DNA Chips
A Genetics Lab in the Palm of Your Hand

Welcome

About Snapshots

We designed Snapshots to provide exactly what its title implies—a snapshot of a single area of biomedical research that lets students see how science, people, ethics, and history all fit together. Each issue has four main departments:

- **Research in the News:** an overview
- **Story of Discovery:** a brief history of the featured research
- **People Doing Science:** a career profile of a scientist or two
- **Social Impact:** a bioethics exercise

Each issue also includes a classroom activity to help students understand the scientific concepts, a short summary of the whole issue, Web links, and some good diagrams (suitable for turning into overheads) and other teaching aids.

The Revolutionary DNA Chip

This second issue of Snapshots is all about a fantastic new tool called the DNA microarray, also known as the DNA chip. You and your students really need to know about DNA chips because

- **They are astonishingly powerful.** They allow scientists to quickly and inexpensively do experiments they could only dream about just a few years ago—like tracking how the expression of every single gene in the human genome changes in response to an experimental variable.
- **They have consumer applications.** Sometime in the coming decade, you will likely see these devices popping up in your doctor's office, or at your local police station for use in criminal investigations.
- **They are becoming big business.** Analysts estimate that the annual sales of DNA chips could exceed 1 billion dollars within just a few years.

Basic Concept Reinforcement

Learning about DNA chips is a great way to review and reinforce the fundamentals of molecular biology. The facts this issue of Snapshots highlights include

- The nucleotide sequence of DNA encodes instructions for making proteins.
- Double-stranded DNA contains two complementary DNA sequences, which can be separated and recombined.
- mRNA carries protein-making instructions from the nucleus to the ribosome.
- A cell's genes change in activity in response to outside stimuli.
- Small differences in a DNA sequence between individual people affect everything from their eye color to their chances of getting heart disease.

This issue of *Snapshots* also helps meet the standards described in the National Academy of Sciences' *National Science Education Standards* (National Academy Press, 1995). The content standards for grades 9-12 that this issue of Snapshots helps achieve include

- scientific inquiry (Standard A, Science as Inquiry)
- the cell (Standard C, Life Science)
- the molecular basis of heredity (Standard C, Life Science)
- personal and community health ((Standard F, Science in Personal and Social Perspectives)
- science as a human endeavor (Standard G, History and Nature of Science)
- historical perspectives (Standard G, History and Nature of Science)

Using Snapshots in Class

As long as you can download, print, and photocopy the pdf version of Snapshots for your students, you can use it in class—you don't need a classroom full of computers. Some features, such as the Web links and the animated explanations, obviously demand a computer, but you don't need one in the classroom.

Class Schedule

Here's a full-blown, 3-day schedule. You could cut this back by having students do the "Understanding DNA Chips" activity as homework or by skipping the optional third-day activity. Before you begin, give the students copies of the pdf printout as homework (or, if they have convenient Internet access, save some paper and have them read it from the Web site).

- **Day 1:** Review the Research in the News article—using the diagram "How Chips Work"—and conduct the activity "Understanding DNA Microarrays." Assign the "Fast Fact Summary" as review reading. (This activity could be spread over two shorter periods.)
- **Day 2:** Conduct the "Social Impact" activity. Ask students to summarize their personal views in writing as homework.
- **Day 3:** Plan an optional activity. Some possibilities include
  - **Guest speaker.** A molecular biologist or other scientist who uses microarrays, a representative from a company that carries out DNA analysis, a bioethicist, a law-enforcement officer involved with DNA analysis, a civil liberties activist, or someone else. You will have to find this person locally. Try any local university or research outfit, starting in the Public Affairs office. Research institutions mostly want to help out with education, and scientists usually love to talk about their work. Be aggressive.
  - **Student presentations.** Assign (or lure with extra credit) a few students to prepare short presentations on any of various topics, such as recent discoveries made with the microarray approach or on issues related to the Social Impact section.
  - **Field trip** to a local laboratory involved in molecular biology research, commercial DNA analysis, or forensic testing. Microarrays are spreading like wildfire, but they won't necessarily be part of every molecular biology lab's standard techniques yet. Students could, however, ask questions about how microarrays might affect that work in the future.

