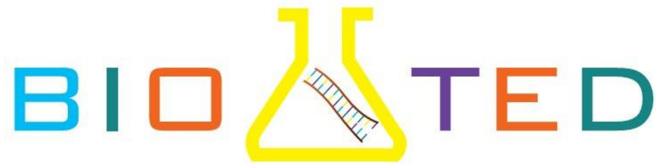


MORPHOLOGY OF CANCER CELLS

This protocol is based on the EDVOTEK® protocol "Morphology of Cancer Cells".

6 groups of students

1. EXPERIMENT OBJECTIVE

In this lab, students will explore the morphology of normal and cancerous cells by staining pre-fixed cells with methylene blue and eosin. The cell morphology will then be examined using light microscopy. After comparing the two cell types, students will identify the cancerous cells.

2. EXPERIMENT COMPONENTS for 6 groups of students

This practice is designed for six groups of 2-4 students per group.

COMPONENTES	Conservación
Ready-to-Stain slides containing normal and cancer cells	Room temperature
Rehydration buffer	Room temperature
Eosin stain solution	Room temperature
Methylene Blue stain solution	Room temperature
Mounting medium	Room temperature
Plastic slide coverslips	Room temperature
Transfer pipets	Room temperature

NOTE: Store entire experiment at room temperature upon receipt.

NOTE: All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

2.1 Requeriments (Not included with this kit)

- Distilled water
- Microscopes (400x total magnifications recommended)
- Forceps
- Gloves
- Timers
- Paper Towels
- Kimwipes
- Beakers

3. BACKGROUND INFORMATION

UNDERSTANDING THE GENETIC AND MOLECULAR BASIS FOR CANCER

Cancer is one of the leading causes of death in the United States of America, contributing to almost one in every four deaths. Almost everyone has been touched by cancer, either through personal experience or through the impact of the disease on someone they know. Fortunately, progress continues to be made and the life expectancies for many cancer patients have steadily increased over the past few decades. Researchers are continually improving our understanding of the molecular and genetic mechanisms behind the development of cancer. These new discoveries, combined with traditional microscopic observations, have increased our understanding of the distinctions between normal and cancer cells.

THE CELL CYCLE DURING CANCER PROGRESSION

The **cell cycle** is a series of biochemical signaling pathways that drive cells through growth and proliferation. This process, involving the duplication of DNA during **mitosis** and the division of the cell through **cytokinesis**, results in two identical daughter cells. Normal cell division has four discrete phases known as **M, G1, S, and G2**. In between each of these phases are tightly controlled molecular roadblocks known as **checkpoints** that regulate passage through cell division (**Figure 1**). This series of coordinated events controls the proliferation, development, and maintenance of cells in an organism.

