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)
```

produces a data fram with 4 different ids like "plank01,02 biofilm01,02" and creates a stage column that idntifies if it is 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 the bg Ballgown object to keep only transcripts whose expression variance across samples is greater than 1, removing low-variance transcripts. The result is a new, smaller object called bg_filt for further analysis.

```
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-PA0143", ]
```

```
##     geneNames transcriptNames    feature  id         fc      pval      qval
## 147       nuh     gene-PA0143 transcript 147 0.03458844 0.6277342 0.9471885
```

This transcript is named nuh, and its id is 147 with a fc 0.0345884, a pval of 0.6277342, and a qval of 0.9471885

This code filters the results_transcripts data frame to keep only rows where the p-value (pval) is less than 0.05, storing them in sigdiff. Then, dim(sigdiff) shows the number of rows and columns in the filtered results.

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

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

organize the table. The table is being organized first by smallest p-value, and within that, by largest absolute fold change (|fc|)

```
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 match the sample IDs (plank01, plank02, biofilm01, biofilm02), so that the column names are easier to understand and match our phenotype information.

Then it shows the first few rows (head()) and checks the dimensions (dim()) of the updated table.

```
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: there is 5 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))
```

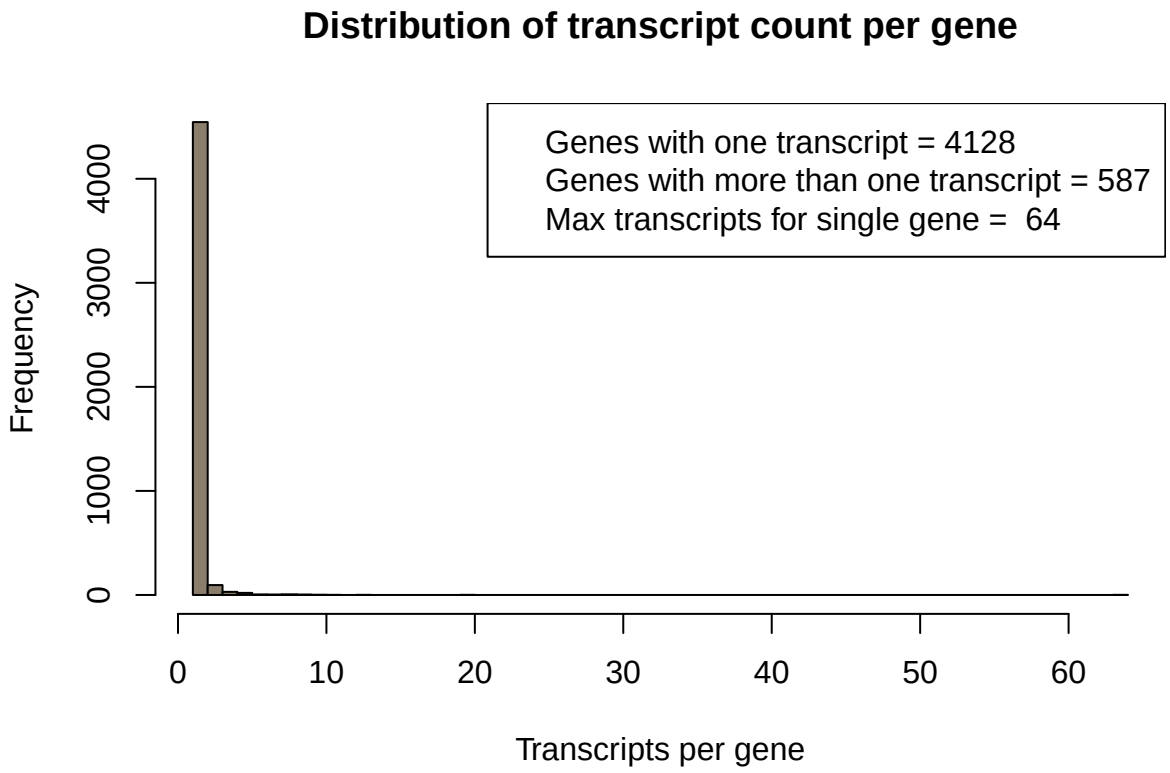
```
## [1] 5737
```

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

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

plot the number of transcripts per gene

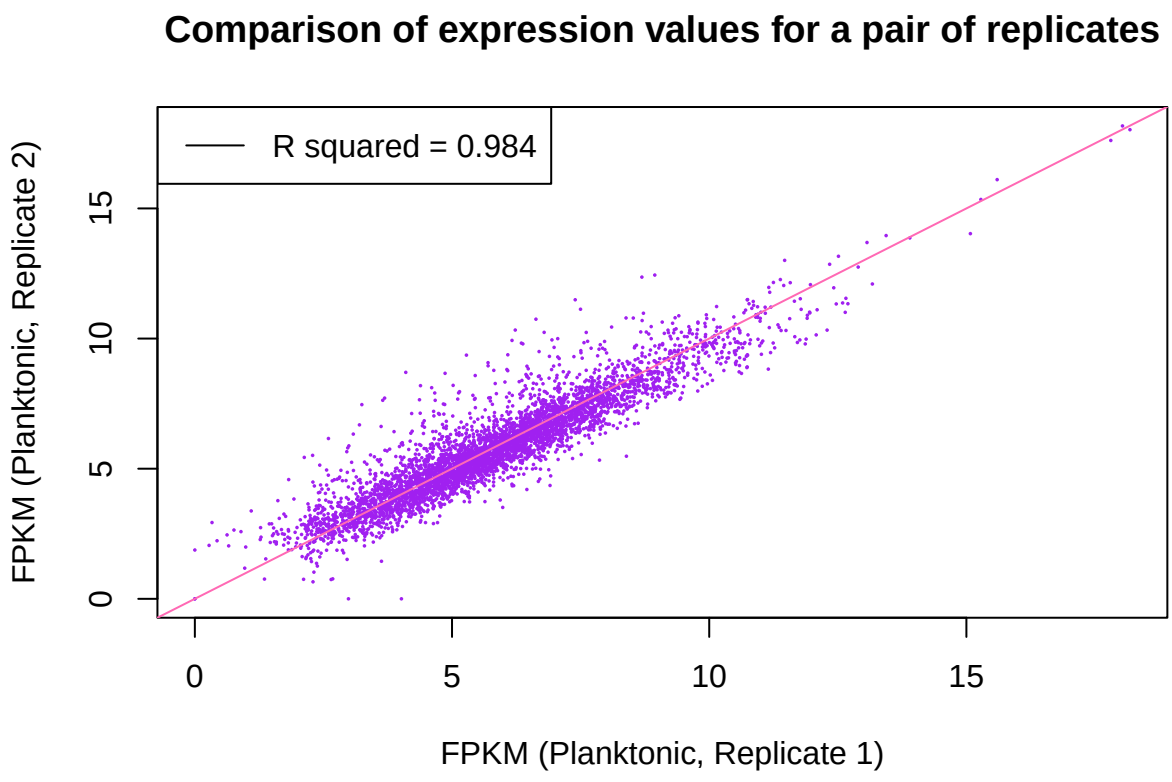
```
counts=table(transcript_gene_table[,"g_id"])
c_one = length(which(counts == 1))
c_more_than_one = length(which(counts > 1))
c_max = max(counts)
hist(counts, breaks=50, col="bisque4", xlab="Transcripts per gene",
main="Distribution of transcript count per gene")
legend_text = c(paste("Genes with one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



Since most genes (4128 genes) have one transcript this is why the leftmost bar is very tall. SO it is very common for genes to have one transcript than more than one. Anything past around 12 are very uncommon.

create a plot of how similar the two replicates are for one another. To create a plot for the other dataset (biofilm replicates), you modify the code by changing plank01 and plank02 to biofilm01 and biofilm02 in the gene_expression table.