Enrichment Reading

If you have no class time to spare for DNA chips, try using this issue of Snapshots as enrichment reading. Possible follow-up assignments include

- Making a short oral presentation to the class, or writing a summary, of one or more of the articles.
- Doing the activity "Understanding DNA Chips" alone, without a partner.
- Doing the Social Impact section and submitting either a short oral presentation to the class or written answers to the questions on the "decision form" that accompanies the section.

Social Impact: DNA Dragnets

Social Impact lets students grapple with one of the ethical, legal, or social issues biomedical research so often creates. These issues are complex, difficult, and often so new that no consensus has emerged on how society should deal with them. Arriving at a workable consensus for these questions will require much public debate and discussion.

For this issue, the question Social Impact addresses is, How far should police go in using DNA- identification technology when seeking to identify possible suspects for a serious crime? The scenario presented is fictional, but something like it could easily happen soon—a similar case occurred in Australia in 1999 (see "Fast Facts About DNA Dragnets" and Web links for more information). Civil libertarians would undoubtedly object to any proposal to carry out a similar DNA dragnet in the United States, arguing that such a sweep would erode constitutional protection against unreasonable searches. It's not at all clear how American society and courts will resolve the question.

The "decision form*" models the kind of back-and-forth discussion society at large must go through to solve bioethical issues. After reading the fictional scenario and picking a question to answer, students must come up with a range of possible solutions. Then they must choose the one they think will provide the best outcome (using their own ideas of what "best" means), consider possible counter arguments, and justify their answer in the face of those arguments. They must also consider how their solution will affect different people with a stake in the outcome.

When conducting this exercise, remember that these questions are deliberately open-ended and don't have a "right" or "wrong" answer. What's important is that students engage the question, clearly express their ideas, and try to understand other viewpoints.

Procedure

1. Form students into small groups and pass out the student handout.
2. Instruct each group to read the scenario and choose one question from the list or come up with another one.
3. Stand back and watch the interaction. You may need to steer the students along if they get stuck. Insist on reasoned and polite discussion if arguments become heated.
4. Have a spokesperson from each group report the group's conclusion to the class.
5. Leave time for a whole-class discussion at the end. We suggest that you hand out the DNA Dragnets: Arguments and Issues" summary on the following page only at the end of the period, after the students have attempted to work the questions through.
6. Some possible concluding assignments include

   - Have each student write a paragraph summarizing their own thinking on the question their group answered.
   - Have each student write down three questions of fact, the answers to which would help them make a more informed decision.
   - Have students research the topic and write a short report about what other people have had to say about the use of DNA dragnets (see Web links for some starting points).

*This decision form is adapted from the book s "Case Studies in Bioethics I & II" by Ronee Yashon. If you like the approach, go to http://home.earthlink.net/~roneey/index.html.

DNA Dragnets: Arguments and Issues

Here are some arguments, both pro and con, that might come up in the discussion.

In favor of DNA dragnets:

- They can help police catch people who have committed horrible crimes.
- They deter crime. The fear of being asked to provide a sample will deter would-be criminals from committing crimes.
- Police need not compel people to provide a sample. Instead, they can merely ask citizens to voluntarily give a sample.
- Giving a sample is simple—just a cotton swab wiped inside a cheek—and painless, and it only takes a moment.
- DNA fingerprinting is no more threatening to privacy rights than paper-and-ink fingerprinting. This is doubly so if only the results of the identification analysis are stored, and the original sample is destroyed.

Against DNA dragnets:

- They amount to an unconstitutional search. The Fourth Amendment to the U.S. Constitution prohibits unreasonable searches. Police should not be allowed to collect DNA from someone unless they have some good reason to suspect that individual.
- They are not truly voluntary. Even if you can in principle decline to give a sample, such a request from the police is difficult to refuse without looking like you have something to hide.
- Police don't do mass fingerprint collection from innocent people they have no reason to suspect. DNA identification should be no different.
- Genetic samples potentially reveal much more about a person than a regular fingerprint. Allowing government officials to demand DNA samples from ordinary citizens sets a terrible precedent, as this power may come to be abused in the future.

