Basics on Analyzing Next Generation Sequencing Data with R and Bioconductor

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Overview

String Handling Utilities in R’s Base Distribution

Sequence Handling with Bioconductor

Range Operations

Exercises
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Exercises
Biosequence Analysis in R and Bioconductor

R Base

- Some basic string handling utilities. Wide spectrum of numeric data analysis tools.

Bioconductor

- Bioconductor packages provide much more sophisticated string handling utilities for sequence analysis.
  - Biostrings: general sequence analysis environment
  - ShortRead: pipeline for short read data
  - IRanges: low-level infrastructure for range data
  - GenomicRanges: high-level infrastructure for range data
  - BSgenome: genome annotation data
  - biomaRt: interface to BioMart annotations
  - rtracklayer: Annotation imports, interface to online genome browsers

Interface for non-R sequence analysis tools

- e.g. short read aligners
Outline

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Exercises
Basic String Matching and Parsing

String matching.

```r
myseq <- c("ATGCAGACATAGTG", "ATGAACATAGATCC", "GTACAGATCAC") # Sample sequence data set.
myseq[grep("AT", myseq)] # String searching with regular expression support.
[1] "ATGCAGACATAGTG" "ATGAACATAGATCC"
```

```r
pos1 <- regexpr("AT", myseq) # Searches 'myseq' for first match of pattern "AT".
as.numeric(pos1); attributes(pos1)$match.length # Returns position information of matches.
[1] 1 1 7
[1] 2 2 2
```

```r
pos2 <- gregexpr("AT", myseq) # Searches 'myseq' for all matches of pattern "AT".
as.numeric(pos2[[1]]); attributes(pos2[[1]])$match.length # Returns positions of matches in first sequence.
[1] 1 9
[1] 2 2
```

```r
gsub("^ATG", "atg", myseq) # String substitution with regular expression support.
[1] "atgCAGACATAGTG" "atgAACATAGATCC" "GTACAGATCAC"
```

Positional parsing.

```r
nchar(myseq) # Computes length of strings.
[1] 14 14 11
```

```r
substring(myseq[1], c(1,3), c(2,5)) # Positional parsing of several fragments from one string.
[1] "AT" "GCA"
```

```r
substring(myseq, c(1,4,7), c(2,6,10)) # Positional parsing of many strings.
[1] "AT" "AAC" "ATCA"
```
Random Sequence Generation

Create any number of random DNA sequences of any length.

```r
> rand <- sapply(1:100, function(x) paste(sample(c("A","T","G","C"), sample(10:20), replace=T), collapse=""))
> rand[1:3]

[1] "GAAGGCAAGAG" "TGCTAATGGTTGGG" "ACACTGGCGACTTACC"
```

Enumerate sequences to check for duplicates.

```r
> table(c(rand[1:4], rand[1]))

ACACTGGCGACCTACC CGCTAATGGCTGAAA GAAGGCAAGAG TGCTAATGGTTGGG
1 1 2 1
```

Extract any number of pseudo reads from the following reference. Note: this requires Biostrings.

```r
> library(Biostrings)
> ref <- DNAString(paste(sample(c("A","T","G","C"), 100000, replace=T), collapse=""))
> randstart <- sample(1:(length(ref)-15), 1000)
> randreads <- Views(ref, randstart, width=15)
> rand_set <- DNAStringSet(randreads)
> unlist(rand_set)

15000-letter "DNAString" instance
seq: ACAACTCCCATTTGACAATAAGCCGGAATGCTGTCACACCACTATTGCGGTCAGTAAGGTACAGTACGACGTATACCAGACACCGAAGAGATTGCAAGATCAG
```
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Important Data Objects in Biostrings

**XString for single sequence**
- **DNAString**: for DNA
- **RNAString**: for RNA
- **AAString**: for amino acid
- **BString**: for any string

