Heatmap y PCA

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2025-04-03

```r
if(!require("pacman"))
  install.packages("pacman")
```

```
## Loading required package: pacman
```

```r
library("pacman")

p_load("pheatmap",
       "RColorBrewer",
       "ggplot2",
       "dplyr",
       "vroom",
       "FactoMineR",
       "factoextra",
       "tibble")
```

```r
Datos_PCR <- vroom("https://raw.githubusercontent.com/ManuelLaraMVZ/Heatmaps/refs/heads/main/miRNA_qPCR_
```

```
## `curl` package not installed, falling back to using `url()`
## Rows: 22 Columns: 10
## -- Column specification -----------------------------------------------------
## Delimiter: ","
## chr (2): Gene, Condition
## dbl (8): Control_1, Control_2, Control_3, Control_4, Tratamiento_1, Tratamie...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
head(Datos_PCR)
```

```
## # A tibble: 6 x 10
##   Gene  Condition Control_1 Control_2 Control_3 Control_4 Tratamiento_1
##   <chr> <chr>         <dbl>     <dbl>     <dbl>     <dbl>         <dbl>
## 1 Gen_1 Target         26.4      26.8      28.6      27.1          30.1
## 2 Gen_2 Target         29.3      29.6      31.2      30.4          27.4
## 3 Gen_3 Target         27.5      25.0      27.7      26.5          28.9
## 4 Gen_4 Target         29.4      28.3      30.8      30.2          25.9
## 5 Gen_5 Target         27.9      27.9      27.8      27.7          30.6
## 6 Gen_6 Target         29.3      29.8      28.7      32.2          28.2
## # i 3 more variables: Tratamiento_2 <dbl>, Tratamiento_3 <dbl>,
## #   Tratamiento_4 <dbl>
```

```r
Ref_gen_prom <- Datos_PCR %>%
  filter(Condition== "Reference") %>%
  select(-1,-2) %>%
```

```r
  summarise(across(everything(),mean, na.rm = T))
