct2 <- filterDirect(y, reference=y2)
sum(direct2$abundances > direct2$threshold + log2(1.5))
trended2 <- filterTrended(y, reference=y2)
sum(trended2$abundances > trended2$threshold)
```

filterPeaks	<i>Filter bin pairs for likely peaks</i>
-------------	--

Description

Identify bin pairs that are likely to represent punctate peaks in the interaction space.

Usage

```
filterPeaks(data, enrichment, min.enrich=log2(1.5), min.count=5, min.diag=2L, ...)
```

Arguments

data	an InteractionSet object produced by squareCounts or neighborCounts
enrichment	a numeric vector of enrichment values, produced by enrichedPairs or neighborCounts
min.enrich	a numeric scalar indicating the minimum enrichment score for a peak
min.count	a numeric scalar indicating the minimum average count for a peak
min.diag	an integer scalar specifying the minimum diagonal in the interaction space with which to consider a peak
...	other arguments to be passed to aveLogCPM for the average count filter

Details

Filtering on the local enrichment scores identifies high-intensity islands in the interaction space. However, this alone is not sufficient to identify sensible peaks. Filtering on the absolute average counts prevents the calling of low-abundance bin pairs with high enrichment scores due to empty neighborhoods. Filtering on the diagonals prevents calling of high-abundance short-range interactions that are usually uninteresting. If either `min.count` or `min.diag` are NULL, no filtering will be performed on the average counts and diagonals, respectively.

Value

A logical vector indicating whether or not each bin pair is to be considered as a peak.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [enrichedPairs](#), [neighborCounts](#)

Examples

```
# Setting up the object.
a <- 10
b <- 20
regions <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)))

set.seed(23943)
all.anchor1 <- sample(length(regions), 50, replace=TRUE)
all.anchor2 <- as.integer(runif(50, 1, all.anchor1+1))
data <- InteractionSet(matrix(rnbinom(200, mu=10, size=10), 50, 4),
  GIInteractions(anchor1=all.anchor1, anchor2=all.anchor2, regions=regions, mode="reverse"),
  colData=DataFrame(totals=runif(4, 1e6, 2e6)), metadata=List(width=1))

# Getting peaks.
enrichment <- enrichedPairs(data)
summary(filterPeaks(data, enrichment, min.enrich=0.5))
summary(filterPeaks(data, enrichment, min.enrich=0.5, min.count=10))
summary(filterPeaks(data, enrichment, min.enrich=0.5, min.diag=NULL))
```

getArea

*Get interaction area***Description**

Compute area in the interaction space for each pair of regions.

Usage

```
getArea(data, bp=TRUE)
```

Arguments

data	an InteractionSet object
bp	a logical scalar indicating whether areas should be reported in base-pair terms

Details

The `getArea` function returns the area in the interaction space for each pair of regions. If `bp=TRUE`, the area is reported in terms of squared base pairs. This tends to be the easiest to interpret. Otherwise, the area is reported as the number of pairs of restriction fragments. This may be more relevant to the actual resolution of the Hi-C experiment.

Some special consideration is required for areas overlapping the diagonal. This is because counting is only performed on one side of the diagonal, to avoid redundancy. Base-pair areas are automatically adjusted to account for this feature, based on the presence of partial overlaps between interacting regions.

For fragment-based areas, some additional work is required to properly compute areas around the diagonal for partially overlapping regions. This is only necessary when data is produced by [connectCounts](#). This is because bins will not partially overlap in any significant manner when counts are generated with [squareCounts](#).

Value

A numeric vector is returned containing the area in the interaction space for each pair of regions in data.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [connectCounts](#)

Examples

```
# Making up an InteractionSet for binned data.
nfrags <- 50
frag.sizes <- as.integer(runif(nfrags, 5, 10))
ends <- cumsum(frag.sizes)
cuts <- GRanges("chrA", IRanges(c(1, ends[-nfrags]+1), ends))
param <- pairParam(cuts)

regions <- diffHic:::.getBinID(cuts, 20)$region
all.combos <- combn(length(regions), 2)
y <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,],
    regions=regions, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param, width=20))

# Generating partially overlapping regions.
set.seed(3424)
re <- sample(nfrags, 20)
rs <- as.integer(runif(20, 1, re+1))
regions <- GRanges("chrA", IRanges(start(cuts)[rs], end(cuts)[re]))
regions$nfrags <- re - rs + 1L
regions <- sort(regions)
all.combos <- combn(length(regions), 2)
y2 <- InteractionSet(matrix(0, ncol(all.combos), 1),
  GInteractions(anchor1=all.combos[2,], anchor2=all.combos[1,],
    regions=regions, mode="reverse"),
  colData=DataFrame(lib.size=1000), metadata=List(param=param))

#### Getting areas. ####

getArea(y)
getArea(y, bp=FALSE)

getArea(y2)
getArea(y2, bp=FALSE)
```

getDistance	<i>Get the linear distance for each interaction</i>
-------------	---

Description

Compute the distance between interacting regions on the linear genome.

NOTE: This function is deprecated in favour of the `pairdist` function from the `InteractionSet` package.

Usage

```
getDistance(data, type=c("mid", "gap", "span"))
```

Arguments

<code>data</code>	a <code>DIList</code> object
<code>type</code>	a character string specifying the type of distance to compute

Details

The `getDistance` function examines each interaction in `data$pairs` and computes the distance between the corresponding regions on the linear genome. Options to compute the distances are `mid`, for the distance between the midpoints of the regions; `gap`, for the distance of the interval lying between the regions (set to zero, if regions are overlapping); and `span`, for the total distance spanned by the interaction, including both regions. Interchromosomal interactions are marked with `NA`.

Value

An integer vector is returned containing the distances between interacting regions for each pair in `data`.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [connectCounts](#)

`getPairData`*Get read pair data*

Description

Extract diagnostics for each read pair from an index file

Usage

```
getPairData(file, param)
```

Arguments

<code>file</code>	character string, specifying the path to the index file produced by preparePairs
<code>param</code>	a <code>pairParam</code> object containing read extraction parameters

Details

This is a convenience function to extract read pair diagnostics from an index file, generated from a Hi-C library with [preparePairs](#). The aim is to examine the distribution of each returned value to determine the appropriate cutoffs for [prunePairs](#).

The `length` refers to the length of the DNA fragment used in sequencing. It is computed for each read pair by adding the distance of each read to the closest restriction site in the direction of the read.

The `insert` simply refers to the insert size for each read pair. This is defined as the distance between the extremes of each read on the same chromosome. Values for interchromosomal pairs are set to NA.

For orientation, setting 0x1 or 0x2 means that the read mapped into the first or second anchor fragment respectively is on the reverse strand. For intrachromosomal reads, an orientation value of 1 represents inward-facing reads whereas a value of 2 represents outward-facing reads.

Note that a `pairParam` object is only used here for consistency. Specifically, the restriction fragment coordinates in `param$fragments` are required. No removal of read pairs will be performed here, so the values of `param$restrict` or `param$discard` will be ignored.

Value

A dataframe is returned containing integer fields for `length`, `orientation` and `insert` for each read pair.

Author(s)

Aaron Lun

See Also

[preparePairs](#), [prunePairs](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

tmpf <- "gunk.h5"
invisible(preparePairs(hic.file, param, tmpf))
getPairData(tmpf, param)
```

loadData	<i>Load data from an index file</i>
----------	-------------------------------------

Description

Load read pair data and chromosome names from a HDF5 index file.

