

Activity #6. Mitosis, Meiosis, and Mendelian Genetics

Learning Goals:

- To follow the stages of mitosis and meiosis and calculate the mitotic index in onion root tip sections
- To simulate mitosis and meiosis using pipe cleaners
- To understand the differences and similarities between mitosis and meiosis
- To build comprehension of Mendelian genetics by analyzing test crosses in corn
- To study human chromosomes and understand the consequences of chromosomal abnormalities that occur during meiosis

Lab Background:

Mitosis is the mechanism by which the chromosomes of eukaryotes are segregated so that the **two** daughter cells formed by cell division receive the **same** number of chromosomes that the parent cell contained. Thus, all daughter cells formed by mitosis have an identical set of genes. This process of mitosis is the primary means by which all eukaryotic organisms grow and replace damaged cells. After fertilization of an egg by a sperm, mitosis must occur many millions of times to produce an adult organism such as you.

Meiosis is a specialized division of sex cells that results in the production of **four** cells, each with **one half** the number of chromosomes contained by the parent cell. In animals, these cells serve exclusively as gametes for sexual reproduction. **It is essential that gametes contain only half the number of chromosomes in order to maintain a constant chromosome number in the zygotes that result from fertilization.** Meiosis is accomplished by two consecutive divisions in which the genetic material is only duplicated once. This process of meiosis occurs in the male's testis to produce sperm and in the female's ovary to produce the egg. Although there are differences in the process of meiosis in the male and female, we will concern ourselves with the general features of meiosis in this lab. To understand how our bodies grow, repair damage, and reproduce, it is essential to understand the similarities and differences between mitosis and meiosis.

Different goals for mitosis versus meiosis: In mitosis, it's important that the daughter cells each end up with a full set of chromosomes—the diploid number ($2n$)—so that they can divide normally and do their jobs like any other body cell they may become. In meiosis, however, the chromosome number in sex cells (eggs and sperm) must be reduced to one-half of the number in our normal body cells—the haploid number (n). This reduction during meiosis means that when the egg and sperm meet to form a zygote, that zygote will have a full set of chromosomes: $n + n = 2n$. Although some plant species can tolerate variations in chromosome number, most animal zygotes with abnormal numbers of chromosomes will not survive. So the process of meiosis is literally a life-or-death issue during development of animals, including *Homo sapiens*.

How do the chromosomes move? The chromosomes contain a constricted region called a centromere in their DNA sequence. The centromere allows attachment of a group of kinetochore proteins that hold the replicated copies of the chromosomal DNA together. These kinetochore proteins also allow attachment of microtubules that pull the chromosomes around the cell during either mitosis or meiosis. The microtubules are like a fly's tongue, and the kinetochore proteins are like the sticky stuff on the fly's tongue that allows it to catch the fly/centromere. If the microtubules fail to attach to a chromosome at its centromere, sensors in the cell will usually pause mitosis until all chromosomes are "captured" by microtubules. If this "checkpoint" fails, the unattached chromosome will not be moved into daughter cells correctly. And once again—for most diploid animal cells—missing chromosomes are usually a death sentence for the affected cell.

In this lab, you will first observe mitosis and meiosis under the microscope. Then you will simulate these processes yourself in order to ensure that you understand their mechanism and function. While some new terms will be introduced for convenience (it is much easier to refer to "metaphase" than "the stage where the chromosomes line up in the middle"), the terms **are not** the most important thing to learn. Be sure to focus your attention on the *processes* of mitosis and meiosis. How are the cellular products different? Why are these differences important? When does each process occur? How does it work? Can you think of a more elegant strategy to accomplish the same goals?

Mendelian genetics: The mechanism of evolution itself remained unexplained until the principles of heredity were worked out about 100 years ago by the Austrian monk Gregor Mendel who, interestingly enough, had no concept of genes or chromosomes. At the beginning of the twentieth century, the "laws" Mendel proposed for inheritance, the visible effects of breeding, and the (then) recently discovered information about the behavior of chromosomes in meiosis all were joined to explain the phenomenon of heredity. Shortly thereafter, changes in heredity (mutations) were recognized as the basic raw material of evolution. The selection of mutations or groups of mutations by nature or man could explain the evolution of new species from old.

The study of heredity is significant not only to the science of which it is a part, but also to the practical world of affairs. The principles of heredity can be used in many practical plant and animal breeding procedures. They can be used to give a partial basis for understanding and eliminating some social and personal problems such as race prejudice, some diseases, appearance of offspring, and determination of sex. The study of heredity is one of the most important subjects in biology, and it all has its roots in mitosis and meiosis.

I. Mitosis

A Mitosis in Plants

Study the diagrams of various stages of cell division shown in Figure 7.1 and then using slides of the root tip of onion (*Allium*), locate cells in all the five major stages of division. Compare each stage under the microscope with that illustrated in Figure

7.1. The instructor may spot-check your slides to see if you have correctly identified the various stages. Examine 100 cells, and count the number of cells in each stage of mitosis. Enter your data in Table 1 below.

For convenience, mitosis has been classified into four stages: prophase, metaphase, anaphase and telophase. The period of the cell cycle between divisions (G_1 , S, G_2) was previously thought to be a resting stage, and is referred to as interphase. It is now clear that this period is characterized by a high level of metabolic activity in which the cell undergoes specialization or performs its normal functions. Regulatory events control whether or not the DNA will be copied, a process that commits the cell to undergoing mitosis again. It should be kept in mind that the process is dynamic and continuous and each stage actually passes imperceptibly into the next.

1. Interphase. Nuclei in interphase show little definable structure except the prominent, darkly stained spherical nucleoli. The chromosomes are uncondensed and give the nuclei a relatively homogeneous appearance. During the S (synthesis) phase of the cell cycle, the chromosomes undergo replication to produce two sister chromatids attached to each other at a single point called the centromere.
2. Prophase. The first microscopically visible evidence of division is when the chromosomes condense such that individual chromosomes become distinguishable. As prophase progresses, the chromosomes become shorter, thicker and more distinct. Coiling becomes more pronounced and eventually the coils take on a regular, smooth appearance. As the chromosomes become shorter and thicker, the nucleoli progressively disappear.
3. Metaphase. At the end of prophase, the nuclear membrane disappears and a spindle composed of microtubules develops from the two poles toward the equator. Some of the spindle fibers extend from pole to pole while others become attached to the chromosomes. Spindle fibers become attached to the centromere from each pole of the spindle. The chromosomes migrate to the middle of the spindle, equidistant from the poles. There the chromosomes become aligned in a single plane.
4. Anaphase. Anaphase begins with the poleward movement of sister chromatids. The centromere may be in the middle of the chromosome or at any position between the middle and the ends. Thus, chromosomes in anaphase may be V-, J- or rod-shaped as they trail behind the centromere. As soon as the chromatids separate in anaphase, they represent separate chromosomes.
5. Telophase. The chromosomes are reorganized into a nuclear structure with a membrane and the nucleoli reappear. The chromosomes uncoil, become thinner and more thread-like and gradually return to their interphase state.

