DNA MICROARRAYS
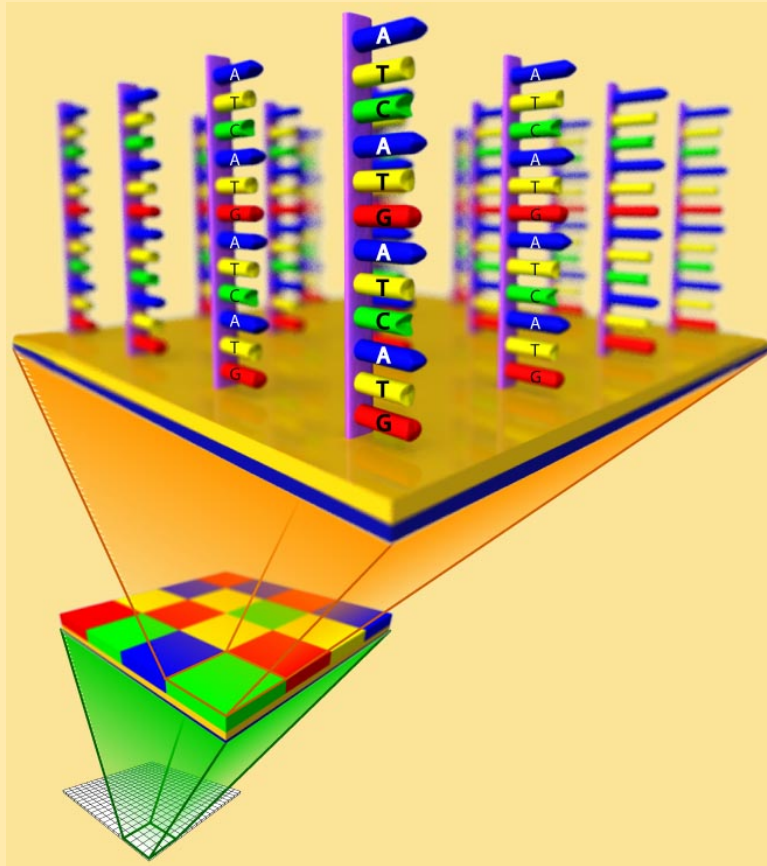
DNA Microarray

Unit Overview

This unit explains

❖ what a DNA Microarray does,

❖ how it works,

❖ how it is used,

❖ how it is fabricated, and

❖ how it is interpreted.

Objectives

❖ Describe three applications of the DNA microarray.

❖ Explain how a DNA microarray works from hybridization to interpretation.

Introduction

DNA microarrays look at our genes.

❖ They can identify the presence or absence of a gene,

❖ they can compare our genes with those from another source, and

❖ they can see how our genes are affected by external stimuli.

Two GeneChips© by Affymetrix with projected results. Image courtesy of Affymetrix.

The Human Genome

❖ The human genome is the complete set of human DNA.

❖ This set consists of approximately 30,000 genes.

❖ A person's specific genes are stored in each of one's cells. In fact, every single cell in a human body contains the exact same genes.

❖ However, the "activity" of genes varies from cell to cell. A gene is active when its mRNA can make a copy or cDNA.

❖ Genes are not the same between different human bodies and between different species.

DNA Microarrays

❖ DNA microarrays help us to learn more about our genes.

❖ They help us learn more about human diseases, what causes them, how to identify them, and how to treat them.

❖ We now know more about complex diseases such as diabetes, multiple sclerosis, heart disease, and cancer than we have ever known before.

❖ For some diseases researchers have been able to identify specific genes that influence the risk of getting a disease.

❖ They have found that most diseases that are affected by one's genes are influenced by many, many genes and not just one or two.

DNA Microarrays

DNA microarrays are not only used in the medical field, but in other industries such as forensics, agriculture, and toxicology.

SNP (single nucleotide polymorphisms) chips – a type of DNA microarray
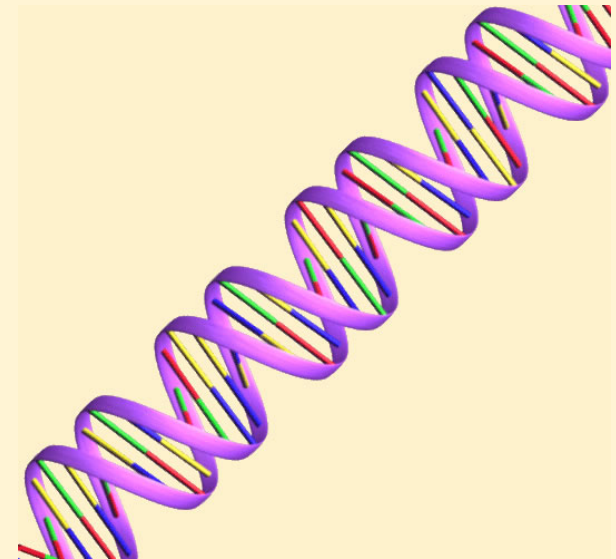
The SNP chips in the photograph are bovine assays (or analysis) that "easily and quickly identify regions within the bovine genome that harbor variants that cause the animals to differ in the outward expression of important traits, allowing scientists to predict an animal's genetic merit from its SNP profile."

[Courtesy of Jeremy Taylor, Animal Genomics, University of Missouri]
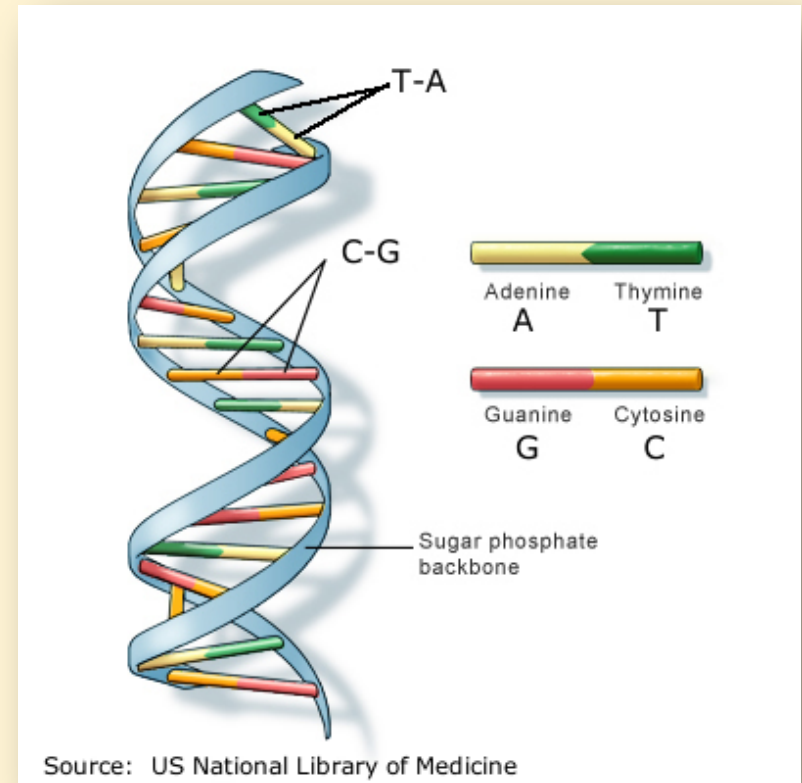
Review of DNA

Before jumping into the DNA microarray, let's review the Deoxyribonucleic acid (DNA) molecule, DNA transcription, and DNA hybridization.

Please refer to the glossary at the end of your guide if you get stuck on terminology.
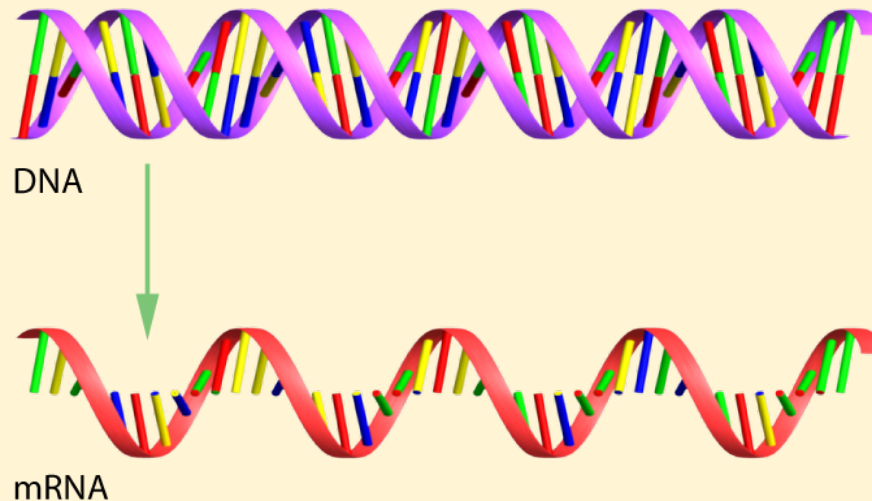
What is DNA?

❖ DNA is a long polymeric molecule that functions in the chromosome as the carrier of genetic information.

❖ The genetic information is stored in the linear sequences of the base pairs:

 ▪ Adenine-Thymine (A-T or T-A)

 ▪ Guanine-Cytosine (G-C or C-G)

❖ One DNA molecule may consists of millions of base pairs and thus, millions of linear sequences (genes).

