# Package 'Rsamtools'

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|---|
| Title Binary alignment (BAM), variant call (BCF), or tabix file import  |
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| Maintainer Bioconductor Package Maintainer < maintainer@bioconductor.org>   |
| <b>Description</b> This package provides an interface to the 'samtools', 'bcftools', and 'tabix' utilities (see 'LICENCE') for manipulating SAM (Sequence Alignment / Map), binary variant call (BCF) and compressed indexed tab-delimited (tabix) files. |
| $\textbf{URL} \ \ \text{http://bioconductor.org/packages/release/bioc/html/Rsamtools.html}$   |
| License Artistic-2.0 + file LICENSE   |
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| biocViews DataImport, Sequencing, HighThroughputSequencing  R topics documented:  |
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This package provides facilities for parsing samtools BAM (binary) files representing aligned sequences.

### **Details**

Description

See package Description ('Rsamtools') for package details. A useful starting point is the  $\frac{\text{scanBam}}{\text{manual page}}$ 

### Note

This package documents the following classes for purely internal reasons, see help pages in other packages: bzfile, fifo, gzfile, pipe, unz, url.

### Author(s)

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### References

The current source code for samtools and bcftools is from https://github.com/samtools/samtools. Additional material is at http://samtools.sourceforge.net/.

### **Examples**

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Create summary pile-up statistics across multiple BAM files.

### **Description**

applyPileups scans one or more BAM files, returning position-specific sequence and quality summaries.

### Usage

```
applyPileups(files, FUN, ..., param)
```

### **Arguments**

files A PileupFiles instances.

FUN

A function of 1 argument, x, to be evaluated for each yield (see yieldSize, yieldBy, yieldAll). The argument x is a list, with elements describing the current pile-up. The elements of the list are determined by the argument what, and include:

**seqnames:** (Always returned) A named integer() representing the seqnames corresponding to each position reported in the pile-up. This is a run-length encoding, where the names of the elements represent the seqnames, and the values the number of successive positions corresponding to that seqname.

**pos:** Always returned) A integer() representing the genomic coordinate of each pile-up position.

**seq:** An array of dimensions nucleotide x file x position.

The 'nucleotide' dimension is length 5, corresponding to 'A', 'C', 'G', 'T', and 'N' respectively.

Entries in the array represent the number of times the nucleotide occurred in reads in the file overlapping the position.

**qual:** Like seq, but summarizing quality; the first dimension is the Phred-encoded quality score, ranging from '!' (0) to '~' (93).

... Additional arguments, passed to methods.

param An instance of the object returned by PileupParam.

#### Value

applyPileups returns a list equal in length to the number of times FUN has been called, with each element containing the result of FUN.

PileupParam returns an object describing the parameters.

### Author(s)

Martin Morgan

### References

http://samtools.sourceforge.net/

#### See Also

PileupParam.

### **Examples**

```
fl <- system.file("extdata", "ex1.bam", package="Rsamtools")
fls <- PileupFiles(c(fl, fl))
calcInfo <-
   function(x)
   ## information at each pile-up position
   info <- apply(x[["seq"]], 2, function(y) {
      y <- y[c("A", "C", "G", "T"),]
      y < -y + 1L
                                   \# continuity
      cvg <- colSums(y)
      p < -y / cvg[col(y)]
      h < -colSums(p * log(p))
      ifelse(cvg == 4L, NA, h)
   })
   list(seqnames=x[["seqnames"]], pos=x[["pos"]], info=info)
which <- GRanges(c("seq1", "seq2"), IRanges(c(1000, 1000), 2000))
param <- PileupParam(which=which, what="seq")
res <- applyPileups(fls, calcInfo, param=param)
str(res)
head(res[[1]][["pos"]]) \# positions matching param
\label{eq:head} \mbox{head}(\mbox{res}[[1]][["\mbox{info}"]]) \; \# \; \mbox{inforamtion in each file}
## 'param' as part of 'files'
fls1 <- PileupFiles(c(fl, fl), param=param)
res1 <- applyPileups(fls1, calcInfo)
identical(res, res1)
## yield by position, across ranges
param <- PileupParam(which=which, yieldSize=500L, yieldBy="position",
                what="seq")
res <- applyPileups(fls, calcInfo, param=param)
sapply(res, "[[", "seqnames")
```

BamFile

Maintain SAM and BAM files

### **Description**

Use BamFile() to create a reference to a BAM file (and optionally its index). The reference remains open across calls to methods, avoiding costly index re-loading.

BamFileList() provides a convenient way of managing a list of BamFile instances.

### Usage

## Constructors

BamFile(file, index=file, ..., yieldSize=NA integer )

```
BamFileList(..., yieldSize=NA integer )
## Opening / closing
## S3 method for class 'BamFile'
open(con, ...)
## S3 method for class 'BamFile'
close(con, ...)
## accessors; also path(), index(), yieldSize()
## S4 method for signature 'BamFile'
isOpen(con, rw="")
\#\# actions
## S4 method for signature 'BamFile'
scanBamHeader(files, ...)
## S4 method for signature 'BamFile'
seqinfo(x)
## S4 method for signature 'BamFile'
scanBam(file, index=file, ..., param=ScanBamParam(what=scanBamWhat()))
## S4 method for signature 'BamFile'
countBam(file, index=file, ..., param=ScanBamParam())
## S4 method for signature 'BamFileList'
countBam(file, index=file, ..., param=ScanBamParam())
## S4 method for signature 'BamFile'
filterBam(file, destination, index=file, ...,
   indexDestination=TRUE, param=ScanBamParam(what=scanBamWhat()))
## S4 method for signature 'BamFile'
indexBam(files, ...)
## S4 method for signature 'BamFile'
sortBam(file, destination, ..., byQname=FALSE, maxMemory=512)
## S4 method for signature 'BamFileList'
mergeBam(files, destination, ...)
## S4 method for signature 'BamFile'
readBamGappedAlignments(file, index=file, use.names=FALSE, param=NULL)
## S4 method for signature 'BamFile'
readBamGappedReads(file, index=file, use.names=FALSE, param=NULL)
## S4 method for signature 'BamFile'
readBamGappedAlignmentPairs(file, index=file, use.names=FALSE, param=NULL)
\#\# counting
\#\# S4 method for signature 'GRanges,BamFileList'
summarizeOverlaps(features, reads, mode, ignore.strand=FALSE, ..., singleEnd=TRUE, param=ScanBamPara
## S4 method for signature 'GRangesList,BamFileList'
```

summarizeOverlaps(features, reads, mode, ignore.strand=FALSE, ..., singleEnd=TRUE, param=ScanBamPara

## S4 method for signature 'character, ANY'

find Splice Overlaps (query, subject, ignore.strand = FALSE, ..., param = ScanBamParam(), paired End = FALSE)

## S4 method for signature 'BamFile, ANY'

findSpliceOverlaps(query, subject, ignore.strand=FALSE, ..., param=ScanBamParam(), pairedEnd=FALSE)

## S4 method for signature 'BamFile' coverage(x, shift=0L, width=NULL, weight=1L, ...)

### **Arguments**

... Additional arguments. For BamFileList, this can either be a single character

vector of paths to BAM files, or several instances of BamFile objects. When a character vector of paths, a second named argument 'index' can be a character() vector of length equal to the first argument specifying the paths to the index files, or character() to indicate that no index file is available. See BamFile. For coverage, the arguments are passed to the coverage method for GappedAlignments

objects.

con An instance of BamFile.

x, file, files A character vector of BAM file paths (for BamFile) or a BamFile instance (for

other methods).

index A character vector of indices (for BamFile); ignored for all other methods on

this page.

yieldSize Number of records to yield each time the file is read from using scanBam.

Only valid when length(bamWhich(param)) == 0. yieldSize does not alter existing yield sizes, include NA, when creating a BamFileList from BamFile

instances.

destination character(1) file path to write filtered reads to.

indexDestination

logical(1) indicating whether the destination file should also be indexed.

 $by Qname,\ maxMemory$ 

See sortBam.

param An optional ScanBamParam instance to further influence scanning, counting,

or filtering.

use.names Construct the names of the returned object from the query template names (QNAME

field)? If not (the default), then the returned object has no names.

rw Mode of file; ignored.

reads A BamFileList that represents the data to be counted by summarizeOverlaps.

features A GRanges or a GRangesList object of genomic regions of interest. When

a GRanges is supplied, each row is considered a feature. When a GRanges-List is supplied, each higher list-level is considered a feature. This distinction is important when defining an overlap between a read and a feature. See

?summarizeOverlaps for details.

mode A function that defines the method to be used when a read overlaps more than

one feature. Pre-defined options are "Union", "IntersectionStrict", or "IntersectionNotEmpty" and are designed after the counting modes available in the

HTSeq package by Simon Anders (see references).

• "Union": (Default) Reads that overlap any portion of exactly one feature are counted. Reads that overlap multiple features are discarded.

- "IntersectionStrict": A read must fall completely "within" the feature to be counted. If a read overlaps multiple features but falls "within" only one, the read is counted for that feature. If the read is "within" multiple features, the read is discarded.
- "IntersectionNotEmpty": A read must fall in a unique disjoint region of a
  feature to be counted. When a read overlaps multiple features, the features
  are partitioned into disjoint intervals. Regions that are shared between the
  features are discarded leaving only the unique disjoint regions. If the read
  overlaps one of these remaining regions, it is assigned to the feature the
  unique disjoint region came from.

ignore.strand A logical value indicating if strand should be considered when matching.

singleEnd A logical value indicating if reads are single or paired-end.

A logical value indicating if reads are single or paired-end.

A logical value indicating if reads are single or paired-end.

query character name of a Bam file, a BamFile, GappedAlignments, GappedAlign-

mentPairs or a GRangesList object containing the reads.

Paired-end reads can be supplied in a Bam file or GappedAlignmentPairs object. Single-end may be in a Bam file, GappedAlignments or GRanges object.

subject A TranscriptDb, or GRangesList containing the annotations.

shift, width, weight

See coverage.

### **Objects from the Class**

Objects are created by calls of the form BamFile().

### **Fields**

The BamFile class inherits fields from the RsamtoolsFile class.

### **Functions and methods**

BamFileList inherits methods from RsamtoolsFileList and SimpleList.

Opening / closing:

**open.BamFile** Opens the (local or remote) path and index (if bamIndex is not character(0)), files. Returns a BamFile instance.

**close.BamFile** Closes the BamFile con; returning (invisibly) the updated BamFile. The instance may be re-opened with open.BamFile.

Accessors:

**path** Returns a character(1) vector of BAM path names.

index Returns a character(1) vector of BAM index path names.

yieldSize, yieldSize<- Return or set an integer(1) vector indicating yield size.

Methods:

**scanBamHeader** Visit the path in path(file), returning the information contained in the file header; see scanBamHeader.

**seqinfo** Visit the path in path(file), returning a Seqinfo instance containing information on the lengths of each sequence.

scanBam Visit the path in path (file), returning the result of scanBam applied to the specified path.

**countBam** Visit the path(s) in path(file), returning the result of countBam applied to the specified path.

**filterBam** Visit the path in path(file), returning the result of filterBam applied to the specified path.

indexBam Visit the path in path(file), returning the result of indexBam applied to the specified path.

sortBam Visit the path in path(file), returning the result of sortBam applied to the specified path.

**mergeBam** Merge several BAM files into a single BAM file. See mergeBam for details; additional arguments supported by mergeBam, character-method are also available for BamFileList.

readBamGappedAlignments, readBamGappedReads, readBamGappedAlignmentPairs Visit the path in path(file), returning the result of readBamGappedAlignments, readBamGappedReads, or readBamGappedAlignmentPairs applied to the specified path. See readBamGappedAlignments. show Compactly display the object.

