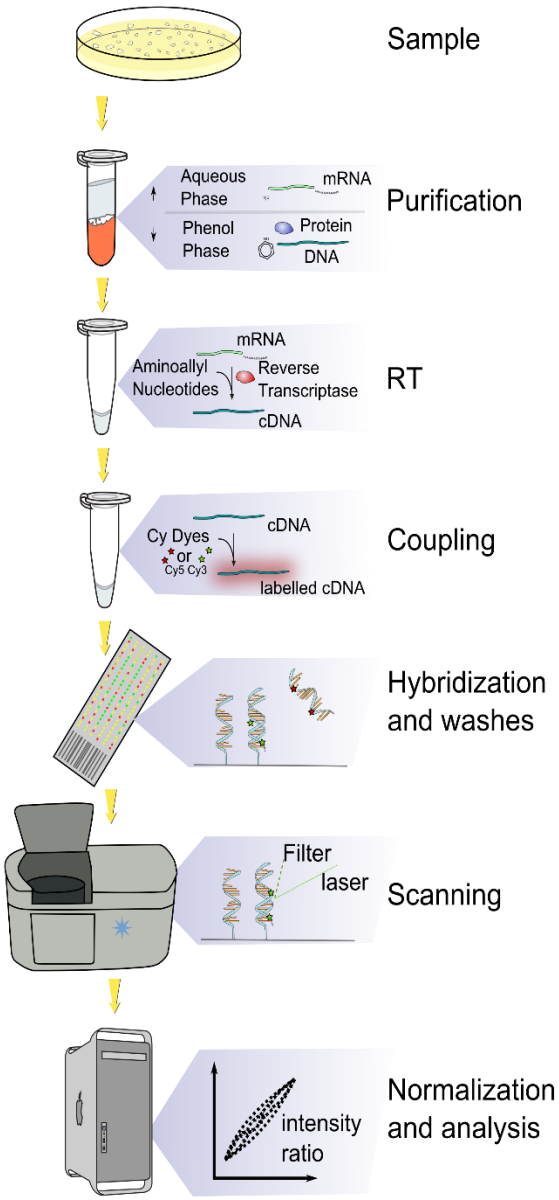
3.1.4 DNA Microarray Technology

Scientists have discovered that one of the differences between healthy cells and cancer cells is which genes are turned on in each. Scientists can compare the gene expression patterns between healthy and cancer cells through the use of DNA microarray technology.

Every cell in the human body contains the same 20,000 or so genes (with the exception of red blood cells, which contain no DNA). However, not every gene is active in each cell. The gene for melanin (a protein that gives your skin color) is only active, or turned on, in skin cells. The gene for myosin is only turned on in muscle cells.



Messenger RNA (or mRNA) is only produced in a cell when the cell is constructing a protein. Therefore, if mRNA is produced from a particular gene, scientists can infer that this gene is turned on within the cell. If mRNA is not produced from a particular gene, scientists can infer that this gene is turned off within the cell. Scientists use DNA microarrays to scan multiple genes (sometimes even thousands at a time) to quantitatively measure the gene expression for each of these genes.

DNA microarrays are glass, plastic, or silicon slides that have been spotted with thousands of short segments of DNA. These short segments of DNA are single stranded and each contains a portion of a gene of interest to the scientist.

The following steps outline the process used to develop a DNA microarray slide:

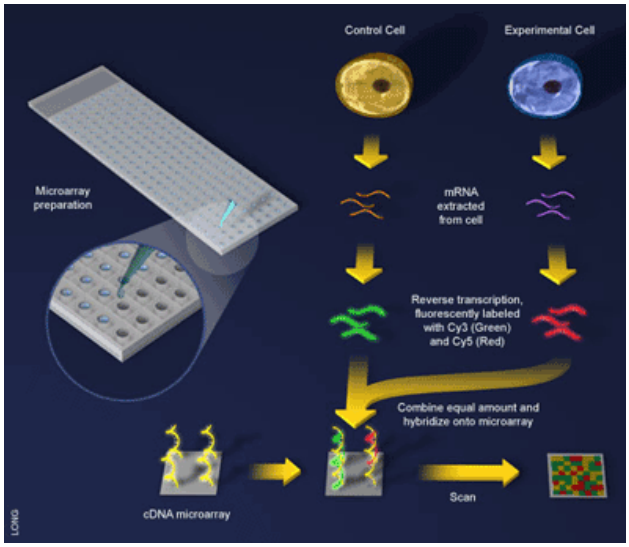
1) A gene thought to be involved in a particular type of cancer is located within the human genome sequence. (Specifically, the portion of the gene of interest is located.)
2) Primers are designed to run PCR reactions that will make copies of the portion of the gene of interest.
3) The double-stranded DNA from each DNA copy is separated into single strands.
4) Microscopic droplets of each single-stranded DNA sample are placed onto a specific spot on the microarray slide.
5) Steps 1 through 4 are followed to produce single-stranded DNA samples for each gene of interest the researcher wants to investigate. These samples are spotted in ordered rows and columns on the microarray slide.
6) Computers are used to keep track of all the gene spots on the microarray and ensure that each spot contains equal amounts of DNA.

A good short video explaining how DNA microarray slides are created are found at the McGraw-Hill websites, found at **http://highered.mcgraw-hill.com/olc/dl/120078/micro50.swf**.

The following steps outline a DNA microarray experiment:

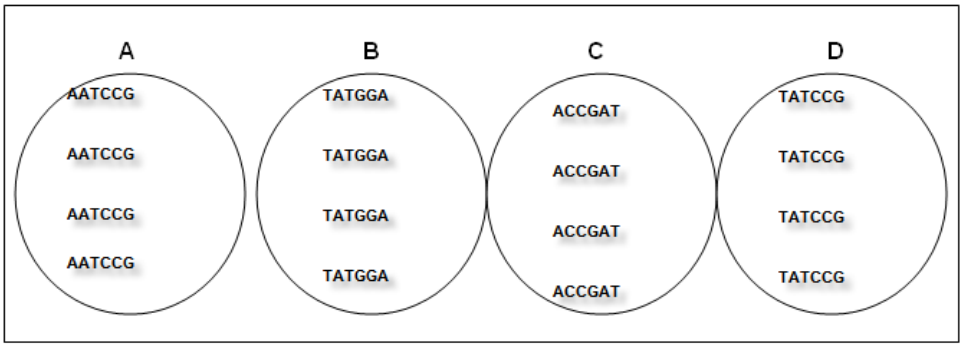
1) Collect tissue samples (for example, collect normal lung tissue and malignant lung tissue from a patient). Dissolve both samples in solvents to separate, for example, the DNA, proteins, and RNA.
2) Wash the samples over small beads that will only bind to the poly-A tails of the mRNA. The mRNA will attach itself to the small beads. Discard everything except the RNA samples.
3) Make a complementary DNA strand for every mRNA molecule in the sample, incorporating fluorescent labels. These fluorescent labeled, complementary DNA strands are called cDNA.
    - Complementary DNA (cDNA) is used instead of the RNA because DNA is more stable than RNA.
    - Poly-T primers bind to the poly-A mRNA tails. Nucleotides with either green or red fluorescent molecules attached to them are added and reverse transcriptase enzyme is used to produce cDNA strands. (The sample isolated from the healthy lung tissue is labeled with green fluorescent molecules; whereas, the sample isolated from the cancerous lung tissue is labeled with red fluorescent molecules).
4) Add the single-stranded cDNA to the microarray slide.
    - For every molecule of cDNA, there is a matching spot of single-stranded DNA on the microarray.
    - When two complementary DNA strands are mixed together, they will find and base pair with each other, forming a double-stranded DNA molecule. This process is called *hybridization*.
5) Wash off any cDNA that did not bind to the slide.
6) Scan the DNA microarray with a microarray scanner.
7) Analyze the gene expression data. The computer will complete this analysis. The hybridization that occurs between the cDNA produced from the mRNA sample and the DNA probes on the microarray indicate what genes are active and how active they are. The computer will analyze the saturation of the colors in the assay to determine the amount of gene expression for each gene of interest.
    - A saturated red color indicates a gene is highly expressed in the cancerous cells.
    - A saturated green color indicates a gene is highly expressed in the healthy cells.
    - A saturated yellow color indicates a gene is highly expressed in both the healthy cells and the cancerous cells.

