

# Package ‘contibAIT’

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**Type** Package

**Title** Improves Early Build Genome Assemblies using Strand-Seq Data

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**Description** Using strand inheritance data from multiple single cells from the organism whose genome is to be assembled, contibAIT can cluster unbridged contigs together into putative chromosomes, and order the contigs within those chromosomes.

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**Depends** BH (>= 1.51.0-3), Rsamtools (>= 1.21)

**LinkingTo** Rcpp, BH

**Imports** data.table, grDevices, clue, cluster, gplots, BiocGenerics (>= 0.31.6), S4Vectors, IRanges, GenomicRanges, Rcpp, TSP, GenomicFiles, gtools, rtracklayer, BiocParallel, DNACopy, colorspace, reshape2, ggplot2, methods, exomeCopy, GenomicAlignments, diagram

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'barplotLinkageGroupCalls.R' 'clusterContigs.R'  
'computeConsensus.R' 'computeSim.R' 'contibAIT.R'  
'findSexGroups.R' 'findSimilarLibraries.R' 'fixLinkageGroups.R'  
'flipOrderedLinkageGroups.R' 'highlightAssemblyErrors.R'  
'ideogramPlot.R' 'locateMisorients.R' 'makeBoxPlot.R'  
'makeChrTable.R' 'mapGapFromOverlap.R' 'mergeFlankedLGs.R'  
'orderAllLinkageGroups.R' 'orderContigsGreedy.R'  
'orderContigsTSP.R' 'plotContigOrder.R' 'plotLGDistances.R'  
'plotWCdistribution.R' 'preprocessStrandTable.R'  
'reorientAndMergeLGs.R' 'strandSeqFreqTable.R' 'thoroughBed.R'  
'writeBed.R'

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BAIT	<i>BAIT – master function to process strand-seq libraries into BAIT ideograms</i>
------	---

---

### Description

BAIT – master function to process strand-seq libraries into BAIT ideograms

### Usage

```
BAIT(path = ".", splitBy = 2e+05, readQual = 10, pairedEnd = TRUE,
      plotBy = "lib", plotName = NULL, chroms = "all", verbose = TRUE)
```

### Arguments

path	String denoting location of Strand-seq bam files (default is ".")
splitBy	numeric value of binsize for plotting. default is 200000
readQual	Integer dictating the minimal mapping quality required for a read to be accepted. Default is 10.
pairedEnd	Whether the bam files being read are in paired end format. Default is TRUE. Note,
plotBy	Whether to plot by library ('lib') or chromosome ('chr')
plotName	character which determines file name to be saved. Default is to open an R plot from the terminal
chroms	vector of chromosome number to prevent contig plotting. eg for humans use 1:24. Default is 'all'
verbose	prints messages to the terminal (default is TRUE)

**Value**

ideogram plots

**Examples**

```
## Not run:

#Not run because this takes a minute or two:
bamPath=file.path(system.file(package='contiBAIT'), 'extdata')

BAIT(bamPath, pairedEnd = FALSE) #our example data is single-end

## End(Not run)
```

---

*barplotLinkageGroupCalls,LinkageGroupList,ChrTable-method*

*Bar plot all linkage groups, with the true chromosomes of contigs coloured.*

---

**Description**

Bar plot all linkage groups, with the true chromosomes of contigs coloured.

**Usage**

```
## S4 method for signature 'LinkageGroupList,ChrTable'
barplotLinkageGroupCalls(object, chrTable,
  by = "lg", bySize = TRUE, returnTable = NULL, whichGroup = NULL,
  percentage = NULL)
```

**Arguments**

object	LinkageGroupList, as generated by clusterContigs
chrTable	GRanges object containing assembly information about the contigs, including a meta column called 'name' that has names matching the object. Note that the rownames of chrTable should be the contig names, as they are used in object, and the first column (chromosome name) will be used to order by chromosome if 'chr' option used in by parameter. To use a bam file header, the product of makeChrTable(bamFile) is suitable for input
by	whether to plot by linkage group (if 'lg') or chromosomes ('chr')
bySize	logical value to return barplot either with LGs sorted by number of contigs or size (in Mb). Default is TRUE.
returnTable	Logical to return chromosome length matrix. Default is NULL

whichGroup	Numeric vector of groups to analyse. If by='lg', then only the subset of LGs selected by whichGroup will be analyzed. If by='chr', then only subset of chromosomes selected by whichGroup will be analyzed. Note to include legend, use legend=rownames(chr.table) for by='lg', and legend=colnames(chr.table) for by='chr'
percentage	Logical that returns the percentage of different chromosomes or LG within the barplot. Default is NULL

**Value**

a matrix of lengths of each chromosome (rows) in each linkage group (columns)

**Examples**

```
data("exampleLGList")
data("exampleDividedChr")

barplotLinkageGroupCalls(exampleLGList, exampleDividedChr)
```

---

ChrTable-class      *A class for storing chromosome/fragment lengths*

---

**Description**

This class is a GRanges object with a meta column called name, which represents the fragment name.

**Usage**

```
ChrTable(chrRanges = GRanges())
```

**Arguments**

chrRanges	a GRanges object with a meta column called name, which represents the fragment name
-----------	---

**Value**

a ChrTable

---

clusterContigs,StrandStateMatrix-method

*clusterContigs – agglomeratively clusters contigs into linkage groups based on strand inheritance*

---

## Description

clusterContigs – agglomeratively clusters contigs into linkage groups based on strand inheritance

## Usage

```
## S4 method for signature 'StrandStateMatrix'
clusterContigs(object, similarityCutoff = 0.7,
  recluster = NULL, minimumLibraryOverlap = 5, randomise = TRUE,
  randomSeed = NULL, randomWeight = NULL, clusterParam = NULL,
  clusterBy = "hetero", verbose = TRUE)
```

## Arguments

object	data.frame containing strand inheritance information for every contig (rows) in every library (columns). This should be the product of strandSeqFreqTable
similarityCutoff	place contigs in a cluster when their strand state is at least this similar
recluster	Number of times to recluster and take the consensus of. If NULL, clustering is run only once.
minimumLibraryOverlap	for two contigs to be clustered together, the strand inheritance must be present for both contigs in at least this many libraries (in addition to their similarity being at least similarityCutoff)
randomise	whether to reorder contigs before clustering
randomSeed	random seed to initialize clustering
randomWeight	vector of weights for contigs for resampling. If NULL, uniform resampling is used. Typically this should be a measure of contig quality, such as library coverage, so that clustering tends to start from the better quality contigs.
clusterParam	optional BiocParallelParam specifying cluster to use for parallel execution. When NULL, execution will be serial.
clusterBy	Method for performing clustering. Default is 'hetero' (for comparing heterozygous calls to homozygous). Alternative is 'homo' (for comparison between the two homozygous calls)
verbose	prints function progress

**Details**

Note that a more stringent similarity cutoff will result in more clusters, and a longer run time, since at every iteration a distance is computed to the existing clusters. However, in lower-quality data, a more stringent cutoff may be necessary to reduce the number of contigs that are erroneously grouped.