T-A

C-G

Adenine   Thymine
A   T

Guanine   Cytosine
G   C

Sugar phosphate backbone

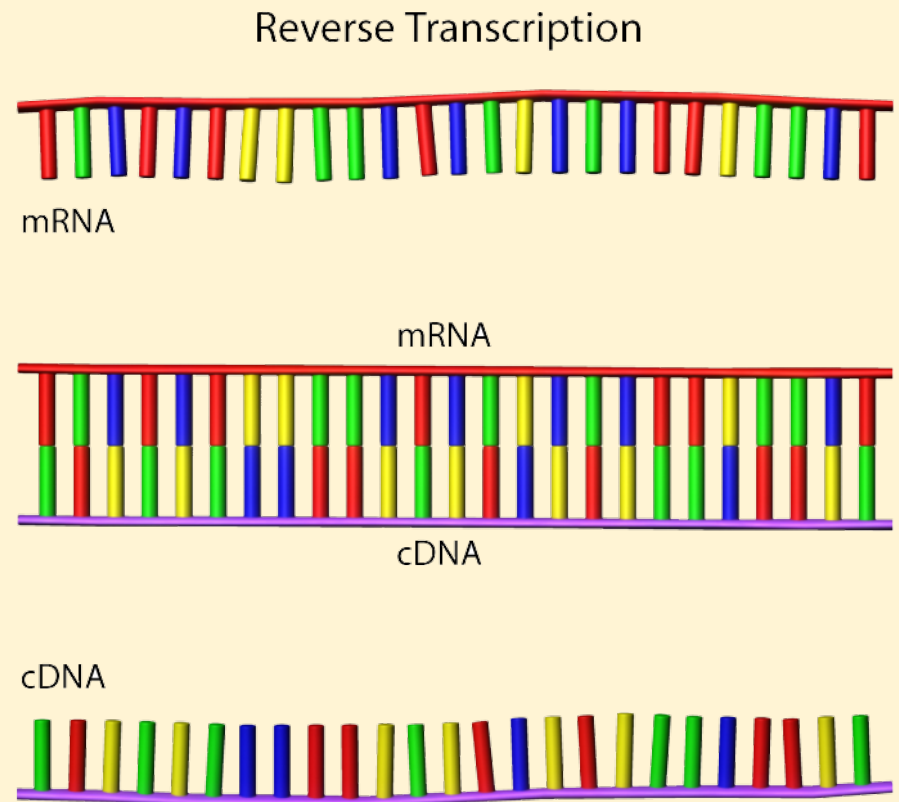Source: US National Library of Medicine

DNA Transcription

❖ DNA copies are made through DNA Transcription followed by reverse transcription.

❖ Transcription creates a messenger Ribonucleic acid molecule (mRNA).

❖ The DNA sequences (genes) are "copied" into the mRNA.

DNA

mRNA

Reverse Transcription

The mRNA sequences are transferred into a DNA copy or cDNA.

DNA microarrays require the cDNA rather than the mRNA, because mRNAs are unstable, thus leading to inaccurate and unreliable results. cDNA are less likely to degrade during the hybridization process in a microarray.

Reverse Transcription

mRNA

mRNA

cDNA

cDNA

DNA Hybridization

❖ DNA hybridization is when a single-stranded DNA molecule (ssDNA) reanneals with another ssDNA from another source.

❖ If the original ssDNA strand has sequences that are complementary to the introduced ssDNA strand, the two strands form a dsDNA hybrid molecule with one strand from each.

❖ Complementary DNA bases (A–T, T–A, C–G, and G-C)

G - C   A - T

The dsDNA is denatured by heating

A new ssDNA molecule from another source

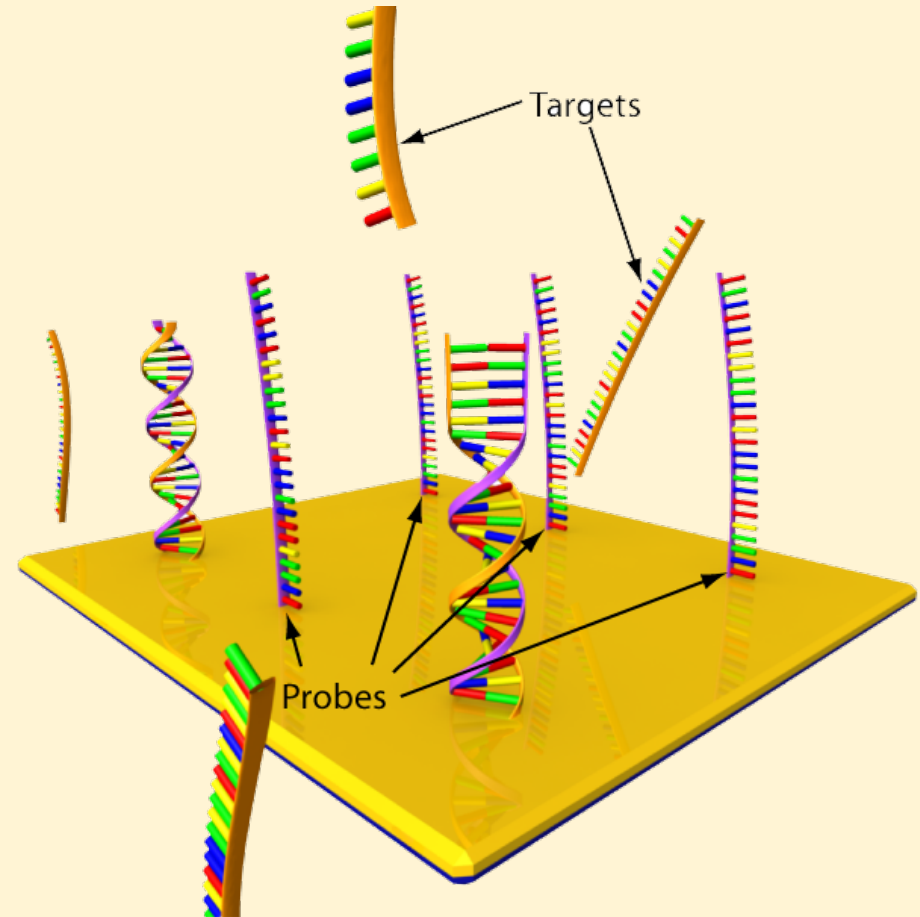During reannealing, the new ssDNA joins to a complementary ssDNA from the original source.

Southern Blot and PCR

The ability to make DNA hybrids is used in molecular genetics using devices called the Southern blot and PCR (polymerase chain reaction).

❖ Southern blot is a technique used to detect a specific DNA fragment; in other words, to locate a specific DNA sequence within an entire genome. (e.g., A-T-T-C-G-C)

❖ PCR is used to amplify DNA sequences and to make numerous copies of specific DNA segments quickly and accurately.
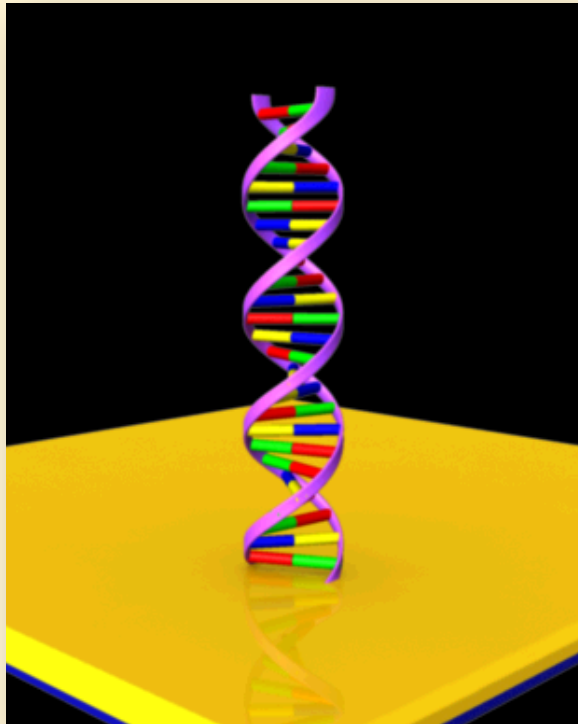
Search, Find, and Hybridize

In each case (Southern blot or PCR) a synthetic single-stranded oligonucleotide or oligo (a short nucleic acid polymer, typically with 20 – 50 nucleotide bases called a probe) is designed to search and find a complementary DNA sequence from a source (target) and form a DNA hybrid. *The graphic illustrates the oligo probes and the targets (cDNA).*

DNA Hybridization Animation
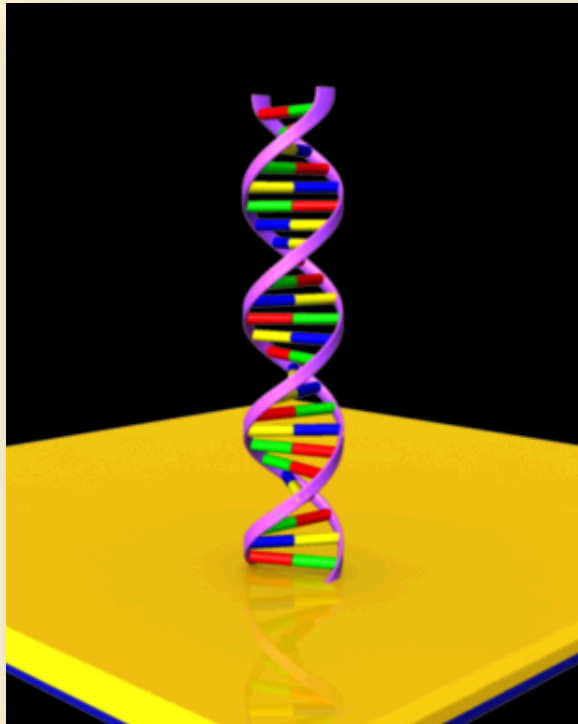
❖ Open the animation called "DNA_Hybridization"