**XStringSet for many sequences**
- **DNAStringSet**: for DNA
- **RNAStringSet**: for RNA
- **AAStringSet**: for amino acid
- **BStringSet**: for any string

**QualityScaleXStringSet for many sequences plus quality data**
- **QualityScaledDNAStringSet**: for DNA
- **QualityScaledRNAStringSet**: for RNA
- **QualityScaledAAStringSet**: for amino acid
- **QualityScaledBStringSet**: for any string
Download the following sequences to your current working directory and then import them into R:

```r
> myseq <- readDNAStringSet("AE004437.ffn")
> myseq[1:3]

A DNAStringSet instance of length 3
   width  seq
[1] 1206 ATGACTCGCGGTCTCGTGCGTCGGTGCCGGCCTCGAGCAGCCATTTGACTTGCCCTGGCGCTCGGTGCTGCCGTCCGATTTGCGGCGGCGCAG...AGCGGTGGCGGGTTACCGCTGTTCAAGATCGGGGGCGCTGTCGCTGTGATTGCGATCGTCGTCGTCGTTGTTCGACGCTGGCGGAACCCATGA gb|AE004437.1|:24...
[2] 666 ATGAGCATCATCGGAACTCGAAGGCGTGGTCAAACGGTACGAAACCGGTGCCGAGACAGTCGAGGCGCTGAAAGGCGTTGACTTCTCGGCGGCG...AACATCGCCGTGGTTGCGATCACTCACGACACGCAACTCGAGGAGTTCTCCGACCGCGCAGTCAACCTCGTCGATGGGGTGTTACACACGTGA gb|AE004437.1|:14...
[3] 1110 ATGGCGTGGCGGAACCTCGGGCGGAACCGCGTGCGGACTGCGCTGGCCGCGCTCGGGATCGTGATCGGTGTGATCTCGATCGCATCGATGGGG...TTCCTGTTCGCGGTCTTCGCCAGCCTGCTCAGCGGGCTCTATCCGGCGTGGAAAGCAGCCAACGATCCGCCCGTCGAGGCGCTCGGCGAATGA gb|AE004437.1|:21...

> sub <- myseq[grep("99.*", names(myseq))]
> length(sub)

[1] 185

> writeXStringSet(sub, file="AE004437sub.ffn", width=80)

Open exported sequence file AE004437sub.ffn in a text editor.
Working with XString Containers

The XString stores the different types of biosequences in dedicated containers:

```r
> library(Biostrings)
> d <- DNAString("GCATAT-TAC")
> d

10-letter "DNAString" instance
seq: GCATAT-TAC

> d[1:4]

4-letter "DNAString" instance
seq: GCAT

> r <- RNAString("GCAUAU-UAC")
> r <- RNAString(d) # Converts d into RNAString object.
> p <- AAString("HCWYHH")
> b <- BString("I store any set of characters. Other XString objects store only the IUPAC characters.")
```
Working with XStringSet Containers

XStringSet containers allow to store many biosequences in one object:

```r
> dset <- DNAStringSet(c("GCATATTAC", "AATCGATCC", "GCATATTAC"))
> names(dset) <- c("seq1", "seq2", "seq3") # Assigns names
> dset[1:2]

A DNAStringSet instance of length 2
  width seq
[1]  9 GCATATTAC
[2]  9 AATCGATCC

> width(dset) # Returns the length of each sequences
[1]  9  9  9

> d <- dset[[1]] # The [[ subsetting operator returns a single entry as XString object
> dset2 <- c(dset, dset) # Appends/concatenates two XStringSet objects
> dsetchar <- as.character(dset) # Converts XStringSet to named vector
> dsetone <- unlist(dset) # Collapses many sequences to a single one stored in a DNAString container

Sequence subsetting by positions:

> DNAStringSet(dset, start=1:2, end=4:5)

