Volcano Plot using ggplot2

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Introduction

This document demonstrates how to generate a volcano plot using `ggplot2` by reading a CSV file that contains gene expression data. The dataset must include at least three mandatory columns:

- `log2FC` (Log2 Fold Change)
- `p_value` (P-value for statistical significance)
- `Gene_symbol` (or Gene EntrezID or Gene ENSEMBL ID)

Each step is explained in detail, with code chunks for clarity.

Installing and Loading Required Libraries

Check if required packages are installed, if not install them

```
if (!requireNamespace("ggplot2", quietly = TRUE)) {
  install.packages("ggplot2")
}

if (!requireNamespace("ggrepel", quietly = TRUE)) {
  install.packages("ggrepel")
}

if (!requireNamespace("dplyr", quietly = TRUE)) {
  install.packages("dplyr")
}
```

Load libraries

```
library(ggplot2)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library(ggrepel)
```

Setting Up Project Directories

```
project_dir <- "/Users/debda22/Projects/core_facility_projects/ggplot_basics"
data_dir <- paste0(project_dir, "/input_data")
result_dir <- paste0(project_dir, "/results")
```

Create directories if they don't exist

```
if (!dir.exists(result_dir)) dir.create(result_dir, recursive = TRUE)
```

Reading the Input Data

```
input_file <- paste0(data_dir, "/test_input_file.csv")
data <- read.csv(input_file)
```

Display first few rows of the dataset

```
head(data)
```

```
##   Gene_symbol    log2FC neg_log10pval log2FC_sq      p_value
## 1       Gene7  2.267283     1.7250171  5.140572 0.0188357482
## 2       Gene9  3.027636     3.8045651  9.166577 0.0001568321
## 3      Gene11  1.957304     0.2616621  3.831041 0.5474417710
## 4      Gene12  3.429968     3.7879732 11.764681 0.0001629396
## 5      Gene13 -2.083291     3.8993947  4.340102 0.0001260681
## 6      Gene18 -3.984683     3.9222951 15.877700 0.0001195928
```

Transforming Data for Visualization

Before plotting, we transform the data: - Convert the `p_value` column to `-log10(p_value)` to emphasize small p-values. - Define upregulated and downregulated genes based on cutoff values. - Reverse the `log2FC` values for visualization.

We define thresholds for classification: - `p_value` cutoff: 0.05 - `log2FC` cutoff: 1

Define cutoffs

```
pval_cutoff <- 0.05
log2fc_cutoff <- 1
```

Classify genes into upregulated, downregulated, or Non-significant

```r
data <- data %>%
  mutate(
    logP = -log10(p_value),
    negLog2FC = -log2FC,
    regulation = case_when(
      p_value < pval_cutoff & negLog2FC > log2fc_cutoff ~ "Upregulated",
      p_value < pval_cutoff & negLog2FC < -log2fc_cutoff ~ "Downregulated",
      TRUE ~ "Non-significant"
    )
  )
```

Selecting Top Genes for Labeling

To highlight important genes, we select the top `n` genes from the upregulated and downregulated groups.

```r
top_n <- 5  # Number of genes to label

top_up <- data %>%
  filter(regulation == "Upregulated") %>%
  arrange(log2FC) %>%
  head(top_n)

top_down <- data %>%
  filter(regulation == "Downregulated") %>%
  arrange(desc(abs(log2FC))) %>%
  head(top_n)

# Combine top genes
top_genes <- bind_rows(top_up, top_down)
```