Note that clusterParam requires BiocParallel to be installed.

**Value**

LinkageGroupList of vectors containing labels of contigs belonging to each linkage group

**Examples**

```
data("exampleWCMatrix")

clusteredContigs <- clusterContigs(exampleWCMatrix, verbose=FALSE)
show(clusteredContigs)
show(clusteredContigs[[1]])
```

---

contiBAIT	<i>contiBAIT – master function to process strand-seq libraries into ordered linkage groups</i>
-----------	--

---

**Description**

contiBAIT – master function to process strand-seq libraries into ordered linkage groups

**Usage**

```
contiBAIT(path = ".", cluster = 1, clusterParam = NULL,
  saveName = FALSE, filter = FALSE, readQual = 10, readLimit = 10,
  pairedEnd = TRUE, makePlots = FALSE, verbose = TRUE)
```

**Arguments**

- path                   String denoting location of Strand-seq bam files (default is ".")
- cluster               Integer denoting the number of reclusterings to be performed for creating linkage groups (default is 1)
- clusterParam       Number of parallel processors to use when clustering contigs. Default is NULL.
- saveName            String denoting the file name for saved data. If FALSE, no intermediate files are saved (default is FALSE)
- filter              additional file to split chromosomes based on locations. If this parameter is blank, a filter table will be automatically generated from the header of the first file in bamFileList
- readQual            Integer dictating the minimal mapping quality required for a read to be accepted. Default is 10.

readLimit	Minimum number of reads on a contig to make a strand call. Default is 10
pairedEnd	Whether the bam files being read are in paired end format. Default is TRUE. Note, since paired reads will be the same direction, only first mate read of pair is used in output
makePlots	Logical determining whether plots should be created. Default is TRUE
verbose	prints messages to the terminal (default is TRUE)

**Value**

ordered contigs in bed format. Depending on options, intermediate files and plots will also be generated

**Examples**

```
#Get a list of BAM files containing libraries for cells from the same organism, aligned to the same genome
#In this case these are the example BAM files provided with the package (hence the call to system.file);
data("exampleDividedChr")
library(BiocParallel)

example.dir <- file.path(system.file(package='contigBAIT'), 'extdata')

orderedContigs <- contigBAIT(path=example.dir,
cluster=1,
clusterParam=MulticoreParam(),
filter=exampleDividedChr,
pairedEnd=FALSE)
```

---

ContigOrdering-class *A class for storing contig ordering of a linkage group*

---

**Description**

This class is a matrix of two character vectors that represent the calculated ordering of a linkage group. The first element of this matrix is the Linkage Group sub-setted by contigs with equal strand states across all libraries in the calculated order. The second element is the names of names of each contig in the calculated order.

**Usage**

```
ContigOrdering(ordering = character())
```

**Arguments**

ordering a matrix of two character vectors that represent the calculated ordering of a linkage group. The first element of this matrix is the Linkage Group sub-setted by contigs with equal strand states across all libraries in the calculated order. The second element is the names of names of each contig in the calculated order.



**Value**

a ContigOrdering

**Examples**

```
thisOrdering <- ContigOrdering(matrix(ncol=2, c( "LG1.11", "chr2:1000820-2001640",
"LG1.1", "chr2:3002461-4003281")))
```

---

exampleChrTable	<i>Example of a ChromosomeTable, containing contigs and their lengths</i>
-----------------	---

---

**Description**

Example of a ChromosomeTable, containing contigs and their lengths

---

exampleContigOrder	<i>Example of a ContigOrdering table, containing a list with a matrix of ordered groups element and a StrandStateList element</i>
--------------------	---

---

**Description**

Example of a ContigOrdering table, containing a list with a matrix of ordered groups element and a StrandStateList element

---

exampleCrickFreq	<i>An example Crick strand frequencies extracted from BAMS by strandSeqFreqTable where BAITtables=TRUE</i>
------------------	--

---

**Description**

An example Crick strand frequencies extracted from BAMS by strandSeqFreqTable where BAITtables=TRUE

---

exampleDividedChr	<i>Example of a divided chromosome, containing contigs and their lengths</i>
-------------------	--

---

**Description**

Example of a divided chromosome, containing contigs and their lengths

---

exampleLGList	<i>Example of a LinkageGroupList output from clusterContigs</i>
---------------	---

---

**Description**

Example of a LinkageGroupList output from clusterContigs

---

exampleLibList	<i>Example of a LibraryGroupList, containing library names</i>
----------------	--

---

**Description**

Example of a LibraryGroupList, containing library names

---

exampleReadCounts	<i>Example of read counts extracted from BAMS by strandSeqFreqTable</i>
-------------------	---

---

**Description**

Example of read counts extracted from BAMS by strandSeqFreqTable

---

exampleStrandFreq	<i>Example of strand frequencies extracted from BAMS by strandSeqFreqTable</i>
-------------------	--

---

**Description**

Example of strand frequencies extracted from BAMS by strandSeqFreqTable

---

exampleWatsonFreq	<i>An example Watson strand frequencies extracted from BAMS by strandSeqFreqTable where BAITables=TRUE</i>
-------------------	--

---

**Description**

An example Watson strand frequencies extracted from BAMS by strandSeqFreqTable where BAITables=TRUE

---

exampleWCMatrix	<i>An example StrandStateMatrix containing WW, CC and WC calls for contigs</i>
-----------------	--

---

**Description**

An example StrandStateMatrix containing WW, CC and WC calls for contigs

---

findSexGroups,LinkageGroupList,StrandStateMatrix-method	<i>Bar plot all linkage groups, with the true chromosomes of contigs coloured.</i>
---	--

---

**Description**

Bar plot all linkage groups, with the true chromosomes of contigs coloured.

