Installation

To install R for windows:
http://cran.r-project.org/bin/windows/base/

To install DESeq2 package, start R and enter:
source("http://bioconductor.org/biocLite.R")
biocLite("DESeq2")

To download the experimental dataset:
source("http://bioconductor.org/workflows.R")
workflowInstall("rnaseqGene")

Execution

1) Reading from read count matrix:

```
# changing to work directory
setwd("C:/Users/Braskem/Desktop/Marcelo/bioinfo/Cenapad/curso_bioinfo_facil
ity/Curso_RNA-seq_1sem2015/Aula_Pratica")

#reading read count matrix from file
rnaseqMatrix <- read.table(“read_count.matrix”, header=T, row.names=1)
rnaseqMatrix <= round(rnaseqMatrix)

#getting information from read count matrix
dim(rnaseqMatrix)
head(rnaseqMatrix)
colnames(rnaseqMatrix)
rownames(rnaseqMatrix)

#generating condition matrix
colData <- data.frame(condition=factor(c("control","control",”control”,
"treated","treated",”treated”)))
rownames(colData) = colnames(rnaseqMatrix)
```

```
#getting information from condition matrix
dim(colData)
head(colData)
colnames(colData)
rownames(colData)

#importing read count and condition matrix into DESeq2 object
dds <- DESeqDataSetFromMatrix(
        countData = rnaseqMatrix,
        colData = colData,
        design = ~ condition
        )

#getting information from DESeq2 object
dds
colData(dds)
colnames(dds)
rownames(dds)
assays(dds)$counts
```

2) Working on experimental dataset available at repository

```
Opening RNAseq data (experimental):
library("airway")
data("airway")
se <- airway

#getting information about experimental dataset (se object)
se
colnames(se)
rownames(se)
rownames(se)[1:10]
assays(se)$counts[1:10,1:8]
```

```
assays(se)$counts[1:10,]
colData(se)

#Importing se object into DESe2
library("DESeq2")
ddsSE <- DESeqDataSet(
        se,
        design = ~ cell + dex
        )

ddsSE
colnames(ddsSE)
rownames(ddsSE)
rownames(ddsSE)[1:10]
assays(ddsSE)$counts[1:10,]
colData(ddsSE)

#Performing differential expressed analysis
ddsSE <- DESeq(ddsSE)
res <- results(ddsSE)

#getting information from results (res)
res
colnames(res)
mcols(res)$description
rownames(res)
rownames(res)[1:10]
res$log2FoldChange[1:10]
res$pvalue[1:10]
res$padj[1:10]
head(res,4)

#ordering by most significant results
resOrdered <- res[order(res$padj),]
```

```
head(resOrdered)

#filtrating by only differential expressed genes (adjusted p-value <= 0.1)
resSig <- subset(resOrdered, padj < 0.1)
resSig

#saving all results in a file
write.csv(as.data.frame(resOrdered), file="condition_treated_results.csv")

#summarizing differential expressed analysis
summary(res)

# MA plot. Points which fall out of the window are plotted as open triangles
pointing either up or down
plotMA(res, main="DESeq2", ylim=c(-2,2))
```