Some other issues related to forensic DNA-identification technology:

- Most states currently retain DNA-identification data on all convicted violent felons. Should police routinely acquire and store DNA-identification data from everyone arrested (but not yet convicted) of a crime?
- Should police routinely acquire and store DNA-identification data from everyone, whether they have ever been arrested or not?
- Crime labs are swamped with requests for DNA testing. Many samples go months—even years—without analysis. Who should provide the money to clear this backlog?
- Should the technicians who perform DNA analysis be aware of the facts of the crime the testing is being used to investigate? Some people argue that just as researchers are "blind" to whether a patient is in the treatment or control group of a clinical trial, technicians should not know the sometimes horrific details of the crime their work might help solve, in order to avoid unintentionally biasing the results.

Activity: Understanding DNA Chips

This activity is designed to help students understand the basic science of DNA chips. It can be done in two 30-to-45-minute segments, or in one longer period; it could also be given as homework.

Before beginning the activity, review the flow of genetic information from DNA to mRNA to protein. Discuss the terms *nucleotide sequence, complementary sequences, hybridization, probe, and target* as they apply to the DNA-chip technology.

Objectives

Students will understand

- The basic principles of DNA microarrays
    - A microarray is made of many different known sequences—the **probes**—tied to known locations on a flat sheet glass or silicon.
    - A **probe** on the array surface will bind its complementary **target** if it's present in the solution that's washed over the chip surface.
    - When the array surface is scanned with a laser, fluorescent labels attached to the targets reveal which probes found their targets in the experimental solution.
- How researchers use DNA microarrays
    - To determine the nucleotide sequence of a DNA molecule.
    - To determine whether specific sequences are present in a mixture of DNA molecules.

Solutions to Questions

I. Using Chips for Sequencing

A. B-2 will fluoresce; the target adenine (in the unknown solution) binds the probe thymine.

B. G-5 will fluoresce; the target CTA binds the complementary probe GAT.

C. Each probe that lights up has found its complement within the seven-nucleotide unknown target sequence. The probes corresponding to the spots on the chip that fluoresce are

ATC
CAT
GTA
TAT
TCA

Therefore, the three-nucleotide targets, all of which must be contained within the seven-nucleotide sequence, are the complements of the probes:

TAG
GTA
CAT
ATA
AGT

The hard part is to see how all these three-nucleotide bits overlap to build up the overall sequence. We see only one way to do that:

```
CAT
 ATA
  TAG
   AGT
    GTA
CATAGTA
```

This means the entire seven-nucleotide unknown sequence would be CATAGTA. Real-life sequencing chips have thousands of probes, and researchers use computer programs to deduce the sequences.

II. Using Microarrays for Gene-Expression Analysis

A. The Doctor's Diagnosis

Patient 1: ALL (*Note:* D4 is fluorescent in this patient and represents an example of the variability that can occur in mutated cancer-cell genes.)

Patient 2: Neither

Patient 3: AML (*Note:* B4 is illuminated in this patient and represents yet another example of the variability that can occur in cancer cells as they mutate during replication.)

B. An Illuminating Challenge

Answers will vary according to genes chosen by students.

Summary Questions

1. a) The complementary sequence is TAATCCATGTGCCCTACCGGATCAATGGCGTTA.

   b) To look for changes in gene expression upon exposure to creatine phosphate, a researcher would first use an expression microarray to find out which genes are active when cultured human kidney cells are resting. Then she would expose the cells to creatine phosphate and look at the expression pattern again. Comparing the pattern before and after exposure would reveal which genes activate in response to creatine phosphate.

2. Students should discuss the cDNA probes bound to the chip and the use of mRNA target solutions extracted from cells. They should explain the process of hybridization between the targets and probes and, finally, the fluorescent tags that provide a positive identification for indicating which genes are being expressed.

3. Students should explain that a microarray could be set up to compare the gene expression in Mylotarg-resistant with Mylotarg-sensitive patients. The patient population that's sensitive to Mylotarg will express the cd33 receptor on the membrane and allow the lethal antibiotic into the AML cancer cells. As cancer cells divide and mutate, there's a chance that the cd33-receptor gene will mutate or that other gene mutations are enabling the resistant cells to destroy the antibiotic once it's in the cell. The microarray should help the researcher identify the new subset of genes being expressed in the drug-resistant patient population.

