DNA Microarrays (Gene Chips) and Cancer

Cancer Education Project

University of Rochester
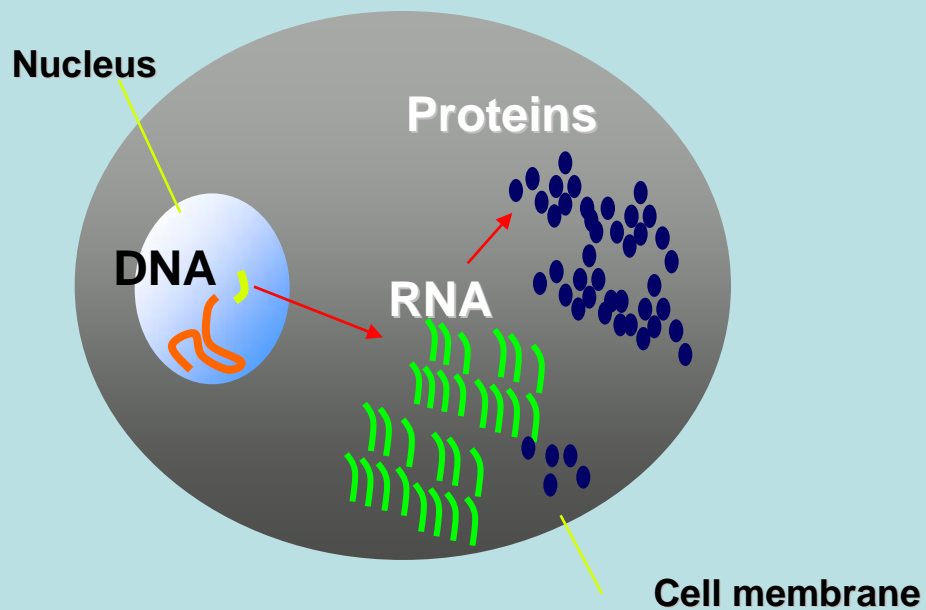
Life Sciences Learning Center

DNA Microarrays (Gene Chips) and Cancer

Part 1
Gene Expression and Cancer

Nucleus

Proteins

DNA

RNA

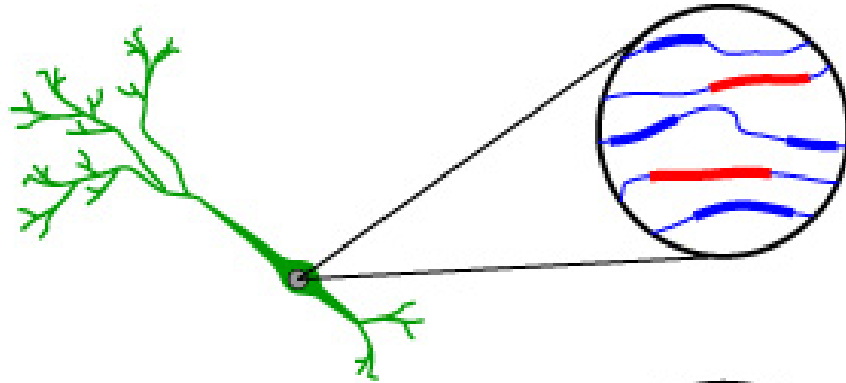Cell membrane
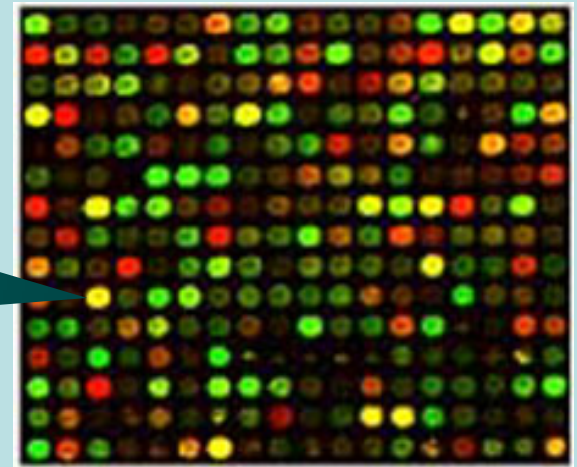
All your cells have the same DNA

Sperm

Egg

Embryo

Fertilized Egg - Zygote
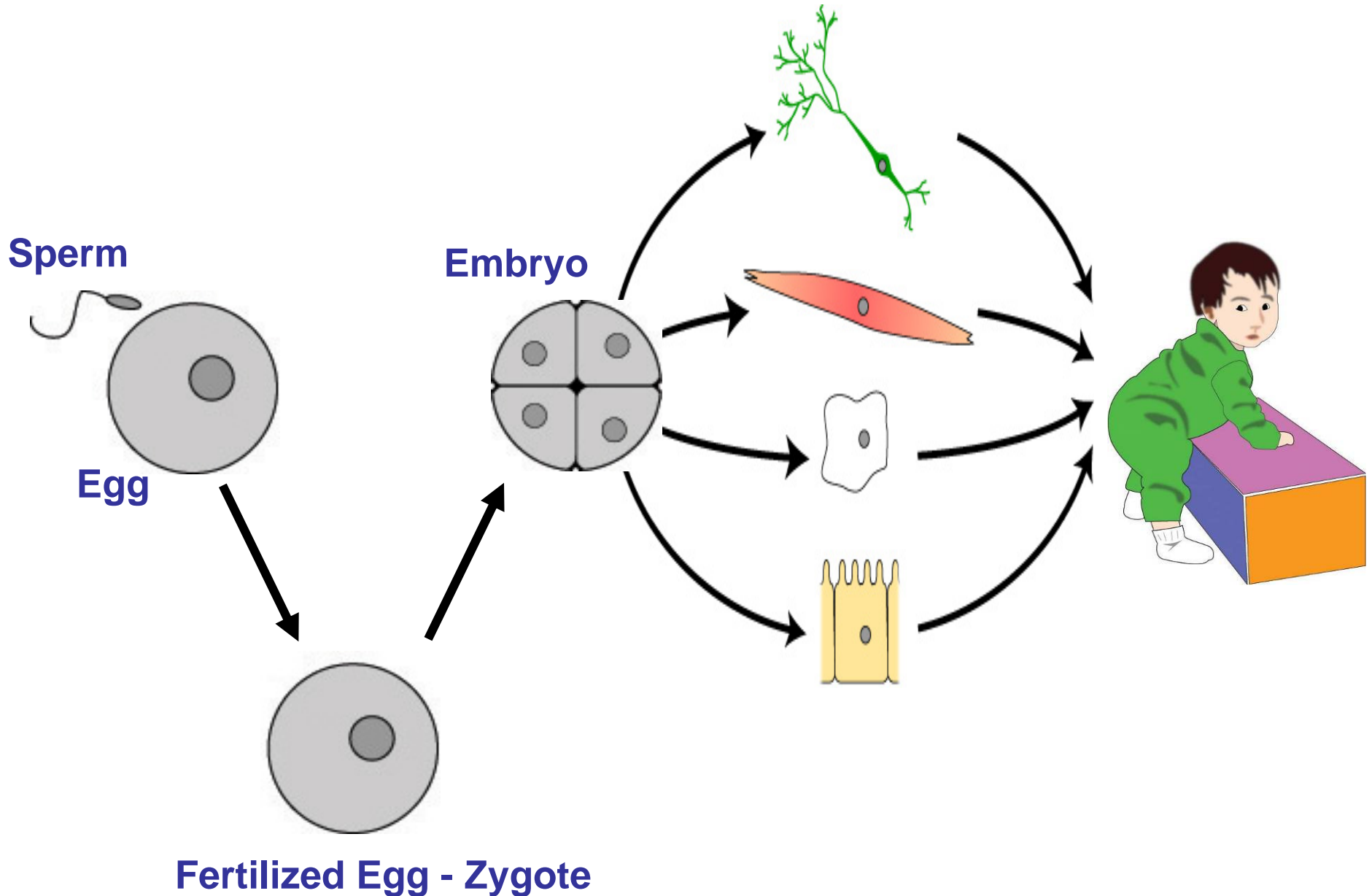
How do cells that have the same DNA (genes) end up having different structures and functions?

DNA in the nucleus
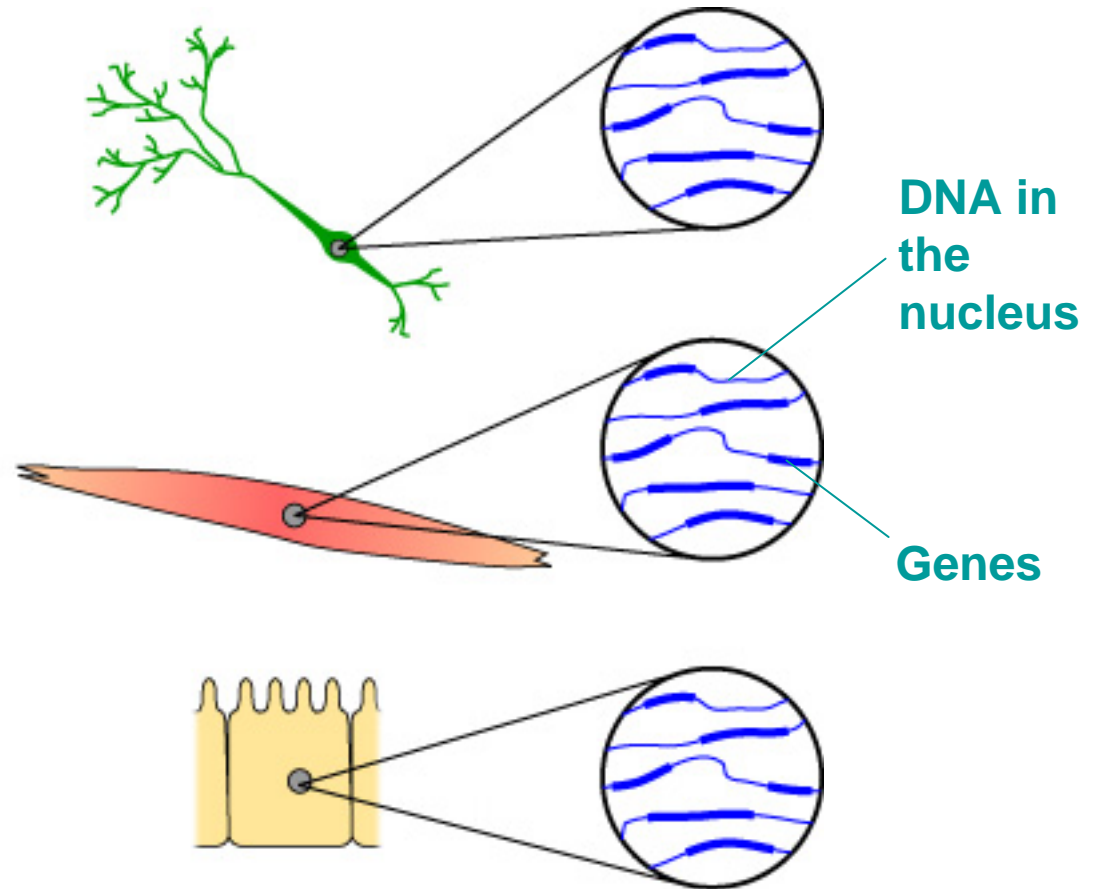
Genes
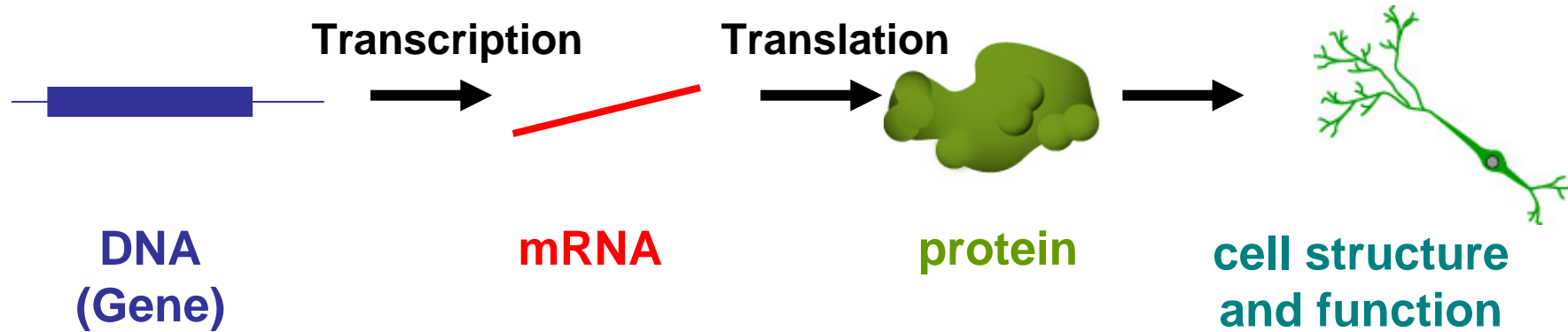
Different genes are turned on in different cells.
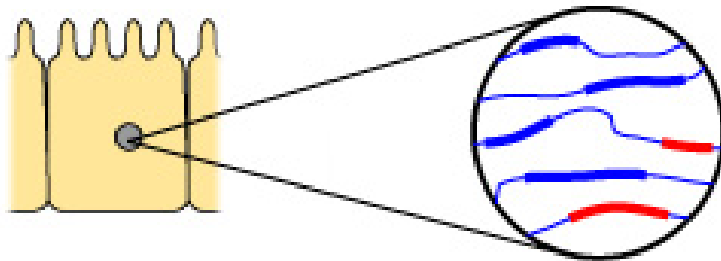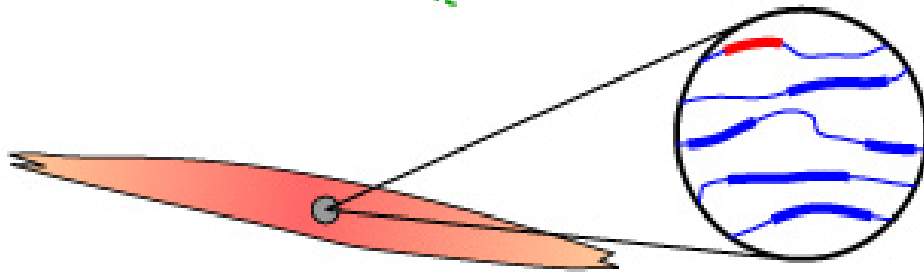
DIFFERENTIAL GENE EXPRESSION

GENE EXPRESSION (Genes are "on")

Transcription → Translation

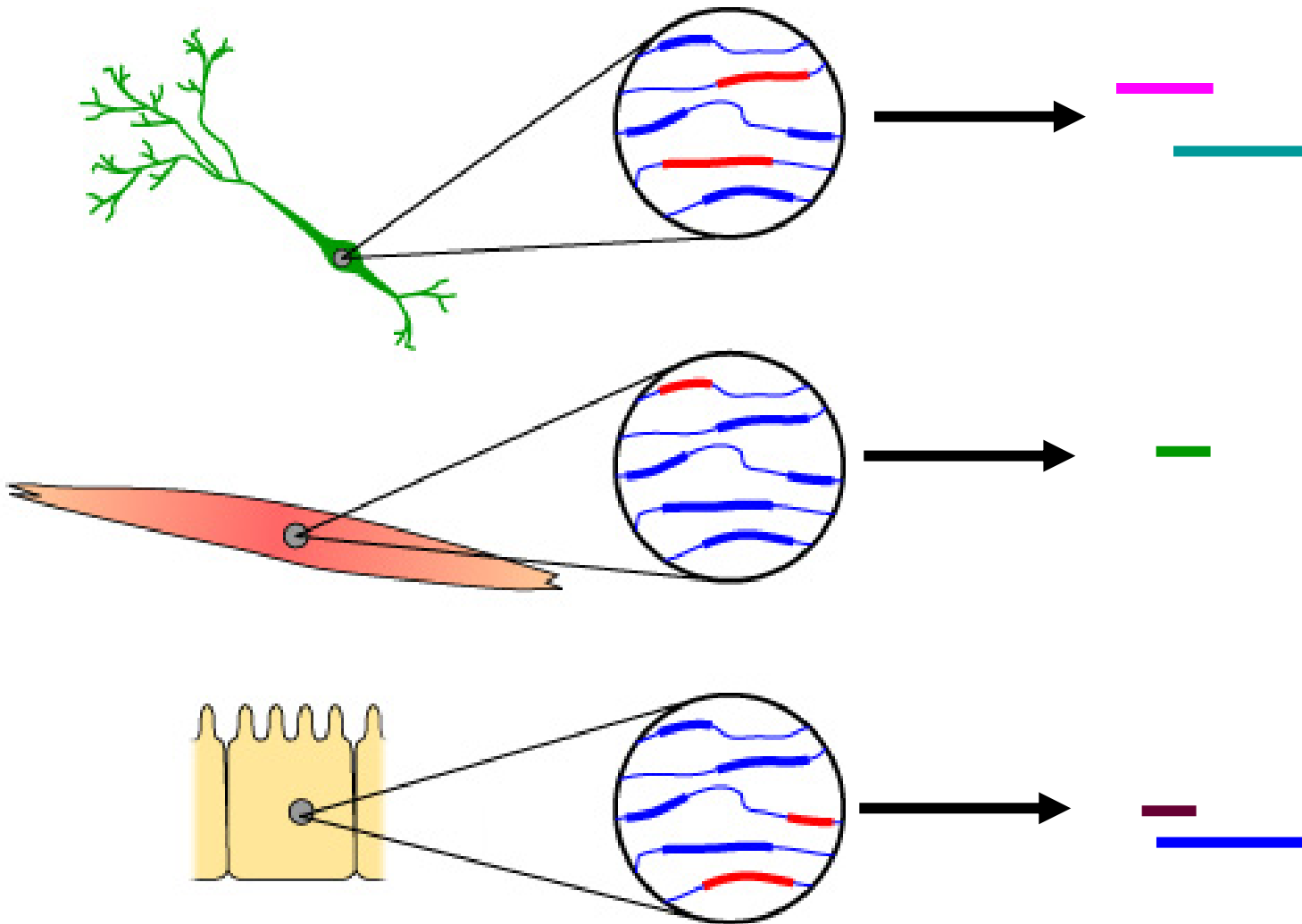DNA (Gene) → mRNA → protein → cell structure and function

Converts the DNA (gene) code into cell structure and function

Differential Gene Expression

Different genes
are turned on in different cells
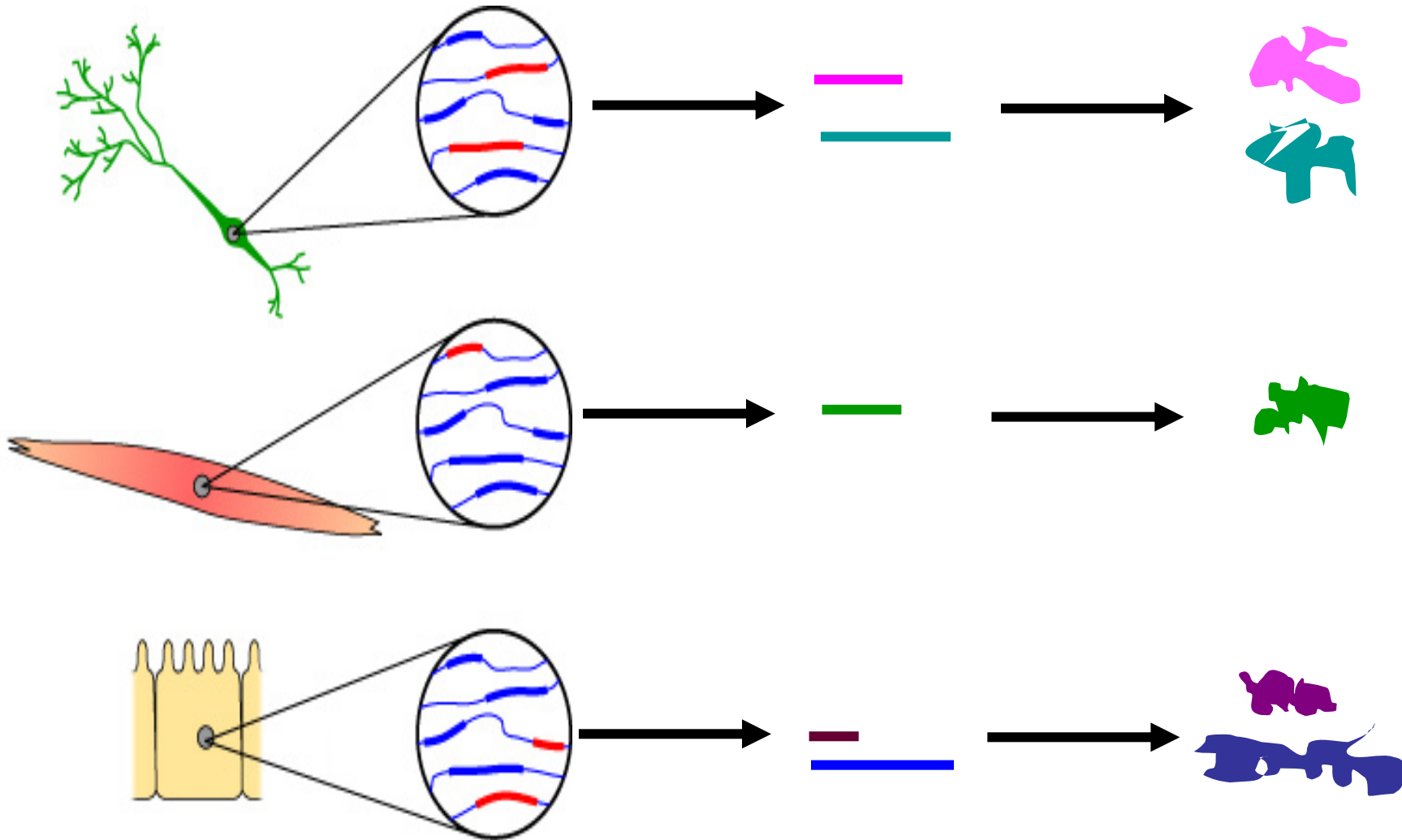
Different genes
make different **mRNA's**