Usage

```
loadChromos(file)
loadData(file, anchor1, anchor2)
```

Arguments

`file` a character string containing a path to a index file
`anchor1, anchor2` a character string, specifying the name of the chromosomes in a pair

Details

The purpose of these function is to allow users to perform custom analyses by extracting the data manually from each index file. This may be desirable, e.g., when preparing data for input into other tools. To extract all data, users are advised to run `loadData` iteratively on each pair of chromosomes as obtained with `loadChromos`.

Note that `loadData` will successfully operate even if the `anchor1/anchor2` specification is permuted. In this case, it will return a warning to inform the user that the names should be switched.

Value

The `loadChromos` function will return a dataframe with character fields `anchor1` and `anchor2`. Each row represents a pair of chromosomes, the names of which are stored in the fields. The presence of a row indicates that the data for the corresponding pair exists in the file.

The `loadData` function will return a dataframe where each row contains information for one read pair. Refer to [preparePairs](#) for more details on the type of fields that are included.

Author(s)

Aaron Lun

Examples

```

hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

tmpf <- "gunk.h5"
preparePairs(hic.file, param, tmpf)

loadChromos(tmpf)
loadData(tmpf, "chrA", "chrA")
loadData(tmpf, "chrB", "chrA")
loadData(tmpf, "chrA", "chrB")
try(loadData(tmpf, "chrA2", "chrB2"))

```

marginCounts

*Collect marginal counts for each bin***Description**

Count the number of read pairs mapped to each bin across multiple Hi-C libraries.

Usage

```
marginCounts(files, param, width=50000)
```

Arguments

files	a character vector containing paths to the index files
param	a pairParam object containing read extraction parameters
width	an integer scalar specifying the width of each bin

Details

The genome is first split into non-overlapping adjacent bins of size width, which are rounded to the nearest restriction site. The marginal count for each bin is defined as the number of reads in the library mapped to the bin. This acts as a proxy for genomic coverage by treating Hi-C data as single-end.

Each row of the output RangedSummarizedExperiment refers to a single bin in the linear genome, instead of a bin pair in the interaction space. The count matrix for all row can be extracted using the assay method. Bin coordinates can be extracted using the rowRanges method.

Larger marginal counts can be collected by increasing the width value. However, this comes at the cost of spatial resolution as adjacent events in the same bin can no longer be distinguished. Note that filtering is automatically performed to remove empty bins.

Counting will consider the values of restrict, discard and cap in param. See [pairParam](#) for more details.

Value

A RangedSummarizedExperiment object containing the marginal counts for each bin.

Author(s)

Aaron Lun

See Also

[squareCounts](#), [RangedSummarizedExperiment-class](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(fragments=cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, fout))

# Collating to count combinations.
mar <- marginCounts(fout, param, width=10)
head(assay(mar))
mar <- marginCounts(fout, param, width=50)
head(assay(mar))
mar <- marginCounts(fout, param, width=100)
head(assay(mar))

# Attempting with other parameters.
mar <- marginCounts(fout, reform(param, restrict="chrA"), width=50)
head(assay(mar))
mar <- marginCounts(fout, reform(param, cap=1), width=50)
head(assay(mar))
mar <- marginCounts(fout, reform(param, discard=GRanges("chrA", IRanges(1, 50))), width=50)
head(assay(mar))
```

`mergePairs`*Merge read pairs*

Description

Merge index files for multiple Hi-C libraries into a single output file.

Usage

```
mergePairs(files, file.out)
```

Arguments

`files` a character vector containing the paths to the index files to be merged
`file.out` a character string specifying the path to the output index file

Details

Hi-C libraries are often split into technical replicates. This function facilitates the merging of said replicates into a single library for downstream processing. Index files listed in `files` should be produced by [preparePairs](#), with or without pruning by [prunePairs](#).

Value

A merged index file is produced at the specified location. A NULL object is invisibly returned.

Author(s)

Aaron Lun

See Also

[preparePairs](#), [prunePairs](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

fout <- "temp.h5"
fout2 <- "temp2.h5"
fout3 <- "temp3.h5"
invisible(preparePairs(hic.file, param, fout))
invisible(prunePairs(fout, param, fout2))
invisible(prunePairs(fout, param, fout3, max.frag=50))

mout <- "merged"
mergePairs(c(fout2, fout3), mout)
```

```
require(rhdf5)
h5read(fout2, "chrA/chrA")
h5read(fout3, "chrA/chrA")
h5read(mout, "chrA/chrA")
```

neighborCounts	<i>Load Hi-C interaction counts</i>
----------------	-------------------------------------

Description

Collate count combinations for interactions between pairs of bins across multiple Hi-C libraries.

Usage

```
neighborCounts(files, param, width=50000, filter=1L, flank=NULL,
               exclude=NULL, prior.count=NULL)
```

Arguments

files	a character vector containing paths to the index files generated from each Hi-C library
param	a pairParam object containing read extraction parameters
width	an integer scalar specifying the width of each square in base pairs
filter	an integer scalar specifying the minimum count for each square
flank	an integer scalar, specifying the number of bins to consider as the local neighborhood
exclude	an integer scalar, specifying the number of bins to exclude from the neighborhood
prior.count	a numeric scalar indicating the prior count to use in computing the log-fold increase

Details

This function combines the functionality of [squareCounts](#) and [enrichedPairs](#). The idea is to allow calculation of local enrichment values when there is insufficient memory to load all bin pairs with `filter=1L` in [squareCounts](#). Here, the interaction space around each bin pair is examined as the counts are loaded for that bin pair, avoiding the need to hold the entire interaction space at once. Only the counts and local enrichment values for those bin pairs with row sums above `filter` are reported to save memory. The returned enrichment values are equivalent to that computed with [enrichedPairs](#) with the default settings.

Value

An InteractionSet object is returned with the number of read pairs for each bin pair across all libraries. In the `mcols`, the `enrichment` field contains a numeric vector of enrichment statistics for each bin pair.

Author(s)

Aaron Lun

References

Rao S et al. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*. 159, 1665-1690.

