

Package ‘Rsamtools’

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Type Package

Title Binary alignment (BAM), FASTA, variant call (BCF), and tabix file import

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Description This package provides an interface to the 'samtools', 'bcftools', and 'tabix' utilities (see 'LICENCE') for manipulating SAM (Sequence Alignment / Map), FASTA, binary variant call (BCF) and compressed indexed tab-delimited (tabix) files.

URL <http://bioconductor.org/packages/release/bioc/html/Rsamtools.html>

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LazyLoad yes

Depends methods, S4Vectors (>= 0.5.11), IRanges (>= 1.99.17), GenomeInfoDb (>= 1.1.3), GenomicRanges (>= 1.17.19), XVector (>= 0.5.3), Biostrings (>= 2.33.11)

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Video https://www.youtube.com/watch?v=Rfon-DQYbWA&list=UUqaMSQd_h-2EDGsU6WDiX0Q

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Rsamtools-package *'samtools' aligned sequence utilities interface*

Description

This package provides facilities for parsing samtools BAM (binary) files representing aligned sequences.

Details

See `packageDescription('Rsamtools')` for package details. A useful starting point is the [scanBam](#) 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)

Author: Martin Morgan

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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

```
packageDescription('Rsamtools')
```

applyPileups	<i>Apply a user-provided function to calculate pile-up statistics across multiple BAM files.</i>
--------------	--

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 <code>yieldSize</code> , <code>yieldBy</code> , <code>yieldAll</code>). The argument x is a list, with elements describing the current pile-up. The elements of the list are determined by the argument <code>what</code> , and include: <ul style="list-style-type: none"> seqnames: (Always returned) A named <code>integer()</code> 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 <code>integer()</code> 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 <code>seq</code>, 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 ApplyPileupsParam.

Details

Regardless of param values, the algorithm follows samtools by excluding reads flagged as unmapped, secondary, duplicate, or failing quality control.

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.

ApplyPileupsParam returns an object describing the parameters.

Author(s)

Martin Morgan

References

<http://samtools.sourceforge.net/>

See Also

[ApplyPileupsParam](#).

Examples

```
f1 <- system.file("extdata", "ex1.bam", package="Rsamtools",
                 mustWork=TRUE)

fls <- PileupFiles(c(f1, f1))

calcInfo <-
  function(x)
  {
    ## information at each pile-up position
    info <- apply(x[["seq"]], 2, function(y) {
      y <- y[c("A", "C", "G", "T"),,drop=FALSE]
      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 <- ApplyPileupsParam(which=which, what="seq")
res <- applyPileups(fls, calcInfo, param=param)
```

```

str(res)
head(res[[1]][["pos"]]) # positions matching param
head(res[[1]][["info"]]) # information 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 <- ApplyPileupsParam(which=which, yieldSize=500L,
                           yieldBy="position", what="seq")
res <- applyPileups(fls, calcInfo, param=param)
sapply(res, "[", "seqnames")

```

ApplyPileupsParam

Parameters for creating pileups from BAM files

Description

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

Usage

```

# Constructor
ApplyPileupsParam(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 'ApplyPileupsParam'
show(object)

```

Arguments

flag	An instance of the object returned by <code>scanBamFlag</code> , restricting various aspects of reads to be included or excluded.
minBaseQuality	The minimum read base quality below which the base is ignored when summarizing pileup information.
minMapQuality	The minimum mapping quality below which the entire read is ignored.
minDepth	The minimum depth of the pile-up below which the position is ignored.
maxDepth	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 (<code>yieldAll=TRUE</code>), or just those passing the filtering criteria of <code>flag</code> , <code>minBaseQuality</code> , etc. When <code>yieldAll=TRUE</code> , positions not passing filter criteria have '0' entries in <code>seq</code> or <code>qual</code> .
which	A <code>GRanges</code> or <code>RangesList</code> instance restricting pileup calculations to the corresponding genomic locations.
what	A <code>character()</code> instance indicating what values are to be returned. One or more of <code>c("seq", "qual")</code> .
object	An instance of class <code>ApplyPileupsParam</code> .
value	An instance to be assigned to the corresponding slot of the <code>ApplyPileupsParam</code> instance.

Objects from the Class

Objects are created by calls of the form `ApplyPileupsParam()`.

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).
minMapQuality 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().

Functions and methods

See 'Usage' for details on invocation.

Constructor:

ApplyPileupsParam: Returns a ApplyPileupsParam 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 minimum base qualities.

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

plpMinDepth, plpMinDepth<- Returns or sets an integer(1) vector of minimum 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(applyPileups)
```

BamFile

Maintain and use 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_, obeyQname=FALSE,
        asMates=FALSE, qnamePrefixEnd=NA, qnameSuffixStart=NA)
```

```
BamFileList(..., yieldSize=NA_integer_, obeyQname=FALSE, asMates=FALSE,
            qnamePrefixEnd=NA, qnameSuffixStart=NA)
```

```
## 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="")
```

```
## S4 method for signature 'BamFile'
isIncomplete(con)
```

```
## S4 method for signature 'BamFile'
obeyQname(object, ...)
```

```
obeyQname(object, ...) <- value
```

```
## S4 method for signature 'BamFile'
```



```

asMates(object, ...)
asMates(object, ...) <- value
## S4 method for signature 'BamFile'
qnamePrefixEnd(object, ...)
qnamePrefixEnd(object, ...) <- value
## S4 method for signature 'BamFile'
qnameSuffixStart(object, ...)
qnameSuffixStart(object, ...) <- value

## actions

## S4 method for signature 'BamFile'
scanBamHeader(files, ..., what=c("targets", "text"))
## S4 method for signature 'BamFile'
seqinfo(x)
## S4 method for signature 'BamFileList'
seqinfo(x)
## S4 method for signature 'BamFile'
filterBam(file, destination, index=file, ...,
           filter=FilterRules(), 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, ...)

## reading

## S4 method for signature 'BamFile'
scanBam(file, index=file, ..., param=ScanBamParam(what=scanBamWhat()))

## counting

## 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'
quickBamFlagSummary(file, ..., param=ScanBamParam(), main.groups.only=FALSE)

