Introduction to R and Data Visualisation

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Installation of R and RStudio

Please go these websites to download and install R and RStudio.

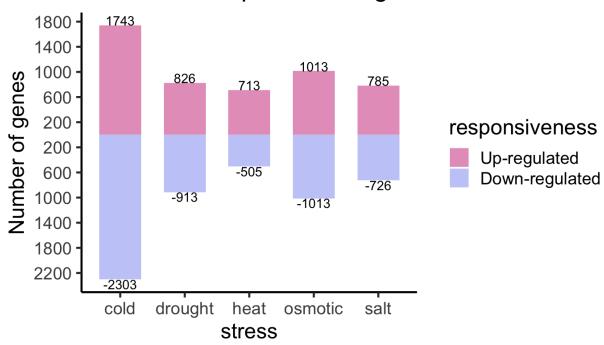
R - https://www.r-project.org

RStudio - https://posit.co/downloads/

For Rstudio, download the free version available on the downloads page.

Goal of our workshop is to create this plot that we see below:

Differential Expression of genes



Basic operations

Some very basic operations you can can carry out in R.

1 + 1 # addition

[1] 2

```
2 - 1 # subtraction
## [1] 1
2 * 2 # multiplication
## [1] 4
6 / 2 # division
## [1] 3
3 ** 2 # exponential
## [1] 9
3 ^ 2 # exponential
## [1] 9
Here R works like a calculator. Apart from numbers, R can also help us print letters or a string of letters.
## [1] "a"
'a'
## [1] "a"
"language"
## [1] "language"
"R is my favourite programming language"
## [1] "R is my favourite programming language"
When working with large numbers such as 1934929292 and 23992343, we cannot keep them in mind, or for
that matter, remember complex computations. So, we have the concept of object or variable.
a <- 1934929292
b <- 23992343
a
## [1] 1934929292
## [1] 23992343
Here, we assign "<-" the first number to "a" and the second to "b". The "<-" is called the "assignment
operator". "a" and "b" are called objects or variables. This now enables us to actually use the variables for
doing further operations as seen below.
a + b
## [1] 1958921635
## [1] 1910936949
a * b
```

[1] 4.642349e+16

```
## [1] Inf
We can do something similar with strings too.
x <- "language"
y <- "R is my favourite programming language"
x

## [1] "language"
y

## [1] "R is my favourite programming language"
print(x)

## [1] "language"
print(y)

## [1] "R is my favourite programming language"</pre>
```

Creating a vector

a ^ b

In R, vectors are the most basic data objects. Let us create vectors \mathbf{x} and \mathbf{y} . We will do that in two ways. One, using the c() function, and the other using the seq() function. 'c' combines values into a vector. 'seq' is a sequence generator.

```
x <- c(1, 2, 3, 4, 5, 6, 7, 8, 9, 10)
y <- seq(11, 20)
x

## [1] 1 2 3 4 5 6 7 8 9 10
y
## [1] 11 12 13 14 15 16 17 18 19 20</pre>
```

The code above creates two vectors, **x** and **y**. Adding the two vectors gives:

```
x + y
## [1] 12 14 16 18 20 22 24 26 28 30
x * y
```

The elements are added element-wise. The operations in R are element-wise. As an exercise you can try doing the other mathematical operations on the two vectors.

Indexing

The elements in the vectors are indexed. So, to extract an element you need only know its position. To extract the first element in x and in y:

```
x[1]
```

[1] 1

This returns 1. Try the following and see what you get.