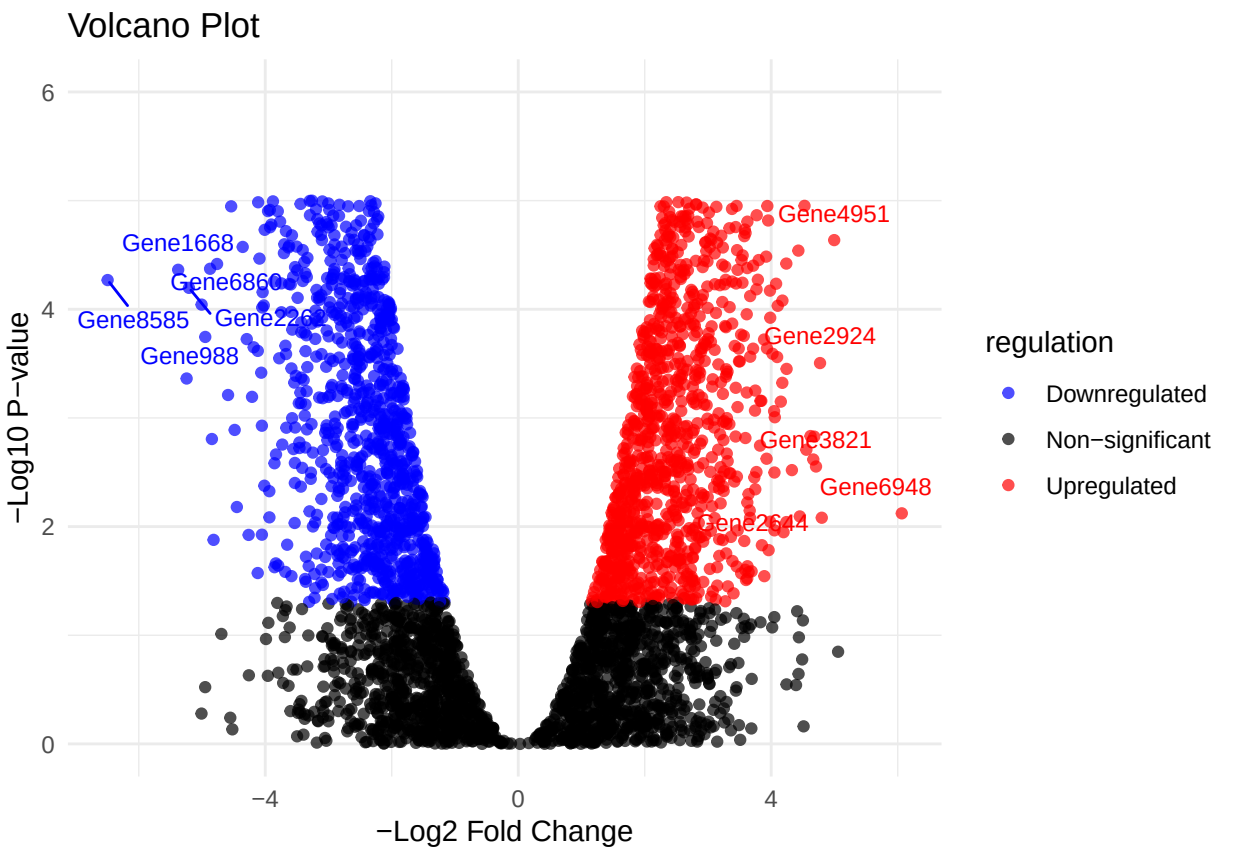
Creating the Volcano Plot

We use `ggplot2` to create a volcano plot with color distinctions:

```r
volcano <- ggplot(data, aes(x = negLog2FC, y = logP, color = regulation)) +
  geom_point(alpha = 0.7) +
  scale_color_manual(values = c("Non-significant" = "black", "Upregulated" = "red", "Downregulated" = "
  labs(title = "Volcano Plot", x = "-Log2 Fold Change", y = "-Log10 P-value") +
  theme_minimal() +
  geom_text_repel(
    data = top_genes,
    aes(label = Gene_symbol),
    vjust = -1,
    size = 3,
    show.legend = FALSE
    ) +
  ylim(c(0,6))
```

Display plot

```
print(volcano)
```



Saving the Plot

```
output_file <- paste0(result_dir, "/volcano_plot.png")
ggsave(output_file, plot = volcano, width = 8, height = 6)
```

Conclusion

This document demonstrated how to load, process, and visualize gene expression data using a volcano plot. We added classification for upregulated and downregulated genes, highlighted top genes, and saved the final plot.