Class 17: Analyzing Sequencing Data

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Downstream Analysis

After downloading the `tximport` package, we can directly and easily read Kallisto results.

```
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

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

```
1 2 3 4
```

```
# Taking a look:
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
```

> (Q): How many transcripts do we have for each sample?

The results are shown by `colSums()`.

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

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

> (Q): How many transcripts are in at least one sample?

There were 94,561 transcripts that were present in at least one sample.

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

```
[1] 94561
```

Principal Component Analysis

Here is some code to filter the results:

```
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,]
```

…Before moving onto 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
```

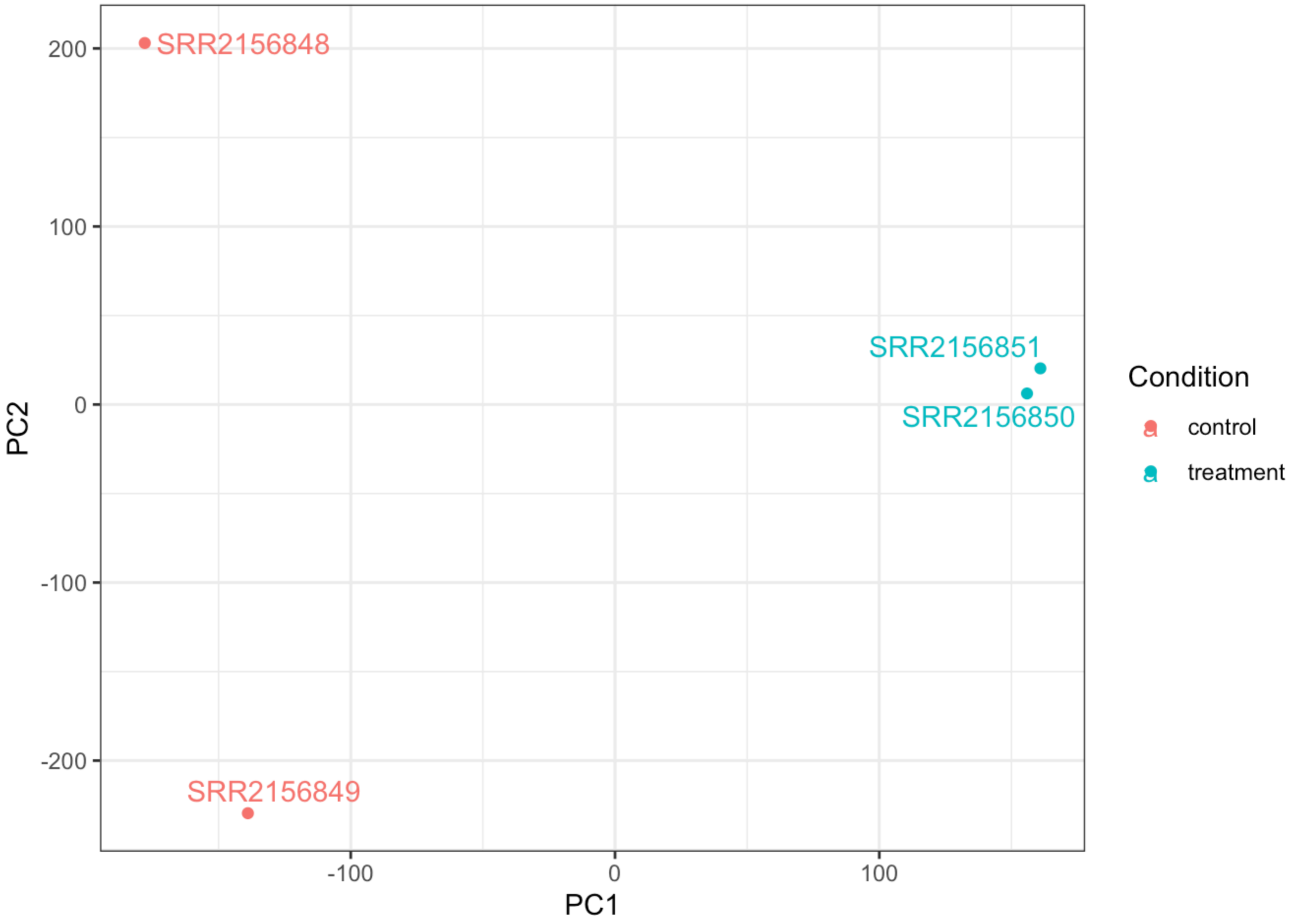
> (Q): Make PCA Plots of PC1 vs PC2.

