



Laboratório de
Imunobiologia

RNA-Seq

Aula 4: Análise Diferencial e Visualização

<http://bit.ly/2IXMbRn>

Edgar Kozlova
Gabriela Luiz

O Curso

Pré requisitos:

Notebook, WiFi, Notepad++, R e Rstudio

Programação das aulas:

1. Banco de dados:

NCBI/SRA
NCBI/GEO

2. RStudio e Instalação de pacotes

edgeR, limma, pheatmap, gplots, ROTS

3. Normalização e Análise Diferencial

voom, RPKM, FPKM, TPM, CPM, counts

4. Análise Diferencial e Visualização

Script, MAplot, VolcanoPlot, Heatmap, Venn

Objetivo

Terminar a análise diferencial

efit

tfit

Explicar as principais visualizações

MAplot

VolcanoPlot

Heatmap

Venn

Aplicar as funções do ROTS

VISUALIZAÇÕES

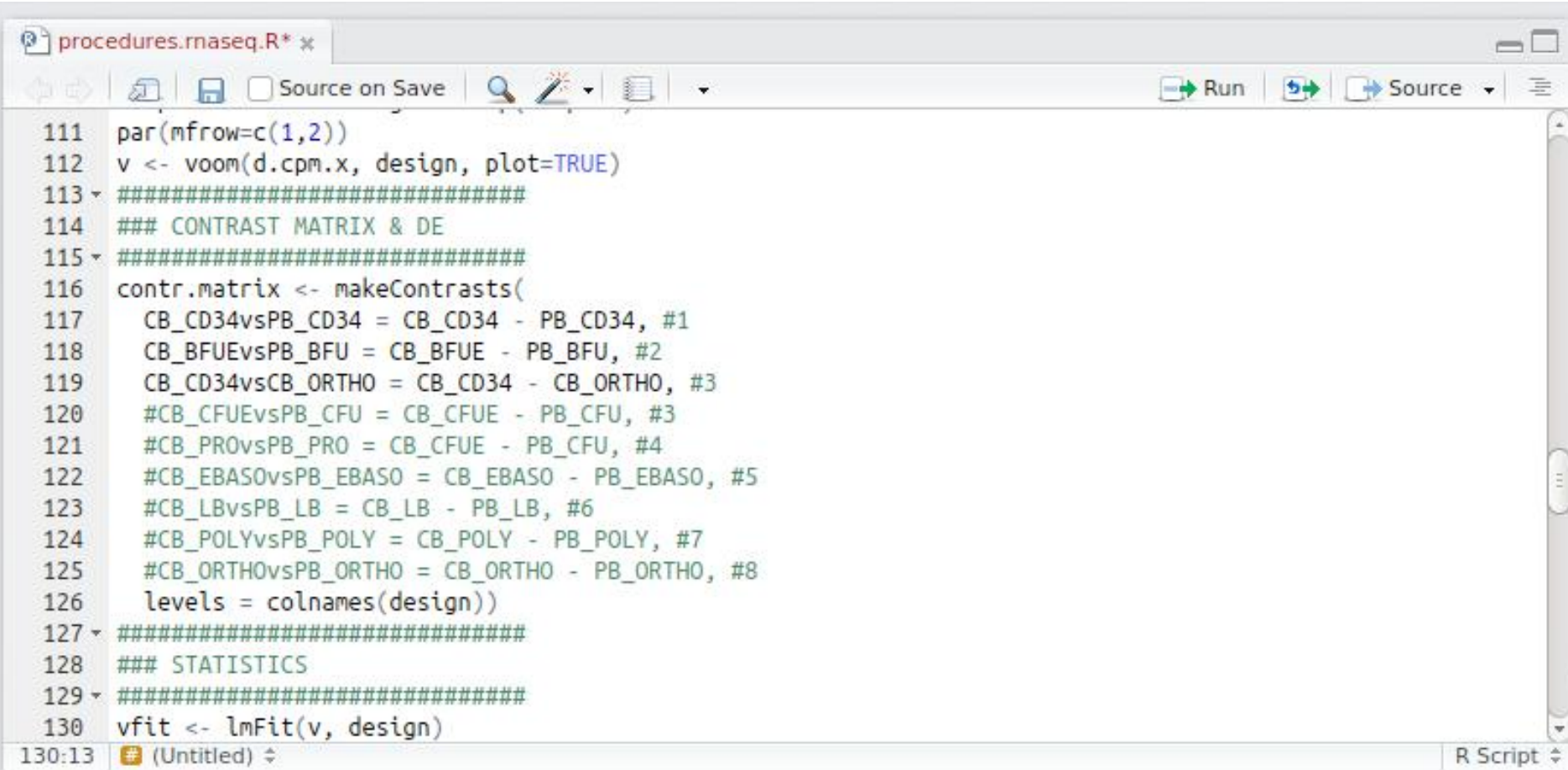
MAplot

VolcanoPlot

Heatmap

Venn