### Author(s)

Martin Morgan and Marc Carlson

### See Also

The GenomicRanges package is where the summarizeOverlaps method originates.

### **Examples**

```
fl <- system.file("extdata", "ex1.bam", package="Rsamtools")
length(scanBam(fl)[[1]][[1]]) # all records
bf <- open(BamFile(fl))
                             # implicit index
identical(scanBam(bf), scanBam(fl))
\#\# chunks of size 1000
bf <- open(BamFile(fl, yieldSize=1000))
while (nrec <- length(scanBam(bf)[[1]][[1]]))
   cat("records:", nrec, "\n")
close(bf)
rng <- GRanges(c("seq1", "seq2"), IRanges(1, c(1575, 1584)))
## repeatedly visit 'bf'
bf <- open(BamFile(fl))
sapply(seq_len(length(rng)), function(i, bamFile, rng) {
   param <- ScanBamParam(which=rng[i], what="seq")
   bam <- scanBam(bamFile, param=param)[[1]]
   alphabetFrequency(bam[["seq"]], baseOnly=TRUE, collapse=TRUE)\\
}, bf, rng)
close(bf)
```

```
## summarizeOverlaps with BamFileList
##
## single-end reads
library ("TxDb.Dmelanogaster.UCSC.dm3.ensGene") \\
exbygene <- exonsBy(TxDb.Dmelanogaster.UCSC.dm3.ensGene, "gene")
fl <- system.file("extdata", "untreated1 chr4.bam",
   package="pasillaBamSubset")
## When 'yieldSize' is specified the file is processed by chunks.
## Otherwise the complete file is read into memory.
bfl <- BamFileList(fl, yieldSize=50000)
trace(readBamGappedAlignments)
counts <- assays(summarizeOverlaps(exbygene, bfl, singleEnd=TRUE))$counts
untrace(readBamGappedAlignments)
## paired-end reads
fl <- system.file("extdata", "untreated3 chr4.bam",
   package="pasillaBamSubset")
## Complete file is read into memory.
bfl <- BamFileList(fl, index=character())
res <- summarizeOverlaps(exbygene, bfl, singleEnd=FALSE)
stopifnot(length(assays(res)\$counts) == length(exbygene))
\#\# findSpliceOverlaps
##
## See ?'findSpliceOverlapsd' for examples
```

BamInput

Import, count, index, filter, sort, and merge 'BAM' (binary alignment) files.

### Description

Import binary 'BAM' files into a list structure, with facilities for selecting what fields and which records are imported, and other operations to manipulate BAM files.

```
scanBam(file, index=file, ..., param=ScanBamParam(what=scanBamWhat()))
countBam(file, index=file, ..., param=ScanBamParam())
scanBamHeader(files, ...)
## S4 method for signature 'character'
scanBamHeader(files, ...)
asBam(file, destination, ...)
## S4 method for signature 'character'
asBam(file, destination, ...,
```

```
overwrite=FALSE, indexDestination=TRUE)
filterBam(file, destination, index=file, ...)
## S4 method for signature 'character'
filterBam(file, destination, index=file, ...,
   indexDestination=TRUE, param=ScanBamParam(what=scanBamWhat()))
sortBam(file, destination, ...)
## S4 method for signature 'character'
sortBam(file, destination, ..., byQname=FALSE, maxMemory=512)
indexBam(files, ...)
## S4 method for signature 'character'
indexBam(files, ...)
mergeBam(files, destination, ...)
## S4 method for signature 'character'
mergeBam(files, destination, ..., region = RangedData(),
   overwrite = FALSE, header = character(), byQname = FALSE,
   addRG = FALSE, compressLevel1 = FALSE, indexDestination = FALSE)
```

#### **Arguments**

file The character(1) file name of the 'BAM' ('SAM' for asBam) file to be pro-

cessed.

files The character() file names of the 'BAM' file to be processed. For mergeBam,

must satisfy length(files) >= 2.

index The character(1) name of the index file of the 'BAM' file being processed; this

is given without the '.bai' extension.

destination The character(1) file name of the location where the sorted, filtered, or merged

output file will be created. For asBam and sortBam this is without the ".bam"

file suffix.

region A RangedData() instance with >= 1 rows, specifying the region of the BAM

files to merged.

... Additional arguments, passed to methods.

overwrite A logical(1) indicating whether the destination can be over-written if it already

exists.

indexDestination

A logical(1) indicating whether the created destination file should also be in-

dexed.

by Qname A logical(1) indicating whether the sorted destination file should be sorted by

Query-name (TRUE) or by mapping position (FALSE).

header A character(1) file path for the header information to be used in the merged

BAM file.

addRG A logical(1) indicating whether the file name should be used as RG (read group)

tag in the merged BAM file.

compressLevel1 A logical(1) indicating whether the merged BAM file should be compressed to

zip level 1.

maxMemory A numerical(1) indicating the maximal amount of memory (in MB) that the

function is allowed to use.

param An instance of ScanBamParam. This influences what fields and which records

are imported.

#### **Details**

The scanBam function parses binary BAM files; text SAM files can be parsed using R's scan function, especially with arguments what to control the fields that are parsed.

countBam returns a count of records consistent with param.

scanBamHeader visits the header information in a BAM file, returning for each file a list containing elements targets and text, as described below. The SAM / BAM specification does not require that the content of the header be consistent with the content of the file, e.g., more targets may be present that are represented by reads in the file.

asBam converts 'SAM' files to 'BAM' files, equivalent to the samtools view -Sb file > destination. The 'BAM' file is sorted and an index created on the destination (with extension '.bai') when indexDestination=TRUE.

filterBam parses records in file satisfying the bamWhich of param, writing each record satisfying the bamFlag and bamSimpleCigar criteria of param to file destination. An index file is created on the destination when indexDestination=TRUE.

sortBam sorts the BAM file given as its first argument, analogous to the "samtools sort" function.

indexBam creates an index for each BAM file specified, analogous to the 'samtools index' function.

mergeBam merges 2 or more sorted BAM files. As with samtools, the RG (read group) dictionary in the header of the BAM files is not reconstructed.

Details of the ScanBamParam class are provide on its help page; several salient points are reiterated here. ScanBamParam can contain a field what, specifying the components of the BAM records to be returned. Valid values of what are available with scanBamWhat. ScanBamParam can contain an argument which that specifies a subset of reads to return. This requires that the BAM file be indexed, and that the file be named following samtools convention as <br/>bai. ScanBamParam can contain an argument tag to specify which tags will be extracted.

### Value

The scanBam,character-method returns a list of lists. The outer list groups results from each Ranges list of bamWhich(param); the outer list is of length one when bamWhich(param) has length 0. Each inner list contains elements named after scanBamWhat(); elements omitted from bamWhat(param) are removed. The content of non-null elements are as follows, taken from the description in the samtools API documentation:

- qname: This is the QNAME field in SAM Spec v1.4. The query name, i.e., identifier, associated with the read.
- flag: This is the FLAG field in SAM Spec v1.4. A numeric value summarizing details of the read. See ScanBamParam and the flag argument, and scanBamFlag().
- rname: This is the RNAME field in SAM Spec v1.4. The name of the reference to which the read is aligned.
- strand: The strand to which the read is aligned.
- pos: This is the POS field in SAM Spec v1.4. The genomic coordinate at the start of the alignment. Coordinates are 'left-most', i.e., at the 3' end of a read on the '-' strand, and 1-based. The position *excludes* clipped nucleotides, even though soft-clipped nucleotides are included in seq.

• qwidth: The width of the query, as calculated from the cigar encoding; normally equal to the width of the query returned in seq.

- mapq: This is the MAPQ field in SAM Spec v1.4. The MAPping Quality.
- cigar: This is the CIGAR field in SAM Spec v1.4. The CIGAR string.
- mrnm: This is the RNEXT field in SAM Spec v1.4. The reference to which the mate (of a paired end or mate pair read) aligns.
- mpos: This is the PNEXT field in SAM Spec v1.4. The position to which the mate aligns.
- isize: This is the TLEN field in SAM Spec v1.4. Inferred insert size for paired end alignments.
- seq: This is the SEQ field in SAM Spec v1.4. The query sequence, in the 5' to 3' orientation. If aligned to the minus strand, it is the reverse complement of the original sequence.
- qual: This is the QUAL field in SAM Spec v1.4. Phred-encoded, phred-scaled base quality score, oriented as seq.

scanBamHeader returns a list, with one element for each file named in files. The list contains two element. The targets element contains target (reference) sequence lengths. The text element is itself a list with each element a list corresponding to tags (e.g., '@SQ') found in the header, and the associated tag values.

asBam returns the file name of the BAM file.

sortBam returns the file name of the sorted file.

indexBam returns the file name of the index file created.

filterBam returns the file name of the destination file created.

#### Author(s)

Martin Morgan <a href="mtmorgan@fhcrc.org">mtmorgan@fhcrc.org</a>. Thomas Unterhiner <a href="mthorgan">thomas.unterthiner@students.jku.at</a> (sortBam).

#### References

http://samtools.sourceforge.net/

### See Also

ScanBamParam, scanBamWhat, scanBamFlag

### **Examples**

```
fl <- system.file("extdata", "ex1.bam", package="Rsamtools") \\ res0 <- scanBam(fl)[[1]] \# always list-of-lists \\ names(res0) \\ length(res0[["qname"]]) \\ lapply(res0, head, 3) \\ table(width(res0[["seq"]])) \# query widths \\ table(res0[["qwidth"]], useNA="always") \# query widths derived from cigar \\ table(res0[["cigar"]], useNA="always") \\ table(res0[["strand"]], useNA="always") \\ table(res0[["flag"]], useNA="always") \\ which <- RangesList(seq1=IRanges(1000, 2000), \\ \\
```

```
seq2 = IRanges(c(100, 1000), c(1000, 2000)))
p1 <- ScanBamParam(which=which, what=scanBamWhat())
res1 <- scanBam(fl, param=p1)
names(res1)
names(res1[[2]])
p2 <- ScanBamParam(what=c("rname", "strand", "pos", "qwidth"))
res2 <- scanBam(fl, param=p2)
p3 <- ScanBamParam(flag=scanBamFlag(isMinusStrand=FALSE))
length(scanBam(fl, param=p3)[[1]])
sorted <- sortBam(fl, tempfile())
## map mcols(gwhich) to output, e.g., of countBam
gwhich <- as(which, "GRanges")[c(2, 1, 3)]
mcols(gwhich)[["OriginalOrder"]] <- 1:3
cnt <- countBam(fl, param=ScanBamParam(which=gwhich))
cntVals <- unlist(split(mcols(gwhich), seqnames(gwhich)))
cbind(cnt, as.data.frame(cntVals))
```

