Ocular disease mechanisms elucidated by genetics of human fetal retinal pigment epithelium gene expression

Lab Journal Theme07 - Gene Expression Analysis

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Contents

1 Loading the data **2**

2 Exploratory Data Analysis **3**
2.1 Data sample 3
2.2 Plots for insight 4

3 Normalization **7**

4 Discovering Differentially Expressed Genes (DEGs) **9**
4.1 Using Bioconductor Packages 9

1 Loading the data

```
knitr::opts_chunk$set(cache = TRUE)
knitr::opts_chunk$set(echo = TRUE)

# Load packages
packages <- c("pander", "dplyr", "affy", "knitr", "ggplot2", "DESeq2", "pheatmap",
              "PoiClaClu", "scales")
invisible(lapply(packages, library, character.only = T))
```

For decompressing the data, run the code chunks in the Rmd file that deem fit for your situation:

- If you downloaded the data from the official site: Decompress the data and run the Rscript `data_loading.R`.
- If you want to use the dataset delivered with the project: Run the `decompress-dataset` code chunk.

```
#' Decompress the complete dataset
#' Use this chunk if you did not download the data from the site and want to use
#'  the delivered gzipped dataset

## Set the count.file variable to the full path of the gene file
count.file <- ""
system(paste("gzip -d", count.file))
```

After decompressing the data, the data can be read:

```
## Read the dataset
dataset <- read.table("./gene_count.txt", sep = "\t", header = TRUE)
## Set rownames of the dataset to first column
row.names(dataset) <- dataset$Gene
## Remove the Gene column
dataset <- dataset[-1]

## Indices for dataset
glucose.data <- seq(1, 48, 2)
galactose.data <- seq(2, 49, 2)
groups <- factor(rep(1:2, times=24), labels = c("Glucose", "Galactose"))

## Colors for the two sample groups (red = galactose, blue = glucose)
group.cols <- hue_pal()(2)
```

2 Exploratory Data Analysis

2.1 Data sample

```
pander(dataset[0:5, 0:4], split.tables = 64)
```

Table 1: Table continues below

	X1_glucose	X1_galactose
___alignment_not_unique	0	0
___ambiguous	73052	71663
___no_feature	6143654	3901459
___not_aligned	0	0
___too_low_aQual	0	0

	X2_glucose	X2_galactose
___alignment_not_unique	0	0
___ambiguous	90130	114748
___no_feature	4560099	10675855
___not_aligned	0	0
___too_low_aQual	0	0

```
pander(summary(dataset[,0:6]), split.tables = 64)
```