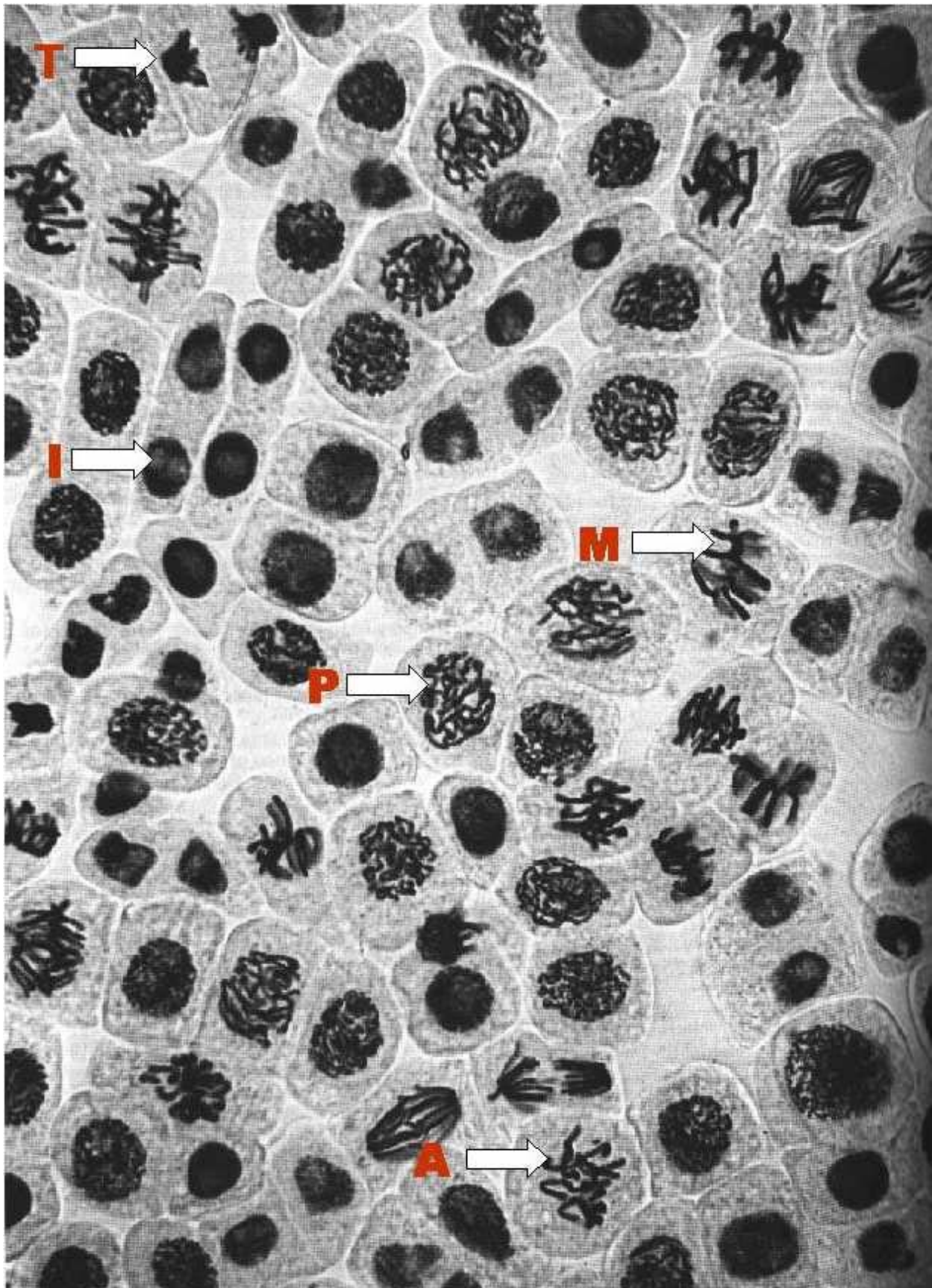


Figure 7.1 Appearance of different phases of mitosis in onion root tip.

B. Mitotic Index in the Onion Root Tip

The mitotic index is defined as the proportion of cells that are in the process of dividing. The onion root tip is an area of active growth and, therefore, one would expect that mitosis is occurring at a rapid rate. What the mitotic index will do is to tell us how active this tissue is. Using a slide of the onion root tip, **count 100 cells** and indicate which stage of mitosis each of these cells are in. Enter your data in the following table.

Table 1, Calculation of Mitotic Index

Observer	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Totals						

$$\text{Mitotic index} = \frac{\begin{array}{c} \# \text{ of cells in mitosis} \\ \text{(PMAT)} \end{array}}{\begin{array}{c} \text{Total \# of cells} \\ \text{counted} \end{array}} = \underline{\hspace{2cm}} \%$$

What is the mitotic index of your sample? _____

Why is the mitotic index less than 100%? _____

What does this tell you about how long it takes mitosis to occur?


C. Cytokinesis in Plants

In most organisms, cell division consists of two processes; namely, nuclear division (**Karyokinesis**) and cytoplasmic division (**cytokinesis**). In plants, cytoplasmic division starts before nuclear division is complete and the two processes finish simultaneously. In a few instances, however, they are separated by a considerable period.

Re-observe the later stages of nuclear division and look for evidence of cytoplasmic division. The first indication is the appearance of a continuous fluid film or cell plate at the equator. Pectic substances and other compounds are then deposited and the cell plate becomes a rigid layer, the middle lamella, separating the two protoplasts. Cellulose and other materials are produced on either side of the middle lamella and the new cell walls are formed.

