

## BIOLOGY 1101

### LAB 4: MITOSIS AND MEIOSIS

**READING:** Please read chapter 8 in your text.

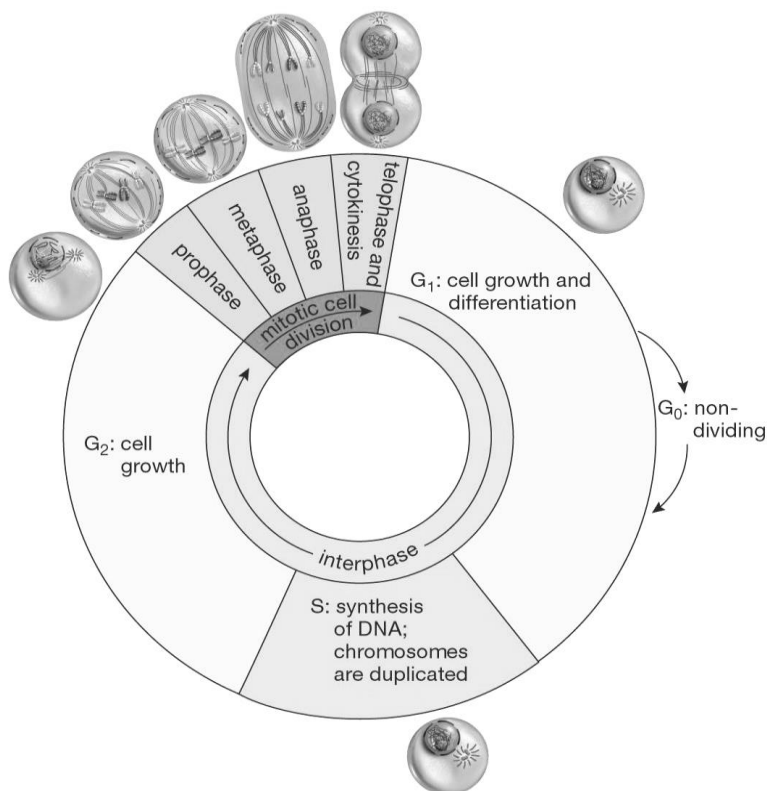
**INTRODUCTION:** The nuclei of eukaryotic cells contain chromosomes along which genes are arranged. **Genes** are sections of double stranded deoxyribonucleic acid (DNA) that form discrete units of hereditary information, which means they influence traits (e.g., eye color or hair color). **Diploid** cells contain two copies of each chromosome. Structural proteins in the chromosomes (called **histones**) organize the DNA and participate in DNA folding and condensation. Before cells divide, chromosomes are duplicated. When cells divide, chromosomes are passed on to daughter cells carrying with them genes and preserving the diploid condition. This process is the mechanism for passing on hereditary information to the next generation.

In single-celled organisms and the **somatic** cells (cells other than a sperm or egg cell) of multicellular organisms, the nucleus divides by **mitosis** into two daughter nuclei. These daughter nuclei have the same number of chromosomes with the same genes as the parent cell. **Meiosis** is a special type of nuclear division that occurs only in multicellular organisms in preparation for sexual reproduction. In meiosis, diploid nuclei of certain cells in ovaries or testes (or sporangia in plants) divide twice, but the chromosomes replicate only once. This process results in four daughter nuclei with differing chromosomes and genes. These daughter nuclei are **haploid**, containing only one copy of each chromosome.

When two of these cells fuse during fertilization, the diploid condition is preserved.

Generally in both mitosis and meiosis, after nuclear division the cytoplasm divides by a process called **cytokinesis**.

In somatic cells, events from the beginning of one cell division to the beginning of the next are collectively called the **cell cycle** (see figure). The cell cycle is divided into four major phases: G<sub>1</sub>, S, G<sub>2</sub>, and M. The **M phase (mitotic phase)** represents the division of the nucleus and cytoplasm and is subdivided into four phases: **prophase**, **metaphase**, **anaphase**, and **telophase**, which concludes in cytokinesis.