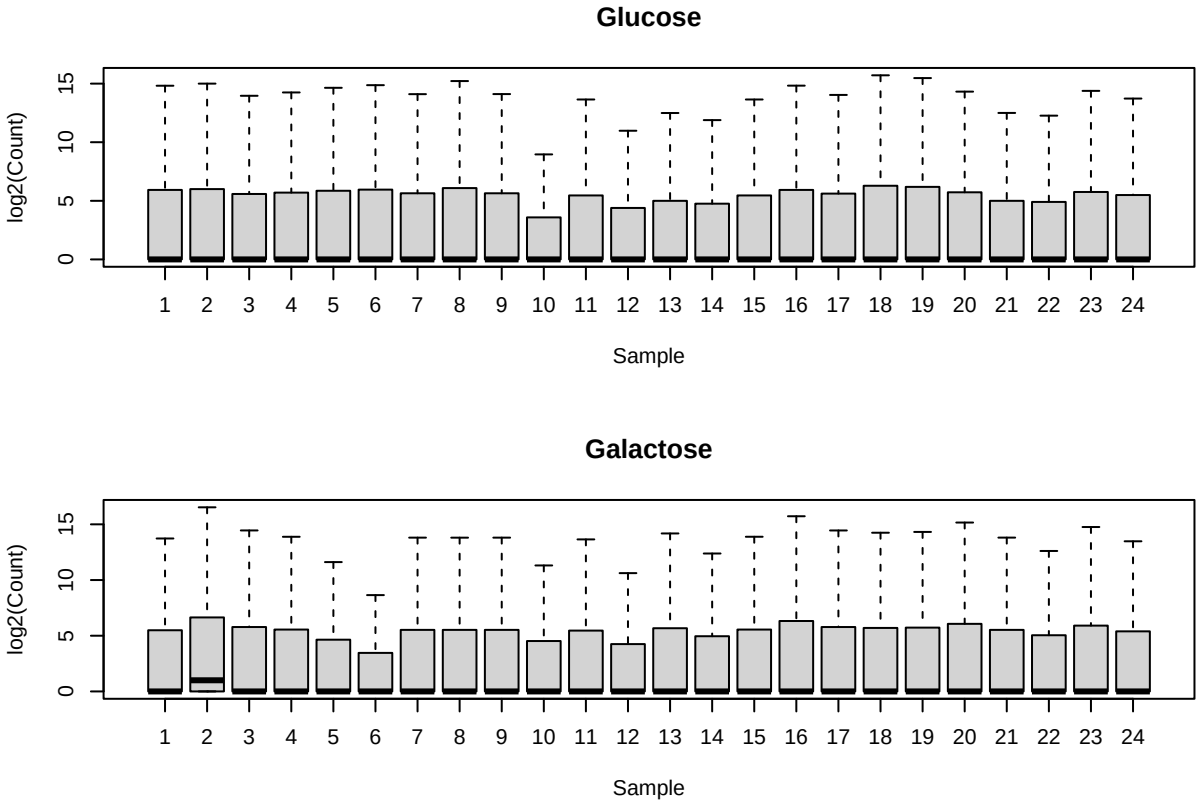
Table 3: Table continues below

X1_glucose	X1_galactose	X2_glucose
Min. : 0	Min. : 0	Min. : 0
1st Qu.: 0	1st Qu.: 0	1st Qu.: 0
Median : 0	Median : 0	Median : 0
Mean : 719	Mean : 549	Mean : 750
3rd Qu.: 60	3rd Qu.: 44	3rd Qu.: 63
Max. :6143654	Max. :3901459	Max. :4560099

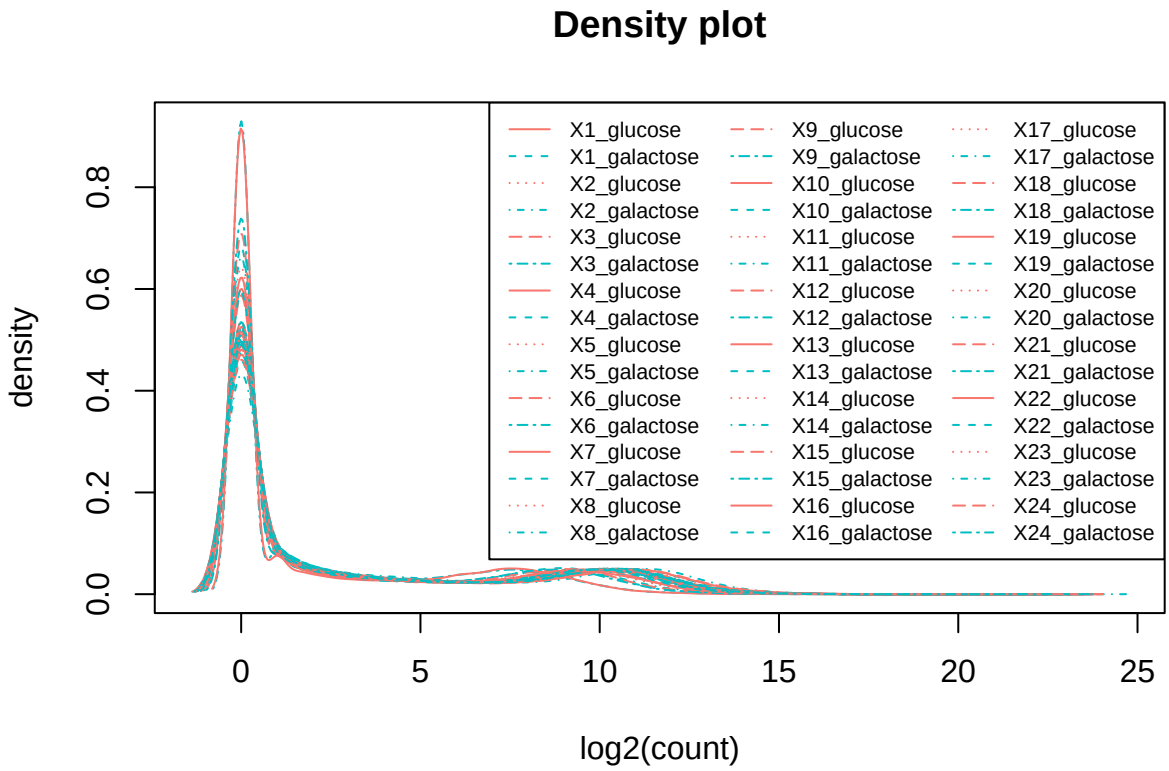
X2_galactose	X3_glucose	X3_galactose
Min. : 0	Min. : 0	Min. : 0
1st Qu.: 0	1st Qu.: 0	1st Qu.: 0
Median : 1	Median : 0	Median : 0
Mean : 1147	Mean : 622	Mean : 679
3rd Qu.: 99	3rd Qu.: 47	3rd Qu.: 54
Max. :10675855	Max. :5017129	Max. :5650847

