# BioC for HTS - PDCB topic
# Infrastructure and Input/Output 02

LCG Leonardo Collado Torres
lcollado@wintergenomics.com – lcollado@ibt.unam.mx

October 13th, 2010

Exercises

IRanges

GenomicRanges

# Some practice

- From the aln object, extract the dinucleotide frequency for the last 2 cycles.

```
> library(ShortRead)
> exptPath <- system.file("extdata",
+     package = "ShortRead")
> sp <- SolexaPath(exptPath)
> aln <- readAligned(sp, "s_2_export.txt")
> di <- colSums(dinucleotideFrequency(sread(narrow(aln,
+     start = 34, width = 2))))
> di
```

# Some practice

| AA | AC | AG | AT | CA | CC | CG | CT | GA | GC |
|---|---|---|---|---|---|---|---|---|---|
| 85 | 52 | 50 | 74 | 72 | 57 | 23 | 63 | 76 | 43 |
| GG | GT | TA | TC | TG | TT | | | | |
| 44 | 49 | 64 | 67 | 65 | 115 | | | | |

1. Given the GC percentage of all cycles, did you expect the results you observe?

```
> gc <- alphabetByCycle(sread(aln))
> gc <- colSums(gc[c("C", "G"), ])/colSums(gc)
> diGC <- unlist(lapply(strsplit(names(di),
+     ""), function(x) {
+     sum(x %in% c("C", "G"))
+ }))
> diGC
```

```
 [1] 0 1 1 0 1 2 2 1 1 2 2 1 0 1 1 0
```

# Some practice

```
> expected <- lapply(gc, function(x) {
+     res <- (x * diGC) + ((1 - x) *
+         (2 - diGC))
+     res <- res/sum(res) * length(aln)
+     return(res)
+ })
> expected[[1]]
 [1] 61.0 62.5 62.5 61.0 62.5 64.0 64.0
 [8] 62.5 62.5 64.0 64.0 62.5 61.0 62.5
[15] 62.5 61.0

> res <- unlist(lapply(expected,
+     function(x) {
+         t.test(di, round(x), paired = TRUE)$p.value
+     }))
> sum(res < 0.05)
```

# Some practice

```
[1] 0
```

2. Which is the read with NN at the end?

```
> sum(di)
```

```
[1] 999
```

```
> table(as.character(sread(narrow(aln,
+     start = 34, width = 2))))
```

```
 AA  AC  AG  AT  CA  CC  CG  CT  GA  GC
 85  52  50  74  72  57  23  63  76  43
 GG  GT  NN  TA  TC  TG  TT
 44  49   1  64  67  65 115
```

```
> i <- which(as.character(sread(narrow(aln,
+     start = 34, width = 2))) ==
+     "NN")
> i
```

# Some practice

```
[1] 882
```

```
> sread(aln[i])
```

```
  A DNAStringSet instance of length 1
    width seq
[1]    35 GNNNNNNNNNNNNNN...NNNNNNNNNNNNNN
```

3. Is there a significative difference vs the dinucleotide frequency of cycles 15 and 16?

```
> res[15:16]
```

```
[1] 0.9886094 0.9627654
```

```
> res[15:16] < 0.05
```

```
[1] FALSE FALSE
```

# Some practice

- Load the na19240url object (note that fpt doesn't wort at IBt).

```
> library(Rsamtools)
> load("na19240.Rda")
```

- Are all reads of the same length? If not, what is the distribution? Make a cumulative plot.

```
> unlist(lapply(na19240bam[[1]],
+     class))
```

# Some practice

```
          qname           flag
    "character"      "integer"
          rname         strand
       "factor"       "factor"
            pos         qwidth
      "integer"      "integer"
           mapq          cigar
      "integer"    "character"
           mrnm           mpos
       "factor"      "integer"
            isize            seq
      "integer" "DNAStringSet"
           qual
"PhredQuality"
```