BamViews

Views into a set of BAM files

### **Description**

Use BamViews() to reference a set of disk-based BAM files to be processed (e.g., queried using scanBam) as a single 'experiment'.

```
## Constructor
BamViews(bamPaths=character(0),
   bamIndicies=bamPaths,
   bamSamples=DataFrame(row.names=make.unique(basename(bamPaths))),
   bamRanges, bamExperiment = list(), ...)
\#\# S4 method for signature 'missing'
BamViews(bamPaths=character(0),
   bamIndicies=bamPaths,
   bamSamples=DataFrame(row.names=make.unique(basename(bamPaths))),
   bamRanges, bamExperiment = list(), ..., auto.range=FALSE)
## Accessors
bamPaths(x)
bamSamples(x)
bamSamples(x) < - value
bamRanges(x)
bamRanges(x) <- value
bamExperiment(x)
## S4 method for signature 'BamViews'
names(x)
```

```
## S4 replacement method for signature 'BamViews'
names(x) < -value
\#\# S4 method for signature 'BamViews'
dimnames(x)
## S4 replacement method for signature 'BamViews, ANY'
dimnames(x) < - value
bamDirname(x, ...) <- value
## Subset
## S4 method for signature 'BamViews, ANY, ANY'
x[i, j, ..., drop=TRUE]
## S4 method for signature 'BamViews, ANY, missing'
x[i, j, ..., drop=TRUE]
## S4 method for signature 'BamViews,missing,ANY'
x[i, j, ..., drop=TRUE]
## Input
\#\# S4 method for signature 'BamViews'
\operatorname{scanBam}(\operatorname{file}, \operatorname{index} = \operatorname{file}, ...,
   param = ScanBamParam(what=scanBamWhat()))
\#\# S4 method for signature 'BamViews'
countBam(file, index = file, ..., param = ScanBamParam())
## S4 method for signature 'BamViews'
readBamGappedAlignments(file, index=file, use.names=FALSE, param=NULL)
\#\# Show
\#\# S4 method for signature 'BamViews'
show(object)
\#\# Counting
## S4 method for signature 'BamViews,missing'
summarizeOverlaps(
  features, reads, mode, ignore.strand=FALSE, ..., singleEnd=TRUE, param=ScanBamParam())
```

### **Arguments**

bamPaths

| Sami aciis          | Tremander () vector of Britis pain manies.   |
|---------------------|--|
| ${\bf bamIndicies}$ | A character() vector of BAM index file path names, without the '.bai' extension.   |
| bamSamples          | A DataFrame instance with as many rows as length(bamPaths), containing sample information associated with each path.                                 |
| bamRanges           | A GRanges, RangedData or missing instance with ranges defined on the spaces of the BAM files. Ranges are <i>not</i> validated against the BAM files. |
| bamExperiment       | A list() containing additional information about the experiment.   |
| auto.range          | If TRUE and all bamPaths exist, populate the ranges with the union of ranges returned in the target element of scanBamHeader.                        |
|                     | Additional arguments.  |
| X                   | An instance of BamViews.   |
| object              | An instance of BamViews.   |

A character() vector of BAM path names.

value An object of appropriate type to replace content.

i During subsetting, a logical or numeric index into bamRanges.

j During subsetting, a logical or numeric index into bamSamples and bamPaths.

drop A logical(1), *ignored* by all BamViews subsetting methods.

file An instance of BamViews.

index A character vector of indices, corresponding to the bamPaths(file).

param An optional ScanBamParam instance to further influence scanning or counting.

use.names Construct the names of the returned object from the query template names (QNAME

field)? If not (the default), then the returned object has no names.

reads Missing when a BamViews is the only argument supplied to summarizeOverlaps.

reads are the files specified in bamPaths of the BamViews object.

features A BamFileList. features are extracted from the bamRanges of the BamViews

object.

Metadata from bamPaths and bamSamples are stored in the colData slot of the SummarizedExperiment object. bamExperiment metadata are in the

exptData slot.

A function that defines the method to be used when a read overlaps more than one feature. Pre-defined options are "Union", "IntersectionStrict", or "IntersectionNotEmpty" and are designed after the counting modes available in the

HTSeq package by Simon Anders (see references).

• "Union": (Default) Reads that overlap any portion of exactly one feature are counted. Reads that overlap multiple features are discarded.

- "IntersectionStrict": A read must fall completely "within" the feature to be counted. If a read overlaps multiple features but falls "within" only one, the read is counted for that feature. If the read is "within" multiple features, the read is discarded.
- "IntersectionNotEmpty": A read must fall in a unique disjoint region of a
  feature to be counted. When a read overlaps multiple features, the features
  are partitioned into disjoint intervals. Regions that are shared between the
  features are discarded leaving only the unique disjoint regions. If the read
  overlaps one of these remaining regions, it is assigned to the feature the
  unique disjoint region came from.

ignore.strand A logical value indicating if strand should be considered when matching.

singleEnd A logical value indicating if the bam files contain single or paired-end reads.

### **Objects from the Class**

mode

Objects are created by calls of the form BamViews().

#### Slots

bamPaths A character() vector of BAM path names.

**bamIndicies** A character() vector of BAM index path names.

**bamSamples** A DataFrame instance with as many rows as length(bamPaths), containing sample information associated with each path.

**bamRanges** A GRanges instance with ranges defined on the spaces of the BAM files. Ranges are *not* validated against the BAM files.

**bamExperiment** A list() containing additional information about the experiment.

#### **Functions and methods**

See 'Usage' for details on invocation.

Constructor:

BamViews: Returns a BamViews object.

Accessors:

**bamPaths** Returns a character() vector of BAM path names.

bamIndicies Returns a character() vector of BAM index path names.

**bamSamples** Returns a DataFrame instance with as many rows as length(bamPaths), containing sample information associated with each path.

**bamSamples<-** Assign a DataFrame instance with as many rows as length(bamPaths), containing sample information associated with each path.

**bamRanges** Returns a GRanges instance with ranges defined on the spaces of the BAM files. Ranges are *not* validated against the BAM files.

**bamRanges<-** Assign a GRanges instance with ranges defined on the spaces of the BAM files. Ranges are *not* validated against the BAM files.

bamExperiment Returns a list() containing additional information about the experiment.

**names** Return the column names of the BamViews instance; same as names(bamSamples(x)).

names<- Assign the column names of the BamViews instance.

**dimnames** Return the row and column names of the BamViews instance.

**dimnames<-** Assign the row and column names of the BamViews instance.

Methods:

"[" Subset the object by bamRanges or bamSamples.

**scanBam** Visit each path in bamPaths(file), returning the result of scanBam applied to the specified path. bamRanges(file) takes precedence over bamWhich(param).

**countBam** Visit each path in bamPaths(file), returning the result of countBam applied to the specified path. bamRanges(file) takes precedence over bamWhich(param).

readBamGappedAlignments Visit each path in bamPaths(file), returning the result of readBamGappedAlignments applied to the specified path. When index is missing, it is set equal to bamIndicies(file). Only reads in bamRanges(file) are returned (if param is supplied, bamRanges(file) takes precedence over bamWhich(param)). The return value is a SimpleList, with elements of the list corresponding to each path. bamSamples(file) is available as metadata columns (accessed with mcols) of the returned SimpleList.

show Compactly display the object.

#### Author(s)

Martin Morgan

### See Also

 ${\bf readBamGappedAlignments.}\ \ {\bf The}\ \ {\bf GenomicRanges}\ \ {\bf package}\ \ {\bf is}\ \ {\bf where}\ \ {\bf the}\ \ {\bf summarizeOverlaps}\ \ {\bf method}\ \ {\bf originates}.$ 

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#### **Examples**

```
fls <- system.file("extdata", "ex1.bam", package="Rsamtools")
rngs <- GRanges(seqnames = Rle(c("chr1", "chr2"), \, c(9, \, 9)), \\
           ranges = c(IRanges(seq(10000, 90000, 10000), width=500),
                   IRanges(seq(100000, 900000, 100000), width=5000)),
           Count = seq len(18L)
v <- BamViews(fls, bamRanges=rngs)
v[1:5,]
bamRanges(v[c(1:5, 11:15),])
bamDirname(v) < - getwd()
bv <- BamViews(fls,
           bamSamples=DataFrame(info="test", row.names="ex1"),
           auto.range=TRUE)
aln < - \ readBamGappedAlignments(bv)
aln
aln[[1]]
aln[colnames(bv)]
mcols(aln)
## summarizeOverlaps with BamViews
## bamSamples and bamPaths metadata are put into the colData
## and the bamExperiment metadata is put into the exptData slot
\#\# of the resulting Summarized
Experiment.
{\it fl} < - system. {\it file}("extdata", "ex1.bam", package="Rsamtools")
rng <- GRanges(c("seq1", "seq2"), IRanges(1, c(1575, 1584)))
bv <- BamViews(fl, bamSamples=DataFrame(info="test", row.names="ex1"),
           bamRanges=rng)
se <- summarizeOverlaps(bv, mode=Union, ignore.strand=TRUE)
colData(se)
```

BcfFile

Manipulate BCF files.

### Description

Use BcfFile() to create a reference to a BCF (and optionally its index). The reference remains open across calls to methods, avoiding costly index re-loading.

BcfFileList() provides a convenient way of managing a list of BcfFile instances.

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```
## Opening / closing
## S3 method for class 'BcfFile'
open(con, ...)
\#\# S3 method for class 'BcfFile'
close(con, ...)
## accessors; also path(), index()
## S4 method for signature 'BcfFile'
isOpen(con, rw="")
bcfMode(object)
\#\# actions
## S4 method for signature 'BcfFile'
scanBcfHeader(file, ...)
## S4 method for signature 'BcfFile'
scanBcf(file, ..., param=ScanBcfParam())
## S4 method for signature 'BcfFile'
indexBcf(file, ...)
```

### **Arguments**

con, object

An instance of BcfFile.

A character(1) vector of the BCF file path or, (for indexBcf) an instance of BcfFile point to a BCF file.

index

A character(1) vector of the BCF index.

mode

A character(1) vector; mode="rb" indicates a binary (BCF) file, mode="r" a text (VCF) file.

param

An optional ScanBcfParam instance to further influence scanning.

Additional arguments. For BcfFileList, this can either be a single character

vector of paths to BCF files, or several instances of BcfFile objects.

rw Mode of file; ignored.

### **Objects from the Class**

Objects are created by calls of the form BcfFile().

### **Fields**

The BcfFile class inherits fields from the RsamtoolsFile class.

### **Functions and methods**

BcfFileList inherits methods from RsamtoolsFileList and SimpleList.

Opening / closing:

**open.BcfFile** Opens the (local or remote) path and index (if bamIndex is not character(0)), files. Returns a BcfFile instance.

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**close.BcfFile** Closes the BcfFile con; returning (invisibly) the updated BcfFile. The instance may be re-opened with open.BcfFile.

Accessors:

```
path Returns a character(1) vector of the BCF path name.
```

index Returns a character(1) vector of BCF index name.

**bcfMode** Returns a character(1) vector BCF mode.

Methods:

**scanBcf** Visit the path in path(file), returning the result of scanBcf applied to the specified path. **show** Compactly display the object.

### Author(s)

Martin Morgan

### **Examples**

```
{\it fl} < - system. {\it file}("extdata", "ex1.bcf", package = "Rsamtools")
bf <- BcfFile(fl)
                      \# implicit index
bf
identical(scanBcf(bf), scanBcf(fl))
rng <- GRanges(c("seq1", "seq2"), IRanges(1, c(1575, 1584)))
param <- ScanBcfParam(which=rng)
bcf <- scanBcf(bf, param=param) ## all ranges
\#\# ranges one at a time 'bf'
open(bf)
sapply(seq_len(length(rng)), function(i, bcfFile, rng) {
   param <- ScanBcfParam(which=rng)
   bcf <- scanBcf(bcfFile, param=param)[[1]]
   \#\# do extensive work with bcf
   isOpen(bf) ## file remains open
}, bf, rng)
```

BcfInput

Operations on 'BCF' files.

### Description

Import, coerce, or index variant call files in text or binary format.

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#### Usage

```
scanBcfHeader(file, ...)
## S4 method for signature 'character'
scanBcfHeader(file, ...)

scanBcf(file, ...)
## S4 method for signature 'character'
scanBcf(file, index = file, ..., param=ScanBcfParam())

asBcf(file, dictionary, destination, ...,
    overwrite=FALSE, indexDestination=TRUE)

## S4 method for signature 'character'
asBcf(file, dictionary, destination, ...,
    overwrite=FALSE, indexDestination=TRUE)

indexBcf(file, ...)

