Virtual Learning

# Medical Interventions

April 10, 2020

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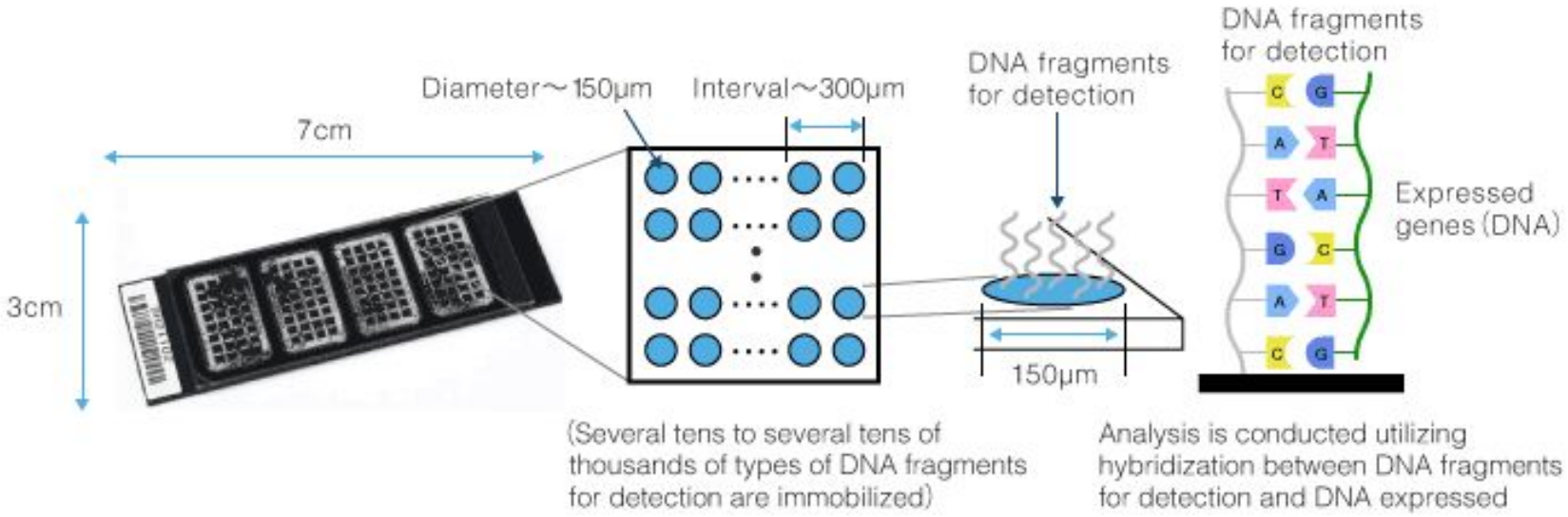
Lesson: April 10, 2020

**Objective/Learning Target:**
Recognize that DNA microarrays measure the amount of mRNA for genes that is present in a cell sample. (3.1.4)

# Let's Get Started

1. Click on the link to the video and view the animation over DNA microarrays.
2. In your own words, describe what a DNA microarray is used for.



