

Paper Microarray Student Procedure

You are part of a research group studying human genes involved in cancer. You have assisted with the human genome project and have identified putative genes via genetic database comparisons and other computer analyses. Many of these human genes share similar protein sequences with those of other organisms, and the putative functions have been thus identified by this shared homology. However, there are many sequences with unknown functions. Your research group believes that many of these unknown genes play a role in either the prevention of cancer in normal cells or the proliferation of cancer cells in abnormal tissues. *Note that we have changed the color green to blue and yellow to purple because when you conduct the wet lab simulation, you will see blue and purple rather than green and yellow.*

In these post-genomics studies (research done after the human genome sequencing has been completed), your group has decided to use microarrays to study genes expressed in normal cells vs. abnormal cancerous cells. Your goal is to identify these unique genes.

1. Each research group will work with two different tissue samples (either normal or cancerous). First, you must extract the mRNA from each tissue sample. A problem with mRNA is that it is very unstable. Also, the mRNA from each of the different samples can't be distinguished. In order to distinguish the mRNA from each tissue sample, the mRNA must be converted into cDNA (complimentary DNA). cDNA is made in a process similar to the transcription of mRNA from DNA. However, in this process, the DNA is copied from the mRNA template, and it is called "reverse transcription". In preparation for microarrays, the cDNA is labeled with different fluorescent nucleotides (either blue Cy3 dye [we have changed this from green to blue in order to match the wet lab simulation you will do soon]; or red Cy5 dye).

To review the process of converting mRNA to cDNA, complete the following problem in the space provided. You extracted the following mRNA sequence (among thousands of other mRNAs) from cancerous cells:

5' CCUAUUGGAAUCGG 3'

What is the cDNA sequence that would be synthesized from this mRNA?

2. Your group has done an excellent job carefully extracting mRNA and preparing fluorescently labeled cDNA from your tissue samples! Your teacher will give you copies of these labeled single-stranded cDNAs. The normal cDNA was labeled with Cy3 (blue), and the cDNA from the cancerous cells was labeled with Cy5 (red).
3. Your research group of 4-6 scientists has obtained a microarray slide containing closeups of 6 spots of DNA oligonucleotides representing different human genes with unknown functions. (*How does this compare with an actual microarray slide?*)

4. Mix the fluorescently-labeled cDNA from the two cell samples (if they aren't already mixed). Hybridize the microarray slide with these labeled cDNAs. Each cDNA will bind to the spots that have totally complementary sequences. While you do this for all of the cDNAs, try to neatly stack your hybridized cDNAs to keep the microarray sequence in view. You may tape these onto the microarray slide using a small piece of removable tape.
5. Wash the slide to remove excess fluorescent cDNA not bound to spots. (You may simply remove the unbound cDNAs left on the slide.)
6. Read the microarray using an instrument that measures the fluorescence of each spot at two different wavelengths for blue (Cy3) or red (Cy5). Analyze the data to determine which genes (represented by spots on the slide page) are expressed in each tissue sample (and which are expressed in both). On the microarray slide sheet, use markers to color each spot. The color of the spots are as follows:

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

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

purple = equally bound to BOTH Cy3- and Cy5-labeled cDNA (these represent genes such as "housekeeping genes" that are required by all cells; we have changed yellow to purple since this color matches what you will see when you perform the wet lab simulation)

7. Your group has obtained interesting results that may be useful in determining how cancer cells differ from normal cells! The next step in functional genomics studies is to further study those genes that appear to be important in your treated cells.

Which unknown gene sequences (#1-6) appear to be genes used in all cells?

Which unknown gene sequences (#1-6) might be cancer-preventing genes?

Which unknown gene sequences (#1-6) might be genes that cause cells to become cancerous?

Are all of the genes expressed at the same level? How do you know this? What could this mean?

What additional questions do you have regarding your microarray results?

In the space below, describe further research that your group would like to accomplish using microarrays. Your study can be related to the cancer study that you just carried out or be totally unique. Be sure to describe what samples you will use and what will be spotted on the microarray.

Single-stranded, Blue-labeled cDNA from normal cells

Copy on BLUE colored paper or cardstock.

Cut out each cDNA.

Combine these cDNAs with one sheet of the red cDNA from tumor cells.

