Proyecto_RNAseq

Análisis de Expresión Diferencial

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Contents

Introducción

Instalar y cargas paquetes

```
# Cargar el paquete de R que incluye a SummarizedExperiment y todas las demás dependencias
library("recount3")
library("limma")
library("edgeR")
```

Selección de Proyecto

```
# Obtener la lista de proyectos disponibles
human_projects <- available_projects()
```

```
## 2025-02-05 23:17:32.411146 caching file sra.recount_project.MD.gz.
## 2025-02-05 23:17:33.344082 caching file gtex.recount_project.MD.gz.
```

```
## 2025-02-05 23:17:33.948647 caching file tcga.recount_project.MD.gz.
```

```r
# Ver los proyectos disponibles
dim(human_projects)
```

```
## [1] 8742    6
```

```r
# Mostrar las primeras filas para inspeccionar su estructura y contenido
head(human_projects)
```

```
##     project organism file_source     project_home project_type n_samples
## 1 SRP107565    human         sra data_sources/sra data_sources       216
## 2 SRP149665    human         sra data_sources/sra data_sources         4
## 3 SRP017465    human         sra data_sources/sra data_sources        23
## 4 SRP119165    human         sra data_sources/sra data_sources         6
## 5 SRP133965    human         sra data_sources/sra data_sources        12
## 6 SRP096765    human         sra data_sources/sra data_sources         7
```

```r
# Seleccionar un estudio de interés
human_projects[709, ]
```

```
##       project organism file_source     project_home project_type n_samples
## 709 SRP075398    human         sra data_sources/sra data_sources        18
```

```r
# Filtrar el dataframe para seleccionar un proyecto específico basado en su ID y tipo
project_info <- subset(
  human_projects,
  project == "SRP075398" & project_type == "data_sources"
)

# Mostrar la información del proyecto seleccionado para confirmar que se ha filtrado correctamente
project_info
```

```
##       project organism file_source     project_home project_type n_samples
## 709 SRP075398    human         sra data_sources/sra data_sources        18
```

```r
# Crear un objeto de tipo RangedSummarizedExperiment (RSE) con la información a nivel de genes
rse_gene_SRP075398 <- create_rse(project_info)
```

```
## 2025-02-05 23:17:43.167174 downloading and reading the metadata.
```

```
## 2025-02-05 23:17:43.979862 caching file sra.sra.SRP075398.MD.gz.
```

```
## 2025-02-05 23:17:44.617157 caching file sra.recount_project.SRP075398.MD.gz.
```

```
## 2025-02-05 23:17:45.267356 caching file sra.recount_qc.SRP075398.MD.gz.
## 2025-02-05 23:17:45.881848 caching file sra.recount_seq_qc.SRP075398.MD.gz.
```

```
## 2025-02-05 23:17:46.502263 caching file sra.recount_pred.SRP075398.MD.gz.
```

```
## 2025-02-05 23:17:46.757173 downloading and reading the feature information.
```

```
## 2025-02-05 23:17:47.242048 caching file human.gene_sums.G026.gtf.gz.
```

```
## 2025-02-05 23:17:48.482346 downloading and reading the counts: 18 samples across 63856 features.
```

```
## 2025-02-05 23:17:49.052022 caching file sra.gene_sums.SRP075398.G026.gz.
```

```
## 2025-02-05 23:17:49.722031 constructing the RangedSummarizedExperiment (rse) object.
```

```
# Explorar el objeto RSE
rse_gene_SRP075398
```

```
## class: RangedSummarizedExperiment
## dim: 63856 18
## 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(18): SRR3544525 SRR3544526 ... SRR3544537 SRR3544540
## colData names(175): rail_id external_id ...
##   recount_pred.curated.cell_line BigWigURL
```

```
## Información sobre el RSE creado
metadata(rse_gene_SRP075398)
```

```
## $time_created
## [1] "2025-02-05 23:17:49 CST"
##
## $recount3_version
##           package ondiskversion loadedversion
## recount3 recount3        1.16.0        1.16.0
##                                            path
## recount3 /usr/local/lib/R/site-library/recount3
##                                      loadedpath attached is_base       date
## recount3 /usr/local/lib/R/site-library/recount3     TRUE   FALSE 2024-10-29
##                               source md5ok                       library
## recount3 Bioconductor 3.20 (R 4.4.2)    NA /usr/local/lib/R/site-library
##
## $project
## [1] "SRP075398"
##
## $project_home
## [1] "data_sources/sra"
##
## $type
## [1] "gene"
##
## $organism
## [1] "human"
##
## $annotation
## [1] "gencode_v26"
##
## $recount3_url
## [1] "http://duffel.rail.bio/recount3"
```

```
## Número de genes y número de muestras
dim(rse_gene_SRP075398)
```

```
## [1] 63856    18
```

El estudio **SRP068565** se compuso de **20 muestras**, para las cuales tenemos **63,856 genes** en GENCODE v26. La información específica de la anotación está disponible rowRanges() como se muestra a continuación con la columna gene_id utilizada para identificar genes en cada una de las anotaciones.

```
# Información sobre los genes
rowRanges(rse_gene_SRP075398)
```

```
## GRanges object with 63856 ranges and 10 metadata columns:
##                              seqnames            ranges strand |   source
##                                 <Rle>         <IRanges>  <Rle> | <factor>
##          ENSG00000278704.1 GL000009.2       56140-58376      - |  ENSEMBL
##          ENSG00000277400.1 GL000194.1      53590-115018      - |  ENSEMBL
##          ENSG00000274847.1 GL000194.1      53594-115055      - |  ENSEMBL
##          ENSG00000277428.1 GL000195.1       37434-37534      - |  ENSEMBL
##          ENSG00000276256.1 GL000195.1       42939-49164      - |  ENSEMBL
##                        ...        ...               ...    ... .      ...
##   ENSG00000124334.17_PAR_Y       chrY 57184101-57197337      + |   HAVANA
##   ENSG00000185203.12_PAR_Y       chrY 57201143-57203357      - |   HAVANA
##    ENSG00000270726.6_PAR_Y       chrY 57190738-57208756      + |   HAVANA
##   ENSG00000182484.15_PAR_Y       chrY 57207346-57212230      + |   HAVANA
##    ENSG00000227159.8_PAR_Y       chrY 57212184-57214397      - |   HAVANA
##                                type bp_length     phase                gene_id
##                            <factor> <numeric> <integer>            <character>
##          ENSG00000278704.1     gene      2237      <NA>      ENSG00000278704.1
##          ENSG00000277400.1     gene      2179      <NA>      ENSG00000277400.1
##          ENSG00000274847.1     gene      1599      <NA>      ENSG00000274847.1
##          ENSG00000277428.1     gene       101      <NA>      ENSG00000277428.1
##          ENSG00000276256.1     gene      2195      <NA>      ENSG00000276256.1
##                        ...      ...       ...       ...                    ...
##   ENSG00000124334.17_PAR_Y     gene      2504      <NA> ENSG00000124334.17_P..
##   ENSG00000185203.12_PAR_Y     gene      1054      <NA> ENSG00000185203.12_P..
##    ENSG00000270726.6_PAR_Y     gene       773      <NA> ENSG00000270726.6_PA..
##   ENSG00000182484.15_PAR_Y     gene      4618      <NA> ENSG00000182484.15_P..
##    ENSG00000227159.8_PAR_Y     gene      1306      <NA> ENSG00000227159.8_PA..
##                                         gene_type   gene_name       level
##                                       <character> <character> <character>
##          ENSG00000278704.1         protein_coding  BX004987.1           3
##         ENSG00000277400.1         protein_coding  AC145212.2           3
##         ENSG00000274847.1         protein_coding  AC145212.1           3
##         ENSG00000277428.1               misc_RNA       Y_RNA           3
##         ENSG00000276256.1         protein_coding  AC011043.1           3
##                       ...                    ...         ...         ...
##   ENSG00000124334.17_PAR_Y         protein_coding        IL9R           2
##   ENSG00000185203.12_PAR_Y              antisense      WASIR1           2
##    ENSG00000270726.6_PAR_Y   processed_transcript AJ271736.10           2
##   ENSG00000182484.15_PAR_Y transcribed_unproces..      WASH6P           2
##    ENSG00000227159.8_PAR_Y unprocessed_pseudogene    DDX11L16           2
##                                     havana_gene         tag
##                                     <character> <character>
##         ENSG00000278704.1                 <NA>        <NA>
##         ENSG00000277400.1                 <NA>        <NA>
##         ENSG00000274847.1                 <NA>        <NA>
##         ENSG00000277428.1                 <NA>        <NA>
##         ENSG00000276256.1                 <NA>        <NA>
##                       ...                  ...         ...
##   ENSG00000124334.17_PAR_Y OTTHUMG00000022720.1         PAR
##   ENSG00000185203.12_PAR_Y OTTHUMG00000022676.3         PAR
##    ENSG00000270726.6_PAR_Y OTTHUMG00000184987.2         PAR
##   ENSG00000182484.15_PAR_Y OTTHUMG00000022677.5         PAR
##    ENSG00000227159.8_PAR_Y OTTHUMG00000022678.1         PAR
##   -------
##   seqinfo: 374 sequences from an unspecified genome; no seqlengths
```