A DNAStringSet instance of length 3
  width seq
[1]  4 GCAT
[2]  7 ATCGATC
[3]  3 ATA
The XMultipleAlignment class stores the different types of multiple sequence alignments:

```
> origMAlign <- read.DNAMultipleAlignment(filepath = system.file("extdata",
+ "msx2_mRNA.aln", package = "Biostrings"), format = "clustal")
> origMAlign
DNAMultipleAlignment with 8 rows and 2343 columns

[1] -----TCCCGTCTCCGAGCAAAAAAGTTTGAGTCGCCGCTGCCGGGTTGCCAGCGGAGTCGCGCGTCGGGAGCTACGTAGGGCAGAGAAGTCA-T...GAAGAGTTATCTCTTATTCTGAATT--AAATTAAGC--ATTTGTTTTATTGCAGTAAAGTTTGTCCAAACTCACAATTAAAAAAAAAAAAAAAAA
[8] ---------------------------------------------------------------------------------------------A-T...-----------------------------------------------------------------------------------------------
```
Basic Sequence Manipulations

Complement, reverse, and reverse & complement of sequences:

```r
> randset <- DNAStringSet(rand)
> complement(randset[1:2])

A DNAStringSet instance of length 2
  width seq
[1]  11 CTTCCGTTCTC
[2]  14 ACGATTACAAACCC
```

```r
> reverse(randset[1:2])

A DNAStringSet instance of length 2
  width seq
[1]  11 GAGAACGGAAG
[2]  14 GGGTTTGTAATCGT
```

```r
> reverseComplement(randset[1:2])

A DNAStringSet instance of length 2
  width seq
[1]  11 CTCTTGCCTTC
[2]  14 CCCAAACATTAGCA
```

Translate DNA sequences into proteins:

```r
> translate(randset[1:2])

A AAStringSet instance of length 2
  width seq
[1]  3 EGK
[2]  4 C*CL
Pattern Matching

Pattern matching with mismatches

> mypos <- matchPattern("ATGGTG", myseq1[[1]], max.mismatch=1) # Finds pattern matches in reference
> countPattern("ATGGCT", myseq1[[1]], max.mismatch=1) # Counts only the corresponding matches
> tmp <- c(DNAStringSet("ATGGTG"), DNAStringSet(m ypos)) # Results sho en in DNAStringSet object
> consensusMatrix(tmp) # Returns a consensus matrix for query and hits.
> myvpos <- vmatchPattern("ATGGCT", myseq1, max.mismatch=1) # Finds all pattern matches in reference
> myvpos # The results are stored as MIndex object.
> Views(myseq1[[1]], start(myvpos[[1]]), end(myvpos[[1]])) # Retrieves the result for single entry
> sapply(seq(along=myseq1), function(x)
+ as.character(Views(myseq1[[x]], start(myvpos[[x]]), end(myvpos[[x]])))) # All matches.

Pattern matching with regular expression support

> myseq <- DNAStringSet(c("ATGCAGACATAGTG", "ATGAACATAGATCC", "GTACAGATCAC"))
> myseq[grep("ATG", myseq, perl=TRUE)] # String searching with regular expression support
> pos1 <- regexpr("AT", myseq) # Searches 'myseq' for first match of pattern "AT"
> as.numeric(pos1); attributes(pos1)$match.length # Returns position information of matches
> pos2 <- gregexpr("AT", myseq) # Searches 'myseq' for all matches of pattern "AT"
> as.numeric(pos2[[1]]); attributes(pos2[[1]])$match.length # Match positions in first sequence
> DNAStringSet(gsub("~ATG", "NNN", myseq)) # String substitution with regular expression support
> pwm <- PWM(DNAStringSet(c("GCT", "GGT", "GCA")))
> library(seqLogo); seqLogo(t(t(pwm) * 1/colSums(pwm)))

Views on a 21-letter DNAString subject
subject: AAAGCTAAAGGTAAAGCAAAA
views:
    start  end  width   [sequence]
[1]    4    6     3 [GCT]
[2]   10  12     3 [GGT]
[3]   16  18     3 [GCA]
Sequence and Quality Data: FASTQ Format

4 lines per sequence
1. ID
2. Sequence
3. ID
4. Base call qualities (Phred scores) as ASCII characters

Example of 3 Illumina reads in FASTQ format:

```
@SRR038845.3  HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCCACACCTTGCGGCACAGGCCGGGTTAA
+SRR038845.3  HWI-EAS038:6:1:0:1938 length=36
BA@7>7@B=>:>>7@7@>>9=BAA?=52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTCCGTTTTCAAGATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAATAAATTGTGTCATTAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBBB@@BAB?BBBBCBC>BBBAA@BBBAA@
```
Phred quality scores are integers from 0-50 that are stored as ASCII characters after adding 33. The basic R functions `rawToChar` and `charToRaw` can be used to interconvert among their representations.

```r
> phred <- 1:9
> phreda <- paste(sapply(as.raw((phred)+33), rawToChar), collapse=""); phreda
[1] "\"#\$\&\('\)\*"
> as.integer(charToRaw(phreda))-33
[1] 1 2 3 4 5 6 7 8 9
```

```r
dset <- DNAStringSet(sapply(1:100, function(x) paste(sample(c("A","T","G","C"), 20, replace=T), collapse=""))) # Creates random sample sequence.
myqlist <- lapply(1:100, function(x) sample(1:40, 20, replace=T)) # Creates random Phred score list.
myqual <- sapply(myqlist, function(x) toString(PhredQuality(x))) # Converts integer scores into ASCII characters.
myqual <- PhredQuality(myqual) # Converts to a PhredQuality object.
dsetq1 <- QualityScaledDNAStringSet(dset, myqual) # Combines DNAStringSet and quality data in QualityScaledDNAStringSet object.
dsetq1[1:2]
```

A QualityScaledDNAStringSet instance containing:

A DNAStringSet instance of length 2

<table>
<thead>
<tr>
<th>width</th>
<th>seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>GTGAAAAGTGTTCACTCATG</td>
</tr>
<tr>
<td>20</td>
<td>TCCATCGCGTAACATCGCG</td>
</tr>
</tbody>
</table>

A PhredQuality instance of length 2

<table>
<thead>
<tr>
<th>width</th>
<th>seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>@;7D3@#47&amp;9I-=1%A+I&lt;</td>
</tr>
<tr>
<td>20</td>
<td>.#CF7G6571/6-7&amp;e=2C0)</td>
</tr>
</tbody>
</table>
Basic usage of ShortReadQ objects. To make the following sample code work, download and unzip this file to your current working directory.

```r
> library(ShortRead)
> fastq <- list.files("data", ".*fastq$"); fastq <- paste("data/", fastq, sep="")
> names(fastq) <- paste("flowcell6_lane", 1:length(fastq), sep="_")
> (fq <- readFastq(fastq[1])) # Imports first FASTQ file

class: ShortReadQ
length: 1000 reads; width: 36 cycles

> countLines(dirPath="./data", pattern=".fastq$") # Counts numbers of reads in FASTQ files
SRR038845.fastq SRR038846.fastq SRR038848.fastq SRR038850.fastq
4000 4000 4000 4000

> id(fq)[1] # Returns ID field

A BStringSet instance of length 1
width seq

> sread(fq)[1] # Returns sequence

A DNAStringSet instance of length 1
width seq
[1] 36 CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA

> quality(fq)[1] # Returns Phred scores

class: FastqQuality
quality:
  A BStringSet instance of length 1
  width seq
[1] 36 BA@7>B=>:>>7@7@>>>9=BAA?;>52;>;9=8.=A

> as(quality(fq), "matrix")[1,1:12] # Coerces Phred scores to numeric matrix
[1] 33 32 31 22 29 33 28 29 25 29 29 22

> ShortReadQ(sread=sread(fq), quality=quality(fq), id=id(fq)) # Constructs a ShortReadQ from components

class: ShortReadQ
length: 1000 reads; width: 36 cycles
```
Quality Reports of FASTQ Files

The following `seeFastq/seeFastqPlot` functions generate and plot a series of useful quality statistics for a set of FASTQ files.