See Also

[squareCounts](#), [enrichedPairs](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(fragments=cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, file=fout))

# Collating to count combinations.
y <- neighborCounts(fout, param, width=50, filter=2,
  flank=5, prior.count=2)
y
mcols(y)$enrichment

# Practically identical to a more memory-intensive call.
ref <- squareCounts(fout, param, width=50, filter=1)
keep <- rowSums(assay(ref)) >= 2
enriched <- enrichedPairs(ref, flank=5, prior.count=2)

stopifnot(identical(anchors(ref[keep,]), anchors(y)))
stopifnot(all(abs(enriched[keep] - mcols(y)$enrichment) < 1e-6))
```

normalizeCNV

Normalize CNV biases

Description

Compute normalization offsets to remove CNV-driven and abundance-dependent biases

Usage

```
normalizeCNV(data, margins, prior.count=3, span=0.3, maxk=500, ...)
matchMargins(data, margins)
```

Arguments

<code>data</code>	an <code>InteractionSet</code> object produced by <code>squareCounts</code>
<code>margins</code>	a <code>RangedSummarizedExperiment</code> object produced by <code>marginCounts</code>
<code>prior.count</code>	a numeric scalar specifying the prior count to use in computing marginal log-ratios
<code>span</code>	a numeric scalar between 0 and 1, describing the span of the fit
<code>maxk</code>	a integer scalar specifying the number of vertices to use during local fitting
<code>...</code>	other arguments to pass to <code>locfit</code>

Details

Each bin pair in `data` is associated with three covariates. The first two are the marginal log-ratios of the corresponding bins, i.e., the log-ratio of the marginal counts between two libraries. These represent the relative CNVs in the interacting regions between libraries. To avoid redundancy, the first covariate is the larger marginal log-ratio whereas the second is the smaller. The third covariate is the average abundance across all libraries.

Each bin pair is also associated with a response, i.e., the log-ratio of the interaction counts between two libraries. A loess-like surface is fitted to the response against the three covariates, using the `locfit` function. The aim is to eliminate systematic differences between libraries at any combination of covariate values. This removes CNV-driven biases as well as trended biases with respect to the abundance. The fitted value can then be used as a GLM offset for each bin pair.

The objects in `data` and `margins` should be constructed with the same `width` and `param` arguments in their respective functions. This ensures that the regions are the same, so that the marginal counts can be directly used. Matching of the bins in each bin pair in `data` to indices of `margins` is performed using `matchMargins`. Note that the marginal counts are not directly computed from `data` as filtering of bin pairs may be performed beforehand.

In practice, normalization offsets are computed for each library relative to a single reference “average” library. This average library is constructed by using the average abundance as the (log-)count for both the bin pair and marginal counts. The space of all pairs of CNV log-ratios is also rotated by 45 degrees prior to smoothing. This improves the performance of the approximations used by `locfit`.

The fit parameters can be changed by varying `span`, `maxk` and additional arguments in `locfit`. Higher values of `span` will increase smoothness, at the cost of sensitivity. Increases in `maxk` may be required to obtain a more accurate approximation when fitting large datasets. In all cases, a loess fit of degree 1 is used.

For use by downstream functions, the offset matrix can be stored as an assay named “offset” in the `data` object.

Value

For `normalizeCNV`, a numeric matrix is returned with the same dimensions as `counts(data)`. This contains log-based GLM offsets for each bin pair in each library.

For `matchMargins`, a data frame is returned with integer fields `anchor1` and `anchor2`. Each field specifies the index in `margins` corresponding to the bin regions for each bin pair in `data`.

Author(s)

Aaron Lun

See Also[locfit](#), [lp](#), [squareCounts](#), [marginCounts](#)**Examples**

```

# Dummying up some data.
set.seed(3423746)
npts <- 100
npairs <- 5000
nlibs <- 4
anchor1 <- sample(npts, npairs, replace=TRUE)
anchor2 <- sample(npts, npairs, replace=TRUE)

data <- InteractionSet(
  list(counts=matrix(rpois(npairs*nlibs, runif(npairs, 10, 100)), nrow=npairs)),
  GInteractions(anchor1=anchor1, anchor2=anchor2,
    regions=GRanges("chrA", IRanges(1:npts, 1:npts)), mode="reverse"),
  colData=DataFrame(totals=runif(nlibs, 1e6, 2e6)))

margins <- SummarizedExperiment(matrix(rpois(npts*nlibs, 100), nrow=npts),
  colData=DataFrame(totals=data$totals), rowRanges=regions(data))

# Running normalizeCNV.
out <- normalizeCNV(data, margins)
head(out)
head(normalizeCNV(data, margins, prior.count=1))
head(normalizeCNV(data, margins, span=0.5))

# Store offsets as the 'offset' assay for use by, e.g., asDGEList.
assays(data)$offset <- out
data

# Occasionally locfit will complain; increase maxk to compensate.
data <- InteractionSet(matrix(rpois(npairs*nlibs, 20), nrow=npairs),
  GInteractions(anchor1=anchor1, anchor2=anchor2,
    regions=GRanges("chrA", IRanges(1:npts, 1:npts)), mode="reverse"),
  colData=DataFrame(totals=runif(nlibs, 1e6, 2e6)))
tryCatch(head(normalizeCNV(data, margins, maxk=100)), error=function(e) e)
head(normalizeCNV(data, margins, maxk=1000))

# Matching margins.
matched <- matchMargins(data, margins)
head(matched)
anchor1.counts <- margins[matched$anchor1,]
anchor2.counts <- margins[matched$anchor2,]

```

pairParam

pairParam class and methods

Description

Class to specify read pair loading parameters

Details

Each pairParam object stores a number of parameters to extract reads from a BAM file. Slots are defined as:

fragments: a GRanges object containing the coordinates of the restriction fragments

restrict: a character vector or 1-by-2 matrix containing the names of allowable chromosomes from which reads will be extracted

discard: a GRanges object containing intervals in which any alignments will be discarded

cap: an integer scalar, specifying the maximum number of read pairs per pair of restriction fragments

The fragments object defines the genomic interval spanned by each restriction fragment. All reads are generated around restriction sites, so the spatial resolution of the experiment depends on such sites. The object can be obtained by applying [cutGenome](#) on an appropriate BSgenome object.

If restrict is supplied, reads will only be extracted for the specified chromosomes. This is useful to restrict the analysis to interesting chromosomes, e.g., no contigs/scaffolds or mitochondria. restrict can also be a n-by-2 matrix, specifying n pairs of chromosomes over which read pairs are to be counted.

If discard is set, a read will be removed if the corresponding alignment is wholly contained within the supplied ranges. Any pairs involving reads discarded in this manner will be ignored. This is useful for removing unreliable alignments in repeat regions.

If cap is set to a non-NA value, an upper bound will be placed on the number of read pairs that are counted for each fragment pair (after any removal due to discard). This protects against spikes in the read pair density throughout the interaction space. Such spikes may be caused by technical artifacts like PCR duplication or repeats, which were not successfully removed in prior processing steps.

Constructor

`pairParam(fragments, discard=GRanges(), restrict=NULL, cap=NA)`: Creates a pairParam object. Each argument is placed in the corresponding slot, with coercion into the appropriate type.

Subsetting

In the code snippets below, x is a pairParam object.

`x$name`: Returns the value in slot name.

Other methods

In the code snippets below, `x` is a `pairParam` object.

`show(x)`: Describes the parameter settings in plain English.

`reform(x, ...)`: Creates a new `pairParam` object, based on the existing `x`. Any named arguments in `...` are used to modify the values of the slots in the new object, with type coercion as necessary.

Author(s)

Aaron Lun

See Also

[cutGenome](#), [squareCounts](#)

Examples

