Part 2

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2023-04-26

#Dseq Data Construction

```
# load library
library(tidyverse)
```

```
## ── Attaching core tidyverse packages ──────────────────────── tidyverse
2.0.0 ──
## ✔ dplyr     1.1.1     ✔ readr     2.1.4
## ✔ forcats   1.0.0     ✔ stringr   1.5.0
## ✔ ggplot2   3.4.1     ✔ tibble    3.2.1
## ✔ lubridate 1.9.2     ✔ tidyr     1.3.0
## ✔ purrr     1.0.1
## ── Conflicts ────────────────────────────────────────── tidyverse_conflicts() ──
## ✖ dplyr::filter() masks stats::filter()
## ✖ dplyr::lag()    masks stats::lag()
## ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force
all conflicts to become errors
```

```
library(DESeq2)
```

```
## Loading required package: S4Vectors
## Loading required package: stats4
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'
##
## The following objects are masked from 'package:lubridate':
##
##     intersect, setdiff, union
##
## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union
##
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
##
## The following objects are masked from 'package:base':
##
```

```
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min
##
##
## Attaching package: 'S4Vectors'
##
## The following objects are masked from 'package:lubridate':
##
##     second, second<-
##
## The following objects are masked from 'package:dplyr':
##
##     first, rename
##
## The following object is masked from 'package:tidyr':
##
##     expand
##
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
##
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
##
## The following object is masked from 'package:lubridate':
##
##     %within%
##
## The following objects are masked from 'package:dplyr':
##
##     collapse, desc, slice
##
## The following object is masked from 'package:purrr':
##
##     reduce
##
## Loading required package: GenomicRanges
## Loading required package: GenomeInfoDb
## Loading required package: SummarizedExperiment
## Loading required package: MatrixGenerics
## Loading required package: matrixStats
##
## Attaching package: 'matrixStats'
##
```

```
## The following object is masked from 'package:dplyr':
##
##     count
##
##
## Attaching package: 'MatrixGenerics'
##
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
##
## Loading required package: Biobase
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
##
##
## Attaching package: 'Biobase'
##
## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
##
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

```
# read count data
rna_cts <- read.csv("Marra2014_count_table_spleen.tsv", sep = "\t", row.names
= "gene_id")
rna_cts <- as.matrix(rna_cts)
colnames(rna_cts)
```

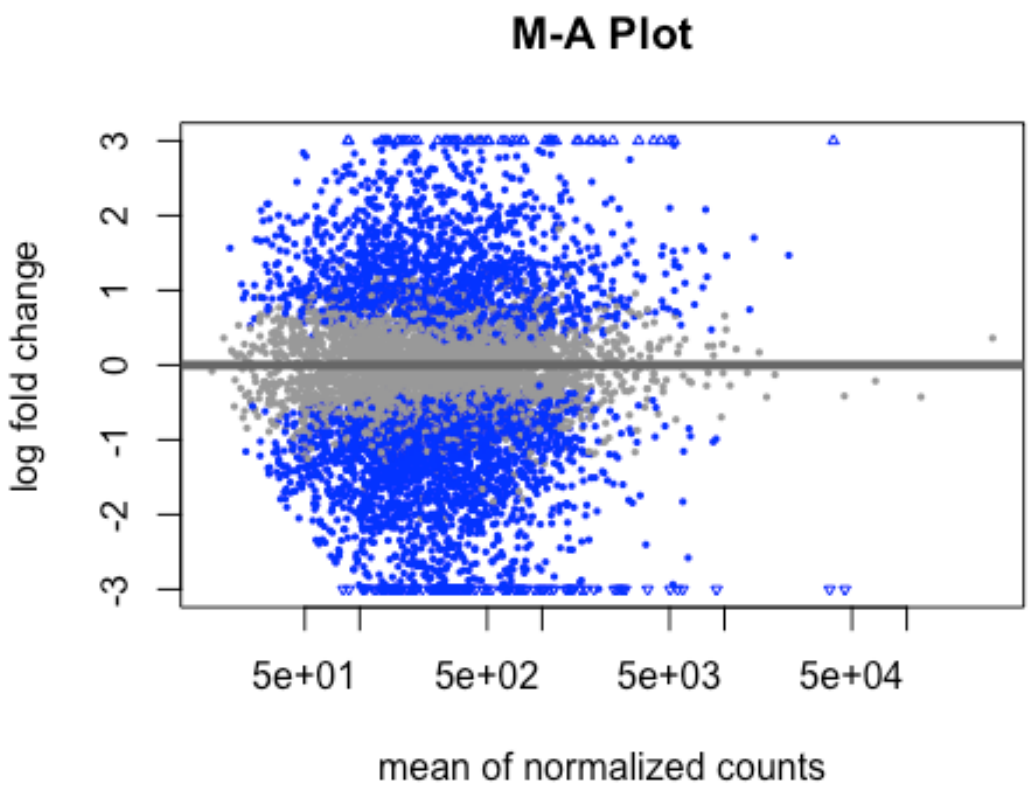
```
##  [1] "D1" "D2" "D3" "D4" "C1" "C2" "C3" "C4" "H1" "H2" "H3" "H4"
```

