

Análisis transcriptómicos de la expresión génica

Máster Universitario en Bioinformática

Sesión 11

The logo consists of the lowercase letters "viu" in white, sans-serif font, centered within a solid orange rounded circle.

viu

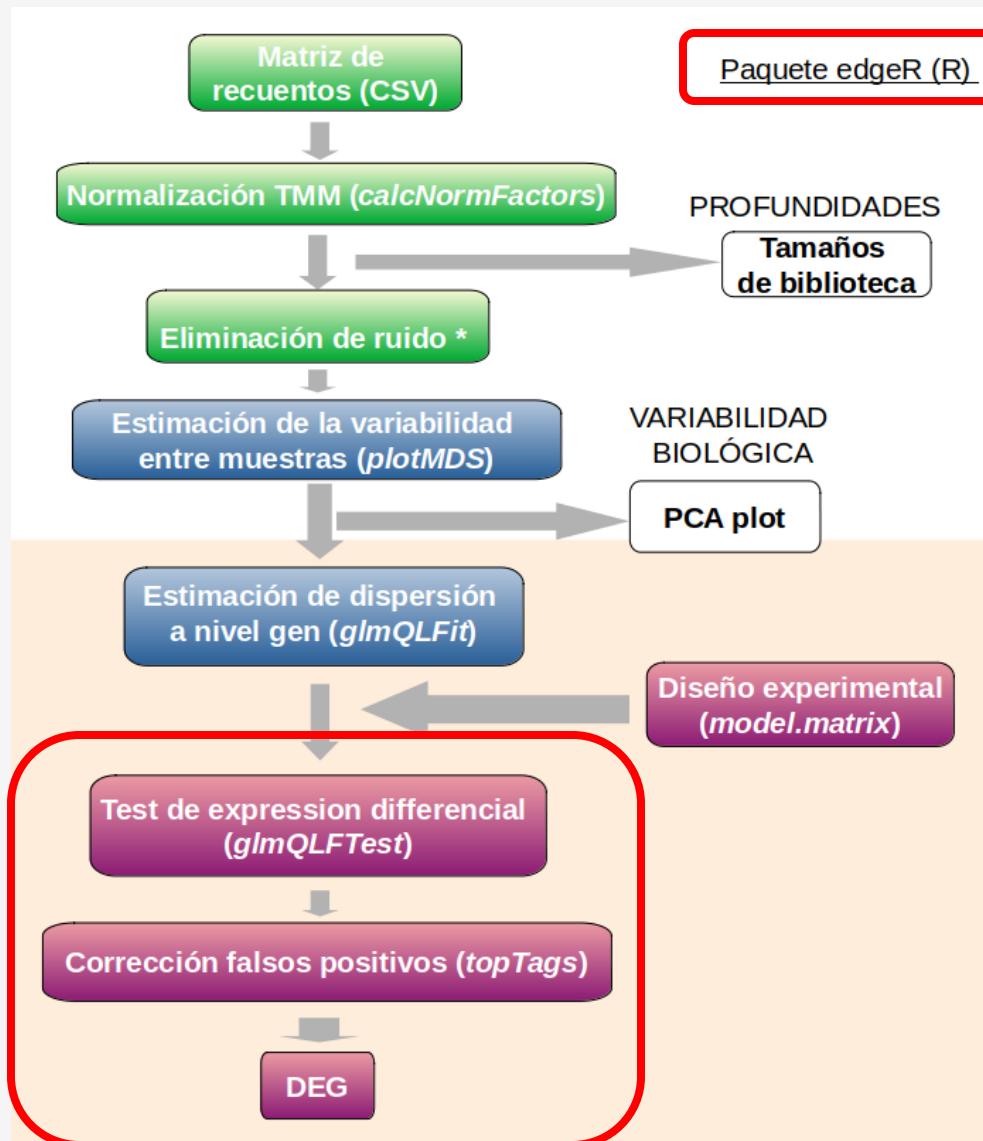
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De:
 Planeta Formación y Universidades



Bloque IV: Análisis estadístico de la diferencia de expresión



Objetivos

1

Extracción del conjunto de genes diferencialmente expresados entre los grupos lactantes vs embarazados en células basales.

- Función **glmQLFTest**
- Función **topTags**

2

Filtrar el conjunto de genes diferencialmente detectados aplicando criterios de significancia estadística.

- **LogFold-change**
- **FDR (p-value ajustado)**

PRACTIQUEMOS



Genes
diferencialmente
expresados

Estimación de los DEG

```
# Libraries
library(ggplot2)
library(edgeR)
library(statmod)

# 1 Testing
B.LvsP <- makeContrasts(basal.lactate-basal.pregnant, levels = design)
B.LvsP

res <- glmQLFTest(fit,contrast = B.LvsP)
dim(res$table)

res_corrected <- topTags(res, n = Inf)

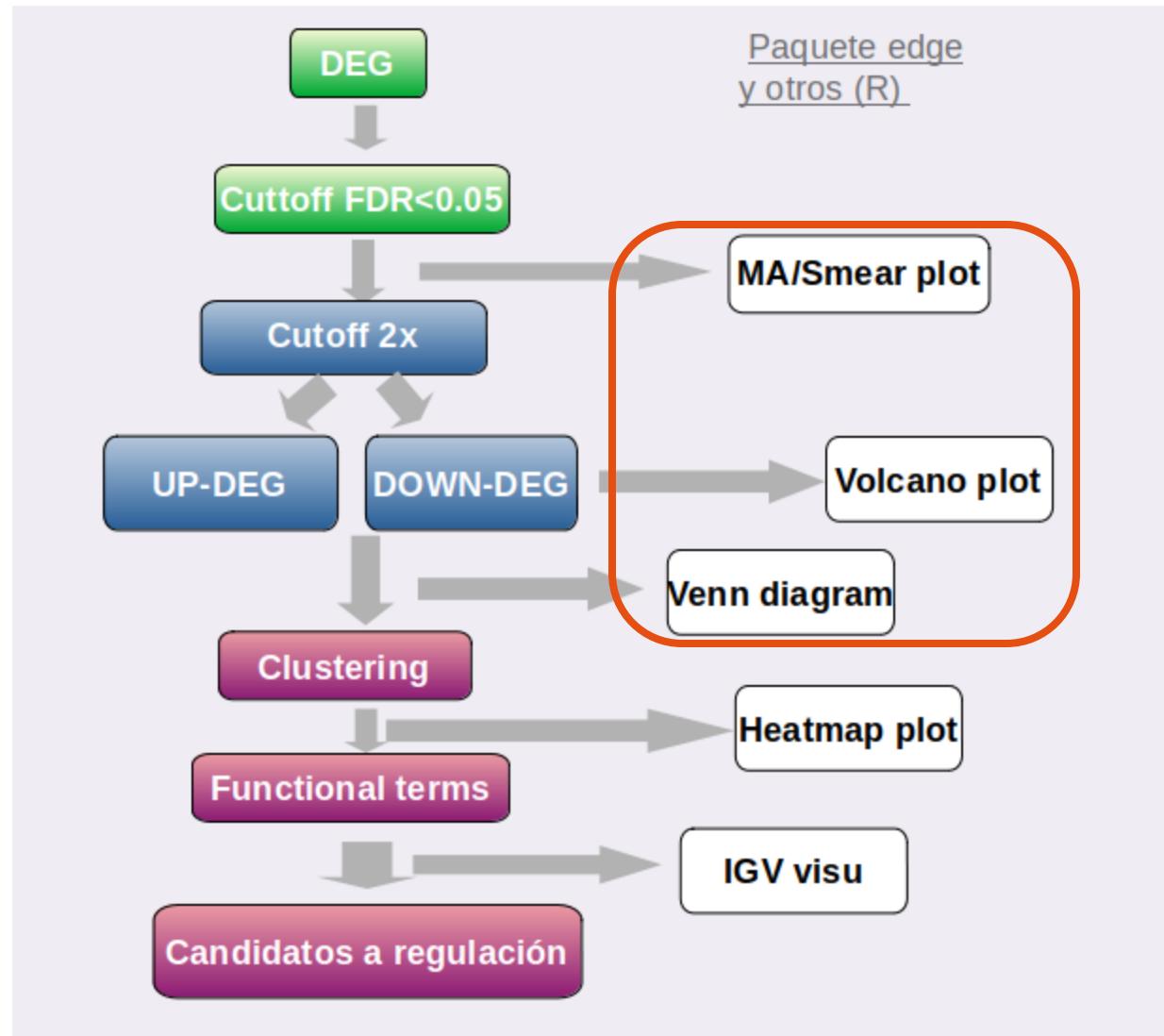
# 2 Filtering
nrow(res_corrected$table[res_corrected$table$FDR <= 0.05,])
is.de <- decideTests(res, adjust.method="BH", p.value=0.05)
head(is.de)
summary(is.de)

nrow(res_corrected$table[res_corrected$table$FDR <= 0.05 & abs(res_corrected$table$logFC)>=1,])
is.de1 <- decideTests(res, adjust.method="BH", p.value=0.05, lfc = 1)
summary(is.de1)
```



Bloque V: Exploración y visualización de resultados

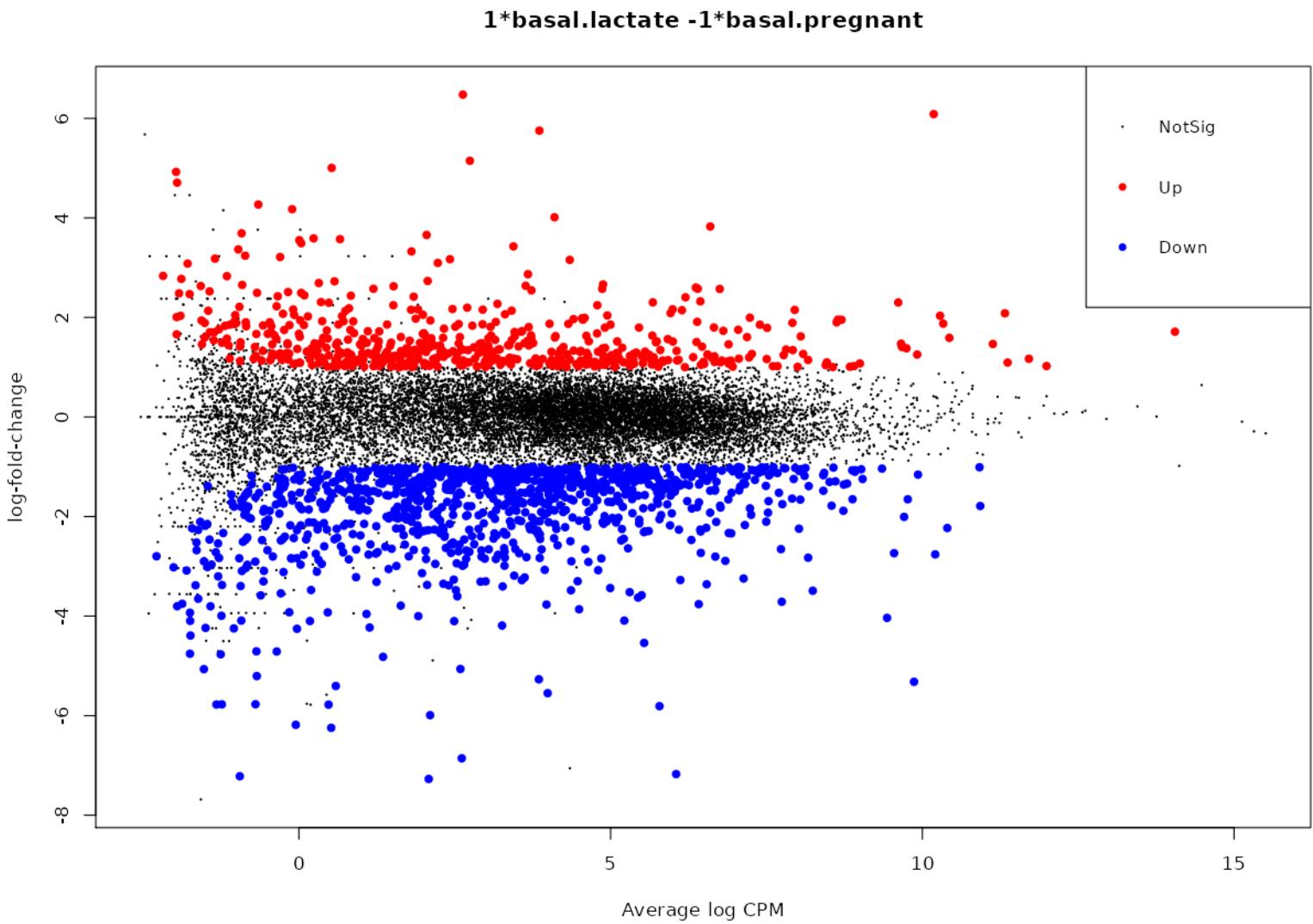
Flujo de trabajo para la exploración y visualización de resultados



1. plotMD



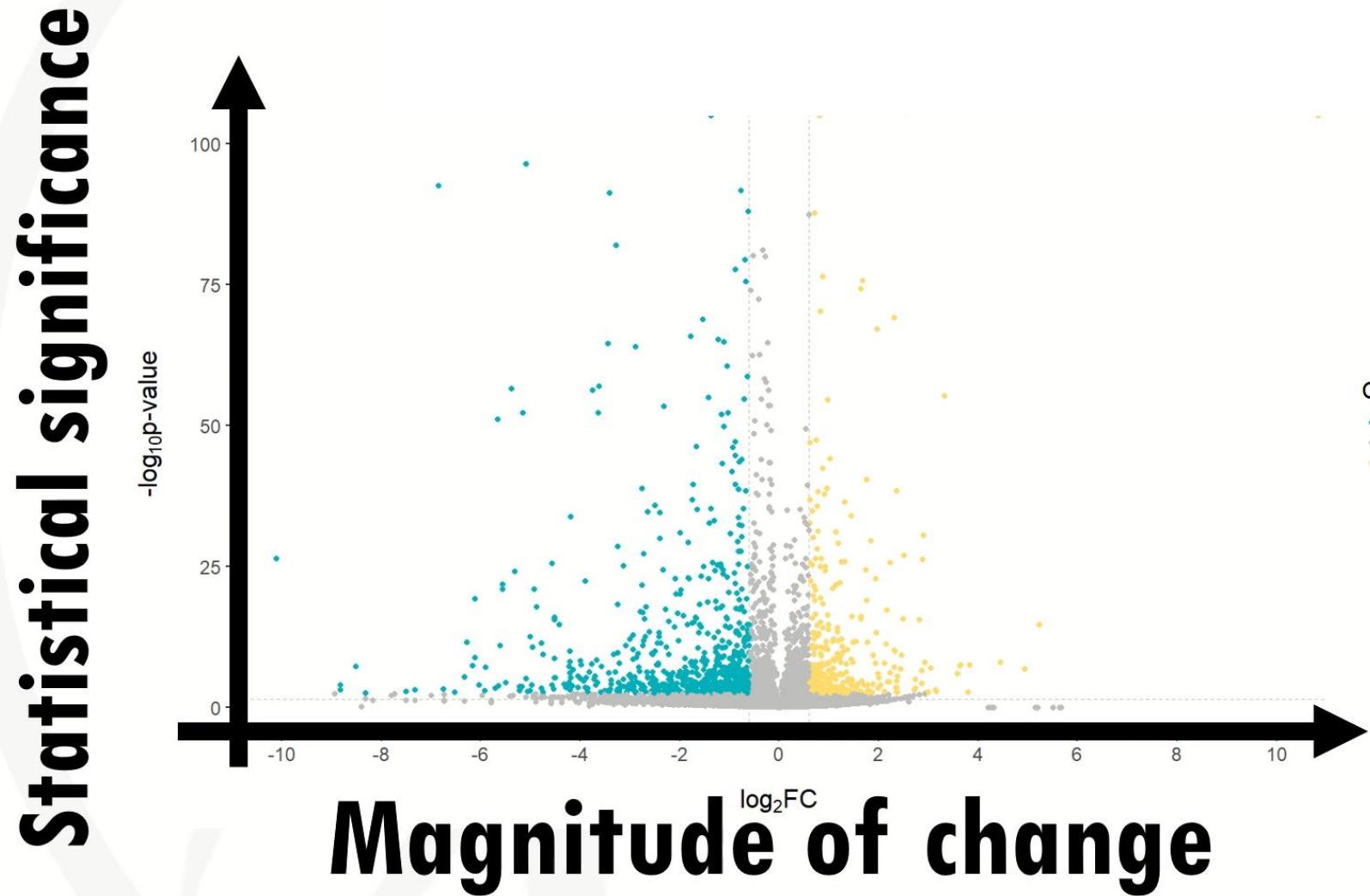
- Gráfico de dispersión
- Relación entre la **expresión** promedio de los genes y su **cambio** de expresión entre dos condiciones
- **plotMD(res, status=is.de)**



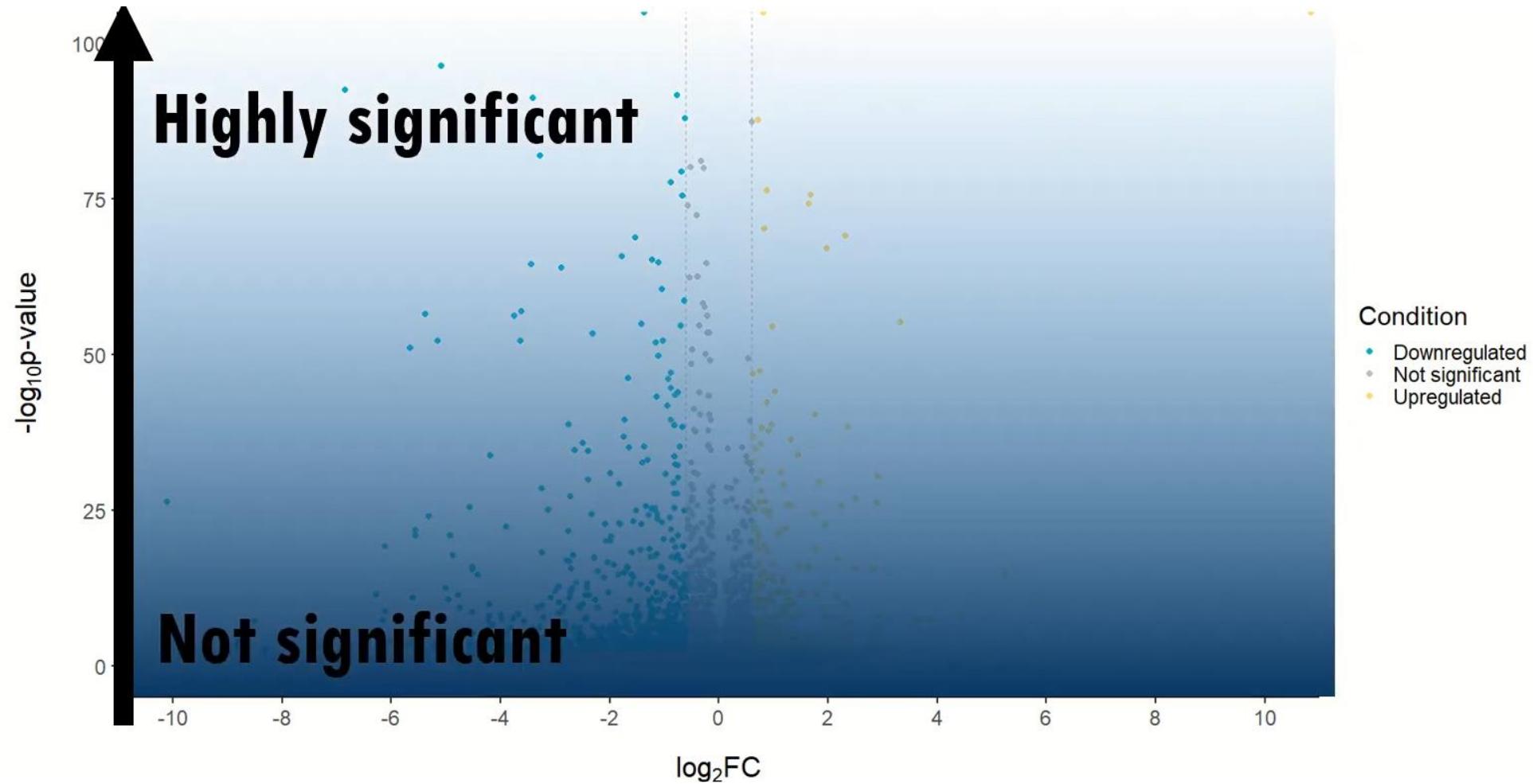
PRACTIQUEMOS



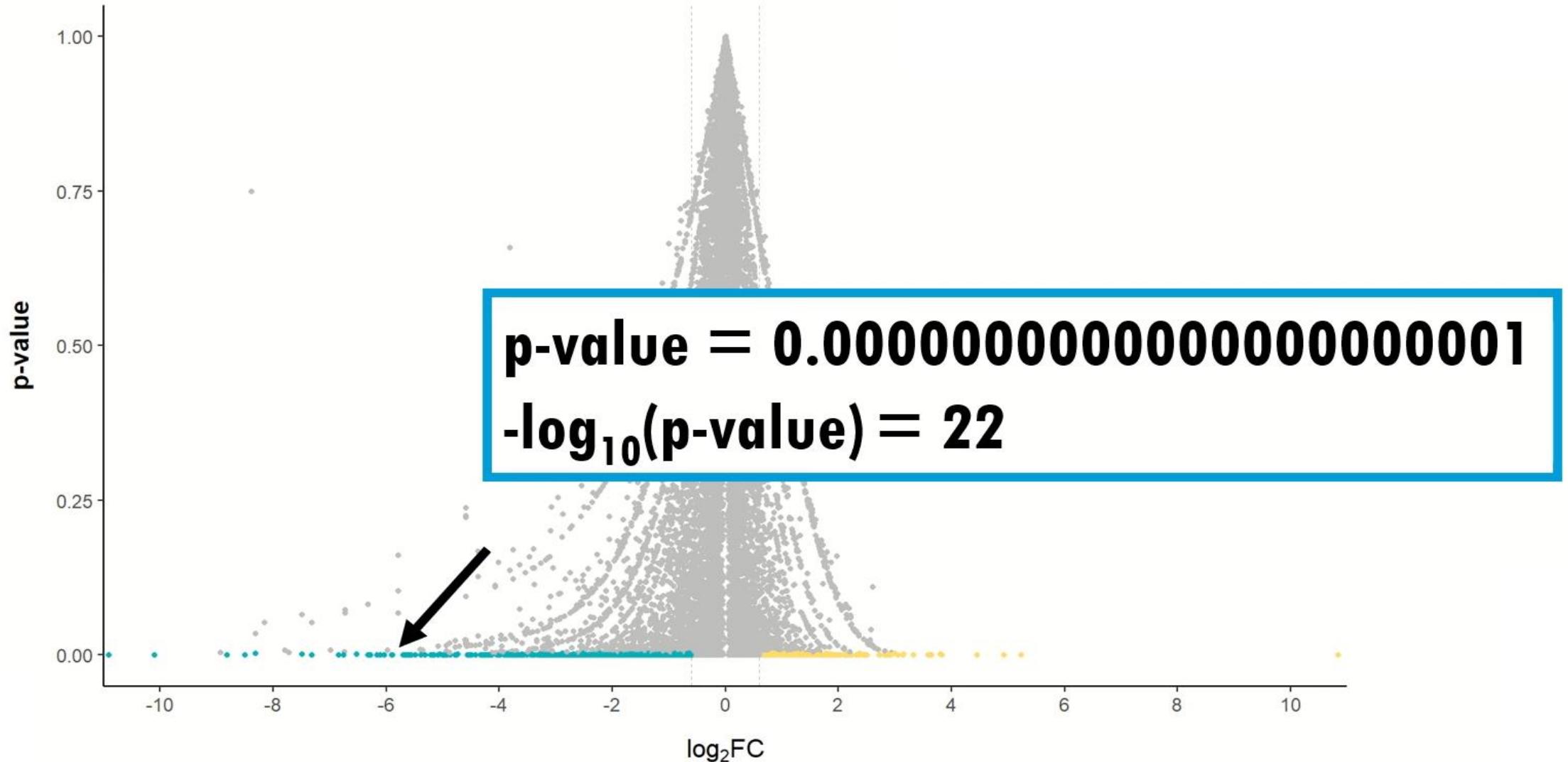
2. Gráfico de Volcán



2. Gráfico de Volcán: significancia estadística

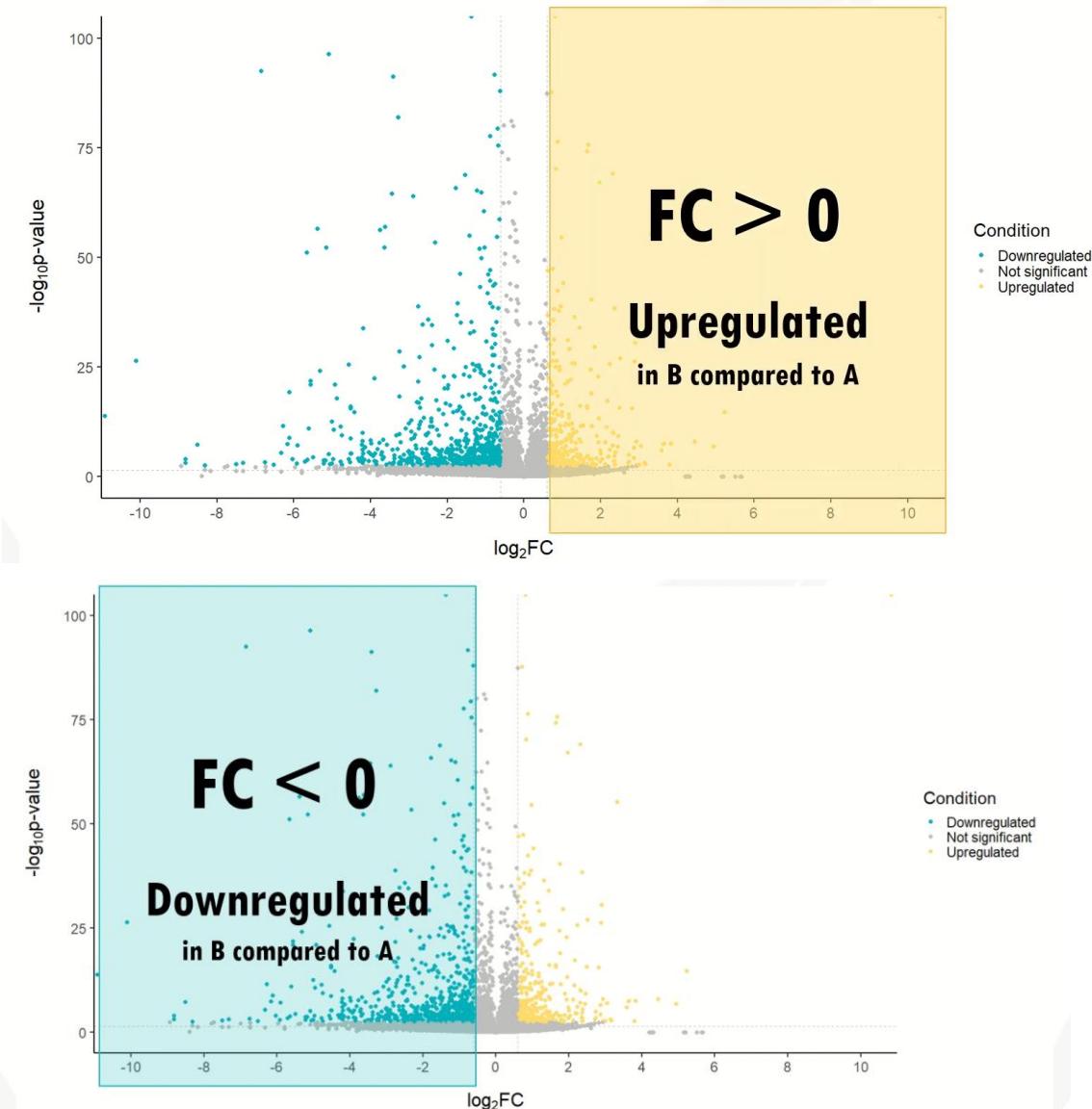
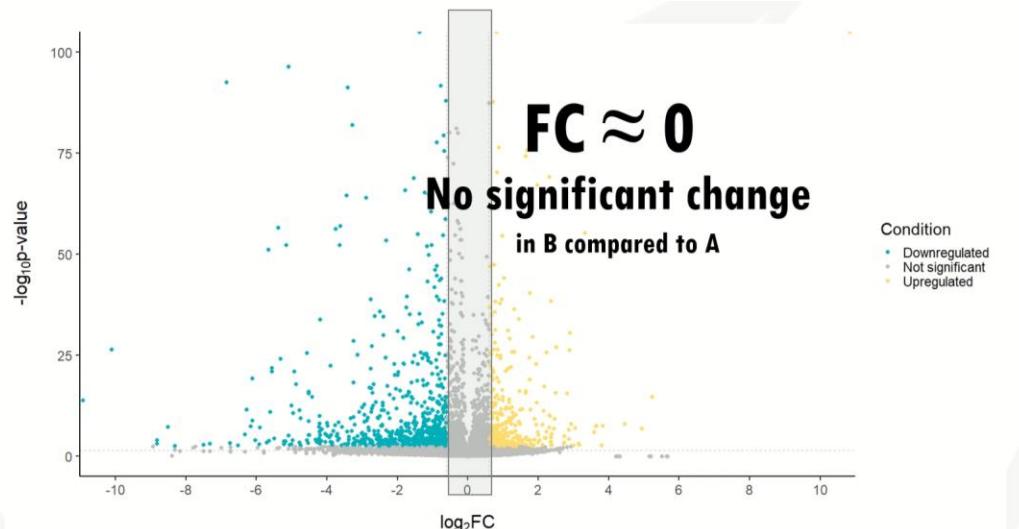


2. Gráfico de Volcán: significancia estadística



2. Gráfico de Volcán: magnitud del cambio

$$FC = \frac{\text{expression of gene X in B}}{\text{expression of gene X in A}}$$



2. Gráfico de Volcán: magnitud del cambio

$$FC = \frac{\text{expression of gene X in B}}{\text{expression of gene X in A}}$$

	Gene	Expression_CondA	Expression_CondB	FC	logFC
1	Gene1	100	200	2.0	1.0
2	Gene2	150	50	0.33	-1.585

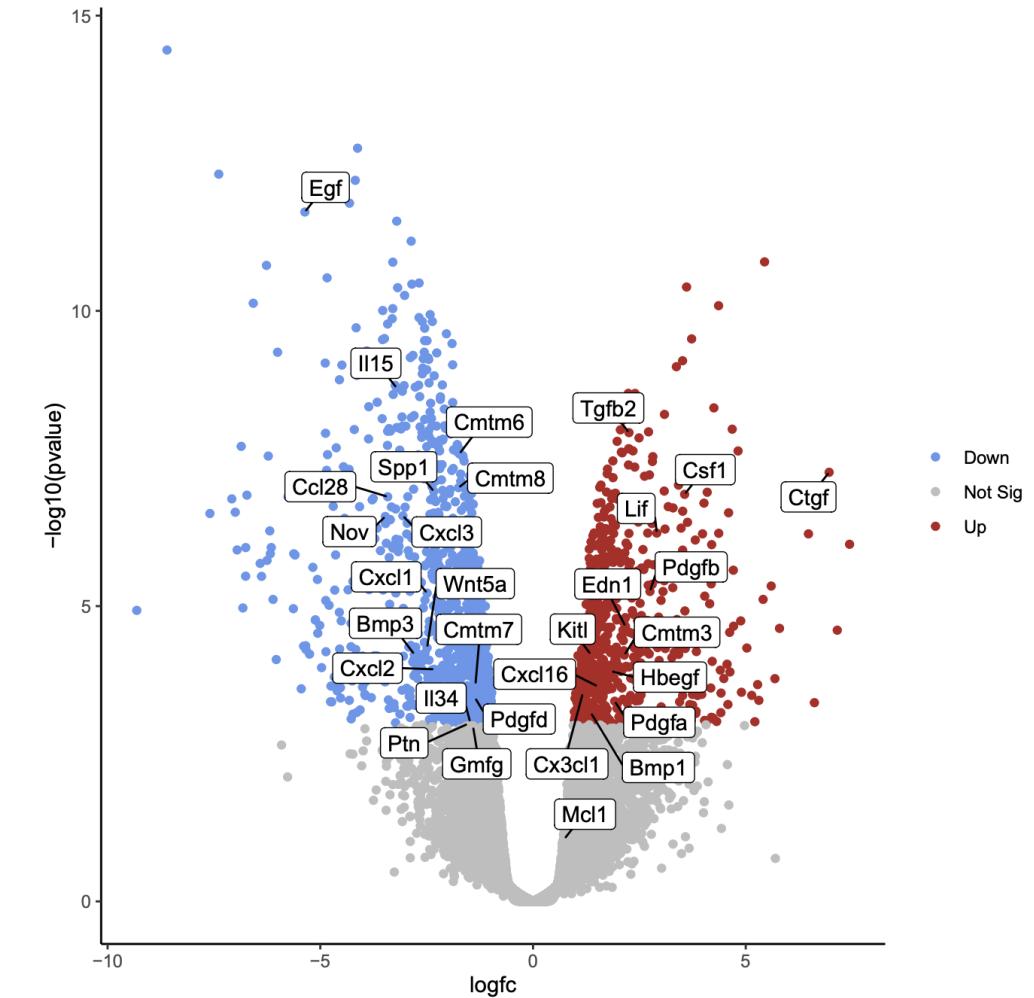
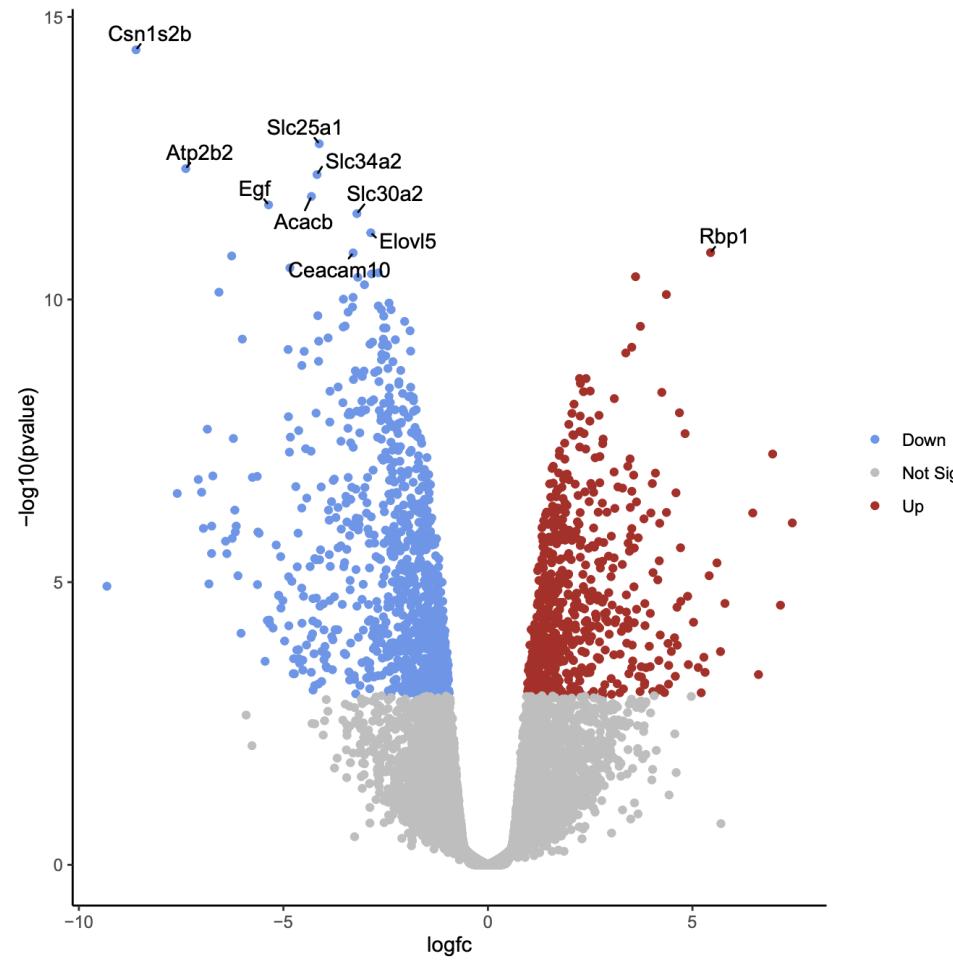
El valor de 1 estará indicando que la expresión se está duplicando, mientras que si el valor es -1.585 indica que su expresión se está reduciendo un tercio de la condición B a la A.

1 Gene1 FC = $\frac{200}{100} = 2$

Gene2 FC = $\frac{50}{150} \approx 0.33$

- 2
- Gene1 logFC = $\log_2(2) = 1$
 - Gene2 logFC = $\log_2(0.33) \approx -1.585$

2. Gráfico de Volcán: significancia y nivel de cambio



ggplot (paquete *ggplot2*), *VolcanoPlot* (paquete *limma*), o *enhancedVolcano*,

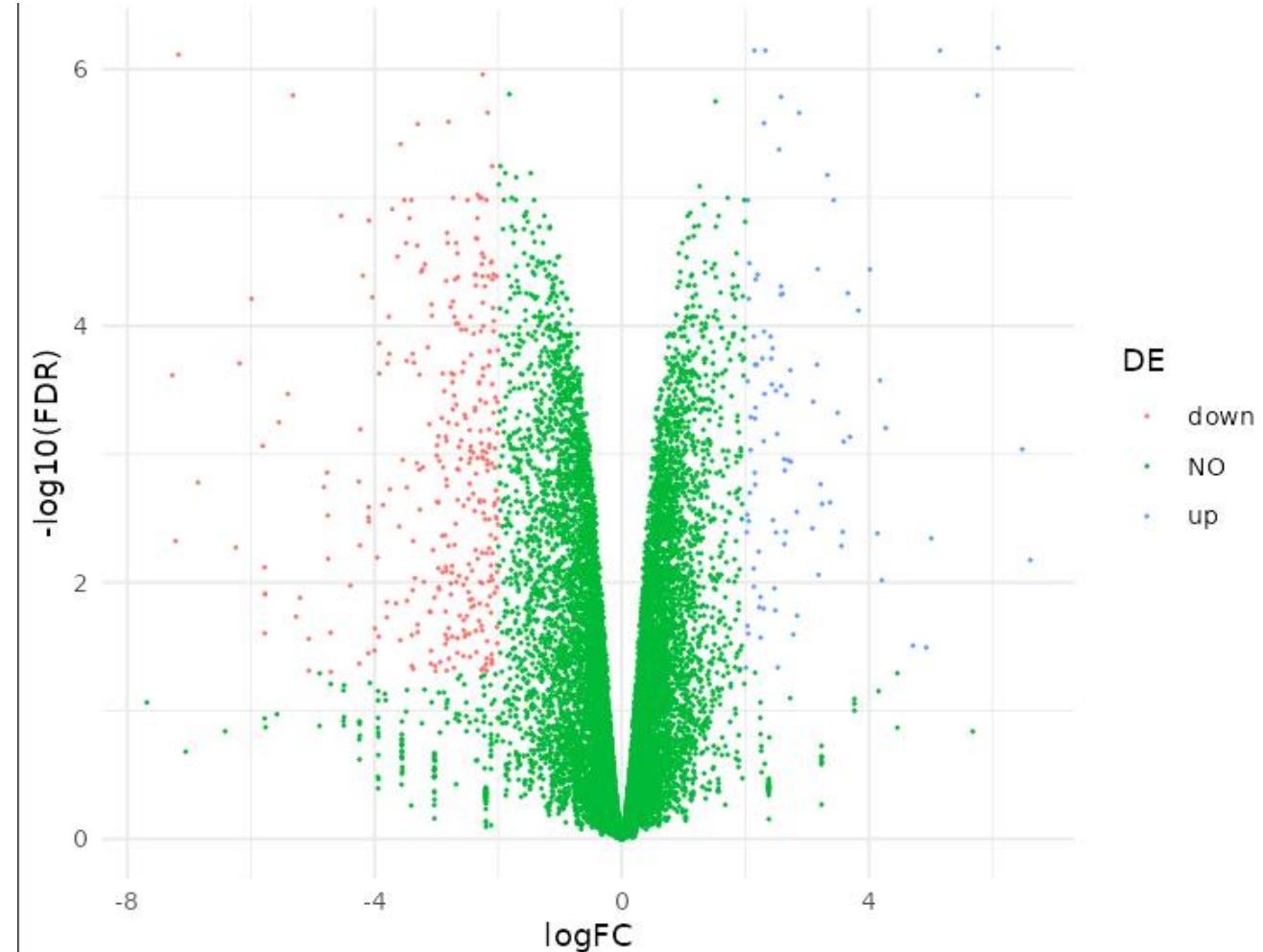
PRACTIQUEMOS



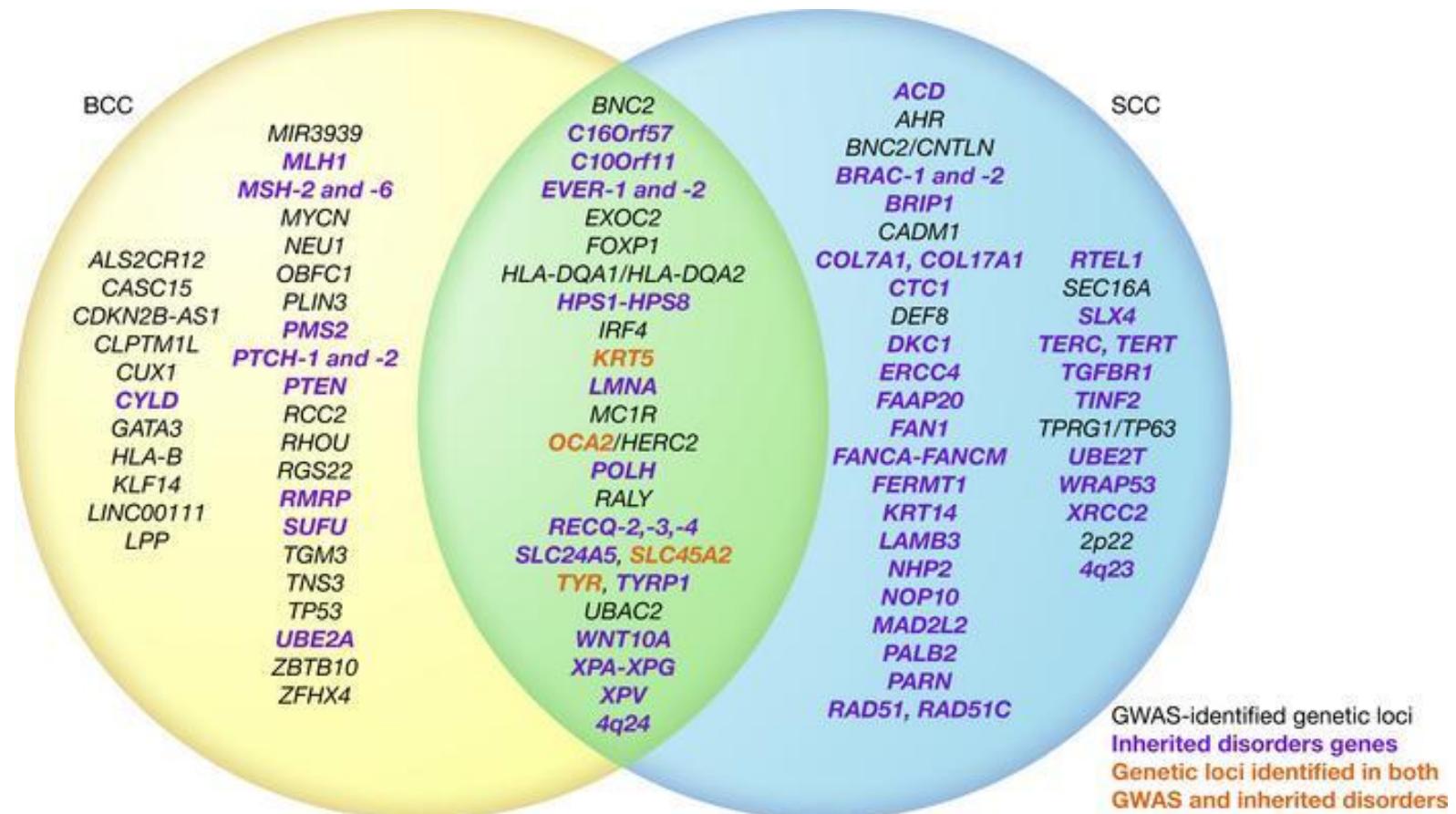
Gráfico de
Volcán

Gráfico de Volcán: significancia y nivel de cambio

```
data <- res_corrected$table  
  
ggplot(data, aes(x=logFC, y = -log10(FDR))) +  
  geom_point()  
  
data$DE <- "NO"  
  
data$DE[data$logFC > 1 & data$FDR < 0.05] <- "up"  
data$DE[data$logFC < -1 & data$FDR < 0.05] <- "down"  
  
ggplot(data, aes(x=logFC, y = -log10(FDR), col=DE)) +  
  geom_point(size=0.2) + theme_minimal()
```



3. Diagrama de Venn: Comparación de conjuntos de genes



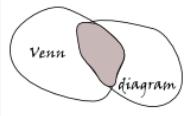
JCI Insight. 2020;5(10):e134783. <https://doi.org/10.1172/jci.insight.134783>.

`library(venn), library(vennDiagramm), library(ggvenn), library("ggVennDiagram")...`

3. Diagrama de Venn: Comparación de conjuntos de genes

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 Calculate and draw custom Venn diagrams

WHAT?
With this tool you can calculate the intersection(s) of list of elements. It will generate a textual output indicating which elements are in each intersection or are unique to a certain list. If the number of lists is lower than 7 it will also produce a graphical output in the form of a venn/Euler diagram. You have the choice between symmetric (default) or non symmetric venn diagrams. Currently you are able to calculate the intersections of at maximum 30 lists.
The graphical output is produced in SVG and PNG format.
Downloading the figure in SVG format will allow you to further customise it with SVG compatible software such as for instance Inkscape (which is freeware).

HOW?
Enter files (in plain text format!) with a list of elements and/or copy-paste lists in the appropriate fields. The lists can contain only a single element on each line, but there is no limit on the number of lines. The elements are processed in a case-sensitive manner (so lowercase and uppercase are seen as two different elements). The input lists will be processed and made non-redundant (= duplicated elements in each list will be removed such that only one remains). You can make extra fields for entering files/lists by clicking the 'Add Another...' button. The style of the graphical output can be specified in the output control section. Choose either symmetric or non-symmetric.
Click 'submit' to start the analysis

Cite?
Unfortunately there is no publication yet describing this tool. In the meantime we would be grateful if you can mention the URL where one can access the tool.

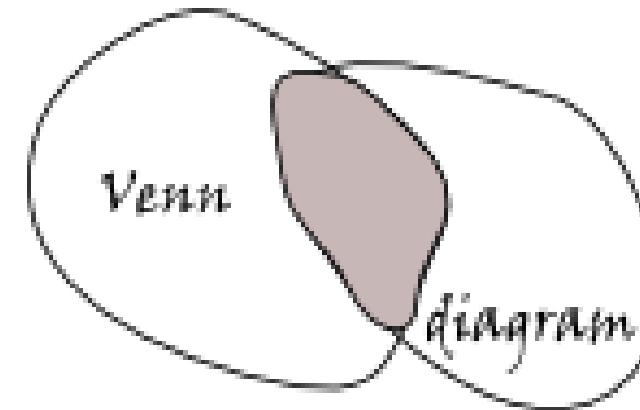
INPUT section

upload files:

file 1: Ninguno archivo selec. Provide name for file (optional):
file 2: Ninguno archivo selec. Provide name for file (optional):
file 3: Ninguno archivo selec. Provide name for file (optional):

upload lists:

list 1: Empty Provide name for list (optional): user_list1
list 2: Empty Provide name for list (optional): user_list2



<https://bioinformatics.psb.ugent.be/webtools/Venn/>

Diagrama de Venn: Comparación de conjuntos de genes

```
## Venn diagram
```

```
gene_1_basal_up <- nombres de genes 1 con un FC positivo y un FDR filtrado
```

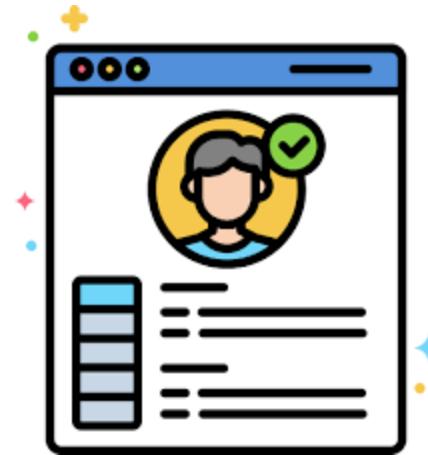
```
gene_1_basal_down <- nombres de genes 1 con un FC negativo y un FDR filtrado
```

```
gene_2_luminal_up <- nombres de genes 2 con un FC positivo y un FDR filtrado
```

```
gene_2_luminal_down <- nombres de genes 2 con un FC negativo y un FDR filtrado
```

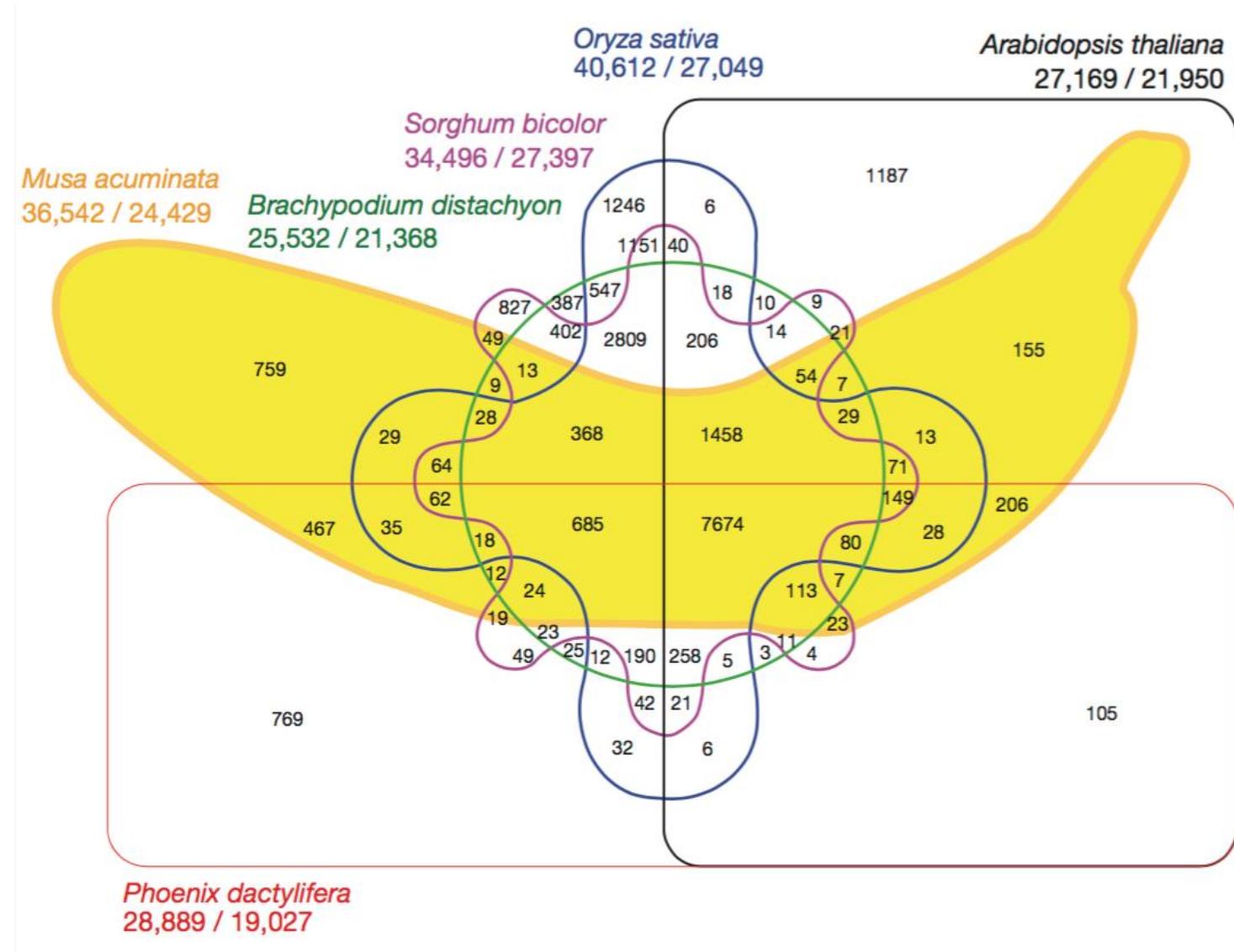
```
library(venn)
```

```
venn(list(gene_1_up , gene_1_down , gene_2_up , gene_2_down))
```

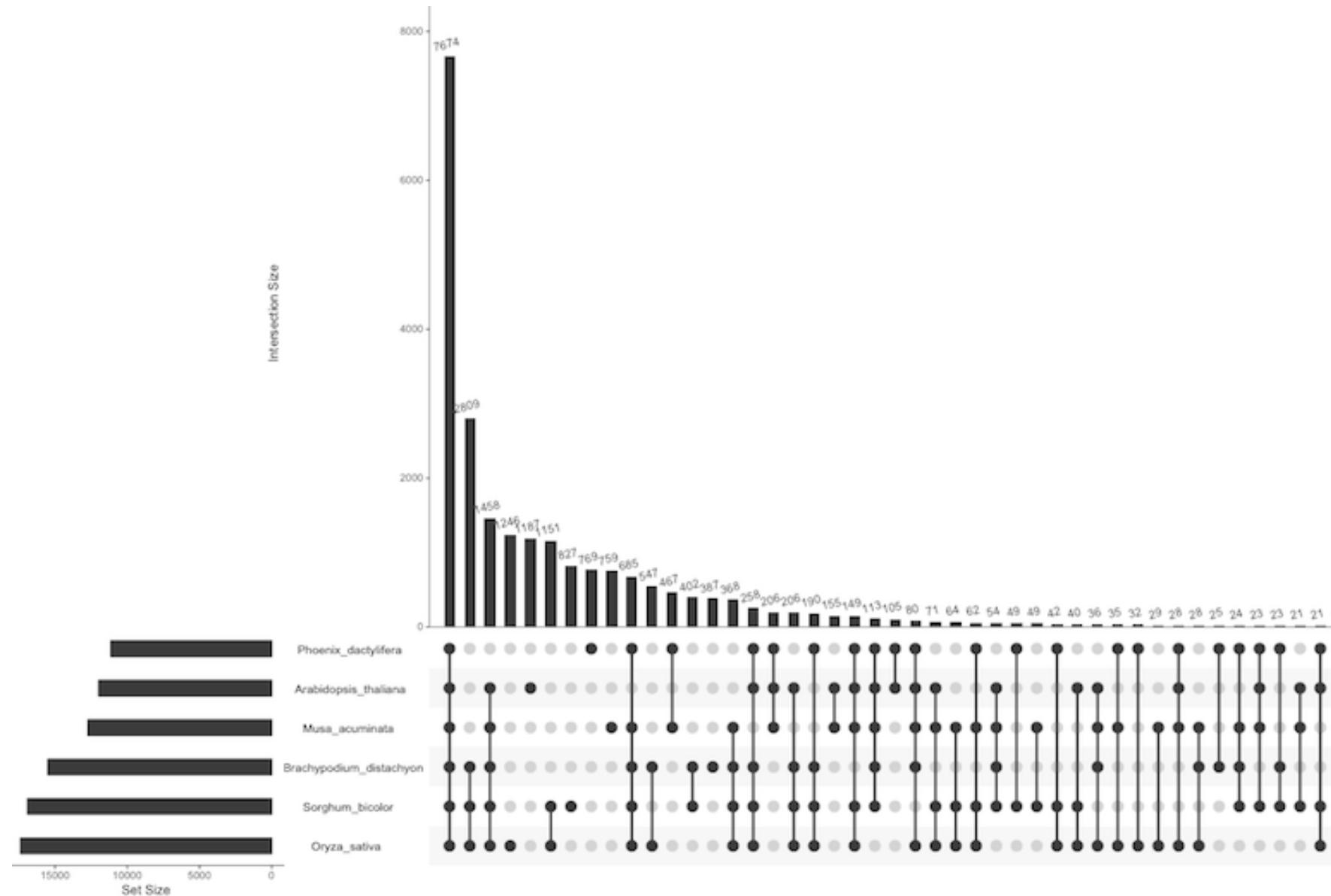


Actividad 2

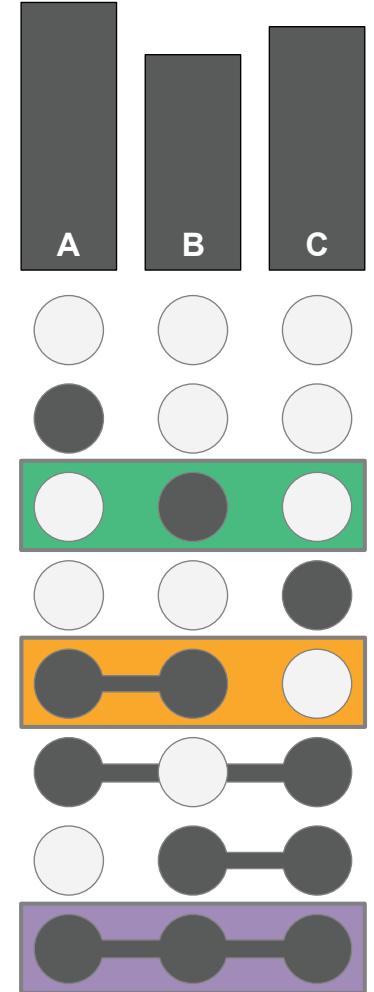
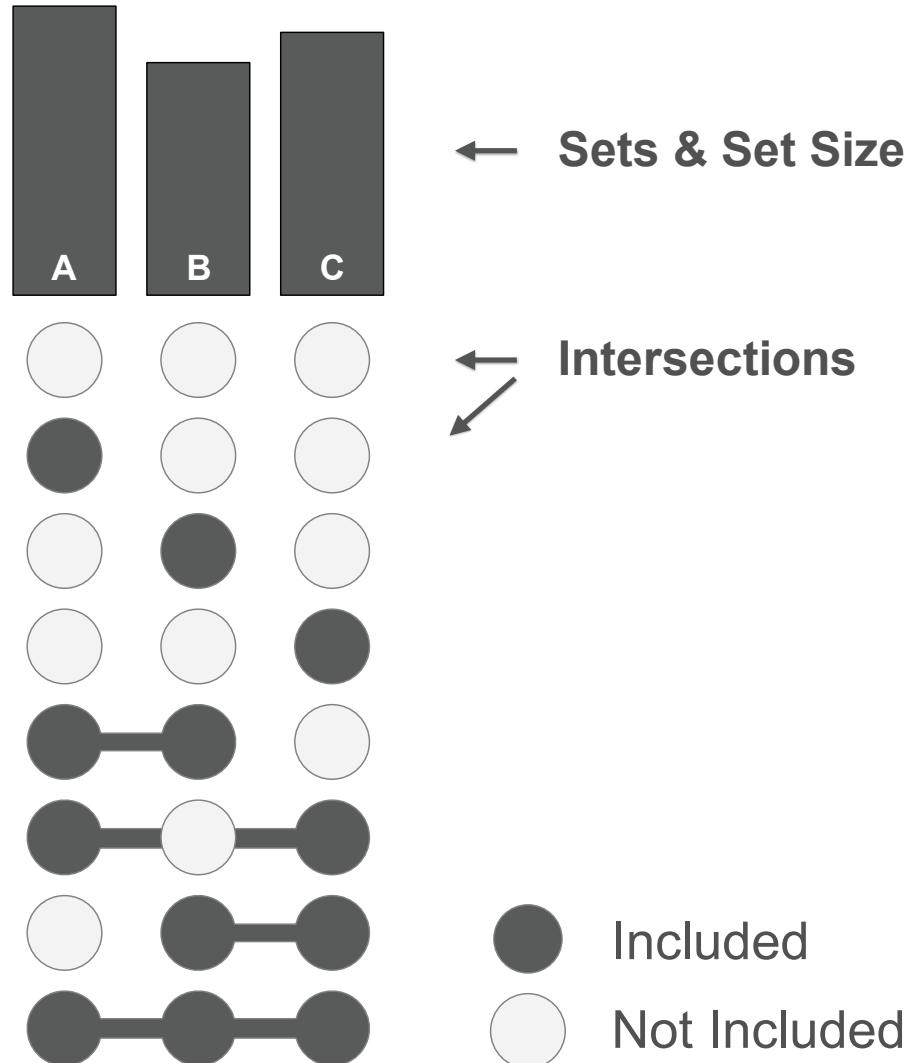
Diagrama de Venn: Problemática



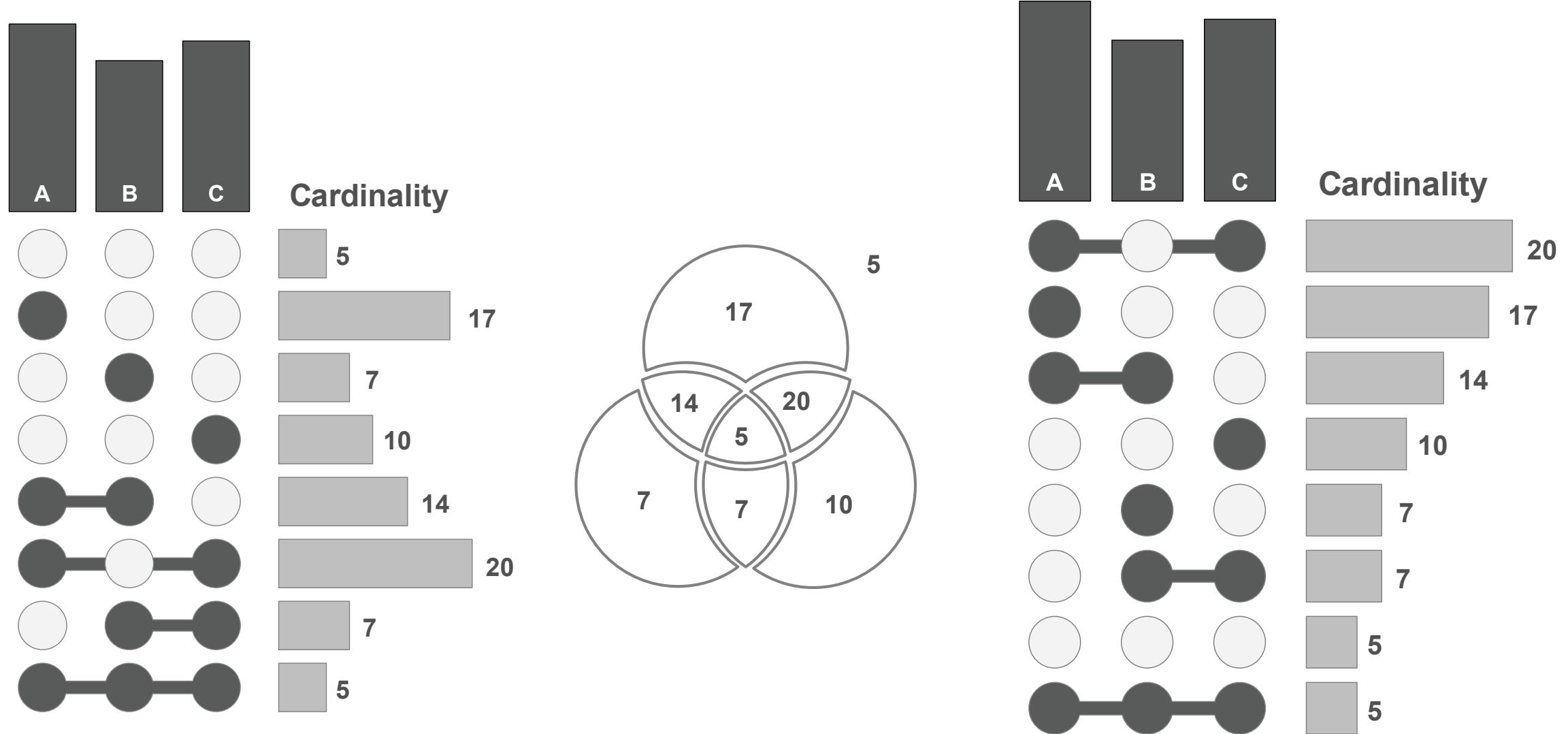
Upset Plot : Comparación de conjuntos de genes



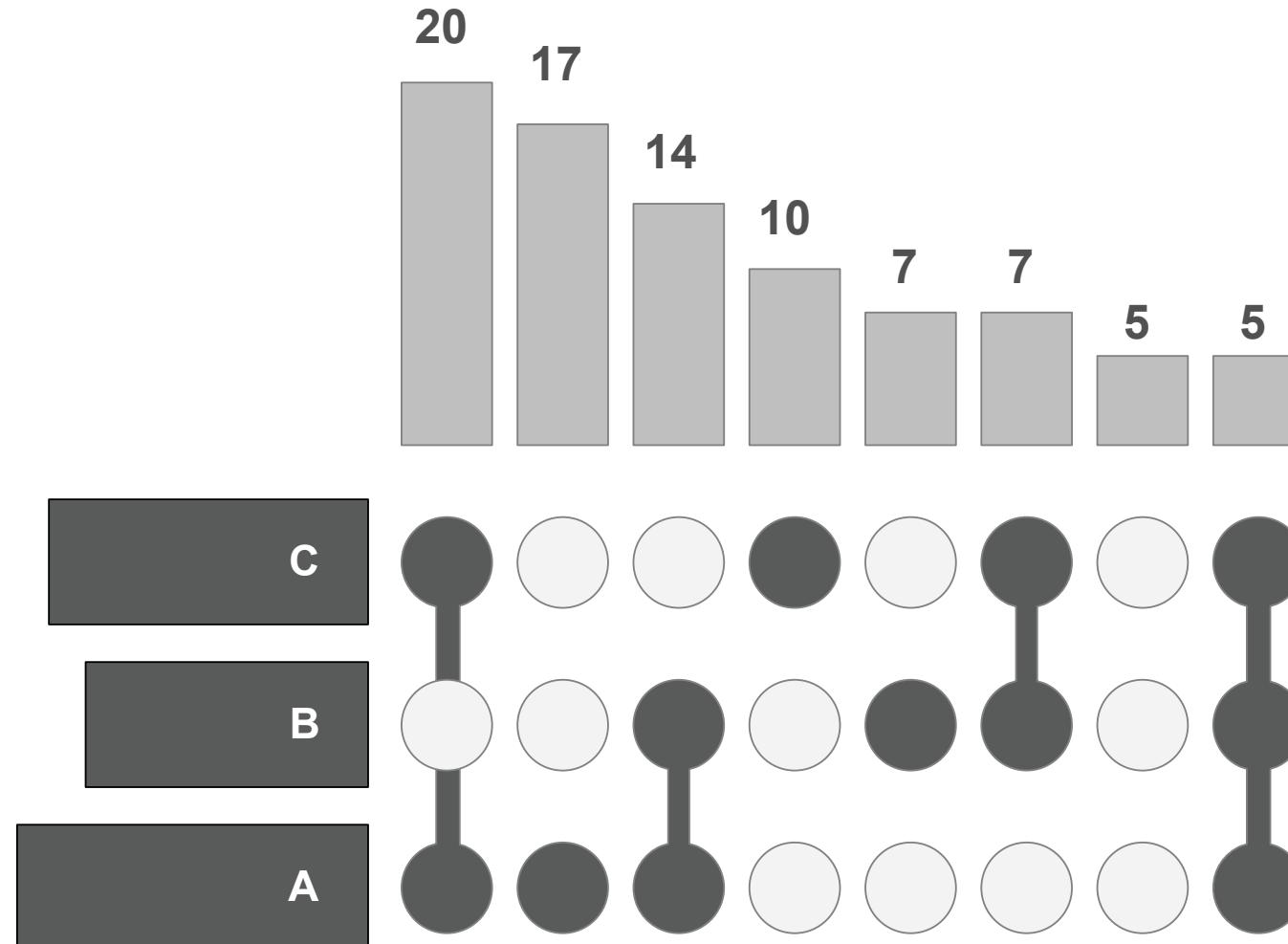
Upset Plot : Comparación de conjuntos de genes



Upset Plot : Comparación de conjuntos de genes



Upset Plot : Comparación de conjuntos de genes



upSetR package



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