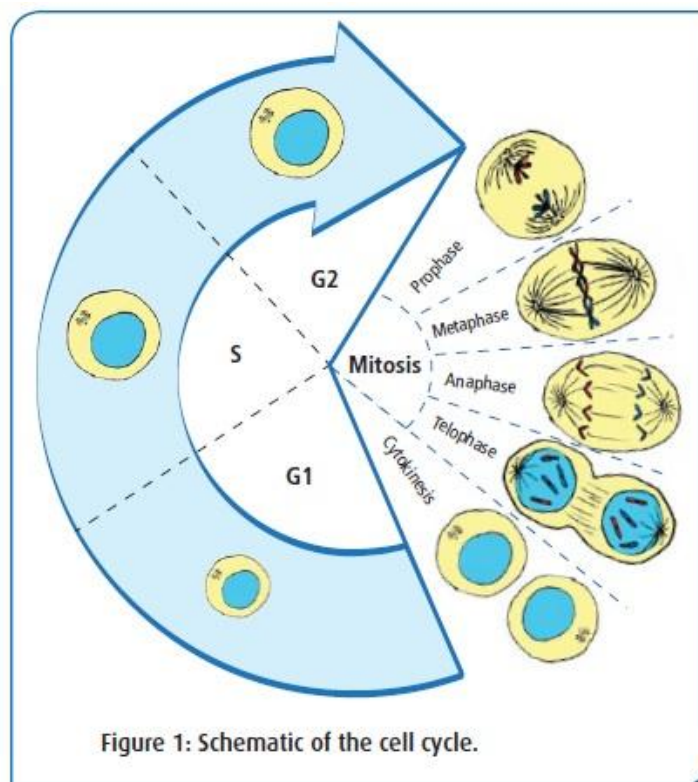


Figure 1: Schematic of the cell cycle.

The growth and death of eukaryotic cells is strictly controlled. Cells use extracellular signals to regulate the rate and location of division. Additionally, if severe DNA damage occurs, normal cells can trigger **apoptosis**, or regulated cell death (**Figure 2A**). In contrast, **mutations** can provide a mechanism that allows cells to bypass the cell cycle checkpoints and inhibit apoptosis, leading to cancer. In cancerous cells mitosis can take place continuously, generating an over-abundance of cells that can form tumors and spread throughout the body.

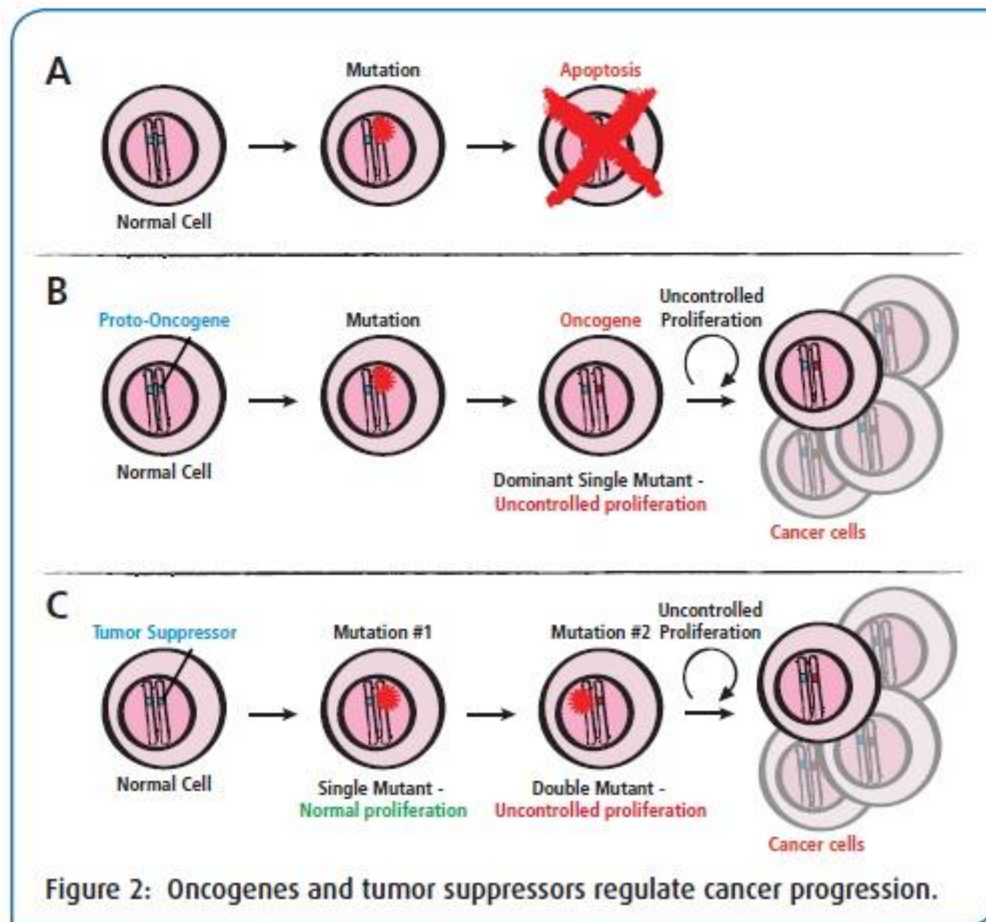


Figure 2: Oncogenes and tumor suppressors regulate cancer progression.

