

# NGS Analysis Basics

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## Overview

## Sequence 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 (Lawrence et al. 2013, Huber et al. (2015)).

- [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
- [GenomicFeatures](#): managing transcript centric annotations
- [GenomicAlignments](#): handling short genomic alignments
- [Rsamtools](#): interface to `samtools`, `bcftools` and `tabix`
- [BSgenome](#): genome annotation data
- [biomaRt](#): interface to BioMart annotations
- [rtracklayer](#): Annotation imports, interface to online genome browsers
- [HelloRanges](#): Bedtools semantics in Bioc's Ranges infrastructure

## Package Requirements

Several Bioconductor packages are required for this tutorial. To install them, execute the following lines in the R console. Please also make sure that you have a recent R version installed on your system. R versions 3.3.x or higher are recommended.

```
source("https://bioconductor.org/biocLite.R")
biocLite(c("Biostrings", "GenomicRanges", "GenomicRanges", "rtracklayer", "systemPipeR", "seqLogo", "Sho
```

## Strings in R Base

### Basic String Matching and Parsing

#### String matching

Generate sample sequence data set

```
myseq <- c("ATGCAGACATAGTG", "ATGAACATAGATCC", "GTACAGATCAC")
```

String searching with regular expression support

```
myseq[grep("ATG", myseq)]
```

```
## [1] "ATGCAGACATAGTG" "ATGAACATAGATCC"
```

Searches myseq for first match of pattern “AT”

```
pos1 <- regexpr("AT", myseq)  
as.numeric(pos1); attributes(pos1)$match.length # Returns position information of matches
```

```
## [1] 1 1 7
```

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

Searches myseq for all matches of pattern “AT”

```
pos2 <- gregexpr("AT", myseq)  
as.numeric(pos2[[1]]); attributes(pos2[[1]])$match.length # Returns positions of matches in first sequence
```

```
## [1] 1 9
```

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

String substitution with regular expression support

```
gsub("^ATG", "atg", myseq)
```

```
## [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

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] "CCACCGCCGGGTTTCGC" "ATGATAGCGTAGC" "CTTCTACTGT"
```

Count identical sequences

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

```
##
## ACCTGGCTCTAGCCTA ATGATAGCGTAGC CCACCGCCGGGTTTCGC CTTCTACTGT
##                1                1                2                1
```

Extract reads from reference

Note: this requires Biostrings package.

```
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: TCACTGCAGGTCTCCTAAGAGTCGAGTGACCGAGTCCGATTGCGTG...TGTGTCATTGTGCAACCTCCGTATCATAAGTCCTCAGAATAACAC
```

## Sequences in Bioconductor

### Important Data Objects of 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 sequences with 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.nlm.nih.gov/genomes/archive/old\\_genbank/Bacteria/Halobacterium\\_sp\\_uid217/AE004437.ffn](ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/Halobacterium_sp_uid217/AE004437.ffn)

```
dir.create("data", showWarnings = FALSE)
# system("wget ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/Halobacterium_sp_uid217/AE004437.ffn")
download.file("ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/Halobacterium_sp_uid217/AE004437.ffn", "data/AE004437.ffn")
```

Import FASTA file with readDNAStringSet

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

```
## A DNAStringSet instance of length 3
## width seq names
## [1] 1206 ATGACTCGGCGGTCTCGTGTGCGGTGCCGGCCTC...GTCGTCGTTGTTTCGACGCTGGCGGAACCCATGA gi|12057215|gb|AE004437.1
## [2] 666 ATGAGCATCATCGAACTCGAAGGCGTGGTCAAA...GTCAACCTCGTCGATGGGGTGTTACACACGTGA gi|12057215|gb|AE004437.2
## [3] 1110 ATGGCGTGGCGGAACCTCGGGCGGAACCGCGTG...AACGATCCGCCCGTCGAGGCGCTCGGCGAATGA gi|12057215|gb|AE004437.3
```

Subset sequences with regular expression on sequence name field

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

```
## [1] 170
```

Export subsetted sequences to FASTA file

```
writeXStringSet(sub, file="./data/AE004437sub.ffn", width=80)
```

Now inspect 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
```

RNA sequences

```
r <- RNAString("GCAUAU-UAC")
r <- RNAString(d) # Converts d to RNAString object
r
```

```
## 10-letter "RNAString" instance
## seq: GCAUAU-UAC
```

Protein sequences

```
p <- AString("HCWYHH")
p
```

```
## 6-letter "AString" instance
## seq: HCWYHH
```

Any type of character strings

```
b <- BString("I store any set of characters. Other XString objects store only the IUPAC characters.")
b
```

```
## 85-letter "BString" instance
## seq: 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 names
## [1] 9 GCATATTAC seq1
## [2] 9 AATCGATCC seq2
```

Important utilities for XStringSet containers

```
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 names
## [1] 4 GCAT seq1
## [2] 7 ATCGATC seq2
## [3] 3 ATA seq3
```

## Multiple Alignment Class

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

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

```
## DNAMultipleAlignment with 8 rows and 2343 columns
## aln names
## [1] -----TCCCGTCTCCGCAGCAAAAAAGTTTGAGTCG...TTGTCCAAACTCACAATTAATAAAAAAAAAAAAAAAAAA gi|84452153|ref|N...
## [2] -----..... gi|208431713|ref|...
## [3] -----..... gi|118601823|ref|...
## [4] -----AAAAGTTGGAGTCT... gi|114326503|ref|...
## [5] -----..... gi|119220589|ref|...
## [6] -----..... gi|148540149|ref|...
## [7] -----CGGCTCCGCAGCGCCTCACTCG... gi|45383056|ref|N...
## [8] GGGGGAGACTTCAGAAGTTGTTGTCCTCTCCGCTGA... gi|213515133|ref|...
```

## Basic Sequence Manipulations

### Reverse and Complement

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

```
## A DNASTringSet instance of length 2
## width seq
## [1] 16 GGTGGCGGCCCAAGCG
## [2] 13 TACTATGCATCG
```

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

```
## A DNASTringSet instance of length 2
## width seq
## [1] 16 CGCTTGGGCCGCCACC
## [2] 13 CGATGCGATAGTA
```

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

```
## A DNASTringSet instance of length 2
## width seq
## [1] 16 GCGAACCCGGCGGTGG
## [2] 13 GCTACGCTATCAT
```

## Translate DNA into Protein

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

```
## Warning in .Call2("DNASTringSet_translate", x, skip_code, dna_codes[codon_alphabet], : in 'x[[1]]':
## last base was ignored
```

```
## Warning in .Call2("DNASTringSet_translate", x, skip_code, dna_codes[codon_alphabet], : in 'x[[2]]':
## last base was ignored
```

```
## A AAStringSet instance of length 2
## width seq
## [1] 5 PPPGS
## [2] 4 MIA*
```

## Pattern Matching

### Pattern matching with mismatches

Find pattern matches in reference

```
myseq1 <- readDNASTringSet("./data/AE004437.ffn")
mypos <- matchPattern("ATGGTG", myseq1[[1]], max.mismatch=1)
```

Count only the corresponding matches