While watching the animation answer the following questions:
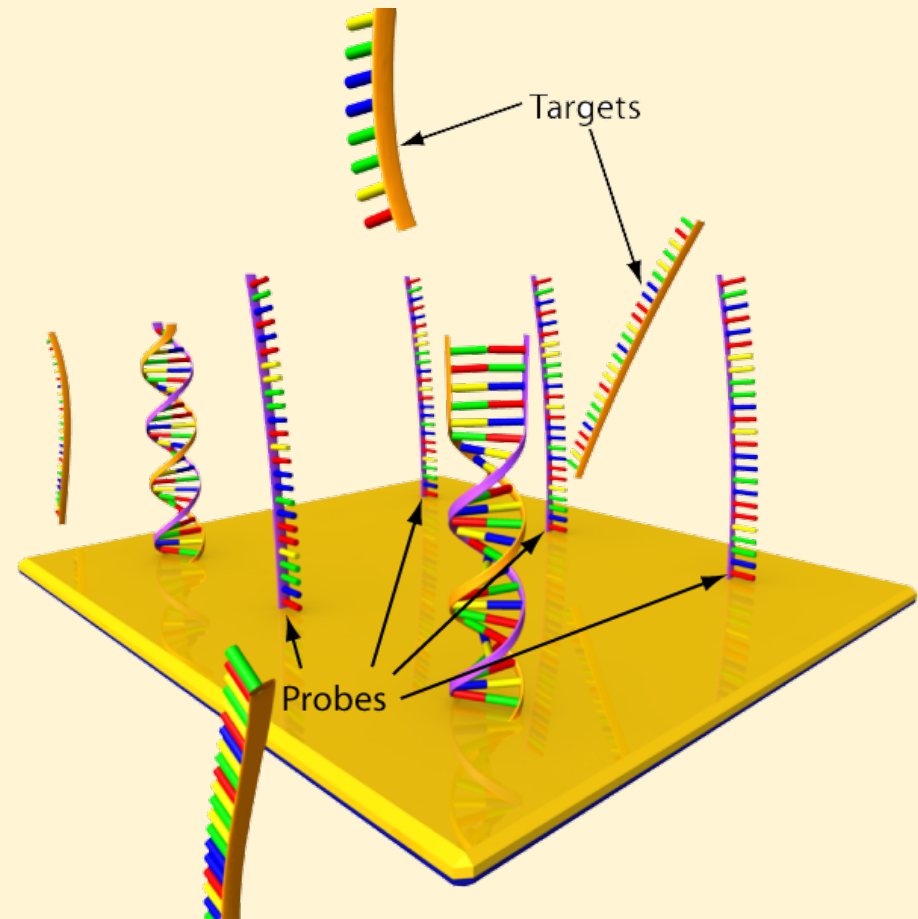
❖ What type of bonds are broken when the dsDNA divides?

❖ What process parameters of the buffered solution allow for hybridization to occur?

❖ Why is the final dsDNA referred to as a hybrid?

❖ (https://youtu.be/0qoqzErrae4)

DNA Hybridization Animation

❖ Open the animation called "DNA_Hybridization"

While watching the animation answer the following questions:

❖ What type of bonds are broken when the dsDNA divides? *Hydrogen*

❖ What process parameters of the buffered solution allow for hybridization to occur? *Cooler temperature and pH and salt concentrations returned to normal.*

❖ Why is the final dsDNA referred to as a hybrid? *The strands of the final dsDNA are from two different sources.*

Activity – DNA Hybridization

- ❖ In your learning modules, locate the DNA Microarray Activity – DNA Hybridization.
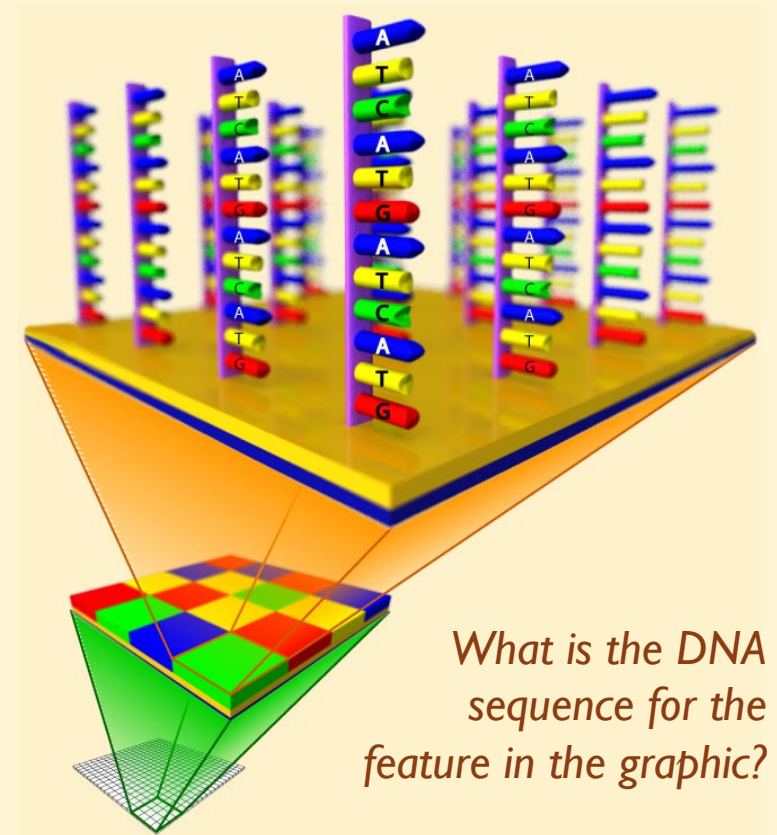
- ❖ Complete this on-line tutorial.

So what exactly is a DNA Microarray?

❖ The DNA microarray relies on cDNA fragments from a sample to hybridize with synthetic ssDNA sequences (specific A, C, G, T combinations).

❖ The synthetic fragments (oligonucleotides or oligos) are the probes.

❖ The cDNA fragments are the targets.

DNA Microarrays

❖ A DNA microarray is a grid on a substrate (e.g., glass, silicon).

❖ Each position in the grid is an "address" or "feature" as small as 200 nm square.

❖ Each feature may contain hundreds or thousands of identical probes (oligos).

❖ Each array may contain tens of thousands of features.

❖ Each feature is looking for a specific gene sequence of nucleotide bases

❖ Thousands of specific genes can be identified simultaneously.

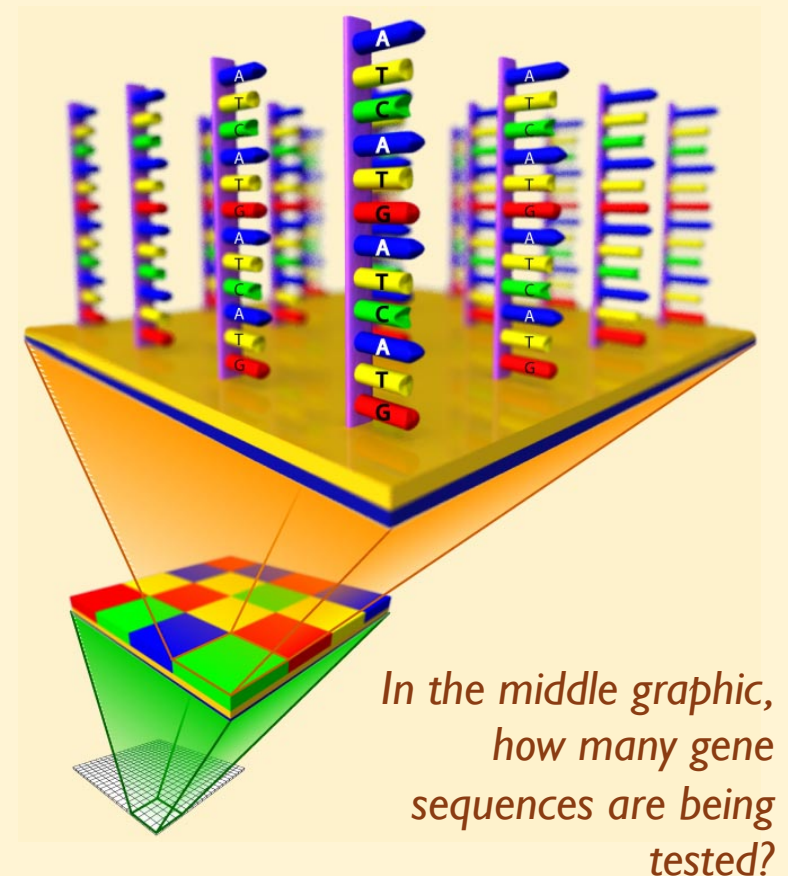*What is the DNA sequence for the feature in the graphic?*