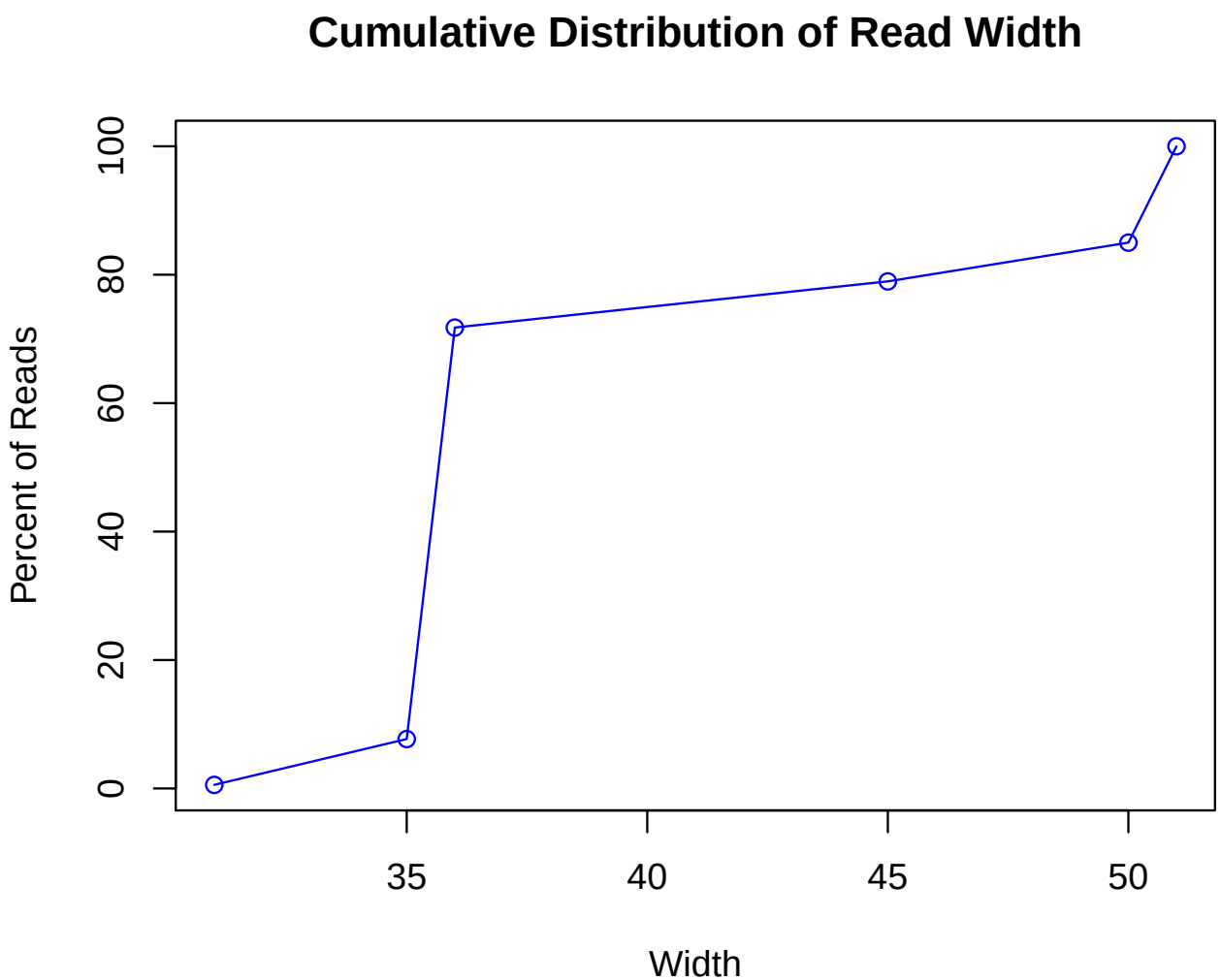
# Some practice

```
> l.seqs <- width(na19240bam[[1]]$seq)
> t.seqs <- table(l.seqs)
> t.seqs

l.seqs
  31   35   36   45   50   51
  61  776 6966  782  656 1631

> table(na19240bam[[1]]$qwidth)

  31   35   36   45   50   51
  60  719 6646  753  633 1619
```

# Some practice

```
> plot(x = as.numeric(names(t.seqs)),
+      y = cumsum(t.seqs)/sum(t.seqs) *
+          100, type = "o", col = "blue",
+      xlab = "Width", ylab = "Percent of Reads",
+      main = "Cumulative Distribution of Read Width")
```