2.2 Plots for insight

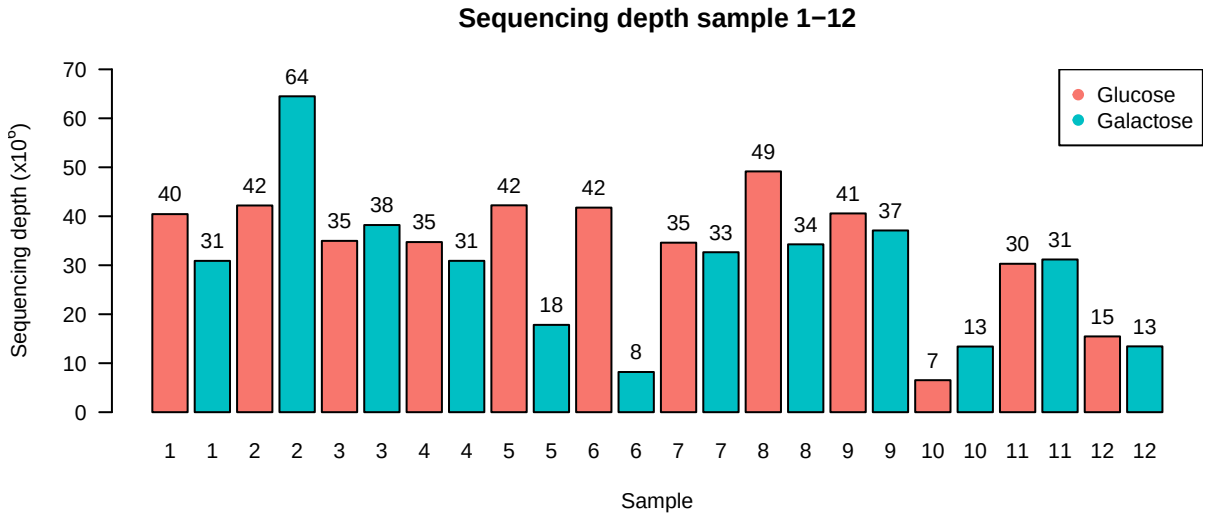
```
layout(matrix(c(1,1,2,2), nrow = 4, ncol = 1, byrow = T))
## Glucose plot
boxplot(log2(dataset[glucose.data]+1), main = "Glucose", names = seq(1, 24),
        xlab = "Sample", ylab = "log2(Count)", outline = FALSE)
## Galactose plot
boxplot(log2(dataset[galactose.data]+1), main = "Galactose", names = seq(1, 24),
        xlab = "Sample", ylab = "log2(Count)", outline = FALSE)
```