Preparación de los datos

```
# Convertir las cuentas por nucleotido a cuentas por lectura usando compute_read_counts().
assay(rse_gene_SRP075398, "counts") <- compute_read_counts(rse_gene_SRP075398)

rse_gene_SRP075398$sra.sample_attributes[]
```

```
##  [1] "cell line;;LCC9|source_name;;LCC9 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-29b
##  [2] "cell line;;LCC9|source_name;;LCC9 cell line Anti-miR-29a transfected|transfection;;Anti-miR-29a
##  [3] "cell line;;LCC9|source_name;;LCC9 cell line Anti-miR-29a transfected|transfection;;Anti-miR-29a
##  [4] "cell line;;LCC9|source_name;;LCC9 cell line Anti-miR-29a transfected|transfection;;Anti-miR-29a
##  [5] "cell line;;LCC9|source_name;;LCC9 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a"
##  [6] "cell line;;LCC9|source_name;;LCC9 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a"
##  [7] "cell line;;LCC9|source_name;;LCC9 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a"
##  [8] "cell line;;MCF-7|source_name;;MCF-7 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-2
##  [9] "cell line;;MCF-7|source_name;;MCF-7 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-2
## [10] "cell line;;MCF-7|source_name;;MCF-7 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a
## [11] "cell line;;MCF-7|source_name;;MCF-7 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a
## [12] "cell line;;LCC9|source_name;;LCC9 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-29b
## [13] "cell line;;LCC9|source_name;;LCC9 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-29b
## [14] "cell line;;MCF-7|source_name;;MCF-7 cell line pre-miR-29b-1 transfected|transfection;;Pre-miR-2
## [15] "cell line;;MCF-7|source_name;;MCF-7 cell line Anti-miR-29a transfected|transfection;;Anti-miR-2
## [16] "cell line;;MCF-7|source_name;;MCF-7 cell line Anti-miR-29a transfected|transfection;;Anti-miR-2
## [17] "cell line;;MCF-7|source_name;;MCF-7 cell line Anti-miR-29a transfected|transfection;;Anti-miR-2
## [18] "cell line;;MCF-7|source_name;;MCF-7 cell line Pre-miR-29a transfected|transfection;;Pre-miR-29a
```

```r
# Hacer más fácil de usar la información del experimento
rse_gene_SRP075398 <- expand_sra_attributes(rse_gene_SRP075398)

colData(rse_gene_SRP075398)[
  ,
  grepl("^sra_attribute", colnames(colData(rse_gene_SRP075398)))
]
```

```
## DataFrame with 18 rows and 3 columns
##            sra_attribute.cell_line sra_attribute.source_name
##                        <character>               <character>
## SRR3544525                    LCC9    LCC9 cell line pre-m..
## SRR3544526                    LCC9    LCC9 cell line Anti-..
## SRR3544527                    LCC9    LCC9 cell line Anti-..
## SRR3544528                    LCC9    LCC9 cell line Anti-..
## SRR3544529                    LCC9    LCC9 cell line Pre-m..
## ...                            ...                       ...
## SRR3544534                   MCF-7    MCF-7 cell line pre-..
## SRR3544535                   MCF-7    MCF-7 cell line Anti..
## SRR3544536                   MCF-7    MCF-7 cell line Anti..
## SRR3544537                   MCF-7    MCF-7 cell line Anti..
## SRR3544540                   MCF-7    MCF-7 cell line Pre-..
##            sra_attribute.transfection
##                           <character>
## SRR3544525              Pre-miR-29b-1
## SRR3544526               Anti-miR-29a
## SRR3544527               Anti-miR-29a
## SRR3544528               Anti-miR-29a
## SRR3544529                Pre-miR-29a
## ...                               ...
## SRR3544534              Pre-miR-29b-1
## SRR3544535               Anti-miR-29a
## SRR3544536               Anti-miR-29a
## SRR3544537               Anti-miR-29a
## SRR3544540                Pre-miR-29a
```

```r
colnames(colData(rse_gene_SRP075398))
```

```
##   [1] "rail_id"
##   [2] "external_id"
##   [3] "study"
##   [4] "sra.sample_acc.x"
##   [5] "sra.experiment_acc"
##   [6] "sra.submission_acc"
##   [7] "sra.submission_center"
##   [8] "sra.submission_lab"
##   [9] "sra.study_title"
##  [10] "sra.study_abstract"
##  [11] "sra.study_description"
##  [12] "sra.experiment_title"
##  [13] "sra.design_description"
##  [14] "sra.sample_description"
##  [15] "sra.library_name"
##  [16] "sra.library_strategy"
##  [17] "sra.library_source"
##  [18] "sra.library_selection"
##  [19] "sra.library_layout"
##  [20] "sra.paired_nominal_length"
##  [21] "sra.paired_nominal_stdev"
##  [22] "sra.library_construction_protocol"
##  [23] "sra.platform_model"
##  [24] "sra.sample_attributes"
##  [25] "sra.experiment_attributes"
##  [26] "sra.spot_length"
##  [27] "sra.sample_name"
##  [28] "sra.sample_title"
##  [29] "sra.sample_bases"
##  [30] "sra.sample_spots"
##  [31] "sra.run_published"
##  [32] "sra.size"
##  [33] "sra.run_total_bases"
##  [34] "sra.run_total_spots"
##  [35] "sra.num_reads"
##  [36] "sra.num_spots"
##  [37] "sra.read_info"
##  [38] "sra.run_alias"
##  [39] "sra.run_center_name"
##  [40] "sra.run_broker_name"
##  [41] "sra.run_center"
##  [42] "recount_project.project"
##  [43] "recount_project.organism"
##  [44] "recount_project.file_source"
##  [45] "recount_project.metadata_source"
##  [46] "recount_project.date_processed"
##  [47] "recount_qc.aligned_reads%.chrm"
##  [48] "recount_qc.aligned_reads%.chrx"
##  [49] "recount_qc.aligned_reads%.chry"
##  [50] "recount_qc.bc_auc.all_reads_all_bases"
##  [51] "recount_qc.bc_auc.all_reads_annotated_bases"
##  [52] "recount_qc.bc_auc.unique_reads_all_bases"
##  [53] "recount_qc.bc_auc.unique_reads_annotated_bases"
##  [54] "recount_qc.bc_auc.all_%"
##  [55] "recount_qc.bc_auc.unique_%"
##  [56] "recount_qc.bc_frag.count"
##  [57] "recount_qc.bc_frag.kallisto_count"
##  [58] "recount_qc.bc_frag.kallisto_mean_length"
##  [59] "recount_qc.bc_frag.mean_length"
##  [60] "recount_qc.bc_frag.mode_length"
##  [61] "recount_qc.bc_frag.mode_length_count"
##  [62] "recount_qc.exon_fc.all_%"
##  [63] "recount_qc.exon_fc.unique_%"
##  [64] "recount_qc.exon_fc_count_all.total"
##  [65] "recount_qc.exon_fc_count_all.assigned"
##  [66] "recount_qc.exon_fc_count_unique.total"
##  [67] "recount_qc.exon_fc_count_unique.assigned"
##  [68] "recount_qc.gene_fc.all_%"
##  [69] "recount_qc.gene_fc.unique_%"
##  [70] "recount_qc.gene_fc_count_all.total"
##  [71] "recount_qc.gene_fc_count_all.assigned"
##  [72] "recount_qc.gene_fc_count_unique.total"
##  [73] "recount_qc.gene_fc_count_unique.assigned"
##  [74] "recount_qc.intron_sum"
##  [75] "recount_qc.intron_sum_%"
##  [76] "recount_qc.star.