

An introduction to *R* and *Bioconductor* for the analysis of high-throughput sequencing data

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1 Introduction

1.1 What is *R/Bioconductor*?

R is a language and environment for statistical computing and graphics. The latest news and updates can be found on the [R website](#). More than 6000 packages are available on the Comprehensive R Archive Network ([CRAN](#)) to extend the functionalities of *R*. *R* is increasingly used in different fields, including life science [1].

With more than 2000 packages, *Bioconductor* is the largest project associated to *R*. It is dedicated to bioinformatics [2] and proposes 4 types of packages:

- [Software packages](#) are classical *R* packages containing new functions
- [Annotation Data packages](#) contain biological annotations wrapped in convenient *R* objects
- [Experiment Data packages](#) contain Experimental data
- [Workflow packages](#) were introduced more recently and contain examples of workflows based on *Bioconductor* packages

The [Bioconductor website](#) is a great resource to get the latest (frequent) updates and to find help and tutorials.

One of the most notable advantage of *R* and *Bioconductor* is the vast amount of learning resources available. In addition to package vignettes (i.e. documentation), the [Bioconductor workflows](#) and [Courses and Conferences section](#) are great resources.

1.2 Core and specialized packages

An enormous amount of work has been done by *Bioconductor* developpers to provide efficient tools for the analysis of genomic data (see for example this excellent overview of the use of *Bioconductor* in the context of genomic data [3]).

Some packages provide a core infrastructure for genomics by defining key classes / containers and the associated accessors. Others provide complete pipelines or integrated solutions for a general task. These packages allow to perform the main steps involved in the analysis of genomic data:

- Work with sequences and strings: [Biostrings](#), [BSgenome](#) and [BSgenome*](#) annotation packages
- Manipulate raw FASTQ data files: [ShortRead](#)
- Align reads on a reference: [Rsubread](#), [QuasR](#)
- Manipulate aligned reads: [Rsamtools](#), [GenomicAlignments](#)
- Work with genomic ranges: [IRanges](#), [GenomicRanges](#)
- Access annotations: [AnnotationDbi](#), [OrganismDbi](#), [GenomicFeatures](#), etc.
- Importing/exporting tracks: [rtracklayer](#), [AnnotationHub](#), [biomaRt](#), etc.
- Visualize genomic data: [ggbio](#), [Gviz](#), [Sushi](#), etc.

Other packages are more focused on specific applications, such as:

- RNA-seq and differential analysis: *limma*, *DESeq2*, *edgeR*, *DEXseq*, *spliceR*, *rnaSeqMap*, etc.
- ChIP-seq: *ChIPQC*, *chipseq*, *NarrowPeaks*, *DiffBind*, *MMDiff*, *epigenomix*, *jmosaics*, etc.
- DNA methylation: *bsseq*, *BiSeq*, *methylumi*, *minfi*, *Rnbeads*, etc.
- ATAC-seq: *ATACseqQC*, *esATAC*, etc.
- CAGE-seq: *TSSi*, *CAGEr*, etc.
- DNase-seq: *DNaseR*, etc.
- MNase-seq: *PING*, etc.
- 3C/4C/Hi-C: *r3Cseq*, *FourCSeq*, *HiTC*, *InteractionSet*, *GenomicInteractions*, etc.

Some packages such as *QuasR* or *systemPipeR* aim at providing complete pipelines to be run from the *R* environment.

1.3 Content and usage of this document

This document presents several packages that constitute the core *Bioconductor* tools dedicated to the analysis of functional genomics data. The chapters correspond to general tasks commonly undertaken in genomic studies and for each task the main functions from the core packages are illustrated. 'R code' is easily recognized by the grey background and text outputs are color-coded and preceded by the `##` symbols. There is a progression in the document and objects created in a chapter are often used in subsequent chapters. It is thus necessary to save your R session when you make a pause in the document:

```
save.image("MySession.RData")
```

and to load it back when you start again:

```
load("MySession.RData")
```

This document does not document core packages to perform genetic studies (i.e. analyze SNPs, CNVs, GWAS, etc.) because I am less familiar with them. This might be a future development of this document.

This document is largely inspired and borrows a lot from the documents, courses and vignettes written by others. I am grateful to them for sharing their knowledge and skills. I hope this document will also be useful to others and I invite the reader to embrace the attitude of sharing and respect that generally characterizes the *R* and *Bioconductor* community (`#rstats`, `@Bioconductor`).

2 Manipulating strings and sequences in Bioconductor

In this section, we will provide examples on the use of the following packages:

- *Biostrings*

- [Rsamtools](#)
- [BSgenome](#)
- [BSgenome.Dmelanogaster.UCSC.dm3](#)

2.1 Containers and accessors

First, we will see how to import sequences from a [fasta](#) file. We will use a file provided with the [Biostrings](#) package which contains the sequences 2000 bases upstream of the annotated transcription start sites (TSS) for the *Drosophila melanogaster* genome.

Get the file path:

```
dm3_upstream_filepath = system.file("extdata",  
                                     "dm3_upstream2000.fa.gz",  
                                     package="Biostrings")
```

Import the sequences as a DNASTringSet:

```
dm3_upstream = readDNASTringSet(dm3_upstream_filepath)  
dm3_upstream  
  
## A DNASTringSet instance of length 26454  
##      width seq                                     names  
## [1] 2000 GTTGGTGGCCACCAAGTGCC...AGTTTACGGTGCACGGT NM_078863_up_2000...  
## [2] 2000 TTATTTATGTAGGCGCCCGT...ACGGAAAGTCATCCTCGAT NM_001201794_up_2...  
## [3] 2000 TTATTTATGTAGGCGCCCGT...ACGGAAAGTCATCCTCGAT NM_001201795_up_2...  
## [4] 2000 TTATTTATGTAGGCGCCCGT...ACGGAAAGTCATCCTCGAT NM_001201796_up_2...  
## [5] 2000 TTATTTATGTAGGCGCCCGT...ACGGAAAGTCATCCTCGAT NM_001201797_up_2...  
## ... ..  
## [26450] 2000 ATTTACAAGACTAATAAAGA...AATTAATTTCAATAAAAC NM_001111010_up_2...  
## [26451] 2000 GATATACGAAGGACGACCTG...GTTTGAGTTGTTATATATT NM_001015258_up_2...  
## [26452] 2000 GATATACGAAGGACGACCTG...GTTTGAGTTGTTATATATT NM_001110997_up_2...  
## [26453] 2000 GATATACGAAGGACGACCTG...GTTTGAGTTGTTATATATT NM_001276245_up_2...  
## [26454] 2000 CGTATGTATTAGTAACTCT...AAAGTGTAAGAACAAATTG NM_001015497_up_2...
```

Create a random sequence:

```
randomSeq = DNASTring(paste(sample(DNA_ALPHABET[1:4],  
                                   size=24,  
                                   replace=TRUE),  
                           collapse=""))  
  
randomSeq  
  
## 24-letter "DNASTring" instance  
## seq: GTCTAAGTAAGGCTACAGTGTCG
```

Load the whole *Drosophila* genome sequence:

```
## library(BSgenome.Dmelanogaster.UCSC.dm3)  
Dmelanogaster
```

BioC for NGS data analysis

```
## Fly genome:
## # organism: Drosophila melanogaster (Fly)
## # provider: UCSC
## # provider version: dm3
## # release date: Apr. 2006
## # release name: BDGP Release 5
## # 15 sequences:
## #   chr2L    chr2R    chr3L    chr3R    chr4    chrX    chrU
## #   chrM     chr2LHet chr2RHet chr3LHet chr3RHet chrXHet chrYHet
## #   chrUextra
## # (use 'seqnames()' to see all the sequence names, use the '$' or '['
## # operator to access a given sequence)

names(Dmelanogaster)

## [1] "chr2L"    "chr2R"    "chr3L"    "chr3R"    "chr4"    "chrX"
## [7] "chrU"     "chrM"     "chr2LHet" "chr2RHet" "chr3LHet" "chr3RHet"
## [13] "chrXHet"  "chrYHet"  "chrUextra"

Dmelanogaster$chr2L

## 23011544-letter "DNAString" instance
## seq: CGACAATGCACGACAGAGGAAGCAGAACAGATATT...ATATTTGCAAATTTTGATGAACCCCTTTCAAA
```

Accessors:

```
dm3_upstream[[5]]

## 2000-letter "DNAString" instance
## seq: TTATTTATGTAGGCGCCCGTTCCCGCAGCCAAAGC...AATTAATCGATAGATACGGAAAGTCATCCTCGAT

toString(dm3_upstream[[5]][2:30])

## [1] "TATTTATGTAGGCGCCCGTTCCCGCAGCC"

subseq(dm3_upstream[[5]],start=2,end=30)

## 29-letter "DNAString" instance
## seq: TATTTATGTAGGCGCCCGTTCCCGCAGCC

Views(dm3_upstream[[5]],start=c(1,11,21),end=c(10,20,30))

## Views on a 2000-letter DNAString subject
## subject: TTATTTATGTAGGCGCCCGTTCCCGCAGCCAAA...TTAATCGATAGATACGGAAAGTCATCCTCGAT
## views:
##   start end width
## [1]    1  10    10 [TTATTTATGT]
## [2]   11  20    10 [AGGCGCCCGT]
## [3]   21  30    10 [TCCCGCAGCC]
```

Views objects are used to store a sequence together with ranges defined on this sequence. The ranges are said to represent *views* onto the sequence (see paragraph 3.4 for details on these objects).

Working with large FASTA files

The *Rsamtools* package (presented in paragraph 5) also provides interesting functions to work on large indexed FASTA files (e.g. containing a whole genome sequence). The short example below illustrates how to extract from a FASTA file a set of sequences defined by a *GRanges* (see paragraph 3.5 for details on *GRanges*):

```
## library(Rsamtools)
indFaEx_path=system.file("extdata","ce2dict1.fa",package="Rsamtools")
indFaEx=FaFile(indFaEx_path)

getSeq(indFaEx,
       GRanges(c("pattern01:3-10",
                  "pattern04:10-24")))

## A DNAStringSet instance of length 2
##      width seq                      names
## [1]      8 GAAACTAG                  pattern01
## [2]     15 TTGTTGCAATTGA            pattern04
```

2.2 Sequence analysis and masks

Reverse complement of a sequence (see also the *reverse* and *complement* functions):

```
reverseComplement(dm3_upstream[[5]])

## 2000-letter "DNAString" instance
## seq: ATCGAGGATGACTTTCGTATCTATCGATTAATTC...CTTGGCTGCGGGAACGGGCGCCTACATAAATAA
```

Count the occurrence of each base:

```
alphabetFrequency(dm3_upstream[1:2],baseOnly=TRUE,as.prob=TRUE)

##      A      C      G      T other
## [1,] 0.323 0.191 0.1875 0.2985    0
## [2,] 0.300 0.207 0.2230 0.2700    0
```

Get the GC content of a sequence:

```
letterFrequency(Dmelanogaster$chr2L,"CG",as.prob=TRUE)

##      C|G
## 0.41835
```

Masked versions of BSgenome packages are generally available:

```
library("BSgenome.Dmelanogaster.UCSC.dm3.masked")
```

Activate/deactivate the masks:

```
maskedgenome <- BSgenome.Dmelanogaster.UCSC.dm3.masked #A MaskedBSgenome object
chrU <- maskedgenome$chrU #A MaskedDNAString object
active(masks(chrU)) #Only some masks are active

## AGAPS  AMB  RM  TRF
```

```
## TRUE TRUE FALSE FALSE

active(masks(chrU)) <- TRUE #turn on all masks
chrUmask=injectHardMask(chrU) #Replaces the masked nucleotides by "+"
as(chrU,"XStringViews") #Get the unmasked regions

## Views on a 10049037-letter DNAString subject
## subject: TGC GTGCTACCACATCATGCAGTTTTC AAAGAA...AGCGCCTTTTACGACCAACTGAGCGTACCAG
## views:
##      start      end width
## [1]    3295    3309    15 [GTGAGGCATCACAAC]
## [2]    3537    3858   322 [CTGAAATTACGTTATAATTTA...ACTAATTTGCGGAATTCGAC]
## [3]    4410    5172   763 [AGCGCGCAAGCAAGAGAGGGA...TATGTCGGTGAAATATTAAT]
## [4]    5904    5991    88 [CAGGTGCCCTTCCAAAGCAA...TTTGCACTGGATAAGACAAG]
## [5]    6109    6393   285 [CAAATTTGTAGAGGGTGAGT...AAGCAACGCACCTCGACGTG]
## ...      ...      ...
## [8109] 10039126 10039126     1 [A]
## [8110] 10039540 10040228   689 [CAGCCATTTATTCTTATTTTC...ATTTTTAGTCGTCAGCGTTG]
## [8111] 10042946 10043532   587 [TATATAGAATATATTCGCCAA...ATTGGCAGGACAAGGCACAC]
## [8112] 10045835 10046264   430 [CTGTTTCCGTTGATTCCCGTT...TTTGCAAATTGAGCTCTAAA]
## [8113] 10046809 10046835    27 [TTTTATGGTTCGGTCAATTGTTGGAT]
```

Hard masking changes the sequence itself while soft masking does not, as illustrated when extracting [Views](#) (see paragraph 3.4):

```
toString(Views(Dmelanogaster$chrU,start=1714848, width=12))

## [1] "GAGAGAGAGAGA"

toString(Views(chrU,start=1714848, width=12))

## Warning in Views(chrU, start = 1714848, width = 12): masks were dropped

## [1] "GAGAGAGAGAGA"

toString(Views(chrUmask,start=1714848, width=12))

## [1] "+++++++"
```

Some functions take the masks into account without requiring hard masking. For example [alphabetFrequency](#) or, as shown below, the [matchPattern](#) function (see paragraph 2.3 for details on this function):

```
length(matchPattern('GAGAGAGAGAGA',maskedgenome$chrU))

## [1] 104

length(matchPattern('GAGAGAGAGAGA',chrU))

## [1] 8

length(matchPattern('GAGAGAGAGAGA',chrUmask))

## [1] 8

active(masks(chrU))=F #deactivate all masks
length(matchPattern('GAGAGAGAGAGA',chrU))

## [1] 104
```



```
active(masks(chrU))['RM']=T #activate only RepeatMasker
length(matchPattern('GAGAGAGAGAGA',chrU))

## [1] 14
```

2.3 Pattern matching

Pattern matching is also frequently used in ChIP-seq experiments to search e.g. for binding sites of transcription factors.

[Sequence motifs](#) are typically used to represent the DNA regions bound by transcription factors and other regulatory proteins. There are several packages in *Bioconductor* to search for and identify such patterns in strings and sequences. A complete [Bioconductor workflow](#) illustrates the use of pattern matching for the identification of transcription factor binding sites.

Here, we will use the following additional packages:

- [MotifDb](#)
- [seqLogo](#)
- [motifStack](#)
- [TFBSTools](#)

Other packages of interest include [rGADEM](#), [TFBSTools](#), [MotIV](#), [BCRANK](#) and [motifRG](#).

FixMe: add a section on TFBSTools which has improved a lot in last version. Mention also motifbreakR PMID 26272984 for the impact of a SNP on a TF binding site

2.3.1 Searching and plotting motifs.

The [MotifDb](#) package allows to query different databases containing DNA motifs.

We search these databases for the response element of the ecdysone receptor (EcR):

```
EcRMotifs=MotifDb::query(MotifDb,"EcR")
EcRMotifs

## MotifDb object of length 3
## | Created from downloaded public sources: 2013-Aug-30
## | 3 position frequency matrices from 3 sources:
## |   FlyFactorSurvey:    1
## |   JASPAR_2014:       1
## |   jasper2016:        1
## | 1 organism/s
## |   Dmelanogaster:     3
## Dmelanogaster-FlyFactorSurvey-EcR_SANGER_5_FBgn0000546
## Dmelanogaster-JASPAR_2014-EcR::usp-MA0534.1
## Dmelanogaster-jasper2016-EcR::usp-MA0534.1

EcRMotifs[[1]]
```

```
##           1 2 3 4           5 6           7 8
## A 0.6428571 0 0 1 0.00000000 0 0.0000000 0.0
## C 0.0000000 0 0 0 0.92857143 1 0.1428571 0.5
## G 0.0000000 0 1 0 0.07142857 0 0.0000000 0.0
## T 0.3571429 1 0 0 0.00000000 0 0.8571429 0.5
```

The motif is represented as a [Position-weight matrix](#)

The package [seqLogo](#) allows to easily plot [sequence logos](#):

Figure 1 shows EcR motif:

```
seqLogo(EcRMotifs[[1]])
```

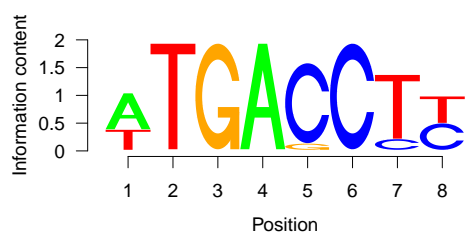


Figure 1: [Sequence logo for EcR motif](#)

Figure 2 its reverse complement:

```
seqLogo(reverseComplement(EcRMotifs[[1]]))
```

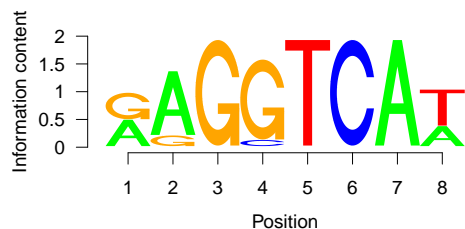


Figure 2: [Sequence logo for EcR motif reverse complement](#)

And Figure 3 the logo for the response element obtained from [JASPAR](#):

```
seqLogo(EcRMotifs[[2]])
```

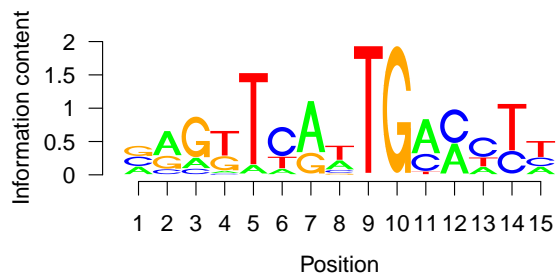


Figure 3: [Sequence logo for EcR:Usp heterodimer](#)

The second motif (from [JASPAR](#)) represented in Figure 3 is a binding site for the heterodimer composed of the ecdysone receptor (EcR) and its binding partner Ultraspiracle protein (Usp). This imperfect palindromic ecdysone response element is an inverted repeat of the consensus motif AGGTCA separated by 1 nucleotide (IR1).

The package [motifStack](#) provides additional plotting functionalities for sequence logos as shown in Figure 4:

```
par(mfrow=c(2,1))
plot(new("pcm",
  as.matrix(reverseComplement(EcRMotifs[[1]])),
  name="EcR_FlyFactorSurvey"))
plot(new("pcm",
  as.matrix(reverseComplement(EcRMotifs[[2]])),
  name="EcR_JASPAR"))
```

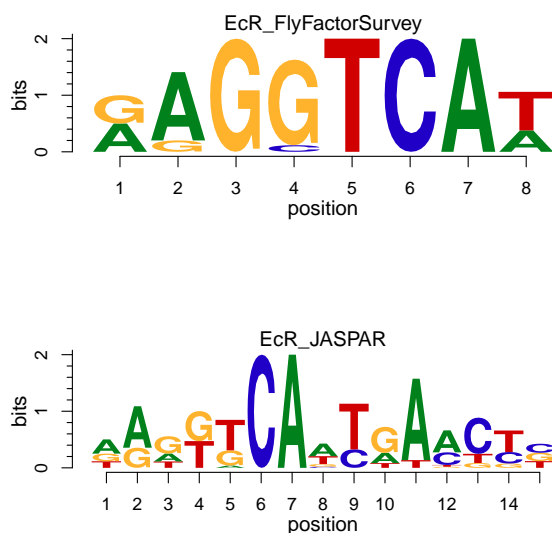


Figure 4: Sequence logos for EcR motifs using `motifStack`

2.3.2 TFBSTools

TFBS stands for Transcription Factor Binding Site. The [TFBSTools](#) package [?] includes several features to manipulate and analyze sequence motifs or potential TFBS:

- Containers for motifs (`XMatrix`), collections of motifs (`XMatrixList`), sets of motifs obtained with [MEME](#) (`MotifSet`) or from a `motifSearch` on a sequence (`SiteSet`)
- Containers and graphical representations of the most recent [TFFM](#) (Transcription Factor Flexible Model) motif representations
- Functions to convert between PWM (position-weight matrix), PFM (position frequency matrix) and ICM (information content matrix)
- Functions to compare motifs as matrices (PFMs/PWMs) or with IUPAC strings
- Functions to scan sequences or whole genome (`searchSeq`) and to scan aligned sequences (`searchAln`) or genomes (`searchPairBSgenome`)

- Functions to query the [JASPAR](#) database, using *Bioconductor* packages such as [JASPAR2018](#)
- Wrapper function for the de novo motif discovery tool [MEME](#)

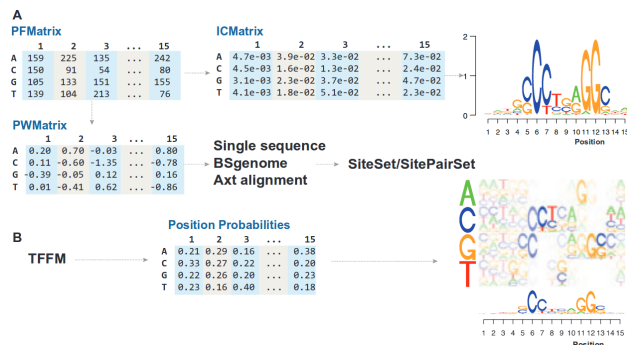


Figure 5: Common workflow and classes in TFBSTools
Taken from the [TFBSTools](#) paper

2.3.3 Scanning a sequence with a Position-weight matrix.

We select the JASPAR motif:

```
EcrJASP=EcrMotifs[[grep("JASPAR_2014",names(EcrMotifs))]]
```

And search for this motif on both strands of chromosome 2L:

```
EcrJASP_2L=matchPWM(EcrJASP,
                    Dmelanogaster$chr2L,
                    min.score='85%')
EcrJASP_2L_rev=matchPWM(reverseComplement(EcrJASP),
                        Dmelanogaster$chr2L,
                        min.score='85%')
EcrJASP_2L_all <- Views(Dmelanogaster$chr2L,
                      union(ranges(EcrJASP_2L),
                           ranges(EcrJASP_2L_rev)))
```

We can use the `matchPWM` function to search a motif directly in a `BSeqGen` object. In this case, the search is automatically done on both strands and the function returns a convenient `GRanges` object (see 3.5 below).

```
EcrJASP_all <- matchPWM(EcrJASP, Dmelanogaster)
EcrJASP_all

## GRanges object with 74551 ranges and 2 metadata columns:
##           seqnames           ranges strand |       score       string
##           <Rle>             <IRanges> <Rle> | <numeric> <DNAStringSet>
##      [1]   chr2L           [ 5749,  5763]   + |   8.221154 AAGTTTATTGCAATG
##      [2]   chr2L           [ 8746,  8760]   + |   8.375000 GAGGTCATTAACCTT
##      [3]   chr2L          [14077, 14091]   + |   8.384615 AAATTCATTGAAATG
```

```
##      [4]      chr2L      [27494, 27508]      + |      8.201923 TAGATCAATGCCTTT
##      [5]      chr2L      [29314, 29328]      + |      8.057692 CAATTCATTAACCTTT
##      ...      ...      ...      ...      ...
## [74547] chrUextra [28864987, 28865001]      - |      8.028846 ACGTTTATTGAAGCT
## [74548] chrUextra [28947220, 28947234]      - |      8.144231 GAATTAAATGAGCTT
## [74549] chrUextra [28961112, 28961126]      - |      8.076923 CAGTTCAGTGCTCTG
## [74550] chrUextra [28971605, 28971619]      - |      8.019231 GAGCTCAGTGAAGTA
## [74551] chrUextra [28977930, 28977944]      - |      8.076923 CAGTTCAGTGCTCTG
## -----
## seqinfo: 15 sequences from an unspecified genome
```

2.3.4 Scanning sequences with strings.

Scanning multiple sequences with one string.

