Sequences

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Contents

1 Introduction to Bioconductor Classes and objects for String manipulation

Aim of this section

- get familiar with various containers for sequences
- read and display sequences from a FASTA file
- simple manipulations on sequences stores in a FASTA file such as reverse(), reverseComplement(), translate()
- calculate gc content

```
library(Biostrings)
library(ShortRead)
```

1.1 Containers for sequences

Bioconductor has various classes for storing sequences. You can find out the possible containers using:

```
showMethods(complement)
```

```
## Function: complement (package Biostrings)
## x="DNAString"
## x="DNAStringSet"
## x="MaskedDNAString"
## x="MaskedRNAString"
```

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```
## x="RNAString"
## x="RNAStringSet"
## x="XStringViews"
```

A quick example for each of them is:

```
b <- BString("I store any set of characters!" )
d <- DNAString("GCATAT-TAC") # Creates DNAString object.
r <- RNAString("GCAUAU-UAC") # Creates RNAString object.
r <- RNAString(d) # Converts d into RNAString object.
p <- AAString("HCWYHH")
```

Lets look at how you can use these on a daily basis:

You're studying Breast Cancer genes -BRCA1 and BRCA2 - Inherited mutations in BRCA1 and BRCA2, confer increased lifetime risk of developing breast or ovarian cancer

We want to do the following:

For BRCA1: We can learn more about the gene at - http://www.ncbi.nlm.nih.gov/gene/672 We can download the FASTA sequence as a file at: http://www.ncbi.nlm.nih.gov/nuccore/NC_000017.11?report=fasta&from=43032116&to=43137660&strand=true

Similarly, for BRCA2 , We can learn more about the gene at http://www.ncbi.nlm.nih.gov/gene/675 we can get the FASTA sequence from : http://www.ncbi.nlm.nih.gov/nuccore/NC_000013.11?report=fasta&from=32302850&to=32412300

These files are already saved for you and you can access them using

```
fls <- list.files(system.file("extdata", package="BiocIntro"),
                  pattern =".txt",full=TRUE)
fls
```

```
## [1] "/tmp/RtmpKcoIsX/Rinst2338456f7a12/BiocIntro/extdata/brca1_cds.txt"
## [2] "/tmp/RtmpKcoIsX/Rinst2338456f7a12/BiocIntro/extdata/brca2_cds.txt"
```

So lets begin by reading them into an R session using DNAStringSet, a container for storing DNAString objects. For this we use the readFASTA from the ShortReads package in Biocondcutor. readFasta reads all FASTA-formated files stored in fls. It returns a DNAStringSet containing sequences and qualities contained in the given file. We will then use sread from the ShortRead package to create a DNAStringSet and diaply the sequence for one of the genes in a nice, user fiendly format.

```
#Approach- 1
# read in the FATSA file
seq <- readFasta(fls)

##Lets create a DNAStringSet which is a container for storing a set of DNAString
dna <- sread(seq)

#Approach-2
dna <- readDNAStringSet(fls)

# let us look at the first DNAString, brca1 stored at [1]
# This [[]] operation converts a DNAStringSet to DNAString
brca1 <- dna[[1]]
brca2 <- dna[[2]]


# making the output more understandable.
```

```
# A sequence in FASTA format is represented as a series of lines, each of which
# usually do not exceed 80 characters.
successiveViews(brca1,  width=rep(50,length(dna[[1]])/50+1))
```

```
##   Views on a 5592-letter DNAString subject
## subject: ATGGATTTATCTGCTCTTCGCGTTGAAGAAGTACAAAAT...ACCTACCTGATACCCCAGATCCCCCACAGCCACTACTGA
## views:
##       start  end width
##   [1]     1   50    50 [ATGGATTTATCTGCTCTTCGCGTTGAAGAAGTACAAAATGTCATTAATGC]
##   [2]    51  100    50 [TATGCAGAAAATCTTAGAGTGTCCCATCTGTCTGGAGTTGATCAAGGAAC]
##   [3]   101  150    50 [CTGTCTCCACAAAGTGTGACCACATATTTTGCAAATTTTGCATGCTGAAA]
##   [4]   151  200    50 [CTTCTCAACCAGAAGAAAGGGCCTTCACAGTGTCCTTTATGTAAGAATGA]
##   [5]   201  250    50 [TATAACCAAAAGGAGCCTACAAGAAAGTACGAGATTTAGTCAACTTGTTG]
##   ...   ...  ...   ... ...
## [108]  5351 5400    50 [TACAGCTGTGTGGTGCTTCTGTGGTGAAGGAGCTTTCATCATTCACCCTT]
## [109]  5401 5450    50 [GGCACAGGTGTCCACCCAATTGTGGTTGTGCAGCCAGATGCCTGGACAGA]
## [110]  5451 5500    50 [GGACAATGGCTTCCATGCAATTGGGCAGATGTGTGAGGCACCTGTGGTGA]
## [111]  5501 5550    50 [CCCGAGAGTGGGTGTTGGACAGTGTAGCACTCTACCAGTGCCAGGAGCTG]
## [112]  5551 5600    50 [GACACCTACCTGATACCCCAGATCCCCCACAGCCACTACTGA        ]
```

By default we show only the top 5 and last 5 in a given View. But we can set `options(showHeadLines=Inf)` to display everything

