

# Class 16: Mini Project

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## Downstream Analysis

```
#BiocManager::install("tximport")
#BiocManager::install("rhdf5")

library(tximport)

folders <- dir(pattern="SRR21568*")
samples <- sub("_quant", "", folders)
files <- file.path( folders, "abundance.h5" )
names(files) <- samples

txi.kallisto <- tximport(files, type = "kallisto", txOut = TRUE)

## 1 2 3 4
head(txi.kallisto$counts)

##           SRR2156848 SRR2156849 SRR2156850 SRR2156851
## ENST00000539570      0          0    0.00000      0
## ENST00000576455      0          0    2.62037      0
## ENST00000510508      0          0    0.00000      0
## ENST00000474471      0          1    1.00000      0
## ENST00000381700      0          0    0.00000      0
## ENST00000445946      0          0    0.00000      0

colSums(txi.kallisto$counts)

## SRR2156848 SRR2156849 SRR2156850 SRR2156851
##   2563611   2600800   2372309   2111474

sum(rowSums(txi.kallisto$counts)>0)

## [1] 94561

to.keep <- rowSums(txi.kallisto$counts) > 0
kset.nonzero <- txi.kallisto$counts[to.keep,]

keep2 <- apply(kset.nonzero,1,sd)>0
x <- kset.nonzero[keep2,]
```

# Principal Component Analysis

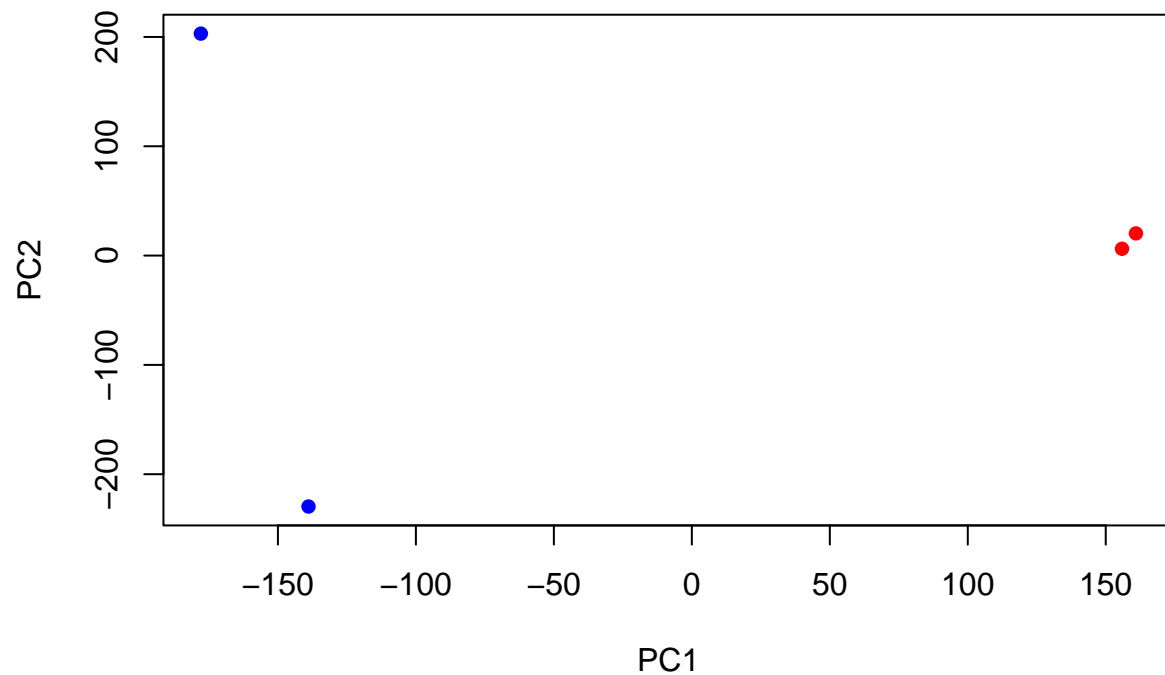
```
pca <- prcomp(t(x), scale=TRUE)
```

```
summary(pca)
```

