

Class 17

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```
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

```
## Install BiocManager::install("rhdf5") if needed
```

```
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
```

Filtering out annotated transcripts with no reads

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

and those without change in samples

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

PCA

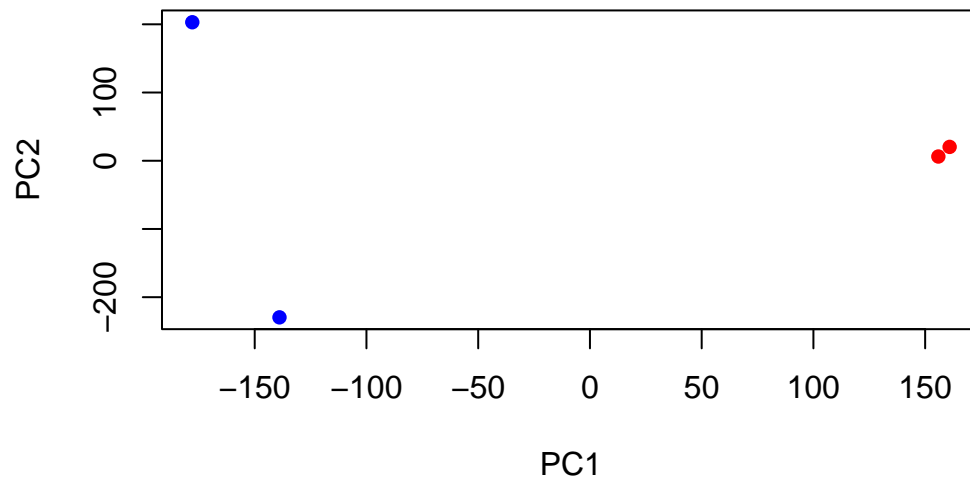
```
pca <- prcomp(t(x), scale=TRUE)
```

```
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

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



ggplot

```
library(ggplot2)
```

```
attributes(pca)
```

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

```
$class
[1] "prcomp"
```

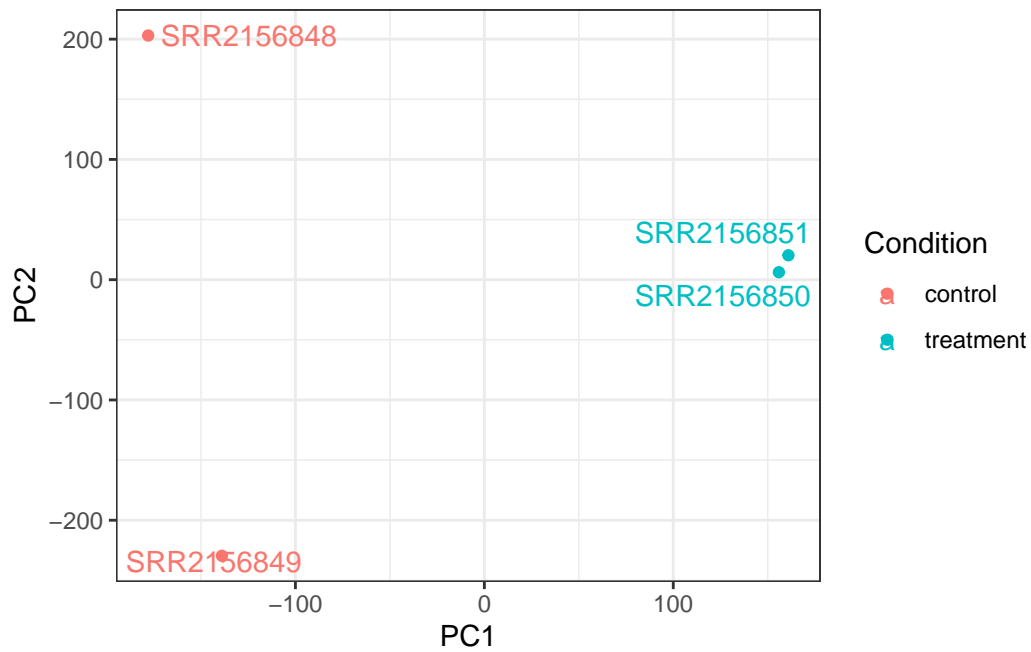
PCA1 vs PCA2

```
library(ggplot2)
library(ggrepel)

