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)
```

This code is creating a data frame called pheno_data with some sample IDs. This also includes their corresponding stages whether it is a planktonic or biofilm.

```
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
```

This code filters bg to keep rows with high expression variability and stores these results in a file titled bg_filt. This means it reduces extra information and only includes the most valiable.

```
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-PA0140", ]
```

```
##     geneNames transcriptNames    feature  id       fc      pval      qval
## 144      ahpF     gene-PA0140 transcript 144 1.768738 0.7673868 0.9658145
```

Here we are given the ID number, the fold change, p value and adjusted p value (qval). Fold change the value of changes in gene expression between two conditions. P value is the measure of statistical significance of the fold change. The adjusted p value is just a corrected verson of the p value and controls for multiple testing.

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

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

organize the table <This table is being organized by p value ranging from smallest to largest. Then it takes into account the absolute fold change ranging from largest to smallest, when p values are the same.>

```
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
```

<This code renames the columns of the "gene_expression" data frame to represent a specific sample. Then this code will check the data. In comparison to the code above, where it will take expression data from the bg_filt file and convert it to a data frame.>

```
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))
```

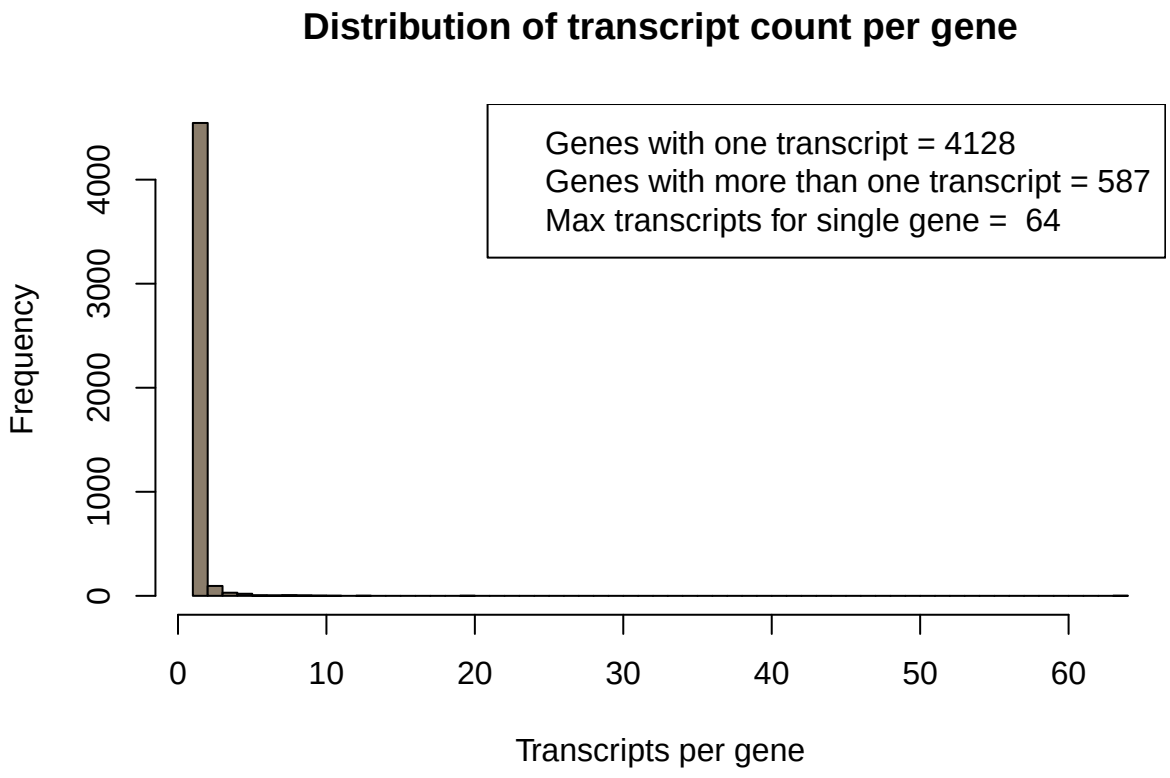
```
## [1] 5737
```

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

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

plot the number of transcripts per gene

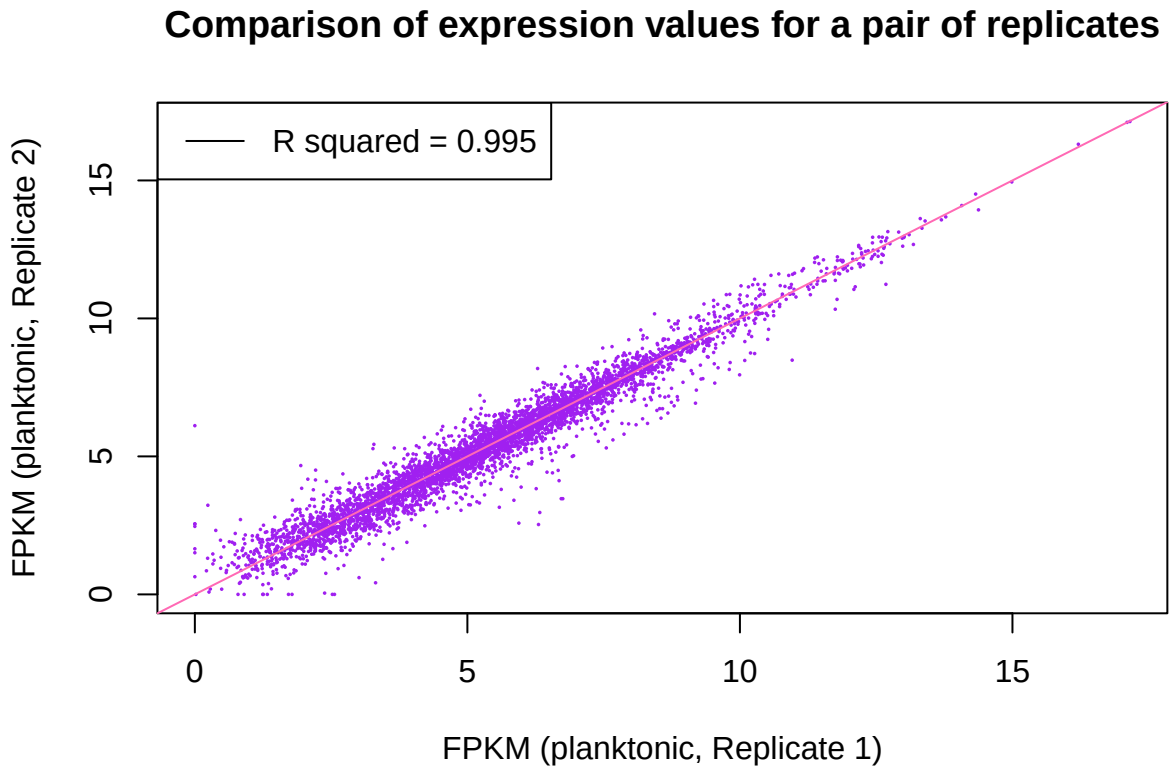
```
counts=table(transcript_gene_table[,"g_id"])
c_one = length(which(counts == 1))
c_more_than_one = length(which(counts > 1))
c_max = max(counts)
hist(counts, breaks=50, col="bisque4", xlab="Transcripts per gene",
main="Distribution of transcript count per gene")
legend_text = c(paste("Genes with one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