```
countPattern("ATGGCT", myseq1[[1]], max.mismatch=1)
```

```
## [1] 3
```

Count matches in many sequences

```
vcountPattern("ATGGCT", myseq1, max.mismatch=1)[1:20]
```

```
## [1] 3 0 5 4 1 2 2 1 4 3 0 0 1 2 0 1 4 0 0 1
```

Results shown in DNASTringSet object

```
tmp <- c(DNAStringSet("ATGGTG"), DNAStringSet(mypos))
```

Return a consensus matrix for query and hits

```
consensusMatrix(tmp)[1:4,]
```

```
##      [,1] [,2] [,3] [,4] [,5] [,6]
## A      3    0    0    0    0    0
## C      1    1    0    0    0    0
## G      0    0    4    4    1    4
## T      0    3    0    0    3    0
```

Find all pattern matches in reference

```
myvpos <- vmatchPattern("ATGGCT", myseq1, max.mismatch=1)
myvpos # The results are stored as MIndex object.
```

```
## MIndex object of length 2058
## $`gi|12057215|gb|AE004437.1|:248-1453 Halobacterium sp. NRC-1, complete genome`
## IRanges object with 3 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         1         6         6
## [2]       383       388         6
## [3]       928       933         6
##
## $`gi|12057215|gb|AE004437.1|:1450-2115 Halobacterium sp. NRC-1, complete genome`
## IRanges object with 0 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
##
## $`gi|12057215|gb|AE004437.1|:2145-3254 Halobacterium sp. NRC-1, complete genome`
## IRanges object with 5 ranges and 0 metadata columns:
##      start      end      width
##      <integer> <integer> <integer>
## [1]         1         6         6
## [2]        94        99         6
## [3]       221       226         6
## [4]       535       540         6
## [5]       601       606         6
##
## ...
## <2055 more elements>
```

```
Views(myseq1[[1]], start(myvpos[[1]]), end(myvpos[[1]])) # Retrieves the result for single entry
```

```
## Views on a 1206-letter DNAString subject
## subject: ATGACTCGGCGGTCTCGTGTGCGTGCCGGCCTCGCAGCCATTGT...TTGCGATCGTCGTCGTTGTTTCGACGCTGGCGGAACCCATGA
## views:
##      start end width
## [1]     1   6     6 [ATGACT]
## [2]   383 388     6 [ATGGCA]
## [3]   928 933     6 [ATGACT]
```



Return all matches

```
sapply(seq(along=myseq1), function(x)
  as.character(Views(myseq1[[x]], start(myvpos[[x]]), end(myvpos[[x]]))))[1:4]
```

## Pattern matching with regular expression support

```
myseq <- DNASTringSet(c("ATGCAGACATAGTG", "ATGAACATAGATCC", "GTACAGATCAC"))
myseq[grep("^ATG", myseq, perl=TRUE)] # String searching with regular expression support
```

```
## A DNASTringSet instance of length 2
## width seq
## [1] 14 ATGCAGACATAGTG
## [2] 14 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 # Match positions in first sequence
```

```
## [1] 1 9
```

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

```
DNASTringSet(gsub("^ATG", "NNN", myseq)) # String substitution with regular expression support
```

```
## A DNASTringSet instance of length 3
## width seq
## [1] 14 NNNCAGACATAGTG
## [2] 14 NNNAACATAGATCC
## [3] 11 GTACAGATCAC
```

## PWM Viewing and Searching

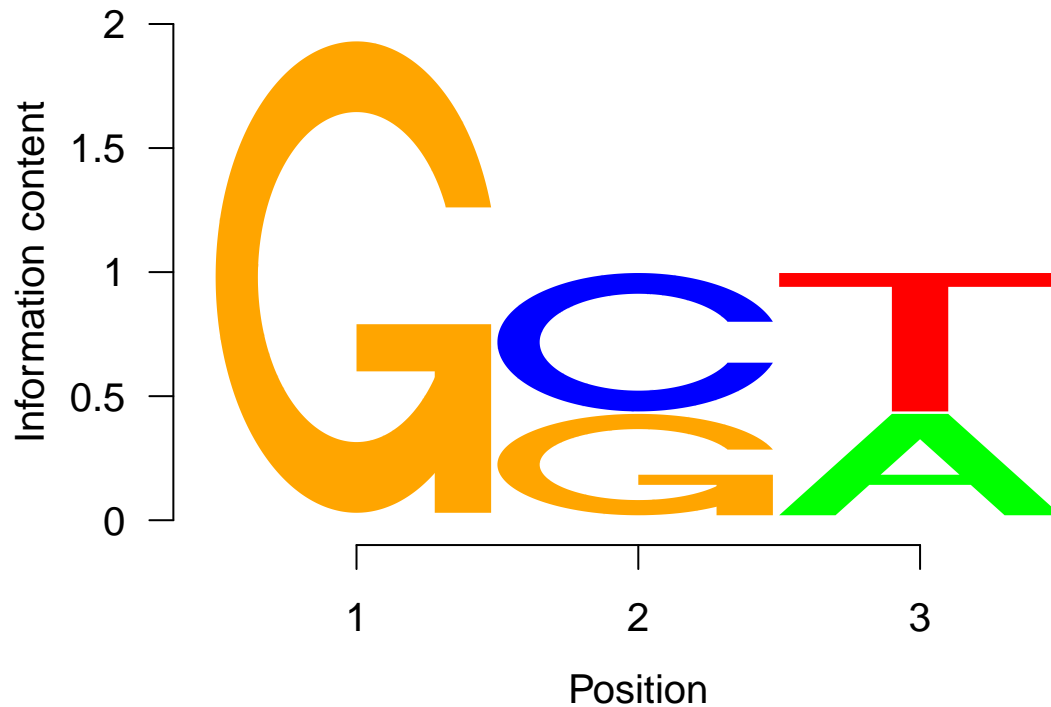
```
library(seqLogo)
```

```
## Loading required package: grid
```

```
pwm <- PWM(DNASTringSet(c("GCT", "GGT", "GCA")))
pwm
```

```
##      [,1]      [,2]      [,3]
## A 0.0000000 0.0000000 0.2312611
## C 0.0000000 0.3157205 0.0000000
## G 0.3685591 0.2312611 0.0000000
## T 0.0000000 0.0000000 0.3157205
```

```
seqLogo(t(t(pwm) * 1/colSums(pwm)))
```



Search sequence for PWM matches with score better than `min.score`

```
chr <- DNASTring("AAAGCTAAAGGTAAAGCAAAA")
matchPWM(pwm, chr, min.score=0.9)
```

```
## 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]
```

## NGS Sequences

### Sequence and Quality Data: FASTQ Format

Four lines per sequence:

1. ID

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

The following gives an example of 3 Illumina reads in a FASTQ file. The numbers at the beginning of each line are not part of the FASTQ format. They have been added solely for illustration purposes.