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**LABORATORY OBJECTIVES:** The purpose of this set of laboratory exercises is to introduce you to the basics of cellular reproduction. In this lab, and from your readings, you should learn:

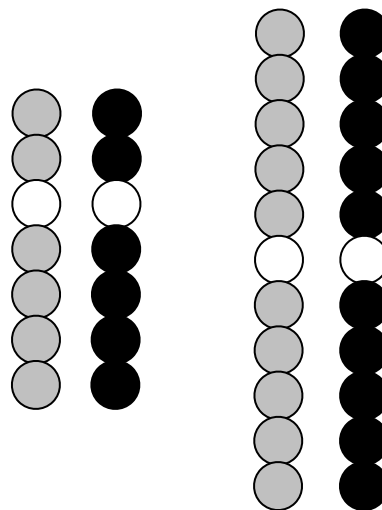
1. To describe the activities of the chromosomes, centrioles, and microtubules in the cell cycle, including all phases of mitosis and meiosis.
2. To identify the phases of mitosis in root tip cells and vertebrate blastula cells.
3. To describe how cytokinesis is different in plant and animal cells.
4. To describe differences between mitosis and meiosis.
5. To identify the phases of meiosis in vertebrate ovary and/or testis cells.
6. The role of mitosis and meiosis in passing hereditary information from one generation to the next.

**EXERCISES:** Today, we will first watch a video or two that describe the processes of mitosis and meiosis, and their significance. We know they aren't the best movies, but they are a good overview. Following the videos, we will model mitosis and observe plant and animal cells in various stages of mitosis. We will then model the events of meiosis and observe animal cells undergoing meiotic cell division.

**A. Modeling the cell cycle and mitosis in an animal cell.** Scientists often use models to represent natural structures and processes that are too small, too large, too slow, too fast, or too complex to investigate directly. Scientists develop their models from observations and experimental data, usually accumulated from a variety of resources. Today in lab you will work in groups to observe some computer models of cell division, and then you will build your own models of cell division. Using these models (while they may seem a bit simple) will enhance your understanding of the structure of cells and of the behavior of chromosomes, centrosomes, membranes, and microtubules during the cell cycle. Your group should discuss activities in each stage of the cell cycle as you build your model. After going through the exercise together, each group member should demonstrate the model to the rest of the group (and maybe the instructor) to reinforce your understanding. Someone in the group should have their book open to chapter 8 as a helpful reference. In the "model of mitosis" you will build, your cell will be a diploid cell ( $2n$ ) with four chromosomes, just like the one you observed online. This means that you will have two homologous pairs of chromosomes. One pair will be long chromosomes and the other pair will be short chromosomes.

**Materials:**

- Laptop computer for the group
- 120 pop beads total, 60 each of two colors
- 8 white pop beads to use as paired centromeres
- 4 white pop beads to use as paired centrioles (two paired centrioles make up a centrosome)
- Open your book to pages 126-127 for reference



Procedure: (Read the entire section before you start.)

1. Work in groups today, we only have eight laptops to go around.
2. Working with digital models, part 1. Now that you've watched the movies about cell division, it's time to start exploring some models of the cell cycle to reinforce your understanding. Let's have a look at an interactive website to begin.
  - a. Start your computer, open up Internet Explorer, and navigate to the following web page: <http://www.cellsalive.com>. Keep in mind that this website is used by students from around the world and some of the terms or spelling may not look familiar to you. Just go with it; they're talking about the same processes/structures even though they are spelled differently. You should use the terms and spelling from your lab manual and text on exams.
  - b. Cell Structure. Back to the task at hand. On the left side of the page that opens, there is a list of links to follow. Click on "*Cell Models*" under the heading "Interactive". Read the brief description and then click on the link in the lower left of the page "Take me to the Plant & ANIMAL CELL Animation". Follow the instructions to review the basic structure of animal and plant cells. Make sure you can identify all the appropriate parts of each cell without using the pointer as a reminder.
  - c. Cell Cycle. When everybody in the group is comfortable with basic cell structure, click on the link "Cell Cycle" on the left side of the page, again under "Interactive". Fair warning, this animation has an annoying soundtrack that plays over and over. You might want to hit "stop" on the animation until you are ready. Use the mouse to scan down and read the description below the animation then restart the animation (if you stopped it) and observe what happens during the cycle.
  - d. Model of mitosis. Now proceed to the "*Mitosis*" link in the list on the left side of the webpage under "Interactive". Scan down the page first, using the mouse, and read over the full description of events that occur during mitosis. Notice something different from what you've learned so far? This site adds a new phase called "prometaphase". We tend to lump this phase into prophase so that you don't have so many phases to remember, isn't that nice of us? Now that you have read the description of each phase, hit "play" on the animation. Wow, that goes fast, huh? Now try going through the model step by step. Can you follow where each phase ends and the next begins? Sometimes this is difficult with online models. They are a nice tool, but having some direct experience working with a physical model of chromosomes might help you understand what's happening. Let's try that now – set the laptop aside and move on to the next section. Don't turn off your computer, you are still going to need it.
3. Model Interphase. During **interphase**, a cell performs its specialized functions (e.g., liver cells produce bile, intestinal cells absorb nutrients, skin cells produce keratin, etc.). Interphase consists of three subphases, G<sub>1</sub>, S, and G<sub>2</sub>, with G<sub>1</sub> beginning as cell division ends. As interphase begins, there is approximately half