## S4 method for signature 'character'
indexBcf(file, ...)
```

### Arguments

| file | For scanBo | of and s | canBcfHe | ader, | the | character() | file name o | of the | 'BCF' | file to |
|------|------------|----------|----------|-------|-----|-------------|-------------|--------|-------|---------|
|      |            |          |          |       |     |             |             |        |       |         |

be processed, or an instance of class BcfFile.

index The character() file name(s) of the 'BCF' index to be processed.
dictionary a character vector of the unique "CHROM" names in the VCF file.

destination The character(1) file name of the location where the BCF output file will be

created. For asBcf this is without the ".bcf" file suffix.

param A instance of ScanBcfParam influencing which records are parsed and the

'INFO' and 'GENO' information returned.

... Additional arguments, e.g., for scanBcfHeader, character-method, mode of

BcfFile.

overwrite A logical(1) indicating whether the destination can be over-written if it already

exists.

indexDestination

A logical(1) indicating whether the created destination file should also be in-

dexed.

#### **Details**

bcf\* functions are restricted to the GENO fields supported by 'bcftools' (see documentation at the url below). The argument param allows portions of the file to be input, but requires that the file be BCF or bgzip'd and indexed as a TabixFile. For similar functions operating on VCF files see ?scanVcf in the VariantAnnotation package.

### Value

scanBcfHeader returns a list, with one element for each file named in file. Each element of the list is itself a list containing three elements. The reference element is a character() vector with names of

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reference sequences. The sample element is a character() vector of names of samples. The header element is a character() vector of the header lines (preceded by "##") present in the VCF file.

scanBcf returns a list, with one element per file. Each list has 9 elements, corresponding to the columns of the VCF specification: CHROM, POS, ID, REF, ALTQUAL, FILTER, INFO, FORMAT, GENO.

The GENO element is itself a list, with elements corresponding to fields supported by 'bcftools' (see documentation at the url below).

asBcf creates a binary BCF file from a text VCF file.

indexBcf creates an index into the BCF file.

### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

#### References

http://vcftools.sourceforge.net/specs.html outlines the VCF specification.

http://samtools.sourceforge.net/mpileup.shtml contains information on the portion of the specification implemented by bcftools.

http://samtools.sourceforge.net/ provides information on samtools.

#### See Also

BcfFile, TabixFile

### **Examples**

```
fl <- system.file("extdata", "ex1.bcf", package="Rsamtools") scanBcfHeader(fl) bcf <- scanBcf(fl) ## value: list-of-lists str(bcf[1:8]) names(bcf[["GENO"]]) str(head(bcf[["GENO"]][["PL"]])) example(BcfFile)
```

Compression

File compression for tabix (bgzip) and fasta (razip) files.

### Description

These functions compress files for use in other parts of **Rsamtools**: bgzip for tabix files, razip for random-access fasta files.

```
bgzip(file, dest=sprintf("%s.gz", file), overwrite = FALSE) razip(file, dest=sprintf("%s.rz", file), overwrite = FALSE)
```

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### **Arguments**

file A character(1) path to an existing file. This file will be compressed.

dest A character(1) path to a file. This will be the compressed file. If dest exists,

then it is only over-written when overwrite=TRUE.

overwrite A logical(1) indicating whether dest should be over-written, if it already exists.

### Value

The full path to dest.

### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>

### References

http://samtools.sourceforge.net/

### See Also

TabixFile, FaFile.

### **Examples**

```
\label{lem:condition} from <- \ system.file("extdata", "ex1.sam", package="Rsamtools") to <- \ tempfile() zipped <- \ bgzip(from, to)
```

defunct

Defunct functions

### Description

Functions listed on this page have been removed.

### **Details**

For bgzipTabix, use bgzip.

### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

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deprecated

Deprecated functions

### **Description**

Functions listed on this page are no longer supported.

### **Details**

For yieldTabix, use the yieldSize argument of TabixFiles.

### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

FaFile

Manipulate indexed fasta files.

### Description

Use FaFile() to create a reference to an indexed fasta file. The reference remains open across calls to methods, avoiding costly index re-loading.

FaFileList() provides a convenient way of managing a list of FaFile instances.

```
## Constructors

FaFile(file, ...)
FaFileList(...)

## Opening / closing

## S3 method for class 'FaFile'
open(con, ...)

## S3 method for class 'FaFile'
close(con, ...)

## accessors; also path(), index()

## S4 method for signature 'FaFile'
isOpen(con, rw="")

## actions

## S4 method for signature 'FaFile'
indexFa(file, ...)
```

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```
## S4 method for signature 'FaFile'
scanFaIndex(file, ...)
\#\# S4 method for signature 'FaFileList'
scanFaIndex(file, ..., as=c("GRangesList", "GRanges"))
\#\# S4 method for signature 'FaFile'
countFa(file, ...)
## S4 method for signature 'FaFile,GRanges'
scanFa(file, param, ...)
## S4 method for signature 'FaFile,RangesList'
scanFa(file, param, ...)
## S4 method for signature 'FaFile,RangedData'
scanFa(file, param, ...)
## S4 method for signature 'FaFile, missing'
scanFa(file, param, ...)
\#\# S4 method for signature 'FaFile'
getSeq(x, param, ...)
\#\# S4 method for signature 'FaFileList'
getSeq(x, param, ...)
```

### **Arguments**

| con, x | An instance of FaFile or (for getSeq) FaFileList.   |
|--------|---|
| file   | A character(1) vector of the fasta file path (for FaFile), or an instance of class FaFile or FaFileList (for scanFaIndex, getSeq).  |
| param  | An optional GRanges, RangesList, or RangedData instance to select reads (and sub-sequences) for input. See Methods, below.  |
|        | Additional arguments. For FaFileList, this can either be a single character vector of paths to BAM files, or several instances of FaFile objects.   |
| rw     | Mode of file; ignored.  |
| as     | character(1) specifying the return type, selected from specified options. When GRangesList, index information from each file is returned as an element of the list. When GRangesList, index information is collapsed across files into the unique index elements. |

### **Objects from the Class**

Objects are created by calls of the form FaFile().

### **Fields**

The FaFile class inherits fields from the RsamtoolsFile class.

#### **Functions and methods**

FaFileList inherits methods from RsamtoolsFileList and SimpleList.

Opening / closing:

open.FaFile Opens the (local or remote) path and index files. Returns a FaFile instance.

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**close.FaFile** Closes the FaFile con; returning (invisibly) the updated FaFile. The instance may be re-opened with open.FaFile.

Accessors:

**path** Returns a character(1) vector of the fasta path name.

index Returns a character(1) vector of fasta index name (minus the '.fai' extension).

Methods:

indexFa Visit the path in path(file) and create an index file (with the extension '.fai').

**scanFaIndex** Read the sequence names and and widths of recorded in an indexed fasta file, returning the information as a GRanges object.

countFa Return the number of records in the fasta file.

scanFa Return the sequences indicated by param as a DNAStringSet instance. seqnames(param) selects the sequences to return; start(param) and end{param} define the (1-based) region of the sequence to return. Values of end(param) greater than the width of the sequence are set to the width of the sequence. When param is missing, all records are selected. When length(param)==0 no records are selected.

getSeq Returns the sequences indicated by param from the indexed fasta file(s) of file.

For the FaFile method, the return type is a DNAStringSet. The getSeq,FaFile and scanFa,FaFile,GRanges methods differ in that getSeq will reverse complement sequences selected from the minus strand.

For the FaFileList method, the param argument must be a GRangesList of the same length as file, creating a one-to-one mapping between the ith element of file and the ith element of param; the return type is a SimpleList of DNAStringSet instances, with elements of the list in the same order as the input elements.

**show** Compactly display the object.

### Author(s)

Martin Morgan

### **Examples**

```
fl <- system.file("extdata", "ce2dict1.fa", package="Rsamtools")
fa <- open(FaFile(fl)) # open
countFa(fa)
(idx <- scanFaIndex(fa))
(dna <- scanFa(fa, param=idx[1:2]))
ranges(idx) <- narrow(ranges(idx), -10) # last 10 nucleotides
(dna <- scanFa(fa, param=idx[1:2]))
```

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FaInput Operations on indexed 'fasta' files.

### **Description**

Scan indexed fasta (or compressed fasta) files and their indicies.

### Usage

```
indexFa(file, ...)
## S4 method for signature 'character'
indexFa(file, ...)
scanFaIndex(file, ...)
## S4 method for signature 'character'
scanFaIndex(file, ...)
countFa(file, ...)
## S4 method for signature 'character'
countFa(file, ...)
scanFa(file, param, ...)
## S4 method for signature 'character,GRanges'
scanFa(file, param, ...)
## S4 method for signature 'character,RangesList'
scanFa(file, param, ...)
## S4 method for signature 'character,RangedData'
scanFa(file, param, ...)
## S4 method for signature 'character, missing'
scanFa(file, param, ...)
```

### **Arguments**

file A character(1) vector containing the fasta file path.

param An optional GRanges, RangesList, or RangedData instance to select reads

(and sub-sequences) for input.

.. Additional arguments, currently unused.

#### Value

indexFa visits the path in file and create an index file at the same location but with extension '.fai'). scanFaIndex reads the sequence names and and widths of recorded in an indexed fasta file, returning the information as a GRanges object.

countFa returns the number of records in the fasta file.

scanFa return the sequences indicated by param as a DNAStringSet instance. seqnames(param) selects the sequences to return; start(param) and  $end\{param\}$  define the (1-based) region of the sequence to return. Values of end(param) greater than the width of the sequence are set to the width of the sequence. When param is missing, all records are selected. When param is GRanges(), no records are selected.

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#### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

#### References

http://samtools.sourceforge.net/ provides information on samtools.

### **Examples**

```
\label{eq:count_fa} $$fa <- system.file("extdata", "ce2dict1.fa", package="Rsamtools")$$ countFa(fa)$$ (idx <- scanFaIndex(fa))$$ (dna <- scanFa(fa, idx[1:2]))$$ ranges(idx) <- narrow(ranges(idx), -10) $$ \#$ last 10 nucleotides$$$ (dna <- scanFa(fa, idx[1:2]))$$
```

findMateAlignment

Pairing the elements of a GappedAlignments object

### **Description**

Utilities for pairing the elements of a GappedAlignments object.

### Usage

```
findMateAlignment(x, verbose=FALSE)
makeGappedAlignmentPairs(x, use.names=FALSE, use.mcols=FALSE)
## Related low-level utilities:
getDumpedAlignments()
countDumpedAlignments()
flushDumpedAlignments()
```

## Arguments

A named GappedAlignments object with metadata columns flag, mrnm, and mpos. Typically obtained by loading aligned paired-end reads from a BAM file with:

```
\begin{array}{l} param <- \; ScanBamParam(what = c("flag", \; "mrnm", \; "mpos")) \\ x <- \; readBamGappedAlignments(..., use.names = TRUE, param = param) \end{array}
```

verbose If TRUE, then findMateAlignment will print some details about what is currently using an Mostly useful for debugging

rently going on. Mostly useful for debugging.

use.names Whether the names on the input object should be propagated to the returned

object or not.

use.mcols Names of the metadata columns to propagate to the returned GappedAlignment-

Pairs object.

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#### **Details**

**Pairing algorithm used by findMateAlignment:** findMateAlignment is the power horse used by higher-level functions like makeGappedAlignmentPairs and readBamGappedAlignmentPairs for pairing the records loaded from a BAM file containing aligned paired-end reads.