```

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 .
<code>con</code>	An instance of <code>BamFile</code> .
<code>x, object, file, files</code>	A character vector of BAM file paths (for <code>BamFile</code>) or a <code>BamFile</code> instance (for other methods).
<code>index</code>	<code>character(1)</code> ; the BAM index file path (for <code>BamFile</code>); ignored for all other methods on this page.
<code>yieldSize</code>	Number of records to yield each time the file is read from with <code>scanBam</code> . See ‘Fields’ section for details.
<code>asMates</code>	Logical indicating if records should be paired as mates. See ‘Fields’ section for details.
<code>qnamePrefixEnd</code>	Single character (or NA) marking the end of the <code>qname</code> prefix. When specified, all characters prior to and including the <code>qnamePrefixEnd</code> are removed from the <code>qname</code> . If the prefix is not found in the <code>qname</code> the <code>qname</code> is not trimmed. Currently only implemented for mate-pairing (i.e., when <code>asMates=TRUE</code> in a <code>BamFile</code>).
<code>qnameSuffixStart</code>	Single character (or NA) marking the start of the <code>qname</code> suffix. When specified, all characters following and including the <code>qnameSuffixStart</code> are removed from the <code>qname</code> . If the suffix is not found in the <code>qname</code> the <code>qname</code> is not trimmed. Currently only implemented for mate-pairing (i.e., when <code>asMates=TRUE</code> in a <code>BamFile</code>).
<code>obeyQname</code>	Logical indicating if the BAM file is sorted by <code>qname</code> . In Bioconductor > 2.12 paired-end files do not need to be sorted by <code>qname</code> . Instead use <code>asMates=TRUE</code> for reading paired-end data. See ‘Fields’ section for details.
<code>value</code>	Logical value for setting <code>asMates</code> and <code>obeyQname</code> in a <code>BamFile</code> instance.
<code>what</code>	For <code>scanBamHeader</code> , a character vector specifying that either or both of <code>c("targets", "text")</code> are to be extracted from the header; see scanBam for additional detail.
<code>filter</code>	A FilterRules instance. Functions in the <code>FilterRules</code> instance should expect a single <code>DataFrame</code> argument representing all information specified by <code>param</code> . Each function must return a logical vector, usually of length equal to the number of rows of the <code>DataFrame</code> . Return values are used to include (when <code>TRUE</code>) corresponding records in the filtered BAM file.
<code>destination</code>	<code>character(1)</code> file path to write filtered reads to.
<code>indexDestination</code>	<code>logical(1)</code> indicating whether the destination file should also be indexed.
<code>byQname, maxMemory</code>	See sortBam .
<code>param</code>	An optional ScanBamParam instance to further influence scanning, counting, or filtering.
<code>rw</code>	Mode of file; ignored.
<code>main.groups.only</code>	See quickBamFlagSummary .

Objects from the Class

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

Fields

The `BamFile` class inherits fields from the `RsamtoolsFile` class and has fields:

yieldSize: Number of records to yield each time the file is read from using `scanBam` or, when `length(bamWhich()) != 0`, a threshold which yields records in complete ranges whose sum first exceeds `yieldSize`. Setting `yieldSize` on a `BamFileList` does not alter existing `yieldSize`s set on the individual `BamFile` instances.

asMates: A logical indicating if the records should be returned as mated pairs. When `TRUE` `scanBam` attempts to mate (pair) the records and returns two additional fields `groupid` and `mate_status`. `groupid` is an integer vector of unique group ids; `mate_status` is a factor with level `mated` for records successfully paired by the algorithm, `ambiguous` for records that are possibly mates but cannot be assigned unambiguously, or `unmated` for reads that did not have valid mates.

Mate criteria:

- Bit 0x40 and 0x80: Segments are a pair of first/last OR neither segment is marked first/last
- Bit 0x100: Both segments are secondary OR both not secondary
- Bit 0x10 and 0x20: Segments are on opposite strands
- mpos match: `segment1 mpos` matches `segment2 pos` AND `segment2 mpos` matches `segment1 pos`
- tid match

Flags, tags and ranges may be specified in the `ScanBamParam` for fine tuning of results.

obeyQname: A logical(0) indicating if the file was sorted by `qname`. In Bioconductor > 2.12 paired-end files do not need to be sorted by `qname`. Instead set `asMates=TRUE` in the `BamFile` when using the `readGAlignmentsList` function from the **GenomicAlignments** package.

Functions and methods

`BamFileList` inherits additional 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`.

isOpen Tests whether the `BamFile` con has been opened for reading.

isIncomplete Tests whether the `BamFile` con is neither closed nor at the end of the file.

Accessors:

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

index Returns a `character(0)` or `character(1)` vector of BAM index path names.

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

obeyQname, obeyQname<- Return or set a logical(0) indicating if the file was sorted by qname.
asMates, asMates<- Return or set a logical(0) indicating if the records should be returned as mated pairs.

Methods:

scanBamHeader Visit the path in `path(file)`, returning the information contained in the file header; see [scanBamHeader](#).

seqinfo, seqnames, seqlength Visit the path in `path(file)`, returning a [Seqinfo](#), character, or named integer vector containing information on the names and / or lengths of each sequence. Seqnames are ordered as they appear in the file.

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`.

show Compactly display the object.

Author(s)

Martin Morgan and Marc Carlson

See Also

- The [readGAlignments](#), [readGAlignmentPairs](#), and [readGAlignmentsList](#) functions defined in the **GenomicAlignments** package.
- [summarizeOverlaps](#) and [findSpliceOverlaps-methods](#) in the **GenomicAlignments** package for methods that work on a [BamFile](#) and [BamFileList](#) objects.

Examples

```
##
## BamFile options.
##

fl <- system.file("extdata", "ex1.bam", package="Rsamtools")
bf <- BamFile(fl)
bf

## When 'asMates=TRUE' scanBam() reads the data in as
```

```

## pairs. See 'asMates' above for details of the pairing
## algorithm.
asMates(bf) <- TRUE

## When 'yieldSize' is set, scanBam() will iterate
## through the file in chunks.
yieldSize(bf) <- 500

## Some applications append a filename (e.g., NCBI Sequence Read
## Archive (SRA) toolkit) or allele identifier to the sequence qname.
## This may result in a unique qname for each record which presents a
## problem when mating paired-end reads (identical qnames is one
## criteria for paired-end mating). 'qnamePrefixEnd' and
## 'qnameSuffixStart' can be used to trim an unwanted prefix or suffix.
qnamePrefixEnd(bf) <- "/"
qnameSuffixStart(bf) <- "."

##
## Reading Bam files.
##

fl <- system.file("extdata", "ex1.bam", package="Rsamtools",
                  mustWork=TRUE)
(bf <- BamFile(fl))
head(seqlengths(bf))                # sequences and lengths in BAM file

if (require(RNAseqData.HNRNPC.bam.chr14)) {
  bf1 <- BamFileList(RNAseqData.HNRNPC.bam.chr14_BAMFILES)
  bf1
  bf1[1:2]                          # subset
  bf1[[1]]                          # select first element -- BamFile
  ## merged across BAM files
  seqinfo(bf1)
  head(seqlengths(bf1))
}

length(scanBam(fl)[[1]][[1]]) # all records

bf <- open(BamFile(fl))           # implicit index
bf
identical(scanBam(bf), scanBam(fl))
close(bf)

## Use 'yieldSize' to iterate through a file in chunks.
bf <- open(BamFile(fl, yieldSize=1000))
while (nrec <- length(scanBam(bf)[[1]][[1]]))
  cat("records:", nrec, "\n")
close(bf)

## Repeatedly visit multiple ranges in the BamFile.
rng <- GRanges(c("seq1", "seq2"), IRanges(1, c(1575, 1584)))
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)
```

