

**The result on the  
microarray slide is  
BLUE.**

**Is this gene turned on  
or off in a cancer cell?**

**Normal cell?**

**The result on the  
microarray slide is  
RED.**

**Is this gene turned on  
or off in a cancer cell?**

**Normal cell?**

**The result on the  
microarray slide is  
Purple.**

**Is this gene turned on or  
off in a cancer cell?**

**Normal cell?**

**The result on the  
microarray slide is  
Clear.**

**Is this gene turned on  
or off in a cancer cell?**

**Normal cell?**

Move this to decide for trial 1:

Is this color blue or purple?

Move this to decide for trial 2

- **Get out Microarray Paper Simulation from Friday**

**Pick up: Microarray simulation**

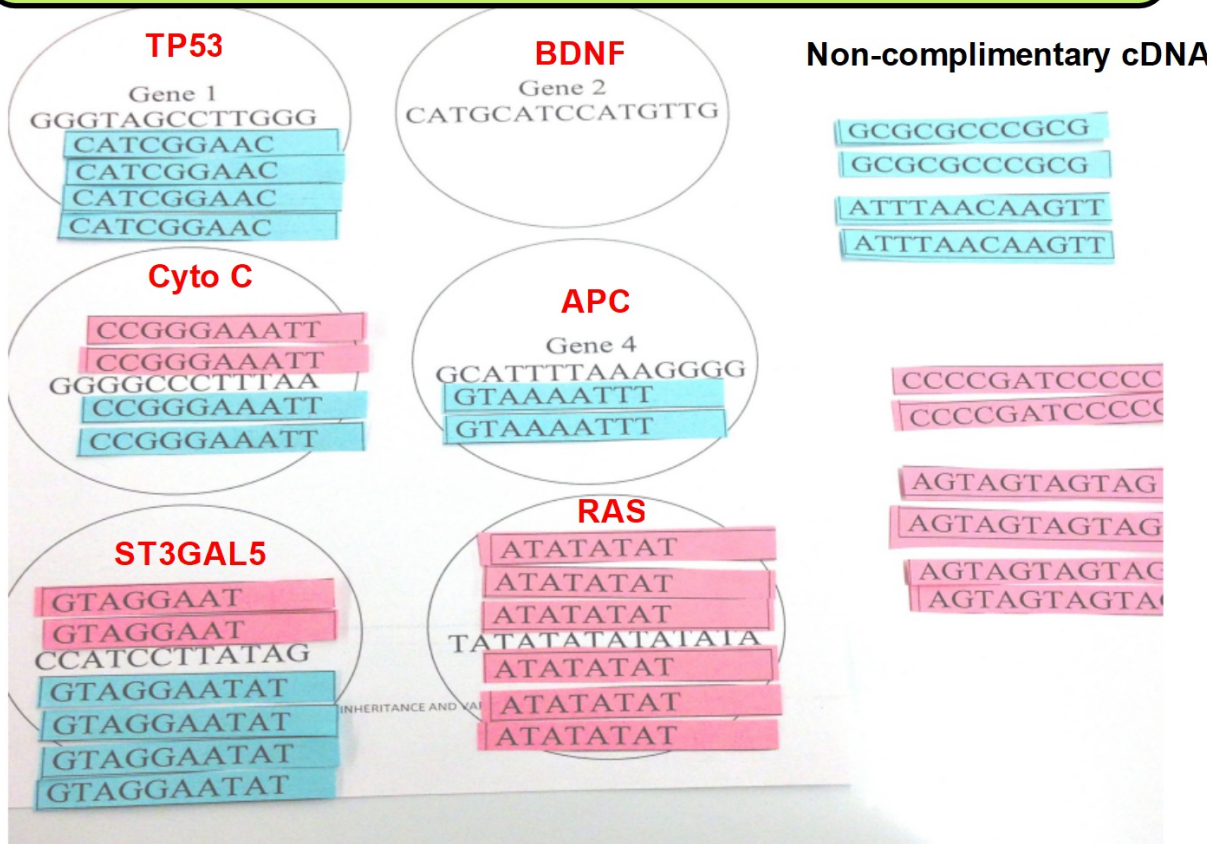
**Homework: Finish microarray lab questions**

**Open Notes Lab Evaluation Thursday on the following  
Labs/Activities:**

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- Cell Differentiation and Gene Expression
- Microarray Background Information
- Microarray Paper Simulation
- Red Light Green Light (Microarray lab)

# Microarray Paper Simulation



# Microarray Paper Simulation

## Microarray Results

Gene 1: TP53



Gene 2: BDNF



Gene 3: Cyto C



Gene 4: APC



Gene 5: ST3GAL5



Gene 6: RAS



# Microarray Paper Simulation

## **Analysis of Results:**      **Review homework**

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)

## 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)

## Microarray Simulation (Wet Lab)

This wet lab uses the same genes and color schemes as in the paper lab.

