



Cancer Education Project

DNA Microarrays (Gene Chips) and Cancer

Overview:

This series of activities explores the use of DNA Microarray (Gene Chips) technology in investigations to understand the role of genes involved in causing cancer. It also illustrates how the results of these investigations can be applied to diagnosing and treating cancer patients.

Overview of Microarray Activities (**Note: Part 3 may be done for homework*)

- **Part 1: Gene Expression and Cancer (40 minutes)**
Students view a PowerPoint presentation that illustrates and helps them understand the concepts of gene expression and cancer as a change in gene expression. This PowerPoint is followed by a reading activity that introduces students to the concepts of cancer genes and differential gene expression. Students read about the action of some genes involved in cancer and predict whether increased or decreased expression of the genes would lead to cancer.
- **Part 2: DNA Microarray Technology (40 minutes)**
Students are introduced to microarray technology through a PowerPoint and a simulated paper microarray activity. They analyze the microarray results to compare gene expression in normal and cancerous cells.
- **Part 3: Apply Your Understanding of Microarray Technology (20 minutes)***
This worksheet reviews concepts of microarray technology and allows students to apply these concepts to analyze how a cancer drug affects gene expression.
- **Part 4: Personalized Medicine (40 minutes)**
Students read about a new microarray test that makes personalized medicine a reality for breast cancer patients. This activity may be used as an introduction to the laboratory activity.

- **Part 5: Microarrays and Personalized Medicine (60 minutes)**
This simulated microarray “wet lab” illustrates how personalized medicine could be used to select breast cancer patients who are likely to need chemotherapy. Students prepare a microarray and analyze the results to determine which patients might benefit most from chemotherapy.
- **Part 6: Microarrays and Cancer Diagnosis (20 minutes)**
This data analysis activity illustrates how microarrays can be used in cancer diagnosis.

The DNA Microarrays (Gene Chips) activities introduce (or reinforce) the following student understandings:

- Genes contain coded information for the production of proteins.
- When a gene is transcribed in a cell, the coded information in the gene is copied to produce messenger RNA (mRNA)
- The coded information in messenger RNA is then translated to produce a protein.
- “Gene Expression” refers to the processes by which a gene is transcribed and translated to produce a functional protein.
- All cells in an organism contain the same genes, but different genes are expressed (transcribed or “turned on”) in different cells under different conditions. This is also known as “differential gene expression.”
- Differential gene expression results in different cells having different structures and functions.
- Differential gene expression allows cells to maintain homeostasis when they are exposed to different environmental conditions.
- Cell structures and functions may change when gene expression is turned on, off, increased or decreased.
- Cancer can develop when gene mutations lead to changes in genes or gene expression that disrupt the regulation of cell division.
- DNA microarrays (gene chips) are a new technology that scientists use to measure the expression of thousands of genes at one time.
- Microarrays illustrate important connections between genetics (genes, DNA, RNA, and proteins) and cancer.
- Microarrays technology has uses in many areas of biology and medicine.

Citations:

- Parts 1 and 2 were adapted from *Fotodyne MolecuLab™ 40: Paper Microarrays - A Classroom Exercise*.
- Parts 3 and 5 were adapted from *Using Gene Chips to Study the Genetics of Lung Cancer: A DNA Microarray Lab* by Carolyn Zanta, UIUC-HHMI Biotechnology Education and Outreach Program with A. Malcom Campbell, Kathleen Gabric, and Ben Kittinger.

- Part 6 was adapted from *DNA Chips: A Genetics Lab in the Palm of Your Hand* (in *Snapshots* magazine from *Modern Biology* for High School Classrooms)
http://science.education.nih.gov/newsnapshots/toc_chips/toc_chips.html

Suggested Supply Order List:

If materials are recycled, this list will supply enough materials for multiple classes of 30 students working in teams of 3 students.

- Thin stem plastic pipets—approximately 250
- Microcentrifuge tubes (1.5 mL)—approximately 250
- Microcentrifuge tube racks—10 racks that hold at least 9 microcentrifuge tubes. Hint: make inexpensive ones by using a cork borer to punch widely spaced holes into styrofoam meat trays or cups.
- Bromothymol Blue (0.5 gram)
- Methyl Red (0.5 gram)
- Buffer pH 5 (1 buffer capsule or 100 mL premixed)
- Distilled water
- Buffer pH 8 (1 buffer capsule or 500 mL premixed)
- Yellow Food Coloring - McCormick (1 small bottle)
- Plastic overhead transparency sheets for copiers - heavy duty if possible (10)
- Colored pencils or crayons - red, green, yellow, black (1 of each per student or team)
- Small labels for microcentrifuge tubes and pipets (approximately 500)
- Plastic zip-lock snack bags (30)
- Plastic zip-lock quart bags (10)

Other Microarray Teaching Resources:

These Internet sites provide additional teaching materials on microarray technology for high school classrooms:

- [Affymetrix Educator Outreach](http://www.affymetrix.com/corporate/outreach/educator.affx): Curricula, image library, Power Points, and information on how to order demonstration GeneChip arrays.
<http://www.affymetrix.com/corporate/outreach/educator.affx>
- [Gensphere Teacher Resources](http://www.genisphere.com/ed_lung_cancer_scenario.html): A laboratory kit (DNA Chips: Genes to Disease, A Hands-on Wet Lab) that costs approximately \$70. Also includes an excellent list of references. http://www.genisphere.com/ed_lung_cancer_scenario.html
- [DNA Microarray Wet Lab Simulation](http://www.lifescied.org/cgi/content/abstract/5/4/332): DNA Microarray Wet Lab Simulation Brings Genomics into the High School Curriculum provides teacher instructions and student handouts. <http://www.lifescied.org/cgi/content/abstract/5/4/332>

- Profiling Technique: Microarray Analysis: An online tutorial on pharmacogenomics and personalized medicine. Includes an excellent interactive virtual microarray laboratory activity. <http://learn.genetics.utah.edu/units/pharma/phmicroarray/>

Microarray computer activities:

- DNA Microarray Methodology Flash Animation by Malcolm Campbell that illustrates how microarrays can be used to compare genes expressed in yeast under aerobic and anaerobic conditions <http://www.bio.davidson.edu/courses/genomics/chip/chip.html>
- DNA microarray: A virtual biotechnology laboratory that walks students through a microarray experiment. <http://learn.genetics.utah.edu/units/biotech/microarray>
- Microarray Experiment: Animation of microarray technology from Rediscovering Biology. <http://learner.org/channel/courses/biology/units/genom/images.html>
- Microarrays: Part 1 Chipping Away at the Mysteries of Science and Medicine and Part 2 The Promise of Microarrays in Environmental Medicine A webquest on microarray technology. http://www.fastol.com/~renkwitz/microarray_chips.htm
- HHMI Scanning Life's Matrix Biointeractives Analyzing Microarray Data at <http://hhmi.org/biointeractive/genomics/genechipdata/index.html> and Life Matrix Video lecture at <http://hhmi.org/biointeractive/genomics/genechip.mov>

Note: Teacher answer keys can be found at the end of this document.

Teacher Instructions - Part 1 Gene Expression and Cancer

Before class:

1. Download the Microarray PowerPoint Presentation. Review Part 1 of this PowerPoint presentation.
2. Copy pages 4-7 of *Your World: Fighting Cancer with Biotechnology* available at http://biotechinstitute.org/resources/pdf/yw11_1.pdf
3. For supplementary teacher resources see transparencies available at http://biotechinstitute.org/resources/pdf/yw11_1_oh.pdf and a teacher's guide at http://biotechinstitute.org/resources/pdf/yw11_1_tg.pdf

During class:

1. Distribute one copy of pages of 4-7 of *Your World: Fighting Cancer with Biotechnology* (http://biotechinstitute.org/resources/pdf/yw11_1.pdf) to each student.
2. Use "Part 1: Gene Expression and Cancer" of the Microarray PowerPoint presentation to explain the concept of gene expression and relate cancer to changes in gene expression.
3. Use the PowerPoint presentation samples at the end of "Part 1: Gene Expression and Cancer" to model (for the whole class) how to do the predictions **for the first two A genes**.
4. Allow time for students to work in pairs to do the predictions **for the last two A genes**. Then use the PowerPoint presentation samples to have students check their work.
5. Assign the completion of the chart for the B and C genes and the questions as individual (or group) work. You may wish to refer students to the reading on pages 4-7 of *Your World: Fighting Cancer with Biotechnology*.
6. Allow time in the following class for class or small group discussion of students' answers.

Part 1: Gene Expression and Cancer

For the purposes of this activity, the term “Gene Expression” refers to the cellular processes by which a gene is transcribed and translated to produce a functional protein.

All the cells in an organism have identical genetic instructions, but different genes are expressed (turned on) in different cells under different conditions. When a gene is expressed, the genetic instructions coded in its DNA are transcribed (copied) to make messenger RNA (mRNA) molecules; then the coded instructions in messenger RNA are translated by the ribosome to make a specific protein.

Cells become differentiated (specialized) because different genes are either expressed (turned on) or not expressed (turned off). The different proteins resulting from different genes being expressed give cells their different structures and functions.

Many diseases are caused by mutations in genes. In cancers, these mutations may result in genes being expressed too much, too little, or not at all. Scientists believe that the uncontrolled cell division that leads to cancer is a result of changes in the expression of genes whose proteins normally regulate the cell cycle of growth and cell division.

By studying which genes are expressed differently in cancer cells compared to normal cells, and learning what these genes do, scientists hope to understand the gene changes that lead to cancer. They hope to use this understanding to develop ways to treat or prevent cancer.

Understanding Genes Involved In Colon Cancer: A Hypothetical Study

A researcher wants to compare the expression of genes in cancerous colon cells with the expression of the same genes in normal colon cells. She hopes that an understanding of gene expression differences can be applied, in the future, to selection of chemotherapy drugs that would be more effective in curing colon cancer.

Table 1 lists some genes that scientists have found to be abnormally expressed in other kinds of cancer cells. The researcher has selected these genes because she wants to determine if any of them are abnormally expressed in cancerous colon cells.

Your teacher will model how to do the activity by demonstrating how to complete the table for the first two "A" genes.

1. Work with a partner. For each of the last two "A" genes found in column 1 of Table 1:
 - Read the information about the gene's function in column 2 and in the reading provided by your teacher.
 - Use this information to predict whether the gene will be more or less expressed in cancer cells than in normal cells. Write your prediction in column 3.
 - Briefly explain the reasons for your prediction in column 4. Your teacher may ask you to explain these reasons to your classmates.
2. Work individually to complete the chart for the "B" and "C" genes.
3. The researcher plans to use gene C3 as a positive control gene. Explain why gene C3 is an appropriate positive control.
4. The researcher plans use gene C4 as a negative control gene. Explain why gene C4 is an appropriate negative control.
5. All human body cells express gene C3. Explain why you would expect gene C3 to be expressed in all body cells.
6. All cells contain gene C4. This gene is not expressed in colon cells. Explain why the C4 gene is not expressed in colon cells.
7. Abnormal expression of some genes may lead to an increased mutation rate in other genes. From the genes listed on the chart, choose two genes that, if mutated, might lead to additional mutations. Support your choices with information from the chart.