```
options(showHeadLines=Inf)
successiveViews(brca1,  width=rep(50,length(dna[[1]])/50+1))
```

1.2 Basic Manipulations

Exercise:1

a) Create the complement, the reverse and the reverse and complement sequences for brca1

```
reverse(brca1)
```

```
##   5592-letter "DNAString" instance
## seq: AGTCATCACCGACACCCCCTAGACCCCATAGTCCATCCACA...TGTAAAACATGAAGAAGTTGCGCTTCTCGTCTATTTAGGTA
```

```
complement(brca1)
```

```
##   5592-letter "DNAString" instance
## seq: TACCTAAATAGACGAGAAGCGCAACTTCTTCATGTTTTACA...TGTGGATGGACTATGGGGTCTAGGGGGTGTCGGTGATGACT
```

```
reverseComplement(brca1)
```

```
##   5592-letter "DNAString" instance
## seq: TCAGTAGTGGCTGTGGGGGATCTGGGGTATCAGGTAGGTGT...ACATTTTGTACTTCTTCAACGCGAAGAGCAGATAAATCCAT
```

b) Translate your random DNA sequences into proteins.

```
translate(brca1)
```

```
##   1864-letter "AAString" instance
## seq: MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDH...IGQMCEAPVVTREWVLDSVALYQCQELDTYLIPQIPHSHY*
```

1.3 Pre-defined constants

Bioconductor also has some predefined constants which you can use.

```
DNA_BASES
```

```
## [1] "A" "C" "G" "T"
```

```
DNA_ALPHABET
```

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

```
IUPAC_CODE_MAP
```

```
##      A      C      G      T      M      R      W      S      Y      K      V      H
##    "A"    "C"    "G"    "T"   "AC"   "AG"   "AT"   "CG"   "CT"   "GT"  "ACG"  "ACT"
##      D      B      N
##  "AGT"  "CGT" "ACGT"
```

1.4 Frequencies

We can also find out various frequencies for our given GOI, lets look at brca2:

```
# what are the unique letter ?
uniqueLetters(brca2)
```

```
## [1] "A" "C" "G" "T"
```

```
alphabetFrequency(brca2)
```

```
##    A    C    G    T    M    R    W    S    Y    K    V    H    D    B    N    -    +    .
## 3769 1784 1882 2822    0    0    0    0    0    0    0    0    0    0    0    0    0    0
```

```
alphabetFrequency(brca2, baseOnly=TRUE)
```

```
##     A     C     G     T other
##  3769  1784  1882  2822     0
```

```
dinucleotideFrequency(brca2)
```

```
##   AA   AC   AG   AT   CA   CC   CG   CT   GA   GC   GG   GT   TA   TC   TG   TT
## 1562  554  805  847  774  339   74  597  765  333  313  471  667  558  690  907
```

```
trinucleotideFrequency(brca2)
```

```
## AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT ATA ATC ATG ATT CAA CAC CAG CAT CCA CCC
## 687 206 334 334 233 110  31 180 336 146 133 190 225 143 216 263 278 106 229 161 161  64
## CCG CCT CGA CGC CGG CGT CTA CTC CTG CTT GAA GAC GAG GAT GCA GCC GCG GCT GGA GGC GGG GGT
##  12 102  22  15  12  25 116 107 184 190 369 102 122 172 146  71  12 104 152  59  36  66
## GTA GTC GTG GTT TAA TAC TAG TAT TCA TCC TCG TCT TGA TGC TGG TGT TTA TTC TTG TTT
## 124  90 109 148 228 140 120 179 234  94  19 211 255 113 132 190 202 218 181 306
```

you can also have oligonucleotideFrequency()

Exercise:2 Can you find the GC content for BRCA1 and BRCA2? Hint: use alphabetFrequency

Solution:

```
gcContent <-
    function(x)
{
    alf <- alphabetFrequency(x, as.prob=TRUE)
    sum(alf[c("G","C")])
}

gcContent(brca1)
```

```
## [1] 0.4122
```

```
gcContent(brca2)
```

```
## [1] 0.3574
```

1.5 Bioconductor has data pacakges for your favourite organism

BSgenome Data Packages

- Full genomes stored in Biostrings containers
- Currently 16 organisms supported (Human, Mouse, Worm, Yeast, etc...)
- acilities for supporting new genomes (BSgenomeForge)

Exercise:3 a. Can you find out the gc content for chromosome 17 (home of BRCA1) and the gc content for chromosome 13 (home of BRCA2)

```
library(BSgenome.Hsapiens.UCSC.hg19)
```

```
gcContent(Hsapiens[["chr17"]])
```

```
## [1] 0.4363
```

```
gcContent(Hsapiens[["chr13"]])
```

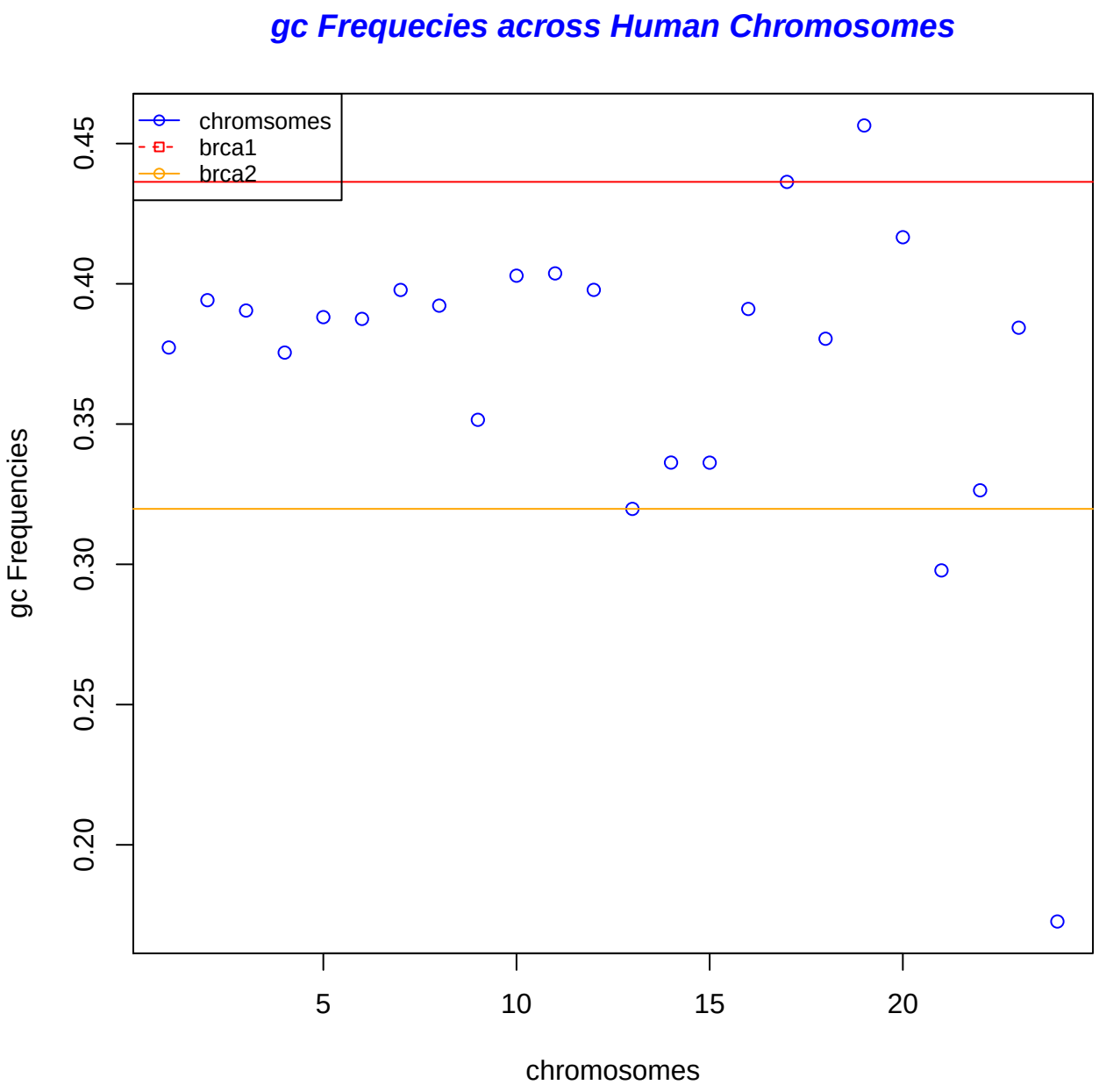
```
## [1] 0.3198
```

b. Please create a plot of the GC frequencies for all the primary chromosomes Please superimpose the frequencies of BRCA1 and BRCA2 on this plot Add title , legend and appropriate labels for the axes.