TUMOR SUPPRESSOR PROTEINS AND ONCOGENES

There are many theories explaining how a healthy cell can become cancerous. In general, most cancer cells seem to result from mutations in genes controlling cellular growth. These mutations then lead to either the gain or loss of important protein functions, the destabilization of the cell, and eventually to the development of cancer.

Proto-oncogenes are a group of genes which code for proteins that regulate cell growth and division.

During the early steps in cancer progression a mutation, or combination of multiple mutations, can lead to the conversion of a proto-oncogene into an **oncogene**. Once converted, oncogenes become more active and repeatedly signal for the cell to divide. Many oncogenes are dominant, meaning that a mutation is needed in only one copy of the gene in diploid cells. Cells with oncogenic mutations are unable to control cell proliferation, one of the primary promoters of cancer formation (**Figure 2B**). For example, the **Myc oncogene** is mutated in many different cancers, including breast, lung, colon, and stomach. The activated **Myc oncogene** can control up to 15% of the genes in a cell, making it a primary driver of cell proliferation.

In contrast to oncogenes, **tumor suppressor proteins** are able to inhibit cell growth and prevent tumor formation. Mutations in tumor suppressor genes can inactivate or destroy the corresponding proteins. In this way, a mutation in a tumor suppressor gene removes one of the barriers preventing uncontrolled cell proliferation and tumor formation (**Figure 2C**). It is important to remember that normal human cells are diploid, containing two copies of every gene. Because of this, a single tumor suppressor mutation can often be compensated for by the remaining normal gene on the duplicate chromosome. In these cases both copies of the gene must be mutated in cancerous cells.

One example of a tumor suppressor protein is **p53**, encoded by the **TBP53 gene**. **P53** is an important protein in tumor suppression, regulating DNA damage repair, arresting cell growth, and initiating apoptosis in unhealthy cells. Homozygous loss of **p53**, where mutations have arisen in both copies of the gene, is found in 65% of colon cancers, 30-50% of breast cancers, and 50% of lung cancers. Due to the prevalence of **p53** mutations, and the importance of the gene in driving cancer progression, it has become one of the most well studied cancer genes.

Mutations in oncogenes and tumor suppressors are often the driving force behind cancer formation, and it is rare for cancer cells to contain only a single mutation. In fact, the initial mutations in pre-cancerous cells can lead to additional genetic instability, leading to further mutations in that cell and its daughter cells. Cancer cells will often contain one or more oncogenes combined with the inactivation of an important tumor suppressor.

THE CHARACTERISTICS OF CANCER CELLS

While uncontrolled cell growth is the defining characteristic of cancer, there are several additional qualities that distinguish cancer cells from their normal counterparts. For example, normal cells are difficult for scientists to grow in the laboratory. These cells are generally very sensitive to the cell culture conditions and demand specialized treatments and media.

Additionally, normal cells will only divide a few times before arresting. In contrast, tumor cells are often much easier to culture, readily proliferate in the laboratory and divide indefinitely.

Another important distinction between normal and cancer cells concerns a mechanism known as **contact inhibition**. Normal cells will divide until they are in contact with the neighboring cells, at which point they stop growing. Thus, contact inhibition results in a sheet of cells just one layer thick, referred to as a **monolayer**. Cancer cells typically lose contact inhibition, causing them to pile up and form tumors (**Figure 3**). In addition, cancer cells often become less adherent, both to other cells and to the extracellular matrix. This occurs as a result of changes in cell-surface glycoproteins, altering a cell's ability to form proper connections.