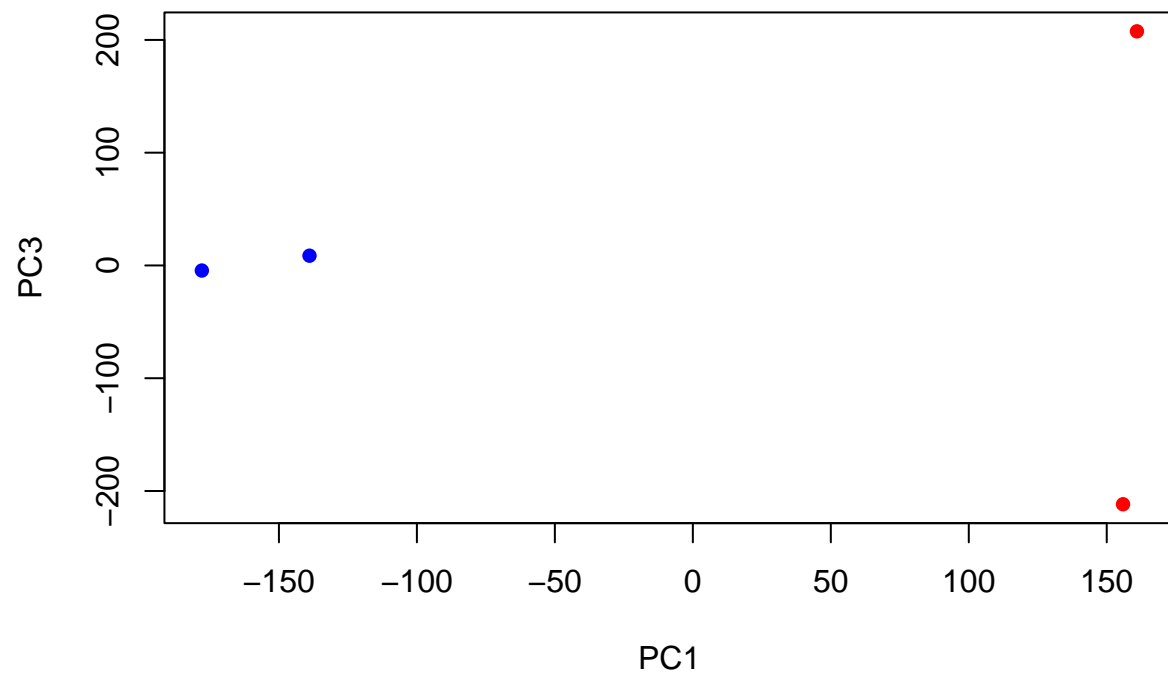
```
## Importance of components:
```

```
##              PC1      PC2      PC3  PC4
## Standard deviation 183.6379 177.3605 171.3020 1e+00
## Proportion of Variance 0.3568 0.3328 0.3104 1e-05
## Cumulative Proportion 0.3568 0.6895 1.0000 1e+00
```

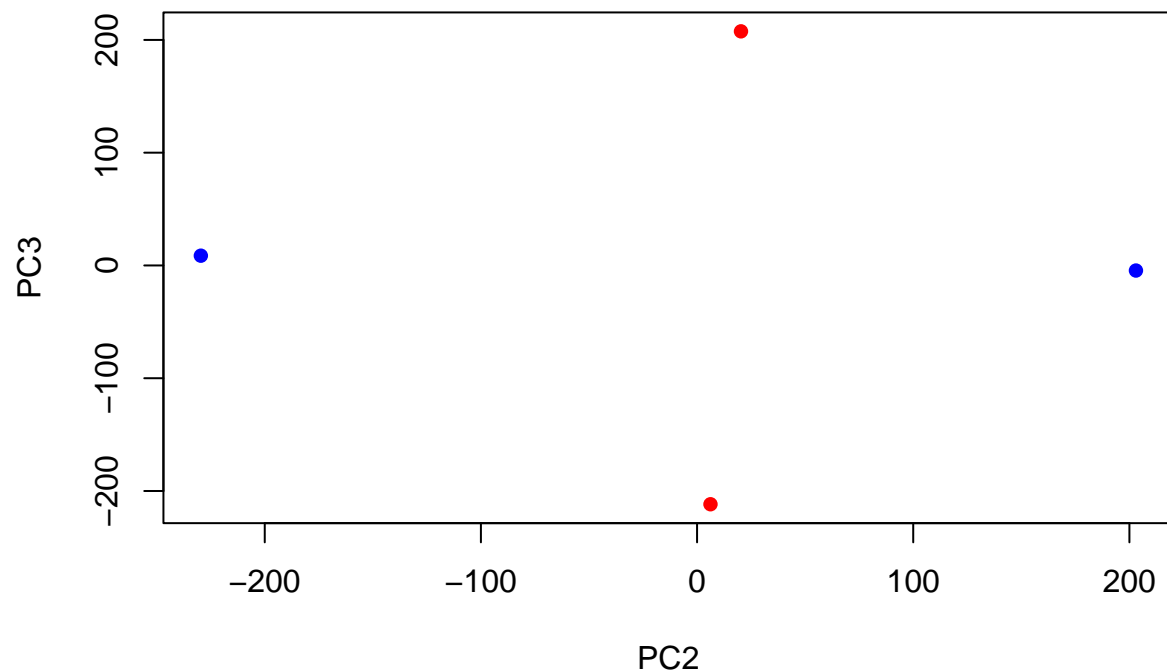
```
plot(pca$x[,1], pca$x[,2],
     col=c("blue", "blue", "red", "red"),
     xlab="PC1", ylab="PC2", pch=16)
```



```
plot(pca$x[,1], pca$x[,3],
     col=c("blue", "blue", "red", "red"),
     xlab="PC1", ylab="PC3", pch=16)
```



