

Class 17: Analyzing sequencing data in the cloud

Sion Kang (PID: A17052234)

2025-03-04

Installed package `tximport` in console

```
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

```
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

We can see how many transcripts we have for each sample:

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

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

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

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

```
[1] 94561
```

Filter out annotated transcripts with no reads:

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

Filter out those with no change over the samples:

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

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

PC1 vs PC2

```

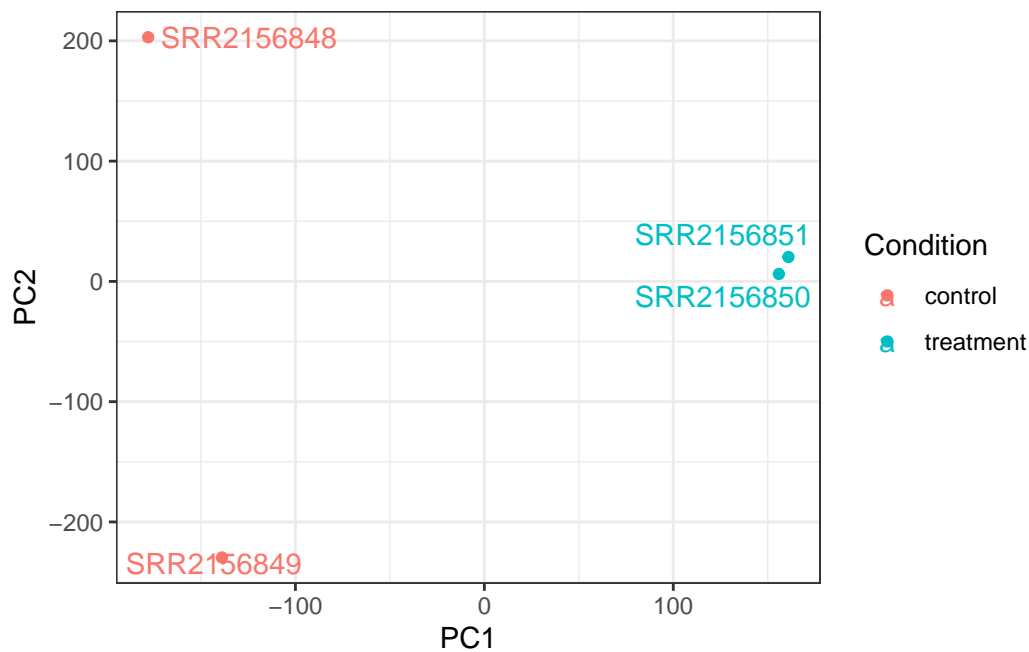
library(ggplot2)
library(ggrepel)

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()

```

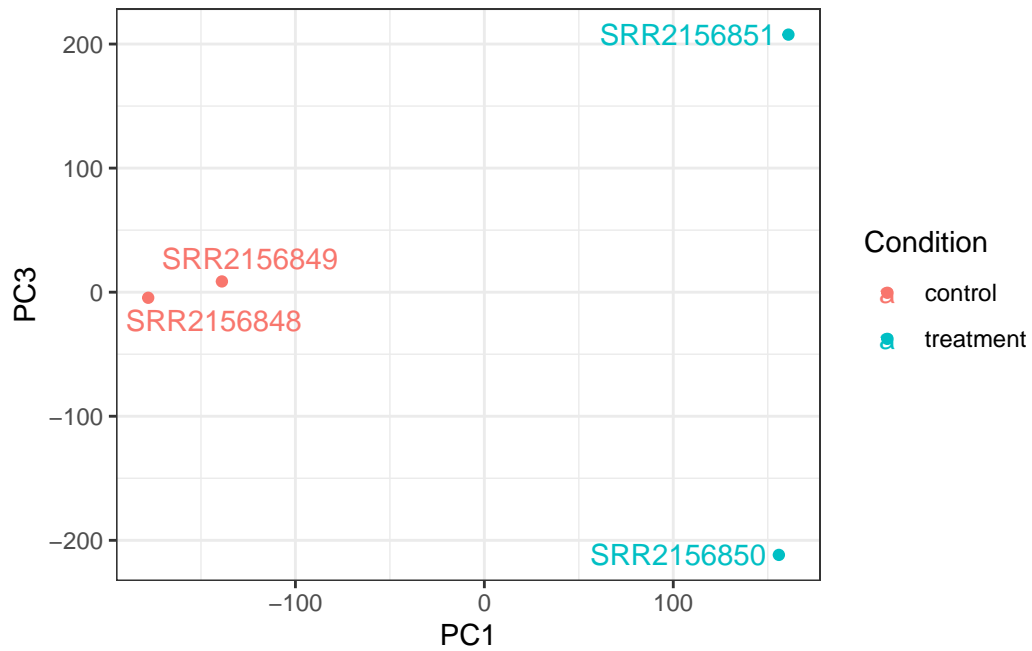


PC1 vs PC3

```

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

```



PC2 vs PC3

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