BamInput	<i>Import, count, index, filter, sort, and merge 'BAM' (binary alignment) files.</i>
----------	--

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.

Usage

```
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)
```

```
asSam(file, destination, ...)
## S4 method for signature 'character'
asSam(file, destination, ..., overwrite=FALSE)
```

```
filterBam(file, destination, index=file, ...)
## S4 method for signature 'character'
filterBam(file, destination, index=file, ...,
          filter=FilterRules(), 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 processed.
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 <i>without</i> 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 asSam, 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.
filter	A FilterRules instance allowing users to filter BAM files based on arbitrary criteria, as described below.
indexDestination	A logical(1) indicating whether the created destination file should also be indexed.
byQname	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. An optional character vector argument containing one or two elements of `what=c("targets", "text")` can be used to specify which elements of the header are returned.

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

`asSam` converts 'BAM' files to 'SAM' files, equivalent to `samtools view file > destination`.

`filterBam` parses records in `file`. Records satisfying the `bamWhich`, `bamFlag` and `bamSimpleCigar` criteria of `param` are accumulated to a default of `yieldSize = 1000000` records (change this by specifying `yieldSize` when creating a `BamFile` instance; see `BamFile`-class). These records are then parsed to a `DataFrame` and made available for further filtering by user-supplied `FilterRules`. Functions in the `FilterRules` instance should expect a single `DataFrame` argument representing all information specified by `param`. Each function must return a logical vector equal to the number of rows of the `DataFrame`. Return values are used to include (when `TRUE`) corresponding records in the filtered BAM file. The BAM file is created at destination. An index file is created on the destination when `indexDestination=TRUE`. It is more space- and time-efficient to filter use `bamWhich`, `bamFlag`, and `bamSimpleCigar`, if appropriate, than to supply `FilterRules`.

`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 `<bam_filename>.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`.
- `groupid`: This is an integer vector of unique group ids returned when `asMates=TRUE` in a `BamFile` object. `groupid` values are used to create the partitioning for a `GAlignmentsList` object.
- `mate_status`: Returned (always) when `asMates=TRUE` in a `BamFile` object. This is a factor indicating status (mated, ambiguous, unmated) of each record.

`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`, `asSam` return the file name of the destination 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 <mtmorgan@fhcrc.org>. Thomas Unterhiner <thomas.unterthiner@students.jku.at> (`sortBam`).

References

<http://samtools.sourceforge.net/>

See Also

[ScanBamParam](#), [scanBamWhat](#), [scanBamFlag](#)

Examples

```
f1 <- system.file("extdata", "ex1.bam", package="Rsamtools",
                 mustWork=TRUE)

##
## scanBam
##

res0 <- scanBam(f1)[[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(f1, param=p1)
names(res1)
names(res1[[2]])

p2 <- ScanBamParam(what=c("rname", "strand", "pos", "qwidth"))
res2 <- scanBam(f1, param=p2)

p3 <- ScanBamParam(
  what="flag", # information to query from BAM file
  flag=scanBamFlag(isMinusStrand=FALSE))
length(scanBam(f1, param=p3)[[1]]$flag)

##
## filterBam
##

param <- ScanBamParam(
  flag=scanBamFlag(isUnmappedQuery=FALSE),
  what="seq")
dest <- filterBam(f1, tempfile(), param=param)
countBam(dest) ## 3271 records
filt <- list(MinWidth = function(x) width(x$seq) > 35)
dest <- filterBam(f1, tempfile(), param=param, filter=FilterRules(filt))
countBam(dest) ## 398 records
res3 <- scanBam(dest, param=ScanBamParam(what="seq"))[[1]]
table(width(res3$seq))
```

```
##
## sortBam
##

sorted <- sortBam(fl, tempfile())

##
## scanBamParam re-orders 'which'; recover original order
##

gwhich <- as(which, "GRanges")[c(2, 1, 3)] # example data
cnt <- countBam(fl, param=ScanBamParam(which=gwhich))
reorderIdx <- unlist(split(seq_along(gwhich), seqnames(gwhich)))
cnt
cnt[reorderIdx,]
```

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’.

Usage

```
## Constructor
BamViews(bamPaths=character(0),
         bamIndices=bamPaths,
         bamSamples=DataFrame(row.names=make.unique(basename(bamPaths))),
         bamRanges, bamExperiment = list(), ...)
## S4 method for signature 'missing'
BamViews(bamPaths=character(0),
         bamIndices=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'
scanBam(file, index = file, ..., param = ScanBamParam(what=scanBamWhat()))
## S4 method for signature 'BamViews'
countBam(file, index = file, ..., param = ScanBamParam())

## Show
## S4 method for signature 'BamViews'
show(object)

```

Arguments

<code>bamPaths</code>	A <code>character()</code> vector of BAM path names.
<code>bamIndicies</code>	A <code>character()</code> vector of BAM index file path names, <i>without</i> the <code>‘.bai’</code> extension.
<code>bamSamples</code>	A DataFrame instance with as many rows as <code>length(bamPaths)</code> , containing sample information associated with each path.
<code>bamRanges</code>	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.
<code>bamExperiment</code>	A <code>list()</code> containing additional information about the experiment.
<code>auto.range</code>	If TRUE and all <code>bamPaths</code> exist, populate the ranges with the union of ranges returned in the <code>target</code> element of <code>scanBamHeader</code> .
<code>...</code>	Additional arguments.
<code>x</code>	An instance of <code>BamViews</code> .
<code>object</code>	An instance of <code>BamViews</code> .
<code>value</code>	An object of appropriate type to replace content.
<code>i</code>	During subsetting, a logical or numeric index into <code>bamRanges</code> .
<code>j</code>	During subsetting, a logical or numeric index into <code>bamSamples</code> and <code>bamPaths</code> .

drop	A logical(1), <i>ignored</i> 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.

Objects from the Class

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).

show Compactly display the object.

Author(s)

Martin Morgan

Examples