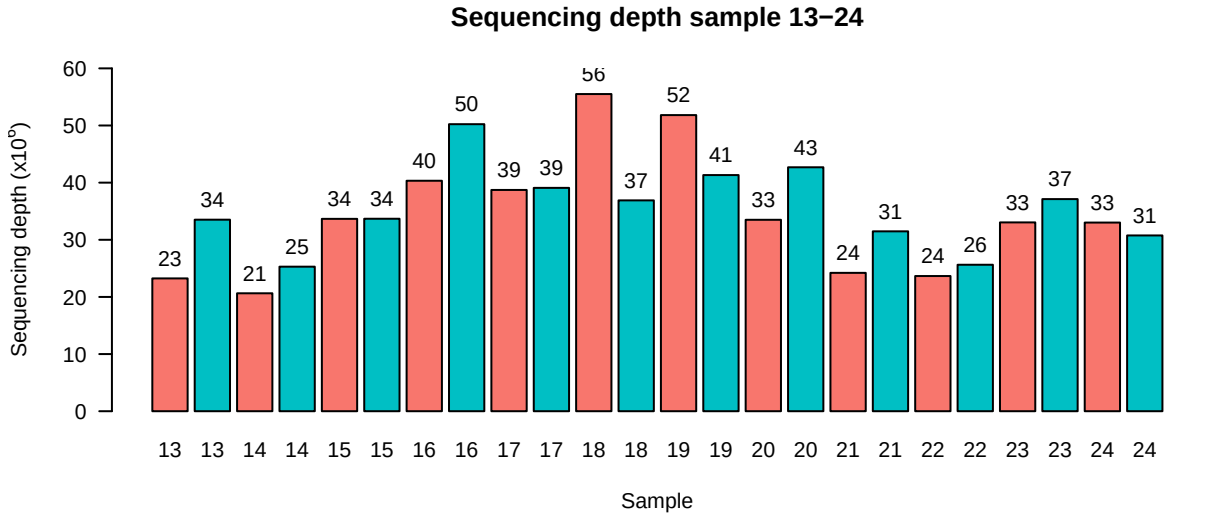


```
plotDensity(log2(dataset+1), main = "Density plot", col = group.cols,
            lty = 1:48, xlab = "log2(count)")
legend("topright", names(dataset), lty = 1:48, col = group.cols,
       cex = 0.7, ncol = 3)
```



```
layout(matrix(c(1,1,1,2,2,2), nrow = 6, ncol = 1, byrow = T))
## Barplot of first half of the data
x1 <- barplot(colSums(dataset[1:24]/ 1e6), main = "Sequencing depth sample 1-12",
              xlab = "Sample", ylab = expression("Sequencing depth (x10"^6*")"),
              ylim = c(0, 70), las = 2, col = group.cols, xaxt = 'n')
text(x = x1, y = colSums(dataset[1:24]/ 1e6),
     label = round(colSums(dataset[1:24]/ 1e6),0), pos = 3)
axis(1, at = x1, labels = rep(1:12, each = 2), tick = FALSE, cex = 0.6)
legend("topright", c("Glucose", "Galactose"), col = group.cols, pch = 19)

## Rest of the data
x2 <- barplot(colSums(dataset[25:48]/ 1e6), main = "Sequencing depth sample 13-24",
              xlab = "Sample", ylab = expression("Sequencing depth (x10"^6*")"),
              ylim = c(0, 60), las = 2, col = group.cols, xaxt = 'n')
text(x = x2, y = colSums(dataset[25:48]/ 1e6),
     label = round(colSums(dataset[25:48]/ 1e6), 0), pos = 3)
axis(1, at = x1, labels = rep(13:24, each = 2), tick = FALSE, cex = 0.6)
```