```
# create a dataframe of sample info
col_data <- data.frame(
  row.names=colnames(rna_cts),
  condition=c("desert", "desert", "desert", "desert", "desert", "desert",
"desert", "desert","mesic","mesic","mesic", "mesic"),
  species=c("Dipodomys", "Dipodomys", "Dipodomys", "Dipodomys",
"Chaetodipus", "Chaetodipus", "Chaetodipus", "Chaetodipus", "Heteromys",
"Heteromys", "Heteromys", "Heteromys")
)

# Construct a DESeq data set
de_obj <- DESeqDataSetFromMatrix(
  countData = rna_cts,
  colData = col_data,
  design = ~ condition)

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables
in
## design formula are characters, converting to factors

# Run differential gene expression analysis
dds <- DESeq(de_obj)

## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## -- note: fitType='parametric', but the dispersion trend was not well
captured by the
##    function: y = a/x + b, and a local regression fit was automatically
substituted.
##    specify fitType='local' or 'mean' to avoid this message next time.
## final dispersion estimates
## fitting model and testing
## -- replacing outliers and refitting for 32 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
## estimating dispersions
## fitting model and testing

# get results
res <- results(dds)
```

MA Plot

In DESeq2, the function plotMA shows the log2 fold changes attributable to a given variable over the mean of normalized counts for all the genes in the DESeqDataSet

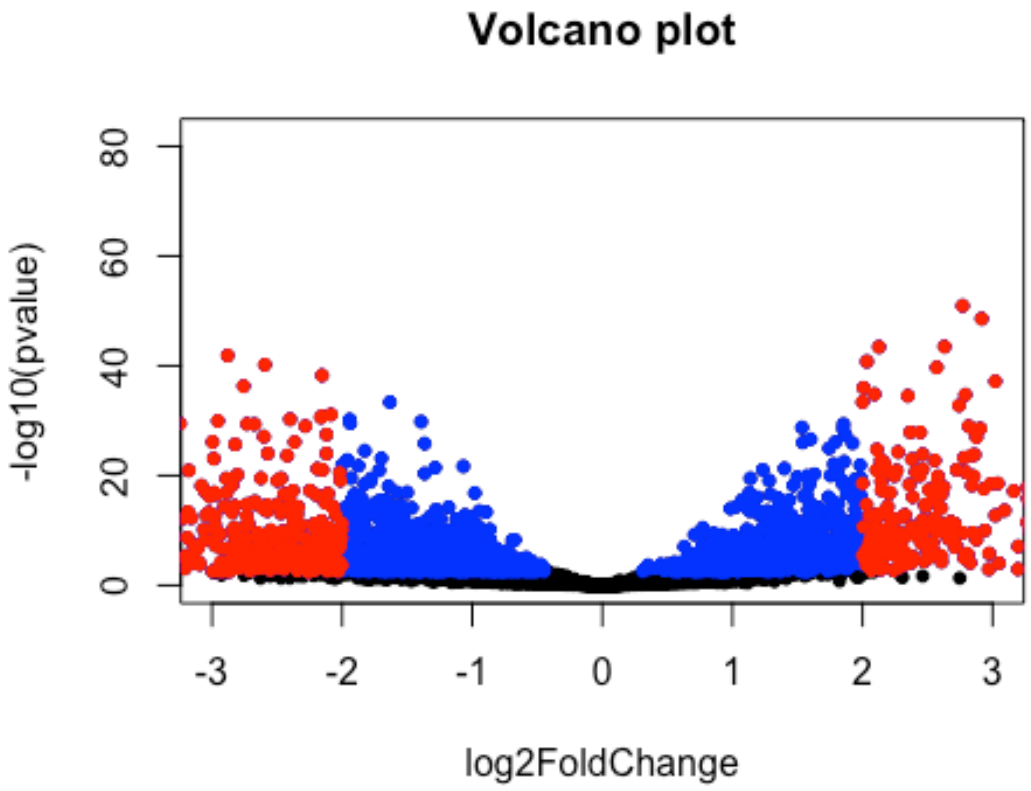
```
plotMA(res, main="M-A Plot", ylim=c(-3,3))
```



Volcano Plot

A volcano plot is a type of scatterplot that shows statistical significance (P value) versus magnitude of change (fold change). It enables quick visual identification of genes with large fold changes that are also statistically significant. These may be the most biologically significant genes.

```
with(res, plot(log2FoldChange, -log10(pvalue), pch=20, main="Volcano plot",
xlim=c(-3,3)))

# Add colored points: blue if padj<0.01, red if log2FC>1 and padj<0.05)
with(subset(res, padj<.01 ), points(log2FoldChange, -log10(pvalue), pch=20,
col="blue"))
with(subset(res, padj<.01 & abs(log2FoldChange)>2), points(log2FoldChange, -
log10(pvalue), pch=20, col="red"))
```


Volcano plot
-log10(pvalue)
0
20
40
60
80
-3
-2
-1
0
1
2
3
log2FoldChange