```
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))

blah <- pairParam(cuts)
blah <- pairParam(cuts, discard=GRanges("chrA", IRanges(1, 10)))
blah <- pairParam(cuts, restrict='chr2')
blah$fragments
blah$restrict
blah$cap

# Use 'reform' if only some arguments need to be changed.
blah
reform(blah, restrict='chr3')
reform(blah, discard=GRanges())
reform(blah, cap=10)

# Different restrict options.
pairParam(cuts, restrict=c('chr2', 'chr3'))
pairParam(cuts, restrict=cbind('chr2', 'chr3'))
pairParam(cuts, restrict=cbind(c('chr1', 'chr2'), c('chr3', 'chr4')))
```

plotDI

Construct a plaid plot of differential interactions

Description

Plot differential interactions in a plaid format with informative coloring.

Usage

```
plotDI(data, fc, first.region, second.region=first.region, col.up="red",
       col.down="blue", background="grey70", zlim=NULL, xlab=NULL, ylab=NULL,
       diag=TRUE, ...)
```

```
rotDI(data, fc, region, col.up="red", col.down="blue", background="grey70",
      zlim=NULL, xlab=NULL, max.height=NULL, ylab="Gap", ...)
```

Arguments

<code>data</code>	an <code>InteractionSet</code> object
<code>fc</code>	a numeric vector of log-fold changes
<code>first.region</code>	a <code>GRanges</code> object of length 1 specifying the first region
<code>second.region</code>	a <code>GRanges</code> object of length 1 specifying the second region
<code>region</code>	a <code>GRanges</code> object of length 1 specifying the region of interest
<code>col.up</code>	any type of R color to describe the maximum color for positive log-fold changes
<code>col.down</code>	any type of R color to describe the maximum color for negative log-fold changes
<code>background</code>	any type of R color, specifying the background color of the interaction space
<code>zlim</code>	a numeric scalar indicating the maximum absolute log-fold change
<code>xlab</code>	character string for the x-axis label on the plot, defaults to the first chromosome name
<code>max.height</code>	a numeric scalar indicating the y-axis limit for <code>rotPlaid</code>
<code>ylab</code>	character string for the y-axis label on the plot, defaults to the second chromosome name in <code>plotDI</code>
<code>diag</code>	a logical scalar specifying whether boxes should be shown above the diagonal for intra-chromosomal plots in <code>plotDI</code>
<code>...</code>	other named arguments to be passed to <code>plot</code>

Details

The `plotDI` function constructs a plaid plot on the current graphics device. The intervals of `first.region` and `second.region` are represented by the x- and y-axes, respectively. Each bin pair is represented by a box in the plotting space, where each side of the box represents a bin. Plotting space that is not covered by any bin pair is shown in background.

The color of the box depends on the magnitude and sign of the log-fold change in `fc`. Positive log-FCs will range from white to `col.up`, whereas negative log-FCs will range from white to `col.down`. The chosen color is proportional to the magnitude of the log-FC, and the most extreme colors are only obtained at the maximum absolute log-FC in `fc`. The maximum value can be capped at `zlim` for better resolution of small log-FCs.

If `diag=TRUE`, boxes will also be plotted above the diagonal for intra-chromosomal plots. This is set as the default to avoid confusion when `first.region` is not set as the anchor range, i.e., it has a lower sorting order than `second.region`. However, this can also be turned off to reduce redundancy in visualization around the diagonal.

The `rotDI` function constructs a rotated plot of differential interactions, for visualization of local changes. See `rotPlaid` for more details.

Value

A (rotated) plaid plot of differential interactions is produced on the current graphics device. A function is also invisibly returned that converts log-FCs into colors. This is useful for coordinating the colors, e.g., when constructing a separate color bar.

Author(s)

Aaron Lun

References

Lieberman-Aiden E et al. (2009). Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome. *Science* 326, 289-293.

See Also

[plotPlaid](#), [rotPlaid](#), [squareCounts](#)

Examples

```
# Setting up the objects.
a <- 10
b <- 20
regions <- GRanges(rep(c("chrA", "chrB"), c(a, b)), IRanges(c(1:a, 1:b), c(1:a, 1:b)),
  seqinfo=Seqinfo(seqlengths=c(chrA=a, chrB=b), seqnames=c("chrA", "chrB")))

set.seed(3423)
all.anchor1 <- sample(length(regions), 500, replace=TRUE)
all.anchor2 <- as.integer(runif(500, 1, all.anchor1+1))
out <- InteractionSet(matrix(0, 500, 1), colData=DataFrame(lib.size=1000),
  GInteractions(anchor1=all.anchor1, anchor2=all.anchor2,
    regions=regions, mode="reverse"), metadata=List(width=1))
fc <- runif(nrow(out), -2, 2)

# Constructing intra-chromosomal DI plots around various regions
plotDI(out, fc, first.region=GRanges("chrA", IRanges(1, 10)),
  second.region=GRanges("chrA", IRanges(1, 10)), diag=TRUE)
plotDI(out, fc, first.region=GRanges("chrA", IRanges(1, 10)),
  second.region=GRanges("chrA", IRanges(1, 10)), diag=FALSE)

# Constructing inter-chromosomal DI plots around various regions
xxx <- plotDI(out, fc, first.region=GRanges("chrB", IRanges(1, 10)),
  second.region=GRanges("chrA", IRanges(1, 20)), diag=TRUE)
plotDI(out, fc, first.region=GRanges("chrB", IRanges(1, 100)),
  second.region=GRanges("chrA", IRanges(1, 200)), diag=TRUE, zlim=5)

# Making colorbars.
xxx((-10):10/10)
xxx((-20):20/20)

# Rotated.
```

```
rotDI(out, fc, region=GRanges("chrA", IRanges(1, 200)))
rotDI(out, fc, region=GRanges("chrB", IRanges(1, 200)))
```

plotPlaid	<i>Construct a plaid plot of interactions</i>
-----------	---

Description

Plot interactions between two sequences in a plaid format with informative coloring.

Usage

```
plotPlaid(file, param, first.region, second.region=first.region, width=10000,
  col="black", max.count=20, xlab=NULL, ylab=NULL, diag=TRUE, count=FALSE,
  count.args=list(), ...)
```

