Sequence Analysis with R and Bioconductor

Overview

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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
  - 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 sequenc

[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] "TTATATATAGCT"      "TTAAGATGTCCCTCGGG" "ACGACCATGAAC"
```

Enumerate sequences to check for duplicates.

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

       ACGACCATGAAC CCGATGCAAGCCATCGGGA   TTAAGATGTCCCTCGGG        TTATATATAGCT
                  1                    1                   1                   2
```

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: TAATAGGCCCTCCTAGTCTCTAAGTGTTGTGGATGGTGTCGGTGAACAACGCCTCGATGTAGTACCTAGGCCATTAAGAGCAAGACACAACGAAGCTATCC
```

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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

Sequence Import and Export

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

```
> # system("wget ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE004437.ffn")
> myseq <- read.DNAStringSet("AE004437.ffn")
> myseq[1:3]

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

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

[1] 185

> write.XStringSet(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 <- read.DNAMultipleAlignment(filepath = system.file("extdata",
+               "msx2_mRNA.aln", package = "Biostrings"), format = "clustal")
> origMAlign

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

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

  A QualityScaledDNAStringSet instance containing:
  A DNAStringSet instance of length 2
    width seq
[1]    20 AGACTGGACTCATGGAATGG
[2]    20 AGTAGTATCAAGTATTCACC

  A PhredQuality instance of length 2
    width seq
[1]    20 CB?2<71+#749CG#7D+<>
[2]    20 I'37299)1*C)F7&'@.B)
```

See ShortRead for additional utilities!

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]    12 AATATATATCGA
[2]    17 AATTCTACAGGGAGCCC

> reverse(randset[1:2])

  A DNAStringSet instance of length 2
    width seq
[1]    12 TCGATATATATT
[2]    17 GGGCTCCCTGTAGAATT

> reverseComplement(randset[1:2])

  A DNAStringSet instance of length 2
    width seq
[1]    12 AGCTATATATAA
[2]    17 CCCGAGGGACATCTTAA
```

Translate DNA sequences into proteins:

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

  A AAStringSet instance of length 2
    width seq
[1]     4 LYIA
[2]     5 LRCPS
```

Pattern Matching

Pattern matching with mismatches

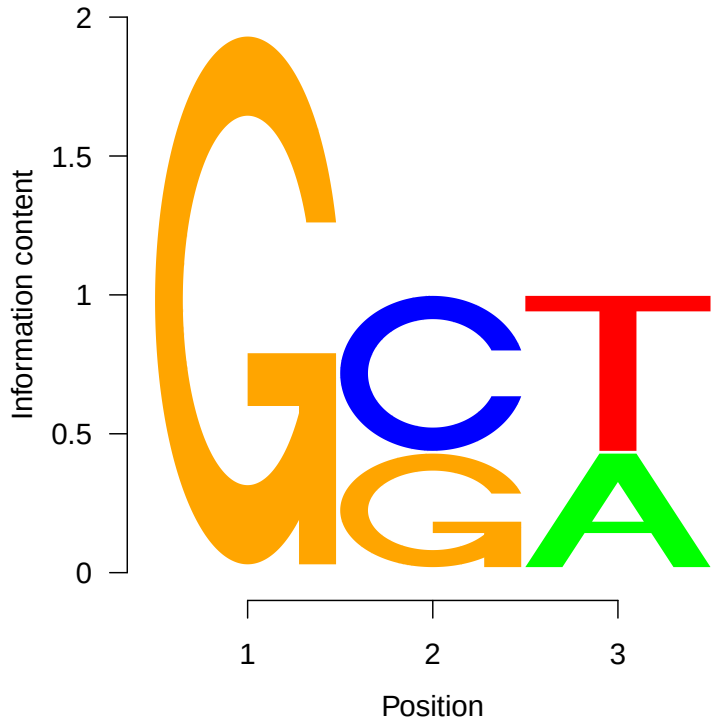
```
> myseq1 <- read.DNAStringSet("ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Halobacterium_sp_uid217/AE00
> mypos <- matchPattern("ATGGTG", myseq1[[1]], max.mismatch=1) # Finds pattern matches in reference
> countPattern("ATGGCT", myseq1[[1]], max.mismatch=1) # Counts only the corresponding matches
> tmp <- c(DNAStringSet("ATGGTG"), DNAStringSet(mypos)) # Results shoen 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 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]
```

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, en
> 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(elementMetadata(gff)[,"type"]=="chromosome"),]))
> names(gff) <- 1:length(gff) # Assigns names to corresponding slot.
> gff[1:4,]

GRanges with 4 ranges and 4 elementMetadata values:
    seqnames           ranges strand |       type   source    phase
       <Rle>        <IRanges>  <Rle> |   <factor> <factor> <factor>
  1     Chr1 [   1, 30427671]      + | chromosome   TAIR10     <NA>
  2     Chr1 [3631,     5899]      + |       gene   TAIR10     <NA>             ID=AT1G01010;Note=protein_
  3     Chr1 [3631,     5899]      + |       mRNA   TAIR10     <NA>         ID=AT1G01010.1;Parent=AT1G0101
  4     Chr1 [3760,     5630]      + |    protein   TAIR10     <NA> ID=AT1G01010.1-Protein;Name=AT1G01010.
  ---
  seqlengths:
       Chr1     Chr2     Chr3     Chr4     Chr5     ChrC     ChrM
   30427671 19698289 23459830 18585056 26975502   154478   366924

> 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
> 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) # 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
```

Outline

Exercise

GFF from *Halobacterium sp* Link

Genome from *Halobacterium sp* Link

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

```
> chr <- read.DNAStringSet("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"]
> translate(gene[names(gene) %in% pos])
> neg <- elementMetadata(gffgene[strand(gffgene) == "-"])[,"group"]
> translate(reverseComplement(gene[names(gene) %in% neg]))
```