• 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,"RGLList")`

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

- Kolmogorov-Smirnov test of normality:
  ```r
  ks.test(swirl.ma$M[47,],"pnorm",mean(swirl.ma$M[47,]),sd(swirl.ma$M[47,]))
  ```
  ```r
  for (i in 1:length(rand.gn)){
    junk <- ks.test(swirl.ma$M[rand.gn[i],],"pnorm",mean(swirl.ma$M[rand.gn[i],]),sd(swirl.ma$M[rand.gn[i],]))
    print(junk$p)
  }
  ```

- qq-plot:
  ```r
  qnorm(swirl.ma$M[47,])
  cor(qnorm(norm.data$M[47,]$x,qnorm(norm.data$M[47,]$y)
  ```
  ```r
  for (i in 1:length(rand.gn)){
    junk <- cor(qnorm(swirl.ma$M[rand.gn[i],]$x,qnorm(swirl.ma$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.