Class17

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Done in Class

The command that worked to log into the AWS server today: `ssh -i ~/Downloads/BIMM143_GTan.pem ubuntu@ec2-54-214-189-205.us-west-2.compute.amazonaws.com`

To get it, we need to install the software to get the SRA.

Download

```
curl -O https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/current/sratoolkit.current-ubuntu64.tar.gz
```

Unzip and Untar

`gunzip sratoolkit.current-ubuntu64.tar.gz` `tar -xvf sratoolkit.current-ubuntu64.tar`
^ `tar` = tape archive

Typing the full path is annoying and it will make u type something wrong.

So, let's make it easier.

- echo: will print out things `echo $PATH` > /usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/sbin:/bin:/usr

this shows every commands we have in unix.

Now we want to make SRAtoolkit as one of our commands, so it makes it easier for us to go back here.

```
export PATH=$PATH:/home/ubuntu/class17/sratoolkit.3.2.1-ubuntu64/bin
```

To get a dataset from a study in NCBI, we need the Accession number. > `prefetch SRR600956`
> `fastq-dump SRR600956/` #will download the fastq files!
> `fastq-dump --split-3 SRR2156848` #will download the files into separate files.

For the fastq file, we use: > `grep -c "@SRR" [SRR600956.fastq]`
we want to add the @SRR because @ is also a QC character.
Import data to R

Kallisto

Then, we download Kallisto program to read the fastq files and see how much of each annotated genes are expressed in our sample – kind of like Galaxy, but faster.

Build an index using a reference genome (have to download file first)

kallisto index -i hg19.ensembl Homo_sapiens.GRCh37.67.cdna.all.fa

Getting a quantification of expression of each genes:

kallisto quant -i hg19.ensembl -o SRR2156848_quant SRR2156848_1.fastq SRR2156848_2.fastq

Making this prev. command simultaneously by using a nano file ([FILENAME].sh) !

Write down the three commands for each files. Then, we can put in `&` if we want it to run in the background, all three simultaneously.

> chmod +x [FILENAME].sh # to tell UNIX that this file is a run-able program.
> ./[FILENAME].sh #run!!!

Downloading the file into our local computer

Once we get all the quant data back, we can send it to our **local computaH**!!! We type in this command in our LOCAL terminal:

```
scp -r -i /Users/abel/Downloads/BIMM143_GTan.pem ubuntu@ec2-54-214-189-205.us-west-2.com
.
```

^ the `.` is to make it download to the directory we are at

HOMEWORK:

Importing downloaded files to R

Importing the files we obtained from cloud supercomputer data-processing:

```
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 peek into our imported kallisto files:

```
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
```

Total of gene expressions on each columns:

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

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

There are about 94,561 genes that are expressed in at least one of the samples.

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

```
[1] 94561
```

Cleaning out the data

Filtering out transcripts with no reads:

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

And filter out the ones without the changes over the samples:

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

Plotting the PCA

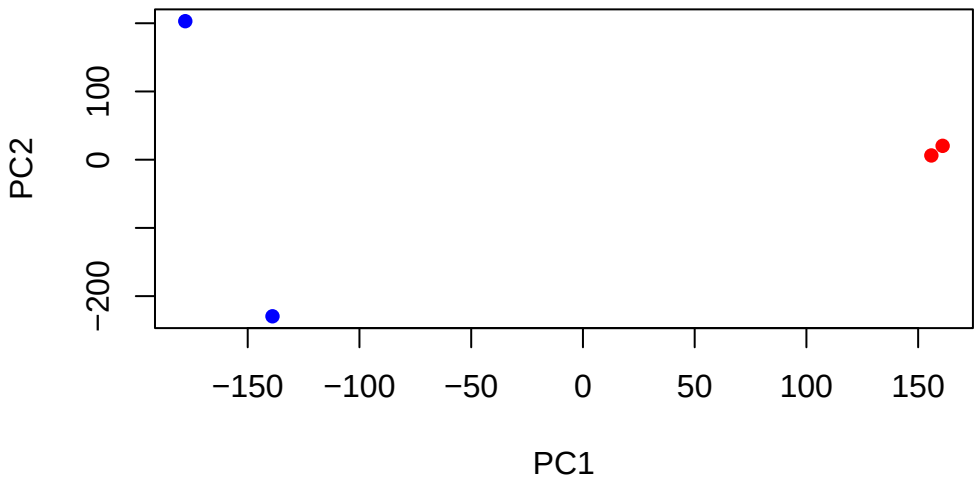
Making the PCA from the filtered dataset:

```
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
```

Plotting the PCA, with `x = PC1` and `y = PC2`.

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



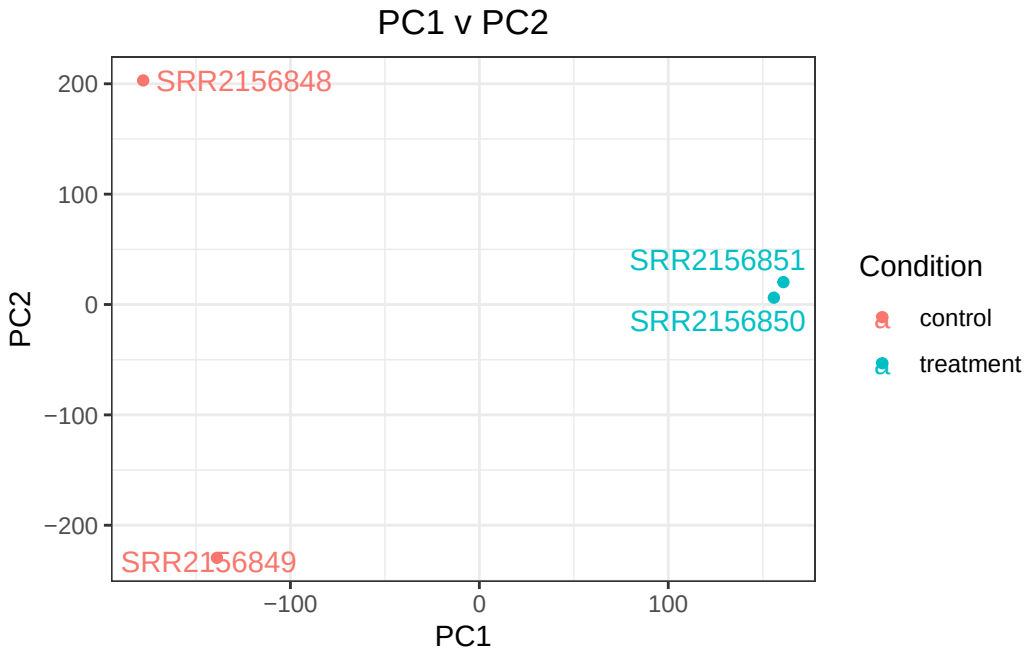
Q. Use ggplot to make a similar figure of PC1 vs PC2 and a seperate figure PC1 vs PC3 and PC2 vs PC3.

```
library(ggplot2)
library(ggrepel)

