True/False:

Muscle cells have the same genes as intestinal lining cells

True/False:

Muscle cells make the same proteins as intestinal lining cells.

True/False

Muscle cells always make the same amounts of each protein.

True/False:

There are some proteins that are never made in muscle cells.

Objective: The student will be able to simulate how a microarray works

Agenda: Warm Up
Microarray Background Info
Microarray Paper Simulation

Homework: Complete ALL questions on BOTH Packets

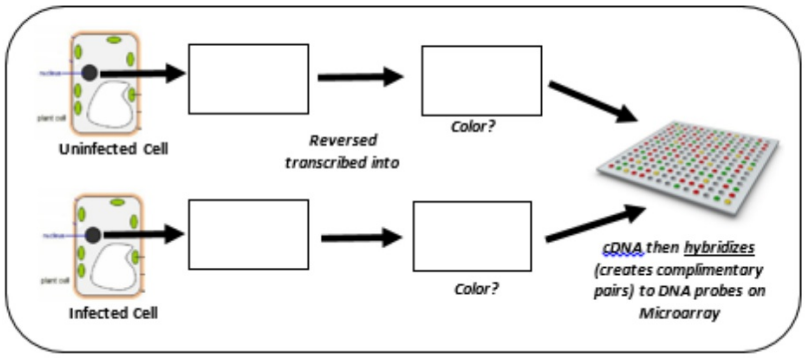
Big Lab on Tuesday

Open Notes Lab Evaluation on Thursday...All Packets must be done.

Name: _____ Period: _____

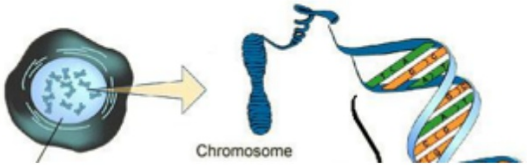
Microarray Background Information
Capture Sheet

Use the video clip to fill in the flowchart below.



A Review of Protein Synthesis

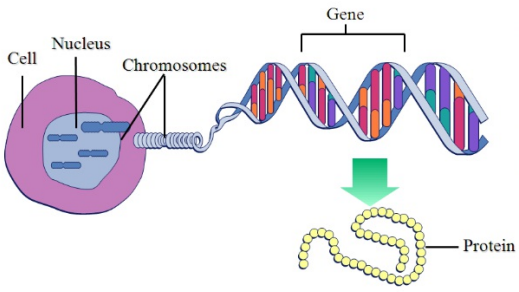
DNA is contained in the nucleus and coiled into chromosomes. In humans, each chromosome contains about 1000 genes. Genes code for proteins which give organisms their characteristics or traits.

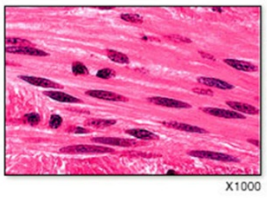


Get This Paper Out.....

Introduction to Microarray

How can we actually study what genes are being expressed in a cell, such as a muscle cell or skin cell or blood cell?