```
chrs <- paste0("chr", c(1:22,"X","Y"))
data <- sapply(chrs, function(x) gcContent(Hsapiens[[x]]))
names(data) <- chrs

plot(data,
     xlab="chromosomes",ylab="gc Frequencies",
     xlim=c(1,24),
        col="blue")
abline(h=gcContent(Hsapiens[["chr17"]]),col="red")
abline(h=gcContent(Hsapiens[["chr13"]]),col="orange")
title(main="gc Frequecies across Human Chromosomes", col.main="blue",
      font.main=4)

legend("topleft",c("chromsomes","brca1","brca2"), cex=0.8,
   col=c("blue","red","orange"), pch=21:22, lty=1:2)
```



2 Session Info

Here is the output of sessionInfo on the system on which this document was compiled:

```
sessionInfo()
```

```
## R Under development (unstable) (2014-02-23 r65064)
## Platform: x86_64-unknown-linux-gnu (64-bit)
##
## locale:
```

```
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C               LC_TIME=en_US.UTF-8
##  [4] LC_COLLATE=C               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] parallel  stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] quantreg_5.05                          SparseM_1.03
##  [3] BiocIntro_0.0.4                        rtracklayer_1.23.12
##  [5] AnnotationHub_1.3.18                   BSgenome.Hsapiens.UCSC.hg19_1.3.99
##  [7] BSgenome_1.31.11                       TxDb.Hsapiens.UCSC.hg19.knownGene_2.10.1
##  [9] GenomicFeatures_1.15.7                 org.Hs.eg.db_2.10.1
## [11] RSQLite_0.11.4                         DBI_0.2-7
## [13] AnnotationDbi_1.25.9                   Biobase_2.23.5
## [15] RNAseqData.HNRNPC.bam.chr14_0.1.6      ggplot2_0.9.3.1
## [17] VariantAnnotation_1.9.41               ShortRead_1.21.14
## [19] GenomicAlignments_0.99.24              Rsamtools_1.15.29
## [21] GenomicRanges_1.15.31                  Biostrings_2.31.14
## [23] XVector_0.3.7                          IRanges_1.21.32
## [25] BiocParallel_0.5.14                    BiocGenerics_0.9.3
## [27] knitr_1.5
##
## loaded via a namespace (and not attached):
##  [1] BBmisc_1.5               BatchJobs_1.2            BiocInstaller_1.13.3
##  [4] BiocStyle_1.1.17         Category_2.29.1          GSEABase_1.25.2
##  [7] MASS_7.3-29              Matrix_1.1-2             RBGL_1.39.1
## [10] RColorBrewer_1.0-5       RCurl_1.95-4.1           RJSONIO_1.0-3
## [13] Rcpp_0.11.0              XML_3.98-1.1             annotate_1.41.1
## [16] biomaRt_2.19.3           bitops_1.0-6             brew_1.0-6
## [19] codetools_0.2-8          colorspace_1.2-4         dichromat_2.0-0
## [22] digest_0.6.4             evaluate_0.5.1           fail_1.2
## [25] foreach_1.4.1            formatR_0.10             genefilter_1.45.1
## [28] graph_1.41.2             grid_3.1.0               gridSVG_1.4-0
## [31] gtable_0.1.2             highr_0.3                httpuv_1.2.3
## [34] httr_0.2                 hwriter_1.3              interactiveDisplay_1.0.30
## [37] iterators_1.0.6          labeling_0.2             lattice_0.20-24
## [40] latticeExtra_0.6-26      munsell_0.4.2            plyr_1.8
## [43] proto_0.3-10             reshape2_1.2.2           rjson_0.2.13
## [46] scales_0.2.3             sendmailR_1.1-2          shiny_0.8.0.99
## [49] splines_3.1.0            stats4_3.1.0             stringr_0.6.2
## [52] survival_2.37-7          tools_3.1.0              xtable_1.7-1
## [55] zlibbioc_1.9.0
```