

# Volcano plot

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## Introduction

The RNA seq data provides information on the differential gene expression under biofilm and planktonic conditions in *Salmonella enterica serovar Enteritidis*. The RNA extraction was carried out using Qiagen RNA extraction kit and the samples were processed via Illumina Miseq for transcriptomic studies. The raw reads were processed and the DESeq2 package was used for normalization, visualization, and differential analysis of differentially expressed genes.

## Volcano Plot

This plot identifies that genes that are upregulated and downregulated under a the given conditions.

```
#Read the table
RNA_Data <- read.table("results.txt", header=TRUE, sep = '\t')
head(RNA_Data)

##           Gene log2FoldChange      pvalue      padj
## 1  gene-16S rRNA      5.798025 0.012471394 0.071767204
## 2  gene-16S rRNA-2     8.412940 0.001014382 0.008232104
## 3  gene-16S rRNA-3     1.571388 0.398503872 0.713176495
## 4  gene-16S rRNA-4     5.923779 0.003882010 0.027183085
## 5  gene-16S rRNA-5     3.195232 0.213500712 0.522375292
## 6  gene-16S rRNA-6     5.898077 0.003883298 0.027183085

# Making volcano plot

library(BiocManager)

## Bioconductor version '3.12' is out-of-date; the current release version '3.13'
##   is available with R version '4.1'; see https://bioconductor.org/install

library(EnhancedVolcano)

## Warning: package 'EnhancedVolcano' was built under R version 4.0.3

## Loading required package: ggplot2

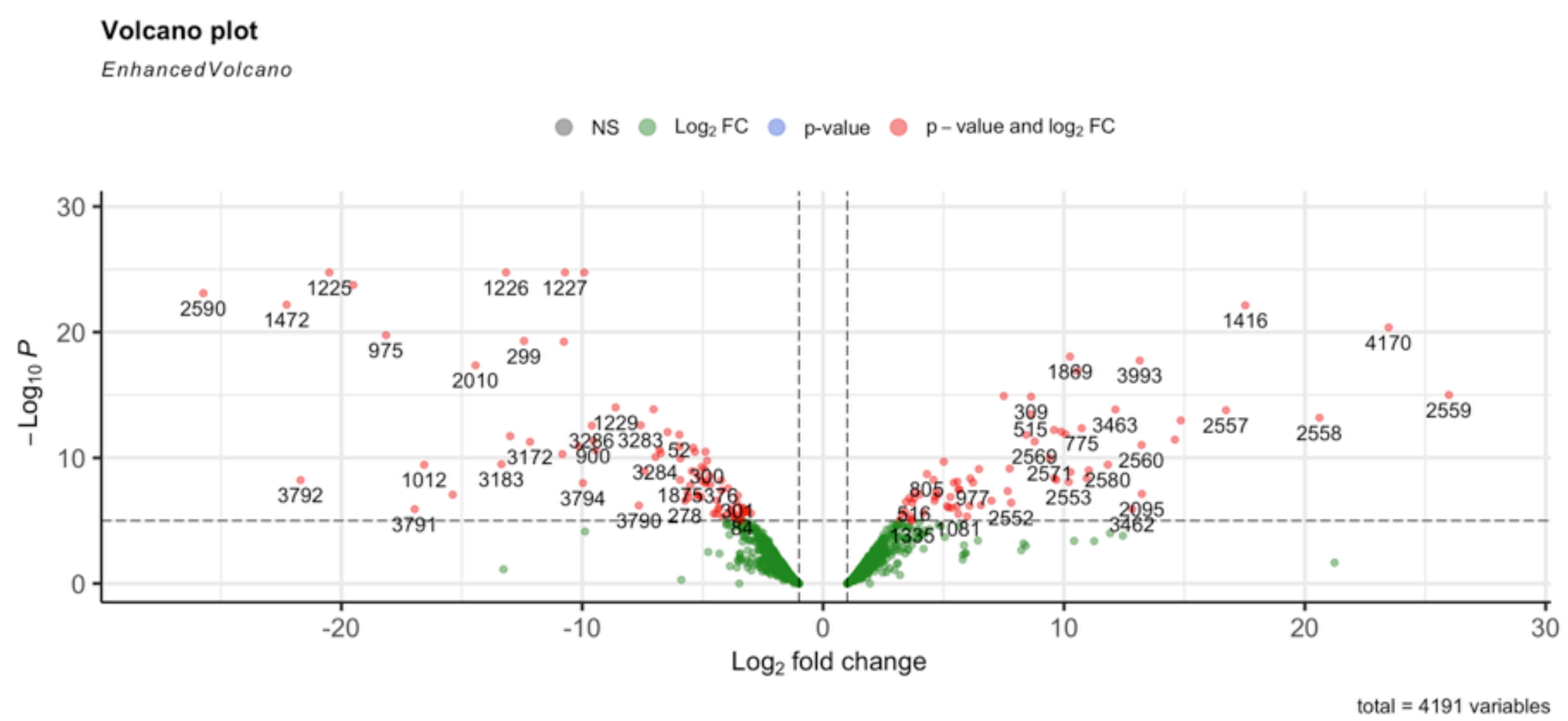
## Loading required package: ggrepel

## Registered S3 methods overwritten by 'ggalt':
##   method                from
##   grid.draw.absoluteGrob ggplot2
##   grobHeight.absoluteGrob ggplot2
##   grobWidth.absoluteGrob  ggplot2
##   grobX.absoluteGrob      ggplot2
##   grobY.absoluteGrob      ggplot2

# Plot 1

EnhancedVolcano(RNA_Data,
  lab = rownames(RNA_Data),
  x = 'log2FoldChange',
  y = 'pvalue')

## Warning: One or more p-values is 0. Converting to 10^-1 * current lowest non-
## zero p-value...
```



```
# Plot 2
keyvals <- ifelse(
  RNA_Data$log2FoldChange < -20.5, 'green',
  ifelse(RNA_Data$log2FoldChange > 15.5, 'red',
    'black'))
keyvals[is.na(keyvals)] <- 'black'
names(keyvals)[keyvals == 'red'] <- 'high'
names(keyvals)[keyvals == 'black'] <- 'mid'
names(keyvals)[keyvals == 'green'] <- 'low'

EnhancedVolcano(RNA_Data,
  lab = rownames(RNA_Data),
  x = 'log2FoldChange',
  y = 'pvalue',
  selectLab = rownames(RNA_Data)[which(names(keyvals) %in% c('high', 'low'))],
  xlab = bquote(~Log[2]~ 'fold change'),
  title = 'Volcano Plot',
  pCutoff = 10e-14,
  FCcutoff = 5.0,
  pointSize = 4.5,
  labSize = 4.5,
  shape = c(6, 4, 2, 11),
  colCustom = keyvals,
  colAlpha = 1,
  legendPosition = 'right',
  legendLabSize = 15,
  legendIconSize = 4.0,
  drawConnectors = TRUE,
  widthConnectors = 1.0,
  colConnectors = 'black',
  arrowheads = FALSE,
  gridlines.major = TRUE,
  gridlines.minor = FALSE,
  border = 'partial',
  borderWidth = 1.0,
  borderColour = 'black')

## Warning: One or more p-values is 0. Converting to 10^-1 * current lowest non-
## zero p-value...
```