```r
> library(ggplot2)
> source("http://faculty.ucr.edu/~tgirke/Documents/R_BioCond/My_R_Scripts/fastqQuality.R")
> fqlist <- seeFastq(fastq=fastq, batchsize=800, klength=8) # For real data set batchsize to at least 10^5
> seeFastqPlot(fqlist[1:2], arrange=c(1,4,7))
```

Handles many samples in on PDF file. For more details see here [Link](#).
ShortRead contains various FASTQ quality report functions

```r
> sp <- SolexaPath(system.file('extdata', package='ShortRead'))
> fl <- file.path(analysisPath(sp), "s_1_sequence.txt")
> fls <- c(fl, fl)
> coll <- QACollate(QAFastqSource(fls), QAReadQuality(), QAAdapterContamination(),
+                   QANucleotideUse(), QAQualityUse(), QASequenceUse(), QAFrequentSequence(n=10),
+                   QANucleotideByCycle(), QAQualityByCycle())
> x <- qa2(coll, verbose=TRUE)
> res <- report(x)
> if(interactive())
+ browseURL(res)
```
Adaptor trimming

```r
> fqtrim <- trimLRPatterns(Rpattern="GCCCGGGTAA", subject=fq)
> sread(fqtrim)[1:2]

A DNAStringSet instance of length 2
  width seq
[1]  26 CAACGAGTTACACCTTGGCCGACAG
[2]  36 CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
```

Read counting and duplicate removal

```r
> tables(fq)$distribution # Counts read occurrences

           nOccurrences nReads
1          1         948
2          2          26

> sum(srduplicated(fq)) # Identifies duplicated reads

[1] 26

>fq[!srduplicated(fq)]

class: ShortReadQ
length: 974 reads; width: 36 cycles
>
Trimming low quality tails

```r
> cutoff <- sapply(as.raw((30)+33), rawToChar)
> sread(trimTails(fq, k=2, a=cutoff, successive=FALSE))[1:2]
```

A DNAStringSet instance of length 2

<table>
<thead>
<tr>
<th>width</th>
<th>seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>4 CAAC</td>
</tr>
<tr>
<td>[2]</td>
<td>20 CCAATGATTTTTTTCCGTGT</td>
</tr>
</tbody>
</table>

Removal of reads with ≥ Ns and/or low complexity segments

```r
> filter1 <- nFilter(threshold=1) # Keeps only reads without Ns
> filter2 <- polynFilter(threshold=20, nuc=c("A","T","G","C")) # Removes reads with >=20 of one nucleotide
> filter <- compose(filter1, filter2)
> fq[filter(fq)]
```

class: ShortReadQ
length: 989 reads; width: 36 cycles
Memory Efficient FASTQ Processing

Streaming through FASTQ files with FastqStreamer and random sampling reads with FastqSampler

```r
> fq <- yield(FastqStreamer(fastq[1], 50)) # Imports first 50 reads from FASTQ file
> fq <- yield(FastqSampler(fastq[1], 50)) # Random samples 50 reads from entire FASTQ file
```

Streaming through a FASTQ file while applying filtering/trimming functions and writing the results to a new file.

```r
> f <- FastqStreamer(fastq[1], 50)
> while(length(fq <- yield(f))) {
+    fqsub <- fq[grepl("^TT", sread(fq))]
+    writeFastq(fqsub, paste(fastq[1], "sub", sep="_"), mode="a")
+ }
> close(f)
```