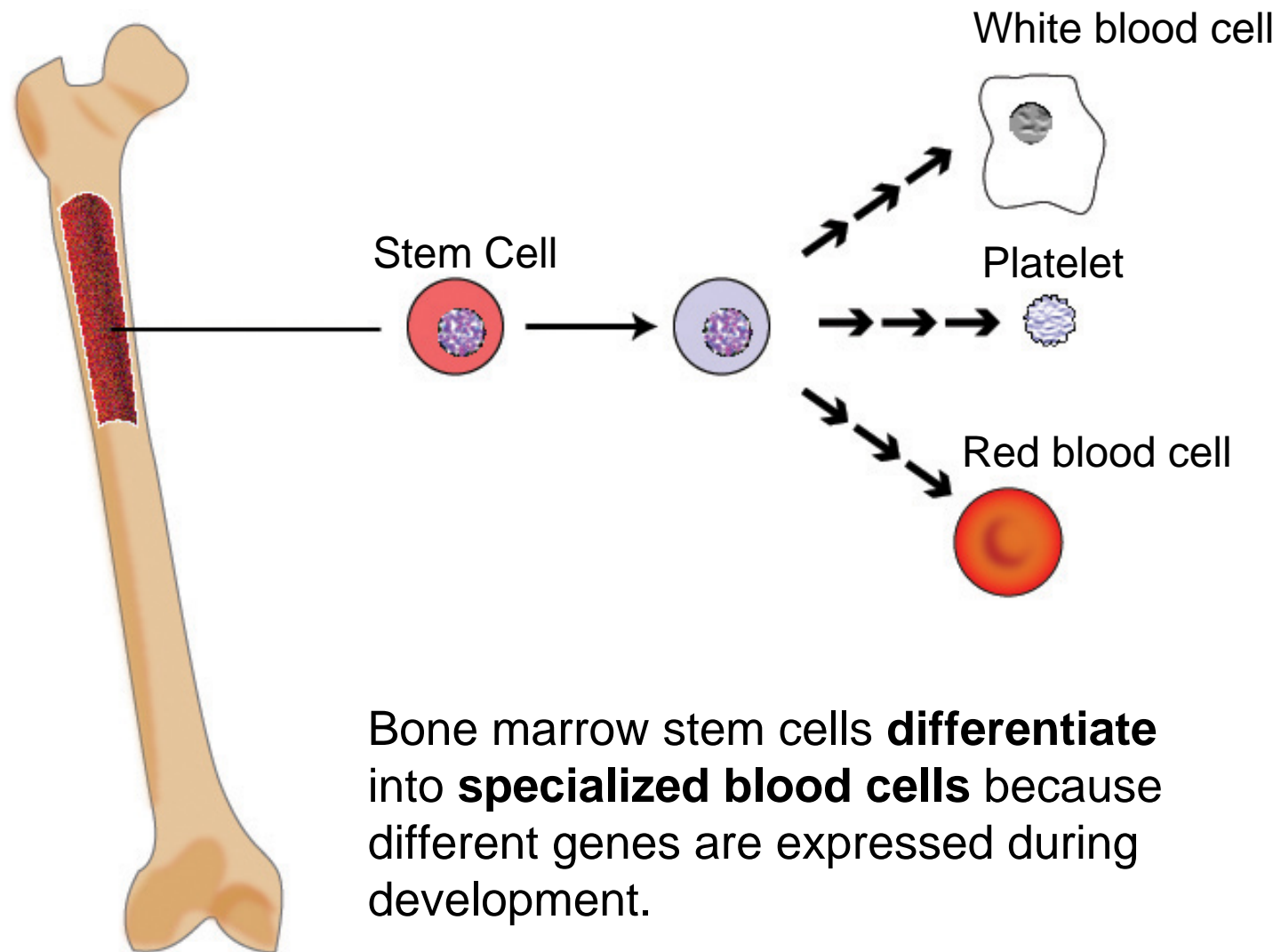# Differential Gene Expression

Different genes are turned
on in different cells

Different genes
make different **mRNA's**

Different mRNA's
make different **Proteins**
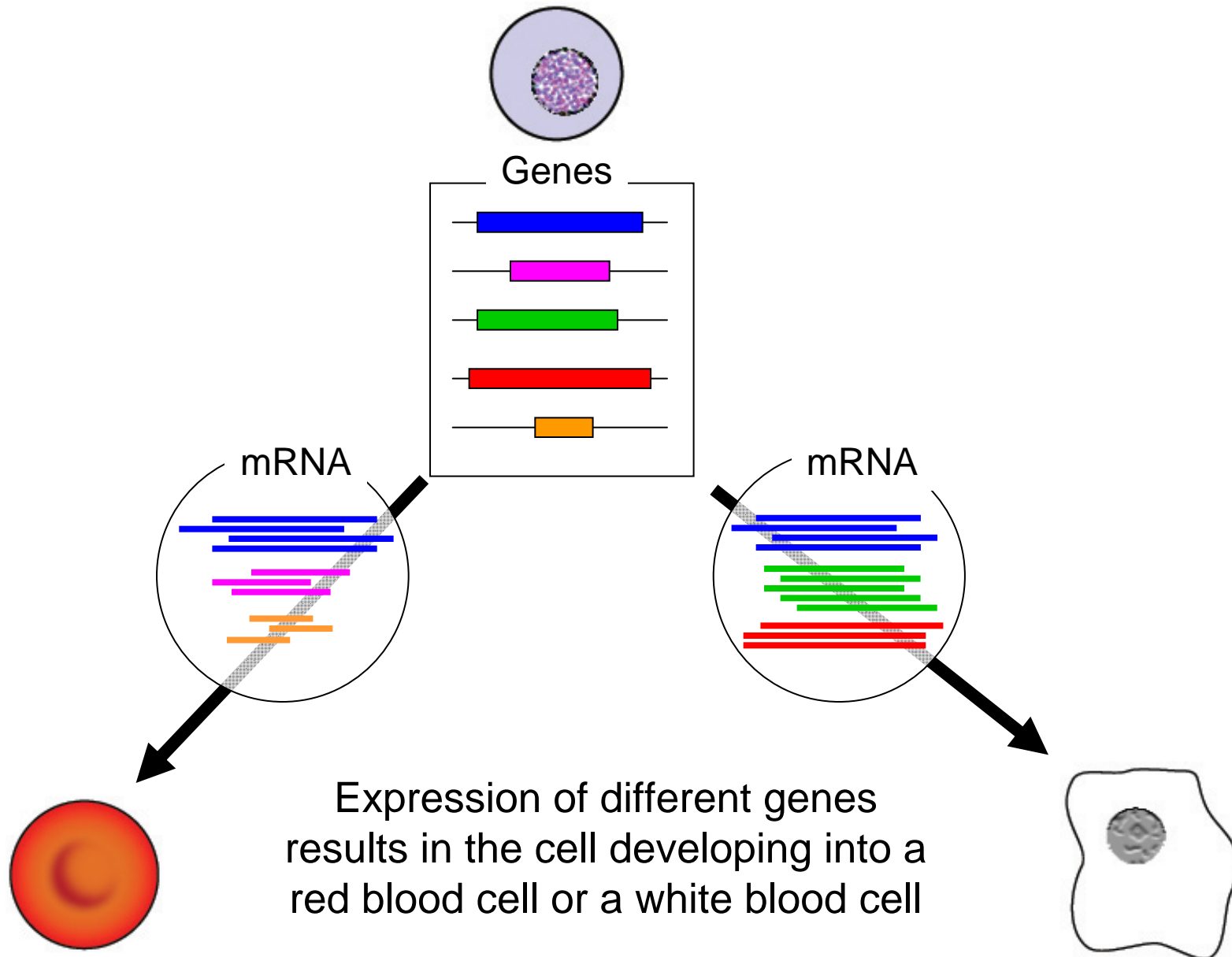
An example of differential gene expression

White blood cell

Stem Cell

Platelet

Red blood cell

Bone marrow stem cells **differentiate** into **specialized blood cells** because different genes are expressed during development.

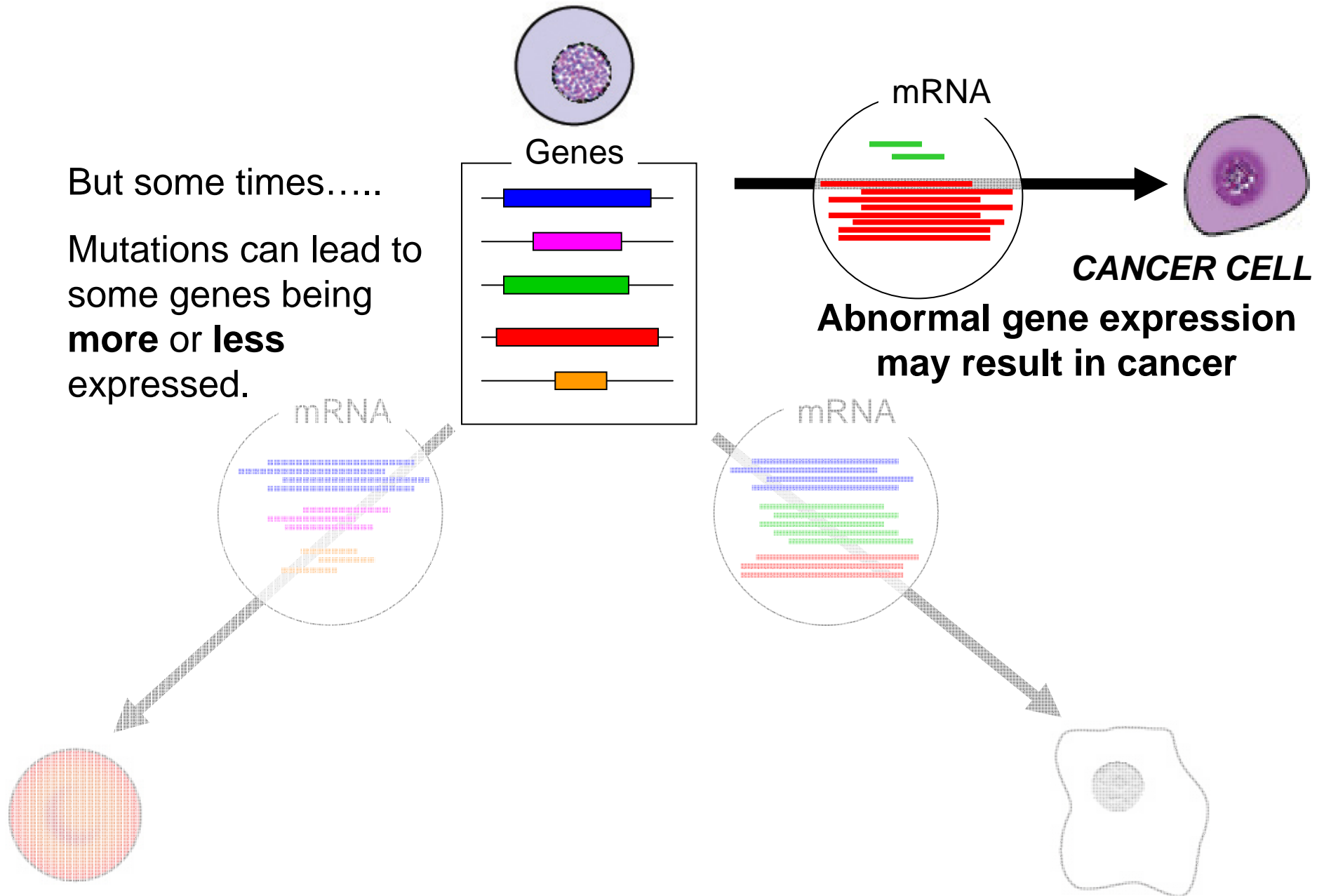# Normal Differential Gene Expression

Genes

mRNA

mRNA

Expression of different genes results in the cell developing into a red blood cell or a white blood cell

Cancer and Differential Gene Expression

But some times…..

Mutations can lead to some genes being **more** or **less** expressed.

Genes

mRNA

CANCER CELL

Abnormal gene expression may result in cancer

Gene Expression and Cancer

Table 1: Predicting Gene Expression in Genes Involved in Cancer
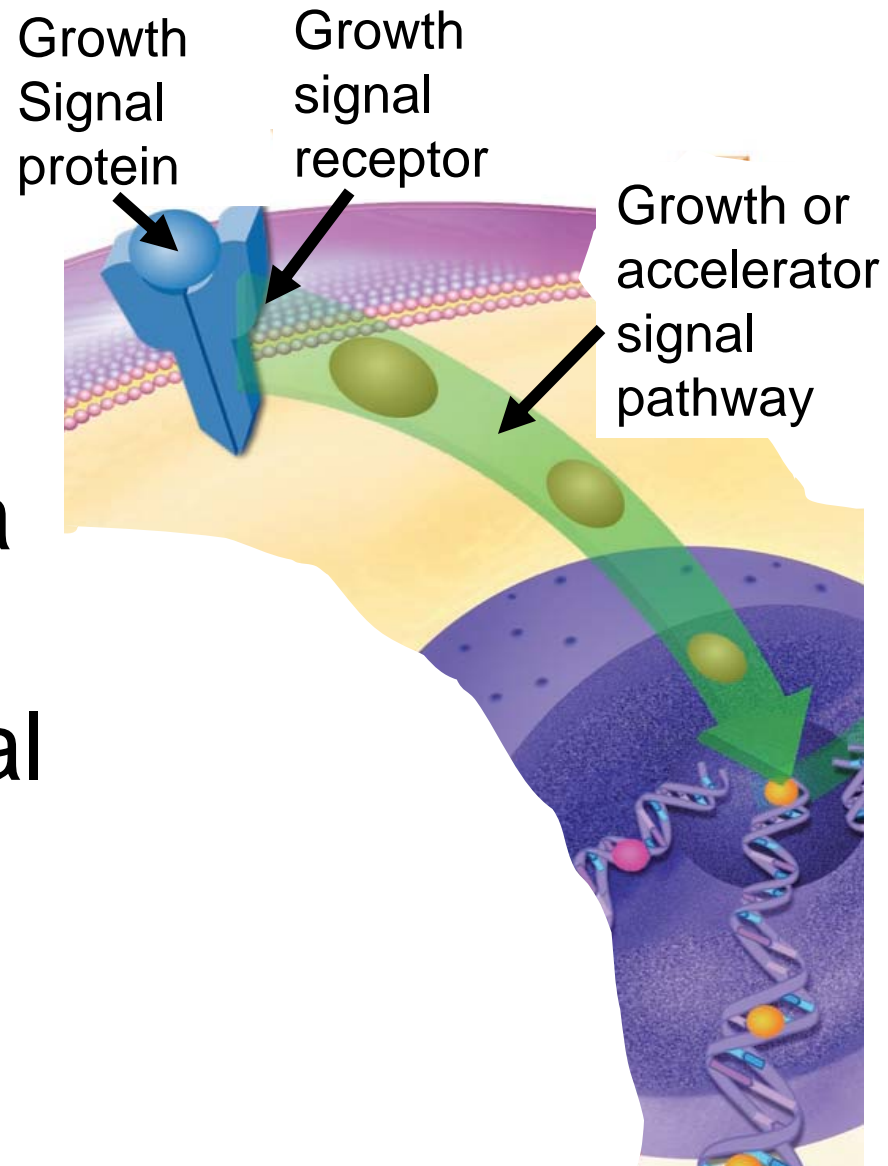
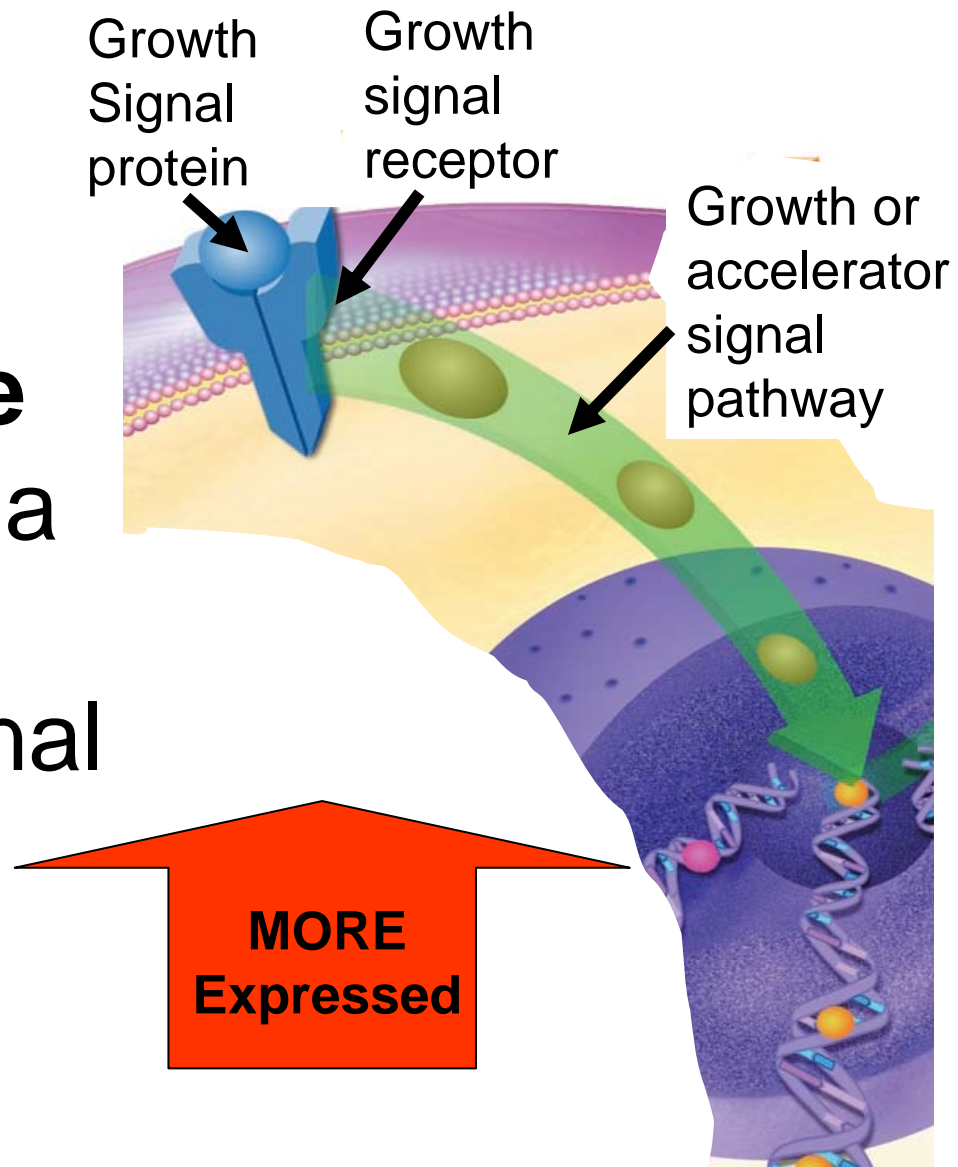| 1	2	3	4
Gene Location	Gene Function	Prediction: Will this gene be **More or Less** expressed in cancer cells?	Explanation for Prediction
A1	An **oncogene** that produces a protein in an accelerator signal pathway		
A2	A **tumor suppressor gene** that produces a protein in a brake signal pathway		
A3	A **guardian gene** that produces p53 protein that inspects for DNA damage, calls in repair enzymes and triggers apoptosis (cell death) if DNA damage cannot be repaired		
A4	A gene that produces DNA repair enzymes that corrects mutations when they occur		
B1	A gene that produces telomerase, an enzyme that rebuilds chromosome ends resulting in cells that can divide indefinitely		

Your Task:

Use the information in the second column of the chart to predict whether each gene will be MORE expressed or LESS expressed in cancer cells than in normal cells.

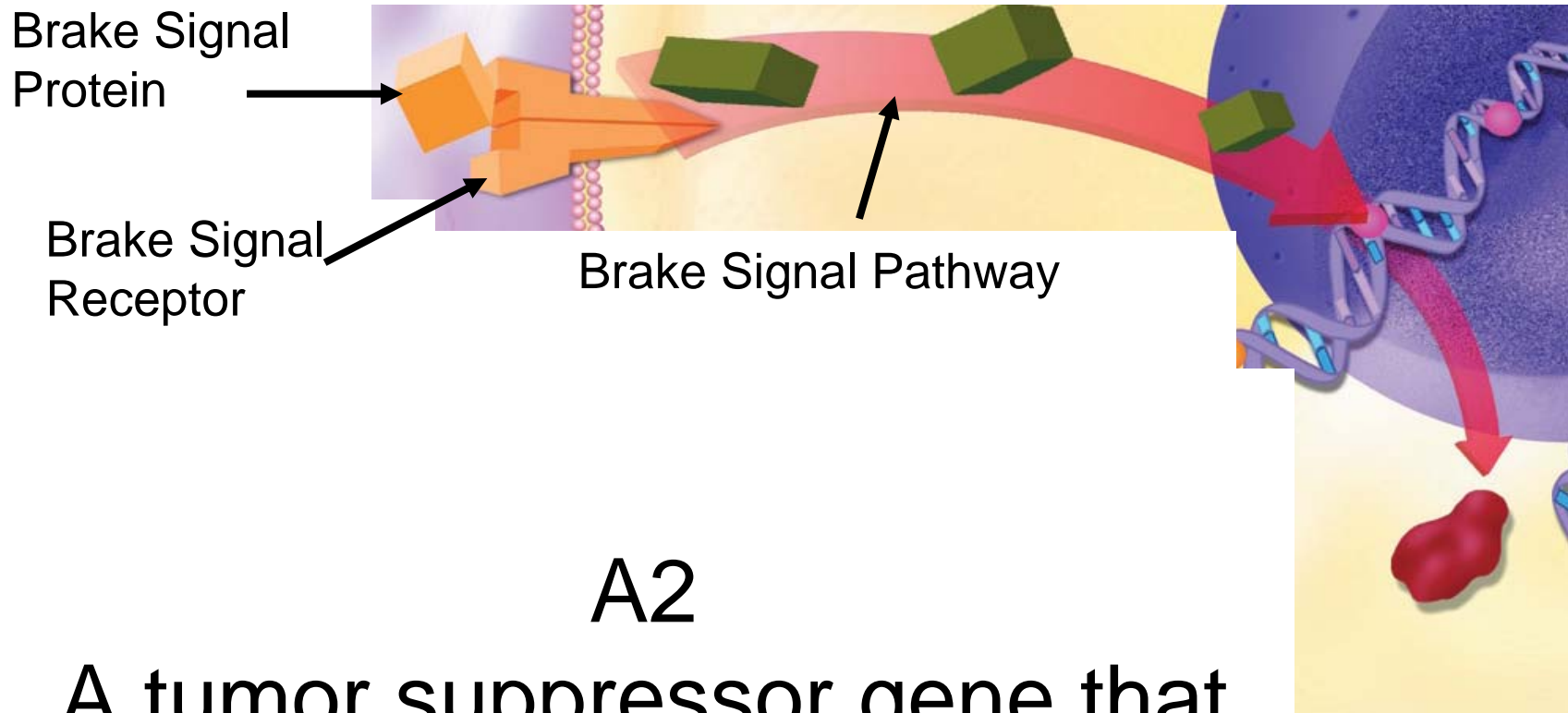| 1	2	3	4
Gene Location	Gene Function	Prediction: Will this gene be **More or Less** expressed in cancer cells?	Explanation for Prediction
A1	An **oncogene** that produces a protein in an accelerator signal pathway		
A2	A **tumor suppressor gene** that produces a protein in a brake signal pathway		

A1
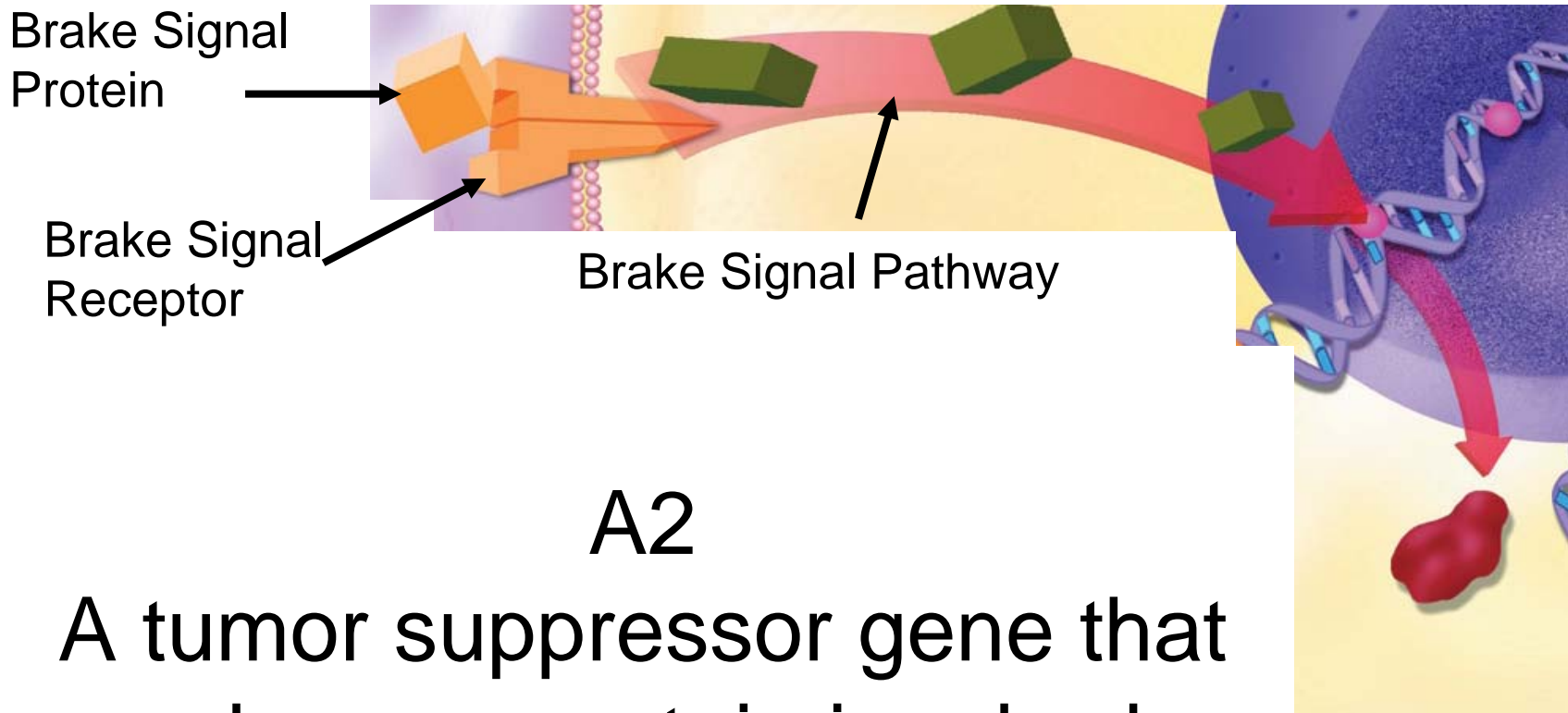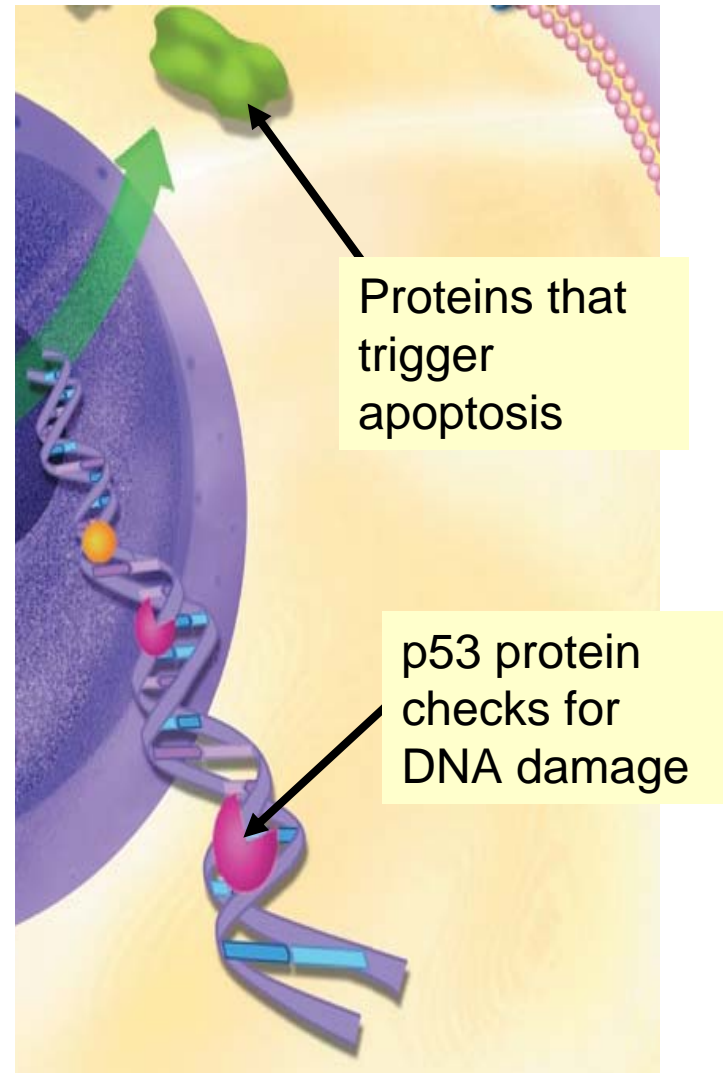An **oncogene** that produces a protein in an accelerator signal pathway.

