Reporte del Análisis de la expresión diferencial de células endoteliales dérmicas de pacientes diabéticos tipo 2

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Introducción

La prevalencia de diabetes mellitus tipo 2 (DT2) aumenta constantemente y se han identificado diversos factores de riesgo como la obesidad, el envejecimiento, los estados nutricionales y la inactividad física, además de predisposiciones genéticas en diferentes poblaciones. Las consecuencias de la alta glucosa en sangre incluyen vasos sanguíneos dañados, lo que lleva a arteriosclerosis y microangiopatías diabéticas crónicas.

Para obtener las muestras y realizar el análisis con los datos obtenidos se aislaron células endoteliales dérmicas de pacientes diabéticos (Pat) e individuos de control (Ctrl) y se realizaron RNASeq para comparar genes expresados diferencialmente. Diseño general: Se tomaron muestras quirúrgicas de piel humana de pacientes diabéticos tipo 2 y pacientes no diabéticos.**Cita** https://jhubiostatistics.shinyapps.io/recount3-study-explorer/

Este proyecto tiene como objetivo analizar la expresión diferencial de genes en células endoteliales de personas diabéticas y sanas, utilizando datos de RNA-seq obtenidos a través del paquete Recount3 de bioconductor. Utilizamos herramientas bioinformáticas para identificar genes diferencialmente expresados y visualizamos los resultados con gráficos y heatmaps. Con la finalidad de tener una propuesta biológica de los cambios observados en la expresión de los genes.

Datos

- Número de identificación de los datos en Recount3 : **SRP095512**
- GEO accession a los datos : **GSE92724**

Cargar los paquetes necesarios para todo el análisis

```
library("recount3")
```

```
## Cargando paquete requerido: SummarizedExperiment
```

```
## Cargando paquete requerido: MatrixGenerics
```

```
## Cargando paquete requerido: matrixStats
```

```
##
## Adjuntando el paquete: 'MatrixGenerics'
```

```
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars

## Cargando paquete requerido: GenomicRanges

## Cargando paquete requerido: stats4

## Cargando paquete requerido: BiocGenerics

##
## Adjuntando el paquete: 'BiocGenerics'

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

## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, 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, saveRDS, setdiff,
##     table, tapply, union, unique, unsplit, which.max, which.min

## Cargando paquete requerido: S4Vectors

##
## Adjuntando el paquete: 'S4Vectors'

## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
```

```
## Cargando paquete requerido: IRanges

##
## Adjuntando el paquete: 'IRanges'

## The following object is masked from 'package:grDevices':
##
##     windows

## Cargando paquete requerido: GenomeInfoDb

## Cargando paquete requerido: Biobase

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

##
## Adjuntando el paquete: 'Biobase'

## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians

## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

```
library("edgeR") # BiocManager::install("edgeR", update = FALSE)
```

```
## Cargando paquete requerido: limma

##
## Adjuntando el paquete: 'limma'

## The following object is masked from 'package:BiocGenerics':
##
##     plotMA
```

```
library("ggplot2")
library("limma")
library("pheatmap")
library("RColorBrewer")
```

Obtención y análisis de los datos

```r
## Revisemos todos los proyectos con datos de humano en recount3
human_projects <- available_projects()
```

```
## 2025-02-08 19:48:34.67445 caching file sra.recount_project.MD.gz.

## 2025-02-08 19:48:36.366116 caching file gtex.recount_project.MD.gz.

## 2025-02-08 19:48:37.799615 caching file tcga.recount_project.MD.gz.
```

```r
## Identificador de mi proyecto de interes:SRP095512
##Obtencion del proyecto de interes
proj_info <- subset(
  human_projects,
  project == "SRP095512" & project_type == "data_sources"
)
```

```r
## create_rse Crea un objeto de tipo RangedSummarizedExperiment (RSE)
## con la información a nivel de genes
rse_gene_SRP095512 <- create_rse(proj_info)
```

```
## 2025-02-08 19:48:55.017177 downloading and reading the metadata.