DNA Microarrays

This graphic illustrates one feature of a DNA microarray expanding from many features (bottom grid) to a few features (middle grid), to a single feature (top grid) depicting a unique DNA sequence (G-T-A-C-T-A…).
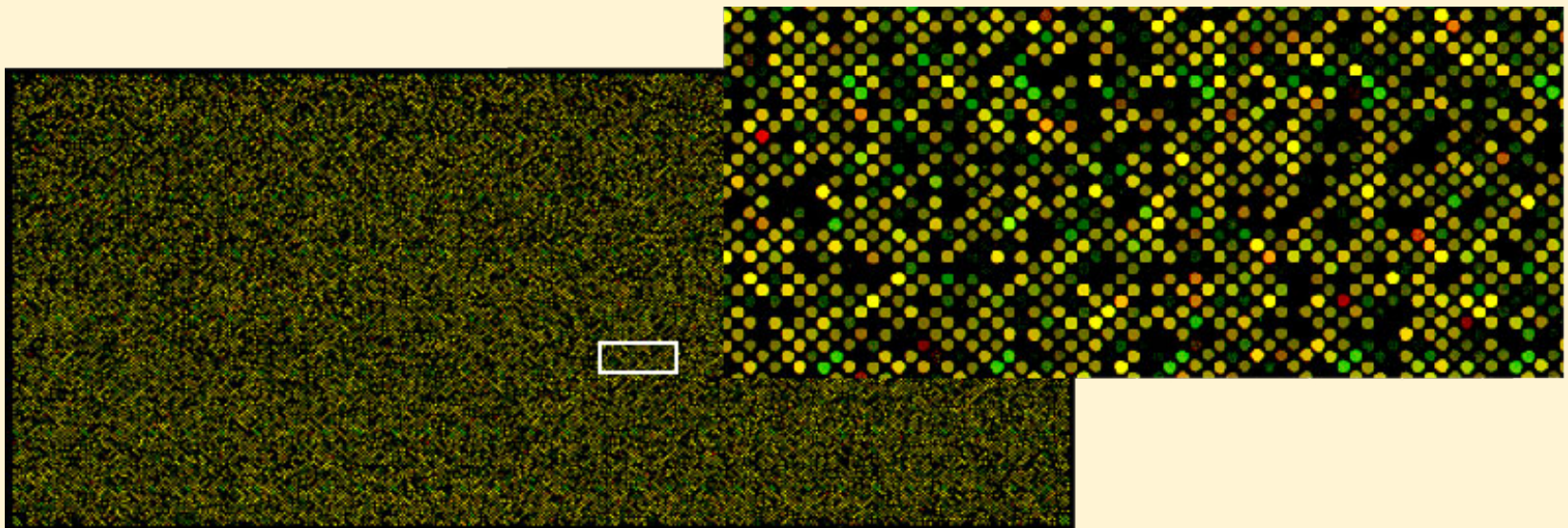
The coloration in this graphic is strictly to illustrate different locations (features) of ssDNA sequences (oligonucleotides) in a DNA microarray.

DNA, and thus a DNA microarray is actually colorless.

In the middle graphic, how many gene sequences are being tested?

DNA Microarrays "chips"

The image shows a DNA microarray with tens of thousands of features (left) and an exploded section (right). Each color dot is one "feature" containing hundreds/thousands of the same oligo probe that, in many features, has hybridized with cDNA from a sample. *[Courtesy of Affymetrix] We'll explain the colors in a later slide.*

DNA Microarray Controls

Controls qualify test results by ensuring the accuracy of the microarray's fabrication and the preparation of the samples.

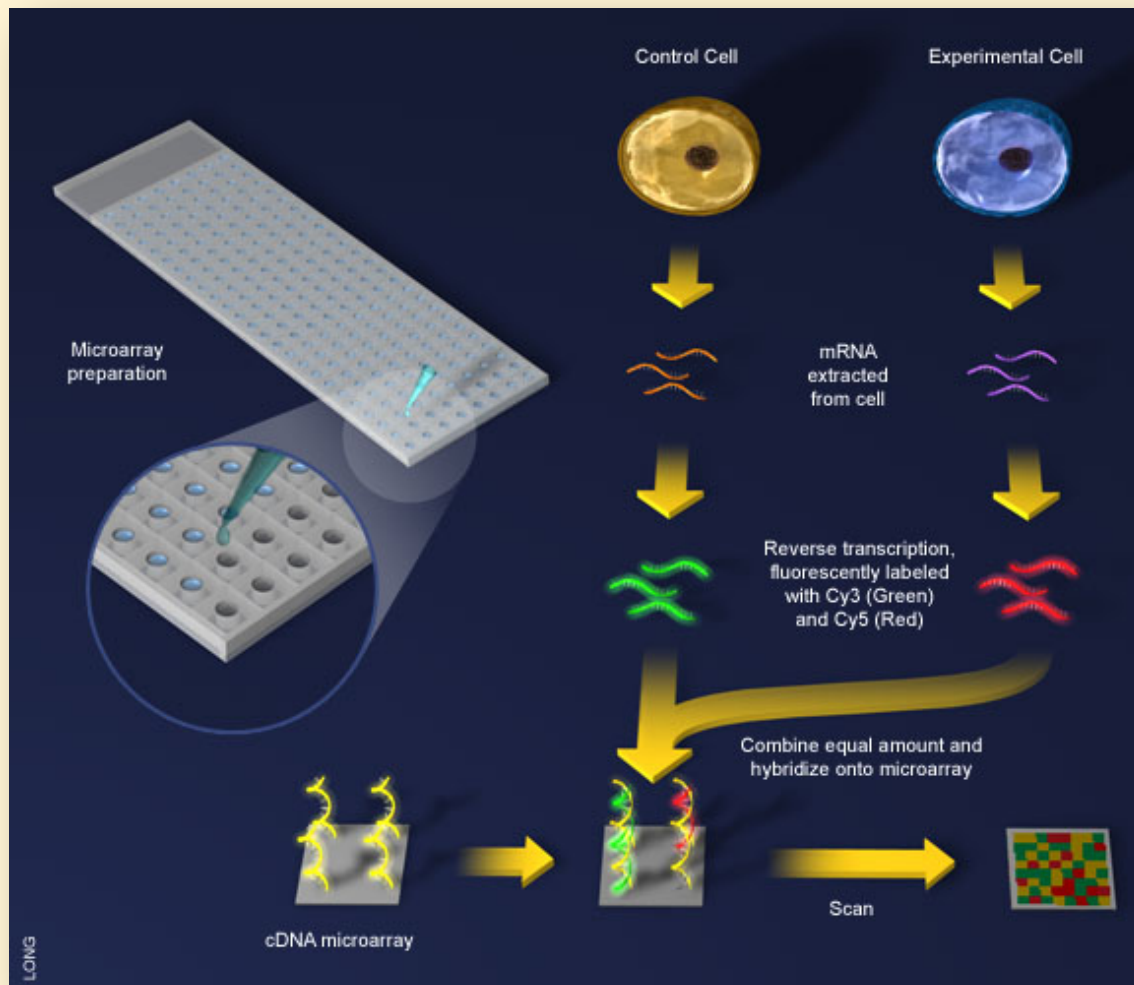Control sample – Set of cDNA from tissue for which the genes are known.

Test sample – Set of cDNA from tissue that is to be analyzed.

Positive control – Feature that must show hybridization with both the control sample and test sample.

Negative control – Feature that must show NO hybridization with cDNA from either sample.

Direct Comparison controls – Each feature of the array is a comparison between the control sample and the test sample.
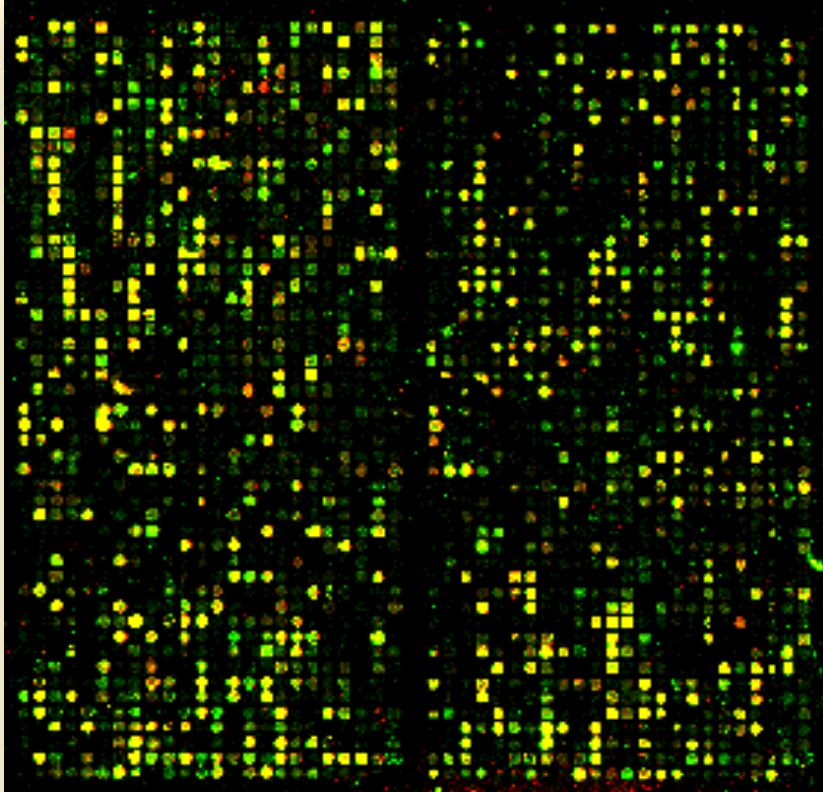
What is a DNA Microarray test?

Start with a "control" and "test" sample (experimental cell).
1. mRNA is extracted from the DNA in each cell.
2. Reverse transcription - cDNA from the mRNA.
3. Each cDNA is fluorescently labeled green (control) and red (test).
4. Samples are combined and washed over the array.
5. Complementary genes hybridize with the synthetic probes on the array.
6. Array is scanned to see the results.