Growth Signal protein

Growth signal receptor

Growth or accelerator signal pathway

A1
An **oncogene** that produces a protein in an accelerator signal pathway.

Growth Signal protein

Growth signal receptor

Growth or accelerator signal pathway

MORE Expressed

Brake Signal Protein

Brake Signal Receptor

Brake Signal Pathway

A2
A tumor suppressor gene that produces a protein in a brake signal pathway

Brake Signal Protein

Brake Signal Receptor

Brake Signal Pathway

A2
A tumor suppressor gene that produces a protein in a brake signal pathway

LESS Expressed

A3
A guardian gene that produces p53 protein that inspects for DNA damage and triggers apoptosis if DNA damage cannot be repaired
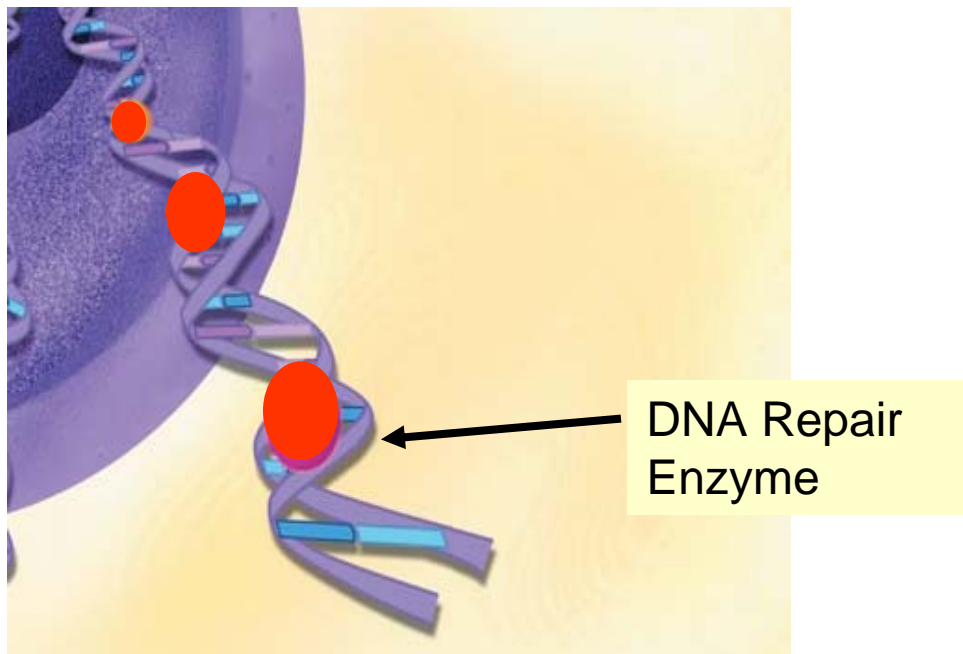

Proteins that trigger apoptosis

p53 protein checks for DNA damage

A3
A guardian gene that produces p53 protein that inspects for DNA damage and triggers apoptosis if DNA damage cannot be repaired

Proteins that trigger apoptosis

p53 protein checks for DNA damage

LESS Expressed

A4
A gene that produces DNA repair enzymes that correct mutations

DNA Repair Enzyme

A4
A gene that produces DNA repair enzymes that correct mutations

DNA Repair Enzyme

LESS Expressed
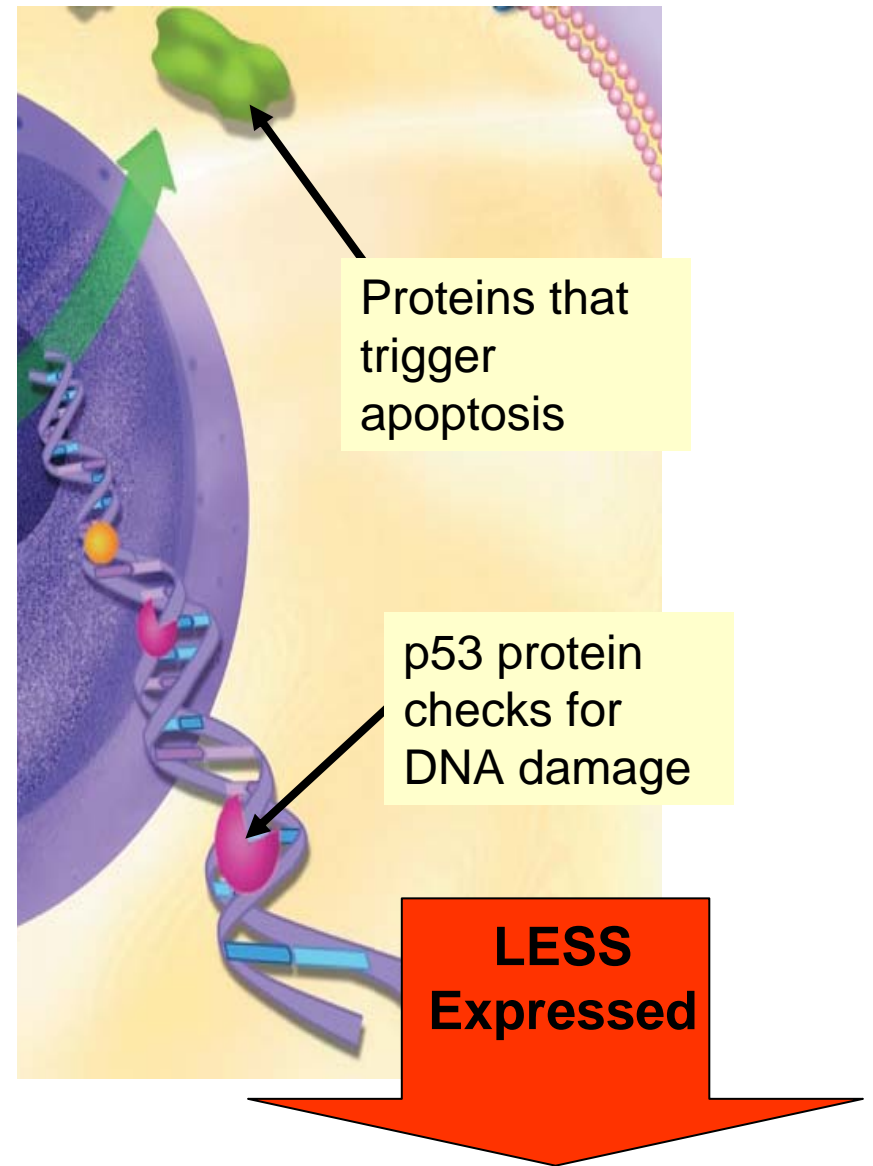
Work individually
Complete Table 1

Part 2
DNA Microarray Technology

Gene expression in colon cancer cells

Colon Cancer:

Normal colon cells

Cancerous colon cells
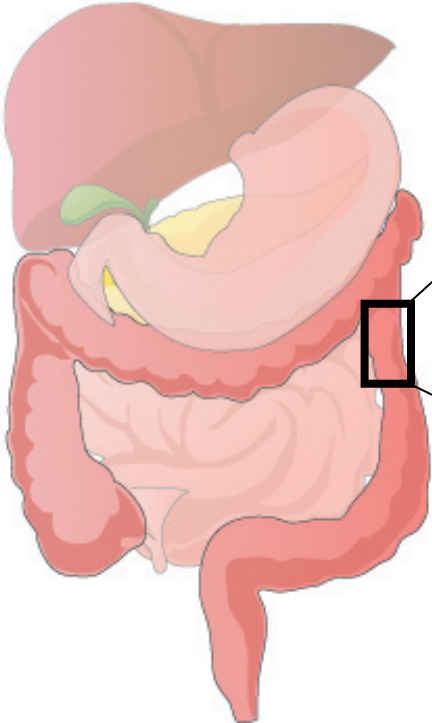
The colon (large intestine) is lined with cells that absorb water and secrete mucous.

Colon Cancer:
Uncontrolled Cell Division
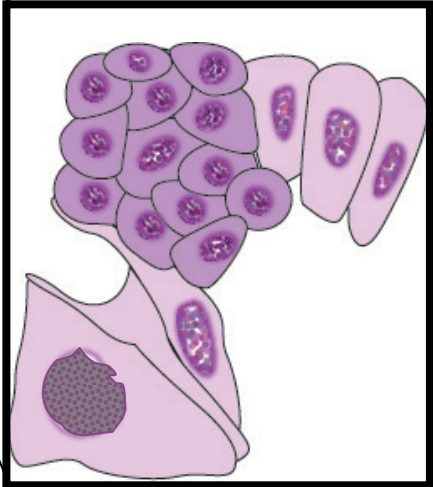
Cancerous Colon Cells

Cancer cells divide rapidly to form a tumor
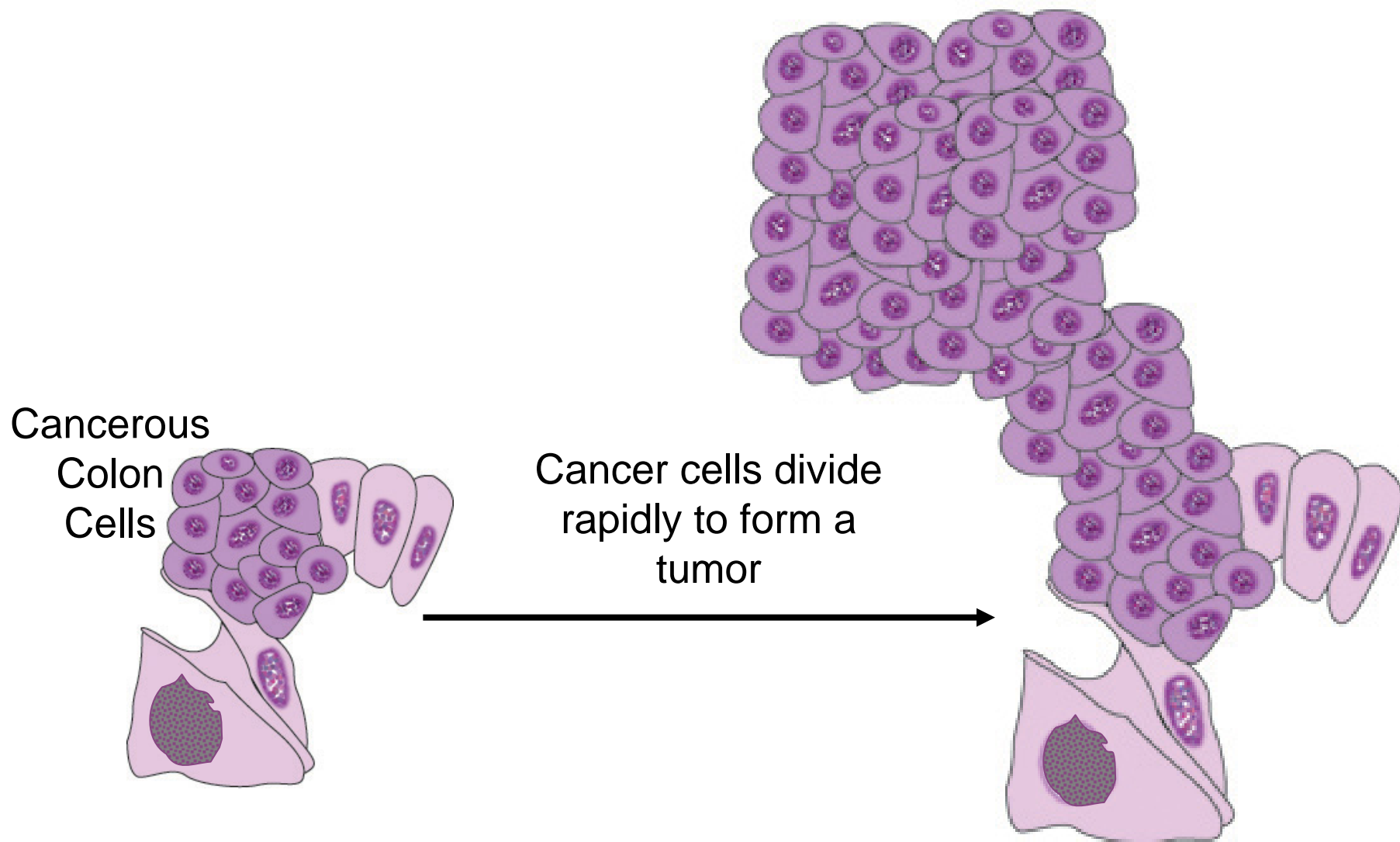
Expression of **cancer causing** genes

Cancer causing gene

Cancer causing protein
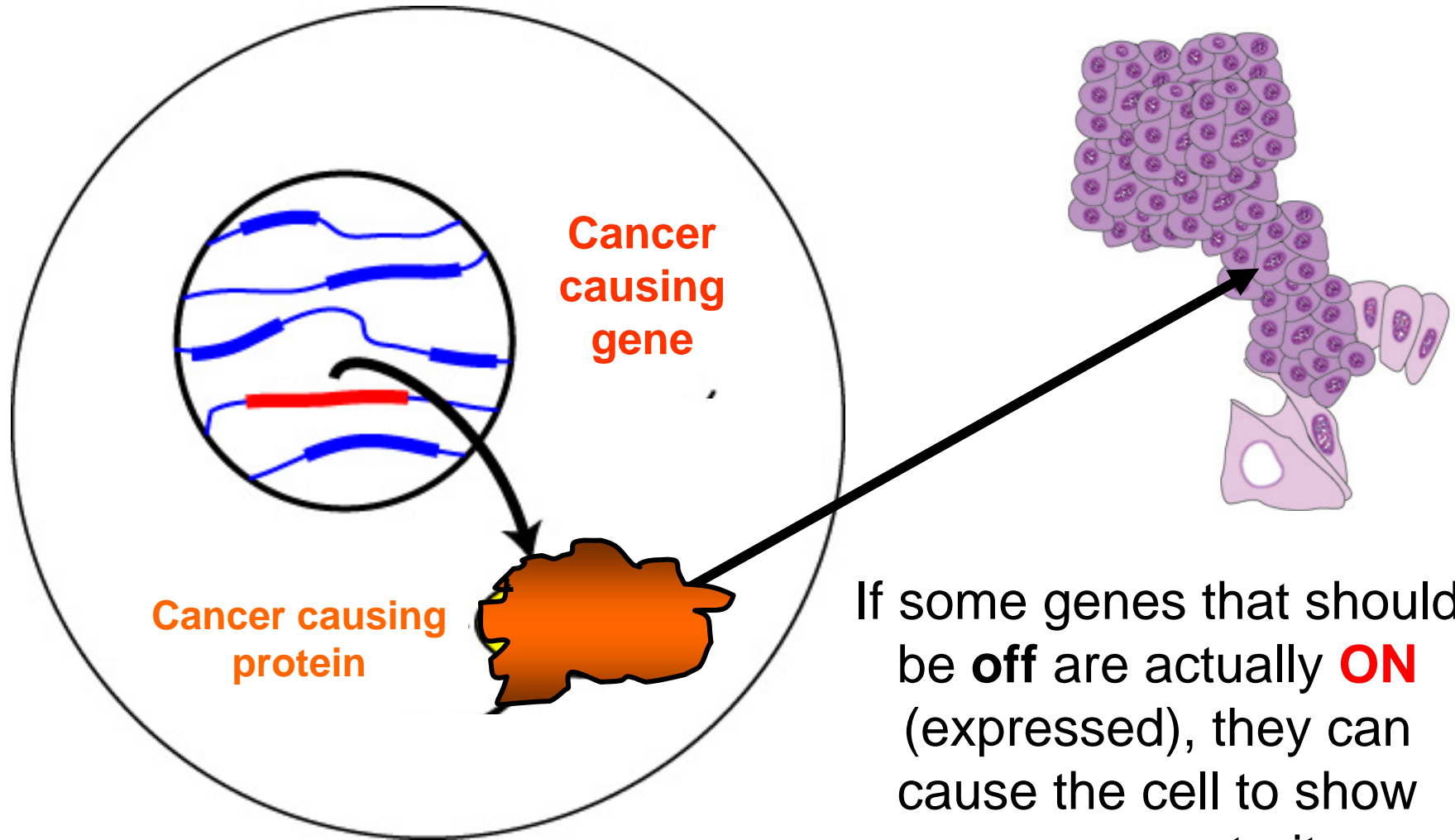
If some genes that should be **off** are actually **ON** (expressed), they can cause the cell to show cancerous traits

Expression of **cancer preventing** genes

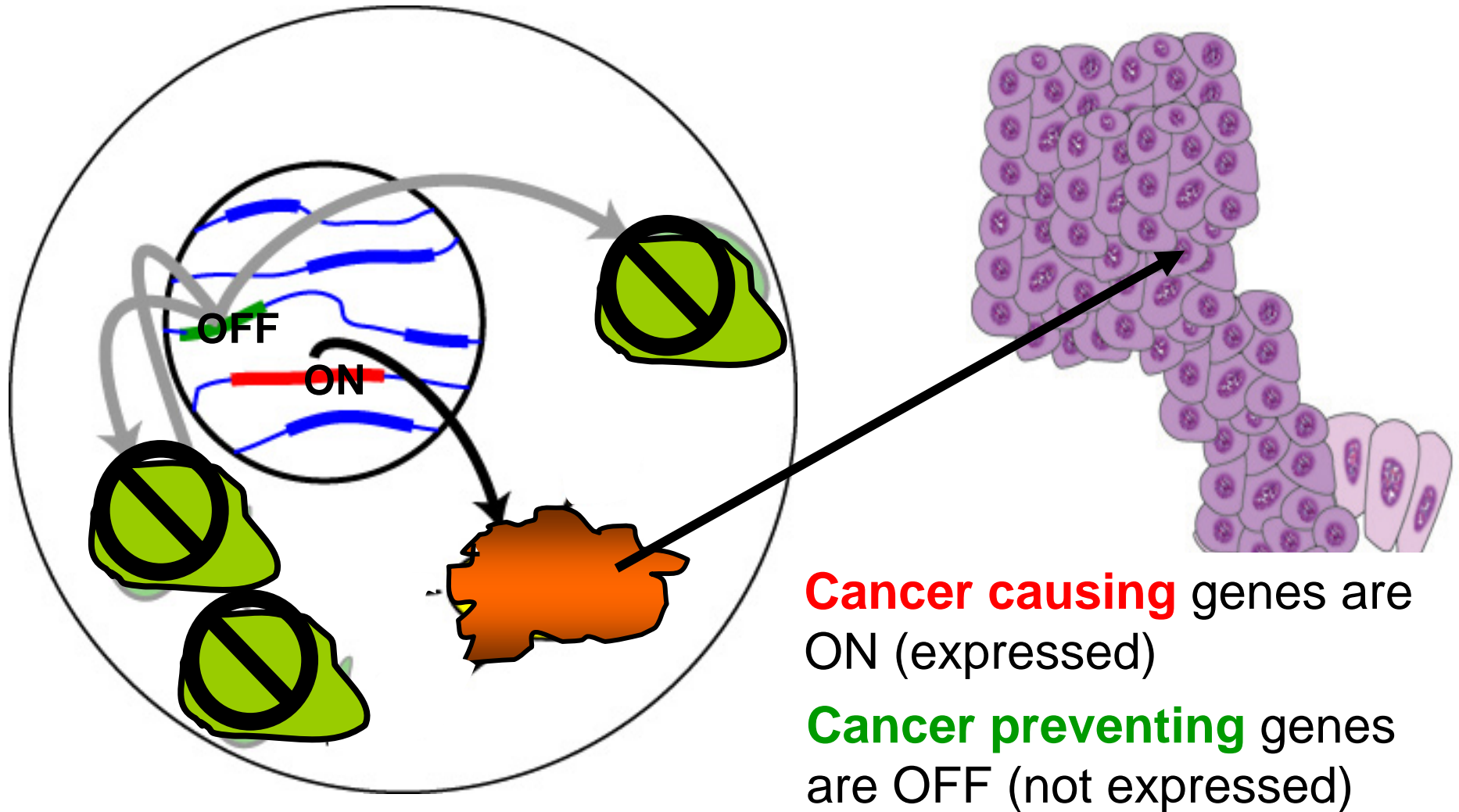*Cancer preventing gene*

Cancer preventing protein
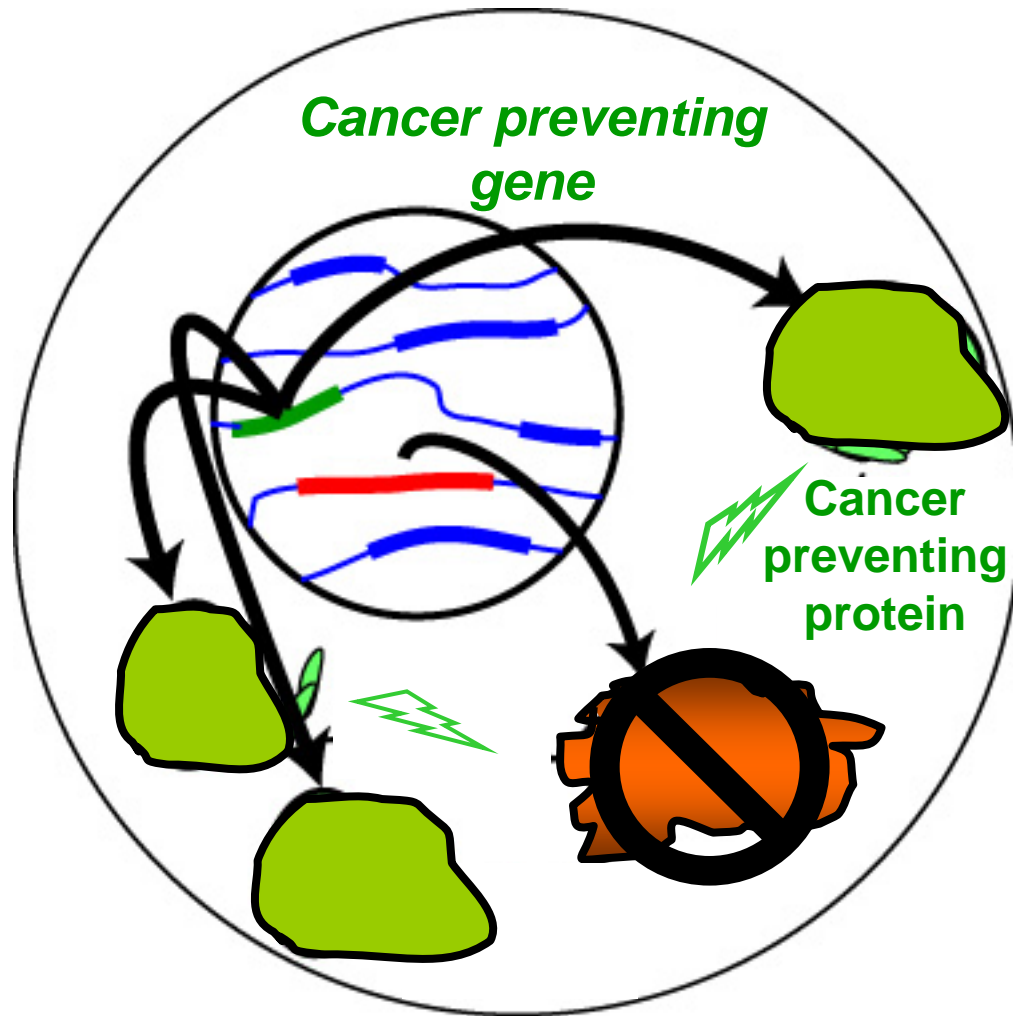
Expression of **cancer preventing** genes can block cancer causing proteins.