# Background Info

## How to make 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.

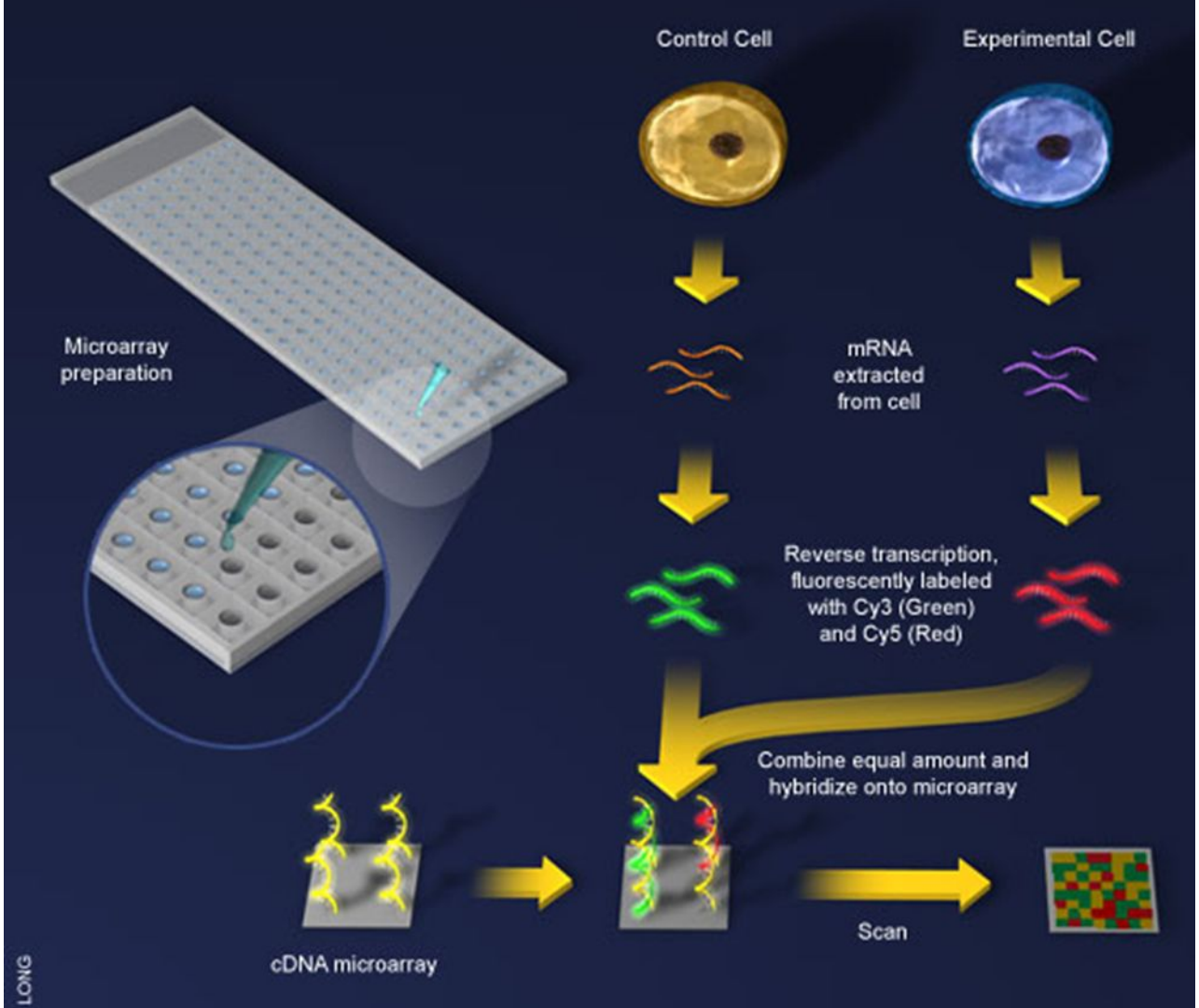
# Background Info

## How to conduct 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 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).

# Background Info

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.


Control Cell
Experimental Cell
Microarray preparation
mRNA extracted from cell
Reverse transcription, fluorescently labeled with Cy3 (Green) and Cy5 (Red)
Combine equal amount and hybridize onto microarray
Scan
cDNA microarray
LONG

# Activity

Complete the following DNA Microarray virtual lab by going through all 3 parts; genomics, measuring gene expression, and the experiment. As you do, answer the following in your notebooks or on a piece of paper:

1. Explain how production of mRNA relates to gene expression.
2. What happens after you apply the DNA from the two samples to the DNA microarray?
3. What does the red color indicate?
4. What does the green color indicate?
5. What does the yellow color indicate? Draw, label and explain what is happening on a yellow spot in a microarray.
6. What conclusions can you make from microarray data?
7. What are the limitations of DNA microarray technology?

# Activity - Answers

1. mRNA is transcribed from DNA to produce a protein. The quantity of mRNA produced gives a good indication of whether or not a particular gene is “on” and is being transcribed and translated to produce the coded protein product. If no mRNA is produced for a particular gene, this indicates a lack of protein production for this gene in the particular sample.
2. Because two complementary strands of DNA can rejoin, the cDNA that is applied to the microarray will pair up, or hybridize, with the single-stranded DNA molecules that are stuck to the spots of the microarray. The microarray can be scanned by a computer to determine which cDNA molecules hybridized with which genes on the card.
3. Red spots show genes that produce more mRNA in cancer cells than in healthy cells. These genes are “turned up” or “upregulated” in cancer.
4. Green spots show genes whose expression is “turned down” or “downregulated” in cancer.
5. Any spot that contains both red and green cDNA shows up as yellow. This means that the particular gene is expressed in both cancer cells and healthy cells. Student drawings should show both red and green cDNA attached to the DNA on the microarray.
6. Microarray data allows you to determine the level of gene expression in a patient for many genes at one time. In this case, the data provides some insight into which genes may be affected in the development of cancer.
7. In some cancer cells, mRNA is produced (as it would be in a normal cell). However, a defect may prevent this mRNA from being translated into a protein. This type of defect cannot be detected with a microarray. Microarrays can tell us if mRNA is present, but they cannot tell us whether that mRNA does in fact get translated. Microarray data cannot identify every gene that is malfunctioning, nor can it cure disease.