Purpose: To study the expression levels of four genes in both healthy and cancerous skin cells.

Question : “How can we determine what genes are expressed in a healthy cell compared to genes expressed in a cancerous cell?”



# Microarray Simulation (Wet Lab)

The basic idea of a microarray is that if a gene is active in an organism, a lot of RNA is produced and therefore more single-stranded labeled DNA is available to bind to the microarray. If a gene is not as active in a cell then very little RNA is produced. If a gene is knocked out or completely repressed in an organism, no RNA is produced and no single-stranded cDNA is available to bind to the microarray.

The cDNA's from the four genes below are the focus of this study. The cDNA's for each gene are located in the tubes containing agarose. These tubes can be melted using a hot water bath. Shake each tube vigorously or vortex to ensure agarose is completely melted.

Tube	Gene	
1	APC	Tumor Suppressor
2	Ras	Accelerates Cell Division
3	P53	Tumor Suppressor
4	Cytochrome c	Needed for cells to make energy (cellular respiration)

## **Materials:**

- “*The Microarray Paper Simulation*” lab paper with gene descriptions of: APC, Ras, P53, & Cytochrome C
- Water bath set to 95°C
- cDNA Tubes (containing agarose and cDNA gene):
  - Tube 1 – APC gene
  - Tube 2 – Ras gene
  - Tube 3 – P53 gene
  - Tube 4 – Cytochrome c
  - Tube R – (+) control – red
  - Tube B – (+) control – blue
  - Tube P – (+) control – purple
  - Tube N – (-) control – no color



**Push tables together:**

**Each table needs:**

- card with labels**
- micropipette**
- micropipette tip box**
- cup**

## How to use a micropipette

- Make sure the pipette has a tip on it!



**Do NOT use the same tip in different samples!!!**

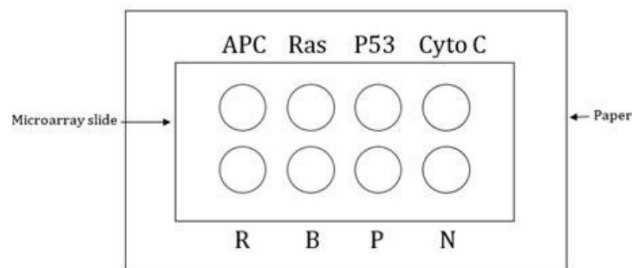
- 1) Press down on top plunger to the **FIRST STOP**
- 2) Place the tip in the sample
- 3) Release the top plunger completely
- 4) Take the pipette out of the sample
- 5) Hold the pipette over the spot where you want the sample to be placed
- 6) Press **ALL THE WAY** down on the top plunger
- 7) Move the pipette out of the sample
- 8) Release the top plunger

**\*\*\*\*\*Do NOT use the same tip in different samples!!!**

## **Procedure:**

1. Place the microarray slide flat on laboratory surface on top of a piece of paper to label the circles or wells on both sides of the slide.

**Do not move your slide or you may mix up your genes!**



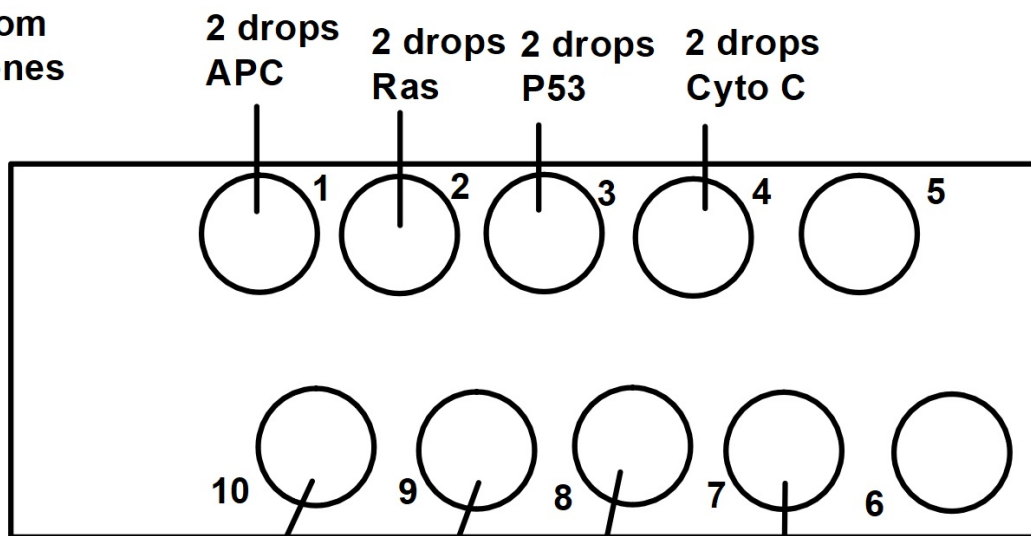
2. Using the corresponding pipette to each tube, add two drops each of the cDNA at the designated well on the microarray slide.