This is an example of a mechanism for maintaining homeostasis

However, if **cancer preventing genes** are turned **OFF (not expressed)**, then the cell can become cancerous
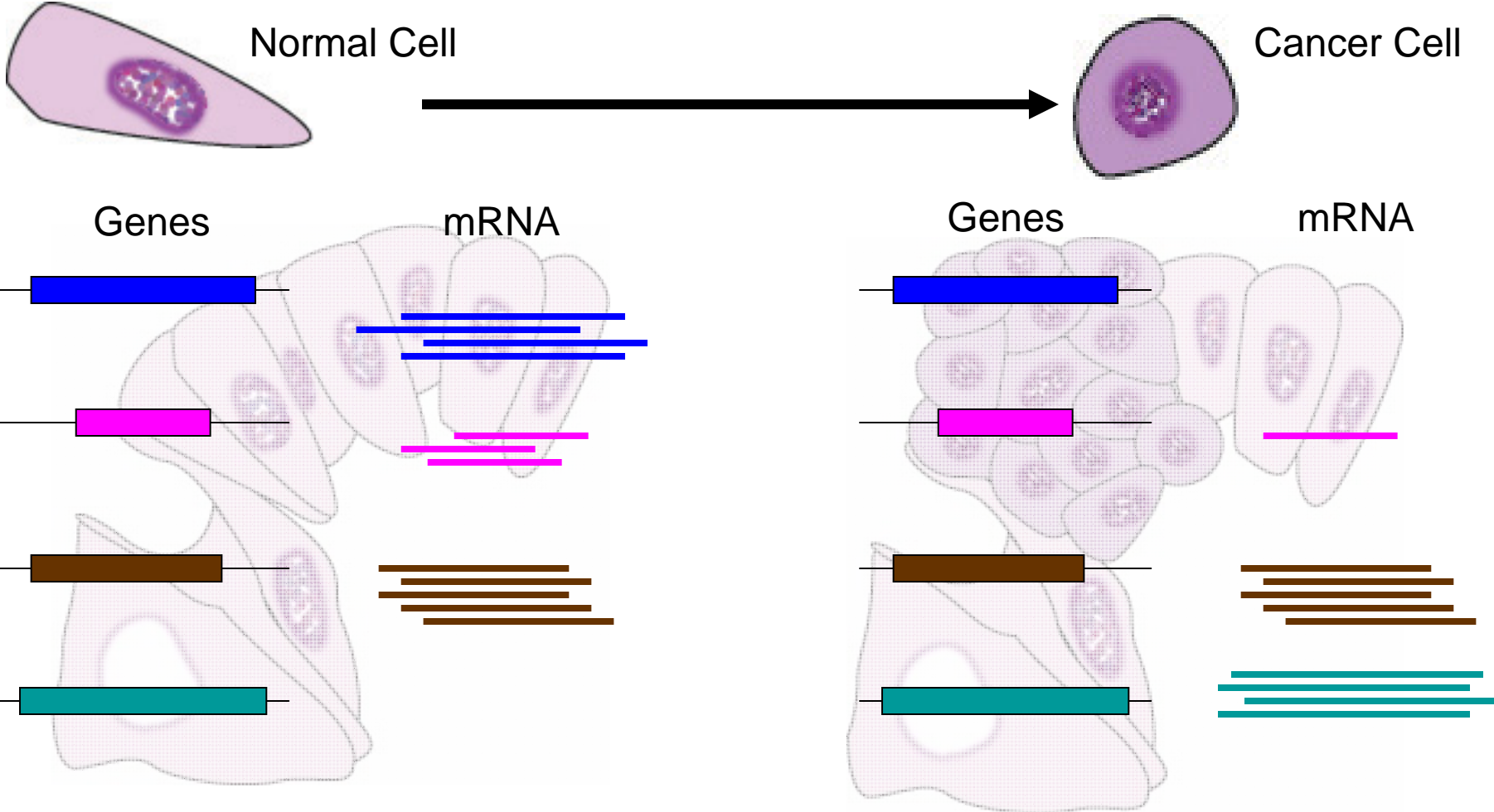
CANCEROUS CELLS

OFF

ON

Cancer causing genes are ON (expressed)

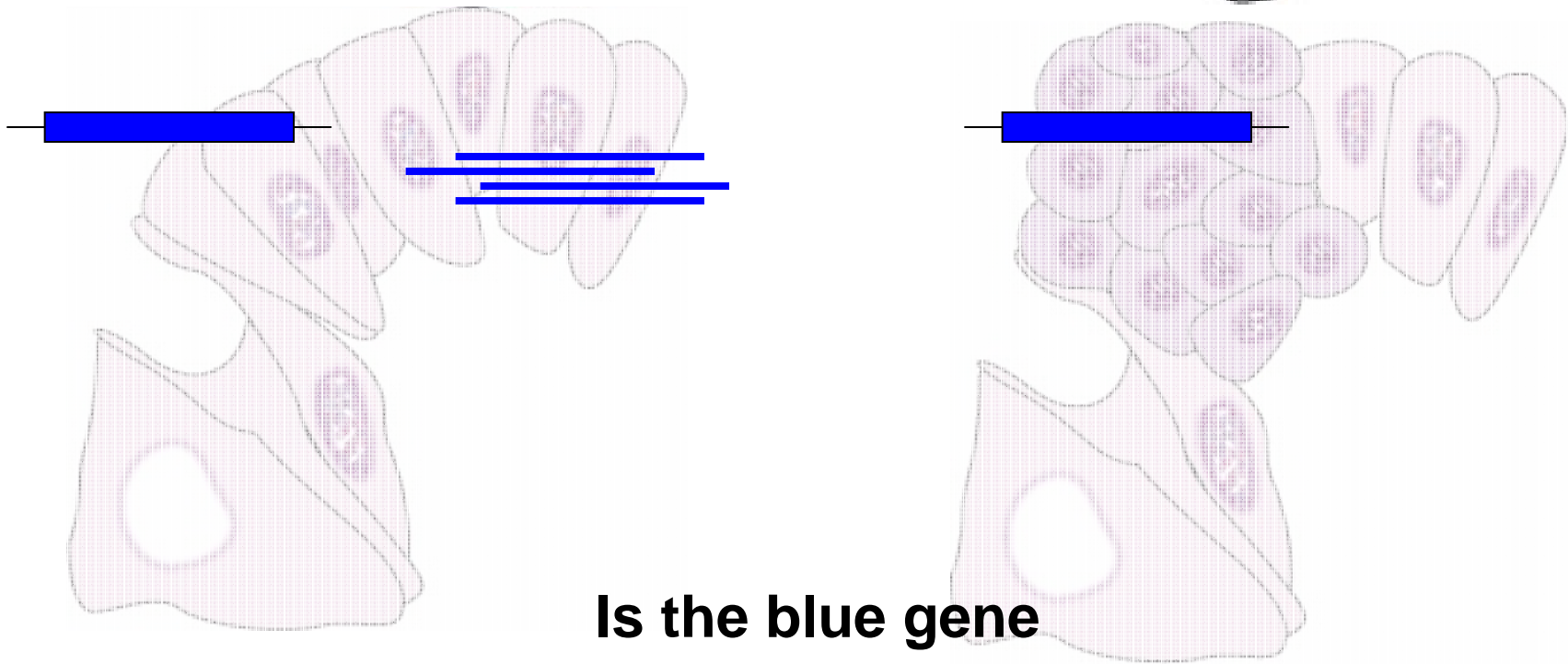**Cancer preventing** genes are OFF (not expressed)
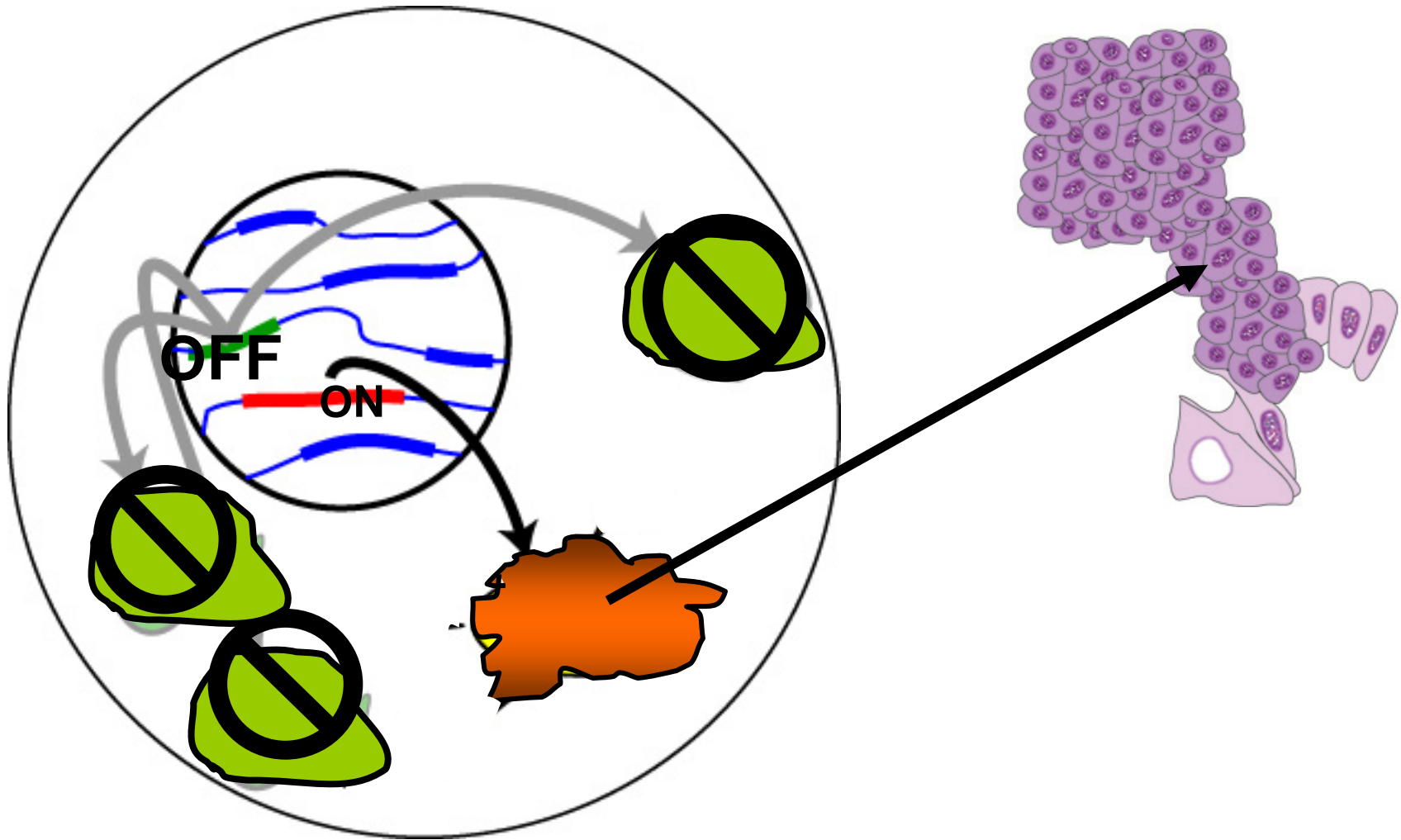
Changes in gene expression may lead to uncontrolled cell division

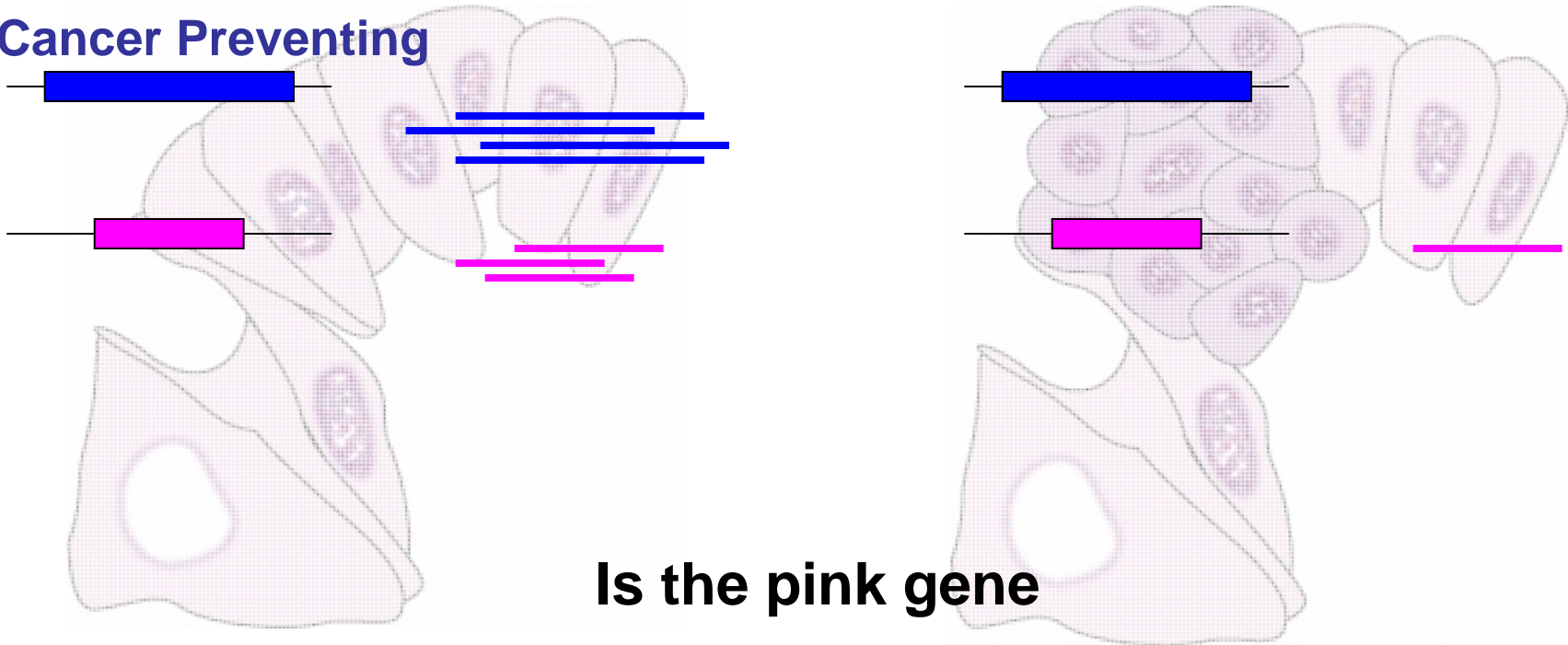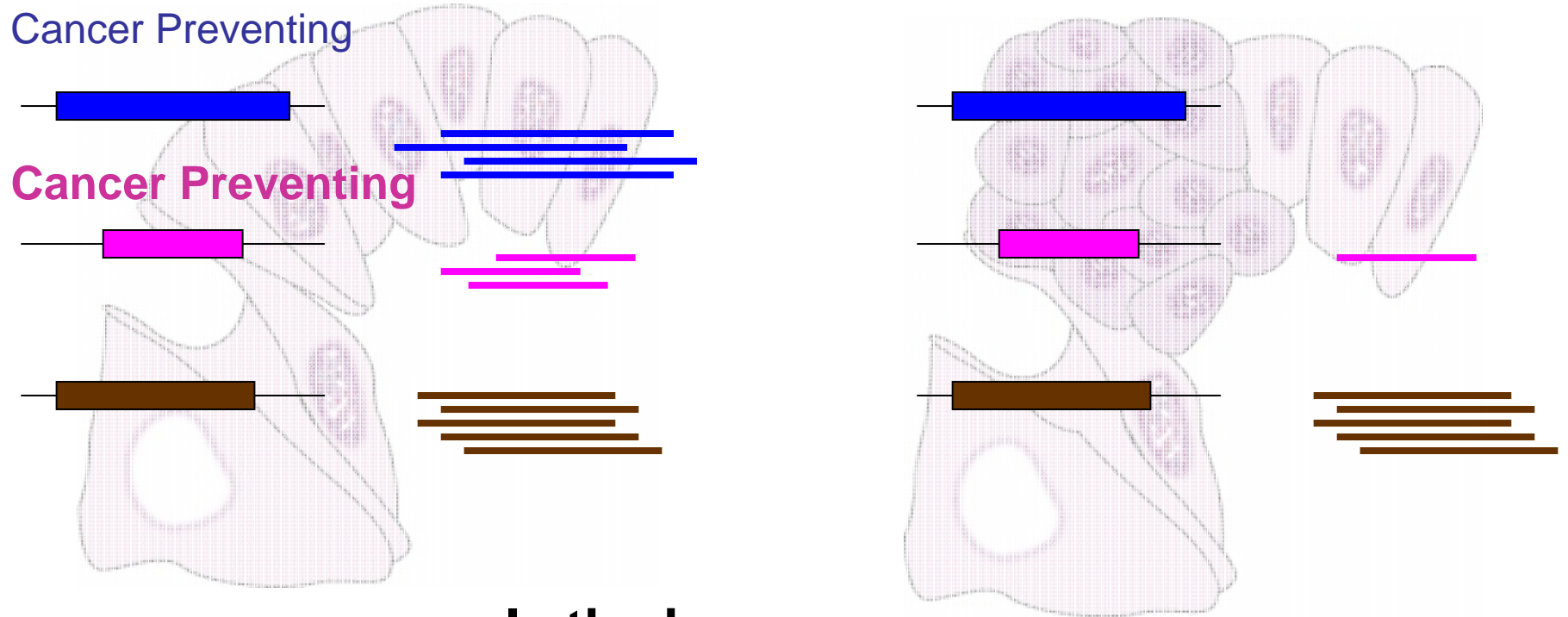# Changes in gene expression may lead to uncontrolled cell division

Normal Cell
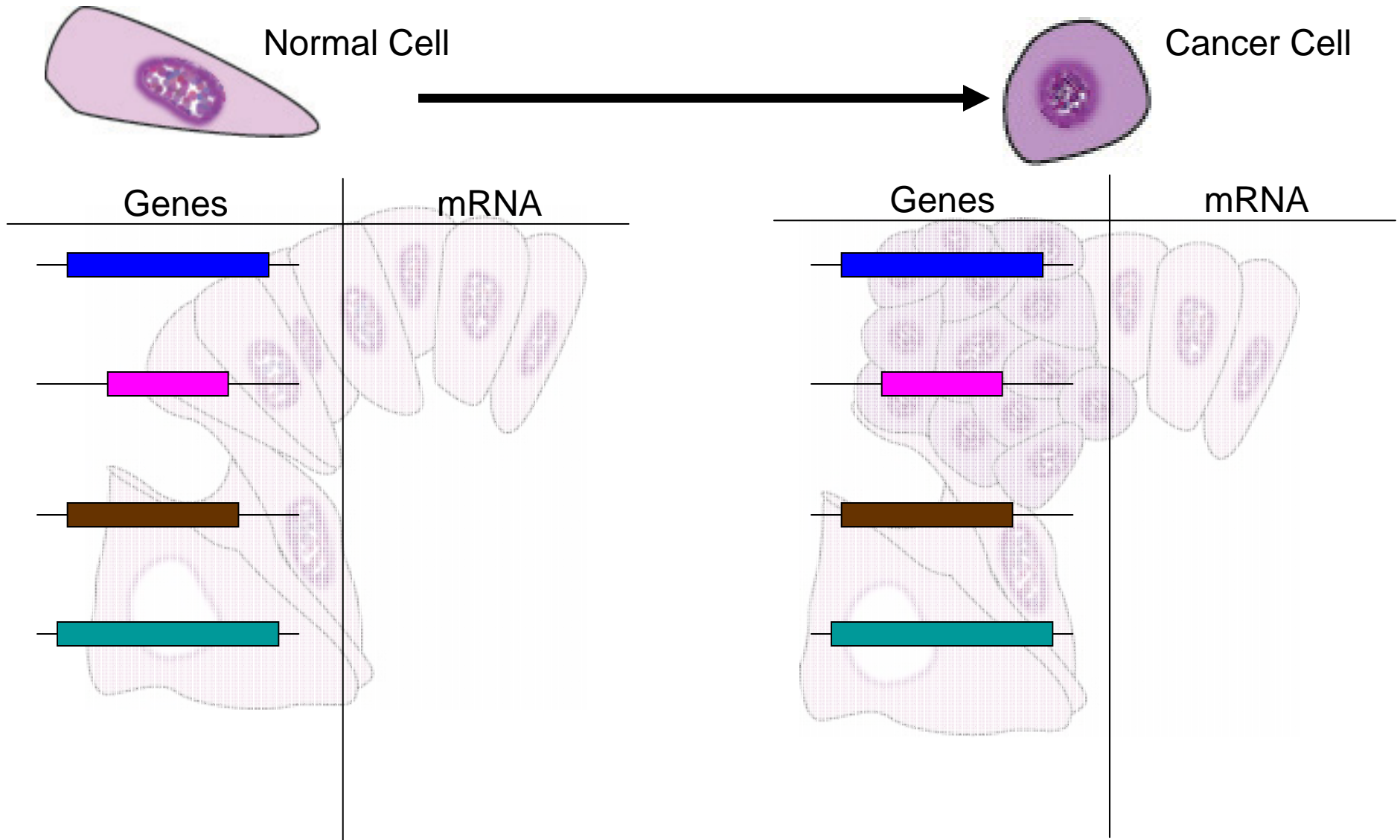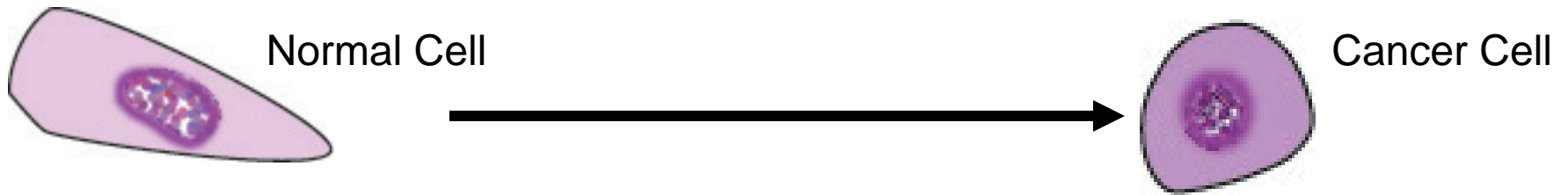
Cancer Cell

Is the blue gene

a cancer causing or cancer preventing gene?

Normal Cell

Cancer Cell

Cancer Preventing

Is the pink gene a cancer causing or cancer preventing gene?
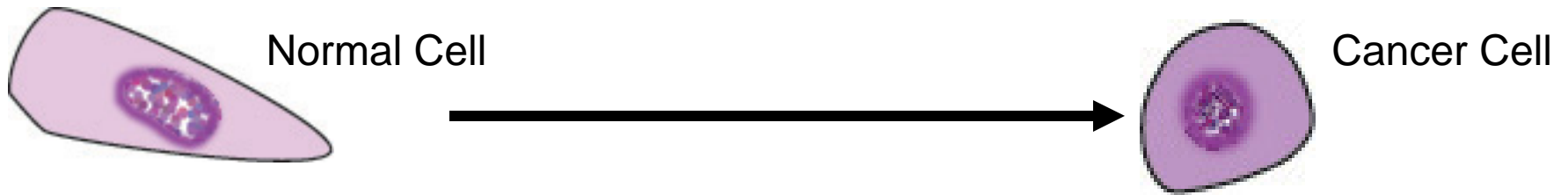
Normal Cell

Cancer Cell

Cancer Preventing

Cancer Preventing

Is the brown gene

a cancer causing or cancer preventing gene?