**Usage**

```
## S4 method for signature 'LinkageGroupList,StrandStateMatrix'
findSexGroups(linkageGroupList,
  strandStateMatrix, callThreshold = 0.2)
```

**Arguments**

linkageGroupList	a LinkageGroupList object as generated by clusterContigs
strandStateMatrix	a StrandStateMatrix object as generated by preprocessStrandTable
callThreshold	a value between 0 and 1 to threshold the proportion of libraries in which scaffolds are heterozygous (ie unlikely to be a monosome sex chromosome). Default value is 0.2

**Value**

the same LinkageGroupList entered into the function, but with potential sex chromosomes highlighted in the name attribute

---

```
findSimilarLibraries,StrandStateMatrix,StrandReadMatrix,ChrTable-method
  findSimilarLibraries – function to identify libraries that hare similar
  WC patterns on chromosomes
```

---

**Description**

`findSimilarLibraries` – function to identify libraries that hare similar WC patterns on chromosomes

**Usage**

```
## S4 method for signature 'StrandStateMatrix,StrandReadMatrix,ChrTable'
findSimilarLibraries(strandStateMatrix,
  strandReadMatrix, chrGrange, chrNum, cluster = 1, clusterParam = NULL,
  verbose = TRUE)
```

**Arguments**

<code>strandStateMatrix</code>	A <code>strandStateMatrix</code> object for all libraries across split fragments, derived from <code>preprocessStrandTable</code>
<code>strandReadMatrix</code>	The number of reads present for each <code>strandStateMatrix</code> element. An object of type <code>strandReadMatrix</code> from <code>strandSeqFreqTable</code>
<code>chrGrange</code>	File of type <code>ChrTable</code> (a <code>GRanges</code> object with a meta column titled 'name' determining contig name) to split chromosomes based on locations. name meta should match the rownames of <code>strandStateMatrix</code> and <code>strandReadMatrix</code>
<code>chrNum</code>	The chromosome number to analyse
<code>cluster</code>	Number of times to recluster and take the consensus of. If <code>NULL</code> , clustering is run only once
<code>clusterParam</code>	optional <code>BiocParallelParam</code> specifying cluster to use for parallel execution. When <code>NULL</code> , execution will be serial.
<code>verbose</code>	prints messages to the terminal (default is <code>TRUE</code> )

**Value**

a list of type `LinkageGroupList` with two elements; libraries that are mostly Watson, and those that are mostly Crick

**Examples**

```
#Get a list of BAM files containing libraries for cells from the same organism, aligned to the same genome
#In this case these are the example BAM files provided with the package (hence the call to system.file);
data("exampleDividedChr")
data("exampleWCMatrix")
data("exampleReadCounts")
```

```

library(BiocParallel)

example.dir <- file.path(system.file(package='contibAIT'), 'extdata')

chrGrange <- exampleDividedChr[which(exampleDividedChr$name %in% rownames(exampleWCMatrix))]

exampleLibList <- lapply(seq_len(length(unique(seqnames(chrGrange)))), function(x) findSimilarLibraries(exampleWCMatrix[chrGrange[x,],]))
exampleLibList <- exampleLibList[!sapply(exampleLibList, is.null)]
exampleLibList <- LibraryGroupList(exampleLibList)

show(exampleLibList)

```

---

```

fixLinkageGroups,ContigOrdering,StrandStateList,LinkageGroupList-method
fixLinkageGroups – searches for discrepancies within ordered contigs
to highlight erroneously merged fragments.

```

---

### Description

`fixLinkageGroups` – searches for discrepancies within ordered contigs to highlight erroneously merged fragments.

### Usage

```

## S4 method for signature 'ContigOrdering,StrandStateList,LinkageGroupList'
fixLinkageGroups(contigOrdering,
  orderFrame, linkageGroupList, whichLG = NULL, relatedCutOff = 0.6,
  verbose = TRUE)

```

### Arguments

<code>contigOrdering</code>	a data.frame of ordered contigs with linkage group names of class <code>ContigOrdering</code>
<code>orderFrame</code>	a list of <code>StrandStateMatrix</code> elements of class <code>StrandStateList</code>
<code>linkageGroupList</code>	List of vectors containing names of contigs belonging to each LG of type <code>LinkageGroupList</code> .
<code>whichLG</code>	vector of integers specifying the element(s) of <code>linkageGroupList</code> to be ordered (i.e. which specific linkage groups to try to order). Default is all LGs.
<code>relatedCutOff</code>	The minimal dissimilarity between adjacent contigs to subset a linkage group into multiple smaller groups. Default is 0.6
<code>verbose</code>	Outputs information to the terminal. Default is TRUE.

### Value

a `LinkageGroupList` with erroneously clustered contigs separated into their own groups

---

*flipOrderedLinkageGroups, ContigOrdering, StrandStateList, LinkageGroupList, StrandStateMatrix-method*  
*flipOrderedLinkageGroups – Simple greedy algorithm to find misorientations within previously ordered LG.*

---

### **Description**

*flipOrderedLinkageGroups* – Simple greedy algorithm to find misorientations within previously ordered LG.

### **Usage**

```
## S4 method for signature
## 'ContigOrdering,StrandStateList,LinkageGroupList,StrandStateMatrix'
flipOrderedLinkageGroups(contigOrdering,
  orderFrame, linkageGroupList, strandStateMatrix, whichLG = NULL,
  maxiter = 100, dissimilarityCutoff = 0.3)
```

### **Arguments**

*contigOrdering* a data.frame of ordered contigs with linkage group names of class *ContigOrdering*

*orderFrame* a list of *StrandStateMatrix* elements of class *StrandStateList*

*linkageGroupList* List of vectors containing names of contigs belonging to each LG of type *LinkageGroupList*. Should be ordered as the output of *orderAllLinkageGroups*[[3]]

*strandStateMatrix* a *StrandStateMatrix* to be reoriented

*whichLG* vector of integers specifying the element(s) of *linkageGroupList* to be ordered (i.e. which specific linkage groups to try to order). Default is all LGs.

