Zebrafish Dataset Practical 2

All of these exercises will be done on the same dataset as yesterday's practicals. Make sure you've read the document that describes the dataset and make sure you've got the four required files (`Amp.counts.tsv`, `Amp.samples.tsv`, `Oxy.counts.tsv` and `Oxy.samples.tsv`) in your home directory. You will also need the significantly differentially expressed gene lists you made in yesterday's practical and also the files that just contain their Ensembl IDs. (If you haven't finished yesterday's practical then please carry on with it, either before or after today's practical.)

1. Use BioMart to get the sequence of the 1000 bp upstream of each transcript of the top 20 (extracted using `head`) most significantly differentially expressed genes. The header information should include the gene Ensembl ID, the gene name, the transcript Ensembl ID, the transcript type and the transcript length.

2. Use `awk` (not `cut`, which doesn't allow you to change the column order) to make a new file that contains the chromosome, start, end and Ensembl ID (in that order) for all the significant genes. Name the file something like "`Amp.sig.bed`" or "`Oxy.sig.bed`". Congratulations - you've made a BED file. See https://genome.ucsc.edu/FAQ/FAQformat.html#format1 for more information about the BED format. Try viewing this file in Ensembl by adding it as a custom track. Have a look at the distribution of the genes in the "Whole genome", "Chromosome summary" and "Region in detail" views.

3. Use BioMart to get the human orthologues of all the significant genes. Your output should include the zebrafish Ensembl ID, the zebrafish gene name, the human Ensembl ID, the human gene name, the human orthology type, the percentage identity (both target to query and query to target) and the human orthology confidence. How many of the zebrafish genes have a human orthologue? How many have a high confidence human orthologue?

   This question is trickier than it seems and you'll need to know that the `sort` command has a "-u" option that stands for "unique" and will remove any duplicates. Your solution will need to use `cut`, `grep`, `cut` (again) and `sort -u` (in that order).

4. Choose one of your significant genes and go to its Gene page on Ensembl. Click on "External references" in the left-hand menu and then click on the Expression Atlas link. The initial view in Expression Atlas isn't very useful because the stages are ordered alphabetically and not developmentally. If you click on "18 White et al" then you'll get a much more useful view. How does the expression of the gene change over development? Note the option above the heatmap for viewing a boxplot. Add the other 20 most significant genes via the box at the left-hand side. Are there any similarities in the developmental expression of the genes?

5. In the "Region in detail" view, go to "Configure this page" and add the merged RNA-seq models, including the merged intron-spanning reads. Can you find any evidence for alternative splicing in any of the significant genes? http://www.ensembl.org/Help/Faq?id=472 might be useful.

6. Yesterday Sam mentioned the improved annotation from the Lawson lab. It's described in this paper:

   Lawson et al. (2020) "An improved zebrafish transcriptome annotation for sensitive and comprehensive detection of cell type-specific genes" https://elifesciences.org/articles/55792

   And also described here: https://www.umassmed.edu/lawson-lab/reagents/zebrafish-transcriptome/

   Try adding https://www.umassmed.edu/globalassets/lawson-lab/downloadfiles/v4.3.2.gtf as a custom track. Warning: the file is very large and so Ensembl will be quite slow. Make sure you disable or delete the track when you're done.

   Have a look at the Lawson annotation for a selection of the significantly differentially expressed genes. How does it differ from the Ensembl annotation? Can you spot any issues with the Lawson annotation? How does it compare to RefSeq annotation? You'll need to turn on the RefSeq track.

   (RefSeq is NCBI's annotated and curated database of reference sequences, including transcripts and proteins. Accessions starting with X are predictions from automatic genome annotation. Those starting with N come from manual annotation.)

7. Now, we're going to take some data from BioMart and plot it in R.

Use BioMart to get all the zebrafish genes annotated to the Gene Ontology (GO) term `GO:0002088`. We'll cover Gene Ontology (GO) terms tomorrow. This particular GO id is for the term 'lens development in camera-type eye'.

