We can use t-test, ebayes, treat or wilcox test in order to determine differentially expressed genes  (DEGs) in microarray data. We use two classes of sample to detect which genes are differentially expressed.

In this case study I, we used Bioconductor project's several packages such as affy, limma, hgu95av2.db to performData analysis using Prostrate cancer Affymetrix data (Affymetrix U95Av2arrays) from D. Singh et al., "Gene expression correlates of clinical prostrate cancer behavior", Cancer Cell 2002, 1(2):203-209. The steps to follow are:

1) Make use of the ReadAffy() function to read data in Affy CEL files.

2) We add a variable indicating normal/tumor group which would be obtained from the sample.

3) We can use Robust Multi-Array Average (RMA) method to preprocess Affymetrix data. Pre-processing involves background correction, normalization, and expression calculation.

Basically, we use rma() and mas5() methods to collapse the measurements across a probe pair set into single measurement for every affyID.

4) We use boxplots to plot and graphically look at normalized data. We will use a log2 transform and plot again.

5) We perform non-specific filtering to remove uninteresting things from the data. We look at various options in nsFilter function to achieve this. Examples are removing duplicate Entrez IDs, not filtering features based on variance, GOBP annotation & variance filtering.

6) Then we will use a suitable method for microarray Differential expression and we create a table of results using multiple comparison adjustments using a method such as:

(i) Ebayes method

(ii) Treat method

(iii) wilcox test

(iv) row t-test

we use limma and genefilter packages to achieve multiple comparisons.

7) Using the appropriate table of result (i.e from eBayes/TREAT,/row t-test/wilcox), we map AffyIDs to Entrez GeneIDs/Gene symbols. This step involves investigating the biology from the table of results.

8) Then we typicallypick a threshold p-value to generate a Gene listand the result is written to a file.

- optional 9) We then plot a venn diagram consisting the four gene list generated using an appropriate method such as ebayes, treat, wilcox and t-test (If result using all 4 methods are generated).