4. Using microarray technology, a researcher could compare the gene-expression patterns for antibiotic-resistant vs. non-resistant bacterial cells growing in culture. Once these differences in gene expression were identified, the researcher could then search for drugs that would interfere with the metabolic pathways used in the resistant population of bacteria.

5. People differ in their response to medications. Serious complications and death can occur from adverse reactions to them. Some people, for instance, are deathly allergic to penicillin, while others tolerate it easily. Often patients who experience negative reactions to drugs do so because of gene variations they have. These genetic differences can be identified using microarrays. A researcher could use a microarray to compare the genes expressed in nonreactive with those in reactive patient groups. This would define a set of genes on a microarray associated with adverse reactions to a drug. Once this microarray pattern in reactive patients is known, doctors can screen new patients to prevent adverse reactions before they happen.

DNA Chips:
A New Recipe for Understanding Life

By Fred Sculco, Morrison Chair in Science, Noble and Greenough School,
and Wright Fellow in Innovative Science Education 1999–2000, Tufts University*

Introduction

DNA chips, also known as DNA microarrays, are a revolutionary new technology for analyzing genetic information in unknown samples. They allow researchers to do experiments in an afternoon that would've taken an army of technicians months to do with older techniques. **Figure 1** (next page) presents a brief overview of how chips work.

Chips can be set up to perform two basic kinds of analysis:

- They can reveal the nucleotide sequence of an unknown DNA molecule.
- They can tell a researcher whether thousands of specific sequences are present in a mixture of many different DNA molecules.

In this activity, you'll do two exercises to help you understand how researchers use DNA chips. First, you'll determine the sequence of an unknown DNA molecule, using the kind of data that researchers get from a DNA-sequencing microarray. Then, you'll analyze gene-expression data to diagnose two kinds of leukemia.

I. Using Chips for Sequencing: A Fictional Exercise

A. Figure 2 represents a DNA microarray made by DNA Oncogene Hybrids (Doh! Inc.). It's the world's simplest array, with only four probes, each one nucleotide long:

	1	2
A	A	C
B	G	T

Figure 2

Doh! CEO and Chief Scientist Homer Simpson places a solution containing the nucleotide adenine (A, his target) on the array surface. After gently washing the surface of the array, at which spot on the array will he find a one-nucleotide bit of double-stranded DNA? (Remember: Adenine (A) binds thymine (T); guanine (G) binds cytosine (C).)

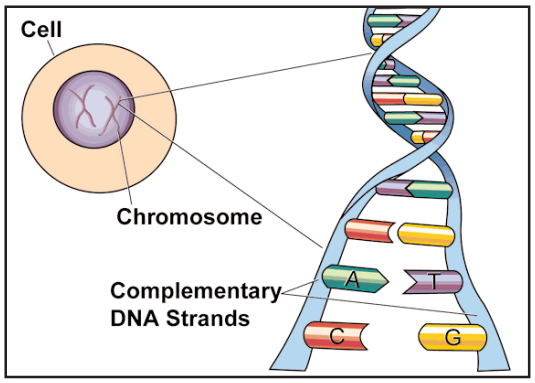
B. Doh! Inc. also makes a more complex chip **(Figure 3)**, this time with 64 different probes, each three nucleotides long.

	1	2	3	4	5	6	7	8
A	AAA	ACA	CAA	CCA	GAA	GCA	TAA	TCA
B	AGA	ATA	CGA	CTA	GGA	GTA	TGA	TTA
C	AAC	ACC	CAC	CCC	GAC	GCC	TAC	TCC
D	AGC	ATC	CGC	CTC	GGC	GTC	TGC	TTC
E	AAG	ACG	CAG	CCG	GAG	GCG	TAG	TCG
F	AGG	ATG	CGG	CTG	GGG	GTG	TGG	TTG
G	AAT	ACT	CAT	CCT	GAT	GCT	TAT	TCT
H	AGT	ATT	CGT	CTT	GGT	GTT	TGT	TTT

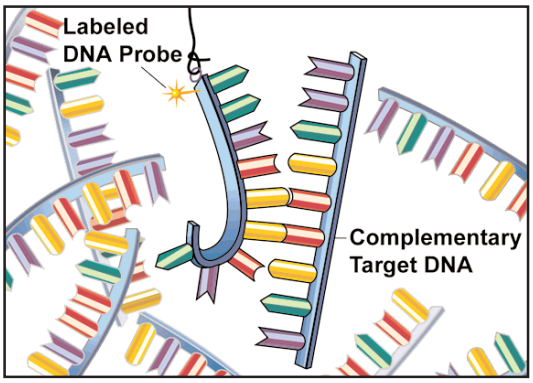
Figure 3

*Special thanks to Christine Huard of Millenium Pharmaceuticals for advice and guidance on Part II of this activity.

