Transcriptome Demo

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Load required packages (you might have to figure out how to install some of these first...)

```
library(ballgown)
library(RColorBrewer)
library(genefilter)
library(dplyr)
library(devtools)
```

What is this code doing?

```
pheno_data<-data.frame(ids = c("plank01", "plank02", "biofilm01", "biofilm02"),
                       stage = c("planktonic", "planktonic", "biofilm", "biofilm"))
```

create Ballgown object and check transcript number

```
samples.c <- paste('ballgown', pheno_data$ids, sep = '/')
bg <- ballgown(samples = samples.c, meas='all', pData = pheno_data)
bg
```

```
## ballgown instance with 5737 transcripts and 4 samples
```

<what is this code doing?>

```
bg_filt = subset(bg,"rowVars(texpr(bg)) >1",genomesubset=TRUE)
bg_filt
```

```
## ballgown instance with 5163 transcripts and 4 samples
```

create a table of transcripts

```
results_transcripts<- stattest(bg_filt, feature = "transcript", covariate = "stage",
getFC = TRUE, meas = "FPKM")
results_transcripts<-data.frame(geneNames=geneNames(bg_filt),
transcriptNames=transcriptNames(bg_filt), results_transcripts)
```

choose a transcript to examine more closely (this is a demo, you need to choose another)

```
results_transcripts[results_transcripts$transcriptNames == "gene-PA0135", ]
```

```
##     geneNames transcriptNames    feature  id           fc      pval      qval
## 139         .     gene-PA0135 transcript 139 2.449214e-11 0.2341011 0.9471885
```

what information are you given about this transcript?

```
sigdiff <- results_transcripts %>% filter(pval<0.05)
dim(sigdiff)
```

```
## [1] 207   7
```

organize the table <by what metrics is the table being organized?>

```
o = order(sigdiff[,"pval"], -abs(sigdiff[,"fc"]), decreasing=FALSE)
output = sigdiff[o,c("geneNames","transcriptNames", "id","fc","pval","qval")]
write.table(output, file="SigDiff.txt", sep="\t", row.names=FALSE, quote=FALSE)
head(output)
```

```
##      geneNames transcriptNames   id           fc         pval      qval
## 4091         .     gene-PA3992 4091 9.886091e+01 0.0003032315 0.9471885
## 4958         .     gene-PA4804 4958 3.563696e-04 0.0006661432 0.9471885
## 2745         .     gene-PA2690 2745 5.783390e-02 0.0014192618 0.9471885
## 2896       tpm     gene-PA2832 2896 1.786570e+03 0.0023414834 0.9471885
## 370          .     gene-PA0365  370 3.964652e-07 0.0023906201 0.9471885
## 3129      pelF     gene-PA3059 3129 1.687425e-03 0.0025838457 0.9471885
```

load gene names

```
bg_table = texpr(bg_filt, 'all')
bg_gene_names = unique(bg_table[, 9:10])
```

pull out gene expression data and visualize

```
gene_expression = as.data.frame(gexpr(bg_filt))
head(gene_expression)
```

```
##            FPKM.plank01 FPKM.plank02 FPKM.biofilm01 FPKM.biofilm02
## .              1.198359    0.9103059       2.526183       2.685373
## MSTRG.1      405.892761  400.8589780     232.324417     181.932617
## MSTRG.10      89.649139   78.5762100      35.010487      59.757320
## MSTRG.100    116.443428  106.2109530      92.206810      95.322479
## MSTRG.1000     7.833186    5.5019700      15.717344      42.342495
## MSTRG.1001     6.845010    4.7381980      38.199095      89.078876
```

<what is this code doing? hint:compare the above output of head(gene_expression) to this output>

```
colnames(gene_expression) <- c("plank01", "plank02", "biofilm01", "biofilm02")
head(gene_expression)
```

```
##                plank01     plank02  biofilm01  biofilm02
## .             1.198359   0.9103059   2.526183   2.685373
## MSTRG.1     405.892761 400.8589780 232.324417 181.932617
## MSTRG.10     89.649139  78.5762100  35.010487  59.757320
## MSTRG.100   116.443428 106.2109530  92.206810  95.322479
## MSTRG.1000    7.833186   5.5019700  15.717344  42.342495
## MSTRG.1001    6.845010   4.7381980  38.199095  89.078876
```

```
dim(gene_expression)
```

```
## [1] 4592    4
```

load the transcript to gene table and determine the number of transcripts and unique genes

```
transcript_gene_table = indexes(bg)$t2g
head(transcript_gene_table)
```

```
##   t_id    g_id
## 1    1 MSTRG.1
## 2    2 MSTRG.2
## 3    3 MSTRG.3
## 4    4 MSTRG.3
## 5    5 MSTRG.4
## 6    6 MSTRG.5
```

```
length(row.names(transcript_gene_table))
```

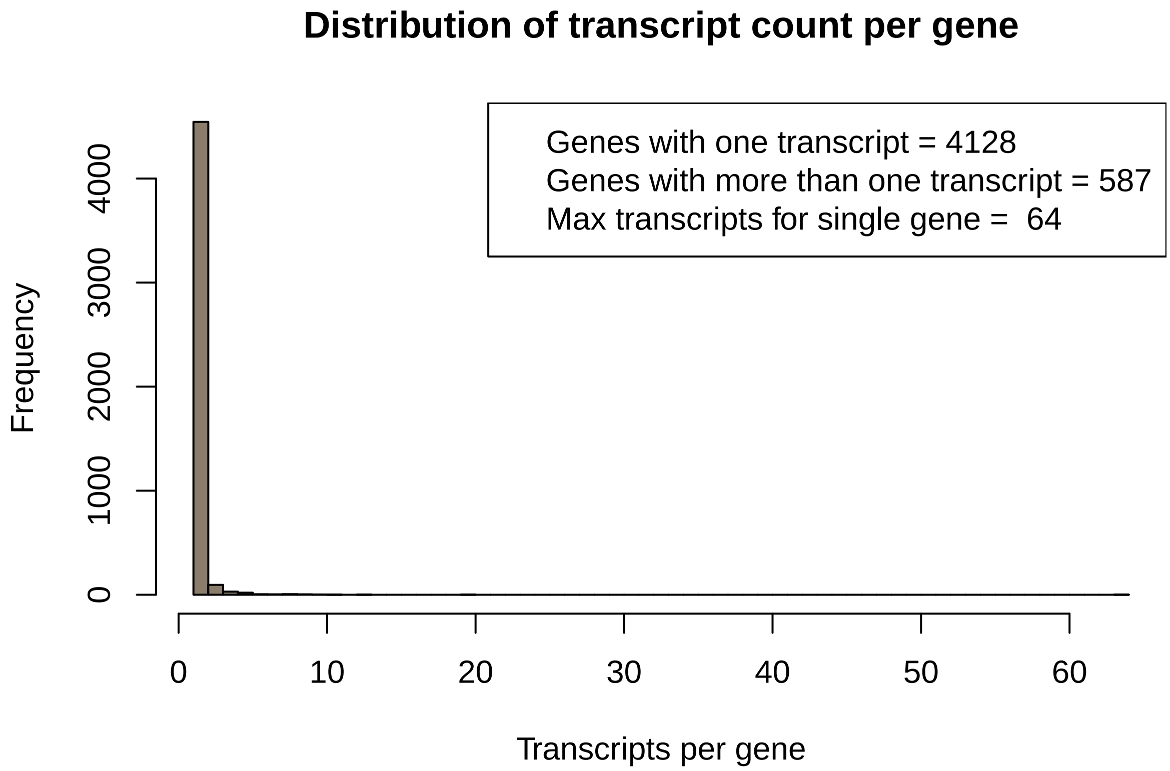
```
## [1] 5737
```

```
length(unique(transcript_gene_table[,"g_id"]))
```

```
## [1] 4715
```

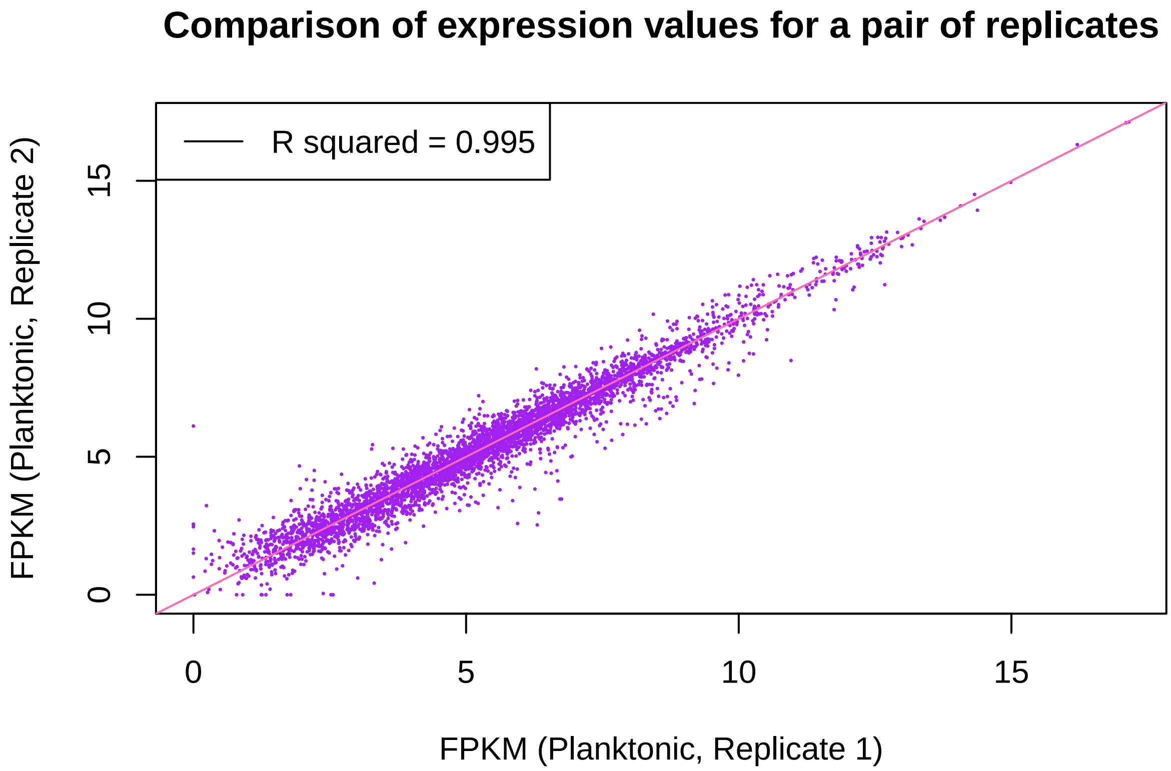
plot the number of transcripts per gene

```
counts=table(transcript_gene_table[,"g_id"])
c_one = length(which(counts == 1))
c_more_than_one = length(which(counts > 1))
c_max = max(counts)
hist(counts, breaks=50, col="bisque4", xlab="Transcripts per gene",
main="Distribution of transcript count per gene")
legend_text = c(paste("Genes with one transcript =", c_one),
paste("Genes with more than one transcript =", c_more_than_one),
paste("Max transcripts for single gene = ", c_max))
legend("topright", legend_text, lty=NULL)
```



create a plot of how similar the two replicates are for one another. We have two data sets. . . how can you modify this code in another chunk to create a plot of the other set?

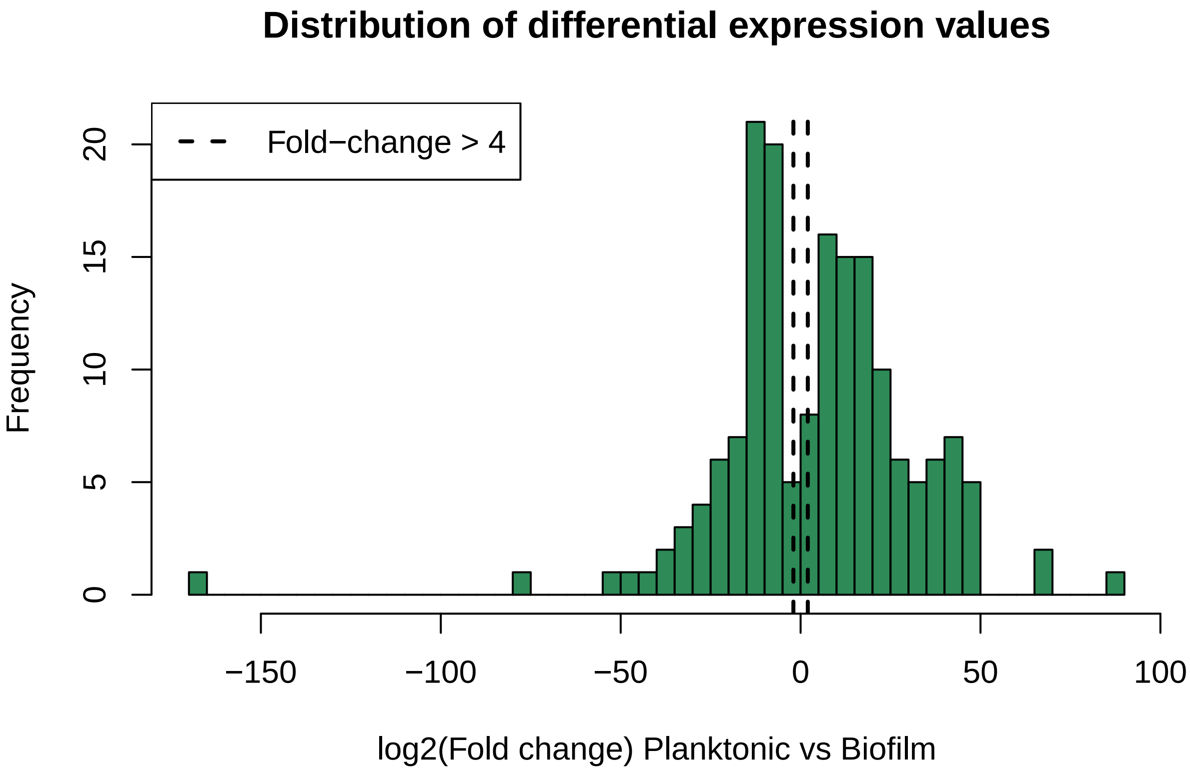
```
x = gene_expression[,"plank01"]
y = gene_expression[,"plank02"]
min_nonzero=1
plot(x=log2(x+min_nonzero), y=log2(y+min_nonzero), pch=16, col="purple", cex=0.25,
xlab="FPKM (Planktonic, Replicate 1)", ylab="FPKM (Planktonic, Replicate 2)",
main="Comparison of expression values for a pair of replicates")
abline(a=0,b=1, col = "hotpink")
rs=cor(x,y)^2
legend("topleft", paste("R squared = ", round(rs, digits=3), sep=""), lwd=1, col="black")
```



What does it mean if the two data sets are similar?

create plot of differential gene expression between the conditions

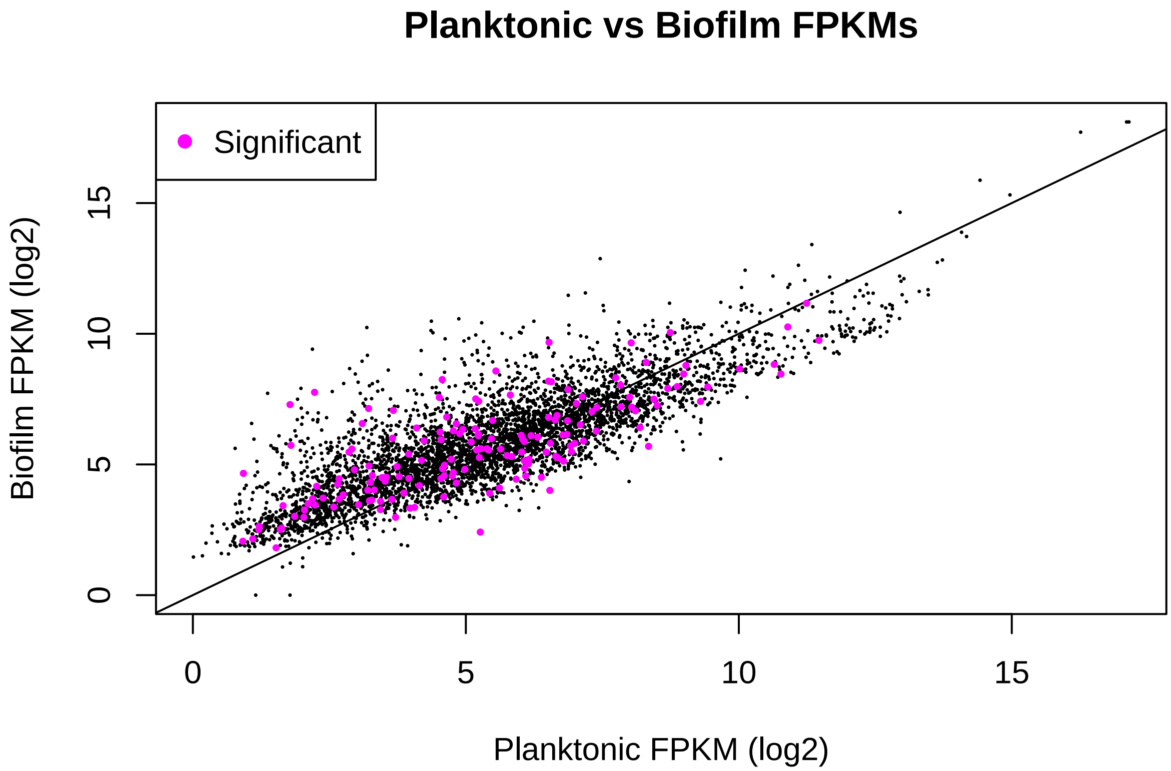
```
results_genes = stattest(bg_filt, feature="gene", covariate="stage", getFC=TRUE, meas="FPKM")
results_genes = merge(results_genes,bg_gene_names,by.x=c("id"),by.y=c("gene_id"))
sig=which(results_genes$pval<0.05)
results_genes[,"de"] = log2(results_genes[,"fc"])
hist(results_genes[sig,"de"], breaks=50, col="seagreen",
xlab="log2(Fold change) Planktonic vs Biofilm",
main="Distribution of differential expression values")
abline(v=-2, col="black", lwd=2, lty=2)
abline(v=2, col="black", lwd=2, lty=2)
legend("topleft", "Fold-change > 4", lwd=2, lty=2)
```



interpret the above figure:

Plot total gene expression highlighting differentially expressed genes

```
gene_expression[,"plank"]=apply(gene_expression[,c(1:2)], 1, mean)
gene_expression[,"biofilm"]=apply(gene_expression[,c(3:4)], 1, mean)
x=log2(gene_expression[,"plank"]+min_nonzero)
y=log2(gene_expression[,"biofilm"]+min_nonzero)
plot(x=x, y=y, pch=16, cex=0.25, xlab="Planktonic FPKM (log2)", ylab="Biofilm FPKM (log2)",
main="Planktonic vs Biofilm FPKMs")
abline(a=0, b=1)
xsig=x[sig]
ysig=y[sig]
points(x=xsig, y=ysig, col="magenta", pch=16, cex=0.5)
legend("topleft", "Significant", col="magenta", pch=16)
```



make a table of FPKM values

```
fpkm = texpr(bg_filt,meas="FPKM")
```

choose a gene to determine individual expression (pick a different number than I did)

```
ballgown::transcriptNames(bg_filt)[2]
```

```
##             2
## "gene-PA0002"
```

```
ballgown::geneNames(bg_filt)[2]
```

```
##      2
## "dnaN"
```

transform to log2

```
transformed_fpkm <- log2(fpkm[2, ] + 1)
```

make sure values are properly coded as numbers

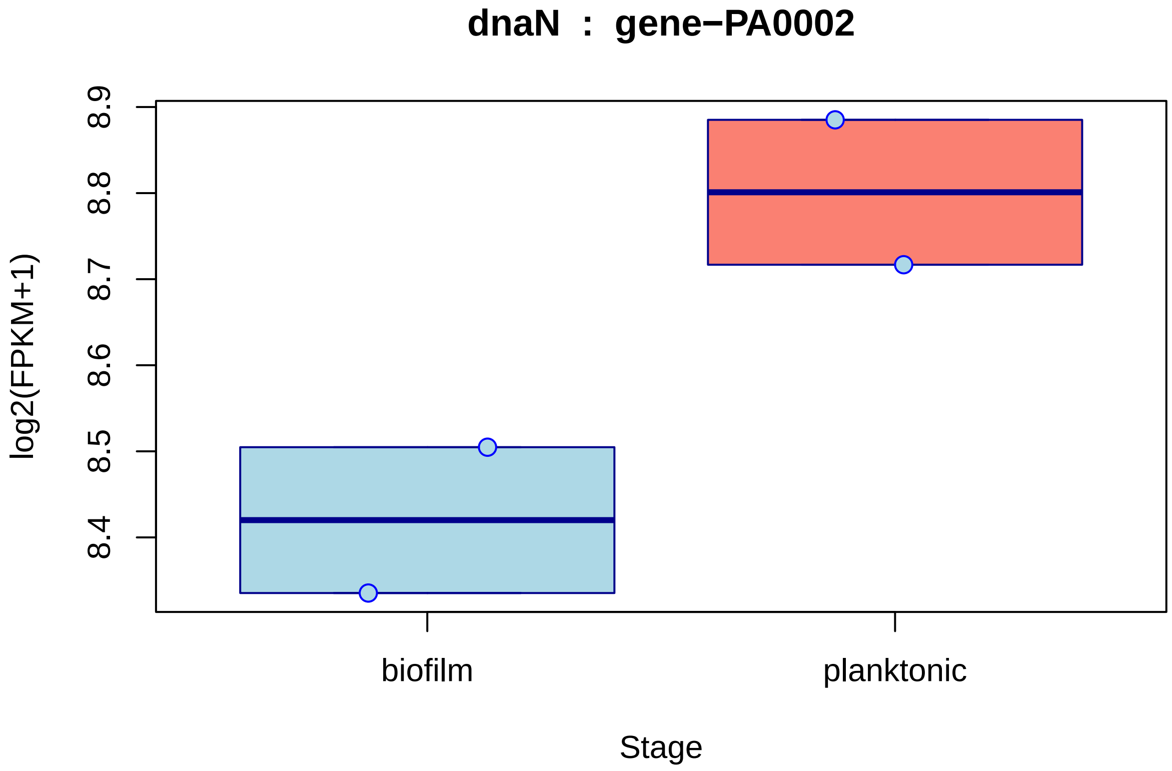
```
numeric_stages <- as.numeric(factor(pheno_data$stage))

jittered_stages <- jitter(numeric_stages)
```

plot expression of individual gene

```
boxplot(transformed_fpkm ~ pheno_data$stage,
        main=paste(ballgown::geneNames(bg_filt)[2], ' : ', ballgown::transcriptNames(bg_filt)[2]),
        xlab="Stage",
        ylab="log2(FPKM+1)",
        col=c("lightblue", "salmon"),
        border="darkblue")

points(transformed_fpkm ~ jittered_stages,
       pch=21, col="blue", bg="lightblue", cex=1.2)
```



interpret the above figure