An Introduction to Rsamtools

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> lil	rary(Rsamtools)	

1 Introduction

The *Rsamtools* package provides an interface to BAM files. BAM files are produced by *samtools* and other software, and represent a flexible format for storing 'short' reads aligned to reference genomes. BAM files typically contain sequence and base qualities, and alignment coordinates and quality measures. BAM files are appealing for several reasons. The format is flexible enough to represent reads generated and aligned using diverse technologies. The files are binary so that file access is relatively efficient. BAM files can be indexed, allowing ready access to localized chromosomal regions. BAM files can be accessed remotely, provided the remote hosting site supports such access and a local index is available. This means that specific regions of remote files can be accessed without retrieving the entire (large!) file. A full description is available in the BAM format specification (http://samtools.sourceforge.net/SAM1.pdf)

The main purpose of the *Rsamtools* package is to import BAM files into *R. Rsamtools* also provides some facility for file access such as record counting, index file creation, and filtering to create new files containing subsets of the original. An important use case for *Rsamtools* is

as a starting point for creating R objects suitable for a diversity of work flows, e.g., Aligne-dRead objects in the ShortRead package (for quality assessment and read manipulation), or GAlignments objects in GenomicAlignments package (for RNA-seq and other applications). Those desiring more functionality are encouraged to explore samtools and related software efforts.

2 Input

The essential capability provided by *Rsamtools* is BAM input. This is accomplished with the scanBam function. scanBam takes as input the name of the BAM file to be parsed. In addition, the param argument determines which genomic coordinates of the BAM file, and what components of each record, will be input. *R*param is an instance of the *ScanBamParam* class. To create a param object, call ScanBamParam. Here we create a param object to extract reads aligned to three distinct ranges (one on seq1, two on seq2). From each of read in those ranges, we specify that we would like to extract the reference name (rname, e.g., seq1), strand, alignment position, query (i.e., read) width, and query sequence:

Additional information can be found on the help page for ScanBamParam. Reading the relevant records from the BAM file is accomplished with

```
> bamFile <-
+ system.file("extdata", "ex1.bam", package="Rsamtools")
> bam <- scanBam(bamFile, param=param)</pre>
```

Like scan, scanBam returns a list of values. Each element of the list corresponds to a range specified by the which argument to ScanBamParam.

```
> class(bam)
[1] "list"
> names(bam)
[1] "seq1:1000-2000" "seq2:100-1000" "seq2:1000-2000"
```

Each element is itself a list, containing the elements specified by the what and tag arguments to ScanBamParam.

```
> class(bam[[1]])
[1] "list"
> names(bam[[1]])
[1] "rname" "strand" "pos" "qwidth" "seq"
```

The elements are either basic R or IRanges data types

```
> sapply(bam[[1]], class)

rname strand pos qwidth

"factor" "integer" "integer"

seq
"DNAStringSet"
```

A paradigm for collapsing the list-of-lists into a single list is

This might be further transformed, e.g., to a DataFrame, with

```
> head(do.call("DataFrame", lst))
DataFrame with 6 rows and 5 columns
     rname strand
                          pos
                                 qwidth
  <factor> <factor> <integer> <integer>
1
                          970
      seq1
                  +
2
                          971
                                     35
      seq1
3
                          972
                                     35
      seq1
4
      seq1
                          973
                                     35
5
      seq1
                          974
                                     35
                                     35
      seq1
                          975
                      seq
           <DNAStringSet>
1 TATTAGGAAA...ACTATGAAGA
2 ATTAGGAAAT...CTATGAAGAG
3 TTAGGAAATG...TATGAAGAGA
4 TAGGAAATGC...ATGAAGAGAC
5 AGGAAATGCT...TGAAGAGACT
6 GGAAATGCTT...GAAGAGACTA
```

Often, an alternative is to use a *ScanBamParam* object with desired fields specified in what as an argument to GenomicAlignments::readGAlignments; the specified fields are added as columns to the returned *GAlignments*.