**DO NOT CROSS CONTAMINATE THE PIPETTES.**

3. Allow the slide to dry. This will take about 3 to 5 minutes for the solution to solidify.



cDNA from these genes



Controls that show color change

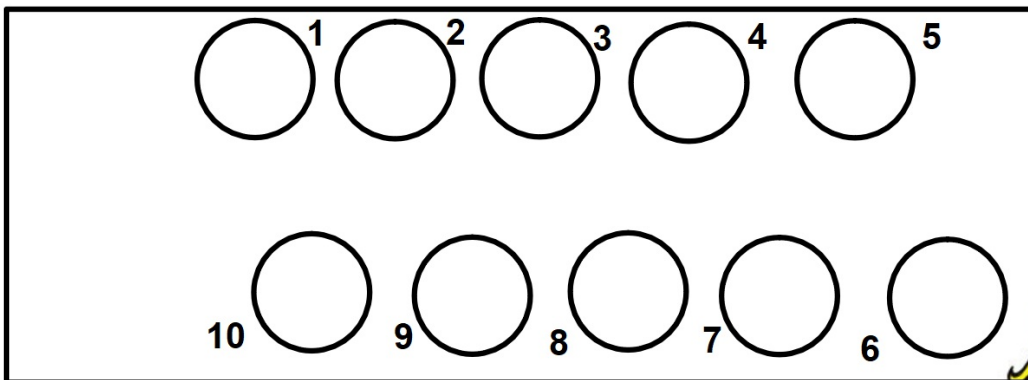
2 drops Red Control  
2 drops Blue Control  
2 drops Purple Control  
2 drops Negative Control

Place your slide on top of the index card on your table.  
Do not move your slide once you have put drops on it.

**Get a NEW tip on your pipette between EACH sample.**

4. Using a clean pipette, add one drop of the probe solution (containing the cDNA from both the healthy and cancerous cells) to each well on the slide.

5. Record the data (colors of each well.)



**Add one drop of probe solution to #1, 2, 3, 4, 10, 9, 8, 7**

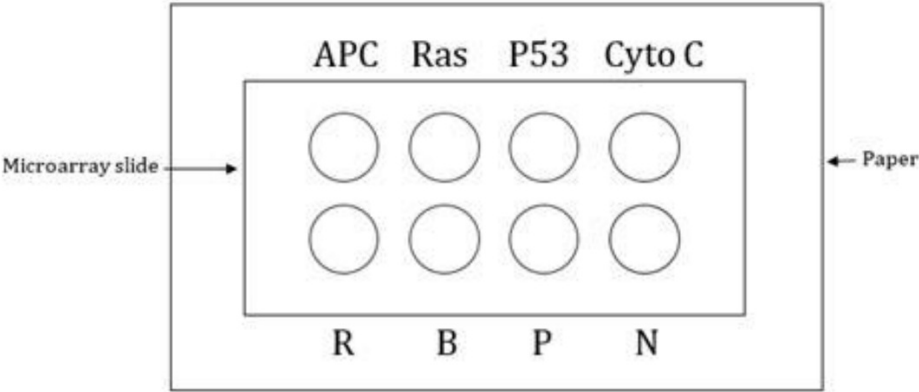


**Use colored pencils to record the color that the drops change.**

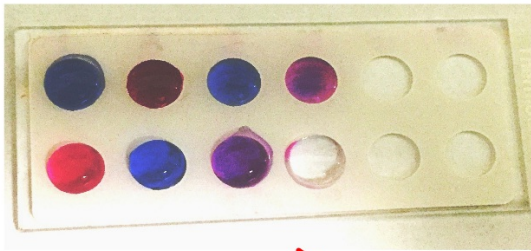
**Data: Color of 4 Different Genes on a Microarray Slide**

