Homework 2

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Part 1

A: What are the first five genomic nucleotides from the first exon of the transcript AK002007?

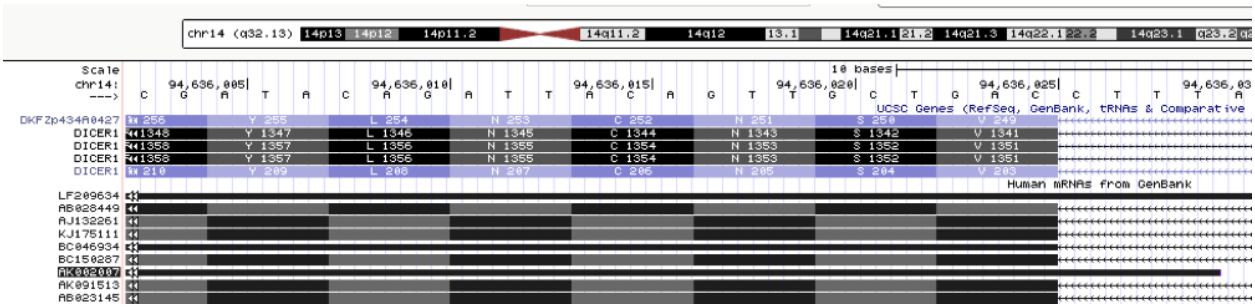


Figure 1: Exon 1 of transcript AK002007 from March 2006 assembly

Examination of the first exon in AK002007 reveals the first five nucleotides to be: AAAGG

B: Look at the raw mRNA sequence of AK002007, from the database it actually comes from. What are the first five nucleotides?

cDNA AK002007

gaagcaaAAA GGTCAGCAAC TGTAATCTGT ATCGCCTTGG AAAAAAGAAG 50
GGACTACCCA GCCGCATGGT GGTGTCAATA TTTGATCCCC CTGTGAATTG 100

Figure 2: Exon 1 of transcript AK002007 from the database

Thus, the first five nucleotides in the raw mRNA sequence of AK002007 is: GAAGC

C: How do you explain the discrepancy (maximum 5 lines)?

First seven nucleotides on the mRNA sequence are not mapped to the genome. The raw sequence is cDNA that then is mapped to the genome to create the mRNA track. The discrepancy could be explained by the fact that cDNA is used during the gene sequencing in which a lot of other factors are also involved. These include adaptors, primers and linkers which are attached to the sequence during the process. Thus, the ends of the sequence are error-prone and therefore need to be trimmed.

Part 2

A: What is the genome coverage (% of base pair covered at each chromosome) for ERB and ERA sites?

In order to compute the genome coverage for ERb and ERa sites we firstly need to sort them. This is done with the following commands:

```
sort -k 1,1 -k 2,2n ERb_hg18.bed > sorted_ERb_hg18.bed
sort -k 1,1 -k 2,2n ERa_hg18.bed > sorted_ERa_hg18.bed
```

The genome coverage is computed using the genomecov command from the BEDtools library. The -max 1 option in the command means that all chromosomes that have 1 or more ERa and ERb sites covering will be counted as one.

```
nice bedtools genomecov -i ERa_hg18.bed -g hg18_chrom_sizes.txt -max 1
```

```
nice bedtools genomecov -i ERb_hg18.bed -g hg18_chrom_sizes.txt -max 1
```

The output of the genomecov for the ERa sites and ERb sites, where the last column shows genome coverage for each chromosomes: ERa genome coverage

```
ERa_coverage <- read.table("genomecoverage_ERa.bed", header = F)
head(ERa_coverage, n=3)
```

```
##      V1 V2        V3        V4          V5
## 1  chr1  0 247150807 247249719 0.999600000
## 2  chr1  1     98912 247249719 0.000400049
## 3 chr21  0  46928331  46944323 0.999659000
```

ERb genome coverage

```
ERb_coverage <- read.table("genomecoverage_ERb.bed", header = F)
head(ERb_coverage, n=3)
```

```
##      V1 V2        V3        V4          V5
## 1  chr1  0 247162506 247249719 0.999647000
## 2  chr1  1     87213 247249719 0.000352732
## 3 chr21  0  46932488  46944323 0.999748000
```

Plot the fractions for all chromosomes as a single barplot in R.

The plot shows the percentage of genome coverage for ERa and ERb sites. The genome rows have been deleted from the genomecoverage_ERa.bed and genomecoverage_ERb.bed files because this data is not relevant in this analysis.