Normal Cell → Cancer Cell

Cancer Preventing

Cancer Preventing

Not involved

Is turquoise gene a cancer causing or cancer preventing gene?
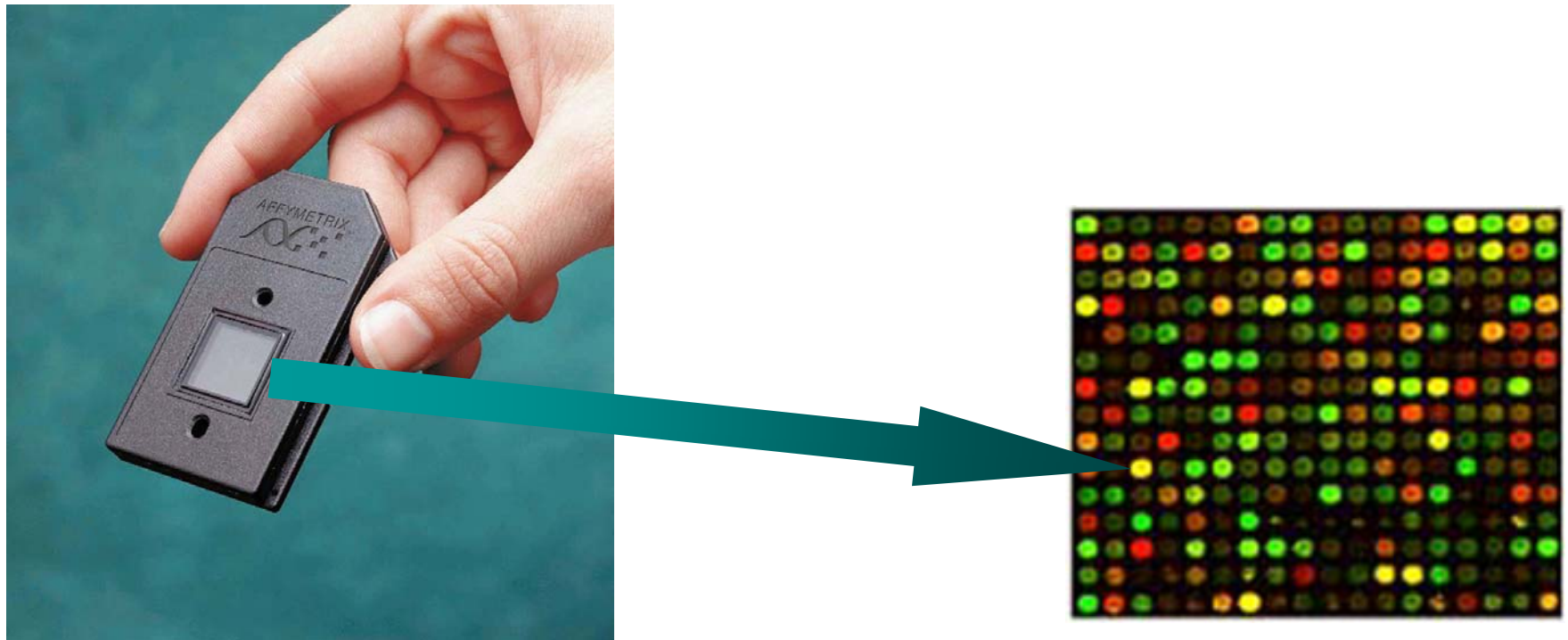
Normal Cell → Cancer Cell

Cancer Preventing

Cancer Preventing

Not involved
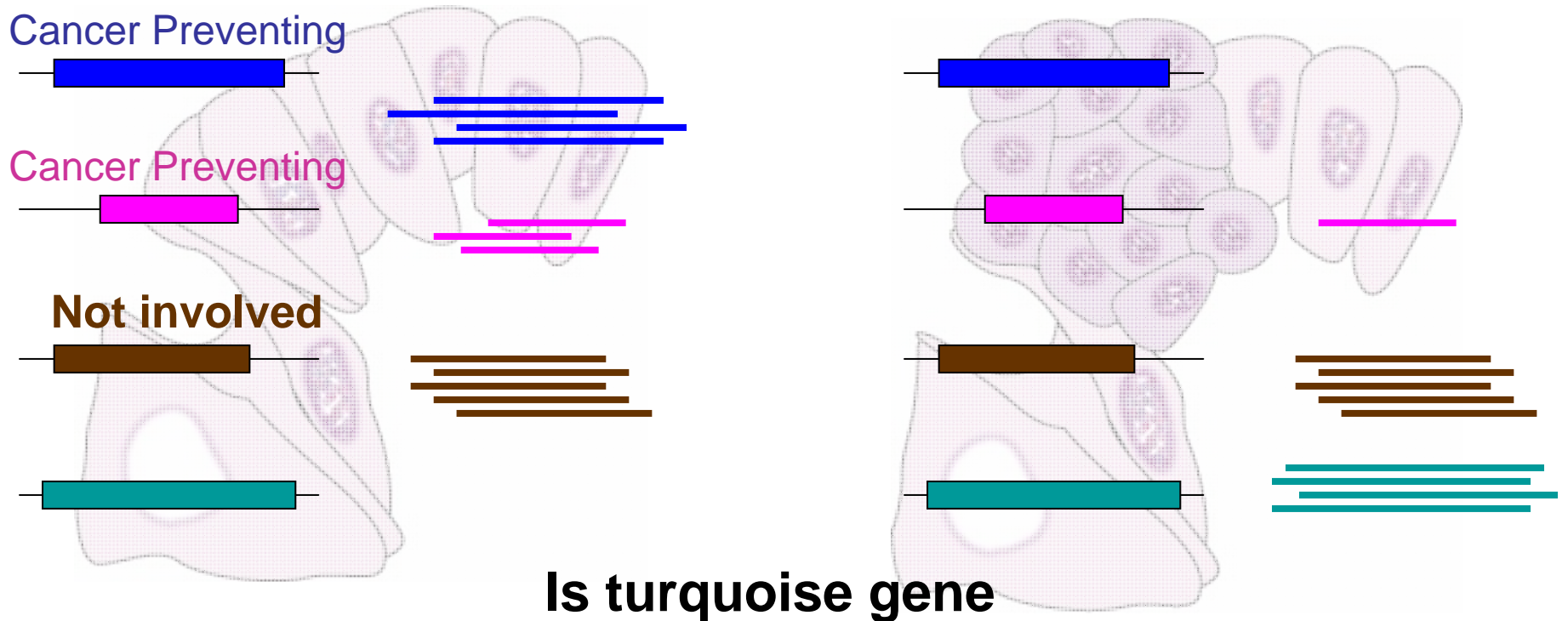
Cancer Causing
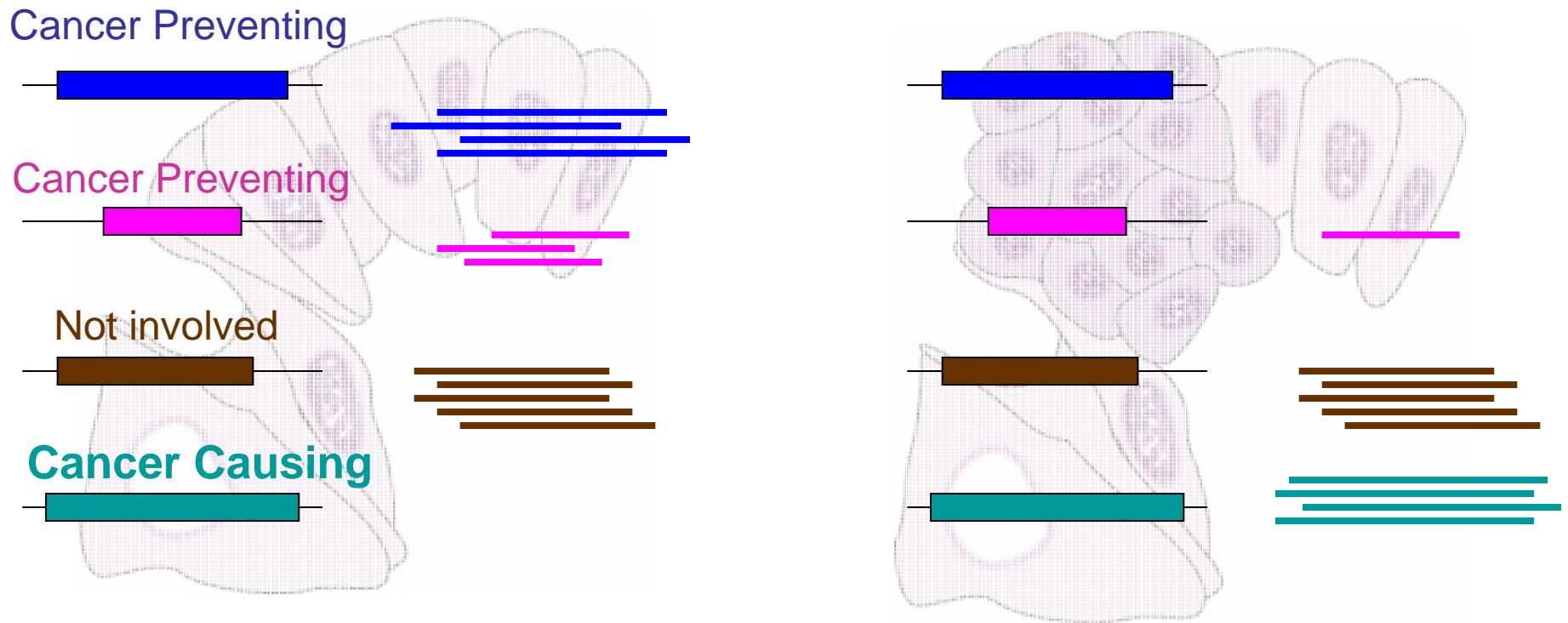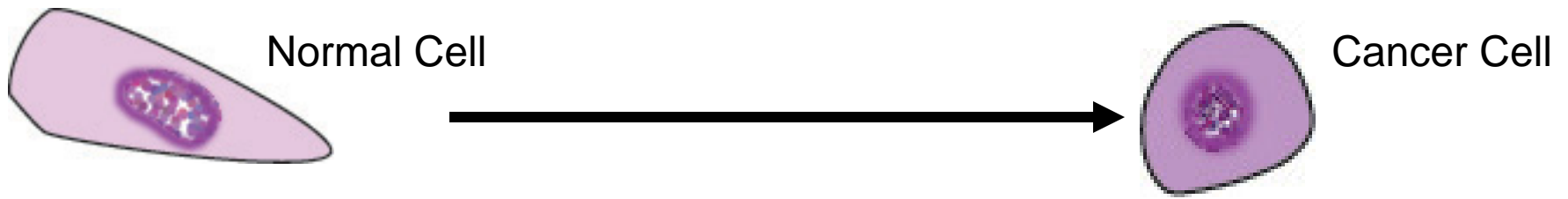
Studying changes in gene expression may lead to ways to prevent, diagnose, or treat cancer

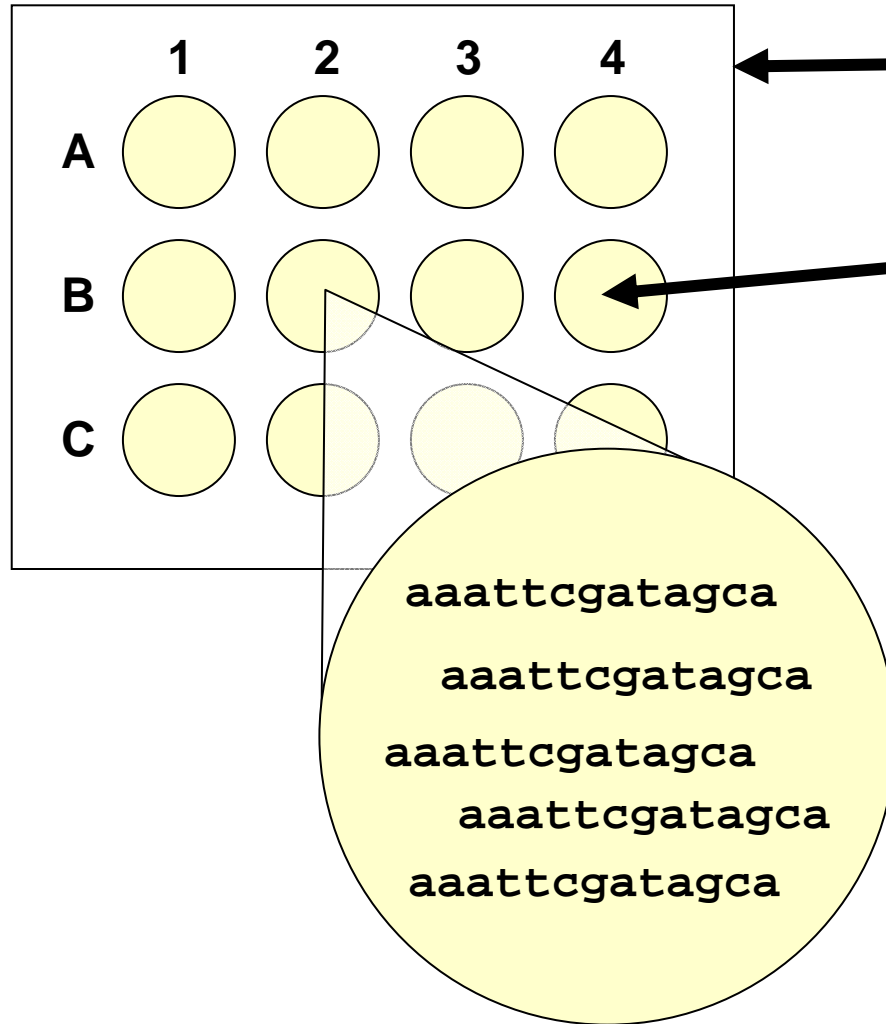# We can use DNA microarray technology to study changes in gene expression that lead to cancer

What is a DNA microarray?

A **DNA microarray** is a plastic chip or glass slide that has been "printed" with thousands of short, single-stranded pieces of DNA for known genes.
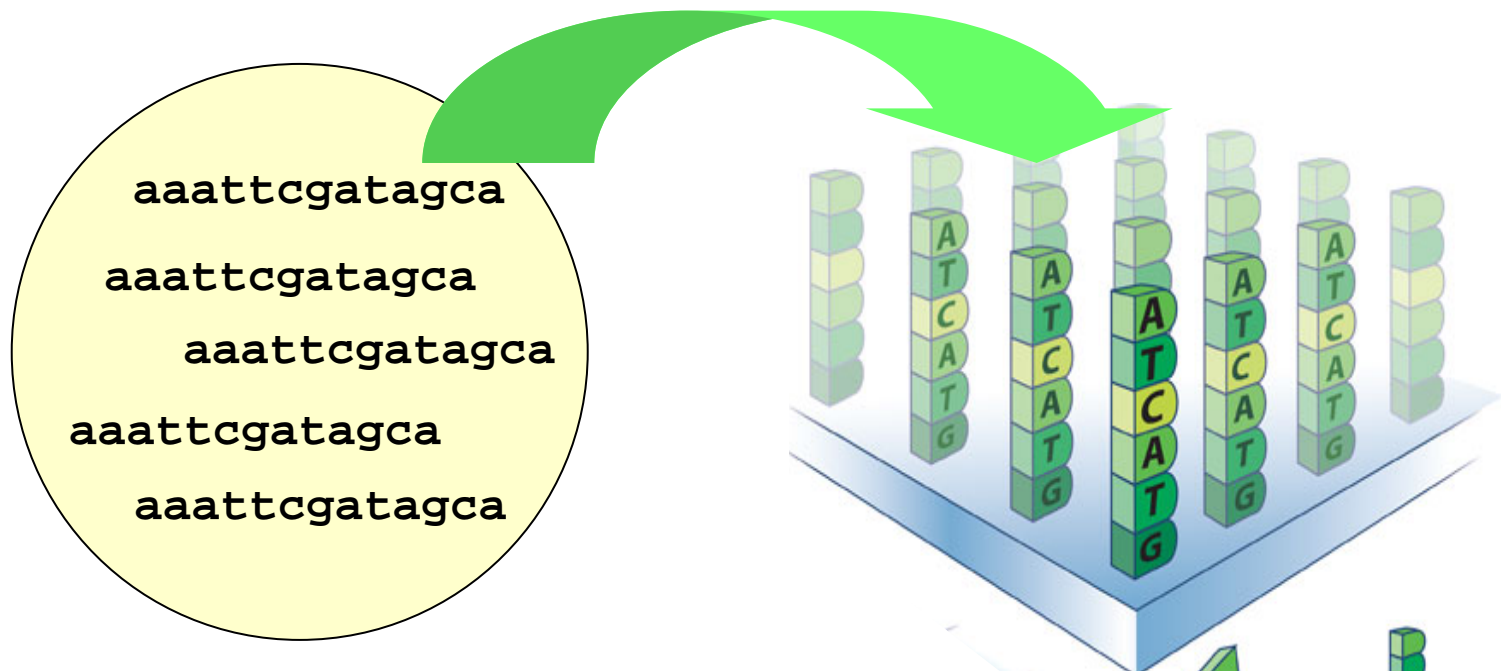
What is a DNA Microarray?

Glass or plastic slide

Each spot on the slide has different DNA sequences

- Microarrays usually have <u>thousands</u> of DNA spots

aaattcgatagca

aaattcgatagca

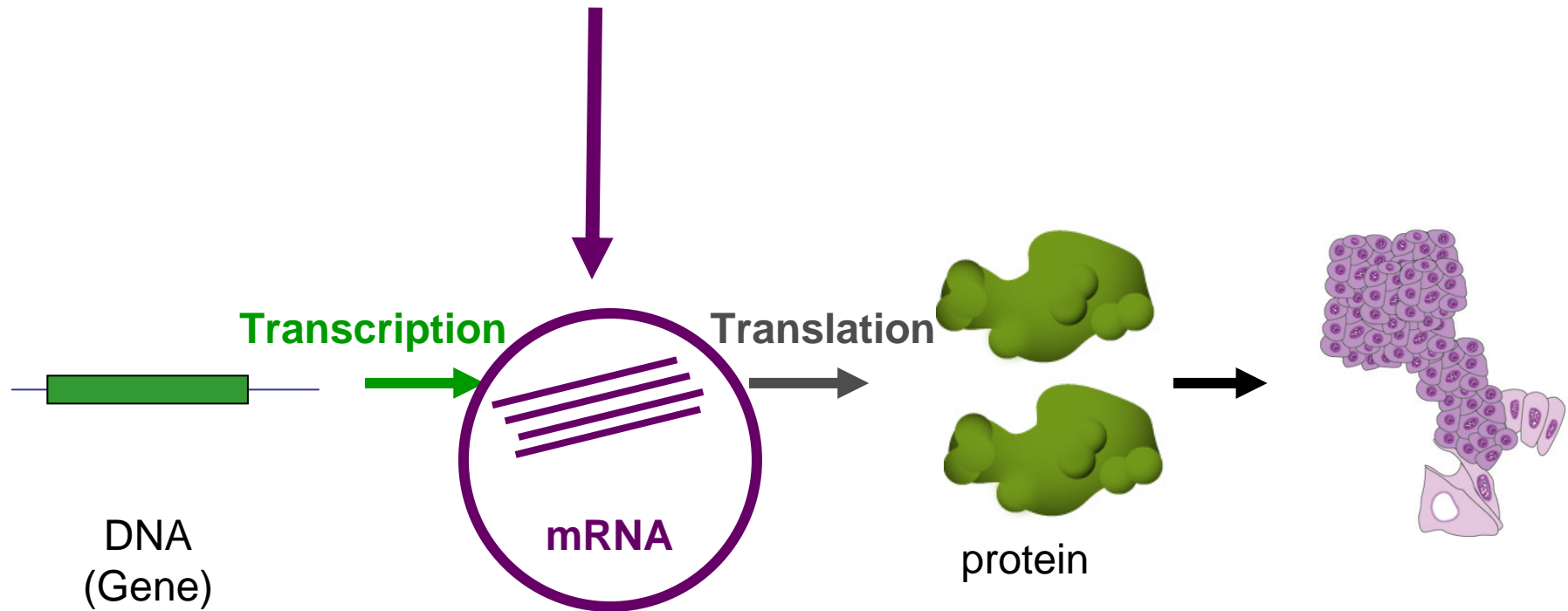aaattcgatagca

aaattcgatagca

aaattcgatagca

- Microscopic spot of single stranded DNA sequences attached to a slide.
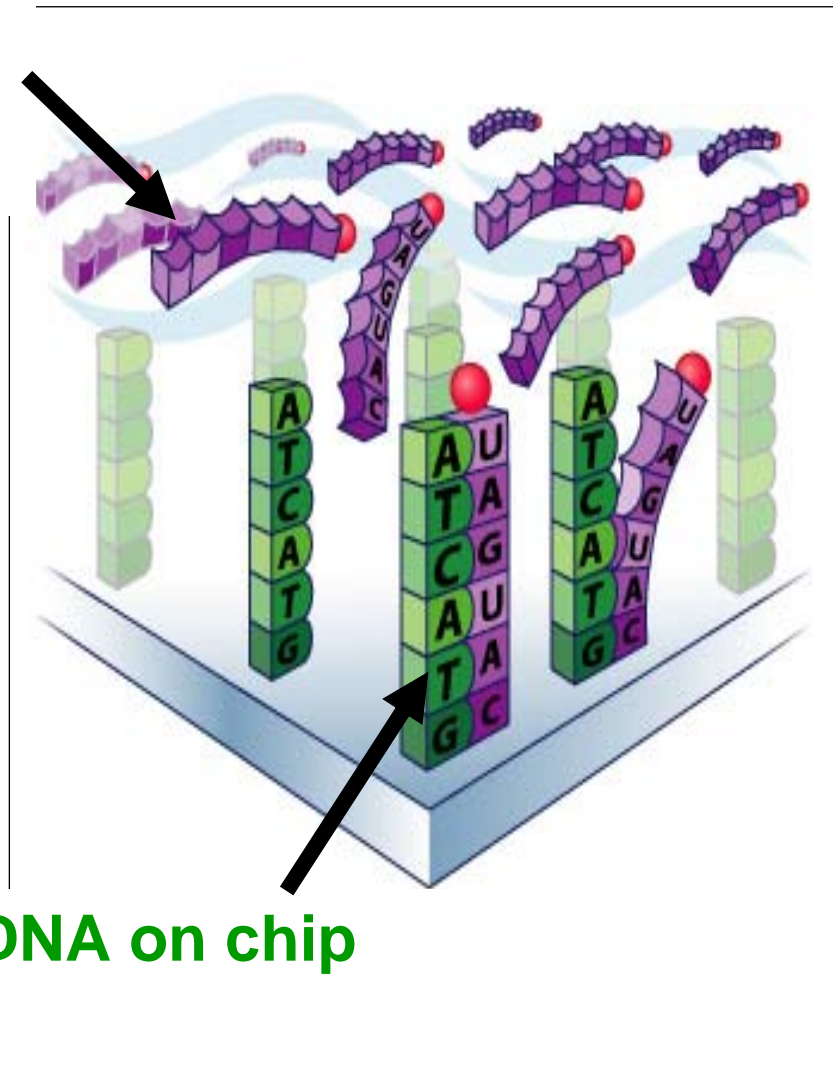
Millions of DNA strands built up in each location

Actual strand = 25 base pairs

Microarrays can be used to determine the types and quantities of **mRNAs** transcribed.

Transcription   Translation

DNA
(Gene)

mRNA

protein

How does a DNA Microarray work?