*maxiter* The number of iterations used to find the optimal path. Default is 100

*dissimilarityCutoff* The minimum amount of dissimilarity needed to start reorienting contigs. Default is 0.3.

### **Value**

a *StrandStateMatrix* with misorientations resolved

---

 highlightAssemblyErrors

*highlightAssemblyErrors – Master function to identify misorientations and chimeras in the assembly*

---

### Description

highlightAssemblyErrors – Master function to identify misorientations and chimeras in the assembly

### Usage

```
highlightAssemblyErrors(path, splitBy = 1e+06, cluster = 1,
  clusterParam = NULL, pairedEnd = TRUE, qual = 10, gapFile = NULL,
  verbose = TRUE)
```

### Arguments

path	String denoting location of Strand-seq bam files
splitBy	integer determining the average size contigs should be split by
cluster	Number of times to recluster and take the consensus of. If NULL, clustering is run only once.
clusterParam	optional BiocParallelParam specifying cluster to use for parallel execution. When NULL, execution will be serial.
pairedEnd	Whether the bam files being read are in paired end format. Default is TRUE. Note,
qual	Mapping quality threshold. Default is 1
gapFile	A GRanges object consisting of start and end locations of assembly gaps (default it NULL)
verbose	prints messages to the terminal (default is TRUE)

### Value

a directional ChrTable object that can be used in downstream functions (strandSeqFreqTable)

---

ideogramPlot,StrandReadMatrix,StrandReadMatrix,ChrTable-method  
*ideogramPlot – plots BAIT-like ideograms*

---

**Description**

ideogramPlot – plots BAIT-like ideograms

**Usage**

```
## S4 method for signature 'StrandReadMatrix,StrandReadMatrix,ChrTable'
ideogramPlot(WatsonFreqList,
  CrickFreqList, chrTable, plotBy = "lib", sizeProportional = NULL,
  showPage = NULL, orderFrame = NULL, orientationData = NULL,
  verbose = TRUE)
```

**Arguments**

WatsonFreqList	data.frame of Watson calls. Product of strandSeqFreqTable[[3]] when BAITtables=TRUE
CrickFreqList	data.frame of Crick calls. Product of strandSeqFreqTable[[4]] when BAITtables=TRUE
chrTable	A data.frame consisting of chromosomes and lengths. Generated by makeChrTable(). Note rownames equal to chromosome names are required
plotBy	Whether to generate a plot for each library ('lib') or a plot for each chromosome ('chr')
sizeProportional	The plotter will divide each fragment into a discrete number of bins of size sizeProportional and distribute reads across them. For example, when set to 200000, the all contigs in each linkage group will be split into 200kb fragments and each fragment will have an equal proportion of the reads derived from that fragment. This ultimately rescales the ideograms such that LGs with the greatest DNA content are the biggest, as opposed to teh LGs with the most contigs. Default value is NULL
showPage	Integer specifying which LG (if plotBy='chr') or libraries (if plotBy='lib') to plot. Useful when not plotting to a file, or when wishing to subset data. Default is NULL
orderFrame	ordered data.frame of contigs (produced by orderAllLinkageGroups). Default is FALSE, where plots will be made from elements in chrTable.
orientationData	ChrTable of contig orientations telling which reads to flip Watson and Crick counts
verbose	prints messages to the terminal (default is TRUE)



**Value**

ordered contigs in bed format. Depending on options, intermediate files and plots will also be generated

**Examples**

```
data("exampleWatsonFreq")
data("exampleCrickFreq")
data('exampleDividedChr')

singleWatsonLibrary <- StrandReadMatrix(exampleWatsonFreq[,2, drop=FALSE])
singleCrickLibrary <- StrandReadMatrix(exampleCrickFreq[,2, drop=FALSE])

ideogramPlot(singleWatsonLibrary, singleCrickLibrary, exampleDividedChr)
```

---

**LibraryGroupList-class**

*A class for storing library group calls for contigs*

---

**Description**

This class is a list of lists, each primary list element is a chromosome/contig, and contains 2 sub-list elements: a list of 'Mostly Watson' and "Mostly Crick" library names.

**Usage**

```
LibraryGroupList(libraryGroups = list(), names = character())
```

**Arguments**

**libraryGroups** a list of lists, with each primary list element representing a chromosome with two internal list elements; a character vector of mostly watson library names, and a character vector of mostly Crick library names

**names** a vector of names of linkage groups

**Value**

a LibraryGroupList

**Examples**

```
lg1 <- LinkageGroupList(list(a=c('library1', 'library2'), b=c('library3')), names=c('chr1_Mostly_Crick', 'chr1_M
lg2 <- LinkageGroupList(list(a=c('library1'), b=c('library6', 'library4')), names=c('chr2_Mostly_Crick', 'chr2_M
libList <- LibraryGroupList(list(lg1, lg2))
```

---

LinkageGroupList-class

*A class for storing linkage group calls for contigs*

---

### Description

This class is simply a list of character strings containing the names of linkage groups.

### Usage

```
LinkageGroupList(linkageGroups = list(), names = character())
```

### Arguments

linkageGroups a list of character vectors of names of contigs in each LG  
names a vector of names of linkage groups

### Value

a LinkageGroupList

### Examples

```
lgList <- LinkageGroupList(list(lg1=c('contig1', 'contig2'), lg2=c('contig3')),  
names=c('lg1', 'lg20'))
```

---

locateMisorients,GRanges-method

*locateMisorients – function to identify libraries that hare similar WC patterns on chromosomes*

---

### Description

locateMisorients – function to identify libraries that hare similar WC patterns on chromosomes

### Usage

```
## S4 method for signature 'GRanges'  
locateMisorients(compiledGrange, gapFile = NULL,  
stateNum = 3, readCutOff = 40, verbose = TRUE)
```

**Arguments**

compiledGrange	A GRanges object consisting of read locations. Can be an individual file or the product of thoroughBed
gapFile	A GRanges object consisting of start and end locations of assembly gaps (default is NULL)
stateNum	The number of expected strand states. Default is 3 (WW, WC and CC). Function may exhibit unusual behaviour is changed
readCutOff	The minimal number of reads required to make an accurate strand state call. Default is 40.
verbose	prints messages to the terminal (default is TRUE)