as much cytoplasm in each cell as there was before cell division. Each new cell has a nucleus that is surrounded by a nuclear envelope and contains 2 copies of each chromosome in an uncoiled state. In this uncoiled state, the mass of DNA and protein is called **chromatin**.

- a. Build a **homologous pair** of single-stranded chromosomes (two copies of the same chromosome) using 10 beads of one color for one member of the long pair and 10 beads of the other color for the other member of the pair. Place the centromere at any position in the chromosome but note that it must be in the same position on the homologous chromosome. Build a shorter pair of homologous chromosomes using the same two colors but use only 6 beads as shown in the figure on page 34. You should have enough beads left over to duplicate each chromosome.
- b. Take a piece of scrap paper and place it in the middle of your work station. Draw a large circle on it to represent the nuclear membrane of your “cell”. The edges of the paper will represent the cell membrane.
- c. Pile the assembled chromosomes in the center of your “nucleus” to represent the uncoiled chromosomes as a mass of chromatin in **G<sub>1</sub> (gap 1)**.
- d. Position two centrosomes as a pair just outside your nucleus. (Recall that most plant cells do not have centrioles.) In the G<sub>1</sub> phase, the cytoplasm mass increases and will continue to do so throughout interphase. Proteins are synthesized, new organelles are formed, and some organelles such as mitochondria and chloroplasts grow and divide in two. Throughout interphase one or more dark, round, bodies, called **nucleoli** (singular, **nucleolus**) are visible in the nucleus (we will not bother to model these).
- e. Duplicate the chromosomes in your model cell to represent DNA replication in the **S (synthesis) phase**. Make a second set of strands that are identical to the first strands, and attach the identical strands together at the centromere. In the replicated chromosomes, two white beads will have to be used to form the new centromere, but recall that the centromere in a cell is a single unit until it splits in metaphase. In your model, consider each pair of white beads to be the single centromere. During the S phase of the cell cycle, chromosomal DNA is replicated and synthesis continues until the chromosomes have been duplicated, and additionally, the synthesis of chromosomal proteins occurs. Each chromosome is now described as double-stranded, and each strand is called a **sister chromatid**.
- f. The cell is growing throughout interphase. To represent this growth, we need to modify our model cell. Pull out two additional pieces of scrap paper and lay them next to one another, forming a large square. This is your new cell with the edges again representing the cell membrane. Draw the nucleus *in pencil*, as before, but have half of it on one page and half on the other. The reason for this will become clear later. Pile your duplicated chromosomes in the nucleus.
- g. Duplicate the centrioles (add a second pair of centrioles to your model). Centriole duplication actually begins in late G<sub>1</sub> or early S phase.
- h. Here’s a tough step... Do not disturb the chromosomes to represent **G<sub>2</sub> (gap 2)**. During this phase, in addition to continuing cell activities, cells prepare for

mitosis. Enzymes and other proteins necessary for cell division are synthesized during this phase.

- i. Separate your centrosomes, moving them to opposite poles of the nucleus to represent that the G<sub>2</sub> phase is coming to an end and mitosis is about to begin. Life will be easier for you if you have the “poles” to the left and right sides of the nucleus, one on each page of scrap paper.

*How many pairs of homologous chromosomes are present in your cell during this stage of the cell cycle? \_\_\_\_\_*