```
rotPlaid(file, param, region, width=10000, col="black", max.count=20,
  xlab=NULL, max.height=NULL, ylab="Gap", ...)
```

Arguments

file	character string specifying the path to an index file produced by preparePairs
param	a pairParam object containing read extraction parameters
first.region	a GRanges object of length 1 specifying the first region
second.region	a GRanges object of length 1 specifying the second region
region	a GRanges object of length 1 specifying the region of interest
width	an integer scalar specifying the width of each bin in base pairs
col	any type of R color to describe the color of the plot elements
max.count	a numeric scalar specifying the count for which the darkest color is obtained
xlab	character string for the x-axis label on the plot, defaults to the chromosome name of first.region
max.height	a numeric scalar indicating the y-axis limit for rotPlaid
ylab	character string for the y-axis label on the plot, defaults to the chromosome name of second.regeion in plotPlaid
diag	a logical scalar specifying whether boxes should be shown above the diagonal for intra-chromosomal plots in plotPlaid
count	a logical scalar specifying whether the count for each bin should be plotted in plotPlaid
count.args	a named list of arguments to be passed to text for plotting of bin counts, if count=TRUE
...	other named arguments to be passed to plot

Details

The `plotPlaid` function constructs a plaid plot on the current graphics device. The intervals of the `first.region` and `second.region` are represented by the x- and y-axes, respectively. Each region is partitioned into bins of size `width`. Each bin pair is represented by a box in the plotting space, where each side of the box represents a bin. The color of the box depends on the number of read pairs mapped between the corresponding bins.

The resolution of colors can be controlled by varying `max.count`. All boxes with counts above `max.count` will be assigned the maximum intensity. Other boxes will be assigned a color of intensity proportional to the size of the count, such that a count of zero results in white space. Smaller values of `max.count` will improve contrast at low counts at the cost of contrast at higher counts. Scaling `max.count` is recommended for valid comparisons between libraries of different sizes (e.g., larger `max.count` for larger libraries).

If `count=TRUE`, the number of read pairs will be shown on top of each bin. This will be slower to plot but can be useful in some cases, e.g., when more detail is required, or when the range of colors is not sufficient to capture the range of counts in the data. If `diag=TRUE`, boxes will also be plotted above the diagonal for intra-chromosomal plots. This is set as the default to avoid confusion when `first.region` is not set as the anchor range, i.e., it has a lower sorting order than `second.region`. However, this can also be turned off to reduce redundancy in visualization around the diagonal.

The `rotPlaid` function constructs a plaid plot that has been rotated by 45 degrees. This is useful for visualizing local interactions within a specified region. In a rotated plot, the x-coordinate of a box in the plotting space represents the midpoint between two interacting bins, while the y-coordinate represents the distance between bins. More simply, the interacting bins of a box can be identified by tracing diagonals from the edges of the box to the x-axis.

By default, `max.height` is chosen to include the interaction between the boundaries `region` in `rotPlaid`. This is equivalent to the width of `region`. Smaller values can be chosen to focus on interactions closer to the diagonal. Larger values can also be used, but this is less useful as the interacting bins cannot be easily traced (as they will lie outside the x-axis limits).

Note that the plotted boxes for the bin pairs may overwrite the bounding box of the plot. This can be fixed by running `box()` after each `plotPlaid` call.

Value

A (rotated) plaid plot is produced on the current graphics device. For both functions, a function is invisibly returned that converts counts into colors. This is useful for coordinating the colors, e.g., when constructing a separate color bar.

Author(s)

Aaron Lun

References

Lieberman-Aiden E et al. (2009). Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome. *Science* 326, 289-293.

See Also

[preparePairs](#)

Examples

```

hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
originals <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(originals)

# Setting up parameters
fout <- "temp_saved.h5"
invisible(preparePairs(hic.file, param, fout))

# Constructing intra-chromosomal plaid plots around various regions.
plotPlaid(fout, param, first.region=GRanges("chrA", IRanges(1, 100)),
          second.region=GRanges("chrA", IRanges(1, 200)), width=50, diag=TRUE)
box()
xxx <- plotPlaid(fout, param, first.region=GRanges("chrA", IRanges(1, 100)),
                second.region=GRanges("chrA", IRanges(1, 200)), width=50, diag=FALSE)

# Making colorbars.
xxx(1:2)
xxx(1:5)
xxx(1:10)

# Constructing inter-chromosomal plaid plots around various regions
plotPlaid(fout, param, first.region=GRanges("chrB", IRanges(1, 100)),
          second.region=GRanges("chrA", IRanges(1, 200)), width=50)
plotPlaid(fout, param, first.region=GRanges("chrB", IRanges(1, 100)),
          second.region=GRanges("chrA", IRanges(1, 200)), width=100)

# For a hypothetical second library which is half the size of the previous one:
plotPlaid(fout, param, first.region=GRanges("chrB", IRanges(1, 100)),
          second.region=GRanges("chrA", IRanges(1, 200)), width=100, max.count=20, count=TRUE)
plotPlaid(fout, param, first.region=GRanges("chrB", IRanges(1, 100)),
          second.region=GRanges("chrA", IRanges(1, 200)), width=100, max.count=40,
          count=TRUE, count.args=list(col="blue"))

# Rotated
rotPlaid(fout, param, region=GRanges("chrA", IRanges(1, 200)), width=50)
rotPlaid(fout, param, region=GRanges("chrA", IRanges(1, 200)), width=100)

```

```
preparePairs
```

Prepare Hi-C pairs

Description

Identifies the interacting pair of restriction fragments corresponding to each read pair in a Hi-C library.

Usage

