library(DESeq2)

library(gplots)

library( "RColorBrewer” )

library(“genefilter”)

count\_data=read.csv("LUSC\_NORM.csv", row.names = 1, header = TRUE)

count\_data=as.matrix(count\_data)

countData <- as.matrix(read.csv("LUSC\_NORM.csv",row.names=2))

countData=countData[,c(2:10)]

colData <- read.csv("Conditions.csv", row.names=1)

all(rownames(colData) %in% colnames(countData))

dds <- DESeqDataSetFromMatrix(countData = countData, colData = colData, design=~condition, ignoreRank = TRUE)

dds <- DESeq(dds)

res <- results( dds )

mcols(res, use.names=TRUE)

sum( res$pvalue < 0.01, na.rm=TRUE )

sum( res$padj < 0.1, na.rm=TRUE )

resSig <- res[ which(res$padj < 0.1 ), ]

head(resSig)

tail( resSig[ order( resSig$log2FoldChange ), ] )

plotDispEsts( dds, ylim = c(1e-6, 1e1) )

rld <- rlog( dds )

head( assay(rld) )

plot( log2( 1+counts(dds, normalized=TRUE)[, 1:2] ), col="#00000020", pch=20, cex=0.3 )

plot( assay(rld)[, 1:2], col="#00000020", pch=20, cex=0.3 )

sampleDists <- dist( t( assay(rld) ) )

sampleDists

sampleDistMatrix <- as.matrix( sampleDists )

rownames(sampleDistMatrix) <- paste( rld$condition)

colnames(sampleDistMatrix) <- NULL

library( "gplots" )

library( "RColorBrewer" )

colours = colorRampPalette( rev(brewer.pal(9, "Blues")) )(255)

heatmap.2( sampleDistMatrix, trace="none", col=colours)

topVarGenes <- head( order( rowVars( assay(rld) ), decreasing=TRUE ), 35 )

heatmap.2( assay(rld)[ topVarGenes, ], scale="row",

+            trace="none", dendrogram="column",

+            col = colorRampPalette( rev(brewer.pal(9, "RdBu")) )(255))