The output should include the zebrafish Ensembl ID, Gene name, Chromosome/scaffold name, Gene start, Gene End and Strand. Download the data as a tab-separated values file and load into R.

The column names have spaces and other characters that R doesn't like such as '('.
Use the `col_names =` argument to `read_tsv` to set the column names to
`c('Gene', 'Name', 'Chr', 'Start', 'End', 'Strand')`

You'll also need to skip over the header row of the file with the `skip = 1` argument

```r
# load the tidyverse packages
library(tidyverse)
# read in the BioMart data
# skip the header row
# set the column names manually
lens_genes <-
  read_tsv('mart_export.txt', skip = 1,
           col_names = c('Gene', 'Name', 'Chr', 'Start',
                         'End', 'Strand'))
```

We're going to plot the data by chromsomal location. So we want to filter out any non-numeric chromosomes to simplify this. Use `filter` on the data along with the `%in%` operator and the vector `1:25` to filter the `Chr` column.

`%in%` returns TRUE if the value in the vector on the left-hand side is in the vector on the right-hand side. For example:

```r
# letters is a vector of the 26 lowercase letters
# letters[1:6] is the first six letters
# so 'a' and 'd' in this vector evaluate to TRUE
# everything else is FALSE
c('a', 'z', 't', 'd', NA, 'tyu') %in% letters[1:6]
```

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

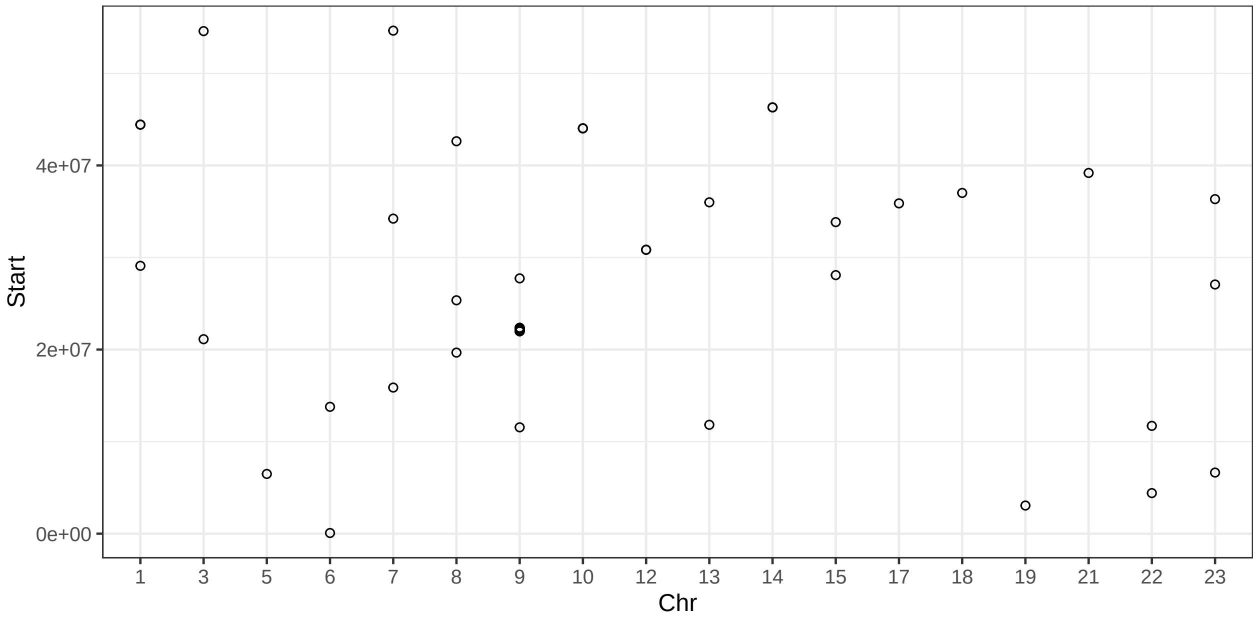
Pipe the result of `filter` into `mutate` and make `Chr` a factor with levels `1:25`.