```

```
## Warning: There was 1 warning in `summarise()`.
## i In argument: `across(everything(), mean, na.rm = T)`.
## Caused by warning:
## ! The `...` argument of `across()` is deprecated as of dplyr 1.1.0.
## Supply arguments directly to `.fns` through an anonymous function instead.
##
##   # Previously
##   across(a:b, mean, na.rm = TRUE)
##
##   # Now
##   across(a:b, \(x) mean(x, na.rm = TRUE))
```

```r
head (Ref_gen_prom)
```

```
## # A tibble: 1 x 8
##   Control_1 Control_2 Control_3 Control_4 Tratamiento_1 Tratamiento_2
##       <dbl>     <dbl>     <dbl>     <dbl>         <dbl>         <dbl>
## 1      25.9      24.5      25.2      25.0          24.8          25.3
## # i 2 more variables: Tratamiento_3 <dbl>, Tratamiento_4 <dbl>
```

```r
DCt <- Datos_PCR %>%
  filter(Condition == "Target") %>%
  select(-2) %>%
  mutate(across(-1, ~ -(. - Ref_gen_prom[[cur_column()]][[1]]),
                .names = "DCt_{.col}")) %>%
  select(Gene, starts_with("DCt_"))
head (DCt)
```

```
## # A tibble: 6 x 9
##   Gene  DCt_Control_1 DCt_Control_2 DCt_Control_3 DCt_Control_4
##   <chr>         <dbl>         <dbl>         <dbl>         <dbl>
## 1 Gen_1        -0.498         -2.29         -3.32         -2.12
## 2 Gen_2        -3.37          -5.07         -5.98         -5.41
## 3 Gen_3        -1.56          -0.550        -2.46         -1.57
## 4 Gen_4        -3.43          -3.83         -5.59         -5.20
## 5 Gen_5        -1.95          -3.39         -2.58         -2.73
## 6 Gen_6        -3.36          -5.31         -3.49         -7.21
## # i 4 more variables: DCt_Tratamiento_1 <dbl>, DCt_Tratamiento_2 <dbl>,
## #   DCt_Tratamiento_3 <dbl>, DCt_Tratamiento_4 <dbl>
```

```r
miRNA_escalado <- DCt %>%