## 2025-02-08 19:48:56.904536 caching file sra.sra.SRP095512.MD.gz.

## 2025-02-08 19:48:58.483073 caching file sra.recount_project.SRP095512.MD.gz.

## 2025-02-08 19:48:59.582339 caching file sra.recount_qc.SRP095512.MD.gz.

## 2025-02-08 19:49:00.694474 caching file sra.recount_seq_qc.SRP095512.MD.gz.

## 2025-02-08 19:49:02.374732 caching file sra.recount_pred.SRP095512.MD.gz.

## 2025-02-08 19:49:02.771871 downloading and reading the feature information.

## 2025-02-08 19:49:03.885973 caching file human.gene_sums.G026.gtf.gz.

## 2025-02-08 19:49:06.526395 downloading and reading the counts: 10 samples across 63856 features.

## 2025-02-08 19:49:07.474551 caching file sra.gene_sums.SRP095512.G026.gz.

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8456-AF82' to a wide string

## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8456-AF82' to a wide string
```

```
## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8456-AF82' to a wide string

## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

## 2025-02-08 19:49:10.069973 constructing the RangedSummarizedExperiment (rse) object.
```

```
## Explorar el objeto RSE
rse_gene_SRP095512
```

```
## class: RangedSummarizedExperiment
## dim: 63856 10
## metadata(8): time_created recount3_version ... annotation recount3_url
## assays(1): raw_counts
## rownames(63856): ENSG00000278704.1 ENSG00000277400.1 ...
##   ENSG00000182484.15_PAR_Y ENSG00000227159.8_PAR_Y
## rowData names(10): source type ... havana_gene tag
## colnames(10): SRR5125028 SRR5125029 ... SRR5125036 SRR5125037
## colData names(175): rail_id external_id ...
##   recount_pred.curated.cell_line BigWigURL
```

```
## Convirtamos las cuentas por nucleotido a cuentas por lectura
## usando compute_read_counts().
assay(rse_gene_SRP095512, "counts") <- compute_read_counts(rse_gene_SRP095512)

## Visualizar la informacion de los atributos del objeto "rse_gene_SRP095512"

rse_gene_SRP095512 <- expand_sra_attributes(rse_gene_SRP095512)
colData(rse_gene_SRP095512)[
  ,
  grepl("^sra_attribute", colnames(colData(rse_gene_SRP095512)))
]
```

```
## DataFrame with 10 rows and 4 columns
##            sra_attribute.cell_type sra_attribute.disease_state
##                        <character>                 <character>
## SRR5125028        endothelial cell             Healthy control
## SRR5125029        endothelial cell            Diabetic Patient
## SRR5125030        endothelial cell            Diabetic Patient
## SRR5125031        endothelial cell            Diabetic Patient
## SRR5125032        endothelial cell            Diabetic Patient
## SRR5125033        endothelial cell             Healthy control
## SRR5125034        endothelial cell             Healthy control
## SRR5125035        endothelial cell             Healthy control
## SRR5125036        endothelial cell             Healthy control
## SRR5125037        endothelial cell             Healthy control
##            sra_attribute.gender sra_attribute.source_name
##                     <character>               <character>
## SRR5125028               female    dermal blood endothe..
## SRR5125029                 male    dermal blood endothe..
```

```
## SRR5125030               female    dermal blood endothe..
## SRR5125031               female    dermal blood endothe..
## SRR5125032                 male    dermal blood endothe..
## SRR5125033               female    dermal blood endothe..
## SRR5125034               female    dermal blood endothe..
## SRR5125035               female    dermal blood endothe..
## SRR5125036               female    dermal blood endothe..
## SRR5125037               female    dermal blood endothe..
```

```
## Formato correcto en el que debemos tener la informacion
## Pasar de character a numeric o factor
rse_gene_SRP095512$sra_attribute.disease_state <- factor(tolower(rse_gene_SRP095512$sra_attribute.disea

rse_gene_SRP095512$sra_attribute.gender <- factor(rse_gene_SRP095512$sra_attribute.gender)

