Statistical Analysis

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##Dependencies

```
library(SummarizedExperiment)
library(genefilter)
library(limma)
library(dbplyr)
library(ggplot2)
```

Plot colour palette

```
tropical=  c('darkorange', 'dodgerblue', 'hotpink', 'limegreen', 'yellow')
palette(tropical)
par(pch=19)
```

Load the data

Data is loaded and edata transformed and filtered as in "Exploratory Data Analysis.Rmd".

Linear Regression

The null hypothesis is that there is no relationship between group and gene expression.

Model matrix is made to include both the variable of interest, Group, and the covariate RIN.

```
mod1 = model.matrix( ~ pdata$RIN + pdata$Group)
fit1 = lmFit(filt_edata, mod1)
fit1 = eBayes(fit1)
```

```
## Warning: Zero sample variances detected, have been offset away from zero
```

```
tt = topTable(fit1, number = Inf, coef = 3)
genes_logFC = subset(tt, select = c(logFC, P.Value, adj.P.Val))
```

Volcano Plot

Create labels for whether genes are up-regulated, down-regulated or neither. (+-)1.5 was chosen as a cut-off for

```
genes_logFC$diffexp = "NO"
genes_logFC$diffexp[genes_logFC$logFC > 1.5 & genes_logFC$adj.P.Val < 0.05] = "UP"
genes_logFC$diffexp[genes_logFC$logFC < -1.5 & genes_logFC$adj.P.Val < 0.05] = "DOWN"
```

Set colour scheme for differential expression.

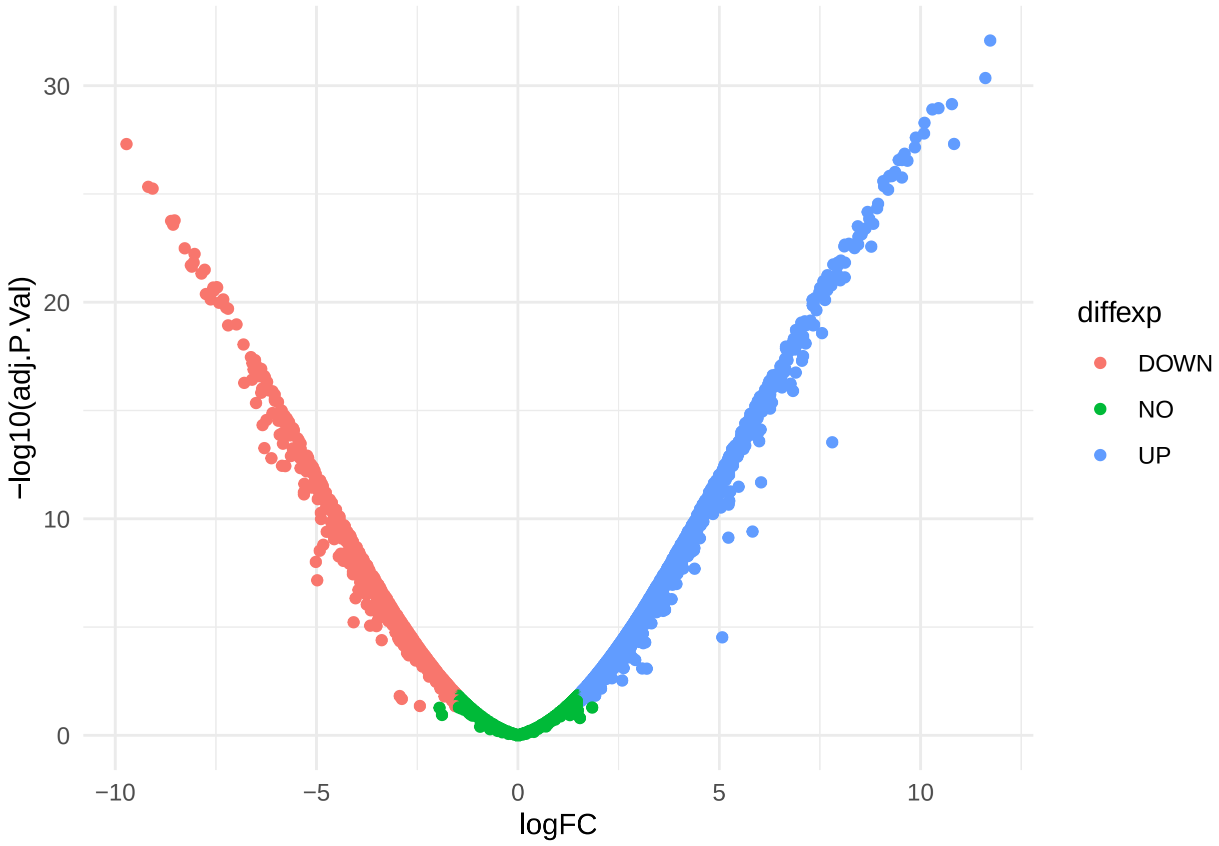
```
mycolors = c("blue", "red", "black")
names(mycolors) = c("DOWN", "UP", "NO")
```