[Courtesy of "The Science Creative Quarterly". scq.ubc.ca. Artist: Jiang Long}

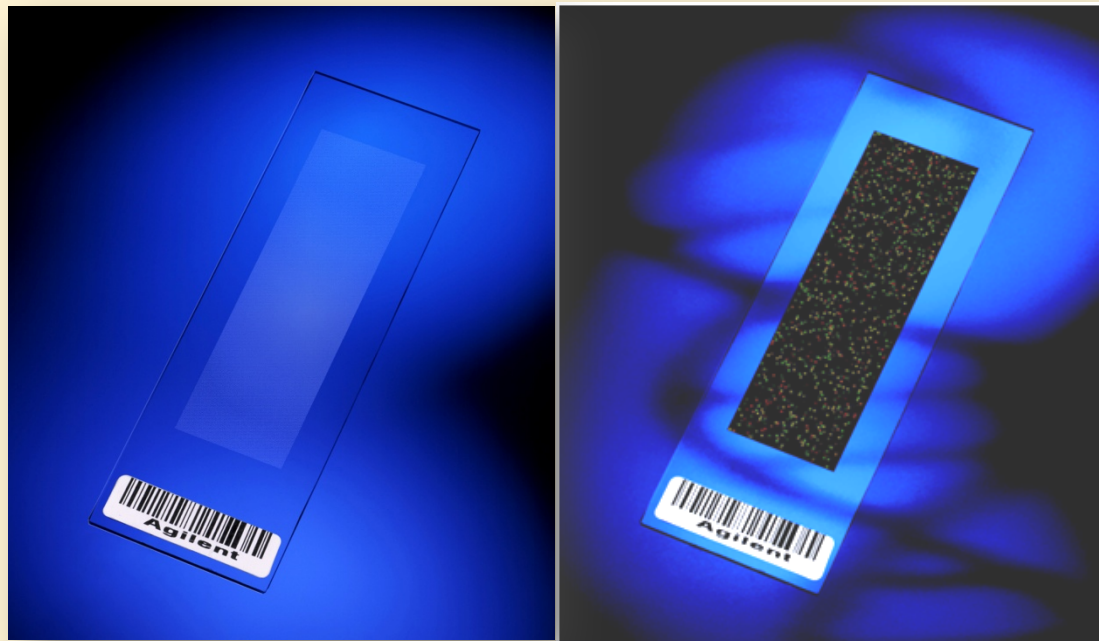# Interpretation of Microarray Results

[Image courtesy of NASA]

Flourescening DNA microarray showing results of DNA hybridization between the probes and target DNAs.

When cDNA is prepared from a test sample (red) and from a control sample (green), and both hybridize with probes, the color of the dot indicates the activity level or the presence of that gene in one or both samples.

❖ Green dot shows activity or presence in the control only

❖ Red shows activity or presence in the test tissue only

❖ Yellow shows activity/presence in both.

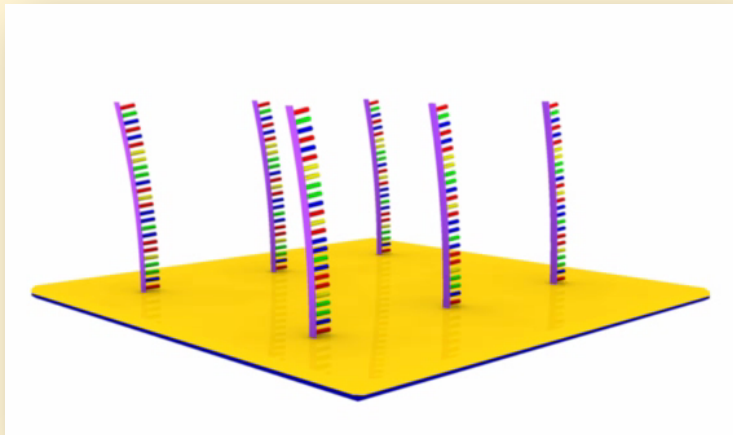❖ Black is activity/presence with neither sample.

DNA Microarray – Before and After

Images show an Agilent Technologies microarray printed on a 1" x 3" glass slide format. Image on the right shows the microarray after hybridization and the fluorescence of hybridization while being scanned with a laser. *[Images courtesy of Agilent Technologies]*

DNA Microarray Test Animation

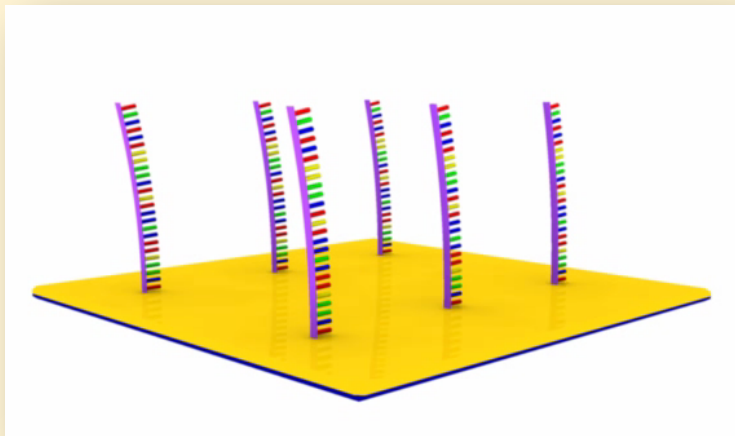❖ Open the animation called "DNA_Microarray"

❖ https://youtu.be/9U-9mlOzoZ8

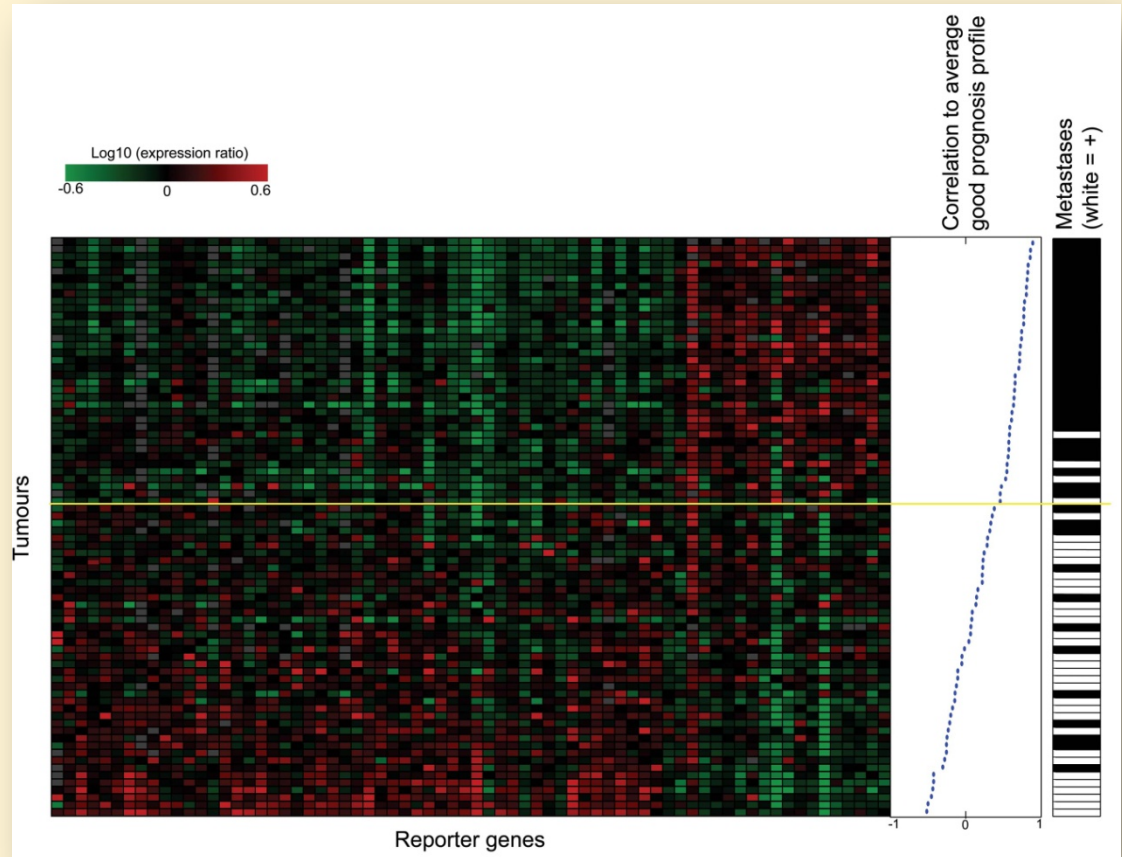

While watching the animation answer the following questions:

❖ The starting single feature is showing hybridization with cDNA from which sample (control or test)?

❖ What do the four colors indicate?

DNA Microarray Test Animation

❖ Open the animation called "DNA_Microarray"

While watching the animation answer the following questions:

❖ The starting single feature is showing hybridization with cDNA from which sample (control or test)? Test sample

❖ What do the four colors indicate? Red (test only), green (control only), yellow (both control and test), black (no hybrids)

Example of DNA Microarray Analysis

A reoccurrence of cancer is partly dependent on the activation and suppression of certain genes located in the tumor.
Prognostic tests like the MammaPrint can measure the activity of these genes and help physicians understand their patient's odds of the cancer spreading."

[Image courtesy of the FDA]

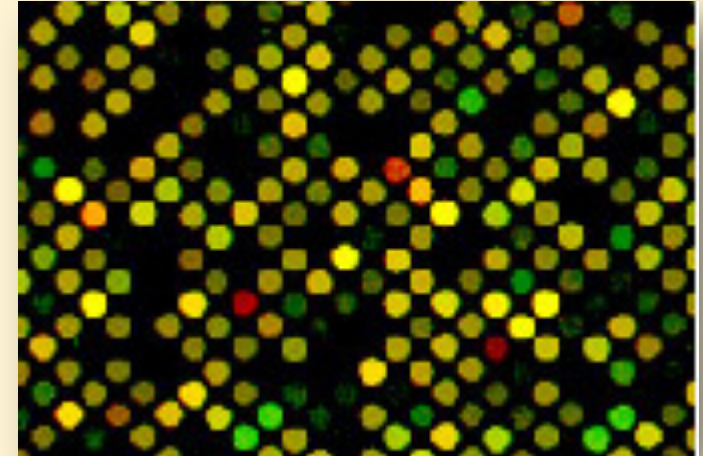# Two basic types of DNA Microarrays

Direct Detection and Gene Expression

Direct detection – detects specific genes or gene mutations in a sample. *(RED identifies a specific gene in test sample but not in control sample)*

Applications:

❖ Medical – Identify a gene or gene mutation that may cause a specific disease or genetic disorder, and identify DNA-based drugs.