## Resumen de las variables de interés
summary(as.data.frame(colData(rse_gene_SRP095512)[
  ,
  grepl("^sra_attribute.[gender|disease_state]", colnames(colData(rse_gene_SRP095512)))
]))
```

```
##    sra_attribute.disease_state sra_attribute.gender sra_attribute.source_name
##  diabetic patient:4            female:8             Length:10
##  healthy control :6            male  :2             Class :character
##                                                     Mode  :character
```

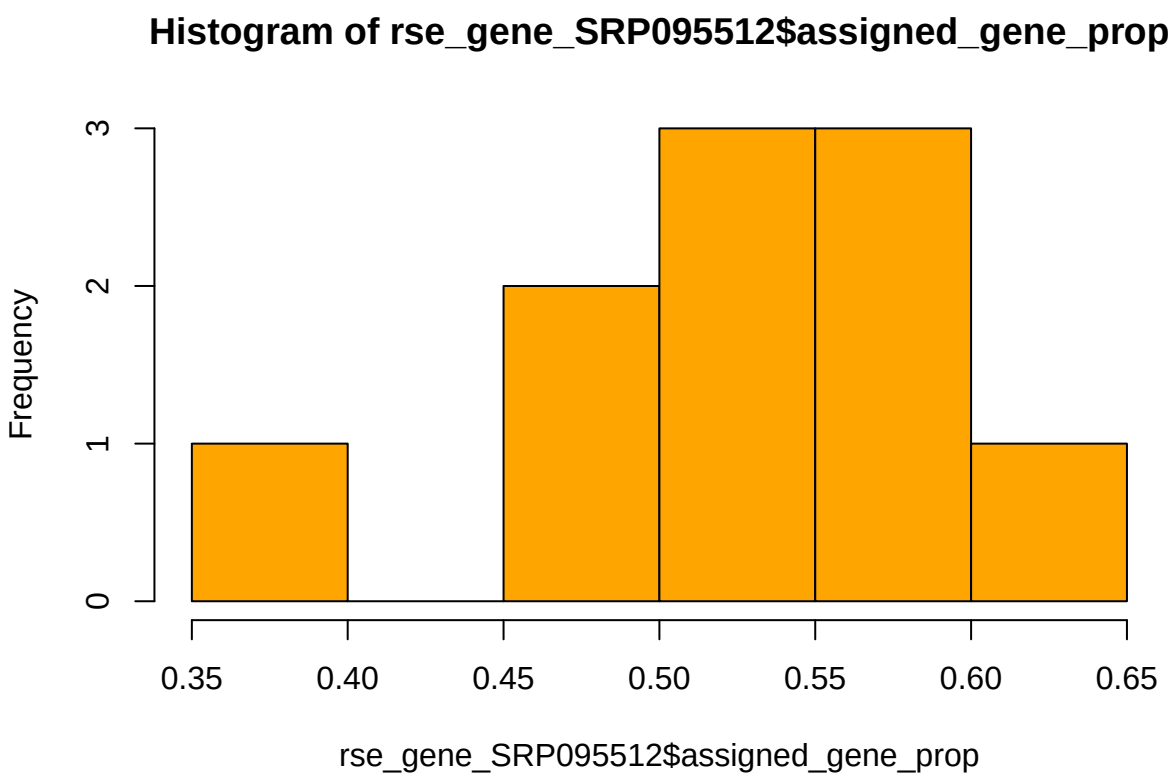
```
# Checar la calidad de los datos
rse_gene_SRP095512$assigned_gene_prop <-
  rse_gene_SRP095512$recount_qc.gene_fc_count_all.assigned /
  rse_gene_SRP095512$recount_qc.gene_fc_count_all.total
summary(rse_gene_SRP095512$assigned_gene_prop)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.3665  0.4902  0.5349  0.5189  0.5608  0.6074
```