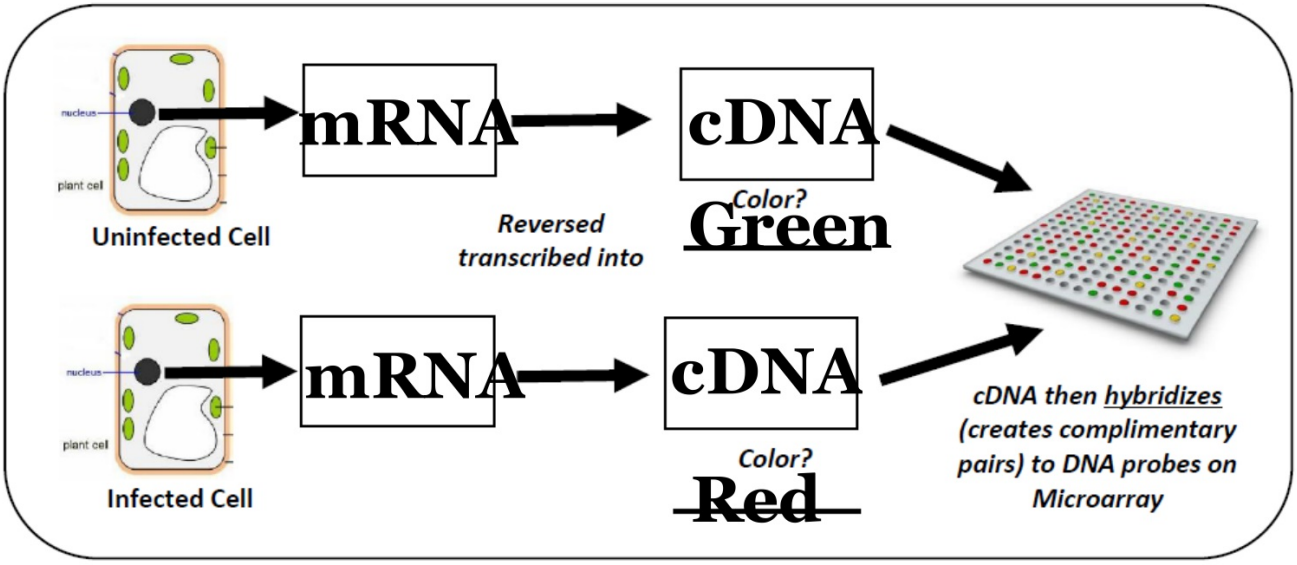


Microarray Background Information

Capture Sheet

Watch Video Clip: Introduction to Microarray (1:30 min.)

Use the video clip to fill in the flowchart below.

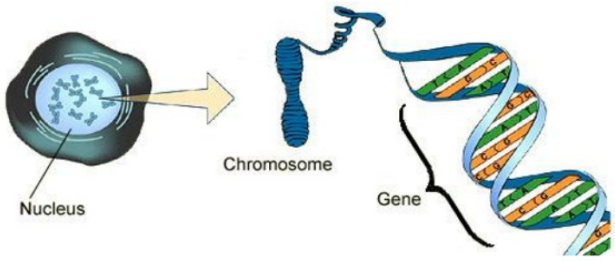


Word Bank for boxes above:

Read the "A Review of Protein Synthesis" and "How Microarrays are made." portion of *The Microarray Background Information* capture sheet **individually** and **answer the questions within the reading**.

A Review of Protein Synthesis

DNA is contained in the nucleus and coiled into chromosomes. In humans, each chromosome contains about 1000 genes. Genes code for proteins which give organisms their characteristics or traits.

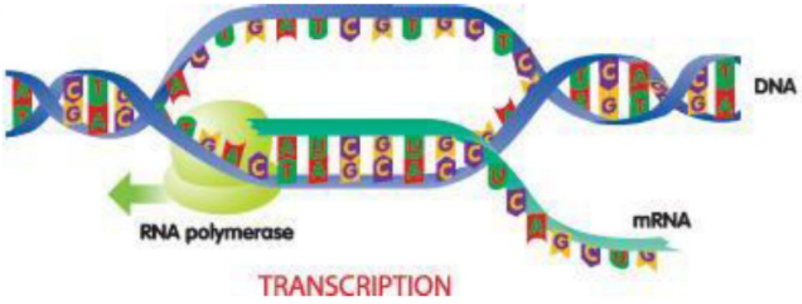


1. What do genes do?

2. Where is DNA located?

Introduction to Microarray

During gene expression, a segment (part) of DNA called a gene, separates and is copied into mRNA. This process is called transcription. The amount of mRNA copied indicates how much of the protein is needed by the cell. Only the proteins required for a particular cell to function will be transcribed. A heart cell will only make the proteins necessary for a heart cell to function, even though every cell holds the information to make all the genes for every protein in its' genome.



