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Revised Analysis of the Pasilla dataset

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The counts for the pasilla dataset were read from the pasilla data package.

The first few lines of the file are shown.

```
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
```

A design matrix was used to compare treated and untreated samples.

```
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 ]
```

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

Prior to filtering, there are 14599 genes

```
countsPerMillion <- cpm(y)
summary(countsPerMillion)
```

```
##    untreated3          untreated4           treated2
##  Min.   :    0.000   Min.   :    0.000   Min.   :    0.000
##  1st Qu.:    0.000   1st Qu.:    0.000   1st Qu.:    0.000
##  Median :    2.154   Median :    1.931   Median :    2.089
##  Mean   :   68.498   Mean   :   68.498   Mean   :   68.498
##  3rd Qu.:   41.994   3rd Qu.:   42.017   3rd Qu.:   43.461
##  Max.   :15701.760   Max.   :16981.030   Max.   :15293.843
##     treated3
##  Min.   :    0.000
##  1st Qu.:    0.000
##  Median :    2.127
##  Mean   :   68.498
##  3rd Qu.:   44.084
##  Max.   :15869.130
```

```
#'summary' is a useful function for exploring numeric data; eg. summary(1:100)
countCheck <- countsPerMillion > 1
head(countCheck)
```

```
##             untreated3 untreated4 treated2 treated3
## FBgn0000003      FALSE      FALSE    FALSE    FALSE
## FBgn0000008       TRUE       TRUE     TRUE     TRUE
## FBgn0000014      FALSE      FALSE    FALSE    FALSE
## FBgn0000015      FALSE      FALSE    FALSE    FALSE
## FBgn0000017       TRUE       TRUE     TRUE     TRUE
## FBgn0000018       TRUE       TRUE     TRUE     TRUE
```

```
keep <- which(rowSums(countCheck) >= 2)
y <- y[keep,]
summary(cpm(y))
```

```
##    untreated3          untreated4           treated2
##  Min.   :    0.12   Min.   :    0.102   Min.   :    0.104
##  1st Qu.:   10.53   1st Qu.:   10.873   1st Qu.:   11.283
##  Median :   36.13   Median :   36.377   Median :   37.401
##  Mean   :  126.91   Mean   :  126.925   Mean   :  126.918
##  3rd Qu.:  103.25   3rd Qu.:   98.767   3rd Qu.:  102.593
##  Max.   :15701.76   Max.   :16981.030   Max.   :15293.843
##     treated3
##  Min.   :    0.00
```

```
##  1st Qu.:   11.31
##  Median :   37.90
##  Mean   :  126.92
##  3rd Qu.:  102.28
##  Max.   :15869.13
```

After filtering, 7873 genes remain.

The analysis will use 3 Treated and 4 Untreated samples. Normalisation was performed with a standard edgeR protocol

```
y <- calcNormFactors(y)
y <- estimateCommonDisp(y)
y <- estimateTagwiseDisp(y)
```

Differential expression was performed between treated and untreated samples using the exact test in edgeR. The top hits are shown below.

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

```
## Comparison of groups:  untreated-treated
##                 logFC    logCPM        PValue           FDR
## FBgn0039155  4.385238  5.589470 5.135176e-197 4.042924e-193
## FBgn0025111 -2.935480  7.156088 4.089145e-154 1.609692e-150
## FBgn0003360  2.968460  8.060906 2.963278e-146 7.776628e-143
## FBgn0039827  4.136724  4.282611 1.518899e-103 2.989573e-100
## FBgn0035085  2.506729  5.543742 3.977713e-103 6.263307e-100
## FBgn0026562  2.453695 11.905324  4.727494e-95  6.203260e-92
## FBgn0029167  2.234275  8.065584  1.403281e-82  1.578290e-79
## FBgn0000071 -2.557948  5.031865  3.418496e-79  3.364227e-76
## FBgn0029896  2.553932  5.133395  8.862470e-77  7.752691e-74
## FBgn0034897  2.069850  6.098928  2.280255e-76  1.795245e-73
```

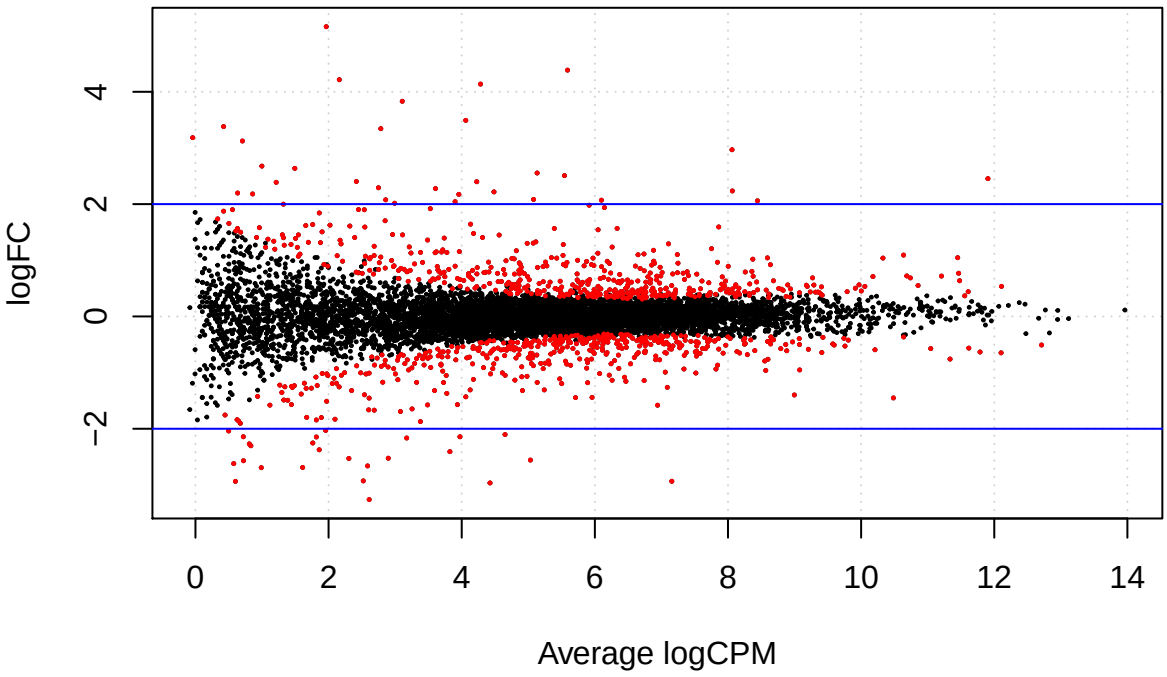
```
p <- 0.01
summary(de <- decideTestsDGE(et, p=p))
```

```
##    [,1]
## -1  477
## 0  6891
## 1   505
```

```
detags <- rownames(y)[as.logical(de)]
```

The total number of differentially-expressed genes at a cutoff of 0.01 was 982, and 505 genes were up-regulated. The logFC and CPM of these differentially-expressed genes is shown below.

```
plotSmear(et, de.tags=detags)
abline(h = c(-2, 2), col = "blue")
```


logFC
4
2
0
−2
0
2
4
6
8
10
12
14
Average logCPM