Table 1: Predicting Gene Expression in Genes Involved in Cancer

1	2	3	4
Gene Location	Gene Function	Prediction: Will this gene be More or, Less , expressed in cancer cells?	Explanation for Prediction
A1	An oncogene that produces a protein in an accelerator signal pathway		
A2	A tumor suppressor gene that produces a protein in a brake signal pathway		
A3	A guardian gene that produces p53 protein that inspects for DNA damage, calls in repair enzymes and triggers apoptosis (cell death) if DNA damage cannot be repaired		
A4	A gene that produces telomerase, an enzyme that rebuilds chromosome ends resulting in cells that can divide indefinitely		
B1	A gene that produces DNA repair enzymes that corrects mutations when they occur		
B2	A gene that produces an angiogenesis factor, a protein that triggers the growth of blood vessels to tumors		

B3	A gene that produces a cell membrane protein that links cells to their neighbors and prevents metastasis (the spread of cancer)		
B4	A gene that produces a cell surface receptor protein for growth signals		
C1	A gene that produces cell surface receptor protein for brake signals		
C2	A gene that produces a protein that promotes invasion and metastasis by allowing cells to move to other parts of the body		
C3	A gene that produces a protein that converts ADP to ATP	Expressed the same in cancer and normal cells	This gene is expressed in all cells because all cells need ATP as a source of energy for their life activities
C4	A gene that produces hemoglobin, an oxygen carrying protein in red blood cells	Not produced by colon cells	This gene is expressed in red blood cells but not in other cells of the body.

Teacher Instructions - Part 2

DNA Microarray Technology

Before class:

1. Download and review the Microarray PowerPoint presentation.
2. Print one copy of “Part 2: DNA Microarray Technology” for each student.
3. Consider providing one color copy of the *Putting together a Microarray* review sheet (see Appendix A) for students to refer to as they complete each step of this activity. If put in sheet protectors and collected at the end of class, these may be used for many classes. Consider providing one black and white copy of the *Putting together a Microarray* review sheet for each student.
4. Print ONE copy of Appendix B that includes pieces for the microarray class simulation.
5. Make a transparency of the microarray chart from Appendix B. For each class (32 students or less), make one copy of the mRNA strips from normal cells (see Appendix B), cut these into strips, and place strips in a large envelope labeled “mRNA from Normal Cells.” You will need enough “normal cell” mRNA strips so that each student in one half of your class receives at least one mRNA strip.
6. For each class (32 students or less), make one copy of the mRNA strips from cancer cells (see Appendix B), cut these into strips, and place strips in a large envelope labeled “mRNA from Cancer Cells.” You will need enough “cancer cell” mRNA strips so that each student in one half of your class receives at least one mRNA strip.
7. For each student on the “normal cell” side of the room prepare a small plastic bag that contains **green stickers**, scissors, tape, a pencil (label the pencil “Reverse Transcriptase Enzyme”) and colored pencils (red, green, yellow, and black).
8. For each student on the “cancerous cell” side of the room prepare a small plastic bag that contains **red stickers**, scissors, tape, a pencil (label the pencil “Reverse Transcriptase Enzyme”) and colored pencils (red, green, yellow, and black).
9. Prepare one box or bucket labeled “WASH bucket” and on box or bucket labeled “HYBRIDIZATION SOLUTION”.
10. Obtain access to a computer and projector for showing the “Microarray” PowerPoint.
11. Copy or download the PowerPoint file (DNA Microarrays). Part 1 illustrates and explains gene expression. Part 2 illustrates how microarrays can be used to study gene expression. Part 2 also provides instructions for creating the class microarray model.

During class:

1. If possible, show students a GeneChip microarray sample (may be ordered from outreach@affymetrix.com). Explain that this chip contains thousands of different gene sequences and can be used to study the expression of thousands of different genes.
2. Explain that today they are going to work as a class to do a class simulation to show how microarray technology works. Instead of using a real microarray with thousands of microscopic gene fragments, they will be using the paper microarray with 12 gene fragments posted in the classroom.
3. Hand the “mRNA from Normal Colon Cell” envelope to a student on one side of the room.

4. Hand the “mRNA from Cancerous Colon Cell” envelope to a student on the other side of the room.
5. Distribute to each student on the “normal cell” side of the room, a small plastic bag that contains green stickers, scissors, tape, and a pencil.
6. Distribute to each student on the “cancerous cell” side of the room, a small plastic bag that contains red stickers, scissors, tape, and a pencil.
7. Distribute one copy of “Part 2: DNA Microarray Technology” to each student.
8. Distribute, if available, one color copy of the “Putting together a Microarray” review sheet for each student. May be used for multiple classes if collected at the end of class.
9. Distribute one black and white copy of the “Putting together a Microarray” review sheet for each student.
10. Show the “Microarrays (DNA Chips)” PowerPoint. This PowerPoint provides information on microarray technology (in the left column) and provides instructions for students to complete the microarray simulation (in the right column).
11. HINT: It is important that ALL of the mRNA strips be attached to the microarray chart. After each student gets one mRNA strip, distribute any remaining strips to students who will be responsible for taping more than one strip to the microarray chart.
12. HINT for large classes: If you have a large class, ask students to look at their cDNA strips and find the complementary circle on the microarray wall chart before they move to the chart. You may want to have students take turns (one or two at a time) or call students to the microarray chart by rows.

Part 2: DNA Microarray Technology

There is a new technology, called a **DNA micorarry (chip)**, that allows scientists to efficiently measure the expression of thousands of genes at the same time by looking at the amount of RNA that is transcribed from those genes. A DNA microarray is a plastic chip or glass slide that has been "printed" (spotted at precise locations) with thousands of short, single-stranded pieces of DNA for known genes.



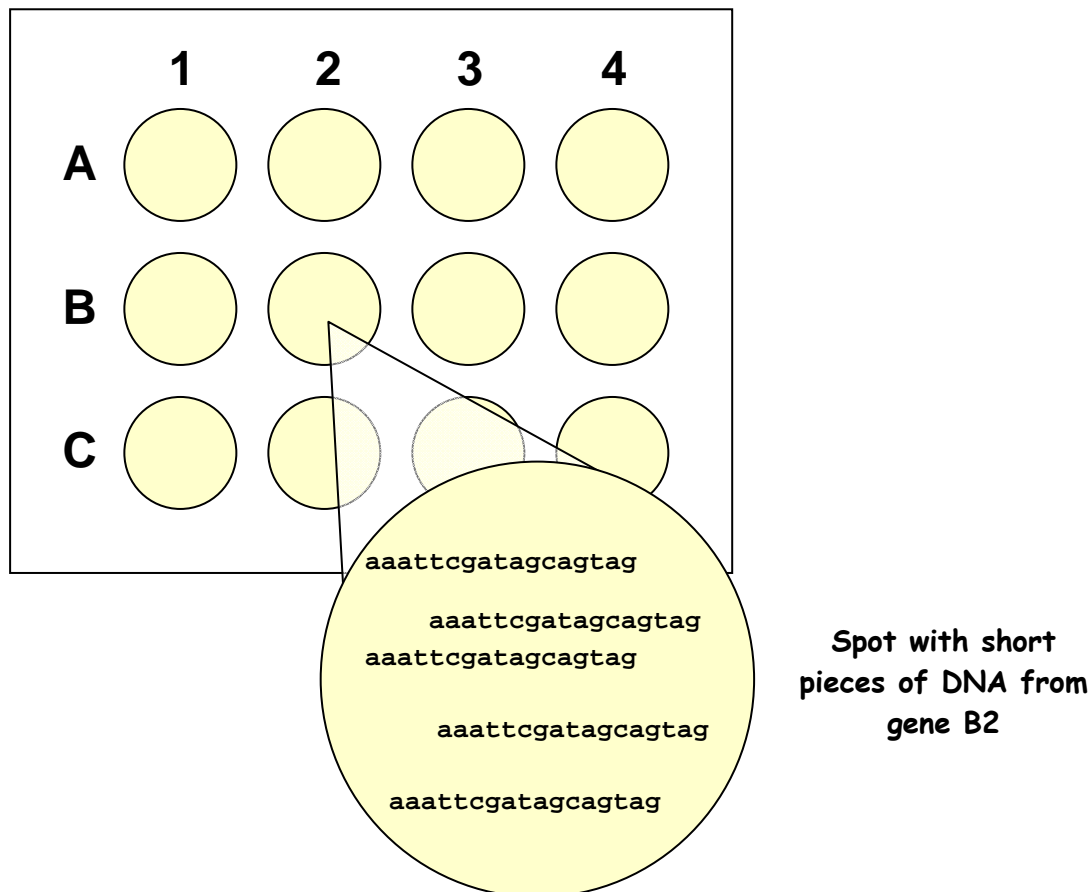
A researcher wants to study how changes in gene expression might lead to cancer (uncontrolled cell division). She plans to use DNA microarray (chip) technology to compare the messenger RNA produced in cancerous colon cells with the messenger RNA produced in normal colon cells. The researcher has access to both normal cells and cancerous cells from a patient who has colon cancer. She will use a DNA Microarray (Chip) to determine which genes might be:

- Cancer preventing genes because they are less expressed in cancerous colon cells than in normal colon cells, or
- Cancer causing genes because they are more expressed in cancerous colon cells than in normal colon cells.

To help you understand how DNA microarray technology works, your class will use a paper simulation of the researcher's experiment. In this activity, the class will simulate the steps in a DNA microarray experiment to compare the expression of genes in cancerous colon cells with the expression of genes in normal colon cells.