```
# Observar la diferencia entre las muestras
with(colData(rse_gene_SRP095512),
     tapply(assigned_gene_prop,
            sra_attribute.disease_state,
            summary))
```

```
## $`diabetic patient`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.4544  0.5260  0.5503  0.5298  0.5541  0.5641
##
## $`healthy control`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.3665  0.4902  0.5156  0.5116  0.5659  0.6074
```

```
# Grafica de la distribucion de los genes asignados
hist(rse_gene_SRP095512$assigned_gene_prop, col = "orange")
```



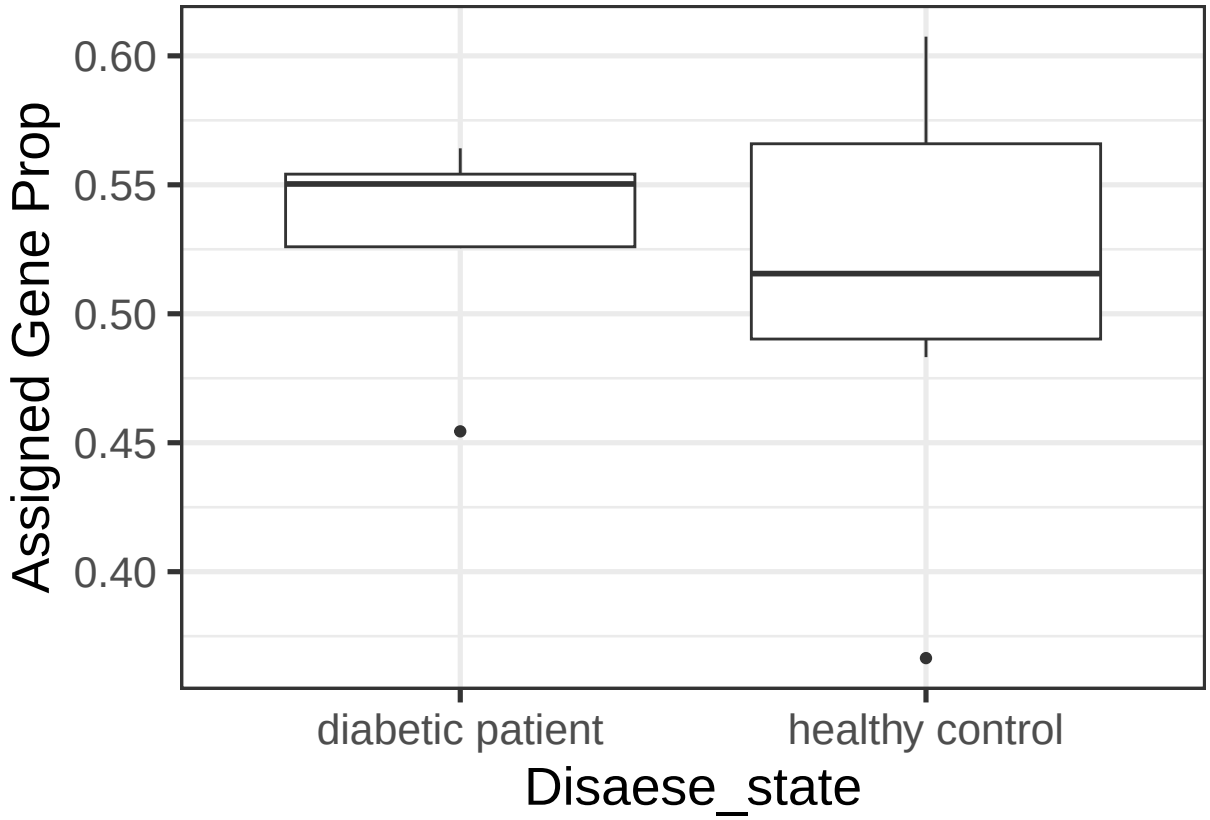

```
# Guardad los datos sin filtrar, en caso de querer recuperarlos despues
rse_gene_SRP095512_unfiltered <- rse_gene_SRP095512
```

Normalización de los datos