D. Simulation of Mitosis Using Pipe Cleaners

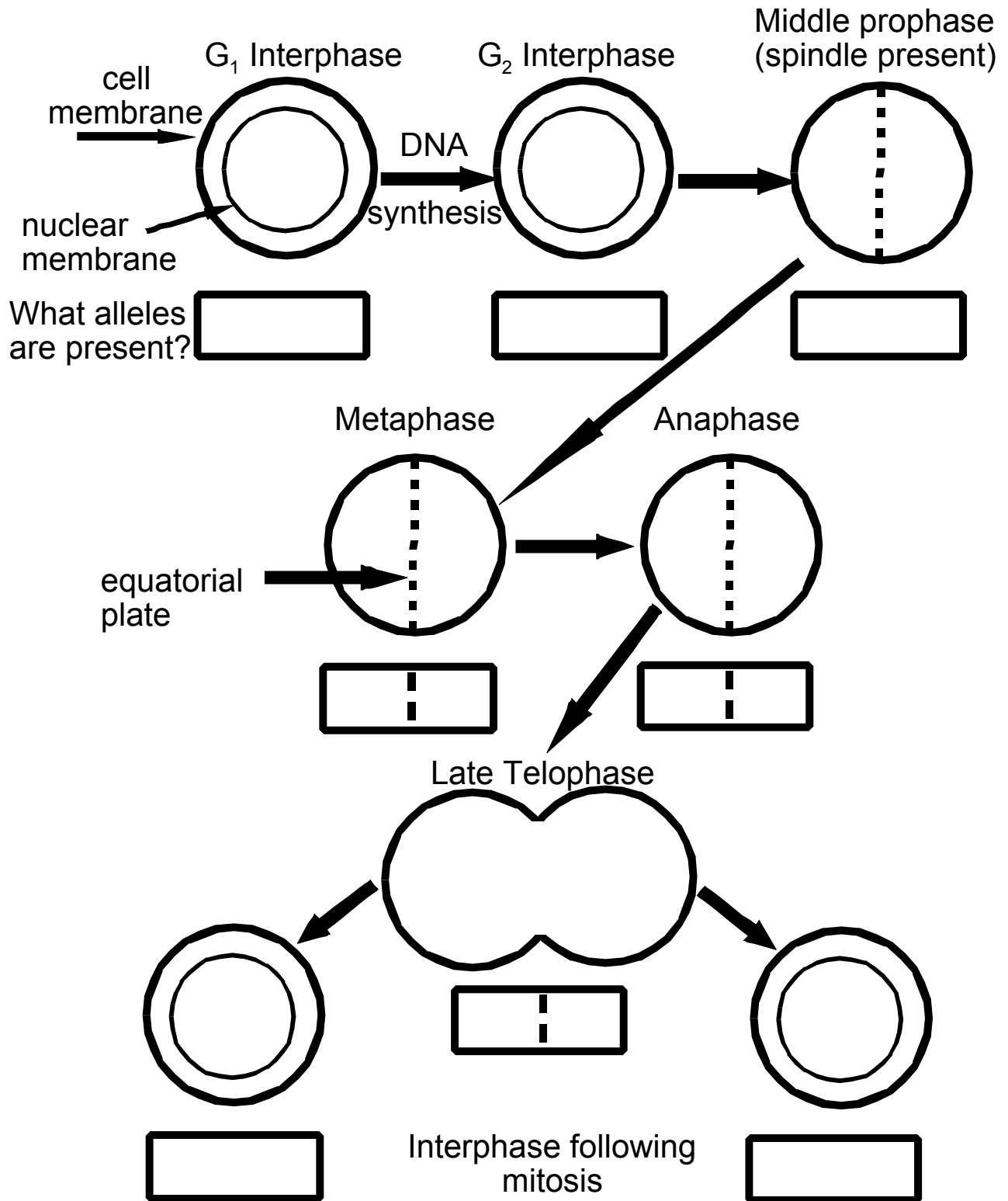
On the front desk, you will find colored pipe cleaners that will be used to simulate chromosomes. Starting with four chromosomes ($2N$ (diploid number) = 4), you should be able to move these "chromosomes" through the sequence of events necessary to produce two identical daughter cells. Your instructor will check that you can simulate mitosis prior to leaving the laboratory. You may want to use figures from your textbook as a guide. Remember that somatic (body) cells have 2 copies of each chromosome, one from the father and one from the mother, and that each chromosome is one helix or strand of DNA.

You can begin by representing the chromosomes like this  (2 copies of 2 chromosomes that differ in length). Note that a single pipe cleaner can represent a single chromosome/helix/strand of DNA here, and you'll have another set of pipe cleaners in reserve.

As the DNA replicates, you should attach the matching pipe cleaner to its partner with the Velcro in the middle—now each chromosome looks like an "X". You should have 2 big "X" molecules and 2 little "x" molecules after the DNA replicates.

Follow the diagrams in your book to simulate the other stages of mitosis. Sketch what the chromosomes look like on the Mitosis diagrams on the next page. Have your instructor check to be sure you have this correct before you go on.

MITOSIS



II. Meiosis

- A. Study the photographs of the various stages of meiosis. As with mitosis, meiosis has been divided into several stages. You should understand the sequence of events necessary to form a haploid gamete and not become overwhelmed with the exact stages that are to be described below.

MEIOSIS -PART I:

1. Interphase. Nuclei in interphase show little definable structure except the prominent, darkly stained spherical nucleoli. The chromosomes are uncondensed and give the nuclei a relatively homogeneous appearance. During the S (synthesis) phase of the cell cycle, the chromosomes undergo replication to produce two sister chromatids attached to each other at a single point called the centromere. This is the same as occurred for cells entering mitosis.
2. Prophase I - The highlight of this stage is the *unique pairing (synapsis) of homologous chromosomes to form a **tetrad***. This pairing permits an exchange of DNA between homologous chromosomes resulting in crossing over, or recombination. In the best preparations, it is possible to identify the four chromatids involved in forming the tetrad.
3. Metaphase I - Tetrads are lined up on the equatorial plate and will often appear as loops or circles.
4. Anaphase I - *Homologous chromosomes move to opposite poles*. Movement is a direct result of the attachment of the spindle fibers to the centromere.
5. Telophase I - Each of the two new nuclei that start to re-form here have only **one-half the number of chromosomes** as the original cell, but each is still duplicated.

MEIOSIS - PART II:

6. Interphase II - The nuclei will uncoil and often there is some delay prior to the start of the second part of meiosis.
IMPORTANT: NO replication of genetic material occurs in the second phase of meiosis!
7. Prophase II - Chromosomes start to contract and are visible again.
8. Metaphase II - The duplicated chromosomes line up on the equatorial plate.
9. Anaphase II - Centromeres separate which permits the duplicated chromatids to move toward opposite poles

10. Telophase II - the nuclei start to re-form. Each contains a haploid number of chromosomes (see photograph h).

The actual production of a functional egg or sperm often requires some morphological change after the completion of meiosis. It can be seen, for example, that sperm are not the immediate product of Meiosis II, but are formed only after the cytoplasm is transformed into a long extension to form the tail.

B. Comparison of Mitosis and Meiosis

Using figure 7.2 (on the next page), work with your lab partner to compare the processes of mitosis and meiosis.

Similarities

- 1.
- 2.
- 3.
- 4.