STEP 1: Print or purchase a preprinted DNA microarray	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Prepare DNA microarrays by selecting and "printing" known sequences of DNA (approximately 20-70 bases long) that represent specific genes from an organism. Because "printing" a chip is a very time-consuming process, most scientists order pre-printed microarrays from companies that automate the microarray printing process.	<ul style="list-style-type: none"> <i>Figure 1 and the chart posted on the board in the classroom show a simplified, simulated microarray with DNA base sequences from twelve different genes attached to the spots.</i> <i>Most of these genes were selected because scientists suspect that these genes might be involved in causing colon cancer.</i>

Figure 1. Microarray with DNA Sequences from Known Genes



Step 2: Collect cancerous colon cells and normal colon cells from a patient.	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Identify genes involved in colon cancer, by comparing cancerous colon cells with normal (control) colon cells from the same patient.	<ul style="list-style-type: none"> • <i>One side of the room will be given an envelope representing cancerous colon cells.</i> • <i>The other side of the room will be given an envelope with paper strips representing normal colon cells.</i>

STEP 3: Isolate mRNA from two types of cells	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Isolate the messenger RNA from normal cells and from cancer cells. Remember that when a gene is expressed in a cell, the DNA is transcribed (copied) to make messenger RNA molecules.	<ul style="list-style-type: none"> • <i>The paper strips inside the envelope represent messenger RNA molecules.</i> • <i>Distribute one messenger RNA molecule to each student on your side of the room.</i>

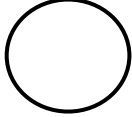
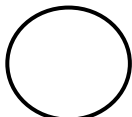
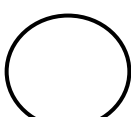
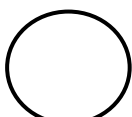
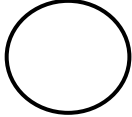
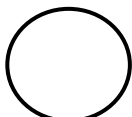
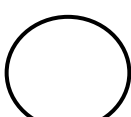
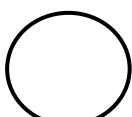
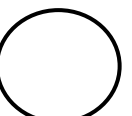
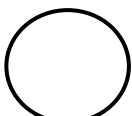
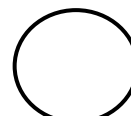
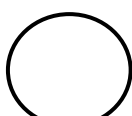
STEP 4: Use reverse transcriptase to synthesize cDNA from mRNA	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Treat the messenger RNA from both cell types with reverse transcriptase, an enzyme that copies the base sequence on mRNA molecules to make complementary, single-stranded DNA known as cDNA (complementary DNA) molecules.	<ul style="list-style-type: none"> • <i>Act like a reverse transcriptase enzyme. Look at the mRNA base sequence on the top half of your mRNA strip. Use the pencil to "copy" the <u>complementary</u> cDNA base sequence on the bottom half of the strip. (Hint: Use T not U)</i> • <i>Use scissors to separate the cDNA part from the mRNA part. Save the cDNA base strip and discard the mRNA base strip.</i>

STEP 5: Label the cDNA's	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Label the cDNA's from the two kinds of cells with different colors of fluorescent dye. For example, scientists may label the cDNA from cancer cells with red dye and the cDNA from normal cells with green dye.	<ul style="list-style-type: none"> • <i>Label the cDNA strips by attaching: A red sticker to the cDNA from cancerous cells A green sticker to the cDNA from normal cells.</i> • <i>NOTE: Place the sticker on the end of the strip that is labeled cDNA.</i>
STEP 6: Mix the labeled cDNA's together	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Mix together the labeled cDNAs from the normal and cancer cells in a single test tube. This mixture is called a "hybridization solution."	<ul style="list-style-type: none"> • <i>Place all of the labeled cDNA base strips into the "hybridization solution" bucket in the center of the room.</i>
STEP 7: Hybridize the microarray with labeled cDNA's.	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Soak the microarray in the hybridization solution (mixture of labeled cDNAs). The cDNAs that are complementary to the DNA (gene) sequences on the microarray spots will hybridize (bind) to the spots.	<ul style="list-style-type: none"> • <i>Randomly select one cDNA strip from the bucket and move to the microarray posted on the board.</i> • <i>Tape (hybridize) your cDNA strip to a complementary base sequence on the microarray.</i> • <i>If your cDNA strip is NOT complementary to any of base sequences on the microarray, remain near the microarray and hold onto your cDNA strip until the next step.</i>

STEP 8: Remove unbound (unhybridized) cDNA's	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Wash the microarray to remove the cDNA strips that are not hybridized to gene spots.	<ul style="list-style-type: none"> • <i>Put unattached (unhybridized) cDNAs into the "wash" bucket.</i>

STEP 9: Scan to make the the microarray results visible	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Scan the microarray with two laser lights that cause the fluorescent labels on the cDNA to emit colored light. Scanning with one laser causes the cDNA from cancer cells to emit red light. Scanning with a second laser causes the cDNA from normal cells to emit green light.	<ul style="list-style-type: none"> • <i>Look at the class microarray and count the number of red cDNA strips taped to each spot. Use a <u>red</u> pencil to record these numbers in the corresponding spots on Figure 2.</i> • <i>Repeat for green cDNA strips. Use a <u>green</u> pencil to record these numbers.</i>

Figure 2. DNA Microarray Results

	1	2	3	4
A				
B				
C				

Remember: the cDNA's from cancerous cells have a red label and the cDNAs from normal cells have a green label.

- *On the line below, write the symbols (letters and numbers on the microarray) for the gene spots on the microarray that are expressed in cancerous colon cells.*
-

- *On the line below, write the symbols (letters and numbers on the microarray) for the gene spots on the microarray that are expressed in normal colon cells.*
-

Step 10: Computer Merging of Images	
Scientists doing a microarray:	<i>Students doing this simulation:</i>
Use a computer to merge the red and green scanned images. If both red and green labeled cDNA are bound to the same spot, this results in a YELLOW spot. If no cDNAs are bound to a spot, this will result in a black spot.	<ul style="list-style-type: none"> • Use a green pencil to color in the microarray spots that have <u>only green cDNA</u>. • Use a red pencil to color in the microarray spots that have <u>only red cDNA</u>. • Use a yellow pencil to color in the microarray spots that would appear yellow because they have <u>both red and green cDNA</u>. • Use a black pencil to color in the microarray spots that do not have any cDNA attached to them.

Step 11: Analyze the results of the gene expression study

The microarray color pattern is analyzed to identify which genes are expressed differently in the two types of cells and which genes might be involved in causing or preventing colon cancer.

- *A **green** spot means that the gene is not expressed in cancer cells but is expressed in normal cells. **This gene may be involved in preventing colon cancer***
- *A **red** spot means that the gene is expressed in cancer cells but not expressed in normal cells. **This gene may be involved in causing colon cancer.***
- *A **yellow** spot mean that the gene is expressed in both normal and cancer cells. **This gene is probably not involved in causing colon cancer.***
- *A **black** spot means that the gene is not expressed in either type of cell. **This gene is probably not involved in causing colon cancer.***
- *Answer questions 1 through 10. To identify genes, write the gene location on the microarray (e.g., A-1, B-3, C-2).*

Questions:

1. Which genes are expressed in cancerous colon cells but not expressed in normal cells?

2. Which genes are not expressed in cancerous colon cells but are expressed in normal colon cells? _____
3. Which genes are expressed in both cancerous and normal colon cells? _____
4. Which genes are expressed in neither cancerous nor normal colon cells? _____

5. Which genes might be cancer **causing** genes? What evidence do you have to support this? _____

6. Which genes might be cancer **preventing** genes? What evidence do you have to support this? _____

7. Explain why it is unlikely that genes with yellow microarray spots are associated with causing cancer.

8. Refer to the chart in **Part 1: Genes and Cancer** activity and to the results of **Part 2: DNA Microarray Technology**. What kinds of proteins would you hypothesize would be present in the cancerous colon cells and not present in normal colon cells?

9. Refer to the chart in **Part 1: Genes and Cancer** activity and to the results of **Part 2: DNA Microarray Technology**. What kinds of proteins would you hypothesize would be present in the normal colon cells and not present in cancerous colon cells?

10. Some gene spots on a microarray may have a yellowish-green color. What do you think a yellow-green spot on a microarray indicates about the gene expression of these genes?

Teacher Instructions - Part 3
Apply Your Understanding of Microarray Technology

Before class:

- Print one copy of *Part 3: Apply Your Understanding of Microarray Technology* for each student.

During class:

- Distribute one copy of *Part 3: Apply Your Understanding of Microarray Technology* to each student. Students work individually to read and answer questions for homework.

Part 3: Apply Your Understanding of Microarray Technology

1. What is a DNA microarray (DNA chip)?

2. Create an analogy that could be used to explain the structure and function of a microarray. For example: A DNA microarray is like a _____ because _____

3. Explain what causes the cDNA to bind to the gene spots on the microarray?

Base your answers to questions 4 and 5 on the information below.

A researcher studying tumor cell function wants to study how treatment with a cancer drug affects expression of the gene for a DNA repair enzyme. He knows that the mRNA for the enzyme contains the base sequence

AAU AGG UAC ACG

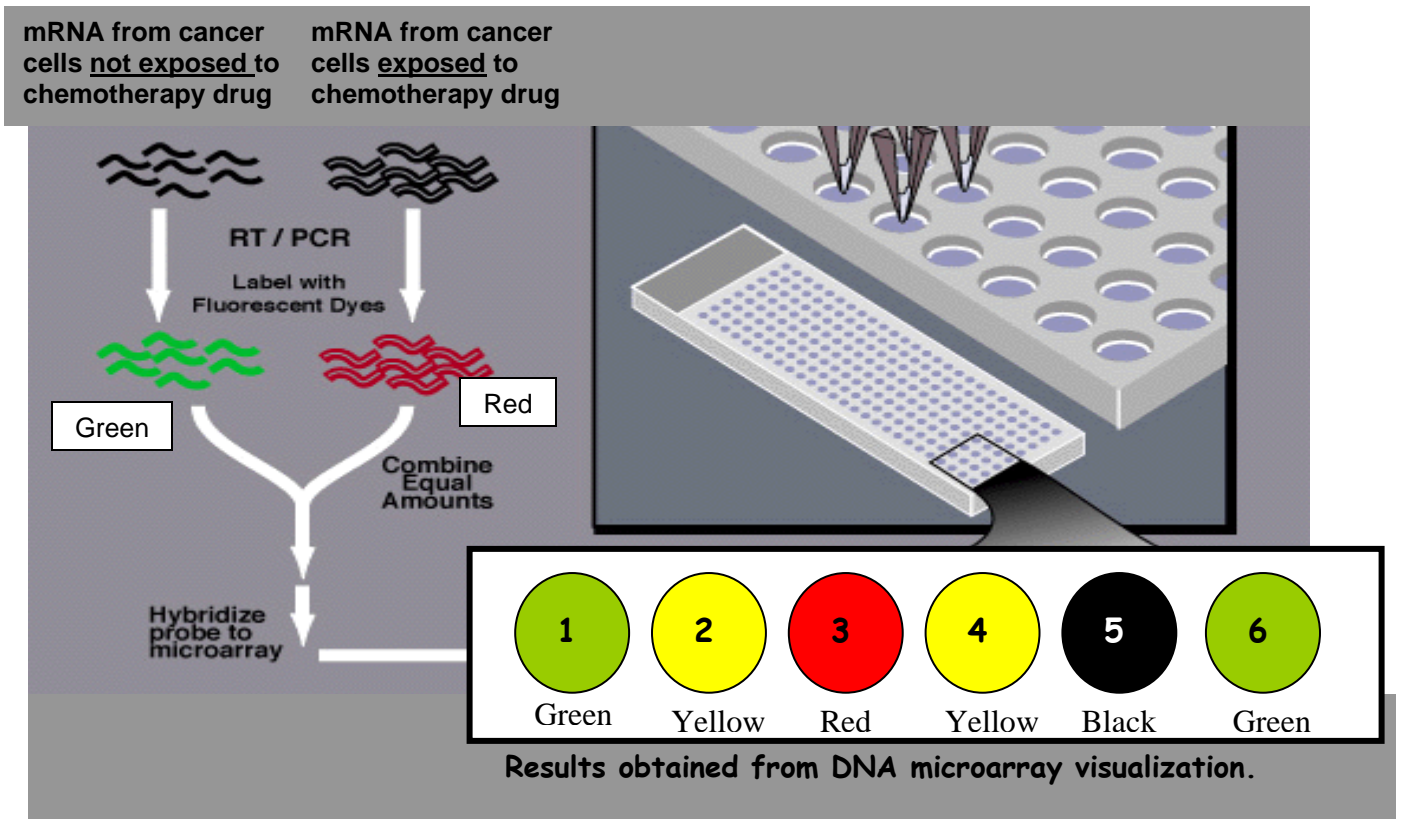
4. What sequence of cDNA bases would result if the researcher mixed this mRNA with reverse transcriptase? _____

5. What gene sequence on the DNA chip would hybridize with (bind to) this cDNA?

Base your answers to question 6-9 on the information below.