# Make metadata object for the samples
colData <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
rownames(colData) <- colnames(tx1.kallisto$counts)
```

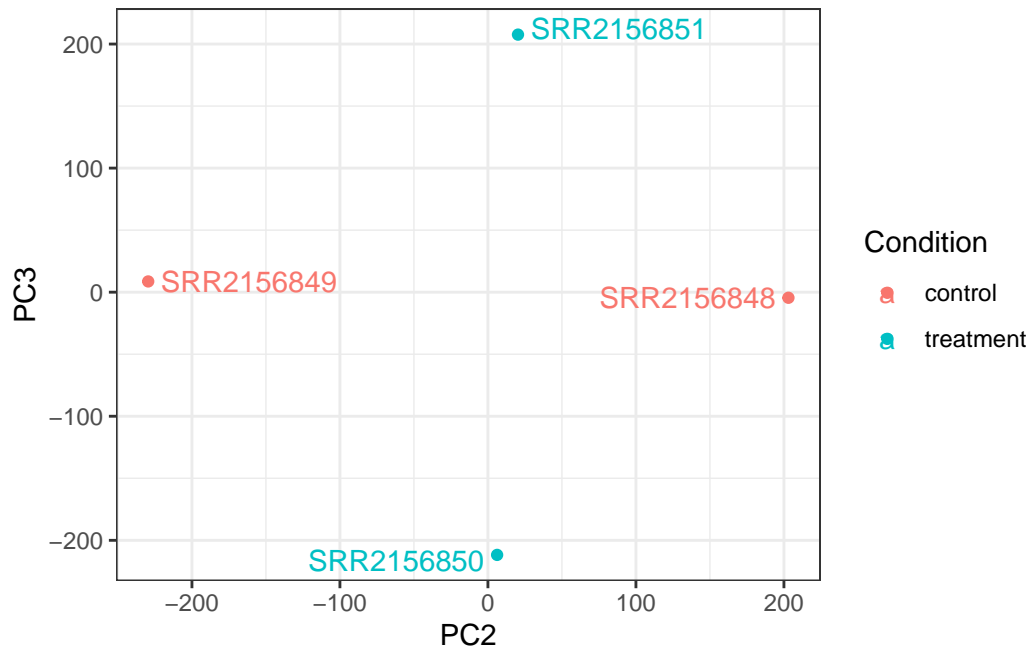
```
# Make the data.frame for ggplot
y <- as.data.frame(pca$x)
y$Condition <- as.factor(colData$condition)

ggplot(y) +
  aes(PC1, PC2, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```



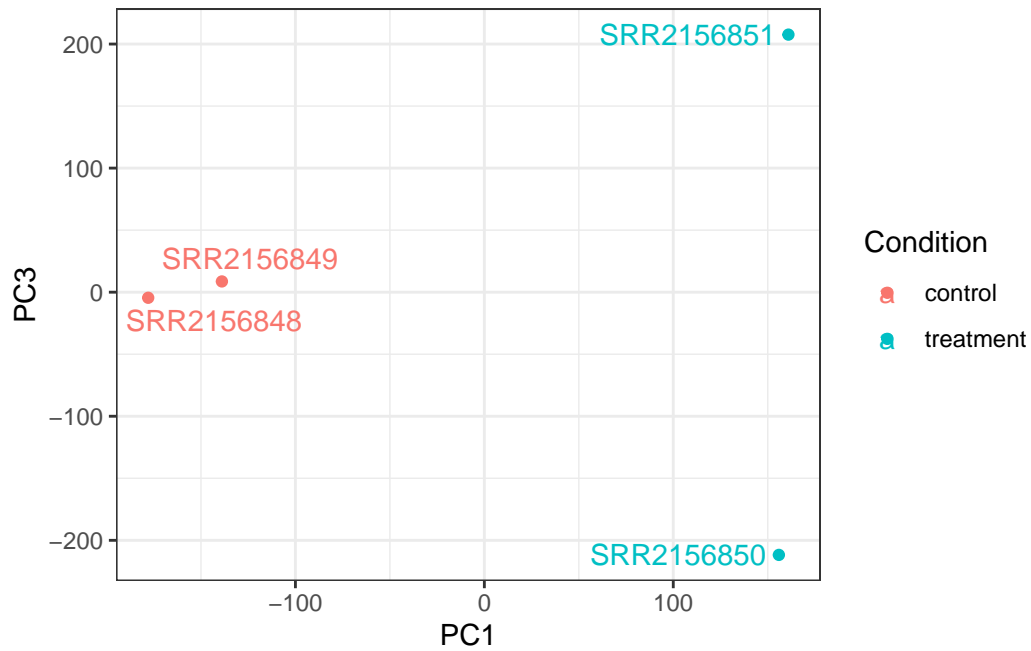
PCA2 vs PCA3

```
ggplot(y) +
  aes(PC2, PC3, col=Condition) +
  geom_point() +
  geom_text_repel(label=rownames(y)) +
  theme_bw()
```



PCA1 vs PCA3

```
ggplot(y) +  
  aes(PC1, PC3, col=Condition) +  
  geom_point() +  
  geom_text_repel(label=rownames(y)) +  
  theme_bw()
```



DESeq2

```
library(DESeq2)
```

Loading required package: S4Vectors

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, saveRDS, setdiff,  
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

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

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
```

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



```
dds <- DESeqDataSetFromTximport(txi.kallisto,
                                sampleTable,
                                ~condition)
```

using counts and average transcript lengths from tximport

```
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

```
res <- results(dds)
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				