# Some practice



# Some practice

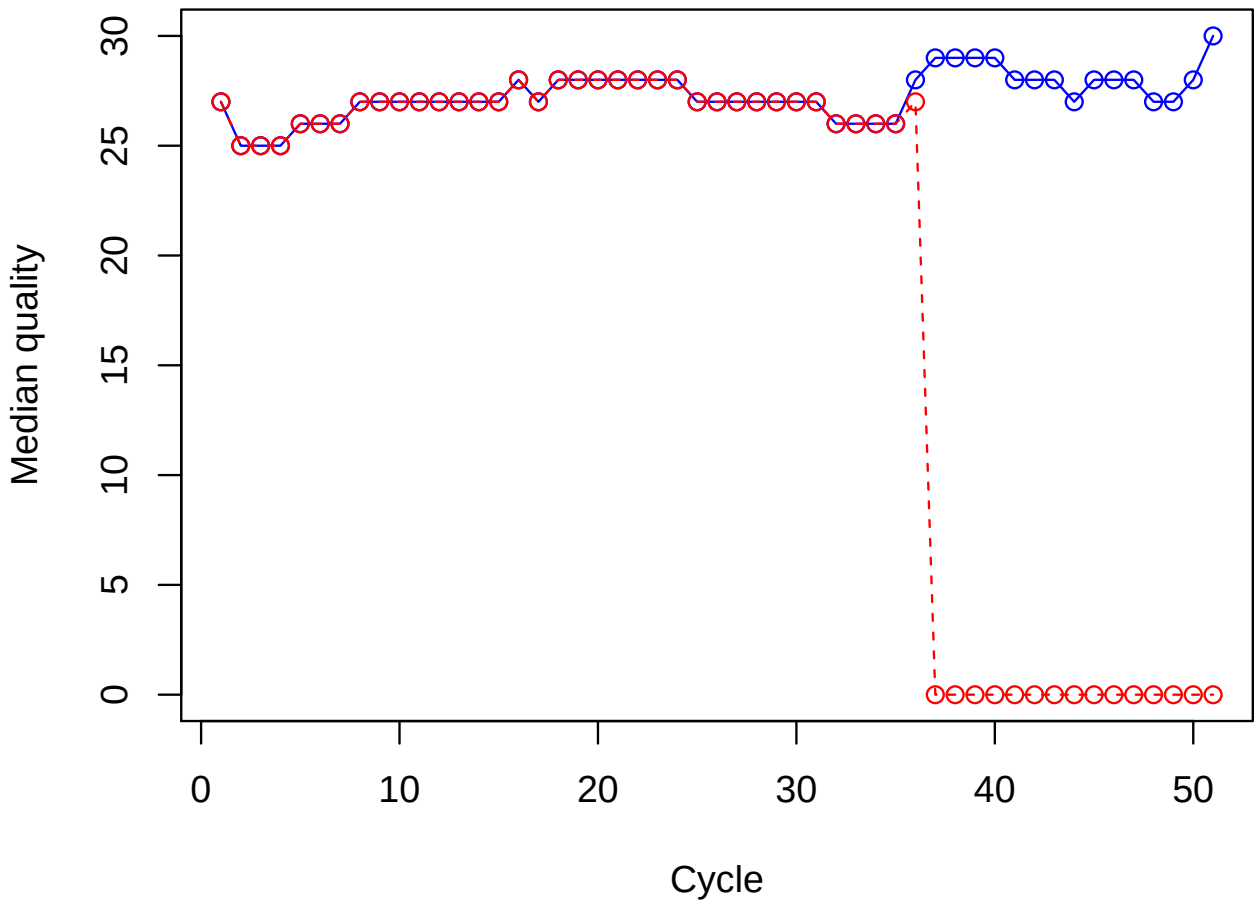
- Convert the PhredQuality instance to a quality matrix and make a plot of the median quality per cycle. Is there any trend in the quality?

```
> as(na19240bam[[1]]$qual, "matrix")

> qual <- lapply(na19240bam[[1]]$qual,
+     as.integer)

> mat <- matrix(NA, nrow = length(qual),
+     ncol = max(unlist(lapply(qual,
+         length))))
> for (i in 1:length(qual)) mat[i,
+     1:length(qual[[i]])] <- qual[[i]] -
+     33
```

# Some practice

```
> mat2 <- mat
> for (i in 1:nrow(mat2)) mat2[i,
+     is.na(mat2[i, ])] <- 0
> medians <- apply(mat, 2, median,
+     na.rm = TRUE)
> medians2 <- apply(mat2, 2, median)
> plot(1:length(medians), medians,
+     type = "o", xlab = "Cycle",
+     ylab = "Median quality", col = "blue",
+     ylim = c(min(medians, medians2),
+         max(medians, medians2)))
> lines(medians2, type = "o", col = "red",
+     lty = 2)
```