%_of_chimeric_reads"
##  [77] "recount_qc.star.%_of_chimeric_reads2"
##  [78] "recount_qc.star.%_of_reads_mapped_to_multiple_loci"
##  [79] "recount_qc.star.%_of_reads_mapped_to_multiple_loci2"
##  [80] "recount_qc.star.%_of_reads_mapped_to_too_many_loci"
##  [81] "recount_qc.star.%_of_reads_mapped_to_too_many_loci2"
##  [82] "recount_qc.star.%_of_reads_unmapped:_other"
##  [83] "recount_qc.star.%_of_reads_unmapped:_other2"
##  [84] "recount_qc.star.%_of_reads_unmapped:_too_many_mismatches"
##  [85] "recount_qc.star.%_of_reads_unmapped:_too_many_mismatches2"
##  [86] "recount_qc.star.%_of_reads_unmapped:_too_short"
##  [87] "recount_qc.star.%_of_reads_unmapped:_too_short2"
##  [88] "recount_qc.star.all_mapped_reads"
##  [89] "recount_qc.star.all_mapped_reads2"
##  [90] "recount_qc.star.average_input_read_length"
##  [91] "recount_qc.star.average_input_read_length2"
##  [92] "recount_qc.star.average_mapped_length"
##  [93] "recount_qc.star.average_mapped_length2"
##  [94] "recount_qc.star.deletion_average_length"
##  [95] "recount_qc.star.deletion_average_length2"
##  [96] "recount_qc.star.deletion_rate_per_base"
##  [97] "recount_qc.star.deletion_rate_per_base2"
##  [98] "recount_qc.star.insertion_average_length"
##  [99] "recount_qc.star.insertion_average_length2"
## [100] "recount_qc.star.insertion_rate_per_base"
## [101] "recount_qc.star.insertion_rate_per_base2"
## [102] "recount_qc.star.mapping_speed,_million_of_reads_per_hour"
## [103] "recount_qc.star.mapping_speed,_million_of_reads_per_hour2"
## [104] "recount_qc.star.mismatch_rate_per_base,_%"
## [105] "recount_qc.star.mismatch_rate_per_base,_%2"
## [106] "recount_qc.star.number_of_chimeric_reads"
## [107] "recount_qc.star.number_of_chimeric_reads2"
## [108] "recount_qc.star.number_of_input_reads"
## [109] "recount_qc.star.number_of_input_reads2"
## [110] "recount_qc.star.number_of_reads_mapped_to_multiple_loci"
## [111] "recount_qc.star.number_of_reads_mapped_to_multiple_loci2"
## [112] "recount_qc.star.number_of_reads_mapped_to_too_many_loci"
## [113] "recount_qc.star.number_of_reads_mapped_to_too_many_loci2"
## [114] "recount_qc.star.number_of_reads_unmapped:_other"
## [115] "recount_qc.star.number_of_reads_unmapped:_other2"
## [116] "recount_qc.star.number_of_reads_unmapped:_too_many_mismatches"
## [117] "recount_qc.star.number_of_reads_unmapped:_too_many_mismatches2"
## [118] "recount_qc.star.number_of_reads_unmapped:_too_short"
## [119] "recount_qc.star.number_of_reads_unmapped:_too_short2"
## [120] "recount_qc.star.number_of_splices:_at/ac"
## [121] "recount_qc.star.number_of_splices:_at/ac2"
## [122] "recount_qc.star.number_of_splices:_annotated_(sjdb)"
## [123] "recount_qc.star.number_of_splices:_annotated_(sjdb)2"
## [124] "recount_qc.star.number_of_splices:_gc/ag"
## [125] "recount_qc.star.number_of_splices:_gc/ag2"
## [126] "recount_qc.star.number_of_splices:_gt/ag"
## [127] "recount_qc.star.number_of_splices:_gt/ag2"
## [128] "recount_qc.star.number_of_splices:_non-canonical"
## [129] "recount_qc.star.number_of_splices:_non-canonical2"
## [130] "recount_qc.star.number_of_splices:_total"
## [131] "recount_qc.star.number_of_splices:_total2"
## [132] "recount_qc.star.uniquely_mapped_reads_%"
## [133] "recount_qc.star.uniquely_mapped_reads_%2"
## [134] "recount_qc.star.uniquely_mapped_reads_number"
## [135] "recount_qc.star.uniquely_mapped_reads_number2"
## [136] "recount_qc.junction_count"
## [137] "recount_qc.junction_coverage"
## [138] "recount_qc.junction_avg_coverage"
## [139] "recount_qc.star.number_of_input_reads_both"
## [140] "recount_qc.star.all_mapped_reads_both"
## [141] "recount_qc.star.number_of_chimeric_reads_both"
## [142] "recount_qc.star.number_of_reads_mapped_to_multiple_loci_both"
## [143] "recount_qc.star.number_of_reads_mapped_to_too_many_loci_both"
## [144] "recount_qc.star.number_of_reads_unmapped:_other_both"
## [145] "recount_qc.star.number_of_reads_unmapped:_too_many_mismatches_both"
## [146] "recount_qc.star.number_of_reads_unmapped:_too_short_both"
## [147] "recount_qc.star.uniquely_mapped_reads_number_both"
## [148] "recount_qc.star.%_mapped_reads_both"
## [149] "recount_qc.star.%_chimeric_reads_both"
## [150] "recount_qc.star.%_reads_mapped_to_multiple_loci_both"
## [151] "recount_qc.star.%_reads_mapped_to_too_many_loci_both"
## [152] "recount_qc.star.%_reads_unmapped:_other_both"
## [153] "recount_qc.star.%_reads_unmapped:_too_many_mismatches_both"
## [154] "recount_qc.star.%_reads_unmapped:_too_short_both"
## [155] "recount_qc.star.uniquely_mapped_reads_%_both"
## [156] "recount_seq_qc.min_len"
## [157] "recount_seq_qc.max_len"
## [158] "recount_seq_qc.avg_len"
## [159] "recount_seq_qc.#distinct_quality_values"
## [160] "recount_seq_qc.#bases"
## [161] "recount_seq_qc.%a"
## [162] "recount_seq_qc.%c"
## [163] "recount_seq_qc.%g"
## [164] "recount_seq_qc.%t"
## [165] "recount_seq_qc.%n"
## [166] "recount_seq_qc.avgq"
## [167] "recount_seq_qc.errq"
## [168] "recount_pred.sample_acc.y"
## [169] "recount_pred.curated.type"
## [170] "recount_pred.curated.tissue"
## [171] "recount_pred.pattern.predict.type"
## [172] "recount_pred.pred.type"
## [173] "recount_pred.curated.cell_type"
## [174] "recount_pred.curated.cell_line"
## [175] "BigWigURL"
## [176] "sra_attribute.cell_line"
## [177] "sra_attribute.source_name"
## [178] "sra_attribute.transfection"
```

```r
# Ajustar el tipo de dato de las variables
## Pasar de character a factor

rse_gene_SRP075398$sra_attribute.cell_line <- factor(rse_gene_SRP075398$sra_attribute.cell_line)

rse_gene_SRP075398$sra_attribute.source_name <- factor(tolower(rse_gene_SRP075398$sra_attribute.source_

rse_gene_SRP075398$sra_attribute.transfection <- factor(rse_gene_SRP075398$sra_attribute.transfection)

# Resumen de las variables
summary(as.data.frame(colData(rse_gene_SRP075398)[
    ,
    grepl("^sra_attribute.[cell_line|source_name|transfection]", colnames(colData(rse_gene_SRP075398)))
]))
```

