lab16

Nate Tran

Reading in data using tximport

```
library(tximport)
library(rhdf5)

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
```

Filtering for transcripts with no reads in any sample or with no change over any samples

```
data_idx <- rowSums(txi.kallisto$counts) > 0
data <- txi.kallisto$counts[data_idx,]

data_idx2 <- apply(data, 1, sd) > 0
data2 <- data[data_idx2,]
```

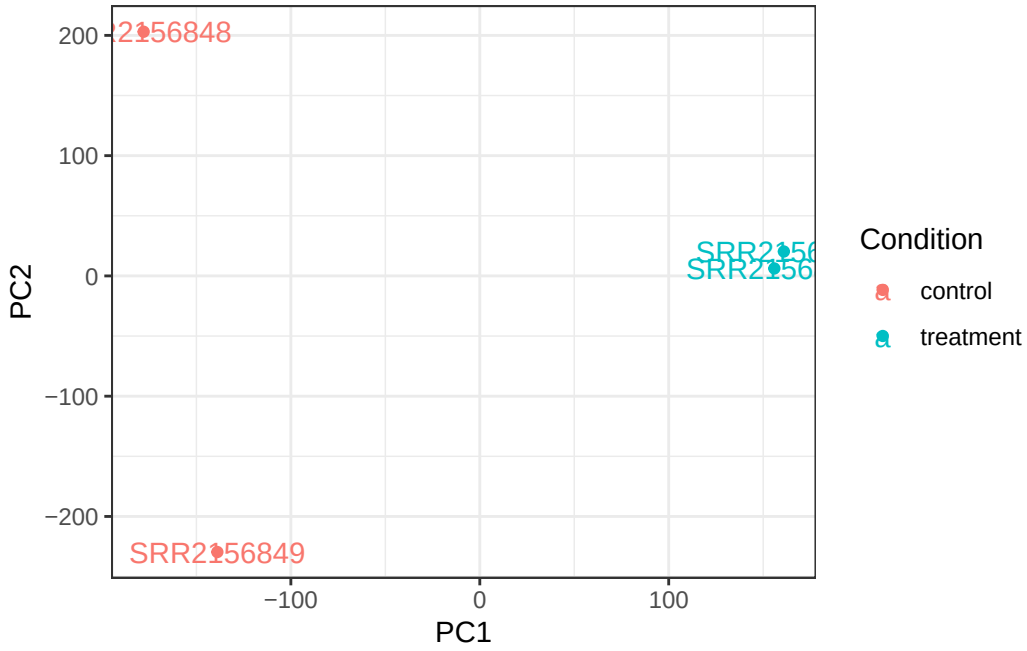
Running PCA

```
pca_data2 <- prcomp(t(data2), scale=T)
```

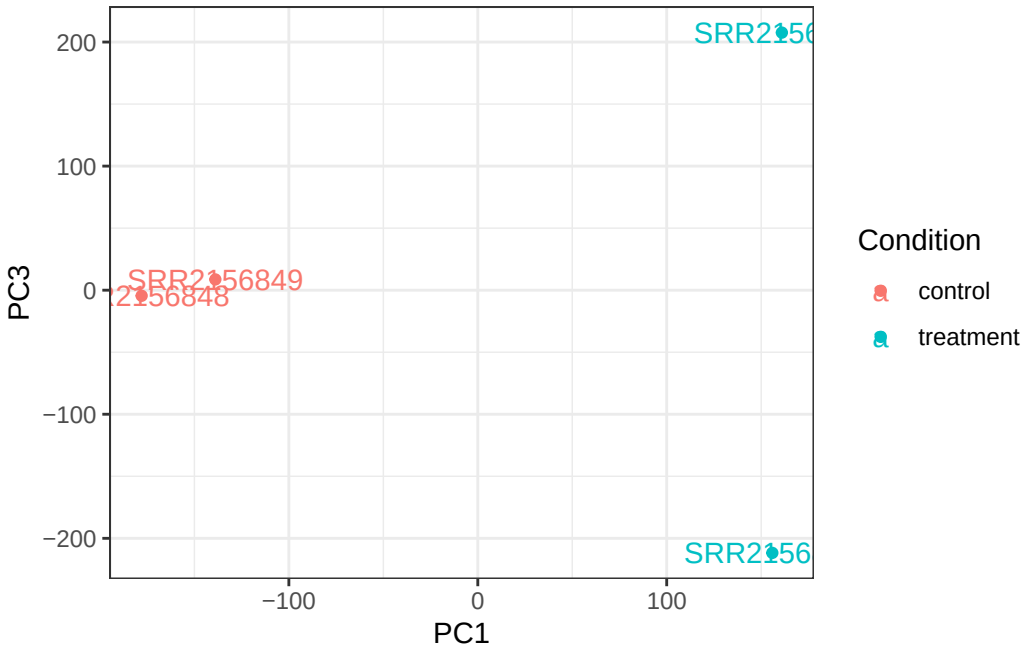
Plotting PCA Results

```
library(ggplot2)

columns <- data.frame(condition = factor(rep(c("control", "treatment"), each = 2)))
```

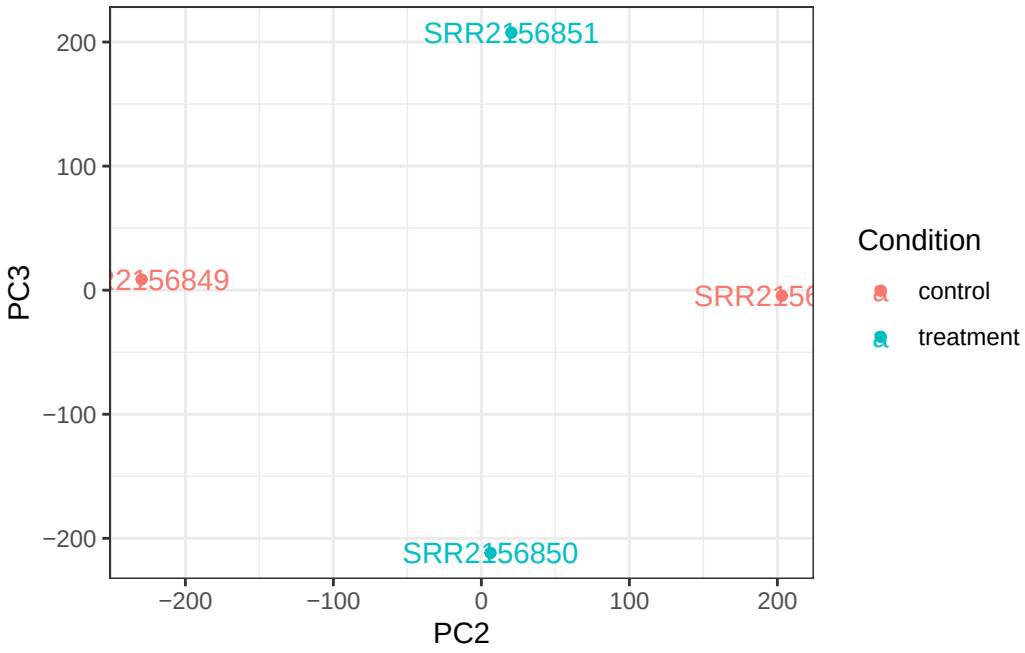
```
rownames(columns) <- colnames(txi.kallisto$counts)

input <- as.data.frame(pca_data2$x)
input$Condition <- as.factor(columns$condition)

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




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




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



Using DESeq2 for Differential Gene Expression Analysis

```
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, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min


Attaching package: 'S4Vectors'

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

    expand.grid, I, unname

Loading required package: IRanges


Attaching package: 'IRanges'

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

    windows

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
```

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

```
#confirm that rownames of colData and colnames of countsData are the same

all.equal(rownames(test), colnames(txi.kallisto$counts))
```

```
[1] TRUE
```

```
dds <- DESeqDataSetFromTximport(txi.kallisto, test, ~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
```

Viewing results of DESeq analysis

```
res <- results(dds)

library(ggrepel)
```

```r
colors_DE <- rep("black", nrow(res))
colors_DE[res$log2FoldChange > 2 & res$padj < 0.05] <- "blue"
colors_DE[res$log2FoldChange < -2 & res$padj < 0.05] <- "red"

res_df <- as.data.frame(res)

ggplot(res_df) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(color=colors_DE, alpha=0.3) +
  geom_vline(xintercept = c(-2,2), linetype="dashed", color = "red") +
  geom_hline(yintercept=-log(0.05), linetype="dashed", color = "red")
```

```
Warning: Removed 147246 rows containing missing values (`geom_point()`).
```