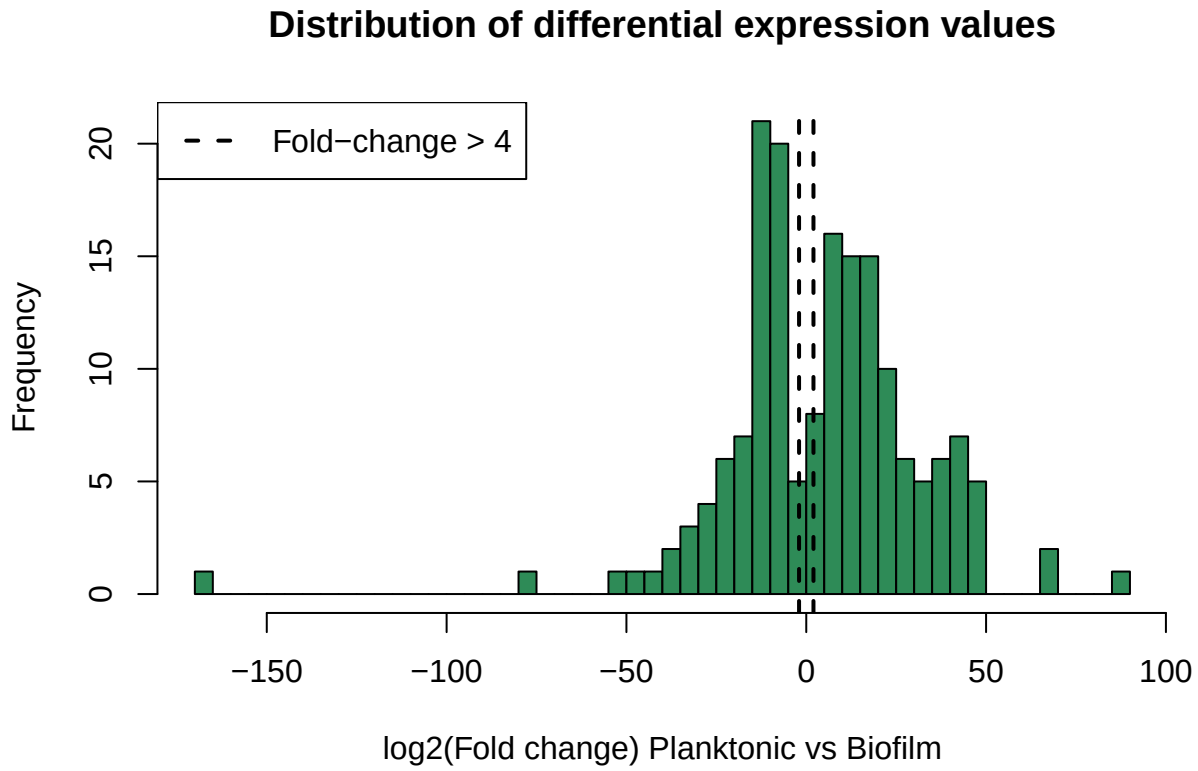
```
x = gene_expression[,"biofilm01"]
y = gene_expression[,"biofilm02"]
min_nonzero=1
plot(x=log2(x+min_nonzero), y=log2(y+min_nonzero), pch=16, col="purple", cex=0.25,
xlab="FPKM (Planktonic, Replicate 1)", ylab="FPKM (Planktonic, Replicate 2)",
main="Comparison of expression values for a pair of replicates")
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")
```



If both are similar that would mean there is no signifigant difference between the two groups.

create plot of differential gene expression between the conditions

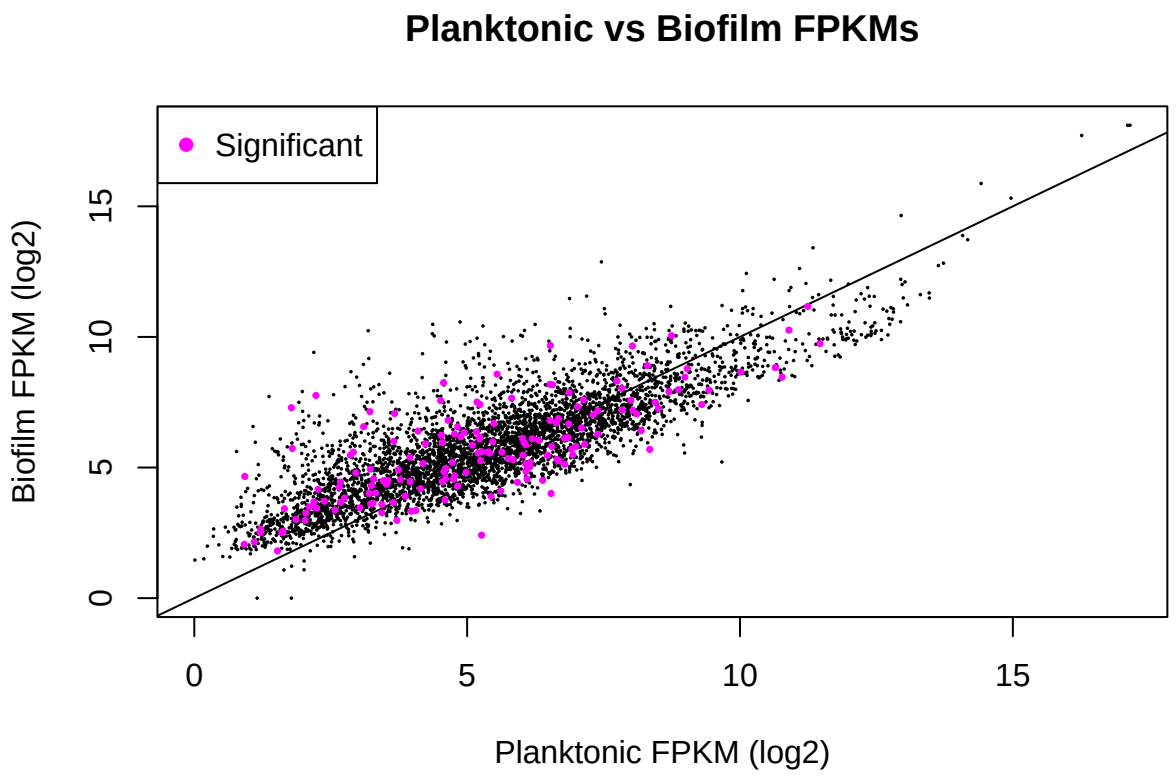
```
results_genes = stattest(bg_filt, feature="gene", covariate="stage", getFC=TRUE, meas="FPKM")
results_genes = merge(results_genes,bg_gene_names,by.x=c("id"),by.y=c("gene_id"))
sig=which(results_genes$pval<0.05)
results_genes[,"de"] = log2(results_genes[,"fc"])
hist(results_genes[sig,"de"], breaks=50, 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: This is showing the distribution of log2 fold changes in gene expression between planktonic and biofilm conditions eith a p value lower than 0.05. I noticed that there are a 2 drastic outliers on the graph, one at -70 and one way beyond -150 on the x-axis.