**Value**

a list of ChrTable objects. The first is a ChrTable of misorientations detected. The second is a ChrTable of chimera detected. Output can be used in downstream functions (strandSeqFreqTable)

---

makeBoxPlot,ChrTable,LinkageGroupList-method

*makeBoxPlot – creates boxplot of contigs included in the analysis vs those excluded*

---

**Description**

makeBoxPlot – creates boxplot of contigs included in the analysis vs those excluded

**Usage**

```
## S4 method for signature 'ChrTable,LinkageGroupList'
makeBoxPlot(chrTable, linkage.contigs)
```

**Arguments**

chrTable	A GRanges object consisting of contigs and positions. A meta column called 'names' must be present with names in the same format as those from linkage.contigs. Generated by makeChrTable().
linkage.contigs	A list of clustered contigs, generated by clusterContigs()

**Value**

a box plot of included and excluded contigs

**Examples**

```
#make an example barplot of data

data("exampleLGList")
data("exampleChrTable")
makeBoxPlot(exampleChrTable, exampleLGList)
```

---

makeChrTable	<i>makeChrTable – Pulls out chromosome and length data from the header of a bam file</i>
--------------	--

---

**Description**

makeChrTable – Pulls out chromosome and length data from the header of a bam file

**Usage**

```
makeChrTable(bamFile, splitFile = NULL, splitBy = NULL, verbose = TRUE)
```

**Arguments**

bamFile	string of location of a bam file to extract header data from
splitFile	GRanges object (of type chr, start and end: no strand or meta columns) of locations in which to split the assembly, such as previously determined locations of contig chimerism
splitBy	integer determining the average size contigs should be split by
verbose	if FALSE, no messages appear on terminal

**Details**

makeChrTable creates a table with chromosome name and chromosome length by extracting header data from the supplied bam file.

**Value**

a GRanges object of class ChrTable, containing information on the organism's chromosomes as extracted from the BAM file header.

**Examples**

```
#Get an example BAM file and generate a chromosome table featuring fragment names and lengths

example.bam <- list.files(file.path(system.file(package='contibAIT'), 'extdata'), full.names=TRUE)[1]

chrTable <- makeChrTable(example.bam)
```

```

show(chrTable)

dividedChr <- makeChrTable(example.bam, splitBy=1000000)

show(dividedChr)

```

---

mapGapFromOverlap	<i>mapGapFromOverlap – function to co-localize strand state changes with assembly gaps</i>
-------------------	--

---

### Description

mapGapFromOverlap – function to co-localize strand state changes with assembly gaps

### Usage

```

mapGapFromOverlap(sceFile, gapFile, chrTable, verbose = TRUE,
  overlapNum = 4)

```

### Arguments

sceFile	GRanges object of strand state change locations in BED format
gapFile	GRanges object of assembly gaps in BED format (can be downloaded from UCSC table browser)
chrTable	GRanges object of chromosome table (product of makeChrTable)
verbose	prints messages to the terminal (default is TRUE)
overlapNum	Minimal number of strand state changes that overlap with a gap before assembly is cut at that location

### Value

a GRanges object of all contigs split by regions where the sceFile and gapFile GRanges objects overlap.

---

mergeFlankedLGs, LinkageGroupList, StrandStateMatrix-method	<i>mergeFlankedLGs – searches for similarities at the ends of ordered linkage groups to chain groups together</i>
---	---

---

### Description

mergeFlankedLGs – searches for similarities at the ends of ordered linkage groups to chain groups together

## Usage

```
## S4 method for signature 'LinkageGroupList,StrandStateMatrix'  
mergeFlankedLGs(linkageGroupList,  
  strandStateMatrix, buildConsensus = 1, cluster = NULL,  
  clusterParam = NULL, similarityCutoff = 0.7, verbose = TRUE)
```

## Arguments

linkageGroupList	List of ordered vectors containing names of contigs belonging to each LG, of type LinkageGroupList
strandStateMatrix	Table of type strandStateMatrix encompassing strand state for all contigs. Product of StrandSeqFreqTable.
buildConsensus	number of contigs to take at the end of the linkage group to build a consensus strand state. Default is 5
cluster	Number of times to recluster and take the consensus of. If NULL, clustering is run only once.
clusterParam	optional BiocParallelParam specifying cluster to use for parallel execution. When NULL, execution will be serial.
similarityCutoff	merge contigs that are more similar this this
verbose	Outputs information to the terminal. Default is TRUE.

## Value

a list containing a revised LinkageGroupList with merged groups, if appropriate, and a StrandStateMatrix with contigs reoriented, if newly merged groups were in opposite orientations.

---

orderAllLinkageGroups,LinkageGroupList,StrandStateMatrix,StrandFreqMatrix,StrandReadMatrix-method  
*Function to call contig ordering algorithms iteratively across each linkage group element*

---

## Description

Function to call contig ordering algorithms iteratively across each linkage group element

## Usage

```
## S4 method for signature  
## 'LinkageGroupList,StrandStateMatrix,StrandFreqMatrix,StrandReadMatrix'  
orderAllLinkageGroups(linkageGroupList,  
  strandStateMatrix, strandFreqMatrix, strandReadCount, whichLG = NULL,  
  saveOrdered = NULL, orderCall = "greedy", randomAttempts = 75,  
  nProcesses = 1, verbose = TRUE)
```

## Arguments

<code>linkageGroupList</code>	list of vectors, each specifying which contigs belong in which linkage group (product of <code>clusterContigs</code> )
<code>strandStateMatrix</code>	table of strand calls for all contigs (product of <code>preprocessStrandTable</code> )
<code>strandFreqMatrix</code>	table of W:C read proportions (used for QC) (product of <code>strandSeqFreqTable[[1]]</code> )
<code>strandReadCount</code>	table of read counts (product of <code>strandSeqFreqTable[[2]]</code> )
<code>whichLG</code>	vector of integers specifying the element(s) of <code>linkageGroupList</code> to be ordered (i.e. which specific linkage groups to try to order). Default is all LGs.
<code>saveOrdered</code>	Will return a pdf of heatmaps for each linkage group; String entered becomes the <code>fileName</code> (default is <code>saveOrderedPDF=FALSE</code> )
<code>orderCall</code>	currently either 'greedy' for greedy algorithm or 'TSP' for travelling salesperson algorithm (default is 'greedy')
<code>randomAttempts</code>	integer specifying number of iterations of either the Monte Carlo or the 2-opt TSP solver to identify the best ordering. Default is 75, but higher values (eg 1000) make more sense for the TSP.
<code>nProcesses</code>	number of processes to attempt ordering in parallel
<code>verbose</code>	Prints messages to the terminal. Default is TRUE

## Value

a list containing a `ContigOrdering` object consisting of ordered contigs with linkage group names, a `StrandStateList` consisting of `StrandStateMatrices` for each LG, and a `LinkageGroupList` consisting of reordered contigs within each list element.

