Bioconductor Basics: Granges and Biostrings

- Core Bioconductor structures for representing genes and genetic sequences

Motivation and Introduction

- Case study: given genomic DNA extracted from human cells, where on the genome does the nuclear protein ESRRA (estrogen related receptor alpha) bind?
- Role of estrogen receptors in breast cancer
- Data comes from analysis of ChIP-seq experiments: performed in ENCODE project - import info for files in "narrowPeak" format and analyze in Bioconductor GRanges object
- Identifying nearest transcriptional start site for each binding peak - assess whether regulatory activity of ESRRA occurs in transcriptional promoter regions

```
library(ERBS)
data(HepG2)
class(HepG2)
```

```
## [1] "GRanges"
## attr(,"package")
## [1] "GenomicRanges"
```

GenomicRanges

- ERBS library from github repo
- Load two datasets - GM12878, HepG2. Estrogen receptor binding site datasets from two cell lines (cell-type dependent outcome).
- Contains: Chromosome start + end (1 row / region), strand information, score from peaks
- Access the GRanges objects as a matrix, i.e. subsetting is okay.
- **seqnames** function to access chromosome for each row. Returns object of type *Rle* - more efficient to save ordered by chromosome with counts. Can turn into character using **as.character**
- Most of analysis is focused on first 23 chromosomes
- Function to order by genomic region
- Iranges function not specific to genomics - Granges builds on Iranges in relation to genomics

```
# install ERBS
library(devtools)
```

```
## Loading required package: usethis
```

```
install_github("genomicsclass/ERBS")
```

```
## Skipping install of 'ERBS' from a github remote, the SHA1 (9f16eb6a) has not changed since last insta
##   Use `force = TRUE` to force installation
```

```
library(GenomicRanges)
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:base':
##
##     expand.grid

## Loading required package: IRanges

## Loading required package: GenomeInfoDb

## Warning: package 'GenomeInfoDb' was built under R version 4.0.4
```

```
# load GM12878 and HepG2 objects from ERBS package
library(ERBS)
data(GM12878)
data(HepG2)

# inspect HepG2 GRanges object
class(HepG2)
```

```
## [1] "GRanges"
## attr(,"package")
## [1] "GenomicRanges"
```

```
HepG2
```

```
## GRanges object with 303 ranges and 7 metadata columns:
##         seqnames              ranges strand |      name     score       col
##            <Rle>           <IRanges>  <Rle> | <numeric> <integer> <logical>
##     [1]     chr2   20335378-20335787      * |        NA         0      <NA>
##     [2]    chr20       328285-329145      * |        NA         0      <NA>
##     [3]     chr6 168135432-168136587      * |        NA         0      <NA>
##     [4]    chr19     1244419-1245304      * |        NA         0      <NA>
##     [5]    chr11   64071828-64073069      * |        NA         0      <NA>
##     ...      ...                 ...    ... .       ...       ...       ...
##   [299]     chr4     1797182-1797852      * |        NA         0      <NA>
##   [300]     chr1 110198573-110199126      * |        NA         0      <NA>
##   [301]    chr17   17734052-17734469      * |        NA         0      <NA>
```

```
##   [302]     chr1   48306453-48306908      * |        NA         0      <NA>
##   [303]    chr12 123867207-123867554      * |        NA         0      <NA>
##         signalValue    pValue      qValue      peak
##           <numeric> <numeric>   <numeric> <integer>
##     [1]      58.251    75.899 6.14371e-72       195
##     [2]      10.808    69.685 5.02806e-66       321
##     [3]      17.103    54.311 7.93067e-51       930
##     [4]      12.427    43.855 1.35976e-40       604
##     [5]      10.850    40.977 7.33386e-38       492
##     ...         ...       ...         ...       ...
##   [299]       9.681    10.057 1.42334e-08       402
##   [300]       7.929    10.047 1.44208e-08       197
##   [301]       5.864     9.990 1.63892e-08       227
##   [302]       5.660     9.948 1.79941e-08       211
##   [303]      13.211     9.918 1.92180e-08       163
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
values(HepG2)
```

```
## DataFrame with 303 rows and 7 columns
##          name     score       col signalValue    pValue      qValue      peak
##     <numeric> <integer> <logical>   <numeric> <numeric>   <numeric> <integer>
## 1          NA         0        NA      58.251    75.899 6.14371e-72       195
## 2          NA         0        NA      10.808    69.685 5.02806e-66       321
## 3          NA         0        NA      17.103    54.311 7.93067e-51       930
## 4          NA         0        NA      12.427    43.855 1.35976e-40       604
## 5          NA         0        NA      10.850    40.977 7.33386e-38       492
## ...       ...       ...       ...         ...       ...         ...       ...
## 299        NA         0        NA       9.681    10.057 1.42334e-08       402
## 300        NA         0        NA       7.929    10.047 1.44208e-08       197
## 301        NA         0        NA       5.864     9.990 1.63892e-08       227
## 302        NA         0        NA       5.660     9.948 1.79941e-08       211
## 303        NA         0        NA      13.211     9.918 1.92180e-08       163
```

```
# seqnames extracts chromosome names
seqnames(HepG2)     # stored as type Rle
```

```
## factor-Rle of length 303 with 292 runs
##   Lengths:     1     1     1     1     1 ...     1     1     1     1     1
##   Values : chr2  chr20 chr6  chr19 chr11 ... chr4  chr1  chr17 chr1  chr12
## Levels(93): chr1 chr2 chr3 ... chrUn_gl000247 chrUn_gl000248 chrUn_gl000249
```

```
chr = seqnames(HepG2)
as.character(chr)    # view as character type
```

```
##   [1] "chr2"  "chr20" "chr6"  "chr19" "chr11" "chr20" "chr19" "chr2"  "chr16"
##  [10] "chr3"  "chr6"  "chr20" "chr7"  "chr16" "chr9"  "chr11" "chr22" "chrX"
##  [19] "chr8"  "chr16" "chr16" "chr19" "chr17" "chr17" "chr16" "chr1"  "chr16"
##  [28] "chr9"  "chr17" "chr16" "chr12" "chr6"  "chr2"  "chr3"  "chr11" "chr16"
##  [37] "chr6"  "chr2"  "chr8"  "chr1"  "chr17" "chr20" "chr4"  "chr14" "chr19"
##  [46] "chr20" "chr9"  "chr2"  "chr2"  "chr19" "chr8"  "chr14" "chr22" "chr2"
##  [55] "chr14" "chr6"  "chr20" "chr2"  "chr19" "chr8"  "chr2"  "chr19" "chr12"
##  [64] "chr2"  "chr2"  "chr11" "chr12" "chr7"  "chr19" "chr22" "chr17" "chr3"
##  [73] "chr8"  "chr3"  "chr15" "chr6"  "chr9"  "chr10" "chr6"  "chr2"  "chr19"
##  [82] "chr11" "chr8"  "chr17" "chr15" "chr21" "chr7"  "chr2"  "chr2"  "chr3"
```

```
##  [91] "chr2"  "chr16" "chr10" "chr20" "chr17" "chr13" "chr2"  "chr5"  "chr14"
## [100] "chr11" "chr8"  "chr20" "chr3"  "chr7"  "chr1"  "chr1"  "chr3"  "chr17"
## [109] "chrX"  "chr19" "chr20" "chr6"  "chr7"  "chr16" "chr7"  "chr17" "chr20"
## [118] "chr2"  "chr5"  "chrX"  "chr7"  "chr6"  "chr19" "chr17" "chr16" "chr5"
## [127] "chr12" "chr9"  "chr20" "chr2"  "chr12" "chr3"  "chr7"  "chr2"  "chr20"
## [136] "chr20" "chr17" "chr12" "chr19" "chr1"  "chr7"  "chr20" "chr14" "chr12"
## [145] "chr10" "chr6"  "chr9"  "chr6"  "chr1"  "chr18" "chr8"  "chr15" "chr6"
## [154] "chr2"  "chr1"  "chr18" "chr16" "chr9"  "chr20" "chr19" "chr17" "chr10"
## [163] "chr6"  "chr2"  "chrX"  "chr16" "chr20" "chr16" "chr20" "chr16" "chr20"
## [172] "chr5"  "chr16" "chr17" "chr17" "chr3"  "chr8"  "chr18" "chr18" "chr7"
## [181] "chr20" "chr16" "chr19" "chr11" "chr12" "chr2"  "chr17" "chr1"  "chr20"
## [190] "chr4"  "chr17" "chr1"  "chr6"  "chr5"  "chr13" "chr7"  "chr20" "chr2"
## [199] "chr16" "chr6"  "chr11" "chr5"  "chr20" "chr1"  "chr9"  "chr2"  "chr16"
## [208] "chr10" "chr9"  "chr2"  "chr2"  "chr21" "chr1"  "chr16" "chr18" "chr10"
## [217] "chr16" "chr3"  "chr6"  "chr16" "chr2"  "chr6"  "chr10" "chr16" "chr22"
## [226] "chr2"  "chr16" "chr8"  "chr20" "chr19" "chr16" "chr20" "chr2"  "chr3"
## [235] "chr10" "chr14" "chr6"  "chr18" "chr15" "chr9"  "chr14" "chr7"  "chr20"
## [244] "chr3"  "chr6"  "chr10" "chr4"  "chr1"  "chr9"  "chr15" "chr6"  "chr16"
## [253] "chr2"  "chr3"  "chr14" "chr19" "chr2"  "chr5"  "chr22" "chr16" "chr6"
## [262] "chr16" "chr17" "chr11" "chr8"  "chr3"  "chr1"  "chr16" "chr21" "chr12"
## [271] "chr16" "chr1"  "chr2"  "chr2"  "chr9"  "chr2"  "chr16" "chr17" "chr12"
## [280] "chr17" "chr7"  "chr20" "chr7"  "chr6"  "chr12" "chr2"  "chr1"  "chr5"
## [289] "chr6"  "chr2"  "chr1"  "chr12" "chr2"  "chr6"  "chr20" "chr2"  "chr17"
## [298] "chr3"  "chr4"  "chr1"  "chr17" "chr1"  "chr12"
```

```
# make a table of numbers of sequences on each chromosome
table(chr)
```

```
## chr
##                  chr1                  chr2                  chr3
##                    18                    38                    15
##                  chr4                  chr5                  chr6
##                     4                     8                    24
##                  chr7                  chr8                  chr9
##                    14                    11                    12
##                 chr10                 chr11                 chr12
##                     9                     9                    13
##                 chr13                 chr14                 chr15
##                     2                     8                     5
##                 chr16                 chr17                 chr18
##                    31                    21                     6
##                 chr19                 chr20                 chr21
##                    16                    27                     3
##                 chr22                  chrX                  chrY
##                     5                     4                     0
##                  chrM  chr1_gl000191_random  chr1_gl000192_random
##                     0                     0                     0
##        chr4_ctg9_hap1  chr4_gl000193_random  chr4_gl000194_random
##                     0                     0                     0
##         chr6_apd_hap1         chr6_cox_hap2         chr6_dbb_hap3
##                     0                     0                     0
##        chr6_mann_hap4         chr6_mcf_hap5         chr6_qbl_hap6
##                     0                     0                     0
##        chr6_ssto_hap7  chr7_gl000195_random  chr8_gl000196_random
##                     0                     0                     0
```

```
##  chr8_gl000197_random  chr9_gl000198_random  chr9_gl000199_random
##                     0                     0                     0
##  chr9_gl000200_random  chr9_gl000201_random chr11_gl000202_random
##                     0                     0                     0
##       chr17_ctg5_hap1 chr17_gl000203_random chr17_gl000204_random
##                     0                     0                     0
## chr17_gl000205_random chr17_gl000206_random chr18_gl000207_random
##                     0                     0                     0
## chr19_gl000208_random chr19_gl000209_random chr21_gl000210_random
##                     0                     0                     0
##        chrUn_gl000211        chrUn_gl000212        chrUn_gl000213
##                     0                     0                     0
##        chrUn_gl000214        chrUn_gl000215        chrUn_gl000216
##                     0                     0                     0
##        chrUn_gl000217        chrUn_gl000218        chrUn_gl000219
##                     0                     0                     0
##        chrUn_gl000220        chrUn_gl000221        chrUn_gl000222
##                     0                     0                     0
##        chrUn_gl000223        chrUn_gl000224        chrUn_gl000225
##                     0                     0                     0
##        chrUn_gl000226        chrUn_gl000227        chrUn_gl000228
##                     0                     0                     0
##        chrUn_gl000229        chrUn_gl000230        chrUn_gl000231
##                     0                     0                     0
##        chrUn_gl000232        chrUn_gl000233        chrUn_gl000234
##                     0                     0                     0
##        chrUn_gl000235        chrUn_gl000236        chrUn_gl000237
##                     0                     0                     0
##        chrUn_gl000238        chrUn_gl000239        chrUn_gl000240
##                     0                     0                     0
##        chrUn_gl000241        chrUn_gl000242        chrUn_gl000243
##                     0                     0                     0
##        chrUn_gl000244        chrUn_gl000245        chrUn_gl000246
##                     0                     0                     0
##        chrUn_gl000247        chrUn_gl000248        chrUn_gl000249
##                     0                     0                     0
```

```
table(chr)[1:24]    # restrict to autosomes, X and Y
```

```
## chr
##  chr1  chr2  chr3  chr4  chr5  chr6  chr7  chr8  chr9 chr10 chr11 chr12 chr13
##    18    38    15     4     8    24    14    11    12     9     9    13     2
## chr14 chr15 chr16 chr17 chr18 chr19 chr20 chr21 chr22  chrX  chrY
##     8     5    31    21     6    16    27     3     5     4     0
```

```
# GRanges can be subsetted and ordered
HepG2[chr=="chr20",]
```

```
## GRanges object with 27 ranges and 7 metadata columns:
##        seqnames            ranges strand |      name     score       col
##           <Rle>         <IRanges>  <Rle> | <numeric> <integer> <logical>
##    [1]    chr20     328285-329145      * |        NA         0      <NA>
##    [2]    chr20 22410891-22411863      * |        NA         0      <NA>
##    [3]    chr20 56039583-56040249      * |        NA         0      <NA>
##    [4]    chr20 16455811-16456232      * |        NA         0      <NA>
```

```
##     [5]    chr20   3140243-3140774      * |        NA         0      <NA>
##     ...      ...               ...    ... .       ...       ...       ...
##    [23]    chr20   5591571-5592037      * |        NA         0      <NA>
##    [24]    chr20 25519664-25520238      * |        NA         0      <NA>
##    [25]    chr20 19900951-19901275      * |        NA         0      <NA>
##    [26]    chr20 35156796-35157140      * |        NA         0      <NA>
##    [27]    chr20 25036720-25037716      * |        NA         0      <NA>
##         signalValue    pValue      qValue      peak
##           <numeric> <numeric>   <numeric> <integer>
##     [1]      10.808    69.685 5.02806e-66       321
##     [2]       6.419    41.020 7.74961e-38       660
##     [3]       7.796    36.977 3.66693e-34       315
##     [4]       7.351    21.831 1.59668e-19       199
##     [5]       7.296    21.587 2.62536e-19       315
##     ...         ...       ...         ...       ...
##    [23]       8.766    11.433 7.67742e-10       249
##    [24]       3.300    11.419 7.89520e-10       206
##    [25]       4.809    11.155 1.37954e-09       140
##    [26]      10.154    10.313 8.30971e-09       163
##    [27]       4.381    10.087 1.33278e-08       170
##    -------
##    seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
x = HepG2[order(HepG2),]
seqnames(x)     # demonstrate usefulness of Rle type
```

```
## factor-Rle of length 303 with 23 runs
##    Lengths:    18    38    15     4     8 ...    16    27     3     5     4
##    Values : chr1  chr2  chr3  chr4  chr5  ... chr19 chr20 chr21 chr22 chrX
## Levels(93): chr1 chr2 chr3 ... chrUn_gl000247 chrUn_gl000248 chrUn_gl000249
```

```
as.character(seqnames(x))
```

```
##   [1] "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"
##  [10] "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"  "chr1"
##  [19] "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"
##  [28] "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"
##  [37] "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"
##  [46] "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"  "chr2"
##  [55] "chr2"  "chr2"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"
##  [64] "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr3"  "chr4"
##  [73] "chr4"  "chr4"  "chr4"  "chr5"  "chr5"  "chr5"  "chr5"  "chr5"  "chr5"
##  [82] "chr5"  "chr5"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"
##  [91] "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"
## [100] "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr6"  "chr7"
## [109] "chr7"  "chr7"  "chr7"  "chr7"  "chr7"  "chr7"  "chr7"  "chr7"  "chr7"
## [118] "chr7"  "chr7"  "chr7"  "chr7"  "chr8"  "chr8"  "chr8"  "chr8"  "chr8"
## [127] "chr8"  "chr8"  "chr8"  "chr8"  "chr8"  "chr8"  "chr9"  "chr9"  "chr9"
## [136] "chr9"  "chr9"  "chr9"  "chr9"  "chr9"  "chr9"  "chr9"  "chr9"  "chr9"
## [145] "chr10" "chr10" "chr10" "chr10" "chr10" "chr10" "chr10" "chr10" "chr10"
## [154] "chr11" "chr11" "chr11" "chr11" "chr11" "chr11" "chr11" "chr11" "chr11"
## [163] "chr12" "chr12" "chr12" "chr12" "chr12" "chr12" "chr12" "chr12" "chr12"
## [172] "chr12" "chr12" "chr12" "chr12" "chr13" "chr13" "chr14" "chr14" "chr14"
## [181] "chr14" "chr14" "chr14" "chr14" "chr14" "chr15" "chr15" "chr15" "chr15"
## [190] "chr15" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16"
```

```
## [199] "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16"
## [208] "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16" "chr16"
## [217] "chr16" "chr16" "chr16" "chr16" "chr16" "chr17" "chr17" "chr17" "chr17"
## [226] "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr17"
## [235] "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr17" "chr18"
## [244] "chr18" "chr18" "chr18" "chr18" "chr18" "chr19" "chr19" "chr19" "chr19"
## [253] "chr19" "chr19" "chr19" "chr19" "chr19" "chr19" "chr19" "chr19" "chr19"
## [262] "chr19" "chr19" "chr19" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20"
## [271] "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20"
## [280] "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20" "chr20"
## [289] "chr20" "chr20" "chr20" "chr21" "chr21" "chr21" "chr22" "chr22" "chr22"
## [298] "chr22" "chr22" "chrX"  "chrX"  "chrX"  "chrX"
```