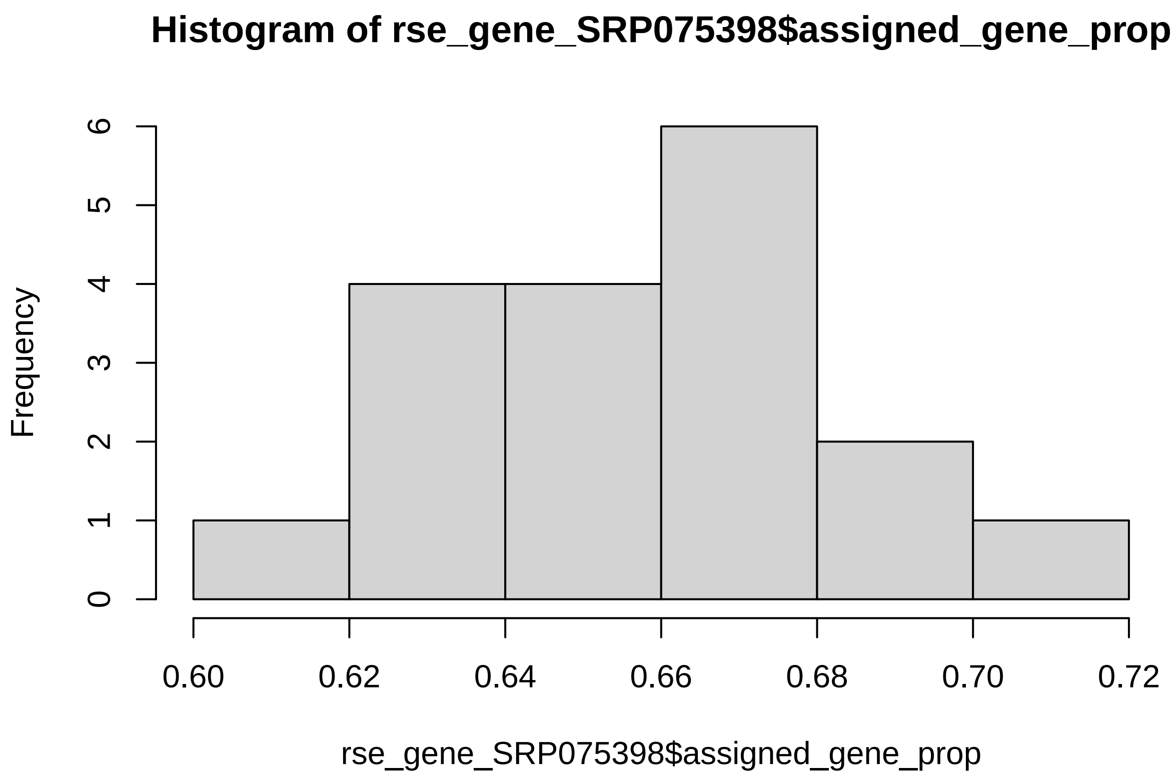
```
##  sra_attribute.cell_line                              sra_attribute.source_name
##  LCC9 :9                 lcc9 cell line anti-mir-29a transfected  :3
##  MCF-7:9                 lcc9 cell line pre-mir-29a transfected   :3
##                          lcc9 cell line pre-mir-29b-1 transfected :3
##                          mcf-7 cell line anti-mir-29a transfected :3
##                          mcf-7 cell line pre-mir-29a transfected  :3
##                          mcf-7 cell line pre-mir-29b-1 transfected:3
##  sra_attribute.transfection
##  Anti-miR-29a :6
##  Pre-miR-29a  :6
##  Pre-miR-29b-1:6
##
##
##
```

```r
# Calcular la proporción de lecturas asignadas a genes
rse_gene_SRP075398$assigned_gene_prop <-
  rse_gene_SRP075398$recount_qc.gene_fc_count_all.assigned /
  rse_gene_SRP075398$recount_qc.gene_fc_count_all.total

# Resumen de la nueva variable
summary(rse_gene_SRP075398$assigned_gene_prop)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.6076  0.6405  0.6603  0.6585  0.6696  0.7017
```