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)[4]
```

```
##             4
## "gene-PA0004"
```

```
ballgown::geneNames(bg_filt)[4]
```

```
##      4
## "gyrB"
```

transform to log2

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

make sure values are properly coded as numbers

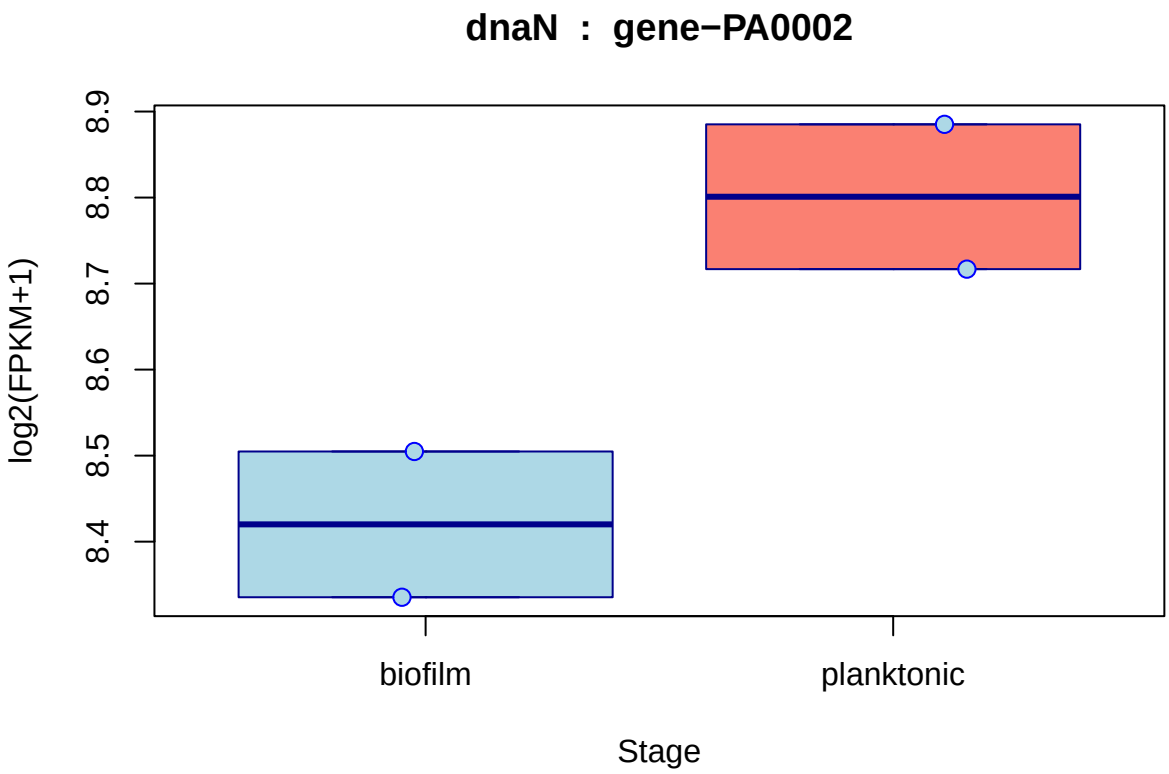
```
numeric_stages <- as.numeric(factor(pheno_data$stage))

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)
```



The planktonic condition shows higher expression levels (approximately 8.8) compared to biofilm (approximately 8.4). Both show high expression overall. Thus the planktonic has a higher amount of dnaN gene expresion.