Assessment: Genomic Ranges

```
library(GenomicRanges)
paste("median of signal value column for HepG2 data: ")
```

```
## [1] "median of signal value column for HepG2 data: "
```

```
median(mcols(HepG2)$signalValue)
```

```
## [1] 7.024
```

```
paste("chromosome in region with highest signal value: ")
```

```
## [1] "chromosome in region with highest signal value: "
```

```
max_index <- which.max(mcols(HepG2)$signalValue)
chr = seqnames(HepG2)
as.character(chr)[max_index]
```

```
## [1] "chrX"
```

```
paste("Number of regions from chromosome 16: ")
```

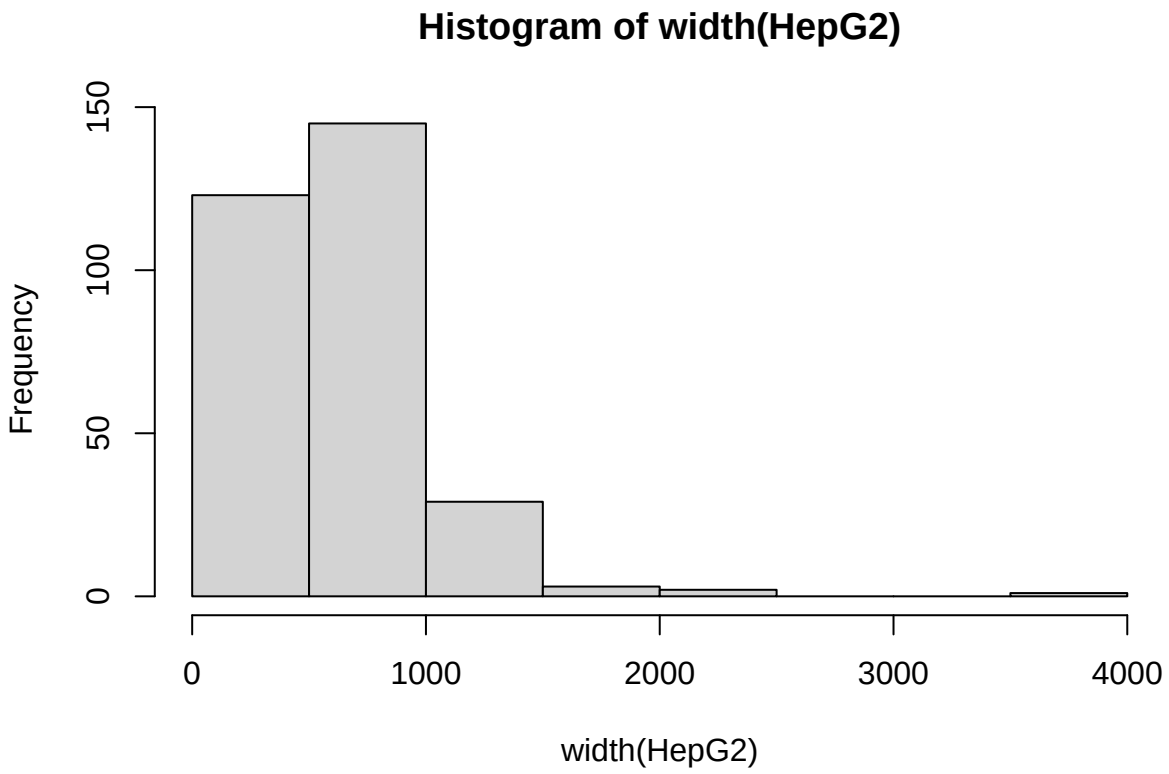
```
## [1] "Number of regions from chromosome 16: "
```

```
HepG2[chr == "chr16",]
```

```
## GRanges object with 31 ranges and 7 metadata columns:
##        seqnames            ranges strand |      name     score       col
##           <Rle>         <IRanges>  <Rle> | <numeric> <integer> <logical>
##    [1]    chr16 70191209-70192150      * |        NA         0      <NA>
##    [2]    chr16   1701039-1702137      * |        NA         0      <NA>
##    [3]    chr16 25189109-25190026      * |        NA         0      <NA>
##    [4]    chr16 85325101-85325686      * |        NA         0      <NA>
##    [5]    chr16 29986461-29986872      * |        NA         0      <NA>
##    ...      ...               ...    ... .       ...       ...       ...
##   [27]    chr16 57481218-57481854      * |        NA         0      <NA>
##   [28]    chr16 85322504-85322950      * |        NA         0      <NA>
##   [29]    chr16 19134897-19135280      * |        NA         0      <NA>
##   [30]    chr16   2586101-2586737      * |        NA         0      <NA>
##   [31]    chr16 29975932-29976255      * |        NA         0      <NA>
##        signalValue    pValue      qValue      peak
##          <numeric> <numeric>   <numeric> <integer>
##    [1]       8.371    37.774 8.19277e-35       688
```

```
##     [2]      16.157    36.264 1.65696e-33       783
##     [3]       5.979    31.808 3.44356e-29       606
##     [4]       7.664    31.429 7.88321e-29       223
##     [5]      14.795    29.018 1.73008e-26       198
##     ...         ...       ...         ...       ...
##    [27]       5.126    10.761 3.20978e-09       196
##    [28]       4.331    10.725 3.43494e-09       223
##    [29]       5.380    10.562 4.92563e-09       203
##    [30]       6.521    10.514 5.42123e-09       472
##    [31]       6.897    10.436 6.37196e-09       145
##    -------
##    seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
hist(width(HepG2))
```




```
median_width <- median(width(HepG2))
paste("Median width of all chromosomes: ", median_width)
```

```
## [1] "Median width of all chromosomes:  560"
```