# Some practice

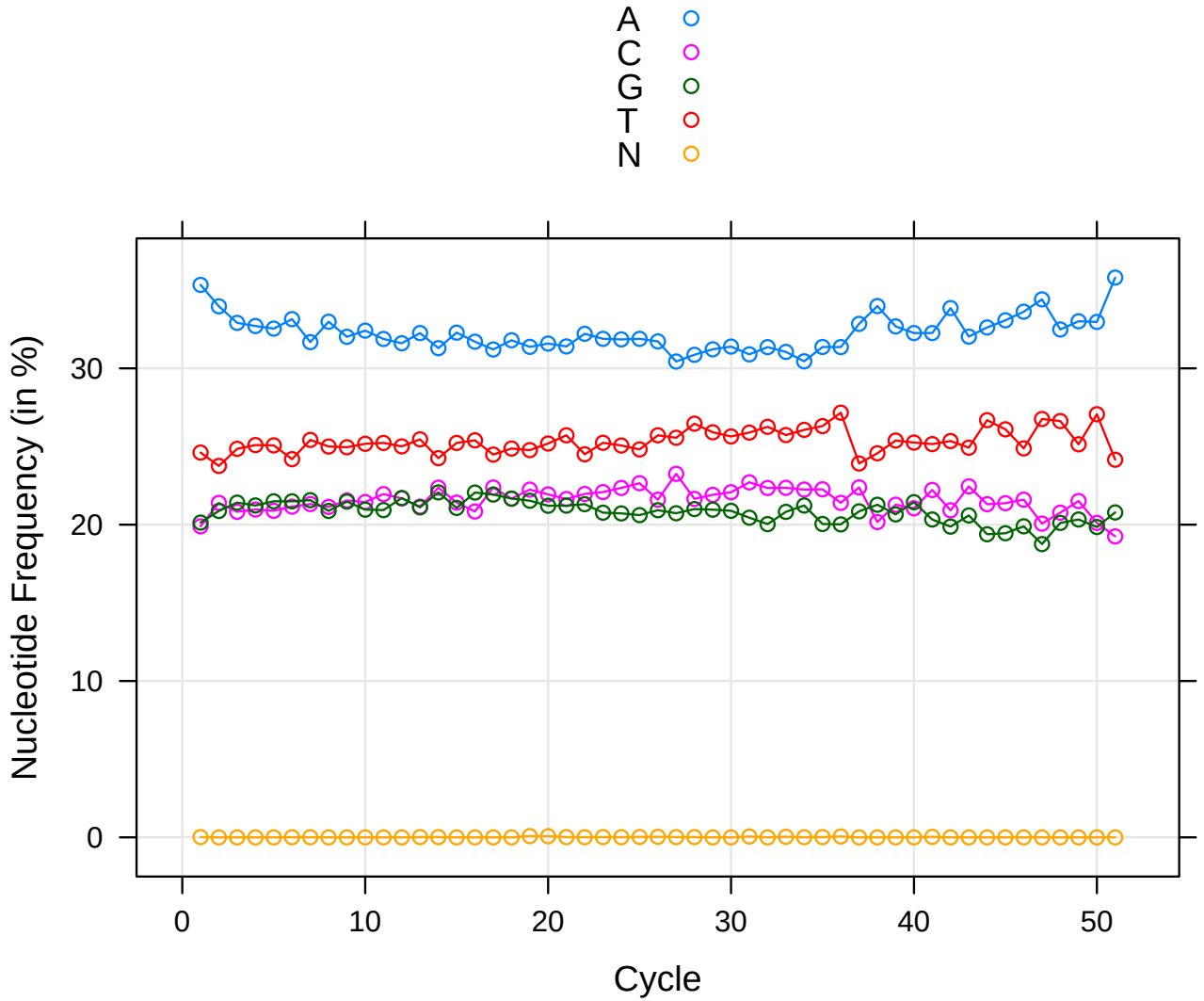


# Some practice

- Make a third plot for the alphabet by cycle relative frequency (in percent). Do you observe anything unexpected?

```
> abc <- alphabetByCycle(na19240bam[[1]]$seq)
> abc <- t(abc[rowSums(abc) > 0,
+     ])
> abc <- abc/rowSums(abc) * 100
> abc <- as.data.frame(abc)
> library(lattice)
> print(xyplot(A + C + G + T + N ~
+     1:nrow(abc), data = abc, auto.key = TRUE,
+     type = c("o", "g"), xlab = "Cycle",
+     ylab = "Nucleotide Frequency (in %)"))
```

# Some practice



# Overview

IRanges is an infraestructure package that

- will help us save memory
- allows us to manipulate data in ranges
- is the backbone for manipulating our HTS data

# Rle

- Rle or Run length encoded objects are the main source of memory usage reduction in IRanges
- The idea: if neighbor values are frequently repeated in a vector, we can now construct a kind of matrix where we have each value in one row and the number of times it appears on the second row.
- For example, we have the vector x which is made up of 0s and 1s:

```
> x <- round(runif(10000))
> table(x)

x
   0    1
4991 5009
```

# Rle

```
> head(x)
[1] 0 0 1 0 1 1
> tail(x)
[1] 0 1 0 0 1 0
```

- We can observe that 0s are 1s are adjecent to each other quite frequently. This kind of vector is a good candidate to transform into an Rle:

```
> library(IRanges)
> y <- Rle(x)
> y
```

# Rle

```
'numeric' Rle of length 10000 with 5075 runs
  Lengths: 2 1 1 4 3 1 ... 1 1 1 2 1 1
  Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0
```

- This allows us to save some memory:

```
> print(object.size(x) - object.size(y),
+       units = "Kb")
```

```
17.4 Kb
```

- With larger vectors, we save more memory :)

```
> x2 <- round(runif(4e+06))
> y2 <- Rle(x2)
> print(object.size(x2) - object.size(y2),
+       units = "Kb")
```

```
7793.4 Kb
```

# More on Rle

- Just like with vectors, several basic accessors have been implemented:

```
> x[2:3]

[1] 0 1

> y[2:3]

'numeric' Rle of length 2 with 2 runs
  Lengths: 1 1
  Values : 0 1

> c(x[2:3], x[10])

[1] 0 1 0

> c(y[2:3], y[10])
```

# More on Rle

```
'numeric' Rle of length 3 with 3 runs
  Lengths: 1 1 1
  Values : 0 1 0
```

```
> identical(c(y[2:3], y[10]), append(y[2:3],
+     y[10]))
```

```
[1] TRUE
```

- And if needed, you can always return to a vector:

```
> as.vector(y[1:10])
```

```
 [1] 0 0 1 0 1 1 1 1 0 0
```

```
> identical(x, as.vector(y))
```

```
[1] TRUE
```

# More on Rle

- Our object y is a numeric Rle. We can also create other type of Rles:

```
> z <- y > 0
> m <- Rle(sample(c("A", "C", "T",
+     "G"), 10000, replace = TRUE))
> n <- Rle(as.factor(sample(1:10,
+     10000, replace = TRUE)))
> o <- Rle(as.integer(x))
> identical(y, o)
```

```
[1] FALSE
```

- Using the function strand we can create a special kind of factor:

# More on Rle

```
> str <- strand(sample(c("+", "-"),
+     1000, replace = TRUE))
> class(str)

[1] "factor"

> levels(str)

[1] "+" "-" "*"

> head(str)

[1] - - - + + +
Levels: + - *
```

- Without any problems, we can convert this strand factor into an Rle:

```
> Rle(str)
```

