# Calss17: Cloud SRA data analysis

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## Downstream Analysis

```
folders <- list.files(pattern = "_quant")
files <- paste0(folders, "/abundance.h5")
```

```
file.exists(files)
```

```
[1] TRUE TRUE TRUE TRUE
```

```
names(files) <- sub("_quant","",folders)
files
```

```
                      SRR2156848                       SRR2156849
"SRR2156848_quant/abundance.h5" "SRR2156849_quant/abundance.h5"
                      SRR2156850                       SRR2156851
"SRR2156850_quant/abundance.h5" "SRR2156851_quant/abundance.h5"
```

Load up the tximport package

```
library(tximport)

txi.kallisto <- tximport(files, type = "kallisto", txOut = TRUE)
```

```
1 2 3 4
```

## Remove zero count genes

Before subsequent analysis, we might want to filter out those annotated transcripts with no reads:

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

```
nrow(kset.nonzero)
```

```
[1] 94561
```

and thos 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)
```

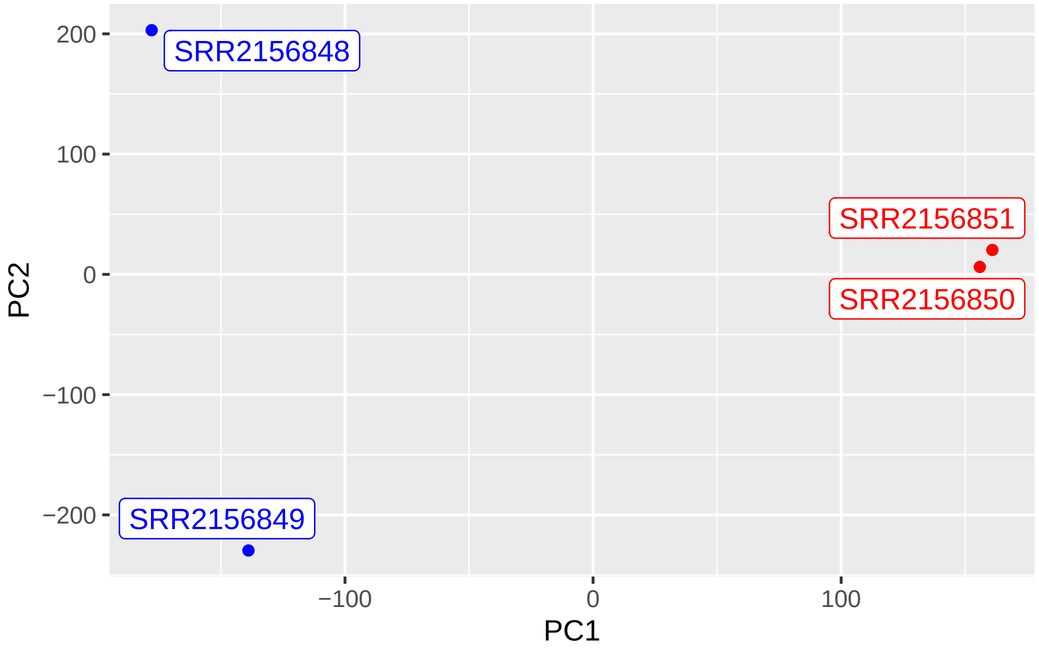
```
Importance of components:
                            PC1      PC2      PC3   PC4
Standard deviation     183.6379 177.3605 171.3020 1e+00
Proportion of Variance   0.3568   0.3328   0.3104 1e-05
Cumulative Proportion    0.3568   0.6895   1.0000 1e+00
```

```
mycols <- c("blue","blue","red","red")

library(ggplot2)
library(ggrepel)
```

```
Warning: package 'ggrepel' was built under R version 4.3.3
```

```
ggplot(pca$x) +
  aes(PC1, PC2) +
  geom_point(col=mycols)+
  geom_label_repel(label = rownames(pca$x), col=mycols)
```



## DESeq

```
library(DESeq2)
```

```
Warning: package 'DESeq2' was built under R version 4.3.3
```

```
Loading required package: S4Vectors

Warning: package 'S4Vectors' was built under R version 4.3.2

Loading required package: stats4

Loading required package: BiocGenerics

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min

Attaching package: 'S4Vectors'

The following object is masked from 'package:utils':

    findMatches

The following objects are masked from 'package:base':

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb
```

```
Warning: package 'GenomeInfoDb' was built under R version 4.3.3

Loading required package: SummarizedExperiment

Warning: package 'SummarizedExperiment' was built under R version 4.3.2

Loading required package: MatrixGenerics

Loading required package: matrixStats

Warning: package 'matrixStats' was built under R version 4.3.3

Attaching package: 'MatrixGenerics'

The following objects are masked from 'package:matrixStats':

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
Attaching package: 'Biobase'
```

```
The following object is masked from 'package:MatrixGenerics':

    rowMedians
```

```
The following objects are masked from 'package:matrixStats':

    anyMissing, rowMedians
```

```r
sampleTable <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(sampleTable) <- colnames(txi.kallisto$counts)
```

```r
sampleTable
```

```
           condition
SRR2156848   control
SRR2156849   control
SRR2156850 treatment
SRR2156851 treatment
```

```r
dds <- DESeqDataSetFromTximport(txi.kallisto, colData = sampleTable, design = ~condition)
```

```
using counts and average transcript lengths from tximport
```

```r
dds <- DESeq(dds)
```

```
estimating size factors
```

```
using 'avgTxLength' from assays(dds), correcting for library size
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
-- note: fitType='parametric', but the dispersion trend was not well captured by the
   function: y = a/x + b, and a local regression fit was automatically substituted.
   specify fitType='local' or 'mean' to avoid this message next time.
```

```
final dispersion estimates
```

```
fitting model and testing
```

```r
res <- results(dds)
```

```r
head(res)
```

```
log2 fold change (MLE): condition treatment vs control
Wald test p-value: condition treatment vs control
DataFrame with 6 rows and 6 columns
                 baseMean log2FoldChange     lfcSE      stat    pvalue
                <numeric>      <numeric> <numeric> <numeric> <numeric>
ENST00000539570  0.000000             NA        NA        NA        NA
ENST00000576455  0.761453       3.155061   4.86052 0.6491203  0.516261
ENST00000510508  0.000000             NA        NA        NA        NA
ENST00000474471  0.484938       0.181923   4.24871 0.0428185  0.965846
ENST00000381700  0.000000             NA        NA        NA        NA
ENST00000445946  0.000000             NA        NA        NA        NA
                     padj
                <numeric>
ENST00000539570        NA
ENST00000576455        NA
ENST00000510508        NA
ENST00000474471        NA
ENST00000381700        NA
ENST00000445946        NA
```