A pattern or motif can also be represented as a character string possibly using the [IUPAC ambiguity code](#). Here, for simplicity, we will represent ambiguities as 'N'.

To perform pattern matching, we define a consensus sequence for the IR1 ecdysone response element:

```
EcR_IR1_cons=consensusString(EcrJASP,ambiguityMap="N")
EcR_IR1_cons

## [1] "NAGTTCATTGACCTT"

EcR_IR1_cons=substring(EcR_IR1_cons,first=2)
EcR_IR1_cons

## [1] "AGTTCATTGACCTT"
```

Similarly, we can build consensus sequences for the EcR itself or for its binding partner Ultraspiracle (Usp):

```
EcR_cons=substring(consensusString(reverseComplement(EcRMotifs[[1]]),
                                ambiguityMap="N"),
                  first=2)
Usp_cons=substring(consensusString(MotifDb::query(MotifDb,"Usp")[[5]],
                                ambiguityMap="N"),
                  first=1,
                  last=7)
```

Now, we search for the EcR consensus in chromosome 2L:

```
EcR_on_2L=matchPattern(EcR_cons,Dmelanogaster$chr2L)
EcR_on_2L_rev=matchPattern(reverseComplement(DNAString(EcR_cons)),
                           Dmelanogaster$chr2L)
EcR_on_2L_all=Views(Dmelanogaster$chr2L,union(ranges(EcR_on_2L),
                                                ranges(EcR_on_2L_rev)))
```

Note that we have found a perfect palindrome:

BioC for NGS data analysis

```
EcR_on_2L_all[width(EcR_on_2L_all)!=7]

## Views on a 23011544-letter DNAString subject
## subject: CGACAATGCACGACAGAGGAAGCAGAACAGATA...ATTTGCAAATTTTGATGAACCCCTTTCAAA
## views:
##      start      end width
## [1] 7208722 7208733    12 [AGGTCATGACCT]
```

Scanning multiple sequences with one string.

It is also possible to search for a single pattern in several subject sequences using the `vmatchPattern`.

Search for the EcR pattern in TSS upstream sequences:

```
EcR_on_up=vmatchPattern(EcR_cons, dm3_upstream)
```

Get the number of matches per subject element:

```
nmatch_per_seq = elementNROWS(EcR_on_up)
table(nmatch_per_seq)

## nmatch_per_seq
##      0      1      2      3
## 24765 1638   48    3
```

Let's take look at one of the upstream sequence with the maximum number of matches:

```
i0=which.max(nmatch_per_seq)
Views(dm3_upstream[[i0]], EcR_on_up[[i0]])

## Views on a 2000-letter DNAString subject
## subject: AAATATCTATTTTCTTAGTTAAGTCCCATCAAA...TTCAAATAACTGTATTAGTGGACTTTTCTGG
## views:
##      start end width
## [1] 1633 1639    7 [AGGTCAT]
## [2] 1809 1815    7 [AGGTCAT]
## [3] 1829 1835    7 [AGGTCAT]
```

Scanning a sequence with multiple strings.

One may also search for multiple patterns in a single subject sequence using `matchPDict`. Get all PWM matrices available for *Drosophila melanogaster*:

```
dm_matrices = MotifDb::query(MotifDb,"dmelanogaster")
```

Keep only the motifs that are 8bp-long and get their consensus sequences:

```
motif_ln = sapply(dm_matrices,ncol)
dm_matrices = dm_matrices[motif_ln==8]
dm_motifs=DNAStringSet(sapply(dm_matrices,consensusString,ambiguityMap="N"))
```

Search for all these motifs in chromosome 2L:

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```
mot8_on_2L=matchPDict(dm_motifs,Dmelanogaster$chr2L,fixed=FALSE)
summary(elementNROWS(mot8_on_2L)) #Number of matches

##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      268     867     3289   57508   13143 4815391

head(unlist(mot8_on_2L)) #first 6 matches

## IRanges object with 6 ranges and 0 metadata columns:
##
##              start      end    width
##              <integer> <integer> <integer>
## Dmelanogaster-cispb_1.02-M0111_1.02      118      125      8
## Dmelanogaster-cispb_1.02-M0111_1.02      196      203      8
## Dmelanogaster-cispb_1.02-M0111_1.02      576      583      8
## Dmelanogaster-cispb_1.02-M0111_1.02      654      661      8
## Dmelanogaster-cispb_1.02-M0111_1.02     1034     1041      8
## Dmelanogaster-cispb_1.02-M0111_1.02     1112     1119      8
```

The motif most frequently found on chromosome 2L:

```
names(dm_motifs[which.max(elementNROWS(mot8_on_2L))])
## [1] "Dmelanogaster-cispb_1.02-M5115_1.02"
toString(dm_motifs[[which.max(elementNROWS(mot8_on_2L))]])
## [1] "NNNNGNNN"
```

The motif less frequently found on chromosome 2L:

```
names(dm_motifs[which.min(elementNROWS(mot8_on_2L))])
## [1] "Dmelanogaster-cispb_1.02-M5018_1.02"
toString(dm_motifs[[which.min(elementNROWS(mot8_on_2L))]])
## [1] "CGCGCGAT"
```

Scanning multiple sequence with multiple strings.

Finally, some functions, still under development, are available to search for multiple patterns in multiple sequences.

Remove the motifs containing N bases and create a dictionary of motifs (the motifs must have the same length):

```
dm_mot8_dict=PDict(dm_motifs[sapply(dm_motifs,hasOnlyBaseLetters)])
```

Search for the motifs in TSS upstream sequences:

```
mot8_count_upstream=vcountPDict(dm_mot8_dict,dm3_upstream)
```

Number of motifs found:

```
apply(mot8_count_upstream,1,sum)
## [1] 975 566 1133 733 1445 1295 1133 920 498 521 9411 4366 399 292
```

```
## [15] 1281 1698 1698 761 475 2536 706 706 290 6162 2327 542 3063 724
## [29] 708 5781 1579 1340 702 4384 1939 6411 473 823 4560 742 714 952
## [43] 432 432 7916 6411 414 419 1284 1103 321 321 324 324 574 574
## [57] 6411 2527 3027 2549 469 469 885 1461 532 6411 724 1284 2527 6411
## [71] 724 1284 2527 885 515 6411 724 1284 2527 885
```

Number of motif1 per upstream sequence:

```
table(mot8_count_upstream[1,])
```

```
##
##      0      1      2      3      5
## 25527  887   36    2    2
```

Number of motifs per upstream sequence:

```
nMot8_perSeq=apply(mot8_count_upstream,2,sum)
names(nMot8_perSeq)=names(dm3_upstream)
```

Plot in Figure 6 the number of upstream sequences as a function of the number of motifs:

```
nMot8_perSeq=nMot8_perSeq[nMot8_perSeq>=1]
plot(as.integer(names(table(nMot8_perSeq))),
     as.integer(table(nMot8_perSeq)),
     pch=15,type="b",log="y",col="blue",
     xlab="Number of 8bp-long motifs",
     ylab="Number of upstream sequences")
```

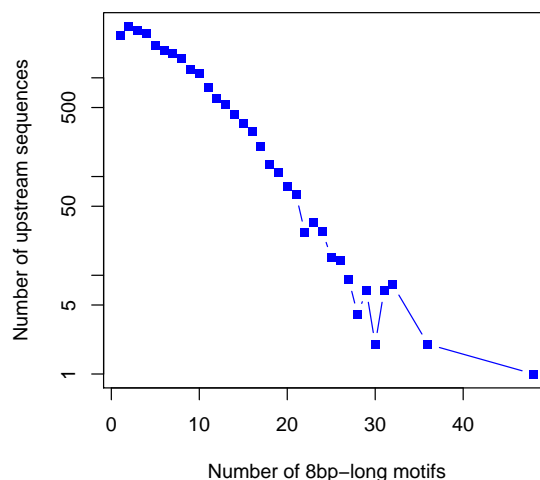


Figure 6: Motifs per upstream sequence

2.4 Sequence alignment

Sequence alignment is a common task in NGS bioinformatic pipelines. Most of the time, alignment of short or long reads is performed outside *R*, using dedicated NGS aligner.

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Widely used short read aligners include:

- BWA [4, 5]
- Bowtie/Bowtie2 [6, 7]

Some aligners are also adapted to RNA-seq which often generates spliced reads:

- HiSat2 [8, 9]
- STAR [10]
- Subread [11]

There are *R* packages that provide interfaces to some of these aligners, such as *Rbowtie*, *Rbowtie2*, *QuasR* or *Rsubread*.

Here, we only briefly present classical sequence alignment tools available in *Bioconductor*, mainly via the *Biostrings* package (see the vignette of this package for further examples, including on NGS data). In NGS analysis, these tools can be used to search for adapters or primer sequences for example.

2.4.1 Pairwise alignment.

The main function `pairwiseAlignment` provides functionalities to perform global (*Needleman-Wunsch*), local (*Smith-Waterman*) and overlap (ends-free) pairwise alignments while tuning *substitution scoring* and *gap penalties*.

Align the consensus ecdysone receptor response element to a region of chromosome 2L where such a motif is present:

```
pairwiseAlignment(EcR_IR1_cons,
                  Dmelanogaster$chr2L[14067:14101],
                  type='global-local')

## Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern:  [1] AGTTCATTGACCTT
## subject:  [12] AATTCATTGAAATG
## score: -3.779581
```

Align the consensus motifs for EcR and Usp on a TSS upstream sequence:

```
paln_EcRUsp=pairwiseAlignment(c(EcR_cons,Usp_cons),
                              dm3_upstream[[1780]],
                              type='global-local')

paln_EcRUsp[1]

## Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern:  [1] AGGTCAT
## subject:  [779] AGGGCAT
## score: 5.991251

paln_EcRUsp[2]

## Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern:  [1] GGGGTCA
## subject:  [772] GAGGTCA
```

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```
## score: 5.991251
Views(paln_EcRUp)

## Views on a 2000-letter DNAString subject
## subject: CCATGCACTGGCCAGCGATAGCCCCATCTATCG...AGCCGCGAAATGAGGGGAAACCGAGCTGGAGC
## views:
##      start end width
## [1]   779 785     7 [AGGGCAT]
## [2]   772 778     7 [GAGGTCA]

Views(dm3_upstream[[1780]],start=772,end=785)

## Views on a 2000-letter DNAString subject
## subject: CCATGCACTGGCCAGCGATAGCCCCATCTATCG...AGCCGCGAAATGAGGGGAAACCGAGCTGGAGC
## views:
##      start end width
## [1]   772 785    14 [GAGGTCAAGGGCAT]
```

Global vs local alignment:

```
pairwiseAlignment('GTGTCAATACGACAGCAATCTG',
                  'AGTGTGAATTACAGCAAATCTCTGT',
                  type='global')

## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAATACGACAGCAA---TCTG
## subject: [2] GTGTGAATT--ACAGCAAATCTCTG
## score: -48.12697

pairwiseAlignment('GTGTCAATACGACAGCAATCTG',
                  'AGTGTGAATTACAGCAAATCTCTGT',
                  type='local')

## Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [12] ACAGCAA
## subject: [11] ACAGCAA
## score: 13.87229

pairwiseAlignment('GTGTCAATACGACAGCAATCTG',
                  'AGTGTGAATTACAGCAAATCTCTGT',
                  type='global-local')

## Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAATACGACAGCAA-TCTG
## subject: [2] GTGTGAATT--ACAGCAAATCTC
## score: -16.008
```

Playing with gap penalties:

```
pairwiseAlignment('GTGTCAATACGACAGCAATCTG', 'AGTGTGAATTACAGCAAATCTCTGTTCAATTTCTG',
                  type='global')

## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAATACGACAGCAA-----TCTG
## subject: [2] GTGTGAATT--TACAGCAAATCTCTGTTCAATTTCTG
## score: -74.12697
```

```
pairwiseAlignment('GTGTCAATACGACAGCAATCTG', 'AGTGTGAATTACAGCAAATCTCTGTTCAATTTCTG',
                  gapExtension=-6, type='global')

## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAAT-ACGACAG-----CAAT--CTG
## subject: [2] GTGTGAATTACAGCAAATCTCTGTTCAATTTCTG
## score: -105.9255

pairwiseAlignment('GTGTCAATACGACAGCAATCTG', 'AGTGTGAATTACAGCAAATCTCTGTTCAATTTCTG',
                  gapOpening=-60, type='global')

## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAATACGACAGCAATCTG
## subject: [14] GCAAATCTCTGTTCAATTTCTG
## score: -186.617
```

Accessors and methods:

```
paln=pairwiseAlignment(DNAStringSet(c('GTGTCAATACGACAGCAATCTG', 'TAAGGTCATAGTGT')),
                      DNAString('TCGCCATAGGTCAATAGTGTGAATTACAGCAAATCTCTGTTCAATTTCTG'),
                      type='global-local')

pattern(paln)

## QualityAlignedXStringSet (1 of 2)
## [1] GTGTCAATACGACAGCAA-TCTG

subject(paln)

## QualityAlignedXStringSet (1 of 2)
## [17] GTGTGAATT--ACAGCAAATCTC

aligned(paln)

## A DNAStringSet instance of length 2
## width seq
## [1] 50 -----GTGTCAATAACAGCAA-TCTG-----
## [2] 50 -----TAAGGTC-ATAGTGT-----

Biostrings::score(paln)

## [1] -16.008003 -2.017499

pid(paln) # percentage identity

## [1] 73.91304 80.00000

compareStrings(paln) #symbolic representation of the alignment

## [1] "GTGT?AAT?++ACAGCAA-TCT?" "??AGGTC-ATAGTGT"

nedit(paln) #Levenshtein edit distance

## [1] 6 3

nmatch(paln) #also nmismatch(paln)

## [1] 17 12

insertion(paln)[[1]] #also deletion(paln)
```

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```
## IRanges object with 1 range and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      10      11         2

Views(paln)

## Views on a 50-letter DNAString subject
## subject: TCGCCATAGGTCAATAGTGTGAATTACAGCAAATCTCTGTTCAATTTCTG
## views:
##      start end width
## [1]    17  37    21 [GTGTGAATTACAGCAAATCTC]
## [2]     6  20    15 [ATAGGTCAATAGTGT]

coverage(paln)

## integer-Rle of length 50 with 5 runs
## Lengths:  5 11  4 17 13
## Values :  0  1  2  1  0
```

See the 'pairwise alignment' vignette of [Biostrings](#) for other utilities and accessors.

The `stringDist` function computes the Levenshtein edit distance or pairwise alignment score for a set of strings:

```
paln[1]

## Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] GTGTCAATACGACAGCAA-TCTG
## subject: [17] GTGTGAATT--ACAGCAAATCTC
## score: -16.008

nedit(paln[1])

## [1] 6

#Can also be obtained with:
stringDist(c('GTGTCAATACGACAGCAATCTG',
             'GTGTGAATTACAGCAAATCTC'),
           method = "levenshtein")

## 1
## 2 6
```

Using substitution matrices based on evolutionary models (other matrices are available):

```
data(BLOSUM62)
paln <- pairwiseAlignment(AAString("ALAKHLYDSYIKSFPLTKAKARAILTGKTTDKS"),
                          AAString("AQQFNDIVCAMTQEDLEKFWKRCRPFTHM"),
                          substitutionMatrix = BLOSUM62)
```

Visualizing the alignment with the DECIPHER package

```
library(DECIPHER)
alnseqs <- c(aligned(pattern(paln)), aligned(subject(paln)))
BrowseSeqs(alnseqs)
```

2.4.2 Multiple alignment.

Several tools and algorithms are available to perform multiple sequence alignments (e.g. [Multalin](#), [ClustalW](#), [T-Coffee](#) or [MUSCLE](#)). [Biostrings](#) essentially allows to import and explore the resulting alignments. Examples are provided in the vignette of [Biostrings](#) and [here](#).

FixMe: mention the msa BioC package, PMID 26315911

3 Manipulating genomic ranges

So far, we have essentially manipulated sequence data. However, we have already noticed that start-end coordinates along a sequence are also useful in some situations. These start-end coordinates define a range onto the sequence. As underlined by [Martin Morgan](#): 1) ranges allow to represent a wide array of genomic data and annotations and 2) several biological questions reflect range-based queries. [Bioconductor](#) implements a number of tools to manipulate and analyze ranges and specifically genomic ranges [12].

In this section, we will mainly use the following 2 packages:

- [IRanges](#)
- [GenomicRanges](#)

We will also present the packages providing annotations as genomic ranges.

3.1 IRanges and accessors

Definition.

An *IRanges* object is defined as follow:

- defined by 2 vectors out of start, end, width (SEW ; $end = start + width - 1$)
- closed intervals (i.e. include end points)
- zero-width convention: $width \geq 0$; $end = start - 1 \Leftrightarrow width = 0$
- can be named

A simple *IRanges*:

```
eg = IRanges(start = c(1, 10, 20),
             end = c(4, 10, 19),
             names = c("A", "B", "C"))

eg

## IRanges object with 3 ranges and 0 metadata columns:
##      start      end  width
##   <integer> <integer> <integer>
##    A         1         4     4
##    B        10        10     1
##    C        20        19     0
```

A bigger *IRanges*:

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```
set.seed(123) #For reproducibility
start = floor(runif(10000, 1, 1000))
end = start + floor(runif(10000, 0, 100))
ir = IRanges(start, end)
ir

## IRanges object with 10000 ranges and 0 metadata columns:
##           start      end    width
##      <integer> <integer> <integer>
##      [1]      288      319      32
##      [2]      788      820      33
##      [3]      409      496      88
##      [4]      883      915      33
##      [5]      940      952      13
##      ...      ...      ...      ...
## [9996]      466      493      28
## [9997]      899      984      86
## [9998]      114      125      12
## [9999]      571      596      26
## [10000]      900      966      67
```

Accessors and methods.

IRanges accessors:

```
length(ir)
## [1] 10000

ir[1:4]

## IRanges object with 4 ranges and 0 metadata columns:
##           start      end    width
##      <integer> <integer> <integer>
##      [1]      288      319      32
##      [2]      788      820      33
##      [3]      409      496      88
##      [4]      883      915      33

start(ir[1:4])
## [1] 288 788 409 883

width(ir[1:4])
## [1] 32 33 88 33

names(eg)
## [1] "A" "B" "C"
```

Other useful methods for *IRanges*

```
c(ir[1:2],ir[5:6]) #combining
## IRanges object with 4 ranges and 0 metadata columns:
```

```
##           start      end      width
##      <integer> <integer> <integer>
##   [1]      288      319        32
##   [2]      788      820        33
##   [3]      940      952        13
##   [4]       46       81        36

sort(ir[1:4])

## IRanges object with 4 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##   [1]      288      319        32
##   [2]      409      496        88
##   [3]      788      820        33
##   [4]      883      915        33

rank(ir[1:4],ties="first")

## [1] 1 3 2 4

mid(ir[1:4]) # midpoints

## [1] 303 804 452 899

tile(ir[1:2],n=2) #returns an IRangesList (see below)

## IRangesList of length 2
## [[1]]
## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##   [1]      288      303        16
##   [2]      304      319        16
##
## [[2]]
## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##   [1]      788      803        16
##   [2]      804      820        17

ir[[1]]

## [1] 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305
## [19] 306 307 308 309 310 311 312 313 314 315 316 317 318 319

as.integer(ir[1]) #equivalent but works on multiple ranges

## [1] 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305
## [19] 306 307 308 309 310 311 312 313 314 315 316 317 318 319

unlist(ir[1]) #also equivalent but names can be added

## [1] 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305
## [19] 306 307 308 309 310 311 312 313 314 315 316 317 318 319

rep(ir[1:2],each=2)
```

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```
## IRanges object with 4 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      288      319        32
## [2]      288      319        32
## [3]      788      820        33
## [4]      788      820        33

isNormal(ir[1:4])

## [1] FALSE

isNormal(sort(ir[1:4])) #see '?Ranges-class' for Normality definition

## [1] TRUE

isDisjoint(ir[1:4])

## [1] TRUE

match(ir[1:4],ir[4:1]) #see '?Ranges-comparison' for Ranges comparison methods

## [1] 4 3 2 1

ir[1:4]>ir[4:1]

## [1] FALSE  TRUE FALSE  TRUE
```

Other methods creating IRanges:

```
as(c(2:10,8,90:100),"IRanges") #from a vector of integers

## IRanges object with 3 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         2      10         9
## [2]         8       8         1
## [3]        90     100        11

successiveIRanges(width=rep(10,5),gap=10)

## IRanges object with 5 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         1      10        10
## [2]        21      30        10
## [3]        41      50        10
## [4]        61      70        10
## [5]        81      90        10

whichAsIRanges(c(19, 5, 0, 8, 5)>=5) #transforms a logical vector in IRanges

## NormalIRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         1       2         2
## [2]         4       5         2
```


IRangesList.

It can be convenient to group ranges in a list (e.g. exons grouped by genes). *IRangesList* objects serve this purpose. Accessors and functions for *IRanges* generally work on *IRangesList* objects.