Filtrar genes de baja expresión

```r
# Guardar el objeto original
rse_gene_SRP075398_unfiltered <- rse_gene_SRP075398

# Filtrar muestras de baja calidad
hist(rse_gene_SRP075398$assigned_gene_prop)
```



```
table(rse_gene_SRP075398$assigned_gene_prop < 0.3)
```

```
##
## FALSE
##    18
```

```
rse_gene_SRP075398 <- rse_gene_SRP075398[, rse_gene_SRP075398$assigned_gene_prop > 0.3]

# Filtrar genes de baja expresión usando edgeR
dge <- DGEList(counts = assay(rse_gene_SRP075398, "counts"))
keep <- filterByExpr(dge, group = rse_gene_SRP075398$sra_attribute.transfection)
rse_gene_SRP075398 <- rse_gene_SRP075398[keep, ]

# Dimensiones finales
dim(rse_gene_SRP075398)
```

```
## [1] 22789    18
```

```
# Porcentaje de genes retenidos
round(nrow(rse_gene_SRP075398) / nrow(rse_gene_SRP075398_unfiltered) * 100, 2)
```

```
## [1] 35.69
```

Normalización de los datos

```r
# Crear un objeto DGEList para normalización
dge <- DGEList(
    counts = assay(rse_gene_SRP075398, "counts"),
    genes = rowData(rse_gene_SRP075398)
)

# Normalización TMM
dge <- calcNormFactors(dge)

dge
```

```
## An object of class "DGEList"
## $counts
##                    SRR3544525 SRR3544526 SRR3544527 SRR3544528 SRR3544529
## ENSG00000223972.5         44         31         54         44         62
## ENSG00000227232.5        297        264        405        352        242
## ENSG00000238009.6         32         27         19         32         20
## ENSG00000268903.1         17          7          9          6         17
## ENSG00000269981.1         14         19         25         23         16
##                    SRR3544530 SRR3544531 SRR3544532 SRR3544533 SRR3544538
## ENSG00000223972.5         37         51         13         18         16
## ENSG00000227232.5        215        277        200        204        245
## ENSG00000238009.6         15         25         19         30         48
## ENSG00000268903.1          6         13          8          3          4
## ENSG00000269981.1         12         34         20         22         25
##                    SRR3544539 SRR3544523 SRR3544524 SRR3544534 SRR3544535
## ENSG00000223972.5         13         71         52         10         11
## ENSG00000227232.5        105        509        353        217        123
## ENSG00000238009.6         23         45         28         26         31
## ENSG00000268903.1          1         35         24          0          4
## ENSG00000269981.1         10         37         33         17         10
##                    SRR3544536 SRR3544537 SRR3544540
## ENSG00000223972.5          7          9         25
## ENSG00000227232.5         74        163        183
## ENSG00000238009.6         32         27         45
## ENSG00000268903.1          0          2          3
## ENSG00000269981.1          6         13         26
## 22784 more rows ...
##
## $samples
##            group lib.size norm.factors
## SRR3544525     1 40704527    1.0515272
## SRR3544526     1 44710538    1.0305267
## SRR3544527     1 55789726    0.9938596
## SRR3544528     1 61289070    1.0017792
## SRR3544529     1 34508615    1.0395402
## 13 more rows ...
##
## $genes
##                    source type bp_length phase           gene_id
## ENSG00000223972.5 HAVANA gene      1735    NA ENSG00000223972.5
## ENSG00000227232.5 HAVANA gene      1351    NA ENSG00000227232.5
## ENSG00000238009.6 HAVANA gene      3726    NA ENSG00000238009.6
## ENSG00000268903.1 HAVANA gene       755    NA ENSG00000268903.1
## ENSG00000269981.1 HAVANA gene       284    NA ENSG00000269981.1
##                                            gene_type     gene_name level
## ENSG00000223972.5 transcribed_unprocessed_pseudogene       DDX11L1     2
## ENSG00000227232.5             unprocessed_pseudogene        WASH7P     2
## ENSG00000238009.6                            lincRNA  RP11-34P13.7     2
## ENSG00000268903.1               processed_pseudogene RP11-34P13.15     2
## ENSG00000269981.1               processed_pseudogene RP11-34P13.16     2
##                             havana_gene               tag
## ENSG00000223972.5 OTTHUMG00000000961.2              <NA>
## ENSG00000227232.5 OTTHUMG00000000958.1              <NA>
## ENSG00000238009.6 OTTHUMG00000001096.2 overlapping_locus
## ENSG00000268903.1 OTTHUMG00000182518.2              <NA>
## ENSG00000269981.1 OTTHUMG00000182738.2              <NA>
## 22784 more rows ...
```

Determinar el modelo estadístico

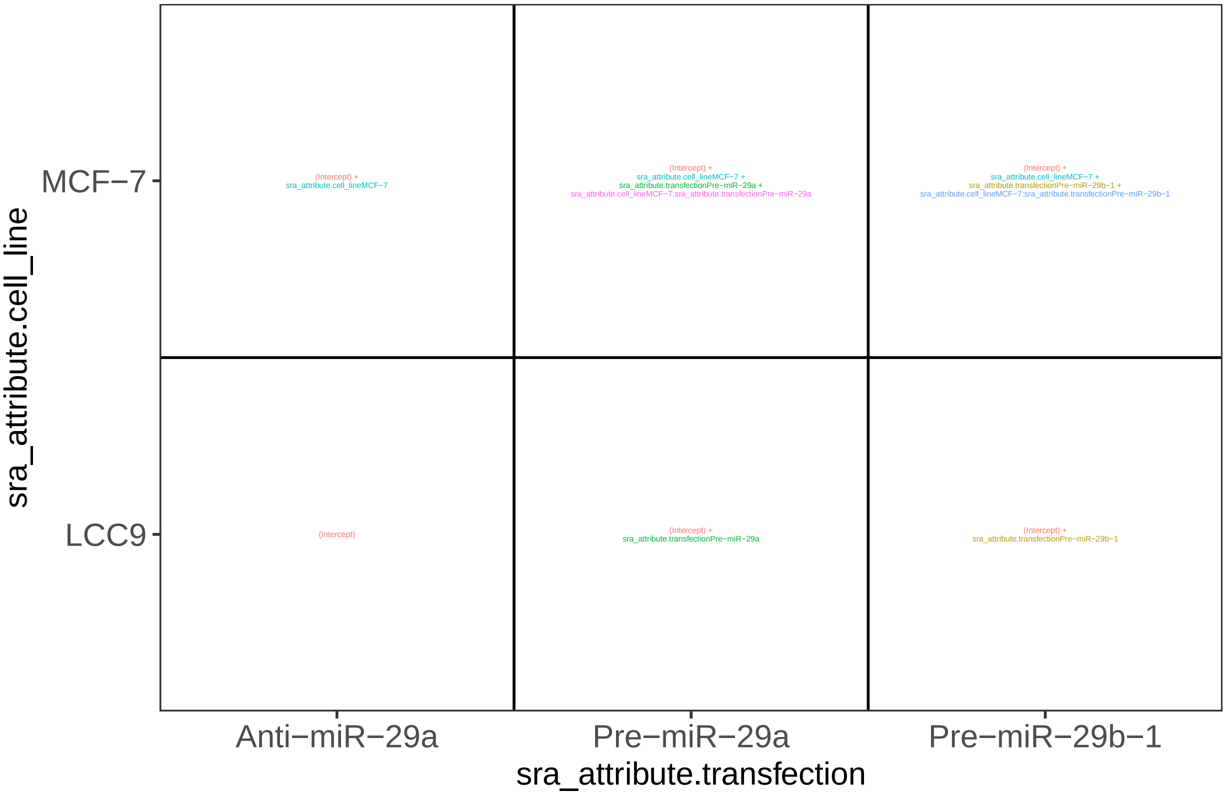
```
mod <- model.matrix(
  ~ sra_attribute.cell_line * sra_attribute.transfection,
  data = colData(rse_gene_SRP075398)
)

colnames(mod)
```

```
## [1] "(Intercept)"
## [2] "sra_attribute.cell_lineMCF-7"
## [3] "sra_attribute.transfectionPre-miR-29a"
## [4] "sra_attribute.transfectionPre-miR-29b-1"
## [5] "sra_attribute.cell_lineMCF-7:sra_attribute.transfectionPre-miR-29a"
## [6] "sra_attribute.cell_lineMCF-7:sra_attribute.transfectionPre-miR-29b-1"
```

```
# Simplificar nombres de la columna

#colnames(mod) <- c("Intercept", "CellLine_MCF7", "Transfection_miR29a",
                   #"Transfection_miR29b1", "Interaction_MCF7_miR29a",
                   #"Interaction_MCF7_miR29b1")
```