❖ Forensics

❖ Genotyping

SNP (single nucleotide polymorphisms) chips are a type of direct detection microarray
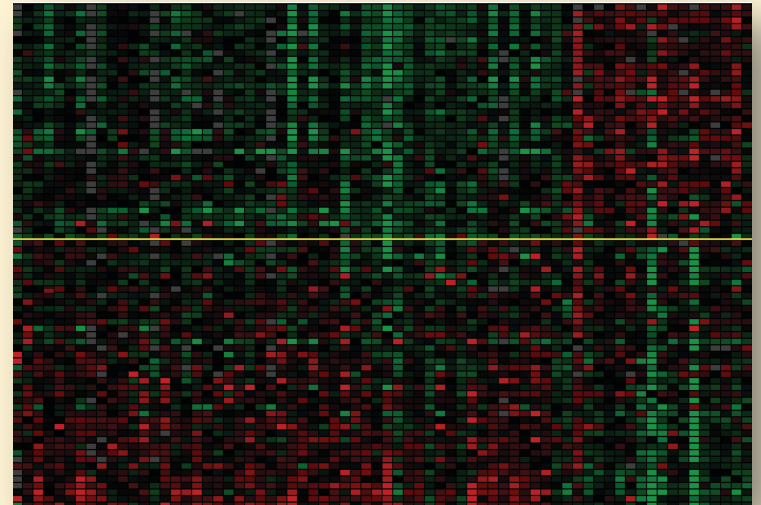
Two basic types of DNA Microarrays

Gene Expression Mircoarrays

Create gene expression profiles by detecting "expression levels" in a sample (when mRNA copies to cDNA (i.e., which genes are "active" or "inactive".))

Each of your cells contain the exact same genes, but different genes may be active in different cells. Gene expression arrays identify which genes are active and inactive.

They detect how cells and organisms change and adapt to stimuli (e.g., changes in the environment or a disease state.

The MammaPrint (discussed in a previous slide) is an example of a gene expression array. It provides a profile of the activity of breast cancer related genes within different patients.
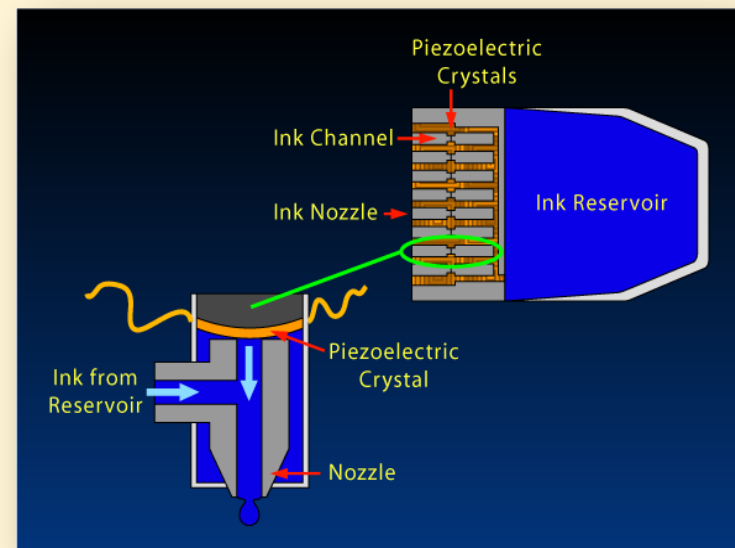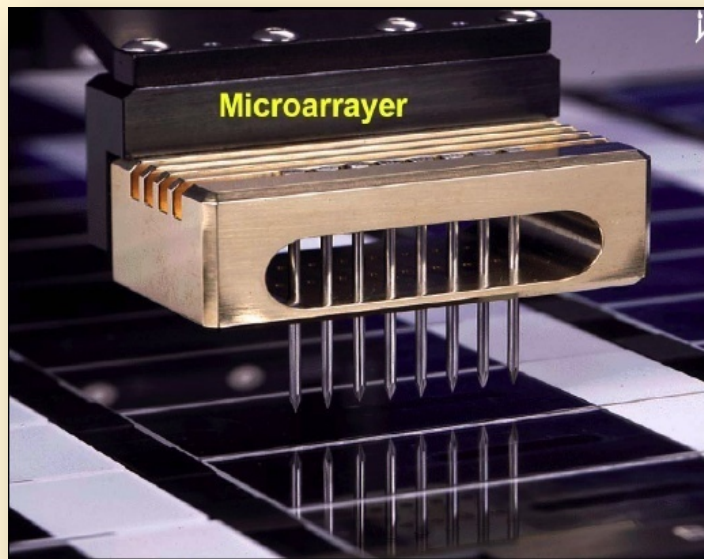
DNA Microarray Fabrication

So how on earth do we fabricate a DNA microarray?
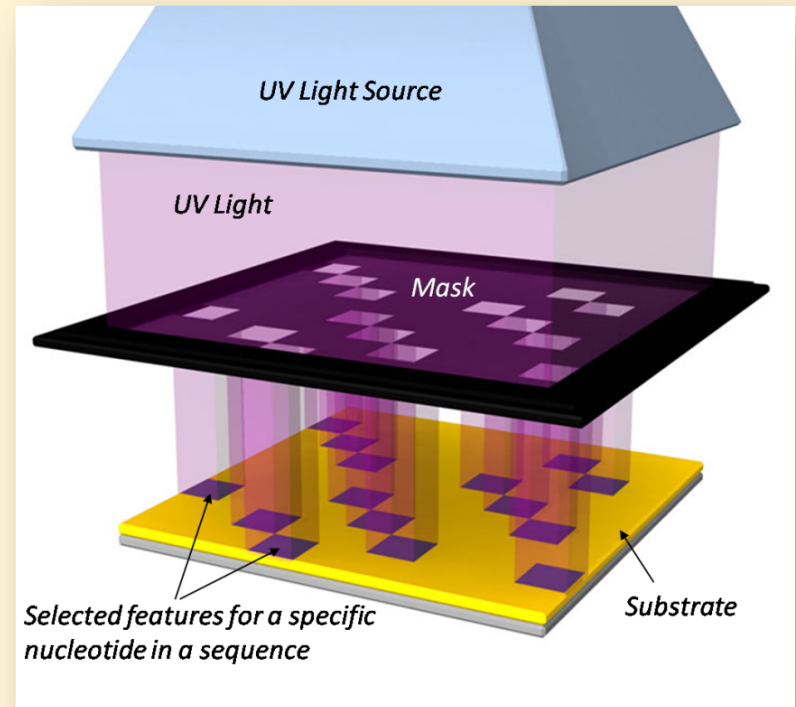
DNA Microarray Fabrication

One type of microarray fabrication technique uses a printer similar to an ink-jet printhead (a micro-size device) to print the addresses onto the microarray slide. However, the "ink" is an solution with millions of a single nucleotide. There are four "ink" reservoirs, each containing a different nucleotide. The printhead deposits nucleotides at a specific location time, creating the desired nucleotide sequences (or oligonucleotides).

DNA Microarray Fabrication

A photolithography process along with chemical reactions between silicon and a nucleotide, and between different nucleotides.

❖ A patterned mask and ultraviolet (UV) light "expose" specific nano-size features of the microarray. Each feature is a location for identical oligonucleotides or nucleotide sequences.

❖ The graphic shows the UV light, mask, and substrate.

❖ Each square in the mask is a select feature or address in the array.

❖ Inside each feature are hundreds/thousands of silicon molecules that "link" with the initial nucleotides.

This is the method that we will explore further in the GeneChip® Model Activity. This method was developed by Affymetrix and is used to fabricate synthetic oligonucleotides on a silicon substrate.

Food for Thought

The DNA microarray may be small, but when it comes to career potential, it's huge!

❖ Discuss at least three applications or potential applications of DNA microarrays.

❖ How does a DNA microarray identify a target DNA?

❖ What are some of the careers that one might look into that involve the use of or the fabrication of DNA microarrays?

Summary

The DNA microarray has opened up a whole new frontier for exploration in medical research, drug development, forensics, toxicology, and food production, just to name a few.

All of the information derived from DNA microarrays affect us all in one way or another.

Through the hybridization of synthetic oligos and target DNA molecules we can identify the presence of specific genes, mutation and pathogens.

We are getting closer and closer to knowing what makes us tick, what makes us sick and what can make us well.

Acknowledgements

Website: www.scme-nm.org

DNA MICROARRAY MODEL ACTIVITY

Activity Description

In this activity you study how DNA (Deoxyribonucleic acid) microarrays are fabricated using a photolithography process developed by the semiconductor manufacturing industry.

You then apply this knowledge to building a macro-size DNA microarray model.