A scientist used DNA microarray analysis to study how a potential chemotherapy drug affected gene expression in cancer cells. During his experiment, the scientist isolated RNA from two kinds of cancer cells: one type was grown in a normal culture medium and the other type was grown in the same culture medium that also contained the chemotherapy drug. He used the messenger RNA from the cancer cells that were not exposed to the chemotherapy drug to make green labeled cDNA. He used the RNA from the cancer cells that were exposed to the chemotherapy drug to make red labeled cDNA.

Figure 1.



Modified from: http://www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/Images/microarray_technology.gif

The scientist mixed both kinds of cDNA together to produce a hybridization solution that he applied to a microarray that was printed with six different gene sequences. Then he washed the microarray to remove unbound cDNA and scanned the microarray to visualize the colors of the spots. Figure 1 shows the color of the spots on the DNA microarray.

Based on the results of his experiment, which gene or genes appear to be:

6. Expressed only in cells exposed to the chemotherapy drug? _____
7. Expressed only in cells not exposed to the chemotherapy drug? _____
8. Expressed in both types of cells? _____
9. Not expressed in either type of cells? _____

Teacher Instructions - Part 4 Personalized Medicine

Before class:

1. Prepare one copy of *Part 4: Personalized Medicine* for each student.
2. Arrange for computer access or prepare copies of the information found at each of the URL's listed in the student handout. Prepare one set of articles for each team of four students. Resources available at:
 - Genetic tests get approval: An article on FDA approval for MammaPrints, a new microarray test to determine if breast cancer patients have a high or low risk of cancer reoccurrence <http://www.bioedonline.org/news/news-print.cfm?art=3131>
 - Agendia About MammaPrints: Information about the Mammaprint test provided by the company that produced and sells the test http://www.agendia.com/index.php?option=com_content&task=view&id=15&Itemid=137 . Follow the links at the left side of the screen.
 - FDA News February 6, 2007: FDA Clears Breast Cancer Specific Molecular Prognostic Test: An FDA statement regarding the approval for MammaPrints, as a test to determine if breast cancer patients have a high or low risk of cancer reoccurrence <http://www.fda.gov/bbs/topics/NEWS/2007/NEW01555.html>
 - Personalized Medicine: A new approach to staying well by Francis S. Collins published in the Boston Globe, July 17, 2005: An article by Francis Collins about the ethical, legal, and social issues associated with personalized medicine. http://www.boston.com/news/globe/editorial_opinion/oped/articles/2005/07/17/personalized_medicine?mode=PF

During class:

1. Distribute one copy of *Part 4: Personalized Medicine* to each student.
2. Write the phrase "Personalized Medicine" on the board and ask students to brainstorm (as a class or in small groups) what they think of when they hear that phrase.
3. Explain that today they will learn about one case that involves the use of personalized medicine.
4. Ask students to listen and follow along as two students in the class read the case study scenario aloud. They should underline the facts of the case.
5. Ask students to work individually to write one question that they have about the case.
6. Work as a class to list some facts and questions about the case on the board.
7. Form teams of 4 students.
8. Ask students to turn the Part 4 handout over and read the instructions.

9. Assign each student to read ONE of the articles. Each member of the team should read a different article. You may provide them with print copies or refer them to the appropriate website.
10. Explain that they should be prepared to share the answers to the questions with their team members during the next class.
11. The next day, students meet with their team members to share information and prepare team answers to the questions.

Part 4: Microarrays and Personalized Medicine

Kate: What's the matter Bob? You look upset...

Bob: Oh.... Hi Kate...Yes, I am kind of upset right now. My mom was diagnosed with stage 2 breast cancer. She's got to have surgery. I'm really worried about her.

Kate: That's terrible, I'm sorry. How's your family doing?

Bob: Mom's upbeat about it, but she and Dad were talking about whether or not she should have chemotherapy after her surgery. If she has chemo, she might get really sick and lose her hair. But what's worse is that she can't be sure the chemo will even work.

Kate: Sounds like your Mom is facing a really scary decision.

Bob: I went online to look up stuff about chemo, just to feel like I was doing something. I found something about a personalized medicine test called a *MammaPrint*. It's a microarray test.

Kate: Is that like the microarray we learned about in biology class?

Bob: Yeah, scientists use a microarray to see what genes are expressed in cancer cells. The test results might help the doctor decide whether Mom needs to get chemo at all.

Kate: Is your Mom going to get a *MammaPrint*?

Bob: I'm going to tell her about it, but wouldn't you want a *MammaPrint* if you had breast cancer?

Kate: I don't know...How accurate is this *MammaPrint* test? I mean, what if the test said you were OK, but you really weren't? That might mean that you didn't get the right treatment.

Bob: I didn't think of that...but the *MammaPrint* is FDA approved, so that must mean that it works, right?

Work individually to write one question that you have about this case.

Directions:

Bob found 4 articles about MammaPrints at these Internet sites.

- Genetic tests get approval: <http://www.bioedonline.org/news/news-print.cfm?art=3131>
- Agendia About MammaPrints:
http://www.agendia.com/index.php?option=com_content&task=view&id=15&Itemid=137
. Follow the links at the left side of the screen.
- FDA News February 6, 2007: FDA Clears Breast Cancer Specific Molecular Prognostic Test: <http://www.fda.gov/bbs/topics/NEWS/2007/NEW01555.html>
- Personalized Medicine: A new approach to staying well by Francis S. Collins published in the Boston Globe, July 17, 2005:
http://www.boston.com/news/globe/editorial_opinion/oped/articles/2005/07/17/personalized_medicine?mode=PF

Read the ONE article assigned to you. Use the information from your reading to answer questions 1-5. Tomorrow in class you will share your answers to the questions with your team members who have read the other three articles.

Questions:

1. What does it mean to have a cancer "Recurrence"?
2. What is meant by the phrase "personalized medicine"?
3. Why is using a *MammaPrint* considered a form of personalized medicine?
4. State two (2) benefits of using *MammaPrint* technology.
5. State two (2) risks/concerns that patients or doctors might have about the use of *MammaPrint* technology.
6. If a MammaPrint indicates that a patient has a low risk of recurrence, why might that patient choose to have chemotherapy treatments?

Teacher Instructions - Part 5 Microarrays and Personalized Medicine

Before Class:

1. Prepare 1 copy of Part 5: Microarrays and Personalized Medicine for each student.
2. Prepare the following solutions:
 - 0.04% Methyl red solution:
0.04 grams methyl red + 100 mL distilled water. Add drops of household ammonia (approximately 1-4 drops) until this solution just turns yellowish.
 - 0.02% Bromothymol blue solution:
0.02 grams bromothymol blue + 2 mL yellow food coloring (McCormicks) + 100 mL distilled water. Add drops of white vinegar (approximately 1-3 drops) and stir until this solution just turns yellowish.
 - Yellow solution:
20 drops yellow food coloring (McCormicks) + 2 drops red food coloring (McCormicks) + 100 mL distilled water.
 - pH 5 buffer:
Purchase prepared solutions or make using pH 5 buffer capsule. White vinegar could be used instead. You just need a solution with a pH of 5 or below.
 - pH 8 buffer:
Purchase prepared solutions or make using pH 8 buffer capsule. A 1% solution of baking soda or household ammonia could be used instead. You just need a solution with a pH of 8 or higher.
3. For each team of three students, prepare a microcentrifuge tube rack with one set of the following tubes. Fill each microcentrifuge tube with approximately 1 mL of the appropriate solution.

Label	Solutions in tubes (1mL)
cDNA Patient A	pH 5 buffer
cDNA Patient B	Distilled water
cDNA Patient C	pH 8 buffer
Gene 1	Methyl red solution
Gene 2	Bromothymol blue solution
Gene 3	Methyl red solution
Gene 4	Bromothymol blue solution
Gene 5	Methyl red solution
Gene 6	Yellow solution

Hint: To avoid the need for sharing the tubes containing genes 1-6 you may want to consider providing 3 sets of the tubes containing genes 1-6 for each team.

4. Reduce the number of droppers needed by providing each team with a set of labeled droppers. If droppers are labeled, provide 9 plastic thin stem (Beral) droppers per team. Cut the dropper stems so that only 1 inch of dropper stem remains. Label to match the labels on the microcentrifuge tubes. If droppers are not labeled, each student will need 7 plastic thin stem (Beral) droppers. Cut the dropper stems so that only 1-2 inches of dropper stem remains attached to the bulb.
5. Prepare one plastic microarray slide for each student. See the Microarray Pieces file for an original that can be photocopied, not printed, onto heavy duty transparency sheets and cut into microarray slides. Each member of a team should have a different microarray slide (A, B, or C).
6. Provide for each student:
 - One plastic microarray slide (see separate “Microarray” Pieces file)
 - Safety goggles
 - White paper
 - Colored pencils (red, green, yellow, black)

During Class:

1. Form teams of three students.
2. Distribute to each team:
 - One set of 9 microcentrifuge tubes containing patient samples and genes for spotting on microarrays.
 - 9 Labeled droppers
 - One set of 3 microarray slides (A-C)
3. Distribute to each student:
 - One copy of Part 5: Microarrays and Personalized Medicine
 - Safety goggles
 - White paper
 - Colored pencils (red, green, yellow)
4. Read the first two paragraphs of Part 5 aloud. Then ask students to follow the laboratory instructions.

Part 5: Microarrays and Personalized Medicine: A Simulation

Today your class will conduct a simulated microarray analysis to determine whether several breast cancer patients have a high or a low risk for recurrence of breast cancer. Your team will use a microarray chip to test samples from one of these breast cancer patients. The microarray test results will be analyzed to determine which patients are most likely to be in a high risk group.

Because microarray technology is expensive and requires complex procedures and equipment, we will use a simulation. This simulation will be different from an actual microarray experiment because you will study only six genes instead of 70 genes.

Safety: Wear safety goggles during this laboratory activity. Avoid contact with the solutions used in this laboratory activity.