Labeled RNA or DNA in a sample

Binds to DNA on chip

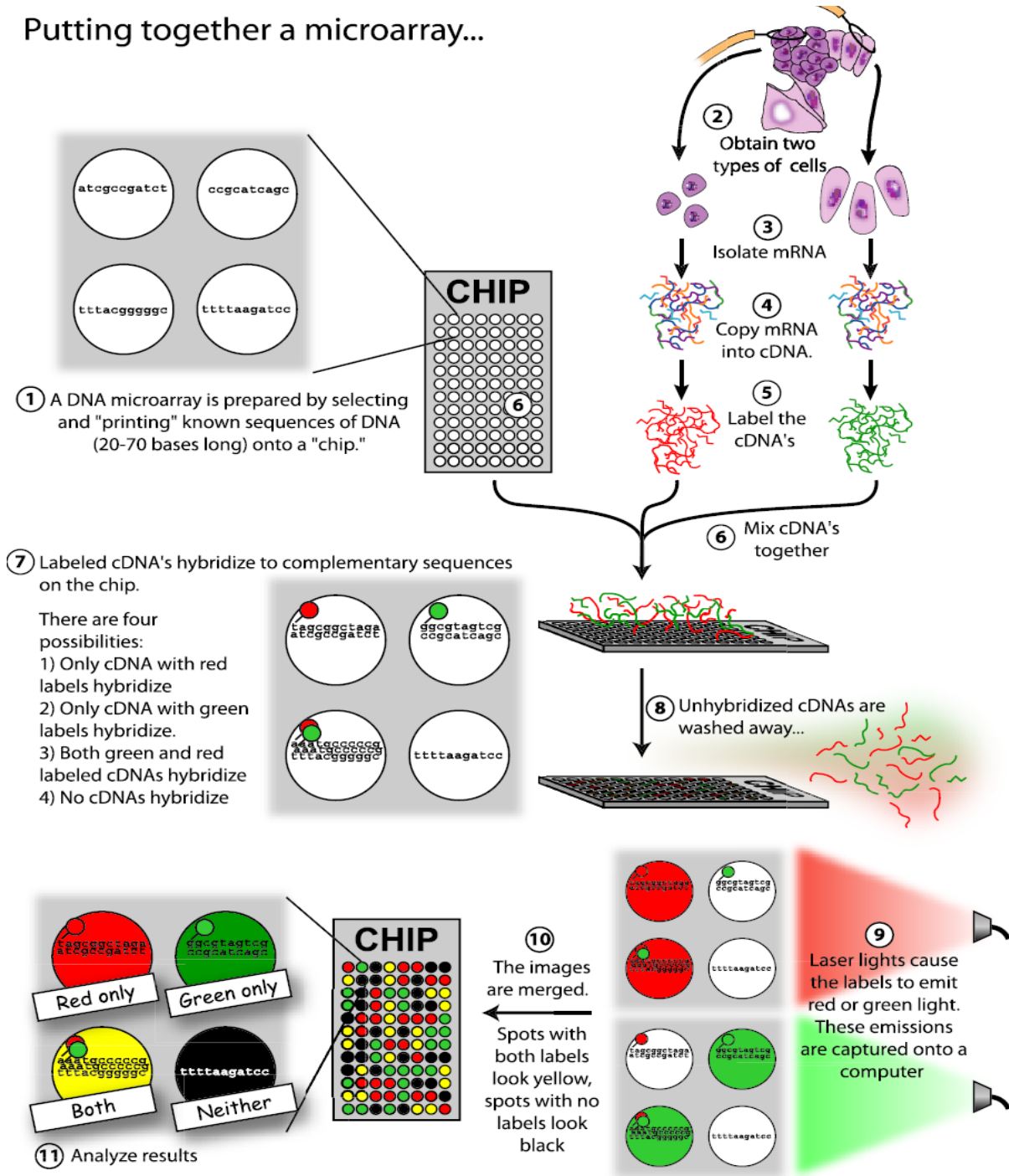# A Class Model of a Microarray

Studying gene expression in colon cancer cells
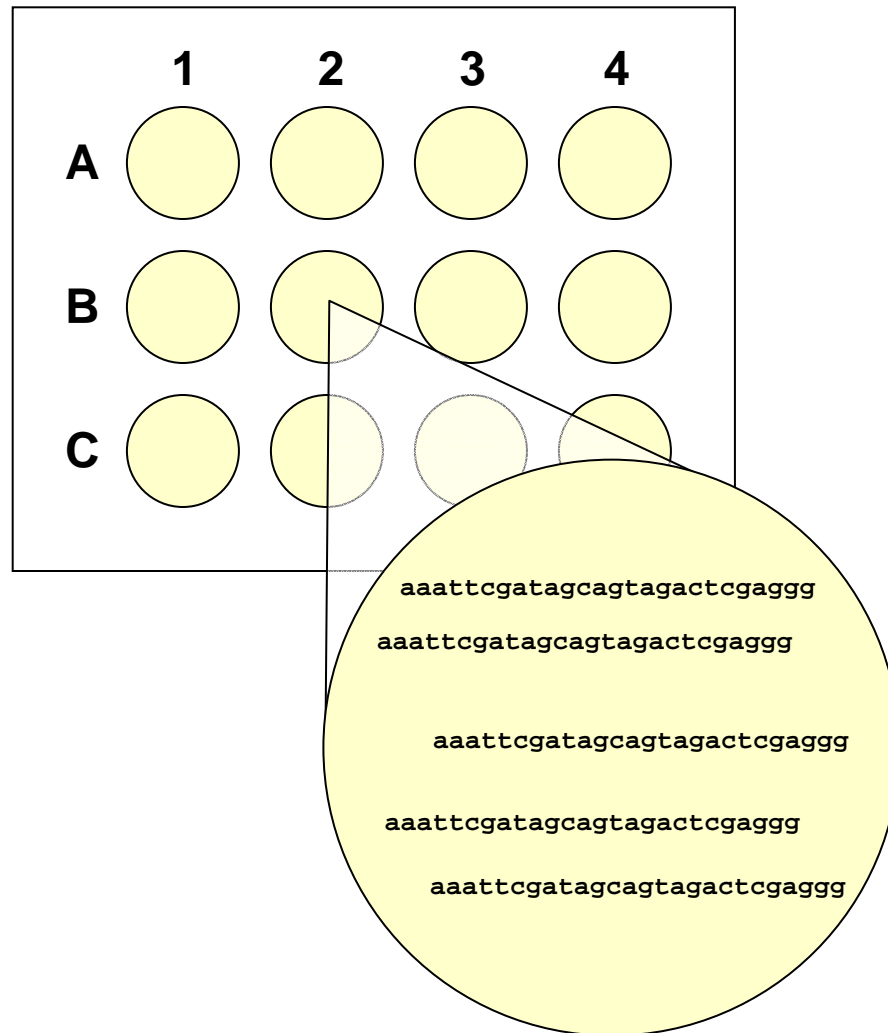
Summary Sheet

Follow the steps on the summary sheet as the class models the steps in a microarray experiment
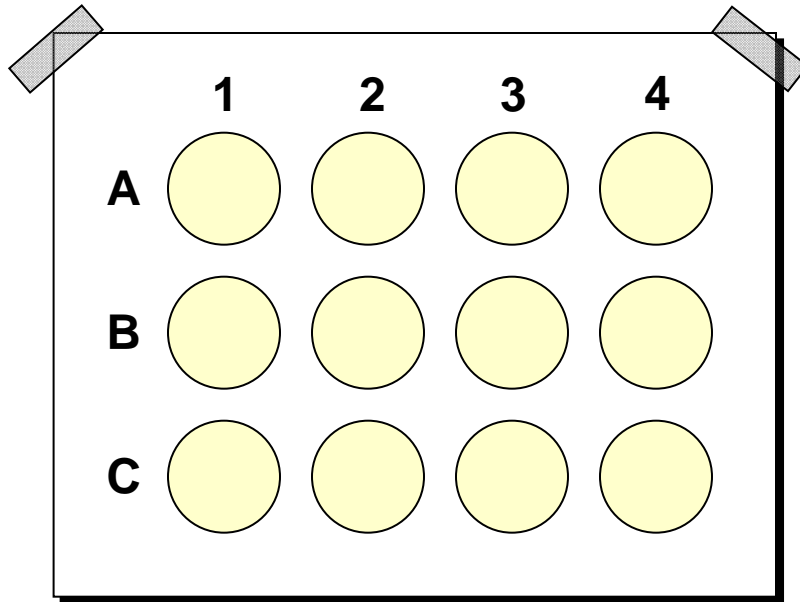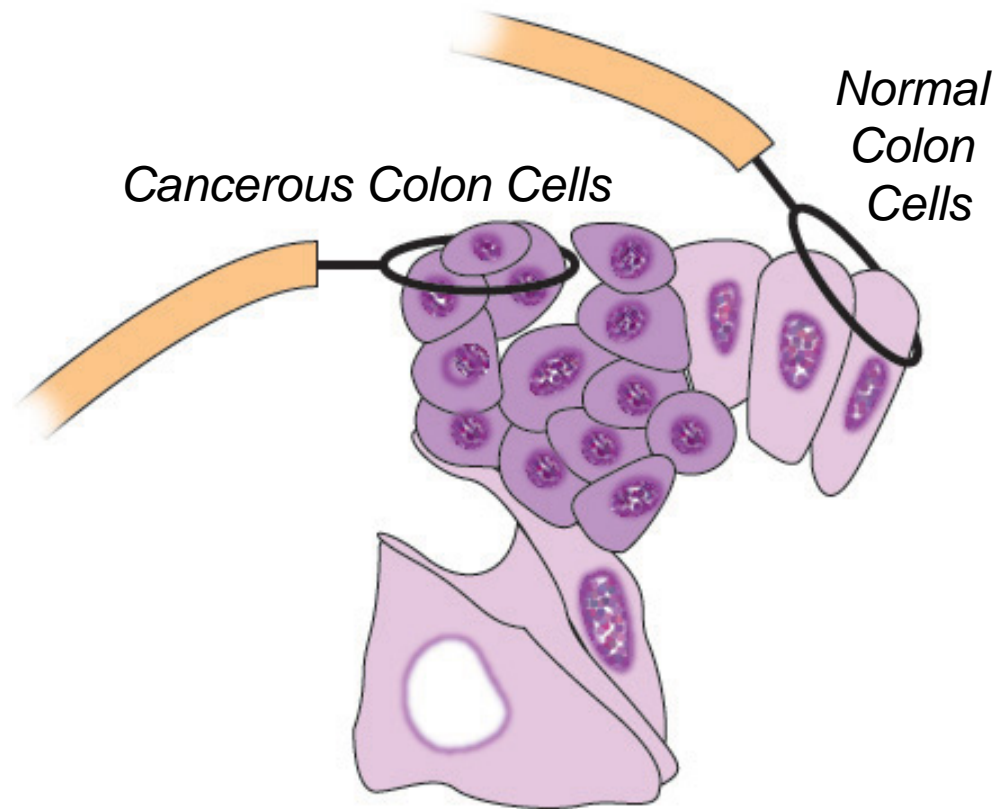

Putting together a microarray...

① A DNA microarray is prepared by selecting and "printing" known sequences of DNA (20-70 bases long) onto a "chip."

② Obtain two types of cells

③ Isolate mRNA

④ Copy mRNA into cDNA.

⑤ Label the cDNA's

⑥ Mix cDNA's together

⑦ Labeled cDNA's hybridize to complementary sequences on the chip.

There are four possibilities:
1) Only cDNA with red labels hybridize
2) Only cDNA with green labels hybridize.
3) Both green and red labeled cDNAs hybridize
4) No cDNAs hybridize

⑧ Unhybridized cDNAs are washed away...

⑨ Laser lights cause the labels to emit red or green light. These emissions are captured onto a computer

⑩ The images are merged.

Spots with both labels look yellow, spots with no labels look black

Red only   Green only
Both   Neither

⑪ Analyze results

STEP 1

- **"Print" genes that might be involved in causing colon cancer onto the chip.**

- **Also "print" positive and negative control genes.**

aaattcgatagcagtagactcgaggg

aaattcgatagcagtagactcgaggg

aaattcgatagcagtagactcgaggg

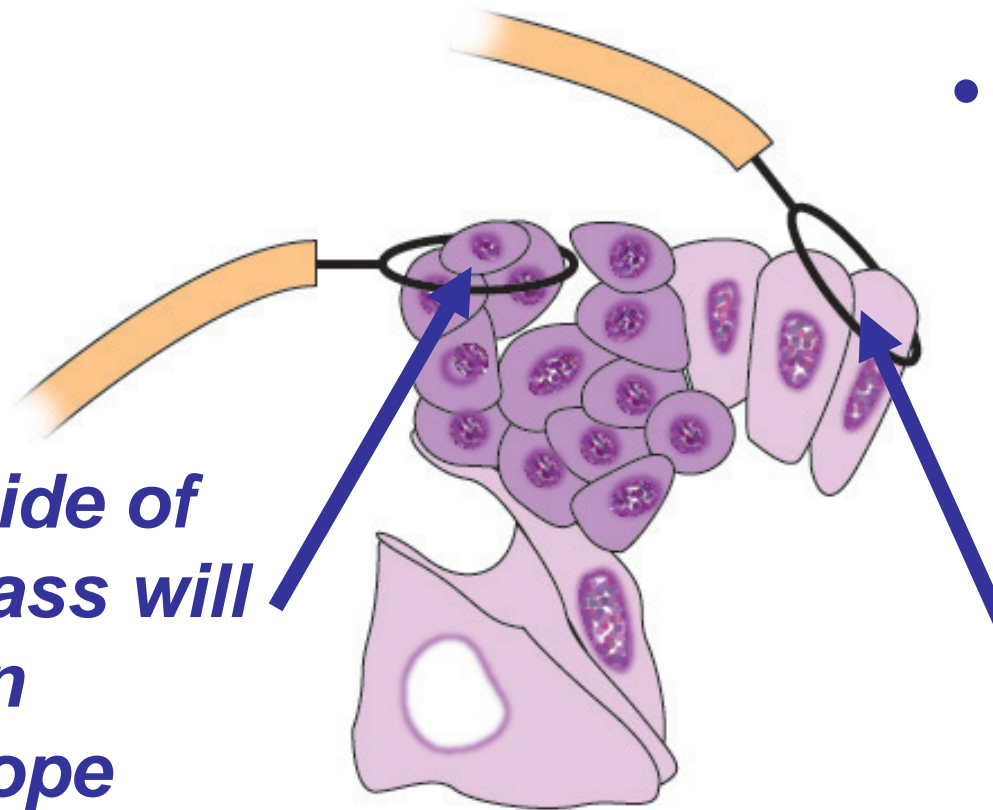aaattcgatagcagtagactcgaggg

aaattcgatagcagtagactcgaggg

STEP 1

- *A "wall-mounted" microarray has been pre-printed with genes that we will study.*

STEP 2

Cancerous Colon Cells

Normal Colon Cells

- **Collect cancerous colon cells and normal colon cells from a patient.**

STEP 2

- **One side of the class will use an envelope representing cancerous colon cells**

- **The other side of the class will use an envelope representing normal colon cells**

STEP 3
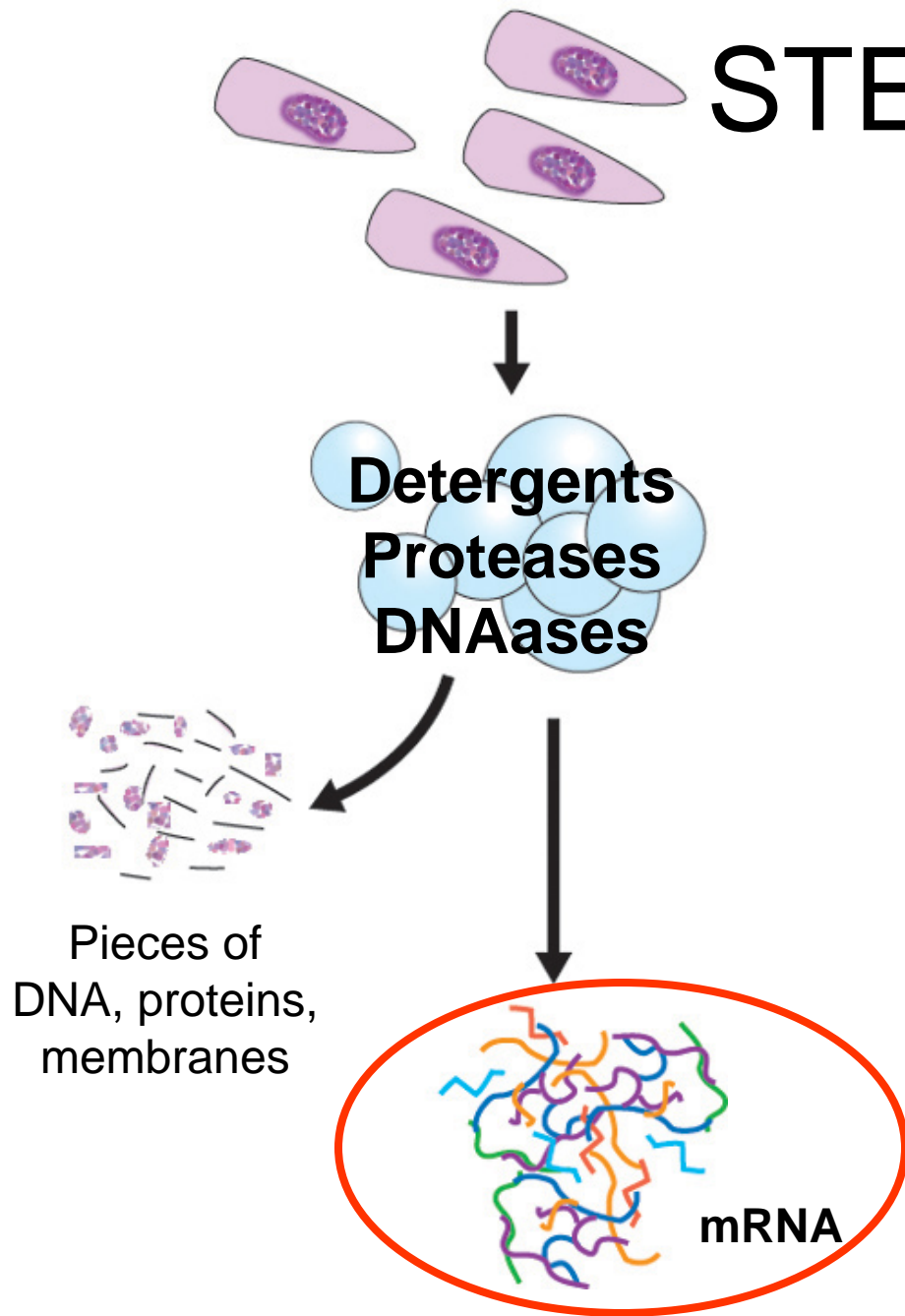
- **Isolate mRNA from the two types of cells**

Remember, when a gene is expressed, the DNA is transcribed (copied) to make messenger RNA (mRNA)

Detergents Proteases DNAases

Pieces of DNA, proteins, membranes

mRNA

STEP 3
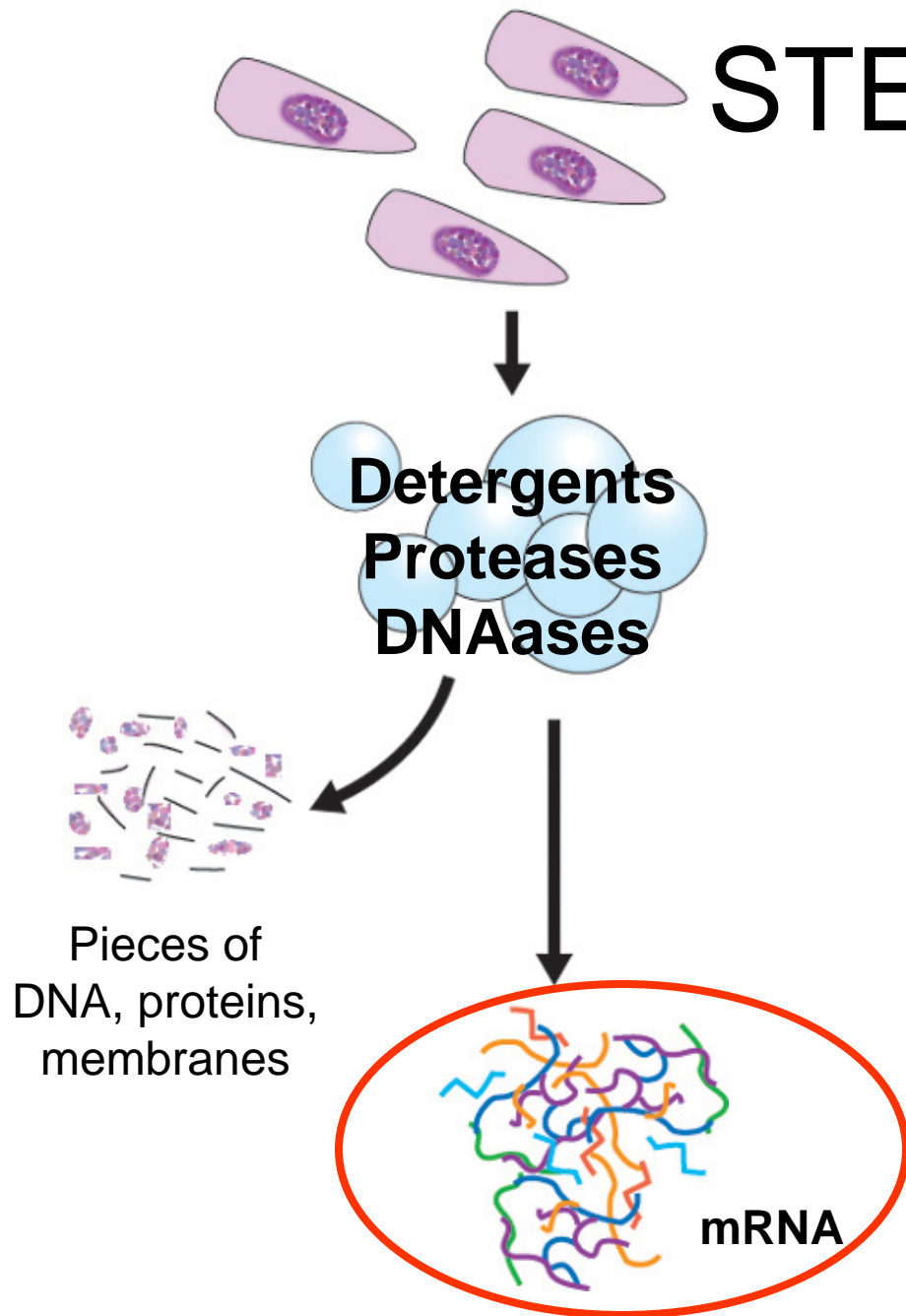
- *Remove the mRNA strips from the envelope*
- *Distribute one mRNA strip to each student on your side of the room.*

Detergents
Proteases
DNAases

Pieces of DNA, proteins, membranes

mRNA

STEP 4

- **Use reverse transcriptase enzyme to synthesize cDNA from mRNA**
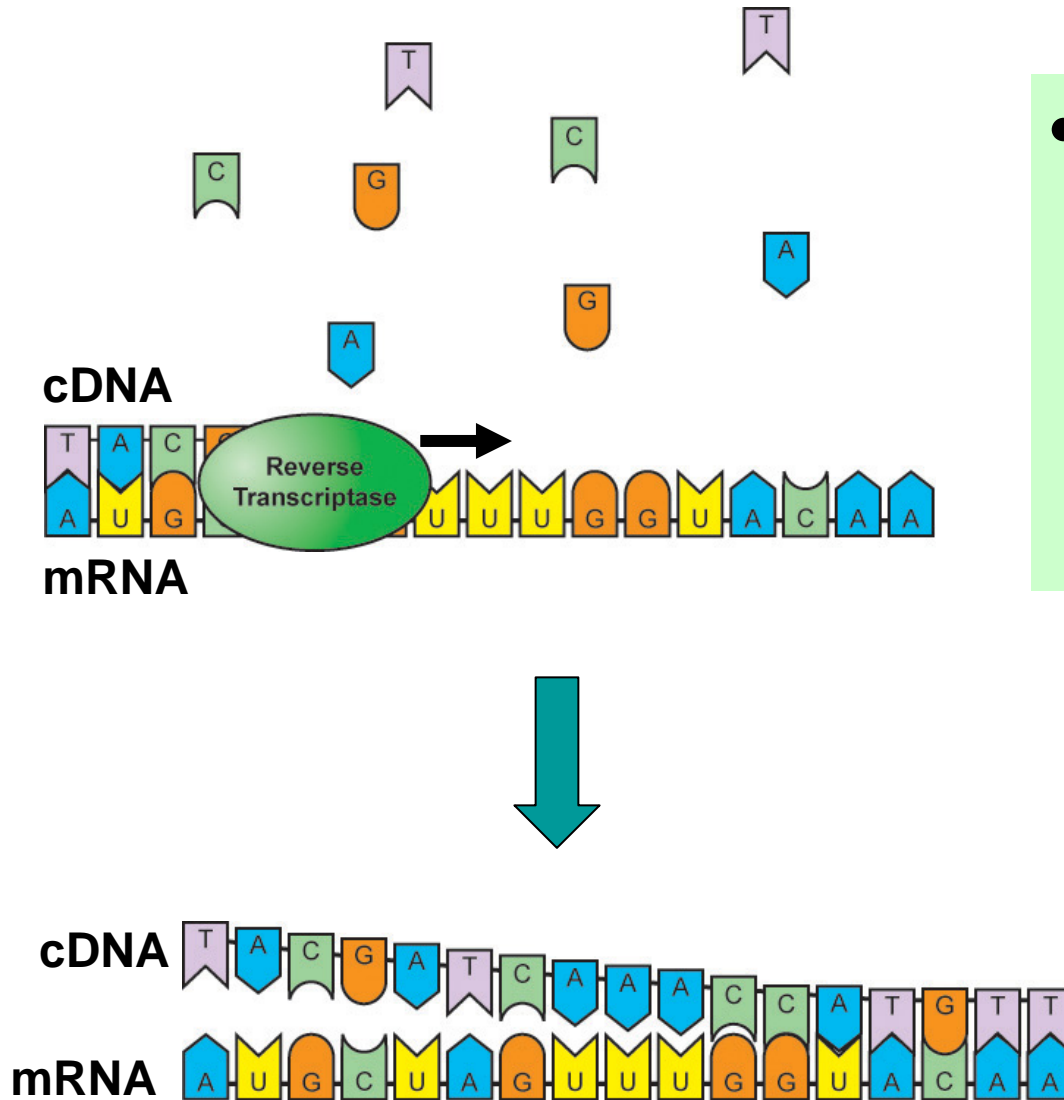
Reverse Transcriptase copies mRNA to make complementary, single stranded cDNA