Remember to assign the new data.frame created by `mutate` back to the data object.

```
lens_genes <- lens_genes %>%
  # filter for rows with Chr in the vector 1:25
  filter(Chr %in% 1:25) %>%
  # change the Chr column to a factor with levels 1:25
  mutate(Chr = factor(Chr, levels = 1:25))
```

Plot the data with `Chr` on the x-axis and `Start` on the y. Also use `shape = 21`. Make sure you put `shape = 21` outside of the `aes()` function.

```
# plot the lens_gene data
ggplot(data = lens_genes) +
  # Chr on the x-axis and Start on the y
  geom_point(aes(x = Chr, y = Start),
             shape = 21) +
  theme_bw()
```

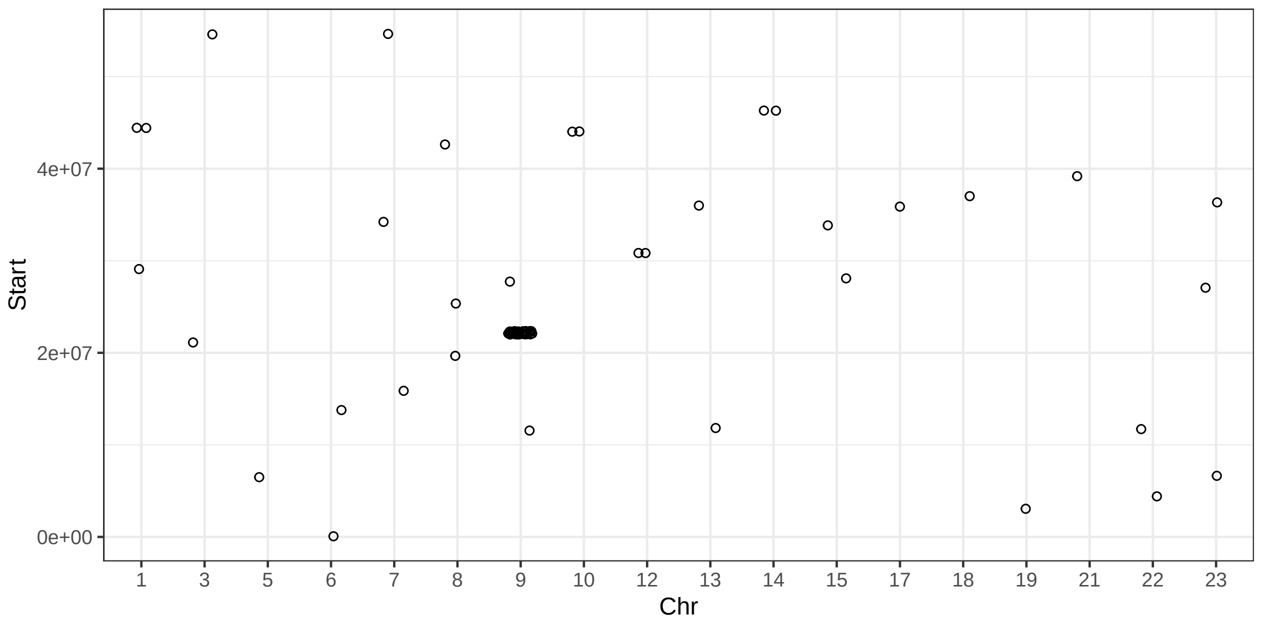


You may notice that there is a region with multiple points plotted on top of each other. We can make this clearer by using `geom_jitter` to move the points a small random amount along the x-axis so they are more obvious. `geom_jitter` has arguments `width` and `height` to control how much jitter there is and in which direction. The default values for `width` and `height` are 40% of the resolution of the data. The resolution is defined as the smallest non-zero distance between adjacent values in the data.

Play around with different values to see how they affect things.