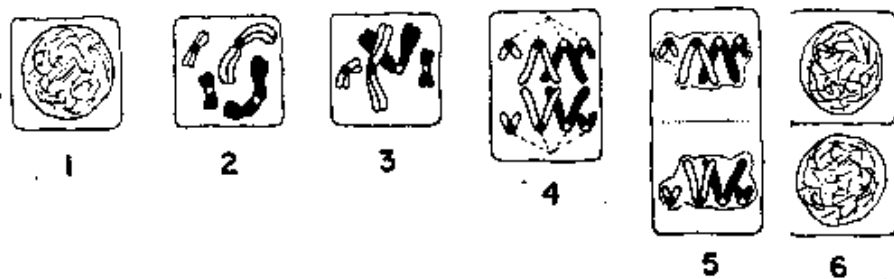
Differences

- 1.
- 2.
- 3.
- 4.

C. Simulation of Meiosis

In order to make sure that you understand the sequence of events that occur in meiosis, each student is expected to simulate meiosis with pipe cleaners prior to leaving the lab. We will use a "cell" that contains a diploid number of 4 (2 copies of 2 different chromosomes). After you have successfully moved the pipe cleaner chromosomes through meiosis, complete the worksheets using different colored markers to draw the 4 chromosomes.

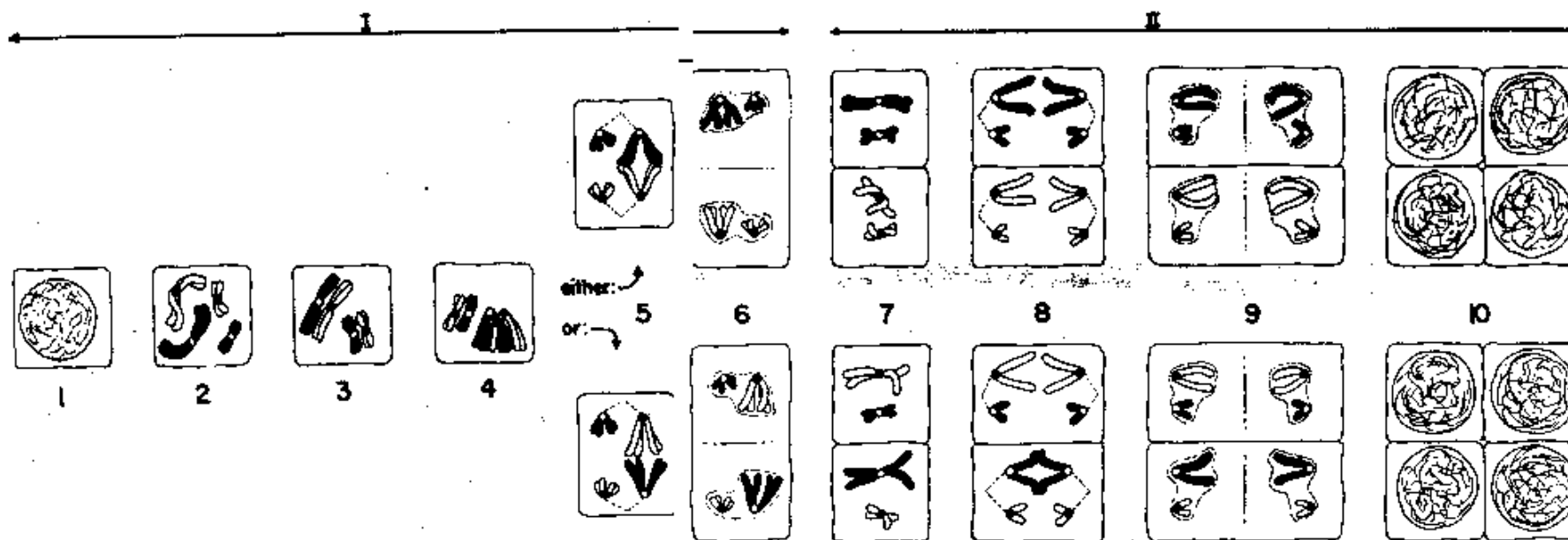
I. Mitosis -



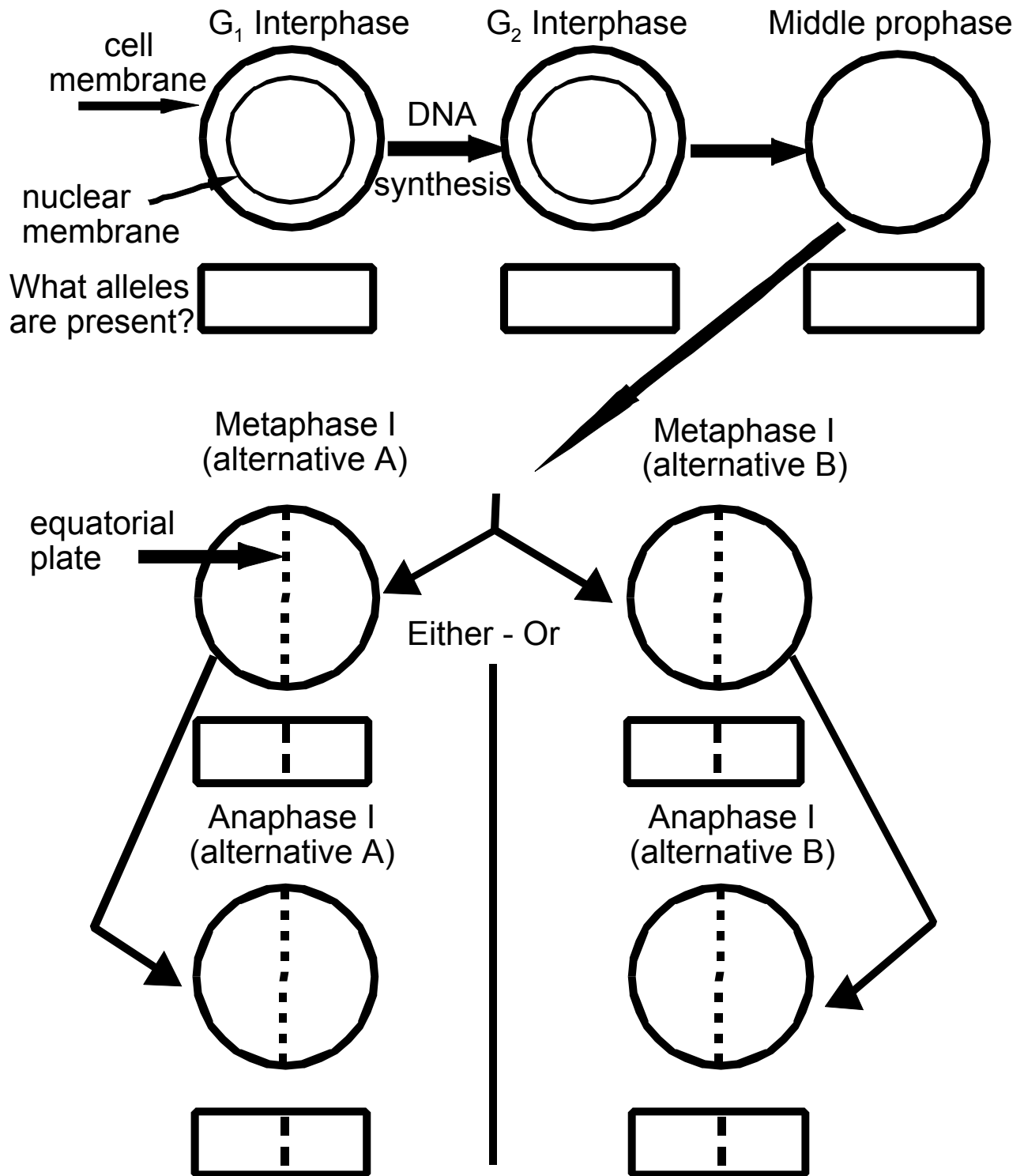
This is a longitudinally duplicated chromosome: 