# More on Rle

```
'factor' Rle of length 1000 with 490 runs
  Lengths: 3 3 3 3 3 3 ... 1 4 1 1 1 1
  Values : - + - + - + ... - + - + - +
Levels(3): + - *
```

# Lets practice a bit

- Using the following vectors a and b, what are the mean and median a values for each level of the b factor. Transform them into Rle objects.

```
> a <- round(runif(1e+05, min = 0,
+     max = 10000))
> b <- sample(c("+", "-"), 1e+05,
+     replace = TRUE)
```

# Solutions

- First, a long solution where we transform our objects back to vectors in order to use the functions mean and median:

```
> e <- Rle(a)
> f <- Rle(b)
> sapply(unique(f), function(x) {
+     set <- as.vector(e[f == x])
+     res <- c(mean(set), median(set))
+ })
```

```
             -        +
[1,] 5006.663 4992.109
[2,] 5017.000 4990.000
```

# Solutions

- However, the above is not necessary since both mean and median have methods for Rle objects. Hence, we can solve it like this:

```
> sapply(unique(f), function(x) {
+     set <- e[f == x]
+     res <- c(mean(set), median(set))
+ })
```

```
          -        +
[1,] 5006.663 4992.109
[2,] 5017.000 4990.000
```

- Yet, the ideal scenario is to use the tapply function :) Either one by one or all together.

```
> tapply(e, f, mean)
```

# Solutions

```
       -        +
5006.663 4992.109
```

```
> tapply(e, f, median)
```

```
   -    +
5017 4990
```

```
> tapply(e, f, function(x) {
+     c(mean(x), median(x))
+ })
```

```
$`-`
[1] 5006.663 5017.000

$`+`
[1] 4992.109 4990.000
```

# Solutions

- In this case we could have used tapply from the start with the vectors a and b:

```
> tapply(a, b, function(x) {
+     c(mean(x), median(x))
+ })

$`-`
[1] 5006.663 5017.000

$`+`
[1] 4992.109 4990.000
```

- Basically, we can use Rle's just as we would use vectors yet we get the memory advantage :)[1]
- Btw, this was another solution:

# Solutions

```
> tapply(e, f, function(x) {
+     summary(x)[3:4]
+ })

$`-`
Median   Mean
  5017   5007

$`+`
Median   Mean
  4990   4992
```

[1] To make full use of the advantage we shouldn't create the vectors, just create the Rles directly.

# More on Rles

- Just like with vectors, we can reverse or access a subsection of an Rle

```
> y
```

```
'numeric' Rle of length 10000 with 5075 runs
  Lengths: 2 1 1 4 3 1 ... 1 1 1 2 1 1
  Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0
```

```
> rev(y)
```

```
'numeric' Rle of length 10000 with 5075 runs
  Lengths: 1 1 2 1 1 1 ... 1 3 4 1 1 2
  Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0
```

```
> window(y, 2, 4)
```

# More on Rles

```
'numeric' Rle of length 3 with 3 runs
  Lengths: 1 1 1
  Values : 0 1 0
```

- We can also get into the parts of an Rle object using:

```
> head(runLength(y))
```

```
[1] 2 1 1 4 3 1
```

```
> head(runValue(y))
```

```
[1] 0 1 0 1 0 1
```

- Remember the matrix idea that lead to Rles? Well, we can build that said matrix:

# More on Rles

```
> mat <- matrix(0, nrow = nrun(y),
+     ncol = 2)
> mat[, 1] <- runLength(y)
> mat[, 2] <- runValue(y)
> head(mat)
```

```
     [,1] [,2]
[1,]    2    0
[2,]    1    1
[3,]    1    0
[4,]    4    1
[5,]    3    0
[6,]    1    1
```

```
> y
```

# More on Rles

```
'numeric' Rle of length 10000 with 5075 runs
  Lengths: 2 1 1 4 3 1 ... 1 1 1 2 1 1
  Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0
```

- We can also get the start and end positions for each run:

```
> head(start(y))
```

```
[1]  1  3  4  5  9 12
```

```
> head(end(y))
```

```
[1]  2  3  4  8 11 12
```

- There are plenty of other numerical and character methods for Rles which you find on the help page for Rle. For example:

```
> cor(y, e[1:10000])
```

```
[1] 0.001470080
```

# More on Rles

```
> range(e)
```

```
[1]     0 10000
```

- We can also create list of Rle objects:

```
> rlelist <- RleList(y, e[1:10000])
> rlelist
```

```
SimpleRleList of length 2
[[1]]
'numeric' Rle of length 10000 with 5075 runs
  Lengths: 2 1 1 4 3 1 ... 1 1 1 2 1 1
  Values : 0 1 0 1 0 1 ... 1 0 1 0 1 0

[[2]]
'numeric' Rle of length 10000 with 10000 runs
```

# More on Rles

```
Lengths:    1    1    1 ...    1    1
Values : 5614 7993 5994 ... 4163 9976
```

# IRanges

- Another fundamental piece of IRanges is the ability to construct matrixes of ranges using IRanges. For example:

```
> IR <- IRanges(start = 1:5, end = 6:10)
```