Task 1 Write a demultiplexing function that accepts any number of barcodes and splits a FASTQ file into as many subfiles as there are barcodes. At the same time the function should remove low quality tails from the reads. The following function accomplishes the first step. Expand this function so that it performs the second step as well.

```r
> demultiplex <- function(x, barcode, nreads) {
+   f <- FastqStreamer(x, nreads)
+   while(length(fq <- yield(f))) {
+     for(i in barcode) {
+       pattern <- paste("^", i, sep="")
+       fqsub <- fq[grepl(pattern, sread(fq))]
+       if(length(fqsub) > 0) writeFastq(fqsub, paste(x, i, sep="_"), mode="a")
+     }
+   }
+   close(f)
+ }
> demultiplex(x=fastq[1], barcode=c("TT", "AA", "GG"), nreads=50)
```
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Exercises
Important Data Objects for Range Operations

- **IRanges**: stores range data only (IRanges library)
- **GRanges**: stores ranges and annotations (GenomicRanges library)
- **GRangesList**: list version of GRanges container (GenomicRanges library)
Constructing GRanges Objects

```r
> library(GenomicRanges); library(rtracklayer)
> gr <- GRanges(seqnames = Rle(c("chr1", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)),
               ranges = IRanges(1:10, end = 7:16, 
               score = 1:10, GC = seq(1, 0, length = 10)) # Example of creating a GRanges object with its constructor function.
             + asRangedData=FALSE) # Imports a simplified GFF3 genome annotation file.
> seqlengths(gff) <- end(ranges(gff[which(elementMetadata(gff)[,"type"]=='chromosome'
             + )]))
> names(gff) <- 1:length(gff) # Assigns names to corresponding slot.
> gff[1:4,]
```

GRanges with 4 ranges and 5 metadata columns:

<table>
<thead>
<tr>
<th>seqnames</th>
<th>ranges</th>
<th>strand</th>
<th>source</th>
<th>type</th>
<th>score</th>
<th>phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr1</td>
<td>[1, 30427671]</td>
<td>+</td>
<td>TAIR10</td>
<td>chromosome</td>
<td>&lt;NA&gt;</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>Chr1</td>
<td>[3631, 5899]</td>
<td>+</td>
<td>TAIR10</td>
<td>gene</td>
<td>&lt;NA&gt;</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>Chr1</td>
<td>[3631, 5899]</td>
<td>+</td>
<td>TAIR10</td>
<td>mRNA</td>
<td>&lt;NA&gt;</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>Chr1</td>
<td>[3760, 5630]</td>
<td>+</td>
<td>TAIR10</td>
<td>protein</td>
<td>&lt;NA&gt;</td>
<td>&lt;NA&gt;</td>
</tr>
</tbody>
</table>

---

seqlengths:

|Chr1|Chr2|Chr3|Chr4|Chr5|ChrC|ChrM|
|130427671|19698289|23459830|18585056|26975502|154478|366924|

```r
> gff_rd <- as(gff, "RangedData") # Coerces GRanges object to RangedData class.
> gff_gr <- as(gff_rd, "GRanges") # Coerces RangedData object to GRanges class.
```
Utilities for Range Containers

Accessor and subsetting methods for GRanges objects

> c(gff[1:2], gff[401:402]) # GRanges objects can be concatenated with the c() function.
> seqnames(gff); ranges(gff); strand(gff); seqlengths(gff) # Accessor functions
> start(gff[1:4]); end(gff[1:4]); width(gff[1:4]) # Direct access to IRanges components
> elementMetadata(gff); elementMetadata(gff)[, "type"] # Accessing metadata component.
> gff[elementMetadata(gff)[, "type"] == "gene"] # Returns only gene ranges.