```
# Normalización de los datos
dge <- DGEList(
  counts = assay(rse_gene_SRP095512, "counts"),
  genes = rowData(rse_gene_SRP095512)
)
dge <- calcNormFactors(dge)
```

Análisis de la expresión diferencial

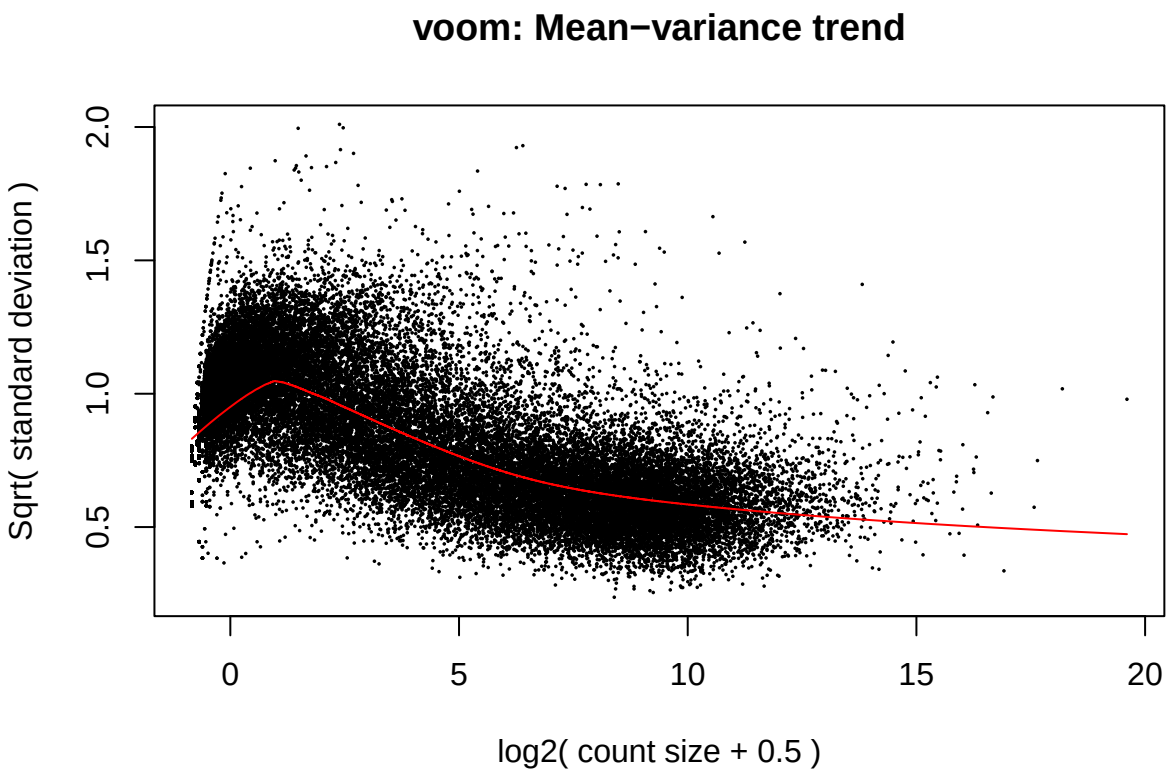
```
## Creacion de un boxplot para analizar la distribución de los genes en muestras
## de pacientes enfermos y sanos

ggplot(as.data.frame(colData(rse_gene_SRP095512)), aes(y = assigned_gene_prop, x = sra_attribute.diseas
  geom_boxplot() +
  theme_bw(base_size = 20) +
  ylab("Assigned Gene Prop") +
  xlab("Disaese_state")
```




```
# Modelo estadístico

mod <- model.matrix(~ sra_attribute.gender +sra_attribute.disease_state + assigned_gene_prop,
                    data = colData(rse_gene_SRP095512)
)
colnames(mod)
```

```
## [1] "(Intercept)"
## [2] "sra_attribute.gendermale"
## [3] "sra_attribute.disease_statehealthy control"
## [4] "assigned_gene_prop"
```

