class 16 EC

Gen Dantay (BIMM143)

```r
library(tximport)

# setup the folder and filenames to read
folders <- dir(pattern="SRR21568*")
samples <- sub("_quant", "", folders)
files <- file.path( folders, "abundance.h5" )
names(files) <- samples
```

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

```
1 2 3 4
```

```r
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
```

Above we now have our estimated transcript counts for each sample in R. We can see how many transcripts we have for each sample:

```r
colSums(txi.kallisto$counts)
```

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

And now how many transcripts are detected in at least one sample:

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

```
[1] 94561
```

Before subsequent analysis, we might want to filter out those annotated transcripts with no reads:

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

and those with no change over the samples:

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

Principle Component Analysis

We can now apply an exploratory analysis technique to this counts matrix. As an example, we will perform a PCA of the transcriptomic profiles of these samples. Now we compute the principal components, centering and scaling each transcript's measured levels so that each feature contributes equally to the PCA:

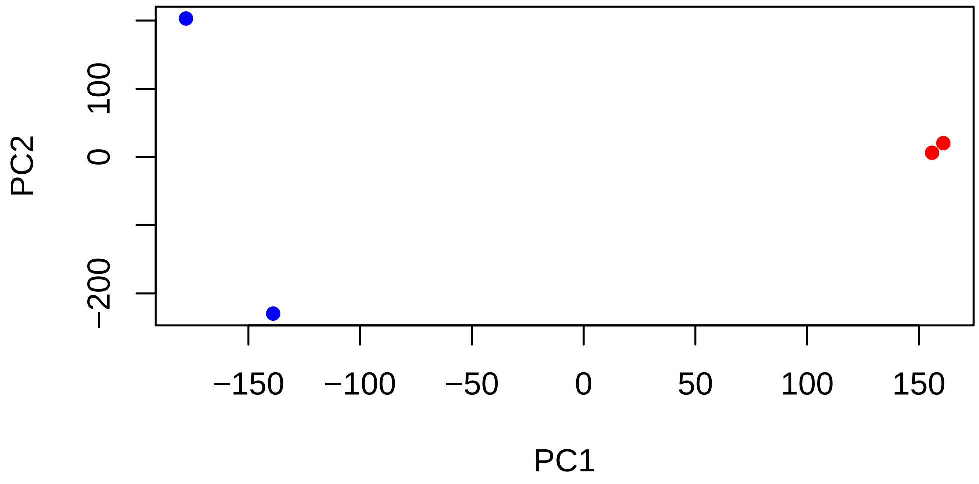
```
pca <- prcomp(t(x),scale=TRUE)
```

```
summary(pca)
```

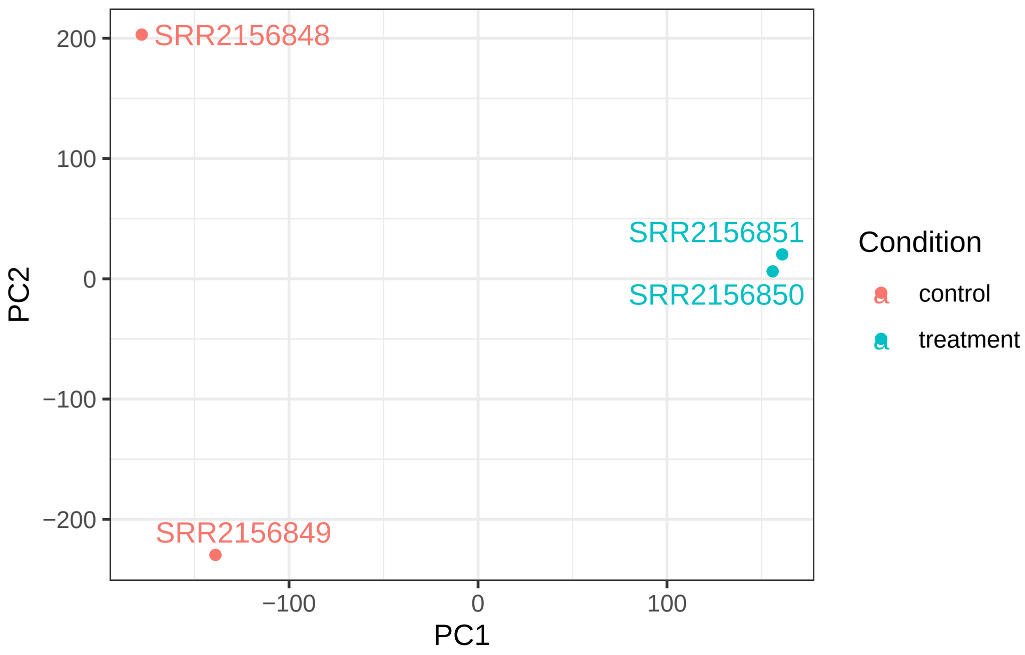
```
Importance of components:
                            PC1      PC2      PC3   PC4
Standard deviation     183.6379 177.3605 171.3020 1e+00
Proportion of Variance   0.3568   0.3328   0.3104 1e-05
Cumulative Proportion    0.3568   0.6895   1.0000 1e+00
```