[1] 11 24 39 56 75 96 119 144 171 200

```
y[1:4]

## [1] 11 12 13 14

y[c(1, 3, 5)]

## [1] 11 13 15

y[c(-1, -3, -5)]

## [1] 12 14 16 17 18 19 20

y[-c(1, 3, 5)]

## [1] 12 14 16 17 18 19 20
```

Data types

What are the important data types? They can be listed as:

- integer
- numeric
- logical
- character Let us first create some vectors

```
n <- 1 # numeric
i <- 1L # integer
l <- TRUE # logical
c <- "Some string" #character</pre>
```

Here we have four vectors created. x is a numeric vector, y an integer vector, t a logical vector, and c is a character vector. Remember, in R everything is a vector. There are no scalars. Therefore, all these vectors that we have created are all vectors of length one. To check the length of the vector, use the length() function:

```
length(n)
```

```
## [1] 1
length(i)
```

```
## [1] 1
length(1)
```

```
## [1] 1
length(c)
```

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

You can see that all objects created are of length one.

Now let us check these vectors using the $\mathit{class}()$ function:

```
class(n)
```

```
## [1] "numeric" class(i)
```

```
## [1] "integer"
```

```
class(1)
## [1] "logical"
class(c)
## [1] "character"
Now let us create vectors of length > 1
num_vr <- c(1, 3.0, 5.0) # numeric vector
int_vr <- c(1L, 3L, 5L) # integer vector</pre>
log_vr <- c(TRUE, FALSE, TRUE) # logical vector</pre>
char_vr <- c("I am", "a", "string.") # character vector</pre>
Now get their class.
class(num_vr); length(num_vr)
## [1] "numeric"
## [1] 3
class(int_vr); length(int_vr)
## [1] "integer"
## [1] 3
class(log_vr); length(log_vr)
## [1] "logical"
## [1] 3
class(char_vr); length(char_vr)
## [1] "character"
## [1] 3
We learnt to make vectors before, and now we have learnt to understand them a bit more. We now move on
to matrices. First, let us create some vectors.
v1 <- 1:5
v2 <- 6:10
v3 <- 11:15
We have three vectors v1, v2, and v3 and we are going to bind them column-wise.
cbind(v1, v2, v3)
##
        v1 v2 v3
## [1,]
         1 6 11
## [2,]
         2 7 12
## [3,]
         3 8 13
## [4,]
         4 9 14
## [5,] 5 10 15
The output just spews out to the console, which is not helpful. Let us create a variable, my_mat, and store
the output
my_mat <- cbind(v1, v2, v3)</pre>
```

my_mat

```
##
        v1 v2 v3
## [1,]
          1
             6 11
             7 12
  [2,]
         2
## [3,]
         3
             8 13
## [4,]
         4
             9 14
## [5,]
         5 10 15
```

Here, we used a function, cbind(), to bind three vectors into three columns. Now let us use the class function on the my mat variable.

```
class(my_mat)
```

```
## [1] "matrix" "array"
```

my_matrix is a matrix. It has three columns, v1, v2, and v3. And, as it should be clear now, we used three vectors to create a matrix. Let us now see an alternate method for creating a matrix.

```
trial_mat <- matrix(1:20, nrow=5, ncol=4, byrow = TRUE)
trial_mat</pre>
```

```
[,1] [,2] [,3] [,4]
##
## [1,]
                  2
             1
                        3
## [2,]
            5
                  6
                        7
                              8
## [3,]
            9
                             12
                 10
                       11
## [4,]
           13
                 14
                       15
                             16
## [5,]
            17
                 18
                       19
                             20
```

This creates a matrix with 4 rows and 5 columns. The [1,] refers to the first row. The [,1] refers to the first column.

Let us now talk about another kind of data structure, data frame. So, a data frame is similar to a matrix, but it can hold vectors of different classes. Let us create the same vectors we created previously even though they are still in memory.

```
num_vr <- c(1, 3.0, 5.0) # numeric vector
int_vr <- c(1L, 3L, 5L) # integer vector
log_vr <- c(TRUE, FALSE, TRUE) # logical vector
char_vr <- c("I am", "a", "string.") # character vector

# Let us use the cbind() function to put them together.

new_mat <- cbind(num_vr, int_vr, log_vr, char_vr)
new_mat</pre>
```

```
## num_vr int_vr log_vr char_vr
## [1,] "1" "1" "TRUE" "I am"
## [2,] "3" "3" "FALSE" "a"
## [3,] "5" "5" "TRUE" "string."
```