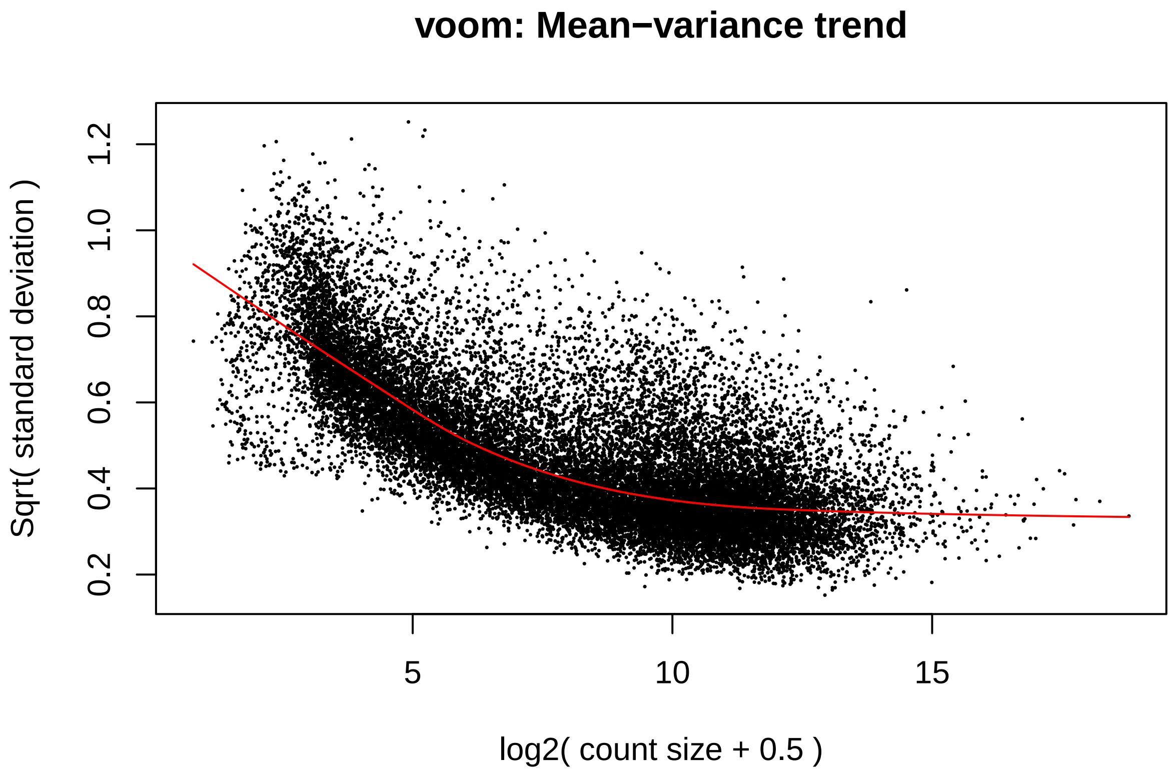
Visualizar matriz (REVISAR ESTO AL FINAL)

```
library(ExploreModelMatrix)

## Crear las visualizaciones
vd <- ExploreModelMatrix::VisualizeDesign(
    sampleData = colData(rse_gene_SRP075398), # Metadatos de las muestras
    designFormula = ~ sra_attribute.cell_line * sra_attribute.transfection,           # Fórmula del mod
    textSizeFitted = 1                              # Tamaño del texto
)

library(cowplot)
cowplot::plot_grid(plotlist = vd$plotlist)
```



Expresión diferencial

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



```
# Ajuste del modelo lineal y cálculo de estadísticas empíricas de Bayes
eb_results <- eBayes(lmFit(vGene))

de_results <- topTable(
    eb_results,
    coef = 2,
    number = nrow(rse_gene_SRP075398),
    sort.by = "none"
)

# Dimensiones y vista preliminar de los resultados
dim(de_results)
```

```
## [1] 22789    16
```

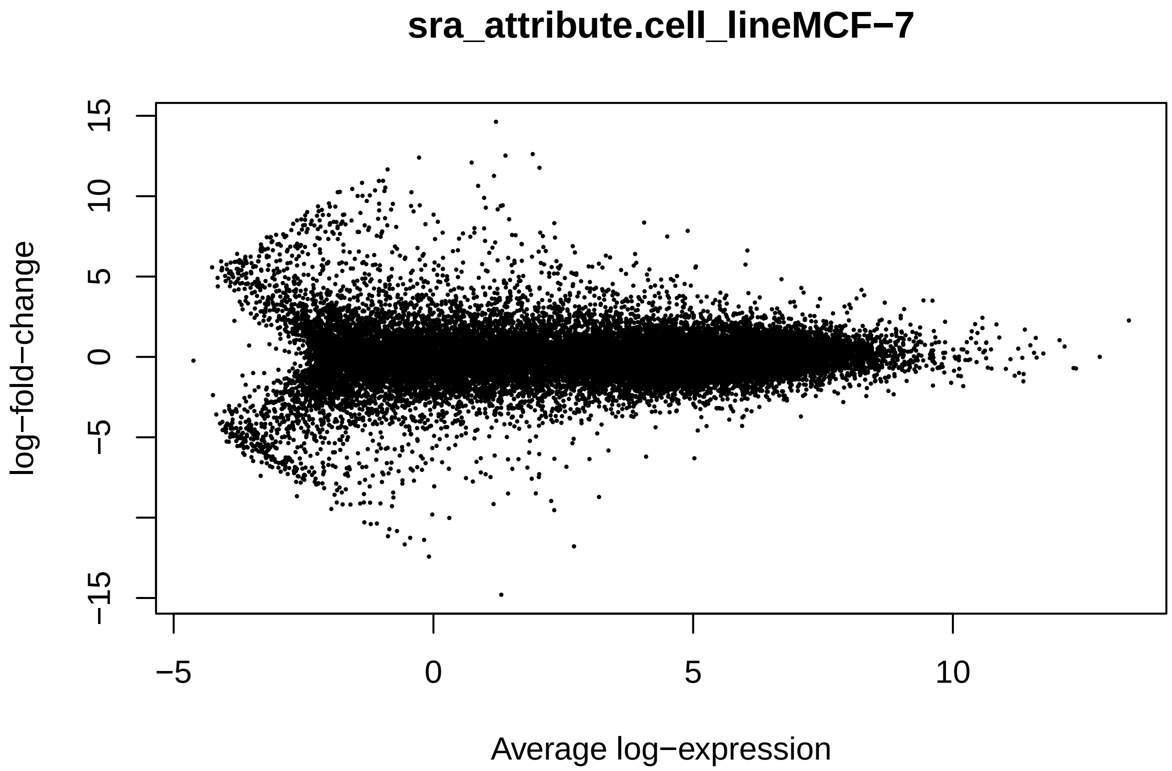
```
head(de_results)
```

```
##                      source type bp_length phase           gene_id
## ENSG00000223972.5 HAVANA gene      1735    NA ENSG00000223972.5
## ENSG00000227232.5 HAVANA gene      1351    NA ENSG00000227232.5
## ENSG00000238009.6 HAVANA gene      3726    NA ENSG00000238009.6
## ENSG00000268903.1 HAVANA gene       755    NA ENSG00000268903.1
## ENSG00000269981.1 HAVANA gene       284    NA ENSG00000269981.1
## ENSG00000239906.1 HAVANA gene       323    NA ENSG00000239906.1
##                                          gene_type     gene_name level
## ENSG00000223972.5 transcribed_unprocessed_pseudogene       DDX11L1     2
## ENSG00000227232.5             unprocessed_pseudogene        WASH7P     2
## ENSG00000238009.6                            lincRNA  RP11-34P13.7     2
## ENSG00000268903.1               processed_pseudogene RP11-34P13.15     2
## ENSG00000269981.1               processed_pseudogene RP11-34P13.16     2
## ENSG00000239906.1                          antisense RP11-34P13.14     2
##                             havana_gene               tag      logFC    AveExpr
## ENSG00000223972.5 OTTHUMG00000000961.2              <NA> -1.6682397 -0.7347566
## ENSG00000227232.5 OTTHUMG00000000958.1              <NA> -1.0300576  2.4023283
## ENSG00000238009.6 OTTHUMG00000001096.2 overlapping_locus  0.7712042 -0.5755998
## ENSG00000268903.1 OTTHUMG00000182518.2              <NA> -1.6043444 -2.9389997
## ENSG00000269981.1 OTTHUMG00000182738.2              <NA> -0.6971305 -1.1724133
## ENSG00000239906.1 OTTHUMG00000002481.1              <NA>  1.5168961 -0.5286964
##                           t      P.Value    adj.P.Val          B
## ENSG00000223972.5 -4.947936 0.0001334750 0.0002696371  0.8157153
## ENSG00000227232.5 -4.097809 0.0007946777 0.0013918923 -1.7013844
## ENSG00000238009.6  3.158990 0.0059046617 0.0088550498 -3.1137314
## ENSG00000268903.1 -1.811961 0.0882499498 0.1089038883 -4.9948247
## ENSG00000269981.1 -1.625628 0.1229935089 0.1480665121 -5.6956305
## ENSG00000239906.1  3.957787 0.0010712285 0.0018297276 -1.3144976
```

