

# Class17

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Instance ID

Open an SSH client.

Locate your private key file. The key used to launch this instance is bimm143\_shg005.pem

Run this command, if necessary, to ensure your key is not publicly viewable. `chmod 400 "bimm143_shg005.pem"`

Connect to your instance using its Public DNS: `ec2-18-237-42-241.us-west-2.compute.amazonaws.com`

Example:

```
ssh -i keyfile ubuntu@ec2-18-237-42-241.us-west-2.compute.amazonaws.com
```

```
scp -r -i keyfile ubuntu@ec2-18-237-42-241.us-west-2.compute.amazonaws.com:~/*_quant .  
>this is ran on local machine
```

```
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

```
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,]
```

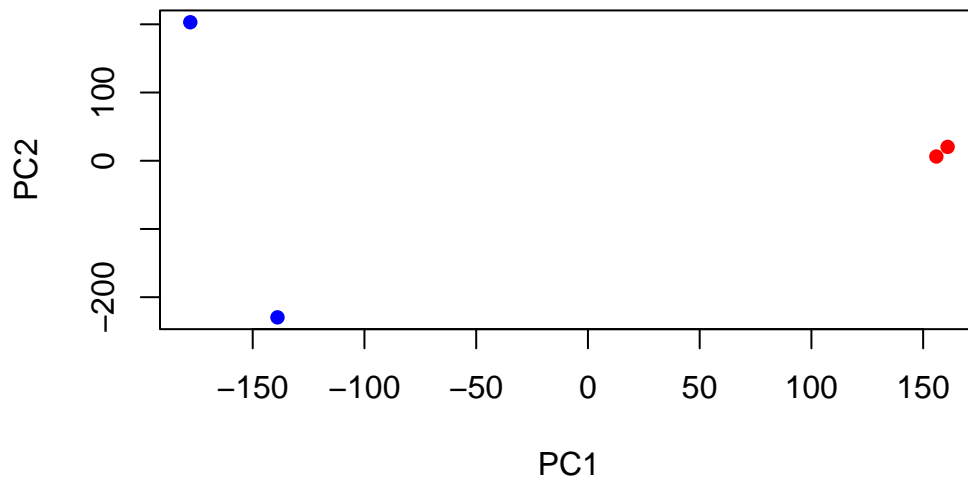
```
pca <- prcomp(t(x), scale=TRUE)
```

```
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)
```



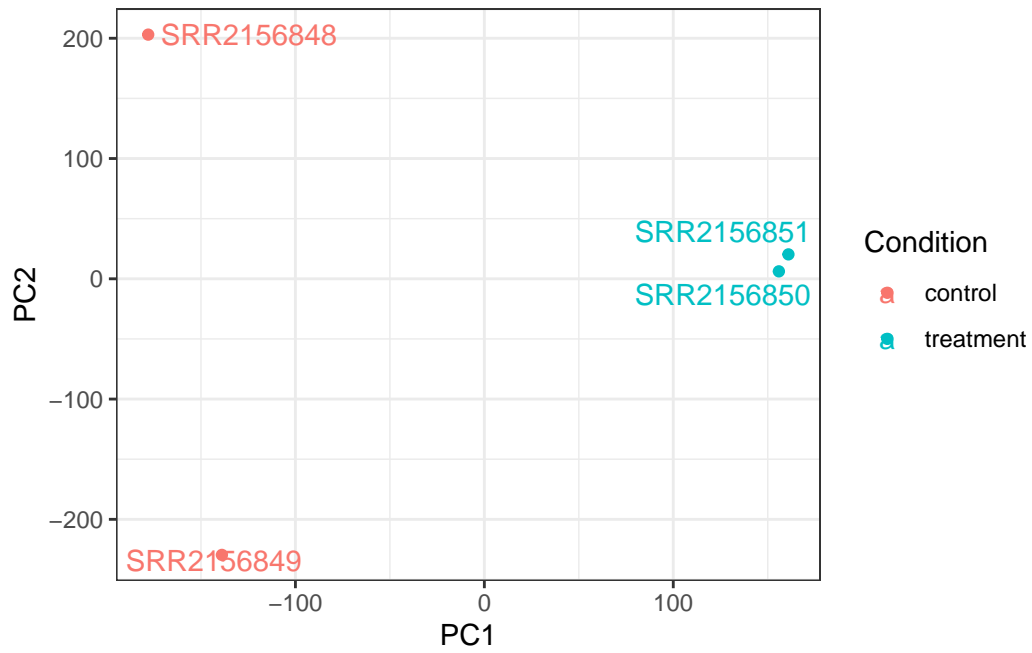
Q. Use ggplot to make a similar figure of PC1 vs PC2 and a separate figure PC1 vs PC3 and PC2 vs PC3.

```
library(ggplot2)
library(ggrepel)

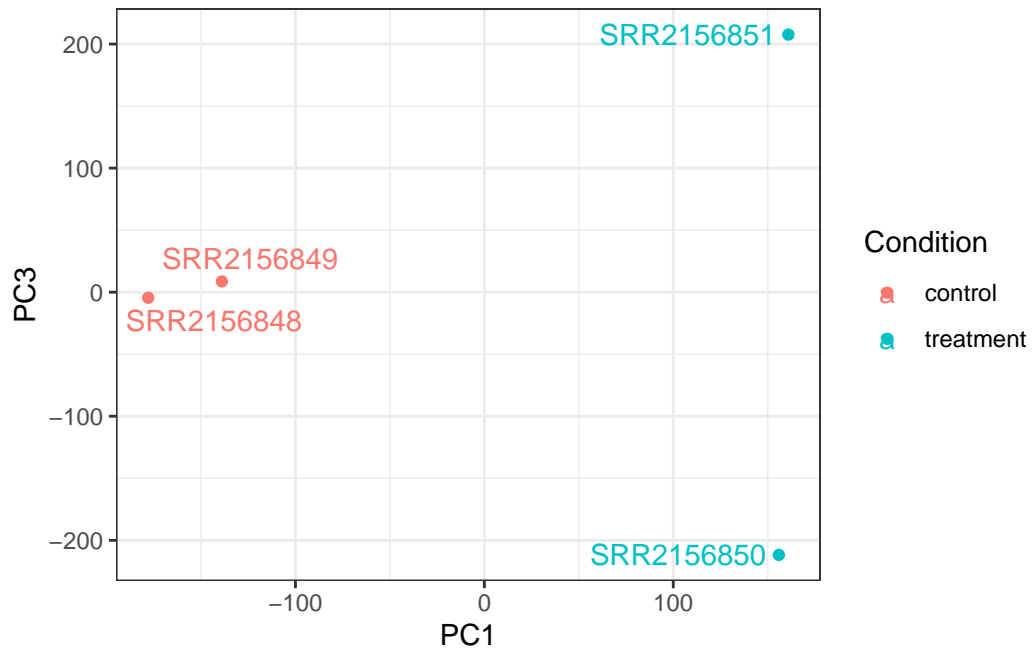
# Make metadata object for the samples
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(txi.kallisto$counts)

# 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()
```

