GS01 0163 **Assignment 3** **Fall 2009**

Due Date: 15 October 2009

Background

In lecture 6, we discussed how Sweave could be used to document the use of R code by combining code and documentation in a single report. Examples are available on the Lecture Notes page. Recall that Sweave itself is already available as part of R. To produce the pdf output, you will need MiKTeX as well (http://miktex.org). Once MiKTeX is installed, you can (a) prepare the template Sweave document (e.g., "fileA.Rnw"), (b) invoke "`Sweave('fileA.Rnw')`", which will produce fileA.tex, and (c) from the windows command prompt in the appropriate directory, invoke "`pdflatex fileA.tex`", which should produce fileA.pdf.

For this assignment, which involves coding in R, you should prepare your reports using Sweave. Submissions will ideally include two files:

1. the **source file**, which we should be able to run on our own machines, and
2. the **final output pdf file**, which should include the results of running the R code.

Questions

1. This assignment uses a subset of the Singh prostate cancer data that was acquired for Homework 1. Download the "subcel.txt" and "subsamples.txt" files for homework 3 from the course web site. These files list the 20 CEL files to be used in this assignment. (You will have to edit "subcel.txt" so the path points to the correct location on your computer.)

   (a) Use the supplied "subsamples.txt" file to create a `phenoData` object. Use short sample names to identify the arrays.

   (b) Load in all 20 CEL files to create an `AffyBatch` object. Create a boxplot and a histogram of the raw data. Do the arrays need to be normalized?

   (c) Look at images of several of the arrays. Do you see anything unusual?

2. Use BioConductor to decide if the RNA used in any of these experiments was exceptionally degraded.

   Bonus: What happens if you plot the degradation slopes as a function of the factors in the `pData` object in the AffyBatch?

3. In Homework 1, we saw that clustering the samples after standard processing with dChip, there were two dominant clusters that did not match the biological division into normal and cancer. The 20 samples used in this homework are evenly balanced

between normal/cancer and between the two dChip clusters. Using the `qc` function in the `simpleaffy` package, explore the various quality control metrics. Do any of the quality control metrics suggest an explanation for how the data clusters?

4. Perform background correction on all the arrays using two different methods: "mas" and "rma". Prepare histograms and boxplots of the AffyBatch objects for both background correction methods. What can you say about the differences? Which background correction method do you think is better? Why?

5. This problem is a continuation of the previous problem. This problem, like several of the later problems, uses the `simpleCluster` function that is available from the course web site. This function produces cluster dendrograms based on the 25% of genes with the highest average expression.

   (a) After processing the arrays using "mas" background correction, use `expresso` to perform "quantiles" normalization, select the "pmonly" features, and quantify using the "medianpolish" summarization method. Use `simpleCluster` to produce a dendrogram.

   (b) Repeat part (a) using "rma" background correction.

   (c) Does the background correction method have any effect on clustering based on the highest expressing genes?