

# Lab 9: Mitosis

## I. Objectives:

In today's lab, you will first observe prepared slides of plant and animal cells in different stages of cell division. In the second part of the lab, you will prepare your own slides of onion root tips and examine the tissue for both interphase and mitotic cells. By the end of today's lab, you should be able to:

- Use a microscope to identify cells in interphase and different stages of mitosis from prepared animal and plant slides.
- Prepare your own temporary mounts of onion root tips and examine them under the microscope for different phases of the cell cycle.

## II. Things to Do:

### PART A: MITOSIS IN PLANT CELLS - ONION (*Allium*) ROOT TIP SLIDES

Examine the prepared slide in your tray of an onion root tip. Since a root tip is an actively growing part of the plant (the tip elongates by mitotic cell divisions), you should be able to see all of the stages of the cell cycle. The root tip has been stained with dyes that concentrate in the chromosomes, so you should be able to see them clearly and be able to distinguish cells in interphase, the different stages of mitosis, and cells undergoing cytokinesis. Photographs of mitosis in onion root tip cells are also available in the lab. You should use these to help you identify the different stages and the structures ON YOUR OWN SLIDE (in Exercise #3). **Record drawings of the stages with labels in your lab notebook.**

### PART B: MITOSIS IN ANIMAL CELLS - WHITEFISH (*Leucichthys*) BLASTULA SLIDES

Examine the prepared slide of Whitefish blastula in your tray. Mitotic activity in animals is most rapid during early development. Therefore, the blastula stage of a Whitefish embryo has been selected for observation. A blastula is a very early embryo stage that consists of a ball of rapidly dividing cells. In preparing the slides, the ball of cells was thinly sliced into a number of sections. Concentrate your attention on those cells that can be seen in full "side-on" view. *You may have to look at more than one slide to see all stages.* Trade views with your lab partners. As in Exercise #1, you should try to identify all of the stages. However, the chromosomes are much smaller and cannot be seen in all cells because of how the cells have been sectioned. Study the photographs to be sure that you can identify the stages and structures in animal cells. Compare these cells to those you examined in the onion root tip. **Record drawings of the stages with labels in your lab notebook.**

### PART C: SMEAR TECHNIQUE TO PREPARE ROOT TIP SLIDES OF MITOSIS

Prior to this laboratory period, onion bulbs were soaked in water to stimulate the growth of roots. The preparation of material for microscopic study consists of three stages in which the root-tips, containing many actively dividing cells, are cut off, killed, and hardened in an acid solution.

1. The root-tip is prepared for staining; it is treated with hydrochloric acid to expose the aldehydes present in the chromosomal DNA.
2. The root-tip is stained with Schiff's Reagent, a dye mixture that adheres to the aldehyde groups of DNA, and thereby colors the chromosomes a deep magenta.
3. The root-tip cells are dispersed into a single layer on a slide and the slide prepared for microscopic examination.

Making slides requires a great deal of care to get worthwhile results. Since you will have only one opportunity to make a good slide, read the directions VERY CAREFULLY before you begin and follow them EXACTLY!

*Procedure for making a temporary mount:*

1. Obtain 3-4 root tips (or number provided by instructor). From the onion bulb soaking in water, simply cut 3-4 roots about 1 cm from the tips and place them in a clean vial.
2. Add **6N HCl** (50% HCl) just to cover root tips and let soak for exactly three minutes. This exposes parts of the chromosomes. **CAUTION: Handle the Hydrochloric Acid (HCl) with care. Do not inhale or contact it with your bare hands.**
3. With a dropper, remove the acid and discard it into the beaker labeled "waste."
4. Add deionized water to the vial to rinse the root tips, gently agitating for one minute. Discard the water with a dropper into your waste beaker.
5. Add just enough **Schiff's Reagent to the vial to cover the root tips**. Note the time and leave the tips in the Schiff's for 20 minutes. **CAUTION: Handle Schiff's Reagent with care. It is a strong acid. Do not inhale it or contact it with your bare hands (it will permanently stain anything it contacts).**
6. While the tips are staining in the Schiff's reagent, add a dropper full of 45% acetic acid a new clean vial. **CAUTION: Do not inhale or contact the acetic acid.**
7. After the 20 minutes of staining in the Schiff's Reagent have elapsed, place the root tips in the vial with the acetic acid for one minute. This will help the stain set.
8. Remove a root tip from the acetic acid and place it on a clean microscope slide. Add a small drop of 45% acetic acid to the root tip. Using forceps and a razor blade, cut and save the bright pink part of the root tip (about 1-2 mm from the tapered end) and discard the rest. Save all of the other stained root tips in case you need to prepare another slide.
9. Use a razor blade to tease or chop the root tip into very small pieces in a drop of 45% acetic acid on the glass slide. If necessary, add another drop of acetic acid to completely cover the cells. Gently place a cover slip over the mashed root tip, taking care to eliminate air bubbles.
10. Put a small clean cork upside down on the cover slip and press down firmly, in a vertical direction. This operation should smear the cells into a very thin layer. It takes a great deal of pressure to make a proper smear, so don't hesitate to apply it. Applying vertical pressure rarely

breaks the cover slip. Remove excess acid from the edges of the cover slip with absorbent paper.

11. Examine your slide under high power to locate cells in various stages of the cell cycle. Draw and label the stages you observed.

CLEAN UP:

- Check to ensure all dropper bottles have been closed tightly.
- Discard all waste beaker contents into the designated container in the lab.
- Empty and rinse your glass vials
- Put up microscopes according to instructor's directions.

NOTE: *DO NOT THROW AWAY UNUSED ROOT TIPS.*

## **PART D: PIPECLEANER MODELS OF MITOSIS**

Your instructor will provide you with colored pipecleaners, representing chromosomes, and yarn that you will use to define the cell membrane.

1. On your lab bench, set up your pipecleaners to represent a cell in G1 of Interphase.
2. Use the pipecleaner chromosomes to walk through each step of the cell cycle - Interphase: G1, S, G2, and Mitosis: Prophase, Metaphase, Anaphase and Telophase.
3. Finish the cell cycle with cytokinesis.

While you are conducting the exercise above, fill out the following table:

	# of cells	# of chromosomes	# of chromatids
Interphase: G1			
Interphase: end of S and G2			
Mitosis: Prophase			
Mitosis: Metaphase			
Mitosis: Anaphase			
Mitosis: Telophase			
After Cytokinesis			