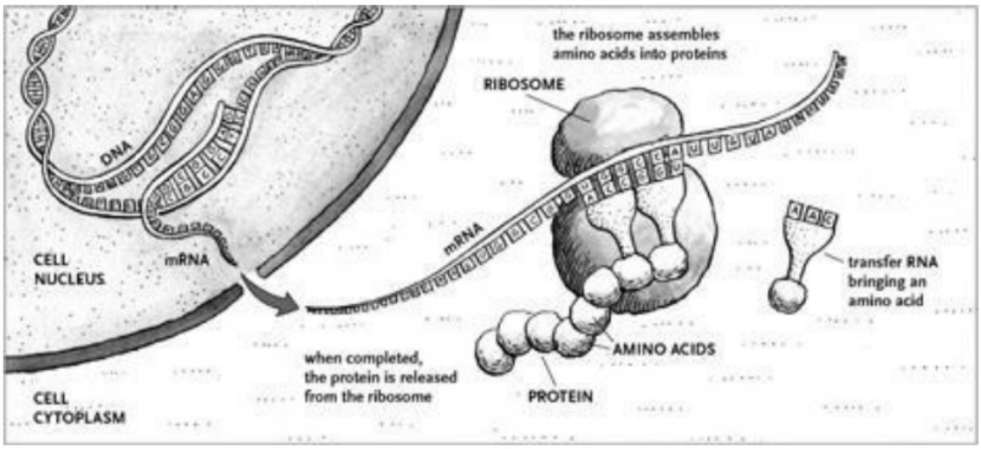
TRANSCRIPTION

3. What is transcription?

4. Why don't all cells make all proteins?

Introduction to Microarray

After the mRNA is made it leaves the nucleus and goes to the ribosome where the mRNA matches up with tRNA to construct the polypeptide chain or protein. This process is called translation. Transcription and translation are the two processes of protein synthesis.



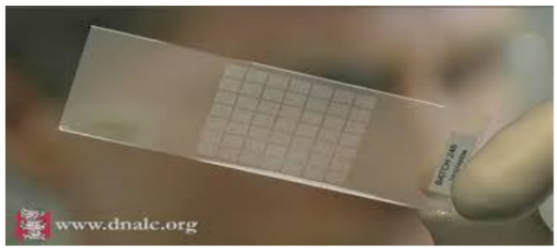
5. Where are proteins made?

6. What processes make up protein synthesis?

Introduction to Microarray

How Microarrays are Made

If a cell is expressing a gene to make a protein then it has to make mRNA. Scientists use this cellular information to study which genes are being expressed by measuring the amount of mRNA present in a cell. This tool is known as a **microarray**, which allows a scientist to measure the gene activity in a cell all at one time.



A microarray slide can hold an organisms' entire genome with small sections of DNA from every gene attached to a section or circle on the slide. These attached sections of genes are called probes. They are single-stranded DNA, so they are ready to pair up with their complimentary strands.

7. What must a cell make in order to make a protein?

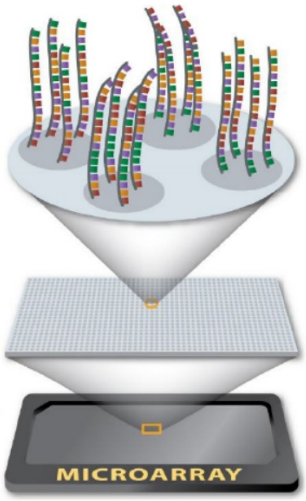
8. What does a microarray measure?

9. What is a DNA probe? What is unique about a DNA probe compared to normal DNA?

Introduction to Microarray

If a person has cancer, a doctor can take mRNA from both their cancer cells and healthy cells in their body. The mRNA taken from both the healthy and cancerous cells is converted to cDNA (complimentary DNA) and tagged with a fluorescent dye. The healthy cells' cDNA is tagged with a green dye and will fluoresce a bright green spot if a gene is being expressed. The cancerous cells cDNA is tagged with a red dye and will show a red spot if a gene from the cancer cell is expressed.