First, let’s load in some additonal code. We are loading in the metadata as well as creating teh dataframe so we can make a `ggplot` rather than a `base R` plot.

```
# 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)
```

Now, here’ PC1 vs. PC2:

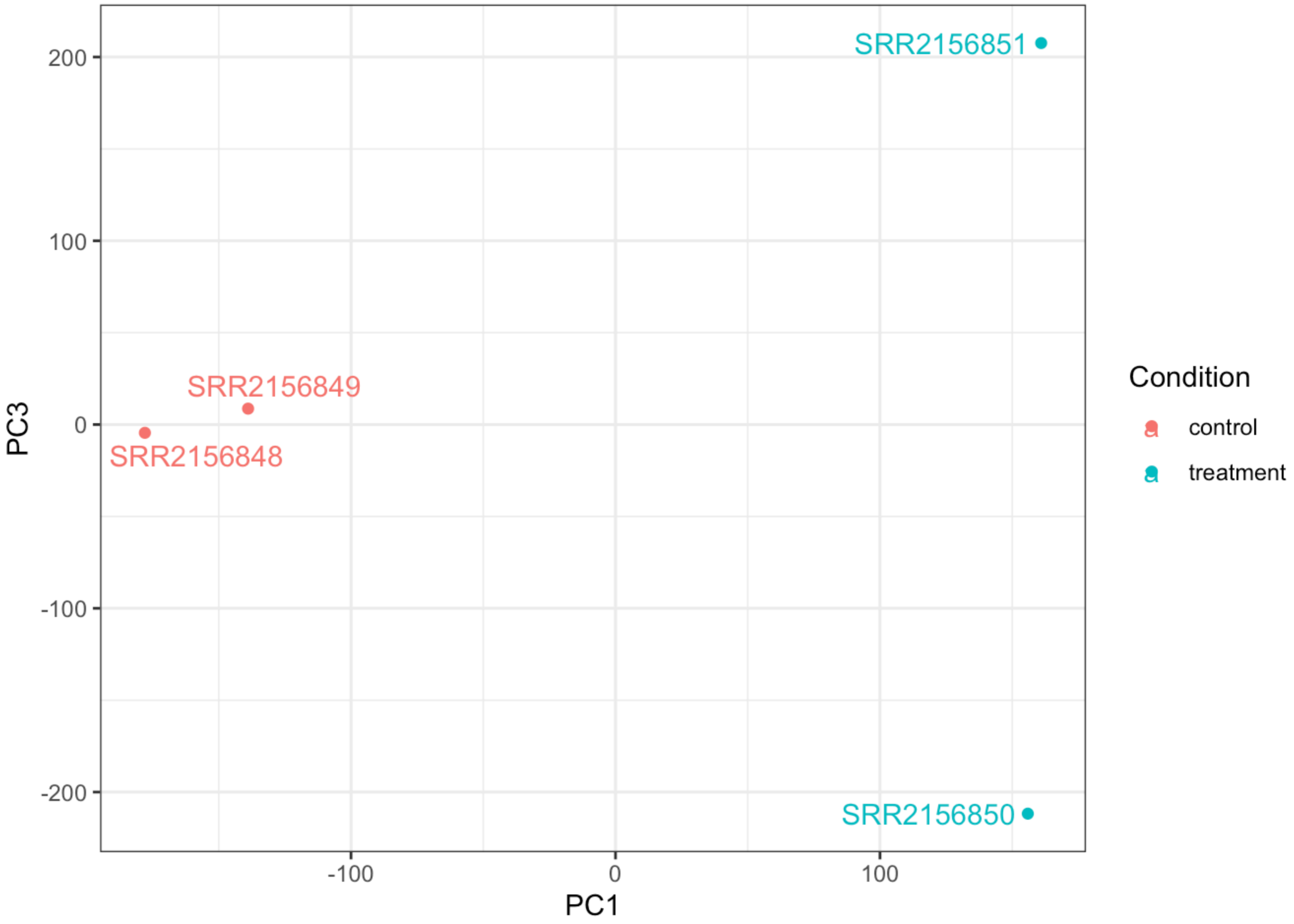
```
library(ggplot2)
library(ggrepel)

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