You may notice other regions of the genome that have more than one gene in a short space.

```
ggplot(data = lens_genes) +
  # change geom_point to geom_jitter
  geom_jitter(aes(x = Chr, y = Start),
              # jitter in the x-axis but not the y
              width = 0.2, height = 0,
              shape = 21) +
  theme_bw()
```

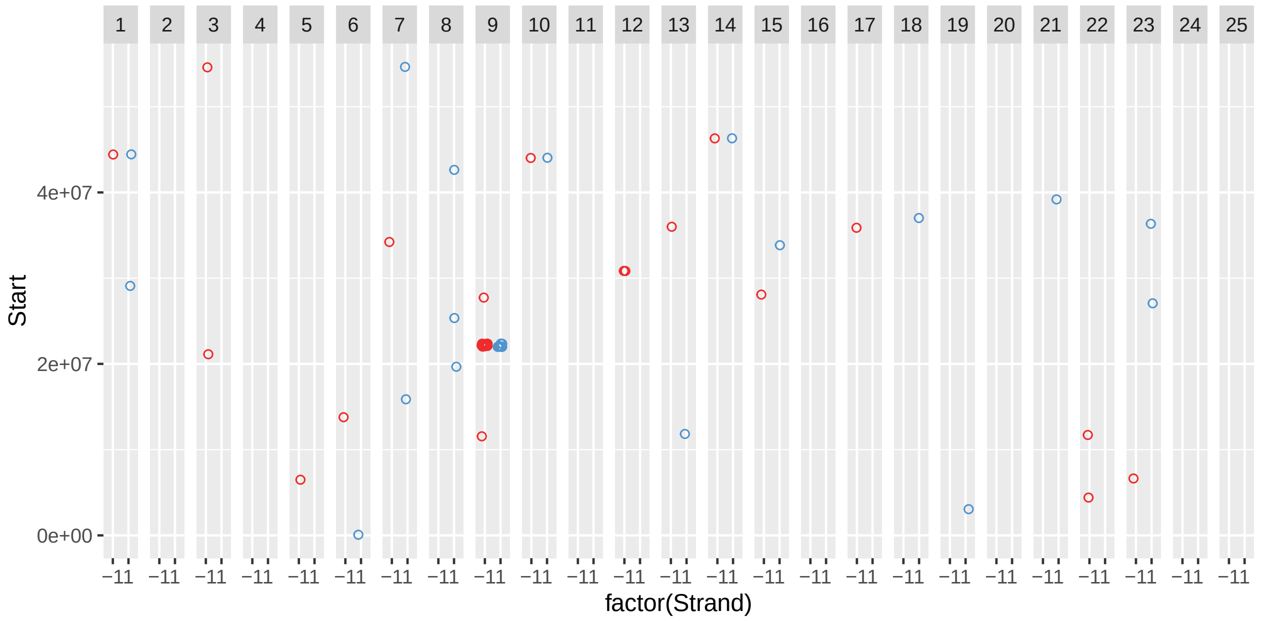


If we wanted to make this look more like a karyogram we could facet the plot by the `Chr` column to split it up into chromosomes. Below I'm using `facet_wrap`, with `ncol = 25` to arrange the chromosomes in one row.

There aren't genes on every chromosome, but we want all the chromosomes to appear. We can add `drop = FALSE` to `facet_wrap` for this.

Also, I've changed the variable mapped to the x aesthetic to be `Strand` as a factor to spread the points out a bit. I've also coloured the points by `Strand` as well.

```
ggplot(data = lens_genes) +
  geom_jitter(aes(x = factor(Strand), y = Start,
                  colour = factor(Strand)),
              width = 0.2, height = 0,
              shape = 21, show.legend = FALSE) +
  # add colours for Strand
  scale_color_manual(values = c('firebrick2', 'steelblue3')) +
  # facet by Chr column, 25 columns in 1 row
  # don't drop unused levels
  facet_wrap(vars(Chr), ncol = 25, drop = FALSE)
```



