```
#SINA GHASEMY
# my project is about DMD
#step 1
# Import DMD DATA with affy
library(affy)
library(oligo)
cellpath = "D:/R/project/DMD data"
DMD = ReadAffy(celfile.path=cellpath)
class(DMD)
str(DMD)

#import data with oligo
celFiles <- list.celfiles("D:/R/project/DMD data", full.names=TRUE)
DMD1 <- read.celfiles(celFiles)

#step 2
#perfect match and miss matches
pms<-affy::pm(DMD)
head(names(pms))
head(pms)
tail(pms)

#pheno Data
ph=DMD@phenoData
str(ph)
ph
ph@data
ph@data[,1]=c("C1","C2","C3","C4","C5","C6","D1","D2","D3","D4","D5","D6")
ph@data
```

```
#Extraction of probe sets
fname=featureNames(DMD)
head(fname)
length(fname)

#Extraction of probe sets names
pnam<-affy::probeNames(DMD)
head(pnam)

#View specific Probe ID
affy::pm(DMD,"1007_s_at")

#Quality control before normalization
#pseudo image
oligo::image(DMD1[,1])
dev.off()
for (i in 1:12) {
 name=paste("image",i,".jpeg",sep = "")
 jpeg(name)
 oligo::image(DMD1[,i],main=ph@data$sample[i])
 dev.off()
}
for (i in 1:12) {
 name=paste("image",i,".jpeg",sep = "")
 jpeg(name)
 affy::image(DMD[,i],main=ph@data$sample[i])
 dev.off()
}
#histograms
```

```
color=c("green","green","green","green","green","green","red","red","red","red","red","red")

hist(DMD[,1:12],lwd=2,lty=1,which='pm',col=color,ylab='Density',xlab='Log2 intensities',main='Histogram of raw data befor normalize')

-------#box plot----------------

name = "boxplot.jpg"

jpeg(name)

boxplot(DMD,which='pm',col='red',names=ph@data$sample)

dev.off()

-------# MA plot---------------

library(affy)

library(oligo)

for (i in 1:12){

 name = paste("MAplot",i,".jpg",sep="")

 jpeg(name)

 oligo::MAplot(DMD1,which=i)

 dev.off()

}

for (i in 1:12){

 name = paste("MAplot",i,".jpg",sep="")

 jpeg(name)

 affy::MAplot(DMD,which=i)

 dev.off()

}

#normalization
```

```
DMD.rma<-affy::rma(DMD)
DMD.matrix = exprs(DMD.rma)
DMD.matrix["1007_s_at",]
affy::pm(DMD,"1007_s_at")

#for DMD1(oligo data)
DMD1.rma<-oligo::rma(DMD1)
DMD1.matrix<-exprs(DMD1.rma)


---------#Quality control after normalization------

#normal box plot
name = "boxplotnorm.jpg"
jpeg(name)
affy::boxplot(DMD.matrix,col='red',names=ph@data$sample)
dev.off()

#normal histogram

affy::hist(DMD.rma,col=color,lty=1)
oligo::hist(DMD1.rma,col=color,lty=1)

#normal MA plot

for (i in 1:12){
  name = paste("MAplot",i,".jpg",sep="")
  jpeg(name)
```

```
 oligo::MAplot(DMD1.rma,which=i)
 dev.off()
}

for (i in 1:12){
 name = paste("MAplot",i,".jpg",sep="")
 jpeg(name)
 affy::MAplot(DMD.rma,which=i)
 dev.off()
}


#MAIN ANALYSIS
#statistic analysis
library(limma)
dmd=c(0,0,0,0,0,0,1,1,1,1,1,1)
control=c(1,1,1,1,1,1,0,0,0,0,0,0)
design<-cbind(dmd,control)
design

#Processing the linear model by design
fit<-lmFit(DMD1.rma, design);
fit<-eBayes(fit)
contrast.matrix <- makeContrasts(dmd-control, levels=design)
fit2 <- contrasts.fit(fit, contrast.matrix);
fit2 <- eBayes(fit2)

#result of data analyze (View the output )
```

```
results <- decideTests(fit2,adjust.method="BH", p.value=0.05, lfc=1.6)
head(results)
summary(results)

#Increase of Expression
sum(results > 0 , na.norm=dmd)
#Decrease of Expression
sum(results< 0 , na.norm=dmd)
write.csv(results,"D:/R/project/dmd-control p value 0.01.txt lfc=1.6")
write.table(results,"D:/R/project/dmd-control p value 0.01.txt lfc=1.6")
write.fit(fit2, results,"complete table limma", adjust="BH", sep="\t")
str(results)
class(results)

#Extraction of genes with expression differences from these files
finaltable<-cbind(fit2,results)
DEGt<-read.table("complete table limma")
dim(DEGt)
DEG<-DEGt[DEGt[,8]!=0,]
view(DEG)
dim(DEG)

#functional annotation
write.csv(DEG,"DEG.csv")
#we use site named DAVID for functional annotation
#we use affymetrix ID of our DEG in this site
```