

Class 17

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```
# BiocManager::install("tximport") in console
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

How many transcripts we have for each sample:

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

SRR2156848	SRR2156849	SRR2156850	SRR2156851
2563611	2600800	2372309	2111474

How many transcripts detected in at least one sample:

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

```
[1] 94561
```

Filtering out no reads:

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

Filtering out no change over samples:

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

PCA

Now we return to PCA as an exploratory analysis technique.

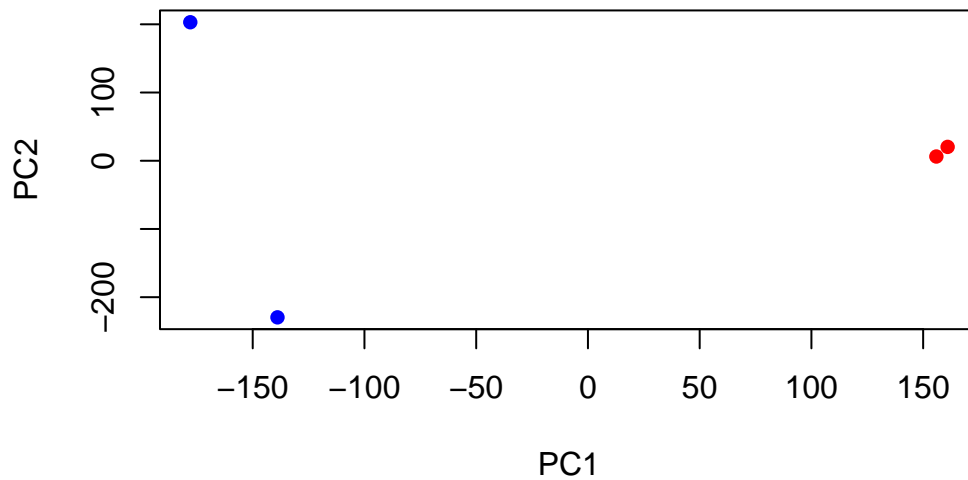
```
pca <- prcomp(t(x), scale=TRUE)
```

```
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)
```



Q1. Use ggplot to make a similar figure of PC1 vs PC2 and a separate figure PC1 vs PC3 and PC2 vs. PC3.

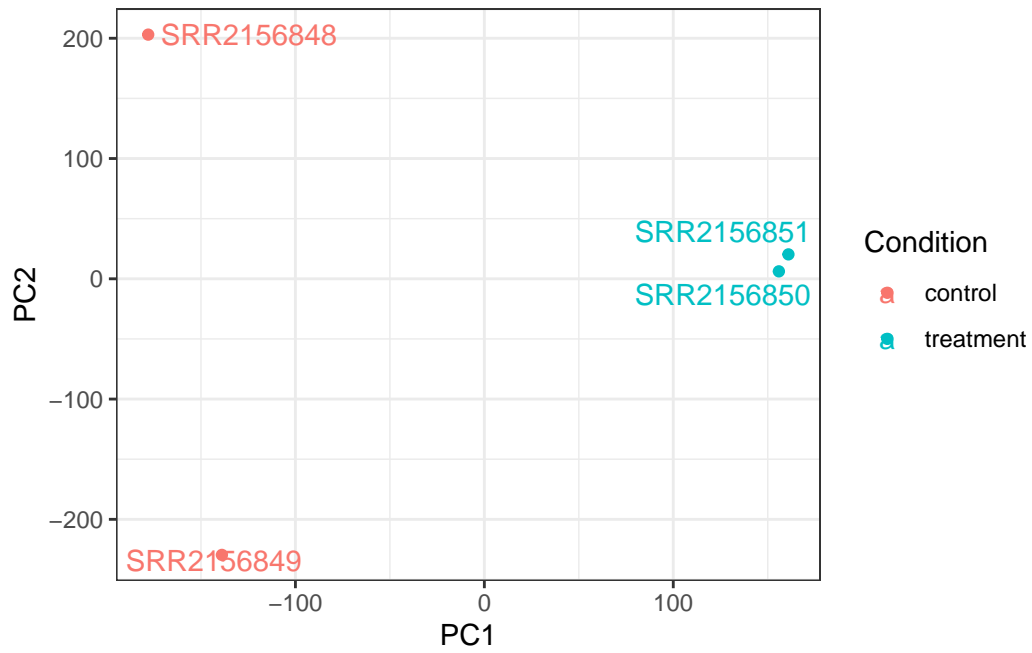
A1. See code below.

```
library(ggplot2)
library(ggrepel)

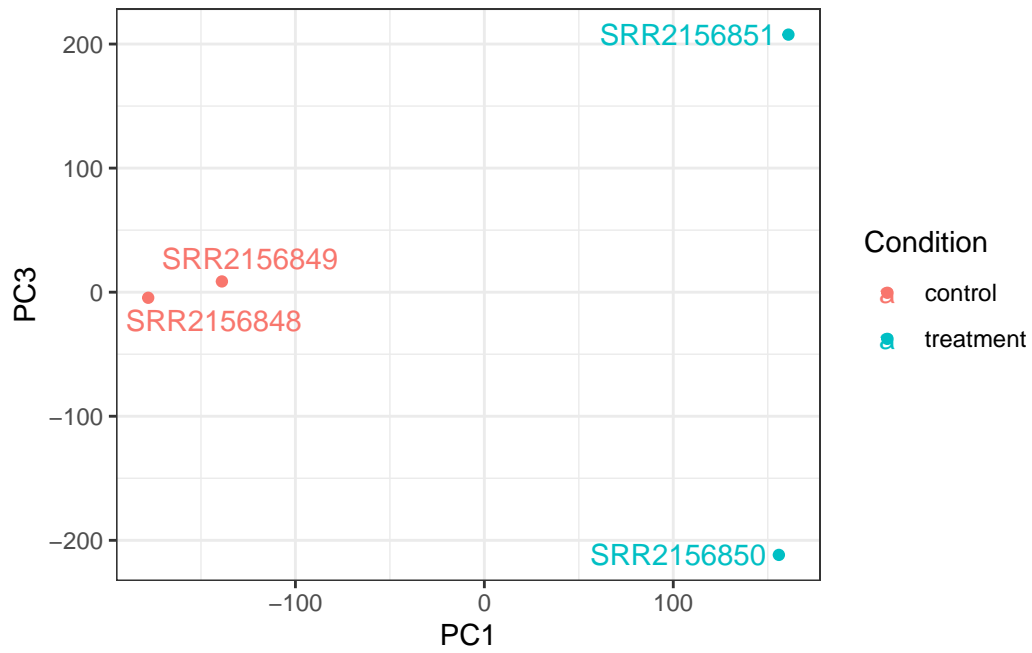
# Make metadata object for the samples (had to use hint for this)
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(tx1.kallisto$counts)

# Make the data.frame for ggplot
df <- as.data.frame(pca$x)
df$Condition <- as.factor(colData$condition)

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



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



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