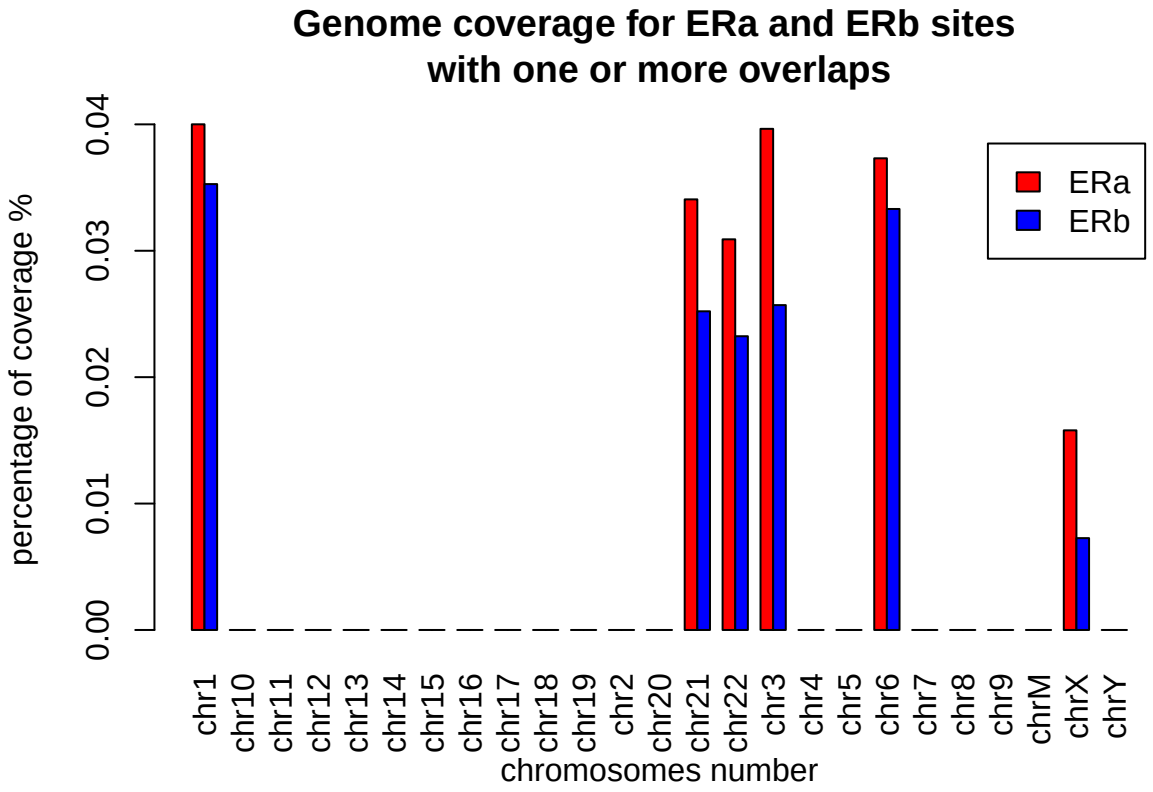
```
ERa_coverage <- read.table("genomecoverage_ERa.bed", header = F)
ERb_coverage <- read.table("genomecoverage_ERb.bed", header = F)

coverage_a <- ERa_coverage[ERa_coverage$V5==1, c(1:5)]
coverage_a$V2=1; coverage_a$V5=0
coverage_b <- ERb_coverage[ERb_coverage$V5==1, c(1:5)]
coverage_b$V2=1; coverage_b$V5=0

new_coverage_ERa <- rbind(ERa_coverage, coverage_a)
new_coverage_ERa_sorted <- new_coverage_ERa[order(new_coverage_ERa$V1),]
new_coverage_ERb <- rbind(ERb_coverage, coverage_b)
new_coverage_ERb_sorted <- new_coverage_ERb[order(new_coverage_ERb$V1),]
```

```
chromosome_number_a <- new_coverage_ERa_sorted$V1[new_coverage_ERa_sorted$V2==1] # labels
chromosome_number_b <- new_coverage_ERb_sorted$V1[new_coverage_ERb_sorted$V2==1] #labels

data <- rbind(new_coverage_ERa_sorted$V5[new_coverage_ERa_sorted$V2==1]*100,
              new_coverage_ERb_sorted$V5[new_coverage_ERb_sorted$V2==1]*100)
rownames(data)<-c("ERa","ERb")
barplot(data,col=c("red","blue"),beside=T,las=3,names.arg = chromosome_number_a,
        legend = rownames(data),xlab = 'chromosomes number',
        ylab='percentage of coverage %',
        main='Genome coverage for ERa and ERb sites \nwith one or more overlaps')
```