Useful utilities for GRanges objects

> strand(gff) <- "*") # Erases the strand information
> reduce(gff) # Collapses overlapping ranges to continuous ranges.
> gaps(gff) # Returns uncovered regions.
> disjoin(gff) # Returns disjoint ranges.
> coverage(gff) # Returns coverage of ranges.
> findOverlaps(gff, gff[1:4]) # Returns the index pairings for the overlapping ranges.
> countOverlaps(gff, gff[1:4]) # Counts overlapping ranges
> subsetByOverlaps(gff, gff[1:4]) # Returns only overlapping ranges

GRangesList Objects

> sp <- split(gff, seq(along=gff)) # Stores every range in separate component of a GRangesList object
> split(gff, seqnames(gff)) # Stores ranges of each chromosome in separate component.
> unlist(sp) # Returns data as GRanges object
> sp[1:4, "type"] # Subsetting of GRangesList objects is similar to GRanges objects.
> lapply(sp[1:4], length); sapply(sp[1:4], length) # Looping over GRangesList objects similar to lists
Efficient Sequence Parsing with `getSeq`

The following parses all annotation ranges provided by `GRanges` object (e.g. `gff`) from a genome sequence stored in a local file.

```r
> rand <- DNAStringSet(sapply(unique(as.character(seqnames(gff))), function(x) paste(sample(c("A","T","G","C"), 200000, replace=T), collapse="")))
> writeXStringSet(DNAStringSet(rand), "/Desktop/test")
> getSeq(FaFile("~/Desktop/test"), gff)
```

A DNAStringSet instance of length 449

```
[1] 200000 TGGGGAAGGAAGATATACTTTTGTTTGCTCTGCTCCCGCCTACTCAACTCTTGCTAGATCGATCGATCTCTCCCGTGCTATTTAG...TCATCCAGGCGGGCCTCCCTGGTGAGTCCGCGACATATTGCTTAGCCAAGGCGGCTTTTGATGATGGAAGGACCAGTAGCGTAATGACCC Chr1
[2] 2269 ACGGACGTGCGGATAGAAGCGCGTCATGTGCCGACAGTTGAGGGGTCAGTGAATAATGGACGGGAGATCGTCTAGTG...GCGTATTGCATTATTTAGGTCAAGGTAATGTGAACAATCTGCTGGAGCATCTGTGGATCAAGATTTAATCCGCTGGCATCGCGCATACGAT Chr1
[3] 2269 ACGGACGTGCGGATAGAAGCGCGTCATGTGCCGACAGTTGAGGGGTCAGTGAATAATGGACGGGAGATCGTCTAGTG...GCGTATTGCATTATTTAGGTCAAGGTAATGTGAACAATCTGCTGGAGCATCTGTGGATCAAGATTTAATCCGCTGGCATCGCGCATACGAT Chr1
[4] 1871 TGGCTAGTACTCGGTTCTTTCGAAGCTATTGTATAATATACAAACACGCATCCATCAATGTGTTTTAGATGGGCTTTTAGACATTAAAGCAC...TGChr1
[5] 283 ACGGACGTGCGGATAGAAGCGCGTCATGTGCCGACAGTTGAGGGGTCAGTGAATAATGGACGGGAGATCGTCTAGTG...GCGTATTGCATTATTTAGGTCAAGGTAATGTGAACAATCTGCTGGAGCATCTGTGGATCAAGATTTAATCCGCTGGCATCGCGCATACGAT Chr1
[6] 129 ACGGACGTGCGGATAGAAGCGCGTCATGTGCCGACAGTTGAGGGGTCAGTGAATAATGGACGGGAGATCGTCTAGTG...GCGTATTGCATTATTTAGGTCAAGGTAATGTGAACAATCTGCTGGAGCATCTGTGGATCAAGATTTAATCCGCTGGCATCGCGCATACGAT Chr1
[7] 154 TGGCTAGTACTCGGTTCTTTCGAAGCTATTGTATAATATACAAACACGCATCCATCAATGTGTTTTAGATGGGCTTTTAGACATTAAAGCAC...TGChr1
[8] 281 GCAATCCAGAACATGCGACTCTGCCACAACTCGTGCGGCCTGGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChr1
[9] 281 GCAATCCAGAACATGCGACTCTGCCACAACTCGTGCGGCCTGGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChr1
... ... ...
[441] 462 AAACATAATAACTAAAGTATGCTGTGGAGGAAAATTGATGTAATTGAGGTGTTCCTAGATTGAAAGTCTCTCTAACCAGACTCCATACTCGGCGGCTGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
[442] 2568 CGATCGTGTTTTTCGAGGTAGCCAAAGTAGGCGAACCTGAGTCGATACAGCATGGTCTACTCCGGAGTCATACCTTAAGCACCAAGGAAACCATGAGGCGTATTGCACCCATTAGGAAACTAAGACTATGCTAGAATCGTAAGTGGACGTCCATCCTTTC ChrM
[443] 2568 CGATCGTGTTTTTCGAGGTAGCCAAAGTAGGCGAACCTGAGTCGATACAGCATGGTCTACTCCGGAGTCATACCTTAAGCACCAAGGAAACCATGAGGCGTATTGCACCCATTAGGAAACTAAGACTATGCTAGAATCGTAAGTGGACGTCCATCCTTTC ChrM
[444] 2568 CGATCGTGTTTTTCGAGGTAGCCAAAGTAGGCGAACCTGAGTCGATACAGCATGGTCTACTCCGGAGTCATACCTTAAGCACCAAGGAAACCATGAGGCGTATTGCACCCATTAGGAAACTAAGACTATGCTAGAATCGTAAGTGGACGTCCATCCTTTC ChrM
[445] 324 TGCCCTAACATGCCCACTTTAATACGGGTCGCAGATACATGGAGGCTGATGATGGAAGATATGGCAATTGCGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
[446] 324 TGCCCTAACATGCCCACTTTAATACGGGTCGCAGATACATGGAGGCTGATGATGGAAGATATGGCAATTGCGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
[447] 324 TGCCCTAACATGCCCACTTTAATACGGGTCGCAGATACATGGAGGCTGATGATGGAAGATATGGCAATTGCGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
[448] 324 TGCCCTAACATGCCCACTTTAATACGGGTCGCAGATACATGGAGGCTGATGATGGAAGATATGGCAATTGCGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
[449] 324 TGCCCTAACATGCCCACTTTAATACGGGTCGCAGATACATGGAGGCTGATGATGGAAGATATGGCAATTGCGTTTCCCGATATATAGCTAAATCGCGTGGGACTTCCGTTCTCAGGAGGGAA...TGChrM
```

