Pasilla Analysis in EdgeR

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Load edgeR and set working directory

```
library(edgeR)
setwd("/home/participant/Course_Materials")
```

Load the count file

```
datafile = system.file( "extdata/pasilla_gene_counts.tsv", package="pasilla" )
```

```
## [1] "/home/participant/R/x86_64-pc-linux-gnu-library/3.2/pasilla/extdata/pasilla_gene_counts.tsv"
```

Read the count table and print out

```
pasillaCountTable = read.table(datafile, header=TRUE, row.names=1)

head(pasillaCountTable)
```

```
##             untreated1 untreated2 untreated3 untreated4 treated1 treated2
## FBgn0000003          0          0          0          0        0        0
## FBgn0000008         92        161         76         70      140       88
## FBgn0000014          5          1          0          0        4        0
## FBgn0000015          0          2          1          2        1        0
## FBgn0000017       4664       8714       3564       3150     6205     3072
## FBgn0000018        583        761        245        310      722      299
##             treated3
## FBgn0000003        1
## FBgn0000008       70
## FBgn0000014        0
## FBgn0000015        0
## FBgn0000017     3334
## FBgn0000018      308
```

Construct the design and setup the conditions. Report how many samples were in the treated and untreated groups

```
pasillaDesign = data.frame(
  row.names = colnames( pasillaCountTable ),
  condition = c( "untreated", "untreated", "untreated",
                 "untreated", "treated", "treated", "treated" ),
  libType = c( "single-end", "single-end", "paired-end",
               "paired-end", "single-end", "paired-end", "paired-end" ) )

pasillaDesign
```

```
##            condition    libType
## untreated1 untreated single-end
## untreated2 untreated single-end
## untreated3 untreated paired-end
## untreated4 untreated paired-end
## treated1     treated single-end
## treated2     treated paired-end
## treated3     treated paired-end
```

```
pairedSamples = pasillaDesign$libType == "paired-end"
countTable = pasillaCountTable[ , pairedSamples ]
condition = pasillaDesign$condition[ pairedSamples ]
```

Normalise

```
y <- DGEList(counts=countTable,group=condition)
y <- calcNormFactors(y)
y <- estimateCommonDisp(y)
y <- estimateTagwiseDisp(y)
```

Do the differential expression test and print the table of top hits

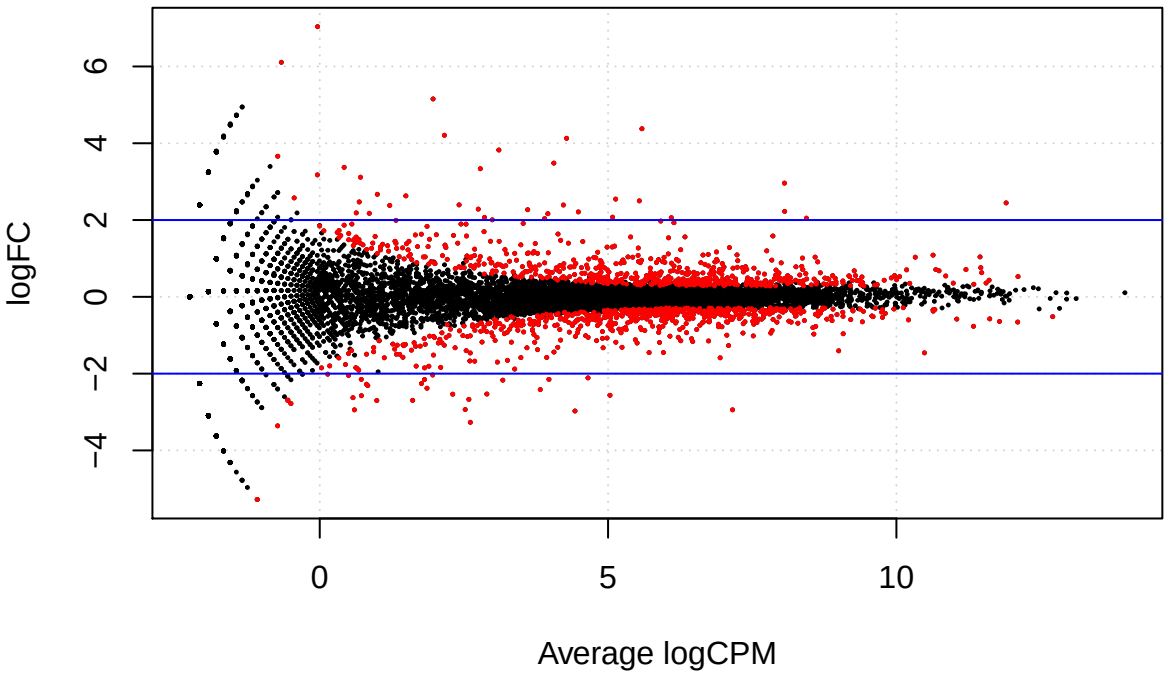
```
et <- exactTest(y)
topTags(et)
```

```
## Comparison of groups:  untreated-treated
##                 logFC    logCPM        PValue           FDR
## FBgn0039155  4.378187  5.587721 1.988561e-183 2.903100e-179
## FBgn0003360  2.961327  8.058804 2.725221e-156 1.989275e-152
## FBgn0025111 -2.943074  7.158666 3.007218e-154 1.463412e-150
## FBgn0026562  2.446889 11.903496 1.955728e-106 7.137917e-103
## FBgn0039827  4.129115  4.281292 1.646830e-105 4.808413e-102
## FBgn0035085  2.499390  5.542361  1.617570e-96  3.935818e-93
## FBgn0029167  2.225726  8.062840  4.295075e-93  8.957687e-90
## FBgn0000071 -2.564871  5.033671  5.473558e-79  9.988558e-76
## FBgn0029896  2.545682  5.131528  1.268123e-77  2.057037e-74
## FBgn0034897  2.061625  6.096982  3.430337e-75  5.007949e-72
```

Use the results of the previous code-chunk to comment on how many up and down regulated genes were found at a p-value cut-off of 0.05. [Embed your answers in this section of text quoting the p-value cut-off and number of genes]

Produce the smear plot, but hide the code

```
##    [,1]
## -1   625
## 0  13349
## 1    625
```


logFC
6
4
2
0
−2
−4
0
5
10
Average logCPM