It implements the following pairing algorithm:

- First only records with flag bit 0x1 set to 1, flag bit 0x4 set to 0, and flag bit 0x8 set to 0 are candidates for pairing (see the SAM Spec for a description of flag bits and fields). findMateAlignment will ignore any other record. That is, records that correspond to single-end reads, and records that correspond to paired-end reads where one or both ends are unmapped, are discarded.
- Then the algorithm looks at the following fields and flag bits:
  - (A) QNAME
  - (B) RNAME, RNEXT
  - (C) POS, PNEXT
  - (D) Flag bits Ox10 and 0x20
  - (E) Flag bits 0x40 and 0x80
  - (F) Flag bit 0x2
  - (G) Flag bit 0x100

2 records rec(i) and rec(j) are considered mates iff all the following conditions are satisfied:

- (A) They have the same QNAME
- (B) RNEXT(i) == RNAME(j) and RNEXT(j) == RNAME(i)
- (C) PNEXT(i) == POS(j) and PNEXT(j) == POS(i)
- (D) Flag bit 0x20 of rec(i) == Flag bit 0x10 of rec(j) and Flag bit 0x20 of rec(j) == Flag bit 0x10 of rec(i)
- (E) rec(i) corresponds to the first segment in the template and rec(j) corresponds to the
  last segment in the template, OR, rec(j) corresponds to the first segment in the template
  and rec(i) corresponds to the last segment in the template
- (F) rec(i) and rec(j) have same flag bit 0x2
- (G) rec(i) and rec(j) have same flag bit 0x100

**Ambiguous pairing:** The above algorithm will find almost all pairs unambiguously, even when the same pair of reads maps to several places in the genome. Note that, when a given pair maps to a single place in the genome, looking at (A) is enough to pair the 2 corresponding records. The additional conditions (B), (C), (D), (E), (F), and (G), are only here to help in the situation where more than 2 records share the same QNAME. And that works most of the times. Unfortunately there are still situations where this is not enough to solve the pairing problem unambiguously.

For example, here are 4 records (loaded in a GappedAlignments object) that cannot be paired with the above algorithm:

Showing the 4 records as a GappedAlignments object of length 4:

GappedAlignments with 4 alignments and 2 metadata columns:

```
segnames strand
                              cigar
                                     qwidth
              <Rle> <Rle> <character> <integer> <integer> <integer>
SRR031714.2658602
                   chr2R.
                            + 21M384N16M
                                                37
                                                    6983850 6984270
SRR031714.2658602
                   chr2R
                             + 21M384N16M
                                                37
                                                    6983850 6984270
SRR031714.2658602
                   chr2R
                            - 13M372N24M
                                                37
                                                   6983858 6984266
SRR031714.2658602
                   chr2R
                            - 13M378N24M
                                                   6983858 6984272
                               mrnm
                       ngap
            <integer> <integer> | <factor> <integer>
```

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| SRR031714.2658602 | 421 | 1 | chr2R | 6983858 |
|-------------------|-----|---|-------|---------|
| SRR031714.2658602 | 421 | 1 | chr2R | 6983858 |
| SRR031714.2658602 | 409 | 1 | chr2R | 6983850 |
| SRR031714.2658602 | 415 | 1 | chr2R | 6983850 |

Note that the BAM fields show up in the following columns:

• QNAME: the names of the GappedAlignments object (unnamed col)

• RNAME: the segnames col

POS: the start colRNEXT: the mrnm colPNEXT: the mpos col

As you can see, the aligner has aligned the same pair to the same location twice! The only difference between the 2 aligned pairs is in the CIGAR i.e. one end of the pair is aligned twice to the same location with exactly the same CIGAR while the other end of the pair is aligned twice to the same location but with slightly different CIGARs.

Now showing the corresponding flag bits:

isPaired isProperPair isUnmappedQuery hasUnmappedMate isMinusStrand

| [1,] | 1 | 1 | 0 | 0 | 0 |
|------|---|---|---|---|---|
| [2,] | 1 | 1 | 0 | 0 | 0 |
| [3,] | 1 | 1 | 0 | 0 | 1 |
| [4,] | 1 | 1 | 0 | 0 | 1 |

isMateMinusStrand isFirstMateRead isSecondMateRead isNotPrimaryRead

| [1,]                 | 1 | 0 | 1 | 0 |
|----------------------|---|---|---|---|
| [1,]<br>[2,]<br>[3,] | 1 | 0 | 1 | 0 |
| [3,]                 | 0 | 1 | 0 | 0 |
| [4,]                 | 0 | 1 | 0 | 0 |

isNotPassingQualityControls isDuplicate

| [1,] | 0 | 0 |
|------|---|---|
| [2,] | 0 | 0 |
| [3,] | 0 | 0 |
| [4,] | 0 | 0 |

As you can see, rec(1) and rec(2) are second mates, rec(3) and rec(4) are both first mates. But looking at (A), (B), (C), (D), (E), (F), and (G), the pairs could be rec(1) <-> rec(3) and rec(2) <-> rec(4), or they could be rec(1) <-> rec(4) and rec(2) <-> rec(3). There is no way to disambiguate! So findMateAlignment is just ignoring (with a warning) those alignments with ambiguous pairing, and dumping them in a place from which they can be retrieved later (i.e. after findMateAlignment has returned) for further examination (see "Dumped alignments" subsection below for the details). In other words, alignments that cannot be paired unambiguously are not paired at all. Concretely, this means that readGappedAlignmentPairs is guaranteed to return a GappedAlignmentPairs object where every pair was formed in an non-ambiguous way. Note that, in practice, this approach doesn't seem to leave aside a lot of records because ambiguous pairing events seem pretty rare.

**Dumped alignments:** Alignments with ambiguous pairing are dumped in a place ("the dump environment") from which they can be retrieved with getDumpedAlignments() after findMateAlignment has returned.

Two additional utilities are provided for manipulation of the dumped alignments: countDumpedAlignments for counting them (a fast equivalent to length(getDumpedAlignments())), and flushDumpedAlignments to flush "the dump environment". Note that "the dump environment" is automatically flushed at the beginning of a call to findMateAlignment.

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#### Value

For findMateAlignment: An integer vector of the same length as x, containing only positive or NA values, where the i-th element is interpreted as follow:

- An NA value means that no mate or more than 1 mate was found for x[i].
- A non-NA value j gives the index in x of x[i]'s mate.

For makeGappedAlignmentPairs: A GappedAlignmentPairs object where the pairs are formed internally by calling findMateAlignment on x.

For getDumpedAlignments: NULL or a GappedAlignments object containing the dumped alignments. See "Dumped alignments" subsection in the "Details" section above for the details.

For countDumpedAlignments: The number of dumped alignments.

Nothing for flushDumpedAlignments.

### Author(s)

H. Pages

### See Also

Gapped A lignments-class, Gapped A lignment Pairs-class, read Bam Gapped A lignments, read Bam Gapped A lignment Pairs-class, read Bam Gapped A lignments, read Bam Gapped A lignment Pairs-class, read Bam Gapped A lignments, read Bam Gapped A lign

### **Examples**

```
\label{lem:bamfile} $$\operatorname{bamfile} <-\operatorname{system.file}("\operatorname{extdata}", "\operatorname{ex1.bam}", \operatorname{package} = "\operatorname{Rsamtools}")$ \\ \operatorname{param} <-\operatorname{ScanBamParam}(\operatorname{what} = \operatorname{c}("\operatorname{flag}", "\operatorname{mrnm}", "\operatorname{mpos}"))$ \\ x <-\operatorname{readBamGappedAlignments}(\operatorname{bamfile}, \operatorname{use.names} = \operatorname{TRUE}, \operatorname{param} = \operatorname{param})$ \\ \operatorname{mate} <-\operatorname{findMateAlignment}(x)$ \\ \operatorname{head}(\operatorname{mate})$ \\ \operatorname{table}(\operatorname{is.na}(\operatorname{mate}))$ \\ \operatorname{galp} <-\operatorname{makeGappedAlignmentPairs}(x)$ \\ \operatorname{galp} <-\operatorname{makeGappedAlignmentPairs}(x, \operatorname{use.name} = \operatorname{TRUE}, \operatorname{use.mcols} = "\operatorname{flag}")$ \\ \operatorname{galp}$ \\ \operatorname{colnames}(\operatorname{mcols}(\operatorname{galp}))$ \\ \operatorname{colnames}(\operatorname{mcols}(\operatorname{first}(\operatorname{galp})))$ \\ \operatorname{colnames}(\operatorname{mcols}(\operatorname{last}(\operatorname{galp})))
```

headerTabix

Retrieve sequence names defined in a tabix file.

### **Description**

This function queries a tabix file, returning the names of the 'sequences' used as a key when creating the file.

```
headerTabix(file, ...)
## S4 method for signature 'character'
headerTabix(file, ...)
```

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### Arguments

file A character(1) file path or TabixFile instance pointing to a 'tabix' file.

... Additional arguments, currently ignored.

### Value

A list(4) of the sequence names, column indicies used to sort the file, the number of lines skipped while indexing, and the comment character used while indexing.

### Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

### **Examples**

```
fl <- system.file
("extdata", "example.gtf.gz", package="R<br/>samtools") header
Tabix(fl)
```

indexTabix

Compress and index tabix-compatible files.

### **Description**

Index (with indexTabix) files that have been sorted into ascending sequence, start and end position ordering.

### Usage

```
\begin{split} & indexTabix(file,\\ & format=c("gff", "bed", "sam", "vcf", "vcf4", "psltbl"),\\ & seq=integer(), \, start=integer(), \, end=integer(),\\ & skip=0L, \, comment="\#", \, zeroBased=FALSE, \ldots) \end{split}
```

### **Arguments**

| file      | A characater(1) path to a sorted, bgzip-compressed file.   |
|-----------|--|
| format    | The format of the data in the compressed file. A characater(1) matching one of the types named in the function signature.            |
| seq       | If format is missing, then $\operatorname{seq}$ indicates the column in which the 'sequence' identifier (e.g., chrq) is to be found. |
| start     | If format is missing, start indicates the column containing the start coordinate of the feature to be indexed.                       |
| end       | If format is missing, end indicates the column containing the ending coordinate of the feature to be indexed.                        |
| skip      | The number of lines to be skipped at the beginning of the file.  |
| comment   | A single character which, when present as the first character in a line, indicates that the line is to be omitted. from indexing.    |
| zeroBased | A logical(1) indicating whether coordinats in the file are zero-based.   |
|           | Additional arguments.  |

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#### Value

The return value of indexTabix is an updated instance of file reflecting the newly-created index file.

### Author(s)

Martin Morgan <a href="mtmorgan@fhcrc.org">mtmorgan@fhcrc.org</a>.

#### References

http://samtools.sourceforge.net/tabix.shtml

### **Examples**

```
from <- system.file("extdata", "ex1.sam", package="Rsamtools")
to <- tempfile()
zipped <- bgzip(from, to)
idx <- indexTabix(zipped, "sam")
tab <- TabixFile(zipped, idx)
```

PileupFiles

Represent BAM files for pileup summaries.