```
f1s <- system.file("extdata", "ex1.bam", package="Rsamtools",
                  mustWork=TRUE)
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(f1s, bamRanges=rngs)
v
v[1:5,]
bamRanges(v[c(1:5, 11:15),])
bamDirname(v) <- getwd()
v
```

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.

Usage

Constructors

```
BcfFile(file, index = file,
        mode=ifelse(grepl("\\.bcf$", file), "rb", "r"))
BcfFileList(...)
```

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.
file	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.

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

```
f1 <- system.file("extdata", "ex1.bcf", package="Rsamtools",
                 mustWork=TRUE)
bf <- BcfFile(f1)      # implicit index
bf
identical(scanBcf(bf), scanBcf(f1))

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.

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 scanBcf and scanBcfHeader, the character() file name 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 indexed.

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 reference sequences. The `sample` element is a `character()` vector of names of samples. The `header` element is a `character()` vector of the header lines (preceeded 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

```
f1 <- system.file("extdata", "ex1.bcf", package="Rsamtools",
                 mustWork=TRUE)
scanBcfHeader(f1)
bcf <- scanBcf(f1)
## value: list-of-lists
str(bcf[1:8])
names(bcf[["GENO"]])
str(head(bcf[["GENO"]][["PL"]]))
example(BcfFile)
```

Compression	<i>File compression for tabix (bgzip) and fasta (razip) files.</i>
-------------	--

Description

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

Usage

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

Arguments

file	A character(1) path to an existing uncompressed or gz-compressed 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

```
from <- system.file("extdata", "ex1.sam", package="Rsamtools",
                  mustWork=TRUE)
to <- tempfile()
zipped <- bgzip(from, to)
```

deprecated *Deprecated functions*

Description

Functions listed on this page are no longer supported.

Details

For yieldTabix, use the yieldSize argument of TabixFiles.

For BamSampler, use REDUCEsampler with reduceByYield in the GenomicFiles package.

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.

Usage

```
## Constructors

FaFile(file, index=sprintf("%s.fai", 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, ...)

## S4 method for signature 'FaFile'
scanFaIndex(file, ...)
## S4 method for signature 'FaFileList'
scanFaIndex(file, ..., as=c("GRangesList", "GRanges"))

## S4 method for signature 'FaFile'
seqinfo(x)

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

## S4 method for signature 'FaFile,GRanges'
scanFa(file, param, ...,
       as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'FaFile,RangesList'
scanFa(file, param, ...,
       as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'FaFile,RangedData'
scanFa(file, param, ...,
       as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'FaFile,missing'
scanFa(file, param, ...,
       as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))

## 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, index	A character(1) vector of the fasta or fasta index 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. <ul style="list-style-type: none"> • For FaFileList, this can either be a single character vector of paths to BAM files, or several instances of FaFile objects. • For scanFa, FaFile, missing-method this can include arguments to readDNAStringSet / readRNAStringSet / readAAStringSet when param is 'missing'.
rw	Mode of file; ignored.

as A character(1) vector indicating the type of object to return.

- For scanFaIndex, default GRangesList, with index information from each file is returned as an element of the list.
- For scanFa, default DNASTringSet.

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.

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 widths of recorded in an indexed fasta file, returning the information as a GRanges object.

seqinfo Consult the index file for defined sequences (seqlevels()) and lengths (seqlengths()).

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 cause an error; use seqlengths(FaFile(file)) to discover sequence widths. 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

```
f1 <- system.file("extdata", "ce2dict1.fa", package="Rsamtools",
                 mustWork=TRUE)
fa <- open(FaFile(f1))           # 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]))
```

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, ...,
       as=c("DNAStrngSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'character,GRanges'
scanFa(file, param, ...,
       as=c("DNAStrngSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'character,RangesList'
scanFa(file, param, ...,
```

```

    as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'character,RangedData'
scanFa(file, param, ...,
    as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))
## S4 method for signature 'character,missing'
scanFa(file, param, ...,
    as=c("DNAStringSet", "RNAStringSet", "AAStringSet"))

```

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.
as	A character(1) vector indicating the type of object to return; default DNAStringSet .
...	Additional arguments, passed to readDNAStringSet / readRNAStringSet / readAAStringSet when param is 'missing'.

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 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](#), [RNAStringSet](#), [AAStringSet](#) 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.

Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

References

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

Examples

```

fa <- system.file("extdata", "ce2dict1.fa", package="Rsamtools",
    mustWork=TRUE)
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]))

```

headerTabix	<i>Retrieve sequence names defined in a tabix file.</i>
-------------	---

Description

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

Usage

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

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 indices 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

```
f1 <- system.file("extdata", "example.gtf.gz", package="Rsamtools",  
                 mustWork=TRUE)  
headerTabix(f1)
```

indexTabix	<i>Compress and index tabix-compatible files.</i>
------------	---

Description

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

Usage

```
indexTabix(file,
           format=c("gff", "bed", "sam", "vcf", "vcf4", "psltbl"),
           seq=integer(), start=integer(), end=integer(),
           skip=0L, comment="#", zeroBased=FALSE, ...)
```

Arguments

file	A character(1) path to a sorted, bgzip-compressed file.
format	The format of the data in the compressed file. A character(1) matching one of the types named in the function signature.
seq	If format is missing, then 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 coordinates in the file are zero-based.
...	Additional arguments.

