# Class 18: Downstream Analysis

Hailey Wheeler (A13312713)

```r
library(tximport)
BiocManager::install("rhdf5n")
```

```
Bioconductor version 3.18 (BiocManager 1.30.22), R 4.3.1 (2023-06-16)

Installing package(s) 'rhdf5n'

Warning: package 'rhdf5n' is not available for Bioconductor version '3.18'

A version of this package for your version of R might be available elsewhere,
see the ideas at
https://cran.r-project.org/doc/manuals/r-patched/R-admin.html#Installing-packages

Old packages: 'BiocVersion', 'bslib', 'cluster', 'crosstalk', 'dplyr',
  'foreign', 'GenomeInfoDb', 'htmlwidgets', 'httr2', 'lifecycle', 'lme4',
  'Matrix', 'MatrixModels', 'matrixStats', 'mvtnorm', 'nlme', 'rgl', 'rhdf5',
  'rhdf5filters', 'rlang', 'rprojroot', 'S4Vectors', 'scales', 'shiny',
  'stringi', 'stringr', 'tinytex', 'XML'
```

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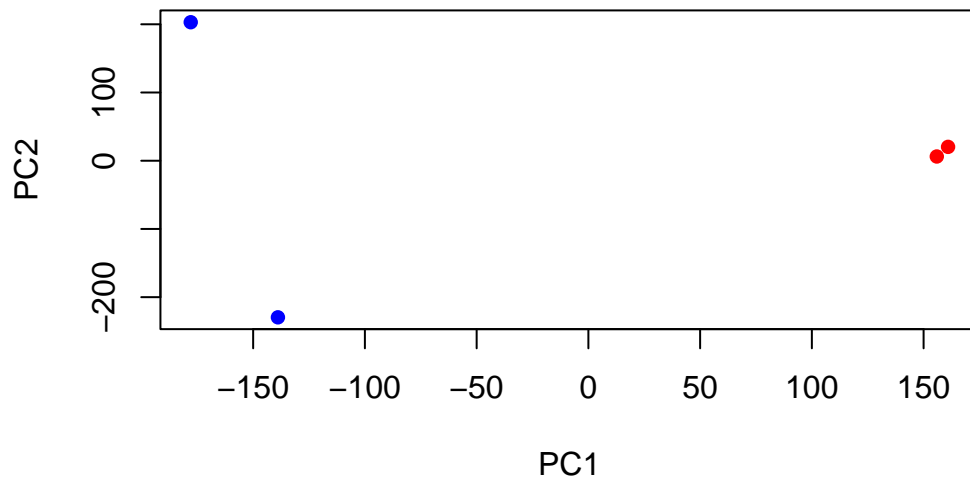
```
pca <- prcomp(t(x), scale=TRUE)
```

```
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
```

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



```r
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()
```