The microarray with the DNA probes is then "washed" with the tagged cDNA and the probes may pair up with the cDNA. If the same gene is expressed in both cells, the spot will glow yellow. If the gene is not expressed in either cell, the spot will appear black. Therefore a microarray slide will show green, red, yellow and black spots, based on what genes are making mRNA, in order to create a protein.



9. Why is mRNA extracted from both healthy and cancerous cells?

10. Microarrays will show green, red, yellow and black spots. What does each color represent?

- Green:
- Red:
- Yellow:
- Black:

Get Out This Worksheet

Microarray Paper Simulation

1. In this lab, we will study gene expression (making mRNA) in skin cancer cells as compared to those in normal, healthy skin cells. Complimentary DNA or cDNA is made from the mRNA of cancer cells and will be labeled red, and the cDNA made from the mRNA from normal cells will be labeled blue. If neither cell is expressing a gene which means it's not making mRNA for that gene, then the spot shows black. What three colors are seen in most microarrays used in scientific research? What type of cells are you studying?

2. If the cDNAs made from the cancer cells' mRNA are labeled red, and the cDNAs made from the normal cells' mRNA are labeled blue, for each of the situations below, describe what color you expect the gene spot to be on a microarray; red, blue, or purple.

GENE DESCRIPTION	COLOR OF SPOT
A gene was expressed (making mRNA) more in cancer cells than in normal cells.	
A gene was expressed the same in both cells.	
A gene wasn't expressed at all in either cell.	
A gene was expressed (making mRNA) more in normal cells than in cancer cells.	

In this lab, we will study gene expression (making mRNA) in **skin cancer cells** as compared to those in normal, **healthy skin cells**. Complimentary DNA or cDNA is made from the mRNA of cancer cells and will be labeled **red**, and the cDNA made from the mRNA from normal cells will be labeled **blue**. If neither cell is expressing a gene which means it's not making mRNA for that gene, then the spot shows **clear**.

- **What three colors are seen in most microarrays used in scientific research (shown in the video)?**

- **What three colors are seen in most microarrays used in in our paper simulation?**

- **What type of cells are you studying?**

Skin

Microarray Paper Simulation

If the cDNAs made from the cancer cells' mRNA are labeled red, and the cDNAs made from the normal cells' mRNA are labeled blue, for each of the situations below, describe what color you expect the gene spot to be on a microarray; red, blue, or purple.

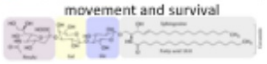
GENE DESCRIPTION	COLOR OF SPOT
A gene was expressed (making mRNA) more in cancer cells than in normal cells.	**Red**
A gene was expressed the same in both cells.	**Purple**
A gene wasn't expressed at all in either cell.	**Clear**
A gene was expressed (making mRNA) more in normal cells than in cancer cells.	**Blue**

Microarray Paper Simulation

Read the description of the 6 genes we will be measuring. Write a simplified function on the next page.

Gene	Definition
'53 Gene vn as **TP53** or **tumor**	This gene codes for a protein that controls the cell cycle and functions as a tumor suppressor. Therefore it keeps tumors or cancers from forming or growing too big.
Brain-derived **phic** vn as **BDNF**	This protein acts on certain nerve cells of the central nervous system, helping to support the survival of existing neurons, and encourage the growth and of new neurons or nerve cells.
Cytochrome C	Cytochrome c is primarily known as an electron-carrying mitochondrial protein. It is known as a catalyst of respiration, forming reactions between the respiration molecules and oxygen.
Adenomatous **s coli (APC)**	*APC* is classified as a tumor suppressor gene. Tumor suppressor genes prevent the uncontrolled growth of cells that may result in cancerous tumors.
actosylceramide **3 -** **nsferase- ST3GAL5**	Ganglioside GM3 helps create lipids used for cell to cell messaging for cell growth and division, transportation and survival.
Ras- p21/Ras	Ras is the name given to a family of related proteins found inside cells and are involved in transmitting signals within cells. When Ras is 'switched on' by incoming signals, it then switches on other proteins, and those proteins turn on genes involved in cell growth, differentiation and survival. As a result, mutations in Ras genes can lead to the production of permanently activated Ras proteins, meaning they are always turned on.

