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

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# Outline

# 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 Link: general sequence analysis environment
  - ShortRead Link: pipeline for short read data
  - IRanges Link: low-level infrastructure for range data
  - GenomicRanges Link: high-level infrastructure for range data
  - GenomicFeatures Link: managing transcript centric annotations
  - BSgenome Link: genome annotation data
  - biomaRt Link: interface to BioMart annotations
  - rtracklayer Link: Annotation imports, interface to online genome browsers

## Interface for non-R sequence analysis tools

- *e.g.* short read aligners

# Outline

# Basic String Matching and Parsing

String matching.

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

[1] "ATGCAGACATAGTG" "ATGAACATAGATCC"

> 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

> 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

> gsub("^ATG", "atg", myseq) # String substitution with regular expression support.

[1] "atgCAGACATAGTG" "atgAACATAGATCC" "GTACAGATCAC"
```

Positional parsing.

```
> nchar(myseq) # Computes length of strings.

[1] 14 14 11

> substring(myseq[1], c(1,3), c(2,5)) # Positional parsing of several fragments from one string.

[1] "AT"  "GCA"

> 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.

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

[1] "CGCCTATCCC"       "TAGCGGGGGTATGGAC" "AGGTTGAACTACA"
```

Enumerate sequences to check for duplicates.

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

   AGGTTGAACTACA       CGCCTATCCC TAGCGGGGGTATGGAC       TCATTGAAAA
               1                2                1                1
```

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

```
> 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: TTATATTTGAAGGTTAAACGCTTACCCACACGCTATTTGTAGCACAACAGATGTTGCCCGAGGCCTGTCTTACCGGCCACGCGGTTGTGTGGGTTGTGTAGGAAC
```

# Outline

# 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

# Sequence Import and Export

Download the following sequences to your current working directory and then import them into R:
ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.ffn

```
> dir.create("data")
> # system("wget ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.ffn")
> download.file("ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.ffn", "data/A
> myseq <- readDNAStringSet("data/AE004437.ffn")
> myseq[1:3]
```

```
  A DNAStringSet instance of length 3
    width seq
[1]  1206 ATGACTCGGCGGTCTCGTGTCGGTGCCGGCCTCGCAGCCATTGTACTGGCCCTGGCCGCAGTGTCGGCTGCCGCTCCGATTGCCGGGGCGCAG...AGCG
[2]   666 ATGAGCATCATCGAACTCGAAGGCGTGGTCAAACGGTACGAAACCGGTGCCGAGACAGTCGAGGCGCTGAAAGGCGTTGACTTCTCGGCGGCG...AACA
[3]  1110 ATGGCGTGGCGGAACCTCGGGCGGAACCGCGTGCGGACTGCGCTGGCCGCGCTCGGGATCGTGATCGGTGTGATCTCGATCGCATCGATGGGG...TTCC
```

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

```
[1] 170
```

```
> 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:

```
> 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:

```
> 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=c(1,2,3), end=c(4,8,5))

  A DNAStringSet instance of length 3
    width seq
[1]     4 GCAT
[2]     7 ATCGATC
[3]     3 ATA
```

# XMultipleAlignment Class

The XMultipleAlignment class stores the different types of multiple sequence alignments:

```
> origMAlign <- readDNAMultipleAlignment(filepath = system.file("extdata",
+                "msx2_mRNA.aln", package = "Biostrings"), format = "clustal")
> origMAlign

DNAMultipleAlignment with 8 rows and 2343 columns
     aln
[1] -----TCCCGTCTCCGCAGCAAAAAAGTTTGAGTCGCCGCTGCCGGGTTGCCAGCGGAGTCGCGCGTCGGGAGCTACGTAGGGCAGAGAAGTCA-T...GAAGAGT
[2] ----------------------------------------------------------------------------------------A-T...-------
[3] ----------------------------------------------------------------------------GAGAGAAGTCA-T...-------
[4] ----------------------AAAAGTTGGAGTCTTCGCTTGAGAGTTGCCAGCGGAGTCGCGCGCCGACAGCTACGCGGCGCAGA-AAGTCA-T...GAAGAGT
[5] ----------------------------------------------------------------------------------------A-T...GAAGAGT
[6] ----------------------------------------------------------------------------------------A-T...-------
[7] --------------CGGCTCCGCAGCGCCTCACTCGCGCAGTCCCCGCGCAGGGCCGGGCAGAGGCGCACGCAGCTCCCCGGGCGGCCCCGCTC-C...-------
[8] GGGGGAGACTTCAGAAGTTGTTGTCCTCTCCGCTGATAACAGTTGAGATGCGCATATTATTATTACCTTTAGGACAAGTTGAATGTGTTCGTCAAC...-------
```