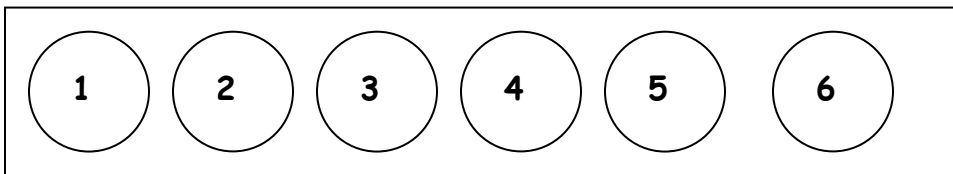
Directions:

1. Prepare (print) a DNA microarray by spotting six different gene sequences onto the paper microarray chip.
 - There are six tubes containing solutions of gene (DNA) sequences from different genes (1-6) that have been related to cancer recurrence risk. Use a dropper to carefully "spot" one drop of each gene solution onto the appropriately labeled circle on your microarray chip.
2. Three patients (A, B, and C) have requested testing to determine whether they have a high or a low risk of breast cancer recurrence. Each member of your team will test a sample of cDNA hybridization solution from ONE of the breast cancer patients (A, B, or C). The cDNA hybridization solution for each patient contains a mixture of:
 - **Red** labeled cDNA from the tumor of one breast **cancer** patient (Patient A, B, or C).
 - **Green** labeled cDNA from the tumor of a patient whose cancer did not recur. This cDNA is used as a standard control sample.
3. Obtain a tube containing the patient's cDNA hybridization solution that you will be testing. Record the patient identification letter. _____
4. Hybridize your microarray with the patient's cDNA hybridization solution. Use a dropper to put one drop of the patient's cDNA hybridization solution onto each circle on your printed microarray chip.

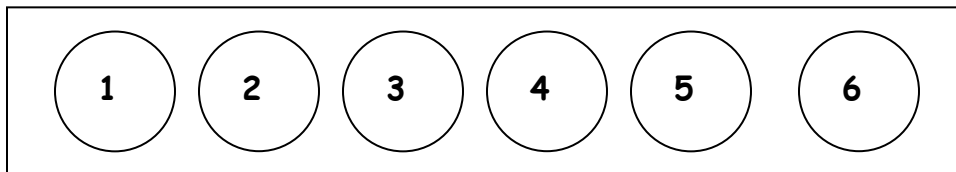
5. Complete Figure 1 by recording the results from your patient and from other patients (tested by your team members) on the diagrams of microarray chips.
- Place your microarray sheet over a sheet of white paper.
 - Record your results on the appropriate microarray for your patient. Use colored pencils to color the circles to represent the colors on your microarray. Also write the name of the color under each circle.
 - Record the results from the other patients tested by your team members.

Figure 1: Results of Gene Expression Microarray Testing

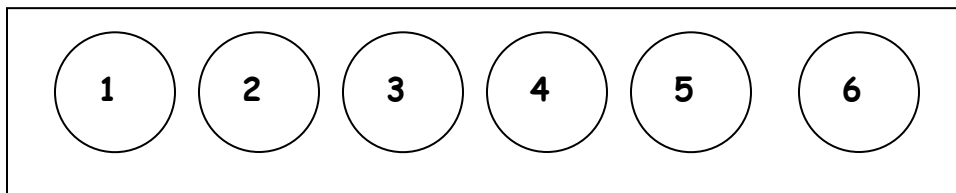
Patient A



Patient B



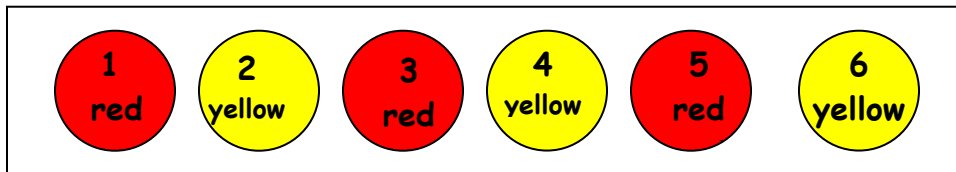
Patient C



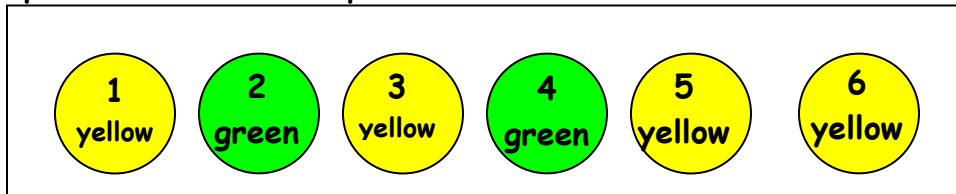
6. Observe Figure 2 that shows the typical microarray patterns for low risk and for high risk cancer patients. Use the information in Figure 1 and Figure 2 to answer questions 7-10.

Figure 2: Gene Expression Microarray Testing

Typical pattern for HIGH risk patients with a risk score of 10.



Typical pattern for LOW risk patients with a risk score of 1



7. Which locations (1-6) represent genes that are **more expressed** in HIGH risk cancer patients than in low risk cancer patients? _____
8. Which locations (1-6) represent genes that are more **expressed** in LOW risk cancer patients than in high risk cancer patients? _____
9. Which location represents a positive control gene used in this test? _____
10. Imagine that you are a doctor who received the micorarray results from Patients A, B, and C. All of these patients have told you they would rather not receive chemotherapy treatments unless these treatments were necessary.
 - a. Which of the patients would you recommend receive chemotherapy? Support your recommendation using the microarray data.
 - b. Which of the patients would you recommend not receive chemotherapy? Support your recommendation using the microarray data.
11. Imagine that you are Patient B. What questions might you want to ask your doctor before deciding whether or not to have chemotherapy treatment?

Teacher Instructions - Part 6 Microarrays and Cancer Diagnosis

Before class:

- Prepare one copy of “Part 6: Microarrays and Cancer Diagnosis for each student. Students work individually to read and answer questions. Important: make single-sided, not back-to-back copies.

During class:

- Distribute one copy of “Part 6: Microarrays and Cancer Diagnosis to each student. Students work individually to read and answer questions for homework.

Part 6: Microarray Technology and Cancer Diagnosis

Two types of leukemia, Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML), have similar symptoms and both affect cells in the bone marrow. However, these cancers respond differently to the same chemotherapy treatments. To choose the right kind of chemotherapy treatments for a patient with acute leukemia, doctors need to know whether a patient has ALL or AML.

Scientists from Harvard University have developed a DNA microarray test that uses gene expression patterns in leukemia cells (isolated from the bone marrow) to distinguish between these two types of leukemia. Table 1 shows the locations of 25 genes (A1-E5) on that DNA chip. These genes have been identified as related to acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). Table 1 indicates whether expression of each gene is associated with ALL or AML.

Table 1: Gene Table

L = Acute Lymphoblastic Leukemia (ALL)

M = Acute Myeloid Leukemia (AML)

GENE	EXPRESSED IN	GENE	EXPRESSED IN	GENE	EXPRESSED IN	GENE	EXPRESSED IN	GENE	EXPRESSED IN
A1	M	B1	L	C1	BOTH	D1	NEITHER	E1	M
A2	L	B2	M	C2	L	D2	M	E2	M
A3	L	B3	L	C3	M	D3	M	E3	L
A4	M	B4	NEITHER	C4	L	D4	NEITHER	E4	L
A5	L	B5	L	C5	M	D5	M	E5	BOTH

The data for three patients were obtained by taking cells from their bone marrow, culturing those cells, and then isolating mRNA from the cells. The mRNA was then copied to make green fluorescent cDNA that was placed on the DNA chip. Expressed genes show up as fluorescent green spots as noted on the diagrams below.

1. Use the DNA microarray data shown below to diagnose patients W and X.

Diagnosis for Patient W: Acute _____ Leukemia

	A	B	C	D	E
1	<input type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
2	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
4	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
5	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>

Diagnosis for Patient X: Acute _____ Leukemia

	A	B	C	D	E
1	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
2	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
3	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
4	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>

2. Why should you be hesitant to give a definitive diagnosis for Patient Y? Be specific!

Patient Y:

	A	B	C	D	E
1	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
2	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
3	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
4	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
5	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>

Teacher answer Keys for DNA Microarrays (Gene Chips) and Cancer

Part 1: Gene Expression and Cancer

8. The researcher plans to use gene C3 as a positive control gene. Explain why gene C3 is an appropriate positive control.

Gene C3 is found in both normal cells and cancerous cells. If the microarray is working properly, C3 should turn yellow to indicate that it is expressed in both types of cells.

9. The researcher plans use gene C4 as a negative control gene. Explain why gene C4 is an appropriate negative control.

Gene C4 is not found in normal colon cells or cancer cells. If the microarray is working properly, C4 should be black.

10. All human body cells express gene C3. Explain why you would expect gene C3 to be expressed in all body cells.

Gene C3 is essential for ATP production. All cells need ATP to provide energy for life activities.

11. All cells contain gene C4. This gene is not expressed in colon cells. Explain why the C4 gene is not expressed in colon cells.

Gene C4 makes hemoglobin. The gene for hemoglobin would only be expressed in red blood cells.

12. Abnormal expression of some genes may lead to an increased mutation rate in other genes. From the genes listed on the chart, choose two genes that, if mutated, might lead to additional mutations. Support your choices with information from the chart.

A3 is a gene that inspects for DNA damage, calls in repair enzymes and triggers apoptosis (cell death) if DNA damage cannot be repaired.

B1 is a gene that produces DNA repair enzymes to correct mutations.

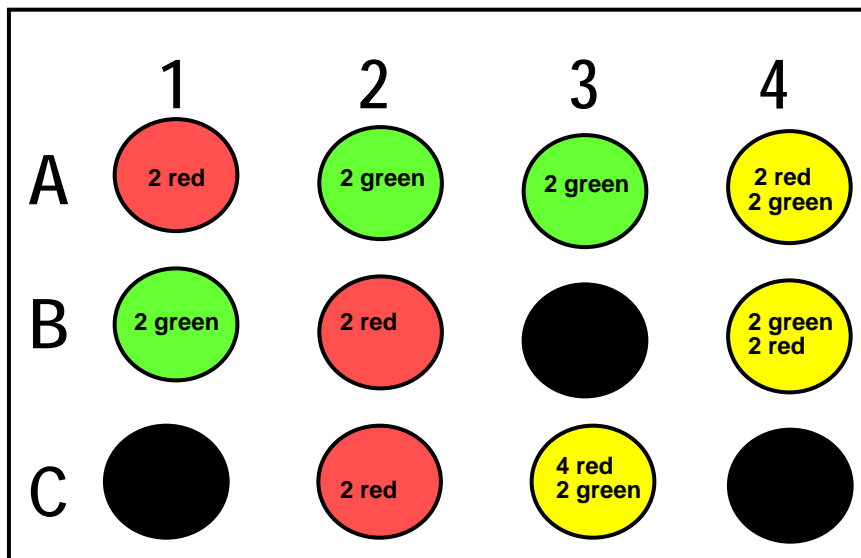
Table 1: Predicting Gene Expression in Genes Involved in Cancer

1	2	3	4
Gene Location	Gene Function	Prediction: Will this gene be More or, Less , expressed in cancer cells?	Explanation for Prediction
A1	An oncogene that produces a protein in an accelerator signal pathway	More	More of this protein could speed up cell division.
A2	A tumor suppressor gene that produces a protein in a brake signal pathway	Less	Less of this protein could mean that cell division is not slowed down.
A3	A guardian gene that produces p53 protein that inspects for DNA damage, calls in repair enzymes and triggers apoptosis (cell death) if DNA damage cannot be repaired	Less	Less of this protein could result in mutations that could lead to cancer.
A4	A gene that produces telomerase, an enzyme that rebuilds chromosome ends resulting in cells that can divide indefinitely	More	More of this protein could make cells continue to divide.
B1	A gene that produces DNA repair enzymes that corrects mutations when they occur	Less	Less of this protein could result in mutations that could lead to cancer.
B2	A gene that produces an angiogenesis factor, a protein that triggers the growth of blood vessels to tumors	More	More of this protein could make tumors grow more rapidly.

