BioSys PhD - Normalization Methods in Microarray Data

EXERCISES

Package `limma` (Linear Models for Microarray Data), from `Bioconductor`, contains functions for exploratory microarray analysis. Here, some of these functions will be explored.

Swirl Zebrafish data are used as an example of data analyzes in package `limma`:

1. Install and load the package:

```
source("http://bioconductor.org/biocLite.R")
biocLite("limma")
library(limma)
```

2. Read the data and check their components
(download at `http://bioinf.wehi.edu.au/limma/`):

```
swirl <- readTargets("SwirlSample.txt")
RG <- read.maimages(swirl$FileName, source="spot")
RG$genes <- readGAL("fish.gal")
RG$printer <- getLayout(RG$genes)
```

3. Graphical representations.

   Visualize the background images for the Red and the Green intensities, for each array (at the same window).

```
imageplot(RG$Rb[,1],RG$printer,low="white",high="red")
```

Visualize the MA plot for each array: (1) considering all the intensities in the array; (2) the intensities by print tip.

```
plotMA(RG)
plotPrintTipLoess(RG)
```

Visualize the M values for all the arrays, by using box plots.

```
MA.raw <- normalizeWithinArrays(RG,method="none")
head(MA.raw)
boxplot(MA.raw$M,names=colnames(MA.raw$M),col=rainbow(4))
```

4. Background correction.

   Correct the background in different ways and compare the resulting graphics.

   ```
   RG.bg.correct <- backgroundCorrect(RG,method="sub")
   ```

5. Normalize within arrays after correcting the background. Visualize the results by using box plots.

   ```
   MA.with <- normalizeWithinArrays(RG.bg.correct,method="printtiploess")
   boxplot(MA.with$M,names=colnames(MA.with$M),col=rainbow(6))
   ```

6. Normalize between arrays. Visualize the results by using box plots.

   ```
   MA.bet <- normalizeBetweenArrays(MA.with$M,method="scale")
   boxplot(MA.bet$M,names=colnames(MA.bet$M),col=rainbow(8))
   ```