procedures.rnaseq.R



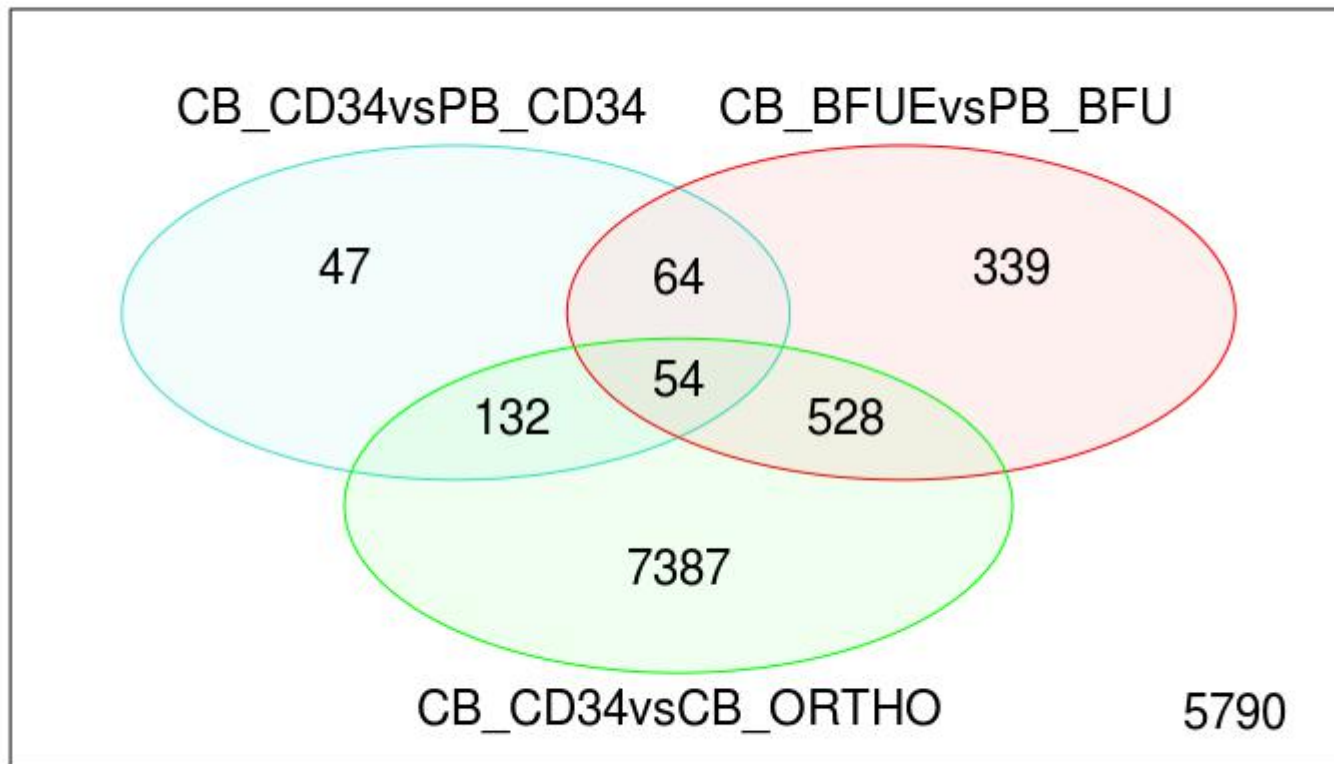
```
111 par(mfrow=c(1,2))
112 v <- voom(d.cpm.x, design, plot=TRUE)
113 #####
114 ### CONTRAST MATRIX & DE
115 #####
116 contr.matrix <- makeContrasts(
117   CB_CD34vsPB_CD34 = CB_CD34 - PB_CD34, #1
118   CB_BFUEvsPB_BFU = CB_BFUE - PB_BFU, #2
119   CB_CD34vsCB_ORTHO = CB_CD34 - CB_ORTHO, #3
120   #CB_CFUEvsPB_CFU = CB_CFUE - PB_CFU, #3
121   #CB_PROvsPB_PRO = CB_CFUE - PB_CFU, #4
122   #CB_EBASOvsPB_EBASO = CB_EBASO - PB_EBASO, #5
123   #CB_LBvsPB_LB = CB_LB - PB_LB, #6
124   #CB_POLYvsPB_POLY = CB_POLY - PB_POLY, #7
125   #CB_ORTHOvsPB_ORTHO = CB_ORTHO - PB_ORTHO, #8
126   levels = colnames(design))
127 #####
128 ### STATISTICS
129 #####
130 vfit <- lmFit(v, design)
```