```
irl=split(ir,width(ir)) # an IRangesList
irl[[1]]

## IRanges object with 96 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##   [1]      321      321          1
##   [2]      600      600          1
##   [3]      184      184          1
##   [4]      297      297          1
##   [5]      276      276          1
##   ...      ...      ...      ...
##  [92]      188      188          1
##  [93]      308      308          1
##  [94]      289      289          1
##  [95]      936      936          1
##  [96]      669      669          1

start(irl)

## IntegerList of length 100
## ["1"] 321 600 184 297 276 816 87 729 407 ... 858 52 85 188 308 289 936 669
## ["2"] 915 576 706 235 678 647 451 138 ... 638 66 979 740 300 869 433 645
## ["3"] 457 415 336 774 487 787 587 352 ... 264 60 607 292 709 418 552 102
## ["4"] 253 75 429 4 785 24 464 869 433 ... 827 240 175 459 130 146 681 267
## ["5"] 498 812 977 120 991 583 959 931 532 ... 157 871 538 209 8 37 39 443
## ["6"] 372 298 241 61 363 351 847 37 916 ... 910 929 656 71 839 223 34 602
## ["7"] 753 198 674 584 850 250 962 14 ... 457 175 731 758 953 212 551 153
## ["8"] 895 803 784 358 366 536 530 323 ... 350 959 592 651 487 406 895 53
## ["9"] 318 478 991 747 769 626 332 436 ... 114 546 928 69 415 219 568 546
## ["10"] 467 674 225 194 464 87 494 364 ... 706 267 938 259 685 700 435 937
## ...
## <90 more elements>

head(elementNROWS(irl))

##   1   2   3   4   5   6
##  96  83 108  95  84 110
```

3.2 Intra-and inter-range operations

3.2.1 Intra-range operations

These operations apply on each range of an *IRanges* object:

```
ir[1:2]

## IRanges object with 2 ranges and 0 metadata columns:
```

```
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      288      319        32
##      [2]      788      820        33

shift(ir[1:2],shift=10)

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      298      329        32
##      [2]      798      830        33

resize(ir[1:2],width=100,fix="start")

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      288      387       100
##      [2]      788      887       100

flank(ir[1:2],width=100,start=T)

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      188      287       100
##      [2]      688      787       100

narrow(ir[1:2],start=1,width=30) #here 'start' is relative

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      288      317        30
##      [2]      788      817        30

ir[1:2]+10

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      278      329        52
##      [2]      778      830        53

ir[1:2]-10

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
##      [1]      298      309        12
##      [2]      798      810        13

ir[1:2]+c(0,10)

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##      <integer> <integer> <integer>
```

```
## [1]      288      319      32
## [2]      778      830      53

ir[1:4]*-10 ; ir[1:4]*10 # acts like a centered zoom

## IRanges object with 4 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      144      463      320
## [2]      639      968      330
## [3]       13      892      880
## [4]      734     1063      330

## IRanges object with 4 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      302      304        3
## [2]      803      805        3
## [3]      449      456        8
## [4]      898      900        3

ir[1:2]*c(1,2) #zoom second range by 2X

## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      288      319      32
## [2]      796      811      16
```

See `help('intra-range-methods', package="IRanges")` for other methods

3.2.2 Inter-range operations

Function to plot ranges (adapted from the *IRanges* vignette):

```
plotRanges <- function(x, xlim = x, main = deparse(substitute(x)),
                      col = "black", sep = 0.5, cex.title=1, cex.axis=1, xaxis=T,...)
{
  height <- 1
  if (is(xlim, "Ranges"))
    xlim <- c(min(start(xlim)), max(end(xlim)))
  bins <- disjointBins(IRanges(start(x), end(x) + 1))
  plot.new()
  plot.window(xlim, c(0, max(bins)*(height + sep)))
  ybottom <- bins * (sep + height) - height
  rect(start(x)-0.5, ybottom, end(x)+0.5, ybottom + height, col = col, ...)
  if (xaxis)
    (axis(1, cex.axis=cex.axis, padj=1))
  title(main, cex.main=cex.title)
}
```

These inter-range operations, called *endomorphisms*, apply on a set of ranges and return a set of ranges:

```

irs=ir[which(start(ir)<=100 & end(ir)<=100)[c(3:4,8,14,17:18)]] #select some ranges in [1:100]
irs

## IRanges object with 6 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      25      44        20
## [2]      46      60        15
## [3]      53      65        13
## [4]       9      33        25
## [5]       1      50        50
## [6]      87      96        10

reduce(irs)

## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]       1      65        65
## [2]      87      96        10

disjoin(irs)

## IRanges object with 10 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]       1       8         8
## [2]       9      24        16
## [3]      25      33         9
## [4]      34      44        11
## [5]      45      45         1
## [6]      46      50         5
## [7]      51      52         2
## [8]      53      60         8
## [9]      61      65         5
## [10]     87      96        10

gaps(irs)

## IRanges object with 1 range and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      66      86        21

coverage(irs)

## integer-Rle of length 96 with 11 runs
##  Lengths:  8 16  9 11  1  5  2  8  5 21 10
##  Values :  1  2  3  2  1  2  1  2  1  0  1

```

See `help('inter-range-methods', package="IRanges")` for other methods.

They are illustrated in Figure 7 using:

```
par(mfrow=c(5,1))
plotRanges(irs,xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(reduce(irs),xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(disjoin(irs),xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(gaps(irs),xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plot(1:100,c(coverage(irs),rep(0,4)),type="l",axes=F,xlab="",ylab="",lwd=3)
title(main="coverage",cex.main=2)
axis(side=2,lwd=2,cex.axis=2,at=0:3,labels=0:3)
axis(1,lwd=2,cex.axis=2,adj=1)
```

3.2.3 Set operations

See `help('setops-methods',package="IRanges")` for details. The `union` and `intersect` functions for *IRanges*:

```
union(irs[1:3],irs[4:6])

## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         1        65        65
## [2]        87        96        10

intersect(irs[1:3],irs[4:6])

## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]        25        44        20
## [2]        46        50         5
```

Illustrated in Figure 8:

```
par(mfrow=c(4,1))
plotRanges(irs[1:3],xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(irs[4:6],xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(union(irs[1:3],irs[4:6]),xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
plotRanges(intersect(irs[1:3],irs[4:6]),xlim=c(0,100),xaxis=T, cex.title=2, cex.axis=1.5)
```

The `setdiff` function for asymmetric differences:

```
setdiff(irs[1:3],irs[4:6])

## IRanges object with 1 range and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]        51        65        15

setdiff(irs[4:6],irs[1:3])

## IRanges object with 3 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
```

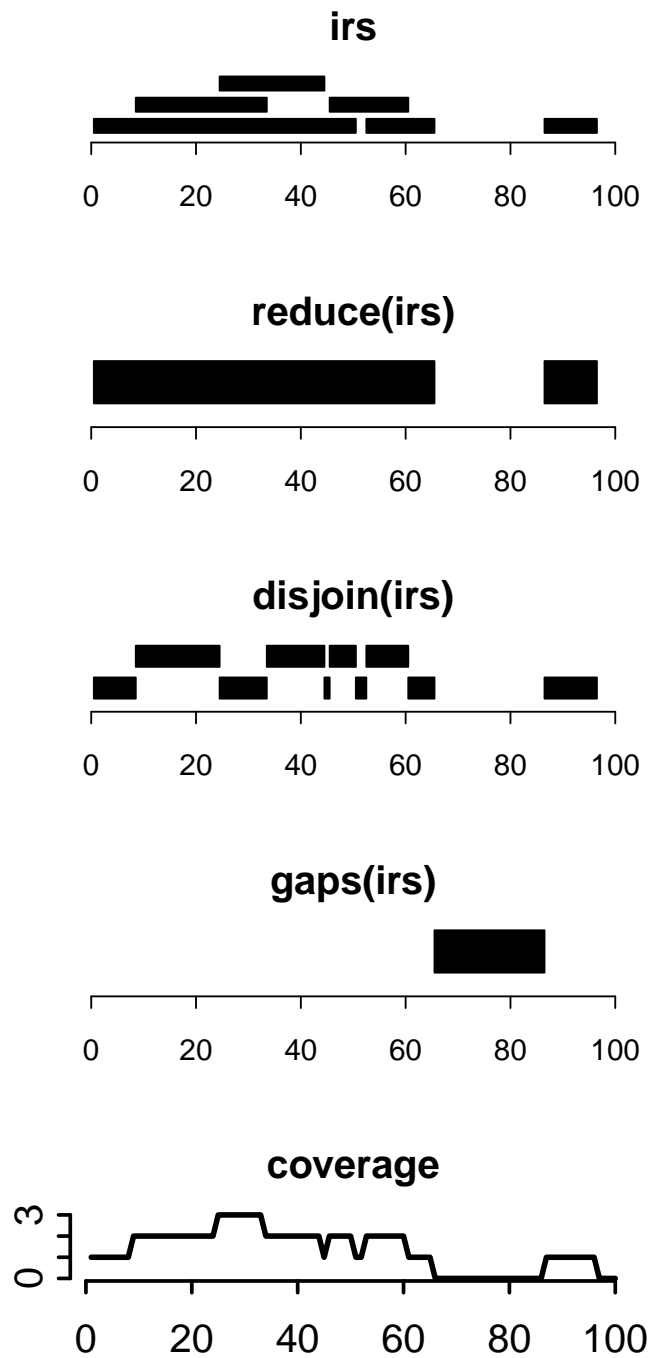


Figure 7: Inter-range operations

##	[1]	1	24	24
##	[2]	45	45	1
##	[3]	87	96	10

Illustrated in Figure 9:

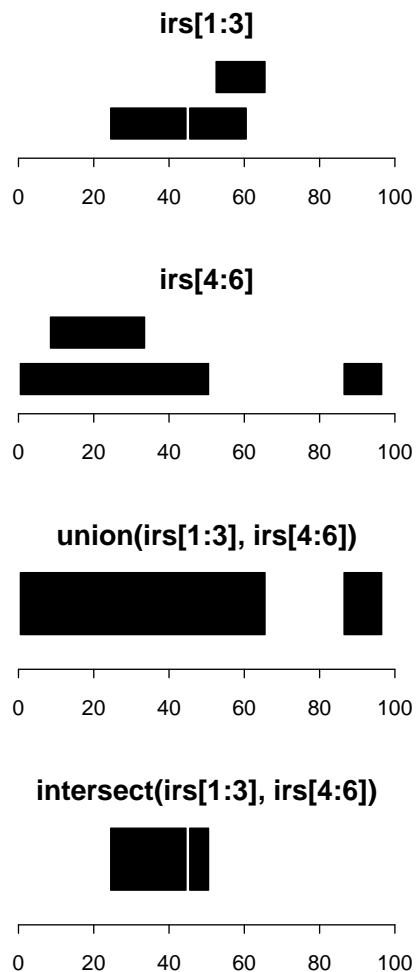


Figure 8: Union and intersect on IRanges

```
par(mfrow=c(4,1))
plotRanges(irs[1:3],xlim=c(0,100),xaxis=T, cex=2, cexaxis=1.5)
plotRanges(irs[4:6],xlim=c(0,100),xaxis=T, cex=2, cexaxis=1.5)
plotRanges(setdiff(irs[1:3],irs[4:6]),xlim=c(0,100),xaxis=T, cex=2, cexaxis=1.5)
plotRanges(setdiff(irs[4:6],irs[1:3]),xlim=c(0,100),xaxis=T, cex=2, cexaxis=1.5)
```

The same functions can be applied in a parallel fashion (i.e. on the first elements of the provided IRanges, then on the second elements, etc.)

```
punion(irs[1:2],irs[4:5]) #element-wise (aka "parallel") union

## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]          9        44        36
## [2]          1        60        60

pintersect(irs[1:2],irs[4:5])
```

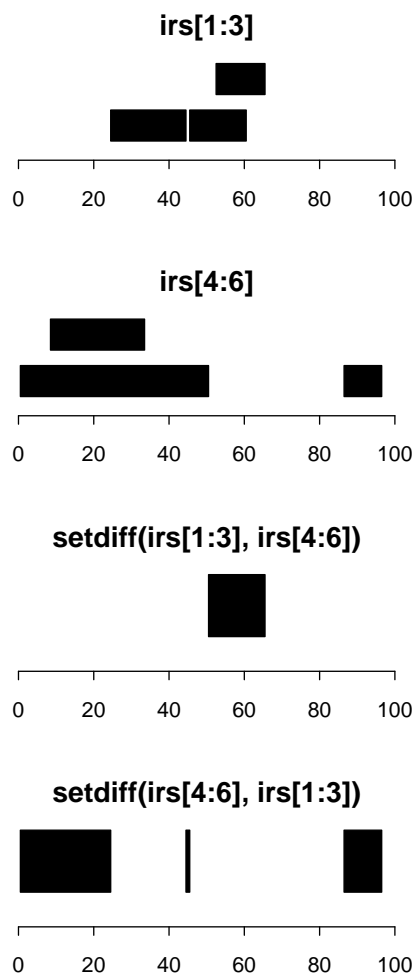


Figure 9: Asymetric differences with `setdiff` on `IRanges`

```
## IRanges object with 2 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      25      33         9
## [2]      46      50         5

psetdiff(irs[1:3],irs[4:6]) # asymmetric! difference

## IRanges object with 3 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      34      44        11
## [2]      51      60        10
## [3]      53      65        13

pgap(irs[1:3],irs[4:6])

## IRanges object with 3 ranges and 0 metadata columns:
##      start      end      width
```



```
##      <integer> <integer> <integer>
## [1]      34      33      0
## [2]      51      50      0
## [3]      66      86     21
```

3.2.4 Nearest methods

See `help('nearest-methods', package="IRanges")` for details and examples:

```
nearest(irs[4:6],irs[1:3])
## [1] 1 1 3
distance(irs[4:6],irs[1:3])
## [1] 0 0 21
```

Other examples are provided in paragraph [3.5.4](#)

Between ranges operations.

These are mainly methods to find overlaps between ranges. These functions are exemplified below in paragraph [3.5.5](#). See `help('findOverlaps-methods', package="IRanges")` for details.

3.3 Rle

[Run-length encoding](#) is a data compression method highly adapted to long vectors containing repeated values (e.g. coverage on a whole chromosome). For example, the sequence $\{1, 1, 1, 2, 3, 3\}$ can be represented as $values = \{1, 2, 3\}$ and the paired $runlengths = \{3, 1, 2\}$. In *IRanges*, the *Rle* class is used to represent run-length encoded (compressed) atomic vectors.

Rle objects:

```
set.seed(123)
lambda = c(rep(0.001, 3500), seq(0.001, 10, length = 500), #From IRanges vignette
           seq(10, 0.001, length = 500))
xRle=Rle(rpois(1e4,lambda))
yRle=Rle(rpois(1e4, lambda[c(251:length(lambda), 1:250)]))
xRle

## integer-Rle of length 10000 with 1630 runs
## Lengths: 326 1 1150 1 772 1 ... 1 170 1 466 1 262
## Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0
yRle
```

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```
## integer-Rle of length 10000 with 1616 runs
##   Lengths: 1984   1 1268   1  15   1 ...   1   1   1   8   1 1264
##   Values :    0    1    0    1    0   1 ...   1   0   1   0   1    0

as.vector(object.size(xRle)/object.size(as.vector(xRle))) #Gain of memory

## [1] 0.3532468

head(runValue(xRle))

## [1] 0 1 0 1 0 1

head(runLength(xRle))

## [1] 326   1 1150   1  772   1

head(start(xRle)) #starts of the runs

## [1]    1  327  328 1478 1479 2251

head(end(xRle)) #ends of the runs

## [1] 326  327 1477 1478 2250 2251

nrun(xRle) #number of runs

## [1] 1630

findRun(as.integer(c(100,200,300,1200)),xRle)

## [1] 1 1 1 3

coverage(irs)

## integer-Rle of length 96 with 11 runs
##   Lengths:  8 16  9 11  1  5  2  8  5 21 10
##   Values :  1  2  3  2  1  2  1  2  1  0  1
```

These objects support a number of basic methods associated with R atomic vectors:

```
xRle+yRle

## integer-Rle of length 10000 with 2111 runs
##   Lengths: 326   1 1150   1  506   1 ...   1 170   1 466   1 262
##   Values :    0    1    0    1    0   1 ...   1   0   1   0   1   0

xRle>0

## logical-Rle of length 10000 with 211 runs
##   Lengths:  326   1 1150   1  772 ... 170   1 466   1 262
##   Values : FALSE  TRUE FALSE  TRUE FALSE ... FALSE  TRUE FALSE  TRUE FALSE

xRle>yRle

## logical-Rle of length 10000 with 367 runs
##   Lengths:  326   1 1150   1  772 ... 170   1 466   1 262
##   Values : FALSE  TRUE FALSE  TRUE FALSE ... FALSE  TRUE FALSE  TRUE FALSE

max(xRle)

## [1] 18

summary(xRle)
```

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```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 0.0000  0.0000  0.0000  0.9932  0.0000 18.0000

sqrt(xRle)

## numeric-Rle of length 10000 with 1630 runs
## Lengths:          326          1 ...          262
## Values :           0          1 ...           0

rev(xRle)

## integer-Rle of length 10000 with 1630 runs
## Lengths: 262   1 466   1 170   1 ...   1 772   1 1150   1 326
## Values :   0   1   0   1   0   1 ...   1   0   1   0   1   0

table(xRle)

## xRle
##   0   1   2   3   4   5   6   7   8   9  10  11  12  13  14
## 8200 201 198 202 212 184 167 146 140 101  89  49  51  25  12
##  15  16  17  18
##   6   7   8   2

union(xRle,yRle)

## integer-Rle of length 20 with 20 runs
## Lengths: 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## Values : 0 1 3 2 4 5 6 7 8 9 11 12 10 13 17 14 16 15 18 22

cor(xRle,yRle)

## [1] 0.5690394
```

See `?'Rle-class'` and `?'Rle-utils'` for other methods.

There are useful functions to perform fixed-width running window summaries:

```
runmean(xRle,k=100) # See '?Rle-runstat' for other examples

## numeric-Rle of length 9901 with 1852 runs
## Lengths: 227 100 1051 100 673 100 ... 100 71 100 367 100 163
## Values :   0 0.01   0 0.01   0 0.01 ... 0.01   0 0.01   0 0.01   0

#same result, more flexible but much slower:
Rle(aggregate(xRle, start = 1:(length(xRle)-99), width = 100, FUN = mean))

## numeric-Rle of length 9901 with 1852 runs
## Lengths: 227 100 1051 100 673 100 ... 100 71 100 367 100 163
## Values :   0 0.01   0 0.01   0 0.01 ... 0.01   0 0.01   0 0.01   0

runq(xRle,k=100,i=10) #10th smallest value in windows of 100

## integer-Rle of length 9901 with 41 runs
## Lengths: 3558   7   2   6   4 102 ...   2   2  80  59  53 1090
## Values :   0   1   0   1   0   1 ...   3   4   3   2   1   0
```

One typical application of *Rle* objects is to store the coverage of NGS reads along a chromosome. The coverage for all chromosomes can be stored in an *RleList* (see `?AtomicList` for details) on which most functions defined for *Rle* objects would also work.

Practically, any variable defined along a genome can be represented as

- an *RleList* with one *Rle* for each chromosome
- the mcols (metadata columns) of a *GRanges* object (see 3.5 below)

Sometimes, it is desirable to manipulate several of these variables in the same object (e.g. for plotting with *Gviz*). The `?genomicvars` help page provides useful functions such as `bindAsGRanges` and `mcolAsRleList` to go from one representation to the other.

3.4 Views

As already mentioned, *Views* objects are used to store a sequence (a *Vector* object called the "subject") together with a set of ranges which define *views* onto the sequence. Specific subclasses exist for different classes of "subject" *Vectors*, such as *RleViews* from the *IRanges* package and *XStringViews* from the *Biostrings* package.

An *RleViews* object stores an *Rle* subject and its *views*:

```
coverage(irs)

## integer-Rle of length 96 with 11 runs
##   Lengths:  8 16  9 11  1  5  2  8  5 21 10
##   Values  :  1  2  3  2  1  2  1  2  1  0  1

irs_views=Views(coverage(irs),start=c(-5,10,20,90),end=c(10,30,50,100))
irs_views #Views can be out of bound

## Views on a 96-length Rle subject
##
## views:
##   start end width
## [1]   -5  10   16 [1 1 1 1 1 1 1 1 2 2 ...]
## [2]   10  30   21 [2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3]
## [3]   20  50   31 [2 2 2 2 2 3 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2 ...]
## [4]   90 100   11 [1 1 1 1 1 1 1 ...]

try(irs_views[[1]]) #but can't be extracted
irs_views[[2]]

## integer-Rle of length 21 with 2 runs
##   Lengths: 15  6
##   Values  :  2  3

start(irs_views)

## [1] -5 10 20 90
```

See `'RleViews-class'` for details.

Other ways to create Views:

```
Views(coverage(irs),irs[c(1,2,6)]) #use an IRanges to extract Views

## Views on a 96-length Rle subject
##
## views:
##      start end width
## [1]    25  44    20 [3 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2 2]
## [2]    46  60    15 [2 2 2 2 2 1 1 2 2 2 2 2 2 2 2]
## [3]    87  96    10 [1 1 1 1 1 1 1 1 1 1]

Views(coverage(irs),coverage(irs)>=1) #or a logical Rle

## Views on a 96-length Rle subject
##
## views:
##      start end width
## [1]     1  65    65 [1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 3 ...]
## [2]    87  96    10 [1 1 1 1 1 1 1 1 1 1]

slice(coverage(irs),3) #use slice

## Views on a 96-length Rle subject
##
## views:
##      start end width
## [1]    25  33     9 [3 3 3 3 3 3 3 3 3]

successiveViews(coverage(irs),width=rep(20,4)) #get successive Views

## Views on a 96-length Rle subject
##
## views:
##      start end width
## [1]     1  20    20 [1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2]
## [2]    21  40    20 [2 2 2 2 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2]
## [3]    41  60    20 [2 2 2 2 1 2 2 2 2 2 1 1 2 2 2 2 2 2 2]
## [4]    61  80    20 [1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0]
```

XStringViews is used to store views on an *XString* object:

```
dmup_views=Views(dm3_upstream[[1]],irs[1:2])
dmup_views

## Views on a 2000-letter DNAString subject
## subject: GTTGGTGGCCACCAGTGCCAAAATACACAAGA...CGTATAAAAGGCAAGTTACCGTTGCACGGT
## views:
##      start end width
## [1]    25  44    20 [TACACAAGAAGAAGAAACAG]
## [2]    46  60    15 [ATCTTGACACTAAAA]

nchar(dmup_views)

## [1] 20 15

toString(dmup_views) #see '?XStringViews-class' for other methods
```

```
## [1] "TACACAAGAAGAAGAAACAG, ATCTTGACACTAAAA"
```

Furthermore there is a *ViewsList* virtual class. Its specialized subclass *RleViewsList* is useful to store coverage vectors along with their specific views over a set of chromosomes.