# Basic Sequence Manipulations

Complement, reverse, and reverse & complement of sequences:

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

  A DNAStringSet instance of length 2
    width seq
[1]    10 GCGGATAGGG
[2]    16 ATCGCCCCCATACCTG

> reverse(randset[1:2])

  A DNAStringSet instance of length 2
    width seq
[1]    10 CCCTATCCGC
[2]    16 CAGGTATGGGGGCGAT

> reverseComplement(randset[1:2])

  A DNAStringSet instance of length 2
    width seq
[1]    10 GGGATAGGCG
[2]    16 GTCCATACCCCCGCTA
```

Translate DNA sequences into proteins:

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

  A AAStringSet instance of length 2
    width seq
[1]     3 RLS
[2]     5 *RGYG
```

# Pattern Matching

## Pattern matching with mismatches

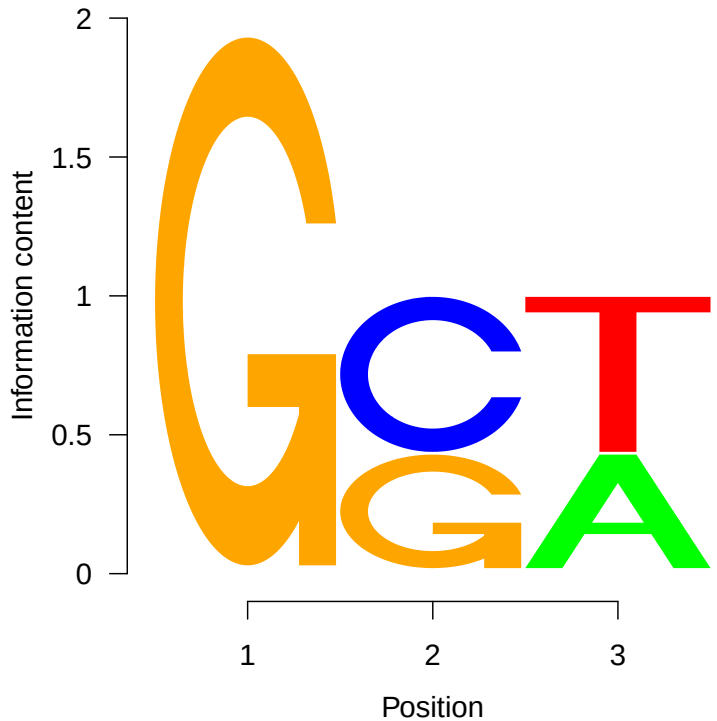
```
> myseq1 <- readDNAStringSet("ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.
> mypos <- matchPattern("ATGGTG", myseq1[[1]], max.mismatch=1) # Finds pattern matches in reference
> countPattern("ATGGCT", myseq1[[1]], max.mismatch=1) # Counts only the corresponding matches
> vcountPattern("ATGGCT", myseq1, max.mismatch=1) # Counts only the matches in many sequences
> tmp <- c(DNAStringSet("ATGGTG"), DNAStringSet(mypos)) # Results shown in DNAStringSet object
> consensusMatrix(tmp)[1:4,] # 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 Viewing and Searching

```
> pwm <- PWM(DNAStringSet(c("GCT", "GGT", "GCA")))
> library(seqLogo); seqLogo(t(t(pwm) * 1/colSums(pwm)))
```




```
> chr <- DNAString("AAAGCTAAAGGTAAAGCAAAA")
> matchPWM(pwm, chr, min.score=0.9) # Searches sequence for PWM matches with score better than min.score.
```

```
  Views on a 21-letter DNAString subject
subject: AAAGCTAAAGGTAAAGCAAAA
views:
    start end width
[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
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+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
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBB@@BAB?BBBBCBC>BBBAA8>BBBAA@
```

# Sequence and Quality Data: QualityScaleXStringSet

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.