130:13 # (Untitled) ↕ R Script ↕

procedures.rnaseq.R

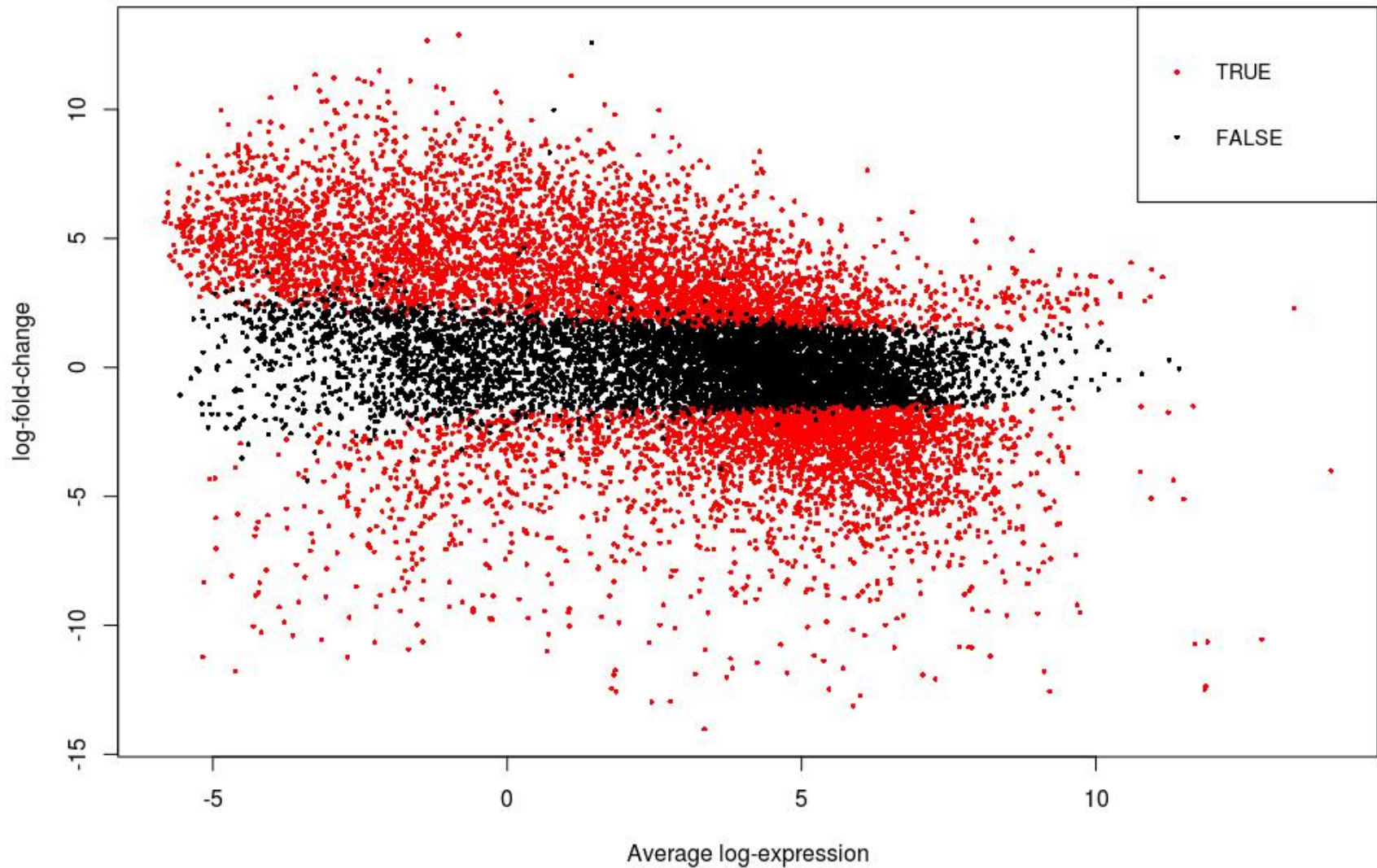
```
Console ~/Dropbox/Docencia/AulaRNASeq/ ↵
> vfit <- contrasts.fit(vfit, contrasts=contr.matrix)
> efit <- eBayes(vfit)
> summary(decideTests(efit))
      CB_CD34vsPB_CD34 CB_BFUEvsPB_BFU CB_CD34vsCB_ORTHO
Down              2585              3395              5107
NotSig            8805              7767              2759
Up                2951              3179              6475
> tfit <- treat(vfit, lfc=1)
> dt <- decideTests(tfit)
> summary(dt)
      CB_CD34vsPB_CD34 CB_BFUEvsPB_BFU CB_CD34vsCB_ORTHO
Down               106               735              3444
NotSig            14044             13356              6240
Up                 191               250              4657
> write.fit(tfit, dt, file="results.csv", sep = ";")
> |
```

