Appendix 3. Student Handout for Reading Assignment Prior to Beginning Module

(For a free downloadable Word file, with comments for teachers, see Zanta, 2006.)

Objective:

To offer students an interactive way to visualize how microarrays are used to study gene expression.

Below is a detailed description of how microarrays are used in research labs. Starting on page 2, we describe a simplified procedure for a paper microarray activity that mimics this wet lab procedure. In this paper activity, you will experience the main parts of working with DNA microarrays.

General Microarray Procedure:

1. Obtain a microarray slide containing 70 bp oligonucleotide sequences representing each gene in the genome of your favorite organism. Most scientists purchase these slides already prepared. The DNA is contained in spots approximately 100 microns in diameter. Each slide contains thousands of microscopic spots of DNA (one for each gene in the genome; humans have ~25,000 genes). While the function may be known for some of these gene sequences, many genes have unknown functions.
2. Extract mRNA from your experimental organism and your control organism for comparison. For example, corn growing under drought conditions vs. a corn growing in normal conditions, or tumor cells vs. normal cells. Each sample will contain thousands of different mRNA sequences representing all of the genes expressed in those cells.
3. Prepare fluorescent labeled cDNA copies of this mRNA. This cDNA from each sample of mRNA will be labeled with different fluorescent nucleotides (either green Cy3 dye; or red

Cy5 dye). The cDNA will have to be denatured to produce single-stranded DNA prior to the next step.

4. Hybridize the microarray slide with both of these labeled cDNAs. Each cDNA will bind to the spots that have complementary sequences. Stringent conditions are used to ensure that the probe sequences are entirely complementary to the microarray spot sequences.

5. Wash the slide to remove excess fluorescent cDNA not bound to spots.

6. Read the microarray using an instrument that measures the fluorescence of each spot at two different wavelengths for green Cy3 or red Cy5. The instrument is connected to a computer which integrates the data into a single image. The color of the spots are as follows:

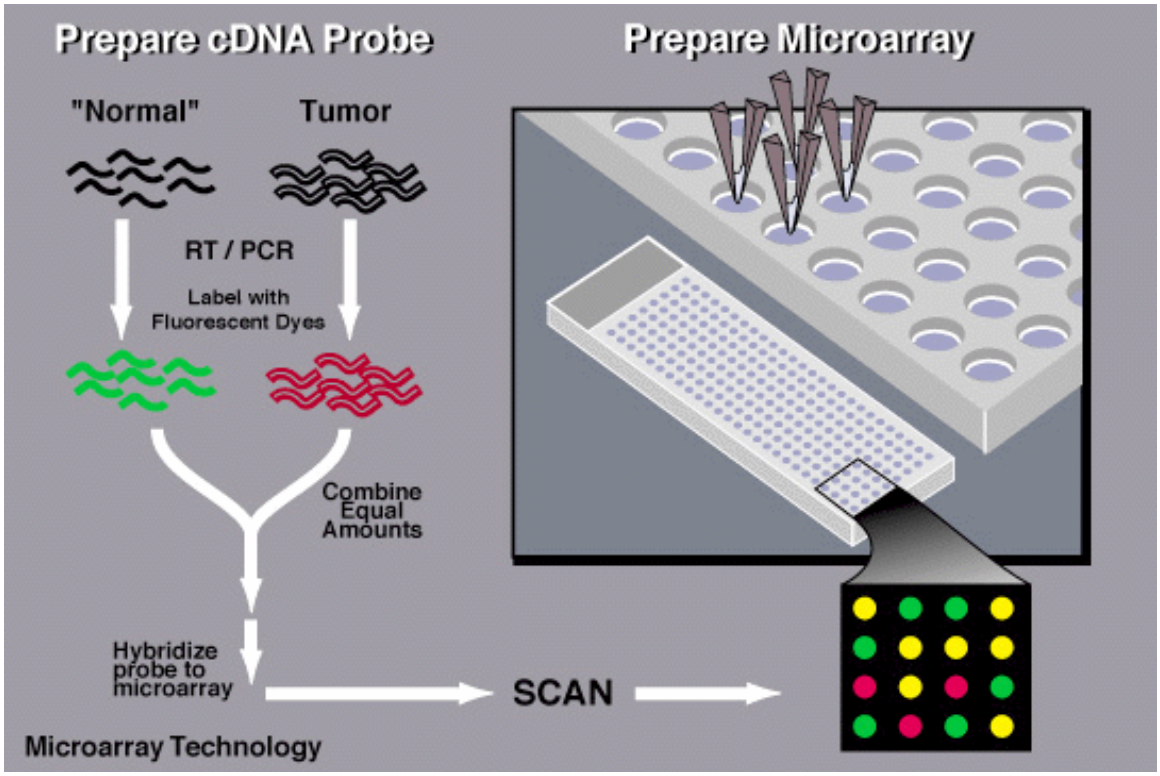
green = bound to Cy3-labeled cDNA (what do these represent?)

red = bound to Cy5-labeled cDNA (what do these represent?)

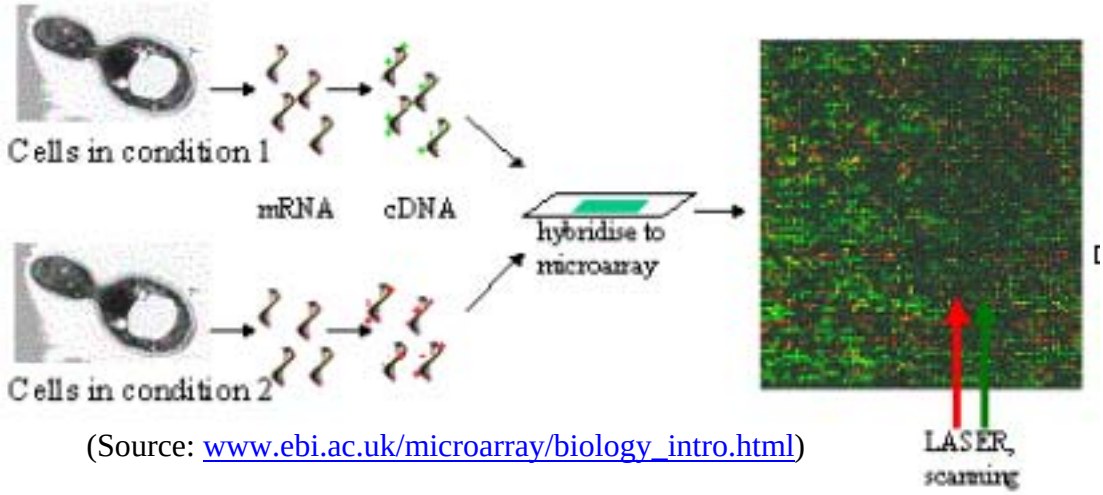
yellow = bound to BOTH Cy3 and Cy5-labeled cDNA (these represent genes such as “housekeeping genes” that are required by all cells)

7. Analyze the data to determine which genes (represented by spots on the slide) are expressed in each cell sample (and which are expressed in both). The next step in functional genomics studies is study in more detail those genes that are differentially expressed in control vs. experimental conditions.

Figure 1. Using Microarray Technology to Study Gene Expression in Normal and Tumor Cells



(NOTE that black spots should also be present where no labeled probe bound to the DNA in the spots; Source: http://www.genome.gov/10000533)



(Source: www.ebi.ac.uk/microarray/biology_intro.html)

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