STEP 4
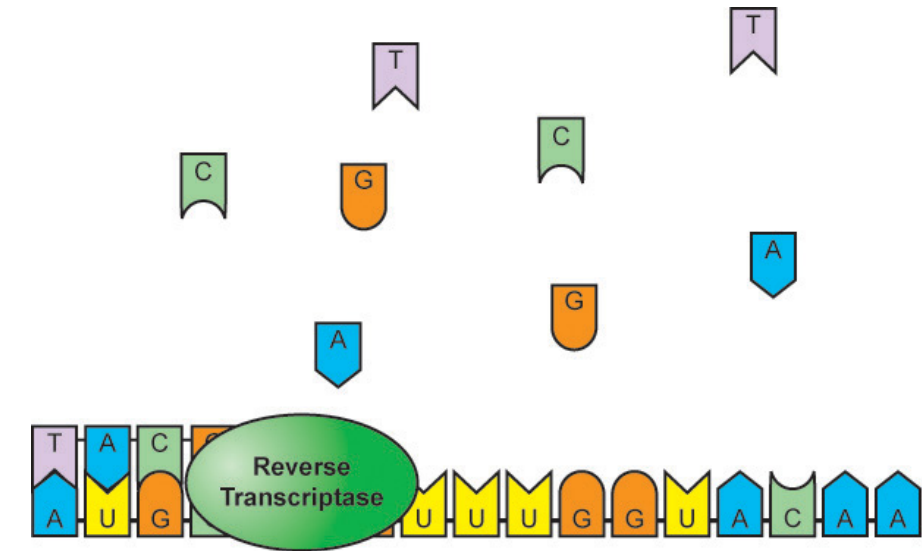
- *Act like a Reverse Transcriptase enzyme; copy the RNA molecule into a cDNA molecule (Remember to use T, not U!)*

- *Use scissors to separate the cDNA from the RNA.*

- *Save the cDNA, and discard the RNA*

STEP 4: What you have
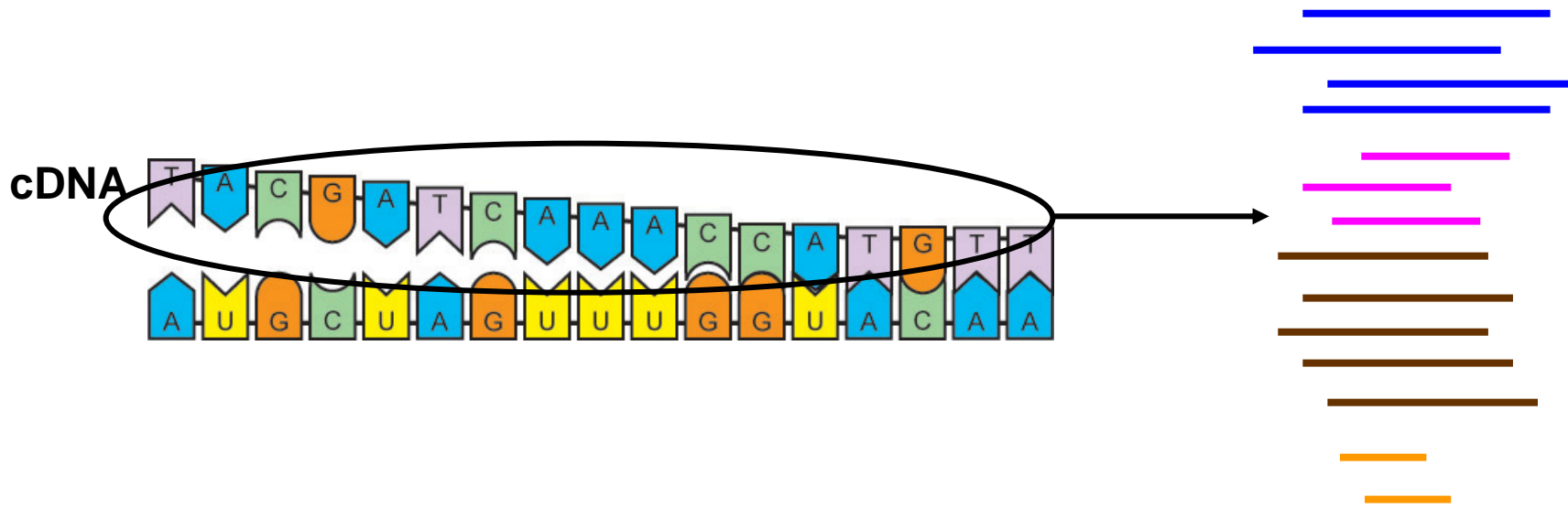
cDNA

cDNA's
from normal cells
or from cancerous cells

STEP 5
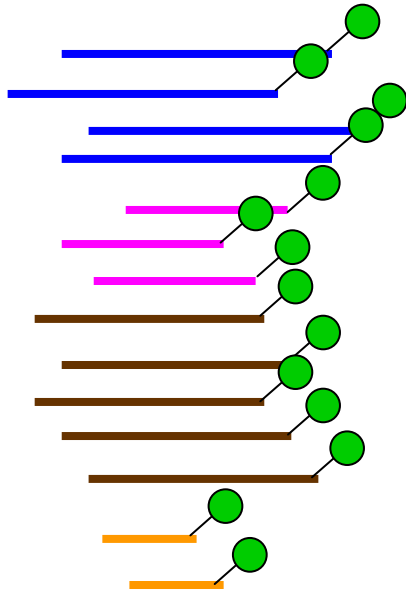
**cDNA from
Normal Colon Cells**
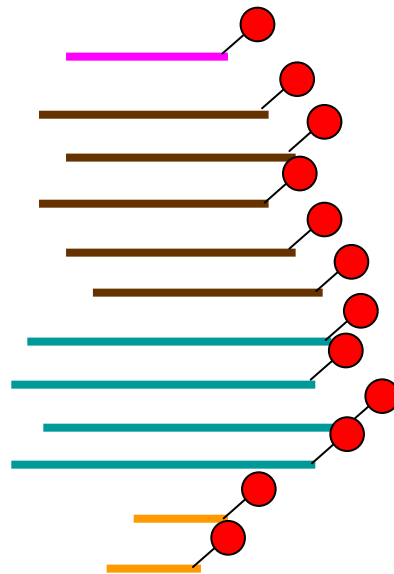
**cDNA from
Cancerous Colon Cells**

- **Label the cDNA from the two kinds of cells with different colored fluorescent labels.**

STEP 5

**cDNA from
Normal Colon Cells**
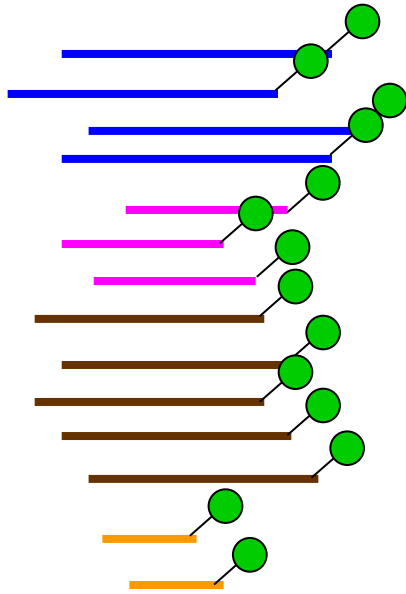
**cDNA from
Cancerous Colon Cells**

Label the cDNA by attaching:

- *a green sticker to the end of cDNA from normal cells*
- *a red sticker to the end of cDNA from cancer cells*
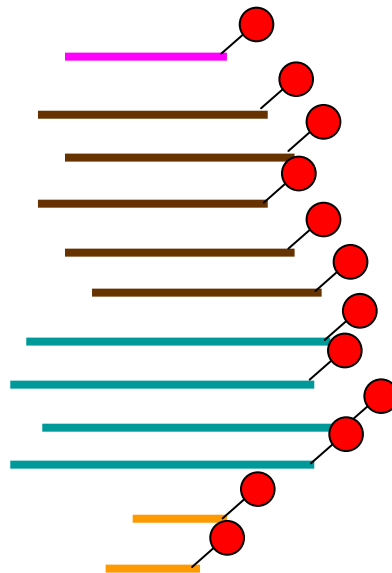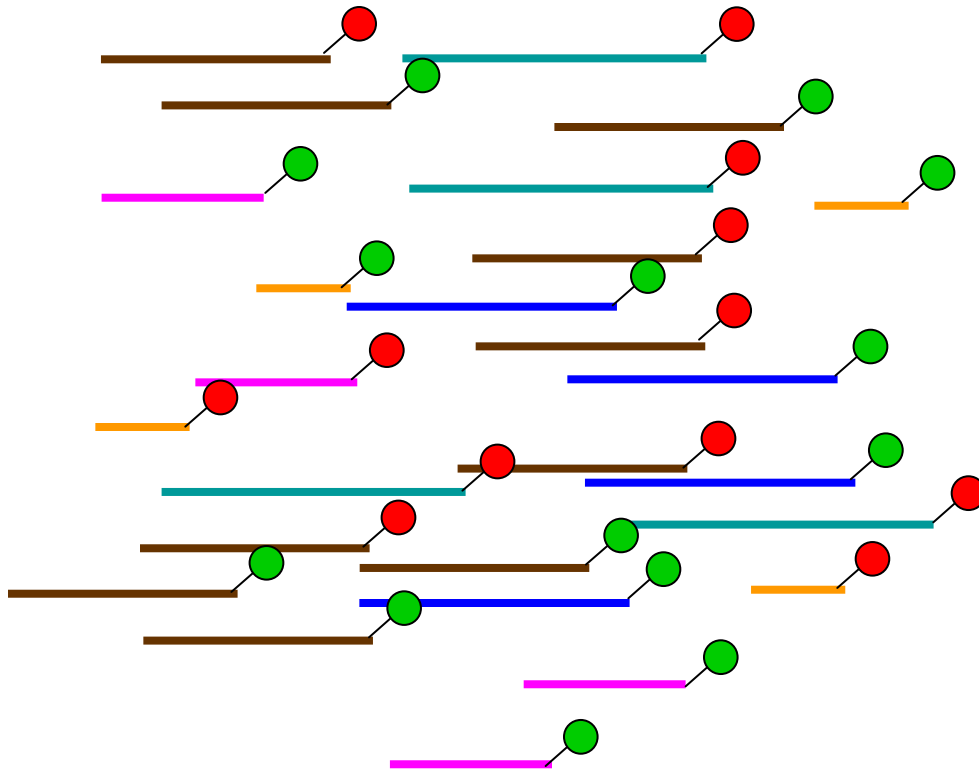
STEP 6

- **Mix the labeled cDNAs from the two kinds of cells together.**

STEP 6
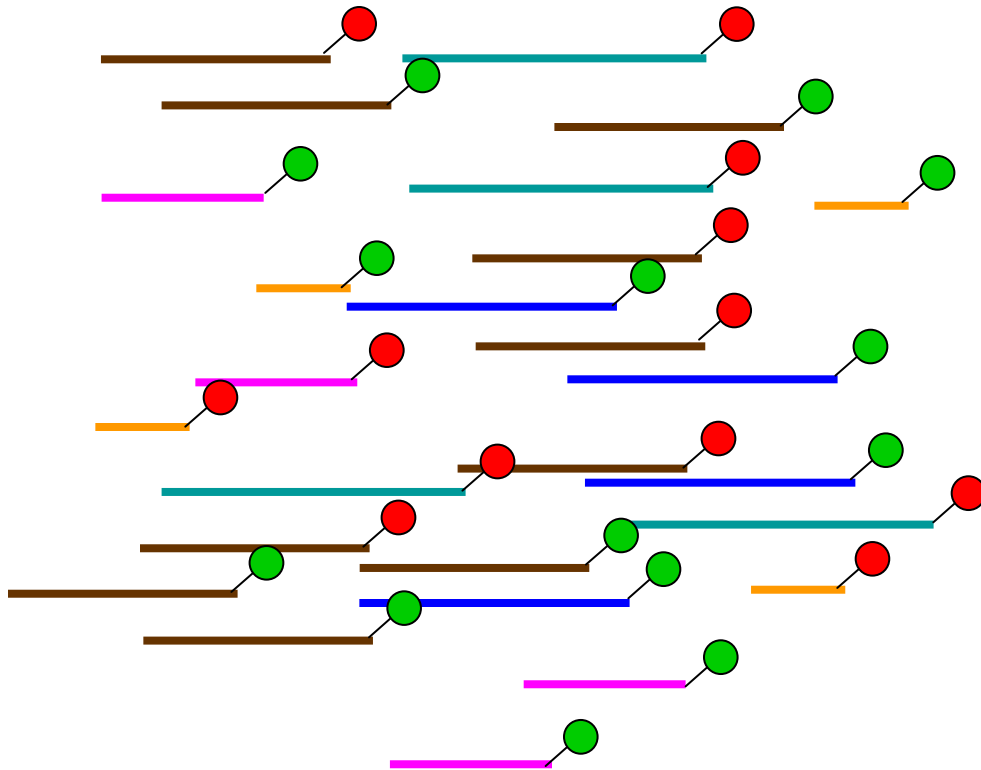
- *Place the labeled cDNA strips into a "hybridization solution" bucket in the center of the room.*

STEP 7

Labeled cDNA

Genes on chip
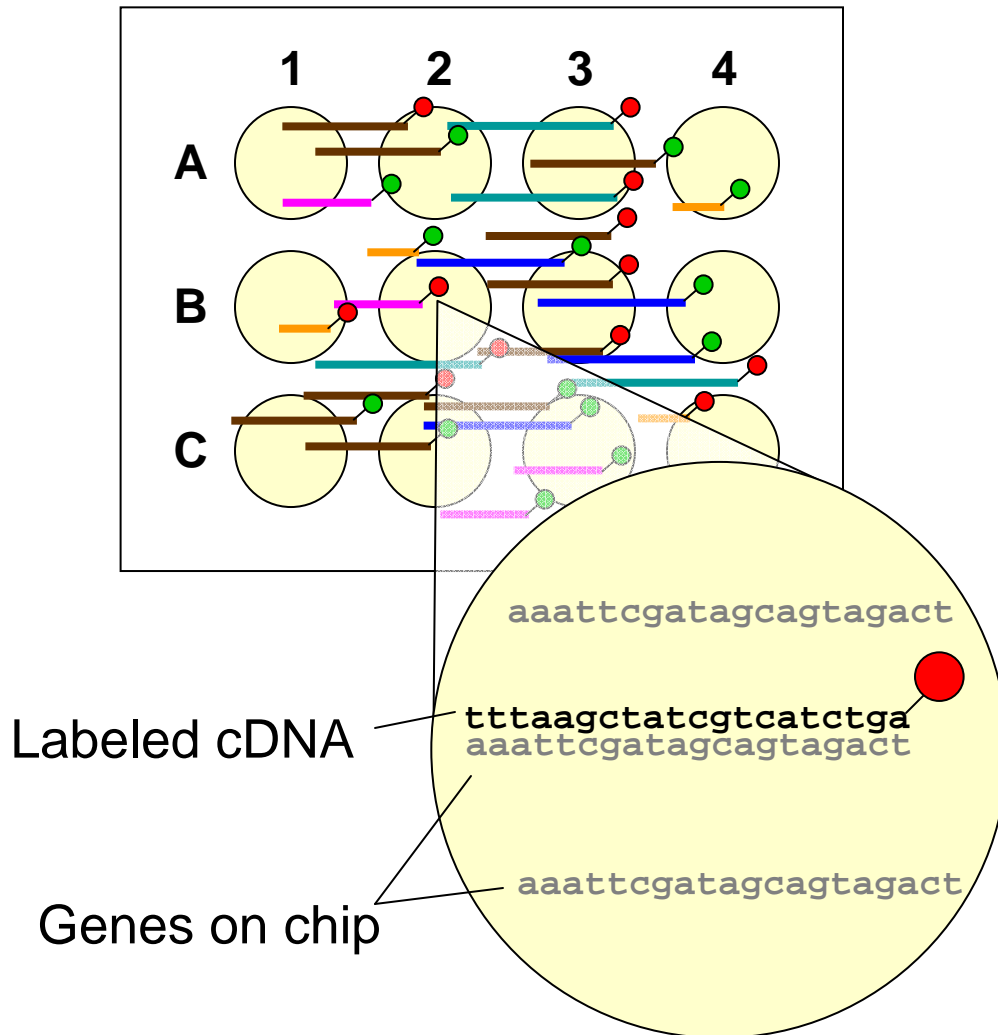
- **Soak the microarray slide in the mixture of labeled cDNA's.**

- **cDNA's that are complementary to sequences on the microarray will hybridize (bind).**

STEP 7

Labeled cDNA

Genes on chip

aaattcgatagcagtagact

tttaagctatcgtcatctga
aaattcgatagcagtagact

aaattcgatagcagtagact
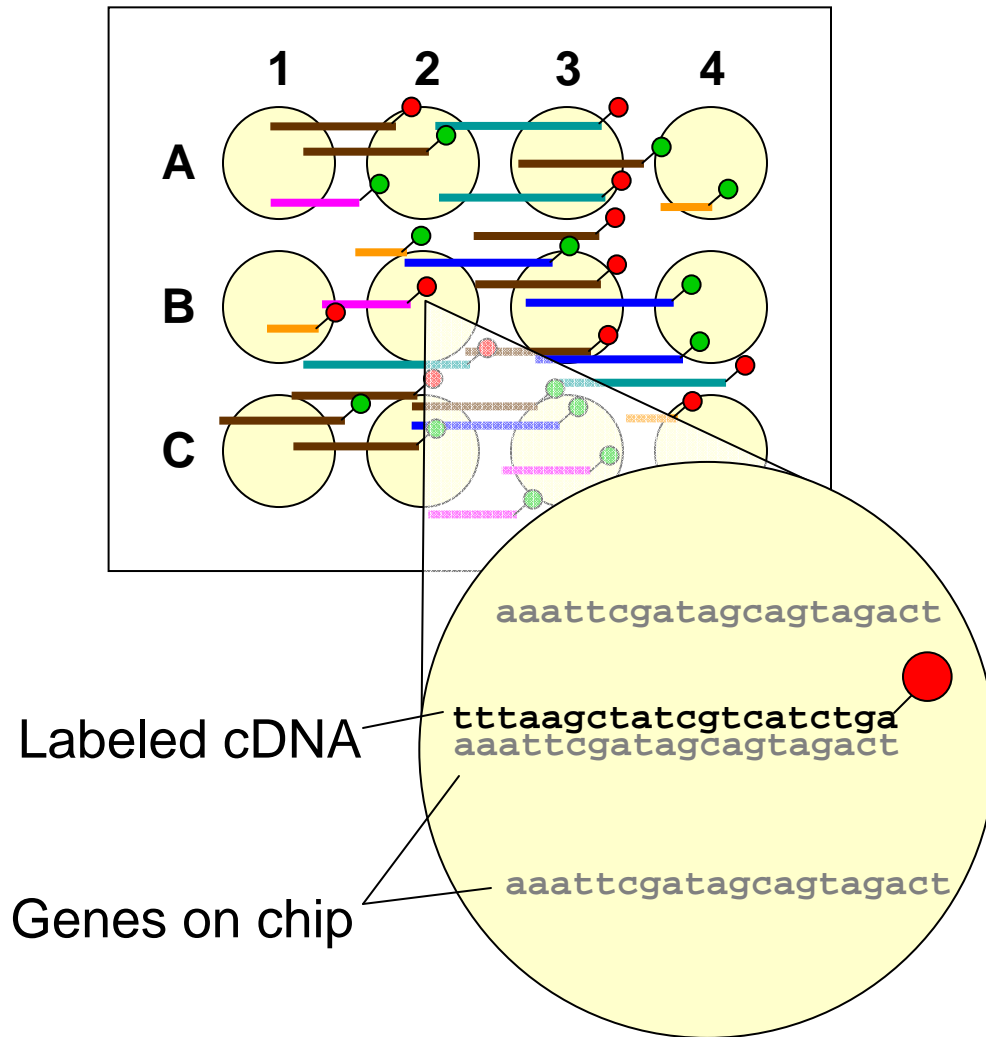
- *Randomly select one cDNA strip from the bucket*

- *Tape (hybridize) your cDNA to a complementary sequence on the microarray*

- *If there is no complementary sequence, stand near the microarray holding your cDNA strip.*

STEP 8
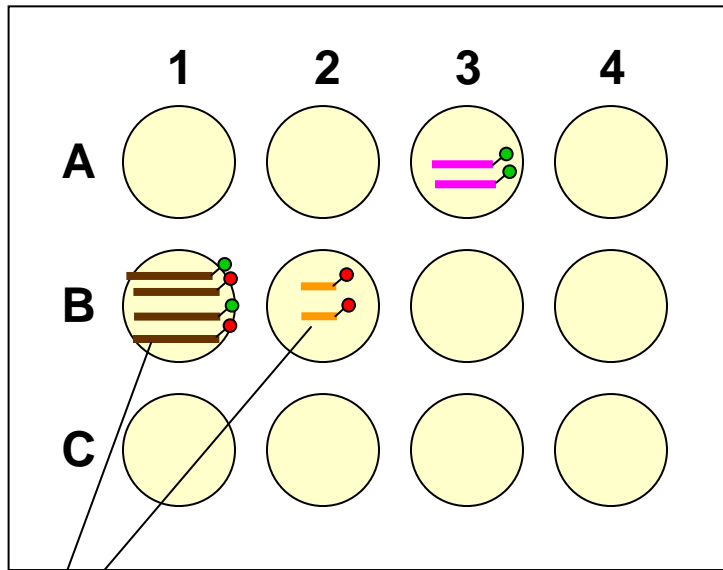
- **Wash the microarray to remove cDNA strips that are not hybridized to any spots.**
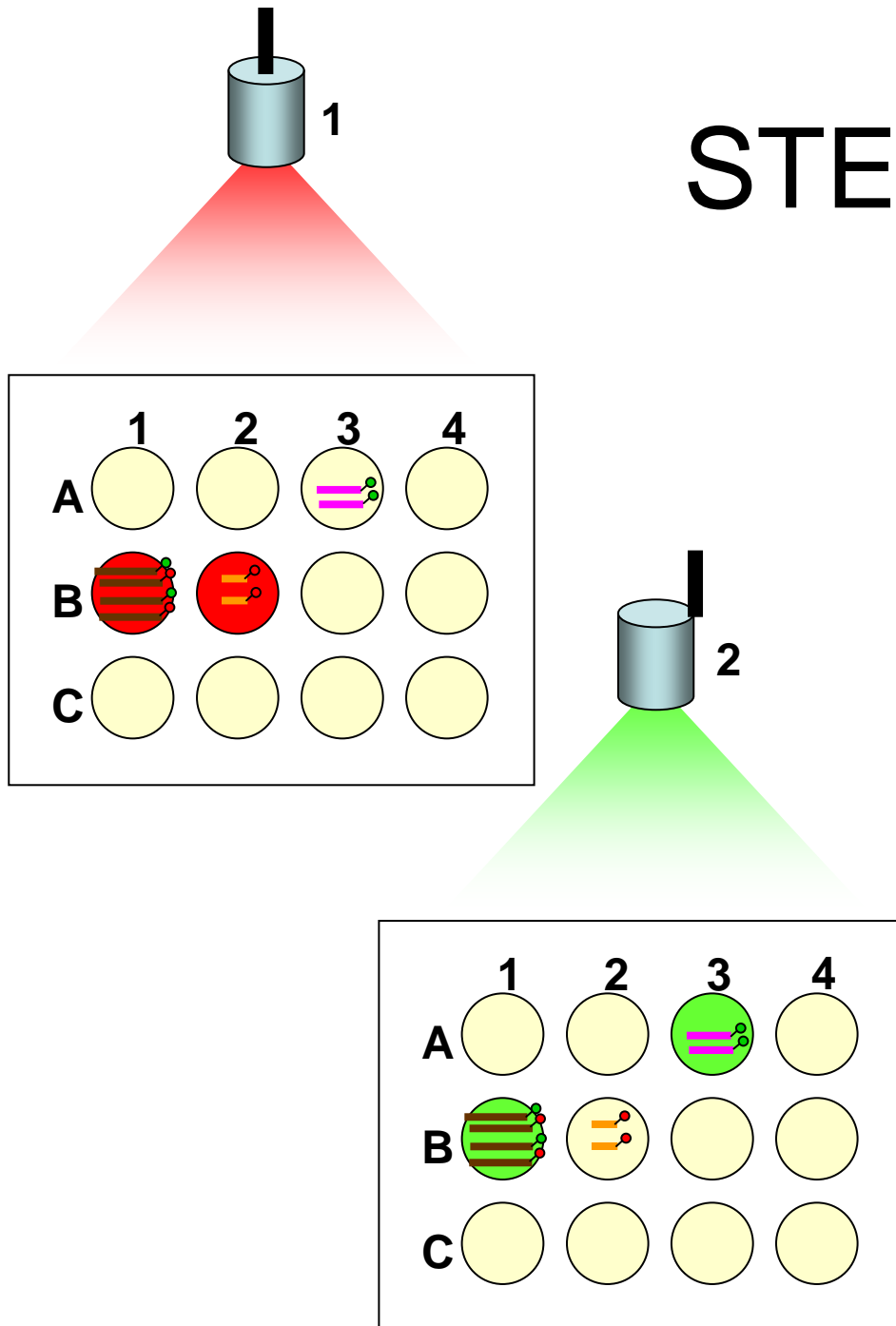
Hybridized cDNA