  column_to_rownames(var = "Gene") %>%
  scale(center = T,
        scale = T) %>%
  as.data.frame()

miRNA_escalado
```

```
##       DCt_Control_1 DCt_Control_2 DCt_Control_3 DCt_Control_4
## Gen_1     1.0273615    0.97484827    0.12356681    0.88604034
## Gen_2    -0.6633067   -0.71178341   -1.42820012   -1.00751528
## Gen_3     0.4047056    2.02664135    0.62271304    1.19880193
## Gen_4    -0.6996741    0.03993184   -1.20330127   -0.88867348
## Gen_5     0.1709725    0.30352416    0.55271855    0.53020016
```

```
## Gen_6       -0.6586858    -0.85578969     0.02116144    -2.04898477
## Gen_7        0.2387261     0.88592374     0.88355813     0.94306346
## Gen_8       -0.1562162    -1.33584014    -0.78765321    -0.92469228
## Gen_9        1.3281891     0.65157277     0.77009342     0.89611697
## Gen_10      -1.6591265    -0.55215317    -0.31499077    -1.39077216
## Gen_11       0.6942198     0.60205424     1.24683527     0.55568146
## Gen_12      -0.8756513    -1.67758263    -1.29395881    -1.11607700
## Gen_13      -0.5892852    -0.09290454     1.16826244     1.51751002
## Gen_14      -0.5075344    -0.95445486    -0.25857850     0.15980360
## Gen_15       1.6494768     0.86908048     0.72864265     0.75326487
## Gen_16      -1.1366262    -0.40782637    -0.42994585    -0.65295644
## Gen_17       1.2635102     0.87861819     0.19002870     0.66671244
## Gen_18      -0.2080565    -1.42992863    -1.82701632     0.03082536
## Gen_19       1.6398595     1.15700531     1.88206557     0.53061664
## Gen_20      -1.2628582    -0.37093692    -0.64600120    -0.63896584
##          DCt_Tratamiento_1 DCt_Tratamiento_2 DCt_Tratamiento_3 DCt_Tratamiento_4
## Gen_1         -0.81362577       -1.55535151       -1.283472848        -0.2403860
## Gen_2          0.81618925        0.69362505        1.152347005        -0.2686596
## Gen_3         -0.09855941       -0.61117243       -0.381782614        -0.5320576
## Gen_4          1.73541997        0.13527056        0.556662808         0.8639210
## Gen_5         -1.06726846       -0.68740035       -0.818426389        -0.7215950
## Gen_6          0.33403296        1.29626131        1.059592095         0.9572610
## Gen_7         -0.71079112       -1.38612435       -0.867055780        -1.7535139
## Gen_8          0.82881193        0.99304339        1.017338673         1.2575005
## Gen_9         -1.28729356       -1.71898941       -0.706198163         0.3275990
## Gen_10         1.22568310        1.34393777        0.705331833         0.7789604
## Gen_11        -0.60469632       -0.87969712       -1.669105648        -1.1653025
## Gen_12         0.91297985        1.05438330       -0.009842471         1.0299405
## Gen_13        -0.31205414       -0.84311362       -0.854369049        -0.7395078
## Gen_14         1.28270001        0.29881009        1.164175623         0.3726777
## Gen_15        -0.79952197       -0.40469254       -0.488693550        -0.3714511
## Gen_16        -0.04580695        1.06612733        0.672535063         0.6609948
## Gen_17        -0.76102830       -0.51127520        0.241152286        -1.5440049
## Gen_18         0.63638628        0.87575564        1.768647889         1.5273701
## Gen_19        -1.99084945       -0.08900012       -1.481662833        -1.3469197
## Gen_20         0.71929209        0.92960218        0.222826070         0.9071733
```

```r
paleta_colores <- colorRampPalette(c("#2153ab", "white", "#d2d801"))(100)
```

```r
Heatmap <- pheatmap(miRNA_escalado,