Gene	Simplified Function
Gene 1. P53 Gene also known as **TP53** or **tumor protein**	**Tumor Suppresso**
Gene 2. Brain-derived neurotrophic also known as **BDNF**	**Helps neurons gr**
Gene 3. Cytochrome C	**Makes Energy**
Gene 4. Adenomatous polyposis coli (APC)	**Tumor Suppress**
Gene 5. Lactosylceramide alpha- 2, 3 - sialyltransferase- ST3GAL5	**Cell Growth**
Gene 6. Ras- p21/Ras	**Cell Division**

Gene 1: P53 Gene	Helps produce lipids for cell division, movement and survival 
Gene 2: Brain-derived neurotrophic	Starts cellular respiration 
Gene 3: Cytochrome C	Helps neurons to survive and grow 
Gene 4: Adenomatous polyposis coli (APC)	Sends messages to turn on genes (It is the "accelerator" for cell division) 
Gene 5: Lactosylceramide alpha- 2, 3 -sialyltransferase- ST3GAL5	Prevents tumor growth (It is the "brakes" for cell division) 
Gene 6: Ras- p21/Ras	Prevents tumor growth (It is the "brakes" for cell division) 

Flip to the last page of your packet.

Match the gene on the left with the simplified function on the right.

Microarray Paper Simulation

Gene 1:
P53 Gene

Gene 2:
Brain-derived neurotrophic

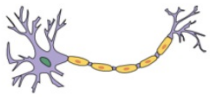
Gene 3:
Cytochrome C

Gene 4:
Adenomatous polyposis coli (APC)

Gene 5:
Lactosylceramide alpha-2, 3 -sialyltransferase-ST3GAL5

Gene 6:
Ras- p21/Ras

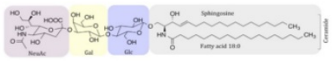
Helps neurons to survive and grow



Prevents tumor growth
(It is the "brakes" for cell division)

Starts cellular respiration

Helps produce lipids for cell division, movement and survival



Sends messages to turn on genes
(It is the "accelerator" for cell division)

Prevents tumor growth
(It is the "brakes" for cell division)

Now, predict which of the two cells (cancerous and non-cancerous) will express the 6 genes on your capture sheet.

Gene	Simplified Function	Prediction: Do you think the two cells will express this gene?		Color
		Cancer	Non-Cancer	
Gene 1. P53 Gene also known as TP53 or tumor protein	Tumor Suppressor		X	Blue
Gene 2. Brain-derived neurotrophic also known as BDNF	Helps neurons grow			Clear
Gene 3. Cytochrome C	Makes Energy	X	X	Purple
Gene 4. Adenomatous polyposis coli (APC)	Tumor Suppressor		X	Blue
Gene 5. Lactosylceramide alpha- 2, 3 - sialyltransferase- ST3GAL5	Cell Growth	X	X	Purple
Gene 6. Ras- p21/Ras	Cell Division	X		Red

Red= expressed only in cancer cells

Blue= expressed only in healthy cells

Purple= expressed in Both cells

Clear= not expressed in these skin cells

Microarray Paper Simulation

Simulation Set-Up

You have a microarray with six circles with a single-stranded DNA probe. A DNA probe is a DNA sequence that codes for one of the six genes listed above. Each of the six probes may have complimentary matches to the cDNA. If the two DNA strands are complimentary, hybridization occurs and the cDNA attaches to the DNA probe.