Value

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

Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

References

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

Examples

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

tab <- TabixFile(zipped, idx)
```

pileup	<i>Use filters and output formats to calculate pile-up statistics for a BAM file.</i>
--------	---

Description

pileup uses PileupParam and ScanBamParam objects to calculate pileup statistics for a BAM file. The result is a data.frame with columns summarizing counts of reads overlapping each genomic position, optionally differentiated on nucleotide, strand, and position within read.

Usage

```
pileup(file, index=file, ..., scanBamParam=ScanBamParam(),
       pileupParam=PileupParam())

## PileupParam constructor
PileupParam(max_depth=250, min_base_quality=0, min_mapq=13,
            min_nucleotide_depth=1, min_minor_allele_depth=0,
            distinguish_strands=TRUE, distinguish_nucleotides=TRUE,
            ignore_query_Ns=TRUE, include_deletions=TRUE, include_insertions=FALSE,
            cycle_bins=numeric())
```

Arguments

file	character(1) or BamFile; BAM file path.
index	When file is character(1), an optional character(1) of BAM index file path; see scanBam .
...	Additional arguments, perhaps used by methods.
scanBamParam	An instance of ScanBamParam .
pileupParam	An instance of PileupParam .
max_depth	integer(1); maximum number of overlapping alignments considered for each position in the pileup.
min_base_quality	integer(1); minimum 'QUAL' value for each nucleotide in an alignment.
min_mapq	integer(1); minimum 'MAPQ' value for an alignment to be included in pileup.
min_nucleotide_depth	integer(1); minimum count of each nucleotide (<i>independent</i> of other nucleotides) at a given position required for said nucleotide to appear in the result.
min_minor_allele_depth	integer(1); minimum count of <i>all</i> nucleotides other than the major allele at a given position required for a particular nucleotide to appear in the result.
distinguish_strands	logical(1); TRUE if result should differentiate between strands.

distinguish_nucleotides	logical(1); TRUE if result should differentiate between nucleotides.
ignore_query_Ns	logical(1); TRUE if non-determinate nucleotides in alignments should be excluded from the pileup.
include_deletions	logical(1); TRUE to include deletions in pileup.
include_insertions	logical(1); TRUE to include insertions in pileup.
cycle_bins	numeric; non-negative, unique positions within a read to delimit bins. At least two values are required so at least one range can be formed. numeric(0) (default) indicates no binning. Sorted order is not required. Floating-point values are coerced to integer.

Details

NB: Use of `pileup` assumes familiarity with [ScanBamParam](#), and use of `cycle_bins` assumes familiarity with [cut](#).

`pileup` visits each position in the BAM file, subject to constraints implied by `which` and `flag` of `scanBamParam`. For a given position, all reads overlapping the position that are consistent with constraints dictated by `flag` of `scanBamParam` and `pileupParam` are used for counting. `pileup` also automatically excludes reads with flags that indicate any of:

- unmapped alignment ([isUnmappedQuery](#))
- secondary alignment ([isSecondaryAlignment](#))
- not passing quality controls ([isNotPassingQualityControls](#))
- PCR or optical duplicate ([isDuplicate](#))

If no `which` argument is supplied to the `ScanBamParam`, behavior reflects that of `scanBam`: the entire file is visited and counted.

Use a `yieldSize` value when creating a `BamFile` instance to manage memory consumption when using `pileup` with large BAM files. There are some differences in how `pileup` behavior is affected when the `yieldSize` value is set on the BAM file. See more details below.

Many of the parameters of the `pileupParam` interact. A simple illustration is `ignore_query_Ns` and `distinguish_nucleotides`, as mentioned in the `ignore_query_Ns` section.

Parameters for `pileupParam` belong to two categories: parameters that affect only the filtering criteria (so-called ‘behavior-only’ policies), and parameters that affect filtering behavior and the schema of the results (‘behavior+structure’ policies).

`distinguish_nucleotides` and `distinguish_strands` when set to TRUE each add a column (nucleotide and strand, respectively) to the resulting `data.frame`. If both are TRUE, each combination of nucleotide and strand at a given position is counted separately. Setting only one to TRUE behaves as expected; for example, if only `nucleotide` is set to TRUE, each nucleotide at a given position is counted separately, but the distinction of on which strand the nucleotide appears is ignored.

`ignore_query_Ns` determines how ambiguous nucleotides are treated. By default, ambiguous nucleotides (typically 'N' in BAM files) in alignments are ignored. If `ignore_query_Ns` is FALSE, ambiguous nucleotides are included in counts; further, if `ignore_query_Ns` is FALSE and `distinguish_nucleotides` is TRUE the 'N' nucleotide value appears in the nucleotide column when a base at a given position is ambiguous.

By default, deletions with respect to the reference genome to which the reads were aligned are included in the counts in a pileup. If `include_deletions` is TRUE and `distinguish_nucleotides` is TRUE, the nucleotide column uses a '-' character to indicate a deletion at a position.

The '=' nucleotide code in the SEQ field (to mean 'identical to reference genome') is supported by pileup, such that a match with the reference genome is counted separately in the results if `distinguish_nucleotides` is TRUE.

CIGAR support: pileup handles the extended CIGAR format by only heeding operations that contribute to counts ('M', 'D', 'I'). If you are confused about how the different CIGAR operations interact, see the SAM versions of the BAM files used for pileup unit testing for a fairly comprehensive human-readable treatment.

- Deletions and clipping:

The extended CIGAR allows a number of operations conceptually similar to a 'deletion' with respect to the reference genome, but offer more specialized meanings than a simple deletion. CIGAR 'N' operations (not to be confused with 'N' used for non-determinate bases in the SEQ field) indicate a large skipped region, 'S' a soft clip, and 'H' a hard clip. 'N', 'S', and 'H' CIGAR operations are never counted: only counts of true deletions ('D' in the CIGAR) can be included by setting `include_deletions` to TRUE.

Soft clip operations contribute to the relative position of nucleotides within a read, whereas hard clip and `refskip` operations do not. For example, consider a sequence in a bam file that starts at 11, with a CIGAR 2S1M and SEQ field ttA. The cycle position of the A nucleotide will be 3, but the reported position will be 11. If using `cycle_bins` it might make sense to first preprocess your files to remove soft clipping.

- Insertions and padding:

pileup can include counts of insertion operations by setting `include_insertions` to TRUE. Details about insertions are effectively truncated such that each insertion is reduced to a single '+' nucleotide. Information about e.g. the nucleotide code and base quality within the insertion is not included.

Because '+' is used as a nucleotide code to represent insertions in pileup, counts of the '+' nucleotide participate in voting for `min_nucleotide_depth` and `min_minor_allele_depth` functionality.

The position of an insertion is the position that precedes it on the reference sequence. *Note:* this means if `include_insertions` is TRUE a single read will contribute two nucleotide codes (+, plus that of the non-insertion base) at a given position if the non-insertion base passes filter criteria.

'P' CIGAR (padding) operations never affect counts.

`cycle_bins` allows users to differentiate pileup counts based on arbitrary regions within a read. pileup relies on `cut` to derive bins, but limits input to non-negative numeric values (coerced to integers), including Inf. If `cycle_bins` is set pileup automatically excludes bases outside the implicit outer range. There are two important factors to consider when specifying bins:

- Because `cut` produces ranges in the form (first,last], '0' should be used to create a bin that includes the first position.
- In order to account for variable-length reads in BAM files, `Inf` should be used to create a bin that extends to the end of all reads.

`pileup` obeys `yieldSize` on `BamFile` objects with some differences from how `scanBam` responds to `yieldSize`. Here are some points to keep in mind when using `pileup` in conjunction with `yieldSize`:

- `BamFiles` with a `yieldSize` value set, once opened and used with `pileup`, *should not be used* with other functions that accept a `BamFile` instance as input. Create a new `BamFile` instance instead of trying to reuse.
- `pileup` only returns genomic positions for which all input has been processed. `pileup` will hold in reserve positions for which only partial data has been processed. Positions held in reserve will be returned upon subsequent calls to `pileup` when all the input for a given position has been processed.
- The correspondence between `yieldSize` and the number of rows in the `data.frame` returned from `pileup` is not 1-to-1. `yieldSize` only limits the number of *alignments processed* from the BAM file each time the file is read. Only a handful of alignments can lead to many distinct records in the result.
- Like `scanBam`, `pileup` uses an empty return object (a zero-row `data.frame`) to indicate end-of-input.
- Sometimes reading `yieldSize` records from the BAM file does not result in any completed positions. In order to avoid returning a zero-row `data.frame` `pileup` will repeatedly process `yieldSize` additional records until at least one position can be returned to the user.

Value

For `pileup` a `data.frame` with 1 row per unique combination of differentiating column values that satisfied filter criteria, with frequency (count) of unique combination. Columns `seqnames`, `pos`, and `count` always appear; optional `strand`, `nucleotide`, and `cycle_bin` columns appear as dictated by arguments to `PileupParam`.

Column details:

- `seqnames`: factor. SAM 'RNAME' field of alignment.
- `pos`: `integer(1)`. Genomic position of base. Derived by offset from SAM 'POS' field of alignment.
- `strand`: factor. 'strand' to which read aligns.
- `nucleotide`: factor. 'nucleotide' of base, extracted from SAM 'SEQ' field of alignment.
- `cycle_bin`: factor. Cycle bin in which base appears.
- `count`: `integer(1)`. Frequency of combination of differentiating fields, as indicated by values of other columns.

See `scanBam` for more detailed explanation of SAM fields.

If a `which` argument is specified for the `scanBamParam`, a `which_label` column (factor in the form 'rname:start-end') is automatically included. The `which_label` column is used to maintain grouping of results, such that two queries of the same genomic region can be differentiated.

Order of rows in data.frame is first by order of seqnames column based on the BAM index file, then non-decreasing order on columns pos, then nucleotide, then strand, then cycle_bin.

PileupParam returns an instance of PileupParam class.

Author(s)

Nathaniel Hayden nhayden@fredhutch.org

See Also

- [Rsamtools](#)
- [BamFile](#)
- [ScanBamParam](#)
- [scanBam](#)
- [cut](#)

Examples