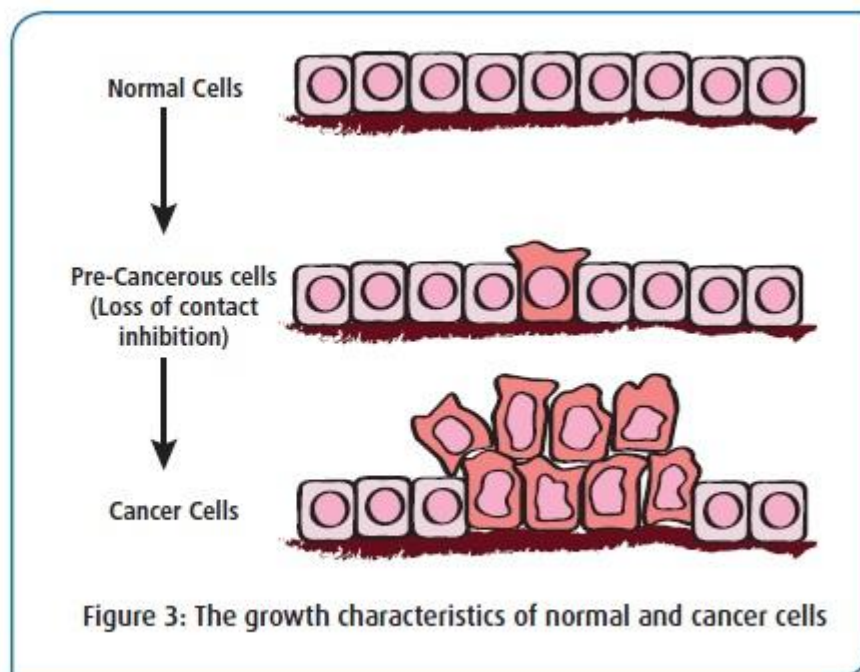


Figure 3: The growth characteristics of normal and cancer cells

Together, these characteristics produce the most devastating effects of cancer. Uncontrolled growth results in painful and dangerous tumors, displacing normal cells and destroying surrounding tissues. Changes in adherence and contact inhibition allow cancer cells to migrate away from the original tumor and grow in other parts of the body, known as **metastasis**. In fact, the spread of cancer throughout the body is responsible for most of the suffering and death seen in cancer patients.

CANCER CELL MORPHOLOGY AND ANALYSIS

Along with genomic alterations and accelerated growth, physical characteristics can be used to classify cancer cells. The nuclear structure of cancer cells undergoes changes that result in a large, irregularly shaped nucleus and modifications to the chromosomes.

These morphological characteristics have been considered the "gold-standard" for diagnosing cancer.

In general, normal cells have a regular and ellipsoid shape while cancer cells are often irregular and contoured. Decreased adherence in cancer cells can lead to disorganized cell spreading and fewer cell- cell contacts, as well as an overall chaotic appearance to the cell population. In contrast, normal cells will grow as a uniform layer of cells with many tight connections between neighboring cells. Structural changes to nuclear lamina proteins, which provide mechanical support to cells, can affect chromatin organization and alter gene expression. Cancer cells also often feature changes to cell structures. For example, the size of the endoplasmic reticulum and mitochondria often decreases, the Golgi apparatus is underdeveloped, and the number of peroxisomes increase (**Figure 4**).