Bioconductor Infrastructure for genomics, microarray and NGS

- IRanges package - representing ranges of integers. Base pair arrangements we want to manipulate in genomics
- Vignette about classes and functions in IRanges package
- Simple functions have good performance
- Summary of most important functions
- IRanges - start, end, width (i.e., 5, 10, 6bP long)

- Start, end, and width functions
- Can specify > 1 range at a time to make IRanges objects of length n
- Intra-range methods:
- **Shift** - Intra range methods for IRanges - doesn't depend on other ranges contained in IRanges object. I.e., shift IRange to the left by 2.
- **Narrow** - relative to start, start at nth base pair
- **Flank** - get flanking sequence 3 base pairs from start or end (start = False). Also bi-directional (both=True)
- Inter-range methods:
- **range** - will give beginning of the IRanges to the end, including gaps in between
- **reduce** - gives us base pairs covered by the original ranges (do not get gaps). Can ask for gaps.
- **disjoint** - set of ranges which has the same coverage as original IRanges object but non-overlapping. Contain union of all endpoints of the original range.

Assessment: IRanges

```
library(IRanges)
ir <- IRanges(101, 200)
paste("*2 zooms in, giving range with half the width. New starting point: ", start(ir*2))
```

```
## [1] "*2 zooms in, giving range with half the width. New starting point:  126"
```

```
n_ir <- narrow(ir, start=20)
paste("narrow function with start of 20. New starting point: ", start(n_ir))
```

```
## [1] "narrow function with start of 20. New starting point:  120"
```

```
paste("+25 operation gives width of resulting range: ", width(ir+25))
```

```
## [1] "+25 operation gives width of resulting range:  150"
```

```
m_ir <- IRanges(start=c(1, 11, 21),end=c(3, 15, 27))
paste("sum of widths of multiple IRanges objects:", sum(width(m_ir)))
```

```
## [1] "sum of widths of multiple IRanges objects: 15"
```

```
x <- IRanges(start=c(101,106,201,211,221,301,306,311,351,361,401,411,501), end=c(150,160,210,270,225,31
library(ph525x)
```

```
## Loading required package: png
```

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

```
## Loading required package: Biobase
```

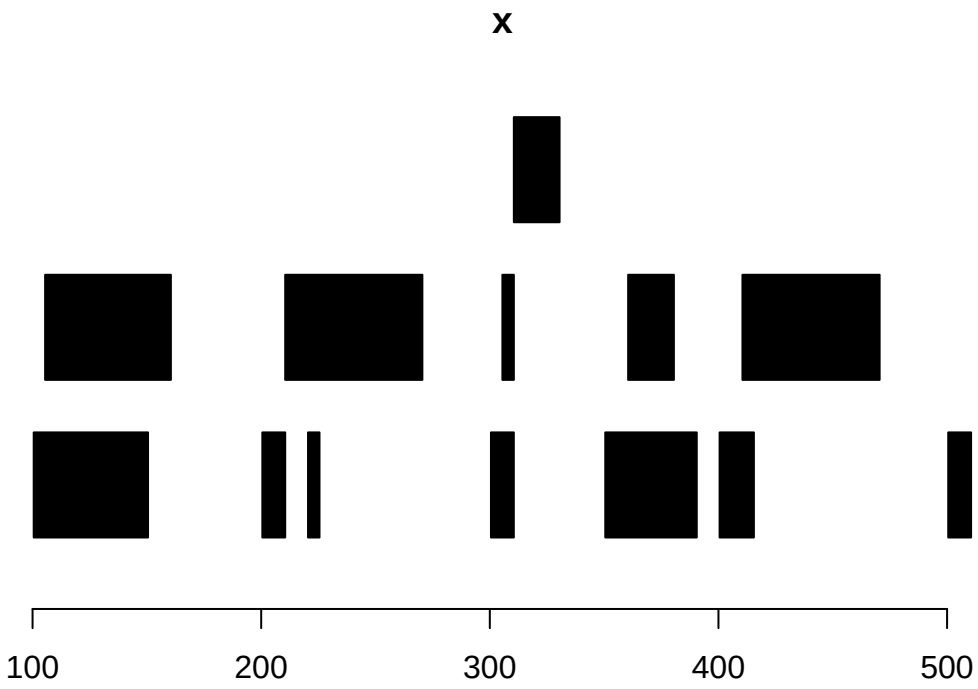
```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
## Loading required package: Homo.sapiens
```

```
## Loading required package: AnnotationDbi
```

```
## Loading required package: OrganismDbi
## Loading required package: GenomicFeatures
## Warning: package 'GenomicFeatures' was built under R version 4.0.4
## Loading required package: GO.db
##
## Loading required package: org.Hs.eg.db
##
## Loading required package: TxDb.Hsapiens.UCSC.hg19.knownGene
```

```
plotRanges(x)
```



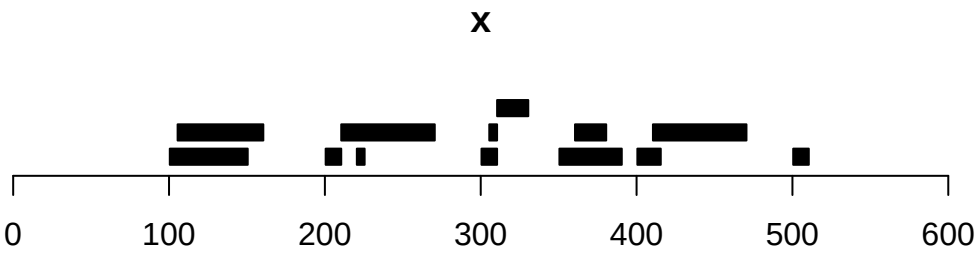

```
paste("Total width not covered by ranges in x:", sum(width(gaps(x))))
```

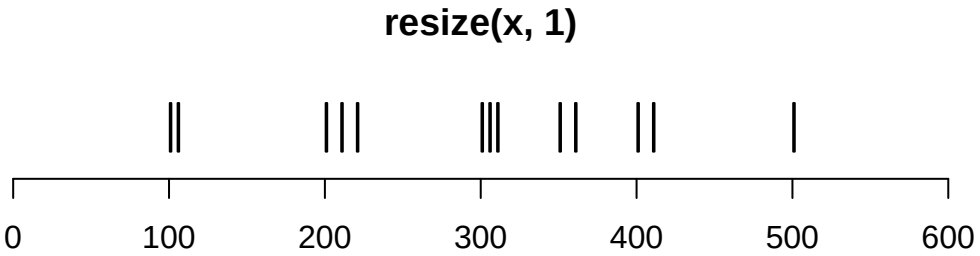
```
## [1] "Total width not covered by ranges in x: 130"
```

```
paste("Number of disjoint ranges within ranges in x:", length(disjoin(x)))
```

```
## [1] "Number of disjoint ranges within ranges in x: 17"
```

```
par(mfrow=c(2, 1))
plotRanges(x, xlim=c(0, 600))
plotRanges(resize(x, 1), xlim=c(0, 600))
```





Genomic ranges: GRanges

- Extension of IRanges
- Contain a sequence name - IRange of chromosome Z.
- Can contain chromosome information and sequence length
- Sequence names as Rle
- IRanges and strange as Rle also
- Can shift similar to IRanges - will go off end of chromosome if exceeds length
- Wrap in trim function to make sure that the end at chromosome end does not exceed
- Metadata accessed with *mcols*
- Can add cols by *mcols$*
- Additional package called *GRangesList* - groups GRanges together by wrapping in function call
- Example of *GRangesList* - grouping exons by gene or by transcript
- Application of package - find overlaps between GRanges objects
- *findOverlaps* function - query and subject (see in help() function)
- output of *findOverlaps* is a hits object with length representing # overlaps
- Same way to get the overlaps is *%over%* function - which returns logical vector
- *Rle* object defined by IRanges but similar object in base R = Run length encoding
- If vector repeats certain values, can save memory by number and number of times repeated
- *str* function gives us the compact representation
- Peering into *Rle* object - can use *Views* object to see *IRanges* from start to end. Only a virtual class - saves *Rle* and number of views / windows into it
- Can also use for *Fasta* files or other objects

Assessment: GRanges

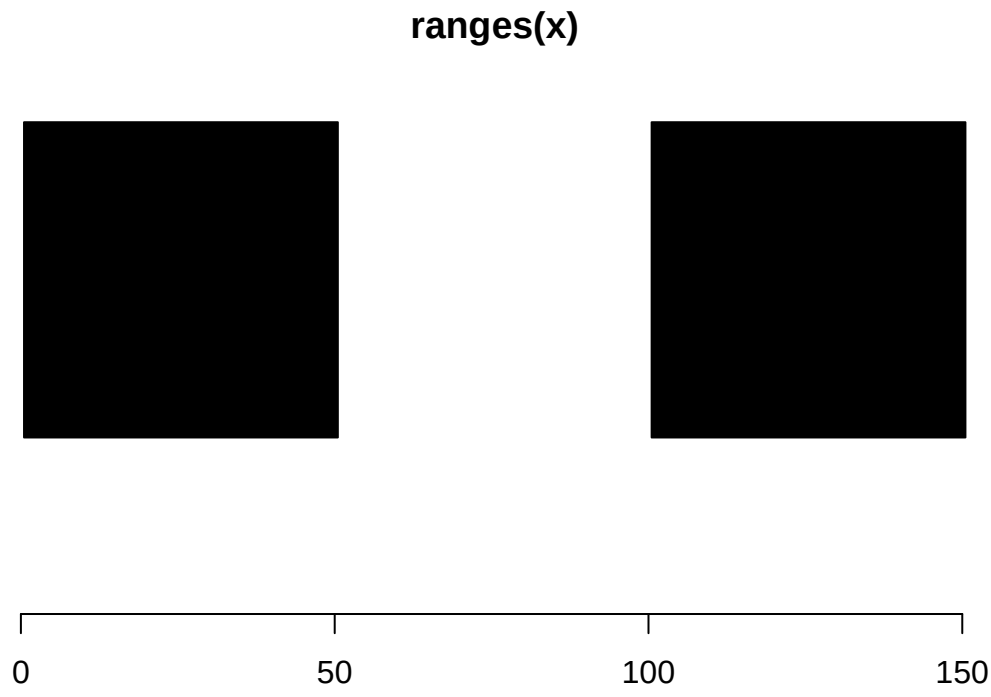
- GRanges object extends concept of interval ranges
- Ranges can be defined by:
  - chromosome we are referring to (seqnames in Bioconductor)
  - strand of DNA we are referring to (+ or -)

- These two pieces of information are necessary for specification of a range of DNA