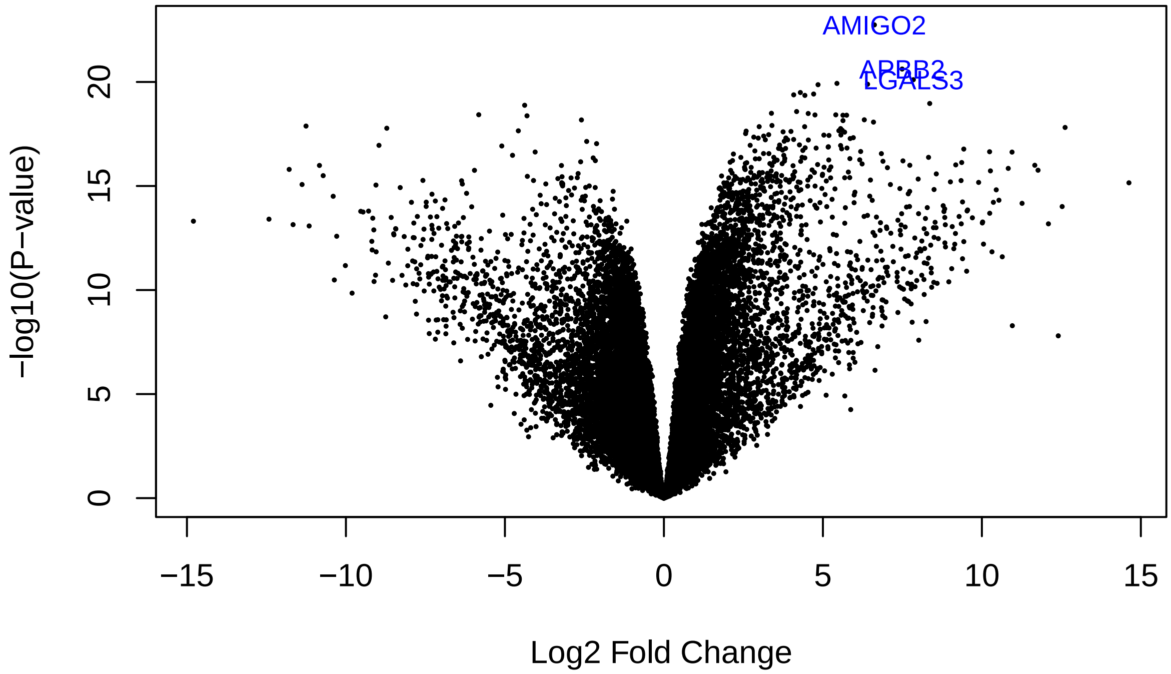
```r
## Genes diferencialmente expresados con FDR < 5%
table(de_results$adj.P.Val < 0.05)
```

```
##
## FALSE  TRUE
##  5408 17381
```

```r
## Visualizar los resultados estadísticos

plotMA(eb_results, coef = 2)
```



```
volcanoplot(eb_results, coef = 2, highlight = 3, names = de_results$gene_name)
```

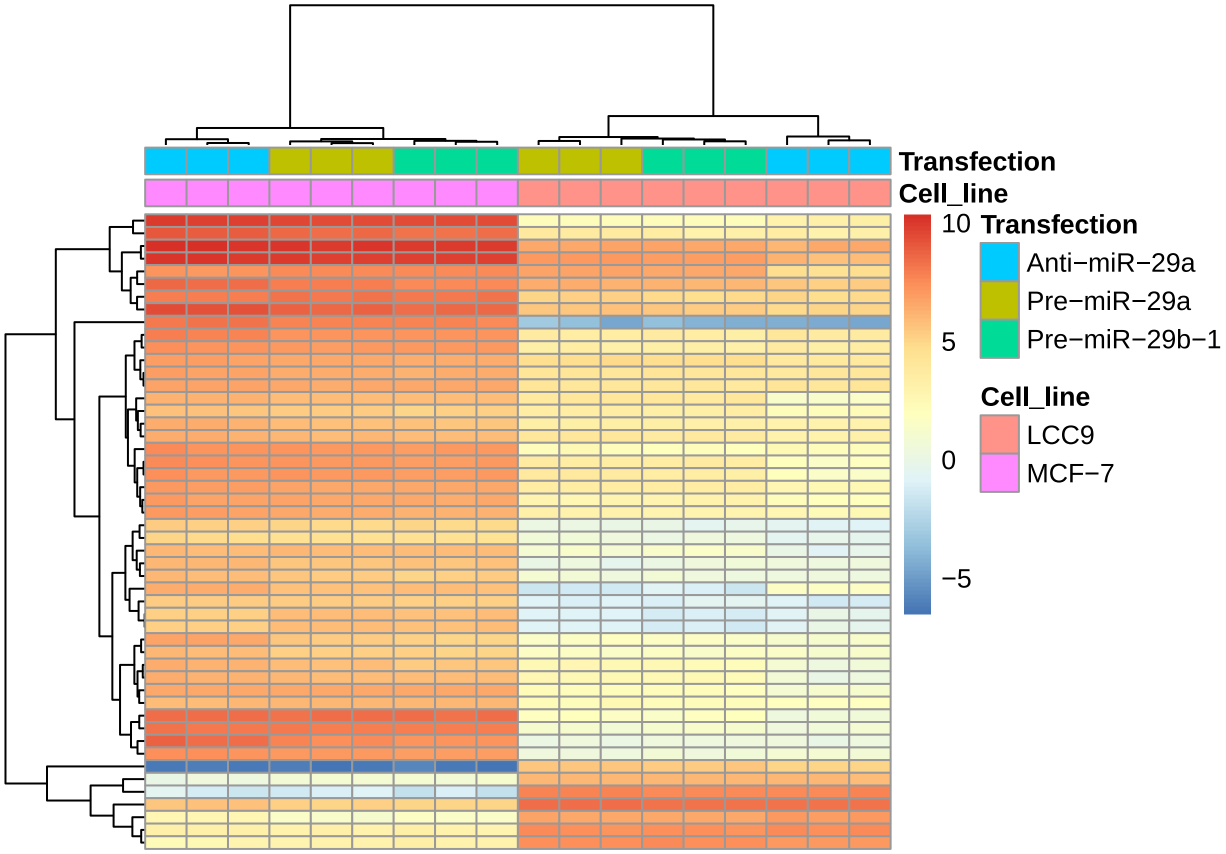


```r
## Información de los 3 genes más significativos
de_results[de_results$gene_name %in% c("AMIGO2", "APBB2", "LGALS3"), ]
```

```
##                       source type bp_length phase            gene_id
## ENSG00000139211.6  HAVANA gene      3956    NA  ENSG00000139211.6
## ENSG00000131981.15 HAVANA gene      2397    NA ENSG00000131981.15
## ENSG00000163697.16 HAVANA gene     12956    NA ENSG00000163697.16
##                         gene_type gene_name level           havana_gene
## ENSG00000139211.6  protein_coding    AMIGO2     2  OTTHUMG00000169616.1
## ENSG00000131981.15 protein_coding    LGALS3     1  OTTHUMG00000171030.4
## ENSG00000163697.16 protein_coding     APBB2     2 OTTHUMG00000160416.11
##                           tag    logFC  AveExpr        t      P.Value
## ENSG00000139211.6        <NA> 6.619379 6.044395 88.02985 1.804171e-23
## ENSG00000131981.15       <NA> 7.844704 4.895663 60.85097 7.793307e-21
## ENSG00000163697.16 ncRNA_host 7.492499 4.501818 65.39220 2.390532e-21
##                       adj.P.Val        B
## ENSG00000139211.6  4.111525e-19 42.91918
## ENSG00000131981.15 5.145767e-17 36.96864
## ENSG00000163697.16 2.723892e-17 37.95833
```