Unfortunately, DNA microarray technology is not a perfect system because it relies on the assumption that if mRNA is present for a gene, the gene will be translated into a protein. Unfortunately, RNA expression does not always indicate protein activity. Scientists cannot tell if the mRNA is actually being translated into a protein or not. For example, a defect in a gene can prevent the RNA from being translated into a protein.



Example Simplified DNA Microarray Experiment

Patient X with lung cancer has samples of cancerous lung tissue and normal lung tissue biopsied. These samples are sent to the lab to be run on a DNA microarray. Four genes of interest are identified as genes potentially associated with lung cancer. The four DNA probes for these genes of interest are shown below. Remember that these are extremely simplified examples, and an actual DNA probe would be much larger than only six nucleotides. Also, note that on an actual DNA microarray, each spot contains numerous copies of the DNA probe. For this example, there are only four copies of the DNA probe for each spot.

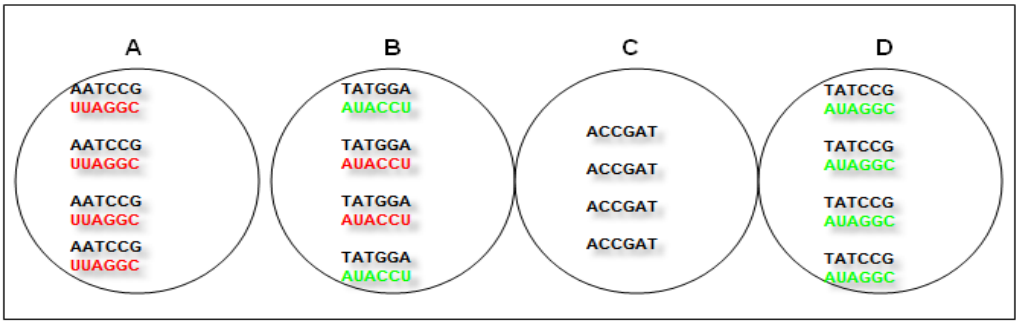


Complementary DNA samples have been produced from the patient's mRNA samples. Note that the cDNA samples produced from the cancerous lung tissue samples are labeled with red fluorescent nucleotides and the cDNA samples produced from the healthy lung tissue samples are labeled with green fluorescent nucleotides.

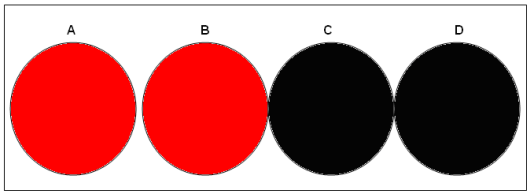
Isolated from cancerous tissue = RED
Isolated from healthy tissue = GREEN

AAUCCG (mRNA) TTAGGC (cDNA)	UAUGGA (mRNA) ATACCT (cDNA)
AAUCCG (mRNA) TTAGGC (cDNA)	UAUGGA (mRNA) ATACCT (cDNA)
AAUCCG (mRNA) TTAGGC (cDNA)	UAUCCG (mRNA) ATAGGC (cDNA)
AAUCCG (mRNA) TTAGGC (cDNA)	UAUCCG (mRNA) ATAGGC (cDNA)
UAUGGA (mRNA) ATACCT (cDNA)	UAUCCG (mRNA) ATAGGC (cDNA)
UAUGGA (mRNA) ATACCT (cDNA)	UAUCCG (mRNA) ATAGGC (cDNA)

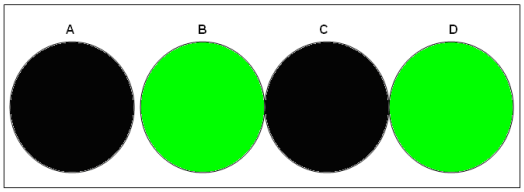
The cDNA samples have been added to the DNA microarray slide. The cDNA samples have been hybridized with their complementary DNA probes on the microarray slide. See the results below.



