lab 4

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Question 1

Run all the R code and reproduce the graphics. Go carefully through the R code and explain in your words what each step does. HINT Recall what a design/model matrix is from linear regression

This chunk gives an initial look at the dataset `airway`, which provides expression values in genes on four human airway muscle cell lines treated with a compound. We see that the data is organised as a matrix or assay with 63677 genes along the rows, and 8 different experiments along the columns. What follows below is a section of the assay,

```
## ----airway-SummarizedExperiment-----------------------------------------------------------------
library(airway)         # An 'ExperimentData' package...
data(airway)            # ...with a sample data set...
airway                  # ...that is a SummarizedExperiment
```

```
## class: RangedSummarizedExperiment
## dim: 63677 8
## metadata(1): ''
## assays(1): counts
## rownames(63677): ENSG00000000003 ENSG00000000005 ... ENSG00000273492 ENSG00000273493
## rowData names(10): gene_id gene_name ... seq_coord_system symbol
## colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
## colData names(9): SampleName cell ... Sample BioSample
```

```
head(assay(airway))     # contains a matrix of counts
```

```
##                 SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516 SRR1039517 SRR1039520
## ENSG00000000003        679        448        873        408       1138       1047        770
## ENSG00000000005          0          0          0          0          0          0          0
## ENSG00000000419        467        515        621        365        587        799        417
## ENSG00000000457        260        211        263        164        245        331        233
## ENSG00000000460         60         55         40         35         78         63         76
## ENSG00000000938          0          0          2          0          1          0          0
##                 SRR1039521
## ENSG00000000003        572
## ENSG00000000005          0
## ENSG00000000419        508
## ENSG00000000457        229
## ENSG00000000460         60
## ENSG00000000938          0
```

```
head(rowRanges(airway)) # information about the genes...
```

```
## GRangesList object of length 6:
## $ENSG00000000003
## GRanges object with 17 ranges and 2 metadata columns:
##        seqnames            ranges strand |   exon_id       exon_name
##           <Rle>         <IRanges>  <Rle> | <integer>     <character>
##    [1]        X 99883667-99884983      - |    667145 ENSE00001459322
##    [2]        X 99885756-99885863      - |    667146 ENSE00000868868
##    [3]        X 99887482-99887565      - |    667147 ENSE00000401072
##    [4]        X 99887538-99887565      - |    667148 ENSE00001849132
##    [5]        X 99888402-99888536      - |    667149 ENSE00003554016
##    ...      ...                 ...    ... .       ...             ...
##   [13]        X 99890555-99890743      - |    667156 ENSE00003512331
##   [14]        X 99891188-99891686      - |    667158 ENSE00001886883
##   [15]        X 99891605-99891803      - |    667159 ENSE00001855382
##   [16]        X 99891790-99892101      - |    667160 ENSE00001863395
##   [17]        X 99894942-99894988      - |    667161 ENSE00001828996
##   -------
##   seqinfo: 722 sequences (1 circular) from an unspecified genome
##
## ...
## <5 more elements>
```

```
colData(airway)[, 1:3]  # ...and samples
```

```
## DataFrame with 8 rows and 3 columns
##             SampleName     cell      dex
##               <factor> <factor> <factor>
## SRR1039508 GSM1275862  N61311     untrt
## SRR1039509 GSM1275863  N61311     trt
## SRR1039512 GSM1275866  N052611    untrt
## SRR1039513 GSM1275867  N052611    trt
## SRR1039516 GSM1275870  N080611    untrt
## SRR1039517 GSM1275871  N080611    trt
## SRR1039520 GSM1275874  N061011    untrt
## SRR1039521 GSM1275875  N061011    trt
```

```
## coordinated subsetting
untrt <- airway[, airway$dex == 'untrt']
head(assay(untrt))
```

```
##                 SRR1039508 SRR1039512 SRR1039516 SRR1039520
## ENSG00000000003        679        873       1138        770
## ENSG00000000005          0          0          0          0
## ENSG00000000419        467        621        587        417
## ENSG00000000457        260        263        245        233
## ENSG00000000460         60         40         78         76
## ENSG00000000938          0          2          1          0
```

```
colData(untrt)[, 1:3]
```

```
## DataFrame with 4 rows and 3 columns
##             SampleName     cell      dex
##               <factor> <factor> <factor>
## SRR1039508 GSM1275862  N61311     untrt
## SRR1039512 GSM1275866  N052611    untrt
## SRR1039516 GSM1275870  N080611    untrt
## SRR1039520 GSM1275874  N061011    untrt
```

```
## ----airway-colData-------------------------------------------------------------------------------
library(airway)          # An 'ExperimentData' package...
data(airway)             # ...with a sample data set...
colData(airway)[, 1:3]   # ...represented as a SummarizedExperiment
```

```
## DataFrame with 8 rows and 3 columns
##             SampleName     cell      dex
##               <factor> <factor> <factor>
## SRR1039508 GSM1275862  N61311     untrt
## SRR1039509 GSM1275863  N61311     trt
## SRR1039512 GSM1275866  N052611    untrt
## SRR1039513 GSM1275867  N052611    trt
## SRR1039516 GSM1275870  N080611    untrt
## SRR1039517 GSM1275871  N080611    trt
## SRR1039520 GSM1275874  N061011    untrt
## SRR1039521 GSM1275875  N061011    trt
```

```
## ----airway-assay---------------------------------------------------------------------------------
head(assay(airway))
```

```
##                 SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516 SRR1039517 SRR1039520
## ENSG00000000003        679        448        873        408       1138       1047        770
## ENSG00000000005          0          0          0          0          0          0          0
## ENSG00000000419        467        515        621        365        587        799        417
## ENSG00000000457        260        211        263        164        245        331        233
## ENSG00000000460         60         55         40         35         78         63         76
## ENSG00000000938          0          0          2          0          1          0          0
##                 SRR1039521
## ENSG00000000003        572
## ENSG00000000005          0
## ENSG00000000419        508
## ENSG00000000457        229
## ENSG00000000460         60
## ENSG00000000938          0
```

