

# CELL STRUCTURE

## INTRODUCTION

Cell types are divided into two major categories: Prokaryotes and Eukaryotes. Structurally these are generally easy to distinguish because the Prokaryotes lack a nucleus, whereas the Eukaryotes possess a nucleus. In addition, there is much more specialized cytoplasmic organization and structure in the Eukaryote. The first part of this lab will be an exploration of the diversity of cell shapes and arrangements found on this planet. There will be representative cells from each of the five Kingdoms of organisms. This part of the lab will also serve as a refresher on slide preparation and microscope use. The second part of this lab will involve using stains to determine the chromosomal content, and sex, of your own cheek cells.

## PART I: CELL DIVERSITY

Choose at least 8 specimens for viewing and drawing. For each, draw a detailed sketch and label as many structures as you can. Include names of organisms and magnifications. You may use your textbook or other resources to help you identify cell structures. Be sure you choose at least one representative from each of the 5 Kingdoms.

### Procedure for Viewing Prepared Slides:

1. Check out a microscope and locate the following: light source; stage; ocular lens; objective lens; course focus; and fine focus.
2. Set the lowest power objective lens (this is labeled 4X and is the smallest) in place. With the course focus knob, lower the lens down as far as it will go. The tip of the lens should be at least 1/2 inch above the stage. If it isn't, check with the instructor.
3. Obtain a prepared slide. Make sure it is clean of smudges or dust and place it on the microscope stage. Turn on the light source.
4. Adjust the stage so that the specimen on the slide is in the light beam. Now look through the ocular lens, and with the coarse focus raise the lens until the specimen comes into focus. Don't touch the fine focus yet.
5. Move the stage around to examine the entire specimen.
6. Choose an area that looks interesting and swing the next highest objective lens, labeled 10X, into place. Be sure you don't swing the highest power objective (1000X) by mistake!
7. Examine the specimen. It will probably be a bit out of focus, but you only need to focus with the fine focus knob. In fact from this point on, don't touch the course focus at all, unless you switch back to the 4X objective.
8. When ready, switch to the next highest objective lens (40X). Again, the image should not be far out of focus and require only fine adjustment. If this is not the case, check with the instructor.
9. The most powerful objective lens (100X) is an oil immersion lens. This means that a drop of optical oil must be placed on the slide before the lens is switched into place. If you try this

lens be very careful not to get any oil on any of the other lenses, and be sure to clean the oil off of the lens and the slide when finished. Also, use only the fine focus!

**Procedure for Onion Epidermis:**

1. Place a drop of iodine on a microscope slide.
2. Cut an onion into eighths. Remove one of the sections from a piece and snap it in two by bending it backwards. This exposes the epidermis on either edge of the break.
3. With forceps, peel off a piece of the thin epidermis (it should be so thin as to be transparent). Spread it evenly on the drop of iodine on a microscope slide. Make sure the epidermis is wetted and not floating on the water.
4. Carefully lower a cover slip on the specimen. If you do this gradually, you won't get air bubbles which interfere with viewing. This is called a wet mount.
5. As outlined above locate the tissue under low power and gradually work up to the 40X objective. Remember, use only the fine focus after the 4X objective.
6. Plant cells have their plasma membranes surrounded by non living cell walls. These structures provide support and prevent the cells from exploding.
7. Most of the central area of the onion cell is occupied by a large, fluid-filled vacuole. The remaining contents of the cytoplasm, including the nucleus, are compressed between the plasma membrane and the central vacuole.
8. Locate several cells in which you can see the cell wall, central vacuole, and nucleus. Make a drawing of these.

**Procedure for Human Cheek Cells:**

1. Obtain a clean toothpick, slide and cover slip. Gently scrape the inside of your cheek with the toothpick and then smear it across the slide. This is also known as a buccal smear.
2. Allow a minute or so for the preparation to dry. Then add several drops of Methylene Blue to stain the cells, and let stand for two minutes.
3. Turn the slide upside down and hold under a gentle stream of water until the blue stain is rinsed away.
4. Quickly dry the bottom of the slide and place a cover slip over the cell smear.
5. Examine these cells under low and higher magnifications. Choose several representative cells and draw them. In addition to the nucleus, you should see cytoplasmic granules in many of the cells. Sometimes these turn out to be bacteria, but are usually glycogen granules.

