

- We're going to work on some data that exists in R already. This experiment was carried out using zebrafish as a model organism to study early development in vertebrates. Swirl is a point mutant in the BMP2 gene that affects the dorsal/ventral body axis. These data were provided by Katrin Wuennenberg-Stapleton from the Ngai Lab at UC Berkeley. The swirl embryos for this experiment were provided by David Kimelman and David Raible at the University of Washington.
- ```
library(limma)
library(marray)
library(convert)
data(swirl)
```
- To make the data look like your project data:  

```
swirl.rg<-as(swirl,"RGList")
```
- Boxplots of the background and foreground data:  

```
par(mfrow=c(2,2))
boxplot(data.frame(swirl.rg$R),names=c("swirl1","swirl2","swirl3","swirl4"),
 main="Red foreground",log="y")
boxplot(data.frame(swirl.rg$Rb),names=c("swirl1","swirl2","swirl3","swirl4"),
 main="Red background",log="y")
boxplot(data.frame(swirl.rg$G),names=c("swirl1","swirl2","swirl3","swirl4"),
 main="Green foreground",log="y")
boxplot(data.frame(swirl.rg$Gb),names=c("swirl1","swirl2","swirl3","swirl4"),
 main="Green background",log="y")
```
- To normalize the data:  

```
swirl.ma <- normalizeWithinArrays(swirl.rg, method="none")
swirl.bgd <- normalizeWithinArrays(swirl.rg, method="median")
swirl.norm <- normalizeWithinArrays(swirl.rg, method="loess")
```
- – MA plots of the raw data:  

```
win.graph()
par(mfrow=c(2,2))
for(i in 1:4){
 plotMA(swirl.ma,array=i, main=paste("swirl",i))}
mtext("Raw Data",outer=T,line=-2)
```

- MA plots of the background subtracted data:

```
win.graph()
par(mfrow=c(2,2))
for(i in 1:4){
 plotMA(swirl.bgd,array=i, main=paste("swirl",i))}
mtext("Background Subtracted Data",outer=T,line=-2)
```

- MA plots of the loess normalized data:

```
win.graph()
par(mfrow=c(2,2))
for(i in 1:4){
 plotMA(swirl.norm,array=i, main=paste("swirl",i))}
mtext("Loess Normalized Data",outer=T,line=-2)
```

- MA plots with the smoothed curve:

```
win.graph()
par(mfrow=c(2,2))
for(i in 1:4){
 scatter.smooth(swirl.ma$A[,i],swirl.ma$M[,i],main=paste("swirl",i),col=4)}
mtext("Raw Data",outer=T,line=-2)
etc... for the other transformations of the data
```

- To find random genes:

```
rand.gns <- sample(1:nrow(swirl.ma),3)
rand.gns
```

- Kolmogorov-Smirnov test of normality:

```
ks.test(swirl.ma$M[47,],"pnorm",mean(swirl.ma$M[47,]),sd(swirl.ma$M[47,]))

for (i in 1:length(rand.gn)){
 junk <- ks.test(swirl.ma$M[rand.gn[i],],"pnorm",mean(swirl.ma$M[rand.gn[i],]),
 sd(swirl.ma$M[rand.gn[i],]))
 print(junk$p)}
```

- qq-plot:

```
qqnorm(swirl.ma$M[47,])
cor(qqnorm(norm.data$M[47,])$x,qqnorm(norm.data$M[47,])$y)
```

```
for (i in 1:length(rand.gn)){
 junk <- cor(qqnorm(swirl.ma$M[rand.gn[i],])$x,qqnorm(swirl.ma$M[rand.gn[i],])$y)
 print(junk)}
```

- Note: it's pretty hard to reject normality with 4 data points... it's hard to reject any null hypothesis with only 4 data points.