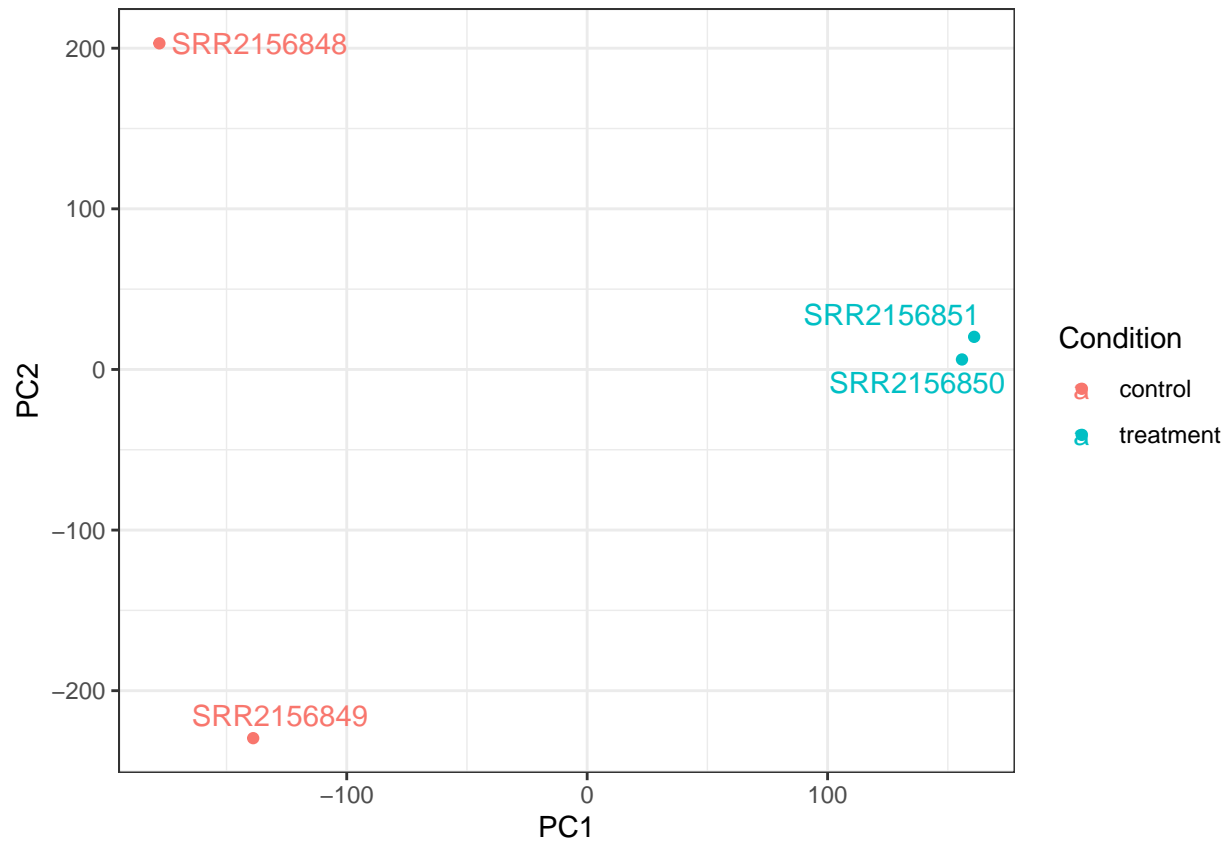
```
plot(pca$x[,2], pca$x[,3],  
     col=c("blue","blue","red","red"),  
     xlab="PC2", ylab="PC3", pch=16)
```



```
library(ggplot2)
library(ggrepel)

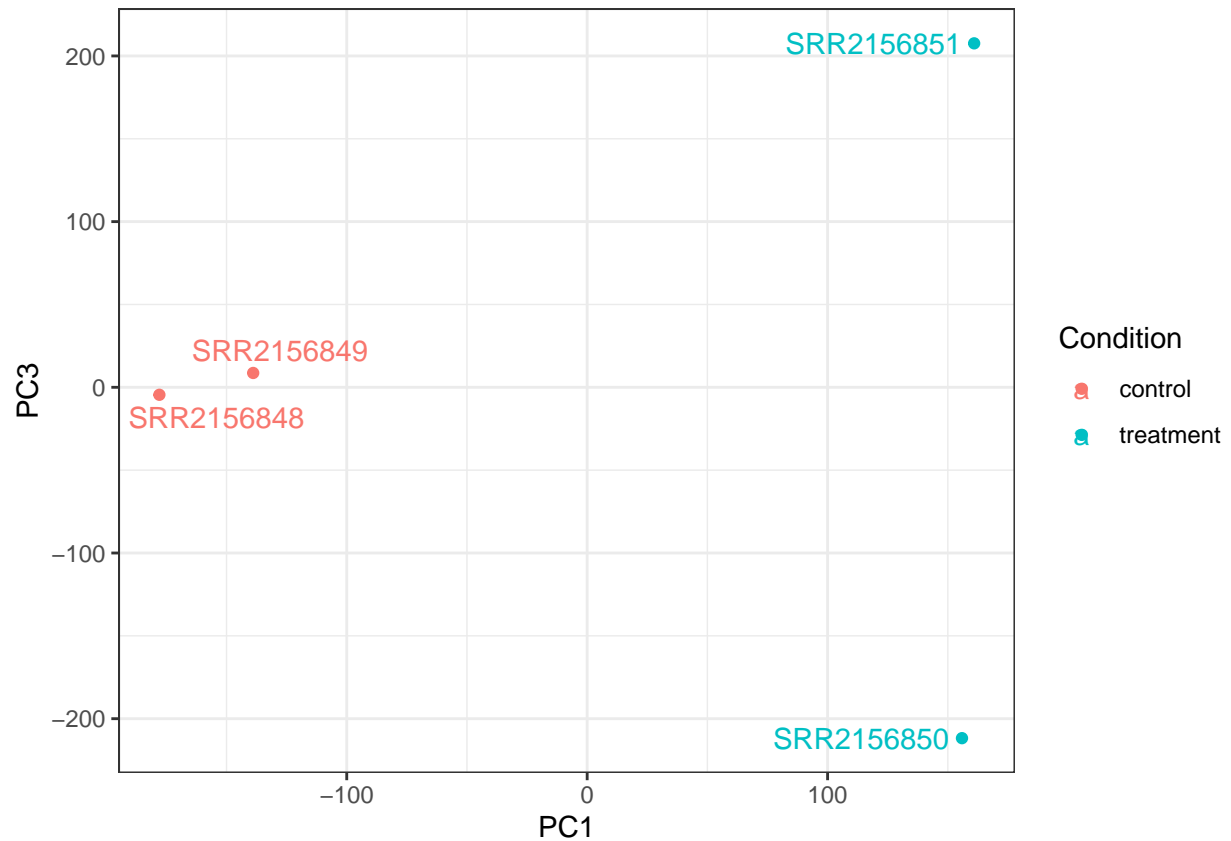
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(txi.kallisto$counts)
y <- as.data.frame(pca$x)
y$Condition <- as.factor(colData$condition)

ggplot(y) +
  aes(PC1, PC2, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```



```
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(txi.kallisto$counts)
y <- as.data.frame(pca$x)
y$Condition <- as.factor(colData$condition)

ggplot(y) +
  aes(PC1, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```



```
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(txi.kallisto$counts)
y <- as.data.frame(pca$x)
y$Condition <- as.factor(colData$condition)

ggplot(y) +
  aes(PC2, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```