Looking at the output, we know that it is something we do not want. What is the class of the new variable? class(new_mat)

```
## [1] "matrix" "array"
```

The class of the object new_mat is "matrix". A matrix can hold data belonging to a particular class. In this case, every data point is converted into a character. This is called coercion. Here we need a different kind of data structure that can hold different classes of data. To demostrate this point, let us create some vectors that we will make use of in creating this structure.

```
set.seed(1234) # since the numbers are random, this will make sure we always
              # get the same set of random numbers
plant_height <- rnorm(100, 110, 10)
head(plant_height)
## [1] 97.92934 112.77429 120.84441 86.54302 114.29125 115.06056
Too many decimals. Let us round it off to two.
plant_height <- round(plant_height, 2)</pre>
head(plant_height)
## [1] 97.93 112.77 120.84 86.54 114.29 115.06
set.seed(237)
flowering_50 <- round(rnorm(100, 100, 10))
head(flowering_50)
## [1] 102 100 101 100 107 101
set.seed(6438)
spikelet_fertility <- round(rnorm(100, 90, 3), 2)</pre>
head(spikelet_fertility)
## [1] 89.05 89.18 93.49 92.28 88.04 93.61
max(spikelet_fertility)
## [1] 95.27
set.seed(345)
thousand seed weight <- round(rnorm(100, 22, 3), 2)
head(thousand_seed_weight)
## [1] 19.65 21.16 21.52 21.13 21.80 20.10
Now let us combine the four vectors into a single data structure.
my_data <- cbind(plant_height, flowering_50, spikelet_fertility, thousand_seed_weight)
head(my_data)
##
        plant_height flowering_50 spikelet_fertility thousand_seed_weight
## [1,]
               97.93
                                                 89.05
                                                                       19.65
                               102
## [2,]
              112.77
                               100
                                                 89.18
                                                                       21.16
## [3,]
              120.84
                               101
                                                 93.49
                                                                       21.52
## [4,]
               86.54
                               100
                                                 92.28
                                                                       21.13
## [5,]
              114.29
                               107
                                                 88.04
                                                                       21.80
                                                                       20.10
              115.06
                               101
                                                 93.61
## [6,]
class(my_data)
```

[1] "matrix" "array"

Let us now create some numbers that we will use as genotype ids. We have 100 observations and that makes it 100 genotypes. We will name the genotypes from "001" to "100". Let us use the paste() function to create these ids.

```
a1 <- paste("00", 1:9, sep = "")
a2 <- paste0("0", 10:99)
genotypes <- c(a1, a2, 100)
genotypes
```

```
[1] "001" "002" "003" "004" "005" "006" "007" "008" "009" "010" "011" "012"
##
##
    [13] "013" "014" "015" "016" "017" "018" "019" "020" "021" "022" "023" "024"
    [25] "025" "026" "027" "028" "029" "030" "031" "032" "033" "034" "035" "036"
##
    [37] "037" "038" "039" "040" "041" "042" "043" "044" "045" "046" "047" "048"
##
    [49] "049" "050" "051" "052" "053" "054" "055" "056" "057" "058" "059" "060"
##
    [61] "061" "062" "063" "064" "065" "066" "067" "068" "069" "070" "071" "072"
##
    [73] "073" "074" "075" "076" "077" "078" "079" "080" "081" "082" "083" "084"
##
    [85] "085" "086" "087" "088" "089" "090" "091" "092" "093" "094" "095" "096"
##
    [97] "097" "098" "099" "100"
##
```

