Prepare MA plots for the E(z) mutant experiment

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Input the data and metadata:

```
inpath <- "~/Desktop/brain"
outpath <- "~/Desktop/brain/_7B_Ez_plotMA"
setwd(inpath)
files <- list.files()
htseq_files <- files[grepl("^JKL.*txt$", files)]
sampleNames <- read.csv("EzSampleNames.csv")
sampleTable <- data.frame(fileName = htseq_files,
                          stringsAsFactors=FALSE)
sampleTable$Library <- gsub("-counts.txt", "", sampleTable$fileName)
sampleTable$Library <- gsub("b", "", sampleTable$Library)
sampleTable$seq.batch <- ifelse(grepl("b", sampleTable$fileName), "B", "A")
sampleTable$seq.batch <- paste(sampleTable$Library, sampleTable$seq.batch)
sampleTable <- merge(sampleTable[,c(1,3)], sampleNames, by = "seq.batch")
sampleTable$genotype <- gsub("-.*$", "", sampleTable$Sample)
sampleTable$genotype <- factor(sampleTable$genotype, levels = c("w", "Ez"))
sampleTable$Temp <- gsub("[A-Z, a-z, -]", "", sampleTable$Sample)
sampleTable$Temp <- factor(sampleTable$Temp, levels = c("25", "29"))
sampleTable$rep <- gsub("^.*-", "", sampleTable$Sample)
sampleTable$condition <- paste(sampleTable$genotype, sampleTable$Temp, sep = "-")
sampleTable
```

```
##    seq.batch          fileName    Sample batch Library genotype Temp rep
## 1     JKL10 A  JKL10-counts.txt   w-25-II     A   JKL10        w   25  II
## 2     JKL10 B JKL10b-counts.txt   w-25-II     B   JKL10        w   25  II
## 3     JKL11 A  JKL11-counts.txt  w-25-III     A   JKL11        w   25 III
## 4     JKL11 B JKL11b-counts.txt  w-25-III     B   JKL11        w   25 III
## 5     JKL12 A  JKL12-counts.txt Ez-25-III     A   JKL12       Ez   25 III
## 6     JKL12 B JKL12b-counts.txt Ez-25-III     B   JKL12       Ez   25 III
## 7     JKL13 A  JKL13-counts.txt    w-29-I     A   JKL13        w   29   I
## 8     JKL13 B JKL13b-counts.txt    w-29-I     B   JKL13        w   29   I
## 9     JKL14 A  JKL14-counts.txt   Ez-29-I     A   JKL14       Ez   29   I
## 10    JKL14 B JKL14b-counts.txt   Ez-29-I     B   JKL14       Ez   29   I
## 11    JKL15 A  JKL15-counts.txt   w-29-II     A   JKL15        w   29  II
## 12    JKL15 B JKL15b-counts.txt   w-29-II     B   JKL15        w   29  II
## 13    JKL16 A  JKL16-counts.txt  Ez-29-II     A   JKL16       Ez   29  II
## 14    JKL16 B JKL16b-counts.txt  Ez-29-II     B   JKL16       Ez   29  II
## 15    JKL17 A  JKL17-counts.txt  