This is a pair of longitudinally duplicated chromosomes: 

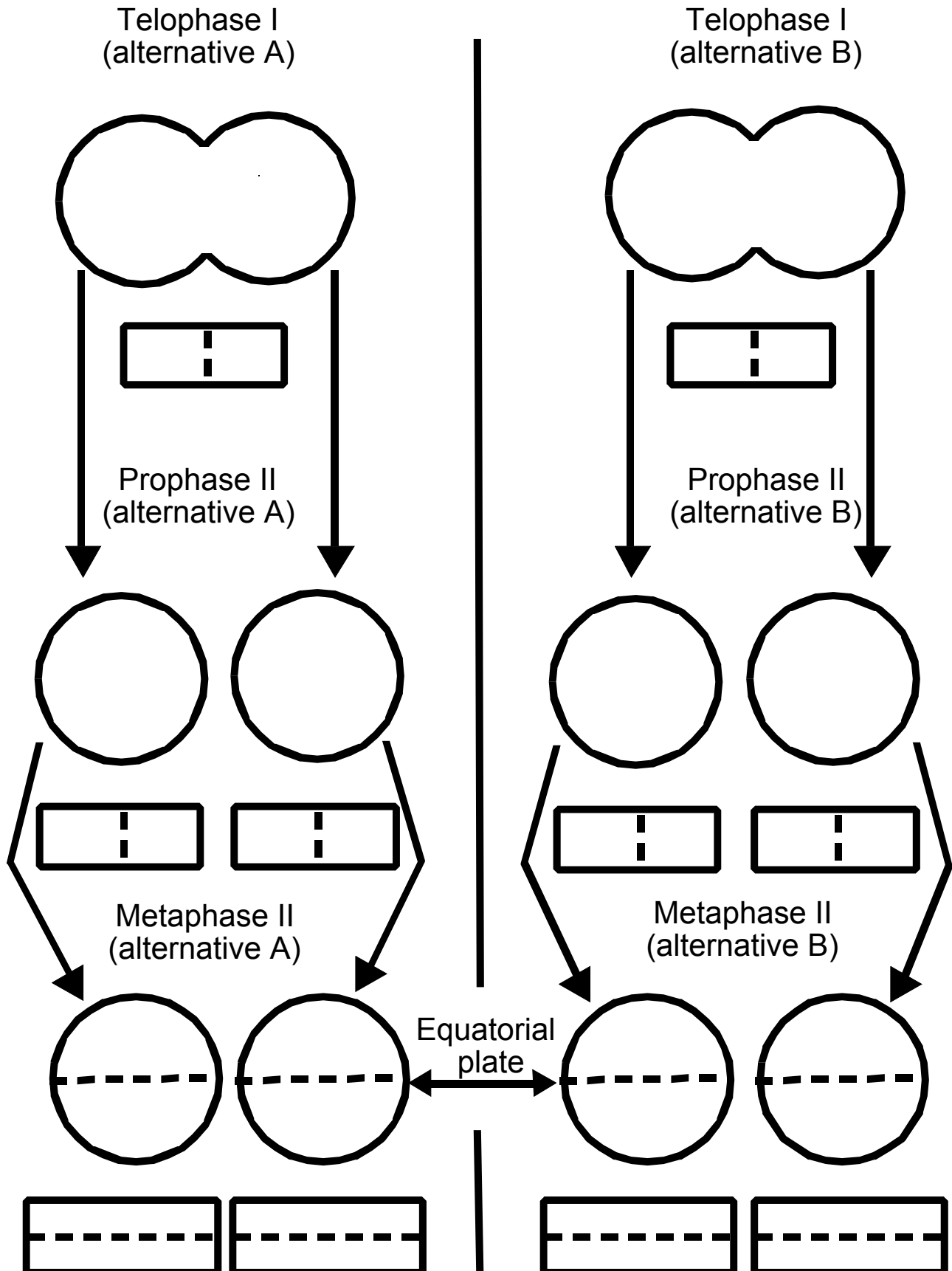
2. Meiosis -



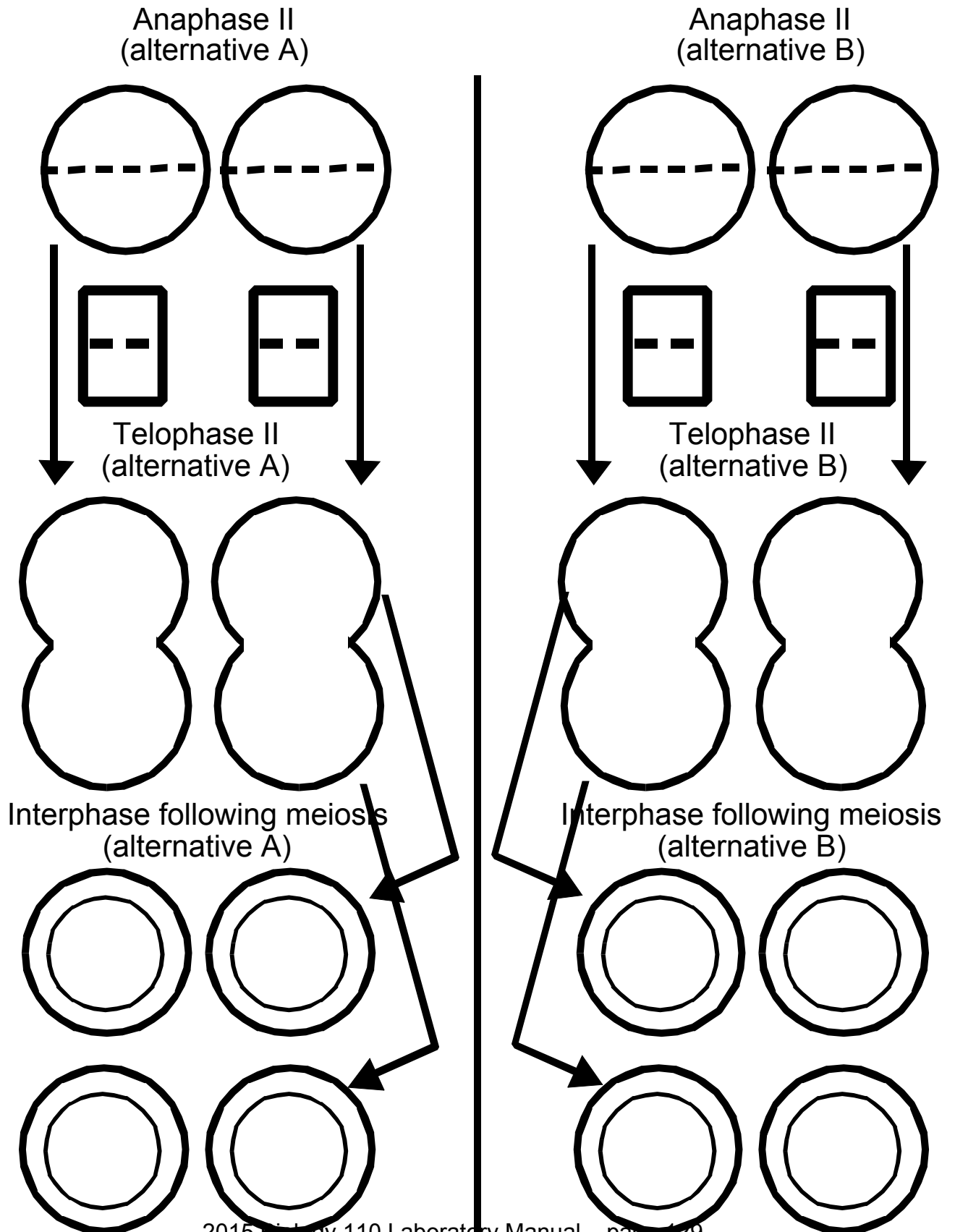
MEIOSIS



MEIOSIS (page 2)



MEIOSIS (page 3)



D. Analysis of corn kernels as offspring of a cross.

The following crosses deal with two characteristics that are inherited independently.

Purple kernel corn (dominant; genetic symbol is **R**) versus
Yellow kernel corn (recessive; genetic symbol is **r**), and

Smooth kernels (dominant; genetic symbol is **S**) versus
Wrinkled kernels (recessive; genetic symbol is **s**).

1. Monohybrid cross: You have been provided with an ear of corn that resulted from a monohybrid cross ($Rr \times Rr$). What phenotypic ratio do you expect find? Create a small Punnett square to find out. **Record this ratio** in the box with a star *. **Count all of the kernels on your cob** and record the number of kernels that show the dominant phenotype, and the number that show the recessive phenotype. Enter the data in the “observed” column of the table below.

Space for your Punnett square for $Rr \times Rr$ cross:

	Observed	Expected	$\frac{(\text{observed}-\text{expected})^2}{\text{expected}}$
Dominant phenotype (purple or smooth)			$\chi^2 =$
Recessive phenotype (yellow or wrinkled)			$\chi^2 =$
Total number counted			total $\chi^2 =$
Ratio (dominant:recessive)		*	P= accept or reject?

Why might your observed results be different from your expected results? When analyzing quantitative data, statistical methods are often used to distinguish between chance variation and real deviation from expected results (due to an incorrect hypothesis). Perform a **Chi-square test** (“chi” is pronounced with a hard k and long i) **to determine the probability that any deviation from the expected results is due to chance variation**. To do this, subtract the expected number from the observed number in the chart above, and square the result. Then divide this new number by the expected number, and enter the result in the chart above. Add up the chi-squared values for each phenotype to get the total chi-square value for the experiment, and enter this in