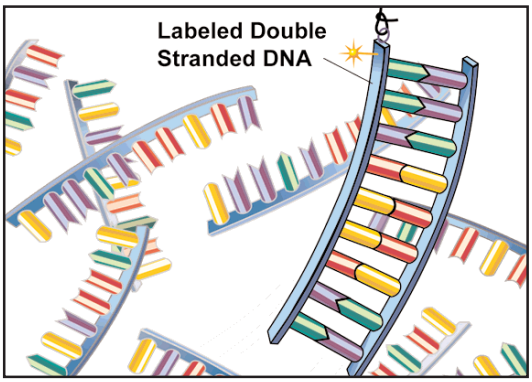
Figure 1. How a DNA microarray works.



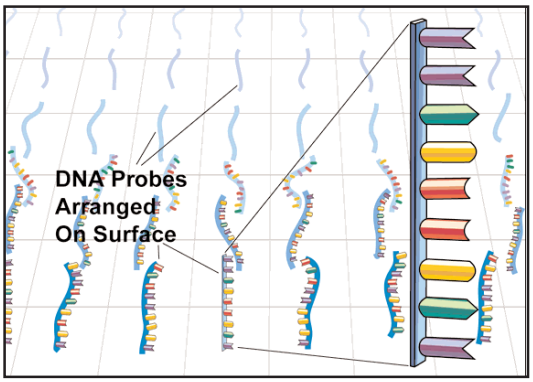
Cellular DNA is double stranded. Chromosomes in a cell's nucleus contain double stranded DNA. The two strands are complementary—A is opposite T, C is opposite G.



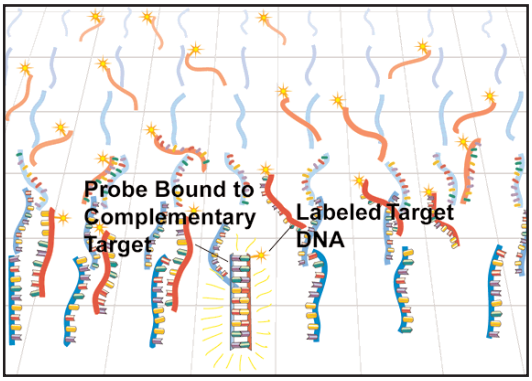
Using a single probe. In the 1970's, scientists learned to use DNA probes to find specific target sequences in solution. First, radioactively label a known DNA sequence, then put it into a mix of unknown sequences. If the probe's complement is there, it will bind.



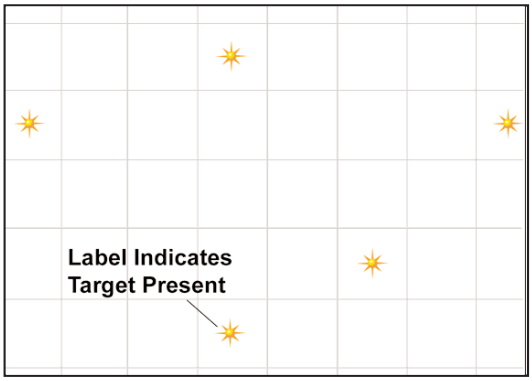
Look for the label. Next, separate the double stranded DNA from the single stranded. If the probe found its target, the radioactive label will be in the double stranded fraction.



DNA Chips: Thousands of Probes at Once. DNA chips allow scientist to use thousands of probes all at once. First, spot the different probes on a surface, noting sequence they put at each spot.



Let The Targets Loose. This time, label the targets in solution and put the solution on the chip. Any targets that find their complementary probes will stick to the surface.