The BAM file in the previous example includes an index, represented by a separate file with extension .bai:

Indexing provides two significant benefits. First, an index allows a BAM file to be efficiently accessed by range. A corollary is that providing a which argument to ScanBamParam requires an index. Second, coordinates for extracting information from a BAM file can be derived from the index, so a portion of a remote BAM file can be retrieved with local access only to the index. For instance, provided an index file exists on the local computer, it is possible to retrieve a small portion of a BAM file residing on the 1000 genomes HTTP server. The url ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/data/NA19240/alignment/NA19240.chrom6.SLX.maq.SRP000032.2009_07.bam points to the BAM file corresponding to individual NA19240 chromosome 6 Solexa (Illumina) sequences aligned using MAQ. The remote file is very large (about 10 GB), but the corresponding index file is small (about 500 KB). With na19240url set to the above address, the following retrieves just those reads in the specified range

```
> which <- IRangesList("6"=IRanges(100000L, 110000L))
> param <- ScanBamParam(which=which, what=scanBamWhat())
> na19240bam <- scanBam(na19240url, param=param)
```

Invoking scanBam without an index file, as above, first retrieves the index file from the remote location, and then queries the remote file using the index; for repeated queries, it is more efficient to retrieve the index file first (e.g., with download.file) and then use the local index as an argument to scanBam. Many BAM files were created in a way that causes scanBam to report that the "EOF marker is absent"; this message can safely be ignored.

BAM files may be read by functions in packages other than *Rsamtools*, in particular the readGAlignments family of functions in *GenomicAlignments*.

Additional ways of interacting with BAM files include scanBamHeader (to extract header information) and countBam (to count records matching param). filterBam filters reads from the source file according to the criteria of the ScanBamParam parameter, writing reads passing the filter to a new file. The function sortBam sorts a previously unsorted BAM, while The function indexBam creates an index file from a sorted BAM file.

readPileup reads a pileup file created by samtools, importing SNP, indel, or all variants into a GRanges object.

2.1 Large bam files

BAM files can be large, containing more information on more genomic regions than are of immediate interest or than can fit in memory. The first strategy for dealing with this is to select, using the what and which arguments to ScanBamParam, just those portions of the BAM file that are essential to the current analysis, e.g., specifying what=c('rname', 'qname', 'pos') when wishing to calculate coverage of ungapped reads.

When selective input of BAM files is still too memory-intensive, the file can be processed in chunks, with each chunk distilled to the derived information of interest. Chromosomes will often be the natural chunk to process. For instance, here we write a summary function that takes a single sequence name (chromosome) as input, reads in specific information from the BAM file, and calculates coverage over that sequence.

```
> summaryFunction <-
+ function(seqname, bamFile, ...)
+ {
+ param <- ScanBamParam(what=c('pos', 'qwidth'),
+ which=GRanges(seqname, IRanges(1, 1e7)),
+ flag=scanBamFlag(isUnmappedQuery=FALSE))
+ x <- scanBam(bamFile, ..., param=param)[[1]]
+ coverage(IRanges(x[["pos"]], width=x[["qwidth"]]))
+ }</pre>
```

This might be used as follows; it is an ideal candidate for evaluation in parallel, e.g., using the *parallel* package and *srapply* function in *ShortRead*.

```
> seqnames <- paste("seq", 1:2, sep="")
> cvg <- lapply(seqnames, summaryFunction, bamFile)
> names(cvg) <- seqnames
> cvg

$seq1
integer-Rle of length 1569 with 1054 runs
    Lengths: 2 2 1 3 4 2 3 4 ... 1 1 1 1 1 1 1
    Values: 1 2 3 4 5 7 8 9 ... 9 7 6 5 3 2 1

$seq2
integer-Rle of length 1567 with 1092 runs
    Lengths: 1 3 1 1 1 3 1 4 ... 1 1 2 1 4 4 1
    Values: 3 4 5 8 12 14 15 16 ... 10 8 7 6 3 2 1
```

The result of the function (a coverage vector, in this case) will often be much smaller than the input.