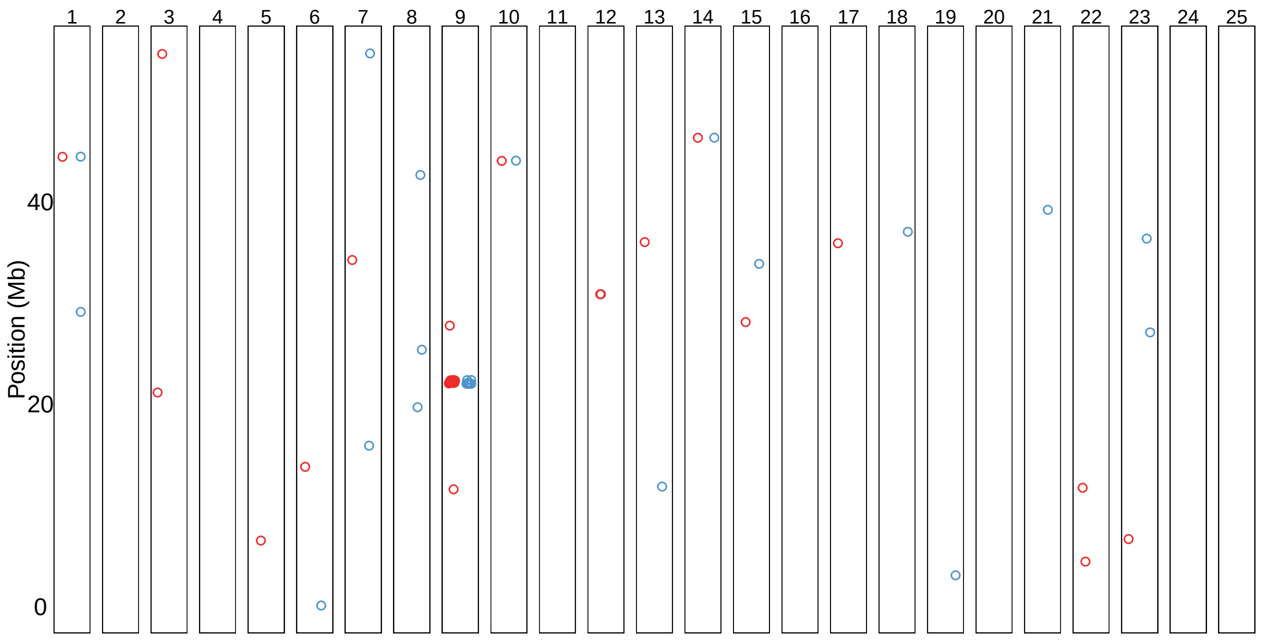
Adding `theme_void` removes the unnecessary axis labels, but I still want the title and labels on the y-axis so I have added those back using `axis.title.y` and `axis.text.y` in `theme()`.

I've also changed the `panel.background` to have a black border colour makes it look more like chromosomes.

And I've written a function to divide the y-axis breaks by 1000000 so that the axis labels are now in Mb.

```
# this function just takes a vector and
# divides each value in the vector by 1,000,000
Mb <- function(positions) {
  positions / 1000000
}

chr_plot <- ggplot(data = lens_genes) +
  geom_jitter(aes(x = factor(Strand), y = Start,
                  colour = factor(Strand)),
              width = 0.2, height = 0,
              # don't show the legend
              shape = 21, show.legend = FALSE) +
  scale_color_manual(values = c('firebrick2', 'steelblue3')) +
  # use name to change the title of the y-axis
  # format the labels using the Mb function
  scale_y_continuous(name = 'Position (Mb)', labels = Mb) +
  facet_wrap(vars(Chr), ncol = 25, drop = FALSE) +
  theme_void() +
  theme(panel.background = element_rect(colour = 'black'),
        axis.title.y = element_text(colour = 'black', angle = 90),
        axis.text.y = element_text(colour = 'black'))
print(chr_plot)
```


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
Position (Mb)
0
20
40