Look For The Label. Next, gently wash the surface, and look for the labeled spots. Because you know the sequences of all the probes, you can easily deduce the sequences present in the solution.

Simpson places a solution containing a single-stranded bit of DNA (his target) with the sequence CTA on the surface of the array. He has also attached to the sequence a fluorescent label that glows red when scanned with a laser. After gently washing the surface, which sector of the chip will glow red when scanned?

C. Now for the hard part. Simpson places a solution on a fresh array containing a labeled, single-stranded target seven nucleotides long. After washing the surface, he scans his chip and observes the pattern in **Figure 4**.

What is the unknown sequence? (Hint: You have to overlap several shorter sequences to get the answer.)

[Editor's Note: Although the chip worked, Doh! Inc. went bankrupt when Simpson, citing better donuts, returned to the nuclear power industry.]

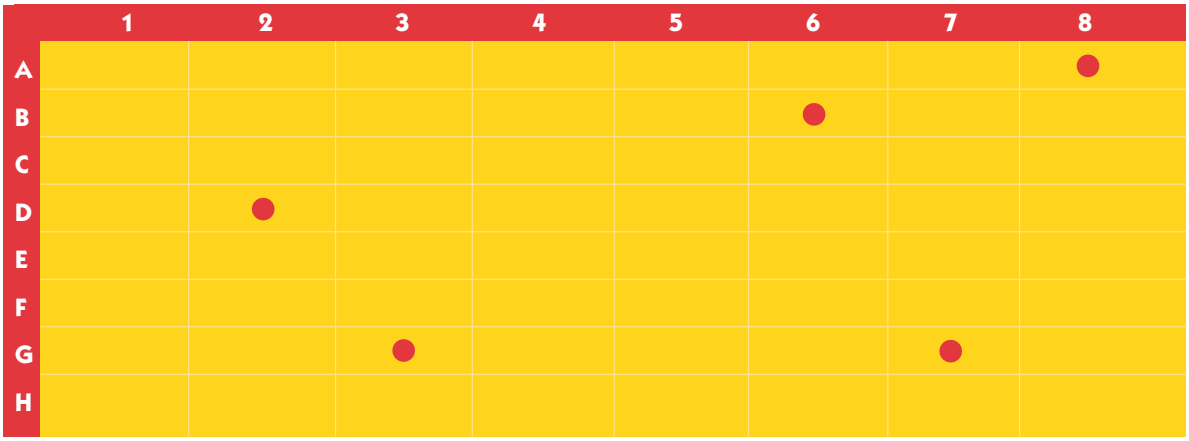


Figure 4

II. Using Microarrays for Gene-Expression Analysis: A Real-Life Story of Cancer Diagnosis

Cancer is caused by damage to the genes that control cell division. The result of this damage is uncontrolled cell division. Cancer cells have different gene-expression patterns from normal cells. Different types of cancer cells have different gene-expression patterns. This can help in identifying what type of cancer a patient might have.

In October 1999, a team of scientists at the Whitehead Institute/MIT Center for Genome Research in Cambridge, Mass., announced that they'd used DNA microarrays to distinguish between two clinically similar types of cancer, **a**cute **l**ymphoblastic **l**eukemia (**ALL**) and **a**cute **m**yeloid **l**eukemia (**AML**). Both affect cells in the bone marrow. ALL is the number one killer of young people below the age of 15. AML is the most common form of leukemia in adults. The sooner a doctor knows which version of leukemia a patient has, the faster the patient can receive the correct treatment.

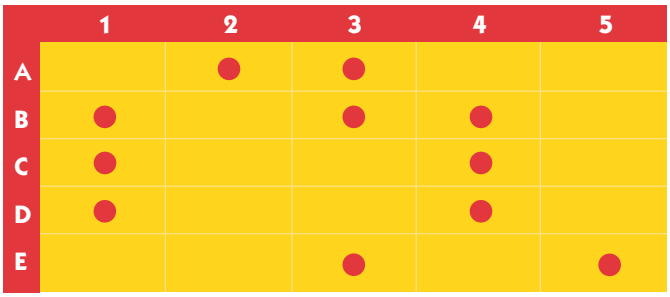
In their research, the Whitehead scientists hypothesized that the gene-expression patterns of AML and ALL would be sufficiently different to tell them apart using a gene-expression microarray. To test this, they allowed AML and ALL cells to multiply in separate cell-culture dishes, disrupted their cell membranes, and collected the mRNA from the cytoplasm. Only mRNA sequences from genes that were active when the membranes were disrupted would be present in the mRNA fraction. The researchers converted all the mRNA into a more stable form called cRNA and probed this mixture of targets with a microarray containing 6,187 different human genetic DNA sequences. Wherever a cRNA target found its matching probe on the microarray, it fluoresced, indicating that gene was being expressed in the cell.