A cancer patient has both normal and cancerous cells removed. The mRNA is extracted from both cells and made into cDNA. The cDNA from the cancer cells are tagged with a RED fluorescent colored dye and the normal cells cDNA are tagged with a BLUE fluorescent colored dye.

Write the complementary sequence below the single strand

A T C G

T A G C

Procedure

1) Work in a Group of 2. One person compare all of the red DNA (from a cancer cell) and one person compare all of the blue DNA (from a healthy cell). Match the strand with the complementary sequence you wrote out.

2) Pick up a red or blue piece of cDNA. Hold it against the first gene. Start with bases (letters) matching at the left side. Move it along to the right to see if it matches. If it matches keep it on the strand. If it doesn't try it on the next gene.

It is possible that multiple strands of DNA will match a gene or no strands of DNA will match a gene.

3) Repeat this process with all of the blue and/or red DNA pieces.

Gene 1:

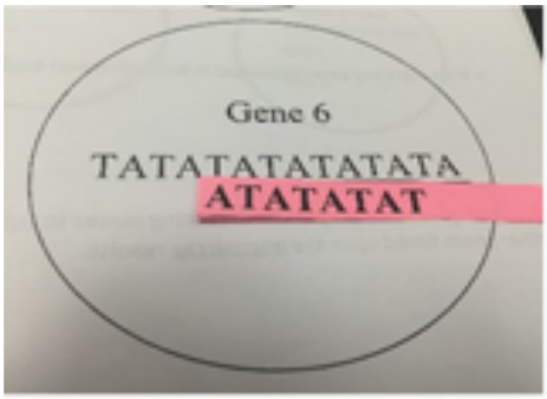
GGGTAGCCTTGGG

CCCATCGGAACCC

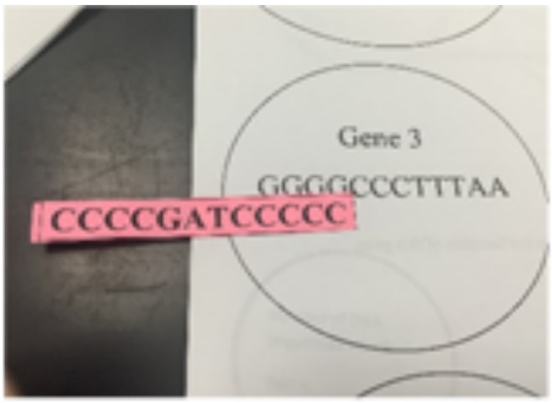
CATCGGAAC

CCGGGAAATT

Microarray Paper Simulation



CORRECT



INCORRECT

The left side of the strand is attached to the bottom of the dish. You can't have your strand attach so that it sticks out on the left side.