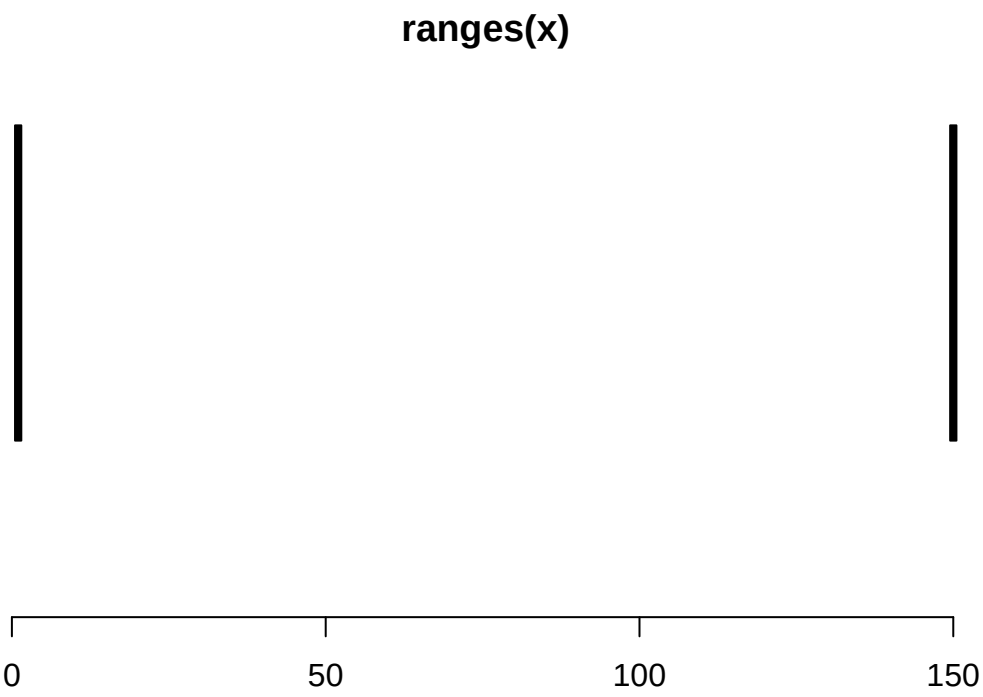
```
library(GenomicRanges)
library(IRanges)
library(ph525x)
x = GRanges("chr1", IRanges(c(1,101),c(50,150)), strand=c("+","-"))
paste("Get the internal IRanges from a GRanges object: ")
```

```
## [1] "Get the internal IRanges from a GRanges object: "
```

```
plotGRanges = function(x) plotRanges(ranges(x))
plotGRanges(x)
```




```
plotGRanges(resize(x, 1))
```



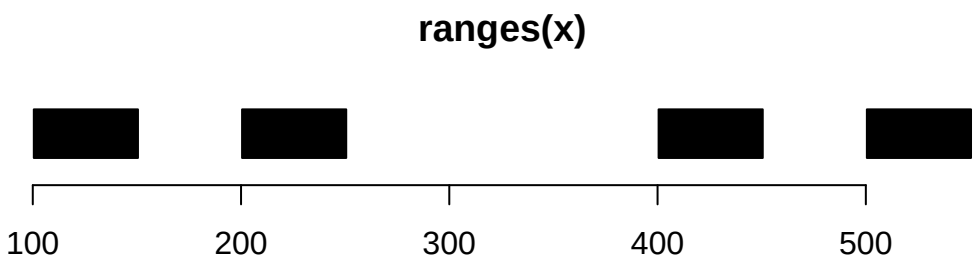

```
paste("Result of running resize(x, 1) is two ranges of width 1 which start at left-most point of the + s
      and the right-most point of the - strand ranges in x (+ -> -)")
```

```
## [1] "Result of running resize(x, 1) is two ranges of width 1 which start at left-most point of the +
```

```
paste("Q2: Intersecting transcripts with basic operations")
```

```
## [1] "Q2: Intersecting transcripts with basic operations"
```

```
x = GRanges("chr1", IRanges(c(101, 201, 401, 501), c(150, 250, 450, 550)), strand="+")
y = GRanges("chr1", IRanges(c(101, 221, 301, 401, 541), c(150, 250, 350, 470, 550)), strand="+")
par(mfrow=c(2,1))
plotGRanges(x)
plotGRanges(y)
```




```
multiple_granges_list <- GRangesList(x,y)
single_granges_list <- GRangesList(c(x, y))

findOverlaps(x, y)
```

```
## Hits object with 4 hits and 0 metadata columns:
##       queryHits subjectHits
##       <integer>   <integer>
##   [1]         1           1
##   [2]         2           2
##   [3]         3           4
##   [4]         4           5
##   -------
##   queryLength: 4 / subjectLength: 5
```

```
paste("width of overlap between x and y: ", sum(width(union(x, y))) - sum(width(intersect(x, y))))
```

```
## [1] "width of overlap between x and y:  130"
```

```
z = GRanges("chr1", range(ranges(x)), strand="-")
```

Operating on GRanges

- Small set of ranges = intervals on chromosome
- Operations:
  - *reduce* - project all of the occupied into contiguous intervals and leaves empty parts with no coverage
  - *disjoin* - set of intervals / ranges generated by disjoin of set of ranges. Same occupancy as original GRanges object
    - Maximal complexity set of intervals where wherever there was an endpoint, we will not cross in a set of ranges.

  - *gap* - set xlim to show the regions that are never expressed. could be regarded as introns, spliced out. (gaps of exons are introns)
- Elaborate the set of intervals by turning it into a GRanges object by specifying seqnames and range information.
- Metadata that should be specified - strand information, genome, seqlengths, seqinfo
- How to pick out transcription start sites - plot overlapping genes. Resize with argument 1 to get down to one base from start. Gives us the addresses of start sites
- Finding promoters - interval of three bases upstream of bases upstream is regarded as a promoter. Use *flank* operation with argument 3 - gives us the locations of the upstream promoters. Use start=FALSE to indicate flank at the end of the interval rather than start.

Finding Overlaps

- Example: finding genes that are close to reported binding sites and add some annotation to those genes
- HepG2 + GM12878 - reported binding sites for 2 cell lines
- Want to find the genes that are nearest to them. Instead of seperately, create a consensus GRanges which includes only sites that are common to both GRanges
- Function: *findOverlaps* uses query and subject - for each range, see if it appears in another range and return pair. Returns object of class *hits*. Only want the ones where there is a hit - use *queryHits* function and subset based on queryHits
- Extract just region information using *granges* function
- Show extraction of genes in next video, and matching of the regions in ERBS dataset to genes

```
# load packages
library(GenomicFeatures)
library(GenomicRanges)
library(IRanges)
library(ERBS)

# load ESRRA ChIP data
data(HepG2)
data(GM12878)

# browseVignettes("GenomicRanges")

# find binding sites common to both HepG2 and GM12878
?findOverlaps
```

```
## Help on topic 'findOverlaps' was found in the following packages:
##
##   Package               Library
##   SummarizedExperiment
##                       /Library/Frameworks/R.framework/Versions/4.0/Resources/library
##   GenomicRanges         /Library/Frameworks/R.framework/Versions/4.0/Resources/library
##   GenomicAlignments     /Library/Frameworks/R.framework/Versions/4.0/Resources/library
##   IRanges               /Library/Frameworks/R.framework/Versions/4.0/Resources/library
##
##
## Using the first match ...
```

```
# for each row in query, return overlapping row in subject
res = findOverlaps(HepG2, GM12878)
class(res)
```

```
## [1] "SortedByQueryHits"
```

```
## attr(,"package")
## [1] "S4Vectors"
```

```
res
```

```
## Hits object with 75 hits and 0 metadata columns:
##        queryHits subjectHits
##        <integer>   <integer>
##    [1]         1          12
##    [2]         2          78
##    [3]         4         777
##    [4]         5           8
##    [5]         8          13
##    ...       ...         ...
##   [71]       285         621
##   [72]       287         174
##   [73]       291        1855
##   [74]       294         512
##   [75]       300         144
##   -------
##   queryLength: 303 / subjectLength: 1873
```

```
# ranges from the query for which we found a hit in the subject
index = queryHits(res)
erbs = HepG2[index,]
erbs
```

```
## GRanges object with 75 ranges and 7 metadata columns:
##        seqnames              ranges strand |      name     score       col
##           <Rle>           <IRanges>  <Rle> | <numeric> <integer> <logical>
##    [1]     chr2   20335378-20335787      * |        NA         0      <NA>
##    [2]    chr20       328285-329145      * |        NA         0      <NA>
##    [3]    chr19     1244419-1245304      * |        NA         0      <NA>
##    [4]    chr11   64071828-64073069      * |        NA         0      <NA>
##    [5]     chr2   16938364-16938840      * |        NA         0      <NA>
##    ...      ...                 ...    ... .       ...       ...       ...
##   [71]    chr12 118558730-118559158      * |        NA         0      <NA>
##   [72]     chr1   35331750-35332300      * |        NA         0      <NA>
##   [73]     chr1   26146200-26147004      * |        NA         0      <NA>
##   [74]     chr6   44224657-44225693      * |        NA         0      <NA>
##   [75]     chr1 110198573-110199126      * |        NA         0      <NA>
##        signalValue    pValue      qValue      peak
##          <numeric> <numeric>   <numeric> <integer>
##    [1]      58.251    75.899 6.14371e-72       195
##    [2]      10.808    69.685 5.02806e-66       321
##    [3]      12.427    43.855 1.35976e-40       604
##    [4]      10.850    40.977 7.33386e-38       492
##    [5]      12.783    38.004 5.36029e-35       255
##    ...         ...       ...         ...       ...
##   [71]       8.292    10.294 8.59089e-09       195
##   [72]      10.458    10.233 9.81822e-09       341
##   [73]       5.742    10.176 1.10429e-08       337
##   [74]       3.525    10.102 1.29621e-08       838
##   [75]       7.929    10.047 1.44208e-08       197
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
# extract only the ranges
granges(erbs)
```

```
## GRanges object with 75 ranges and 0 metadata columns:
##        seqnames              ranges strand
##           <Rle>           <IRanges>  <Rle>
##    [1]     chr2   20335378-20335787      *
##    [2]    chr20       328285-329145      *
##    [3]    chr19     1244419-1245304      *
##    [4]    chr11   64071828-64073069      *
##    [5]     chr2   16938364-16938840      *
##    ...      ...                 ...    ...
##   [71]    chr12 118558730-118559158      *
##   [72]     chr1   35331750-35332300      *
##   [73]     chr1   26146200-26147004      *
##   [74]     chr6   44224657-44225693      *
##   [75]     chr1 110198573-110199126      *
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
erbs
```

```
## GRanges object with 75 ranges and 7 metadata columns:
##        seqnames              ranges strand |      name     score       col
##           <Rle>           <IRanges>  <Rle> | <numeric> <integer> <logical>
##    [1]     chr2   20335378-20335787      * |        NA         0      <NA>
##    [2]    chr20       328285-329145      * |        NA         0      <NA>
##    [3]    chr19     1244419-1245304      * |        NA         0      <NA>
##    [4]    chr11   64071828-64073069      * |        NA         0      <NA>
##    [5]     chr2   16938364-16938840      * |        NA         0      <NA>
##    ...      ...                 ...    ... .       ...       ...       ...
##   [71]    chr12 118558730-118559158      * |        NA         0      <NA>
##   [72]     chr1   35331750-35332300      * |        NA         0      <NA>
##   [73]     chr1   26146200-26147004      * |        NA         0      <NA>
##   [74]     chr6   44224657-44225693      * |        NA         0      <NA>
##   [75]     chr1 110198573-110199126      * |        NA         0      <NA>
##        signalValue    pValue      qValue      peak
##          <numeric> <numeric>   <numeric> <integer>
##    [1]      58.251    75.899 6.14371e-72       195
##    [2]      10.808    69.685 5.02806e-66       321
##    [3]      12.427    43.855 1.35976e-40       604
##    [4]      10.850    40.977 7.33386e-38       492
##    [5]      12.783    38.004 5.36029e-35       255
##    ...         ...       ...         ...       ...
##   [71]       8.292    10.294 8.59089e-09       195
##   [72]      10.458    10.233 9.81822e-09       341
##   [73]       5.742    10.176 1.10429e-08       337
##   [74]       3.525    10.102 1.29621e-08       838
##   [75]       7.929    10.047 1.44208e-08       197
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

Assessment: Finding Overlaps