### **Description**

Use PileupFiles() to create a reference to a BAM files (and their indicies), to be used for calculating pile-up summaries.

```
## Constructors
PileupFiles(files, ..., param=PileupParam())
\#\# S4 method for signature 'character'
PileupFiles(files, ..., param=PileupParam())
\#\# S4 method for signature 'list'
PileupFiles(files, ..., param=PileupParam())
## opening / closing
\#\# S3 method for class 'PileupFiles'
open(con, ...)
## S3 method for class 'PileupFiles'
close(con, ...)
\#\# accessors; also path()
## S4 method for signature 'PileupFiles'
isOpen(con, rw="")
plpFiles(object)
plpParam(object)
```

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```
## actions
## S4 method for signature 'PileupFiles,missing'
applyPileups(files, FUN, ..., param)
## S4 method for signature 'PileupFiles,PileupParam'
applyPileups(files, FUN, ..., param)

## display
## S4 method for signature 'PileupFiles'
show(object)
```

### **Arguments**

files For PileupFiles, a character() or list of BamFile instances representing files

to be included in the pileup. Using a list of BamFile allows indicies to be specified when these are in non-standard format. All elements of ... must be the

same type.

For applyPileups,PileupFiles-method, a PileupFiles instance.

... Additional arguments, currently ignored.

con, object An instance of PileupFiles.

FUN A function of one argument; see applyPileups.

param An instance of PileupParam, to select which records to include in the pileup,

and which summary information to return.

rw character() indicating mode of file; not used for TabixFile.

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

Objects are created by calls of the form PileupFiles().

### **Fields**

The PileupFiles class is implemented as an S4 reference class. It has the following fields:

files A list of BamFile instances.

param An instance of PileupParam.

#### **Functions and methods**

Opening / closing:

**open.PileupFiles** Opens the (local or remote) path and index of each file in the PileupFiles instance. Returns a PileupFiles instance.

**close.PileupFiles** Closes each file in the PileupFiles instance; returning (invisibly) the updated PileupFiles. The instance may be re-opened with open.PileupFiles.

Accessors:

**plpFiles** Returns the list of the files in the PileupFiles instance.

plpParam Returns the PileupParam content of the PileupFiles instance.

Methods:

applyPileups Calculate the pileup across all files in files according to criteria in param (or plpParam(files) if param is missing), invoking FUN on each range or collection of positions. See applyPileups.show Compactly display the object.

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### Author(s)

Martin Morgan

### **Examples**

```
example(applyPileups)
```

PileupParam

Parameters for creating pileups from BAM files

### **Description**

Use PileupParam() to create a parameter object influencing what fields and which records are used to calculate pile-ups, and to influence the values returned.

```
# Constructor
PileupParam(flag = scanBamFlag(),
   minBaseQuality = 13L, minMapQuality = 0L,
   minDepth = 0L, maxDepth = 250L,
   yieldSize = 1L, yieldBy = c("range", "position"), yieldAll = FALSE,
   which = GRanges(), what = c("seq", "qual"))
\# Accessors
plpFlag(object)
plpFlag(object) <- value
plpMaxDepth(object)
plpMaxDepth(object) <- value
plpMinBaseQuality(object)
plpMinBaseQuality(object) <- value
plpMinDepth(object)
plpMinDepth(object) <- value
plpMinMapQuality(object)
plpMinMapQuality(object) <- value
plpWhat(object)
plpWhat(object) <- value
plpWhich(object)
plpWhich(object) <- value
plpYieldAll(object)
plpYieldAll(object) <- value
plpYieldBy(object)
plpYieldBy(object) <- value
plpYieldSize(object)
plpYieldSize(object) <- value
\#\# S4 method for signature 'PileupParam'
show(object)
```

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### **Arguments**

| flag                     | An instance of the object returned by scanBamFlag, restricting various aspects of reads to be included or excluded.  |
|--------------------------|--|
| minBase Quality          | The minimum read base quality below which the base is ignored when summarizing pileup information.   |
| $\min {\it Map Quality}$ | The minimum mapping quality below which the entire read is ignored.  |
| $\min Depth$             | The minimum depth of the pile-up below which the position is ignored.  |
| $\max$ Depth             | The maximum depth of reads considered at any position; this can be used to limit memory consumption.   |
| yieldSize                | The number of records to include in each call to FUN.  |
| yieldBy                  | How records are to be counted. By range (in which case <code>yieldSize</code> must equal 1) means that FUN is invoked once for each range in which. By position means that FUN is invoked whenever pile-ups have been accumulated for <code>yieldSize</code> positions, regardless of ranges in which. |
| yieldAll                 | Whether to report all positions (yieldAll=TRUE), or just those passing the filtering criteria of flag, minBaseQuality, etc. When yieldAll=TRUE, positions not passing filter criteria have '0' entries in seq or qual.   |
| which                    | A GRanges or RangesList instance restricting pileup calculations to the corresponding genomic locations.   |
| what                     | A character() instance indicating what values are to be returned. One or more of c("seq", "qual").   |
| object                   | An instace of class PileupParam.   |
| value                    | An instance to be assigned to the corresponding slot of the PileupParam in-  |

### **Objects from the Class**

Objects are created by calls of the form PileupParam().

stance.

### **Slots**

Slot interpretation is as described in the 'Arguments' section.

```
flag Object of class integer encoding flags to be kept when they have their '0' (keep0) or '1' (keep1) bit set.

minBaseQuality An integer(1).

minDepth An integer(1).

maxDepth An integer(1).

yieldSize An integer(1).

yieldBy An character(1).

yieldAll A logical(1).

which A GRanges or RangesList instance.

what A character().
```

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#### **Functions and methods**

See 'Usage' for details on invocation.

Constructor:

PileupParam: Returns a PileupParam object.

Accessors: get or set corresponding slot values; for setters, value is coerced to the type of the corresponding slot.

plpFlag, plpFlag<- Returns or sets the named integer vector of flags; see scanBamFlag.

**plpMinBaseQuality, plpMinBaseQuality<-** Returns or sets an integer(1) vector of miminum base qualities.

**plpMinMapQuality, plpMinMapQuality<-** Returns or sets an integer(1) vector of miminum map qualities.

**plpMinDepth, plpMinDepth<-** Returns or sets an integer(1) vector of miminum pileup depth.

**plpMaxDepth, plpMaxDepth<-** Returns or sets an integer(1) vector of the maximum depth to which pileups are calculated.

**plpYieldSize, plpYieldSize<-** Returns or sets an integer(1) vector of yield size.

**plpYieldBy, plpYieldBy<-** Returns or sets an character(1) vector determining how pileups will be returned.

**plpYieldAll, plpYieldAll<-** Returns or sets an logical(1) vector indicating whether all positions, or just those satisfying pileup positions, are to be returned.

**plpWhich, plpWhich<-** Returns or sets the object influencing which locations pileups are calculated over.

**plpWhat, plpWhat<-** Returns or sets the character vector describing what summaries are returned by pileup.

Methods:

show Compactly display the object.

#### Author(s)

Martin Morgan

### See Also

applyPileups.

### **Examples**

example (apply Pileups)

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| ${\it quick} {\it Bam} {\it Counts}$ | Group the records of a BAM file based on their flag bits and count the number of records in each group |
|--------------------------------------|--|
|                                      | number of records in each group  |

# Description

quickBamCounts groups the records of a BAM file based on their flag bits and counts the number of records in each group.

# Usage

```
quickBamCounts(file, index=file, param=NULL, main.groups.only=FALSE)
```

# **Arguments**

file, index The path to the BAM file to read, and to the index file of the BAM file to read,

respectively. The latter is given without the '.bai' extension. See scanBam for

more information.

param NULL or an instance of ScanBamParam. This determines which records are

considered in the counting.

main.groups.only

If TRUE, then the counting is performed for the main groups only.

## Value

Nothing is returned. A summary of the counts is printed to the console unless redirected by sink.

# Author(s)

H. Pages

# References

http://samtools.sourceforge.net/

## See Also

```
scanBam, ScanBamParam
```

## **Examples**

```
bamfile <- \ system.file ("extdata", "ex1.bam", package="Rsamtools") \\ quickBamCounts (bamfile)
```

readBamGappedAlignments

Reading a BAM file into a GappedAlignments, GappedReads, or GappedAlignmentPairs object

## **Description**

Read a BAM file into a GappedAlignments, GappedReads, or GappedAlignmentPairs object.

#### Usage

```
readBamGappedAlignments(file, index=file, use.names=FALSE, param=NULL)
readBamGappedReads(file, index=file, use.names=FALSE, param=NULL)
readBamGappedAlignmentPairs(file, index=file, use.names=FALSE, param=NULL)
```

## **Arguments**

The path to the BAM file to read, and to the index file of the BAM file to read, file, index

respectively. The latter is given without the '.bai' extension. See scanBam for

more information.

Use the guery template names (ONAME field) as the names of the returned use.names

object? If not (the default), then the returned object has no names.

param NULL or an instance of ScanBamParam. Like for scanBam, this influences

> what fields and which records are imported. However, note that the fields specified thru this ScanBamParam object will be loaded in addition to any field required for generating the returned object (GappedAlignments, GappedReads, or GappedAlignmentPairs object), but only the fields requested by the user will

actually be kept as metadata columns of the object.

By default (i.e. param=NULL), no additional field is loaded and the flag used is scanBamFlag(isUnmappedQuery=FALSE) for readBamGappedAlignmentsand readBamGappedReads (i.e. only records corresponding to mapped reads are loaded), and scanBamFlag(isUnmappedQuery=FALSE, isPaired=TRUE, hasUnmappedI

for readBamGappedAlignmentPairs (i.e. only records corresponding to paired-

end reads with both ends mapped are loaded).

# **Details**

See ?GappedAlignments-class for a description of GappedAlignments objects.

See ?GappedReads-class for a description of GappedReads objects.

readBamGappedAlignmentPairs proceeds in 2 steps:

- 1. Load the BAM file into a GappedAlignments object with readBamGappedAlignments;
- 2. Turn this GappedAlignments object into a GappedAlignmentPairs object by pairing its elements.

See ?GappedAlignmentPairs-class for a description of GappedAlignmentPairs objects, and ?makeGappedAlignment. for the details of the pairing procedure.

#### Value

A GappedAlignments object for readBamGappedAlignments.

A GappedReads object for readBamGappedReads.

A GappedAlignmentPairs object for readBamGappedAlignmentPairs. Note that a BAM (or SAM) file can in theory contain a mix of single-end and paired-end reads, but in practise it seems that single-end and paired-end are not mixed. In other words, the value of flag bit 0x1 (isPaired) is the same for all the records in a file. So if readBamGappedAlignmentPairs returns a GappedAlignmentPairs object of length zero, this almost certainly means that the BAM (or SAM) file contains alignments for single-end reads (although it could also mean that the user-supplied ScanBamParam is filtering out everything, or that the file is empty, or that all the records in the file correspond to unmapped reads).

## Note

BAM records corresponding to unmapped reads are always ignored.

Starting with Rsamtools 1.7.1 (BioC 2.10), PCR or optical duplicates are loaded by default (use scanBamFlag(isDuplicate=FALSE) to drop them).

#### Author(s)

H. Pages

## See Also

GappedAlignments-class, GappedReads-class, GappedAlignmentPairs-class, makeGappedAlignmentPairs, scanBam, ScanBamParam

### **Examples**

```
## A. readBamGappedAlignments()
\#\# Simple use:
bamfile <- system.file("extdata", "ex1.bam", package="Rsamtools")
gal1 <- readBamGappedAlignments(bamfile)
gal1
names(gal1)
## Using the 'use.names' arg:
gal2 <- readBamGappedAlignments(bamfile, use.names=TRUE)
head(names(gal2))
## Using the 'param' arg to drop PCR or optical duplicates and load
\#\# additional BAM fields:
param <- ScanBamParam(flag=scanBamFlag(isDuplicate=FALSE),
               what=c("qual", "flag"))
gal3 <- readBamGappedAlignments(bamfile, param=param)
gal3
mcols(gal3)
## Using the 'param' arg to load reads from particular regions.
## Note that if we weren't providing a 'what' argument here, all the
```

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```
## BAM fields would be loaded:
which <- RangesList(seq1=IRanges(1000, 2000),
              seq2=IRanges(c(100, 1000), c(1000, 2000)))
param <- ScanBamParam(which=which)
gal4 <- readBamGappedAlignments(bamfile, param=param)
gal4
## Note that a given record is loaded one time for each region it
## belongs to (this is a scanBam() feature, readBamGappedAlignments()
## is based on scanBam()):
which <- IRangesList(seq2=IRanges(c(1563, 1567), width=1))
param <- ScanBamParam(which=which)
gal5 <- readBamGappedAlignments(bamfile, param=param)
gal5
## Using the 'param' arg to load tags. Except for MF and Aq, the tags
\#\# specified below are predefined tags (see the SAM Spec for the list
## of predefined tags and their meaning).
param <- ScanBamParam(tag=c("MF", "Aq", "NM", "UQ", "H0", "H1"),
               what="isize")
gal6 <- readBamGappedAlignments(bamfile, param=param)
mcols(gal6) # "tag" cols always after "what" cols
## B. readBamGappedReads()
{\it greads} 1 < - {\it readBamGappedReads}({\it bamfile})
greads1
names(greads1)
qseq(greads1)
greads2 <- readBamGappedReads(bamfile, use.names=TRUE)
head(greads2)
head(names(greads2))
## C. readBamGappedAlignmentPairs()
galp1 <- readBamGappedAlignmentPairs(bamfile)
head(galp1)
names(galp1)
galp2 <- readBamGappedAlignmentPairs(bamfile, use.names=TRUE)
galp2
head(galp2)
head(names(galp2))
```

readPileup

Import samtools 'pileup' files.