```
> 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

> dset <- DNAStringSet(sapply(1:100, function(x) paste(sample(c("A","T","G","C"), 20, replace=T), collapse="")),
> 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 characte
> myqual <- PhredQuality(myqual) # Converts to a PhredQuality object.
> dsetq1 <- QualityScaledDNAStringSet(dset, myqual) # Combines DNAStringSet and quality data in QualityScaledDNA
> dsetq1[1:2]

  A QualityScaledDNAStringSet instance containing:
  A DNAStringSet instance of length 2
    width seq
[1]    20 CCCTAATCGAGGGCATTGCA
[2]    20 TAGCTATAGACATGCATTTG

  A PhredQuality instance of length 2
    width seq
[1]    20 %,3:(D*$(&,)IE<#*1-2
[2]    20 #<<F<>H:G6D9I)3<F<I?
```

# Processing FASTQ Files with ShortRead

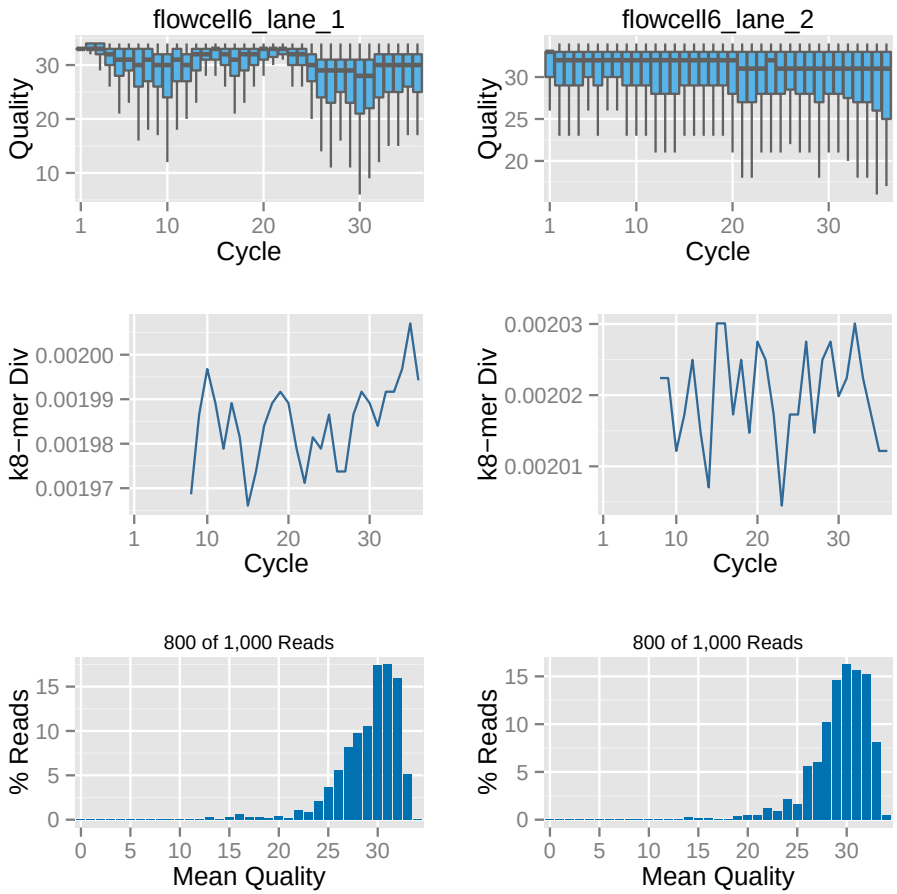
Basic usage of `ShortReadQ` objects. To make the following sample code work, download and unzip this file Link to your current working directory.

```
> library(ShortRead)
> fastq <- list.files("data", "*.fastq$"); fastq <- paste("data/", fastq, sep="")
> names(fastq) <- paste("flowcell6_lane", 1:length(fastq), sep="_")
> (fq <- readFastq(fastq[1])) # Imports first FASTQ file
class: ShortReadQ
length: 1000 reads; width: 36 cycles
> countLines(dirPath="./data", pattern=".fastq$")/4 # Counts numbers of reads in FASTQ files
SRR038845.fastq SRR038846.fastq SRR038848.fastq SRR038850.fastq
           1000            1000            1000            1000
> id(fq)[1] # Returns ID field
  A BStringSet instance of length 1
    width seq
[1]    43 SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
> 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.

```
> 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

# Quality Report from ShortRead

ShortRead contains various FASTQ quality report functions