- Data from an IRanges object can be easily accessed:

```
> length(IR)
[1] 5
> IR[2]
IRanges of length 1
    start end width
[1]     2   7     6
> start(IR[5])
```

# IRanges

```
[1] 5
```

```
> end(IR[3])
```

```
[1] 8
```

```
> width(IR)
```

```
[1] 6 6 6 6 6
```

- Once we have ranges, we can manipulate them:

```
> reduce(IR)
```

```
IRanges of length 1
    start end width
[1]     1  10    10
```

```
> disjoin(IR)
```

# IRanges

```
IRanges of length 9
    start end width
[1]     1   1     1
[2]     2   2     1
[3]     3   3     1
[4]     4   4     1
[5]     5   6     2
[6]     7   7     1
[7]     8   8     1
[8]     9   9     1
[9]    10  10     1
```

- And find overlaps between ranges:

# IRanges

```
> ov <- findOverlaps(IR, reduce(IR))
> as.matrix(ov)
```

```
     query subject
[1,]     1       1
[2,]     2       1
[3,]     3       1
[4,]     4       1
[5,]     5       1
```

# Exercise

- Construct an IRanges object where we'll have 1 range per every read.
- Use the position and width of the read from the aln object

```
> aln
```

```
class: AlignedRead
length: 1000 reads; width: 35 cycles
chromosome: NM NM ... chr5.fa 29:255:255
position: NA NA ... 71805980 NA
strand: NA NA ... + NA
alignQuality: NumericQuality
alignData varLabels: run lane ... filtering contig
```

# Solution

- We just need to be careful with the reads from the minus strand and those that did not map

```
> idx <- which(!is.na(position(aln)))
> start <- position(aln[idx])
> str <- strand(aln[idx]) == "-"
> start[str] <- start[str] - width(aln[idx])[str] +
+     1
> reads <- IRanges(start = start,
+     width = width(aln[idx]))
```

- Once we have our reads in an IRanges object, we can get information such as the coverage:

```
> cov <- coverage(reads)
> cov
```

# Solution

```
'integer' Rle of length 195524766 with 810 runs
  Lengths:   11907      35 ...      35
  Values :       0       1 ...       1
```

- Or manipulate further the ranges:

```
> shift(IR, 10)
```

```
IRanges of length 5
    start end width
[1]    11  16     6
[2]    12  17     6
[3]    13  18     6
[4]    14  19     6
[5]    15  20     6
```

```
> narrow(IR, start = 1, width = 2)
```

# Solution

```
IRanges of length 5
    start end width
[1]     1   2     2
[2]     2   3     2
[3]     3   4     2
[4]     4   5     2
[5]     5   6     2
```

```
> flank(IR, 1)
```

# Solution

```
IRanges of length 5
    start end width
[1]     0   0     1
[2]     1   1     1
[3]     2   2     1
[4]     3   3     1
[5]     4   4     1
```

# Exercise

- Use the function findOverlaps to find the overlaps between our reads.
- Avoid obvious and repetitive overlaps (like range 1 vs range 1).

# Solution

- We need to change the default values for two arguments :)

```
> ovReads <- matchMatrix(findOverlaps(reads,
+     ignoreSelf = TRUE, ignoreRedundant = TRUE))
> ovReads
```

```
     query subject
[1,]     8     156
[2,]    54     104
[3,]    54     374
[4,]   104     374
[5,]   361     371
```

- Which reads overlap with the 100 upstream to other reads? Are the results the same?

# Solution part B

- We'll use flank to get the upstream regions :)

```
> ovReadsUp <- matchMatrix(findOverlaps(reads,
+     flank(reads, 100)))
> ovReadsUp
```

```
     query subject
[1,]    54     104
[2,]    54     374
[3,]   104     374
[4,]   156       8
```

# RangedData

- The third main object from the IRanges package is the RangedData object.
- It is basically a table with an IRanges object inside of it:

```
> rd <- RangedData(ranges = IR, space = rep("chr",
+     5), name = letters[1:5])
```

- Once we have a RangedData object, we can get the names, coverage per space, access the different extra columns (name in this case), or get the IRanges object inside of the RangedData:

```
> names(rd)
```

```
[1] "chr"
```

```
> coverage(rd)
```

# RangedData

```
SimpleRleList of length 1
$chr
'integer' Rle of length 10 with 9 runs
  Lengths: 1 1 1 1 2 1 1 1 1
  Values : 1 2 3 4 5 4 3 2 1
```

```
> rd$name
```

```
[1] "a" "b" "c" "d" "e"
```

```
> rd$space
```

```
[1] chr chr chr chr chr
Levels: chr
```

```
> ranges(rd)
```

# RangedData

```
CompressedIRangesList of length 1
$chr
IRanges of length 5
    start end width
[1]     1   6     6
[2]     2   7     6
[3]     3   8     6
[4]     4   9     6
[5]     5  10     6
```

- Using our object reads, build a RangedData where the space is the chromosome where the read aligned. You might need to use our object idx.

# RangedData

- Get the summary statistics for the coverage of chr5 (exclude bases with coverage equal to 0).