4. Model mitosis and cytokinesis. In the **M phase** the nucleus divides, followed by division of the cytoplasm. Nuclear division is called **mitosis**, and division of the cytoplasm is referred to as **cytokinesis**. Mitosis is further divided into subphases: prophase, metaphase, anaphase, and telophase. It is very important to recognize that while we discuss mitosis (and later meiosis) in terms of discrete phases, the process is actually continuous with no real pause or break between “phases”. Dividing the process into phases makes it easier to understand.
  - a. To represent **prophase**, start with the chromosomes piled in the center of the cell (as described in 3f above). Prophase begins when chromosomes begin to coil and condense, and at this time they become visible using a compound microscope. Centrosomes move to opposite poles of the nucleus, and as they do so, a spindle (a fibrous, rounded structure tapering toward each end) begins to form between them. (Draw this spindle if it helps using figure 8.7 in your book as an example, but you’ll have to erase it and re-draw it several times if you do.) Nucleoli disappear during this phase. Some spindle fibers become associated with the chromosomes, and the push/pull of spindle fibers on the chromosomes ultimately leads to their movement to the equator. The nuclear membrane breaks up during this phase; erase it from your model cell. Move the centromeres of your chromosomes to lie on an imaginary plane (the equator of the cell) midway between the two poles established by the centrioles. In your cell, this plane is defined by the line where the edges of your pieces of paper meet. When the centromeres lie on the equator, prophase ends and metaphase begins.
  - b. To represent **metaphase**, a relatively static phase, leave the chromosomes with the centromeres lying on the equator. In this phase, double-stranded chromosomes lie on the equator (the **metaphase plate**). The two sister chromatids are held together by the centromere, and metaphase ends as the centromeres split.
  - c. Holding onto the centromeres, pull the two white pop beads apart and move the resulting, separated chromosomes to opposite poles. This action represents **anaphase**. After the centromere splits, sister chromatids separate and begin to move toward opposite poles. Chromatids are now called **chromosomes**, and anaphase ends as the chromosomes reach the poles.
  - d. Pile your chromosomes at the poles to represent **telophase**. As the chromosomes reach the poles, anaphase ends and telophase begins. The spindle breaks down, chromosomes begin to uncoil, and nucleoli reappear. A

nuclear envelope forms around each new cluster of chromosomes. Telophase ends when the nuclear envelopes are complete. Draw a new nuclear envelope on each piece of paper and place the chromosomes inside.

*How many chromosomes are in each new nucleus? \_\_\_\_\_*

*How many chromosomes were in the nucleus when the process began? \_\_\_\_\_*

- e. To represent **cytokinesis**, leave the two new chromosome masses at the poles and gently move your two pieces of paper apart. You have now formed two new cells that should look exactly like the cell you started with. The end of telophase marks the end of nuclear division, or mitosis. Sometime during telophase, the division of the cytoplasm, or cytokinesis, results in the formation of two separate cells. In cytokinesis in animal cells, a cleavage furrow forms at the equator and eventually pinches the parent cytoplasm in two. In plant cells, a cell plate begins to form in the center of the equatorial plane and grows until it eventually extends across the cell, dividing the cytoplasm in two. Cell wall materials are secreted into the space between the membranes of the cell plate. **At this stage, do not disassemble your chromosomes. You will need these to model the stages of meiosis later in the lab.**
- f. At this point you are probably asking yourself, "How am I ever going to remember the correct order of all these phases?" Here is a simple method to help: Remember the word **IPMAT**. Each letter represents a phase (Interphase, Prophase, Metaphase, Anaphase, Telophase) and it will keep them in the right order. Of course, remembering what happens in each phase is up to you...

## **B. Observing mitosis and cytokinesis in plant and animal cells.**

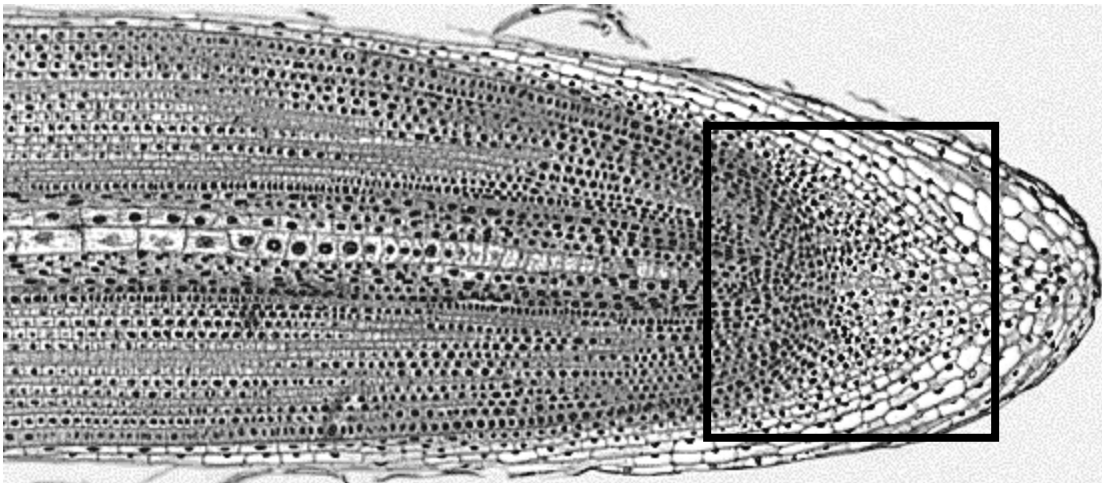
### Materials:

- a prepared slide of onion root tip
- a prepared slide of whitefish blastula cells
- a compound microscope
- Open your book to the picture on page 143 for reference (this is what you should see under the microscope at high power when looking at the plant cells)

Procedure: (Read the entire section before you start.)