3 Views

The functions described in the previous section import data in to R. However, sequence data can be very large, and it does not always make sense to read the data in immediately. Instead, it can be useful to marshal *references* to the data into a container and then act on components of the container. The *BamViews* class provides a mechanism for creating 'views' into a set of BAM files. The view itself is light-weight, containing references to the relevant BAM files and metadata about the view (e.g., the phenotypic samples corresponding to each BAM file).

One way of understanding a *BamViews* instance is as a rectangular data structure. The columns represent BAM files (e.g., distinct samples). The rows represent ranges (i.e., genomic coordinates). For instance, a ChIP-seq experiment might identify a number of peaks of high read counts.

3.1 Assembling a *BamViews* instance

To illustrate, suppose we have an interest in caffeine metabolism in humans. The 'rows' contain coordinates of genomic regions associated with genes in a KEGG caffeine metabolism pathway. The 'columns' represent individuals in the 1000 genomes project.

To create the 'rows', we identify possible genes that KEGG associates with caffeine metabolism:

```
> library(KEGG.db)
> kid <- revmap(KEGGPATHID2NAME)[["Caffeine metabolism"]]
> egid <- KEGGPATHID2EXTID[[sprintf("hsa%s", kid)]]</pre>
```

Then we use the appropriate *TxDb* package to translate Entrez identifiers to obtain ranges of interest (one could also use *biomaRt* to retrieve coordinates for non-model organisms, perhaps making a *TxDb* object as outlined in the *GenomicFeatures* vignette). We'll find that the names used for chromosomes in the alignments differ from those used at Ensembl, so seglevels<- is used to map between naming schemes and to drop unused levels.

Note that bamRanges 'knows' the genome for which the ranges are defined

```
> unique(genome(bamRanges))
[1] "hg18"
```

The details of creating the 'columns' of BAM files. Here we retrieve a vector of BAM file URLs (slxMaq09) from the package.

We now assemble the *BamViews* instance from these objects; we also provide information to annotated the BAM files (with the bamSamples function argument, which is a *DataFrame* instance with each row corresponding to a BAM file) and the instance as a whole (with bamEx periment, a simple named *list* containing information structured as the user sees fit).

```
> bamExperiment <-
+ list(description="Caffeine metabolism views on 1000 genomes samples",
+ created=date())
> bv <- BamViews(slxMaq09, bamRanges=bamRanges,
+ bamExperiment=bamExperiment)
> metadata(bamSamples(bv)) <-
+ list(description="Solexa/MAQ samples, August 2009",
+ created="Thu Mar 25 14:08:42 2010")</pre>
```

3.2 Using BamViews instances

The BamViews object can be queried for its component parts, e.g.,

```
> bamExperiment(bv)
$description
[1] "Caffeine metabolism views on 1000 genomes samples"
$created
[1] "Thu May 02 22:02:03 2019"
```

More usefully, Methods in *Rsamtools* are designed to work with *BamViews* objects, retrieving data from all files in the view. These operations can take substantial time and require reliable network access.

To illustrate, the following code (not evaluated when this vignette was created) downloads the index files associated with the by object

```
> bamIndexDir <- tempfile()
> indexFiles <- paste(bamPaths(bv), "bai", sep=".")
> dir.create(bamIndexDir)
> idxFiles <- mapply(download.file, indexFiles,
+ file.path(bamIndexDir, basename(indexFiles)) ,
+ MoreArgs=list(method="curl"))</pre>
```

and then queries the 1000 genomes project for reads overlapping our transcripts; by loading the *parallel* package, we tell *Rsamtools* to use as many cores as are available on our machine (the *parallel* package does not provide parallel functionality on Windows computers)

```
> library(GenomicAlignments)
> library(parallel)
> options(srapply_fapply="parallel", mc.cores=detectCores())
> olaps <- readGAlignments(bv)</pre>
```