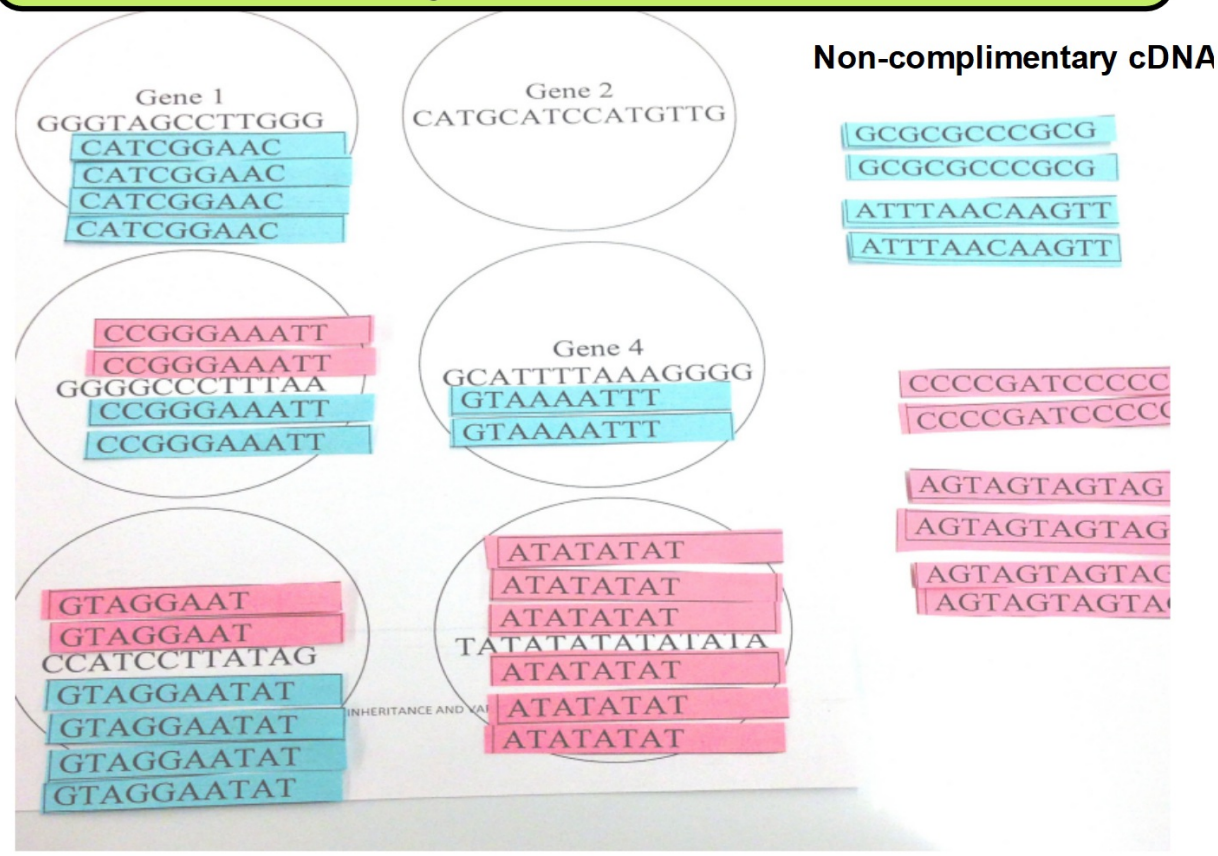
Microarray Paper Simulation

Procedure

4. The region or circle on the microarray will glow red if only cDNA from the cancer cells' hybridizes. The region or circle on the microarray will glow blue if only cDNA from the normal cells' hybridizes. The region or circle on the microarray will glow purple (contain both red and blue paper cDNA strands) if cDNA from both the normal and cancer cells' hybridizes. If cDNA from neither the cancerous or normal cells' hybridizes, the region will not glow at all.

5. Record your results in the "Microarray Results" section below.

Microarray Paper Simulation



Microarray Paper Simulation

Microarray Results

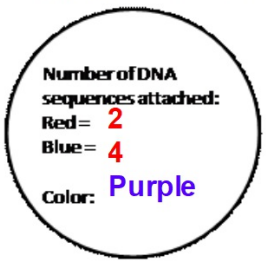
Gene 1: P53

Gene 2: BDNF

Gene 3: Cyto C

Gene 4: APC

Gene 5: ST3GAL5



Gene 6: RAS

[redacted]

Microarray Paper Simulation

Analysis of Results:

1. Which gene(s) were expressed (transcribed) in the skin cancer cells? How do you know?
2. Which gene(s) were not expressed in the skin cancer cells? How do you know?
3. Which gene was not expressed in either skin cell type?
4. Why do think that gene was not expressed? (Hint: Think about the type of cell you are using in the microarray)

K

Microarray Paper Simulation

Analysis of Results:

5. Why do you think that genes 3 and 5 are expressed in both skin cell types, as well as all cells in the body?

6. Choose at least one gene you think may play a role in causing cancer in cells. Explain why you chose that gene and not other genes based upon the microarray results. (on-level)

Choose two or more genes you think may play a role in causing cancer in cells. Explain why you chose those genes and not other genes based upon the microarray results. (honors)