```
preparePairs(bam, param, file, dedup=TRUE, minq=NA,
             ichim=TRUE, chim.dist=NA, output.dir=NULL)
```

Arguments

<code>bam</code>	a character string containing the path to a name-sorted BAM file
<code>param</code>	a <code>pairParam</code> object containing read extraction parameters
<code>file</code>	a character string specifying the path to an output index file
<code>dedup</code>	a logical scalar indicating whether marked duplicate reads should be removed
<code>minq</code>	an integer scalar specifying the minimum mapping quality for each read
<code>ichim</code>	a logical scalar indicating whether invalid chimeras should be counted
<code>chim.dist</code>	an integer scalar specifying the maximum distance between segments for a valid chimeric read pair
<code>output.dir</code>	a character string specifying a directory for temporary files

Value

Multiple dataframe objects are stored within the specified file using the HDF5 format. Each object corresponds to a pair of chromosomes, designated as the `anchor1` (later) or `anchor2` (earlier) chromosome based on the order of their names. Each row of the dataframe contains information for a read pair, with one read mapped to each chromosome. The dataframe contains several integer fields:

`anchor1.id`, `anchor2.id`: index of the `anchor1` or `anchor2` restriction fragment

`anchor1.pos`, `anchor2.pos`: mapping coordinate of the read (or for chimeras, the 5' segment thereof) on the `anchor1` or `anchor2` fragment

`anchor1.len`, `anchor2.len`: length of the alignment on the `anchor1` or `anchor2` fragment, set to a negative value if the alignment is on the reverse strand

See [getPairData](#) for more details on length, orientation and gap.

An integer vector is also returned from the function, containing various diagnostics:

`pairs`: an integer vector containing `total`, the total number of read pairs; `marked`, read pairs with at least one marked 5' end; `filtered`, read pairs where the MAPQ score for either 5' end is below `minq`; `mapped`, read pairs considered as successfully mapped (i.e., not filtered, and also not marked if `dedup=TRUE`)

`same.id`: an integer vector containing `dangling`, the number of read pairs that are dangling ends; and `self.circles`, the number of read pairs forming self-circles

`singles`: an integer scalar specifying the number of reads without a mate

`chimeras`: an integer vector containing `total`, the total number of read pairs with one chimeric read; `mapped`, chimeric read pairs with both 5' ends mapped; `multi`, mapped chimeric pairs with at least one successfully mapped 3' segment; and `invalid`, read pairs where the 3' location of one read disagrees with the 5' location of the mate

Converting to restriction fragment indices

The resolution of a Hi-C experiment is defined by the distribution of restriction sites across the genome. Thus, it makes sense to describe interactions in terms of restriction fragments. This function identifies the interacting fragments corresponding to each pair of reads in a Hi-C library. To save space, it stores the indices of the interacting fragments for each read pair, rather than the fragments themselves.

Indexing is performed by matching up the mapping coordinates for each read with the restriction fragment boundaries in `param$fragments`. Needless to say, the boundary coordinates in `param$fragments` must correspond to the reference genome being used. In most cases, these can be generated using the `cutGenome` function from any given `BSgenome` object. If, for any reason, a modified genome is used for alignment, then the coordinates of the restriction fragments on the modified genome are required.

Each read pair subsequently becomes associated with a pair of restriction fragments. The `anchor1` fragment is that with the higher genomic coordinate, i.e., the larger index in `param$fragments`. The `anchor2` fragment is that with the smaller coordinate/index. This definition avoids the need to consider both permutations of indices in a pair during downstream processing.

Handling of read pairs

For pairs with chimeric reads, the alignment of the 5' end of the read is used to identify the interacting fragments. Invalid chimeras arise when the position of the 3' segment of a chimeric read is not consistent with that of the mate read. Invalid chimeric pairs are generally indicative of mapping errors but can also form due to non-specific ligation events. While they can be explicitly removed, setting `ichim=TRUE` is recommended to avoid excessive filtering of reads when alignment of short chimeric segments is inaccurate.

By default, invalid chimeras are defined as all pairs where the 3' segment and the mate do not map onto the same restriction fragment in an inward-facing orientation. Alternatively, a distance-based threshold can be used by setting `chim.dist` to some reasonable value, e.g., 1000 bp. Chimeras are only considered invalid if the distance between the segment and mate is greater than `chim.dist` (or if the alignments are not inward-facing). This may be more relevant in situations involving inefficient cleavage, where the mapping locations are broadly consistent but do not fall in the same restriction fragment.

Self-circles are outward-facing read pairs mapped to the same restriction fragment. These are formed from inefficient cross-linking and are generally uninformative. Dangling ends are inward-facing read pairs mapped to the same fragment, and are generated from incomplete ligation of blunt ends. Both constructs are detected and discarded within the function. Note that this does not consider dangling ends or self-circles formed from incompletely digested fragments, which must be removed with `prunePairs`.

In all cases, if the 5' end of either read is unavailable (e.g. unmapped, mapping quality score below `minq`, marked as a duplicate), the read pair is recorded as unpaired and discarded. By default, no MAPQ filtering is performed when `minq` is set to NA. Any duplicate read must be marked in the bit field of the BAM file using a tool like Picard's `MarkDuplicates` if it is to be removed with `dedup=TRUE`. For chimeric reads, the recommended approach is to designate the 5' end as the primary alignment. This ensures that the duplicate computations are performed on the most relevant alignments for each read pair.

Miscellaneous information

If `output.dir` is not specified, a directory name is constructed using the `tempfile` command. If it is specified, users should make sure that no file already exists with that name. Otherwise, an error will be raised. This directory is used to store intermediate files that will be eventually processed into the HDF5 output file.

Users should note that the use of a `pairParam` object for input is strictly for convenience. Any non-empty values of `param$discard` and `param$restrict` will be ignored here. Reads will not be discarded if they lie outside the specified chromosomes, or if they lie within blacklisted regions.

Author(s)

Aaron Lun, with use of Rhtslib based on comments from Alessandro Mammana.

References

Imakaev M et al. (2012). Iterative correction of Hi-C data reveals hallmarks of chromosome organization. *Nat. Methods* 9, 999-1003.

Belton, RP et al. (2012). Hi-C: a comprehensive technique to capture the conformation of genomes. *Methods* 58, 268-276.

See Also

[cutGenome](#), [prunePairs](#), [mergePairs](#), [getPairData](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)
```

```
tmpf <- "gunk.h5"
preparePairs(hic.file, param, tmpf)
preparePairs(hic.file, param, tmpf, minq=50)
preparePairs(hic.file, param, tmpf, ichim=TRUE)
preparePairs(hic.file, param, tmpf, dedup=FALSE)
```

prunePairs

Prune read pairs

Description

Prune the read pairs that represent potential artifacts in a Hi-C library

Usage

```
prunePairs(file.in, param, file.out=file.in, max.frag=NA, min.inward=NA, min.outward=NA)
```


Arguments

<code>file.in</code>	a character string specifying the path to the index file produced by preparePairs
<code>param</code>	a <code>pairParam</code> object containing read extraction parameters
<code>file.out</code>	a character string specifying a path to an output index file
<code>max.frag</code>	an integer scalar specifying the maximum length of any sequenced DNA fragment
<code>min.inward</code>	an integer scalar specifying the minimum distance between inward-facing reads on the same chromosome
<code>min.outward</code>	an integer scalar specifying the minimum distance between outward-facing reads on the same chromosome

Details

This function removes potential artifacts from the input index file, based on the coordinates of the reads in each pair. A `pairParam` object is only used here for consistency; only `param$fragments` will be used. Any values of `param$restrict` or `param$discard` will be ignored.

Non-NA values for `min.inward` and `min.outward` are designed to protect against dangling ends and self-circles, respectively. This is particularly true when digestion is incomplete, as said structures do not form within a single restriction fragment and cannot be identified earlier. These can be removed by discarding inward- and outward-facing read pairs that are too close together.

A finite value for `max.frag` also protects against non-specific cleavage. This refers to the length of the actual DNA fragment used in sequencing and is computed from the distance between each read and its nearest downstream restriction site. Off-target cleavage will result in larger distances than expected.

Note the distinction between *restriction* fragments and *sequencing* fragments. The former is generated by pre-ligation digestion, and is of concern when choosing `min.inward` and `min.outward`. The latter is generated by post-ligation shearing and is of concern when choosing `max.frag`.

Suitable values for each parameter can be obtained with the output of [getPairData](#). For example, values for `min.inward` can be obtained by setting a suitable lower bound on the distribution of non-NA values for `gap` with orientation values of 1.