```
1. @SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
2. CAACGAGTTCACACCTTGGCCGACAGGCCCGGTAA
3. +SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
4. BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
1. @SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
2. CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
3. +SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
4. BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
1. @SRR038845.53 HWI-EAS038:6:1:1:360 length=36
2. GTTCAAAAAGAACTAAATTGTGTCAATAGAAAATC
3. +SRR038845.53 HWI-EAS038:6:1:1:360 length=36
4. BBCBBBBBB@B@B@?BBBBBCBC>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 score interconversion

```
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
```

Construct `QualityScaledDNAStringSet` from scratch

```
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 characters.
myqual <- PhredQuality(myqual) # Converts to a PhredQuality object.
dsetq1 <- QualityScaledDNAStringSet(dset, myqual) # Combines DNAStringSet and quality data in QualityScaledDNAStringSet
dsetq1[1:2]
```

```
## A QualityScaledDNAStringSet instance containing:
##
## A DNAStringSet instance of length 2
## width seq
## [1] 20 GTATGATCCTGTACGCTAAT
```

```
## [2]      20 TCGGGCACCACCTATATGCCG
##
##      A PhredQuality instance of length 2
##      width seq
## [1]      20 ADD%(A%A/I8710>6)(G,
## [2]      20 EEGF;B#"@=),*0%#@#G:8
```

## Processing FASTQ Files with ShortRead

The following explains the basic usage of `ShortReadQ` objects. To make the sample code work, download and unzip this [file](http://faculty.ucr.edu/~tgirke/HTML_Presentations/Manuals/Workshop_Dec_6_10_2012/Rsequen) to your current working directory. The following code performs the download for you.

```
library(ShortRead)
download.file("http://faculty.ucr.edu/~tgirke/HTML_Presentations/Manuals/Workshop_Dec_6_10_2012/Rsequen
unzip("data.zip")
```

Important utilities for accessing FASTQ files

```
fastq <- list.files("data", "*.fastq$"); fastq <- paste("data/", fastq, sep="")
names(fastq) <- paste("flowcell16_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")[1:4,1:12] # Coerces Phred scores to numeric matrix
```

```
##      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12]
## [1,]  33  32  31  22  29  33  28  29  25  29  29  22
## [2,]  33  34  34  33  32  31  33  33  31  33  33  33
## [3,]  33  33  34  33  33  33  33  33  33  31  31  33
## [4,]  33  33  33  33  31  33  28  31  28  32  33  33
```

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

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

## FASTQ Quality Reports

### Using systemPipeR

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

```
library(systemPipeR)
fqlist <- seeFastq(fastq=fastq, batchsize=800, klength=8) # For real data set batchsize to at least 10^4
seeFastqPlot(fqlist)
```



Handles many samples in one PDF file. For more details see [here](#)

## Using ShortRead

The ShortRead package contains several FASTQ quality reporting 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

### Adaptor trimming

```
fqtrim <- trimLRPatterns(Rpattern="GCCCAGGTAA", subject=fq)
sread(fq)[1:2] # Before trimming
```

```
## A DNAStringSet instance of length 2
## width seq
## [1] 36 CAACGAGTTCACACCTTGGCCGACAGGCCCGGTAA
## [2] 36 CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
```

```
sread(fqtrim)[1:2] # After trimming
```

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

### Read counting and duplicate removal

```
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

```
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 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 here `SRR038845.fastq_sub` in data directory.

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

```
## Warning: closing unused connection 6 (data/SRR038845.fastq)
```

```
## Warning: closing unused connection 5 (data/SRR038845.fastq)
```

```
close(f)
```

## Range Operations

### 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

### Construct GRanges Object

```
library(GenomicRanges); library(rtracklayer)
gr <- GRanges(seqnames = Rle(c("chr1", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)), ranges = IRanges(1:10, c(
```

### Import GFF into GRanges Object

```
gff <- import.gff("http://faculty.ucr.edu/~tgirke/Documents/R_BioCond/Samples/gff3.gff") # Imports a si
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 10 metadata columns:
```

```
##      seqnames      ranges strand |   source      type      score      phase      ID
##      <Rle>        <IRanges> <Rle> | <factor>    <factor> <numeric> <integer> <character>
##  1      Chr1 [ 1, 30427671]      + |   TAIR10 chromosome    <NA>      <NA>      Chr1
##  2      Chr1 [3631,      5899]      + |   TAIR10      gene    <NA>      <NA>      AT1G01010
##  3      Chr1 [3631,      5899]      + |   TAIR10      mRNA    <NA>      <NA>      AT1G01010.1
##  4      Chr1 [3760,      5630]      + |   TAIR10      protein  <NA>      <NA> AT1G01010.1-Protein
##      Name      Note      Parent      Index Derives_from
##      <character> <CharacterList> <CharacterList> <character> <character>
##  1      Chr1
##  2  AT1G01010 protein_coding_gene
##  3 AT1G01010.1      AT1G01010      1      <NA>
##  4 AT1G01010.1      <NA>  AT1G01010.1
##  -----
##  seqinfo: 7 sequences from an unspecified genome
```

### Coerce GRanges object to data.frame

```
as.data.frame(gff)[1:4, 1:7]
```

```
##      seqnames start      end      width strand source      type
##  1      Chr1      1 30427671 30427671      + TAIR10 chromosome
##  2      Chr1 3631      5899      2269      + TAIR10      gene
##  3      Chr1 3631      5899      2269      + TAIR10      mRNA
##  4      Chr1 3760      5630      1871      + TAIR10      protein
```

### Coerce GRanges to RangedData object and vice versa

```
gff_rd <- as(gff, "RangedData")
```

```
## Warning in cb$putconst(e): closing unused connection 6 (/tmp/RtmpFqpsyR/file79431c974741)
```

```
## Warning in cb$putconst(e): closing unused connection 5 (/tmp/RtmpFqpsyR/file79431c974741)
```

```
gff_gr <- as(gff_rd, "GRanges")
```

## Utilities for Range Containers

### Accessor and subsetting methods for GRanges objects

Subsetting and replacement

```
gff[1:4]
```

```
## GRanges object with 4 ranges and 10 metadata columns:
```

##	seqnames	ranges	strand	source	type	score	phase	ID
##	<Rle>	<IRanges>	<Rle>	<factor>	<factor>	<numeric>	<integer>	<character>
## 1	Chr1	[ 1, 30427671]	+	TAIR10	chromosome	<NA>	<NA>	Chr1
## 2	Chr1	[3631, 5899]	+	TAIR10	gene	<NA>	<NA>	AT1G01010
## 3	Chr1	[3631, 5899]	+	TAIR10	mRNA	<NA>	<NA>	AT1G01010.1
## 4	Chr1	[3760, 5630]	+	TAIR10	protein	<NA>	<NA>	AT1G01010.1-Protein

##	Name	Note	Parent	Index	Derives_from
##	<character>	<CharacterList>	<CharacterList>	<character>	<character>
## 1	Chr1			<NA>	<NA>
## 2	AT1G01010	protein_coding_gene		<NA>	<NA>
## 3	AT1G01010.1		AT1G01010	1	<NA>
## 4	AT1G01010.1			<NA>	AT1G01010.1

```
## -----  
## seqinfo: 7 sequences from an unspecified genome
```

```
gff[1:4, c("type", "ID")]
```

```
## GRanges object with 4 ranges and 2 metadata columns:
```