The resulting object is about 11 MB in size. To avoid having to download this data each time the vignette is run, we instead load it from disk

```
> library(GenomicAlignments)
> load(system.file("extdata", "olaps.Rda", package="Rsamtools"))
> olaps
List of length 24
names(24): NA06986.SLX.maq.SRP000031.2009_08.bam ...
> head(olaps[[1]])
GAlignments object with 6 alignments and 0 metadata columns:
      seqnames strand
                            cigar
                                     qwidth
                                                start
         <Rle> <Rle> <character> <integer> <integer>
             2
  [1]
                              51M
                                         51 31410650
                    +
             2
                              51M
                                         51 31410658
  [2]
                                         51 31410663
  [3]
             2
                              51M
  [4]
             2
                                         51 31410666
                              51M
  [5]
             2
                              51M
                                         51 31410676
```

```
[6]
           2
                                        51 31410676
                            51M
          end
                  width
                            njunc
    <integer> <integer> <integer>
[1] 31410700
                     51
[2] 31410708
                     51
                                 0
[3] 31410713
                     51
                                 0
[4] 31410716
                     51
                                0
                                 0
[5] 31410726
                     51
                     51
                                 0
[6] 31410726
seqinfo: 114 sequences from an unspecified genome
```

There are 34616 reads in NA06986.SLX.maq.SRP000031.2009_08.bam overlapping at least one of our transcripts. It is easy to explore this object, for instance discovering the range of read widths in each individual.

```
> head(t(sapply(olaps, function(elt) range(qwidth(elt)))))

[,1] [,2]

NA06986.SLX.maq.SRP000031.2009_08.bam 51 51

NA06994.SLX.maq.SRP000031.2009_08.bam 36 51

NA07051.SLX.maq.SRP000031.2009_08.bam 51 51

NA07346.SLX.maq.SRP000031.2009_08.bam 48 76

NA07347.SLX.maq.SRP000031.2009_08.bam 51 51

NA10847.SLX.maq.SRP000031.2009_08.bam 36 51
```

Suppose we were particularly interested in the first transcript, which has a transcript id uc002rnv.1. Here we extract reads overlapping this transcript from each of our samples. As a consequence of the protocol used, reads aligning to either strand could be derived from this transcript. For this reason, we set the strand of our range of interest to *. We use the endoapply function, which is like lapply but returns an object of the same class (in this case, SimpleList) as its first argument.

```
> rng <- bamRanges(bv)[1]
> strand(rng) <- "*"
> olap1 <- endoapply(olaps, subsetByOverlaps, rng)</pre>
> olap1 <- lapply(olap1, "seqlevels<-", value=as.character(seqnames(rng)))</pre>
> head(olap1[[24]])
GAlignments object with 6 alignments and 0 metadata columns:
      segnames strand
                                       qwidth
                             cigar
                                                  start
         <Rle>
               <Rle> <character> <integer> <integer>
  [1]
             2
                               36M
                                           36 31410660
  [2]
             2
                               36M
                                           36 31410670
  [3]
             2
                               36M
                                           36 31410683
  [4]
             2
                               36M
                                           36 31410687
  [5]
             2
                               36M
                                           36 31410694
  [6]
             2
                               36M
                                           36 31410701
            end
                    width
                               njunc
      <integer> <integer> <integer>
  [1] 31410695
                        36
                                   0
  [2] 31410705
                        36
                                   0
```

To carry the example a little further, we calculate coverage of each sample:

Since the example includes a single region of uniform width across all samples, we can easily create a coverage matrix with rows representing nucleotide and columns sample and, e.g., document variability between samples and nucleotides

```
> m <- matrix(unlist(lapply(cvg, lapply, as.vector)),</pre>
+
             ncol=length(cvg))
> summary(rowSums(m))
  Min. 1st Qu. Median
                           Mean 3rd Qu.
                                           Max.
  10.00
         74.00
                82.00
                          81.63
                                  91.00 133.00
> summary(colSums(m))
   Min. 1st Qu. Median
                           Mean 3rd Qu.
                                           Max.
 133924 173925 248333 273528 350823 567727
```