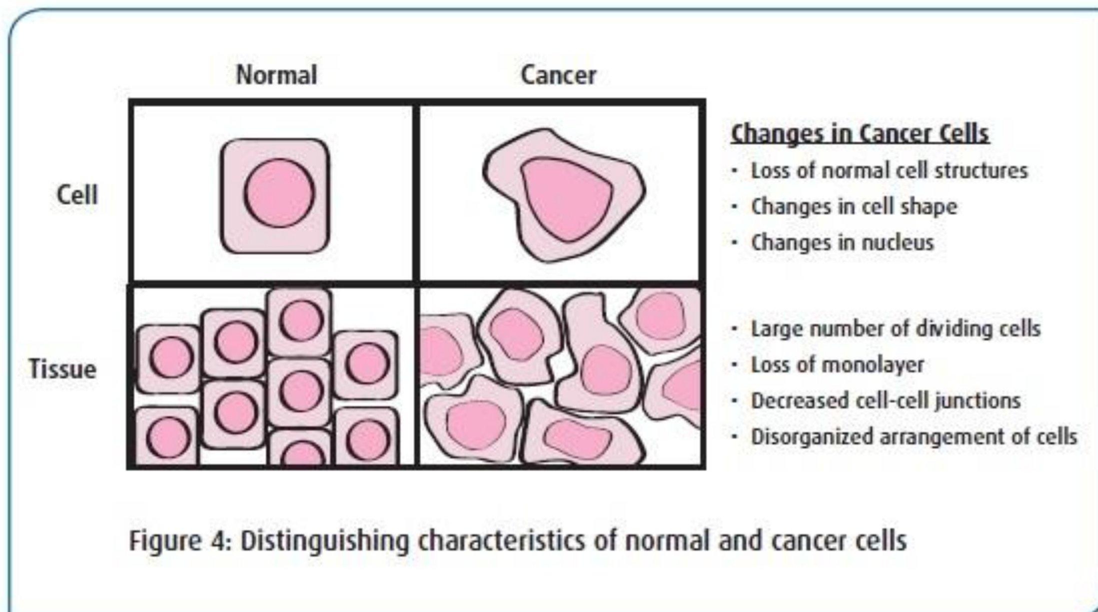


Figure 4: Distinguishing characteristics of normal and cancer cells

Histologists and pathologists routinely use these physical features to identify cancer cells within a patient's tissue sample. For diagnosis, the suspected cancerous tissue is biopsied and then fixed by a chemical or physical procedure to preserve the cells. The fixed tissue is then hardened, cut into very thin sections (one-cell thick), and placed onto a microscope slide. Finally, the prepared sections are treated using a variety of dyes that specifically stain the cellular structures. Methylene blue and eosin are two common dyes used to identify specific cellular features: methylene blue stains the nuclear material a deep blue color, while eosin will stain the cytoplasm and connective tissue a lighter pink (**Figure 5**). Together, these dyes allow a histologist to quickly and easily observe changes in cell structure and composition.

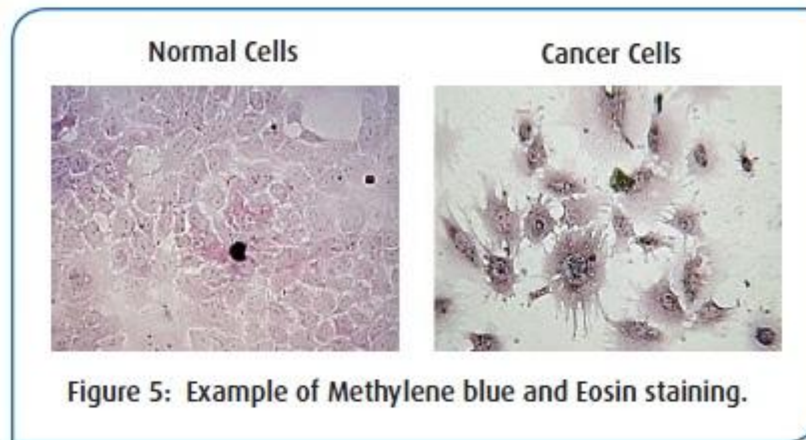


Figure 5: Example of Methylene blue and Eosin staining.

For this activity, students will rehydrate and stain pre-fixed cells provided on a glass slide. They will observe and analyze the morphological differences between normal cells and cancer cells using a compound microscope. Upon completion, the students will be able to describe the physical differences between normal and cancer cells and will understand the functional significance of these structural changes.