```
## Usamos limma para realizar el análisis de expresión diferencial

vGene <- voom(dge, mod, plot = TRUE)
```



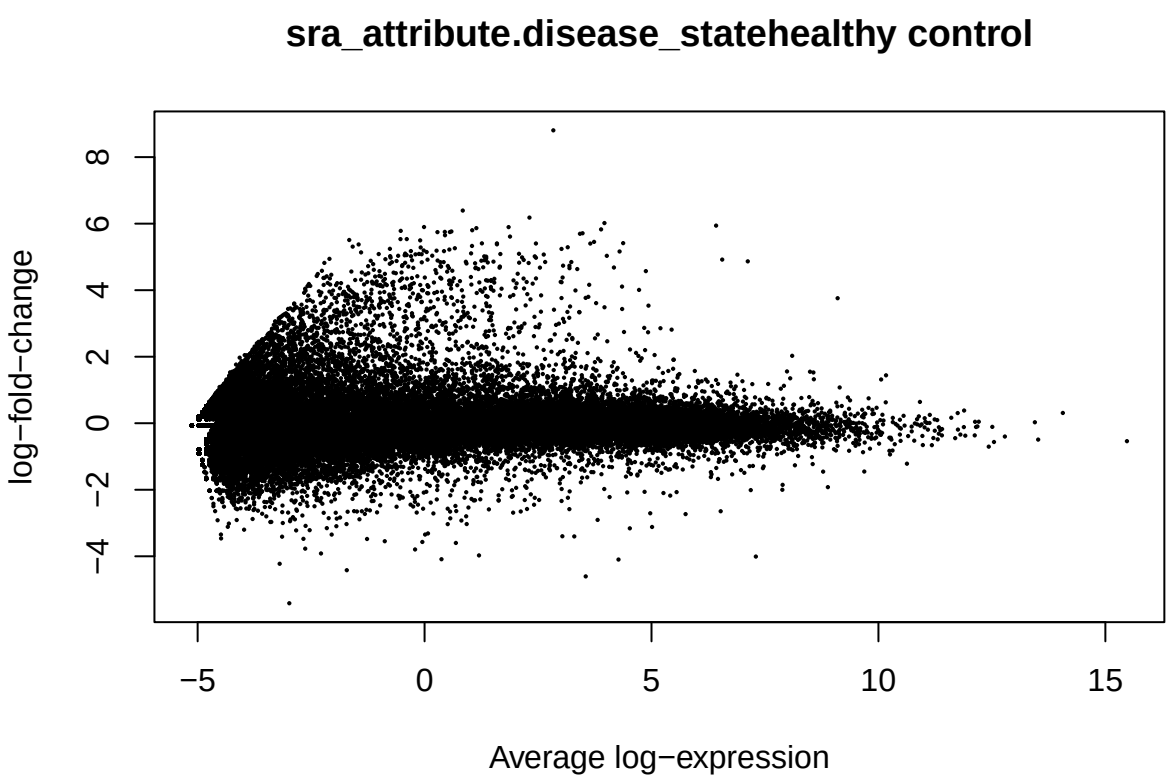

```
eb_results <- eBayes(lmFit(vGene))

de_results <- topTable(
  eb_results,
  coef = 2,
  number = nrow(rse_gene_SRP095512),
  sort.by = "none"
)
dim(de_results)
```