```
xyRleList=RleList(xRle,yRle)
xyRleList_views=Views(xyRleList,
                      IRangesList(IRanges(start=c(3700,4000),width=20),
                                   IRanges(yRle>17)+2))

xyRleList_views[[1]]

## Views on a 10000-length Rle subject
##
## views:
##   start end width
## [1] 3700 3719    20 [1 0 2 7 2 9 5 3 3 6 4 4 3 7 5 5 3 5 8 1]
## [2] 4000 4019    20 [ 9 13 13 8 7 14 3 10 16 8 17 5 8 8 15 17 ...]

xyRleList_views[[2]]

## Views on a 10000-length Rle subject
##
## views:
##   start end width
## [1] 3750 3754     5 [10 13 18 12 14]
## [2] 8250 8254     5 [12 8 22 10 9]

width(xyRleList_views)

## IntegerList of length 2
## [[1]] 20 20
## [[2]] 5 5
```

Specific functions are provided for fast looping over *Views* and *ViewsList* objects:

```
viewMins(irs_views) #same as min(irs_views)

## [1] 1 2 1 1

viewSums(irs_views) #same as sum(irs_views)

## [1] 12 48 70 7

viewWhichMaxs(irs_views) #get the (first) coordinate of viewMaxs (which.max also works)

## [1] 9 25 25 90

viewRangeMins(irs_views) #get the (first) range of viewMins

## IRanges object with 4 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]      1       8       8
## [2]      9      24      16
## [3]     45     45       1
## [4]     87     96      10
```

```
viewApply(irs_views,sd)
## [1] 0.4216370 0.4629100 0.5143113 0.0000000

viewMeans(xyRleList_views)
## NumericList of length 2
## [[1]] 4.15 10.35
## [[2]] 13.4 12.2
```

Note that the `min`, `max`, `sum`, `mean`, `which.min` and `which.max` functions now work on *Views* (but not on *RleViewsList* yet). The corresponding `view*` functions might be deprecated in the future. See `?view-summarization-methods` for details.

3.5 GenomicRanges

3.5.1 GRanges objects

Here, we will present some of the classes and functions defined in the *GenomicRanges* package. This package is central to most *Bioconductor* users who analyze NGS data, so you should consider reading thoroughly the excellent vignettes associated with this package.

The main class defined in *GenomicRanges* is the *GRanges* class which acts as a container for genomic locations and their associated annotations (see `?GRanges`).

GRanges (and *GRangesList*) build on *IRanges* (and *IRangesList* respectively) with the following specificities:

- The informations on `seqnames` (typically chromosomes) and `strand` is stored along with the information on ranges (SEW)
- An optional `seqinfo` slot contains information on the sequences: names, length (`seqlengths`), circularity and genome
- Optional `metadata` columns (`mcols`) containing additional informations on each range (e.g. score, GC content, etc.) which are stored as a *DataFrame*

By convention, in *Bioconductor* genomic coordinates:

- are 1-based
- are *left-most*, i.e. 'start' of ranges on the minus strand are the left-most coordinate, rather than the 5' coordinate
- represent closed intervals, i.e. the intervals contain start and end coordinates

The `GRanges` function can be used to create a *GRanges* object:

```
genes = GRanges(seqnames=c("chr2L", "chrX"),
                ranges=IRanges(start=c(7529, 18962306),
                              end =c(9484, 18962925),
                              names=c("FBgn0031208", "FBgn0085359")),
                strand=c("+", "-"),
                seqlengths=c(chr2L=23011544L, chrX=22422827L))
```

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```
slotNames(genes)

## [1] "seqnames"      "ranges"        "strand"        "elementMetadata"
## [5] "seqinfo"       "metadata"

mcols(genes) = DataFrame(EntrezId=c("33155", "2768869"),
                          Symbol=c("CG11023", "CG34330"))
genome(genes)="dm3" #see ?seqinfo for details
genes

## GRanges object with 2 ranges and 2 metadata columns:
##           seqnames      ranges strand |   EntrezId   Symbol
##           <Rle>        <IRanges> <Rle> | <character> <character>
## FBgn0031208 chr2L [ 7529,   9484]   + |    33155    CG11023
## FBgn0085359 chrX [18962306, 18962925] - |   2768869    CG34330
## -----
## seqinfo: 2 sequences from dm3 genome
```

The *GRanges* accessors include *IRanges* accessors and others:

```
width(genes)

## [1] 1956 620

names(genes)

## [1] "FBgn0031208" "FBgn0085359"

seqnames(genes)

## factor-Rle of length 2 with 2 runs
##   Lengths:    1    1
##   Values : chr2L chrX
## Levels(2): chr2L chrX

strand(genes)

## factor-Rle of length 2 with 2 runs
##   Lengths: 1 1
##   Values : + -
## Levels(3): + - *

ranges(genes)

## IRanges object with 2 ranges and 0 metadata columns:
##           start      end      width
##           <integer> <integer> <integer>
## FBgn0031208    7529    9484    1956
## FBgn0085359 18962306 18962925    620

genes$Symbol

## [1] "CG11023" "CG34330"

mcols(genes)

## DataFrame with 2 rows and 2 columns
##      EntrezId      Symbol
```



```
## <character> <character>
## 1      33155      CG11023
## 2      2768869    CG34330

seqinfo(genes)

## Seqinfo object with 2 sequences from dm3 genome:
##   seqnames seqlengths isCircular genome
##   chr2L      23011544      NA      dm3
##   chrX       22422827      NA      dm3

seqlevels(genes)

## [1] "chr2L" "chrX"
```

3.5.2 GRangesList

As for *IRangesList*, there is a *GRangesList* class which allows to store *GRanges* in a list-type object. This is typically used to store e.g. *GRanges* of exons arranged by transcripts or genes or *GRanges* of transcripts arranged by genes. The vignette of the [GenomicRanges](#) package provides a good example of 2 transcripts, one of which has 2 exons:

```
gr1 = GRanges(seqnames = "chr2", ranges = IRanges(3, 6),
              strand = "+", score = 5L, GC = 0.45)
gr2 = GRanges(seqnames = c("chr1", "chr1"),
              ranges = IRanges(c(7,13), width = 3),
              strand = c("+", "-"), score = 3:4, GC = c(0.3, 0.5))
grl = GRangesList("txA" = gr1, "txB" = gr2)
grl

## GRangesList object of length 2:
## $txA
## GRanges object with 1 range and 2 metadata columns:
##      seqnames  ranges strand |      score      GC
##      <Rle> <IRanges> <Rle> | <integer> <numeric>
## [1]   chr2    [3, 6]    + |         5      0.45
##
## $txB
## GRanges object with 2 ranges and 2 metadata columns:
##      seqnames  ranges strand | score GC
## [1]   chr1 [ 7,  9]    + |    3 0.3
## [2]   chr1 [13, 15]    - |    4 0.5
##
## -----
## seqinfo: 2 sequences from an unspecified genome; no seqlengths

length(grl)

## [1] 2

elementNROWS(grl)

## txA txB
##  1  2
```

```
grl["txB", "GC"]

## GRangesList object of length 1:
## $txB
## GRanges object with 2 ranges and 1 metadata column:
##      seqnames      ranges strand |      GC
##      <Rle> <IRanges> <Rle> | <numeric>
## [1]   chr1 [ 7,  9]      + |      0.3
## [2]   chr1 [13, 15]      - |      0.5
##
## -----
## seqinfo: 2 sequences from an unspecified genome; no seqlengths

unlist(grl)

## GRanges object with 3 ranges and 2 metadata columns:
##      seqnames      ranges strand |      score      GC
##      <Rle> <IRanges> <Rle> | <integer> <numeric>
## txA   chr2 [ 3,  6]      + |          5      0.45
## txB   chr1 [ 7,  9]      + |          3      0.3
## txB   chr1 [13, 15]      - |          4      0.5
##
## -----
## seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

Please refer to the [vignette](#) for further examples.

Most accessors and functions defined for *GRanges* also work on *GRangesList*. However, note that `mcols(grl)` now refers to metadata at the list level rather than at level of the individual *GRanges* objects.

3.5.3 Annotations as GenomicRanges: TxDb* packages

Here, we will briefly present the *Bioconductor* annotation packages which provide genome-wide annotations directly as *GRanges* and *GRangesList* objects. These packages, called *TxDb** can be built using the [GenomicFeatures](#) package.

Here, we show how to extract information from the [TxDb.Dmelanogaster.UCSC.dm3.ensGene](#) package.

A *GRanges* object with the genomic coordinates for the genes can be obtained using:

```
Dmg=genes(TxDb.Dmelanogaster.UCSC.dm3.ensGene, single.strand.genes.only=T)
Dmg

## GRanges object with 15682 ranges and 1 metadata column:
##      seqnames      ranges strand |      gene_id
##      <Rle> <IRanges> <Rle> | <character>
## FBgn0000003 chr3R [ 2648220, 2648518]      + | FBgn0000003
## FBgn0000008 chr2R [18024494, 18060346]      + | FBgn0000008
## FBgn0000014 chr3R [12632936, 12655767]      - | FBgn0000014
## FBgn0000015 chr3R [12752932, 12797958]      - | FBgn0000015
## FBgn0000017 chr3L [16615470, 16640982]      - | FBgn0000017
## ...      ...      ...      ...      ...
## FBgn0264723 chr3L [12238610, 12239148]      - | FBgn0264723
## FBgn0264724 chr3L [15327882, 15329271]      + | FBgn0264724
```

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```
## FBgn0264725 chr3L [12025657, 12026099] + | FBgn0264725
## FBgn0264726 chr3L [12020901, 12021253] + | FBgn0264726
## FBgn0264727 chr3L [22065469, 22065720] + | FBgn0264727
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

Other functions, such as `transcripts`, `exons`, `cds`, `promoters`, `microRNAs` and `tRNAs` allow to extract the corresponding genomic features.

A *GRangesList* object with the coordinates of the transcripts arranged by genes can be obtained using:

```
Dmt=transcriptsBy(TxDb.Dmelanogaster.UCSC.dm3.ensGene,by="gene")
Dmt

## GRangesList object of length 15682:
## $FBgn0000003
## GRanges object with 1 range and 2 metadata columns:
##      seqnames      ranges strand | tx_id tx_name
##      <Rle>         <IRanges> <Rle> | <integer> <character>
## [1] chr3R [2648220, 2648518] + | 17345 FBtr0081624
##
## $FBgn0000008
## GRanges object with 3 ranges and 2 metadata columns:
##      seqnames      ranges strand | tx_id tx_name
## [1] chr2R [18024494, 18060339] + | 7681 FBtr0100521
## [2] chr2R [18024496, 18060346] + | 7682 FBtr0071763
## [3] chr2R [18024938, 18060346] + | 7683 FBtr0071764
##
## $FBgn0000014
## GRanges object with 4 ranges and 2 metadata columns:
##      seqnames      ranges strand | tx_id tx_name
## [1] chr3R [12632936, 12655767] - | 21863 FBtr0306337
## [2] chr3R [12633349, 12653845] - | 21864 FBtr0083388
## [3] chr3R [12633349, 12655300] - | 21865 FBtr0083387
## [4] chr3R [12633349, 12655474] - | 21866 FBtr0300485
##
## ...
## <15679 more elements>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

The functions `exonsBy`, `cdsBy`, `intronsByTranscript`, `fiveUTRsByTranscript`, `threeUTRsByTranscript` allow to extract the corresponding genomic features arranged in a *GRangesList* object.

Other functions including `transcriptsByOverlaps` and `exonsByOverlaps` allow to extract genomic features for genomic locations specified by a *GRanges* object:

```
exonsByOverlaps(TxDb.Dmelanogaster.UCSC.dm3.ensGene, genes)

## GRanges object with 6 ranges and 1 metadata column:
##      seqnames      ranges strand |  exon_id
##      <Rle>        <IRanges> <Rle> | <integer>
## [1] chr2L [ 7529, 8116] + | 1
## [2] chr2L [ 8193, 8589] + | 2
## [3] chr2L [ 8193, 9484] + | 3
## [4] chr2L [ 8229, 9484] + | 4
## [5] chr2L [ 8668, 9484] + | 5
## [6] chrX [18962306, 18962925] - | 75268
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

When a *TxDb* package is paired with an appropriate *BSgenome* object, it is relatively straightforward to extract DNA sequences providing a *GRanges* or a *GRangesList*

```
getSeq(BSgenome.Dmelanogaster.UCSC.dm3, Dmg[1:2])

## A DNAStringSet instance of length 2
##      width seq                                     names
## [1] 299 TCGACTGGAAGGTTGGCAGCTT...GATATGGTTGGACCACAATCT FBgn00000003
## [2] 35853 CGCGGCGGTCGCATCGGAGTCG...TTTACCTGAAAAGCAATATAC FBgn00000008

Dmc=cdsBy(TxDb.Dmelanogaster.UCSC.dm3.ensGene, by="tx")
cds_seq=extractTranscriptSeqs(BSgenome.Dmelanogaster.UCSC.dm3, Dmc[1:2])
cds_seq

## A DNAStringSet instance of length 2
##      width seq                                     names
## [1] 855 ATGGGCGAGCGGGATCAGCCAC...GTATGGCAACGAATATATTGA 1
## [2] 1443 ATGGGCGAGCGGGATCAGCCAC...ATCGTCGACGGAGAGTTGTGA 2

translate(cds_seq)

## A AAStringSet instance of length 2
##      width seq                                     names
## [1] 285 MGERDQPQSSERISIFNPPVYT...HDFNEITQDDKSTVWQRIY* 1
## [2] 481 MGERDQPQSSERISIFNPPVYT...QSEMLYFRKKMALEIVDGEL* 2
```

3.5.4 GRanges methods

Most if not all functions defined for *IRanges* objects are also defined for *GRanges* and *GRangesList* objects and they are generally *seqnames*- and *strand*-aware. These include:

- intra-range operations (`shift`, etc. ; see paragraph 3.2.1 and `help('intra-range-methods', "GenomicRanges")`)
- inter-range operations (`reduce`, etc. ; see paragraph 3.2.2 and `help('inter-range-methods', "GenomicRanges")`)
- set operations (see paragraph 3.2.3 and `help('setops-methods', "GenomicRanges")`)
- nearest methods (`nearest`, etc. ; see paragraph 3.2.4 and `help('nearest-methods', "GenomicRanges")`)

- between ranges ("overlaps") operations presented below in paragraph 3.5.5

Here, we briefly illustrate some of these functions:

```
genes

## GRanges object with 2 ranges and 2 metadata columns:
##           seqnames      ranges strand |   EntrezId   Symbol
##           <Rle>        <IRanges> <Rle> | <character> <character>
##   FBgn0031208   chr2L [ 7529,    9484]   + |    33155   CG11023
##   FBgn0085359   chrX [18962306, 18962925] - |    2768869   CG34330
##   -----
##   seqinfo: 2 sequences from dm3 genome

genes2=Dmg[c(1:2,21:22,36:37)]
genes2

## GRanges object with 6 ranges and 1 metadata column:
##           seqnames      ranges strand |   gene_id
##           <Rle>        <IRanges> <Rle> | <character>
##   FBgn0000003   chr3R [ 2648220,  2648518]   + | FBgn0000003
##   FBgn0000008   chr2R [18024494, 18060346]   + | FBgn0000008
##   FBgn0000052   chr2L [ 6041178,  6045970]   - | FBgn0000052
##   FBgn0000053   chr2L [ 7014861,  7023940]   - | FBgn0000053
##   FBgn0000084   chrX [20065478, 20067552]   - | FBgn0000084
##   FBgn0000092   chrX [ 2586765,  2587919]   - | FBgn0000092
##   -----
##   seqinfo: 15 sequences (1 circular) from dm3 genome

sort(genes2)

## GRanges object with 6 ranges and 1 metadata column:
##           seqnames      ranges strand |   gene_id
##           <Rle>        <IRanges> <Rle> | <character>
##   FBgn0000052   chr2L [ 6041178,  6045970]   - | FBgn0000052
##   FBgn0000053   chr2L [ 7014861,  7023940]   - | FBgn0000053
##   FBgn0000008   chr2R [18024494, 18060346]   + | FBgn0000008
##   FBgn0000003   chr3R [ 2648220,  2648518]   + | FBgn0000003
##   FBgn0000092   chrX [ 2586765,  2587919]   - | FBgn0000092
##   FBgn0000084   chrX [20065478, 20067552]   - | FBgn0000084
##   -----
##   seqinfo: 15 sequences (1 circular) from dm3 genome

c(genes,genes2,ignore.mcols=T) #combine

## GRanges object with 8 ranges and 0 metadata columns:
##           seqnames      ranges strand
##           <Rle>        <IRanges> <Rle>
##   FBgn0031208   chr2L [ 7529,    9484]   +
##   FBgn0085359   chrX [18962306, 18962925] -
##   FBgn0000003   chr3R [ 2648220,  2648518] +
##   FBgn0000008   chr2R [18024494, 18060346] +
##   FBgn0000052   chr2L [ 6041178,  6045970] -
##   FBgn0000053   chr2L [ 7014861,  7023940] -
##   FBgn0000084   chrX [20065478, 20067552] -
##   FBgn0000092   chrX [ 2586765,  2587919] -
```

```
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome

intersect(genes,c(genes2,Dmg['FBgn0031208'])) #set operations

## GRanges object with 1 range and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>      <IRanges> <Rle>
## [1]   chr2L [7529, 9484]      +
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome

nearest(genes,genes2) #nearest-methods

## [1] NA 5

nearest(genes,genes2,ignore.strand=T) #strand-aware by default

## [1] 3 5

precede(genes,genes2,ignore.strand=T)

## [1] 3 5

promoters(genes,upstream=200,downstream=1) #intra-range operations

## GRanges object with 2 ranges and 2 metadata columns:
##      seqnames      ranges strand |   EntrezId   Symbol
##      <Rle>      <IRanges> <Rle> | <character> <character>
## FBgn0031208   chr2L [ 7329, 7529]   + |    33155    CG11023
## FBgn0085359   chrX [18962925, 18963125] - |    2768869    CG34330
## -----
## seqinfo: 2 sequences from dm3 genome

reduce(Dmt[[2]]) #inter-range operations

## GRanges object with 1 range and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>      <IRanges> <Rle>
## [1]   chr2R [18024494, 18060346]      +
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

See also `help('GenomicRanges-comparison', "GenomicRanges")` for other functions for comparing *GenomicRanges*.

3.5.5 Overlaps between ranges

A very common task on genomic ranges is to search for overlaps between sets of genomic ranges which corresponds to an operation between ranges. These functions are defined for several classes of objects, including *IRanges*, *GRanges* and their list-type counterparts *IRangesList* and *GRangesList* but also *Views* and *ViewsList* among others. Details on function definitions can be found using `?findOverlaps-methods`.

As first example, let's count how many of the transcription start sites (TSS) in the *Drosophila melanogaster* genome are located at more than 500bp from another gene:

```
Dm_tss=unlist(reduce(promoters(Dmt,up=0,down=1))) #get all TSS
cov_tss_g500=countOverlaps(Dm_tss,Dmg+500) #strand-aware!

## Warning in valid.GenomicRanges.seqinfo(x, suggest.trim = TRUE): GRanges object
## contains 3 out-of-bound ranges located on sequences
## chrYHet, chr3R, and chr3L. Note that only ranges located on a
## non-circular sequence whose length is not NA can be considered
## out-of-bound (use seqlengths() and isCircular() to get the lengths
## and circularity flags of the underlying sequences). You can use
## trim() to trim these ranges. See ?trim,GenomicRanges-method for
## more information.

table(cov_tss_g500)

## cov_tss_g500
##      1      2      3      4      5      6      7      8      9
## 15586  4488   490   116    65    47    18     6     3

sum(cov_tss_g500>1)

## [1] 5233

cov_tss_g500_bs=countOverlaps(Dm_tss,Dmg+500,ignore.strand=T) #both strands

## Warning in valid.GenomicRanges.seqinfo(x, suggest.trim = TRUE): GRanges object
## contains 3 out-of-bound ranges located on sequences
## chrYHet, chr3R, and chr3L. Note that only ranges located on a
## non-circular sequence whose length is not NA can be considered
## out-of-bound (use seqlengths() and isCircular() to get the lengths
## and circularity flags of the underlying sequences). You can use
## trim() to trim these ranges. See ?trim,GenomicRanges-method for
## more information.

sum(cov_tss_g500_bs>1)

## [1] 10768
```

Getting the corresponding overlaps with `findOverlaps`:

```
fov_tss_g500_bs=findOverlaps(Dm_tss,Dmg+500,ignore.strand=T)

## Warning in valid.GenomicRanges.seqinfo(x, suggest.trim = TRUE): GRanges object
## contains 3 out-of-bound ranges located on sequences
## chrYHet, chr3R, and chr3L. Note that only ranges located on a
## non-circular sequence whose length is not NA can be considered
## out-of-bound (use seqlengths() and isCircular() to get the lengths
## and circularity flags of the underlying sequences). You can use
## trim() to trim these ranges. See ?trim,GenomicRanges-method for
## more information.

Dmg[c(1,1383)]

## GRanges object with 2 ranges and 1 metadata column:
##           seqnames           ranges strand |   gene_id
##           <Rle>           <IRanges> <Rle> | <character>
## FBgn00000003 chr3R [2648220, 2648518]   + | FBgn00000003
## FBgn0011904  chr3R [2648685, 2648757]   - | FBgn0011904
```

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```
## -----  
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

Now, imagine we have a set of 10K NGS reads:

```
set.seed(0)  
randomreads2L=GRanges(seqnames="chr2L",  
                      ranges=IRanges(start=floor(runif(10000,5000,50000)),width=100),  
                      strand="*")
```

And we want to select only the reads overlapping with those two genes:

```
sort(Dmg)[1:2]  
  
## GRanges object with 2 ranges and 1 metadata column:  
##           seqnames      ranges strand |   gene_id  
##           <Rle>        <IRanges> <Rle> | <character>  
## FBgn0031208 chr2L [ 7529,  9484]      + | FBgn0031208  
## FBgn0263584 chr2L [21952, 24237]      + | FBgn0263584  
## -----  
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

We could use:

```
subsetByOverlaps(randomreads2L,sort(Dmg)[1:2])  
  
## GRanges object with 1017 ranges and 0 metadata columns:  
##           seqnames      ranges strand  
##           <Rle>        <IRanges> <Rle>  
## [1] chr2L [ 7780,  7879]      *  
## [2] chr2L [22284, 22383]      *  
## [3] chr2L [22101, 22200]      *  
## [4] chr2L [22375, 22474]      *  
## [5] chr2L [22207, 22306]      *  
## ...      ...              ...      ...  
## [1013] chr2L [22674, 22773]      *  
## [1014] chr2L [23550, 23649]      *  
## [1015] chr2L [22940, 23039]      *  
## [1016] chr2L [22316, 22415]      *  
## [1017] chr2L [ 8623,  8722]      *  
## -----  
## seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

or:

```
randomreads2L[overlapsAny(randomreads2L,sort(Dmg)[1:2])]  
  
## GRanges object with 1017 ranges and 0 metadata columns:  
##           seqnames      ranges strand  
##           <Rle>        <IRanges> <Rle>  
## [1] chr2L [ 7780,  7879]      *  
## [2] chr2L [22284, 22383]      *  
## [3] chr2L [22101, 22200]      *  
## [4] chr2L [22375, 22474]      *
```



```
##      [5] chr2L [22207, 22306] *
##      ...      ...      ...
## [1013] chr2L [22674, 22773] *
## [1014] chr2L [23550, 23649] *
## [1015] chr2L [22940, 23039] *
## [1016] chr2L [22316, 22415] *
## [1017] chr2L [ 8623,  8722] *
## -----
## seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

To count the number of reads overlapping with those 2 genes we could use:

```
assays(summarizeOverlaps(sort(Dmg)[1:2], randomreads2L, mode="Union"))$counts
##              reads
## FBgn0031208    465
## FBgn0263584    552
```

Further examples of counting reads are provided in paragraph 5.6.

3.6 RangedData

The *RangedData* class is defined in the *IRanges* package. It contains genomic coordinates along with some values (typically a numerical variable) defined on these ranges. In practice, metadata (*mcols*) of a *GRanges* object can contain such data and the user would rather work with *GRanges*. However, we mention here the *RangedData* class because it is a more general data structure used in some packages (see for example the *rtracklayer* vignette).

4 Working with FASTQ files

In this paragraph, we present some functions from the *ShortRead* package which is primarily dedicated to manipulating and analyzing raw (not mapped) NGS reads contained in FASTQ files [13].

4.1 FASTQ format

During the sequencing process, raw data are generated (some sort of physical measurements) and processed (image analysis, signal processing, etc.) resulting in sequences and quality informations which are typically presented in FASTQ files. The *FASTQ format* contains both sequence and quality informations ("FASTQ = FASTa + Quality"). Both sequences and *Phred quality scores* are encoded using *ASCII* printable characters. In a FASTQ file, the information for each read occupies 4 lines:

@read01087	<i>read name</i>
GCCTTCTGTACGTTTTTGCTGTGAGT	<i>read sequence</i>
+	<i>separation line</i>
GGGGBB@DB?+>B?B>EE?:E#####	<i>read quality</i>

4.2 Reading FASTQ files

The simplest function to import a FASTQ files in *R* is:

```
fql_path=system.file(package="ShortRead", "extdata", "E-MTAB-1147",
                      "ERR127302_1_subset.fastq.gz")
# A FASTQ file containing 20K reads
myFastq=readFastq(fql_path)
```

The resulting object can be explored using:

```
myFastq
## class: ShortReadQ
## length: 20000 reads; width: 72 cycles

myFastq[1:5]
## class: ShortReadQ
## length: 5 reads; width: 72 cycles

head(sread(myFastq),3)
## A DNASTringSet instance of length 3
## width seq
## [1] 72 GTCTGCTGTATCTGTGTCGGCTGTCTCGCGGG...TCAATGAAGGCCTGGAATGTCACTACCCCCAG
## [2] 72 CTAGGGCAATCTTTGCAGCAATGAATGCCAAT...AGTGGCTTTTGAGGCCAGAGCAGACCTTCGGG
## [3] 72 TGGGCTGTTCTTCTCACTGTGGCCTGACTAA...GGCATTAGAAAGAGTCACGTTTCCAAGTCT

head(quality(myFastq),3)
## class: FastqQuality
## quality:
## A BStringSet instance of length 3
## width seq
## [1] 72 HHHHHHHHHHHHHHHHHHHHEBDBB?B:BBGG...BFEFBDBD@DDECEE3>:?:@@@>?=BAB?##
## [2] 72 IIIIHIIIGIIIIIIHIIIIIEGBGHIIIIHG...IIHIIHIIIIIGIIIEGIIGBGE@DDGGGIG
## [3] 72 GGBHGBGGGHHHHHHHHHHHFGHHHHHH...HHHHHHHGHFHHHHHHHHHHHHHHH8AGDGGG>

head(id(myFastq),3)
## A BStringSet instance of length 3
## width seq
## [1] 53 ERR127302.8493430 HWI-EAS350_0441:1:34:16191:2123#0/1
## [2] 53 ERR127302.21406531 HWI-EAS350_0441:1:88:9330:2587#0/1
## [3] 55 ERR127302.22173106 HWI-EAS350_0441:1:91:10434:14757#0/1

encoding(quality(myFastq))
## ! " # $ % & ' ( ) * + , - . / 0 1 2 3 4 5 6 7 8 9
## 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
```

```
## : ; < = > ? @ A B C D E F G H I J K L M N O P Q R
## 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49
## S T U V W X Y Z [ \ ] ^ _ ` a b c d e f g h i j k
## 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74
## l m n o p q r s t u v w x y z { | } ~
## 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93

alphabet(sread(myFastq))

## [1] "A" "C" "G" "T" "M" "R" "W" "S" "Y" "K" "V" "H" "D" "B" "N" "-" "+" "."
```

However, FASTQ files are often too large to be imported at once in R. The first solution, often used to perform QA/QC, is to obtain a random sample of the reads:

```
set.seed(123)
fqslk = FastqSampler(fq1_path, 1000)
reads_sample = yield(fqslk)
close(fqslk) #close connection
reads_sample

## class: ShortReadQ
## length: 1000 reads; width: 72 cycles
```

The default sample size is 1 million reads which fits easily into memory.

If we need to work on all the reads, we would process the FASTQ file in chunks. In this trivial example we count the number of reads in the FASTQ file:

```
nr_myFastq=0
strm <- FastqStreamer(fq1_path, 1000)
repeat {
  fq <- yield(strm)
  if (length(fq) == 0)
    break
  ## Get FASTQ chunk
  nr_myFastq=nr_myFastq+length(fq)
  ## Do something on the chunk
}
close(strm) #close the connection
nr_myFastq

## [1] 20000
```

4.3 Quality assessment on FASTQ files

One major reason to analyze FASTQ files is to evaluate the quality and potential biases associated with the sequencing process. Outside of R, the [Fastqc](#) tool is widely used to perform such an analysis. It uses a random sample drawn from the FASTQ file to obtain some statistics on the reads. One of its limitation is that it generates one report for each sample which does not simplify comparisons between the samples.

The [ShortRead](#) package provides efficient functions to generate an HTML QA report for multiple samples:

```
fqPath = system.file(package="ShortRead", "extdata", "E-MTAB-1147")
fqFiles = dir(fqPath, pattern="fastq.gz", full=TRUE)
coll = QACollate(QAFastqSource(fqFiles), QAReadQuality(),
                 QAAdapterContamination(), QANucleotideUse(),
                 QAQualityUse(), QASequenceUse(),
                 QAFrequentSequence(n=10), QANucleotideByCycle(),
                 QAQualityByCycle())
qa20nFastq = qa2(coll, BPPARAM=SerialParam(), verbose=FALSE)
## qa_report=report(qa20nFastq) #generate the report
## browseURL(qa_report) #display in your browser
slotNames(qa20nFastq)

## [1] "src"          "filtered"      "flagged"       "listData"
## [5] "elementType" "elementMetadata" "metadata"

names(qa20nFastq)

## [1] "QAReadQuality"      "QAAdapterContamination"
## [3] "QANucleotideUse"    "QAQualityUse"
## [5] "QASequenceUse"      "QAFrequentSequence"
## [7] "QANucleotideByCycle" "QAQualityByCycle"

slotNames(qa20nFastq[["QANucleotideUse"]])

## [1] "addFilter" "useFilter" "values"     "flag"       "html"

qa20nFastq[["QANucleotideUse"]]

## class: QANucleotideUse
## html template: /usr/lib/R/library/ShortRead/template.../QANucleotideUse.html
## useFilter: TRUE; addFilter: TRUE
```

As illustrated, individual results from the quality analysis can be extracted from the `.QA` object but this is not well documented yet, nor particularly easy.

An alternative is to use the `qa` function from which it is relatively easy to extract individual components and to generate separate plots such as Figure 10:

```
qa0nFastq = qa(fqPath, "fastq.gz", BPPARAM=SerialParam()) #collect statistics
qa0nFastq[["readCounts"]]

##               read filter aligned
## ERR127302_1_subset.fastq.gz 20000    NA    NA
## ERR127302_2_subset.fastq.gz 20000    NA    NA

readQuals <- qa0nFastq[["readQualityScore"]]
ggplot(readQuals, aes(x=quality, y=density, colour=lane))+
  geom_line(size=1)+
  scale_colour_discrete(name="Lane",
                        breaks=c("ERR127302_1_subset.fastq.gz",
                                "ERR127302_2_subset.fastq.gz"),
                        labels=c("ERR127302_1", "ERR127302_2"))
```

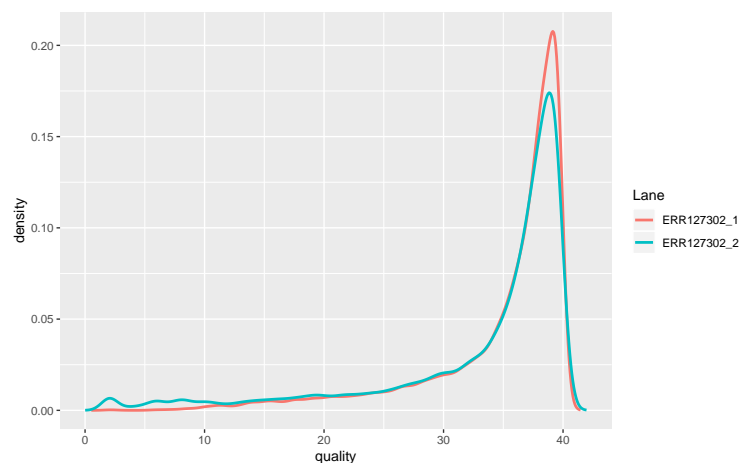


Figure 10: Distribution of average base quality

There are also other *Bioconductor* packages more or less dedicated to generate and plot QA/QC statistics from FASTQ files. These include the *seqTools*, *qrrc* and *Rqc* packages. The latter two packages make use of *ggplot2* graphics. A couple examples using *Rqc* are illustrated in Figure 11

```
rqcResultSet = rqcQA(fqFiles,sample=T)
rqcCycleQualityPlot(rqcResultSet[1])
rqcCycleBaseCallsLinePlot(rqcResultSet[2])
```

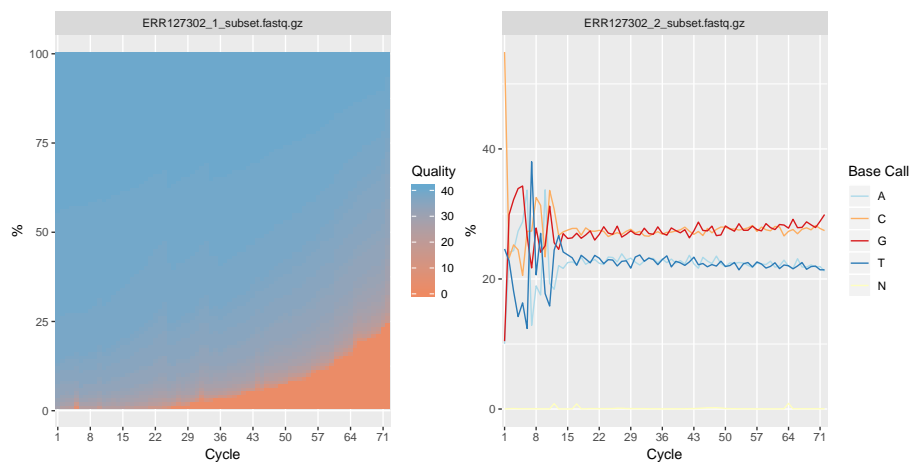


Figure 11: Examples of QC plots using the *Rqc* package

4.4 Reads filtering and trimming

Based on QA/QC it is sometimes advisable to perform read filtering and to trim the extremities of the reads. The *ShortRead* allows to perform such filtering and trimming steps with great flexibility. Note that the choices made below are only to illustrate the different functions, not to recommend some specific preprocessing in the general case.

First, we create some filters:

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```
max1N=nFilter(threshold=1L)
#Remove reads with more than 1N
goodq = srFilter(function(x){
  apply(as(quality(x),"matrix"),
    1,median,na.rm=T)>=30
  },
  name="MedianQualityAbove30")
#Custom filter: Remove reads with median quality<30
myFilter=compose(max1N,goodq) #combine filters
```

Then, we create a function to apply filters and trimming on chunks of a FASTQ file:

```
FilterAndTrim = function(fl,destination=sprintf("%s_filtered",fl))
{
  stream = FastqStreamer(fl)
  on.exit(close(stream))
  ## open input stream

  repeat {
    fq=yield(stream)
    if (length(fq)==0)
      break
    ## get fastq chunk

    ###TRIM
    fq = narrow(fq,start=5,end=70)
    ## trim the first 4 and the last 2 bases

    ####FILTER
    fq = fq[myFilter(fq)]
    ## remove reads that:
    ## contain more than 1 N
    ## have median quality < 30

    writeFastq(fq, destination, mode="a")
    ## Append to fastq file
  }
}
```

Finally we apply this function on a FASTQ file:

```
FilterAndTrim(fqFiles[1],
  destination=file.path(getwd(),"FilteredFastq.fastq"))
FilteredFastq=readFastq("FilteredFastq.fastq")
FilteredFastq
```

5 Working with SAM/BAM files

5.1 Tools for SAM/BAM files

When a reference genome sequence is available, the reads are generally mapped to this reference using one of the NGS read aligners available (see paragraph 2.4). Note that R provides an interface to [bowtie](#) and [SpliceMap](#) [14] aligners via the [Rbowtie](#) package which is used in the pipeline of the [QuasR](#) package. The [Rsubread](#) package also provides a relatively complete pipeline for NGS data analysis. It includes a specific aligner ([Subread](#)) based on an original "seed-and-vote" mapping algorithm [11] as well as tools to count ([featureCounts](#)) the reads within genomic features [15] and to map exon junctions from RNA-seq data.

Once aligned, the reads and their genomic coordinates (defined by chromosome, position and strand) are typically returned in a [SAM](#) file [16]. A [BAM](#) file contains the same information as the corresponding SAM file but BAM is a binary format and thus BAM file size is reduced and its content is not human-readable. These files are often manipulated with [Samtools](#), [Picard Tools](#) and [Python](#) scripts. A nice explanation of what SAM/BAM files contain can be found [here](#).

Here, we will mainly use the following libraries:

- [Rsamtools](#) which provides an interface to samtools, bcftools and tabix (see <https://github.com/samtools>)
- [GenomicAlignments](#) which provides efficient tools to manipulate short genomic alignments

Examples of BAM files from single- and paired-read sequencing are provided in the [passilaBamSubset](#).

5.2 Importing BAM files

5.2.1 Single-end reads

The path for the BAM file is obtained with:

```
sr_bamFile=untreated1_chr4() # from passilaBamSubset package
```

If the BAM file was not indexed yet (i.e. a .bai file present in the same directory and named as the .bam file), we could build such an index using:

```
indexBam(sr_bamFile)
```

Note that a number of other functions are available to manipulate SAM/BAM files, such as [asSam/asBam](#), [sortBam](#) or [mergeBam](#). See [?scanBam](#) for details.

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Now, we could define which regions of the genome we would like to import (*'which'* ; note that here only chr4 is available in the BAM file but for the example we try to extract data from chr2L also), which columns of the BAM file we would like to import (*'what'*) and possibly filters for unwanted reads (*'flag'*). These informations are stored in a *ScanBamParam* object:

```
which = RangesList("chr2L"=IRanges(7000,10000),
                  "chr4"=IRanges(c(75000,1190000),c(85000,1203000)))

scanBamWhat() #available fields

## [1] "qname"      "flag"      "rname"     "strand"    "pos"
## [6] "qwidth"    "mapq"     "cigar"     "mrnm"      "mpos"
## [11] "isize"     "seq"      "qual"     "groupid"   "mate_status"

what = c("rname", "strand", "pos", "qwidth", "seq")
flag=scanBamFlag(isDuplicate=FALSE)
param=ScanBamParam(which=which,what=what,flag=flag)
```

See `?ScanBamParam` for other options and examples. As briefly illustrated it is easy to define filters (based on [SAM flags](#) using `scanBamFlag` or on tags present in the SAM/BAM files) when importing your BAM files.

Now, we use `scanBam` from [Rsamtools](#) to import the data in R:

```
mysrbam=scanBam(sr_bamFile,param=param)
class(mysrbam)

## [1] "list"

names(mysrbam)

## [1] "chr2L:7000-10000"      "chr4:75000-85000"      "chr4:1190000-1203000"

sapply(mysrbam,sapply,length)["rname",] #number of imported reads

##      chr2L:7000-10000      chr4:75000-85000 chr4:1190000-1203000
##                0                304                736
```

The resulting object is a list which is not always easy to manipulate for downstream applications.

So we would rather use the `readGAlignments` from the [GenomicAlignments](#) package:

```
mysrbam2=readGAlignments(sr_bamFile,
                        param=ScanBamParam(which=which,
                                           what="seq",
                                           flag=flag))

mysrbam2[1:2]

## GAlignments object with 2 alignments and 1 metadata column:
##      seqnames strand      cigar  qwidth  start    end
##      <Rle>  <Rle>    <character> <integer> <integer> <integer>
## [1]   chr4      + 21M13615N50M55N4M      75    72990    86734
## [2]   chr4      - 4M13615N50M55N21M      75    73007    86751
```



```
##           width      njunc |           seq
##      <integer> <integer> |      <DNAStrngSet>
## [1]      13745          2 | AAAAAGTGA...CGTAGCCACA
## [2]      13745          2 | ATACCTGTGA...TGGACGGCTG
## -----
## seqinfo: 8 sequences from an unspecified genome
```

Note that we have redefined the `ScanBamParam`. This is because `readGAlignments` comes with predefined fields to import and we just need to add those extra fields we want to import in the `ScanBamParam` parameter object. Here we imported the sequences to show that they are imported as a `DNAStrngSet` but it is generally not necessary to keep these sequences once the reads have been mapped.

So we don't keep them for the next steps:

```
mysrbam2=readGAlignments(sr_bamFile,
                        param=ScanBamParam(which=which))
mysrbam2[1:2]

## GAlignments object with 2 alignments and 0 metadata columns:
##      seqnames strand      cigar      qwidth      start      end
##      <Rle>  <Rle>      <character> <integer> <integer> <integer>
## [1]   chr4      + 21M13615N50M55N4M      75      72990      86734
## [2]   chr4      - 4M13615N50M55N21M      75      73007      86751
##           width      njunc
##      <integer> <integer>
## [1]      13745          2
## [2]      13745          2
## -----
## seqinfo: 8 sequences from an unspecified genome
```

The object returned is a `GAlignments` (see `?'GAlignments-class'` for details and accessors).

These objects are highly similar to `GRanges`. They can be accessed with similar functions:

```
head(start(mysrbam2))
## [1] 72990 73007 73007 73007 73007 73007

head(width(mysrbam2))
## [1] 13745 13745 13745 13745 13745 13745

seqnames(mysrbam2)
## factor-Rle of length 1040 with 1 run
## Lengths: 1040
## Values : chr4
## Levels(8): chr2L chr2R chr3L chr3R chr4 chrM chrX chrYHet

cigar(mysrbam2)[1:3]
## [1] "21M13615N50M55N4M" "4M13615N50M55N21M" "4M13615N50M55N21M"

head(njunc(mysrbam2))
```

```
## [1] 2 2 2 2 2 2
```

They can be converted to *GRanges*:

```
granges(mysrbam2)[1:2]

## GRanges object with 2 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>        <IRanges> <Rle>
## [1]   chr4 [72990, 86734]      +
## [2]   chr4 [73007, 86751]      -
## -----
## seqinfo: 8 sequences from an unspecified genome
```

And one can easily access the details of each read alignment as *GRanges* organized in a *GRangesList* using:

```
grglist(mysrbam2)[[1]] #only first read shown element here

## GRanges object with 3 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>        <IRanges> <Rle>
## [1]   chr4 [72990, 73010]      +
## [2]   chr4 [86626, 86675]      +
## [3]   chr4 [86731, 86734]      +
## -----
## seqinfo: 8 sequences from an unspecified genome

junctions(mysrbam2)[[1]] #and the corresponding junctions

## GRanges object with 2 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>        <IRanges> <Rle>
## [1]   chr4 [73011, 86625]      +
## [2]   chr4 [86676, 86730]      +
## -----
## seqinfo: 8 sequences from an unspecified genome
```

See below and in `help('junctions-methods', "GenomicAlignments")`, `help('findOverlaps-methods', "GenomicAlignments")` and `help('coverage-methods', "GenomicAlignments")` for other methods defined on *GAlignments* objects.

5.2.2 Paired-end reads

An example of paired-end data is available in the *pasillaBamSubset* package:

```
pr_bamFile=untreated3_chr4() # from pasillaBamSubset package
```

We can extract these data using:

```
myprbam=readGAlignmentPairs(pr_bamFile,
```

```

                                param=ScanBamParam(which=which))
myprbam[1:2]

## GAlignmentPairs object with 2 pairs, strandMode=1, and 0 metadata columns:
##      seqnames strand :      ranges --      ranges
##      <Rle> <Rle> :      <IRanges> --      <IRanges>
## [1]   chr4      - : [13711, 13747] -- [ 74403,  77053]
## [2]   chr4      * : [74403, 77053] -- [955236, 964043]
## -----
##      seqinfo: 8 sequences from an unspecified genome

```

The *GAlignmentPairs* class holds only read pairs (reads with no mate or with ambiguous pairing are discarded). Note that the `readGAlignmentPairs` function has a `strandMode` argument to specify how to report the strand of a pair. For stranded protocols, depending how the libraries were generated, `strandMode` should be set to 1 (the default for e.g. directional Illumina protocol by ligation) or 2 (e.g. for dUTP or Illumina stranded TruSeq PE protocol). The individual reads can be accessed as *GAlignments* objects using:

```

myprbam[1] #first record

## GAlignmentPairs object with 1 pair, strandMode=1, and 0 metadata columns:
##      seqnames strand :      ranges --      ranges
##      <Rle> <Rle> :      <IRanges> --      <IRanges>
## [1]   chr4      - : [13711, 13747] -- [74403, 77053]
## -----
##      seqinfo: 8 sequences from an unspecified genome

first(myprbam[1]) #first sequenced fragment

## GAlignments object with 1 alignment and 0 metadata columns:
##      seqnames strand      cigar    qwidth    start      end      width
##      <Rle> <Rle> <character> <integer> <integer> <integer> <integer>
## [1]   chr4      -      37M        37      13711     13747      37
##      njunc
##      <integer>
## [1]      0
## -----
##      seqinfo: 8 sequences from an unspecified genome

last(myprbam[1]) #last sequenced fragment

## GAlignments object with 1 alignment and 0 metadata columns:
##      seqnames strand      cigar    qwidth    start      end      width
##      <Rle> <Rle> <character> <integer> <integer> <integer> <integer>
## [1]   chr4      +    33M2614N4M      37      74403     77053     2651
##      njunc
##      <integer>
## [1]      1
## -----
##      seqinfo: 8 sequences from an unspecified genome

```

We could also use the `readGAlignmentsList` function which returns both mate-pairs and non-mates in a more classical "list-like" structure:

```
myprbam_list=readGAlignmentsList(pr_bamFile,
                                param=ScanBamParam(which=which))

myprbam_list[1:2]

## GAlignmentsList object of length 2:
## [[1]]
## GAlignments object with 2 alignments and 0 metadata columns:
##      seqnames strand cigar qwidth start    end width njunc
## [1]   chr4      +   37M    37 80628 80664    37    0
## [2]   chr4      -   37M    37 80775 80811    37    0
##
## [[2]]
## GAlignments object with 2 alignments and 0 metadata columns:
##      seqnames strand cigar qwidth start    end width njunc
## [1]   chr4      +   37M    37 81002 81038    37    0
## [2]   chr4      -   37M    37 83706 83742    37    0
##
## -----
## seqinfo: 8 sequences from an unspecified genome

table(elementNROWS(myprbam_list))

##
##    1    2
## 74 287

summary(mcols(myprbam_list)$mate_status) #mate status as metadata
##      mated ambiguous    unmated
##      287          0         74
```

Sometimes, it is desirable to process the BAM file in chunk. The `BamFile` allows to create a reference to a BAM file which can be opened, used in a loop and then closed (example taken from the excellent [HOWTO](#) vignette from the [GenomicRanges](#) package):

```
bf = BamFile(sr_bamFile, yieldSize=100000) #create reference
open(bf) #open connection
cvg = NULL #initialize

repeat {
  chunk <- readGAlignments(bf) #loop on the BAM file
  if (length(chunk) == 0L)
    break
  chunk_cvg <- coverage(chunk)
  if (is.null(cvg)) {
    cvg <- chunk_cvg
  } else {
    cvg <- cvg + chunk_cvg
  }
}
close(bf)

cvg$chr4
```

```
## integer-Rle of length 1351857 with 122061 runs
## Lengths: 891 27 5 12 13 45 ... 3 106 75 1600 75 1659
## Values : 0 1 2 3 4 5 ... 6 0 1 0 1 0
```

Note that such a loop is now directly performed by the `coverage` method for *BamFile* class. For details, see `?"coverage,GAlignments-method"`.

5.3 Some QA/QC on aligned reads

The `qa` function from the *ShortRead* also performs quality assessments on BAM files. Alternatively, a quick summary can be generated using:

```
quickBamFlagSummary(pr_bamFile)

##              group |   nb of |   nb of | mean / max
##              of | records | unique | records per
##              records | in group | QNAMEs | unique QNAME
## All records..... A | 175346 | 93620 | 1.87 / 10
## o template has single segment... S | 0 | 0 | NA / NA
## o template has multiple segments. M | 175346 | 93620 | 1.87 / 10
##   - first segment..... F | 88069 | 83413 | 1.06 / 8
##   - last segment..... L | 87277 | 82600 | 1.06 / 9
##   - other segment..... O | 0 | 0 | NA / NA
##
## Note that (S, M) is a partitioning of A, and (F, L, O) is a partitioning of M.
## Indentation reflects this.
##
## Details for group M:
## o record is mapped..... M1 | 175346 | 93620 | 1.87 / 10
##   - primary alignment..... M2 | 175346 | 93620 | 1.87 / 10
##   - secondary alignment..... M3 | 0 | 0 | NA / NA
## o record is unmapped..... M4 | 0 | 0 | NA / NA
##
## Details for group F:
## o record is mapped..... F1 | 88069 | 83413 | 1.06 / 8
##   - primary alignment..... F2 | 88069 | 83413 | 1.06 / 8
##   - secondary alignment..... F3 | 0 | 0 | NA / NA
## o record is unmapped..... F4 | 0 | 0 | NA / NA
##
## Details for group L:
## o record is mapped..... L1 | 87277 | 82600 | 1.06 / 9
##   - primary alignment..... L2 | 87277 | 82600 | 1.06 / 9
##   - secondary alignment..... L3 | 0 | 0 | NA / NA
## o record is unmapped..... L4 | 0 | 0 | NA / NA
```

This could allow to select relevant filters when importing the data in *R*.