4 Directions

This vignette has summarized facilities in the *Rsamtools* package. Important additional packages include *GenomicRanges* (for representing and manipulating gapped alignments), *ShortRead* for I/O and quality assessment of ungapped short read alignments, *Biostrings* and *BSgenome* for DNA sequence and whole-genome manipulation, *IRanges* for range-based manipulation, and *rtracklayer* for I/O related to the UCSC genome browser. Users might also find the interface to the integrative genome browser (IGV) in *SRAdb* useful for visualizing BAM files.

```
> packageDescription("Rsamtools")
Package: Rsamtools
Type: Package
```

```
Title: Binary alignment (BAM), FASTA, variant call
      (BCF), and tabix file import
Version: 2.0.0
Author: Martin Morgan, Herv\'e Pag\`es, Valerie
      Obenchain, Nathaniel Hayden
Maintainer: Bioconductor Package Maintainer
      <maintainer@bioconductor.org>
Description: This package provides an interface to
      the 'samtools', 'bcftools', and 'tabix'
      utilities for manipulating SAM (Sequence
      Alignment / Map), FASTA, binary variant call
      (BCF) and compressed indexed tab-delimited
      (tabix) files.
URL: http://bioconductor.org/packages/Rsamtools
License: Artistic-2.0 | file LICENSE
LazyLoad: yes
Depends: methods, GenomeInfoDb (>= 1.1.3),
      GenomicRanges (>= 1.31.8), Biostrings (>=
      2.47.6)
Imports: utils, BiocGenerics (>= 0.25.1), S4Vectors
      (>= 0.17.25), IRanges (>= 2.13.12), XVector (>=
      0.19.7), zlibbioc, bitops, BiocParallel
Suggests: GenomicAlignments, ShortRead (>= 1.19.10),
      GenomicFeatures,
      TxDb.Dmelanogaster.UCSC.dm3.ensGene, KEGG.db,
      TxDb.Hsapiens.UCSC.hg18.knownGene,
      RNAseqData.HNRNPC.bam.chr14,
      BSgenome.Hsapiens.UCSC.hg19, RUnit, BiocStyle
LinkingTo: Rhtslib (>= 1.15.3), S4Vectors, IRanges,
      XVector, Biostrings
biocViews: DataImport, Sequencing, Coverage,
      Alignment, QualityControl
Video:
      https://www.youtube.com/watch?v=Rfon-DQYbWA&list=UUqaMSQd_h-2EDGsU6WDiX0Q
qit_url:
      https://git.bioconductor.org/packages/Rsamtools
git_branch: RELEASE_3_9
git_last_commit: dc422f7
git_last_commit_date: 2019-05-02
Date/Publication: 2019-05-02
Built: R 3.6.0; x86_64-w64-mingw32; 2019-05-03
      02:01:01 UTC; windows
-- File: C:/Users/biocbuild/bbs-3.9-bioc/tmpdir/RtmpIZf7nr/Rinst29e8265d4ced/Rsamtools/Meta/package.rds
> sessionInfo()
R version 3.6.0 (2019-04-26)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows Server 2012 R2 x64 (build 9600)
Matrix products: default
```

```
locale:
[1] LC_COLLATE=C
[2] LC_CTYPE=English_United States.1252
[3] LC_MONETARY=English_United States.1252
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.1252
attached base packages:
[1] stats4
              parallel stats
                                  graphics grDevices
[6] utils
              datasets methods
                                  base
other attached packages:
 [1] GenomicAlignments_1.20.0
 [2] SummarizedExperiment_1.14.0
 [3] DelayedArray_0.10.0
 [4] BiocParallel_1.18.0
 [5] matrixStats_0.54.0
 [6] TxDb.Hsapiens.UCSC.hg18.knownGene_3.2.2
 [7] GenomicFeatures_1.36.0
 [8] KEGG.db_3.2.3
 [9] AnnotationDbi_1.46.0
[10] Biobase_2.44.0
[11] Rsamtools_2.0.0
[12] Biostrings_2.52.0
[13] XVector_0.24.0
[14] GenomicRanges_1.36.0
[15] GenomeInfoDb_1.20.0
[16] IRanges_2.18.0
[17] S4Vectors_0.22.0
[18] BiocGenerics_0.30.0
loaded via a namespace (and not attached):
 [1] Rcpp_1.0.1
                            compiler_3.6.0
                            prettyunits_1.0.2
 [3] BiocManager_1.30.4
 [5] bitops_1.0-6
                            tools_3.6.0
 [7] zlibbioc_1.30.0
                            progress_1.2.0
 [9] biomaRt_2.40.0
                            digest_0.6.18
[11] bit_1.1-14
                            lattice_0.20-38
[13] evaluate_0.13
                            RSQLite_2.1.1
                            rlang_0.3.4
[15] memoise_1.1.0
[17] pkgconfig_2.0.2
                            Matrix_1.2-17
[19] DBI_1.0.0
                            yaml_2.2.0
[21] xfun_0.6
                            GenomeInfoDbData_1.2.1
[23] rtracklayer_1.44.0
                            httr_1.4.0
[25] stringr_1.4.0
                            knitr_1.22
[27] hms_0.4.2
                            grid_3.6.0
[29] bit64_0.9-7
                            R6_2.4.0
[31] XML_3.98-1.19
                            rmarkdown_1.12
[33] magrittr_1.5
                            blob_1.1.1
[35] htmltools_0.3.6
                            assertthat_0.2.1
```

```
[37] BiocStyle_2.12.0 stringi_1.4.3
[39] RCurl_1.95-4.12 crayon_1.3.4
```