##	seqnames	ranges	strand	type	ID
##	<Rle>	<IRanges>	<Rle>	<factor>	<character>
## 1	Chr1	[ 1, 30427671]	+	chromosome	Chr1
## 2	Chr1	[3631, 5899]	+	gene	AT1G01010
## 3	Chr1	[3631, 5899]	+	mRNA	AT1G01010.1
## 4	Chr1	[3760, 5630]	+	protein	AT1G01010.1-Protein

```
## -----  
## seqinfo: 7 sequences from an unspecified genome
```

```
gff[2] <- gff[3]
```

GRanges objects can be concatenated with the c function

```
c(gff[1:2], gff[401:402])
```

```
## GRanges object with 4 ranges and 10 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      mRNA      <NA>      <NA>
##    401      Chr5 [5516,      5769]      - |      TAIR10      protein      <NA>      <NA>
##    402      Chr5 [5770,      5801]      - |      TAIR10 five_prime_UTR      <NA>      <NA>
##              ID      Name      Note      Parent      Index Derives_from
##              <character> <character> <CharacterList> <CharacterList> <character> <character>
##      1              Chr1      Chr1              AT1G01010      1      <NA>
##      2              AT1G01010.1 AT1G01010.1              AT1G01010      1      <NA>
##    401 AT5G01015.2-Protein AT5G01015.2              <NA>      AT5G01015.2
##    402              <NA>      <NA>              AT5G01015.2      <NA>      <NA>
##    -----
##    seqinfo: 7 sequences from an unspecified genome
```

Accessor functions

```
seqnames(gff)
```

```
## factor-Rle of length 449 with 7 runs
##   Lengths:  72  22  38 118 172  13  14
##   Values : Chr1 Chr2 Chr3 Chr4 Chr5 ChrC ChrM
## Levels(7): Chr1 Chr2 Chr3 Chr4 Chr5 ChrC ChrM
```

```
ranges(gff)
```

```
## IRanges object with 449 ranges and 0 metadata columns:
##       start      end      width
##       <integer> <integer> <integer>
##      1         1 30427671 30427671
##      2      3631      5899      2269
##      3      3631      5899      2269
##      4      3760      5630      1871
##      5      3631      3913       283
##     ...      ...      ...      ...
##    445     11918     12241       324
##    446     11918     12241       324
##    447     11918     12241       324
##    448     11918     12241       324
##    449     11918     12241       324
```

```
strand(gff)
```

```
## factor-Rle of length 449 with 13 runs
##   Lengths:  18  54  28  21  12 117   1 171   1  12   1   8   5
##   Values :   +   -   +   -   +   -   +   -   +   -   +   -   +
## Levels(3): + - *
```

```
seqlengths(gff)
```

```
##      Chr1      Chr2      Chr3      Chr4      Chr5      ChrC      ChrM
## 30427671 19698289 23459830 18585056 26975502 154478 366924
```

```
start(gff[1:4])
```

```
## [1] 1 3631 3631 3760
```

```
end(gff[1:4])
```

```
## [1] 30427671 5899 5899 5630
```

```
width(gff[1:4])
```

```
## [1] 30427671 2269 2269 1871
```

Accessing metadata component

```
values(gff) # or elementMetadata(gff)
```

```
## DataFrame with 449 rows and 10 columns
```

##	source	type	score	phase	ID	Name	Note
##	<factor>	<factor>	<numeric>	<integer>	<character>	<character>	<CharacterList>
## 1	TAIR10	chromosome	NA	NA	Chr1	Chr1	
## 2	TAIR10	mRNA	NA	NA	AT1G01010.1	AT1G01010.1	
## 3	TAIR10	mRNA	NA	NA	AT1G01010.1	AT1G01010.1	
## 4	TAIR10	protein	NA	NA	AT1G01010.1-Protein	AT1G01010.1	
## 5	TAIR10	exon	NA	NA	NA	NA	
## ...	...	...	...	...	...	...	...
## 445	TAIR10	gene	NA	NA	ATMG00030	ATMG00030	protein_coding_gene
## 446	TAIR10	mRNA	NA	NA	ATMG00030.1	ATMG00030.1	
## 447	TAIR10	protein	NA	NA	ATMG00030.1-Protein	ATMG00030.1	
## 448	TAIR10	exon	NA	NA	NA	NA	
## 449	TAIR10	CDS	NA	0	NA	NA	
##		Parent		Index	Derives_from		
##		<CharacterList>		<character>	<character>		
## 1				NA	NA		
## 2		AT1G01010		1	NA		
## 3		AT1G01010		1	NA		
## 4				NA	AT1G01010.1		
## 5		AT1G01010.1		NA	NA		
## ...		...		...	...		
## 445				NA	NA		
## 446		ATMG00030		1	NA		
## 447				NA	ATMG00030.1		
## 448		ATMG00030.1		NA	NA		
## 449	ATMG00030.1,ATMG00030.1-Protein			NA	NA		

```
values(gff)[, "type"][1:20]
```

```
## [1] chromosome mRNA mRNA protein exon five_prime_UTR
## [7] CDS exon CDS exon CDS exon
## [13] CDS exon CDS exon CDS three_prime_UTR
## [19] gene mRNA
## Levels: chromosome gene mRNA protein exon five_prime_UTR CDS three_prime_UTR rRNA tRNA
```

```
gff[values(gff)[, "type"] == "gene"]
```

```
## GRanges object with 21 ranges and 10 metadata columns:
```

```
##      seqnames      ranges strand |   source   type   score   phase      ID
##      <Rle>      <IRanges> <Rle> | <factor> <factor> <numeric> <integer> <character>
##    19      Chr1 [ 5928,  8737]    - |   TAIR10   gene    <NA>    <NA>   AT1G01020
##    64      Chr1 [11649, 13714]    - |   TAIR10   gene    <NA>    <NA>   AT1G01030
##    74      Chr2 [ 1025,  2810]    + |   TAIR10   gene    <NA>    <NA>   AT2G01008
##    84      Chr2 [ 3706,  5513]    + |   TAIR10   gene    <NA>    <NA>   AT2G01010
##    87      Chr2 [ 5782,  5945]    + |   TAIR10   gene    <NA>    <NA>   AT2G01020
##    ...      ...      ...      ... .   ...      ...      ...      ...      ...
##   427      ChrC [  383,  1444]    - |   TAIR10   gene    <NA>    <NA>   ATCG00020
##   432      ChrC [ 1717,  4347]    - |   TAIR10   gene    <NA>    <NA>   ATCG00030
##   437      ChrM [  273,   734]    - |   TAIR10   gene    <NA>    <NA>   ATMG00010
##   442      ChrM [ 8848, 11415]    - |   TAIR10   gene    <NA>    <NA>   ATMG00020
##   445      ChrM [11918, 12241]    + |   TAIR10   gene    <NA>    <NA>   ATMG00030
##
##      Name      Note      Parent      Index Derives_from
##      <character> <CharacterList> <CharacterList> <character> <character>
##    19  AT1G01020 protein_coding_gene    <NA>    <NA>
##    64  AT1G01030 protein_coding_gene    <NA>    <NA>
##    74  AT2G01008 protein_coding_gene    <NA>    <NA>
##    84  AT2G01010          rRNA          <NA>    <NA>
##    87  AT2G01020          rRNA          <NA>    <NA>
##    ...      ...      ...      ...      ...
##   427  ATCG00020 protein_coding_gene    <NA>    <NA>
##   432  ATCG00030          tRNA          <NA>    <NA>
##   437  ATMG00010 protein_coding_gene    <NA>    <NA>
##   442  ATMG00020          rRNA          <NA>    <NA>
##   445  ATMG00030 protein_coding_gene    <NA>    <NA>
## -----
## seqinfo: 7 sequences from an unspecified genome
```