Now we can use the first two principal components as a co-ordinate system for visualizing the summarized transcriptomic profiles of each sample:

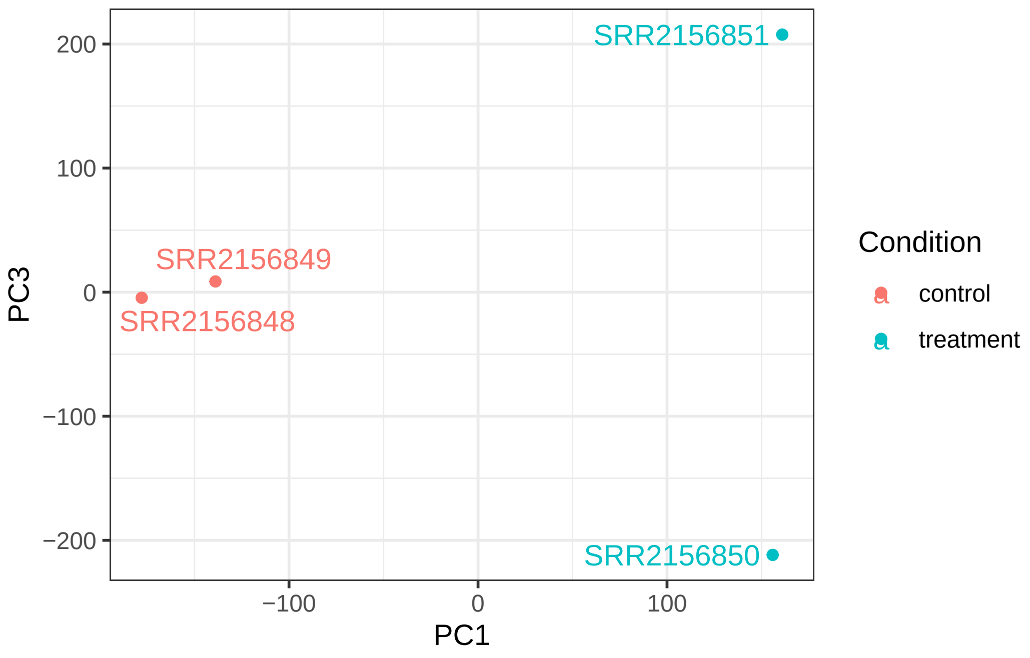
```r
plot(pca$x[,1], pca$x[,2],
     col=c("blue","blue","red","red"),
     xlab="PC1", ylab="PC2", pch=16)
```



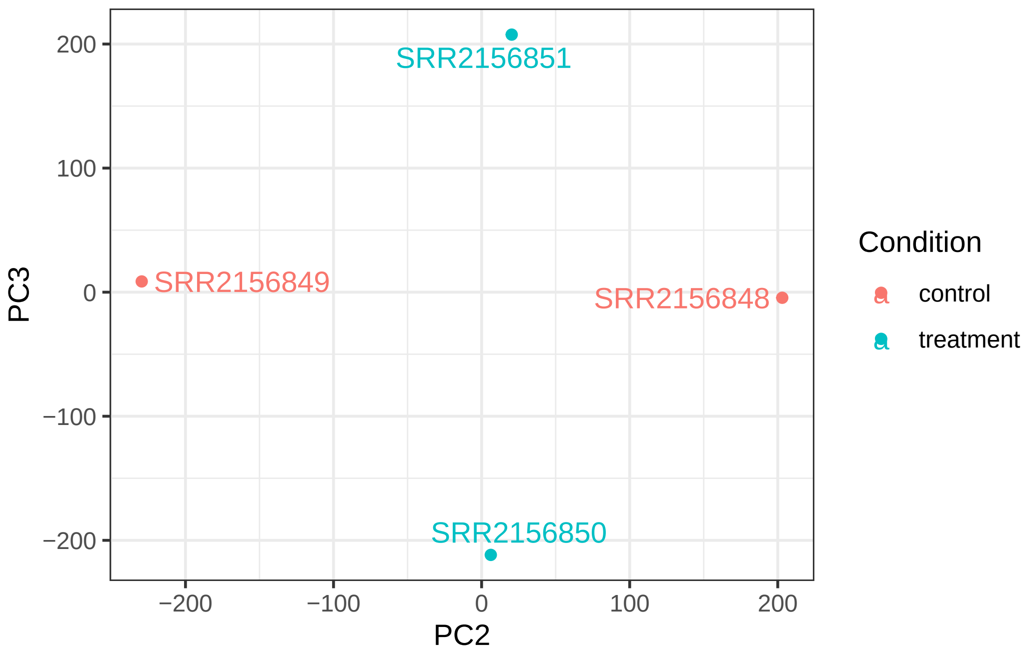
```r
library(ggplot2)
library(ggrepel)

# Make metadata object for the samples
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each =2)))
rownames(colData) <- colnames(txi.kallisto$counts)

# Make the data.frame for ggplot
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()
```



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



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


SRR2156851
SRR2156849
SRR2156848
SRR2156850
PC3
PC2
200
100
0
−100
−200
−200
−100
0
100
200
Condition
control
treatment