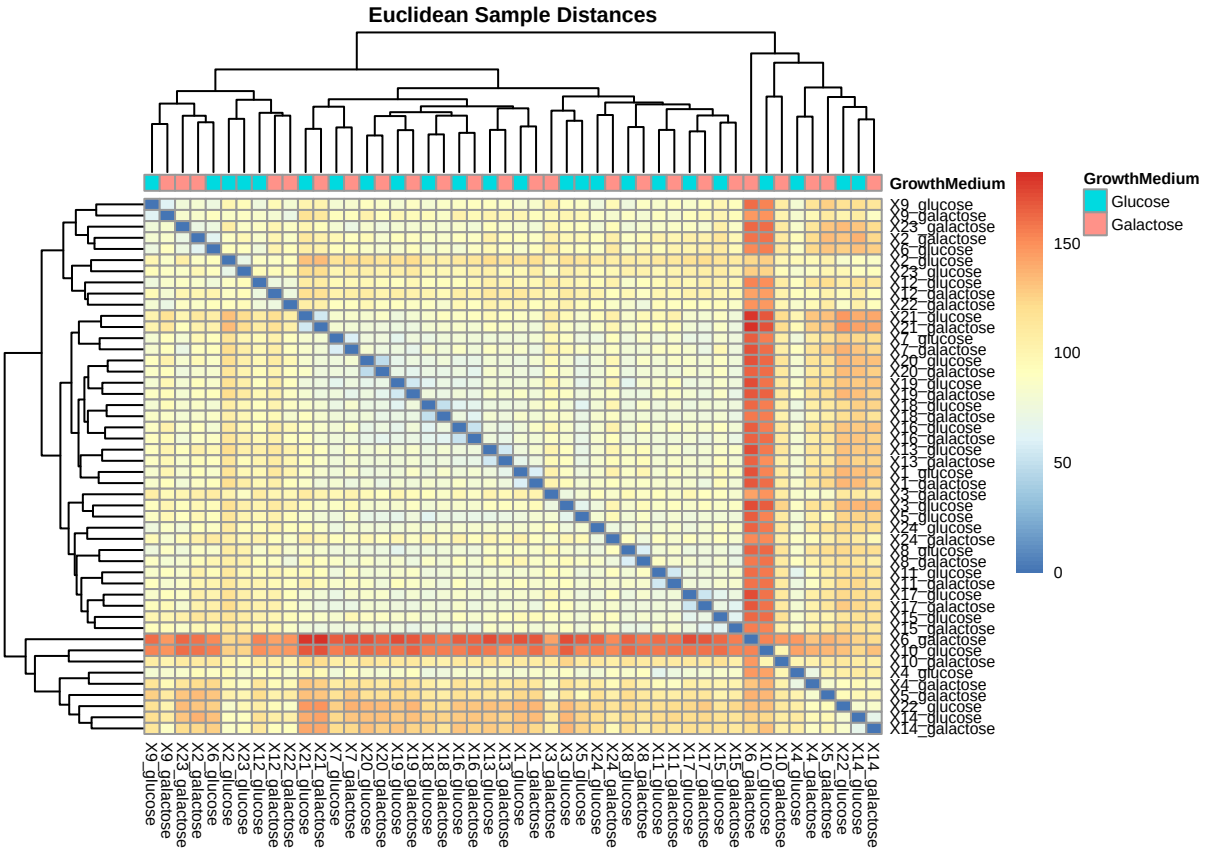




3 Normalization

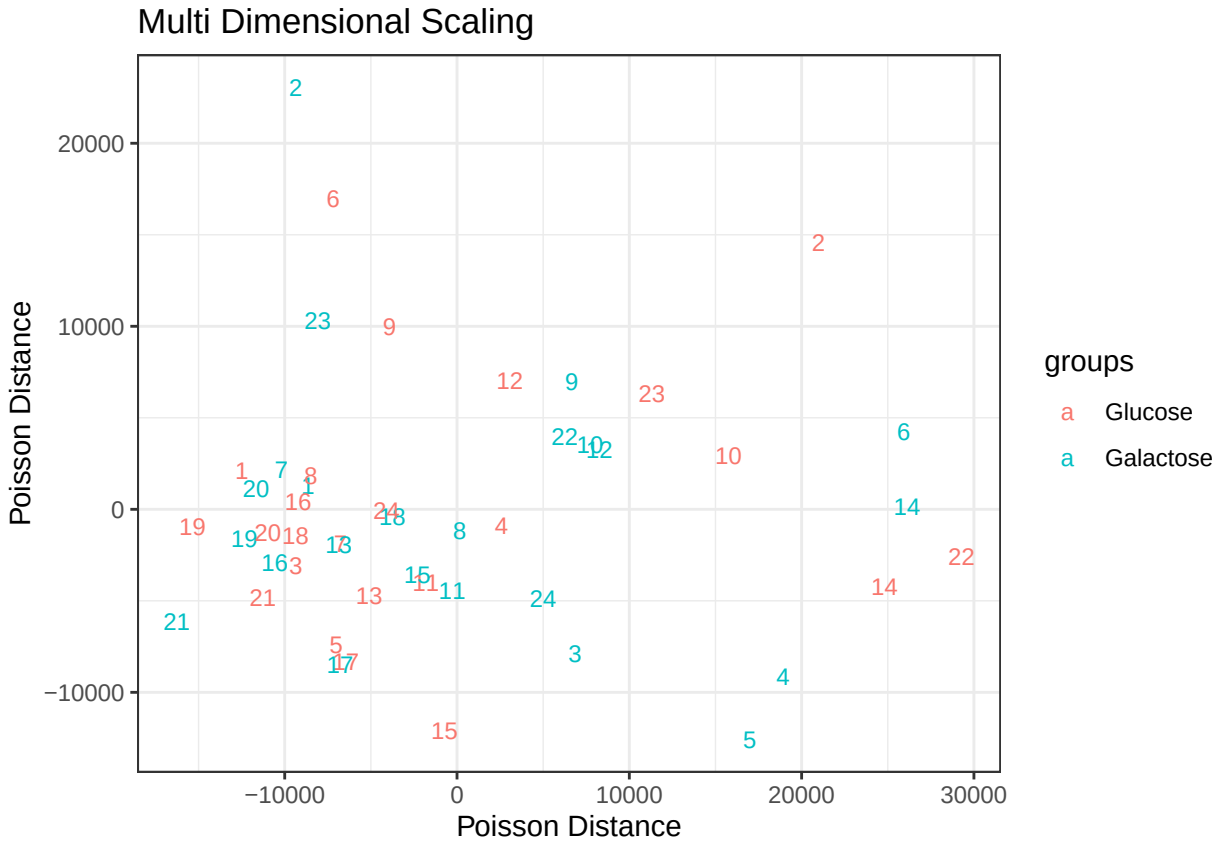
```
ddsMat <- DESeqDataSetFromMatrix(countData = round(dataset),
                                 colData = data.frame(samples = names(dataset)),
                                                      design = ~ 1)
rld.dds <- vst(ddsMat)
rld <- assay(rld.dds)
sampledists <- dist(t(rld))
```

```
distMatrix <- as.matrix(sampledists)

annotation <- data.frame(GrowthMedium = groups)

rownames(annotation) <- names(dataset)

pheatmap(distMatrix, show_colnames = T,
         annotation_col = annotation,
         clustering_distance_rows = sampledists,
         clustering_distance_cols = sampledists,
         main = "Euclidean Sample Distances", fontsize= 6)
```




```
dds <- assay(ddsMat)
poisd <- PoissonDistance(t(dds), type="deseq")
## Extract matrix with distances
poisDistMatrix <- as.matrix(poisd$dd)
## Calculate MDS for X- and Y- coordinates
mdsPoisData <- data.frame(cmdscale(poisDistMatrix))
```

```r
## Readable names
names(mdsPoisData) <- c("x_coord", "y_coord")
## Annotation label
coldata <- rep(1:24, each=2)

ggplot(mdsPoisData, aes(x_coord, y_coord, color = groups, label = coldata)) +
        geom_text(size = 3) +
        ggtitle("Multi Dimensional Scaling") +
        labs(x = "Poisson Distance", y = "Poisson Distance") +
        theme_bw()
```



4 Discovering Differentially Expressed Genes (DEGs)

4.1 Using Bioconductor Packages

```
design <- model.matrix(~ groups)
design
```

```
##    (Intercept) groupsGalactose
## 1            1                0
## 2            1                1
## 3            1                0
## 4            1                1
## 5            1                0
## 6            1                1
## 7            1                0
## 8            1                1
## 9            1                0
## 10           1                1
## 11           1                0
## 12           1                1
## 13           1                0
## 14           1                1
## 15           1                0
## 16           1                1
## 17           1                0
## 18           1                1
## 19           1                0
## 20           1                1
## 21           1                0
## 22           1                1
## 23           1                0
## 24           1                1
## 25           1                0
## 26           1                1
## 27           1                0
## 28           1                1
## 29           1                0
## 30           1                1
## 31           1                0
## 32           1                1
## 33           1                0
## 34           1                1
## 35           1                0
## 36           1                1
## 37           1                0
## 38           1                1
## 39           1                0
## 40           1                1
## 41           1                0
## 42           1                1
## 43           1                0
## 44           1                1
## 45           1                0
## 46           1                1
```

```
## 47           1               0
## 48           1               1
## attr(,"assign")
## [1] 0 1
## attr(,"contrasts")
## attr(,"contrasts")$groups
## [1] "contr.treatment"
```