                color = paleta_colores,
                cluster_rows = T,
                cluster_cols = T,
                show_rownames = T,
                show_colnames = T,
                fontsize_row= 5,
                fontsize_col = 8,
                border_color = "black",
                main = "Heatmap de expresión de miRNAs",
                fontface_row = "bold")
Heatmap
```

Heatmap de expresión de miRNAs
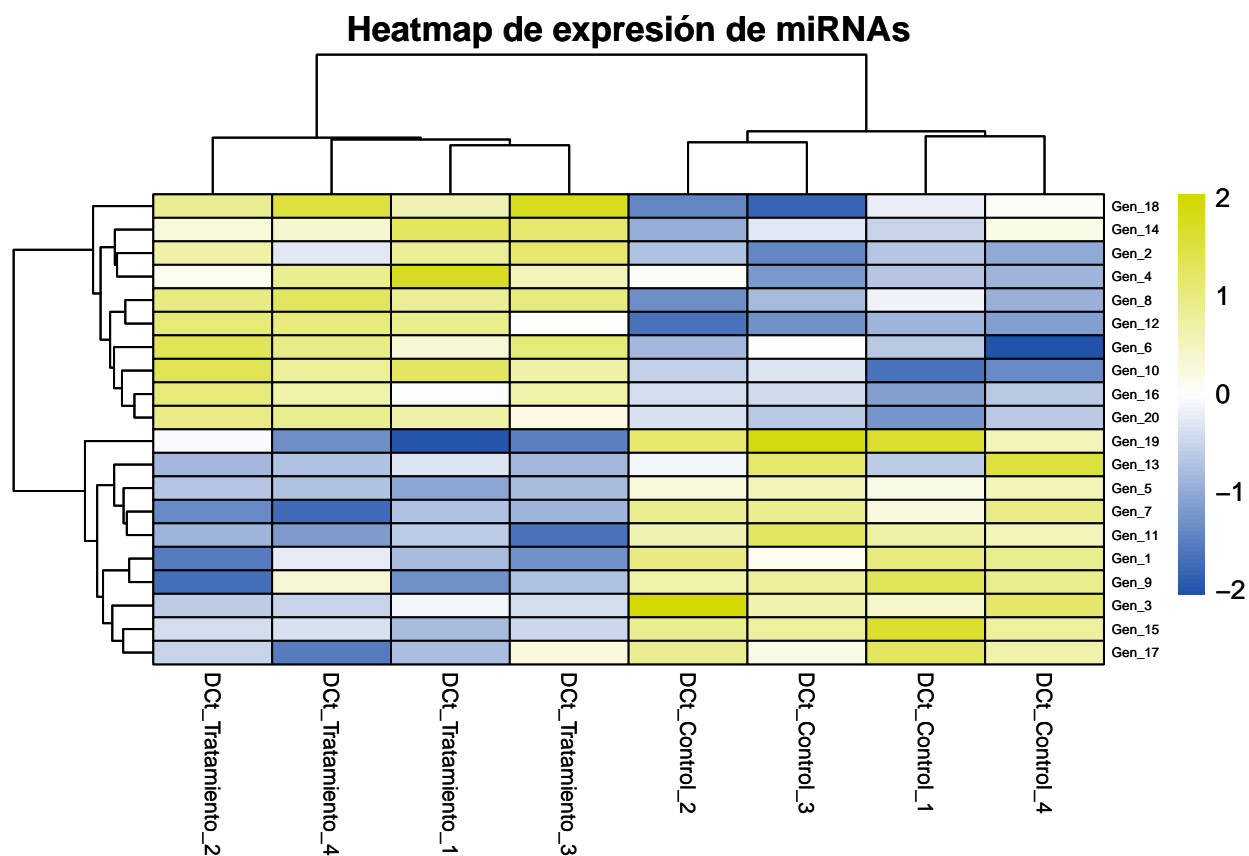

```r
PCA_resultados <- prcomp(t(miRNA_escalado),
                         center = TRUE,
                         scale. = T)
summary (PCA_resultados)
```
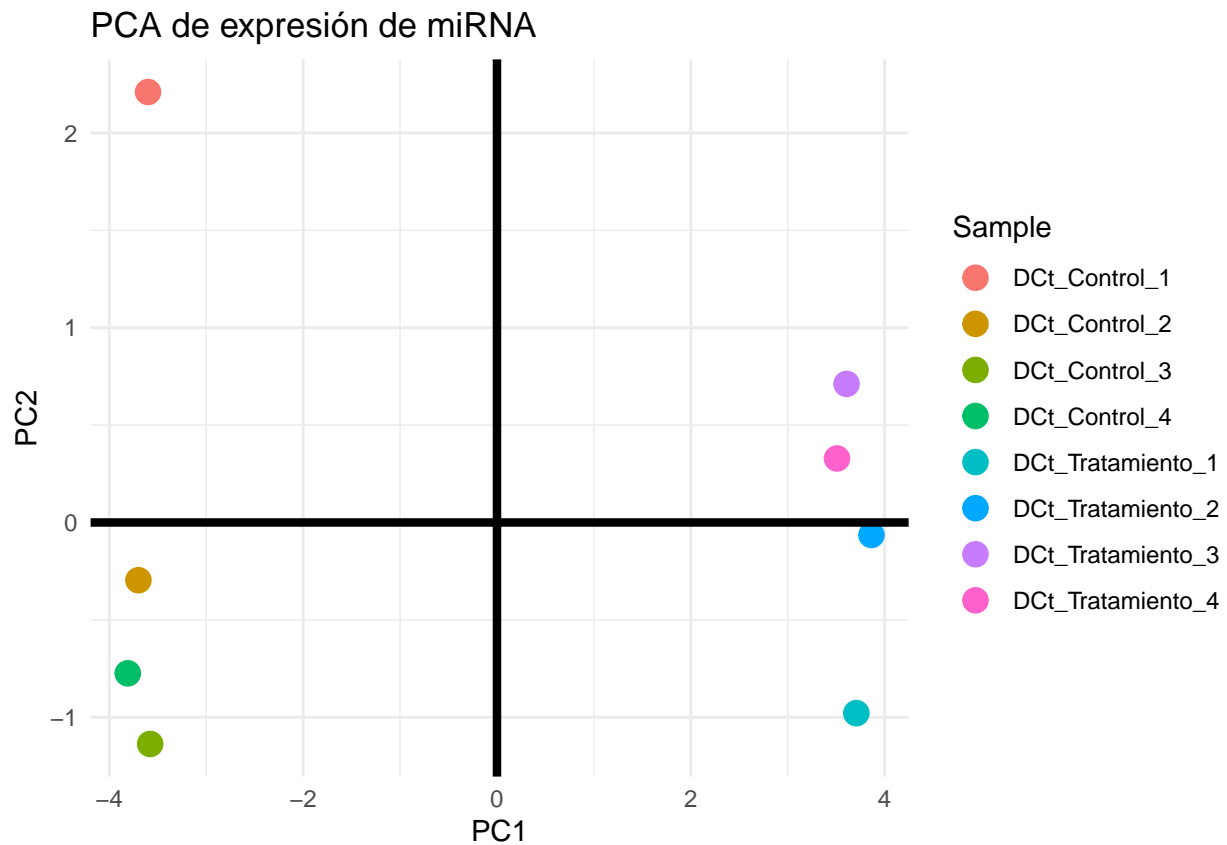
```
## Importance of components:
##                          PC1    PC2     PC3     PC4     PC5     PC6     PC7
## Standard deviation     3.9273 1.0981 1.06384 0.93134 0.86565 0.63804 0.46315
## Proportion of Variance 0.7712 0.0603 0.05659 0.04337 0.03747 0.02035 0.01073
## Cumulative Proportion  0.7712 0.8315 0.88808 0.93145 0.96892 0.98927 1.00000
##                          PC8
## Standard deviation     2.09e-16
## Proportion of Variance 0.00e+00
## Cumulative Proportion  1.00e+00
```

```r
fviz_eig(PCA_resultados,
         addlabels = T,
         barfill = "#CD5C5C",
         barcolor = "#FF00FF")
```

Scree plot
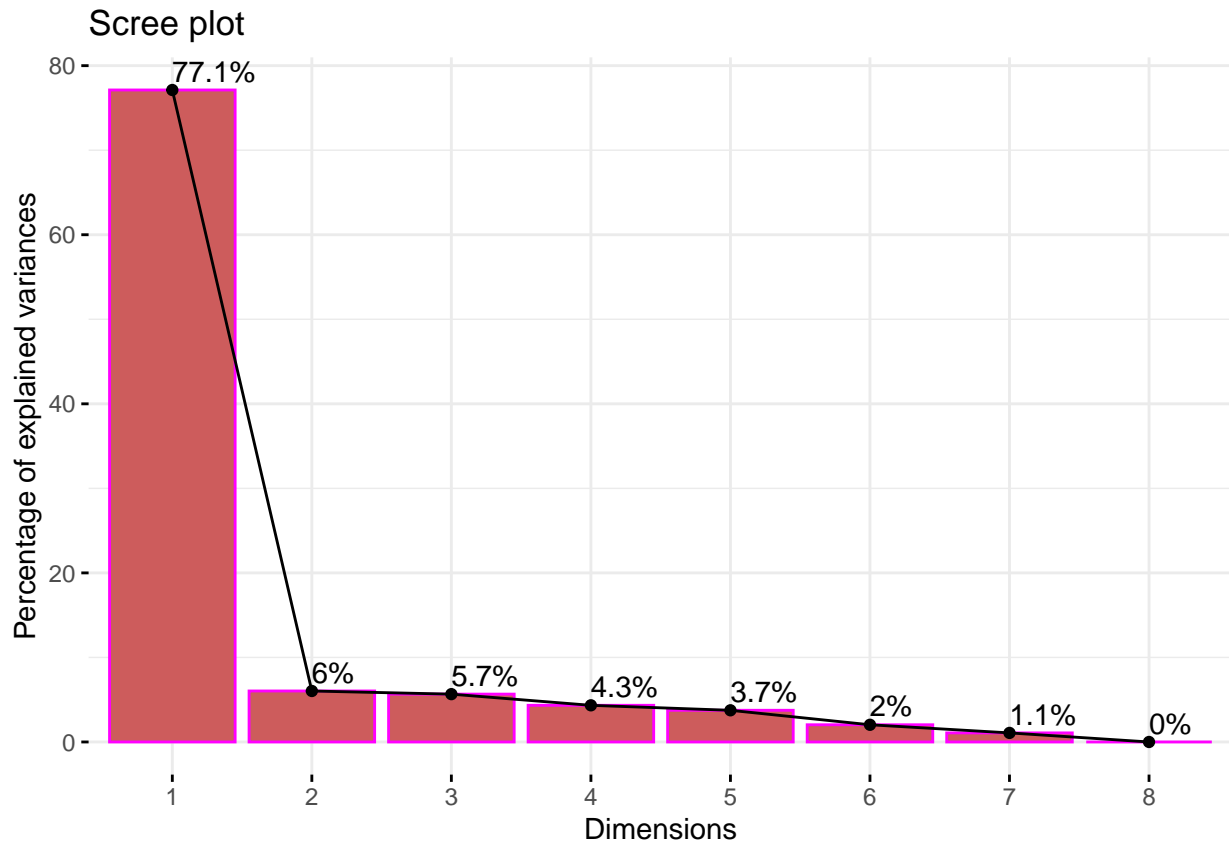
```
PCA_df <- as.data.frame(PCA_resultados $x)
PCA_df$Sample <- row.names(PCA_df)
```

```
PCA_plot <- ggplot(PCA_df,
                   aes(x = PC1,
                       y = PC2,
                       label = Sample))+
  geom_point(size = 4, aes(color = Sample))+
  #geom_text(vjust = -0.1, size = 3)+
  geom_hline(yintercept = 0, linetype = "solid", color = "black", linewidth = 1.5)+