```
> 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)
```

# Filtering and Trimming FASTQ Files with ShortRead I

## Adaptor trimming

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

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

## Read counting and duplicate removal

```
> tables(fq)$distribution # Counts read occurences

  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
```

# Filtering and Trimming FASTQ Files with ShortRead II

### Trimming low quality tails

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

```
  A DNAStringSet instance of length 2
    width seq
[1]     4 CAAC
[2]    20 CCAATGATTTTTTTCCGTGT
```

### Removal of reads with x Phred scores below a threshold value

```
> cutoff <- 30
> qcount <- rowSums(as(quality(fq), "matrix") <= 20)
> fq[qcount == 0] # Number of reads where all Phred scores >= 20
```

```
class: ShortReadQ
length: 349 reads; width: 36 cycles
```

### Removal of reads with x Ns and/or low complexity segments

```
> 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`

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

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

```
> 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", compress=FALS
+ }
> close(f)
```

# Exercise I

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.

Sample code:

```
> 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)
```

# Outline

# 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)

# Range Data Are Stored in IRanges and GRanges Containers

## Constructing GRanges Objects

```
> library(GenomicRanges); library(rtracklayer)
> gr <- GRanges(seqnames = Rle(c("chr1", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)), ranges = IRanges(1:10, end = 
> gff <- import.gff("http://faculty.ucr.edu/~tgirke/Documents/R_BioCond/Samples/gff3.gff",
+                   asRangedData=FALSE) # Imports a simplified GFF3 genome annotation file.
> seqlengths(gff) <- end(ranges(gff[which(values(gff)[,"type"]=="chromosome"),]))
> names(gff) <- 1:length(gff) # Assigns names to corresponding slot.
> gff[1:4,]

GRanges object with 4 ranges and 5 metadata columns:
    seqnames           ranges strand |   source       type     score     phase
       <Rle>        <IRanges>  <Rle> | <factor>   <factor> <numeric> <integer>
  1     Chr1 [   1, 30427671]      + |   TAIR10 chromosome      <NA>      <NA>
  2     Chr1 [3631,     5899]      + |   TAIR10       gene      <NA>      <NA>
  3     Chr1 [3631,     5899]      + |   TAIR10       mRNA      <NA>      <NA>
  4     Chr1 [3760,     5630]      + |   TAIR10    protein      <NA>      <NA>
                                                                                
  1                                                                             
  2                                                ID=AT1G01010;Note=p
  3                                          ID=AT1G01010.1;Parent=A
  4 ID=AT1G01010.1-Protein;Name=AT1
  -------
  seqinfo: 7 sequences from an unspecified genome

> 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

```
> gff[1:4]; gff[1:4, c("type", "group")]; gff[2] <- gff[3] # Subsetting and replacement
> 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
> values(gff); values(gff)[, "type"] # Accessing metadata component.
> gff[elementMetadata(gff)[ ,"type"] == "gene"] # Returns only gene ranges.
```

## Useful utilities for GRanges objects

```
> gff <- gff[values(gff)$type != "chromosome"] # Remove chromosome ranges
> 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
```

# *TranscriptDb*: Managing Transcript Ranges

Storing annotation ranges in *TranscriptDb* databases makes many operations more robust and convenient.

```
> library(GenomicFeatures)
> download.file("http://faculty.ucr.edu/~tgirke/Documents/R_BioCond/Samples/gff3.gff
> txdb <- makeTranscriptDbFromGFF(file="data/gff3.gff",
+         format="gff3",
+         dataSource="TAIR",
+         species="Arabidopsis thaliana")
> saveDb(txdb, file="./data/TAIR10.sqlite")
> txdb <- loadDb("./data/TAIR10.sqlite")
> tr <- transcripts(txdb)
> GRList <- transcriptsBy(txdb, by = "gene")
```

# Generate *TranscriptDb* from BioMart

Alternative sources for creating *TranscriptDb* databases are BioMart, Bioc annotation packages, UCSC, etc. The following shows how to create a *TranscriptDb* from BioMart.

```
> library(GenomicFeatures); library("biomaRt")
> txdb <- makeTranscriptDbFromBiomart(biomart = "plants_mart_23", dataset = "athalia
```

The following steps are useful to find out what is availble on BioMart.