The DNA microarray would be scanned with lasers to locate the red fluorescence.

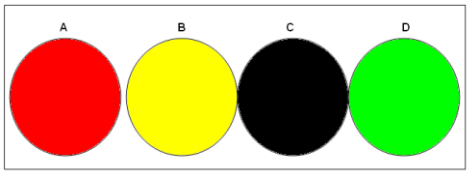


Next, the DNA microarray would be scanned with lasers to locate the green fluorescence.



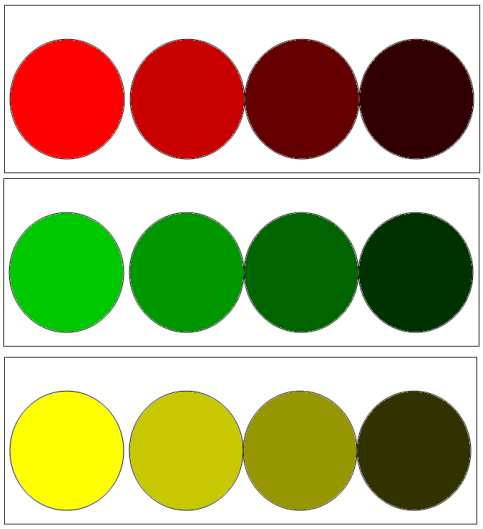
What do these results mean? The reason we added the green and red fluorescent labels was so we would be able to see which DNA probes have hybridized with our samples. Any spot on the microarray which glows green indicates cDNA from the healthy tissue hybridized, indicating these genes were expressed in the tissue. Any spot on the microarray that glows red indicates cDNA from the cancerous tissue hybridized. Any spot on the microarray that does not glow indicates that no cDNA hybridized from either sample.

Finally, the two colors are super-imposed over one another, as shown below.



Any spot on the microarray that glows yellow indicates cDNA from both the healthy and the cancerous samples hybridized with the same gene.

From this example, one might assume that the only three colors one would see on a DNA microarray would be red, green, and yellow. However, each of these colors will show up in varying intensities. Because of this, DNA microarrays can provide more information than just simply if a gene is turned on or off. DNA microarrays can also provide a quantitative measurement of gene expression levels. The saturation or intensity of the color on each spot can be measured to determine the extent a gene is turned on. For example, a very saturated red spot indicates a gene is expressed at a higher level than a light red spot.



The relative color intensities are determined by lasers which scan the DNA microarray and represent the color intensities as numbers. The red and green fluorescence are super-imposed over each other and the ratio of red to green fluorescence is determined. For example, a spot that would appear as bright yellow would be represented with a ratio of one because there is the same amount of red fluorescence as there is green fluorescence. Below are a few examples of how the fluorescent spots would be converted to ratios:

	Gene A:	Gene B:	Gene C:	Gene D:
Red (Tumor Cells)				
Green (Normal Cells)				
Superimposed Image of Green and Red				
Ratio Red: Green (Tumor: Normal)	4 (There are four times the amount of red as there is green.)	0.67 (There are two-thirds the amount of red as there is green.)	1 (There is the same amount of red as there is green.)	0.5 (There is half the amount of red as there is green.)
Meaning of Results:	Higher levels of gene expression in cancer cells than in normal cells	Lower levels of gene expression in cancer cells than in normal cells	Same level of gene expression in both types of cells	Lower levels of gene expression in cancer cells than in normal cells
Therefore:	Gene is induced by tumor formation.	Gene is suppressed by tumor formation.	Gene is not affected by tumor formation.	The gene is suppressed by tumor formation.

The ratios can be used to give meaning to the results:

- When the ratio is greater than one, the gene is induced by tumor formation. This means that the gene transcription was more active in cancer cells than in normal cells.
- When the ratio is less than one, the gene is suppressed by tumor formation. This means that the gene transcription was less active in cancer cells than in normal cells.
- When the ratio is equal to one, the gene is not affected by tumor formation. This means that the gene transcription was the same in cancer cells as it was in normal cells.
- When the ratio is zero, the gene is not expressed in either cell.