attributes(pca)
```

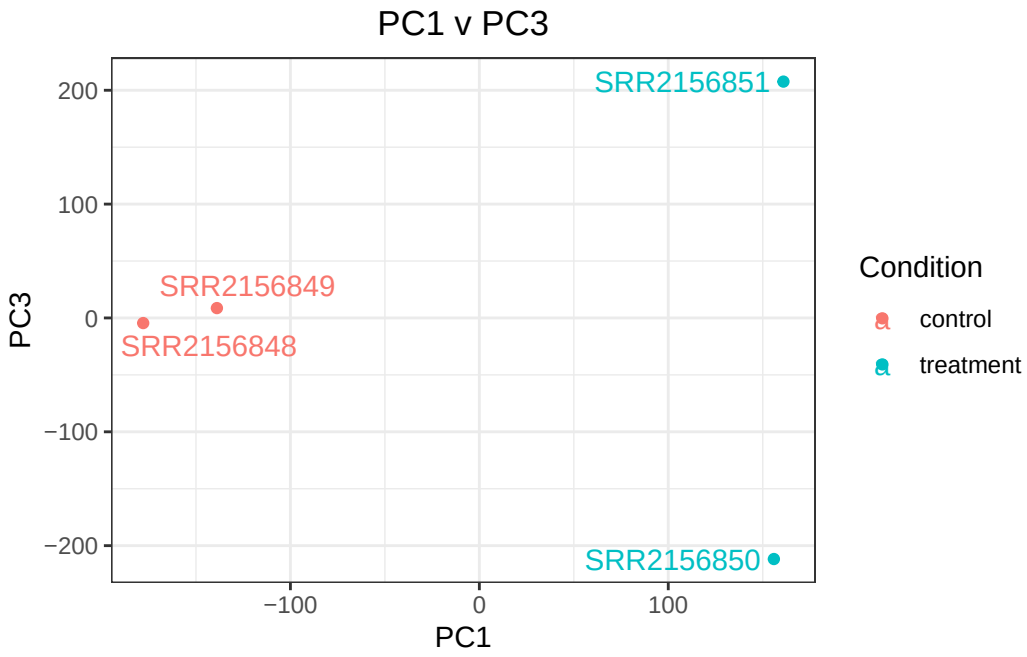
```
$names
[1] "sdev"     "rotation" "center"   "scale"    "x"

$class
[1] "prcomp"
```

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

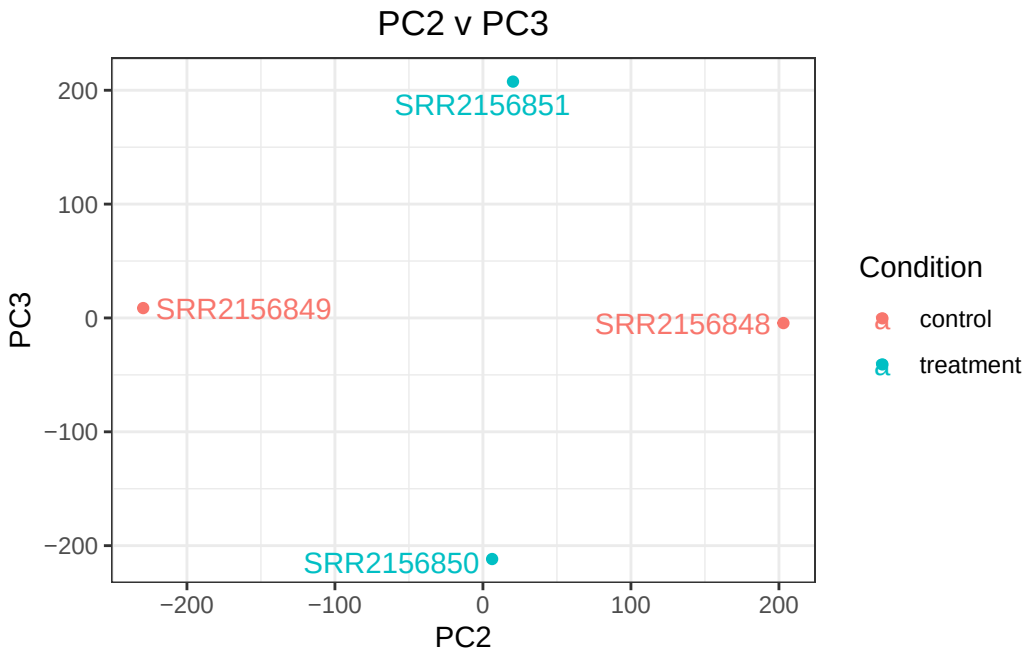
```
# Make the data.frame for ggplot
y <- as.data.frame(pca$x)
y$Condition <- as.factor(colData$condition)

ggplot(y) +
  aes(PC1, PC2, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  ggtitle("PC1 v PC2") + theme_bw() +
  ggeasy::easy_center_title()
```




```
ggplot(y) +
  aes(PC1, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  ggtitle("PC1 v PC3") + theme_bw() +
  ggeasy::easy_center_title()
```




```
ggplot(y) +
  aes(PC2, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  ggtitle("PC2 v PC3") + theme_bw() +
  ggeasy::easy_center_title()
```


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