This activity should improve your understanding of how DNA microarrays are made and how they identify complementary single-stranded DNA (ssDNA) in a sample.
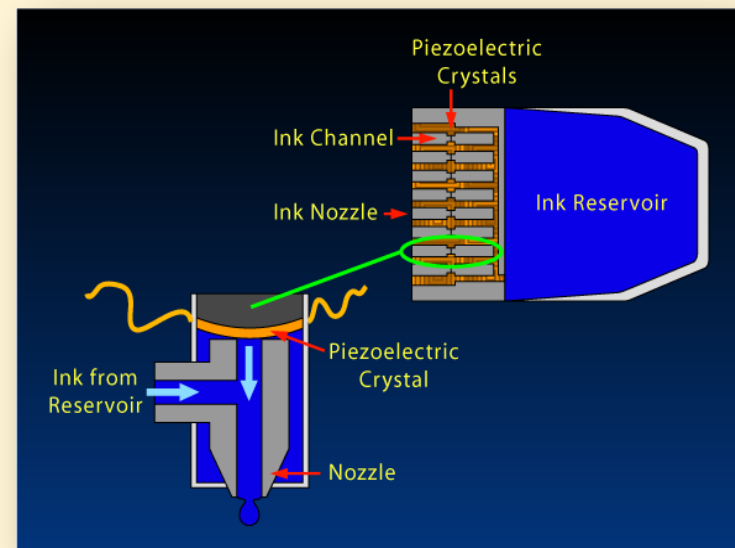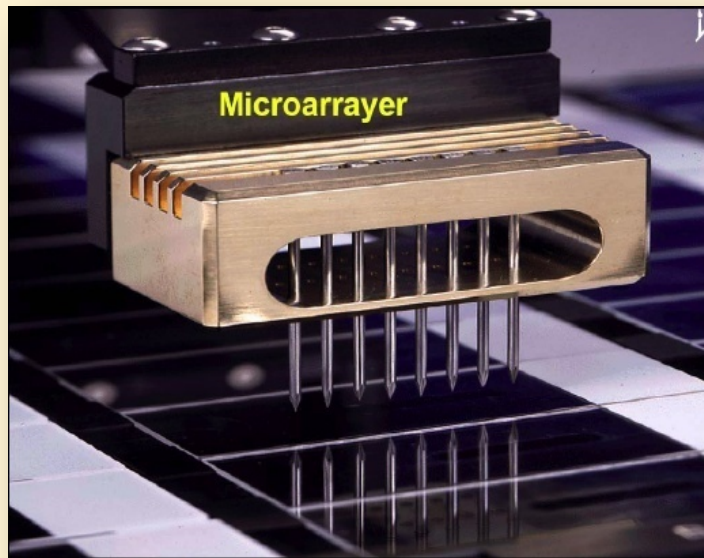
Activity Objectives and Outcomes

❖ Using the components provided in a SCME DNA Microarray kit, build a macro-size DNA microarray with a three (3) nucleotide sequence.

❖ Outline and explain the fabrication steps for an oligonucleotide array or GeneChip®.

At the end of this activity, you will be able to answer the following questions:

❖ How are oligonucleotides used in a DNA microarray?

❖ How are synthetic oligonucleotides fabricated on a DNA microarray?

❖ How does a DNA microarray identify different target molecules simultaneously?

DNA Microarray Fabrication

One type of microarray fabrication technique uses a printer similar to an ink-jet printhead (a micro-size device) to print the addresses onto the microarray slide. However, the "ink" is an oligonucleotide solution that deposits one nano-size nucleotide at a time, creating the desired nucleotide sequences (or oligonucleotides).

Diagnostic Microarrays

Let's watch a video showing the robotic printing of DNA microarrays:

"[Diagnostic Microarrays](#)"

Note that this video was produced in 2007 when it was possible to test for "thousands" of diseases simultaneously. Now we can test for "tens of thousands" using microarrays with one to two million features on one slide!

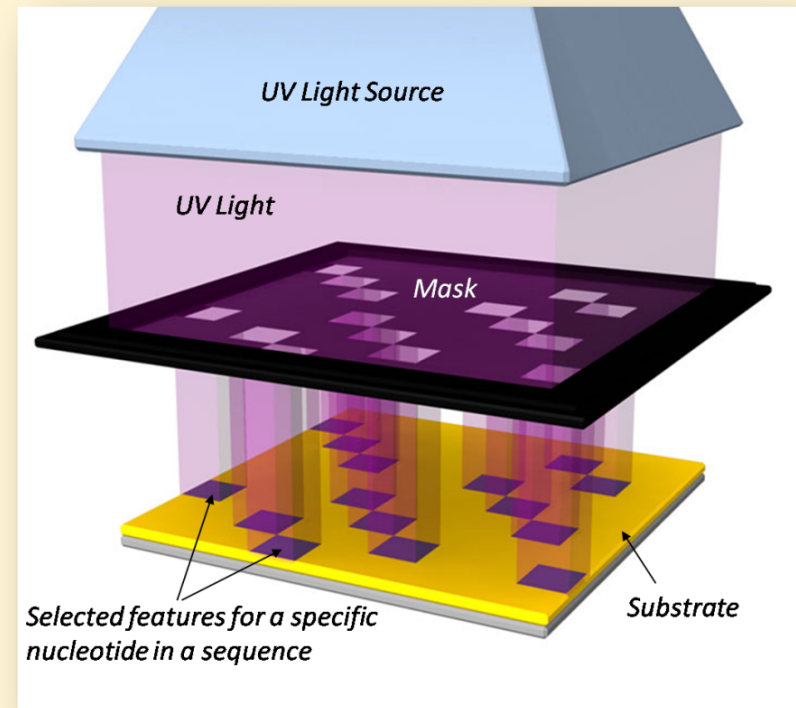We've come a long way in a relatively short period of time!

DNA Microarray Fabrication

❖ The inkjet printing of a DNA microarray is very laborious due to all of the oligonucleotide "inks" that must be prepared for each of the addresses on the microarray.

❖ These oligos are synthesized by chemical methods, one at a time, or are prepared enzymatically by a reverse transcription of mRNA isolated from cells to copy DNA or cDNA.

❖ Alternatively, a photolithography method uses the photolithography process borrowed from the semiconductor fabrication industry in combination with chemical reactions to synthesize oligonucleotide (oligo) probes on a silicon surface.

❖ The oligos on these arrays are generally 20 to 25 nucleotides long and each feature in the array itself may be as small as 50 nm square – almost 2000 times smaller than the width of a strand of hair!

Microarray Fabrication - Photolithography

This method duplicates the photolithography process used in microfabrication along chemical reactions between silicon and a nucleotide, and between different nucleotides.

❖ A patterned mask and ultraviolet (UV) light "expose" specific nano-size features of the microarray. Each feature is a location for several nucleotides of the same sequence.

❖ The graphic illustrates the UV light, mask, and substrate.

❖ Each square in the mask is a select feature or address in the array.

❖ Inside each feature are several silicon molecules that will "link" with the initial nucleotides.
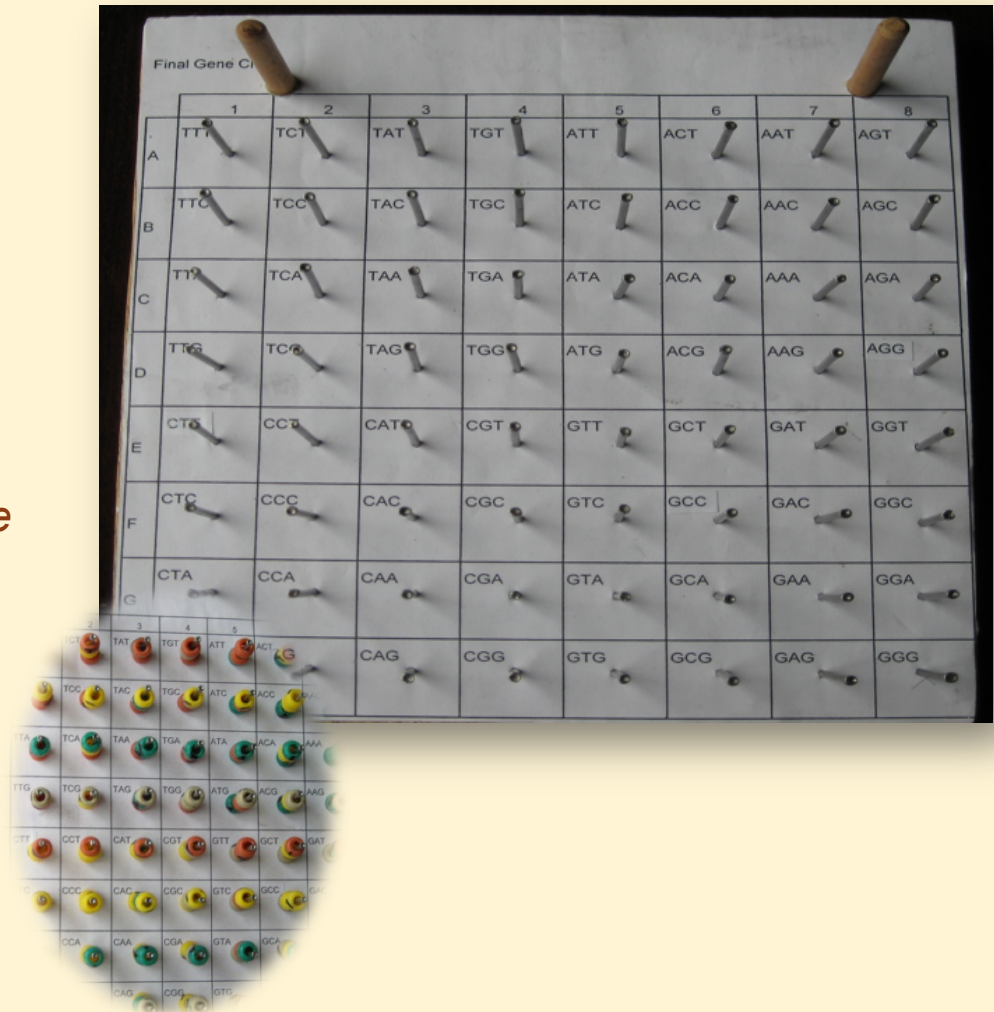

This is the method that we will explore in the GeneChip® Model Activity. This method was developed by Affymetrix and is used to fabricate synthetic oligonucleotides on a silicon substrate.

