Name _____ Class _____ Date_____

You Are A Cytogeneticist

Observing Human Chromosomes

Question What can you learn about chromosome structure and number from observing cultured human cells using cytogenetic (syt oh juh NET ik) techniques?

Lab Overview You will take on the role of a cytogeneticist as you prepare and analyze a chromosome spread from cultured human cells. You will observe human chromosomes and study their different shapes. It is possible that you will observe chromosomal mutations as well.

Background Cytogenetics is the study of the structure and function of chromosomes. A cytogeneticist in a laboratory grows (cultures) cells from human tissues, prepares chromosomes for analysis, and examines the chromosomes for abnormalities. Chromosomes can be prepared from any cells that contain nuclei. To study the chromosomes, the cytogeneticist usually views cells through the microscope and prepares karyotypes. With these techniques, it is possible to discover abnormalities in chromosome structure, such as deletions, translocations, and inversions, as well as errors in chromosome number, such as trisomy 21.

Cytogenetic techniques can also be used to study chromosomal abnormalities in cancer cells. For example, some cancer cells have extra chromosomes, missing chromosomes, or chromosomes with missing pieces. Unlike normal human cells, which stop dividing in the laboratory after a limited number of cell divisions, many types of cancer cells divide as long as nutrients are provided. The cells you will use in this investigation are from a line of human cancer cells that has been grown in laboratories for more than 50 years. These cells are descended from a sample of cancer cells taken from a woman named Henrietta Lacks, who died in 1951. The cells are called "HeLa" cells after her.

Prelab Activity A cytogeneticist in a lab is preparing human cells for a chromosome spread. Read the list of steps on the next page explaining how to prepare the cells. Then study the drawings showing what the cells look like at each step. Afterward, answer the Prelab Questions.

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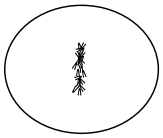
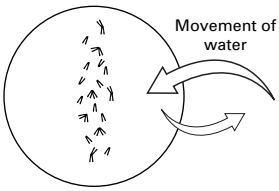
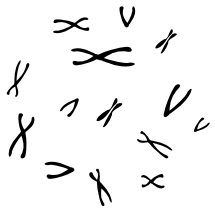
Table 1: Making a Chromosome Spread

Step	View of Cells	What Is Happening
1. Colchicine (a chemical that stops dividing cells in metaphase) is added.		Chromosomes are fully condensed in metaphase. Colchicine prevents the chromatids from separating.
2. Cells are placed in a hypotonic solution to make the cells swell.		Water enters the cells. The cells swell as their volume increases.
3. Cells are flattened by dropping them onto a microscope slide. Then the chromosomes are stained. You will perform this step in the lab activity.		Flattening the cells causes the chromosomes to spread out across the slide. Staining the chromosomes makes them easier to see.

Prelab Questions

1. List the steps to make a chromosome spread. Describe the purpose of each step.

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2. What kinds of chromosomal abnormalities can be found using cytogenetic techniques?

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3. What types of chromosomal abnormalities might be seen in cancer cells, such as the HeLa cells you will observe in the lab?

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Materials

- microscope slide
- marker
- paper towel
- cotton ball or wood block
- microcentrifuge tube of prepared HeLa cells
- 3 transfer pipettes
- stain 1 and stain 2
- petri dish
- microscope
- immersion oil (optional)
- clock or watch with a second hand (optional)

Procedure

Part A: Preparing a Chromosome Spread

1. Mark the microscope slide with your initials in one corner and place it on a paper towel with your initials facing up. Prop the slide at a 45-degree angle with the cotton ball so that your initials are positioned at the upper end of the slide. **CAUTION:** *Handle the slide carefully to avoid breakage.*
2. Carefully open the tube of prepared HeLa cells. Mix up the cells by using a transfer pipette to gently draw them up and replace them several times. **CAUTION:** *The solution in the tube contains an acetic acid fixative that is toxic. Handle the solution with care.*
3. Carefully hold the pipette about 1 meter above the slide. Aiming at the upper third of the slide, slowly drop the cells onto the slide one drop at a time. Upon impact, the cells will slide downward. Try to drop each drop from a slightly different height. Afterward, close the tube tightly and put it aside.
4. Blow gently on the slide to further spread the chromosomes. **CAUTION:** *Be careful not to inhale directly over the slide to avoid breathing in fumes.*
5. Allow the slide to air dry. When the slide is completely dry, place it in a petri dish. Pour Stain 1 into the petri dish until the slide is covered. Leave the slide in the stain for 10 seconds. Pour the stain back into its container. Use a transfer pipette to remove any excess stain from the petri dish. **CAUTION:** *Take care to avoid spilling or touching the stains.*
6. Repeat Step 5 with Stain 2.
7. Rinse the slide by filling the petri dish with water. Repeat until the water is clear. Pick up the slide by the edges and gently shake off excess water. Dry the underside only of the slide.

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Part B: Observing and Analyzing the Chromosomes

1. Place the slide on the microscope stage. Under low power, focus on the flattened cells. These are easily seen as cells stained pink. Use the diaphragm to adjust the light coming into the microscope. Too much light will make it difficult to see the cells.
2. Switch to medium power and search the field of view for dark purple specks. Gently move the slide on the stage to scan the entire slide. Adjust the light as needed.
3. After you locate some dark purple specks, center them in the field of view and switch to 400× power. Adjust the light as needed and use the fine-focus knob to bring the specks into focus.
4. You will be able to see the chromosomes under 400× power. If your microscope does not have a 100× objective lens, skip Step 5 and record your observations as directed below.
5. If your microscope has 4 objective lenses on it, you probably have a 100× objective lens. It is the longest lens on your microscope. If your microscope has a 100× objective lens, follow the procedure below.
   - **a.** Once you have the chromosomes focused under 400×, center the chromosomes in the field of view.
   - **b.** Swing the 40× objective lens out of the way and add 1 drop of oil onto the slide where the light is shining through.
   - **c.** Swing the 100× objective lens in place. The tip of the lens will be immersed in the oil.
   - **d.** Focus only with fine focus knob. Adjust lighting if necessary.

Observations

Count the chromosomes you see under the microscope and sketch them in the space below. Note the location of any centromeres that you can see. Also, pay particular attention to the overall length of each chromosome compared with others that you can see.

Sketch

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Analysis and Conclusions

1. Examine your sketch of the chromosome spread. Consider the abnormalities that cytogeneticists can detect in cancer cells using a light microscope. Do you detect any of these abnormalities?

2. Why do you think making a chromosome spread is an important step in identifying chromosomal mutations?

3. As a cytogeneticist, your next step to further analyze these cells would be to photograph the spread and make a karyotype. How is the photograph used to make a karyotype? What types of abnormalities could you notice using a karyotype?

Extension

If you were unable to find a chromosome spread on your slide to examine, think about possible reasons why the procedure did not work. What part of the procedure was most likely to blame? Propose a way to do that part of the procedure differently and try out your method to see if you get better results. (**NOTE:** *Always check with your teacher before conducting any experiments.*)

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