Value

An integer vector is invisibly returned, containing `total`, the total number of read pairs; `length`, the number of read pairs with fragment lengths greater than `max.frag`; `inward`, the number of inward-facing read pairs with gap distances less than `min.inward`; and `outward`, the number of outward-facing read pairs with gap distances less than `min.outward`.

Multiple data frame objects are also produced within the specified out file, for each corresponding data frame object in `file.in`. For each object, the number of rows may be reduced due to the removal of read pairs corresponding to potential artifacts.

Author(s)

Aaron Lun

References

Jin F et al. (2013). A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* doi:10.1038/nature12644.

See Also

[preparePairs](#), [getPairData](#), [squareCounts](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

require(rhdf5)
fout <- "temp.h5"
fout2 <- "temp2.h5"
invisible(preparePairs(hic.file, param, fout))
x <- prunePairs(fout, param, fout2)
h5read(fout2, "chrA/chrA")

x <- prunePairs(fout, param, fout2, max.frag=50)
h5read(fout2, "chrA/chrA")

x <- prunePairs(fout, param, fout2, min.inward=50)
h5read(fout2, "chrA/chrA")

x <- prunePairs(fout, param, fout2, min.outward=50)
h5read(fout2, "chrA/chrA")
```

savePairs

Save Hi-C interactions

Description

Save a dataframe of interactions into a directory structure for rapid chromosomal access.

Usage

```
savePairs(x, file, param)
```

Arguments

x	a sorted dataframe with integer fields anchor1.id and anchor2.id for each interaction
file	a character string specifying the path for the output index file
param	a pairParam object containing read extraction parameters

Details

This function facilitates the input of processed Hi-C data from other sources into the current pipeline. Each entry in `x$anchor1.id` and `x$anchor2.id` refers to the index of `param$fragments` to denote the interacting regions. The `x` object will be resorted by `anchor1.id`, then `anchor2.id`. If necessary, `anchor1` and `anchor2` indices will be switched such that the former is never less than the latter.

The coordinates of the restriction fragment boundaries in `param$fragments` should correspond to the reference genome being used. In most cases, these can be generated using the [cutGenome](#) function from any given `BSgenome` object. Values of `param$discard` and `param$restrict` will not be used here and can be ignored.

Any additional fields in `x` will also be saved to file. Users are recommended to put in `anchor1.pos`, `anchor1.len`, `anchor2.pos` and `anchor2.len` fields. This will allow removal of reads in `param$discard` during counting (e.g., with [squareCounts](#)). It will also allow proper calculation of statistics with [getPairData](#), and quality control with [prunePairs](#). See the output of [preparePairs](#) for more details on the specification of the fields.

Value

An index file is produced at the specified file location, containing the interaction data. A NULL value is invisibly returned.

Author(s)

Aaron Lun

See Also

[preparePairs](#), [cutGenome](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

n <- 1000
all.a <- as.integer(runif(n, 1L, length(cuts)))
all.t <- as.integer(runif(n, 1L, length(cuts)))
x <- data.frame(anchor1.id=all.a, anchor2.id=all.t,
  anchor1.pos=runif(1:100), anchor1.len=10,
  anchor2.pos=runif(1:100), anchor2.len=-10)

fout <- "temp2.h5"
savePairs(x, fout, param)
require(rhdf5)
head(h5read(fout, "chrA/chrA"))
```

squareCounts

Load Hi-C interaction counts

Description

Collate count combinations for interactions between pairs of bins across multiple Hi-C libraries.

Usage

```
squareCounts(files, param, width=50000, filter=1L)
```

Arguments

files	a character vector containing paths to the index files generated from each Hi-C library
param	a pairParam object containing read extraction parameters
width	an integer scalar specifying the width of each bin in base pairs
filter	an integer scalar specifying the minimum count for each square

Details

The genome is first split into non-overlapping adjacent bins of size `width`. These bins are rounded to the nearest restriction site, forming the sides of potential squares. The number of restriction fragments in each bin is stored as `nfrags` in the metadata of the output region. Each square represents an interaction between the corresponding rounded bins on the genome. The number of read pairs between each pair of sides is counted for each library to obtain the count for the corresponding square.

Larger counts can be collected by increasing the value of `width`. This can improve detection power by increasing the evidence for significant differences. However, this comes at the cost of spatial resolution as adjacent events in the same bin or square can no longer be distinguished. This may reduce detection power if counts for differential interactions are contaminated by counts for non-differential interactions.

Low abundance squares with count sums below `filter` are not reported. This reduces memory usage for large datasets. These squares are probably uninteresting as detection power will be poor for low counts. Another option is to increase `width` to reduce the total number of bins in the genome (and hence, the possible number of bin pairs).

Counting will consider the values of `restrict`, `discard` and `cap` in `param`. See [pairParam](#) for more details.

Value

An `InteractionSet` object is returned containing the number of read pairs for each bin pair across all libraries. Bin pairs are stored as a `ReverseStrictGInteractions` object.

Author(s)

Aaron Lun

References

Imakaev M et al. (2012). Iterative correction of Hi-C data reveals hallmarks of chromosome organization. *Nat. Methods* 9, 999-1003.

Lieberman-Aiden E et al. (2009). Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome. *Science* 326, 289-293.

See Also

[preparePairs](#), [cutGenome](#), [InteractionSet-class](#), [ReverseStrictGInteractions-class](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(fragments=cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, file=fout))

# Collating to count combinations.
y <- squareCounts(fout, param)
head(assay(y))
y <- squareCounts(fout, param, filter=1)
head(assay(y))
y <- squareCounts(fout, param, width=50, filter=1)
head(assay(y))
y <- squareCounts(fout, param, width=100, filter=1)
head(assay(y))

# Attempting with other parameters.
y <- squareCounts(fout, reform(param, restrict="chrA"), width=100, filter=1)
head(assay(y))
y <- squareCounts(fout, filter=1,
  param=reform(param, restrict=cbind("chrA", "chrB")))
head(assay(y))
y <- squareCounts(fout, filter=1,
  param=reform(param, cap=1), width=100)
head(assay(y))
y <- squareCounts(fout, width=100, filter=1,
  param=reform(param, discard=GRanges("chrA", IRanges(1, 50))))
head(assay(y))
```

totalCounts	<i>Get the total counts</i>
-------------	-----------------------------

Description

Get the total number of read pairs in a set of Hi-C libraries.

Usage

```
totalCounts(files, param)
```

Arguments

files	a character vector containing paths to the index files generated from each Hi-C library
param	a pairParam object containing read extraction parameters

Details

As the name suggests, this function counts the total number of read pairs in each index file prepared by [preparePairs](#). Use of param\$fragments ensures that the chromosome names in each index file are consistent with those in the desired genome (e.g., from [cutGenome](#)). Counting will also consider the values of restrict, discard and cap in param.

Value

An integer vector is returned containing the total number of read pairs in each library.

Author(s)

Aaron Lun

See Also

[preparePairs](#), [cutGenome](#), [pairParam](#), [squareCounts](#)

Examples