```
library(ERBS)
data(HepG2)
data(GM12878)
paste("17th region of HepG2 starts at:", start(granges(HepG2[17])))
```

```
## [1] "17th region of HepG2 starts at: 46528596"
```

```
dtn <- distanceToNearest(HepG2[17], GM12878)
gm_idx <- subjectHits(dtn)
start_site <- start(GM12878[gm_idx])
distance_to_closest = mcols(dtn)$distance
paste("Start site of closest region to 17th region of HepG2: ", start_site)
```

```
## [1] "Start site of closest region to 17th region of HepG2:  46524762"
```

```
paste("Distance between closest region to 17th region of HepG2: ", distance_to_closest)
```

```
## [1] "Distance between closest region to 17th region of HepG2:  2284"
```

```
X <- vector(mode="integer", length=length(HepG2))
for(i in seq_along(HepG2)) {
     closest_region = distanceToNearest(HepG2[i], GM12878)
     distance = mcols(closest_region)$distance
     X[i] = distance
}
proportion_lt_2k_bp <- length(X[X < 2000]) / length(X)
paste("proportion of distances < 2000 bp: ", proportion_lt_2k_bp)
```

```
## [1] "proportion of distances < 2000 bp:  0.267326732673267"
```

Genes as GRanges

- Mapping genes to binding sites
- Load Gene information from homo sapiens library and extract genes using function called **genes** - returns GRange

```
library(Homo.sapiens)
library(ERBS)
ghs = genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
res = precede(erbs, ghs)
res
```

```
##  [1] 22817 16173 21772  7870 20199 22131 13257 20469 21943  8564 19061 15836
## [13] 21282  8959   278 14620  1657 22774 21746 10777 14568 20910  6626 12830
## [25]  9838 19991 21643 15740 12911 13326  8303 20834   934 10485 23027  4443
## [37]  6613 21335 21125 14508 15979  2693  4122  9485 17665 18376 17932  2696
## [49] 16990 16952 15735  9386  1642  3634  6037 20891 15062  1779  3316 11746
## [61] 12168 21689 22406 19979 14099 13558  3653  6077 21280  9705 13095  3322
```

```
## [73]  6343  1553  9552
```

```
ghs[res[1:3]]
```

```
## GRanges object with 3 ranges and 1 metadata column:
##         seqnames            ranges strand |          GENEID
##            <Rle>         <IRanges>  <Rle> | <CharacterList>
##    9741     chr2 20232411-20251789      - |            9741
##   57761    chr20     361308-378203      + |           57761
##   90007    chr19   1248552-1259142      + |           90007
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

- Start and end of genes - series of locations. Also have ID used by Homo sapiens database to match gene info across different databases
- Gene with ID 1 is in chromosome 19. Start defined in IRanges.
- **strand** tells you which of two DNA strands the gene is on. When gene expression happens, DNA opens up and code for gene could be in either strand.
- Movement of the transcription is going in a certain direction
- Large -> small if -, small -> large if -.
- Transcription start site - depends on strand information
- Function from **GenomicRanges** package called **precede** - tells you what is ahead of the transcription start site which varies depending on strand
- Finds entry and query closest to subject only when in front of.
- Moving towards report on which genes are closest to binding sites

Assessment: Genes as GRanges

```
library(Homo.sapiens)
ghs = genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

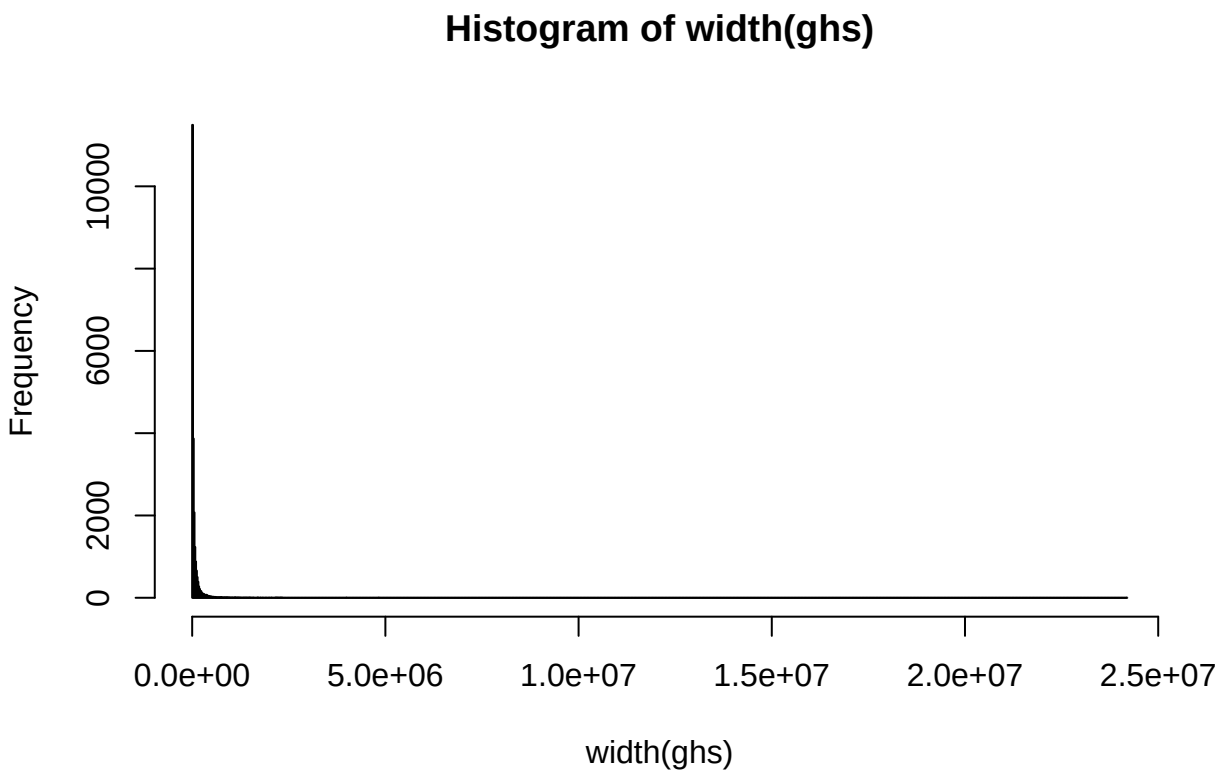
```
number_of_genes <- length(ghs)

paste("number of genes represented: ", number_of_genes)
```

```
## [1] "number of genes represented:  23056"
```

```
chr_most_genes <- names(which.max(table(as.vector(seqnames(ghs)))))
paste("chromosome with most genes: ", chr_most_genes)
```

```
## [1] "chromosome with most genes:  chr1"
```

```
hist(width(ghs), nc=1000)
```




```
median_width <- median(width(ghs))

paste("median gene width: ", median_width)
```

```
## [1] "median gene width:  20115.5"
```

Finding the Nearest Gene

- Compute distance between each binding site and corresponding gene found with precede, use **distance** function
- Takes two GRanges objects, and the genes we have found with precede
- We expect overlaps, however will not show up because we are requiring binding sites precede the genes

```
library(Homo.sapiens)
ghs = genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
library(ERBS)
index = precede(erbs, ghs)
ghs[index[1:3]]
```

```
## GRanges object with 3 ranges and 1 metadata column:
##         seqnames            ranges strand |          GENEID
```

```
##            <Rle>         <IRanges>  <Rle> | <CharacterList>
##    9741     chr2 20232411-20251789      - |            9741
##   57761    chr20     361308-378203      + |           57761
##   90007    chr19   1248552-1259142      + |           90007
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
erbs[1:3]
```

```
## GRanges object with 3 ranges and 7 metadata columns:
##       seqnames            ranges strand |      name     score       col
##          <Rle>         <IRanges>  <Rle> | <numeric> <integer> <logical>
##   [1]     chr2 20335378-20335787      * |        NA         0      <NA>
##   [2]    chr20     328285-329145      * |        NA         0      <NA>
##   [3]    chr19   1244419-1245304      * |        NA         0      <NA>
##       signalValue    pValue      qValue      peak
##         <numeric> <numeric>   <numeric> <integer>
##   [1]      58.251    75.899 6.14371e-72       195
##   [2]      10.808    69.685 5.02806e-66       321
##   [3]      12.427    43.855 1.35976e-40       604
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
distance(erbs, ghs[index])
```

```
##  [1]   83588   32162    3247   12490   91229  125579   95887  203084   26140
## [10]    4342 1158347   30950   82008   20658   32087   19444   74549    5110
## [19]   14252   48739   11043   29669   24061   22090 1989458   77873  343959
## [28]   30283   14601   48298   23414    5533   23832  962904    4678   12616
## [37]   13959  141321   75543   19992   25200   29955   26967   22447   82062
## [46]   12918     880   67725  177905   72543   26399   41808     676  437226
## [55]  270972  105665   60815   82132   40245   14666   74833  127391   10252
## [64]  140320   14696   39814    5507     444  195514   72907   14711    6332
## [73]  201266    2634   31291
```

```
tssgr = resize(ghs, 1) # shrink down to one going in direction towards transcription start site, aware
tssgr
```

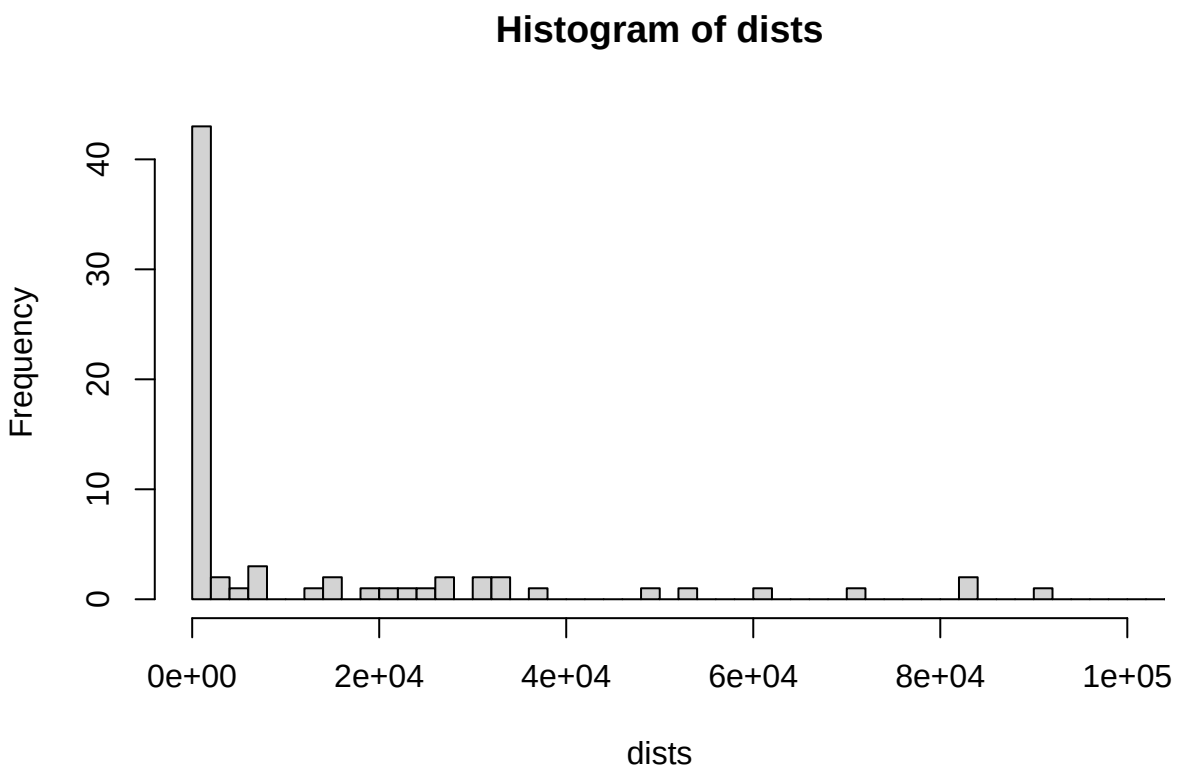
```
## GRanges object with 23056 ranges and 1 metadata column:
##         seqnames    ranges strand |          GENEID
##            <Rle> <IRanges>  <Rle> | <CharacterList>
##       1    chr19  58874214      - |               1
##      10     chr8  18248755      + |              10
##     100    chr20  43280376      - |             100
##    1000    chr18  25757445      - |            1000
##   10000     chr1 244006886      - |           10000
##     ...      ...       ...    ... .             ...
##    9991     chr9 115095944      - |            9991
##    9992    chr21  35736323      + |            9992
##    9993    chr22  19109967      - |            9993
##    9994     chr6  90539619      + |            9994
##    9997    chr22  50964905      - |            9997
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
d=distanceToNearest(erbs, tssgr)
```