Unhybridized cDNA
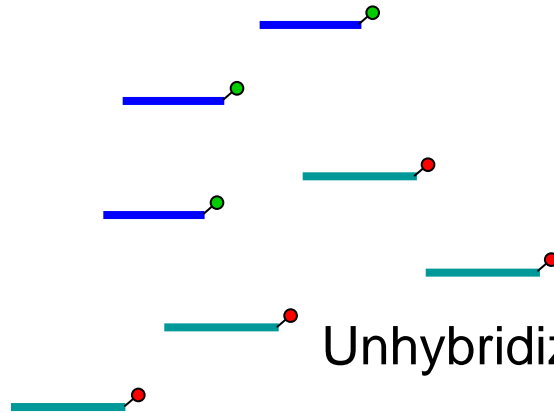
STEP 8
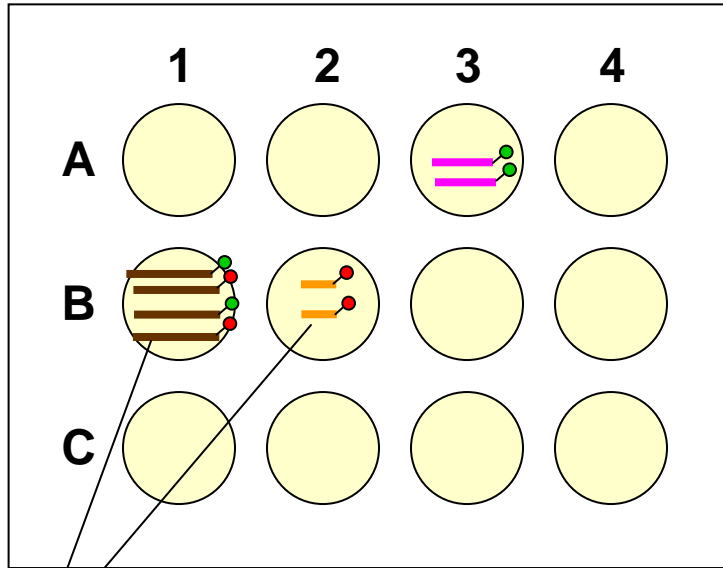
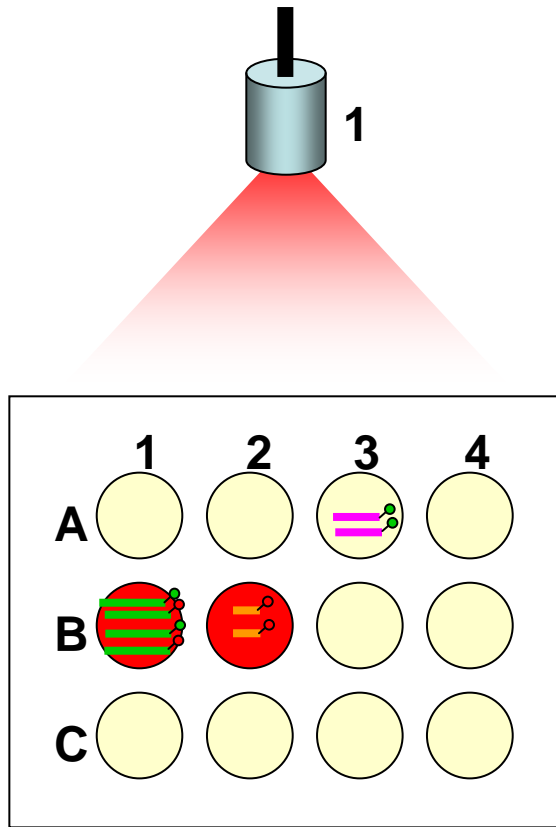- *Put unattached cDNA's into the "Wash Bucket."*

Hybridized cDNA
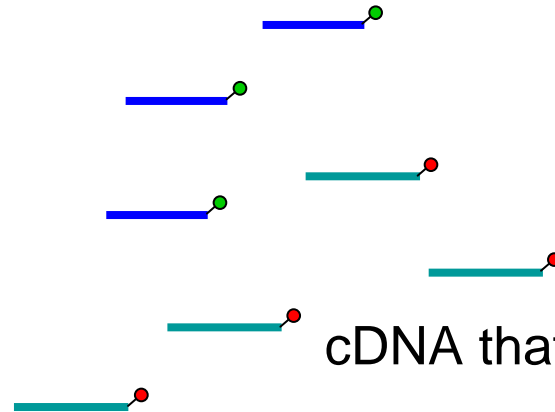
cDNA that is not hybridized

STEP 9

- **Visualize the microarray results using laser lights that cause the fluorescent labels to emit light**
  - **Laser 1 causes the red labels to emit red light**
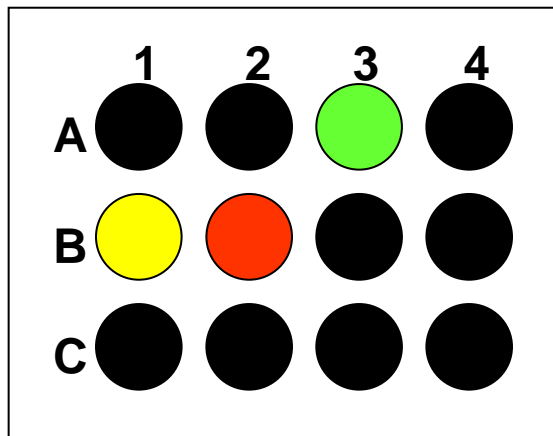 - **Laser 2 causes the green labels to emit green light**

STEP 9
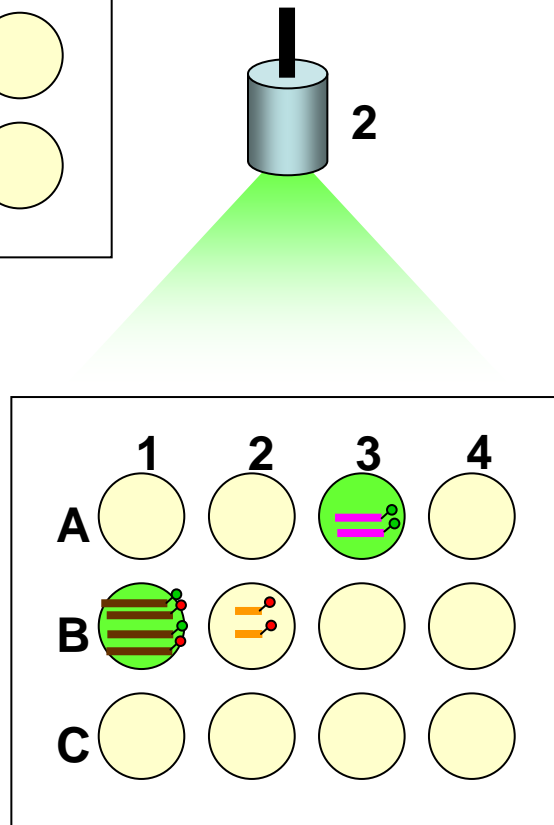
- *Count the number of red cDNA molecules taped to each spot. Use a red pencil to record the numbers on your handout.*

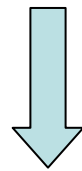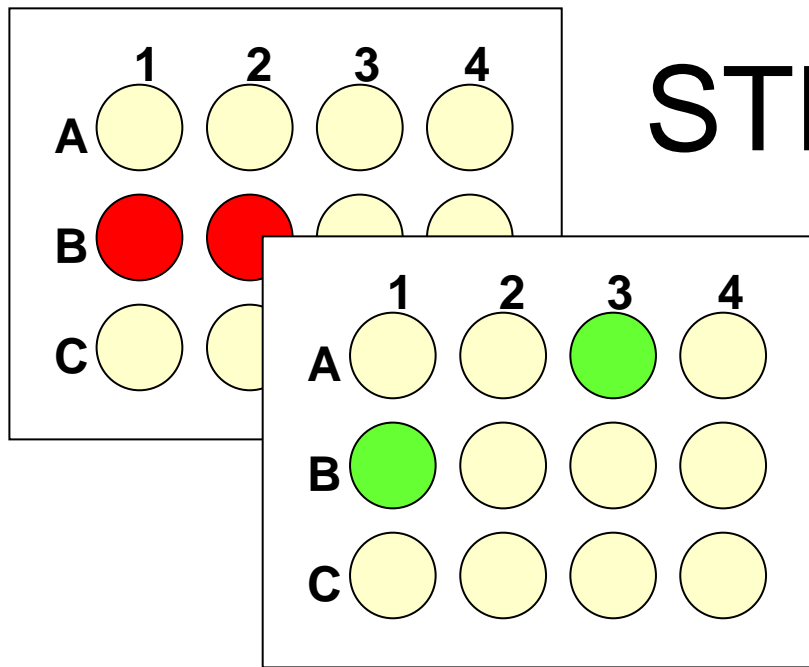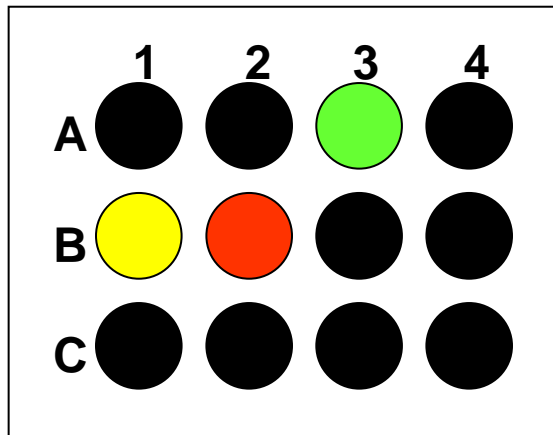- *Repeat for the green cDNA molecules*

STEP 10
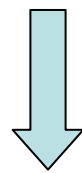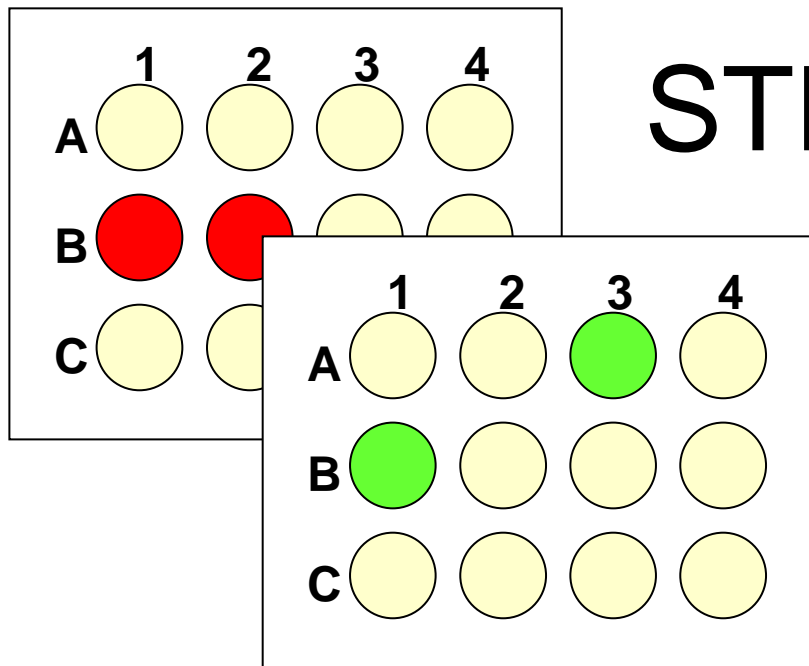
- **Use a computer to merge the red and green scanned images**

- **If red and green are on the same spot, this will be seen as a yellow spot.**

- **If no cDNA's are on the spot, this will be seen as a black spot.**

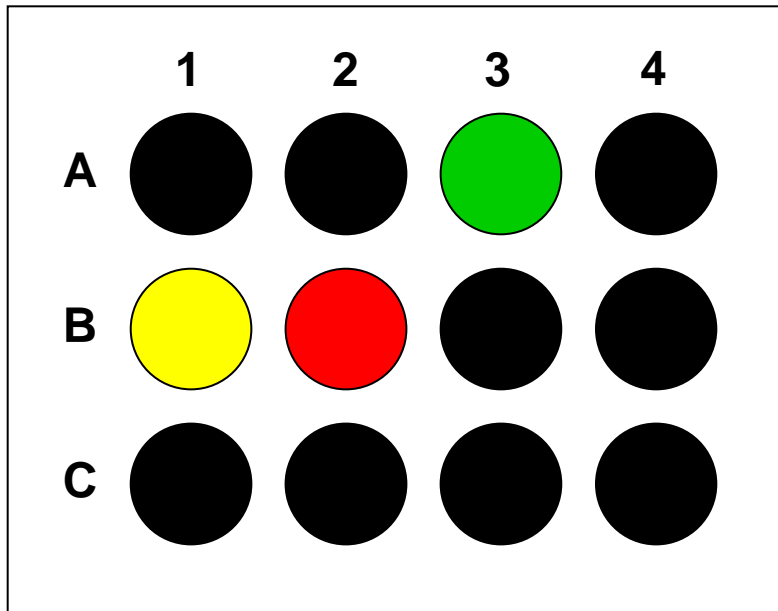Merge Images

STEP 10

- *Use a red pencil to color in the spots that have only red cDNA's attached*

- *Use a green pencil to color in spots that have only green cDNA's attached*

- *Use a yellow pencil to color in spots that have both red and green cDNA's attached.*

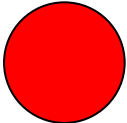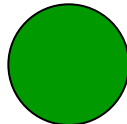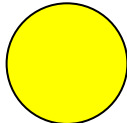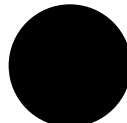- *Use a black pencil to color in spots that have no cDNA's attached*

Merge Images

STEP 11

Analyze the color pattern on the microarray to identify which genes are differently expressed in the two types of cells.

STEP 11

Gene expressed in:

🔴 Cancer cells only

🟢 Normal cells only

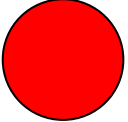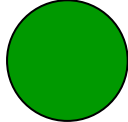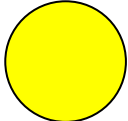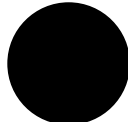🟡 Both types of cells

⚫ Neither type of cell

Analyze the color pattern on the microarray to identify which genes are differently expressed in the two types of cells

Cancer cell cDNA's had red labels
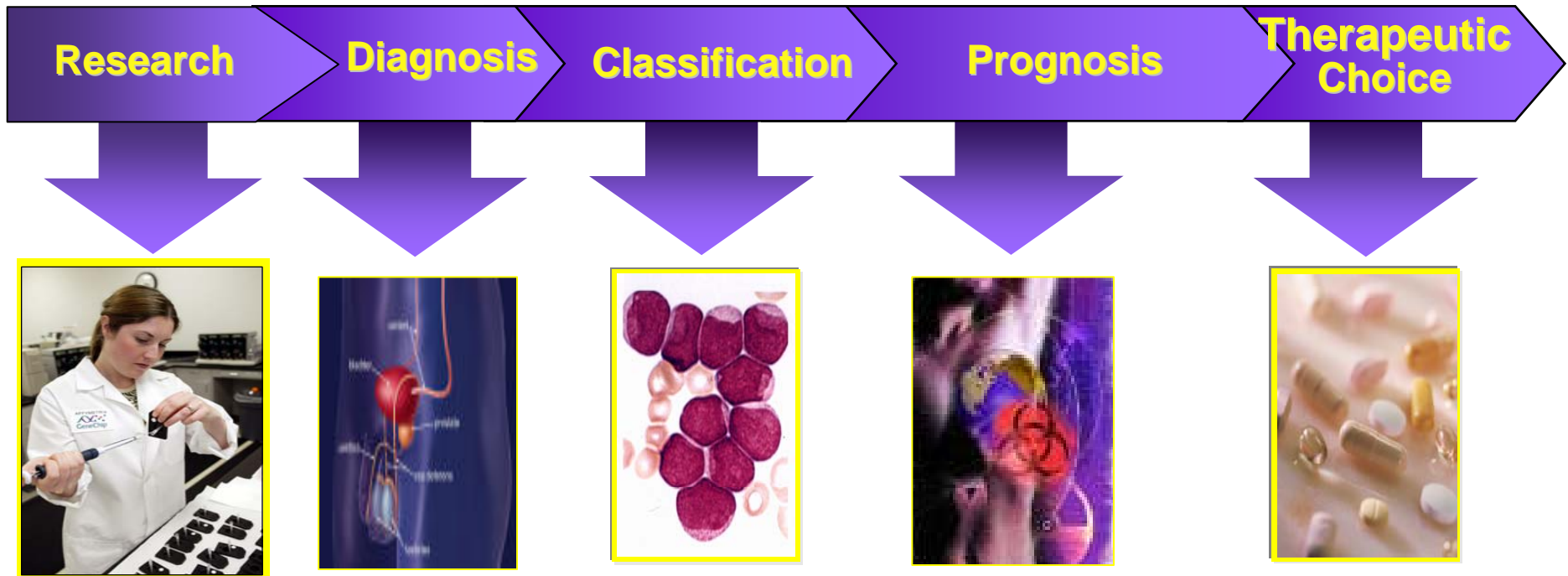Normal cell cDNA's had green labels

STEP 11

Gene expressed in:

🔴 Cancer cells only

🟢 Normal cells only

🟡 Both types of cells

⚫ Neither type of cell

Use the class microarray to answer the questions in your handout…

Cancer cell cDNA's had red labels
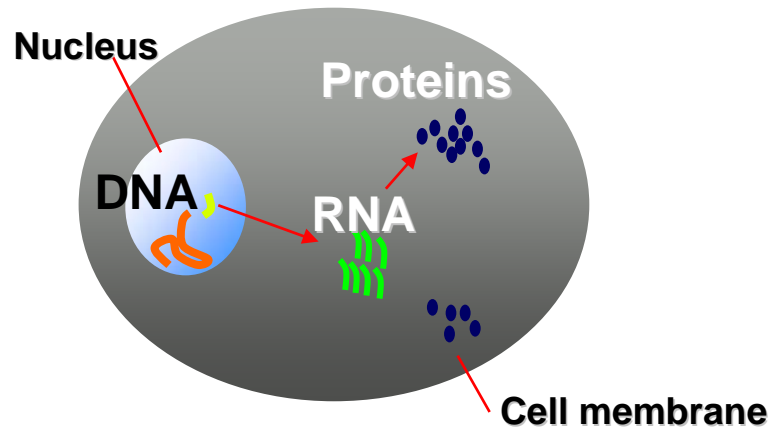Normal cell cDNA's had green labels

Microarrays and Cancer

Research	Diagnosis	Classification	Prognosis	Therapeutic Choice

What causes cancer?
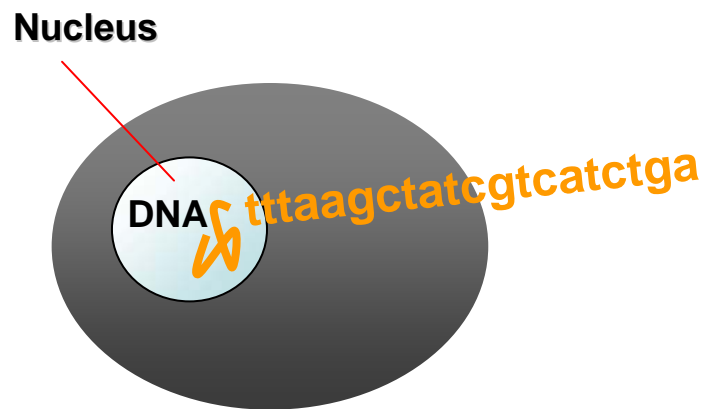
Is it benign?

Which class of cancer?

What are my chances?

Which treatment?
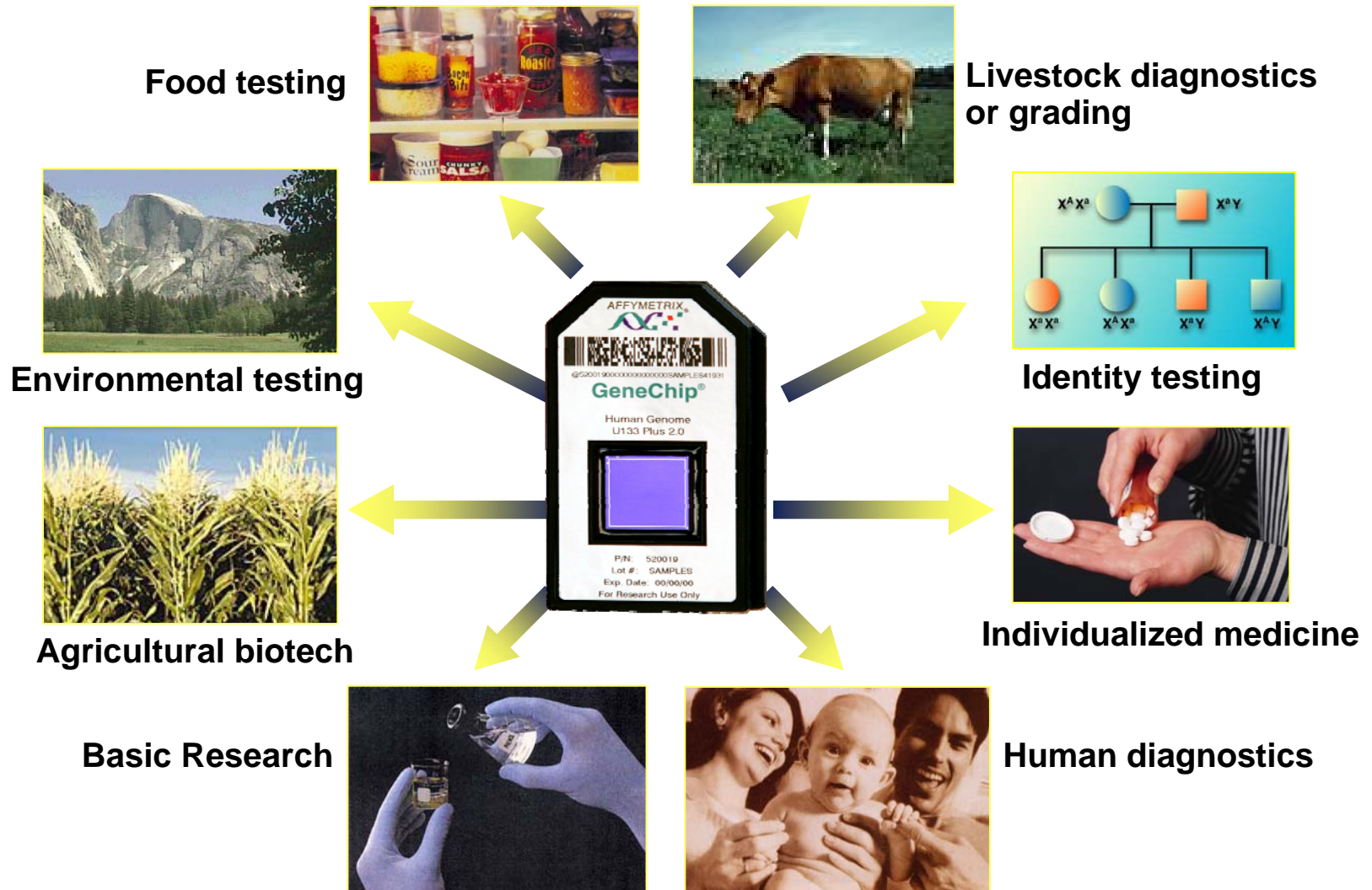
DNA Microarrays are used to

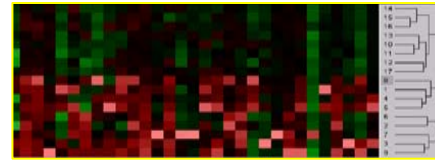**Investigate gene function** - gene expression

Investigate gene structure - sequence variability or resequencing

http://www.affymetrix.com

Other Microarray Applications

Food testing

Livestock diagnostics or grading

Identity testing

Environmental testing

Individualized medicine

Agricultural biotech

Basic Research

Human diagnostics

http://www.affymetrix.com

Types of microarrays
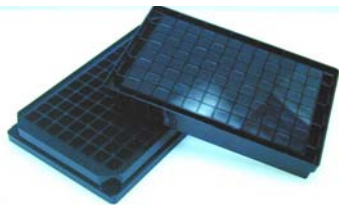
Gene expression
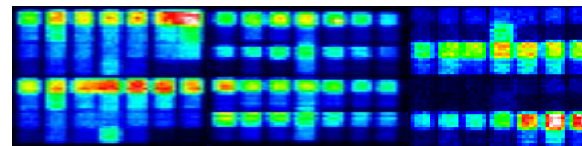
Sequence Polymorphisms

A/A   A/B   B/B

Resequencing

A A A T A G G A T T G G C A T

A
C
G
T
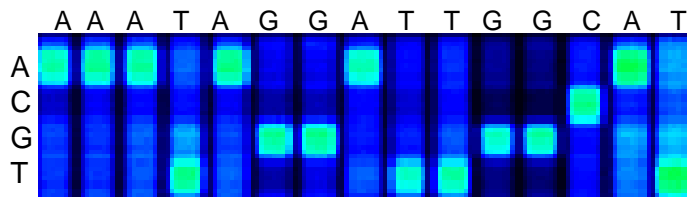
Basic Research

Pre-Clinical Toxicology

Quality Control

Clinical Trials

Diagnosis

Prognosis

Rx Choice

http://www.affymetrix.com