The scientists identified a set of 50 genes that would allow them to distinguish ALL from AML. To prove the diagnostic power of the microarray patterns for these genes, they scanned blood cell samples from 34 patients diagnosed with either ALL or AML diagnosis. They made the right call in 29 out of 34 samples. (The researchers also identified one patient in the test set who had been mistakenly diagnosed as having AML but who in fact had a cancer derived from muscle cells; that patient's treatment was changed accordingly.)

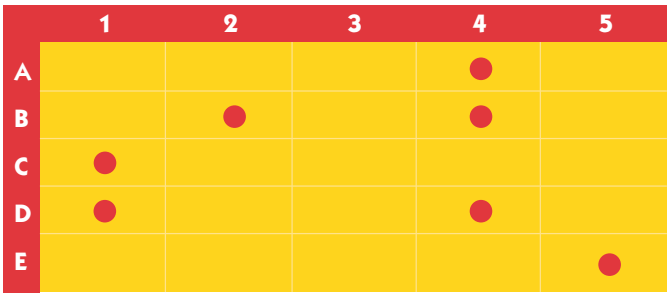
Now it's your turn! Let's see if you can use DNA-microarray data to diagnose several patients. **Table 1**, below, lists 25 gene probes placed on a microarray. The table also identifies whether the genes are expressed by ALL or AML cancer cells. Your task is to use microarray data from four different patients and determine whether each has AML, ALL, or neither type of cancer. Good luck Doc, your patient is counting on you!

A. The doctor's diagnosis is.....

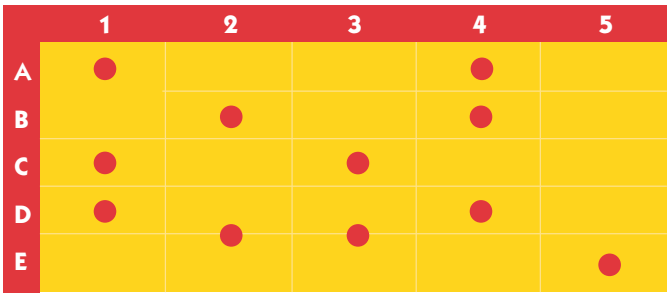
You removed cells from patient 1's bone marrow. You cultured them, then removed the mRNA from the cytoplasm. You converted it to cRNA and used that to probe a microarray containing the genes listed in **Table 1.** The results, or "output," of that probe are shown below in **Figure 5**. In the patient 1 sample below, when the cRNA is probed with a 25-gene array, the genes A2, A3, B1, C1, D1, etc., show a positive fluorescent color for gene expression. What is the diagnosis for this patient? What is your diagnosis for patients 2 and 3?