> (Q): Make PCA Plots of PC1 vs PC3.

The process is the same as before:

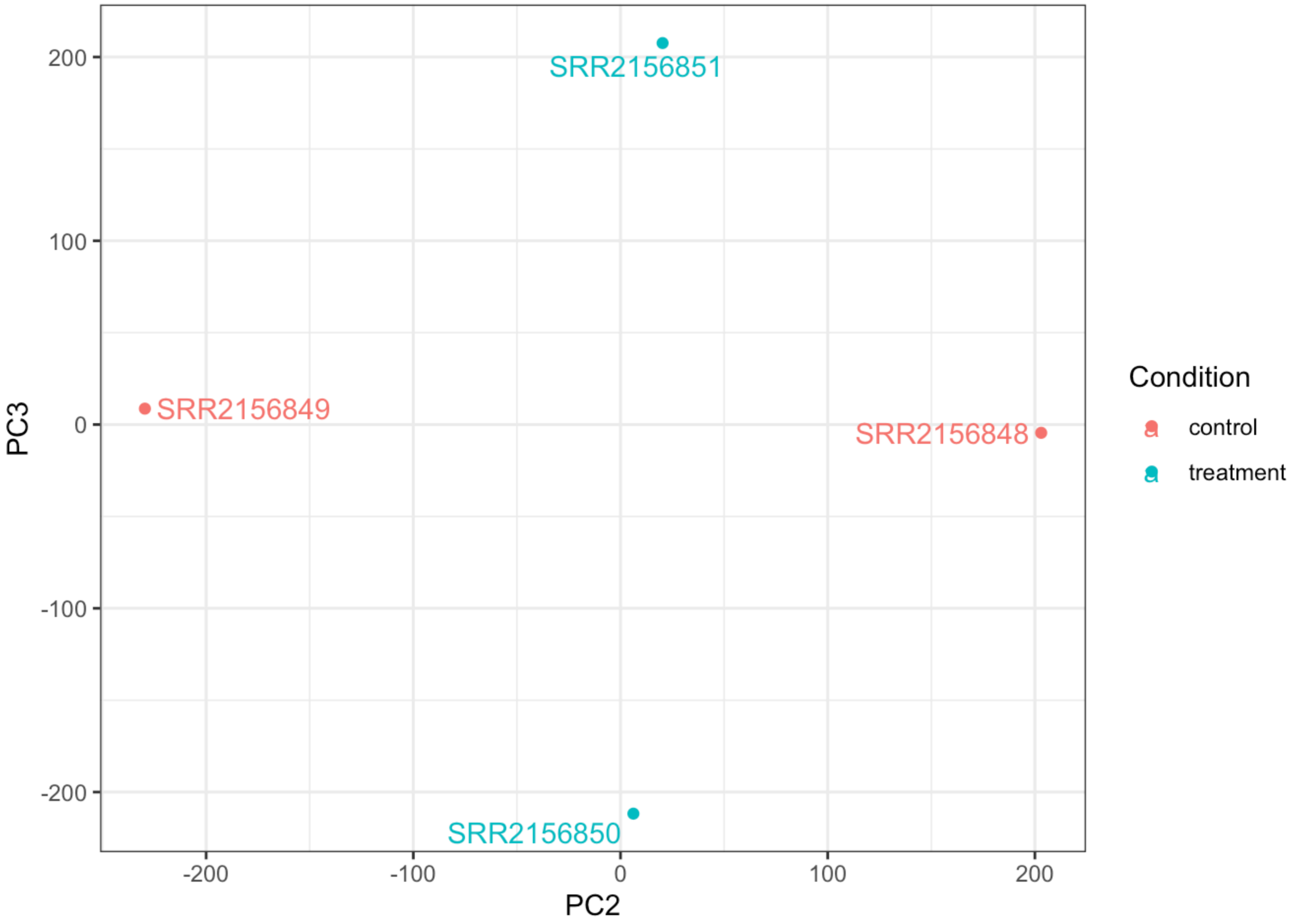
```
ggplot(y) +
  aes(PC1, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```



> (Q): Make PCA Plots of PC2 vs PC3.

The process is the same as before, one final time:

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


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