the space with the “ $\chi^2 =$ ” notation. This total χ^2 value is a numerical estimate of how different the observed results are from the predicted results.

The final step in the chi-square analysis is to interpret the χ^2 value. The total χ^2 value must now be converted into a **probability value (p)**. To do this, you must initially determine the number of the **degrees of freedom (df)**, which is equal to $n - 1$ where n is the number of possible phenotypes. For this monohybrid cross, n is 2 because there were 2 possible phenotypes, so the df is $n - 1 = 2 - 1 = 1$. On the chi-square probability chart below, read across the (first) $df = 1$ row to find where your total χ^2 value falls. Read up from your total χ^2 value to the probability value (0.95-0.001 from left to right across the top of the chart) to determine your probability value p . **If your p is above 0.05, your results are not statistically different from the expected results.** If your p is 0.05 or lower, your results are in fact statistically different from the expected results, and you would need to re-evaluate your hypothesis or your data collection to determine why. Have some of the kernels fallen out/perhaps one type of kernel falls out more easily than another? Did you bias your data collection by choosing a specific kernel type instead of collecting an unbiased sample?

2. Dihybrid Cross. In the next part of today’s lab exercise, you will analyze corn cobs from a dihybrid cross. Dihybrid means there are 2 genes involved. In this case, the genes are:

Purple kernel corn (dominant; genetic symbol is **R**) versus
Yellow kernel corn (recessive; genetic symbol is **r**), and

Smooth kernels (dominant; genetic symbol is **S**) versus
Wrinkled kernels (recessive; genetic symbol is **s**).

Each individual corn kernel is a zygote, the result of a haploid egg cell uniting with a haploid sperm cell to form a new diploid cell. This means that each cell has 2 copies of each gene. One gene controls color (with either purple or yellow alleles), and another gene controls texture (with either smooth or wrinkled alleles). So a pure-breeding purple smooth corn cob would have all of its kernels of genotype **RRSS**.