```
queryHits(d)
```

```
##  [1]  1  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] 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
## [51] 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
```

```
dists = values(d)$distance
hist(dists,nc=1000,xlim=c(0,100000))
```




```
index = subjectHits(d)[dists < 1000]
index
```

```
##  [1] 19883  6316 18488 14325  9348 19622 19678 16536  4171 13017 21240 22438
## [13] 10812 20840 13796  8334  2185  2917 16265  6286 21787 14490 17932 13607
## [25]  3681  1642 21117  2204 14339 12775 13722 16883 19605 15633  7235  6077
## [37] 14605  3119 15380 10503  9555
```

- define another distance: ask for each binding site, find the transcription start site that is closest
- For each of our binding sites, find the closest transcription start site using **distanceToNearest**. Finds distance to nearest given query and subject
- Now, we have zeros because there is overlap. Output is a **hits** object. Need to use **queryHits** rather than subsetting.
- For distance, use **values** function to extract columns and grab distance
- Index of genes that are closest using **subjectHits**
- Find genes that are closer than 1k to binding sites
- Use genes that were found to be close, and get further information

Annotating Genes

- Use **select** function to query Homo sapiens database
- Need to give: key, columns we want to look at, key type
- Example of going from ranges to list of interesting genes

```
library(Homo.sapiens)
library(ERBS)
ghs = genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
tssgr = resize(ghs, 1) # shrink down to one going in direction towards transcription start site, aware
d=distanceToNearest(erbs, tssgr)
dists = values(d)$distance
index = subjectHits(d)[dists < 1000]
tssgr[index,]
```

```
## GRanges object with 41 ranges and 1 metadata column:
##          seqnames    ranges strand |          GENEID
##             <Rle> <IRanges>  <Rle> | <CharacterList>
##    80023    chr20    327370      + |           80023
##     2101    chr11  64073044      + |            2101
##     7086     chr3  53290130      - |            7086
##     5478     chr7  44836241      + |            5478
##   286262     chr9 140095163      - |          286262
##      ...      ...       ...    ... .             ...
##    55090    chr17  17380300      + |           55090
##    11165     chr6  34360457      - |           11165
##    56181     chr1  26146397      + |           56181
##   347734     chr6  44225283      - |          347734
##     2948     chr1 110198698      + |            2948
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
keytypes(Homo.sapiens)
```

```
##  [1] "ACCNUM"       "ALIAS"        "CDSID"        "CDSNAME"      "DEFINITION"
##  [6] "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS" "ENTREZID"     "ENZYME"
## [11] "EVIDENCE"     "EVIDENCEALL"  "EXONID"       "EXONNAME"     "GENEID"
## [16] "GENENAME"     "GO"           "GOALL"        "GOID"         "IPI"
## [21] "MAP"          "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"
## [26] "PFAM"         "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"
## [31] "TERM"         "TXID"         "TXNAME"       "UCSCKG"       "UNIGENE"
## [36] "UNIPROT"
```

```
keys = as.character(values(tssgr[index])$GENEID)
columns(Homo.sapiens)
```

```
##  [1] "ACCNUM"       "ALIAS"        "CDSCHROM"     "CDSEND"       "CDSID"
##  [6] "CDSNAME"      "CDSSTART"     "CDSSTRAND"    "DEFINITION"   "ENSEMBL"
## [11] "ENSEMBLPROT"  "ENSEMBLTRANS" "ENTREZID"     "ENZYME"       "EVIDENCE"
## [16] "EVIDENCEALL"  "EXONCHROM"    "EXONEND"      "EXONID"       "EXONNAME"
```

```
## [21] "EXONRANK"     "EXONSTART"    "EXONSTRAND"   "GENEID"       "GENENAME"
## [26] "GO"           "GOALL"        "GOID"         "IPI"          "MAP"
## [31] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
## [36] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "TERM"
## [41] "TXCHROM"      "TXEND"        "TXID"         "TXNAME"       "TXSTART"
## [46] "TXSTRAND"     "TXTYPE"       "UCSCKG"       "UNIGENE"      "UNIPROT"
```

```
res = select(Homo.sapiens, keys = keys,
             columns = c("SYMBOL", "GENENAME"), keytype="GENEID")
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
res[1:2,]
```

```
##   GENEID                       GENENAME SYMBOL
## 1  80023                    neurensin 2  NRSN2
## 2   2101 estrogen related receptor alpha  ESRRA
```

Assessment: Finding and getting annotation for closest gene

- Find the closest genes to some of our binding sites - use consensus set of regions

```
library(ERBS)
data(HepG2)
data(GM12878)
res = findOverlaps(HepG2,GM12878)
erbs = HepG2[queryHits(res)]
erbs = granges(erbs)

erbs2= intersect(HepG2,GM12878)

erbs
```

```
## GRanges object with 75 ranges and 0 metadata columns:
##        seqnames              ranges strand
##           <Rle>           <IRanges>  <Rle>
##    [1]     chr2   20335378-20335787      *
##    [2]    chr20       328285-329145      *
##    [3]    chr19     1244419-1245304      *
##    [4]    chr11   64071828-64073069      *
##    [5]     chr2   16938364-16938840      *
##    ...      ...                 ...    ...
##   [71]    chr12 118558730-118559158      *
##   [72]     chr1   35331750-35332300      *
##   [73]     chr1   26146200-26147004      *
##   [74]     chr6   44224657-44225693      *
##   [75]     chr1 110198573-110199126      *
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
erbs2
```

```
## GRanges object with 75 ranges and 0 metadata columns:
##        seqnames              ranges strand
##           <Rle>           <IRanges>  <Rle>
##    [1]     chr1   16339152-16339862      *
##    [2]     chr1   26146200-26147004      *
```

```
##     [3]     chr1   32879507-32879907      *
##     [4]     chr1   35331750-35332300      *
##     [5]     chr1 102900017-102900356      *
##     ...      ...                 ...    ...
##    [71]    chr20   62586998-62587997      *
##    [72]    chr22   29977062-29977454      *
##    [73]     chrX     1510388-1511745      *
##    [74]     chrX   26801642-26802021      *
##    [75]     chrX   54466501-54466956      *
##    -------
##    seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
library(Homo.sapiens)
ghs = genes(Homo.sapiens)
```

```
##    403 genes were dropped because they have exons located on both strands
##    of the same reference sequence or on more than one reference sequence,
##    so cannot be represented by a single genomic range.
##    Use 'single.strand.genes.only=FALSE' to get all the genes in a
##    GRangesList object, or use suppressMessages() to suppress this message.
```

```
transcription_start_site=resize(ghs["100113402"], 1)
paste("transcription start site for gene id 100113402: ", transcription_start_site)
```

```
## [1] "transcription start site for gene id 100113402:  chr16:70563402:+"
```

```
library(ERBS)
data(HepG2)
data(GM12878)
res = findOverlaps(HepG2,GM12878)
erbs = HepG2[queryHits(res)]
erbs = granges(erbs)
index = nearest(erbs[4], tssgr)
index
```

```
## [1] 6316
```

```
gene_id <- names(tssgr[index,]$GENEID)

paste("gene id with TSS closest to 4th region of erbs: ", gene_id)
```

```
## [1] "gene id with TSS closest to 4th region of erbs:  2101"
```

```
keys = as.character(values(tssgr[index])$GENEID)
symbol_of_gene <- select(Homo.sapiens, keys=keys, columns=c("SYMBOL"), keytype="GENEID")
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
paste("Symbol of gene id: ", symbol_of_gene$SYMBOL)
```

```
## [1] "Symbol of gene id:  ESRRA"
```

DNAString objects

- **Biostrings** package - efficient handling of DNA, RNA and amino acide sequences in Bioconductor
- Classes for representing individual molecular sequences and optimized functions for performing operations of sequences and sequence sets

- DNA sequences represented as **DNAString** objects. Also **RNAString** and **AAString** classes for representing RNA and protein sequences
- Collectively referred to as **XString** objects

```
library(Biostrings)
```

```
## Loading required package: XVector
```

```
##
## Attaching package: 'Biostrings'
```

```
## The following object is masked from 'package:base':
##
##     strsplit
```

```
dna <- DNAString("TCGAGCAAT")
dna
```

```
## 9-letter DNAString object
## seq: TCGAGCAAT
```

```
length(dna) # number of bases in a DNAString
```

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

```
try(DNAString("JQX")) # Invalid bases
```

```
## Error in .Call2("new_XString_from_CHARACTER", class(x0), string, start,  :
##   key 74 (char 'J') not in lookup table
```

```
try(DNAString("NNNACGCGC-TTA-CGGGCTANN")) # unknowns and gaps
```

```
## 23-letter DNAString object
## seq: NNNACGCGC-TTA-CGGGCTANN
```

```
dna[4:6] # substring
```

```
## 3-letter DNAString object
## seq: AGC
```

```
as.character(dna) # convert DNAString to character
```

```
## [1] "TCGAGCAAT"
```

DNAStringSet objects

- Grouping sets of biostrings in order to operate on them together - **XStringSets**

```
set1 <- DNAStringSet(c("TCA", "AAATCG", "ACGTGCCTA", "CGCGCA", "GTT", "TCA"))
set1
```

```
## DNAStringSet object of length 6:
##     width seq
## [1]     3 TCA
## [2]     6 AAATCG
## [3]     9 ACGTGCCTA
## [4]     6 CGCGCA
## [5]     3 GTT
## [6]     3 TCA
```

```
set1[2:3] # extract subset of sequences
```

```
## DNAStringSet object of length 2:
##     width seq
## [1]     6 AAATCG
## [2]     9 ACGTGCCTA
```

```
set1[[4]] # extract one sequence as a single DNAString
```

```
## 6-letter DNAString object
## seq: CGCGCA
```

```
length(set1) # number of DNAStrings in set
```

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

```
width(set1) # size of each DNAString
```

```
## [1] 3 6 9 6 3 3
```

```
duplicated(set1) # detect which sequences are duplicated
```

```
## [1] FALSE FALSE FALSE FALSE FALSE  TRUE
```

```
unique(set1) # keep only unique sequences
```

```
## DNAStringSet object of length 5:
##     width seq
## [1]     3 TCA
## [2]     6 AAATCG
## [3]     9 ACGTGCCTA
## [4]     6 CGCGCA
## [5]     3 GTT
```