# Description

Import files created by evaluation of samtools' pileup -cv command.

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#### **Usage**

```
readPileup(file, ...)
## S4 method for signature 'connection'
readPileup(file, ..., variant=c("SNP", "indel", "all"))
```

## **Arguments**

file The file name, or connection, of the pileup output file to be parsed.

... Additional arguments, passed to methods. For instance, specify variant for the

readPileup, character-method.

variant Type of variant to parse; select one.

#### Value

readPileup returns a GRanges object.

The value returned by variant="SNP" or variant="all" contains:

space: The chromosome names (fastq ids) of the reference sequence

**position:** The nucleotide position (base 1) of the variant.

**referenceBase:** The nucleotide in the reference sequence.

consensusBase; The consensus nucleotide, as determined by samtools pileup.

consensusQuality: The phred-scaled consensus quality.

**snpQuality:** The phred-scaled SNP quality (probability of the consensus being identical to the reference).

maxMappingQuality: The root mean square mapping quality of reads overlapping the site.

**coverage:** The number of reads covering the site.

The value returned by variant="indel" contains space, position, reference, consensus, consensusQuality, snpQuality, maxMappingQuality, and coverage fields, and:

 $\textbf{alleleOne, alleleTwo} \ \ \text{The first (typically, in the reference sequence) and second allelic variants.}$ 

alleleOneSupport, alleleTwoSupport The number of reads supporting each allele.

additional Indels The number of additional indels present.

## Author(s)

Sean Davis

## References

http://samtools.sourceforge.net/

#### **Examples**

```
fl <- system.file("extdata", "pileup.txt", package="Rsamtools") (res <- readPileup(fl)) xtabs(~referenceBase + consensusBase, mcols(res))[DNA_BASES,] ## Not run: ## uses a pipe, and arguments passed to read.table ## three successive piles of 100 records each
```

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```
\label{eq:cmd} $\operatorname{cmd}<-\operatorname{"samtools\ pileup\ -cvf\ human\_b36\_female.fa.gz\ na19240\_3M.bam"}$ p <-\operatorname{pipe}(\operatorname{cmd},\operatorname{"r"})$ snp <-\operatorname{readPileup}(p,\operatorname{nrow}=100)\ \#\operatorname{variant}=\operatorname{"SNP"}$ indel <-\operatorname{readPileup}(p,\operatorname{nrow}=100,\operatorname{variant}=\operatorname{"indel"})$ all <-\operatorname{readPileup}(p,\operatorname{nrow}=100,\operatorname{variant}=\operatorname{"all"})$ $\#\operatorname{End}(\operatorname{Not\ run})$
```

RsamtoolsFile

A base class for managing file references in Rsamtools

## **Description**

RsamtoolsFile is a base class for managing file references in **Rsamtools**; it is not intended for direct use by users – see, e.g., BamFile.

## Usage

```
## accessors
index(object)
## S4 method for signature 'RsamtoolsFile'
path(object, ...)
## S4 method for signature 'RsamtoolsFile'
isOpen(con, rw="")
## S4 method for signature 'RsamtoolsFile'
yieldSize(object, ...)
yieldSize(object, ...) <- value
## S4 method for signature 'RsamtoolsFile'
show(object)
```

## **Arguments**

con, object An instance of a class derived from RsamtoolsFile.

rw Mode of file; ignored.

... Additional arguments, unused.

value Replacement value.

## **Objects from the Class**

Users do not directly create instances of this class; see, e.g., BamFile-class.

# Fields

The RsamtoolsFile class is implemented as an S4 reference class. It has the following fields:

**.extptr** An externalptr initialized to an internal structure with opened bam file and bam index pointers.

**path** A character(1) vector of the file name.

**index** A character(1) vector of the index file name.

**yieldSize** An integer(1) vector of the number of records to yield.

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## **Functions and methods**

```
Accessors:
```

path Returns a character(1) vector of path names.

**index** Returns a character(1) vector of index path names.

yieldSize, yieldSize<- Return or set an integer(1) vector indicating yield size.

Methods:

**isOpen** Report whether the file is currently open.

show Compactly display the object.

#### Author(s)

Martin Morgan

RsamtoolsFileList

A base class for managing lists of Rsamtools file references

## **Description**

RsamtoolsFileList is a base class for managing lists of file references in Rsamtools; it is not intended for direct use – see, e.g., BamFileList.

## Usage

```
## S4 method for signature 'RsamtoolsFileList' path(object, ...)
## S4 method for signature 'RsamtoolsFileList' isOpen(con, rw="")
## S3 method for class 'RsamtoolsFileList' open(con, ...)
## S3 method for class 'RsamtoolsFileList' close(con, ...)
## S4 method for signature 'RsamtoolsFileList' names(x)
## S4 method for signature 'RsamtoolsFileList' yieldSize(object, ...)
```

## **Arguments**

```
con, object, x An instance of a class derived from RsamtoolsFileList.

rw Mode of file; ignored.

... Additional arguments.
```

# **Objects from the Class**

Users do not directly create instances of this class; see, e.g., BamFileList-class.

#### **Functions and methods**

This class inherits functions and methods for subseting, updating, and display from the SimpleList class.

Methods:

isOpen: Report whether each file in the list is currently open.

**open:** Attempt to open each file in the list. **close:** Attempt to close each file in the list.

**names:** Names of each element of the list or, if names are NULL, the basename of the path of each element.

## Author(s)

Martin Morgan

ScanBamParam

Parameters for scanning BAM files

## **Description**

Use ScanBamParam() to create a parameter object influencing what fields and which records are imported from a (binary) BAM file. Use of which requires that a BAM index file (<filename>.bai) exists.

## Usage

```
# Constructor
ScanBamParam(flag = scanBamFlag(), simpleCigar = FALSE,
  reverseComplement = FALSE, tag = character(0),
  what = character(0), which)
\# Constructor helpers
scanBamFlag(isPaired = NA, isProperPair = NA, isUnmappedQuery = NA,
  hasUnmappedMate = NA, isMinusStrand = NA, isMateMinusStrand = NA,
  isFirstMateRead = NA, isSecondMateRead = NA, isNotPrimaryRead = NA,
  isNotPassingQualityControls = NA, isDuplicate = NA,
  isValidVendorRead = NA)
scanBamWhat()
# Accessors
bamFlag(object, asInteger=FALSE)
bamFlag(object) <- value
bamReverseComplement(object)
bamReverseComplement(object) <- value
bamSimpleCigar(object)
bamSimpleCigar(object) <- value
bamTag(object)
bamTag(object) <- value
```

```
bamWhat(object)
bamWhat(object) <- value
bamWhich(object)
bamWhich(object) <- value

## S4 method for signature 'ScanBamParam'
show(object)

# Flag utils
bamFlagAsBitMatrix(flag, bitnames=FLAG_BITNAMES)
bamFlagAND(flag1, flag2)
bamFlagTest(flag, value)
```

#### **Arguments**

flag For ScanBamParam, an integer(2) vector used to filter reads based on their

'flag' entry. This is most easily created with the scanBamFlag() helper func-

tion.

For bamFlagAsBitMatrix, bamFlagTest an integer vector where each ele-

ment represents a 'flag' entry.

simpleCigar A logical(1) vector which, when TRUE, returns only those reads for which the

cigar (run-length encoded representation of the alignment) is missing or contains

only matches / mismatches ('M').

reverseComplement

A logical(1) vector which, when TRUE, returns the sequence and quality scores of reads mapped to the minus strand in the reverse complement (sequence) and

reverse (quality) of the read as stored in the BAM file.

tag A character vector naming tags to be extracted. A tag is an optional field, with

arbitrary information, stored with each record. Tags are identified by two-letter

codes, so all elements of tag must have exactly 2 characters.

what A character vector naming the fields to return. scanBamWhat() returns a vector

of available fields. Fields are described on the scanBam help page.

which A GRanges, RangesList, RangedData, or missing object, from which a IRangesList

instance will be constructed. Names of the IRangesList correspond to reference sequences, and ranges to the regions on that reference sequence for which matches are desired. Because data types are coerced to IRangesList, which does *not* include strand information (use the flag argument instead). Only records with a read overlapping the specified ranges are returned. All ranges

must have ends less than or equal to 536870912.

isPaired A logical(1) indicating whether unpaired (FALSE), paired (TRUE), or any (NA)

read should be returned.

isProperPair A logical(1) indicating whether improperly paired (FALSE), properly paired

(TRUE), or any (NA) read should be returned. A properly paired read is defined by the alignment algorithm and might, e.g., represent reads aligning to

identical reference sequences and with a specified distance.

isUnmappedQuery

A logical(1) indicating whether unmapped (TRUE), mapped (FALSE), or any

(NA) read should be returned.

hasUnmappedMate

A logical(1) indicating whether reads with mapped (FALSE), unmapped (TRUE),

or any (NA) mate should be returned.

isMinusStrand A logical(1) indicating whether reads aligned to the plus (FALSE), minus (TRUE), or any (NA) strand should be returned.

isMateMinusStrand

A logical(1) indicating whether mate reads aligned to the plus (FALSE), minus (TRUE), or any (NA) strand should be returned.

isFirstMateRead

A logical(1) indicating whether the first mate read should be returned (TRUE) or not (FALSE), or whether mate read number should be ignored (NA).

isSecondMateRead

A logical(1) indicating whether the second mate read should be returned (TRUE) or not (FALSE), or whether mate read number should be ignored (NA).

isNotPrimaryRead

A logical(1) indicating whether reads that are primary (FALSE), are not primary (TRUE) or whose primary status does not matter (NA) should be returned. A non-primary read might result when portions of a read aligns to multiple locations, e.g., when spanning splice junctions).

isNotPassingQualityControls

A logical(1) indicating whether reads passing quality controls (FALSE), reads not passing quality controls (TRUE), or any (NA) read should be returned.

isValidVendorRead

Deprecated; use isNotPassingQualityControls.

isDuplicate A logical(1) indicating that un-duplicated (FALSE), duplicated (TRUE), or any

(NA) reads should be returned. 'Duplicated' reads may represent PCR or optical

duplicates.

object An instance of class ScanBamParam.

value An instance of the corresponding slot, to be assigned to object or, for bamFlagTest,

a character(1) name of the flag to test, e.g., "isUnmappedQuery", from the ar-

guments to scanBamFlag.

asInteger logical(1) indicating whether 'flag' should be returned as an encoded integer

vector (TRUE) or human-readable form (FALSE).

bitnames Names of the flag bits to extract. Will be the colnames of the returned matrix.

flag1, flag2 Integer vectors containing 'flag' entries.

## **Objects from the Class**

Objects are created by calls of the form ScanBamParam().

## Slots

flag Object of class integer encoding flags to be kept when they have their '0' (keep0) or '1' (keep1) bit set.

simpleCigar Object of class logical indicating, when TRUE, that only 'simple' cigars (empty or 'M') are returned.

reverseComplement Object of class logical indicating, when TRUE, that reads on the minus strand are to be reverse complemented (sequence) and reversed (quality).

tag Object of class character indicating what tags are to be returned.

what Object of class character indicating what fields are to be returned.

which Object of class RangesList indicating which reference sequence and coordinate reads must overlap.

#### **Functions and methods**

See 'Usage' for details on invocation.

Constructor:

**ScanBamParam:** Returns a ScanBamParam object. The which argument to the constructor can be one of several different types, as documented above.

Accessors:

**bamTag, bamTag<-** Returns or sets a character vector of tags to be extracted.

bamWhat, bamWhat<- Returns or sets a character vector of fields to be extracted.

**bamWhich, bamWhich<-** Returns or sets a RangesList of bounds on reads to be extracted. A length 0 RangesList represents all reads.

**bamFlag, bamFlag<-** Returns or sets an integer(2) representation of reads flagged to be kept or excluded.

**bamSimpleCigar, bamSimpleCigar<-** Returns or sets a logical(1) vector indicating whether reads without indels or clipping be kept.

**bamReverseComplement, bamReverseComplement<-** Returns or sets a logical(1) vector indicating whether reads on the minus strand will be returned with sequence reverse complemented and quality reversed.

Methods:

show Compactly display the object.

## Author(s)

Martin Morgan

## See Also

scanBam

# **Examples**

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```
## tags; NM: edit distance; H1: 1-difference hits
p4 <- ScanBamParam(tag=c("NM", "H1"), what="flag")
bam4 <- scanBam(fl, param=p4)
str(bam4[[1]][["tag"]])

## flag utils
flag <- scanBamFlag(isUnmappedQuery=FALSE, isMinusStrand=TRUE)
flag
bamFlagAsBitMatrix(flag)
flag4 <- bam4[[1]][["flag"]]
bamFlagAsBitMatrix(flag4[1:9], bitnames=c("isUnmappedQuery", "isMinusStrand"))
```