1. Observe the longitudinal section of root tip.
  - a. Refer to lab 2 for a review of proper microscope techniques. Using low power, locate the area most likely to have dividing cells. You should find this region just behind the root cap (see figure below). At the tip of the root is a cap that protects the tender root tip as it grows through the soil. Just behind the root

- cap is the zone of cell division. As cells divide in this zone, the root tip is pushed farther and farther into the soil.
- Focus on the zone of cell division. Now switch to intermediate power, focus, and switch to high power. Survey the zone of cell division and locate stages of the cell cycle: interphase, prophase, metaphase, anaphase, telophase, and cytokinesis.
  - As you locate dividing cells, think about the stage of division. Read the description of each stage of division in your textbook and verify if your guess is correct. Check with your instructor if you are having trouble locating or identifying dividing cells.



- Observe a prepared slide of whitefish blastula cells.
  - One of the best sources of actively dividing cells in animals is the early embryo, where cells are large and divide rapidly with a short interphase. By examining cross sections of whitefish blastulas, you should be able to locate many dividing cells in various stages of mitosis and cytokinesis.
  - Examine the prepared slide under low power, find a blastula section, focus, switch to intermediate power, focus then switch to high power.
  - As you locate dividing cells, identify the stage of mitosis. Identify the following in several cells:
    - nucleus, nuclear envelope, and nucleolus.
    - chromosomes and centrioles (may be difficult to find and will appear as small dots at the poles)
    - mitotic spindle
    - cleavage furrow

### **C. Modeling meiosis in animal cells.**

#### **Materials:**

- the materials you should still have from modeling mitosis (including the laptop)
- an additional 8 white pop beads to use as centromeres
- Refer to pages 132-133 in the text

Procedure: (Read the entire section before you start.)

1. Working with digital models, part 2. Okay, let's start with the computer models again. If you navigated away, go back to the page: <http://www.cellsalive.com>. This time click on the link to "*Meiosis*", read over the description, then view the animation. Just like last time, this moves very quickly! Did you miss the point when crossing over occurred? Can you tell how this process is different from mitosis? Watch the animation a few times at full speed and then step by step until you are comfortable with the process. When you think you have it, and if you still have time in the lab, move on to model meiosis using beads.
2. Model Interphase. This stage is similar in cells undergoing either mitosis or meiosis and we will repeat the procedure from before.
  - a. Pile all the assembled chromosomes in the nucleus of your original model cell on scrap paper to represent the de-condensed chromosomes as a mass of chromatin in **G<sub>1</sub> (gap 1)**.
  - b. As before, duplicate the chromosomes in your model cell to represent DNA replication in the **S (synthesis) phase**. Make a second strand that is identical to the first strand in each chromosome. In replicating chromosomes, two beads will be used to form the new centromere, but recall that the centromere in a cell is a single unit until it splits in metaphase. In your model, you will still consider each pair of beads to be the single centromere.
  - c. Now switch to the two-page model cell you created for modeling mitosis. This is still representing cell growth.
  - c. Duplicate the centrosome (add a second pair of centrioles to your model).
  - d. Do not disturb the chromosomes to represent **G<sub>2</sub> (gap 2)**. As in mitosis, enzymes and other proteins necessary for cell division are synthesized during this phase.
2. Meiosis I. Meiosis consists of two consecutive nuclear divisions, called **meiosis I** and **meiosis II**. When the first division begins, the chromosomes coil and condense, as in mitosis. Meiosis I is radically different from mitosis, however, and the differences immediately become apparent.
  - a. Meiosis begins with the chromosomes piled in the nucleus of your cell. As chromosomes begin to coil and condense, **prophase I** begins. Each chromosome is double-stranded, made up of two sister chromatids. Centrosomes are located outside the nucleus.
  - b. Separate the two centriole pairs and move them to opposite poles of the nucleus. The nuclear envelope breaks down (erase it as before) and the spindle begins to form as in mitosis.
  - c. Now something very different happens. Move each homologous chromosome to pair with its partner. Because the chromosomes are double-stranded, each paired double chromosome complex is made of four strands. This complex is called a **tetrad** and there should be two tetrads in your cell. Within each tetrad, non-sister chromatids often exchange pieces with each other in a process called "**crossing over**". This effectively shuffles the genetic