<The graph above shows that genes with one transcript have a higher frequency than genes with more than one trascript or transcripts for a single gene. Specifically the frequency for genes with one transcript is 4128. for genes with more than one transcript the frequency is 587. Laslty the frequency for transcripts for a single gene is 64.>

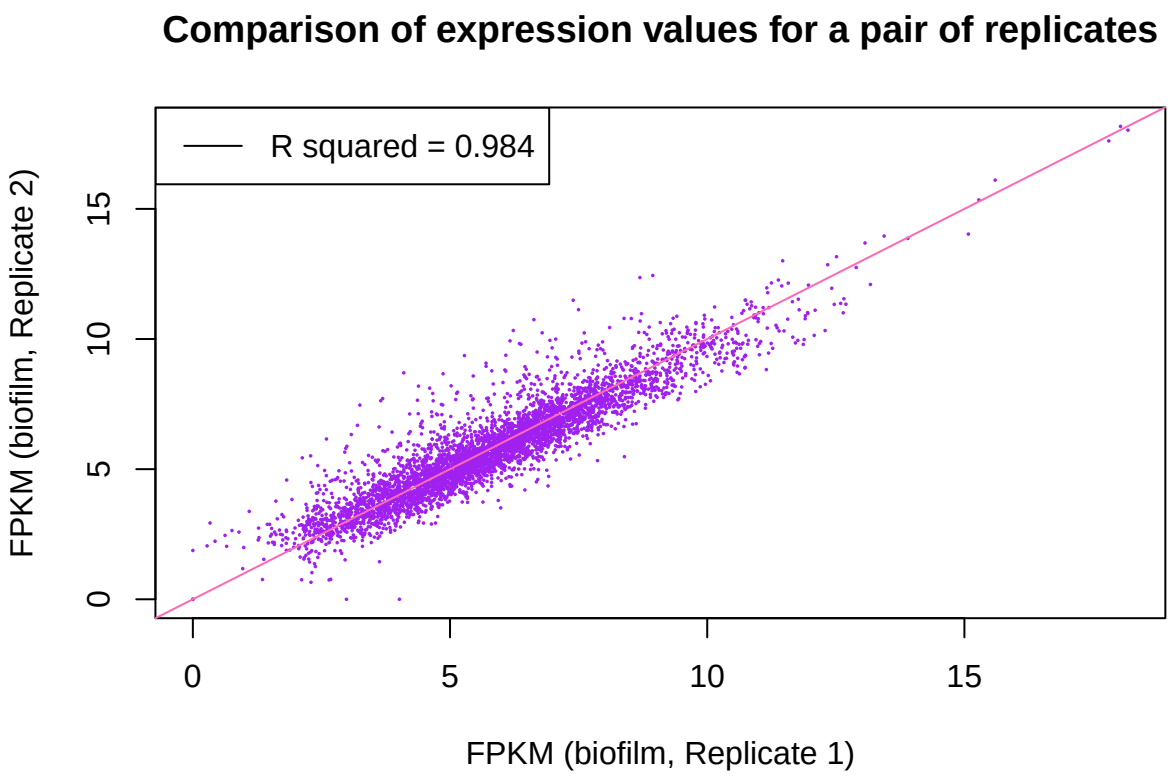
create a plot of how similar the two replicates are for one another. We have two data sets. . . You can modify this data sit by replacing plank with biofilm

```
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")
```




```
x = gene_expression[,"biofilm01"]
y = gene_expression[,"biofilm02"]
min_nonzero=1
plot(x=log2(x+min_nonzero), y=log2(y+min_nonzero), pch=16, col="purple", cex=0.25,
```

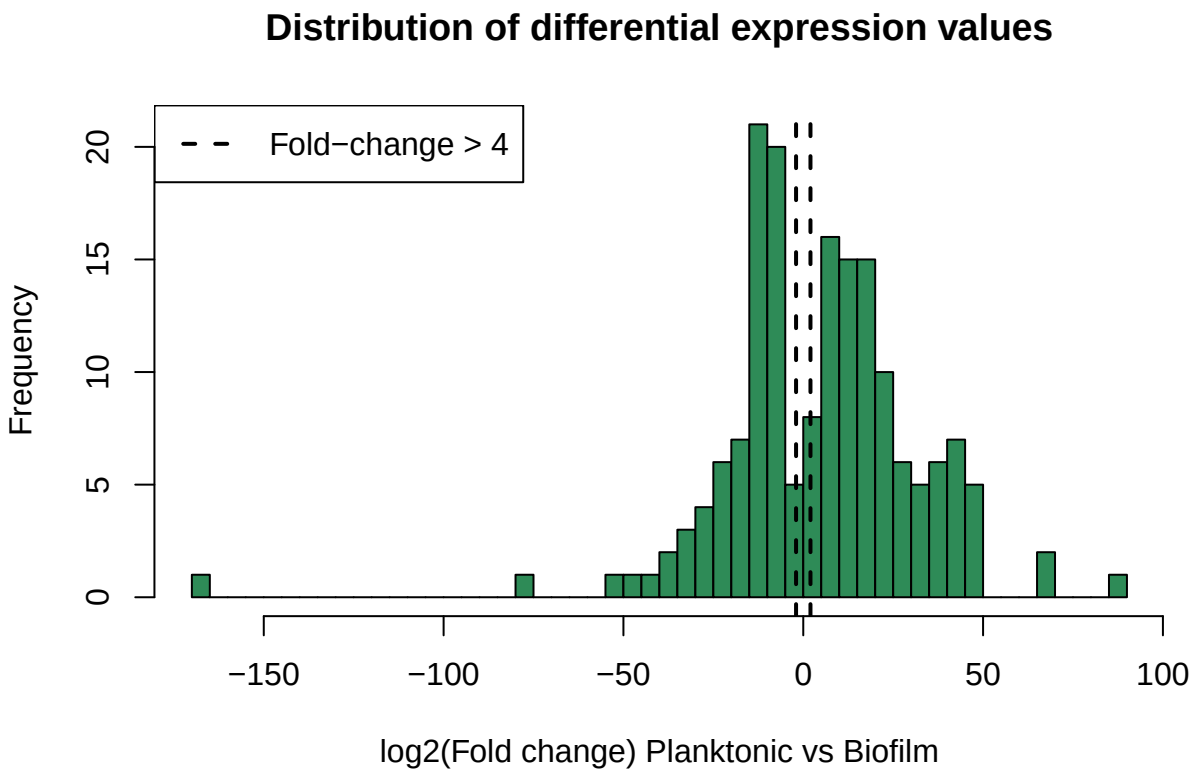
```
xlab="FPKM (biofilm, Replicate 1)", ylab="FPKM (biofilm, Replicate 2)",
main="Comparison of expression values for a pair of replicates")
abline(a=0,b=1, col = "hotpink")
rs=cor(x,y)^2
legend("topleft", paste("R squared = ", round(rs, digits=3), sep=""), lwd=1, col="black")
```



