

Eukaryotic Cells and the Cell Cycle

Mitosis, Meiosis, & Fertilization

Learning Goals:

After completing this laboratory exercise you will be able to:

1. Identify the stages of the cell cycle.
2. Follow the duplication and separation of chromosomes in cell division.
3. Compare and contrast mitosis and meiosis.

Note: This lab will be split between two weeks, mitosis this week and meiosis next week.

Introduction:

In this laboratory session you will study two types of cell division: mitosis and meiosis. Cellular division in which somatic cells (body cells) divide either for growth or for repair of damaged or destroyed cells is called mitosis. Each cell that is undergoing mitosis normally produces two identical daughter cells with the same number of chromosomes as the original cell. In a sexually reproducing organism, another type of cellular division, meiosis takes place at some specific stage in its life cycle. For instance, in many animals and in some plants, meiosis normally occurs during gamete formation (eggs and sperm). In many plants meiosis occurs prior to spore formation. In either case, the process is the same: the daughter cells (usually 4) contain one-half of the original number of chromosomes. For example, each human cell (in the gonads) undergoing meiosis has 46 chromosomes. Each sperm or egg will have only 23 chromosomes. When fertilization occurs, (one sperm and one egg), the original number (46) is restored.

Part I: Mitosis

Chromosomes are composed of chromatin (DNA coated with or complexed with protein). Although the structure of DNA is well understood, the structure of chromosomes is considerably more complex, and is not yet completely understood. Regardless of the intimate details of chromosome structure, it is clear that DNA replication is a vital part of chromosome replication. Chromosome replication is, of course, at the heart of mitosis (meiosis too).

Animals and plants grow by cell multiplication and cell enlargement. Cell multiplication is achieved by the division of pre-existing cells. Today we will study the process of cell division. The type of cell division that is common to both plants and

animals and ensures that the regular diploid (2n) complement of chromosomes is kept constant through successive nuclear divisions is called mitosis.

The process of mitosis is essentially uniform through all the divisions that occur in the somatic (body) cells. Each chromosome duplicates itself and the duplicates are separated from each other at cell division, one going into the nucleus of one daughter cell and its "twin" into the other. The daughter cells are therefore identical with each other and with the parent in chromosome constitution. Similarly these daughter cells may divide and give rise in turn to two cells each. Thus cell multiplication is a geometric progression:

1 → 2 → 4 → 8 → 16 and so on.

In describing the process of nuclear division during mitosis it is customary to arbitrarily break up the process into a series of stages or "phases" for easier description. However, it must be borne in mind that nuclear division is a continuous process, and that any one "phase" passes without interruption into the next phase.

A. Interphase

When a cell is not actively dividing but is carrying out other normal metabolic processes it is said to be in interphase, i.e., it is the stage between 2 successive divisions. During this stage the nucleus is surrounded by a clearly visible nuclear membrane and contains one or two deeply staining nucleoli. Within the nucleus the chromosomes are present as a fine reticular network of chromatin material: individual chromosomes are not usually visible with the light microscope. Two centrioles are visible as darkly staining bodies in the cytoplasm just beside the nucleus. Before the cell begins to visibly undergo mitosis, there is a doubling of the DNA and hence the chromosome material in the nucleus.

B. Phases of mitosis

Study the diagrams in the textbook of the phases of mitosis. Today's lab exercise will help you become familiar with the process of mitosis by simulating it using legos. As you work, try to develop a clear understanding of exactly how chromosomes behave in mitosis.