w-29-III     A   JKL17        w   29 III
## 16    JKL17 B JKL17b-counts.txt  w-29-III     B   JKL17        w   29 III
## 17    JKL18 A  JKL18-counts.txt Ez-29-III     A   JKL18       Ez   29 III
## 18    JKL18 B JKL18b-counts.txt Ez-29-III     B   JKL18       Ez   29 III
## 19    JKL19 A  JKL19-counts.txt   w-29-IV     A   JKL19        w   29  IV
## 20    JKL19 B JKL19b-counts.txt   w-29-IV     B   JKL19        w   29  IV
## 21    JKL20 A  JKL20-counts.txt  Ez-29-IV     A   JKL20       Ez   29  IV
## 22    JKL20 B JKL20b-counts.txt  Ez-29-IV     B   JKL20       Ez   29  IV
## 23    JKL21 A  JKL21-counts.txt    w-29-V     A   JKL21        w   29   V
```

```
## 24   JKL21 B JKL21b-counts.txt    w-29-V     B   JKL21        w   29   V
## 25   JKL22 A  JKL22-counts.txt   Ez-29-V     A   JKL22       Ez   29   V
## 26   JKL22 B JKL22b-counts.txt   Ez-29-V     B   JKL22       Ez   29   V
## 27    JKL7 A   JKL7-counts.txt    w-25-I     A    JKL7        w   25   I
## 28    JKL7 B  JKL7b-counts.txt    w-25-I     B    JKL7        w   25   I
## 29    JKL8 A   JKL8-counts.txt   Ez-25-I     A    JKL8       Ez   25   I
## 30    JKL8 B  JKL8b-counts.txt   Ez-25-I     B    JKL8       Ez   25   I
## 31    JKL9 A   JKL9-counts.txt  Ez-25-II     A    JKL9       Ez   25  II
## 32    JKL9 B  JKL9b-counts.txt  Ez-25-II     B    JKL9       Ez   25  II
##    condition
## 1       w-25
## 2       w-25
## 3       w-25
## 4       w-25
## 5      Ez-25
## 6      Ez-25
## 7       w-29
## 8       w-29
## 9      Ez-29
## 10     Ez-29
## 11      w-29
## 12      w-29
## 13     Ez-29
## 14     Ez-29
## 15      w-29
## 16      w-29
## 17     Ez-29
## 18     Ez-29
## 19      w-29
## 20      w-29
## 21     Ez-29
## 22     Ez-29
## 23      w-29
## 24      w-29
## 25     Ez-29
## 26     Ez-29
## 27      w-25
## 28      w-25
## 29     Ez-25
## 30     Ez-25
## 31     Ez-25
## 32     Ez-25
```

Set up the statistical model:

```
design <- formula(~ Temp + genotype)
```

```
dds <- DESeqDataSetFromHTSeqCount(sampleTable = sampleTable, directory = inpath, design = design)
# Combine the technical replicates (different runs) by adding the count
# totals for each gene across the two runs:
dds <- collapseReplicates(dds, groupby=dds$Library, run = dds$batch)
dds <- DESeq(dds)
# What does the data look like?
```

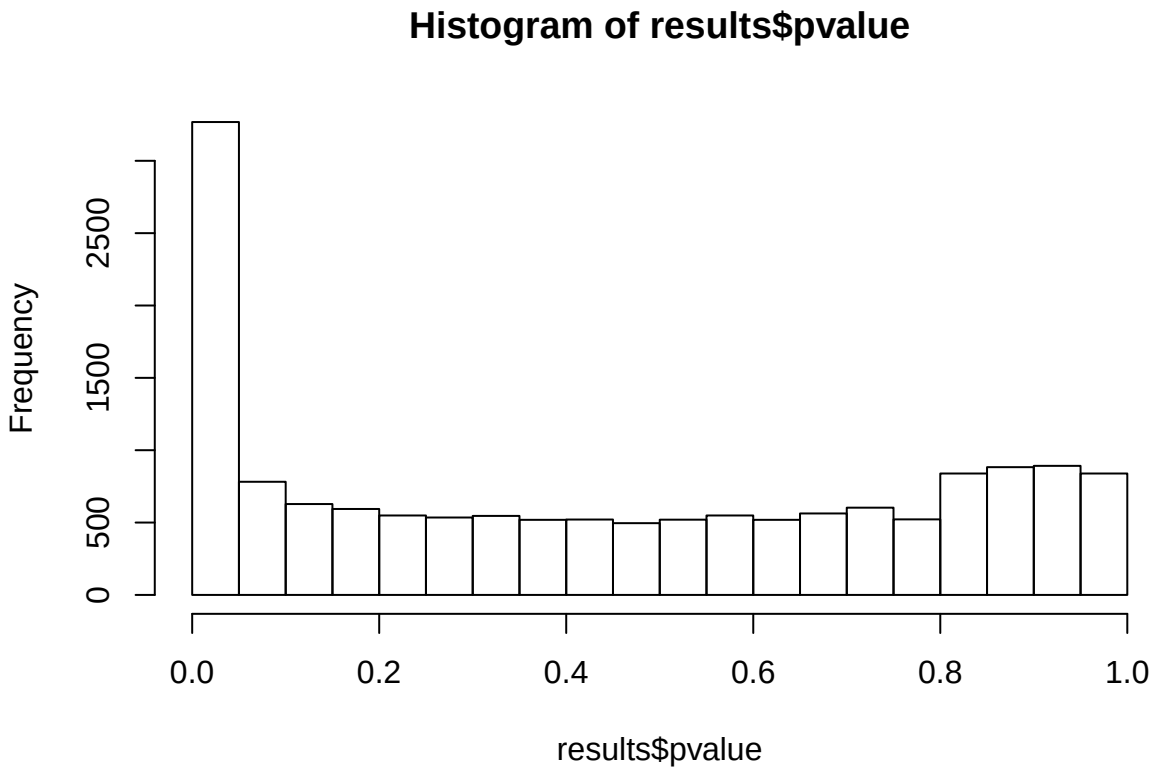
```
head(assay(dds)) # This is the sum of the two runs HTSeq-count output!