```
hic.file <- system.file("exdata", "hic_sort.bam", package="diffHic")
cuts <- readRDS(system.file("exdata", "cuts.rds", package="diffHic"))
param <- pairParam(cuts)

# Setting up the parameters
fout <- "output.h5"
invisible(preparePairs(hic.file, param, file=fout))

# Counting totals, and comparing them.
totalCounts(fout, param)
squareCounts(fout, param, width=10)$totals
```

```
new.param <- reform(param, restrict="chrA")
totalCounts(fout, new.param)
squareCounts(fout, new.param, width=10)$totals

new.param <- reform(param, discard=GRanges("chrA", IRanges(1, 50)))
totalCounts(fout, new.param)
squareCounts(fout, new.param, width=10)$totals

new.param <- reform(param, cap=1)
totalCounts(fout, new.param)
squareCounts(fout, new.param, width=10)$totals
```

Index

- *Topic **annotation**
 - annotatePairs, 2
- *Topic **clustering**
 - boxPairs, 4
 - clusterPairs, 6
 - compartmentalize, 8
- *Topic **counting**
 - connectCounts, 10
 - DIList-class, 21
 - marginCounts, 40
 - neighborCounts, 43
 - pairParam, 47
 - squareCounts, 60
 - totalCounts, 62
- *Topic **diagnostics**
 - getPairData, 38
- *Topic **documentation**
 - diffHicUsersGuide, 20
- *Topic **filtering**
 - enrichedPairs, 27
 - Filtering diagonals, 30
 - Filtering methods, 31
 - getArea, 35
- *Topic **normalization**
 - correctedContact, 14
 - normalizeCNV, 44
- *Topic **preprocessing**
 - cutGenome, 17
 - DNaseHiC, 24
 - loadData, 39
 - mergePairs, 42
 - preparePairs, 53
 - prunePairs, 56
 - savePairs, 58
- *Topic **testing**
 - consolidatePairs, 12
 - diClusters, 19
- *Topic **visualization**
 - plotDI, 48
 - plotPlaid, 51
- [, DIList, ANY, ANY, ANY-method (DIList-class), 21
- [, DIList, ANY, ANY-method (DIList-class), 21
- \$, DIList-method (DIList-class), 21
- \$, pairParam-method (pairParam), 47
- \$<-, DIList-method (DIList-class), 21
- anchors (DIList-class), 21
- anchors, DIList-method (DIList-class), 21
- annotatePairs, 2
- as.matrix, DIList-method (DIList-class), 21
- asDGEList (DIList-wrappers), 23
- asDGEList, DIList-method (DIList-wrappers), 23
- aveLogCPM, 34
- boxPairs, 4, 7, 13
- c, DIList-method (DIList-class), 21
- clusterPairs, 3, 5, 6, 13, 19, 20
- colData, DIList-method (DIList-class), 21
- combineTests, 13
- compartmentalize, 8
- connectCounts, 6, 10, 35–37
- consolidatePairs, 12
- controlClusterFDR, 19, 20
- correctedContact, 14
- counts (DIList-class), 21
- counts, DIList-method (DIList-class), 21
- cutGenome, 17, 25, 47, 48, 55, 56, 59, 61, 62
- DGEList, 24
- DI2IS (DIList-class), 21
- diClusters, 7, 19
- diffHic (diffHicUsersGuide), 20
- diffHicUsersGuide, 20
- DIList (DIList-class), 21

- DIList-class, [21](#)
- DIList-wrappers, [23](#)
- dim, DIList-method (DIList-class), [21](#)
- dimnames, DIList-method (DIList-class), [21](#)
- DNaseHiC, [24](#)
- domainDirections, [26](#)
- enrichedPairs, [27](#), [34](#), [43](#), [44](#)
- exptData, DIList-method (DIList-class), [21](#)
- exptData<- , DIList, SimpleList-method (DIList-class), [21](#)
- filterDiag (Filtering diagonals), [30](#)
- filterDirect (Filtering methods), [31](#)
- Filtering diagonals, [30](#)
- Filtering methods, [31](#)
- filterPeaks, [29](#), [34](#)
- filterTrended, [8](#), [9](#), [29](#)
- filterTrended (Filtering methods), [31](#)
- findOverlaps, [3](#), [11](#)
- getArea, [35](#)
- getDistance, [30](#), [37](#)
- getPairData, [38](#), [54](#), [56–59](#)
- kmeans, [8](#), [9](#)
- loadChromos (loadData), [39](#)
- loadData, [39](#)
- locfit, [45](#), [46](#)
- loessFit, [32](#)
- lp, [46](#)
- marginCounts, [40](#), [45](#), [46](#)
- match, [5](#)
- matchMargins (normalizeCNV), [44](#)
- matchPattern, [18](#)
- mergePairs, [42](#), [56](#)
- mg1mOneGroup, [15](#), [16](#)
- neighborCounts, [34](#), [43](#)
- normalize (DIList-wrappers), [23](#)
- normalize, DIList-method (DIList-wrappers), [23](#)
- normalizeCNV, [44](#)
- normOffsets (DIList-wrappers), [23](#)
- normOffsets, DIList-method (DIList-wrappers), [23](#)
- pairdist, [31](#)
- pairParam, [11](#), [41](#), [47](#), [60](#), [62](#)
- pairParam-class (pairParam), [47](#)
- plot, [49](#), [51](#)
- plotDI, [48](#)
- plotPlaid, [50](#), [51](#)
- preparePairs, [25](#), [38](#), [39](#), [42](#), [51](#), [52](#), [53](#), [57–59](#), [61](#), [62](#)
- prepPseudoPairs (DNaseHiC), [24](#)
- prunePairs, [38](#), [42](#), [55](#), [56](#), [56](#), [59](#)
- reform (pairParam), [47](#)
- reform, pairParam-method (pairParam), [47](#)
- regions (DIList-class), [21](#)
- regions, DIList-method (DIList-class), [21](#)
- rotDI (plotDI), [48](#)
- rotPlaid, [49](#), [50](#)
- rotPlaid (plotPlaid), [51](#)
- savePairs, [58](#)
- scaledAverage, [33](#)
- segmentGenome (DNaseHiC), [24](#)
- show, DIList-method (DIList-class), [21](#)
- show, pairParam-method (pairParam), [47](#)
- squareCounts, [4–9](#), [11](#), [15](#), [16](#), [24](#), [27–30](#), [32–37](#), [41](#), [43–46](#), [48](#), [50](#), [58](#), [59](#), [60](#), [62](#)
- Sweave, [21](#)
- system, [21](#)
- targets (DIList-class), [21](#)
- targets, DIList-method (DIList-class), [21](#)
- tempfile, [56](#)
- text, [51](#)
- totalCounts, [62](#)