Basics on Analyzing Next Generation Sequencing Data with R and Bioconductor
Outline

Overview

String Handling Utilities in R’s Base Distribution

Sequence Handling with Bioconductor

Range Operations

Exercises
Exercise

GFF from *Halobacterium sp* Link
Genome from *Halobacterium sp* Link

Task 2 Extract gene ranges, parse their sequences from genome and translate them into proteins
Task 3 Reduce overlapping genes and parse their sequences from genome
Task 4 Generate intergenic ranges and parse their sequences from genome

Useful commands

```r
> chr <- readDNAStringSet("AE004437.fna")
> writeLines(readLines("AE004437.gff")[-c(1:7)], "AE004437.gff2")
> gff <- import.gff("AE004437.gff2", asRangedData=FALSE)
> gffgene <- gff[elementMetadata(gff)[,"type"]=='gene']
> gene <- DNAStringSet(Views(chr[[1]], IRanges(start(gffgene), end(gffgene))))
> names(gene) <- elementMetadata(gffgene)[,"group"]
> pos <- elementMetadata(gffgene[strand(gffgene) == "+"])[,"group"]
> p1 <- translate(gene[names(gene) %in% pos])
> names(p1) <- names(gene[names(gene) %in% pos])
> neg <- elementMetadata(gffgene[strand(gffgene) == "-"])[,"group"]
> p2 <- translate(reverseComplement(gene[names(gene) %in% neg]))
> names(p2) <- names(gene[names(gene) %in% neg])
> writeXStringSet(c(p1, p2), "mypep.fasta")
```