DNA Microarray Model Activity

So let's step through activity materials and fabrication process together.

The board simulates an array on a silicon substrate. *(Yours may look a little different from the one in the picture.)*
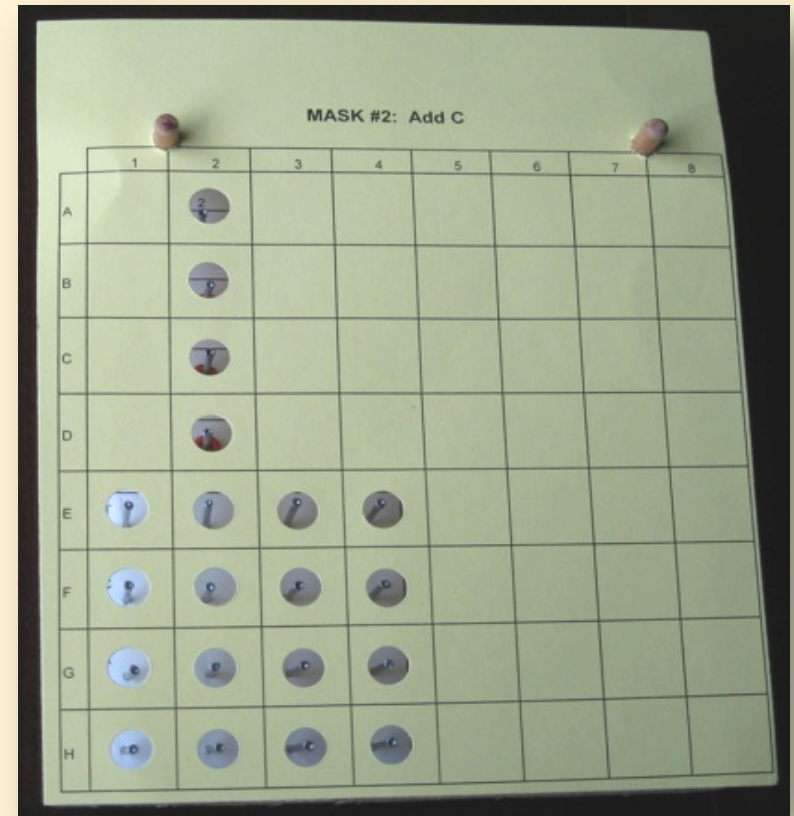
By the end of this activity, each feature in the array will have an oligo of 3 nucleotides.
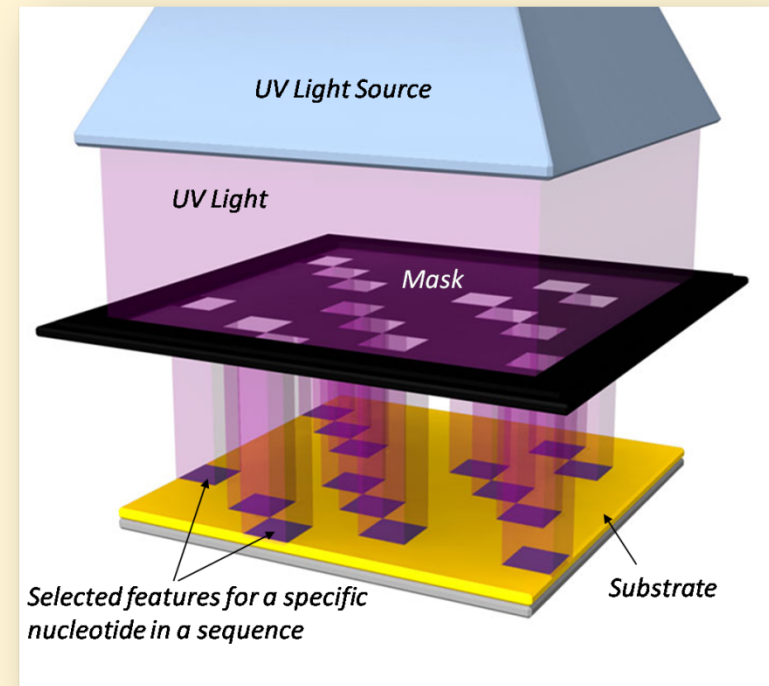
DNA Microarray Model Activity

In this activity you will fabricate a 3 nucleotide oligo (nucleotide sequence) using 12 masks.

❖ Each mask identifies the locations for one of four nucleotides (A, C, T, or G) with a hole or opening.

❖ Colored beads are used for each nucleotide. Refer to the top of your substrate (board) for the color of bead vs. the specific nucleotide.

Fabrication of a Oligonucleotide Array

❖ The light that travels through these holes initiates the growth of a nucleotide chain through a process of deprotection and addition of new bases into specific chains / probes.

❖ The chain begins with a nucleotide linking to an unprotected silicon molecule on the surface of the substrate.

❖ The mask is therefore the tool that ultimately controls the building of the oligo probes.

UV Light Source

UV Light

Mask

Selected features for a specific nucleotide in a sequence

Substrate

Three Step Process

❖ <u>Protect</u> – Initially, a blocking compound is washed over the entire silicon wafer. In subsequent steps, the blocking compound is already attached to the nucleotides in the *Addition* step.

❖ <u>Deprotect (Photolithography)</u> – UV light through the "holes" in the mask, removes the blocking compound, "deprotecting" that area.

❖ <u>Addition</u> – The substrate is washed with a solution of the specific nucleotide being added at that step. The nucleotides in the solution attach to the deprotected areas on the wafer.

❖ This cycle is repeated for each new nucleotide.

"Genechip" Animations

❖ Let's take a look at a couple of animations.

GeneChip Animation on YouTube

DNA Microarray by Affymetrix

Let's to do it!

❖ Are you ready?

❖ Let's build a three nucleotide sequence DNA microarray!

DNA Microarray Model Activity (Fabrication)

1. Set up your station with your substrate, beads (nucleotides), and mask (cards with the pretty flowered holes).

2. Using mask 1, align it over the substrate using the 2 alignment pegs at the top of the board.

3. Mask 1 is for the T nucleotides. Drop a T bead through each hole and onto a nail. (You have just "exposed for" and "added" a T nucleotide.

4. Remove the Mask 1. Align Mask 2 for the C nucleotide.

5. Add C to all of the deprotected areas.

6. Continue this process through all 12 masks.

7. On the array table in your activity, write the oligo for each feature.
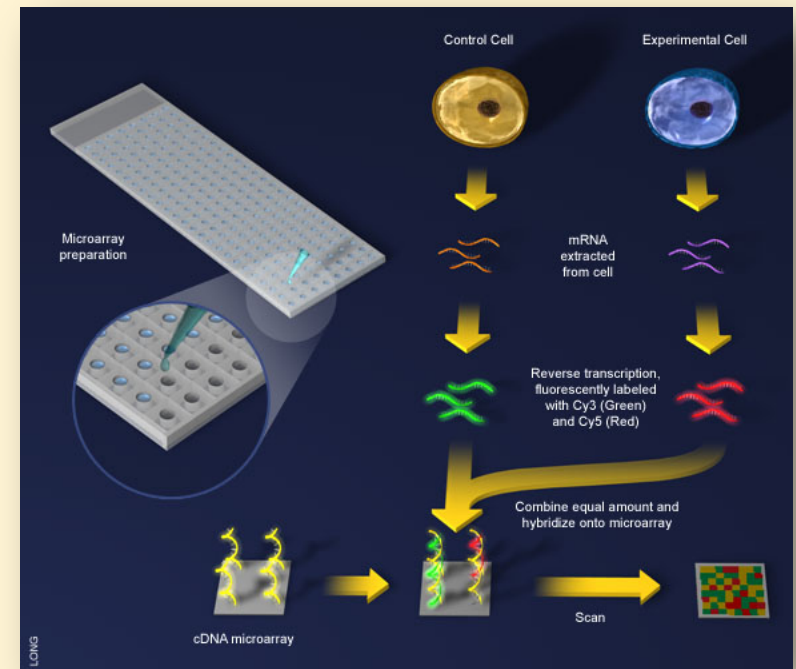
DNA Microarray Model Activity (Interpretation)

Remember this graphic?

Ask your instructor for a sample bag. In your bag are fluorescently labeled nucleotides (red or green sequin with 3 beads).

These are cDNA targets from a control cell and test cell. Which is which?

Match your targets with the oligo probes on your array.

Which genes are detected in the control and in the target?

DNA Microarray Model Activity

❖ Complete the Post-Activity Questions for each part of this activity.

❖ Discuss you answers with you team mates to ensure that everyone understands the concepts explored in this activity.

Summary

❖ DNA microarray fabrication requires the construction of thousands or millions of oligonucleotides within the features of an array.

❖ One fabrication process uses the technology of the inkjet printer while another process uses photolithography.

❖ The photolithography process results in synthetic oligos built one nucleotide at a time using specially designed masks and UV light.

❖ Each of these processes has the ability to construct large microarrays capable of identifying thousands of different genes simultaneously.

Acknowledgements

Website: www.scme-nm.org

Revised May 2018