4. EXPERIMENTAL PROCEDURES

In this lab, students will explore the morphology of normal and cancerous cells by staining pre-fixed cells with methylene blue and eosin. The cell morphology will then be examined using light microscopy. After comparing the two cell types, students will identify the cancerous cells.

4.1 Safety

1. Gloves and safety goggles should be worn at all times as good laboratory practice.

2. NOT PIPETTE WITH THE MOUTH, use appropriate devices.

3. Exercise caution when working with equipment using together heat and mix of reagents.

4. Wash hands with soap and water after working in the laboratory or after using biological reagents and materials.

If you are unsure of something, ASK YOUR INSTRUCTOR

4.2 PreLabs preparations

Notes preparations teacher practice

The class size, length of classes of practices and equipment availability are factors that must be considered in the planning and implementation of this practice with their students. These guidelines can be adapted to fit your specific circumstances.

This lab is designed for six groups of 2-4 students per group.

Laboratory notebooks:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be document- ing your experiment in a laboratory notebook or on a separate worksheet.

Registration laboratory activities
Students must register in their book practices the activities listed below.

Before starting practice:
• Write a hypothesis that reflects practice.
• Predict the experimental results.

During practice:
• Register (drawing) comments, or photograph the results.

At the end of practice:
• Develop an explanation of the results.
• Determine what could change in practice if you repeat.
• Write a hypothesis reflect this change.

Preparations before practice
Pre-lab preparation should take approximately 30 minutes and can be performed any time before the lab period.

1. Label twenty-four (24) 1.5 ml snap-top microcentrifuge tubes as follows:
   - 6 – Rehydration buffer
   - 6 – Methylene blue stain
   - 6 – Eosin Stain
   - 6 – Mounting medium
2. Use a separate transfer pipet for dispensing each component into the appropriately labeled tube.
3. Add approximately 0.5 ml of each solution to the tube.
4. Cap the tubes and store at room temperature.

Prepare beakers and distilled water for washing slides. If beakers are not available, slides can be gently washed under running water.

NOTE: The mounting media will remove non-specific background stain and increase the visibility of nuclei and organelles in stained cells, but can also cause lightly stained cells to fade. If time allows students can visualize slides before and after adding mounting media and coverslips

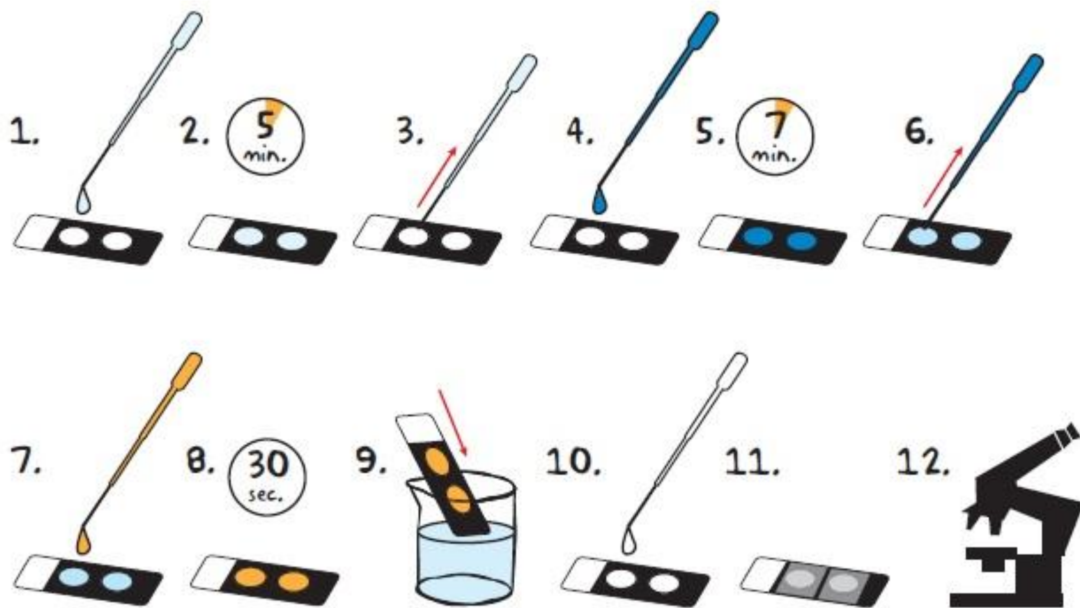
4.3 Material that should receive each group