```

```
##             JKL10 JKL11 JKL12 JKL13 JKL14 JKL15 JKL16 JKL17 JKL18 JKL19
## FBgn0000003     0     0     0     0     0     0     0     0     0     0
## FBgn0000008  1444  1874  1687  1305  1453  1725  1529  1856  1500  1558
## FBgn0000014     0     0     0     0     0     0     1     0     1     0
## FBgn0000015     1     6     1     3     0     1     1     1     2     0
## FBgn0000017  9186 10798  9189  6877  8808  9121  9834 11313  9272  8564
## FBgn0000018   262   306   286   336   288   317   272   346   274   285
##             JKL20 JKL21 JKL22  JKL7  JKL8  JKL9
## FBgn0000003     1     0     0     0     0     1
## FBgn0000008  1353  1589  1367  1980  1861  1884
## FBgn0000014     1     1     1     0     3     0
## FBgn0000015     0     1     0     0     0     2
## FBgn0000017  8697  8196  8612 11453 11360 11139
## FBgn0000018   285   238   284   286   333   333
```

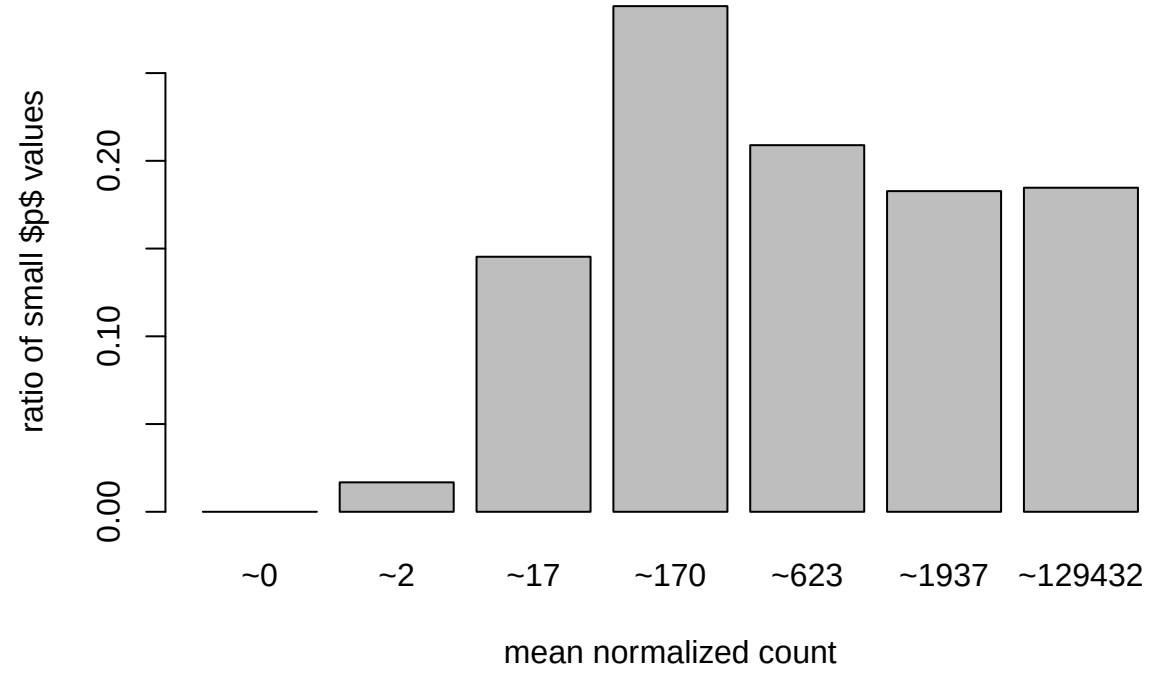
```
# What are the columns?
colData(dds)
```

```
## DataFrame with 16 rows and 9 columns
##          Sample    batch  Library genotype     Temp         rep
##        <factor> <factor> <factor> <factor> <factor> <character>
## JKL10   w-25-II        A    JKL10        w       25          II
## JKL11  w-25-III        A    JKL11        w       25         III
## JKL12 Ez-25-III        A    JKL12       Ez       25         III
## JKL13    w-29-I        A    JKL13        w       29           I
## JKL14   Ez-29-I        A    JKL14       Ez       29           I
## ...         ...      ...      ...      ...      ...         ...