## Useful utilities for GRanges objects

Remove chromosome ranges

```
gff <- gff[values(gff)$type != "chromosome"]
```

Erase the strand information

```
strand(gff) <- "*"
```

Collapses overlapping ranges to continuous ranges.

```
reduce(gff)
```

```
## GRanges object with 22 ranges and 0 metadata columns:
```

```
##      seqnames      ranges strand
##      <Rle>      <IRanges> <Rle>
##    [1]      Chr1 [ 3631,  5899]    *
##    [2]      Chr1 [ 5928,  8737]    *
```

```
##      [3]      Chr1 [11649, 13714]      *
##      [4]      Chr2 [ 1025,  2810]      *
##      [5]      Chr2 [ 3706,  5513]      *
##      ...      ...      ...      ...
##     [18]      ChrC [   383,  1444]      *
##     [19]      ChrC [ 1717,  4347]      *
##     [20]      ChrM [   273,   734]      *
##     [21]      ChrM [ 8848, 11415]      *
##     [22]      ChrM [11918, 12241]      *
## -----
##      seqinfo: 7 sequences from an unspecified genome
```

Return uncovered regions

```
gaps(gff)
```

```
## GRanges object with 43 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>      <IRanges> <Rle>
##      [1]      Chr1 [    1, 30427671]      +
##      [2]      Chr1 [    1, 30427671]      -
##      [3]      Chr1 [    1,    3630]      *
##      [4]      Chr1 [5900,    5927]      *
##      [5]      Chr1 [8738,   11648]      *
##      ...      ...      ...      ...
##     [39]      ChrM [    1, 366924]      -
##     [40]      ChrM [    1,    272]      *
##     [41]      ChrM [  735,   8847]      *
##     [42]      ChrM [11416,  11917]      *
##     [43]      ChrM [12242, 366924]      *
## -----
##      seqinfo: 7 sequences from an unspecified genome
```

Return disjoint ranges

```
disjoin(gff)
```

```
## GRanges object with 211 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>      <IRanges> <Rle>
##      [1]      Chr1 [3631,  3759]      *
##      [2]      Chr1 [3760,  3913]      *
##      [3]      Chr1 [3914,  3995]      *
##      [4]      Chr1 [3996,  4276]      *
##      [5]      Chr1 [4277,  4485]      *
##      ...      ...      ...      ...
##    [207]      ChrC [ 1752,  4310]      *
##    [208]      ChrC [ 4311,  4347]      *
##    [209]      ChrM [   273,   734]      *
##    [210]      ChrM [ 8848, 11415]      *
##    [211]      ChrM [11918, 12241]      *
## -----
##      seqinfo: 7 sequences from an unspecified genome
```

Returns coverage of ranges

```
coverage(gff)
```

```
## RleList of length 7
## $Chr1
## integer-Rle of length 30427671 with 45 runs
##   Lengths:    3630    129    154    82    281 ...    233    161    380 30413957
##   Values :      0      4      5      3      5 ...      4      2      4      0
##
## $Chr2
## integer-Rle of length 19698289 with 14 runs
##   Lengths:    1024    248    185    53    362 ...    164    625    102 19691617
##   Values :      0      5      3      5      3 ...      3      0      5      0
##
## $Chr3
## integer-Rle of length 23459830 with 29 runs
##   Lengths:    1652    145    139    111    95 ...    155    148    156 23453781
##   Values :      0      4      5      3      5 ...      3      5      4      0
##
## $Chr4
## integer-Rle of length 18585056 with 72 runs
##   Lengths:    1179    357    1358    128    872 ...    212    114    74 18571697
##   Values :      0      5      0      5      3 ...      3      5      4      0
##
## $Chr5
## integer-Rle of length 26975502 with 64 runs
##   Lengths:    1222     28     28    109    72 ...    76     55    174 26967058
##   Values :      0      4      7     13    16 ...      3      5      4      0
##
## ...
## <2 more elements>
```

Return the index pairings for overlapping ranges

```
findOverlaps(gff, gff[1:4])
```

```
## Hits object with 55 hits and 0 metadata columns:
##      queryHits subjectHits
##      <integer>  <integer>
## [1]          1          1
## [2]          1          2
## [3]          1          4
## [4]          1          3
## [5]          2          1
## ...          ...          ...
## [51]         16          1
## [52]         16          2
## [53]         16          3
## [54]         17          1
## [55]         17          2
## -----
## queryLength: 442 / subjectLength: 4
```

Counts overlapping ranges

```
countOverlaps(gff, gff[1:4])[1:40]
```

```
##  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
##  4  4  4  4  3  4  3  3  3  3  3  3  3  3  3  3  2  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
## 35 36 37 38 39 40 41
##  0  0  0  0  0  0  0
```

Return only overlapping ranges

```
subsetByOverlaps(gff, gff[1:4])
```

```
## GRanges object with 17 ranges and 10 metadata columns:
##      seqnames      ranges strand |      source      type      score      phase
##      <Rle>        <IRanges> <Rle> | <factor>        <factor> <numeric> <integer>
##    2      Chr1 [3631, 5899]      * | TAIR10          mRNA      <NA>      <NA>
##    3      Chr1 [3631, 5899]      * | TAIR10          mRNA      <NA>      <NA>
##    4      Chr1 [3760, 5630]      * | TAIR10          protein  <NA>      <NA>
##    5      Chr1 [3631, 3913]      * | TAIR10          exon      <NA>      <NA>
##    6      Chr1 [3631, 3759]      * | TAIR10 five_prime_UTR <NA>      <NA>
##    ..      ...      ...      ... | ...      ...      ...      ...
##   14      Chr1 [5174, 5326]      * | TAIR10          exon      <NA>      <NA>
##   15      Chr1 [5174, 5326]      * | TAIR10          CDS      <NA>      0
##   16      Chr1 [5439, 5899]      * | TAIR10          exon      <NA>      <NA>
##   17      Chr1 [5439, 5630]      * | TAIR10          CDS      <NA>      0
##   18      Chr1 [5631, 5899]      * | TAIR10 three_prime_UTR <NA>      <NA>
##              ID      Name      Note      Parent      Index
##              <character> <character> <CharacterList> <CharacterList> <character>
##    2      AT1G01010.1 AT1G01010.1      AT1G01010      1
##    3      AT1G01010.1 AT1G01010.1      AT1G01010      1
##    4 AT1G01010.1-Protein AT1G01010.1      <NA>
##    5      <NA>      <NA>      AT1G01010.1 <NA>
##    6      <NA>      <NA>      AT1G01010.1 <NA>
##    ..      ...      ...      ...      ...
##   14      <NA>      <NA>      AT1G01010.1 <NA>
##   15      <NA>      <NA>      AT1G01010.1,AT1G01010.1-Protein <NA>
##   16      <NA>      <NA>      AT1G01010.1 <NA>
##   17      <NA>      <NA>      AT1G01010.1,AT1G01010.1-Protein <NA>
##   18      <NA>      <NA>      AT1G01010.1 <NA>
##      Derives_from
##      <character>
##    2      <NA>
##    3      <NA>
##    4 AT1G01010.1
##    5      <NA>
##    6      <NA>
##    ..      ...
##   14      <NA>
##   15      <NA>
##   16      <NA>
##   17      <NA>
##   18      <NA>
```



```
## -----
## seqinfo: 7 sequences from an unspecified genome
```