  geom_vline(xintercept = 0, linetype = "solid", color = "black", linewidth = 1.5)+
  labs(title = "PCA de expresión de miRNA",
       x= "PC1",
       y= "PC2")+
  theme_minimal()
PCA_plot
```

PCA de expresión de miRNA


```r
PCA_resultados_genes <- prcomp(miRNA_escalado,
                               center = T,
                               scale. = T)
summary(PCA_resultados_genes)
```

```
## Importance of components:
##                           PC1     PC2     PC3     PC4     PC5    PC6    PC7
## Standard deviation     2.4551 0.73337 0.68254 0.57125 0.50573 0.4317 0.3359
## Proportion of Variance 0.7534 0.06723 0.05823 0.04079 0.03197 0.0233 0.0141
## Cumulative Proportion  0.7534 0.82066 0.87890 0.91969 0.95166 0.9750 0.9891
##                           PC8
## Standard deviation     0.29582
## Proportion of Variance 0.01094
## Cumulative Proportion  1.00000
```

```r
fviz_eig(PCA_resultados_genes, addlabels = T, barfill = "#CD5C5C",
         barcolor = "#FF00FF")
```

Scree plot
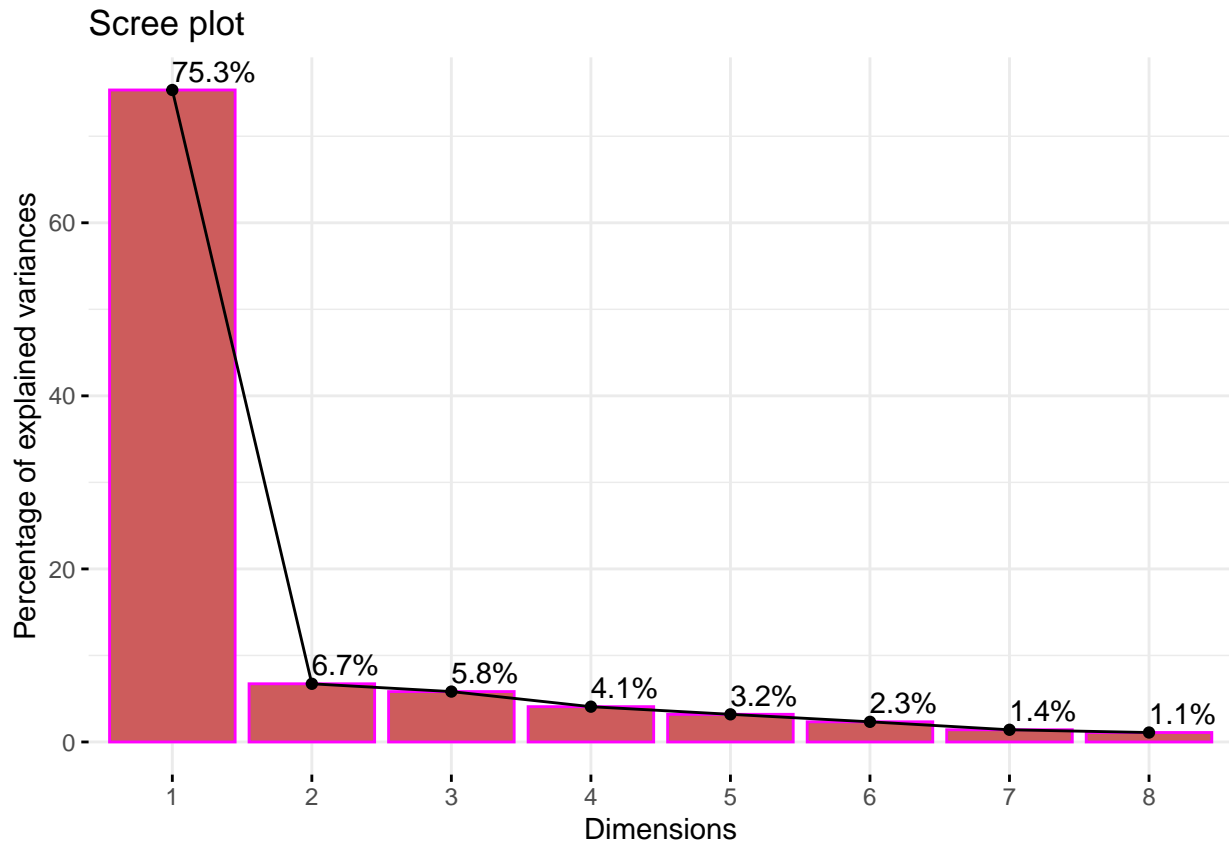

```
PCA_df_genes <- as.data.frame(PCA_resultados_genes$x)
PCA_df_genes$Gene <- row.names(PCA_df_genes)
```

```
PCA_plot_genes <- ggplot(PCA_df_genes,
                         aes(x = PC1,
                             y = PC2,
                             label = Gene))+
  geom_point(size = 4, aes(color = Gene), show.legend = F)+
  geom_text(vjust = -0.05, size = 3)+
  geom_hline(yintercept = 0, linetype = "solid", color = "black", linewidth = 1.5)+
  geom_vline(xintercept = 0, linetype = "solid", color = "black", linewidth = 1.5)+
  labs(title = "PCA de expresión de miRNA",
       x= "PC1",
       y= "PC2")+
  theme_minimal()

PCA_plot_genes
```

PCA de expresión de miRNA