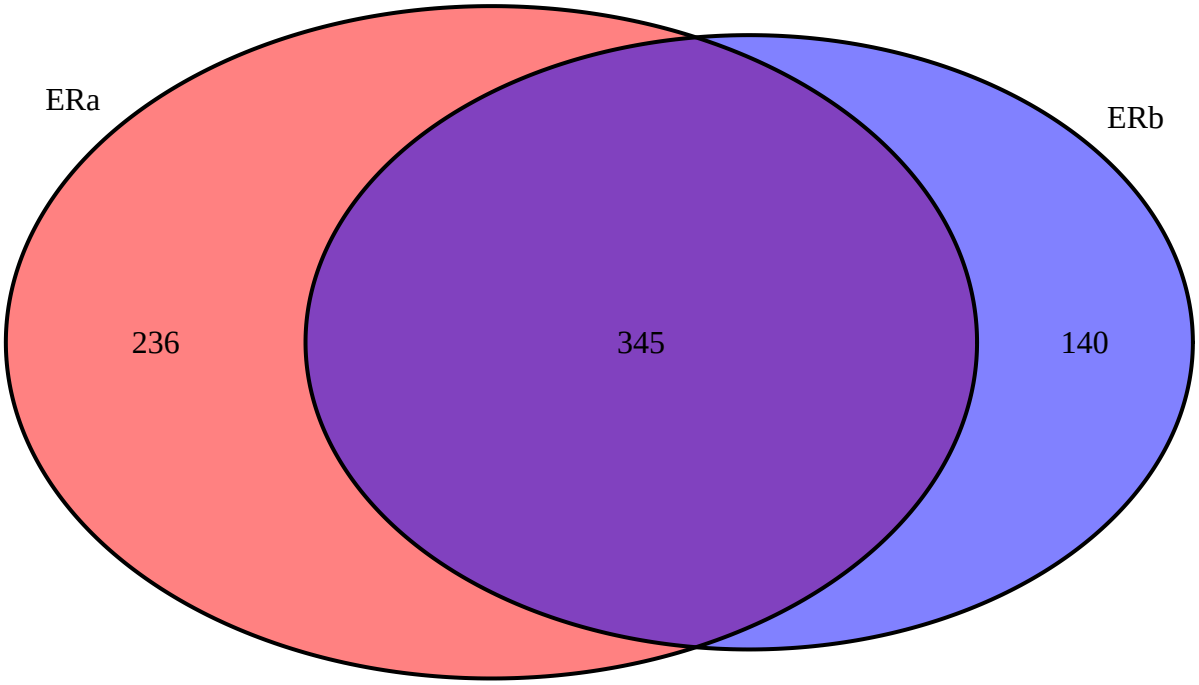
The plot shows the percentage of genome coverage of the chromosomes with 1 or more bindingsites. ERa and ERb have bindingsites on the same chromosomes, though ERa covers a larger percentage of each chromosome.However, in this experiment ERa and ERb only bind to six of the chromosomes. Estrogen receptors are nuclear receptors that are likely to regulate many genes throughout the genome. It is possible that they in fact bind other chromosomes as well, but we did not see it in this dataset. This could partly be explained by economic considerations. As stated in the wikipedia article linked to the question, making ChIP-analysis on the whole chromosome is quite costly, and in order to reduce expenses they might only sequence a minor part of the genome. Based on our data, it is possible that ERa and ERb share bindingsites and thus serve (some of) the same functions. However, both ERA and ERB share structure similarities. Thus, the antibody used for the ChIP assay may have been bound to both proteins giving rise to a skewet interpretation of shared binding sites.

B: How many ERa sites do/do not overlap ERb sites, and vice versa?

```
bedtools intersect -a sorted_ERa.bed -b sorted_ERb.bed -c > a_overlap_b.bed
bedtools intersect -a sorted_ERb.bed -b sorted_ERa.bed -c > b_overlap_a.bed
```

```
a_overlap_b <- read.table("a_overlap_b.bed", header=F)
cross_area_ab <- sum(a_overlap_b$V4)
b_overlap_a <- read.table("b_overlap_a.bed", header=F)
cross_area_ba <- sum(b_overlap_a$V4)

# Venn diagram summarizing the results.
ERa_data<- read.table("ERa_hg18.bed", row.names = NULL)
ERb_data<- read.table("ERb_hg18.bed", row.names = NULL)
area_ERa<-length(ERa_data$track)
area_ERb <- length(ERb_data$track)
library(VennDiagram)
draw.pairwise.venn(area_ERa, area_ERb,
    cross.area=cross_area_ba, c("ERa","ERb"), fill=c("red","blue"))
```




```
## (polygon[GRID.polygon.1], polygon[GRID.polygon.2], polygon[GRID.polygon.3], polygon[GRID.polygon.4],
```

The number of ERa sites overlapping ERb sites is 345 and it is the same as the number of ERb sites overlapping ERa sites.