Microarray Well (Circle)	Color	Gene Expressed? (On or Off)	Cell Type: Normal, Cancerous or Both
1 - APC			
2 - Ras			
3 - P53			
4 - Cytochrome c			
R - (+)			
B - (+)			
P - (+)			
N - (-)			

**Analysis Questions:**







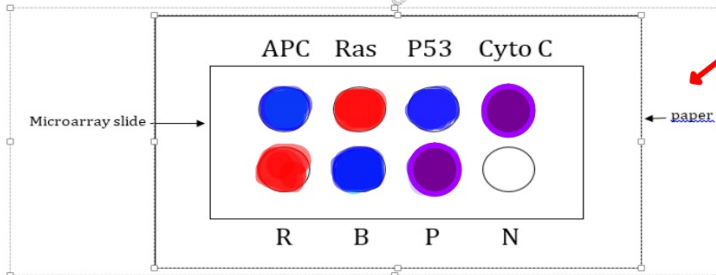
**Student's results.**

Data: Color of 4 Different Genes on a Microarray Slide

Microarray Well (Circle)	Color	Gene Expressed? (On or Off)	Cell Type: Normal, Cancerous or Both
1 - APC	Blue	On	Normal
2 - Ras	Red	On	Cancerous
3 - P53	Blue	On	Normal
4 - Cytochrome c	Purple	On	Both
R - (+)	Red		
B - (+)	Blue		
P - (+)	Purple		
N - (-)	Clear		

**Analysis Questions:**

1. Color the spots on your array as shown by your results.



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## Write question # 3

3. From the four genes studied in this lab, which genes would you expect to be turned on in a cancer cell? Genes turned off in a cancer cell?

Cancer cell	
Genes turned on:	Genes turned off:

4. From the four genes studied in this lab, which genes would you expect to be turned on in a healthy cell? Genes turned off in a healthy cell?

Normal (Non-Cancerous) cell	
Genes turned on:	Genes turned off:

## Microarray Simulation (Wet Lab)

5. Are there any genes turned on in both the normal and the cancerous cells? If so, identify the gene and explain if this is an expected result.
6. Are there any genes turned off in both the normal and the cancerous cells? If so, identify the gene and explain if this is an expected result.
7. Using the following vocabulary, explain the purpose of using the microarray.

*Word Bank: DNA, mRNA, protein, gene and expressed*

## Troubleshooting & Best Practices

### Teacher preparation notes from the lesson plan:

- **Each student station needs:** labeled pipettes for each sample, microarray slide, beaker with sodium hydroxide and pipette (may be shared between two stations or at teacher lab bench).
- **Safety:** Students handling sodium hydroxide will need to wear gloves, goggles and aprons.
- **Set-up a water bath set to 90C.** Place the samples in the water bath using a float. The agarose in the test tubes must melt in order for students to pipette the sample. Be careful; samples will be very warm when removed from the water bath.
- Students need to be aware of the possibility of **cross-contamination** between the samples.
- Teachers may want to provide a **microarray template** with labeled circles for students to place underneath their microarray slide.

## Troubleshooting & Best Practices

- **You will receive in your kit from the DNA Resource Center per section/class:**
  - 1 set of tubes (8 tubes included)
  - 1L Sodium Hydroxide
  - 8 microarray slide?

## Troubleshooting & Best Practices

- **You will need to provide at your school:**
  - **Water bath set to 90°C**
    - Can be a water bath or tea kettle
    - 1 per station or 2 stations can share
  - **Labeled pipettes**
    - 9 pipettes total
      - 1 per each of the 8 reaction tubes
      - 1 for the sodium hydroxide
    - May use a micropipette- will need a large number of tips
  - **Paper towels**

## Troubleshooting & Best Practices

- **You will only get 1 set of tubes (8 tubes included) per section/class**
  - Option #1: Teacher can circulate to each group to allow them to pipette the 8 solutions
  - Option #2: Each group starts with 1 tube and the tubes rotate to each group
  - Option #3: Teachers shares tubes over multiple days, so that all 8 tubes are at each station
  - Suggestion for all options: Use micropipettes to ensure accurate measurements

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## Troubleshooting & Best Practices

### **Teacher preparation notes from the lesson plan:**

Assign each student a role in the group. This role will determine which sample students will pipette on the microarray.

- a. **Student A:** Tube 1 (APC) and Red (R)
- b. **Student B:** Tube 2 (Ras) and Blue (B)
- c. **Student C:** Tube 3 (P53) and Purple (P)
- d. **Student D:** Tube 4 (Cyto C) and No Color (N)