ScanBcfParam-class

Parameters for scanning BCF files

## **Description**

Use ScanBcfParam() to create a parameter object influencing the 'INFO' and 'GENO' fields parsed, and which recrods are imported from a BCF file. Use of which requires that a BCF index file (<filename>.bci) exists.

## Usage

```
ScanBcfParam(fixed=character(), info=character(), geno=character(), trimEmpty=TRUE,
         which, ...)
## S4 method for signature 'missing'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
         trimEmpty=TRUE, which, ...)
## S4 method for signature 'RangesList'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
         trimEmpty=TRUE, which, ...)
## S4 method for signature 'RangedData'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
         trimEmpty=TRUE, which, ...)
## S4 method for signature 'GRanges'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
         trimEmpty=TRUE, which, ...)
\#\# Accessors
bcfFixed(object)
bcfInfo(object)
bcfGeno(object)
bcfTrimEmpty(object)
bcfWhich(object)
```

## **Arguments**

fixed A logical(1) for use with ScanVcfParam only.

info A character() vector of 'INFO' fields (see scanVcfHeader) to be returned.

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geno A character() vector of 'GENO' fields (see scanVcfHeader) to be returned. character(0)

returns all fields, NA\_character\_ returns none.

trimEmpty A logical(1) indicating whether 'GENO' fields with no values should be re-

turned.

which An object, for which a method is defined (see usage, above), describing the

sequences and ranges to be queried. Variants whose POS lies in the interval(s)

[start, end) are returned.

object An instance of class ScanBcfParam.

... Arguments used internally.

## **Objects from the Class**

Objects can be created by calls of the form ScanBcfParam().

#### **Slots**

which: Object of class "RangesList" indicating which reference sequence and coordinate variants must overlap.

info: Object of class "character" indicating portions of 'INFO' to be returned.

geno: Object of class "character" indicating portions of 'GENO' to be returned.

trimEmpty: Object of class "logical" indicating whether empty 'GENO' fields are to be returned.

fixed: Object of class "character". For use with ScanVcfParam only.

## **Functions and methods**

See 'Usage' for details on invocation.

Constructor:

**ScanBcfParam:** Returns a ScanBcfParam object. The which argument to the constructor can be one of several types, as documented above.

Accessors:

bcfInfo, bcfGeno, bcfTrimEmpty, bcfWhich: Return the corresponding field from object.

Methods:

show Compactly display the object.

## Author(s)

Martin Morgan mtmorgan@fhcrc.org

#### See Also

scanVcf ScanVcfParam

# **Examples**

## see ?ScanVcfParam examples

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seqnamesTabix

Retrieve sequence names defined in a tabix file.

## **Description**

This function queries a tabix file, returning the names of the 'sequences' used as a key when creating the file.

## Usage

```
seqnamesTabix(file, ...) ## S4 method for signature 'character' seqnamesTabix(file, ...)
```

## **Arguments**

file A character(1) file path or TabixFile instance pointing to a 'tabix' file.

.. Additional arguments, currently ignored.

## Value

A character() vector of sequence names present in the file.

## Author(s)

Martin Morgan <a href="mtmorgan@fhcrc.org">mtmorgan@fhcrc.org</a>>.

## **Examples**

```
\label{eq:fl} {\it fl} < - system. file ("extdata", "example.gtf.gz", package="Rsamtools") seqnames Tabix (fl)
```

TabixFile

Manipulate tabix indexed tab-delimited files.

# Description

Use TabixFile() to create a reference to a Tabix file (and its index). Once opened, the reference remains open across calls to methods, avoiding costly index re-loading.

TabixFileList() provides a convenient way of managing a list of TabixFile instances.

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#### **Usage**

```
## Constructors
TabixFile(file, index = paste(file, "tbi", sep="."), ...,
        yieldSize=NA integer )
TabixFileList(..., yieldSize=NA integer )
\#\# Opening / closing
\#\# S3 method for class 'TabixFile'
open(con, ...)
## S3 method for class 'TabixFile'
close(con, ...)
## accessors; also path(), index(), yieldSize()
## S4 method for signature 'TabixFile'
isOpen(con, rw="")
\#\# actions
## S4 method for signature 'TabixFile'
seqnamesTabix(file, ...)
## S4 method for signature 'TabixFile'
headerTabix(file, ...)
## S4 method for signature 'TabixFile,GRanges'
scanTabix(file, ..., param)
## S4 method for signature 'TabixFile,RangesList'
scanTabix(file, ..., param)
## S4 method for signature 'TabixFile,RangedData'
scanTabix(file, ..., param)
## S4 method for signature 'TabixFile,missing'
scanTabix(file, ..., param)
## S4 method for signature 'character, ANY'
\operatorname{scanTabix}(\operatorname{file},\,...,\,\operatorname{param})
## S4 method for signature 'character, missing'
scanTabix(file, ..., param)
```

# Arguments

con An instance of TabixFile.

file For TabixFile(), A character(1) vector to the tabix file path; can be remote

Tot Table 10, A character(1) vector to the table file path, can be remote

(http://, ftp://). For others, a TabixFile instance.

index A character(1) vector of the tabix file index.

yieldSize Number of records to yield each time the file is read from using scanTabix.

Only valid when param is unspecified. yieldSize does not alter existing yield sizes, include NA, when creating a TabixFileList from TabixFile instances.

param An instance of GRanges, IRangedData, or RangesList, used to select which

records to scan.

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... Additional arguments. For TabixFileList, this can either be a single character vector of paths to tabix files, or several instances of TabixFile objects.

rw character() indicating mode of file; not used for TabixFile.

## **Objects from the Class**

Objects are created by calls of the form TabixFile().

## **Fields**

The TabixFile class inherits fields from the RsamtoolsFile class.

## **Functions and methods**

TabixFileList inherits methods from RsamtoolsFileList and SimpleList.

Opening / closing:

**open.TabixFile** Opens the (local or remote) path and index. Returns a TabixFile instance. yieldSize determines the number of records parsed during each call to scanTabix; NA indicates that all records are to be parsed.

**close.TabixFile** Closes the TabixFile con; returning (invisibly) the updated TabixFile. The instance may be re-opened with open.TabixFile.

Accessors:

**path** Returns a character(1) vector of the tabix path name.

index Returns a character(1) vector of tabix index name.

yieldSize, yieldSize<- Return or set an integer(1) vector indicating yield size.

Methods:

seqnames Tabix Visit the path in path(file), returning the sequence names present in the file.

**headerTabix** Visit the path in path(file), returning the sequence names, column indicies used to sort the file, the number of lines skipped while indexing, the comment character used while indexing, and the header (preceded by comment character, at start of file) lines.

scanTabix For signature(file="TabixFile"), Visit the path in path(file), returning the result of scanTabix applied to the specified path. For signature(file="character"), call the corresponding method after coercing file to TabixFile.

**indexTabix** This method operates on file paths, rather than TabixFile objects, to index tab-separated files. See indexTabix.

**show** Compactly display the object.

# Author(s)

Martin Morgan

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#### **Examples**

```
{\it fl} < - system. {\it file}("extdata", "example.gtf.gz", package = "Rsamtools")
tbx <- TabixFile(fl)
param <- GRanges(c("chr1", "chr2"), IRanges(c(1, 1), width=100000))
res <- scanTabix(tbx, param=param)
sapply(res, length)
res[["chr1:1-100000"]][1:2]
\#\# parse to list of data.frame's
dff <- Map(function(elt) {
   read.csv(textConnection(elt), sep="\t", header=FALSE)
}, res)
dff[["chr1:1-100000"]][1:5,1:8]
\#\# parse 100 records at a time
length(scanTabix(tbx)[[1]])~\#~total~number~of~records
tbx <- open(TabixFile(fl, yieldSize=100))
while(length(res <- scanTabix(tbx)[[1]]))
  cat("records read:", length(res), "\n")
close(tbx)
```

 ${\bf Tabix Input}$ 

Operations on 'tabix' (indexed, tab-delimited) files.

## **Description**

Scan compressed, sorted, tabix-indexed, tab-delimited files.

## Usage

```
scanTabix(file, ..., param)
## S4 method for signature 'character,RangesList'
scanTabix(file, ..., param)
## S4 method for signature 'character,RangedData'
scanTabix(file, ..., param)
## S4 method for signature 'character,GRanges'
scanTabix(file, ..., param)
```

## **Arguments**

param

file The character() file name(s) of the tabix file be processed, or more flexibly an instance of class TabixFile.

A instance of GRanges, RangedData, or RangesList provide the sequence

names and regions to be parsed.

... Additional arguments, currently ignored.

TabixInput

## Value

scanTabix returns a list, with one element per region. Each element of the list is a character vector representing records in the region.

# Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

# References

http://samtools.sourceforge.net/tabix.shtml

# **Examples**

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