## Examples

```
#Get a data.frame of ordered contigs from cells from the same organism, aligned to the same genome
```

```
data("exampleLGList")
data("exampleWCMatrix")
data("exampleStrandFreq")
data("exampleReadCounts")
contigOrder <- orderAllLinkageGroups(exampleLGList, exampleWCMatrix, exampleStrandFreq, exampleReadCounts)
```

```
show(contigOrder)
```

```
contigOrder <- orderAllLinkageGroups(exampleLGList, exampleWCMatrix, exampleStrandFreq, exampleReadCounts, order=
show(contigOrder)
```

---

orderContigsGreedy	<i>Function to order contigs within a single linkage group using a greedy algorithms Attempt to order contigs within</i>
--------------------	--

---

**Description**

Function to order contigs within a single linkage group using a greedy algorithms Attempt to order contigs within

**Usage**

```
orderContigsGreedy(linkageGroupReadTable, randomAttempts = 75,
  nProcesses = 1, verbose = TRUE)
```

**Arguments**

linkageGroupReadTable	dataframe of strand calls (product of combineZeroDists or preprocessStrandTable)
randomAttempts	number of times to repeat the greedy algorithm with a random restart
nProcesses	number of processes to attempt ordering in parallel
verbose	whether to print verbose messages

**Value**

list of two members: 1) contig names in order, 2) the original data.frame entered into function correctly ordered

---

orderContigsTSP	<i>Attempt to order contigs within linkage groups using travelling salesperson algorithm</i>
-----------------	--

---

**Description**

Attempt to order contigs within linkage groups using travelling salesperson algorithm

**Usage**

```
orderContigsTSP(linkageGroupReadTable, reps)
```

**Arguments**

linkageGroupReadTable	dataframe of strand calls (product of combineZeroDists or preprocessStrandTable)
reps	Number of repetitions for the 2-opt TSP solver algorithm



**Value**

list of two members: 1) contig names in order, 2) the original data.frame entered into function correctly ordered

---

plotContigOrder,ContigOrdering-method

*Plot ordering of contigs within a single linkage group.*

---

**Description**

Plot ordering of contigs within a single linkage group.

**Usage**

```
## S4 method for signature 'ContigOrdering'  
plotContigOrder(contigOrder, lg = "all",  
  verbose = TRUE)
```

**Arguments**

contigOrder	matrix from orderAllContigs with the subdivided linkage groups and the names of the contigs to plot
lg	Integer specifying the linkage group by which to plot. Default is all
verbose	prints messages to the terminal (default is TRUE)

**Value**

A ggplot object (which will be plotted automatically if not assigned).

**Examples**

```
#Get a data.frame of ordered contigs from cells from the same organism, aligned to the same genome  
data("exampleContigOrder")  
plotContigOrder(exampleContigOrder[[1]], lg=1)
```

---

```
plotLGDistances,LinkageGroupList,StrandStateMatrix-method
    plotLGDistances – plots a heatmap of the distances between linkage
    groups
```

---

**Description**

plotLGDistances – plots a heatmap of the distances between linkage groups

**Usage**

```
## S4 method for signature 'LinkageGroupList,StrandStateMatrix'
plotLGDistances(object,
  allStrands, lg = "all", labels = TRUE, state = "all",
  alreadyOrdered = FALSE)
```

**Arguments**

object	LinkageGroupList
allStrands	StrandStateMatrix for all linkageGroups (usually reoriented by reorientStrandTable)
lg	= 'all' vector of integers to determine which linkage group(s) to plot. 'all' will calculate consensus strand calls for all linkage groups and plot them side by side (default is 'all')
labels	= TRUE if TRUE, contig names will be plotted on the axes
state	string denoting whether only homozygous states should be used ('homo'; just WW vs CC), if a comparison should be made between homozygous and heterozygous states only ('hetero'; just homo vs hetero), or if all three states ('all'; WW vs WC vs CC). Default is 'all'
alreadyOrdered	if TRUE, the function will assume that the linkageGroupList is already ordered and not create a dendrogram. Default is FALSE
...	additional parameters to pass to heatmap.2

**Value**

a heatmap of linkage group calls

**Examples**

```
data("exampleLGList")
data("exampleWCMatrix")

plotLGDistances(exampleLGList, exampleWCMatrix)
plotLGDistances(exampleLGList, exampleWCMatrix, lg=1)
```

---

```
plotWCdistribution,StrandFreqMatrix-method
    Creates median distribution boxplots across all libraries and contigs
```

---

**Description**

Creates median distribution boxplots across all libraries and contigs

**Usage**

```
## S4 method for signature 'StrandFreqMatrix'
plotWCdistribution(object, filterThreshold = 0.8)
```

**Arguments**

object	object of class StrandFreqMatrix (product of strandSeqFreqTable)
filterThreshold	numeric value used in assessing the threshold for homozygous strand calls. Default is 0.8.

**Value**

nothing, just plots.