Diagrama de Venn

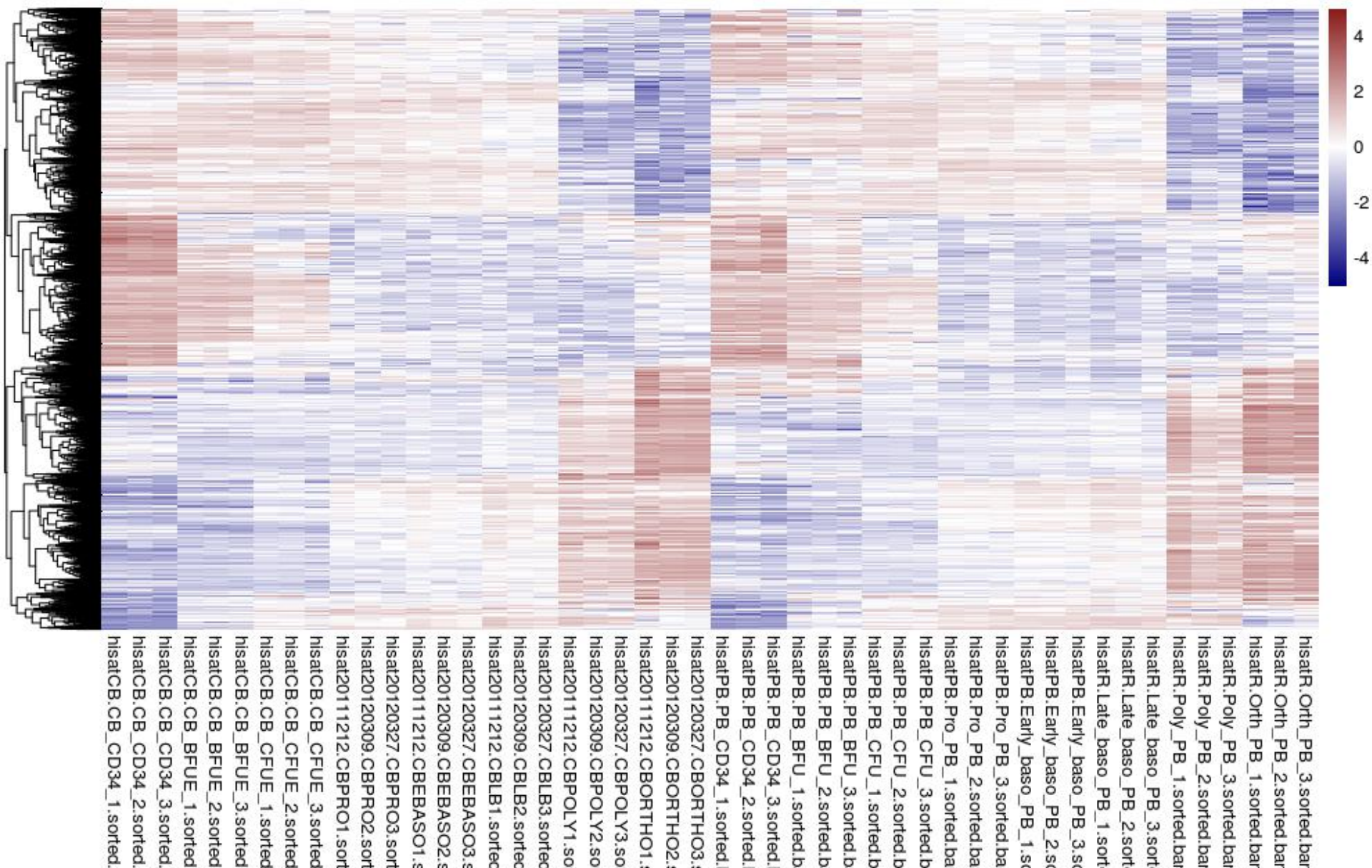


MA plot

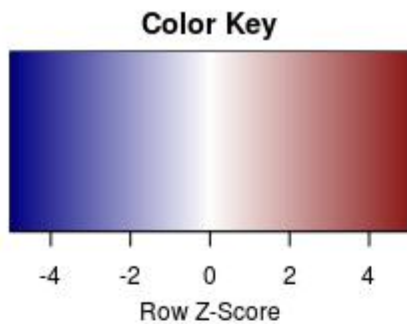
CB_CD34.vs.CB_ORTHO



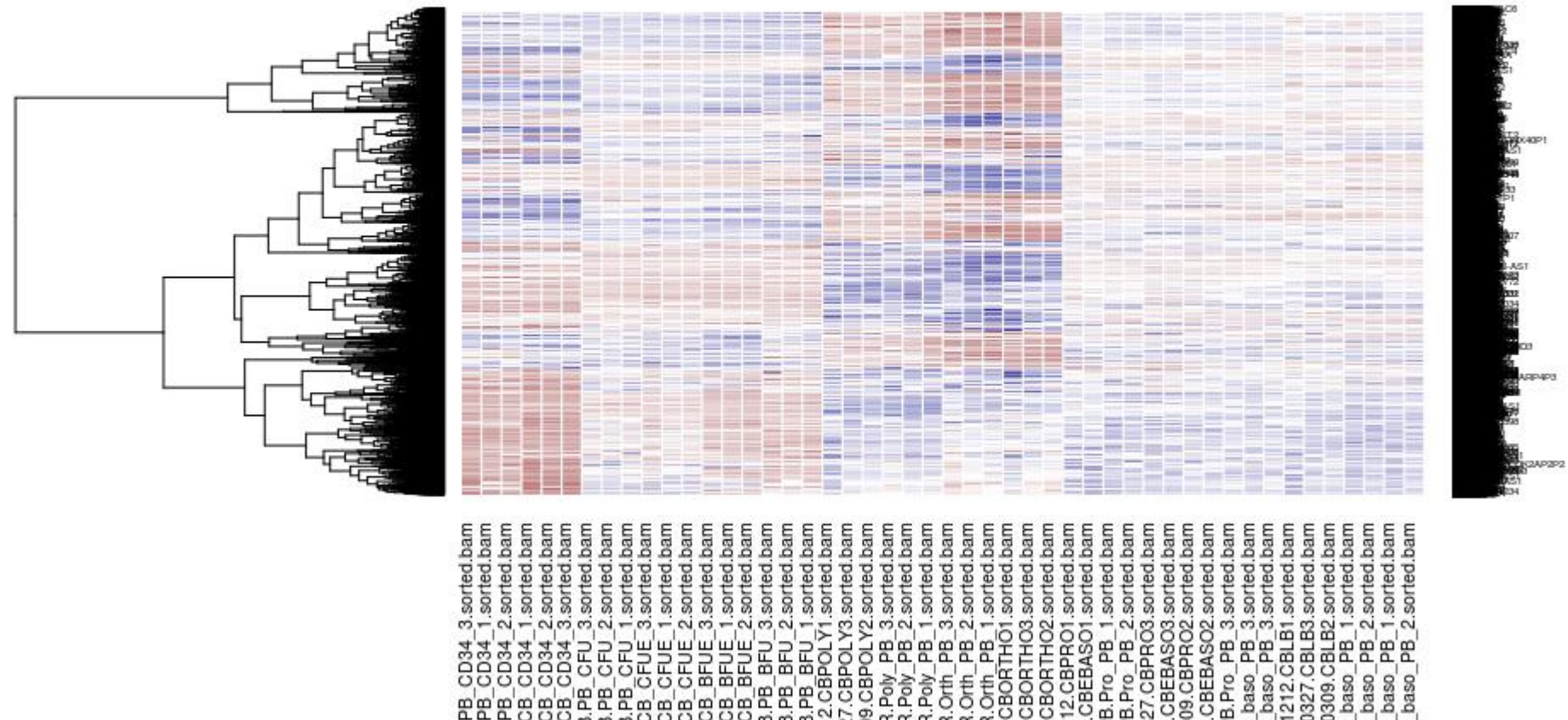
Heatmap



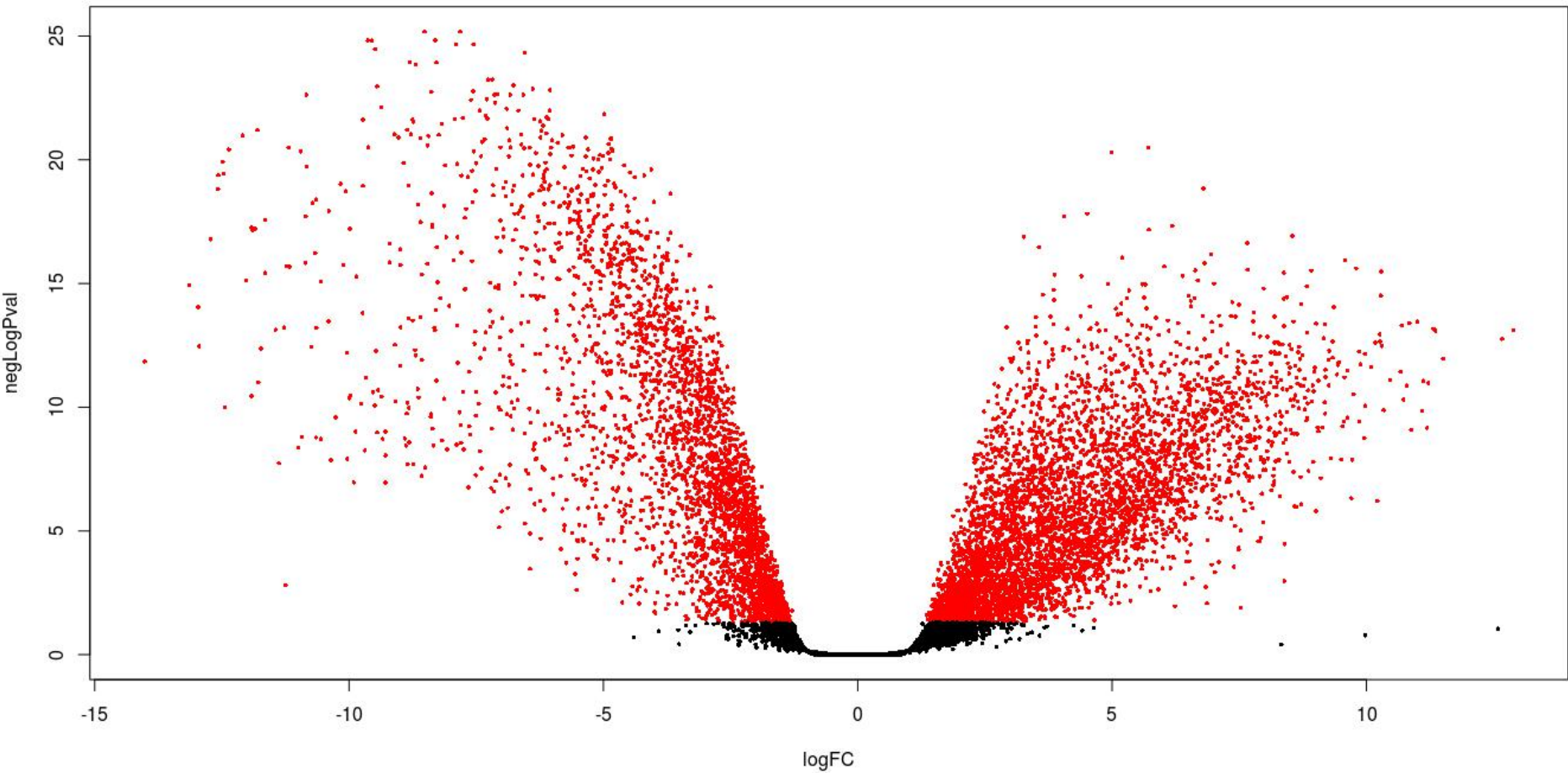
Heatmap



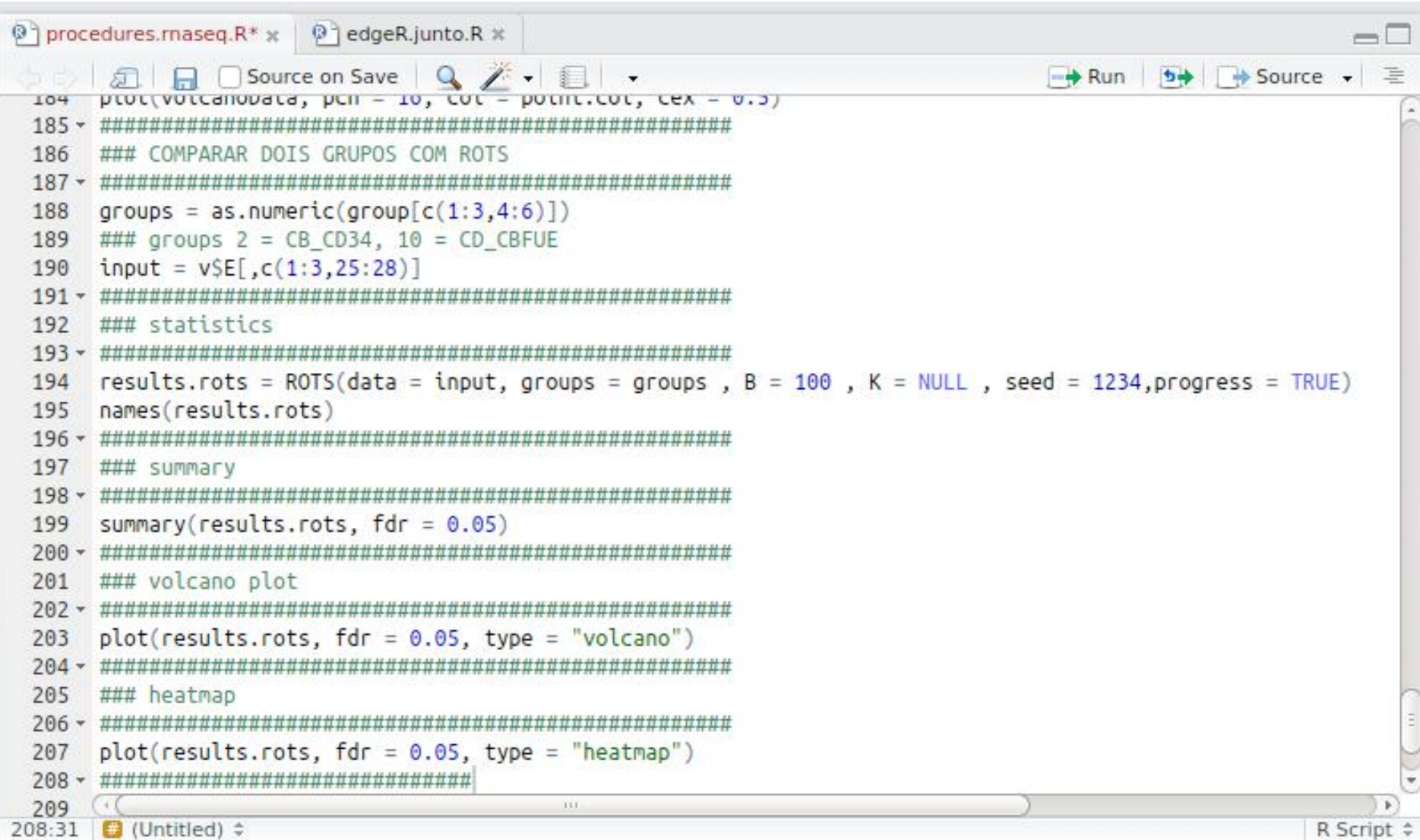
CB_CD34 vs CB_ORTHO Top Genes



Volcano plot



ROTS

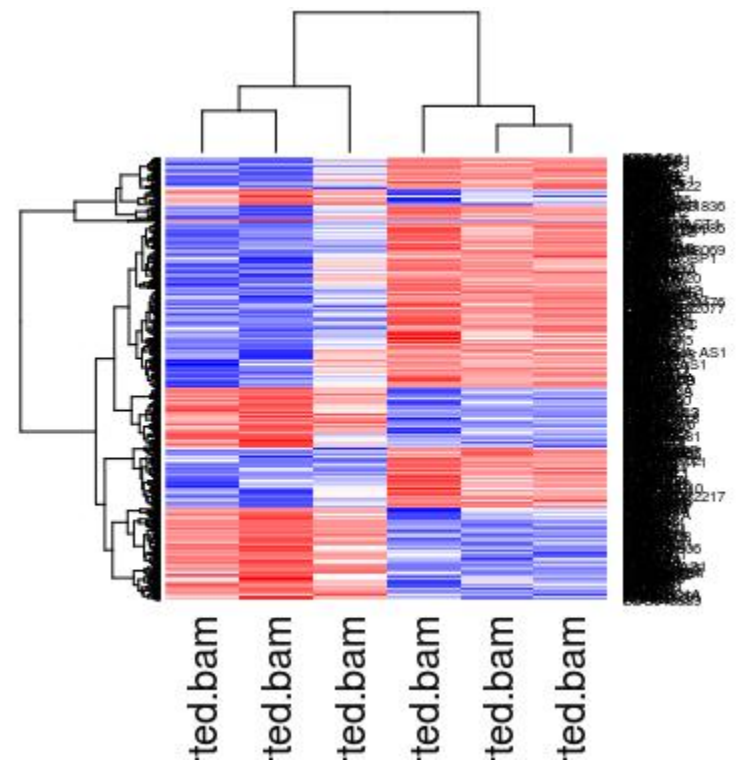