## 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.
```

```
## GRangesList object of length 7:
```

```
## $Chr1
```

```
## GRanges object with 71 ranges and 10 metadata columns:
```

	seqnames	ranges	strand	source	type	score	phase
	<Rle>	<IRanges>	<Rle>	<factor>	<factor>	<numeric>	<integer>
## 2	Chr1	[3631, 5899]	*	TAIR10	mRNA	<NA>	<NA>
## 3	Chr1	[3631, 5899]	*	TAIR10	mRNA	<NA>	<NA>
## 4	Chr1	[3760, 5630]	*	TAIR10	protein	<NA>	<NA>
## 5	Chr1	[3631, 3913]	*	TAIR10	exon	<NA>	<NA>
## 6	Chr1	[3631, 3759]	*	TAIR10	five_prime_UTR	<NA>	<NA>
## ..	...	...	...	...	...	...	...
## 68	Chr1	[13335, 13714]	*	TAIR10	exon	<NA>	<NA>
## 69	Chr1	[12941, 13173]	*	TAIR10	five_prime_UTR	<NA>	<NA>
## 70	Chr1	[11864, 12940]	*	TAIR10	CDS	<NA>	0
## 71	Chr1	[11649, 11863]	*	TAIR10	three_prime_UTR	<NA>	<NA>
## 72	Chr1	[11649, 13173]	*	TAIR10	exon	<NA>	<NA>

	ID	Name	Note	Parent	Index
	<character>	<character>	<CharacterList>	<CharacterList>	<character>
## 2	AT1G01010.1	AT1G01010.1		AT1G01010	1
## 3	AT1G01010.1	AT1G01010.1		AT1G01010	1
## 4	AT1G01010.1-Protein	AT1G01010.1			<NA>
## 5	<NA>	<NA>		AT1G01010.1	<NA>
## 6	<NA>	<NA>		AT1G01010.1	<NA>
## ..	...	...	...	...	...
## 68	<NA>	<NA>		AT1G01030.1	<NA>
## 69	<NA>	<NA>		AT1G01030.1	<NA>
## 70	<NA>	<NA>	AT1G01030.1,AT1G01030.1-Protein		<NA>
## 71	<NA>	<NA>		AT1G01030.1	<NA>
## 72	<NA>	<NA>		AT1G01030.1	<NA>

```
## Derives_from
## <character>
## 2 <NA>
## 3 <NA>
## 4 AT1G01010.1
## 5 <NA>
## 6 <NA>
## .. ...
## 68 <NA>
## 69 <NA>
## 70 <NA>
## 71 <NA>
## 72 <NA>
##
## ...
```

```
## <6 more elements>
## -----
## seqinfo: 7 sequences from an unspecified genome
```

```
unlist(sp) # Returns data as GRanges object
```

```
## GRanges object with 442 ranges and 10 metadata columns:
```

```
##           seqnames      ranges strand | source      type      score      phase
##           <Rle>        <IRanges> <Rle> | <factor>    <factor> <numeric> <integer>
##      1.2      Chr1    [3631, 5899]   * | TAIR10      mRNA      <NA>      <NA>
##      2.3      Chr1    [3631, 5899]   * | TAIR10      mRNA      <NA>      <NA>
##      3.4      Chr1    [3760, 5630]   * | TAIR10      protein    <NA>      <NA>
##      4.5      Chr1    [3631, 3913]   * | TAIR10      exon       <NA>      <NA>
##      5.6      Chr1    [3631, 3759]   * | TAIR10 five_prime_UTR <NA>      <NA>
##      ...      ...      ...         ... | ...        ...      ...      ...
## 438.445      ChrM [11918, 12241]   * | TAIR10      gene       <NA>      <NA>
## 439.446      ChrM [11918, 12241]   * | TAIR10      mRNA      <NA>      <NA>
## 440.447      ChrM [11918, 12241]   * | TAIR10      protein    <NA>      <NA>
## 441.448      ChrM [11918, 12241]   * | TAIR10      exon       <NA>      <NA>
## 442.449      ChrM [11918, 12241]   * | TAIR10      CDS       <NA>      0
##           ID      Name      Note      Parent
##           <character> <character> <CharacterList> <CharacterList>
##      1.2      AT1G01010.1 AT1G01010.1      AT1G01010
##      2.3      AT1G01010.1 AT1G01010.1      AT1G01010
##      3.4 AT1G01010.1-Protein AT1G01010.1
##      4.5      <NA>      <NA>      AT1G01010.1
##      5.6      <NA>      <NA>      AT1G01010.1
##      ...      ...      ...      ...
## 438.445      ATMG00030      ATMG00030 protein_coding_gene
## 439.446      ATMG00030.1 ATMG00030.1      ATMG00030
## 440.447 ATMG00030.1-Protein ATMG00030.1
## 441.448      <NA>      <NA>      ATMG00030.1
## 442.449      <NA>      <NA>      ATMG00030.1,ATMG00030.1-Protein
##           Index Derives_from
##           <character> <character>
##      1.2      1      <NA>
##      2.3      1      <NA>
##      3.4      <NA> AT1G01010.1
##      4.5      <NA>      <NA>
##      5.6      <NA>      <NA>
##      ...      ...      ...
## 438.445      <NA>      <NA>
## 439.446      1      <NA>
## 440.447      <NA> ATMG00030.1
## 441.448      <NA>      <NA>
## 442.449      <NA>      <NA>
## -----
## seqinfo: 7 sequences from an unspecified genome
```

```
sp[1:4, "type"] # Subsetting of GRangesList objects is similar to GRanges objects.
```

```
## GRangesList object of length 4:
## $1
```

```
## GRanges object with 1 range and 1 metadata column:
##      seqnames      ranges strand |      type
##      <Rle>      <IRanges> <Rle> | <factor>
##  2      Chr1 [3631, 5899]      * |      mRNA
##
## $2
## GRanges object with 1 range and 1 metadata column:
##      seqnames      ranges strand | type
##  3      Chr1 [3631, 5899]      * | mRNA
##
## $3
## GRanges object with 1 range and 1 metadata column:
##      seqnames      ranges strand |      type
##  4      Chr1 [3760, 5630]      * | protein
##
## ...
## <1 more element>
## -----
## seqinfo: 7 sequences from an unspecified genome
```

```
lapply(sp[1:4], length) # Looping over GRangesList objects similar to lists
```