Visualizar genes DE

```r
# Revisar los top 50 genes diferencialmente expresados

## Extraer valores de los genes de interés
exprs_heatmap <- vGene$E[rank(de_results$adj.P.Val) <= 50, ]

## Creemos una tabla con información de las muestras
## y con nombres de columnas más amigables
df <- as.data.frame(colData(rse_gene_SRP075398)[, c("sra_attribute.cell_line", "sra_attribute.transfecti
colnames(df) <- c("Cell_line", "Transfection")

## Hagamos un heatmap
library("pheatmap")
pheatmap(
    exprs_heatmap,
    cluster_rows = TRUE,
    cluster_cols = TRUE,
    show_rownames = FALSE,
    show_colnames = FALSE,
    annotation_col = df
)
```

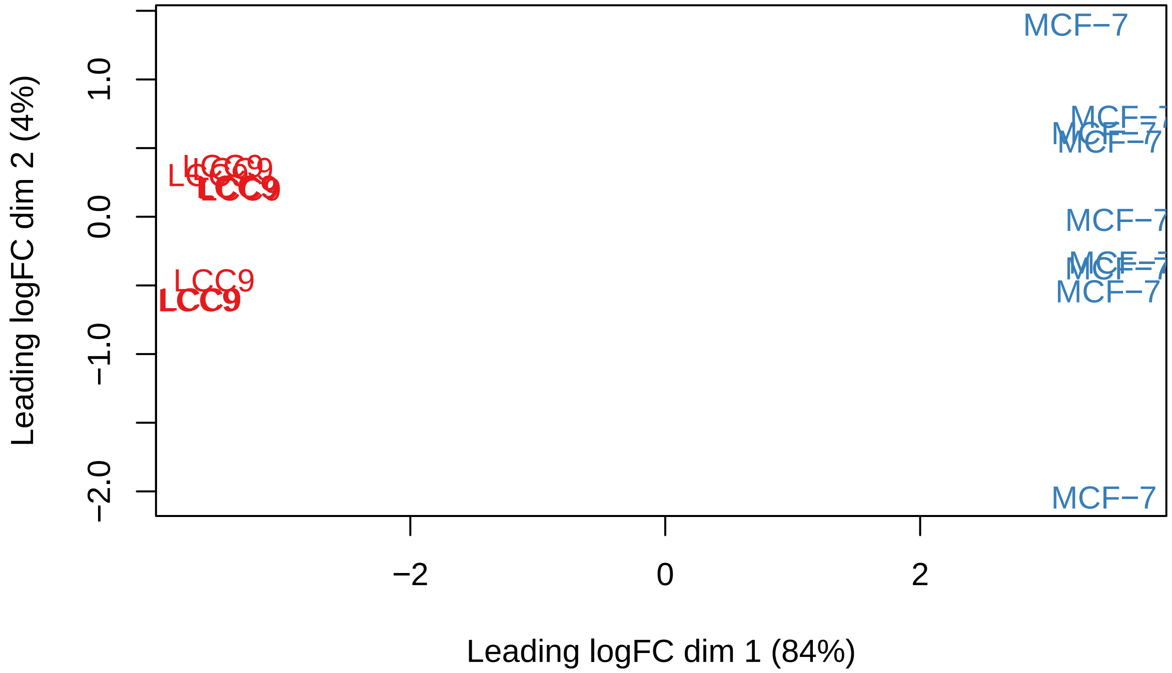


```r
# MDS (multidimensional scaling)

## Para colores
library("RColorBrewer")
```

```r
## Conviertiendo los grupos de Cell_line a colores
col.group <- df$Cell_line
levels(col.group) <- brewer.pal(nlevels(col.group), "Set1")
```

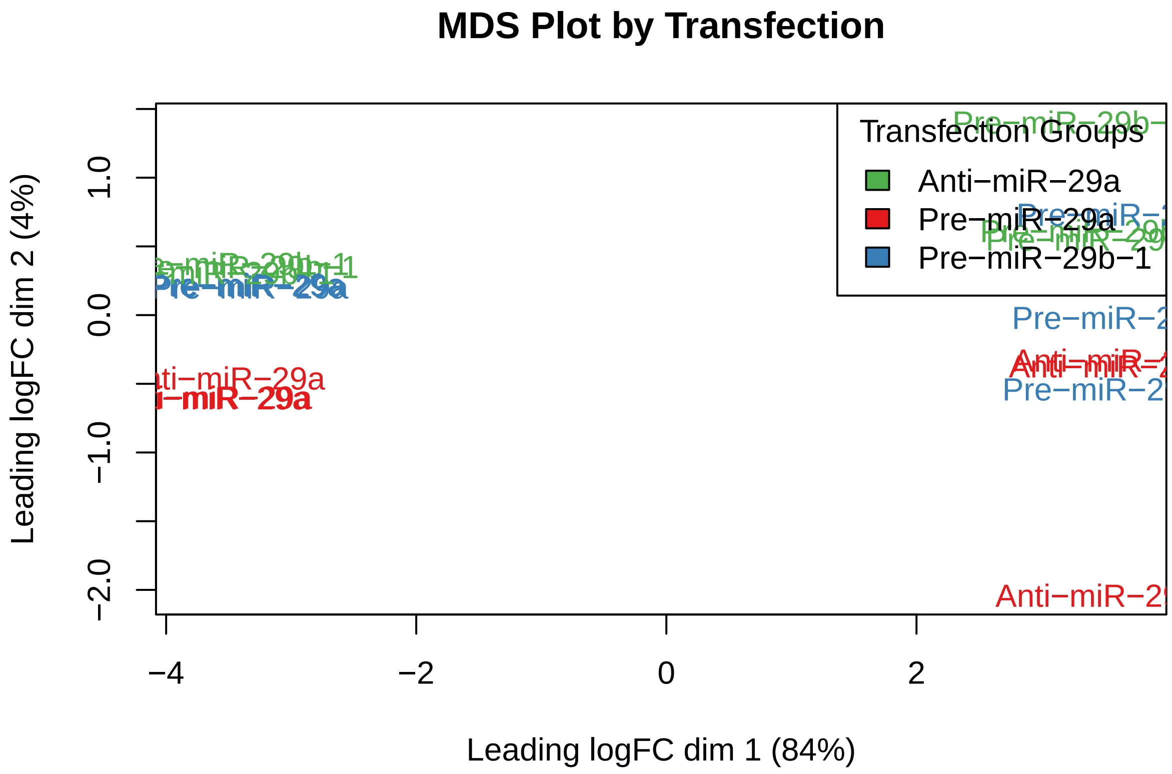
```
## Warning in brewer.pal(nlevels(col.group), "Set1"): minimal value for n is 3, returning requested pal
```

```r
col.group <- as.character(col.group)

## MDS por grupos de Cell_line
plotMDS(vGene$E, labels = df$Cell_line, col = col.group)
```



```r
## Para colores
library("RColorBrewer")

## Convertir Transfection a colores
col.group <- df$Transfection
df$Transfection <- as.factor(df$Transfection) # Asegúrate de que sea un factor
colors <- brewer.pal(nlevels(df$Transfection), "Set1") # Generar paleta de colores
levels(col.group) <- colors # Asignar colores a los niveles
col.group <- as.character(col.group) # Convertir a vector de caracteres

## MDS por grupos de Transfection
plotMDS(vGene$E, labels = df$Transfection, col = col.group,
        main = "MDS Plot by Transfection", pch = 16)
```

```
## Agregar leyenda
legend("topright", legend = levels(df$Transfection), fill = unique(col.group),
       title = "Transfection Groups")
```


MDS Plot by Transfection
Transfection Groups
🟩 Anti−miR−29a
🟥 Pre−miR−29a
🟦 Pre−miR−29b−1
Pre−miR−29b−1
Pre−miR−29a
ti−miR−29a
i−miR−29a
Pre−miR−29b−
Pre−miR−2
Pre−miR−29
Anti−miR−2
Anti−miR−29
Leading logFC dim 2 (4%)
Leading logFC dim 1 (84%)