**Procedure for Other Wet Mounts:**

1. Place a drop of your specimen onto a microscope slide. Be sure you actually have obtained your specimen in your drop by first giving the stock culture a gentle stir.
2. Lower a cover slip onto the specimen.
3. View the specimen as outlined above.

## PART II: BARR BODY STAIN

In 1949 Barr and Bertram reported that nerve cells from cats showed a sexual difference (dimorphism). Cell nuclei from female cells consistently showed a small darkly staining body near the nuclear envelope, while cell nuclei from males showed no such structure. It is now known that this difference is present in most cell nuclei from most of the mammalian groups, including humans. The darkly staining structure found only in the nuclei of cells from females is known as the Barr body.

Further research has shown that the Barr body is actually an inactivated X chromosome. For proper functioning, most cell types must have one and only one active X chromosome at any given time. Since each female cell has two X chromosomes, one of them must be inactivated for normal cell functioning. Male cells have only one X, so they don't have a spare to inactivate and display as a Barr body. Infrequently, some females end up with three X chromosomes (XXX or trisomy X). The cell nuclei of these individuals show two Barr bodies.

The fact that the number of X chromosomes is directly related to the number of Barr bodies in the nucleus is commonly used to confirm the sex of women athletes.

**Table 1**

Condition	Sex Chromosomes	Number of Barr Bodies
Normal Female	XX	1
Normal Male	XY	None
Trisomy X	XXX	2
Turner's Syndrome	X	None
Klinefelter's Syndrome	XXY	1

In this section, you will make a cheek cell smear as above, but use a sensitive DNA stain (Thionin) that will make Barr bodies visible.

### Procedure:

The Thionin staining protocol is a good deal more delicate and involved than the methylene blue stain you used previously. Follow these directions carefully!

1. Wash your slide in detergent and rinse well in deionized water. Rinse the slide in a stream of 70% ethyl alcohol from a squeeze bottle. Allow the slide to air dry on a clean paper towel.
2. To avoid contamination with bacteria, which stain nicely and might look like a Barr body, rinse your mouth very well with tap water at least three times.
3. Immediately after rinsing, Gently scrape the inside of your cheek with the toothpick and then smear it across the slide.
4. Allow the slide to air dry for 1 minute.
5. Treat slides very gently from now on because excess agitation or a direct stream of water will wash away cells.
6. Gently wet the cells with an indirect stream of deionized water.
7. While holding the slide over the sink, cover the cells with several drops of 6N HCl and gently rinse off with an indirect stream of deionized water after 10 seconds. **Caution!** HCl at

this strength will burn eyes and skin! Use protective eyewear. This step breaks down RNA which also stains with thionin and can resemble or obscure Barr bodies.

8. Gently wash the cells with an indirect stream of deionized water for about 1 minute.
9. Cover the cells with several drops of thionin stain and place slide in covered petri dish to prevent evaporation. **Caution!** Stains fingers and clothing.
10. Stain for 15 minutes.
11. Gently wash the cells with an indirect stream of deionized water for about 1 minute.
12. Remove excess water and place cover slip over cells.
13. Examine cells under oil immersion. Barr bodies should be stained dark blue or black and appear near the nuclear envelope.
14. Switch slides with someone of the opposite sex. Make sure you observe Barr bodies in female cells. If you're a male and you find a Barr body in your nuclei, don't panic. About 2% of male cheek cells show dense chromatin that resembles a Barr body.
15. Sketch a representative of each sex.

### **QUESTIONS FOR YOUR LAB REPORT**

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(10 pts total, 2 pts per question)

1. Present an abstract of your work. Your abstract should include a brief description (1/2 page of 1) what you set out to learn, 2) what procedures you performed, and 3) a summary of your data or discoveries.
2. Submit all 10 sketches: properly labeled and with magnifications.
3. Choose two organelles that you observed. Give a detailed explanation of the function of these organelles to a cell.
4. Female athletes are occasionally tested to confirm her sex during competitions. Barr Body tests are no longer used and have been replaced by Y chromosome tests. However, neither of these tests is completely accurate in determining sex. Explain potential errors in each of these tests. (Hint: see Table 1)
5. In your opinion, should gender verification for women's sporting events be discontinued? Support your answer with at least one reference.