```
> listMarts() # Lists BioMart databases
> mymart <- useMart("plants_mart_23") # Select one, here plants_mart_20
> listDatasets(mymart) # List datasets available in the selected BioMart database
> mymart <- useMart("plants_mart_23", dataset="athaliana_eg_gene")
> listAttributes(mymart) # List available features
> getBM(attributes=c("ensembl_gene_id", "description"), mart=mymart)[1:4,]
```

# 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.

```
> gff <- gff[values(gff)$type != "chromosome"] # Remove chromosome ranges
> rand <- DNAStringSet(sapply(unique(as.character(seqnames(gff))), function(x) paste(sample(c("A","T","G","C"),
> writeXStringSet(DNAStringSet(rand), "./data/test")
> getSeq(FaFile("./data/test"), gff)

  A DNAStringSet instance of length 442
      width seq
  [1]  2269 TATATAGTAGGTTCGCCCACTCGGCATGATTCTTGCCGCGATTCTTAAGCCGACCGTGAACCTTACCAGAATACCGGAGTTTCTGCAAAGCT...AAA
  [2]  2269 TATATAGTAGGTTCGCCCACTCGGCATGATTCTTGCCGCGATTCTTAAGCCGACCGTGAACCTTACCAGAATACCGGAGTTTCTGCAAAGCT...AAA
  [3]  1871 TGAAGGCGAATCCTTTCGAGCGCAAAACTTTGGTAAGGGTGGTTAGATCGTAACTTTGACGACGGTCCCGCAATGAGTGCGGGATCAAGCAC...TAG
  [4]   283 TATATAGTAGGTTCGCCCACTCGGCATGATTCTTGCCGCGATTCTTAAGCCGACCGTGAACCTTACCAGAATACCGGAGTTTCTGCAAAGCT...CGG
  [5]   129 TATATAGTAGGTTCGCCCACTCGGCATGATTCTTGCCGCGATTCTTAAGCCGACCGTGAACCTTACCAGAATACCGGAGTTTCTGCAAAGCTAGCGTG
  ...   ... ...
[438]   324 TTAATTAGTTACCGCTTTCTGGCCCGCTATGTCATAAAAACTAAGCCCGCACTGAAAGGTAAAACAGAGAGTGCTGTGCGCGCGGTCCTTCT...TCG
[439]   324 TTAATTAGTTACCGCTTTCTGGCCCGCTATGTCATAAAAACTAAGCCCGCACTGAAAGGTAAAACAGAGAGTGCTGTGCGCGCGGTCCTTCT...TCG
[440]   324 TTAATTAGTTACCGCTTTCTGGCCCGCTATGTCATAAAAACTAAGCCCGCACTGAAAGGTAAAACAGAGAGTGCTGTGCGCGCGGTCCTTCT...TCG
[441]   324 TTAATTAGTTACCGCTTTCTGGCCCGCTATGTCATAAAAACTAAGCCCGCACTGAAAGGTAAAACAGAGAGTGCTGTGCGCGCGGTCCTTCT...TCG
[442]   324 TTAATTAGTTACCGCTTTCTGGCCCGCTATGTCATAAAAACTAAGCCCGCACTGAAAGGTAAAACAGAGAGTGCTGTGCGCGCGGTCCTTCT...TCG
```

# Exercise II

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

```
> download.file("ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.gff", "data/Al
> download.file("ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.fna", "data/Al
> chr <- readDNAStringSet("data/AE004437.fna")
> gff <- import("data/AE004437.gff", asRangedData=FALSE)
> gffgene <- gff[values(gff)[,"type"]=="gene"]
> gene <- DNAStringSet(Views(chr[[1]], IRanges(start(gffgene), end(gffgene))))
> names(gene) <- values(gffgene)[,"locus_tag"]
> pos <- values(gffgene[strand(gffgene) == "+"])[,"locus_tag"]
> p1 <- translate(gene[names(gene) %in% pos])
> names(p1) <- names(gene[names(gene) %in% pos])
> neg <- values(gffgene[strand(gffgene) == "-"])[,"locus_tag"]
> p2 <- translate(reverseComplement(gene[names(gene) %in% neg]))
> names(p2) <- names(gene[names(gene) %in% neg])
> writeXStringSet(c(p1, p2), "mypep.fasta")
```