5.4 Computing a coverage

Computing a coverage (number of reads aligning at a given position on the genome) on a *GAlignments* is straightforward:

```
cvg_sr=coverage(mysrbam2)
cvg_sr$chr4

## integer-Rle of length 1351857 with 1266 runs
##   Lengths: 72989    17    4   2092 ...    5   6175    49 143448
##   Values :    0     1     9     0 ...    1     0     1     0
```

The result is an *RleList* organized by chromosomes.

We can extract specific *Views* from this object:

```
which_chr4_gr=GRanges(seqnames="chr4",
                      ranges=which$chr4,strand="*") #convert imported intervals to GRanges
ex_chr4=exonsByOverlaps(TxDb.Dmelanogaster.UCSC.dm3.ensGene,which_chr4_gr) #get exons
head(Views(cvg_sr$chr4,ranges(ex_chr4))) #extract Views

## Views on a 1351857-length Rle subject
##
## views:
##   start   end width
## [1] 77123 77175    53 [3 3 3 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 ...]
## [2] 77866 78037    172 [1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 ...]
## [3] 80602 81041    440 [2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 ...]
## [4] 81196 81600    405 [3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2 ...]
## [5] 83599 83750    152 [2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 ...]
## [6] 76457 76957    501 [0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ...]
```

Note that the coverage function is not strand specific. If we want the coverage for the minus strand only, we could use:

```
coverage(mysrbam2[strand(mysrbam2)=="-"])$chr4

## integer-Rle of length 1351857 with 724 runs
##   Lengths: 73006    4   3895    75 ...    5   6175    49 143448
##   Values :    0     8     0     1 ...    1     0     1     0
```

It is sometimes necessary to shift or resize the reads before computing coverage. This is a typical situation in ChIP-seq data analysis when the binding of a transcription factor or a chromatin mark with punctuate enrichment is studied. Here, we load some ChIP-seq experimental data from the [MMDiffBamSubset](#) and import the reads as a *GAlignments* objet:

```
# library(MMDiffBamSubset)
ChIPex_path = WT.AB2() #from MMDiffBamSubset package
ChIP_ga=readGAlignments(ChIPex_path,
                       param=ScanBamParam(
                           which=GRanges(seqnames="chr1",
```

```
ranges=IRanges(3e6,5e6),
strand="*"))
```

Then we can compute the coverage after applying some transformation on the reads (shift or resize):

```
coverage(ChIP_ga)$chr1

## integer-Rle of length 197195432 with 63367 runs
## Lengths: 3000431 54 70 ... 9 17 192195393
## Values : 0 1 0 ... 2 1 0

coverage(ChIP_ga,shift=150)$chr1 #directly in coverage (!shift is not strand-aware)

## integer-Rle of length 197195432 with 63367 runs
## Lengths: 3000581 54 70 ... 9 17 192195243
## Values : 0 1 0 ... 2 1 0

coverage(ChIP_ga,shift=150*as.numeric(paste(strand(ChIP_ga),"1",sep="")))$chr1 #now "strand-aware" shift

## integer-Rle of length 197195432 with 63427 runs
## Lengths: 3000494 54 33 ... 45 9 192195260
## Values : 0 1 0 ... 2 1 0

coverage(resize(granges(ChIP_ga),300))$chr1 #resize via a GRanges (strand-aware)

## integer-Rle of length 197195432 with 64106 runs
## Lengths: 3000398 33 124 ... 62 9 192195164
## Values : 0 1 2 ... 2 1 0
```

5.5 Finding peaks in read coverage

In ChIP-seq experiments, one often look for "peaks" (or broad regions in e.g. ChIP of chromatin marks or PolII) in the read coverage. This task, often called "peak calling" or "peak finding" can be performed outside of R with one of the numerous "peak callers" available such as [MACS](#) / [MACS2](#), [SPP](#) or [SICER](#) (for broad regions). A number of specific and advanced tools also exist in R to perform peak calling such as: [BayesPeak](#), [chipseq](#), [bumphunter](#), [exomePeak](#), [CSAR](#), [jmosaics](#) or [PICS](#). Here, we only illustrate a naive approach to get a list of peaks by applying a single threshold on the coverage object using the [slice](#) function:

```
cvg_H3K4me3=coverage(resize(granges(ChIP_ga),300))
slice(cvg_H3K4me3,lower=20)$chr1

## Views on a 197195432-length Rle subject
##
## views:
##      start      end width
## [1] 3027290 3027346    57 [20 21 21 21 21 21 21 21 21 21 21 21 21 21 ...]
## [2] 3042723 3042728     6 [20 20 20 20 20 20]
## [3] 3042793 3042903   111 [20 20 20 20 20 20 20 20 20 20 20 20 20 20 ...]
## [4] 3042911 3042916     6 [20 20 20 20 20 20]
## [5] 3048694 3048705    12 [20 20 20 20 20 20 20 20 20 20 20 20]
## [6] 3048777 3048800    24 [20 20 20 20 20 20 20 20 20 20 20 20 20 20 ...]
```

```
## [7] 3052480 3052488      9 [20 20 20 20 20 20 20 20 20 20]
## [8] 3052492 3052880    389 [20 20 20 20 20 20 20 20 20 20 20 20 21 ...]
## [9] 3129266 3129557   292 [20 20 20 21 21 22 22 22 21 21 21 21 22 24 ...]
## ...      ...      ...    ... ...
## [272] 4964448 4964489    42 [20 20 21 21 21 21 21 21 21 21 21 21 21 21 ...]
## [273] 4976296 4976296     1 [20]
## [274] 4976308 4976317    10 [20 20 20 20 20 21 21 21 21 21]
## [275] 4976482 4976502    21 [20 20 20 20 20 20 20 20 20 20 20 20 20 ...]
## [276] 4977358 4977359     2 [20 20]
## [277] 4977366 4977443    78 [20 20 20 20 20 20 20 20 20 20 20 20 20 ...]
## [278] 4977485 4977599   115 [20 21 21 21 21 21 21 21 21 21 21 21 22 22 ...]
## [279] 4977615 4977630    16 [20 20 20 20 20 20 20 20 20 20 20 20 20 ...]
## [280] 4978067 4978377   311 [20 20 20 21 21 21 21 21 21 21 21 21 22 22 22 ...]
```

FixMe: more examples soon...maybe...

5.6 Counting reads / read summarization

Another common task is to count how many reads align to a set of genomic features. This counting operation is sometimes called read summarization or data reduction. It is typically done in RNA-seq experiments to produce a count matrix for subsequent analysis. The counts can be obtained on e.g. genes, transcripts or exons depending on the aim of the study. Some packages such as [Rsubread](#), [QuasR](#) and [easyRNAseq](#) provide specific functions to perform the counting step. Here we only present the general function `summarizeOverlaps` from [GenomicAlignments](#).

We want to count the reads aligning on the exons. So we first get the exons, organized by genes, which are located in our region of interest:

```
exbygn_chr4=subsetByOverlaps(exonsBy(TxDb.Dmelanogaster.UCSC.dm3.ensGene,
                                     by="gene"),ex_chr4)
```

Then we use the `summarizeOverlaps` function to obtain the counts (*Note that the counting is strand-aware by default!*):

```
count_res=summarizeOverlaps(exbygn_chr4, mysrbam2, mode="Union")
count_res

## class: RangedSummarizedExperiment
## dim: 3 1
## metadata(0):
## assays(1): counts
## rownames(3): FBgn0002521 FBgn0004859 FBgn0264617
## rowData names(0):
## colnames(1): reads
## colData names(2): object records

assays(count_res)$counts
```


##	reads
## FBgn0002521	346
## FBgn0004859	12
## FBgn0264617	117

Different count modes are available, as in HTSeq . These are illustrated in Figure 12.

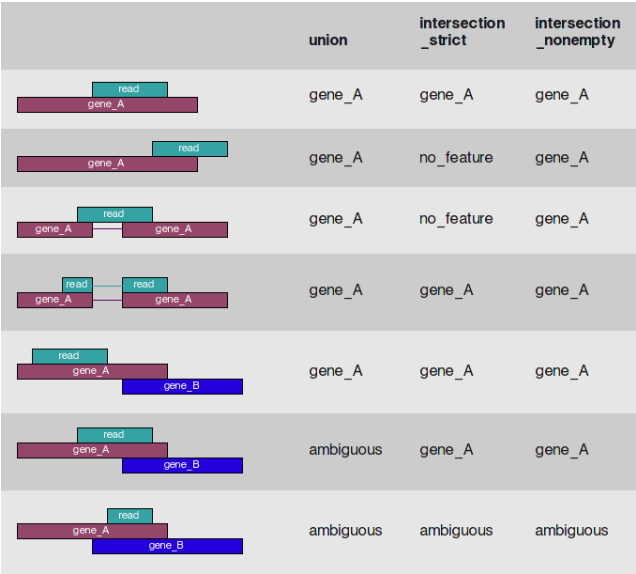


Figure 12: Count modes
Taken from the HTSeq website

The result of `summarizeOverlaps` (here `count_res`) is a *SummarizedExperiment* object (See `help(SummarizedExperiment, package="GenomicRanges")` for details and accessors). This class is very similar to the *eSet* class (from the *Biobase* package) which is often used to analyze microarray data. In *eSet* objects, the features are typically microarray probes, probesets or genes corresponding to these probes. In *summarizedExperiment* objects, the features (i.e. rows) are ranges of interest. Figure 13 illustrates the structure of a *SummarizedExperiment* object.

To count reads from multiple BAM files we just need to enter the path to the BAM files as a character vector:

```
count_res2=summarizeOverlaps(exbygn_chr4,
                             c(sr_bamFile,pr_bamFile),
                             mode="Union")

assays(count_res2)$counts

##          untreated1_chr4.bam untreated3_chr4.bam
## FBgn0002521             346             210
## FBgn0004859             200              96
## FBgn0264617             117              53
```

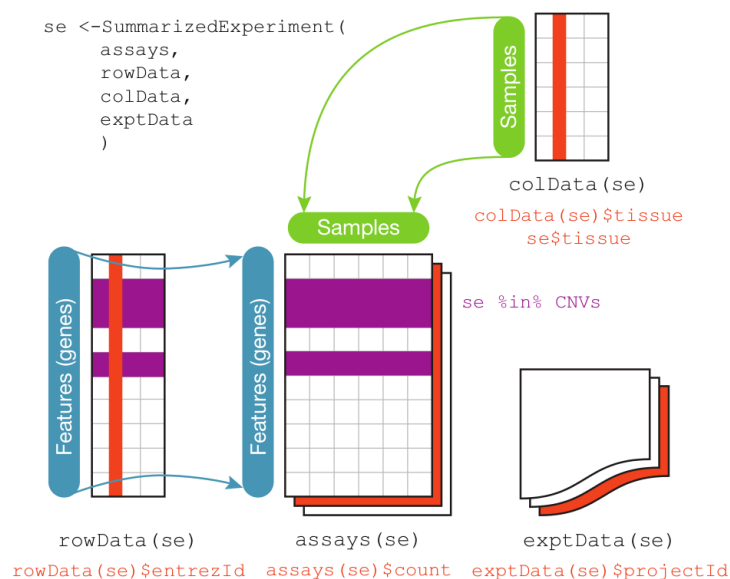


Figure 13: Structure of `SummarizedExperiment` objects

Taken from [3]. The `assays` component contains the data as rectangular matrices. The `rowData` and `colData` components contain metadata on the features and on the samples respectively. The `exptData` component contains experiment-level data. Code examples illustrate how to create a `SummarizedExperiment`, subset it and access the components.

There are several options available to perform read counting with `summarizeOverlaps`. Some examples are provided below:

```

assays(summarizeOverlaps(exbygn_chr4, myprbam, mode="Union"))$counts

##           reads
## FBgn0002521    97
## FBgn0004859     6
## FBgn0264617    32

assays(summarizeOverlaps(exbygn_chr4, myprbam, mode="Union", #ignore strand
                        ignore.strand=T))$counts

##           reads
## FBgn0002521   192
## FBgn0004859    7
## FBgn0264617   50

assays(summarizeOverlaps(exbygn_chr4, mysrbam2, mode="Union"))$counts

##           reads
## FBgn0002521   346
## FBgn0004859   12
## FBgn0264617  117

assays(summarizeOverlaps(exbygn_chr4, mysrbam2, mode="Union", #resize the reads to 100
                        preprocess.reads=function(x){resize(granges(x), 100)}))$counts

##           reads
## FBgn0002521   346
## FBgn0004859    4

```

```
## FBgn0264617 117
```

See `?summarizeOverlaps` for details and examples.

The count matrix could then be used for normalization and differential analysis with the *DESeq2*, *edgeR*, *limma*, *DEXseq* packages for example.

6 More annotation packages in *Bioconductor*

6.1 Types of annotation packages

There are a number of annotation packages in *Bioconductor* which can be browsed [here](#). Annotations are provided as *AnnotationDb* objects and the *AnnotationDbi* provides a common interface to these objects. The vignette of the *AnnotationDbi* provides a good introduction to the different types of package available. Figure 14, adapted from this vignette, illustrates the different types of *gene*- and *genome*-centered annotation packages available in *Bioconductor*.

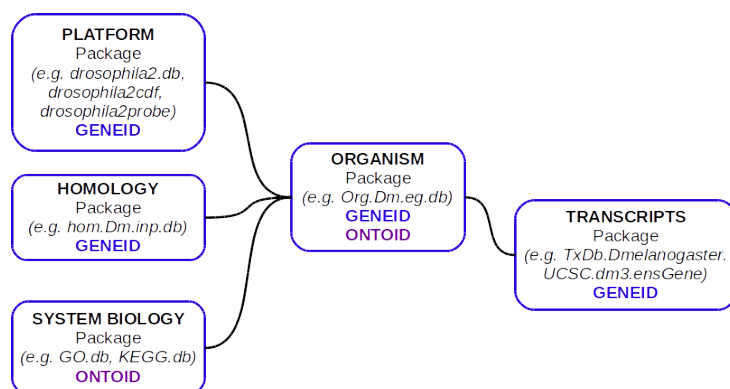


Figure 14: *AnnotationDb* packages
Adapted from the vignette of *AnnotationDbi*

There are gene-centric packages:

- Organism packages (*OrgDb* class ; e.g. *org.Dm.eg.db*)
- Platform-level (essentially microarrays) packages (*ChipDb* class: e.g. *drosophila2.db* and the corresponding *probe* and *cdf* packages: *drosophila2probe* and *drosophila2cdf*)
- Homology packages (*InparanoidDb* class ; e.g. *hom.Dm.inp.db*)
- System-biology packages (e.g. *GO.db*, *KEGG.db*, *reactome.db*)

And genome-centric packages:

- Transcriptome-oriented packages (*TxDb* class ; e.g. *TxDb.Dmelanogaster.UCSC.dm3.ensGene*)
- Generic Genome feature packages (*FeatureDb* class, e.g. *FDb.UCSC.tRNAs*) that can be created with the *GenomicFeatures* package

In addition, *OrganismDb* packages were recently added based on the *OrganismDbi* package. These packages basically combine all the above packages in a single package. These are available for *Homo.sapiens*, *Mus.musculus* and *Rattus.norvegicus*. Finally packages are available in *Bioconductor* to enable the user to build his own annotation packages. These include *AnnotationForge* and *GenomicFeatures*.

6.2 Accessing annotations

There are 4 basic functions to access nearly all annotations:

- The `columns` function shows the fields from which annotations can be extracted
- The `keytypes` function shows the field from which annotations can be extracted AND which can be used as keys to access these annotations
- The `keys` function retrieves the keys themselves (i.e. values from a 'keytype' field used to access the annotation of the corresponding feature)
- The `select` function extracts the data from the *AnnotationDb* object from the *columns* specified and providing a set of *keys* of a given *keytype*.

In our examples, we will explore the main packages for *Drosophila melanogaster*:

- *org.Dm.eg.db*
- *drosophila2.db* and the corresponding *drosophila2probe* and *drosophila2cdf* packages
- *hom.Dm.inp.db*
- *GO.db* (not specific to *D. melanogaster*)
- *TxDb.Dmelanogaster.UCSC.dm3.ensGene*

First, we explore the *OrgDb* package which contains gene-level annotations:

```
## library(org.Dm.eg.db)
columns(org.Dm.eg.db)

## [1] "ACCNUM"      "ALIAS"       "ENSEMBL"     "ENSEMBLPROT"
## [5] "ENSEMBLTRANS" "ENTREZID"    "ENZYME"      "EVIDENCE"
## [9] "EVIDENCEALL"  "FLYBASE"     "FLYBASECG"   "FLYBASEPROT"
## [13] "GENENAME"     "GO"          "GOALL"       "MAP"
## [17] "ONTOLOGY"     "ONTOLOGYALL" "PATH"        "PMID"
## [21] "REFSEQ"       "SYMBOL"      "UNIGENE"     "UNIPROT"

## help("PATH")
## keytypes(org.Dm.eg.db) #same as columns(org.Dm.eg.db) in this case
uniKeys = keys(org.Dm.eg.db, keytype="UNIPROT")[c(5,6,24)]
```

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```
cols = c("SYMBOL", "GO")
select(org.Dm.eg.db, keys=uniKeys[1:2], columns=cols, keytype="UNIPROT")

## 'select()' returned 1:many mapping between keys and columns

##      UNIPROT  SYMBOL      GO EVIDENCE ONTOLOGY
## 1  Q95RU8    G9a GO:0000791    IDA      CC
## 2  Q95RU8    G9a GO:0002165    IMP      BP
## 3  Q95RU8    G9a GO:0005634    IDA      CC
## 4  Q95RU8    G9a GO:0005705    IDA      CC
## 5  Q95RU8    G9a GO:0007614    IMP      BP
## 6  Q95RU8    G9a GO:0007616    IMP      BP
## 7  Q95RU8    G9a GO:0008270    IEA      MF
## 8  Q95RU8    G9a GO:0008345    IMP      BP
## 9  Q95RU8    G9a GO:0010468    IMP      BP
## 10 Q95RU8    G9a GO:0018024    IDA      MF
## 11 Q95RU8    G9a GO:0035076    IGI      BP
## 12 Q95RU8    G9a GO:0035220    IMP      BP
## 13 Q95RU8    G9a GO:0046959    IMP      BP
## 14 Q95RU8    G9a GO:0050688    IMP      BP
## 15 Q95RU8    G9a GO:0050775    IMP      BP
## 16 Q95RU8    G9a GO:0051567    IMP      BP
## 17 Q95RU8    G9a GO:1900111    IMP      BP
## 18 Q9W5H1  CG13377 GO:0002121    IMP      BP
## 19 Q9W5H1  CG13377 GO:0016319    IMP      BP
## 20 Q9W5H1  CG13377 GO:0042048    IMP      BP
```

The [GO.db](#) package can be used to retrieve information on the identified [Gene Ontology](#) categories (chosen also based on [Evidence codes](#)):

```
## library(GO.db)
mygos=c("GO:0002121", "GO:0003858", "GO:0016319")
select(GO.db, columns=columns(GO.db)[1:3],
       keys=mygos, keytype="GOID")

## 'select()' returned 1:1 mapping between keys and columns

##      GOID
## 1 GO:0002121
## 2 GO:0003858
## 3 GO:0016319
##
## 1
## 2
## 3 The process whose specific outcome is the progression of the mushroom body over time, from its formation
##      ONTOLOGY
## 1      BP
## 2      MF
## 3      BP
```

We can also extract a whole table as a *data.frame*:

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```
## ls("package:GO.db")
toTable(GOTERM)[1:3,1:4]

##      go_id    go_id.1      Term Ontology
## 1 GO:0000001 GO:0000001      mitochondrion inheritance      BP
## 2 GO:0000002 GO:0000002      mitochondrial genome maintenance      BP
## 3 GO:0000003 GO:0000003      reproduction      BP
```

We can also search for a specific pattern in the keys:

```
keys(org.Dm.eg.db, keytype="SYMBOL", pattern="EcR")

## [1] "EcR"      "DopEcR"

select(org.Dm.eg.db, keys=c("EcR", "DopEcR"),
       columns=c("ENTREZID", "CHR", "FLYBASE", "GENENAME"), keytype="SYMBOL")

## Warning in .deprecatedColsMessage(): Accessing gene location information via
## 'CHR', 'CHRLLOC', 'CHRLOCEND'
## is deprecated. Please use a range based accessor like genes(), or
## select() with columns values like TXCHROM and TXSTART on a TxDb or
## OrganismDb object instead.

## 'select()' returned 1:1 mapping between keys and columns

##  SYMBOL ENTREZID CHR      FLYBASE      GENENAME
## 1    EcR   35540  2R  FBgn0000546      Ecdysone receptor
## 2 DopEcR   38539  3L  FBgn0035538 Dopamine/Ecdysteroid receptor
```

ChipDb packages contain annotations based on microarray probes:

```
## library(drosophila2.db)
ls("package:drosophila2.db")[1:8]

## [1] "drosophila2"      "drosophila2ACCNUM"
## [3] "drosophila2ALIAS2PROBE" "drosophila2CHR"
## [5] "drosophila2CHRLNGTHS" "drosophila2CHRLOC"
## [7] "drosophila2CHRLOCEND" "drosophila2.db"

## drosophila2.db #provides information on the underlying database
columns(drosophila2.db)[1:7]

## [1] "ACCNUM"      "ALIAS"      "ENSEMBL"      "ENSEMBLPROT"
## [5] "ENSEMBLTRANS" "ENTREZID"      "ENZYME"

select(drosophila2.db, columns=c("ENTREZID", "SYMBOL", "ENSEMBL"),
       keys=c("1639797_at", "1627097_at", "1628020_at"), keytype="PROBEID")

## 'select()' returned 1:1 mapping between keys and columns

##      PROBEID ENTREZID  SYMBOL      ENSEMBL
## 1 1639797_at  4379890 CG34109 FBgn0083945
## 2 1627097_at   318986 t-cup FBgn0051858
## 3 1628020_at   33366 CG15358 FBgn0031373
```

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The associated *probe* and *cdf* packages provide information on microarray probe sequences and on position on the array respectively. They are mainly used by different methods during the analysis of Affymetrix microarray data:

```
## library(drosophila2probe)
## library(drosophila2cdf)
drosophila2probe[1:2,] #a data frame with probe sequences

##           sequence  x  y Probe.Set.Name
## 1 CCTGAATCCTGGCAATGTCATCATC 599 305      1622893_at
## 2 ATCCTGGCAATGTCATCATCAATGG 267  45      1622893_at
##   Probe.Interrogation.Position Target.Strandedness
## 1                               137             Antisense
## 2                               142             Antisense

ls("package:drosophila2cdf")

## [1] "drosophila2cdf" "drosophila2dim" "i2xy"          "xy2i"
```

See the vignettes of the [AnnotationDbi](#) packages for details.