## JKL21    w-29-V        A    JKL21        w       29           V
## JKL22   Ez-29-V        A    JKL22       Ez       29           V
## JKL7     w-25-I        A     JKL7        w       25           I
## JKL8    Ez-25-I        A     JKL8       Ez       25           I
## JKL9   Ez-25-II        A     JKL9       Ez       25          II
##         condition runsCollapsed sizeFactor
##       <character>   <character>  <numeric>
## JKL10        w-25           A,B  0.9090574
## JKL11        w-25           A,B  1.1403424
## JKL12       Ez-25           A,B  1.0027251
## JKL13        w-29           A,B  1.0220507
## JKL14       Ez-29           A,B  0.9570594
## ...           ...           ...        ...
## JKL21        w-29           A,B  0.8750556
## JKL22       Ez-29           A,B  0.9618390
## JKL7         w-25           A,B  1.1341278
## JKL8        Ez-25           A,B  1.1093046
## JKL9        Ez-25           A,B  1.1303920
```

```
results <- results(dds, alpha=0.05)
results$ensembl <- rownames(results)
hist(results$pvalue, breaks=20)
```

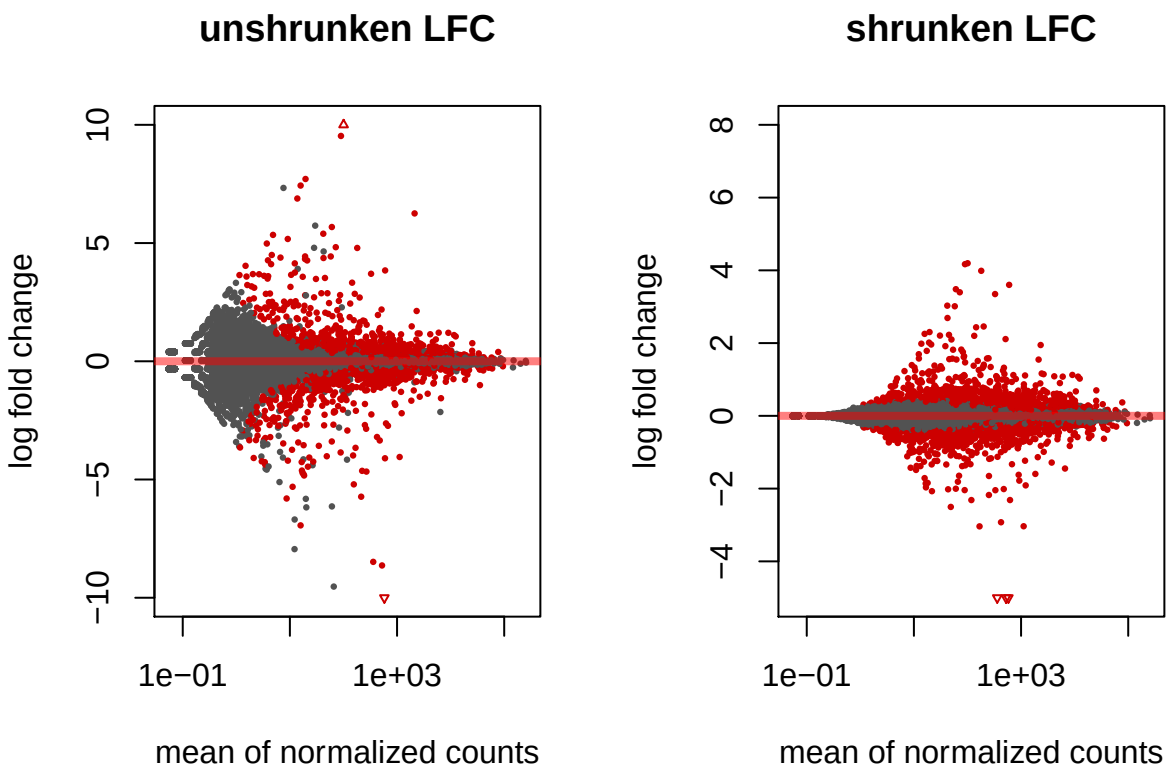



```
qs <- c(0, quantile(results$baseMean[results$baseMean>0], 0:7/7))
bins <- cut(results$baseMean, qs)
levels(bins) <- paste0("~", round(.5*qs[-1] + .5*qs[-length(qs)]))
ratios <- tapply(results$pvalue, bins, function(p) mean(p<.01, na.rm=TRUE))
barplot(ratios, xlab="mean normalized count", ylab="ratio of small $p$ values")
```



Prepare MA plots:

```
# For Maximum likelihood estimates:
resultsMLE <- results(dds, addMLE=TRUE, alpha = 0.05)
par(mfrow=c(1,2))
plotMA(resultsMLE, MLE=TRUE, alpha = 0.05, main="unshrunken LFC", ylim=c(-10,10))
plotMA(results, alpha = 0.05, main="shrunken LFC", ylim=c(-5,8))