If the two datasets are similar this means that the genomes have conserved genes

create plot of differential gene expression between the conditions

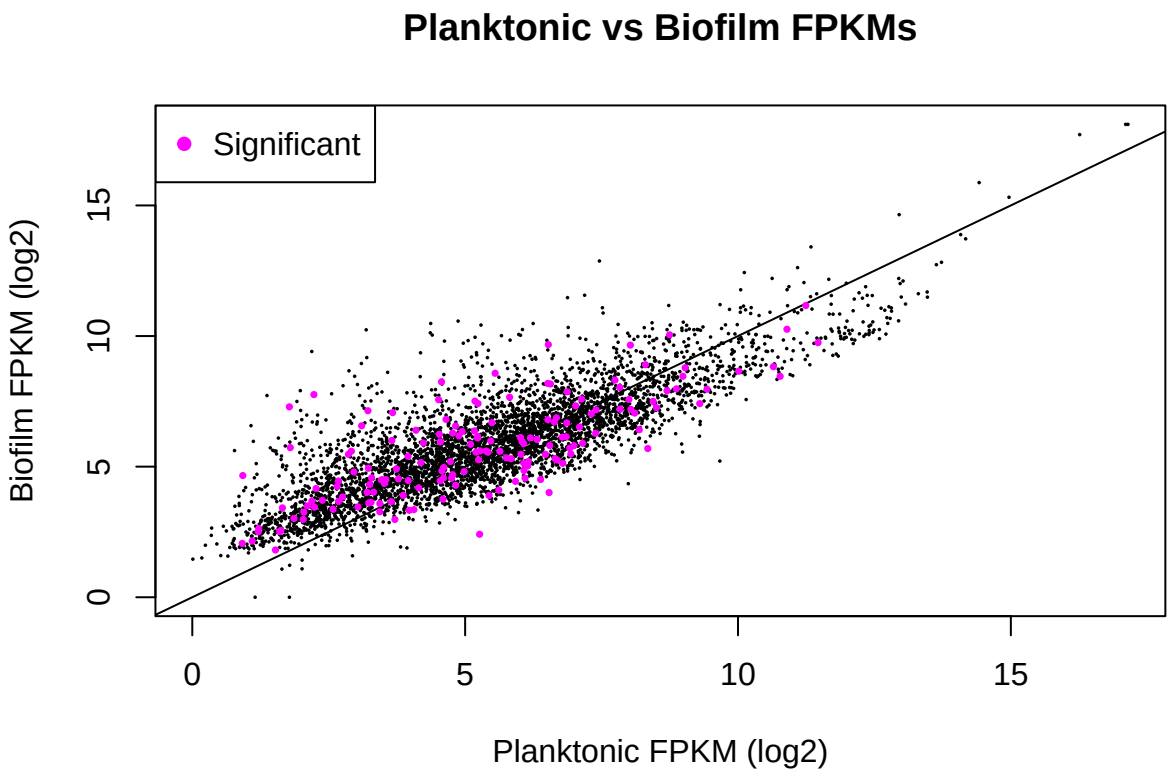
```
results_genes = stattest(bg_filt, feature="gene", covariate="stage", getFC=TRUE, meas="FPKM")
results_genes = merge(results_genes,bg_gene_names,by.x=c("id"),by.y=c("gene_id"))
sig=which(results_genes$pval<0.05)
results_genes[,"de"] = log2(results_genes[,"fc"])
hist(results_genes[sig,"de"], breaks=50, 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: The graph above shows frequency complared to the log2 of fold changes between planktonic and biofilm. As we see in the legend the dotted line shows fold change less than four. As we can see there is a pretty even distribution.

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)[19]
```

```
##            20
## "gene-PA0020"
```

```
ballgown::geneNames(bg_filt)[19]
```

```
##  20
## "."
```