B3	A gene that produces a cell membrane protein that links cells to their neighbors and prevents metastasis (the spread of cancer)	Less	Less of this protein could cause cells to metastasize
B4	A gene that produces a cell surface receptor protein for growth signals	More	More of this protein could speed up cell division.
C1	A gene that produces cell surface receptor protein for brake signals	Less	Less of this protein could speed up cell division.
C2	A gene that produces a protein that promotes invasion and metastasis by allowing cells to move to other parts of the body	More	Less of this protein could cause cells to metastasize
C3	A gene that produces a protein that converts ADP to ATP	Expressed the same in cancer and normal cells	This gene is expressed in all cells because all cells need ATP as a source of energy for their life activities
C4	A gene that produces hemoglobin, an oxygen carrying protein in red blood cells	Not produced by colon cells	This gene is expressed in red blood cells but not in other cells of the body.

Part 2: DNA Microarray Technology

Figure 2. DNA Microarray Results



11. Which genes are expressed in cancerous colon cells but not expressed in normal cells?
A1, B2, C2
12. Which genes are not expressed in cancerous colon cells but are expressed in normal colon cells? **A2, A3, B1**
13. Which genes are expressed in both cancerous and normal colon cells? **A4, B4, C3**
14. Which genes are expressed in neither cancerous nor normal colon cells? **B3, C1, C4**
15. Which genes might be cancer **causing** genes? What evidence do you have to support this? **The red ones (A1, B2, C2) because these genes are expressed in the cancer cells and not the normal cells.**
16. Which genes might be cancer **preventing** genes? What evidence do you have to support this? **The green ones (A2, A3, B1) because these genes are expressed in the normal cells and not in the cancer cells.**
17. Explain why it is unlikely that genes with yellow microarray spots are associated with causing cancer. **They are found in both normal and cancer cells.**

18. Refer to the chart in **Part 1: Genes and Cancer** activity and to the results of **Part 2: DNA Microarray Technology**. What kinds of proteins would you hypothesize would be present in the cancerous colon cells and not present in normal colon cells?

A1 - a protein the growth signal pathway

B2 - angiogenesis factor, a protein that causes blood vessel growth

C2 - a protein that promotes invasion and metastasis

19. Refer to the chart in **Part 1: Genes and Cancer** activity and to the results of **Part 2: DNA Microarray Technology**. What kinds of proteins would you hypothesize would be present in the normal colon cells and not present in cancerous colon cells?

A2 - a protein in a brake signal pathway

A3 - p53 protein that inspects for DNA damage, calls in repair enzymes and triggers apoptosis (cell death) if DNA damage cannot be repaired

B1 - a gene that produces DNA repair enzymes that corrects mutations when they occur

20. Some gene spots on a microarray may have a yellowish-green color. What do you think a yellow-green spot on a microarray indicates about the gene expression of these genes?

The gene is expressed in both normal and cancerous cells but it is expressed more in normal cells than in cancerous cells. OR This gene is expressed mostly in normal cells but there is a small amount in cancer cells.

Part 3: Apply Your Understanding of Microarray Technology

10. What is a DNA microarray (DNA chip)?

A slide with thousands of spots of different single-stranded DNA sequences printed on it.

Create an analogy that could be used to explain the structure and function of a microarray. For example: A DNA microarray is like a ___**Answers will vary**___ because **Accept any answers that provide a logical explanation for the analogy**

11. Explain what causes the cDNA to bind to the gene spots on the microarray?

Base pairing OR A fits with T and C fits with G OR hydrogen bonds OR the bases are complementary

Base your answers to questions 4 and 5 on the information below.

A researcher studying tumor cell function wants to study how treatment with a cancer drug affects expression of the gene for a DNA repair enzyme. He knows that the mRNA for the enzyme contains the base sequence

AAU AGG UAC ACG

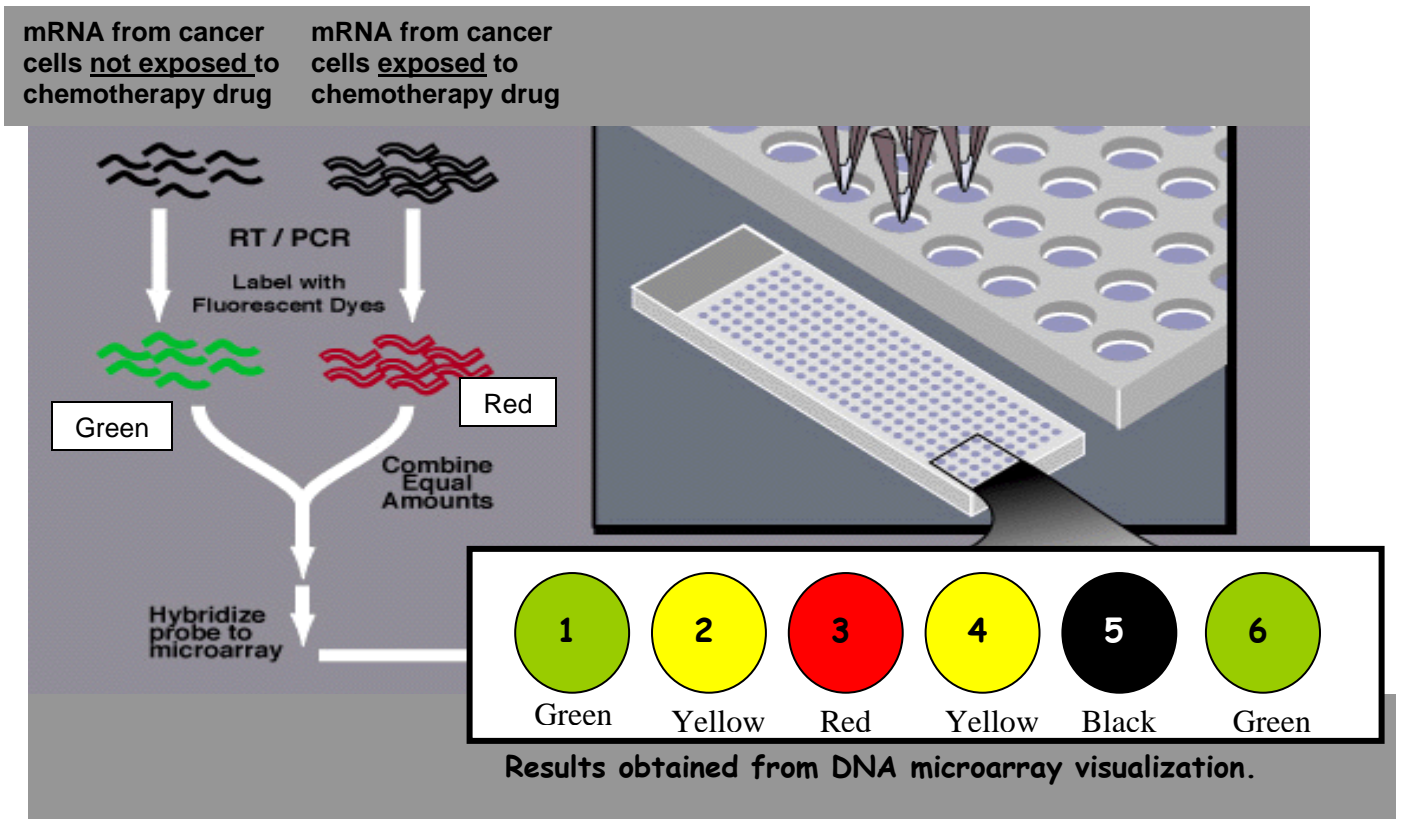
12. What sequence of cDNA bases would result if the researcher mixed this mRNA with reverse transcriptase? **TTA TCC ATG TGC**

13. What gene sequence on the DNA chip would hybridize with (bind to) this cDNA?
AAT AGG TAC ACG

Base your answers to question 6-9 on the information below.

A scientist used DNA microarray analysis to study how a potential chemotherapy drug affected gene expression in cancer cells. During his experiment, the scientist isolated RNA from two kinds of cancer cells: one type was grown in a normal culture medium and the other type was grown in the same culture medium that also contained the chemotherapy drug. He used the messenger RNA from the cancer cells that were not exposed to the chemotherapy drug to make green labeled cDNA. He used the RNA from the cancer cells that were exposed to the chemotherapy drug to make red labeled cDNA.

Figure 1.



Modified from: http://www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/Images/microarray_technology.gif

The scientist mixed both kinds of cDNA together to produce a hybridization solution that he applied to a microarray that was printed with six different gene sequences. Then he washed the microarray to remove unbound cDNA and scanned the microarray to visualize the colors of the spots. Figure 1 shows the color of the spots on the DNA microarray.

Based on the results of his experiment, which gene or genes appear to be:

14. Expressed only in cells exposed to the chemotherapy drug? **_3_**
15. Expressed only in cells not exposed to the chemotherapy drug? **_1 and 6_**
16. Expressed in both types of cells? **_2 and 4_**
17. Not expressed in either type of cells? **_5_**

Part 4: Microarrays and Personalized Medicine

Questions:

7. What does it mean to have a cancer "Recurrence?"

The cancer comes back again or reappears.

8. What is meant by the phrase "personalized medicine"?

The genes expressed in a tumor are analyzed to determine what medicine or treatment should be used.

9. Why is using a *MammaPrint* considered a form of personalized medicine?

It analyzes what genes are expressed to determine whether a patient needs treatment because a cancer recurrence is likely.

10. State two (2) benefits of using *MammaPrint* technology.

A patient can tell whether or not they are at risk for recurrence.

They may be able to avoid the side effects of chemotherapy

They may find out that they don't need chemotherapy.

Helps determine follow-up treatment.

11. State three (3) risks/concerns that patients or doctors might have about the use of *MammaPrint* technology.

The test may not be accurate.

It may be difficult to decide what to do if a patient is at medium risk.

The test may indicate a patient is at low risk but that does not guarantee that the cancer does not recur.

The test may indicate a patient is at high risk but that does not mean that the cancer will definitely recur.

The test may be expensive

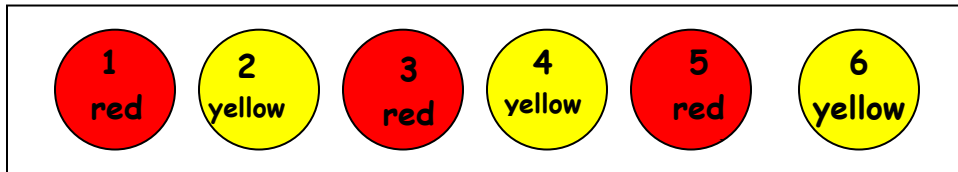
12. If a *MammaPrint* indicates that a patient has a low risk of recurrence, why might that patient choose to have chemotherapy treatments?