What would be the genotype of a pure-breeding yellow wrinkled corn cob? _____

THE PROBABILITIES OF DIFFERENT CHI-SQUARE VALUES FOR DEGREES OF FREEDOM FROM 1 TO 50

df	Probabilities										
	.95	.90	.70	.50	.30	.20	.10	.05	.01	.001	
1	.004	.016	.15	.46	1.07	1.64	2.71	3.84	6.64	10.83	
2	.10	.21	.71	1.39	2.41	3.22	4.61	5.99	9.21	13.82	
3	.35	.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35	16.27	
4	.71	1.06	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47	
5	1.15	1.61	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52	
6	1.64	2.20	3.83	5.35	7.23	8.56	10.65	12.59	16.81	22.46	
7	2.17	2.83	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32	
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.13	
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88	
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59	
11	4.58	5.58	8.15	10.34	12.90	14.63	17.28	19.68	24.73	31.26	
12	5.23	6.30	9.03	11.34	14.01	15.81	18.55	21.03	26.22	32.91	
13	5.89	7.04	9.93	12.34	15.12	16.99	19.81	22.36	27.69	34.53	
14	6.57	7.79	10.82	13.34	16.22	18.15	21.06	23.69	29.14	36.12	
15	7.26	8.55	11.72	14.34	17.32	19.91	22.31	25.00	30.58	37.70	
20	10.85	12.44	16.27	19.34	22.78	25.04	28.41	31.41	37.57	45.32	
25	14.61	16.47	20.87	24.34	23.17	30.68	34.38	37.65	44.31	52.62	
30	18.49	20.60	25.51	29.34	33.53	36.25	40.26	43.77	50.89	59.70	
30	34.76	37.69	44.31	49.34	54.72	58.16	63.17	67.51	76.15	86.66	

← ACCEPT REJECT →
at .05 level

2. Dihybrid cross, continued: A pistillate (female) corn plant homozygous for purple-smooth kernels was crossed with a staminate (male) plant homozygous for yellow-wrinkled. **Supply the following information:**

Pistillate parent

Staminate parent

Phenotype _____

Phenotype _____

Genotype _____

Genotype _____

Ova produced _____ Pollen produced _____

F₁ offspring: fertilize the ova above with the pollen above:

Phenotype _____ (determine from genotype on next line)

Genotype _____ (combine ova + pollen from above)

Some of the F₁ kernels were germinated, grown to maturity and interbred. Below, supply the information regarding the F₁ parents and the production of F₂ offspring:

Pistillate parent

Staminate parent

Phenotype _____

Phenotype _____

Genotype _____

Genotype _____

Punnett Square for dihybrid corn cross:

Ova produced (enter this in first column below) Pollen produced (enter this on first row)

Genotypes of ↓ ova pollen →				

What is the expected phenotypic ratio? _____

Remember to indicate which numbers go with which phenotypes!

Enter this ratio on the next table in the square with the asterisk *.

Each pair of students will now receive an F₂ cob which actually represents the results of crossing the two F₁ plants above. **Count and classify all of the kernels on one cob** according to phenotype and compare the observed with the calculated distributions using a chi-square test. Please handle the cobs with care and do not mar the kernels with ink or pencil.

	Observed	Expected	$\frac{(\text{observed}-\text{expected})^2}{\text{expected}}$
Dominant phenotype (purple and smooth)			$\chi^2 =$
Mixed phenotype (purple and wrinkled)			$\chi^2 =$
Mixed phenotype (yellow and smooth)			$\chi^2 =$
Recessive phenotype (yellow and wrinkled)			$\chi^2 =$
Total number counted			total $\chi^2 =$
Ratio (dominant:mixed: mixed:recessive)		*ratio from prev.pg.	P= accept or reject?

Calculate the χ^2 value for this experiment, and determine your degrees of freedom (number of different phenotypes – 1). Use the probability chart to determine your p value. Are your results statistically different from the expected results? If so, why might this be?

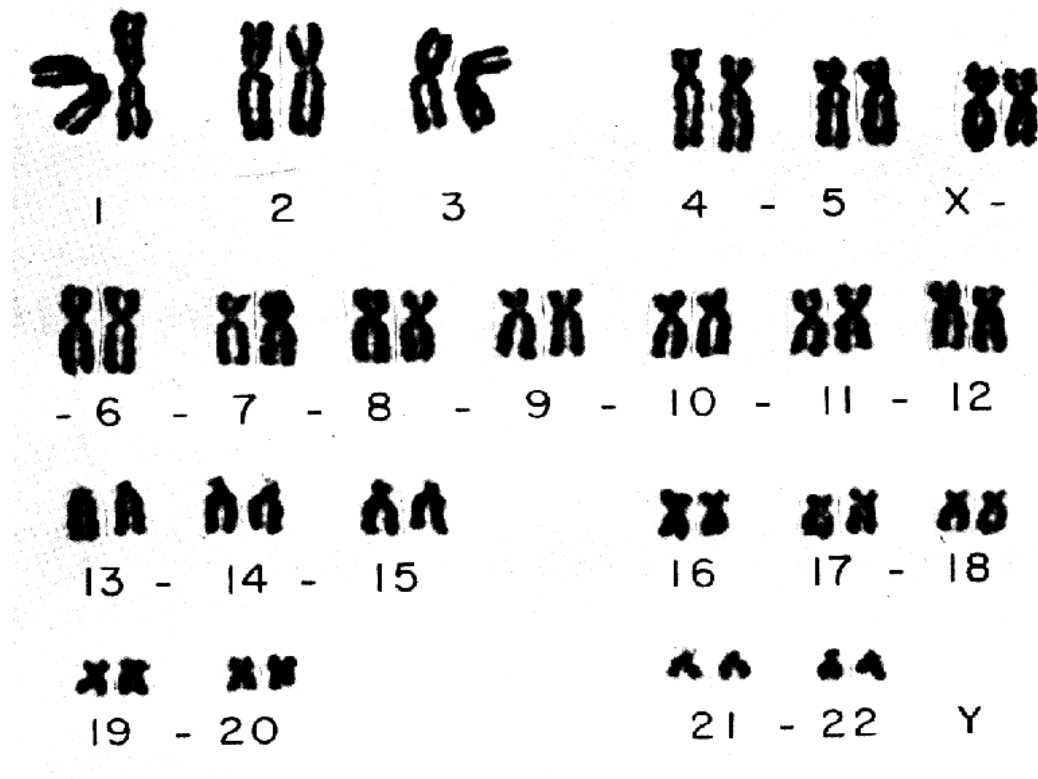
C. Human Chromosomes and Karyotypes

Humans have 46 chromosomes, 23 from the mother and 23 from the father. Chromosomes are analyzed using a karyotype: a picture of all the chromosomes from one cell. Cells containing chromosomes must be photographically enlarged so that the chromosomes are easily visible. The karyotype analysis consists of digitally cutting out the chromosomes from the photograph and then arranging them in descending order by length. Below is a normal karyotype and on the next two pages are karyotypes that show abnormal numbers of chromosomes. Can you identify the differences? Your instructor will discuss with you the implications of these abnormal karyotypes.

How might an individual end up with an extra chromosome? The answer lies in meiosis. During normal sperm or egg production, the chromosomes separate from one another to give gametes that contain only one copy of each chromosome. During abnormal meiosis, however, two copies of a single chromosome may “stick” together, yielding a gamete that has an extra copy of one chromosome. This is called nondisjunction. When that abnormal gamete joins with another gamete that has its

normal one copy of that chromosome, the resulting zygote has a total of 3 copies, a condition known as a trisomy. Many trisomies result in abnormal development of the zygote and spontaneous abortion. One common trisomy in humans is trisomy 21, known as Down Syndrome.