```
## [1] 63856    16
```

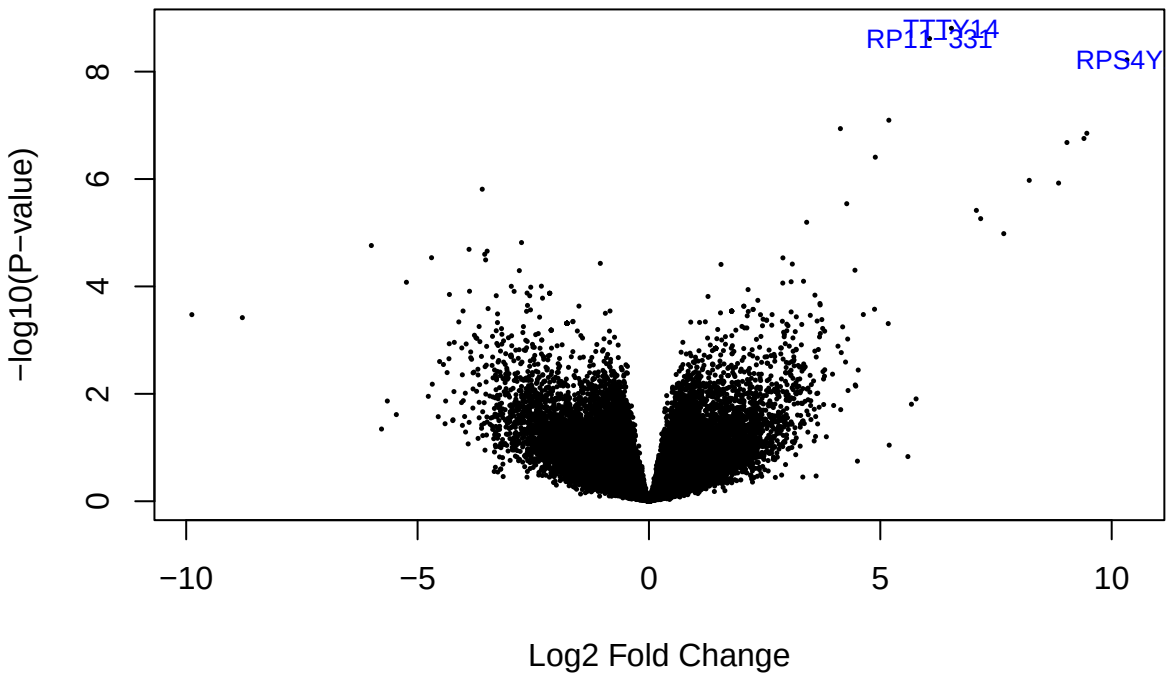
```
## Genes diferencialmente expresados con FDR < 5%
table(de_results$adj.P.Val < 0.05)
```

```
##
## FALSE  TRUE
## 63839    17
```

```
## Visualicemos los resultados estadísticos
plotMA(eb_results, coef = 3)
```



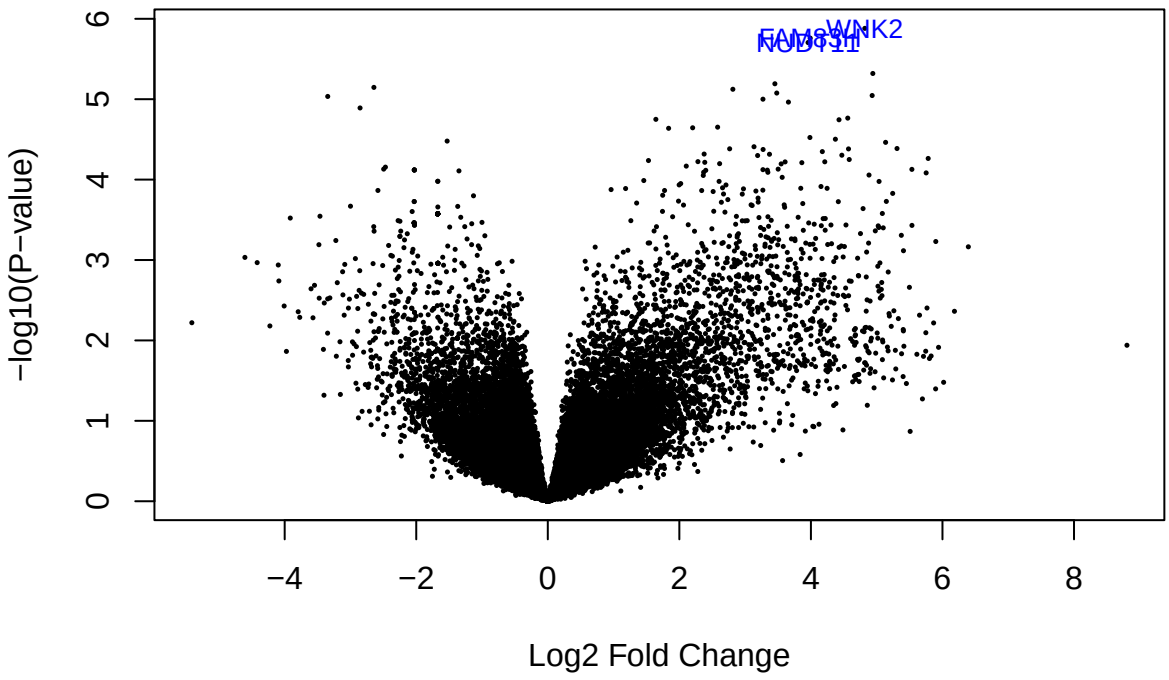