Let us add this vector to our my_data object.

```
newdat <- cbind(genotypes, my_data)
head(newdat)</pre>
```

```
##
        genotypes plant_height flowering_50 spikelet_fertility
   [1,] "001"
                   "97.93"
                                 "102"
                                               "89.05"
   [2,] "002"
                   "112.77"
##
                                 "100"
                                               "89.18"
   [3,] "003"
                   "120.84"
                                 "101"
                                               "93.49"
   [4,] "004"
                                 "100"
                                               "92.28"
                   "86.54"
## [5,] "005"
                   "114.29"
                                 "107"
                                               "88.04"
## [6,] "006"
                   "115.06"
                                 "101"
                                               "93.61"
##
        thousand_seed_weight
## [1,] "19.65"
## [2,] "21.16"
## [3,] "21.52"
## [4,] "21.13"
## [5,] "21.8"
## [6,] "20.1"
```

We have seen this problem before; the entire data getting converted into a character class. To overcome this problem we use the data.frame() function.

```
field_data <- data.frame(genotypes, my_data)
head(field_data)</pre>
```

```
##
     genotypes plant_height flowering_50 spikelet_fertility thousand_seed_weight
## 1
                       97.93
           001
                                        102
                                                          89.05
                                                                                  19.65
## 2
            002
                       112.77
                                        100
                                                           89.18
                                                                                  21.16
## 3
           003
                       120.84
                                        101
                                                           93.49
                                                                                  21.52
## 4
                                        100
                                                           92.28
                                                                                  21.13
            004
                       86.54
## 5
                                        107
                                                          88.04
                                                                                 21.80
            005
                       114.29
            006
                       115.06
                                        101
                                                           93.61
                                                                                  20.10
```

This output is more like it. Let us check the class of the df object.

class(field data)

```
## [1] "data.frame"
```

It is a dataframe. A dataframe, unlike a matrix, can hold vectors of different classes. Using the most important function in R, str(), we get a glimpse of what the field object contains.

```
str(field_data)
```

```
## 'data.frame': 100 obs. of 5 variables:
## $ genotypes : chr "001" "002" "003" "004" ...
## $ plant_height : num 97.9 112.8 120.8 86.5 114.3 ...
```

```
## $ flowering_50 : num 102 100 101 100 107 101 92 95 93 78 ...
## $ spikelet_fertility : num 89 89.2 93.5 92.3 88 ...
## $ thousand seed weight: num 19.6 21.2 21.5 21.1 21.8 ...
```

The "field" object is a data frame with 100 observations and 4 variables. Except for a, which is a factor, plant_height, flowering_50, and spikelet_fertility are numeric. Remember that 'a' is a vector containing the genotype ids. Therefore, "a" is recognised as a factor here.

Writing data to file

```
write.csv(field_data, "field_data.csv", quote = F, row.names = F)
```

Plotting with ggplot2

Introduction

ggplot2 is "A system for 'declaratively' creating graphics, based on The Grammar of Graphics. You provide the data, tell 'ggplot2' how to map variables to aesthetics, what graphical primitives to use, and it takes care of the details."

The Grammar of Graphics, written by Leland Wilkinson, presents a theoretical foundation for producing quantitative graphics. (https://www.amazon.com/Grammar-Graphics-Statistics-Computing/dp/0387245448/ref=as li ss tl)

This book is the foundation for ggplot2 created by Hadley Wickham.

We are going to use the faithful and iris data sets to explore ggplot2. The data sets are part of the R package.

The following are important while using ggplot2.

- 1) Data
- Most important aspect
- Data representation holds the key to what can be done with the data
- 2) Mapping
- Aesthetic mapping Variables in the data linked to graphical properties
- Facet mapping Variables are linked to panels
- 3) Geometries
- geom *()
- 4) Themes
- 5) Scale

Installing and loading the package.

```
# install.packages("ggplot2")
library(ggplot2)
```

The faithful data set contains information on the eruption pattern of the Old Faithful geyser in Yellowstone National Park.

```
# Look at the data
str(faithful)

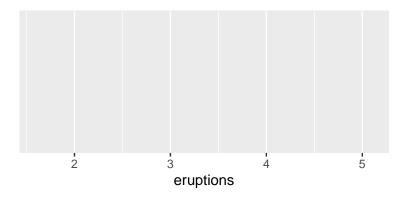
## 'data.frame': 272 obs. of 2 variables:
## $ eruptions: num 3.6 1.8 3.33 2.28 4.53 ...
## $ waiting : num 79 54 74 62 85 55 88 85 51 85 ...
```

head(faithful) ## eruptions waiting ## 1 3.600 79 ## 2 1.800 54 ## 3 3.333 74 ## 4 2.283 62 ## 5 4.533 85 ## 6 2.883 55 1) Data

```
#data("faithful")
ggplot(data = faithful)
```

2) Mapping

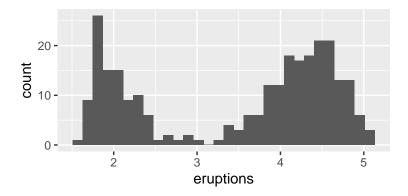
```
# Adding the mapping
ggplot(data = faithful, mapping = aes(x = eruptions))
```



3) Geometry

```
# Basic histogram
ggplot(data = faithful, mapping = aes(x = eruptions)) +
  geom_histogram()
```

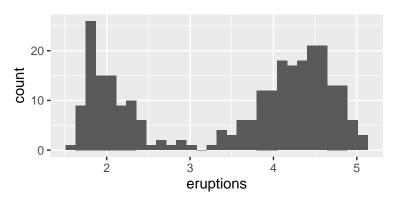
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



The data and the aesthetics can be specified within the layer as well.

```
ggplot() +
  geom_histogram(data = faithful, aes(x = eruptions))
```

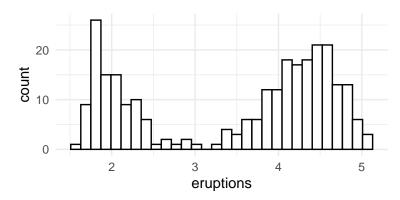
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



4) Theme

```
ggplot(faithful, aes(x = eruptions)) +
  geom_histogram(colour = "black", fill = "white") +
  # theme_classic()
  # theme_bw()
  theme_minimal()
```

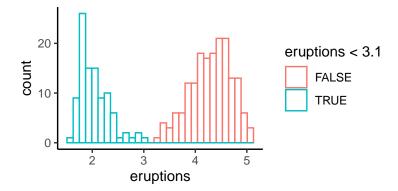
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



Colour based on mapping

```
ggplot(faithful, aes(x = eruptions)) +
  geom_histogram(aes(colour = eruptions < 3.1), fill = "white") +
  theme_classic()</pre>
```

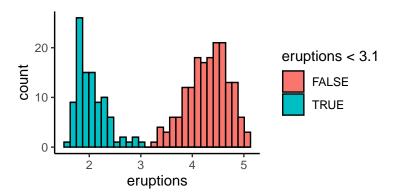
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



Fill based on mapping

```
ggplot(faithful, aes(x = eruptions)) +
  geom_histogram(aes(fill = eruptions < 3.1), colour = "black") +
  theme_classic()</pre>
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



Let us now use the iris data set for further exploration of the ggplot2 package.

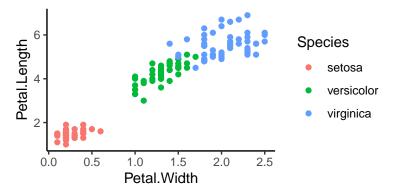
str(iris)

```
## 'data.frame': 150 obs. of 5 variables:
## $ Sepal.Length: num 5.1 4.9 4.7 4.6 5 5.4 4.6 5 4.4 4.9 ...
## $ Sepal.Width : num 3.5 3 3.2 3.1 3.6 3.9 3.4 3.4 2.9 3.1 ...
## $ Petal.Length: num 1.4 1.4 1.3 1.5 1.4 1.7 1.4 1.5 1.4 1.5 ...
## $ Petal.Width : num 0.2 0.2 0.2 0.2 0.2 0.4 0.3 0.2 0.2 0.1 ...
## $ Species : Factor w/ 3 levels "setosa", "versicolor", ..: 1 1 1 1 1 1 1 1 1 1 1 1 ...
head(iris)
```

Sepal.Length Sepal.Width Petal.Length Petal.Width Species ## ## 1 5.1 3.5 1.4 setosa 4.9 3.0 ## 2 1.4 0.2 setosa ## 3 4.7 3.2 1.3 0.2 setosa 0.2 setosa ## 4 4.6 3.1 1.5

```
## 5    5.0    3.6    1.4    0.2 setosa
## 6    5.4    3.9    1.7    0.4 setosa

# Basic scatterplot
ggplot(data = iris, mapping = aes(x = Petal.Width, y = Petal.Length)) +
    geom_point(aes(colour = Species))+
    theme_classic()
```

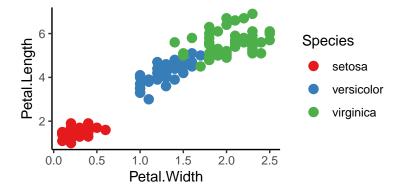


5) Scale Adding a different colour scheme

RColorBrewer::display.brewer.all()



```
ggplot(data = iris, mapping = aes(x = Petal.Width, y = Petal.Length)) +
geom_point(aes(colour = Species), size = 3) +
theme_classic() +
scale_colour_brewer(palette = "Set1")
```

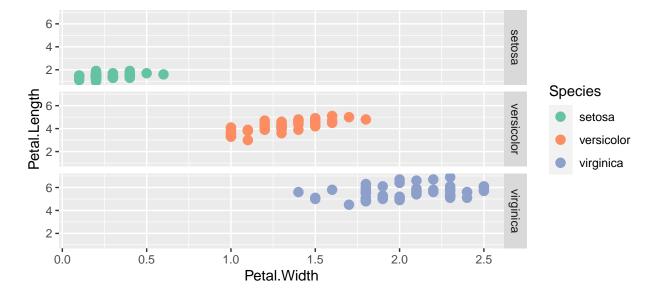


6) Facets

```
ggplot(data = iris, mapping = aes(x = Petal.Width, y = Petal.Length)) +
geom_point(aes(colour = Species), size = 3) +
facet_wrap(~ Species) +
scale_colour_brewer(palette = "Set2")
```



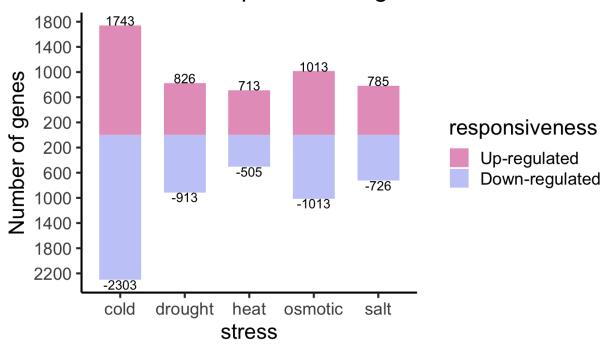
```
ggplot(data = iris, mapping = aes(x = Petal.Width, y = Petal.Length)) +
geom_point(aes(colour = Species), size = 3) +
facet_grid(Species ~ .) +
scale_colour_brewer(palette = "Set2")
```



Creating a mirror plot

In this section we will see how to make the plot shown below.

Differential Expression of genes



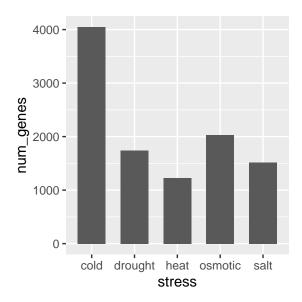
Reading data in:

```
gene_nums_mirror <- read.csv( "up_down_gene_numbers.csv" )
gene_nums_mirror
## stress responsiveness num genes</pre>
```

##		stress	responsiveness	num_genes
##	1	cold	up	1743
##	2	cold	down	2303
##	3	${\tt osmotic}$	up	1013
##	4	${\tt osmotic}$	down	1013
##	5	heat	up	713
##	6	heat	down	505
##	7	salt	up	785
##	8	salt	down	726
##	9	drought	up	826
##	10	drought	down	913

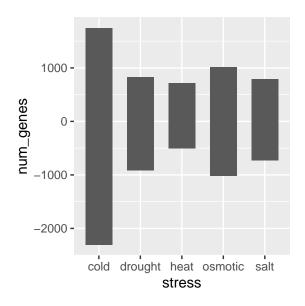
This data set has three columns and 10 rows. It is about differentially expressed genes under different stress conditions. So, let us start plotting with ggplot2.

```
#install.packages("ggplot2")
library( ggplot2 )
ggplot(data = gene_nums_mirror, aes(x = stress, y = num_genes)) +
    # geom_bar(stat = "identity")
geom_bar( stat = "identity", position = "stack", width = 0.65 )
```



```
gene_nums_mirror$num_genes[gene_nums_mirror$responsiveness=="down"] <-
    -gene_nums_mirror$num_genes[gene_nums_mirror$responsiveness=="down"]
gene_nums_mirror</pre>
```

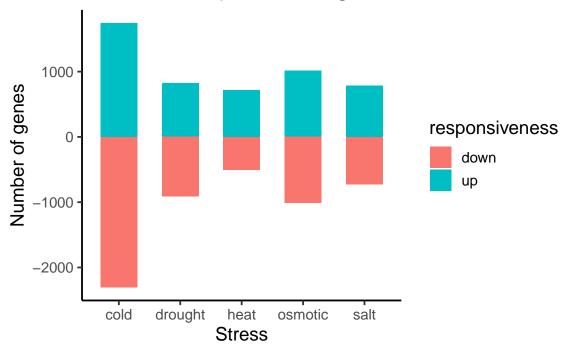
```
##
       stress responsiveness num_genes
## 1
         cold
                                   1743
                           up
## 2
         cold
                                  -2303
                         down
      osmotic
## 3
                                   1013
                           up
      osmotic
                                  -1013
## 4
                         down
## 5
                                    713
         heat
                           up
                                   -505
## 6
         heat
                         down
## 7
         salt
                                    785
                           up
## 8
         salt
                                   -726
                         down
                                    826
## 9 drought
                           up
                                   -913
## 10 drought
                         down
ggplot(data = gene_nums_mirror, aes(x = stress, y = num_genes)) +
  geom_bar(stat = "identity", width = 0.65)
```



Adding colour to the bars, a theme, x- and y-axis labels, and a main title.

```
ggplot(data = gene_nums_mirror, aes(x = stress, y = num_genes, fill = responsiveness)) +
  geom_bar(stat = "identity", width = 0.6) +
  theme_classic(base_size = 14) +
  ylab("Number of genes") +
  xlab("Stress") +
  ggtitle("Differential expression of genes")
```

Differential expression of genes

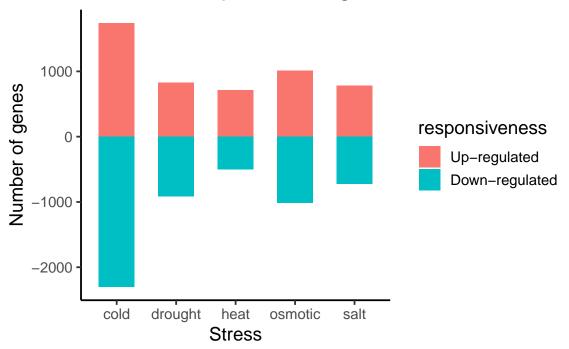


The figure legend has "down" on the top and "up" below. To change that default behavoiur, we need to re-order the levels: "up" first followed by "down", in the data set.