Even with a low risk, there is a chance that cancer might recur if the patient does not have chemotherapy.

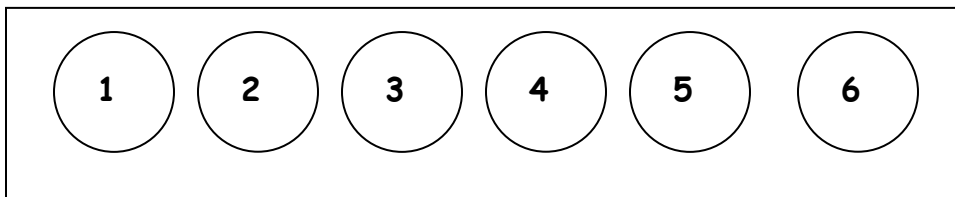
Part 5: Microarrays and Personalized Medicine: A Simulation

Figure 1: Results of Gene Expression Microarray Testing

Patient A



Patient B Results may vary depending on the exact pH of the solution. Be sure to check this one as the students are doing the laboratory activity.



Patient C

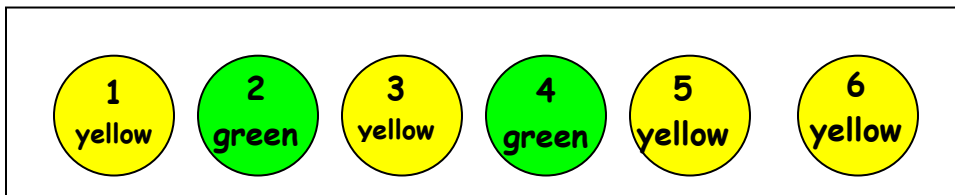


Figure 2: Gene Expression Microarray Testing

12. Which locations (1-6) represent genes that are **more expressed** in HIGH risk cancer patients than in low risk cancer patients? **1, 3, 5**
13. Which locations (1-6) represent genes that are more **expressed** in LOW risk cancer patients than in high risk cancer patients? **2, 4**
14. Which location represents a positive control gene used in this test? **6**
15. Imagine that you are a doctor who received the micorarray results from Patients A, B, and C. All of these patients have told you they would rather not receive chemotherapy treatments unless these treatments were necessary.
- Which of the patients would you recommend receive chemotherapy? Support your recommendation using the microarray data. **Patient A because their microarray is similar to the high risk patient which means that they are at greater risk for a cancer recurrence.**
 - Which of the patients would you recommend not receive chemotherapy? Support your recommendation using the microarray data. **Patient C because their microarray is similar to the low risk patient which means that they have a low risk for a cancer recurrence. Note: depending on test results the students may also indicate patient B.**
16. Imagine that you are Patient B. What questions might you want to ask your doctor before deciding whether or not to have chemotherapy treatment?
- Is it worth the side-effects?**
- Am I really at risk for a recurrence?**
- Are there other tests that could be done?**
- How accurate is this test?**

Part 6: Microarray Technology and Cancer Diagnosis

7. Use the DNA microarray data shown below to diagnose patients W and X.

Diagnosis for Patient W: Acute Lymphoblastic Leukemia

	A	B	C	D	E
1	○	L ●	both ●	○	○
2	L ●	○	○	○	○
3	L ●	L ●	○	○	L ●
4	○	○	L ●	○	○
5	○	○	○	○	both ●

Diagnosis for Patient X: Acute Myeloid Leukemia

	A	B	C	D	E
1	○	○	both ●	○	○
2	○	M ●	○	M ●	○
3	○	○	○	M ●	○
4	M ●	○	○	○	○
5	○	○	○	○	both ●

8. Why should you be hesitant to give a definitive diagnosis for Patient Y? Be specific!

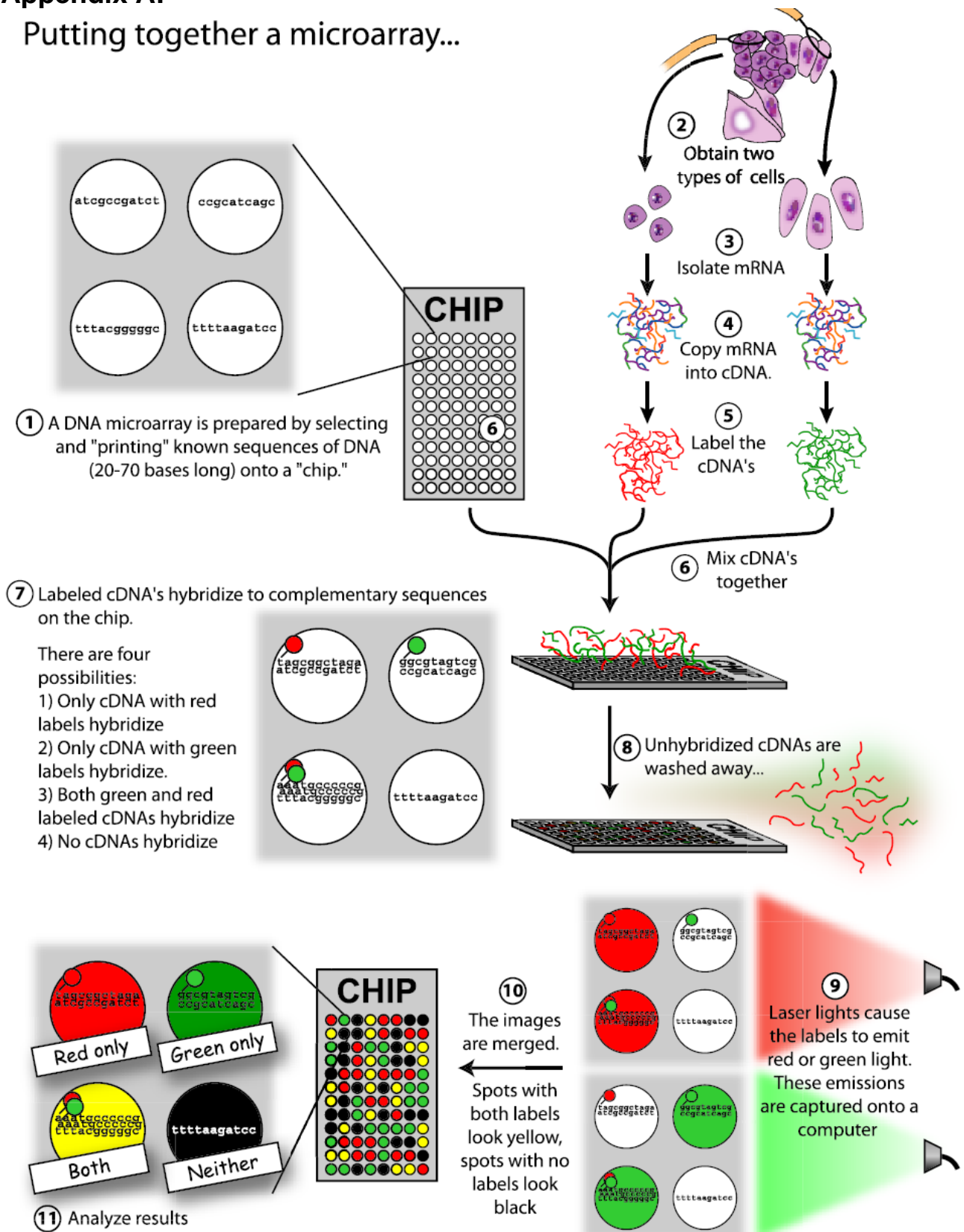
Patient Y:

	A	B	C	D	E
1	M ●	L ●	both ●	neith ●	○
2	○	M ●	○	○	M ●
3	○	○	M ●	○	L ●
4	M ●	neith ●	○	neith ●	○
5	○	○	○	○	both ●

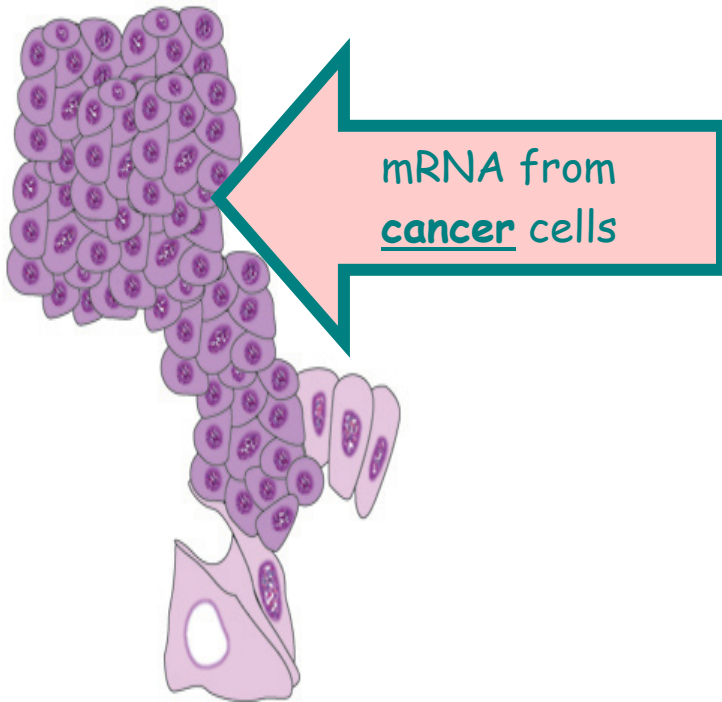
The microarray pattern has some characteristics of both M and L forms of leukemia OR there are three genes that are expressed that are not characteristic of either type of leukemia OR the negative controls show genes that are expressed when they shouldn't be.