```
## Volcano plot Gender
volcanoplot(eb_results, coef = 2, highlight = 3, names = de_results$gene_name)
```




```
de_results[de_results$gene_name %in% c("TTY14", "RPS4Y1", "RP11-331"), ]
```

```
##                     source type bp_length phase            gene_id
## ENSG00000129824.15 HAVANA gene      2275    NA ENSG00000129824.15
##                          gene_type gene_name level          havana_gene
## ENSG00000129824.15 protein_coding    RPS4Y1     2 OTTHUMG00000036152.4
##                                  tag    logFC   AveExpr        t      P.Value
## ENSG00000129824.15 overlapping_locus 10.32998 -2.382076 24.48189 6.047661e-09
##                        adj.P.Val        B
## ENSG00000129824.15 0.0001287265 5.200397
```

```
## Volcao plot Disaese State
volcanoplot(eb_results, coef = 3, highlight = 3, names = de_results$gene_name)
```




```
de_results[de_results$gene_name %in% c("WNK2", "NUD111", "FAM83H"), ]
```

```
##                     source type bp_length phase            gene_id
## ENSG00000273889.2  HAVANA gene      5654    NA  ENSG00000273889.2
## ENSG00000180921.6  HAVANA gene      5654    NA  ENSG00000180921.6
## ENSG00000165238.16 HAVANA gene     13198    NA ENSG00000165238.16
##                          gene_type gene_name level          havana_gene
## ENSG00000273889.2  protein_coding    FAM83H     2 OTTHUMG00000190683.1
## ENSG00000180921.6  protein_coding    FAM83H     2 OTTHUMG00000133559.2
## ENSG00000165238.16 protein_coding      WNK2     1 OTTHUMG00000020247.4
##                           tag       logFC   AveExpr          t     P.Value
## ENSG00000273889.2        <NA> -0.16013602 -5.127480 -0.6052952 0.561392663
## ENSG00000180921.6  ncRNA_host -1.48582887 -2.016150 -3.6851075 0.005936403
## ENSG00000165238.16       <NA>  0.07310596 -2.207875  0.1486222 0.885449682
##                     adj.P.Val         B
## ENSG00000273889.2  0.7356061 -5.770037
## ENSG00000180921.6  0.6400002 -1.938974
## ENSG00000165238.16 0.9355717 -5.947010
```

R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com.

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```r
summary(cars)
```

```
##      speed           dist       
##  Min.   : 4.0   Min.   :  2.00  
##  1st Qu.:12.0   1st Qu.: 26.00  
##  Median :15.0   Median : 36.00  
##  Mean   :15.4   Mean   : 42.98  
##  3rd Qu.:19.0   3rd Qu.: 56.00  
##  Max.   :25.0   Max.   :120.00
```

Including Plots

You can also embed plots, for example:

Note that the `echo = FALSE` parameter was added to the code chunk to prevent printing of the R code that generated the plot.