Create new column which labels differentially expressed genes

```
genes_logFC$diffexplab = NA
genes_logFC$diffexplab[genes_logFC$diffexp != "NO"] = rownames(genes_logFC)[genes_logFC$diffexp != "NO"]
```

Create the volcano plot with colour coding and labels

```
ggplot(data=genes_logFC, aes(x=logFC, y=-log10(adj.P.Val), col=diffexp)) +
    geom_point() +
    theme_minimal()
```



Create TSV file

```
glFC_tsv = genes_logFC[,c(1,2,3)]
glFC_tsv = tibble::rownames_to_column(glFC_tsv, "Entrez.ID")
write.table(glFC_tsv, file = "genes_logFC.tsv", sep = "\t", col.names = colnames(glFC_tsv), row.names =
```

```
sessionInfo()
```

```
## R version 4.0.5 (2021-03-31)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19043)
##
## Matrix products: default
```

```
##
## locale:
## [1] LC_COLLATE=English_United Kingdom.1252
## [2] LC_CTYPE=English_United Kingdom.1252
## [3] LC_MONETARY=English_United Kingdom.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United Kingdom.1252
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] ggplot2_3.3.5               dbplyr_2.1.1
##  [3] limma_3.46.0                genefilter_1.72.1
##  [5] SummarizedExperiment_1.20.0 Biobase_2.50.0
##  [7] GenomicRanges_1.42.0        GenomeInfoDb_1.26.7
##  [9] IRanges_2.24.1              S4Vectors_0.28.1
## [11] BiocGenerics_0.36.1         MatrixGenerics_1.2.1
## [13] matrixStats_0.59.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.7             lattice_0.20-44        assertthat_0.2.1
##  [4] digest_0.6.27          utf8_1.2.1             R6_2.5.0
##  [7] RSQLite_2.2.7          evaluate_0.14          highr_0.9
## [10] httr_1.4.2             pillar_1.6.1           zlibbioc_1.36.0
## [13] rlang_0.4.11           annotate_1.68.0        blob_1.2.2
## [16] Matrix_1.3-4           rmarkdown_2.9          labeling_0.4.2
## [19] splines_4.0.5          stringr_1.4.0          RCurl_1.98-1.3
## [22] bit_4.0.4              munsell_0.5.0          DelayedArray_0.16.3
## [25] compiler_4.0.5         xfun_0.23              pkgconfig_2.0.3
## [28] htmltools_0.5.1.1      tidyselect_1.1.1       tibble_3.1.2
## [31] GenomeInfoDbData_1.2.4 XML_3.99-0.6           fansi_0.5.0
## [34] crayon_1.4.1           dplyr_1.0.7            withr_2.4.2
## [37] bitops_1.0-7           grid_4.0.5             xtable_1.8-4
## [40] gtable_0.3.0           lifecycle_1.0.0        DBI_1.1.1
## [43] magrittr_2.0.1         scales_1.1.1           stringi_1.6.2
## [46] cachem_1.0.5           farver_2.1.0           XVector_0.30.0
## [49] ellipsis_0.3.2         generics_0.1.0         vctrs_0.3.8
## [52] tools_4.0.5            bit64_4.0.5            glue_1.4.2
## [55] purrr_0.3.4            fastmap_1.1.0          survival_3.2-11
## [58] yaml_2.2.1             AnnotationDbi_1.52.0   colorspace_2.0-2
## [61] memoise_2.0.0          knitr_1.33
```