# Solution

- First we build the RangedData object:

```
> readsRD <- RangedData(ranges = reads,
+     space = chromosome(aln[idx]))
> names(readsRD)
```

```
 [1] "chr10.fa"      "chr11.fa"
 [3] "chr12.fa"      "chr13.fa"
 [5] "chr14.fa"      "chr15.fa"
 [7] "chr16.fa"      "chr17.fa"
 [9] "chr18.fa"      "chr19.fa"
[11] "chr1.fa"       "chr2.fa"
[13] "chr3.fa"       "chr4.fa"
[15] "chr5.fa"       "chr6.fa"
[17] "chr7.fa"       "chr8.fa"
```

# Solution

```
[19] "chr9.fa"          "chrM.fa"
[21] "chrUn_random.fa" "chrX.fa"
[23] "chrY_random.fa"
```

- Next we get the coverage for each space (chromosome), and finally we get the summary statistics we wanted:

```
> covRD <- lapply(readsRD, coverage)
> covRD["chr5.fa"]
```

```
SimpleRleList of length 1
$chr5.fa
'integer' Rle of length 140154350 with 58 runs
  Lengths:  3936448 ...       35
  Values :        0 ...        1
```

# Solution

```
> summary(covRD[["chr5.fa"]][[1]][covRD[["chr5.fa"]][[1
+     0])
   Min. 1st Qu.  Median    Mean 3rd Qu.
      1       1       1       1       1
   Max.
      1
```

# Overview

- While built on top of IRanges, GenomicRanges provides a biological-aware framework to work with :)
- The GRanges class outperforms the RangedData class
- Caution: some methods have yet to be implemented for GRanges objects

# GRanges

- It's very similar to RangedData as the minimum information includes an IRanges object.
- Yet, now it requires strand information as well as the names.
- Lets build a GRanges object using our previous IR object:

```
> GR <- GRanges(seqnames = rep("chr",
+     5), ranges = IR, strand = rep("*",
+     5), someVar = letters[1:5])
> GR
```

# GRanges

```
GRanges with 5 ranges and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr   [1,  6]      * |
[2]      chr   [2,  7]      * |
[3]      chr   [3,  8]      * |
[4]      chr   [4,  9]      * |
[5]      chr   [5, 10]      * |
        someVar
    <character>
[1]           a
[2]           b
[3]           c
[4]           d
```

# GRanges

```
[5]            e

seqlengths
 chr
  NA
```

- Note the seqlenghts section. We can specify the length of each unique seqname. This information affects the result from the coverage function:

  `> coverage(GR)`

# GRanges

```
SimpleRleList of length 1
$chr
'integer' Rle of length 10 with 9 runs
  Lengths: 1 1 1 1 2 1 1 1 1
  Values : 1 2 3 4 5 4 3 2 1
```

```
> seqlengths(GR) <- 20
> coverage(GR)
```

```
SimpleRleList of length 1
$chr
'integer' Rle of length 20 with 10 runs
  Lengths:  1  1  1  1  2  1  1  1  1 10
  Values :  1  2  3  4  5  4  3  2  1  0
```

# GRanges

- Similar to RangedData objects, we can access parts of our GRanges object with:

```
> strand(GR)

'factor' Rle of length 5 with 1 run
  Lengths: 5
  Values : *
Levels(3): + - *

> start(GR)

[1] 1 2 3 4 5

> end(GR)

[1]  6  7  8  9 10

> width(GR)
```

# GRanges

```
[1] 6 6 6 6 6
```

```
> ranges(GR)
```

```
IRanges of length 5
    start end width
[1]     1   6     6
[2]     2   7     6
[3]     3   8     6
[4]     4   9     6
[5]     5  10     6
```

```
> GR[2:3]
```

# GRanges

```
GRanges with 2 ranges and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr    [2, 7]      * |
[2]      chr    [3, 8]      * |
        someVar
    <character>
[1]           b
[2]           c

seqlengths
 chr
  20
```

# GRanges

- Do you remember the class DataFrame. Well, that's the class of the part of a GRanges object that contains information for the extra variables. Basically, it's a data.frame where each column can be a vector, an Rle, etc.

```
DataFrame with 5 rows and 1 column
      someVar
  <character>
1             a
2             b
3             c
4             d
5             e
```

# GRanges

```
DataFrame with 5 rows and 1 column
      someVar
  <character>
1           a
2           b
3           c
4           d
5           e
```

```
[1] "a" "b" "c" "d" "e"
```

- Plus, just like IRanges, we can manipulate the ranges:

  `> flank(GR[5], 1)`

# GRanges

```
GRanges with 1 range and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr    [4, 4]      * |
        someVar
    <character>
[1]           e

seqlengths
 chr
  20
```

```
> disjoin(GR[4:5])
```

# GRanges

```
GRanges with 3 ranges and 0 elementMetadata values
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr  [ 4,  4]      * |
[2]      chr  [ 5,  9]      * |
[3]      chr  [10, 10]      * |

seqlengths
 chr
  20
```

```
> shift(GR[3], 2)
```

# GRanges

```
GRanges with 1 range and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr   [5, 10]      * |
        someVar
    <character>
[1]           c

seqlengths
 chr
  20
```

# Exercise

- Lets repeat the previous exercise where we looked for overlaps between
  1. reads
  2. reads and the 100bp upstream of reads
- First, we'll need to construct a GRanges object using the reads from the aln object.