**Examples**

```
data("exampleStrandFreq")

plotWCdistribution(exampleStrandFreq, filterThreshold=0.8)
```

---

```
preprocessStrandTable,StrandFreqMatrix-method
    preprocessStrandTable – remove low quality libraries and contigs before attempting to build a genome
```

---

**Description**

preprocessStrandTable – remove low quality libraries and contigs before attempting to build a genome

**Usage**

```
## S4 method for signature 'StrandFreqMatrix'
preprocessStrandTable(strandTable,
  strandTableThreshold = 0.8, filterThreshold = 0.8,
  orderMethod = "libsAndConc", lowQualThreshold = 0.9, verbose = TRUE,
  minLib = 10)
```

**Arguments**

strandTable	data.frame containing the strand table to use as input
strandTableThreshold	threshold at which to call a contig WW or CC rather than WC
filterThreshold	maximum number of libraries a contig can be NA or WC in
orderMethod	the method to order contigs. currently libsAndConc only option. Set to FALSE to not order contigs based on library quality
lowQualThreshold	background threshold at which to toss an entire library. If NULL, function will not make an overall assessment of library quality. Very chimeric assemblies can appear low quality across all libraries.
verbose	messages written to terminal
minLib	minimum number of libraries a contig must be present in to be included in the output

**Value**

A list of one matrix and three quality data.frames – 1: a matrix of WW/WC/WW calls for all contigs; 2: the quality of libraries used (based on frequencies outside expected ranges); 3: A data.frame of libraries that are of low quality and therefore excluded from analysis; 5: A ChrTable object containing contigs that are present as WC in more libraries than expected. These are excluded from the strandStateMatrix, but are potentially worth investigating for chimerism.

**Examples**

```
data("exampleStrandFreq")

strandStates <- preprocessStrandTable(exampleStrandFreq, lowQualThreshold=0.8)

show(strandStates[[1]]) # WW-WC-CC matrix
```

---

```
reorientAndMergeLGs,LinkageGroupList,StrandStateMatrix-method
reorientAndMergeLGs uses a simple dissimilarity to find misoriented
fragments within linkage groups.
```

---

**Description**

reorientAndMergeLGs uses a simple dissimilarity to find misoriented fragments within linkage groups.

**Usage**

```
## S4 method for signature 'LinkageGroupList,StrandStateMatrix'
reorientAndMergeLGs(object,
  allStrands, cluster = NULL, clusterParam = NULL, similarityCutoff = 0.9,
  verbose = TRUE)
```

**Arguments**

object	List of vectors containing names of contigs belonging to each LG.
allStrands	Table of type strandStateMatrix encompassing strand state for all contigs. Product of StrandSeqFreqTable.
cluster	Number of times to recluster and take the consensus of. If NULL, clustering is run only once.
clusterParam	optional BiocParallelParam specifying cluster to use for parallel execution. When NULL, execution will be serial.
similarityCutoff	merge contigs that are more similar this this
verbose	Outputs information to the terminal. Default is TRUE.

**Value**

a list consisting of a strandStateMatrix (a reoriented version of allStrands), a ChrTable containing contig names and orientations, as '+' or '-' and a merged LinkageGroupList.

**Examples**

```
data(exampleLGList)
data(exampleWCMatrix)

reorientedMatrix <- reorientAndMergeLGs(exampleLGList,
  exampleWCMatrix)

# Note that in this example data, everything is correctly oriented to
# to begin with, so all contigs come out as + orientation
```

---

```
show,ContigOrdering-method
      show-methods
```

---

**Description**

Shows a ContigOrdering

**Usage**

```
## S4 method for signature 'ContigOrdering'
show(object)
```

**Arguments**

object	a ContigOrdering
--------	------------------

**Value**

nothing

---

show,LibraryGroupList-method  
*show-methods*

---

**Description**

Shows a LibraryGroupList

**Usage**

```
## S4 method for signature 'LibraryGroupList'  
show(object)
```

**Arguments**

object            a LibraryGroupList

**Value**

nothing

---

show,LinkageGroupList-method  
*show-methods*

---

**Description**

Shows a LinkageGroupList

**Usage**

```
## S4 method for signature 'LinkageGroupList'  
show(object)
```

**Arguments**

object            a LinkageGroupList

**Value**

nothing

---

show,OrientationFrame-method  
*show-methods*

---

**Description**

Shows a OrientationFrame

**Usage**

```
## S4 method for signature 'OrientationFrame'  
show(object)
```

**Arguments**

object            a OrientationFrame

**Value**

nothing

---

show,StrandFreqMatrix-method  
*show-methods*

---

**Description**

Shows a StrandFreqMatrix

**Usage**

```
## S4 method for signature 'StrandFreqMatrix'  
show(object)
```

**Arguments**

object            a StrandFreqMatrix

**Value**

nothing

---

show,StrandReadMatrix-method  
*show-methods*

---

**Description**

Shows a StrandReadMatrix

**Usage**

```
## S4 method for signature 'StrandReadMatrix'  
show(object)
```

**Arguments**

object            a StrandReadMatrix

**Value**

nothing

---

show,StrandStateList-method  
*show-methods*

---

**Description**

Shows a StrandStateList

**Usage**

```
## S4 method for signature 'StrandStateList'  
show(object)
```

**Arguments**

object            a StrandStateList

**Value**

nothing



---

show,StrandStateMatrix-method  
*show-methods*

---

**Description**

Shows a StrandStateMatrix

**Usage**

```
## S4 method for signature 'StrandStateMatrix'  
show(object)
```

**Arguments**

object            a StrandStateMatrix

**Value**

nothing

---

StrandFreqMatrix-class  
*A class for storing a matrix of frequencies of Watson to Crick reads for a set of contigs over several libraries*

---

**Description**

The strand information stored in this object is the ratio of Watson to Crick reads mapping to each contig in each library (cell). This should fall within the range (-1,1). This class simply extends matrix, but with additional validity checking.

**Usage**

```
StrandFreqMatrix(counts = matrix(double()))
```

**Arguments**

counts            a double matrix of read count ratios

**Value**

a StrandFreqMatrix

**Examples**

```

data("exampleWatsonFreq")
data("exampleCrickFreq")
frequencyMatrix <- sapply(1:ncol(exampleCrickFreq),
function(colNum){exampleCrickFreq[,colNum] / exampleWatsonFreq[,colNum]})

StrandFreqMatrix(frequencyMatrix)

```

---

**StrandReadMatrix-class**

*A class for storing read counts for a set of contigs over several libraries*

---

**Description**

The information stored in this class is simple read counts, so should be integers  $\geq 0$ .