```
## The examples below apply equally to pileup queries for specific
## genomic ranges (specified by the 'which' parameter of 'ScanBamParam')
## and queries across entire files; the entire-file examples are
## included first to make them easy to find. The more detailed examples
## of pileup use queries with a 'which' argument.
```

```
library("RNAseqData.HNRNPC.bam.chr14")
```

```
fl <- RNAseqData.HNRNPC.bam.chr14_BAMFILES[1]
```

```
## no 'which' argument to ScanBamParam: process entire file at once
res <- pileup(fl)
head(res)
table(res$strand, res$nucleotide)
```

```
## no 'which' argument to ScanBamParam with 'yieldSize' set on BamFile
bf <- open(BamFile(fl, yieldSize=5e4))
repeat {
  res <- pileup(bf)
  message(nrow(res), " rows in result data.frame")
  if(nrow(res) == 0L)
    break
}
close(bf)
```

```
## pileup for the first half of chr14 with default Pileup parameters
## 'which' argument: process only specified genomic range(s)
```

```

sbp <- ScanBamParam(which=GRanges("chr14", IRanges(1, 53674770)))
res <- pileup(fl, scanBamParam=sbp)
head(res)
table(res$strand, res$nucleotide)

## specify pileup parameters: include ambiguous nucleotides
## (the 'N' level in the nucleotide column of the data.frame)
p_param <- PileupParam(ignore_query_Ns=FALSE)
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
head(res)
table(res$strand, res$nucleotide)

## Don't distinguish strand, require a minimum frequency of 7 for a
## nucleotide at a genomic position to be included in results.

p_param <- PileupParam(distinguish_strands=TRUE,
                      min_nucleotide_depth=7)
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
head(res)
table(res$nucleotide, res$strand)

## Any combination of the filtering criteria is possible: let's say we
## want a "coverage pileup" that only counts reads with mapping
## quality >= 13, and bases with quality >= 10 that appear at least 4
## times at each genomic position
p_param <- PileupParam(distinguish_nucleotides=FALSE,
                      distinguish_strands=FALSE,
                      min_mapq=13,
                      min_base_quality=10,
                      min_nucleotide_depth=4)
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
head(res)
res <- res[, c("pos", "count")] ## drop seqnames and which_label cols
plot(count ~ pos, res, pch=".")

## Well-supported polymorphic positions (minor allele present in at
## least 5 alignments) with high map quality
p_param <- PileupParam(min_minor_allele_depth=5,
                      min_mapq=40,
                      distinguish_strand=FALSE)
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
dim(res) ## reduced to our biologically interesting positions
head(xtabs(count ~ pos + nucleotide, res))

## cycle_bins

## basic use: single pivot; count bases that appear in first 10
## positions of a read separately from the rest
p_param <- PileupParam(cycle_bins=c(0, 10, Inf))
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)

## basic use: simple range; only include bases in positions 6-10 within

```



```

## read
p_param <- PileupParam(cycle_bins=c(5, 10))
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)

## typical use: beginning, middle, and end segments; because of the
## nature of sequencing technology, it is common for bases in the
## beginning and end segments of each read to be less reliable. pileup
## makes it easy to count each segment separately.