Distribute the following to each student group, or set up a workstation for students to share materials:

- 1 Ready-to-Stain slide
- Rehydration buffer
- Methylene blue stain
- Eosin stain
- Mounting medium
- 3 Transfer pipets
- 1 Pair of forceps (optional)
- 2 Coverslips
- Beaker of distilled water
- Kimwipes
- Microscope

5. STUDENTS EXPERIMENTAL PROCEDURES

Module I: Staining the Pre-fixed Cells



1. Before starting the experiment, gather one slide, a wash beaker, 4 transfer pipets, and tubes containing rehydra- tion buffer, methylene blue, eosin, and mounting medium.

Note: The slide contains two wells, with each well containing a different cell sample.

2. Using a transfer pipet, ADD 8 drops of rehydration buffer to fully cover each well.
3. INCUBATE the slide for 5 minutes at room temperature.
4. ASPIRATE the rehydration buffer using the same transfer pipet as in Step 1.
5. Using a fresh transfer pipet, ADD 8 drops of methylene blue stain to each well.
6. INCUBATE the slide for 7 minutes at room temperature.
7. ASPIRATE the methylene blue using the same transfer pipet as in Step 4.
8. Using a fresh transfer pipet, immediately ADD 8 drops of eosin stain to each well.
9. INCUBATE the slide for 30 seconds at room temperature.
10. RINSE the slide briefly by submerging in distilled water. Gently tap or "wick" the slide on a paper towel to remove excess water.

HINT:
Slides can be washed in a beaker of distilled water by gently submerging for 30 seconds. If residual stain remains, change water and repeat wash step until water no longer turns orange.

OPTIONAL STOPPING POINT:
At this point the stained slides can be stored at room temperature or visualized by moving to Module II. A coverslip can be added by proceeding to step 10 at a later time if desired.

11. OPTIONAL: Using the final transfer pipet, ADD 1 small drop of mounting medium to each cell spot.
12. Carefully PLACE a coverslip on top of the mounting medium in each spot.
13. PROCEED to Module II: Microscopic Observation.

HINT:
Avoid bubbles by placing the cover slip at a 45° angle to the slide and gently lowering. If bubbles are seen, gently press on the cover slip to displace.

OPTIONAL STOPPING POINT:
Once the cover slip has been placed, the stained slides can be stored at 4° C for up to 24 hours before moving to Module II.

Module II: Microscopic Observation

1. LOCATE cells in well #1 (**Figure 6**, below) using the lowest magnification objective. Adjust the slide to find a random field of nicely stained cells that contains at least 15-20 cells.

