Class 17: Seq analysis on the cloud

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Where are my files/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
```

```
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 stimated transcript counts for each sample in R. Let's see how many transcripts we have for each sample:

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

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

how many transcripts are detected in at least one sample?

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

```
[1] 94561
```

Let's filter out annotated transcripts with no reads and with no change over the samples:

```
# No reads
to.keep <- rowSums(txi.kallisto$counts) > 0
kset.nonzero <- txi.kallisto$counts[to.keep,]

# No change (eliminating transripts with SD less than 0)
keep2 <- apply(kset.nonzero,1,sd)>0
x <- kset.nonzero[keep2,]
```

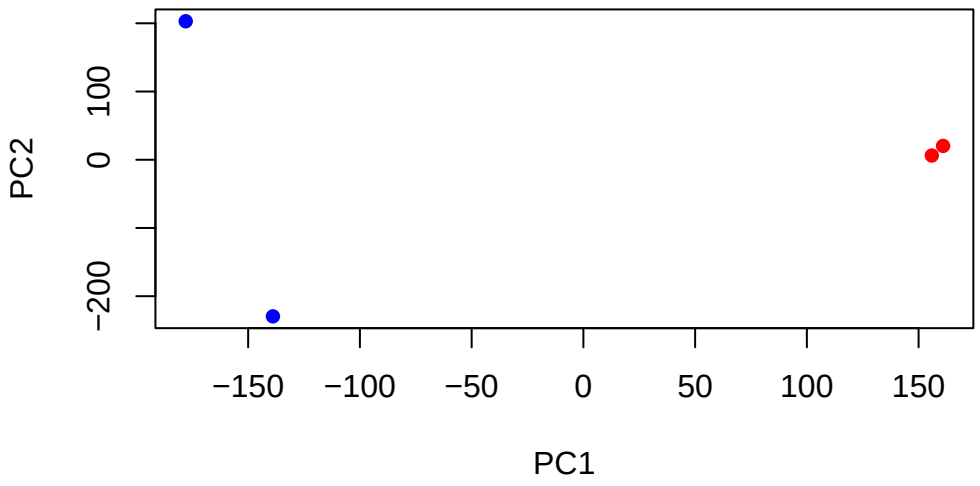
PCA

```
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
```

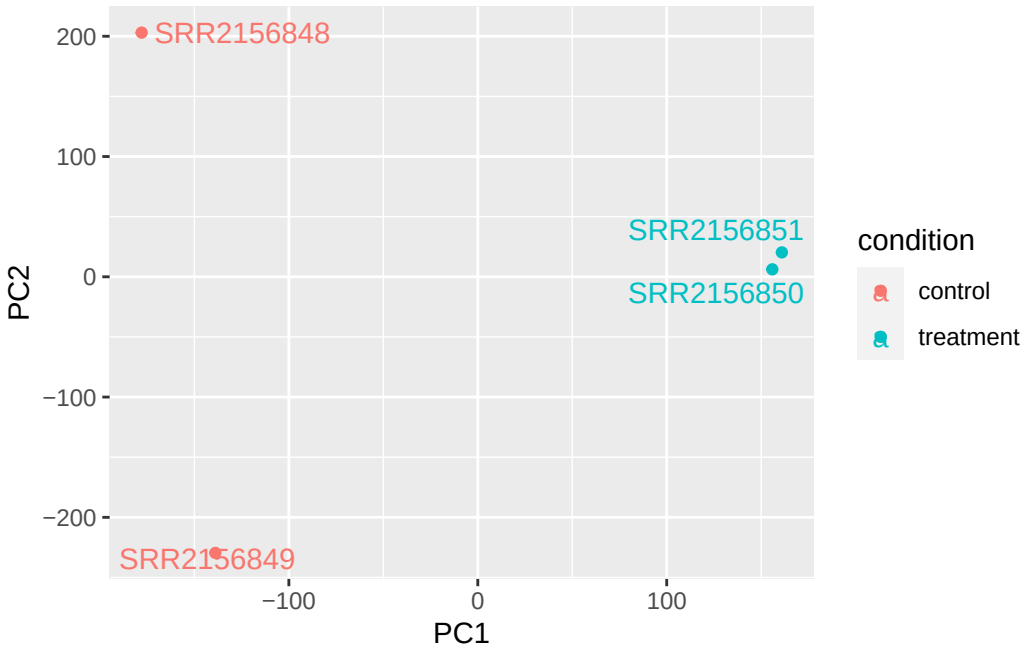
Now we can use the first two principal components as a co-ordinate system for visualizing the summarized transcriptomic profiles of each sample:

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

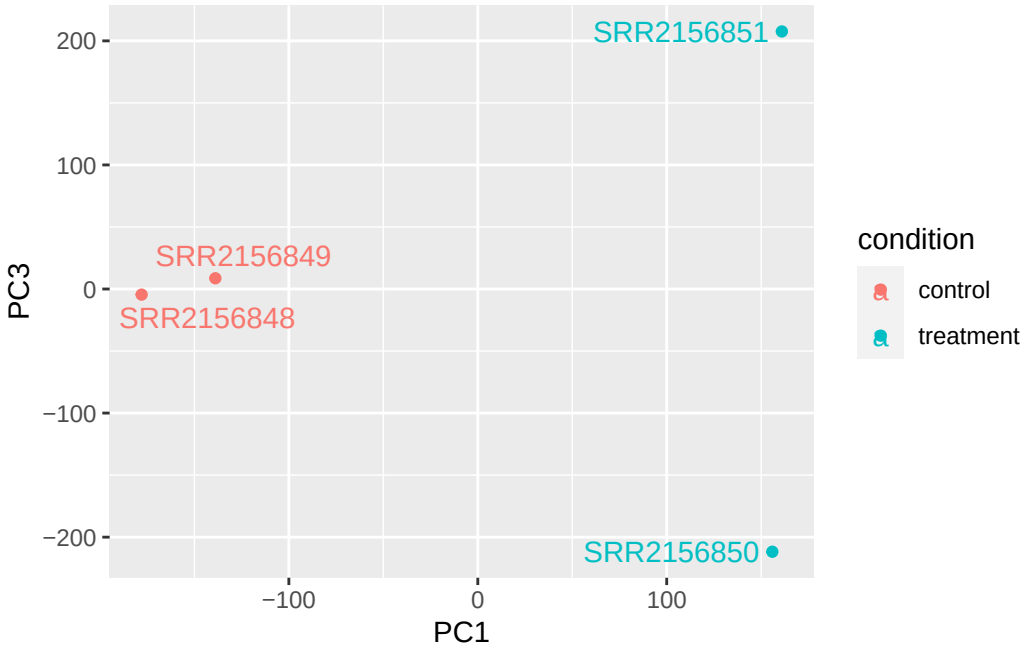


Q. Use ggplot to make a similar figure of PC1 vs PC2 and a seperate figure PC1 vs PC3 and PC2 vs PC3.

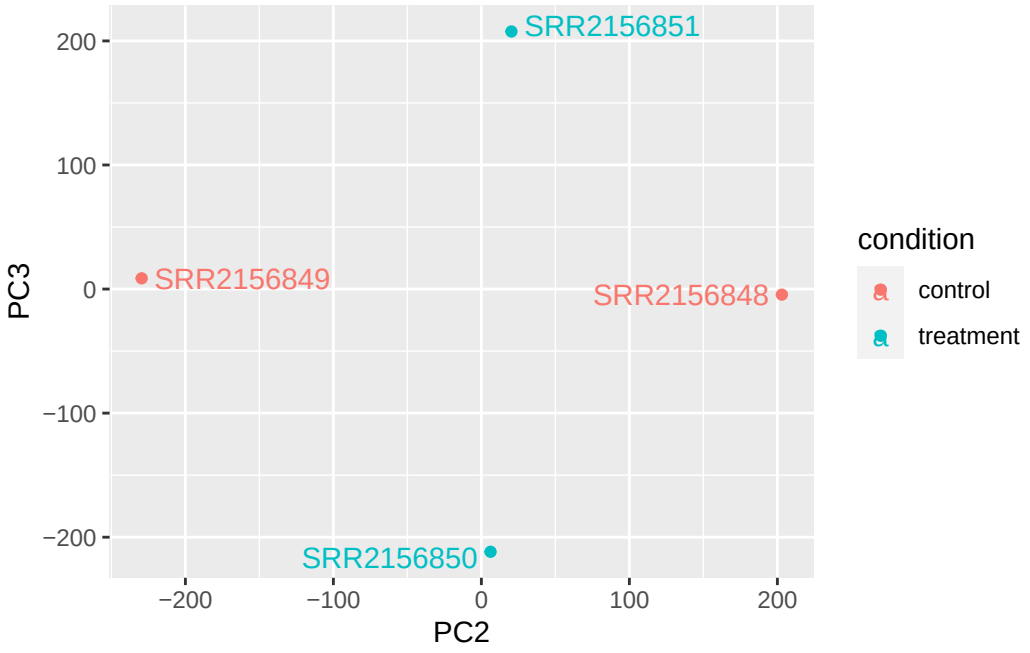
```
library(ggplot2)
library(ggrepel)

samp=colnames(txi.kallisto$counts)
con=c("control","control","treatment","treatment")
metadata<-data.frame(cbind(samp,con))

pca.df<- data.frame(pca$x)
pca.df$condition<-metadata$con
ggplot(pca.df, aes(x=PC1,y=PC2,col=condition)) + geom_point() +
  geom_text_repel(label=metadata$samp)
```




```
ggplot(pca.df, aes(x=PC1,y=PC3,col=condition)) + geom_point() +
  geom_text_repel(label=metadata$samp)
```



```
ggplot(pca.df, aes(x=PC2,y=PC3,col=condition)) + geom_point() +
  geom_text_repel(label=metadata$samp)
```



We can observe that PC1 is separating controls and treatments while PC2 is separating the controls.PC3 separates the two enhancer-targeting CRISPR samples from each other.