Below, we perform a differential expression analysis, based on the Negative Binomial distribution, using the `DESeq2` package. This involves estimating size factors and dispersions, as well as fitting and testing the model. We then extract the results and order them from largest to smallest absolute log fold change. Below we present a table illustrating a subset of the results of the model. Among other things we report the p-value and log fold change for each gene.

```
## ----airway-toptable-----------------------------------------------------------------------------
library(DESeq2)     # package implementing statistical methods
dds <-              # data and experimental design
    DESeqDataSet(airway, design = ~ cell + dex)
dds <- DESeq(dds)   # initial analysis
```

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing
```

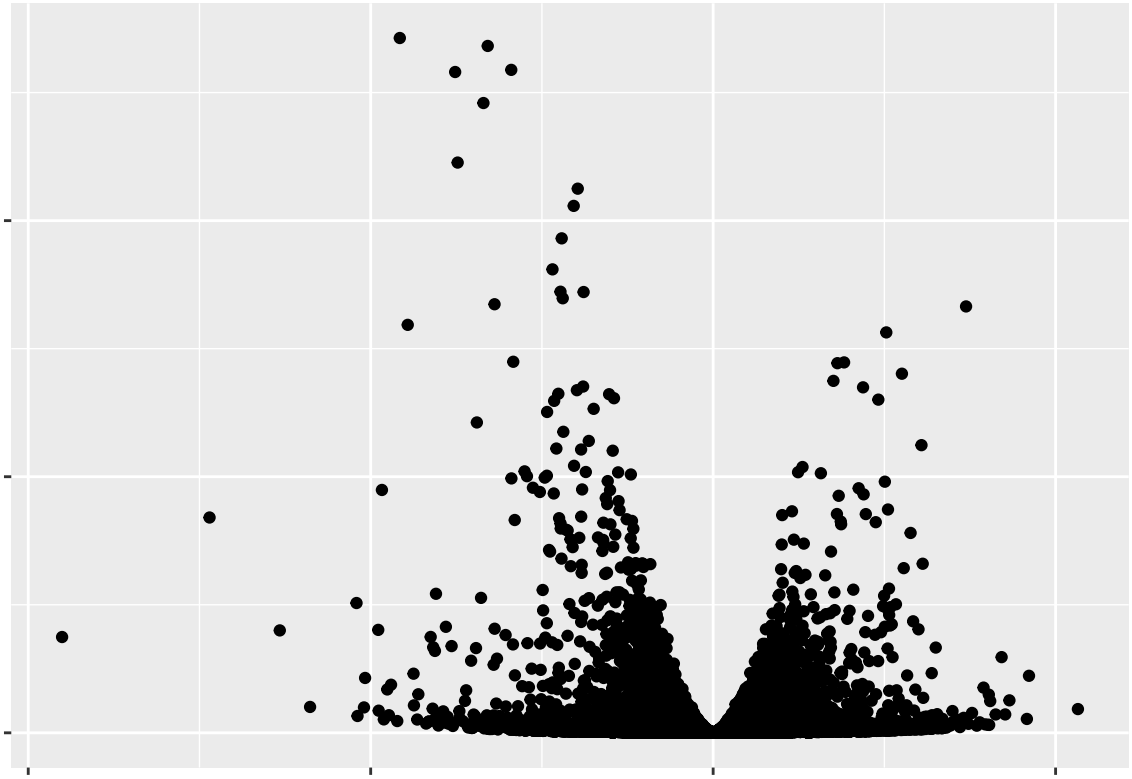
```
res <- results(dds) # summary results
ridx <-             # order from largest to smallest absolute log fold change
    order(abs(res$log2FoldChange), decreasing=TRUE)
res <- res[ridx,]
head(res)           # top-table
```