# Practice

Put the following microarray technology steps in order.

1. Analyze results by comparing colors of the spots on the microarray.
2. Identify genes that are expressed or repressed in malignant bladder tissue.
3. Isolate mRNA from sample tissue
4. Place single-stranded DNA of associated bladder tissue onto a glass slide
5. Produce fluorescent-labeled DNA by reverse transcription from mRNA
6. Collect normal and malignant bladder tissue from patient
7. Bind cDNAs to complementary gene sequence on a glass slide.

# Practice - Answer

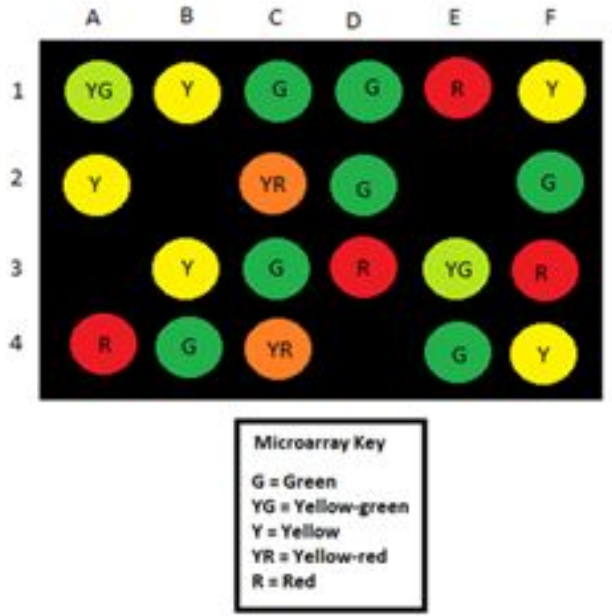
Put the following microarray technology steps in order.

1. Identify genes that are expressed or repressed in malignant bladder tissue.
2. Place single-stranded DNA of associated bladder tissue onto a glass slide
3. Collect normal and malignant bladder tissue from patient
4. Isolate mRNA from sample tissue
5. Produce fluorescent-labeled DNA by reverse transcription from mRNA
6. Bind cDNAs to complementary gene sequence on a glass slide.
7. Analyze results by comparing colors of the spots on the microarray.

# Practice

For each gene on the DNA microarray chip shown below, indicate if the gene is either more active (+), less active (-), equally expressed (=), or not expressed (N/A).

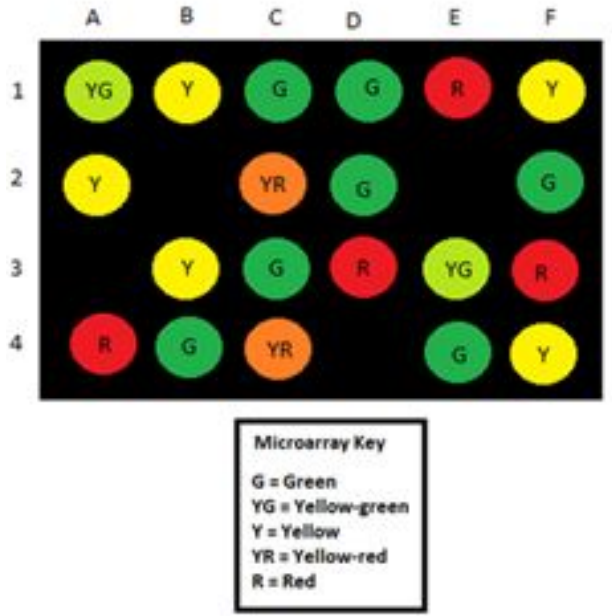
| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |



# Practice - Answer

For each gene on the DNA microarray chip shown below, indicate if the gene is either more active (+), less active (-), equally expressed (=), or not expressed (N/A).

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | = | = | - | - | + | = |
| 2 | = | N/A | + | - | N/A | - |
| 3 | N/A | = | - | + | = | + |
| 4 | + | - | + | N/A | - | = |



# Additional Practice

1. View this lab activity video to learn how we can conduct a simple DNA microarray experiment in class.
2. Check your understanding by creating a flowchart on a piece of paper of the steps of a DNA microarray procedure from beginning (forming the microchip slide) to the analysis of results.

# Additional Resources

Have you or anyone you know completed an at-home genetic test?  Many of these companies use a similar procedure outlined here to determine ancestry.  Check out the link below to learn more about how one specific company, 23andMe, analyzes DNA:

- https://customercare.23andme.com/hc/en-us/articles/202904610-How-does-23andMe-genotype-my-DNA-