Homology *InparanoidDb* packages contain the gene orthologs for a number of species. They are extracted from the [Inparanoid database](#) [17] and can be interrogated both ways:

```
## library(hom.Dm.inp.db)
select(hom.Dm.inp.db, columns=c("HOMO_SAPIENS", "CULEX_PIPPIENS"),
       keys=c("FBpp0084497", "FBpp0077213"), keytype="DROSOPHILA_MELANOGASTER")

## 'select()' returned 1:1 mapping between keys and columns

##   DROSOPHILA_MELANOGASTER CULEX_PIPPIENS   HOMO_SAPIENS
## 1          FBpp0084497    CPIJ009610      <NA>
## 2          FBpp0077213    CPIJ004808  ENSP00000362314

select(hom.Dm.inp.db, columns=c("HOMO_SAPIENS", "DROSOPHILA_MELANOGASTER"),
       keys=c("CPIJ014347", "CPIJ005780"), keytype="CULEX_PIPPIENS")

## 'select()' returned 1:1 mapping between keys and columns

##   CULEX_PIPPIENS DROSOPHILA_MELANOGASTER   HOMO_SAPIENS
## 1    CPIJ014347          FBpp0088446  ENSP00000303147
## 2    CPIJ005780          FBpp0072468  ENSP00000360532
```

Finally, *TxDb* packages can also be interrogated using the same methods:

```
select(TxDb.Dmelanogaster.UCSC.dm3.ensGene,
       columns=c("TXID", "TXCHROM", "TXSTRAND", "TXSTART", "TXEND"),
       keys=c("FBgn0039183", "FBgn0264342", "FBgn0030583"),
       keytype='GENEID')

## 'select()' returned 1:1 mapping between keys and columns

##   GENEID  TXID TXCHROM TXSTRAND TXSTART   TXEND
## 1 FBgn0039183 19327   chr3R      + 20159145 20162971
## 2 FBgn0264342  559   chr2L      +  4647051  4647920
## 3 FBgn0030583 25509   chrX      + 14730758 14731363
```

7 Import/export of genomic data

7.1 *Bioconductor* packages to import/export genomic data

In addition to annotation packages presented in paragraph 6, a number of *Bioconductor* software packages provide interfaces with online databases containing annotations and experimental data.

Here, we will give some examples with the following packages:

- [rtracklayer](#) [18]
- [AnnotationHub](#)
- [biomaRt](#)
- [GEOquery](#)
- [SRADB](#)

7.2 The *rtracklayer* package

The main functionality of the [rtracklayer](#) [18] is to import/export files in a number of different standard formats. These include [GFF](#), [BED](#), [WIG](#), [bigWig](#) and [bedGraph](#). Additionally, [rtracklayer](#) also provides functions to interact with genome browsers and in particular [UCSC Genome Browser](#). However, we do not illustrate these functionalities here.

First we get the path for some example files (from the [Gviz](#) package):

```
bamExFile_path=system.file(package="Gviz", "extdata", "test.bam")
gff3ExFile_path=system.file(package="Gviz", "extdata", "test.gff3")
gtfExFile_path=system.file(package="Gviz", "extdata", "test.gff2")
bedExFile_path=system.file(package="Gviz", "extdata", "test.bed")
wigExFile_path=system.file(package="Gviz", "extdata", "test.wig")
bedGraphExFile_path=system.file(package="Gviz", "extdata", "test.bedGraph")
```

The `import` and `export` functions of [rtracklayer](#) will adapt to the different file formats via the `format` argument. If this argument is missing the format is derived from the file extension. Here, we simply import these files using the `import` function without any other argument than the file path:

```
head(import(bedExFile_path))
```

```
## GRanges object with 6 ranges and 4 metadata columns:
```

##	seqnames	ranges	strand	name	score
##	<Rle>	<IRanges>	<Rle>	<character>	<numeric>
##	[1] chr7	[127471197, 127472363]	+	Pos1	0
##	[2] chr7	[127472364, 127473530]	+	Pos2	0
##	[3] chr7	[127473531, 127474697]	+	Pos3	0
##	[4] chr7	[127474698, 127475864]	+	Pos4	0
##	[5] chr7	[127475865, 127477031]	-	Neg1	0
##	[6] chr7	[127477032, 127478198]	-	Neg2	0


```
##           itemRgb           thick
##      <character>      <IRanges>
## [1]      #FF0000 [127471197, 127472363]
## [2]      #FF0000 [127472364, 127473530]
## [3]      #FF0000 [127473531, 127474697]
## [4]      #FF0000 [127474698, 127475864]
## [5]      #0000FF [127475865, 127477031]
## [6]      #0000FF [127477032, 127478198]
## -----
## seqinfo: 1 sequence from an unspecified genome; no seqlengths

head(import(wigExFile_path)) #binned at 300bp

## GRanges object with 6 ranges and 1 metadata column:
##      seqnames      ranges strand |      score
##      <Rle>      <IRanges> <Rle> | <numeric>
## [1] chr19 [49302001, 49302300]      * |      -1
## [2] chr19 [49302301, 49302600]      * |     -0.75
## [3] chr19 [49302601, 49302900]      * |     -0.2
## [4] chr19 [49302901, 49303200]      * |     -0.1
## [5] chr19 [49303201, 49303500]      * |        0
## [6] chr19 [49303501, 49303800]      * |     0.25
## -----
## seqinfo: 1 sequence from an unspecified genome; no seqlengths

Rle(rep(import(wigExFile_path)$score,each=300)) #convert to Rle

## numeric-Rle of length 2700 with 9 runs
## Lengths:  300  300  300  300  300  300  300  300  300
## Values :   -1 -0.75 -0.2 -0.1   0  0.25  0.4  0.55  1
```

Comment: Try to import the other files and see what you get. Also try to export files to different formats using the `export` function.

Note that a genome-wide coverage as an *RleList* object can be directly exported as WIG or bedGraph files using the `export` function from *rtracklayer*.

7.3 The *AnnotationHub* package

The *AnnotationHub* allows to easily retrieve a number of public datasets and annotation tracks as standard *Bioconductor* objects such as *GRanges* and *GRangesList*. Note however that many datasets have been pre-processed so the user relies on others for these steps. The datasets available come from e.g. [ENSEMBL](#), [NCBI](#), [UCSC](#), [ENCODE](#) and [Inparanoid](#)). Here, we briefly illustrate the main commands used to retrieve results of interest. After loading the library, the user must create an *AnnotationHub* object:

```
library(AnnotationHub)
```

```
ah=AnnotationHub()
```

```
## updating metadata: retrieving 1 resource
```

```
## snapshotDate(): 2017-10-27
```

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To explore this object we take a look at its metadata:

```
annot_ah=mcols(ah) #Informations on the different records
table(annot_ah$rdataclass) #type of files that can be retrieved

##
##      AAStrngSet      BigWigFile      biopax      ChainFile
##           1          10247           9          1113
##      data.frame      EnsDb          FaFile      GRanges
##           24           282          5122      19268
##      Inparanoid8Db    list          MSnSet      mzRident
##           268           2           1           1
##           mzRp wiz      OrgDb          Rle SQLiteConnection
##           1           1018          1586           1
##           TwoBitFile      TxDdb      VcfFile
##           4027           45           8

table(annot_ah$dataprovder) #providers

##
##           BroadInstitute      ChEA
##           18248           1
##           CRIBI           dbSNP
##           1           8
##           Ensembl ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/
##           12003           1018
##           Gencode      Genoscope
##           28           2
##           Haemcode      Inparanoid8
##           945           268
##           MISO, VAST-T00LS, UCSC      NHLBI
##           2           1
##           NIH Pathway Interaction Database      Pazar
##           9           91
##           PRIDE      RefNet
##           4           8
##           Stanford      UCSC
##           24           10337
##           URGI      UWashington
##           1           25
```

orgDb objects are available in the hub:

```
query(ah,"orgDb") #search for available orgDb packages

## AnnotationHub with 1018 records
## # snapshotDate(): 2017-10-27
## # $dataprovder: ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/
## # $species: Escherichia coli, Acanthamoeba castellanii-str._Neff, Acantha...
## # $rdataclass: OrgDb
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1-based, maintainer, rdatadateadded, preparerclass,
## #   tags, rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH57964"]]'
```

```
##
##          title
## AH57964 | org.Ag.eg.db.sqlite
## AH57965 | org.At.tair.db.sqlite
## AH57966 | org.Bt.eg.db.sqlite
## AH57967 | org.Cf.eg.db.sqlite
## AH57968 | org.Gg.eg.db.sqlite
## ...
## AH59987 | org.Pseudoalteromonas_piscicida.eg.sqlite
## AH59988 | org.Bacteroides_fragilis_YCH46.eg.sqlite
## AH59989 | org.Pseudomonas_mendocina_ypm.eg.sqlite
## AH59990 | org.Salmonella_enterica_subsp._enterica_serovar_Typhimurium_s...
## AH59991 | org.Acinetobacter_baumannii.eg.sqlite

query(ah,c("orgDb","Arabidopsis")) #those for Arabidopsis only

## AnnotationHub with 2 records
## # snapshotDate(): 2017-10-27
## # $dataprovder: ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/
## # $species: Arabidopsis lyrata_subsp._lyrata, Arabidopsis thaliana
## # $rdaclass: OrgDb
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass,
## #   tags, rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH57965"]]'
##
##          title
## AH57965 | org.At.tair.db.sqlite
## AH59047 | org.Arabidopsis_lyrata_subsp._lyrata.eg.sqlite

newAt=ah[["AH57965"]] #retrieve the orgDb object

## loading from cache '/home/pmartin/.AnnotationHub/64711'

keytypes(newAt) #explore the object

## [1] "ARACYC"          "ARACYCENZYME" "ENTREZID"      "ENZYME"
## [5] "EVIDENCE"        "EVIDENCEALL"   "GENENAME"      "GO"
## [9] "GOALL"           "ONTOLOGY"      "ONTOLOGYALL"   "PATH"
## [13] "PMID"            "REFSEQ"        "SYMBOL"        "TAIR"

select(newAt,keys="AT1G01010",keytype="TAIR",columns=c("REFSEQ","ENTREZID","GO"))

## 'select()' returned 1:many mapping between keys and columns

##          TAIR      REFSEQ ENTREZID      GO EVIDENCE ONTOLOGY
## 1 AT1G01010 NM_099983  839580 GO:0003700      ISS      MF
## 2 AT1G01010 NM_099983  839580 GO:0005634      ISM      CC
## 3 AT1G01010 NM_099983  839580 GO:0006888      RCA      BP
## 4 AT1G01010 NM_099983  839580 GO:0007275      ISS      BP
## 5 AT1G01010 NM_099983  839580 GO:0043090      RCA      BP
## 6 AT1G01010 NP_171609  839580 GO:0003700      ISS      MF
## 7 AT1G01010 NP_171609  839580 GO:0005634      ISM      CC
## 8 AT1G01010 NP_171609  839580 GO:0006888      RCA      BP
## 9 AT1G01010 NP_171609  839580 GO:0007275      ISS      BP
```

```
## 10 AT1G01010 NP_171609 839580 G0:0043090 RCA BP
```

Note that it is also possible to select resources from the Hub using `subset` and `display`

Another example with data from Roadmap Epigenomics project:

```
epiFiles=query(ah,"EpigenomeRoadMap")
epiFiles

## AnnotationHub with 18248 records
## # snapshotDate(): 2017-10-27
## # $dataProvider: BroadInstitute
## # $species: Homo sapiens
## # $rdaclass: BigWigFile, GRanges, data.frame
## # additional mcalls(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass,
## #   tags, rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH28856"]]'
##
##           title
## AH28856 | E001-H3K4me1.broadPeak.gz
## AH28857 | E001-H3K4me3.broadPeak.gz
## AH28858 | E001-H3K9ac.broadPeak.gz
## AH28859 | E001-H3K9me3.broadPeak.gz
## AH28860 | E001-H3K27me3.broadPeak.gz
## ...
## AH49540 | E058_mCRF_FractionalMethylation.bigwig
## AH49541 | E059_mCRF_FractionalMethylation.bigwig
## AH49542 | E061_mCRF_FractionalMethylation.bigwig
## AH49543 | E081_mCRF_FractionalMethylation.bigwig
## AH49544 | E082_mCRF_FractionalMethylation.bigwig

unique(epiFiles$species) # sanity check

## [1] "Homo sapiens"

unique(epiFiles$genome) # sanity check

## [1] "hg19"

table(epiFiles$sourcetype) #types of files available

##
##   BED BigWig   GTF   tab   Zip
## 8298 9932     3     1    14

head(sort(table(epiFiles$description), decreasing=TRUE)) #more precise description of the files available

##
## Bigwig File containing -log10(p-value) signal tracks from EpigenomeRoadMap Project
##                                     6881
## Bigwig File containing fold enrichment signal tracks from EpigenomeRoadMap Project
##                                     2947
##   Narrow ChIP-seq peaks for consolidated epigenomes from EpigenomeRoadMap Project
##                                     2894
##   Broad ChIP-seq peaks for consolidated epigenomes from EpigenomeRoadMap Project
```

```
## 2534
## Gapped ChIP-seq peaks for consolidated epigenomes from EpigenomeRoadMap Project
## 2534
## Narrow DNasePeaks for consolidated epigenomes from EpigenomeRoadMap Project
## 131

query(ah , c("EpigenomeRoadMap", "H3K36ME3", "broadPeak", "liver")) #a more precise query

## AnnotationHub with 5 records
## # snapshotDate(): 2017-10-27
## # $dataProvider: BroadInstitute
## # $species: Homo sapiens
## # $rdataclass: GRanges
## # additional mcols(): taxonomyid, genome, description,
## # coordinate_1_based, maintainer, rdatadateadded, preparerclass,
## # tags, rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH29351"]]'
##
## title
## AH29351 | E066-H3K36me3.broadPeak.gz
## AH41908 | BI.Adult_Liver.H3K36me3.3.broadPeak.gz
## AH41909 | BI.Adult_Liver.H3K36me3.4.broadPeak.gz
## AH41910 | BI.Adult_Liver.H3K36me3.5.broadPeak.gz
## AH42615 | UCSD.Adult_Liver.H3K36me3.STL011.broadPeak.gz

k36Peaks=ah[["AH29351"]] #retrieve the data

## loading from cache '/home/pmartin/.AnnotationHub/34791'

k36Peaks # a GRanges object

## GRanges object with 199604 ranges and 5 metadata columns:
##      seqnames      ranges strand |      name      score
##      <Rle>      <IRanges> <Rle> | <character> <numeric>
## [1] chr12 [133758469, 133774384] * | Rank_1      158
## [2] chr19 [ 58695221, 58726784] * | Rank_2      134
## [3] chr19 [ 44670183, 44683357] * | Rank_3      133
## [4] chr9 [ 35694083, 35727997] * | Rank_4      131
## [5] chr19 [ 44509434, 44519252] * | Rank_5      131
## ...      ...      ...      ...      ...
## [199600] chr9 [ 3634043, 3634277] * | Rank_199600      0
## [199601] chr1 [182763503, 182763836] * | Rank_199601      0
## [199602] chr2 [173674414, 173674905] * | Rank_199602      0
## [199603] chr1 [117660710, 117661344] * | Rank_199603      0
## [199604] chr3 [ 75749824, 75750091] * | Rank_199604      0
##      signalValue  pValue  qValue
##      <numeric> <numeric> <numeric>
## [1] 7.70772 18.84335 15.87884
## [2] 6.41733 16.12752 13.42749
## [3] 6.30442 15.98875 13.30686
## [4] 6.47104 15.84522 13.15117
## [5] 6.51529 15.79857 13.16733
## ...      ...      ...
## [199600] 1.45074 1.00778 0.00937
```

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```
## [199601] 1.44989 1.00766 0.00929
## [199602] 1.45868 1.00763 0.00937
## [199603] 1.44478 1.00691 0.00885
## [199604] 1.43371 1.00530 0.00789
## -----
## seqinfo: 93 sequences (1 circular) from hg19 genome

metadata(k36Peaks)

## $AnnotationHubName
## [1] "AH29351"
##
## $`File Name`
## [1] "E066-H3K36me3.broadPeak.gz"
##
## $`Data Source`
## [1] "http://egg2.wustl.edu/roadmap/data/byFileType/peaks/consolidated/broadPeak/E066-H3K36me3.broadPeak.g
##
## $Provider
## [1] "BroadInstitute"
##
## $Organism
## [1] "Homo sapiens"
##
## $`Taxonomy ID`
## [1] 9606
```

Another example with a chainfile: Lifting genomic coordinates from one genome build to another, requires a chain file. Here, we use the `liftOver` function from [rtracklayer](#) package to perform the lift over and use the chain file obtained from AnnotationHub.

```
query(ah,c("dm3","dm6","chainfile")) #search for a chain file

## AnnotationHub with 1 record
## # snapshotDate(): 2017-10-27
## # names(): AH15105
## # $dataprovder: UCSC
## # $species: Drosophila melanogaster
## # $rdataclass: ChainFile
## # $rdatadateadded: 2014-12-15
## # $title: dm3ToDm6.over.chain.gz
## # $description: UCSC liftOver chain file from dm3 to dm6
## # $taxonomyid: 7227
## # $genome: dm3
## # $sourcetype: Chain
## # $sourceurl: http://hgdownload.cse.ucsc.edu/goldenpath/dm3/liftOver/dm3T...
## # $sourcesize: NA
## # $tags: c("liftOver", "chain", "UCSC", "genome", "homology")
## # retrieve record with 'object[["AH15105"]]'

chain=ah[["AH15105"]] #retrieve the chain file

## loading from cache '/home/pmartin/.AnnotationHub/19200'
```

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```
genes #Drosophila genes

## GRanges object with 2 ranges and 2 metadata columns:
##           seqnames           ranges strand |   EntrezId   Symbol
##           <Rle>             <IRanges> <Rle> | <character> <character>
## FBgn0031208   chr2L [ 7529, 9484]      + |    33155   CG11023
## FBgn0085359   chrX [18962306, 18962925] - |    2768869   CG34330
## -----
## seqinfo: 2 sequences from dm3 genome

liftOver(genes,chain) #new coordinates

## GRangesList object of length 2:
## $FBgn0031208
## GRanges object with 1 range and 2 metadata columns:
##           seqnames           ranges strand |   EntrezId   Symbol
##           <Rle>             <IRanges> <Rle> | <character> <character>
## [1]   chr2L [7529, 9484]      + |    33155   CG11023
##
## $FBgn0085359
## GRanges object with 1 range and 2 metadata columns:
##           seqnames           ranges strand |   EntrezId   Symbol
## [1]   chrX [19068273, 19068892] - |    2768869   CG34330
##
## -----
## seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

Versions of the hub can be accessed and selected using:

```
possibleDates(ah)[1:10] # available dates for the Hub

## [1] "2013-03-19" "2013-03-21" "2013-03-26" "2013-04-04" "2013-04-29"
## [6] "2013-06-24" "2013-06-25" "2013-06-26" "2013-06-27" "2013-10-29"

snapshotDate(ah) #date currently in use (can be changed using <-)

## [1] "2017-10-27"
```

The location of the downloaded files and the Hub URL are accessed using:

```
hubCache(ah)

## [1] "/home/pmartin//.AnnotationHub"

hubUrl(ah)

## [1] "https://annotationhub.bioconductor.org"
```

The AnnotationHub can also be explored more interactively in a browser using:

```
d=display(ah)
```

7.4 The *biomaRt* package

The *biomaRt* package [19, 20] provides an interface to the [Biomart](#) web services. The so-called "Marts" represent a large family of annotation resources:

```
## library(biomaRt)
listMarts(host="www.ensembl.org")

##           biomart           version
## 1 ENSEMBL_MART_ENSEMBL      Ensembl Genes 93
## 2 ENSEMBL_MART_MOUSE        Mouse strains 93
## 3 ENSEMBL_MART_SNP          Ensembl Variation 93
## 4 ENSEMBL_MART_FUNCGEN      Ensembl Regulation 93
```

Now can select the source (or "Mart") we want to use:

```
ens=useMart('ENSEMBL_MART_ENSEMBL',host='www.ensembl.org')
ens

## Object of class 'Mart':
## Using the ENSEMBL_MART_ENSEMBL BioMart database
## No dataset selected.

listDatasets(ens)[1:5,]

##           dataset           description
## 1 mpahari_gene_ensembl Shrew mouse genes (PAHARI_EIJ_v1.1)
## 2 aplatyrhynchos_gene_ensembl Duck genes (BGI_duck_1.0)
## 3 mauratus_gene_ensembl Golden Hamster genes (MesAur1.0)
## 4 cjacchus_gene_ensembl Marmoset genes (ASM275486v1)
## 5 ptroglodytes_gene_ensembl Chimpanzee genes (Pan_tro_3.0)
##           version
## 1 PAHARI_EIJ_v1.1
## 2 BGI_duck_1.0
## 3 MesAur1.0
## 4 ASM275486v1
## 5 Pan_tro_3.0

rattus=useMart('ENSEMBL_MART_ENSEMBL',
               host='www.ensembl.org',
               dataset='rnorvegicus_gene_ensembl')
```

Now we need to set up filters in order to get results focused on our objects of interest.

```
head(keytypes(rattus)) #see also listFilters(rattus)

## [1] "affy_rae230a" "affy_rae230b" "affy_raex_1_0_st_v1"
## [4] "affy_ragene_1_0_st_v1" "affy_ragene_2_1_st_v1" "affy_rat230_2"

head(columns(rattus)) #see also listAttributes(rattus)

## [1] "3utr" "3utr_end" "3utr_end" "3utr_start" "3utr_start"
## [6] "5utr"
```

To get all the values ('keys') taken by a specific filter ('keytype'), we can use:


```
keys(rattus, keytype="chromosome_name")[1:5]
## [1] "1" "2" "3" "4" "5"
```

Unfortunately, the `keys` method does not work with all keytypes in *biomaRt*.

Once we know where to find the keys corresponding to our subject of interest, we use the `getBM` function to extract the information we selected:

```
goi=c('ENSRNOG00000012586', 'ENSRNOG00000018113') #genes of interest
getBM(attributes=c('ensembl_gene_id', 'strand',
                  'chromosome_name', 'start_position', 'end_position'),
      filters = 'ensembl_gene_id',
      values = goi, mart = rattus)

##      ensembl_gene_id strand chromosome_name start_position end_position
## 1 ENSRNOG00000012586    -1              5      172077282    172078760
## 2 ENSRNOG00000018113     1             17      66548818     66551947
```

Note that when *ENSEMBL* is used as *Mart*, other functions are available, such as `getSequence` and `getGene` which are wrappers to `GetBM`.

The `select` method presented previously also works on *Mart* objects:

```
select(rattus, keys=goi, keytype='ensembl_gene_id',
      columns=c('ensembl_gene_id', 'strand',
                'chromosome_name', 'start_position', 'end_position'))

##      ensembl_gene_id strand chromosome_name start_position end_position
## 1 ENSRNOG00000012586    -1              5      172077282    172078760
## 2 ENSRNOG00000018113     1             17      66548818     66551947
```

The vignette of the *biomaRt* provides several practical examples of queries to Biomart resources.

7.5 The *GEOquery* and *SRADB* packages

The *GEOquery* package [21] retrieves data from the Gene Expression Omnibus (*GEO*) repository and the *SRADB* package retrieves data from the Short Read Archive (*SRA*) repository. These resources are of primary importance for functional genomics and contain thousands of datasets. *SRADB* also provides some functions to open and interact with the Integrative Genome Browser (*IGV*) which are not illustrated here.

Extracting data from *GEO*.

In *GEO*, the data are organized as

- Platforms (GPLxxx identifiers): info on microarray designs and sequencing platforms
- Samples (GSMxxx identifiers): sample-level data and protocols
- Series (GSExxx identifiers): data and information from a same experiment/project
- Dataset (GDSxxx identifiers): statistically comparable data obtained from a unique platform

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The [GEOmetadb](#) package (not used here) aims at facilitating the search for relevant entries in the GEO database.

To automatically download all the raw data from a GEO entry (files can be huge!) in a new folder created in your working directory, use:

```
library(GEOquery)
RawGSE13149=getGEOSuppFiles('GSE13149') #!! large files !!
```

The [getGEO](#) allows to retrieve different types of data from GEO (GPL, GSM, GSE, GDS). Here, for a Series, the data are downloaded to a temporary directory and stored in R as an *ExpressionSet*.