**Usage**

```
StrandReadMatrix(counts = matrix(integer()))
```

**Arguments**

counts            an integer matrix of read counts

**Value**

a StrandReadMatrix

**Examples**

```

data("exampleWatsonFreq")
StrandReadMatrix(exampleWatsonFreq[,2, drop=FALSE])

```

---

strandSeqFreqTable    *strandSeqFreqTable – function to process bam files for contiBAIT*

---

**Description**

strandSeqFreqTable – function to process bam files for contiBAIT

**Usage**

```

strandSeqFreqTable(bamFileList, fieldSep = ".", field = 1, qual = 0,
  rmdup = TRUE, verbose = TRUE, filter = NULL, misorientations = NULL,
  chimeras = NULL, tileChunk = 1e+05, pairedEnd = TRUE,
  BAITtables = FALSE)

```

**Arguments**

bamFileList	vector containing the location of the bams file to be read
fieldSep	The field separator of the bam file to use to define the field. Default is '.'
field	The field of the bam file name to use as an index (default is 1)
qual	Mapping quality threshold. Default is 0
rmdup	remove duplicates in output file. Default is TRUE
verbose	prints messages to the terminal (default is TRUE)
filter	additional file of type ChrTable (GRanges with a meta column titled 'name' determining contig name) to split chromosomes based on locations. If this parameter is NULL (default), a filter table will be automatically generated from the header of the first file in bamFileList.
misorientations	additional file of type ChrTable (GRanges with a meta column titled 'name' determining contig name) with putative misorientation locations. These location's reads are reversed in the regions of filter that they lie within. Product of locateMisorientations[[1]]. Default is NULL
chimeras	additional file of type ChrTable (GRanges with a meta column titled 'name' determining contig name) with putative chimeric locations. These location's reads are masked in the regions of filter that they lie within, and are appended to the end of the output file. Product of locateMisorientations[[2]]. Default is NULL
tileChunk	Number of reads to split bam files into (smaller number requires less RAM). Default is 100000.
pairedEnd	Whether the bam files being read are in paired end format. Default is TRUE. Note, since paired reads will be the same direction, only first mate read of pair is used in output
BAITtables	creates additional matrices in the returned list with just Watson and Crick read counts to be used in downstream BAIT plotting. Default is FALSE

**Value**

a list containing two matrices: a StrandFreqMatrix of W:C read frequencies, and a StrandReadMatrix of read counts

**Examples**

```
#Get a list of BAM files containing libraries for cells from the same organism, aligned to the same genome
#In this case these are the example BAM files provided with the package (hence the call to system.file);

example.dir <- file.path(system.file(package='contiBAIT'), 'extdata')
bam.files <- dir(example.dir, full.names=TRUE)

strand.freq <- strandSeqFreqTable(bam.files, pairedEnd = FALSE)

show(strand.freq[[1]])
show(strand.freq[[2]])
```

---

StrandStateList-class *A class for storing StrandStateList lists for contigs*

---

### Description

This class is a list of StrandStateMatrices, each a subset of the StrandStateMatrix split by linkagegroups

### Usage

```
StrandStateList(strandGroupList = list(), names = character())
```

### Arguments

strandGroupList	a list of StrandStateMatrix elements, with each primary element representing a StrandStateMatrix containing ordered contigs from a LinkageGroupList element
names	a vector of names of StrandStateMatrix elements

### Value

a StrandStateList

---

StrandStateMatrix-class  
*A class for storing a data frame of discrete strand states of a set of contigs over several libraries*

---

### Description

The strand information stored in this object is a call of the strand state of each contig in each library. mapping to each contig in each library (cell). This should fall within the range (-1,1). This class simply extends matrix, but with additional validity checking.

### Usage

```
StrandStateMatrix(states = matrix(integer()))
```

### Arguments

states	an integer matrix of strand states by library
--------	---

### Value

a StrandStateMatrix

**Examples**

```
StrandStateMatrix(matrix(ncol=2, c(1,3,1,2)))
```

---

```
thoroughBed,ANY,LibraryGroupList-method
```

*thoroughBed – function to merge chromosomes from libraries that have the same strand states*

---

**Description**

thoroughBed – function to merge chromosomes from libraries that have the same strand states

**Usage**

```
## S4 method for signature 'ANY,LibraryGroupList'
thoroughBed(bamFileList, relatedLibList,
  qual = 10, pairedEnd = TRUE, rmdup = TRUE, verbose = TRUE)
```

**Arguments**

bamFileList	vector containing the location of the bams file to be read
relatedLibList	list where each element contains all library names that show similar strand pattern. The product of findSimilarLibraries
qual	Mapping quality threshold. Default is 10
pairedEnd	Whether the bam files being read are in paired end format. Default is TRUE. Note, since paired reads will be the same direction, only first mate read of pair is used in output to reduce file size
rmdup	Logical as to whether to remove duplicate reads within each library. Default is TRUE.
verbose	prints messages to the terminal (default is TRUE)

**Value**

a GRanges object comprising merged directional reads from all libraries in relatedLibList.

**Examples**

```
#Get a list of BAM files containing libraries for cells from the same organism, aligned to the same genome
#In this case these are the example BAM files provided with the package (hence the call to system.file);
data("exampleLibList")
```

```
library(BiocParallel)
```

```
example.dir <- file.path(system.file(package='contibAIT'), 'extdata')
```

```
exampleRange <- thoroughBed(example.dir, exampleLibList)

show(exampleRange)
```

---

```
writeBed,ANY,ChrTable,ContigOrdering-method
      function to write contig order to BED file
```

---

## Description

function to write contig order to BED file

## Usage

```
## S4 method for signature 'ANY,ChrTable,ContigOrdering'
writeBed(chrTable, orientationData,
         contigOrder, libWeight = NULL, file = "contibait_assembly.bed")
```

## Arguments

<code>chrTable</code>	a GRanges object with a 'name' meta column matching contig names. Product of <code>makeChrTable</code>
<code>orientationData</code>	ChrTable of contig and strand (with rownames matching contig names). Product of <code>reorientAndMergeLGs[[2]]</code>
<code>contigOrder</code>	an object of type <code>ContigOrdering</code> with ordered Linkage Groups and contigs. Product of <code>orderAllLinkageGroups</code>
<code>libWeight</code>	average quality across all libraries for a contig
<code>file</code>	character string for bed file name to write

## Value

NULL; BED file written to file

## Examples

```
## Not run:

data("exampleDividedChr")

writeBed(exampleDividedChr,
         reorientedMatrix[[2]],
         contigOrder)

## End(Not run)
```

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