```



Add Annotation

```
# Add usefull gene names:
library(biomaRt)

# Get the archived version:
mart = useMart("ENSEMBL_MART_ENSEMBL",
               host="aug2017.archive.ensembl.org")
#x <- listDatasets(mart)
mart = useMart("ENSEMBL_MART_ENSEMBL", host="aug2017.archive.ensembl.org",
               dataset = "dmelanogaster_gene_ensembl")
#listAttributes(mart)
genemap <- getBM(attributes = c("ensembl_gene_id", "entrezgene", "flybasecgid_gene",
                                "external_gene_name"),
                 filters = "ensembl_gene_id",
                 values = results$ensembl,
                 mart = mart)

idx <- match(results$ensembl, genemap$ensembl_gene_id)
results$entrez <- genemap$entrezgene[idx]
```

```
results$geneSymbol <- genemap$external_gene_name[idx]
results$cg <- genemap$flybasecgid_gene[idx]
```

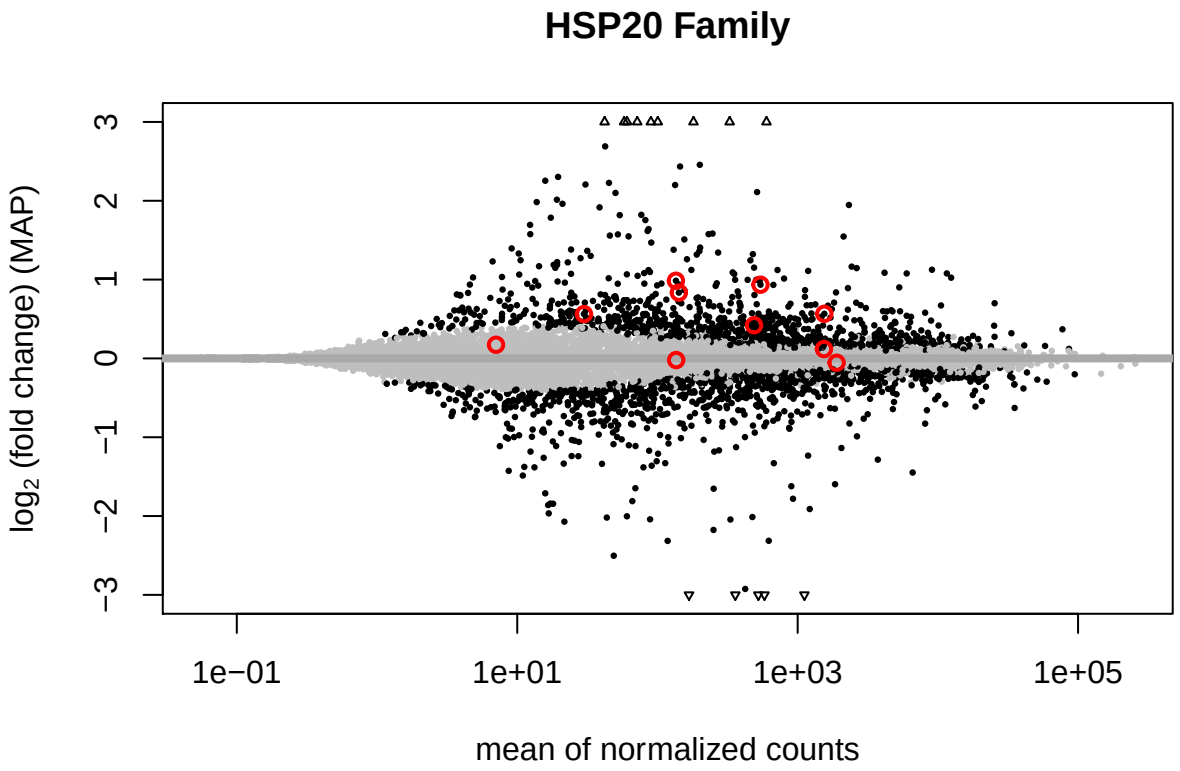
Prepare MA plots of small HSP family

```
small_hsp <- read.delim(file.path(inpath, "FBgg0000507.txt"))
colnames(small_hsp) <- c("FlyBase", "NAME", "SYMBOL")

# Small HSP Family:
jpeg(file=paste(outpath, "Ez_sHSP_MAP.jpg", sep="/"), width=480, height=480)
results$shsp <- results$ensembl %in% small_hsp$FlyBase
plotMA(results, alpha = 0.05, ylim=c(-3,3), colNonSig="gray", colSig="black", colLine="darkgrey",
       ylab=expression("log"[2]*" (fold change) (MAP)"), main="HSP20 Family")
with(results[results$shsp==TRUE,], {points(baseMean, log2FoldChange, col = "red", cex=1, lwd=2)})
dev.off()
```

```
## pdf
##   2
```

```
plotMA(results, alpha = 0.05, ylim=c(-3,3), colNonSig="gray", colSig="black", colLine="darkgrey",