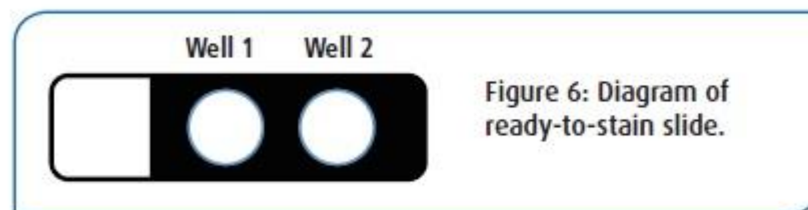


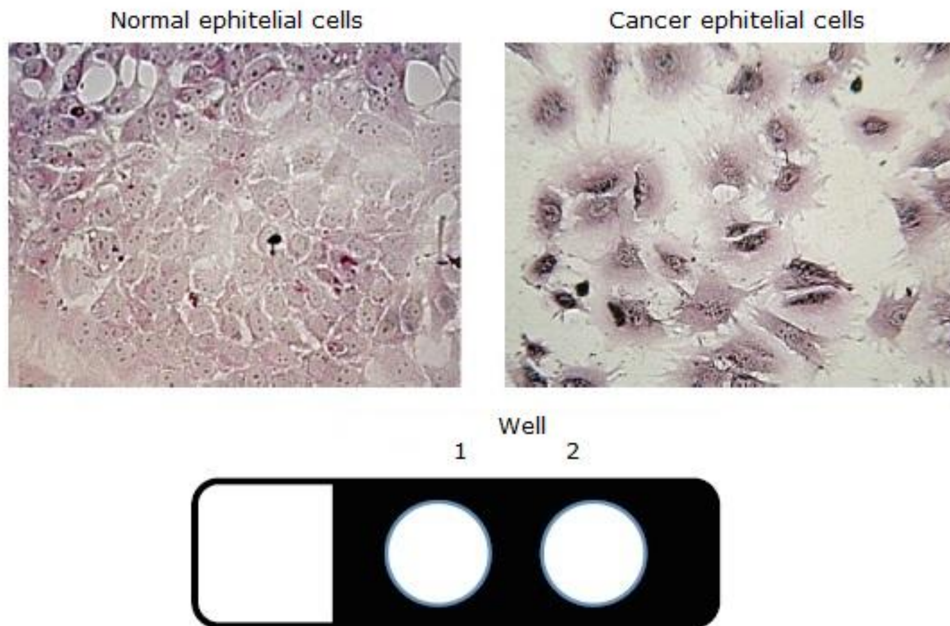
Figure 6: Diagram of ready-to-stain slide.

2. DESCRIBE the overall morphology of the cells in the table below. Features you might record include the number of cells, overall cell morphology, shape and size of the nucleus, and the intensity or color of staining.
3. Using the space provided, DRAW an image of the field of cells that you observe.
4. MOVE the slide and observe a second field of cells. Are your observations from the first area consistent with the second field?
5. SWITCH to a higher magnification and record your observations as in steps 2-3.
6. CHANGE the microscope back to the lower magnification objective and repeat steps 1-5 for the second cell type in well #2.
7. Based on your observations, CLASSIFY each cell type as normal or cancerous.

6. RESULTS AND PRACTICE QUESTIONS

6.1 Experimental Results and Analysis

The cells in Well #1 (closer to the label) are normal mouse cells, while the cells in Well #2 are a mouse cancer cell line. Sample cell images are shown below for both types of cells. These images represent the typical results achieved from these cells; student results will vary due to slight fluctuations in cell preparation and the intensity of staining.



Students should be able to clearly observe the nuclei and cytoplasm of both cell types (see below for examples). In addition, differences in spreading, cell-cell contacts, and contact inhibition should be observed

Cell type	Cell Shape	Spreading	Cell-Cell Contacts	Nuclei
Normal Epithelial Cells.	Uniform, compact.	Some spreading, but more compact. Monolayer.	Many cell-cell junctions.	Uniform, small.
Cancer Epithelial Cells.	Random shapes, larger cells.	Very spread cells, does not form uniform monolayer.	Fewer cell-cell junctions.	Random, larger.

6.2 Study Questions

Answer the following questions in the lab notebook:

1. What is cancer?
2. What is the difference between an oncogene and a tumor suppressor?
3. Describe the characteristics of cancer cells that make them easy to culture in the laboratory.
4. How do pathologists distinguish between normal and cancer cells?
5. Why would you use two different dyes when staining cells?

ANNEX 1

Well	Magnification	Observations	Drawing

Classification

Well #1:_________________

Well #2:_________________