```
## $`1`
## [1] 1
##
## $`2`
## [1] 1
##
## $`3`
## [1] 1
##
## $`4`
## [1] 1
```

## 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", "data/gff3.gff")
txdb <- makeTxDbFromGFF(file="data/gff3.gff", format="gff", dataSource="TAIR", organism="Arabidopsis thaliana")
```

```
## Warning in if (any(opts == 1L)) opts <- unique(c(opts[opts != 1L], 2L, 3L, : closing unused
## connection 6 (data/gff3.gff)
```

```
## Warning in if (any(opts == 1L)) opts <- unique(c(opts[opts != 1L], 2L, 3L, : closing unused
## connection 5 (data/gff3.gff)
```

```
## Warning in .extract_exons_from_GRanges(cds_IDX, gr, ID, Name, Parent, feature = "cds", : The following
## seqid start end strand ID Parent Name
```

```
## 1  Chr1  3760 3913      + <NA> AT1G01010.1-Protein <NA>
## 2  Chr1  3996 4276      + <NA> AT1G01010.1-Protein <NA>
## 3  Chr1  4486 4605      + <NA> AT1G01010.1-Protein <NA>
## 4  Chr1  4706 5095      + <NA> AT1G01010.1-Protein <NA>
## 5  Chr1  5174 5326      + <NA> AT1G01010.1-Protein <NA>
## 6  Chr1  5439 5630      + <NA> AT1G01010.1-Protein <NA>
```

```
saveDb(txdb, file="./data/TAIR10.sqlite")
```

```
## TxDb object:
## # Db type: TxDb
## # Supporting package: GenomicFeatures
## # Data source: TAIR
## # Organism: Arabidopsis thaliana
## # Taxonomy ID: 3702
## # miRBase build ID: NA
## # Genome: NA
## # transcript_nrow: 28
## # exon_nrow: 113
## # cds_nrow: 99
## # Db created by: GenomicFeatures package from Bioconductor
## # Creation time: 2017-04-26 10:52:28 -0700 (Wed, 26 Apr 2017)
## # GenomicFeatures version at creation time: 1.28.0
## # RSQLite version at creation time: 1.1-2
## # DBSCHEMAVERSION: 1.1
```

```
txdb <- loadDb("./data/TAIR10.sqlite")
transcripts(txdb)
```

```
## GRanges object with 28 ranges and 2 metadata columns:
##           seqnames      ranges strand |      tx_id      tx_name
##           <Rle>        <IRanges> <Rle> | <integer> <character>
## [1]      Chr1 [ 3631,  5899]      + |         1 AT1G01010.1
## [2]      Chr1 [  5928,  8737]      - |         2 AT1G01020.1
## [3]      Chr1 [  6790,  8737]      - |         3 AT1G01020.2
## [4]      Chr1 [11649, 13714]      - |         4 AT1G01030.1
## [5]      Chr2 [  1025,  2810]      + |         5 AT2G01008.1
## ...      ...      ...      ... | ...      ...
## [24]     ChrC [   383,  1444]      - |        24 ATCG00020.1
## [25]     ChrC [  1717,  4347]      - |        25 ATCG00030.1
## [26]     ChrM [11918, 12241]      + |        26 ATMG00030.1
## [27]     ChrM [   273,   734]      - |        27 ATMG00010.1
## [28]     ChrM [  8848, 11415]      - |        28 ATMG00020.1
## -----
##      seqinfo: 7 sequences (2 circular) from an unspecified genome; no seqlengths
```

```
transcriptsBy(txdb, by = "gene")
```

```
## GRangesList object of length 22:
## $AT1G01010
## GRanges object with 1 range and 2 metadata columns:
##           seqnames      ranges strand |      tx_id      tx_name
```

```
##          <Rle>      <IRanges>  <Rle> | <integer> <character>
##   [1]      Chr1 [3631, 5899]      + |           1 AT1G01010.1
##
## $AT1G01020
## GRanges object with 2 ranges and 2 metadata columns:
##      seqnames      ranges strand | tx_id      tx_name
##   [1]      Chr1 [5928, 8737]     - |      2 AT1G01020.1
##   [2]      Chr1 [6790, 8737]     - |      3 AT1G01020.2
##
## $AT1G01030
## GRanges object with 1 range and 2 metadata columns:
##      seqnames      ranges strand | tx_id      tx_name
##   [1]      Chr1 [11649, 13714]    - |      4 AT1G01030.1
##
## ...
## <19 more elements>
## -----
## seqinfo: 7 sequences (2 circular) from an unspecified genome; no seqlengths
```

```
exonsBy(txdb, by = "gene")
```

```
## GRangesList object of length 22:
## $AT1G01010
## GRanges object with 6 ranges and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##          <Rle>      <IRanges>  <Rle> | <integer> <character>
##   [1]      Chr1 [3631, 3913]      + |         1      <NA>
##   [2]      Chr1 [3996, 4276]      + |         2      <NA>
##   [3]      Chr1 [4486, 4605]      + |         3      <NA>
##   [4]      Chr1 [4706, 5095]      + |         4      <NA>
##   [5]      Chr1 [5174, 5326]      + |         5      <NA>
##   [6]      Chr1 [5439, 5899]      + |         6      <NA>
##
## $AT1G01020
## GRanges object with 12 ranges and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##   [1]      Chr1 [5928, 6263]     - |         7      <NA>
##   [2]      Chr1 [6437, 7069]     - |         8      <NA>
##   [3]      Chr1 [6790, 7069]     - |         9      <NA>
##   [4]      Chr1 [7157, 7232]     - |        10      <NA>
##   [5]      Chr1 [7157, 7450]     - |        11      <NA>
##   ...      ...      ...      ... .      ...      ...
##   [8]      Chr1 [7762, 7835]     - |        14      <NA>
##   [9]      Chr1 [7942, 7987]     - |        15      <NA>
##  [10]      Chr1 [8236, 8325]     - |        16      <NA>
##  [11]      Chr1 [8417, 8464]     - |        17      <NA>
##  [12]      Chr1 [8571, 8737]     - |        18      <NA>
##
## $AT1G01030
## GRanges object with 2 ranges and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##   [1]      Chr1 [11649, 13173]    - |        19      <NA>
##   [2]      Chr1 [13335, 13714]    - |        20      <NA>
##
```

```
## ...
## <19 more elements>
## -----
## seqinfo: 7 sequences (2 circular) from an unspecified genome; no seqlengths
```

## txdb from BioMart

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

```
library(GenomicFeatures); library("biomaRt")
txdb <- makeTxDbFromBiomart(biomart = "plants_mart", dataset = "athaliana_eg_gene", host="plants.ensembl.org")
```

The following steps are useful to find out what is available in BioMart.

```
listMarts() # Lists BioMart databases
listMarts(host="plants.ensembl.org")
mymart <- useMart("plants_mart", host="plants.ensembl.org") # Select one, here plants_mart_25
listDatasets(mymart) # List datasets available in the selected BioMart database
mymart <- useMart("plants_mart", dataset="athaliana_eg_gene", host="plants.ensembl.org")
listAttributes(mymart) # List available features
getBM(attributes=c("ensembl_gene_id", "description"), mart=mymart)[1:4,]
```

## Efficient Sequence Parsing

### getSeq

The following parses all annotation ranges provided by a 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"), 1000000, replace=TRUE), collapse="")))
writeXStringSet(DNAStringSet(rand), "./data/test")
getSeq(FaFile("./data/test"), gff)
```