A Assembling a *BamViews* instance

A.1 Genomic ranges of interest

A.2 BAM files

Note: The following operations were performed at the time the vignette was written; location of on-line resources, in particular the organization of the 1000 genomes BAM files, may have changed.

We are interested in collecting the URLs of a number of BAM files from the 1000 genomes project. Our first goal is to identify files that might make for an interesting comparison. First, let's visit the 1000 genomes FTP site and discover available files. We'll use the *RCurl* package to retrieve the names of all files in an appropriate directory

```
> library(RCurl)
> ftpBase <-
+    "ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/data/"
> indivDirs <-
+    strsplit(getURL(ftpBase, ftplistonly=TRUE), "\n")[[1]]
> alnDirs <-
+    paste(ftpBase, indivDirs, "/alignment/", sep="")
> urls0 <-
+    strsplit(getURL(alnDirs, dirlistonly=TRUE), "\n")</pre>
```

From these, we exclude directories without any files in them, select only the BAM index (extension .bai) files, and choose those files that exactly six '.' characters in their name.

```
> urls <- urls[sapply(urls0, length) != 0]
> fls0 <- unlist(unname(urls0))
> fls1 <- fls0[grepl("bai$", fls0)]
> fls <- fls1[sapply(strsplit(fls1, "\\."), length)==7]</pre>
```

After a little exploration, we focus on those files obtained form Solexa sequencing, aligned using MAQ, and archived in August, 2009; we remove the .bai extension, so that the URL refers to the underlying file rather than index. There are 24 files.

```
> urls1 <-
+ Filter(function(x) length(x) != 0,
+ lapply(urls,
+ function(x) x[grepl("SLX.maq.*2009_08.*bai$", x)]))
> slxMaq09.bai <-
+ mapply(paste, names(urls1), urls1, sep="", USE.NAMES=FALSE)
> slxMaq09 <- sub(".bai$", "", slxMaq09.bai) #$</pre>
```

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As a final step to prepare for using a *BamViews* file, we create local copies of the *index* files. The index files are relatively small (about 190 Mb total).

```
> bamIndexDir <- tempfile()
> dir.create(bamIndexDir)
> idxFiles <- mapply(download.file, slxMaq09.bai,
+ file.path(bamIndexDir, basename(slxMaq09.bai)) ,
+ MoreArgs=list(method="curl"))</pre>
```