- information contained on a given chromatid. Crossing over takes place only between non-sister chromatids in the tetrad. In this process, a segment from one chromatid will break and exchange with the exact same segment on a non-sister chromatid in the tetrad. Why is this significant? It is significant because crossing over produces new allelic combinations among genes along a chromatid. Genes are often expressed in different forms. For example, when the gene for seed color is expressed in pea plants, the seed may be green or yellow. Alternative forms of genes are called **alleles**. New allele combinations within a particular chromosome occur most often as a result of crossing over. Represent the phenomenon of crossing over by detaching and exchanging identical segments of any two non-sister chromatids in a tetrad. Use the figure on page 132 in your text as a reference, but if this is still unclear, call over your instructor for a demonstration.
- e. Late in **prophase I**, tetrads move to the equator. Move your tetrads to the equator, midway between the two poles (along the seam between pages).
  - f. To represent **metaphase I**, leave the tetrads lying at the equator. During this phase, tetrads lie on the equatorial plane, but centromeres *do not split* as they do in mitosis.
  - g. For **anaphase I**, separate each double-stranded chromosome from its homologue and move one homologue toward each pole.

*How does the structure of chromosomes in anaphase I differ from anaphase in mitosis?*

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- h. To represent **telophase I**, place the chromosomes at the poles. You should have one long and one short chromosome at each pole, representing a homologue from each pair. Two nuclei may form (only in some species), followed by cytokinesis. Separate the pages as before to represent cytokinesis, but do not draw new nuclei; we will not model this step since it doesn't occur in all species.

*How many chromosomes are in each cell?* \_\_\_\_\_

- i. To represent **meiotic interphase**, leave the chromosomes in the two piles formed at the end of meiosis I. This interphase step is usually short. There is little or no cell growth and no synthesis of DNA. All the machinery for a second nuclear division is synthesized, however.
3. **Meiosis II.** The events that take place in meiosis II are similar to the events of mitosis. Meiosis I results in two nuclei with half the number of chromosomes as the parent cell, but the chromosomes are double-stranded (made of two chromatids), just as they are at the beginning of mitosis. The events in meiosis II must change double-stranded chromosomes into single-stranded chromosomes.

As meiosis II begins, two new spindles begin to form, establishing the axes for the dispersal of chromosomes to each new nucleus.

- a. To represent **prophase II**, separate the centrioles and set up the axes of the two new spindles. Pile the chromosomes in the center of each spindle. The events that take place in prophase II are similar to those of a mitosis prophase. In each new cell the centrioles move to the poles, nucleoli break down, the nuclear envelope (if formed) breaks down, and a new spindle forms. The new spindle forms at a right angle to the axis of the spindle in meiosis I.
- b. Align the chromosomes at the equator of their respective spindles. As the chromosomes reach the equator, prophase II ends and **metaphase II** begins.
- c. Leave the chromosomes on the equator to represent metaphase II.
- d. Pull the double-stranded chromosomes apart at the centromere. As metaphase II ends, the centromeres finally split and **anaphase II** begins.
- e. Separate sister chromatids (now chromosomes) and move them to opposite poles. In anaphase II, single-stranded chromosomes move to the poles.
- f. Pile the chromosomes at the poles. As **telophase II** begins, chromosomes arrive at the poles. Spindles break down, nucleoli appear, and nuclear envelopes form around each bunch of chromosomes as the chromosomes uncoil. Draw a nuclear envelope around each pile of chromosomes at each end of both cells. Cytokinesis follows meiosis II. Carefully tear or cut each piece of paper in half to represent cytokinesis.

*What is the total number of nuclei and cells now present?* \_\_\_\_\_

*How many chromosomes are in each cell?* \_\_\_\_\_

Please be able to answer the following questions; you may see them again on a lab practical. You do not need to answer them in class; work on the answers at home.

1. Describe the activity of chromosomes in each stage of mitosis (Prophase, Metaphase, Anaphase, and Telophase)
2. The process of cytokinesis is different in plant and animal cells. How is it different and why would the process of cytokinesis that operates in animal cells not work in plant cells?
3. Compare and contrast Mitosis and Meiosis. How are they similar and how are they different? What are the end products?