Normal Karyotype:



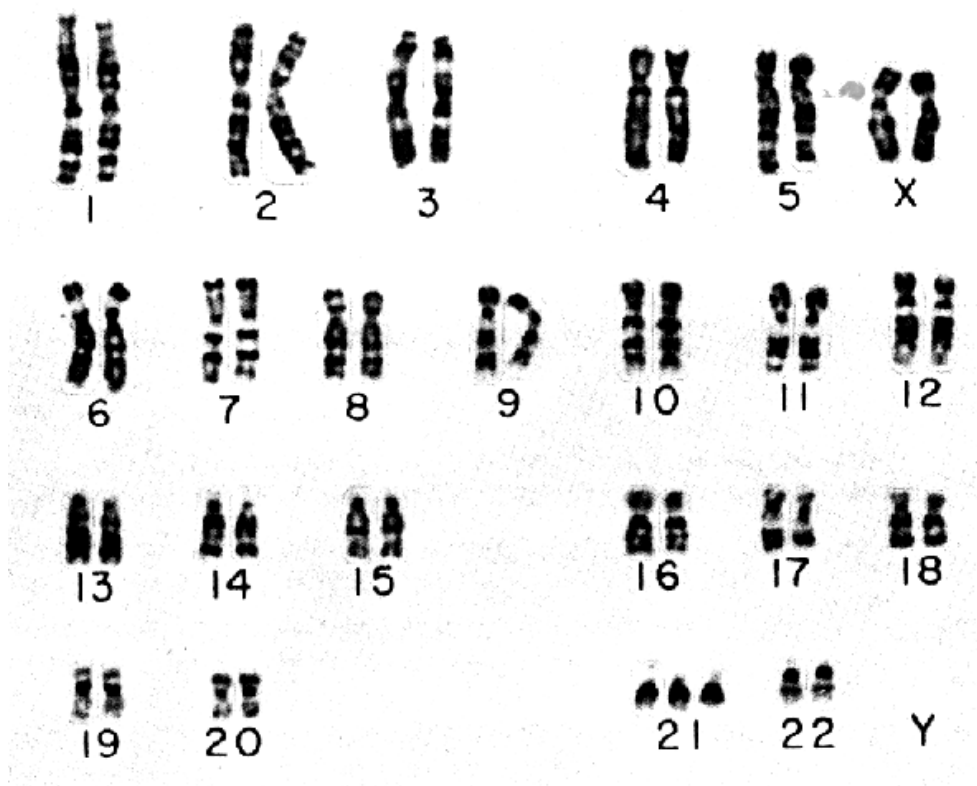
What is the most likely sex of this person?

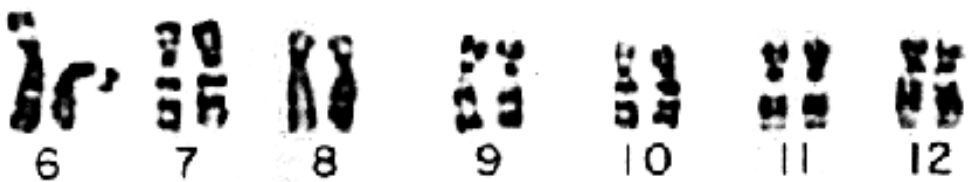
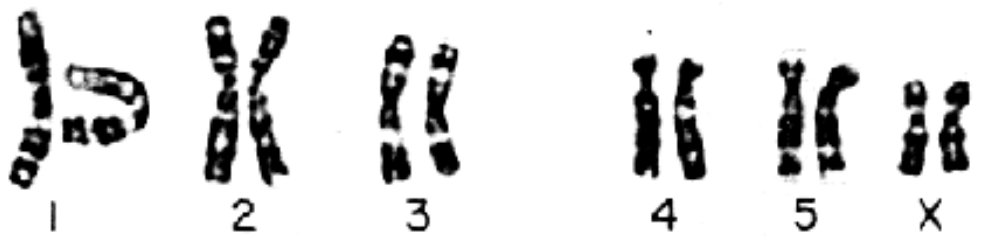
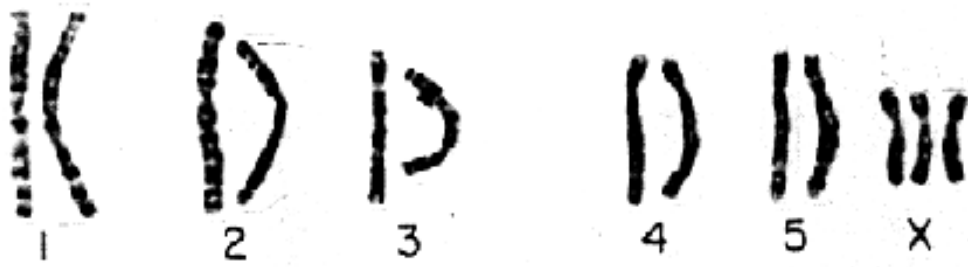
How can you tell?

Practice Karyotype #1

What is the most likely sex of this person?

Is there anything unusual about this person's karyotype?





Summary Questions

1. Why is mitosis essential for eukaryotes?
2. How do the daughter cells formed by mitosis compare genetically to each other?
3. How would you expect the mitotic index of a cancer tissue to compare with that of the onion root tip?
4. What structure(s) is(are) responsible for movement of chromosomes during the mitotic process?
5. Where in your body is mitosis occurring at the fastest rate?
6. What would occur if a cell completed karyokinesis but not cytokinesis?
7. Why do you think so few anaphase and telophase cells were found in your study of mitosis?
8. In the treatment of cancer, a drug called colchicine is sometimes used. Colchicine stops cell division at metaphase. What effect might this treatment have on cancer patients?
9. Why is meiosis essential for sexually-reproducing organisms?
10. Define and draw a tetrad of chromatids.
11. How would a tubal ligation or vasectomy influence meiosis in individuals who elected to have one of these operations?

12. Identify what is accomplished in Meiosis I? Meiosis II?
13. Synapsis of homologous chromosomes is perhaps the most important event in meiosis. What is it and what does it do?
14. When does crossing-over occur and how does it influence the gametes formed?
15. What ratio is expected in the F₂ generation from a monohybrid cross if the gene being examined shows complete dominance?
16. Why was it necessary to perform a X² test on the data we collected?
17. Ideally, the F₂ generation in a dihybrid cross will show a phenotypic ratio of _____?
18. What were the two laws Mendel proposed to account for the phenotypes he observed in his work with garden peas?
19. How would the F₂ results of a dihybrid cross change if the two genes were on the same chromosome?
20. Identify three ways in which it is possible for a child to have traits not found in the biological parents.
21. Make sure you can draw out the steps in Mitosis and Meiosis before next week's quiz. You may want to reproduce them here: