

## Macro-Microarray

Explore the nuts and bolts of gene chips

Construct a simple model of a DNA microarray and learn how they can be used to identify and treat disease.



### Materials and Preparation

For each microarray:

6 screws or bolts that are each unique in size  
peg board or small board with holes drilled  
masking tape to label the screws

For the “cDNA” samples:

2-4 corresponding nuts for each screw  
film cans or other small container

Assemble a film can for each patient sample. Each can will hold an assortment of nuts that represent the “cDNA” collected from each patient. The number of nuts for each screw corresponds to how much a specific gene is expressed in each patient. It may be helpful to label the screws and nuts ahead of time to make sure you assemble the right amounts. Based on real microarray data, we selected 6 genes to model how this technique is used in the laboratory. Because this is a simplified model, the numbers of nuts in each patient sample represents the *qualitative* change in expression for each gene when compared to the control. In addition to labeled sample cans, make some unknown “Patient A” (“B”, etc) samples that match one of the conditions for students to diagnose. An example of some gene expression profiles is given below.

	<i>healthy</i>	<i>Breast cancer</i>	<i>Lung cancer</i>
<b>ACTG</b>	2	2	2
<b>BRCA</b>	2	0	2
<b>ESR1</b>	2	4	2
<b>GLUT1</b>	2	4	4
<b>GST1</b>	3	3	0
<b>VEGF</b>	1	3	3

Copy or post the description of each gene.

ACTG: actin, an important protein for cytoskeleton formation

BRCA: a tumor suppressor that is deficient in many breast cancers

ESR1: receptor for estrogen, a growth factor

GLUT1: glucose transporter important in metabolism

GST1: an enzyme that may help the detoxification of carcinogen metabolites in tobacco smoke

VEGF: growth factor that promotes new blood vessel formation

### To do and notice

1. Prepare your microarray by attaching 6 different screws to a board so that they stick out vertically. You may need to use a nut to secure the screws to the board.

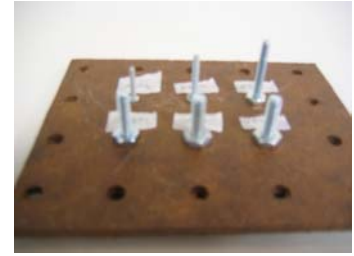
2. Label your microarray with the name of the gene that corresponds to each screw.

3. Look at the descriptions of the genes represented in your microarray. Based on what you know about cells and cancer, predict what genes you think might be expressed more or less in a patient who has cancer.

4. Get a film can of nuts that will be your patient sample. Work with other groups to make sure that at least one of you has a “healthy”, “breast cancer”, “lung cancer”, and unknown patient.

5. Attach the nuts that are in your patient sample to the correct screw on your microarray. Each nut represents a mRNA transcript that came from your patient’s cells. The total number of nuts on each screw corresponds to the level each gene is expressed in your patient.

6. Compare your microarray to other groups’ around you. What genes are expressed more or less in each disease? Can you use the microarrays to diagnose the unknown samples?



### What’s going on?

DNA microarrays, also called gene chips, are slides that are studded with microscopic fragments of DNA that usually correspond to a known gene. These fragments serve as probes for the gene since complementary DNA (cDNA) will bind specifically to each fragment or “spot” on the array. Messenger RNA (mRNA) is extracted from cells and used as a template to create cDNA. This pool of cDNA is passed over a prepared microarray where individual strands bind to their complementary sequences. The cDNA is usually labeled with a fluorescent probe that can be used to measure where on the array it hybridized. Since mRNA transcripts typically (but not always) correspond to the amount of gene expression in a cell, the microarray can be scanned to create a profile of how thousands of individual genes are being expressed.

The power of microarrays lies in their ability to evaluate thousands of parallel hybridization reactions that involve over 20,000 genes with millions of cDNA transcripts. The degree of simplification in our DNA microarray model cannot be stressed enough, since it only looks at the expression of 6 genes with a dozen or so cDNA transcripts that are represented by the various nuts. Even with so few genes, you can see how researchers are able to detect differences in expression levels of individual genes in a given sample. The ability to measure expression levels of so many genes at once allows researchers to gain a better understanding of what is going on in a complicated system. Microarrays are therefore being used to explore many aspects of cell biology, particularly disease, where the gene expression pattern of diseased cells can be analyzed to give a “genetic profile” of the disease. Based on differences in expression of the 6 genes used in this activity, you should be able to use your microarray data to learn about how these genes may play a role in cancer and also see how this technique can be used to make diagnoses.

Cancer is characterized by uncontrolled cell growth. Genetically, this usually involves a disruption of tumor suppressing genes and/or an overexpression of genes related to cell growth, such as growth factors or other proteins related to cell division. Because we are only looking at 6 genes, the expression levels for each one, though based on real data, have been generalized for what is often seen in each form of cancer. Here's a table of *qualitative* expression of each gene in different forms of cancer that we used to generate the numbers of bolts used in the activity.

	<i>Breast cancer</i>	<i>Lung cancer</i>
<b>ACTG</b>	NC	NC
<b>BRCA</b>	---	NC
<b>ESR1</b>	+++	NC
<b>GLUT1</b>	+++	+++
<b>GST1</b>	NC	---
<b>VEGF</b>	+++	+++

+++ : overexpressed  
 --- : underexpressed  
 NC : no change

Real microarrays can detect expression levels *quantitatively* over 4 orders of magnitude from a few transcripts per 10 cells to several thousand transcripts in a single cell. In addition, they look at so many genes that they can actually be used to distinguish between specific subtypes of breast and lung cancer. This information can be useful in determining the best course of treatment for a specific patient.

In this activity, the disease microarrays were compared to a healthy patient microarray to detect whether genes were over or underexpressed. Current microarrays typically use two-colored arrays to do this analysis on a single chip. The cDNA samples collected from the patient are labeled in one color (such as red) and a sample control from a healthy patient is labeled in another color (such as green). Since they are labeled in different colors, the two can be combined and added to the same microarray. The entire array is then read, and genes are evaluated to see if they are expressed more in the patient than the control (red, overexpressed), less (green, underexpressed), the same level in each (yellow, unchanged), or neither (black, unchanged and not expressed)

## References

Alberts, B. et. al. *Molecular Biology of the Cell*. Garland Science, 2002.  
 Free online textbook available at pubmed.org. Here's the section on microarrays:  
<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.section.1618>

Figure showing different cancer microarray signatures  
<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.1215>

Website with information and references to academic papers on cancer gene expression. The data used in this activity are from papers that can be accessed here.  
<http://www.cancerindex.org/geneweb/>