CATCGGAAC
CATCGGAAC
CCGGGAAATT
GTAAAATTT
GTAGGAATAT
GCGCGCCCGCG
GTAGGAATAT
ATTTAACAAGTT

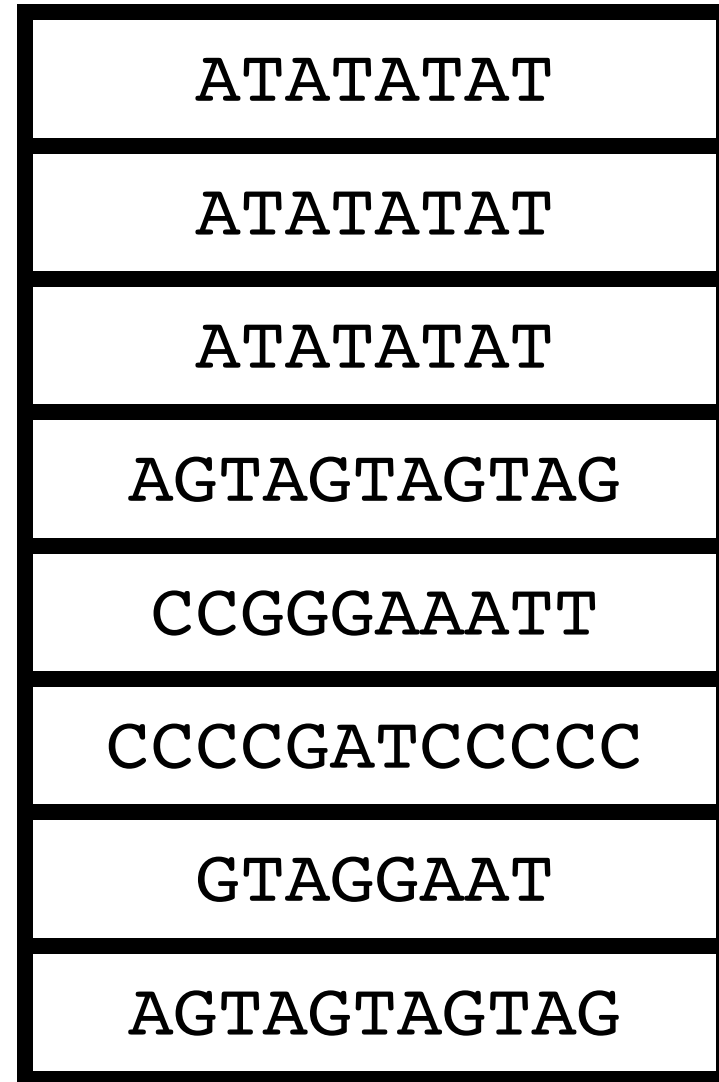
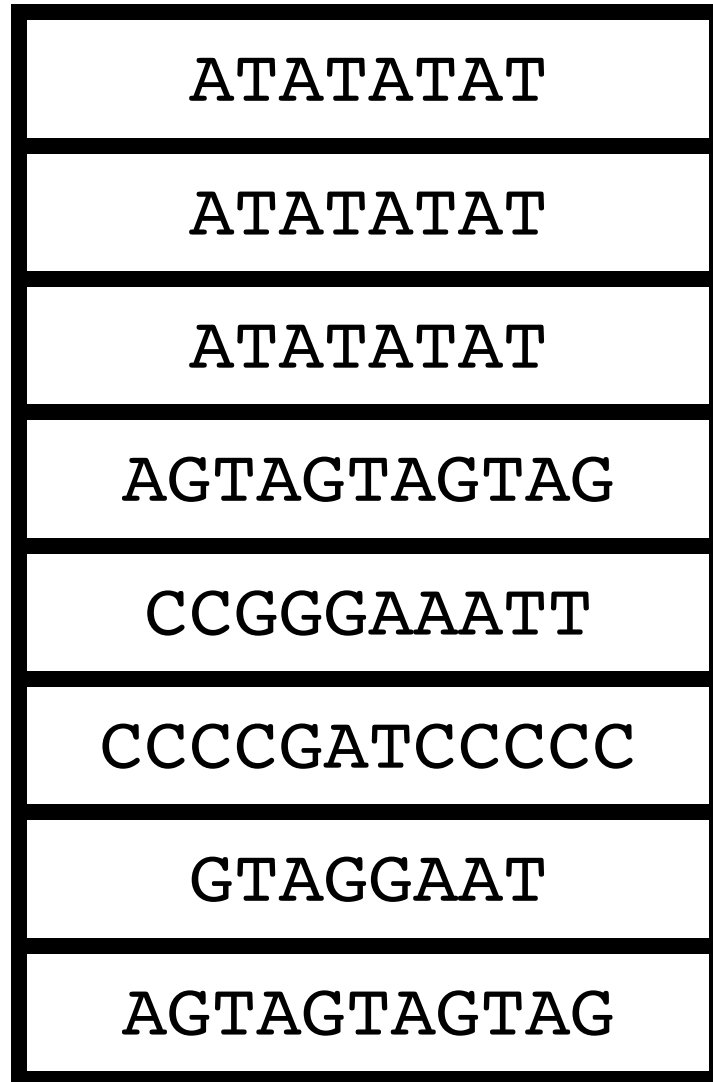
CATCGGAAC
CATCGGAAC
CCGGGAAATT
GTAAAATTT
GTAGGAATAT
GCGCGCCCGCG
GTAGGAATAT
ATTTAACAAGTT

Single-stranded, Red-labeled cDNA from abnormal tumor cells

Copy on RED colored paper or cardstock.

Cut out each cDNA.

Combine these cDNAs with one sheet of the blue cDNA from normal cells.



1

GGGTAGCCTTGG

2

CATGCATCCATG

3

GGGGCCCTTTAA

4

GCATTTTAAAGG

5

CCATCCTTATAG

6

TATATATATATA

Teacher Answer Key

GGGTAGCCTTGG

BLUE
highly expressed in normal cells only (4X)

CATGCATCCATG

BLACK
NOT expressed in either cells

GGGGCCCTTTAA

PURPLE
(constitutively) expressed equally in
normal and cancer cells (2X)

Teacher Answer Key

GCATTTTAAAGG

LIGHT BLUE
expressed in normal cells only (2X)

CCATCCTTATAG

PURPLE/BLUE
expressed in normal cells (4X)
and cancer cells (2X)

TATATATATATA

RED
highly expressed in cancer cells only (6X)