## Assume determined ahead of time that for the data 1-7 makes sense for
## beginning, 8-12 middle, >=13 end (actual positions should be tailored
## to data in actual BAM files).
p_param <- PileupParam(cycle_bins=c(0, 7, 12, Inf)) ## note non-symmetric
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
xt <- xtabs(count ~ nucleotide + cycle_bin, res)
print(xt)
t(t(xt) / colSums(xt)) ## cheap normalization for illustrative purposes

## 'implicit outer range': in contrast to c(0, 7, 12, Inf), suppose we
## still want to have beginning, middle, and end segments, but know
## that the first three positions and any bases beyond the 16th
## position are irrelevant. Hence, the implicit outer range is (3,16];
## all bases outside of that are dropped.
p_param <- PileupParam(cycle_bins=c(3, 7, 12, 16))
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
xt <- xtabs(count ~ nucleotide + cycle_bin, res)
print(xt)
t(t(xt) / colSums(xt))

## single-width bins: count each position within a read separately.
p_param <- PileupParam(cycle_bins=seq(1,20))
res <- pileup(fl, scanBamParam=sbp, pileupParam=p_param)
xt <- xtabs(count ~ nucleotide + cycle_bin, res)
print(xt[ , c(1:3, 18:19)]) ## fit on one screen
print(t(t(xt) / colSums(xt))[ , c(1:3, 18:19)])

```

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.

Usage

```

## Constructors
PileupFiles(files, ..., param=ApplyPileupsParam())
## S4 method for signature 'character'
PileupFiles(files, ..., param=ApplyPileupsParam())
## S4 method for signature 'list'
PileupFiles(files, ..., param=ApplyPileupsParam())

## 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)

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

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

```

Arguments

files	For <code>PileupFiles</code> , a <code>character()</code> or list of <code>BamFile</code> instances representing files to be included in the pileup. Using a list of <code>BamFile</code> allows indices to be specified when these are in non-standard format. All elements of <code>...</code> must be the same type. For <code>applyPileups,PileupFiles</code> -method, a <code>PileupFiles</code> instance.
...	Additional arguments, currently ignored.
con, object	An instance of <code>PileupFiles</code> .
FUN	A function of one argument; see applyPileups .
param	An instance of ApplyPileupsParam , to select which records to include in the pileup, and which summary information to return.
rw	<code>character()</code> indicating mode of file; not used for <code>TabixFile</code> .

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 [ApplyPileupsParam](#).

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 [ApplyPileupsParam](#) 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.

Author(s)

Martin Morgan

Examples

```
example(applyPileups)
```

quickBamFlagSummary *Group the records of a BAM file based on their flag bits and count the number of records in each group*

Description

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

Usage

```
quickBamFlagSummary(file, ..., param=ScanBamParam(), main.groups.only=FALSE)

## S4 method for signature 'character'
quickBamFlagSummary(file, index=file, ..., param=ScanBamParam(),
  main.groups.only=FALSE)

## S4 method for signature 'list'
quickBamFlagSummary(file, ..., param=ScanBamParam(), main.groups.only=FALSE)
```

Arguments

file, index	For the character method, the path to the BAM file to read, and to the index file of the BAM file to read, respectively. For the list() method, file is a named list with elements “qname” and “flag” with content as from scanBam .
...	Additional arguments, perhaps used by methods.
param	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](#).
[BamFile](#) for a method for that class.

Examples

```
bamfile <- system.file("extdata", "ex1.bam", package="Rsamtools",
  mustWork=TRUE)
quickBamFlagSummary(bamfile)
```

```
readPileup          Import samtools 'pileup' files.
```

Description

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

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:

alleleOne, alleleTwo The first (typically, in the reference sequence) and second allelic variants.

alleleOneSupport, alleleTwoSupport The number of reads supporting each allele.

additionalIndels The number of additional indels present.

Author(s)

Sean Davis

References

<http://samtools.sourceforge.net/>

Examples

```
f1 <- system.file("extdata", "pileup.txt", package="Rsamtools",
                 mustWork=TRUE)
(res <- readPileup(f1))
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
cmd <- "samtools pileup -cvf human_b36_female.fa.gz na19240_3M.bam"
p <- pipe(cmd, "r")
snp <- readPileup(p, nrow=100) # variant="SNP"
indel <- readPileup(p, nrow=100, variant="indel")
all <- readPileup(p, nrow=100, variant="all")

## End(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.

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 subsetting, 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), tagFilter = list(),
             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,
           isSecondaryAlignment = NA, isNotPassingQualityControls = NA,
           isDuplicate = 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
bamTagFilter(object)
bamTagFilter(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 function. For bamFlagAsBitMatrix, bamFlagTest an integer vector where each element 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) vectors. BAM files store reads mapping to the minus strand as though they are on the plus strand. Rsamtools obeys this convention by default (reverseComplement=FALSE), but when this value is set to 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. This might be useful if wishing to recover read and quality scores as represented in fastq files, but is NOT appropriate for variant calling or other alignment-based operations.
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.
tagFilter	A named list of atomic vectors. The name of each list element is the tag name (two-letter code), and the corresponding atomic vector is the set of acceptable values for the tag. Only reads with specified tags are included. NULLs, NAs, and empty strings are not allowed in the atomic vectors.
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, any object that can be coerced to a RangesList, 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 <i>not</i> 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 de-

finned 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	Deprecated; use isSecondaryAlignment.
isSecondaryAlignment	A logical(1) indicating whether alignments that are secondary (TRUE), are not (FALSE) or whose secondary status does not matter (NA) should be returned. A non-primary alignment (“secondary alignment” in the SAM specification) might result when a read aligns to multiple locations. One alignment is designated as primary and has this flag set to FALSE; the remainder, for which this flag is TRUE, are designated by the aligner as secondary.
isNotPassingQualityControls	A logical(1) indicating whether reads passing quality controls (FALSE), reads not passing quality controls (TRUE), or any (NA) read should be returned.
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 arguments 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.
- tagFilter** Object of class list (named) indicating tags to filter by, and the set of acceptable values for each tag.
- 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.

bamTagFilter, bamTagFilter<- Returns or sets a named list of tags to filter by, and the set of their acceptable values.