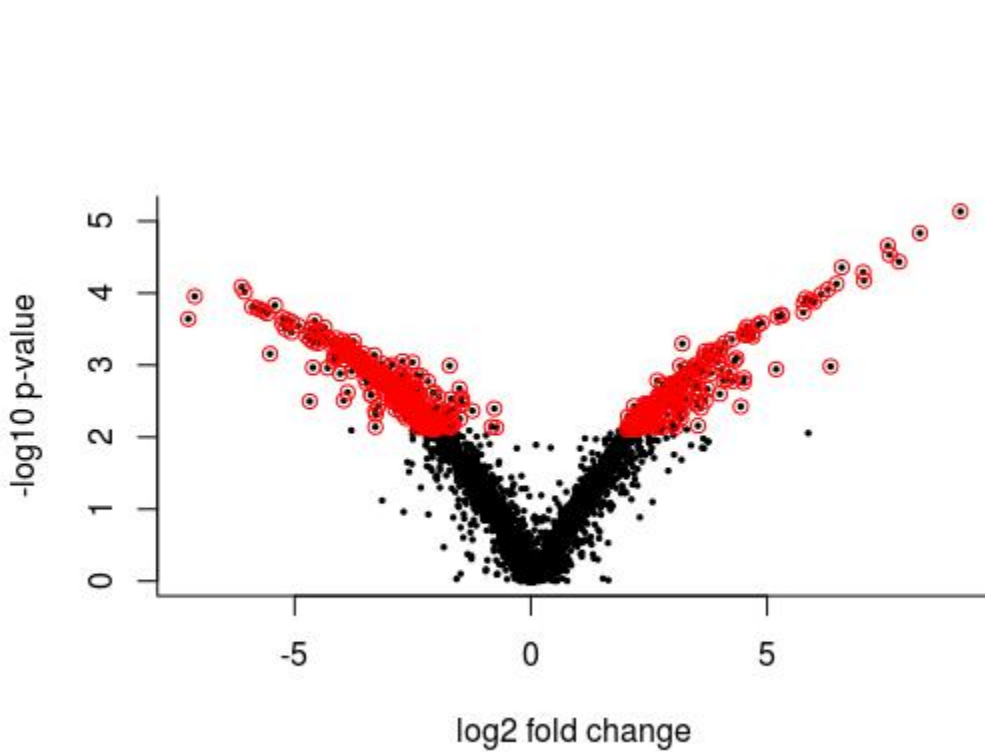


The screenshot shows an RStudio editor window with two tabs: 'procedures.maseq.R*' and 'edgeR.junto.R*'. The active tab is 'procedures.maseq.R*', which contains an R script. The script starts with a plot of volcano data, followed by a series of green hash markers. It then defines two groups (1:3 and 4:6), sets