transform to log2

```
transformed_fpkm <- log2(fpkm[19, ] + 1)
```

make sure values are properly coded as numbers

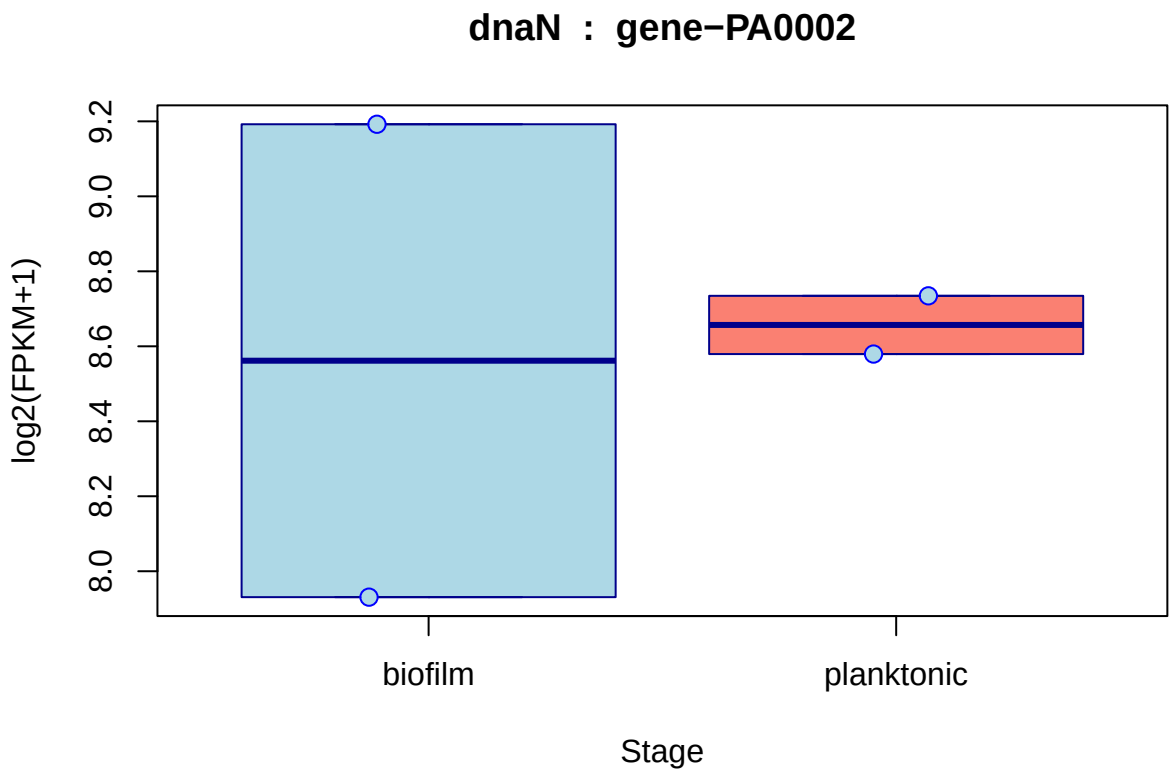
```
numeric_stages <- as.numeric(factor(pheno_data$stage))

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: We can see that the planktonic has a higher log 2 but smaller margins with upper and lower quartile. Biofilm may have a lower mean log 2 but a wider margin meaning upper and lower quartile.