```
## log2 fold change (MLE): dex untrt vs trt
## Wald test p-value: dex untrt vs trt
## DataFrame with 6 rows and 6 columns
##                  baseMean log2FoldChange     lfcSE      stat      pvalue        padj
##                 <numeric>      <numeric> <numeric> <numeric>   <numeric>   <numeric>
## ENSG00000179593  67.24305       -9.50597  1.054503  -9.01465 1.97505e-19 1.25304e-17
## ENSG00000109906 385.07103       -7.35263  0.536389 -13.70764 9.13762e-43 2.25537e-40
## ENSG00000250978  56.31819       -6.32738  0.677797  -9.33521 1.00788e-20 7.20631e-19
## ENSG00000132518   5.65465       -5.88511  1.324044  -4.44480 8.79726e-06 1.00006e-04
## ENSG00000128285   6.62474        5.32590  1.257815   4.23425 2.29314e-05 2.37869e-04
## ENSG00000127954 286.38412       -5.20716  0.493082 -10.56044 4.54548e-26 5.05559e-24
```

Below we finally plot the negative logarithmized p-value against the log fold change.

```
## ----airway-viz----------------------------------------------------------------------------------
library(ggplot2)
g <- ggplot(as.data.frame(res),
       aes(x=log2FoldChange, y=-10 * log10(pvalue))) +
    geom_point()
plot(g)
```

```
## Warning: Removed 30208 rows containing missing values or values outside the scale range
## (`geom_point()`).
```



```
## ----airway-mapids-------------------------------------------------------------------------------
library(org.Hs.eg.db)
ensid <- head(rownames(res))
select(org.Hs.eg.db, ensid, c("SYMBOL", "GENENAME"), "ENSEMBL")
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
##           ENSEMBL  SYMBOL                                   GENENAME
## 1 ENSG00000179593 ALOX15B      arachidonate 15-lipoxygenase type B
## 2 ENSG00000109906  ZBTB16 zinc finger and BTB domain containing 16
## 3 ENSG00000250978    <NA>                                      <NA>
## 4 ENSG00000132518  GUCY2D            guanylate cyclase 2D, retinal
## 5 ENSG00000128285   MCHR1 melanin concentrating hormone receptor 1
## 6 ENSG00000127954  STEAP4                  STEAP4 metalloreductase
```

```
## ----shiny-BAMSpector, eval=FALSE----------------------------------------------------------------
#  app <- system.file(package="BiocUruguay2015", "BAMSpector")
#  shiny::runApp(app)

## ----shiny-MAPlotExplorer, eval=FALSE------------------------------------------------------------
#  app <- system.file(package="BiocUruguay2015", "MAPlotExplorer")
#  shiny::runApp(app)

## ----sessionInfo---------------------------------------------------------------------------------
#sessionInfo()
```

Question 2

In the presented analysis, there are no plots of raw paired data. In the section where the contrasts are defined and the three contrasts. Present the variables versus each other original, log scaled and MAplot for each considered pair both before and after normalization. A cluster analysis is performed on the page but not reported. Present plots and also draw heatmaps.