```
sort(set1)
```

```
## DNAStringSet object of length 6:
##     width seq
## [1]     6 AAATCG
## [2]     9 ACGTGCCTA
## [3]     6 CGCGCA
## [4]     3 GTT
## [5]     3 TCA
## [6]     3 TCA
```

Operations on DNAStrings

- Walkthrough common operations

```
dna_seq <- DNAString("ATCGCGCGCGGCTCTTTTAAAAAAACGCTACTACCATGTGTGTCTATC")
letterFrequency(dna_seq, "A") # count A in sequence
```

```
##  A
## 12
```

```
letterFrequency(dna_seq, "GC") # count G or C in sequence
```

```
## G|C
##  22
```

```
dinucleotideFrequency(dna_seq) # frequencies of all dinucleotides
```

```
## AA AC AG AT CA CC CG CT GA GC GG GT TA TC TG TT
##  6  3  0  3  1  1  5  5  0  5  1  3  4  4  3  3
```

```
trinucleotideFrequency(dna_seq) # frequencies of all trinucleotides
```

```
## AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT ATA ATC ATG ATT CAA CAC CAG CAT
##   5   1   0   0   0   1   1   1   0   0   0   0   0   2   1   0   0   0   0   1
## CCA CCC CCG CCT CGA CGC CGG CGT CTA CTC CTG CTT GAA GAC GAG GAT GCA GCC GCG GCT
##   1   0   0   0   0   4   1   0   3   1   0   1   0   0   0   0   0   0   3   2
## GGA GGC GGG GGT GTA GTC GTG GTT TAA TAC TAG TAT TCA TCC TCG TCT TGA TGC TGG TGT
##   0   1   0   0   0   1   2   0   1   2   0   1   0   0   1   2   0   0   0   3
## TTA TTC TTG TTT
##   1   0   0   2
```

```
# convert DNAStrings

reverseComplement(dna_seq) # find reverse complement
```

```
## 48-letter DNAString object
## seq: GATAGACACACATGGTAGTAGCGTTTTTTTAAAAGAGCCGCGCGCGAT
```

```
translate(dna_seq) # amino acid translation
```

```
## 16-letter AAString object
## seq: IARGSFKKTLLPCVSI
```

Matching and Counting with Biostrings

- Finding all locations or counting matches of a pattern in a molecular seqeunce are common tasks
- **Biostrings** package includes fast function for pattern matching and counting on **XString** and **XStringSet** objects.

```
# count and match on individual Biostrings
dna_seq <- DNAString("ATCGCGCGCGGCTCTTTTAAAAAAACGCTACTACCATGTGTGTCTATC")
dna_seq
```

```
## 48-letter DNAString object
## seq: ATCGCGCGCGGCTCTTTTAAAAAAACGCTACTACCATGTGTGTCTATC
```

```
countPattern("CG", dna_seq) # pattern CG occurs 5x
```

```
## [1] 5
```

```
matchPattern("CG", dna_seq) # locations of the pattern
```

```
## Views on a 48-letter DNAString subject
## subject: ATCGCGCGCGGCTCTTTTAAAAAAACGCTACTACCATGTGTGTCTATC
## views:
##       start end width
##   [1]     3   4     2 [CG]
##   [2]     5   6     2 [CG]
##   [3]     7   8     2 [CG]
##   [4]     9  10     2 [CG]
##   [5]    26  27     2 [CG]
```

```
start(matchPattern("CG", dna_seq)) # start locations of the pattern
```

```
## [1]  3  5  7  9 26
```

```
matchPattern("CTCTTTTAAAAAAACGCTACTACCATGTGT", dna_seq) # match pattern of any length
```

```
## Views on a 48-letter DNAString subject
## subject: ATCGCGCGCGGCTCTTTTAAAAAAACGCTACTACCATGTGTGTCTATC
## views:
##       start end width
##   [1]    12  41    30 [CTCTTTTAAAAAAACGCTACTACCATGTGT]
```

```
# check for pattern and its reverse complement
countPattern("TAG", dna_seq)
```

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

```
countPattern(reverseComplement(DNAString("TAG")), dna_seq)
```

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

```
# count and match on sets of BioStrings
set2 <- DNAStringSet(c("AACCGGTTTCGA", "CATGCTGCTACA", "CGATCGCGCCGG", "TACAACCGTACA"))
set2
```

```
## DNAStringSet object of length 4:
##     width seq
## [1]    12 AACCGGTTTCGA
## [2]    12 CATGCTGCTACA
## [3]    12 CGATCGCGCCGG
## [4]    12 TACAACCGTACA
```

```
vcountPattern("CG", set2) # counts for entire DNAStringSet
```

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

```
vmatchPattern("CG", set2)
```

```
## MIndex object of length 4
## [[1]]
## IRanges object with 2 ranges and 0 metadata columns:
##           start       end     width
##       <integer> <integer> <integer>
##   [1]         4         5         2
##   [2]        10        11         2
##
## [[2]]
## IRanges object with 0 ranges and 0 metadata columns:
##        start       end     width
##    <integer> <integer> <integer>
##
## [[3]]
## IRanges object with 4 ranges and 0 metadata columns:
##           start       end     width
##       <integer> <integer> <integer>
##   [1]         1         2         2
##   [2]         5         6         2
##   [3]         7         8         2
```

```
##   [4]        10        11         2
##
## [[4]]
## IRanges object with 1 range and 0 metadata columns:
##           start       end     width
##       <integer> <integer> <integer>
##   [1]         7         8         2
```

```
vmatchPattern("CG", set2)[[1]] # access matches for first element of DNAStringSet
```

```
## IRanges object with 2 ranges and 0 metadata columns:
##           start       end     width
##       <integer> <integer> <integer>
##   [1]         4         5         2
##   [2]        10        11         2
```

Assessment: Biostrings

- eco seqeuence: short excerpt from E. coli K12 strain genome
- detect and analyze peptide encoded by genome fragment

```
eco <- DNAString("GGTTTCACCGCCGGTAATGAAAAAGGCGAACTGGTGGTGCTTGGACGCAACGGTTCCGACTACTCTGCTGCGGTGCTGGCTGCCT
eco
```

```
## 181-letter DNAString object
## seq: GGTTTCACCGCCGGTAATGAAAAAGGCGAACTGGTG...CGCGTCAGGTGCCCGATGCGAGGTTGTTGAAGTCGA
```

```
number_of_bases=length(eco)
paste("number of bases in eco: ", number_of_bases)
```

```
## [1] "number of bases in eco:  181"
```

```
count_atg <- countPattern("ATG", eco)
paste("Potential start codon in eco sequence: ", count_atg)
```

```
## [1] "Potential start codon in eco sequence:  2"
```

```
start_location_first_atg <- start(matchPattern("ATG", eco))
paste("Start location of the first ATG trinucleotide: ", start_location_first_atg[1])
```

```
## [1] "Start location of the first ATG trinucleotide:  17"
```

```
subset_from_start_location <- eco[start_location_first_atg[1]:length(eco)]
translated <- translate(subset_from_start_location)
paste("Length of resulting subset translated into amino acid: ", length(translated))
```

```
## [1] "Length of resulting subset translated into amino acid:  55"
```

```
location_of_stop_codon <- start(matchPattern("*", translated))
paste("Location of stop codon in AAString: ", location_of_stop_codon)
```

```
## [1] "Location of stop codon in AAString:  53"
```

```
subset_before_stop_codon <- translated[1:location_of_stop_codon-1]
subset_before_stop_codon
```

```
## 52-letter AAString object
## seq: MKKANWWCLDATVPTTLLRCWLPVYAPIVARFGRTLTGSIPATRVRCPMRGC
```

```
num_amino_acids <- length(subset_before_stop_codon)
paste("Number of amino acids in AAString before stop codon: ", num_amino_acids)
```

```
## [1] "Number of amino acids in AAString before stop codon:  52"
```

```
paste("Sequence: ", subset_before_stop_codon)
```

```
## [1] "Sequence:  MKKANWWCLDATVPTTLLRCWLPVYAPIVARFGRTLTGSIPATRVRCPMRGC"
```

```
positive_charge <- sum(countPattern("K", subset_before_stop_codon), countPattern("H", subset_before_sto
                       countPattern("R", subset_before_stop_codon))
paste("Number of positively charged amino acids: ", positive_charge)
```

```
## [1] "Number of positively charged amino acids:  8"
```

```
negative_charge <- sum(countPattern("D", subset_before_stop_codon), countPattern("E", subset_before_sto
paste("Number of negatively charged amino acids: ", negative_charge)
```

```
## [1] "Number of negatively charged amino acids:  1"
```

```
net_charge <- positive_charge - negative_charge
paste("Net charge of peptide at pH 7: ", net_charge)
```

```
## [1] "Net charge of peptide at pH 7:  7"
```

Getting the sequence of Regions

- How to use the whole reference genomic sequence of Homo Sapiens to look at the content of binding sites for the estrogen receptor
- HepG2 is a GRanges telling us where ChIP-seq experiments have identified locations where the estrogen receptor nuclear protein will bind
- Check the occurrence of certain short sequence called "binding motif" in the genomic sequence over which binding peaks are found
- Look up sequence of some genomic feature: match against the DNAString
- **getSeq** function from BioStrings package
- would not in practice use fixed string motif, would use matrix representation or model for this indicating variation in some of the bases
- MotifDb package includes models for binding motifs
- Would also not do direct pattern matching of this type, but something reflecting the probabilistic structure of the binding process. See: program MEME and FIMO
- Summary: we have genomic sequence for all chromosomes of homo sapeins. We have binding peak addresses in a GRanges. We can use getSeq to get the sequence content of the ranges, and search for motifs in those sequences to confirm biological plausibility

```
library(ERBS)
data(HepG2)
library(BSgenome.Hsapiens.UCSC.hg19) # reference build that was used for labeling of peaks
```

```
## Loading required package: BSgenome
```

```
## Loading required package: rtracklayer
```

```
Hsapiens # metadata about the construction of data object
```

```
## Human genome:
## # organism: Homo sapiens (Human)
## # genome: hg19
## # provider: UCSC
## # release date: June 2013
## # 298 sequences:
## #   chr1                  chr2                  chr3
## #   chr4                  chr5                  chr6
## #   chr7                  chr8                  chr9
## #   chr10                 chr11                 chr12
## #   chr13                 chr14                 chr15
## #   ...                   ...                   ...
## #   chr19_gl949749_alt    chr19_gl949750_alt    chr19_gl949751_alt
## #   chr19_gl949752_alt    chr19_gl949753_alt    chr20_gl383577_alt
## #   chr21_gl383578_alt    chr21_gl383579_alt    chr21_gl383580_alt
## #   chr21_gl383581_alt    chr22_gl383582_alt    chr22_gl383583_alt
## #   chr22_kb663609_alt
## # (use 'seqnames()' to see all the sequence names, use the '$' or '[[' operator
## # to access a given sequence)
```

```
ch17 <- Hsapiens$chr17 # sequence of chromosome 17
class(Hsapiens)
```

```
## [1] "BSgenome"
## attr(,"package")
## [1] "BSgenome"
```

```
hepseq = getSeq(Hsapiens, HepG2) # dna string set of length hepg2 (303), one for each of binding peaks
hepseq
```