Part 3

BLAT algorithm has been applied to the mRNA sequence of the fly gene found in mouse genome and that of D. melanogaster (Feb. 2006 assembly/mm8 was chosen to compare these two). The fly gene looks completely odd when mapped to the mouse genome, evident by the fact that the gene only consists of one big exon and does not have either 5' or 3' UTRs, which all are normally present. In addition to that, turning on CpG island tracks in UCSC browser shows that there is no region of CpG islands present in the studied gene region - although CpG islands are not required for promoter regions, the absence seems suspicious. Running BLAT on the mouse sequence from the new-gene region and the mouse genome, gives rise to several perfect hits, and many hits with a relative good score - suggesting the sequence is common in the mouse genome. A particular hit spanning 577 base pairs with a 100% identity maps to a gene called Rpl41 on chromosome 10 in the mouse genome. This can be explained by Rpl14 being greatly conserved throughout species, and that this particular sequence at some point has been replicated and inserted randomly in the genome. Thus, the

postulated ‘new gene’ is actually a processed pseudogene - segment of DNA that is related to a real gene, and that is formed through retrotransposition (does not contain any introns). Since it is highly unlikely that the final processed transcript will be inserted in association with a promoter, it will never be transcribed but it will still resemble the sequence of the original gene due to high conservation.

mRNA/Genomic Alignments

The alignment you clicked on is first in the table below.

BROWSER	SIZE	IDENTITY	CHROMOSOME	STRAND	START	END	QUERY	START	END	TOTAL
browser	81	81.5%	9	+-	24851808	24851888	NM_001014551	44	124	320
browser	81	81.5%	10	+-	43144836	43144916	NM_001014551	44	124	320
browser	81	81.5%	13	+-	112714409	112714489	NM_001014551	44	124	320
browser	81	81.5%	14	++	104567548	104567628	NM_001014551	44	124	320
browser	81	81.5%	16	+-	3932205	3932285	NM_001014551	44	124	320

Figure 3: Fly gene shows 81.5% identify and maps to 70 bases in the mouse genome

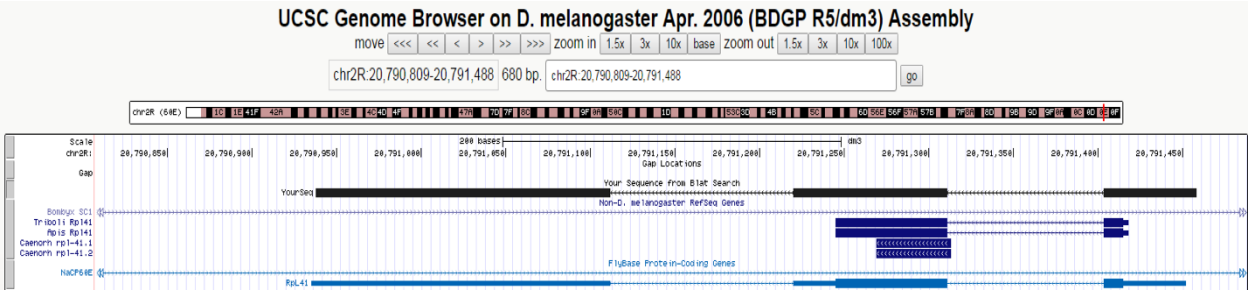


Figure 4: BLAT on D. melanogaster genome

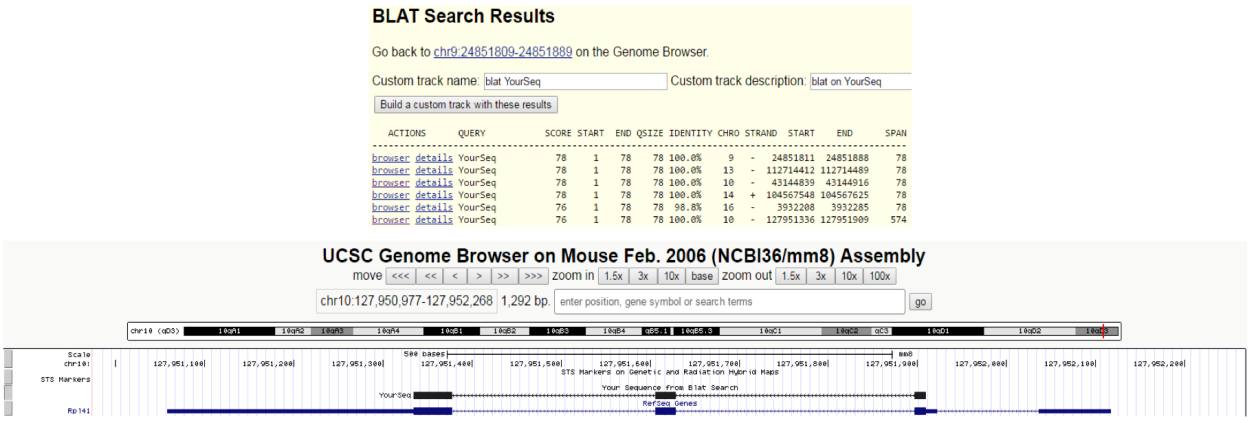


Figure 5: BLAT on mouse genome