Appendix 4. Student Protocol Handout

For a free downloadable Word file of this handout, with comments for teachers, see Zanta, 2006.

The purpose for this simulation is to teach high school students the following:

1) DNA microarrays can measure the activity of many genes simultaneously.

2) Genes are differentially regulated (expressed differently under different conditions).

Materials Required:

Oven or water bath set to 65-70^{o}C to keep spotting solutions warm.

Camera for taking photos to document data (optional).

Procedure for Use:

1) Melt contents of microfuge tubes 1 – 6 containing the 6 genes by microwaving them and then maintain them at 65-70^{o}C prior to use. (Note to instructor: Do this before students arrive.) Loosen caps once or twice during the melting process to release excess pressure. Keep lids on to avoid complete evaporation while waiting to spot the arrays.

2) Spot out the contents of the tubes; you may use pipettors or droppers. Do not overflow the spotting circle. If the drop build up to a dome, that is fine but not necessary. These spots represent the target DNA sequences for 6 genes. The agarose will harden in one to two minutes. One a separate piece of paper or in a notebook, have the students draw a diagram of the slide indicating the position of each spots on the slide. In doing this, they have performed the major steps of printing their own simulated DNA microarray.

3) Place the slide on a white piece of paper. Apply 2 drops of "labeled cDNA Hybridization Solution" to each spot and allow color to develop (~1 minute). The cDNA from the control cells was labeled blue. The cDNA from cancerous cells was labeled red. As with real DNA microarrays, you cannot see the color of the hybridization solution. In a real microarray, the labeled probes are very diffuse and thus invisible. When the probes condense on the spots due to base pairing, you can see the colors. This visualization is very similar to real DNA microarrays.

[In our simulation, we simply convert the pH indicators from clear to blue or red by exposing to high pH.]

4) Record the colors or take a photo – digital cameras work best for fast results and data sharing.

5) Have your students explain which genes were activated and which ones were repressed in the cancer biopsy.

Notes:

SAFETY CONCERNS:

Proper eye protection and gloves should be worn in high school laboratories. Basic solutions (i.e., the cDNA hybridization solution) is caustic. Students should wash their hands after lab and immediately if they have an itching sensation. Students wearing gloves should avoid touching lab surfaces with gloved hands.

The dyes are toxic if swallowed, but basically pretty harmless. (See sections 10 and 11 of MSDS)

http://avogadro.chem.iastate.edu/MSDS/phenolphthalein.htm

http://avogadro.chem.iastate.edu/MSDS/thymolphthalein-0.05pct.htm

Teacher's Page

Key to Spots 1 – 6:

Spot 1 = deep red (gene induced in experimental condition)

Spot 2 = purple (mixed red and blue; gene equally transcribed in both conditions)

Spot 3 = blue (gene repressed in experimental condition)

Spot 4 = clear (gene not transcribed under either condition)

Spot 5 = light red (gene slightly induced in experimental condition)

Spot 6 = light blue (gene slightly repressed in experimental condition)

You can use this simulation to discuss gene regulation qualitatively. However, you could combine this simulation with the free software called MAGIC Tool (www.bio.davidson.edu/GCAT) to bring students to a new, higher level. We are developing a mathematical module to go with this wet lab. Check www.bio.davidson.edu/projects/GCAT/HSChips/HSchips.html for the latest updates (GCAT, 2006b). With the mathematics module, students will learn to:

1) Generate ratios of gene expression that represent fold change in the experimental compared to control cells.

2) Log transform ratios so they can compare induced and repressed genes on comparable scales.

3) Use correlation coefficients to determine which genes are behaving similarly.

For a free downloadable Word file of this handout, with comments for teachers, see Zanta, 2006.