Patient 1 Patient 1 Diagnosis _____



Patient 2 Patient 2 Diagnosis _____

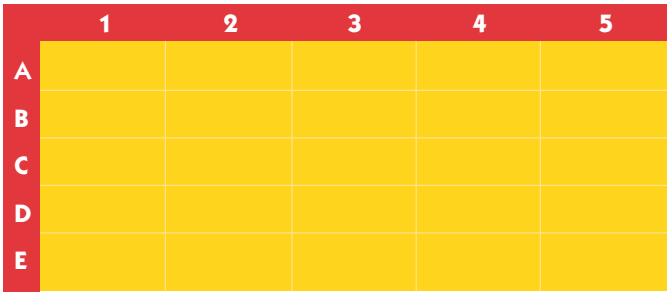


Patient 3 Patient 3 Diagnosis _____

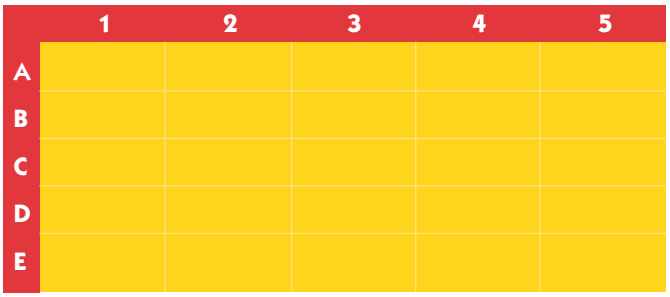
Table 1. Microarray gene locations and expression patterns in AML and ALL

Grid location	Gene name:	Gene highly expressed in:
A1	Zyxin	AML
A2	Cyclin D3	ALL
A3	Myosin light chain	ALL
A4	HOX A-9	AML
A5	SNF 2	ALL
B1	Coenzyme A enzyme	ALL
B2	Leptin receptor	AML
B3	OP 18	ALL
B4	Dynein light chain	Neither (control)
B5	SRP9	ALL
C1	Actin	Both (control)
C2	Il-7 receptor	ALL
C3	CD -33	AML
C4	MCM 3	ALL
C5	LYN	AML
D1	Myc 3	Neither (control)
D2	ATPase	AML
D3	SRP 9	AML
D4	CD 19	Neither (control)
D5	Catalase	AML
E1	Il-8 receptor	AML
E2	Lysozyme	AML
E3	Topoisomerase II	ALL
E4	Acyl-CoA dehydrogenase	ALL
E5	Glucose-6 phosphate	Both (control)

Figure 5. Microarray results for sample patients 1, 2, and 3.



Patient 1 Patient 1 Diagnosis _____



Patient 2 Patient 2 Diagnosis _____

B. An Illuminating Challenge

Now let's move to another level. Use Table 1 to create DNA-microarray data for two more patients. You may make an array that represents a patient with AML, ALL, or neither.

Once you've designed your array pattern on the sample grids, exchange your paper with another student or team, and see if they can properly diagnose your patients.

Summary Questions

1. A research scientist wants to know if a human kidney cell activates the gene for a specific enzyme when the cell is exposed to creatine phosphate. The researcher knows the mRNA for the enzyme contains the sequence:

   AUU AGG UAC ACG GGA UGG CCU AGU UAC CGC AAU

   a. What's the sequence of a DNA probe that would hybridize with the enzyme's mRNA?

   b. How could the researcher use an expression microarray to look for other genes that may turn on or off in response to creatine phosphate?

2. You and your partner have been asked to write an article on microarrays for the school newspaper. Review the steps and then write a one- or two-paragraph description of the sequence of events that occur when one grid on a microarray becomes fluorescent. Start by describing the overall layout of a microarray, and finish with the interpretation of the data from the appearance of a fluorescent grid.

3. Treatment for AML generally involves aggressive combination therapy using a variety of chemotherapeutic agents. These agents are "nonspecific," meaning they can't discriminate between healthy and diseased cells. Relapsed AML patients typically require prolonged hospitalization, and their prognosis is generally poor.

   Mylotarg is a new drug designed to specifically destroy AML cells. It's an antibody molecule linked to a cell-killing antibiotic called calicheamicin that was isolated from a soil bacterium. The antibody portion of Mylotarg binds to the CD-33 antigen, a protein displayed on the surface of myeloid leukemia cells. Some patients, however, don't respond to the drug. You're a research oncologist attempting to explain the reason for this lack of response in certain patients. Design an experiment using microarrays that will help find an answer to this question. (Hint: Cancer cells mutate rapidly.)

4. There's growing concern over the rapid appearance of bacterial strains that are resistant to currently used antibiotics. How might the use of microarray technology help a researcher identify bacterial strains that are resistant to known antibiotics? How might this technology guide scientists in discovering new drugs to destroy bacteria?

5. Some patients have adverse reactions to medications. One example is the allergic reaction some people show to penicillin. How might microarrays be used to reduce the chances of an adverse drug reaction in a patient? (Hint: Think about how cells respond in a negative drug reaction such as an allergic reaction.)