```
gse13149=getGEO('GSE13149')
show(gse13149) #Here only one Expression Set
GEOset=gse13149[[1]] #get the Expression Set
```

[GEOquery](#) is essentially dedicated to microarray data. While [GEO](#) microarray datasets are imported as relevant *ExpressionSet* and *limma* *MAList* objects, NGS data cannot be imported with [GEOquery](#). Note that the [ChIPseeker](#) provides interesting functions to download bed files from ChIP-seq experiment directly from [GEO](#).

Extracting data from SRA.

On the other hand, the [SRADB](#) package is dedicated to retrieve NGS datasets from the [NCBI Short Read Archive](#), many of which are also present in [GEO](#).

The data available in SRA are:

- Studies (SRP/ERP/DRP identifiers): metadata describing a sequencing project or study
- Experiment (SRX/ERX/DRX identifiers): metadata describing the libraries, platform selection and protocols used in a particular sequencing experiment. An experiment may contain several Runs.
- Run (SRR/ERR/DRR identifiers): sequencing data for a particular sequencing experiment
- Sample (SRS/ERS/DRS identifiers): metadata describing the physical sample that has been the subject of the sequencing process
- Analysis (SRZ identifiers): A BAM file resulting from an analysis and the metadata describing the analysis performed

All these 5 types of objects are also regrouped in a virtual container called *Accession* (SRA/ERA/DRA identifiers) which are used to track the submission.

The [SRADB](#) provides an SRADB SQLite file updated regularly which allows to query the database and find relevant content. The corresponding file can be downloaded and uncompressed using:

```
library(SRADb)
sqlfile = 'SRAMetadb.sqlite'
if(!file.exists('SRAMetadb.sqlite')) sqlfile <- getSRADBFile() #large file!!
```

To create a connection to the file use:

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```
sra_con = dbConnect(SQLite(),sqlfile)
```

Explore the content of the database:

```
dbListTables(sra_con) #Tables available in the database
dbListFields(sra_con,"study") #Fields for the study table
colDesc=colDescriptions(sra_con=sra_con) #Description of the fields
```

colDesc contains a description of the different fields and their default values.

Query the database using SQL:

```
rs = dbGetQuery(sra_con,"select * from study limit 3")
rs[, 1:3]
##      study_ID      study_alias study_accession
## 1          1          DRP000001      DRP000001
## 2          2          DRP000002      DRP000002
## 3          3 DLD1_normoxia_nucleosome      DRP000003
```

More examples in the [SRADB](#) vignette.

Convert SRA identifiers and get their links. For examples, which runs and samples are associated with this study?:

```
sraConvert( 'SRP001007', sra_con = sra_con )
##      study submission      sample experiment      run
## 1 SRP001007 SRA009276 SRS004650 SRX007396 SRR020740
## 2 SRP001007 SRA009276 SRS004650 SRX007396 SRR020739
```

Queries with `(getSRA)`:

```
rs = getSRA( search_terms = "RNASeq",
             out_types = c('study'), sra_con )
dim(rs)
## [1] 1172 12
head(rs[,1:2])
##      study_alias      study
## 1 DRP000366 DRP000366
## 2 PRJDB2653 DRP000375
## 3 PRJDB2395 DRP000376
## 4 PRJDB2135 DRP000535
## 5 PRJDB2122 DRP000536
## 6 PRJDB2739 DRP000537
```

More examples in the [SRADB](#) vignette.

The ftp addresses of the files can be obtained with:

```
listSRAfile( c("SRX000122"), sra_con, fileType = 'sra' )
#or: getSRAinfo("SRX000122",sra_con,sraType="sra")
```

The function `getSRAfile` allows to download SRA accessions from [NCBI SRA](#) and Fastq files from [EBI ENA](#).

Disconnect from the database with:

```
dbDisconnect(sra_con)
```

8 Visualization of genomic data

8.1 Introduction to *R* graphics for genomic data

Visualizing NGS data in specific genomic context is an excellent, although not sufficient, mean to assess data quality or the robustness and specificity of findings obtained from data analysis. A number of [Genome Browsers](#) are available for this task, such as [UCSC Genome Browser](#), [Gbrowse](#) or [IGV](#). They allow to interactively 'navigate' the genome and to display several annotation and data types. However, batch extraction of genome views is not always available. Here, we briefly illustrate some tools available in *Bioconductor* to assemble data in genome browser-like plots.

R is quite famous for the excellent quality of its graphics and its graphical capabilities in general (see [CRAN Task view on graphics](#)). *R* base graphics already provide a number of functionalities. The [lattice](#) package has provided a complementary approach. Later, the [ggplot2](#) package [22] has implemented the concepts presented in the book 'The Grammar of Graphics' [23] and is now widely used (see an example at paragraph 4.3). The [ggvis](#) further adds interactivity and web graphics to [ggplot2](#).

In *Bioconductor* specific packages have been developed to plot genomic data and annotations (see [Bioconductor Visualization View](#)), in particular:

- [Gviz](#) which is illustrated in paragraph 8.2
- [ggbio](#) [24] which extends [ggplot2](#) to genomic data visualization
- [genomation](#) which has some interesting functionalities to plot heatmaps and average profiles
- [Sushi](#), which is particularly good to generate multipanel figures

Interactive graphs are increasingly being developed notably based on [shiny](#).

8.2 The *Gviz* package

The [Gviz](#) package vignette is extremely well documented. Here we only briefly illustrate some basic functionalities but the package allows much more and protein-related tracks can also be added using the [Pviz](#) package. [Gviz](#) defines a set of classes which support a wide range of standard formats (see Table 1) and can be created from the typical *Bioconductor* objects used for genomic data (see Table 2).

The first step is to define the region we are interested in. We are going to focus on the region of *Drosophila melanogaster* chromosome 4 previously defined in paragraph 5.2.1. First we define a *GRanges* object corresponding to this region:

Gviz class	File type	Extension	Details
Annotation Track	BED	.bed	Fields <code>chrom</code> , <code>chromStart</code> , <code>chromEnd</code> , <code>strand</code> , <code>name</code> and <code>itemRgb</code> used only (first 3 fields are mandatory)
	GFF	.gff, .gff1	Fields <code>seqname</code> , <code>start</code> , <code>end</code> , <code>strand</code> , <code>feature</code> and <code>group</code> are recognized
	GFF2	.gff2	Same as above. Feature grouping can be provided as <code>Group</code> <code>Parent</code> attribute
	GFF3	.gff3	Same as above but feature grouping information has to be provided as the <code>Parent</code> attribute
	BAM	.bam	Streaming available. <code>Start</code> , <code>end</code> and <code>strand</code> information for reads are used. Reads ids are used for track item grouping
GeneRegion Track	GTF	.gtf	Gene, transcript and exon ids and names can be parsed from <code>gene_id</code> , <code>gene_name</code> , <code>transcript_id</code> , <code>transcript_name</code> , <code>exon_id</code> , <code>exon_name</code> attributes
	GFF	.gff, .gff1	Supports very limited grouping. Not adapted to encode complete gene models.
	GFF2	.gff2	Same as GTF. Files could be renamed .gtf
	GFF3	.gff3	The gene-to-transcript and transcript-to-exon relationships are coded in the <code>parent</code> and <code>type</code> attribute. Most gff3 variants supported.
DataTrack Track	BedGraph WIG BigWig BAM	.bedGraph .wig .bigWig, .bw .bam	Streaming available. Streaming available. Read coverage only is extracted from the BAM file.
Sequence Track	FASTA	.fa, .fasta	Streaming available only if an index file is found in the same directory as the fasta file.
	2Bit	.2bit	Streaming available.
Alignments Track	BAM	.bam	Streaming available. Always needs an index file in the same directory as the BAM file

Table 1: Gviz Track classes and standard NGS formats

Gviz class	Bioconductor class
Annotation Track	<i>data.frame</i>
	<i>IRanges</i>
	<i>GRanges</i>
	<i>GRangesList</i>
GeneRegion Track	<i>data.frame</i>
	<i>IRanges</i>
	<i>GRanges</i>
	<i>GRangesList</i>
	<i>TxDb</i>
DataTrack Track	<i>data.frame</i>
	<i>IRanges</i>
	<i>GRanges</i>
Sequence Track	<i>DNAStringSet</i>
	<i>BSgenome</i>

Table 2: Gviz Track classes and standard Bioconductor objects

```
R0I=GRanges(seqnames="chr4", ranges=which$chr4[1], strand="*")
```

Let's search for the motif 'TATAAA' in this region:

```
TATAAA_on_R0I=shift(union(ranges(matchPattern('TATAAA',
                                             subseq(Dmelanogaster$chr4, start=start(R0I), end=end(R0I)))),
                           ranges(matchPattern('TTTATA',
                                             subseq(Dmelanogaster$chr4, start=start(R0I), end=end(R0I))))),
                    start(R0I))
TATAAAgr=GRanges(seqnames="chr4",
                 ranges=TATAAA_on_R0I,
                 strand="*")
```

From this *GRanges* object, we can define an *AnnotationTrack*:

```
atrack=AnnotationTrack(TATAAAgr, name="TATAAA motif")
```

Next, we create a genome axis (i.e. genomic coordinates) track:

```
gtrack=GenomeAxisTrack()
```

An ideogram of the chromosome can be downloaded from UCSC using:

```
itrack=IdeogramTrack(genome="dm3", chromosome="chr4")
#I'm having some unresolved issues with these ideograms so I don't use them below
```

Let's take a look at our plot at this point (Figure 15):

```
plotTracks(list(gtrack, atrack)) #add itrack if possible
```

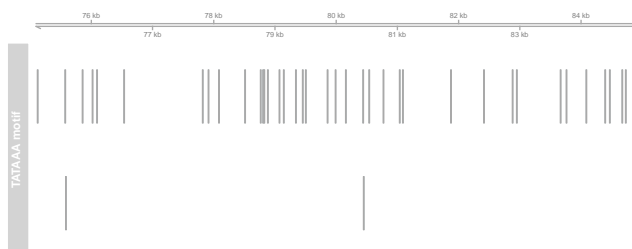


Figure 15: Genome axis and TATAAA Annotationtracks

Then, we import Gene models from the *TxDb.Dmelanogaster.UCSC.dm3.ensGene* using:

```
grtrack=GeneRegionTrack(TxDb.Dmelanogaster.UCSC.dm3.ensGene,
                        start=start(R0I),
                        end=end(R0I),
                        genome="dm3", chromosome="chr4",
                        name="Gene Model")
```

and sequence information using *BS.genome.Dmelanogaster.UCSC.dm3*:

```
strack=SequenceTrack(Dmelanogaster, chromosome="chr4")
```

This gives Figure 16 where the sequence track is not visible (too small):

```
plotTracks(list(gtrack,atrack,grtrack,strack))
```

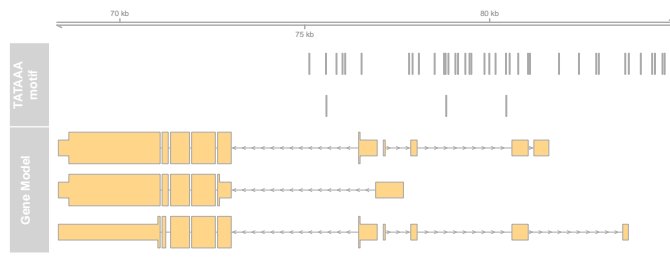


Figure 16: Visualizing Gene and Sequence Tracks

We can zoom in and out using for example (Figure 17):

```
plotTracks(list(gtrack,atrack,grtrack),
            extend.left = 0.5, extend.right = 10000)
```

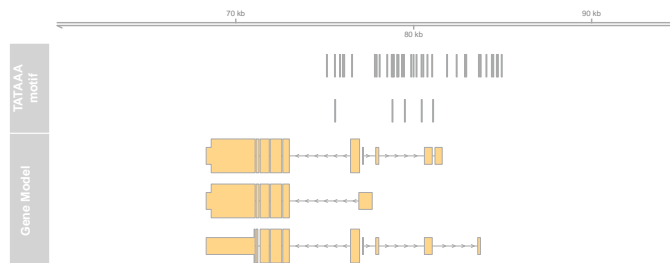


Figure 17: Zooming in and out

or (Figure 18):

```
plotTracks(list(gtrack,atrack,strack),
            from = 79900, to = 80100)
```

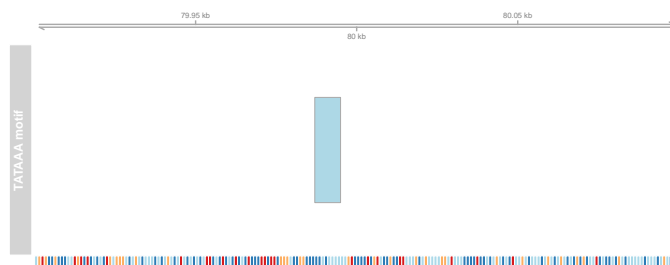


Figure 18: Zooming in

If we zoom enough we get the actual sequence (Figure 19):

```
plotTracks(list(gtrack,atrack,strack),
            from = 79975, to = 80015)
```

Now we import some paired-end reads from a BAM file:

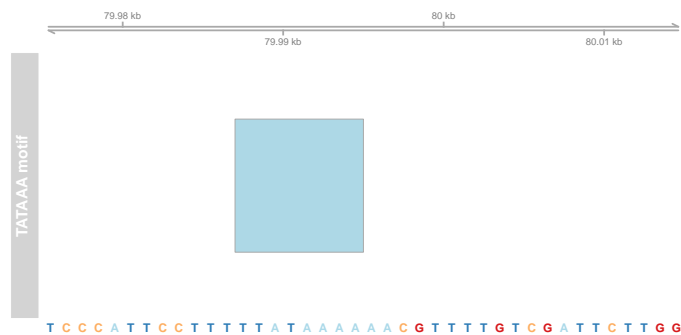


Figure 19: Zooming in some more to read the sequence

```
altrack=AlignmentsTrack(pr_bamFile, isPaired=TRUE)
```

And visualize these reads (Figure 20):

```
plotTracks(list(gtrack,atrack,
               grtrack,altrack)) #use type="coverage" to see only the coverage
```

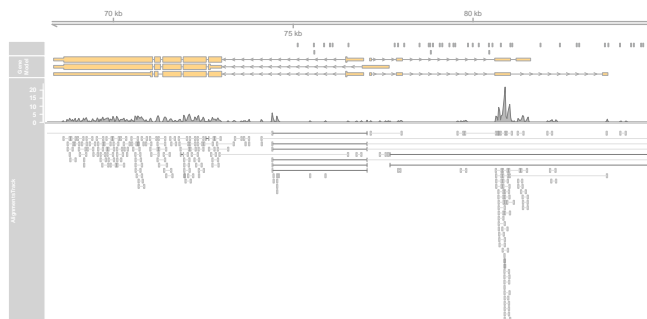


Figure 20: Visualizing Alignment Tracks

With a zoom and some more options (Figure 21):

```
plotTracks(list(gtrack,atrack,grtrack,altrack,stack),
           from=72000,to=73500,
           col.mates="purple",
           col.gaps="orange")
```

Gviz has lots of functionalities to plot quantitative data associated to genomic positions (*DataTrack*). Here we provide a simple illustration based on simulated data:

```
set.seed(255)
lim <- c(start(ROI), end(ROI))
coords <- sort(c(lim[1], sample(seq(from=lim[1], to=lim[2]), 99), lim[2]))
c1=runif(100, min=-10, max=8)
c2=runif(100, min=-10, max=8)
dat=GRanges(seqnames="chr4",strand="*",
            ranges=IRanges(start=coords[-length(coords)], end=coords[-1]),
```


BioC for NGS data analysis

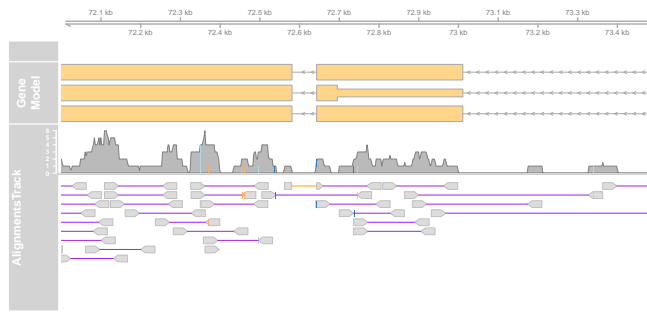


Figure 21: Visualizing Alignment Tracks

```
ctrl1=c1,ctrl2=c2,
treated1=c1+rnorm(100,3),
treated2=c2+rnorm(100,3))
genome(dat)="dm3"
dtrack <- DataTrack(dat,name="Uniform")
```

Which gives Figure 22

```
plotTracks(list(gtrack, atrack, grtrack, dtrack),
  from=lim[1], to=lim[2], type=c("a","p"),
  groups=rep(c("ctrl", "treated"), each=2))
```

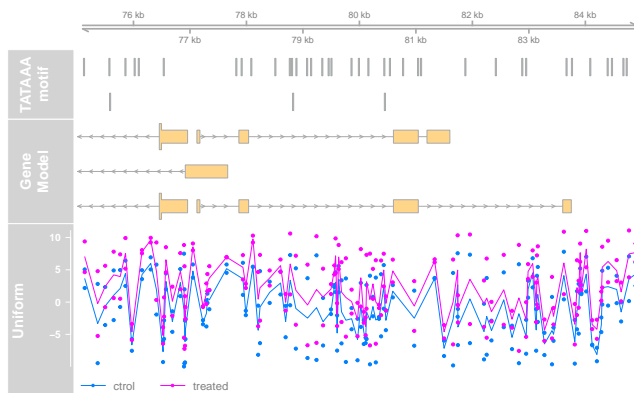


Figure 22: Visualizing Data Tracks

The [Gviz](#) package offers many more functionalities that are well illustrated in the [package vignette](#).

9 Session info

- R version 3.4.4 (2018-03-15), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=fr_FR.UTF-8, LC_NUMERIC=C, LC_TIME=fr_FR.UTF-8, LC_COLLATE=fr_FR.UTF-8, LC_MONETARY=fr_FR.UTF-8, LC_MESSAGES=fr_FR.UTF-8, LC_PAPER=fr_FR.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=fr_FR.UTF-8, LC_IDENTIFICATION=C
- Running under: Ubuntu 14.04.5 LTS
- Matrix products: default
- BLAS: /usr/lib/libblas/libblas.so.3.0
- LAPACK: /usr/lib/lapack/liblapack.so.3.0
- Base packages: base, datasets, graphics, grDevices, grid, methods, parallel, stats, stats4, utils
- Other packages: ade4 1.7-11, AnnotationDbi 1.40.0, AnnotationHub 2.10.1, Biobase 2.38.0, BiocGenerics 0.24.0, BiocParallel 1.12.0, BiocStyle 2.6.1, biomaRt 2.34.2, Biostrings 2.46.0, bitops 1.0-6, BSgenome 1.46.0, BSgenome.Dmelanogaster.UCSC.dm3 1.4.0, BSgenome.Dmelanogaster.UCSC.dm3.masked 1.3.99, DelayedArray 0.4.1, drosophila2cdf 2.18.0, drosophila2.db 3.2.3, drosophila2probe 2.18.0, GenomInfoDb 1.14.0, GenomicAlignments 1.14.2, GenomicFeatures 1.30.3, GenomicRanges 1.30.3, GEOquery 2.46.15, ggplot2 3.0.0, GO.db 3.5.0, graph 1.56.0, grImport 0.9-0, Gviz 1.22.3, hom.Dm.inp.db 3.1.2, IRanges 2.12.0, knitr 1.20, matrixStats 0.53.1, MMDiffBamSubset 1.14.0, MotifDb 1.20.0, motifStack 1.22.4, MotIV 1.34.0, org.Dm.eg.db 3.5.0, pasillaBamSubset 0.16.0, RCurl 1.95-4.11, Rqc 1.12.0, Rsamtools 1.30.0, RSQLite 2.1.0, rtracklayer 1.38.3, S4Vectors 0.16.0, seqLogo 1.44.0, ShortRead 1.36.1, SRADB 1.40.0, SummarizedExperiment 1.8.1, TxDb.Dmelanogaster.UCSC.dm3.ensGene 3.2.2, XML 3.98-1.11, XVector 0.18.0
- Loaded via a namespace (and not attached): acepack 1.4.1, AnnotationFilter 1.2.0, assertthat 0.2.0, backports 1.1.2, base64enc 0.1-3, bindr 0.1.1, bindrcpp 0.2.2, BiocInstaller 1.28.0, biovizBase 1.26.0, bit 1.1-12, bit64 0.9-7, blob 1.1.1, checkmate 1.8.5, cluster 2.0.7-1, colorspace 1.3-2, compiler 3.4.4, crayon 1.3.4, curl 3.2, data.table 1.11.0, DBI 1.0.0, dichromat 2.0-0, digest 0.6.15, dplyr 0.7.4, ensemblDb 2.2.2, evaluate 0.10.1, foreign 0.8-70, Formula 1.2-2, GenomInfoDbData 1.0.0, GenomicFiles 1.14.0, glue 1.3.0, gridExtra 2.3, gtable 0.2.0, highr 0.6, Hmisc 4.1-1, hms 0.4.2, htmlTable 1.11.2, htmltools 0.3.6, htmlwidgets 1.2, httpuv 1.4.1, httr 1.3.1, hwriter 1.3.2, interactiveDisplayBase 1.16.0, later 0.7.2, lattice 0.20-35, latticeExtra 0.6-28, lazyeval 0.2.1, limma 3.34.9, magrittr 1.5, markdown 0.8, MASS 7.3-50, Matrix 1.2-14, memoise 1.1.0, mime 0.5, munsell 0.5.0, nnet 7.3-12, pillar 1.2.2, pkgconfig 2.0.1, plyr 1.8.4, prettyunits 1.0.2, progress 1.2.0, promises 1.0.1, ProtGenerics 1.10.0, purrr 0.2.5, R6 2.2.2, RColorBrewer 1.1-2, Rcpp 0.12.18, readr 1.1.1, reshape2 1.4.3, rGADEM 2.26.0, rlang 0.2.2, rmarkdown 1.9, RMySQL 0.10.15, rpart 4.1-13, rprojroot 1.3-2, rstudioapi 0.7, scales 1.0.0, shiny 1.0.5, splines 3.4.4, splitstackshape 1.4.4, stringi 1.2.4, stringr 1.3.1, survival 2.42-3, tibble 1.4.2, tidyr 0.8.0, tools 3.4.4, VariantAnnotation 1.24.5, withr 2.1.2, xml2 1.2.0, xtable 1.8-2, yaml 2.1.19, zlibbioc 1.24.0

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