       ylab=expression("log"[2]*" (fold change) (MAP)"), main="HSP20 Family")
with(results[results$shsp==TRUE,], {points(baseMean, log2FoldChange, col = "red", cex=1, lwd=2)})
```

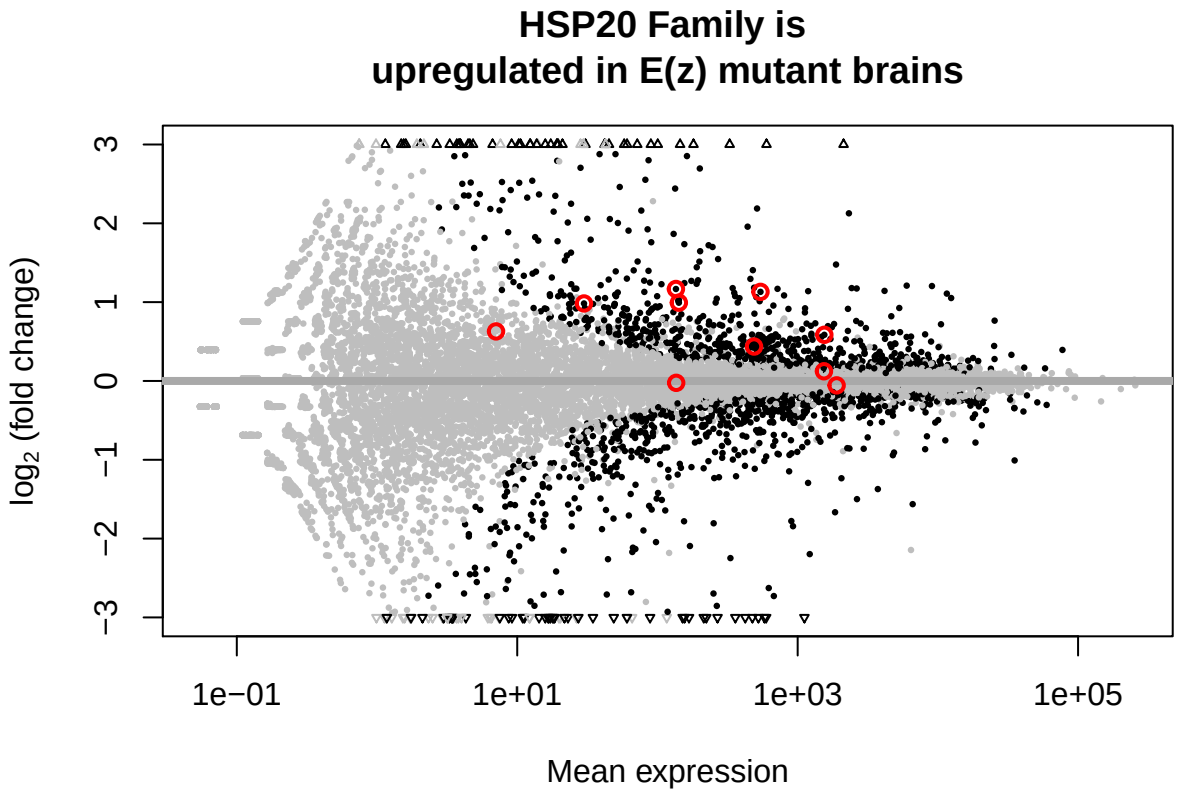


Prepare MA plots using MLE:

```
# Small HSP Family
#####################Prepare Publication Plot#########################
jpeg(file=paste(outpath, "Fig7b_Ez_sHSP_MLE.jpg", sep="/"),
     quality=100,
     res=300,
     width=960,
     height=960)
resultsMLE$shsp <- rownames(resultsMLE) %in% small_hsp$FlyBase
par(mai=c(0,0,0,0), mar=c(2,4.5,3,1)+.5)
plotMA(resultsMLE, alpha = 0.05, ylim=c(-3,3), colNonSig = "gray", colSig = "black",
       MLE=TRUE, colLine = "darkgrey",
       las=1, cex.lab=1, cex.axis=1, cex.main=1.5,
       ylab="", xlab = "", xaxp=c(1,2,1), xaxt="n",
       main="")
axis(1, at=c(1,10, 100, 1000, 10000), labels=c(1,10, 100, 1000, 10000), cex.axis=1)
with(resultsMLE[resultsMLE$shsp==TRUE,], {points(baseMean, lfcMLE, col = "red", cex=1, lwd=3)})
dev.off()
```

```
## pdf
##   2
```

```
#########################################################################
plotMA(resultsMLE, alpha = 0.01, ylim=c(-3,3), colNonSig = "gray", colSig = "black",
       MLE=TRUE, colLine = "darkgrey",
       ylab=expression("log"[2]*" (fold change)"), xlab = "Mean expression",
       main="HSP20 Family is \nupregulated in E(z) mutant brains")
with(resultsMLE[resultsMLE$shsp==TRUE,], {points(baseMean, lfcMLE, col = "red", cex=1, lwd=2)})
```



```
Sys.info()
```

```
##                                                                                                    sysname
##                                                                                                   "Darwin"
##                                                                                                    release
##                                                                                                   "15.6.0"
##                                                                                                    version
## "Darwin Kernel Version 15.6.0: Thu Jun 21 20:07:40 PDT 2018; root:xnu-3248.73.11~1/RELEASE_X86_64"
##                                                                                                   nodename
##                                                                                  "Jasons-MacBook-Pro.local"
##                                                                                                    machine
##                                                                                                   "x86_64"
##                                                                                                      login
##                                                                                               "jasonkennerdell"
##                                                                                                       user
##                                                                                               "jasonkennerdell"
##                                                                                                  effective_user
##                                                                                               "jasonkennerdell"
```