# Solution

- Lets construct the GRanges object:

```
> readsGR <- GRanges(seqnames = chromosome(aln[idx]),
+     ranges = reads, strand = Rle(strand(aln[idx])))
```

- Next, lets find the overlaps between reads:

```
> findOverlaps(readsGR, ignoreSelf = TRUE,
+     ignoreRedundant = FALSE)
```

- Sadly, that doesn't work yet. So lets do it the hard way:

# Solution

```
> ov <- matchMatrix(findOverlaps(readsGR,
+     readsGR))
> removeSelf <- function(ov) {
+     ov2 <- NULL
+     for (i in 1:nrow(ov)) if (ov[i,
+         1] != ov[i, 2])
+         ov2 <- rbind(ov2, ov[i,
+             ])
+     return(ov2)
+ }
> removeRedundant <- function(ov) {
+     index <- apply(ov, 1, function(x) {
+         res <- TRUE
+         x <- as.vector(x)
```

# Solution

```
+         for (j in 1:nrow(ov)) {
+             y <- as.vector(ov[j,
+                 ])
+             if (identical(y, x))
+                 break
+             if (identical(y, rev(x)))
+                 res <- FALSE
+         }
+         return(res)
+     })
+     return(ov[index, ])
+ }
> ov <- removeRedundant(removeSelf(ov))
> ov
```

# Solution

```
     query subject
[1,]     8     156
[2,]    54     104
[3,]   361     371
```

- Our new result is slight different that our original result:

```
> ovReads
```

```
     query subject
[1,]     8     156
[2,]    54     104
[3,]    54     374
[4,]   104     374
[5,]   361     371
```

# Solution

- Next, lets find the overlaps between reads and upstream regions of reads.

```
> matchMatrix(findOverlaps(readsGR,
+     flank(readsGR, 100)))
```

```
     query subject
[1,]     8     156
[2,]    54     104
```

```
> ovReadsUp
```

```
     query subject
[1,]    54     104
[2,]    54     374
[3,]   104     374
[4,]   156       8
```

# Solution

- Just as above, the result is different. The reason: overlaps in GRanges objects takes into account the strand!

# GRangesList

- A follow up class to GRanges is GRangesList. That's the default output of the split function:

```
> grList <- split(GR)
> class(grList)
```

```
[1] "GRangesList"
attr(,"package")
[1] "GenomicRanges"
```

- You don't need double brackets to access the elements of a GRangesList:

```
> grList[1:2]
```

# GRangesList

```
GRangesList of length 2
$1
GRanges with 1 range and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr    [1, 6]      * |
        someVar
    <character>
[1]           a

$2
GRanges with 1 range and 1 elementMetadata value
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
```

# GRangesList

```
[1]       chr    [2, 7]      * |
          someVar
      <character>
[1]             b


seqlengths
 chr
  20
```

- Functions like coverage work with all the elements of a GRangesList. Accessors like strand work with each element individually:

  `> coverage(grList)`

# GRangesList

```
SimpleRleList of length 1
$chr
'integer' Rle of length 10 with 9 runs
  Lengths: 1 1 1 1 2 1 1 1 1
  Values : 1 2 3 4 5 4 3 2 1
```

```
> strand(grList)
```

```
CompressedRleList of length 5
$`1`
'factor' Rle of length 1 with 1 run
  Lengths: 1
  Values : *
Levels(3): + - *
```

# GRangesList

```
$`2`
'factor' Rle of length 1 with 1 run
  Lengths: 1
  Values : *
Levels(3): + - *

$`3`
'factor' Rle of length 1 with 1 run
  Lengths: 1
  Values : *
Levels(3): + - *

$`4`
'factor' Rle of length 1 with 1 run
```

# GRangesList

```
  Lengths: 1
  Values : *
Levels(3): + - *

$`5`
'factor' Rle of length 1 with 1 run
  Lengths: 1
  Values : *
Levels(3): + - *
```

- The idea behind GRangesList is that you can have all the exons of a given gene in a GRanges, and have one element in your GRangesList per every gene.

# Session Information

```
> sessionInfo()

R version 2.12.0 Under development (unstable) (2010-09-08 r52880)
Platform: x86_64-unknown-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.utf8
 [2] LC_NUMERIC=C
 [3] LC_TIME=en_US.utf8
 [4] LC_COLLATE=en_US.utf8
 [5] LC_MONETARY=C
 [6] LC_MESSAGES=en_US.utf8
 [7] LC_PAPER=en_US.utf8
 [8] LC_NAME=C
 [9] LC_ADDRESS=C
[10] LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.utf8
[12] LC_IDENTIFICATION=C

attached base packages:
```

# Session Information

```
[1] stats     graphics  grDevices
[4] utils     datasets  methods
[7] base

other attached packages:
[1] ShortRead_1.7.20
[2] Rsamtools_1.1.15
[3] lattice_0.19-11
[4] Biostrings_2.17.41
[5] GenomicRanges_1.1.25
[6] IRanges_1.7.34

loaded via a namespace (and not attached):
[1] Biobase_2.9.0 grid_2.12.0
[3] hwriter_1.2
```