```
## DNAStringSet object of length 303:
##       width seq
##   [1]   410 GAGACAGGGTTTCACCATGTTGGCCAGGCTGGT...CTTCCAGGAAGCAGAAATGTTCAAGGACTCTC
##   [2]   861 TGGGAAGGACACAACTGAATGAGGCTGTGCAGA...GCAGAACCTCCAACCGTGTGTGTGTGTGTGTG
##   [3]  1156 GACACCTGCCACCCCGGACCCCACAGAATGGGC...CTTCGTGTCTGCTTTCTTATGTGTTTTTGTTT
##   [4]   886 GTGAAGGCCCTGGAGTAGGCGGTGCGTACCCGG...GTGTTTTTGGCACCTCCGTGGGCACCTAGGCT
##   [5]  1242 CATCCTCCACCTTAACACTCAGCACCCTTAGAG...TTTGTGTCCTACAAGCAGCCGGCGGCGCCGCC
##   ...   ... ...
## [299]   671 CACTGGAGCTGGTGAAACAGGTAGTGAGTTGAT...TCATCTAGGGAGGCATGCAGCCCTCACCTGAG
## [300]   554 TCCGGAGAAGAAGAAACGGGGGAAGAACTTTTC...GTGCCGAGCGGCTGGGGACCGGCTCTAGGGAC
## [301]   418 CCACACCTGGAGCCAGTCTCAATGGCTCCCTGA...CATGAATGGTTGGAGACCAGGGGAGTTCTGTG
## [302]   456 TGATGACATTTCTCAAGGATTAAGAAAAAGAGA...CCTGCACCCATTTTGGTTTTGCTGTAGGGCCT
## [303]   348 TCCAAAGCAGACACTCCAGGACACCTGATTCTC...TTCTTTTTTTGAGACGGAGTCTCGTTCTGTCG
```

```
width(HepG2[1:5])
```

```
## [1]  410  861 1156  886 1242
```

```
rhepseq = getSeq(Hsapiens, shift(HepG2, 2500)) # collection of DNA strings which have no principled rel
rhepseq
```

```
## DNAStringSet object of length 303:
##       width seq
##   [1]   410 CTGCAGCCAGAAAGCAGGTCAGGGCTTGCCTGA...TTTGAAGAAATACAGTACCAGGGCATCATAGA
##   [2]   861 ACCTGCTCTGTCAGCATGGCGAAACCCTTTCTC...TGTAATCCCATCATTTTGGGAGGCCAAGGTGG
```

```
##   [3]  1156 AGGGGCATGGCCCACGGGCCCACCGTGGGGTTG...TGTATGCACTCTAGTTTTTATTATTAAACCAA
##   [4]   886 TCCCGGGTTCGAGCAATGCTCCCGCCTCAGCCT...AACCAGGACCATCCTCAAAAAGCCGACGGGGA
##   [5]  1242 AAGGTTGCCTGGGGGCTACGGTTACCCTGCTCC...ATTTTTAGTAGAGACGACGTTTCACCATGTCG
##   ...   ... ...
## [299]   671 GAATGACAAGTGATGGCGCAACCCGCCCAGCTG...CATCGCCCCCCAGACCGTCCACACGGCCACGT
## [300]   554 TTTCAACTTCTTTCTCTGAGCTCCTTTAGTTCT...AGCCCACACATTCTTGGCCTTCTGCAGATCAC
## [301]   418 ATGGTGAAACCCCATCTCTACTAAAAATACAAA...ATGATATACTAAAAATGGGTAAATTTTGTGAT
## [302]   456 TGCAGGGCAGACGCAGGGACCCTGGTCCAGCGG...AGGCAAGCAGTGAAGACAGAGGGGTGGCCCGA
## [303]   348 CATTCAGTAAATATCTATTGGGTCCCTTTGTTT...ACTACTTGTTTTAAAAGTAGTGCATTAATTAA
```

```
mot = "TCAAGGTCA" # one representation of binding motif for ER protein

sum(vcountPattern(mot, hepseq)) # count of times the motif occurs
```

```
## [1] 20
```

```
sum(vcountPattern(mot, reverseComplement(hepseq))) # match reverse compliment of hepseq
```

```
## [1] 35
```

```
total_sum = sum(vcountPattern(mot, hepseq), vcountPattern(mot, reverseComplement(hepseq)))
total_sum
```

```
## [1] 55
```

```
 # compare with the randomly selected equal length collection of DNA string
sum(vcountPattern(mot, rhepseq), vcountPattern(mot, reverseComplement(rhepseq)))
```

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

Assessment: Getting sequences

```
library(ERBS)
library(GenomicRanges)
data(HepG2)
data(GM12878)
res = findOverlaps(HepG2,GM12878)
erbs = HepG2[queryHits(res)]
erbs = granges(erbs)

library(BSgenome.Hsapiens.UCSC.hg19)

hepseq = getSeq(Hsapiens, erbs)
gc_content <- (vcountPattern("C", hepseq) + vcountPattern("G", hepseq)) / width(hepseq)
gc_content
```

```
##  [1] 0.5682927 0.6527294 0.6805869 0.6497585 0.5157233 0.6148282 0.5352324
##  [8] 0.6859903 0.5423112 0.7356322 0.7334315 0.6431227 0.5959596 0.5000000
## [15] 0.5495890 0.4837905 0.4400871 0.6785714 0.6476898 0.6560150 0.7123711
## [22] 0.4853420 0.6709677 0.6157761 0.7180157 0.5075000 0.5202864 0.6960258
## [29] 0.6346968 0.5369863 0.6646778 0.7694175 0.6868009 0.4736842 0.6095238
## [36] 0.5921502 0.6909976 0.6898551 0.6875834 0.6406460 0.5083799 0.6853193
## [43] 0.6818727 0.6701389 0.5657895 0.7370000 0.7053763 0.6149068 0.5191257
## [50] 0.6510417 0.6104418 0.7107558 0.6117886 0.5088235 0.6574713 0.7442197
## [57] 0.6466019 0.7216495 0.7353579 0.5679172 0.6602086 0.6218487 0.5285935
## [64] 0.6358930 0.6938776 0.6601307 0.6694796 0.6525680 0.6888889 0.7030129
```

```
## [71] 0.4778555 0.6860254 0.7130435 0.7087753 0.6552347
```

```
paste("median gc-content: ", median(gc_content))
```

```
## [1] "median gc-content:  0.652567975830816"
```

```
control_set <- getSeq(Hsapiens, shift(erbs, 10000))

gc_content_control <- (vcountPattern("C", control_set) + vcountPattern("G", control_set)) / width(contro
paste("median gc-content of control: ", median(gc_content_control))
```

```
## [1] "median gc-content of control:  0.486017357762777"
```

Assessment: GRanges and Biostrings

```
library(GenomicRanges)
library(Biostrings)
library(Homo.sapiens)
library(BSgenome.Hsapiens.UCSC.hg19)
library(ERBS)

library(Homo.sapiens)
g <- genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
unique_seqlevels <- length(unique(seqlevels(g)))
num_genes <- length(g)
paste("number of genes in build: ", num_genes)
```

```
## [1] "number of genes in build:  23056"
```

```
paste("unique seqlevels: ", unique_seqlevels)
```

```
## [1] "unique seqlevels:  93"
```

```
chr21 <- keepSeqlevels(g, "chr21", pruning.mode = "coarse")
paste("number of genes on chromosome 21: ", length(chr21))
```

```
## [1] "number of genes on chromosome 21:  296"
```

```
num_bp_longest_gene <-  max(width(chr21))
paste("number of base pairs in longest gene on chromosome 21: ", num_bp_longest_gene)
```

```
## [1] "number of base pairs in longest gene on chromosome 21:  1196950"
```

```
prop_positive <- length(strand(chr21)[strand(chr21) == "+"]) / length(strand(chr21))
paste("proportion of genes on chromosome 21 on positive strand: ", prop_positive)
```

```
## [1] "proportion of genes on chromosome 21 on positive strand:  0.462837837837838"
```

```
hepseq = getSeq(Hsapiens, chr21)
gc_content <- (vcountPattern("C", hepseq) + vcountPattern("G", hepseq)) / width(hepseq)
paste("median gc content chr21: ", median(gc_content))
```

```
## [1] "median gc content chr21:  0.461321869758511"
```

```
fifth_seq <- hepseq[5]
possible_start_codons <- vcountPattern("ATG", fifth_seq)
paste("number possible start codons: ", possible_start_codons)
```

```
## [1] "number possible start codons:  6"
```

```
start_locations <- vmatchPattern("ATG", fifth_seq)

real_start_codon_loc <- start(start_locations)[["100151643"]][1]
paste("start location within this gene of first ATG: ", real_start_codon_loc)
```

```
## [1] "start location within this gene of first ATG:  32"
```

```
from_start <- DNAString(substr(as.character(hepseq[5]), real_start_codon_loc, width(hepseq[5])))
translated <- translate(from_start)
```

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

```
location_codons <- matchPattern("*", translated)
location_first_codon <- start(location_codons)[1]
sequence_up_until_first_codon <- translated[1:location_first_codon-1]
paste("sequence up until first codon: ", sequence_up_until_first_codon)
```

```
## [1] "sequence up until first codon:  MSYYSHLSGGLGCGLAVAVTMGRTVAVAEYGRCRHGCHSSYSAR"
```

```
snca <- genes(Homo.sapiens, filter=list(GENEID="6622"))
tss_location = start(snca)
paste("TSS location: ", tss_location)
```

```
## [1] "TSS location:  90645250"
```

```
sequence <- getSeq(Hsapiens, snca)
num_motifs <- sum(vcountPattern("ACTGTGAA", sequence), vcountPattern("ACTGTGAA", reverseComplement(sequ
paste("Number of binding sites: ", num_motifs)
```

```
## [1] "Number of binding sites:  8"
```

```
# Load the GRanges corresponding to human genes from the Homo.sapiens package
g <- genes(Homo.sapiens)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
# Load the ESRRA binding site GRanges in GM12878 cells
data(GM12878)

flanked = flank(g, 2000)

promoter_start = start(flanked[100])
promoter_end = end(flanked[100])

paste("range for promoter of 100th gene in g: ", promoter_start, "-", promoter_end)
```

```
## [1] "range for promoter of 100th gene in g:  70561402 - 70563401"
```

```
res = findOverlaps(flanked, GM12878)

num_overlaps = sum(countOverlaps(flanked, GM12878))
num_unique_promoters_overlap = length(unique(queryHits(res)))
num_unique_gm128_overlap = length(unique(subjectHits(res)))
paste("unique GM12878 ESRRA binding sites overlap with promoters: ", num_unique_gm128_overlap)
```

```
## [1] "unique GM12878 ESRRA binding sites overlap with promoters:  757"
```

```
paste("Unique promoters overlapping with GM12878 ESRRA binding sites: ", num_unique_promoters_overlap)
```

```
## [1] "Unique promoters overlapping with GM12878 ESRRA binding sites:  943"
```

```
shifted = shift(flanked, 10000)
res_shifted = findOverlaps(shifted, GM12878)
num_unique_promoters_overlap_s = length(unique(queryHits(res_shifted)))
num_unique_gm128_overlap_s = length(unique(subjectHits(res_shifted)))
num_overlaps = length(res_shifted)
paste("number of overlaps: ", num_overlaps)
```

```
## [1] "number of overlaps:  132"
```

```
paste("unique GM12878 ESRRA binding sites overlap with promoters post shift: ", num_unique_gm128_overla
```

```
## [1] "unique GM12878 ESRRA binding sites overlap with promoters post shift:  117"
```

```
paste("Unique promoters overlapping with GM12878 ESRRA binding sites post shift: ", num_unique_promoter
```

```
## [1] "Unique promoters overlapping with GM12878 ESRRA binding sites post shift:  131"
```

```
ratio_shifted = num_unique_gm128_overlap / num_unique_gm128_overlap_s
paste("ratio of the number of unique ESRRA binding sites overlapping promoters versus the number of uni
```

```
## [1] "ratio of the number of unique ESRRA binding sites overlapping promoters versus the number of un
```