```
# script to perform differential gene expression analysis using DESeq2 package
# setwd("~/Desktop/demo/DESeq2_tutorial/data")

# load libraries
library(DESeq2)
library(tidyverse)
library(airway)

# Step 1: preparing count data ----------------

# read in counts data
counts_data <- read.csv('counts_data.csv')
head(counts_data)


# read in sample info
colData <- read.csv('sample_info.csv')


# making sure the row names in colData matches to column names in
counts_data
all(colnames(counts_data) %in% rownames(colData))

# are they in the same order?
all(colnames(counts_data) == rownames(colData))


# Step 2: construct a DESeqDataSet object ----------

dds <- DESeqDataSetFromMatrix(countData = counts_data,
                              colData = colData,
                              design = ~ dexamethasone)

dds

# pre-filtering: removing rows with low gene counts
# keeping rows that have at least 10 reads total
keep <- rowSums(counts(dds)) >= 10
dds <- dds[keep,]

dds

# set the factor level
dds$dexamethasone <- relevel(dds$dexamethasone, ref = "untreated")

# NOTE: collapse technical replicates
```

```
# Step 3: Run DESeq ----------------------
dds <- DESeq(dds)
res <- results(dds)

res



# Explore Results ----------------

summary(res)

res0.01 <- results(dds, alpha = 0.01)
summary(res0.01)

# contrasts
resultsNames(dds)

# e.g.: treated_4hrs, treated_8hrs, untreated

results(dds, contrast = c("dexamethasone", "treated_4hrs", "untreated"))

# MA plot
plotMA(res)
```