```
## A DNAStringSet instance of length 442
##      width seq                                     names
## [1] 2269 GGTGCCTTCCTGCGGTCCTCTACGAAATCTTA...GGTTCCACTCTGCCTCATTGGCATCATGGCGC Chr1
## [2] 2269 GGTGCCTTCCTGCGGTCCTCTACGAAATCTTA...GGTTCCACTCTGCCTCATTGGCATCATGGCGC Chr1
## [3] 1871 CTCCAGACAAGTGAGGAAGTCTGCCTCACGGC...GCATAGCACAGGTAGGCAGACCTCTCGGAGTT Chr1
## [4] 283 GGTGCCTTCCTGCGGTCCTCTACGAAATCTTA...AGGCCACGGCCACAAGCCACGCACAGTTCTGC Chr1
## [5] 129 GGTGCCTTCCTGCGGTCCTCTACGAAATCTTA...CATCTCTGATAGGCGGGAGCTCTTACATTTAT Chr1
## ...
## [438] 324 AGTATCGCACTCGCGGTTTCGTGTTTAACTCCT...TTGACGATGCTGACTCAATCGTAATGGCATCT ChrM
## [439] 324 AGTATCGCACTCGCGGTTTCGTGTTTAACTCCT...TTGACGATGCTGACTCAATCGTAATGGCATCT ChrM
## [440] 324 AGTATCGCACTCGCGGTTTCGTGTTTAACTCCT...TTGACGATGCTGACTCAATCGTAATGGCATCT ChrM
## [441] 324 AGTATCGCACTCGCGGTTTCGTGTTTAACTCCT...TTGACGATGCTGACTCAATCGTAATGGCATCT ChrM
## [442] 324 AGTATCGCACTCGCGGTTTCGTGTTTAACTCCT...TTGACGATGCTGACTCAATCGTAATGGCATCT ChrM
```

## extractTranscriptSeqs

Sequences composed of several ranges, such as transcripts (or CDSs) with several exons, can be parsed with `extractTranscriptSeqs`. Note: the following expects the genome sequence in a file called `mygenome.fasta` and a valid `txdb` defining the ranges for that genome.

```
library(GenomicFeatures); library(Biostrings); library(Rsamtools)
extractTranscriptSeqs(FaFile("mygenome.fasta"), exonsBy(txdb, "tx", use.names=TRUE))
```

## Homework 6

### HW6a - Demultiplexing

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.

```
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", compress=FALSE)
      }
    }
  }
  close(f)
}
demultiplex(x=fastq[1], barcode=c("TT", "AA", "GG"), nreads=50)
```

### HW6b - Sequence Parsing

- Download GFF from *Halobacterium* sp [here](#)
- Download genome sequence from *Halobacterium* sp [here](#)
- **Task 1** Extract gene ranges, parse their sequences from genome and translate them into proteins
- **Task 2** Reduce overlapping genes and parse their sequences from genome
- **Task 3** Generate intergenic ranges and parse their sequences from genome

### Useful commands

```
download.file("ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/Halobacterium_sp_uid217/");
download.file("ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/Halobacterium_sp_uid217/");
chr <- readDNASTringSet("data/AE004437.fna")
gff <- import("data/AE004437.gff")
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), "./data/mypep.fasta")
```

## Homework submission

Submit the homework results in one well structured and annotated R script to the instructor. The script should include instructions on how to use the functions.

## Due date

This homework is due on Thu, May 4th at 6:00 PM.

## Homework Solutions

See [here](#).

## Session Info

```
sessionInfo()
```

```
## R version 3.4.0 (2017-04-21)
## Platform: x86_64-pc-linux-gnu (64-bit)
## 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
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C               LC_TIME=en_US.UTF-8
##  [4] LC_COLLATE=en_US.UTF-8    LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8      LC_NAME=C                   LC_ADDRESS=C
## [10] LC_TELEPHONE=C            LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
##  [1] grid      stats4     parallel  methods   stats      graphics  utils      datasets  grDevices
## [10] base
##
## other attached packages:
##  [1] GenomicFeatures_1.28.0      AnnotationDbi_1.38.0        rtracklayer_1.36.0
##  [4] systemPipeR_1.10.0          ShortRead_1.34.0            GenomicAlignments_1.12.0
##  [7] SummarizedExperiment_1.6.0 DelayedArray_0.2.0          matrixStats_0.52.2
## [10] Biobase_2.36.0              Rsamtools_1.28.0            GenomicRanges_1.28.0
```



```

## [13] GenomeInfoDb_1.12.0      BiocParallel_1.10.0      seqLogo_1.42.0
## [16] Biostrings_2.44.0         XVector_0.16.0           IRanges_2.10.0
## [19] S4Vectors_0.14.0         BiocGenerics_0.22.0      ggplot2_2.2.1
## [22] limma_3.32.0              BiocStyle_2.4.0
##
## loaded via a namespace (and not attached):
## [1] edgeR_3.18.0              splines_3.4.0            latticeExtra_0.6-28      RBGL_1.52.0
## [5] GenomeInfoDbData_0.99.0  yaml_2.1.14              Category_2.42.0          RSQLite_1.1-2
## [9] backports_1.0.5          lattice_0.20-35          digest_0.6.12            RColorBrewer_1.1-2
## [13] checkmate_1.8.2         colorspace_1.3-2         htmltools_0.3.5         Matrix_1.2-8
## [17] plyr_1.8.4              GSEABase_1.38.0          XML_3.98-1.6            pheatmap_1.0.8
## [21] biomaRt_2.32.0          genefilter_1.58.0        zlibbioc_1.22.0         xtable_1.8-2
## [25] GO.db_3.4.1             scales_0.4.1            brew_1.0-6              tibble_1.3.0
## [29] annotate_1.54.0         lazyeval_0.2.0          survival_2.41-3         magrittr_1.5
## [33] memoise_1.1.0           evaluate_0.10            fail_1.3                 hwriter_1.3.2
## [37] GOstats_2.42.0          graph_1.54.0            tools_3.4.0             BBmisc_1.11
## [41] stringr_1.2.0           sendmailR_1.2-1         munsell_0.4.3           locfit_1.5-9.1
## [45] compiler_3.4.0          RCurl_1.95-4.8          rjson_0.2.15            AnnotationForge_1.18.0
## [49] labeling_0.3            bitops_1.0-6            base64enc_0.1-3         rmarkdown_1.5
## [53] gtable_0.2.0            codetools_0.2-15        DBI_0.6-1               knitr_1.15.1
## [57] rprojroot_1.2           stringi_1.1.5           BatchJobs_1.6           Rcpp_0.12.10

```

## References

Huber, Wolfgang, Vincent J Carey, Robert Gentleman, Simon Anders, Marc Carlson, Benilton S Carvalho, Hector Corrada Bravo, et al. 2015. “Orchestrating High-Throughput Genomic Analysis with Bioconductor.” *Nat. Methods* 12 (2): 115–21. doi:10.1038/nmeth.3252.

Lawrence, Michael, Wolfgang Huber, Hervé Pagès, Patrick Aboyoun, Marc Carlson, Robert Gentleman, Martin T Morgan, and Vincent J Carey. 2013. “Software for Computing and Annotating Genomic Ranges.” *PLoS Comput. Biol.* 9 (8): e1003118. doi:10.1371/journal.pcbi.1003118.