9. If the gene at 1A on the microarray had the DNA base sequence "**AAATTTC**",
- c) What base sequence of cDNA would bind in this position? **TTTAAAG**
 - d) What base sequence in mRNA would produce this cDNA? **AAAUUUC**
10. For all three patient samples, there is no fluorescent spot at A5 . Use your understanding of DNA and of microarray technology to explain why no fluorescence appeared at A5.
- The DNA sequence at A5 does not match with any of the genes in the cells being sampled OR this gene is not expressed in either type of cells.**
11. Explain why genes C1 and E5 act as positive controls for these tests.
- These genes are expressed in both types of cells.**
12. Explain why genes B4 and D4 act as negative controls for these tests.
- These genes are NOT expressed in both types of cells.**

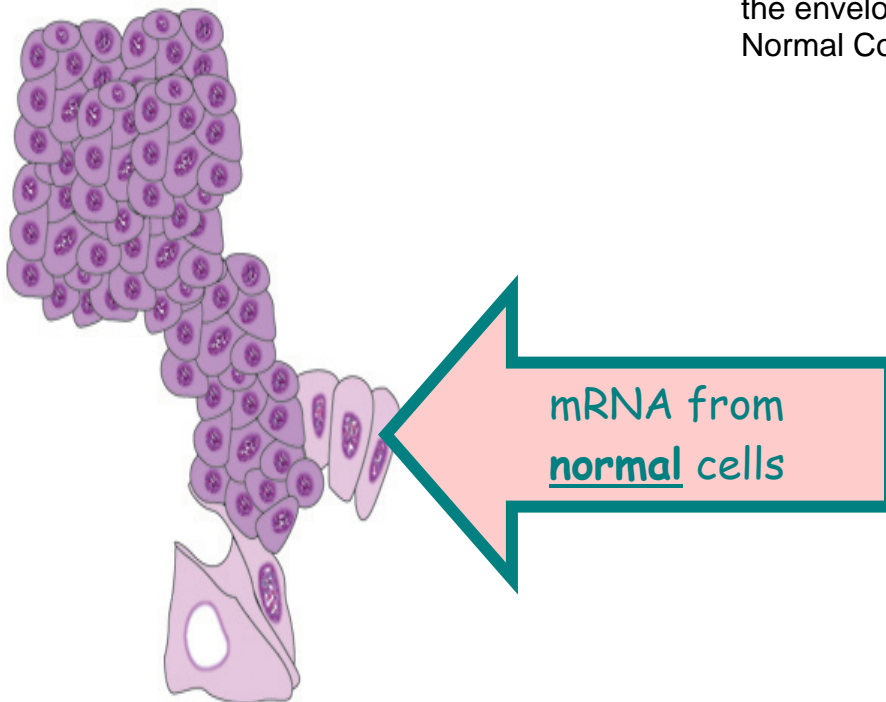
Appendix A: Putting together a microarray...



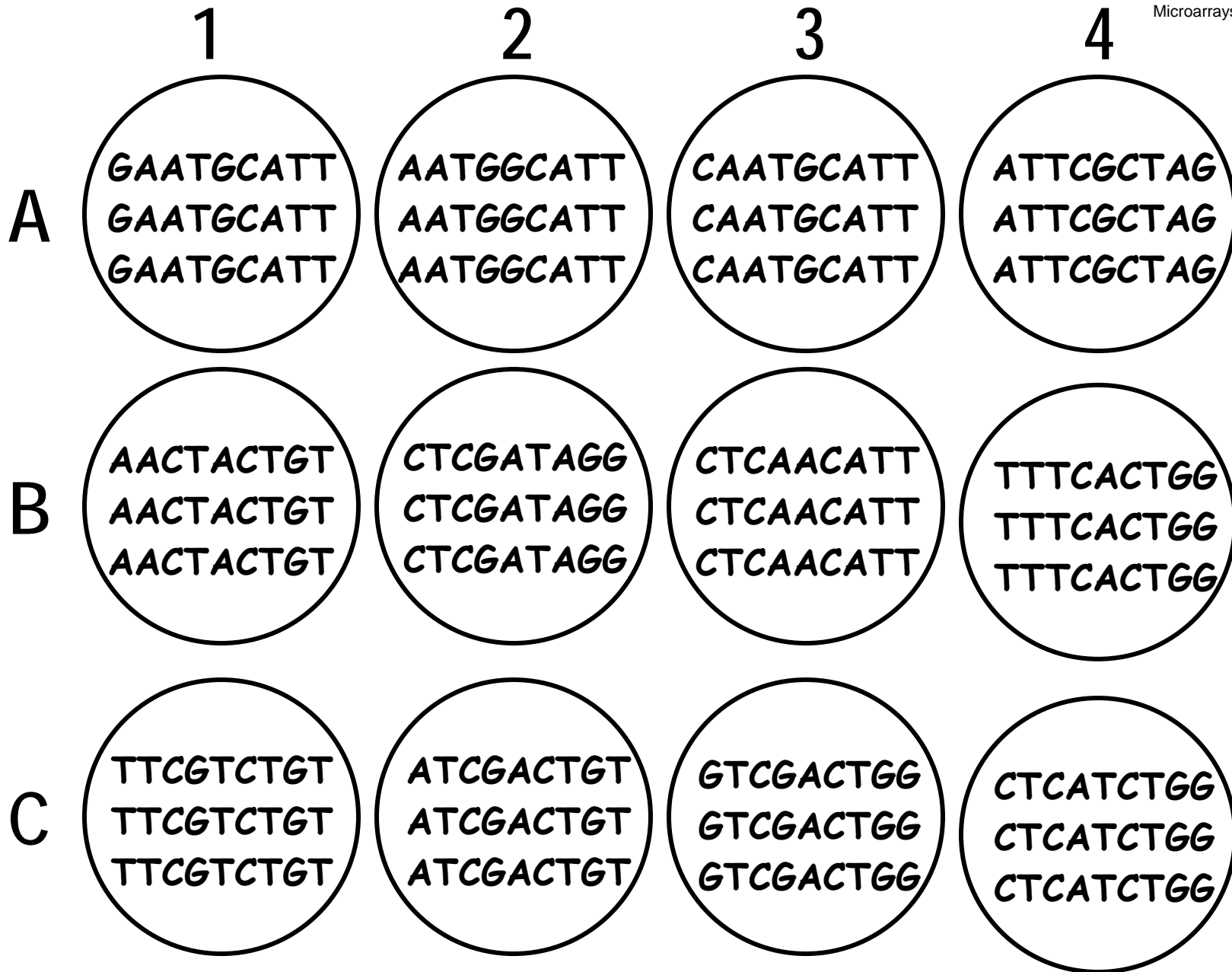
Appendix B: Pieces for Paper Microarray Class Simulation



Envelope label: Print and attach to the envelope labeled "mRNA from Cancerous Colon Cell"



Envelope label: Print and attach to the envelope labeled "mRNA from Normal Colon Cell"



Microarray (DNA Chip) with different gene (DNA) sequences printed at each location

Microarray Strips for envelope labeled "mRNA from Cancerous Colon Cell:
Copy, cut to separate, insert into the envelope labeled "mRNA from Cancerous Colon Cell."

mRNA GAAUGCAUUGUUUAAACGCGUAUAGUCACGUACGGGGGUACGU

cDNA

mRNA GAAUGCAUUGUUUAAACGCGUAUAGUCACGUACGGGGGUACGU

cDNA

mRNA AUUCGCUAGGUAAACACGAUCGAUCGAUCGAUCGGGGGUACGU

cDNA

mRNA AUUCGCUAGGUAAACACGAUCGAUCGAUCGAUCGGGGGUACGU

cDNA

Microarray Strips for envelope labeled "mRNA from Cancerous Colon Cell:
Copy, cut to separate, insert into the envelope labeled "mRNA from Cancerous Colon Cell."

mRNA AUCUACUGUACCCCCCGUGAUCGAUCGAUCGAUCAGGCAUGCU

cDNA

mRNA AUCUACUGUACCCCCCGUGAUCGAUCGAUCGAUCAGGCAUGCU

cDNA

mRNA CUCGAUAGGAAAACGAUCGAUCGAUCGAUCGAUCGAUCGAUGU

cDNA

mRNA CUCGAUAGGAAAACGAUCGAUCGAUCGAUCGAUCGAUCGAUGU

cDNA

Microarray Strips for envelope labeled "mRNA from Cancerous Colon Cell:
Copy, cut to separate, insert into the envelope labeled "mRNA from Cancerous Colon Cell."

mRNA UUUCACUGGAAAAACGUGUGUACGUACGUACGAUCGAUCGGU

cDNA

mRNA UUUCACUGGAAAAACGUGUGUACGUACGUACGAUCGAUCGGU

cDNA

mRNA AUCGACUGUGGGCCCAAUACACUAGCAGCUACGAUCGAUCGAUC

cDNA

mRNA AUCGACUGUGGGCCCAAUACACUAGCAGCUACGAUCGAUCGAUC

cDNA

Microarray Strips for envelope labeled "mRNA from Cancerous Colon Cell."

Copy, cut to separate, insert into the envelope labeled "mRNA from Cancerous Colon Cell."

mRNA GUCGACUGGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU

cDNA

mRNA GUCGACUGGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU

cDNA

mRNA GUCGACUGGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU

cDNA

mRNA GUCGACUGGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU

cDNA

Microarray Strips for Envelope labeled "mRNA from Normal Colon Cell":

Copy, cut to separate, insert into the envelope labeled "mRNA from Normal Colon Cell."

mRNA **AAUGGCAUUACAGUCAGCAUCGAUCGAUCGAUCGAUCGAUCGUG**

cDNA

mRNA **AAUGGCAUUACAGUCAGCAUCGAUCGAUCGAUCGAUCGAUCGUG**

cDNA

mRNA **CAAUGCAUUGUUUAAACGCGUAUAGUCACGUACGGGGGUACGU**

cDNA

mRNA **CAAUGCAUUGUUUAAACGCGUAUAGUCACGUACGGGGGUACGU**

cDNA

Microarray Strips for Envelope labeled "mRNA from Normal Colon Cell":

Copy, cut to separate, insert into the envelope labeled "mRNA from Normal Colon Cell."

mRNA **AUUCGCUAGGUAAACACGAUCGAUCGAUCGAUCGGGGGUACGU**

cDNA

mRNA **AUUCGCUAGGUAAACACGAUCGAUCGAUCGAUCGGGGGUACGU**

cDNA

mRNA **AACUACUGUACCCCCCGUGAUCGAUCGAUCGAUCAGGCAUGCU**

cDNA

mRNA **AACUACUGUACCCCCCGUGAUCGAUCGAUCGAUCAGGCAUGCU**

cDNA

Microarray Strips for Envelope labeled “mRNA from Normal Colon Cell”:

Copy, cut to separate, insert into the envelope labeled “mRNA from Normal Colon Cell.”

mRNA **CCCAACAUUACCCCCCCCCCGACUAGCAACGUUACGAUCGAU**

cDNA

mRNA **CCCAACAUUACCCCCCCCCCGACUAGCAACGUUACGAUCGAU**

cDNA

mRNA **UUUCACUGGAAAAACGUGUGUACGUACGUACGAUCGAUCGGU**

cDNA

mRNA **UUUCACUGGAAAAACGUGUGUACGUACGUACGAUCGAUCGGU**

cDNA

Microarray Strips for Envelope labeled "Normal Colon Cell":

Copy, cut to separate, insert into the envelope labeled "mRNA from Normal Colon Cell."

mRNA UACGUCUGUACUACGAUCAGCUACGAUCGAUCGAUCGAUGCAG

cDNA

mRNA UACGUCUGUACUACGAUCAGCUACGAUCGAUCGAUCGAUGCAG

cDNA

mRNA GUCGACUGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU


cDNA


mRNA GUCGACUGGAGAGAUUAUCACACAUUACAUACUAGGAUUUU


cDNA


Microarray Slides:


- Print one copy.
- **Photocopy** onto overhead project transparencies. Each transparency makes enough microarray slides for 6 students.


	Patient A
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	

	Patient B
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	

	Patient C
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	

	Patient A
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	

	Patient B
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	

	Patient C
<i>Gene 1</i> <i>Gene 2</i> <i>Gene 3</i> <i>Gene 4</i> <i>Gene 5</i> <i>Gene 6</i>	