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**

```
## defaults
p0 <- ScanBamParam()

## subset of reads based on genomic coordinates
which <- RangesList(seq1=IRanges(1000, 2000),
                    seq2=IRanges(c(100, 1000), c(1000, 2000)))
p1 <- ScanBamParam(what=scanBamWhat(), which=which)

## subset of reads based on 'flag' value
p2 <- ScanBamParam(what=scanBamWhat(),
                  flag=scanBamFlag(isMinusStrand=FALSE))

## subset of fields
p3 <- ScanBamParam(what=c("rname", "strand", "pos", "qwidth"))

## use
f1 <- system.file("extdata", "ex1.bam", package="Rsamtools",
                  mustWork=TRUE)
res <- scanBam(f1, param=p2)[[1]]
lapply(res, head)

## tags; NM: edit distance; H1: 1-difference hits
p4 <- ScanBamParam(tag=c("NM", "H1"), what="flag")
bam4 <- scanBam(f1, param=p4)
str(bam4[[1]][["tag"]])

## tagFilter
p5 <- ScanBamParam(tag=c("NM", "H1"), tagFilter=list(NM=c(2, 3, 4)))
bam5 <- scanBam(f1, param=p5)
table(bam5[[1]][["tag"]][["NM"]])

## flag utils
flag <- scanBamFlag(isUnmappedQuery=FALSE, isMinusStrand=TRUE)

p6 <- ScanBamParam(what="flag")
bam6 <- scanBam(f1, param=p6)
flag6 <- bam6[[1]][["flag"]]
head(bamFlagAsBitMatrix(flag6[1:9]))
colSums(bamFlagAsBitMatrix(flag6))
flag
bamFlagAsBitMatrix(flag)
```

Description

Use `ScanBcfParam()` to create a parameter object influencing the ‘INFO’ and ‘GENO’ fields parsed, and which sample records 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(),
             samples=character(), trimEmpty=TRUE, which, ...)

## S4 method for signature 'missing'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
             samples=character(), trimEmpty=TRUE, which, ...)
## S4 method for signature 'RangesList'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
             samples=character(), trimEmpty=TRUE, which, ...)
## S4 method for signature 'RangedData'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
             samples=character(), trimEmpty=TRUE, which, ...)
## S4 method for signature 'GRanges'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
             samples=character(), trimEmpty=TRUE, which, ...)
## S4 method for signature 'GRangesList'
ScanBcfParam(fixed=character(), info=character(), geno=character(),
             samples=character(), trimEmpty=TRUE, which, ...)

## Accessors
bcfFixed(object)
bcfInfo(object)
bcfGeno(object)
bcfSamples(object)
bcfTrimEmpty(object)
bcfWhich(object)
```

Arguments

<code>fixed</code>	A logical(1) for use with <code>ScanVcfParam</code> only.
<code>info</code>	A <code>character()</code> vector of ‘INFO’ fields (see scanVcfHeader) to be returned.
<code>geno</code>	A <code>character()</code> vector of ‘GENO’ fields (see scanVcfHeader) to be returned. <code>character(0)</code> returns all fields, <code>NA_character_</code> returns none.
<code>samples</code>	A <code>character()</code> vector of sample names (see scanVcfHeader) to be returned. <code>character(0)</code> returns all fields, <code>NA_character_</code> returns none.
<code>trimEmpty</code>	A logical(1) indicating whether ‘GENO’ fields with no values should be returned.

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.

samples: Object of class "character" indicating the samples 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

seqnamesTabix	<i>Retrieve sequence names defined in a tabix file.</i>
---------------	---

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 <mtmorgan@fhcrc.org>.

Examples

```
f1 <- system.file("extdata", "example.gtf.gz", package="Rsamtools",  
                 mustWork=TRUE)  
seqnamesTabix(f1)
```

TabixFile	<i>Manipulate tabix indexed tab-delimited files.</i>
-----------	--

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.

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'
scanTabix(file, ..., param)
## S4 method for signature 'character,missing'
scanTabix(file, ..., param)

countTabix(file, ...)

```

Arguments

con	An instance of TabixFile.
file	For TabixFile(), A character(1) vector to the tabix file path; can be remote (http://, ftp://). For countTabix, a character(1) or TabixFile instance. 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.
...	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:

seqnamesTabix 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 indices 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.

countTabix Return the number of records in each range of `param`, or the count of all records in the file (when `param` is missing).

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

Examples

```

fl <- system.file("extdata", "example.gtf.gz", package="Rsamtools",
                  mustWork=TRUE)
tbx <- TabixFile(fl)

param <- GRanges(c("chr1", "chr2"), IRanges(c(1, 1), width=100000))
countTabix(tbx)
countTabix(tbx, param=param)
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)

```

TabixInput

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

file	The character() file name(s) of the tabix file be processed, or more flexibly an instance of class <code>TabixFile</code> .
param	A instance of <code>GRanges</code> , <code>RangedData</code> , or <code>RangesList</code> provide the sequence names and regions to be parsed.
...	Additional arguments, currently ignored.

Value

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

Error

`scanTabix` signals errors using `signalCondition`. The following errors are signaled:

`scanTabix_param yieldSize(file)` must be `NA` when more than one range is specified.

`scanTabix_io` A read error occurred while inputing the tabix file. This might be because the file is corrupt, or of incorrect format (e.g., when path points to a plain text file but index is present, implying that path should be a bgzipped file. The error message may include an error code representing the logical OR of these cryptic signals: 1, `BGZF_ERR_ZLIB`; 2, `BGZF_ERR_HEADER`; 4, `BGZF_ERR_IO`; 8, `BGZF_ERR_MISUSE`.

Author(s)

Martin Morgan <mtmorgan@fhcrc.org>.

References

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

Examples

```
example(TabixFile)
```

testPairedEndBam

Quickly test if a BAM file has paired end reads

Description

Iterate through a BAM file until a paired-end read is encountered or the end of file is reached; report the occurrence of paired-end reads to the user.

Usage

```
testPairedEndBam(file, index=file, ...)
```

Arguments

- file character(1) BAM file name, or a [BamFile](#) instance. Open BamFiles are closed; their yield size is respected when iterating through the file.
- index (optional) character(1) name of the index file of the 'BAM' file being processed; this is given *without* the '.bai' extension.
- ... Additional arguments, currently unused.

Value

A logical vector of length 1 containing TRUE is returned if BAM file contained paired end reads, FALSE otherwise.

Author(s)

Martin Morgan <mailto:mtmorgan@fhcrc.org>, Sonali Arora <mailto:sarora@fhcrc.org>

Examples

```
f1 <- system.file("extdata", "ex1.bam", package="Rsamtools")
testPairedEndBam(f1)
```

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