```
# Define the new level order
new_levels <- c("up", "down", setdiff(levels(gene_nums_mirror$responsiveness), c("up", "down")))
# Modify the "responsiveness" column
gene_nums_mirror$responsiveness <- factor(gene_nums_mirror$responsiveness, levels = new_levels)</pre>
```

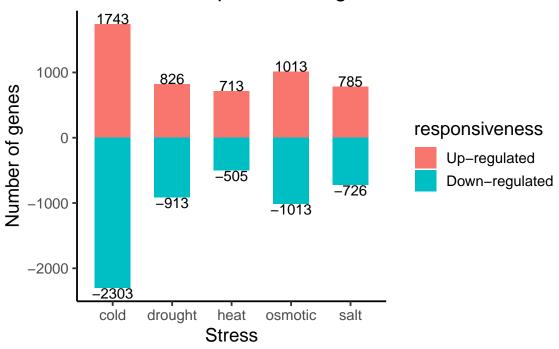
Apart from changing the levels, we provide labels for the legend in the code below.

Differential Expression of genes

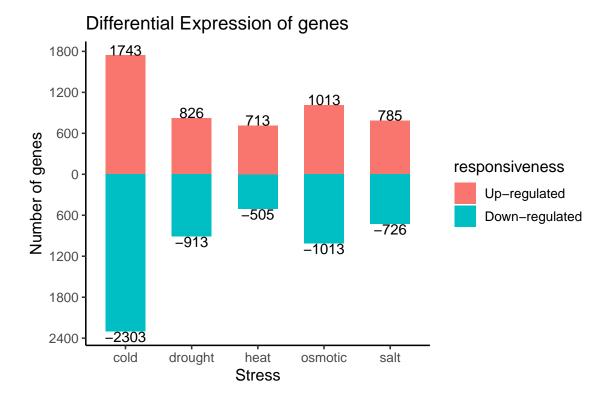


Adding the number of genes on the bars.

Differential Expression of genes

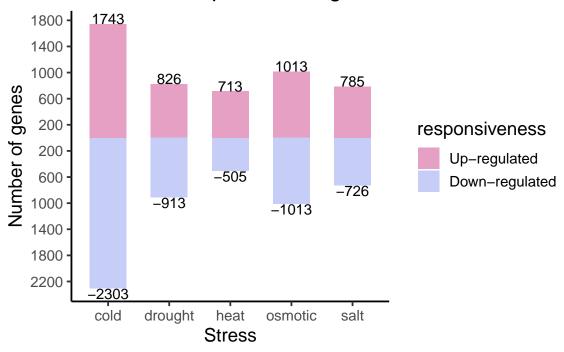


Setting the y-axis ticks:



The final code snippet with a different colour scheme.

Differential Expression of genes



sessionInfo()

```
## R version 4.2.1 (2022-06-23)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
           /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## BLAS:
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
## attached base packages:
  [1] stats
                 graphics grDevices utils
                                                datasets methods
                                                                     base
##
## other attached packages:
## [1] wesanderson_0.3.6 ggplot2_3.4.1
##
## loaded via a namespace (and not attached):
   [1] rstudioapi_0.14
                           knitr 1.42
                                               magrittr_2.0.3
                                                                   tidyselect_1.2.0
##
   [5] munsell_0.5.0
                           colorspace_2.1-0
                                               R6_2.5.1
                                                                   rlang_1.0.6
   [9] fastmap 1.1.0
                           fansi 1.0.4
                                               highr 0.10
                                                                   dplyr 1.1.0
##
## [13] tools 4.2.1
                           grid_4.2.1
                                               gtable_0.3.1
                                                                   xfun 0.38
## [17] utf8_1.2.3
                           cli_3.6.0
                                               withr_2.5.0
                                                                   htmltools_0.5.4
## [21] yaml_2.3.7
                                               tibble_3.1.8
                                                                   lifecycle_1.0.3
                           digest_0.6.31
## [25] RColorBrewer_1.1-3 farver_2.1.1
                                               vctrs_0.5.2
                                                                   glue_1.6.2
## [29] evaluate_0.20
                                               labeling_0.4.2
                           rmarkdown_2.21
                                                                   compiler_4.2.1
## [33] pillar_1.8.1
                           generics_0.1.3
                                               scales_1.2.1
                                                                   pkgconfig_2.0.3
```