input data, and performs a ROTS analysis with parameters B=100, K=NULL, seed=1234, and progress=TRUE. The results are summarized, and two plots are generated: a volcano plot and a heatmap, both with an FDR of 0.05. The script ends with another series of green hash markers. The status bar at the bottom indicates the current line is 208:31 and the file is '(Untitled)'.

```
184 plot(volcanodata, pcut = 10, col = point.col, cex = 0.5)
185 #####
186 ### COMPARAR DOIS GRUPOS COM ROTS
187 #####
188 groups = as.numeric(group[c(1:3,4:6)])
189 ### groups 2 = CB_CD34, 10 = CD_CBFUE
190 input = v$E[,c(1:3,25:28)]
191 #####
192 ### statistics
193 #####
194 results.rots = ROTS(data = input, groups = groups , B = 100 , K = NULL , seed = 1234,progress = TRUE)
195 names(results.rots)
196 #####
197 ### summary
198 #####
199 summary(results.rots, fdr = 0.05)
200 #####
201 ### volcano plot
202 #####
203 plot(results.rots, fdr = 0.05, type = "volcano")
204 #####
205 ### heatmap
206 #####
207 plot(results.rots, fdr = 0.05, type = "heatmap")
208 #####
209
```

208:31 (Untitled) R Script

ROTS



DÚVIDAS?