Part II: Meiosis

In all sexually reproducing organisms there is a doubling of chromosome number at fertilization (one set of chromosomes is provided by the nucleus of an egg and the complementary set comes from a sperm). Since the number of chromosomes in a given species is constant, it follows that there must be in the life cycle a compensatory mechanism, before the next sex cells are produced, in which the chromosome number is reduced to half of the original diploid number. This reduction is accomplished in the

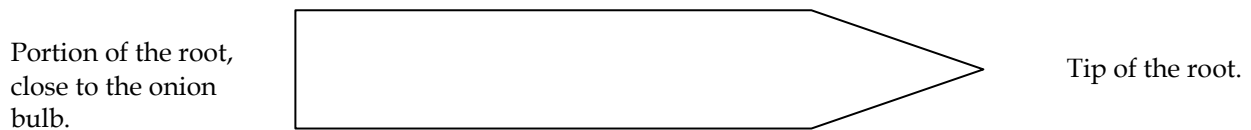
process of meiosis. Thus meiosis and fertilization are two major events which alternate in the life cycles of all sexually reproducing organisms. Meiosis is essentially similar in plants and animals. It consists of 2 consecutive cell divisions but with only one duplication of the chromosomes. Thus the 4 cells resulting have the haploid number (n) of chromosomes. Each of the two meiotic divisions can be separated into phases similar to those occurring in mitosis. However, in the first of these divisions the chromosomes behave differently from those in mitosis.

Study the diagrams in the textbook of the phases of meiosis. In today's lab exercise you will simulate meiotic chromosome movements using legos. Work to develop a clear mental picture of this process. The ability to visualize chromosomes and alleles segregating during meiosis will be very important to your future studies in genetics.

Activity: Phases of Mitosis

1. Today you will use a compound microscope to observe the stages of mitosis in animal (white-fish blastula) and plant (onion root tip) cells, using prepared slides. *For a review of how to use a microscope refer to the appendix of this manual.*

A) Formulate a hypothesis and indicate in the diagrams below where you think the greatest mitotic activity is found in the onion root.



Test your hypothesis by viewing a prepared slide beneath the microscope. Refer to the Mitosis stages described in the textbook above if you must.

B) Was your prediction correct? Describe below how you were able to distinguish the area of most activity:

2. Observe the Demonstrations set up under the microscopes on the counter tops.

Plants

A series of slides will be set up showing various stages of meiosis in the anther of a lily flower. See if you can identify the stages available.

Animals

A series of slides will be set up showing various stages of meiosis in animals using rat testis. See if you can identify the stages available.

Giant Chromosomes of *Drosophila*, (fruit fly). In several tissues of *Drosophila* and other dipteran insects (flies and such) unique giant chromosomes can be seen. Following synapsis the chromosomes replicate about ten times with no division of the nucleus or cell. The result is a chromosome consisting of about one thousand strands which become very rigid and tightly aligned with each other. These giant chromosomes are seen to have alternating light and dark bands of varying widths. The bands have been mapped and it has been demonstrated that the positions of specific bands can be related to chromosome abnormalities and are often reflected in visually apparent rearrangements or deletions of parts of the banding patterns in the giant chromosomes.

Human Chromosomes Separate microscopes will be set up with slides showing human male and female chromosomes. Count the number of chromosomes in one or two cells. Why are they doubled? What do we mean by karyotype? Would it be possible to distinguish between male and female sets of chromosomes? Observe the demonstration of human karyotypes. Count the number of chromosomes in the photomicrograph, also on demonstration.

Mitosis, Meiosis, & Fertilization

Objectives:

1. Follow genes, alleles, and chromosomes through mitosis, meiosis, and fertilization.
2. Get a clearer picture of gene/allele and how they connect to chromosomes.
3. See the connection between the symbols and the mechanism behind them.
4. A 'behind the scenes' view of a Punnett square.

Description:

You will use Lego pieces to model the behavior of genes, alleles, chromosomes, and the mitotic spindle. You will construct demonstrations of these three processes; these demonstrations will be checked off by other students in the lab. For each stage of mitosis or meiosis, once you are satisfied that it is correct, sketch it in your notebook for future reference.

The Genetic System:

You will be modeling the genetics of Furbies - hypothetical diploid animals with only 2 chromosomes. We will consider three genes on these two chromosomes. The chromosomes from the nucleus of a diploid Furby cell are shown below:

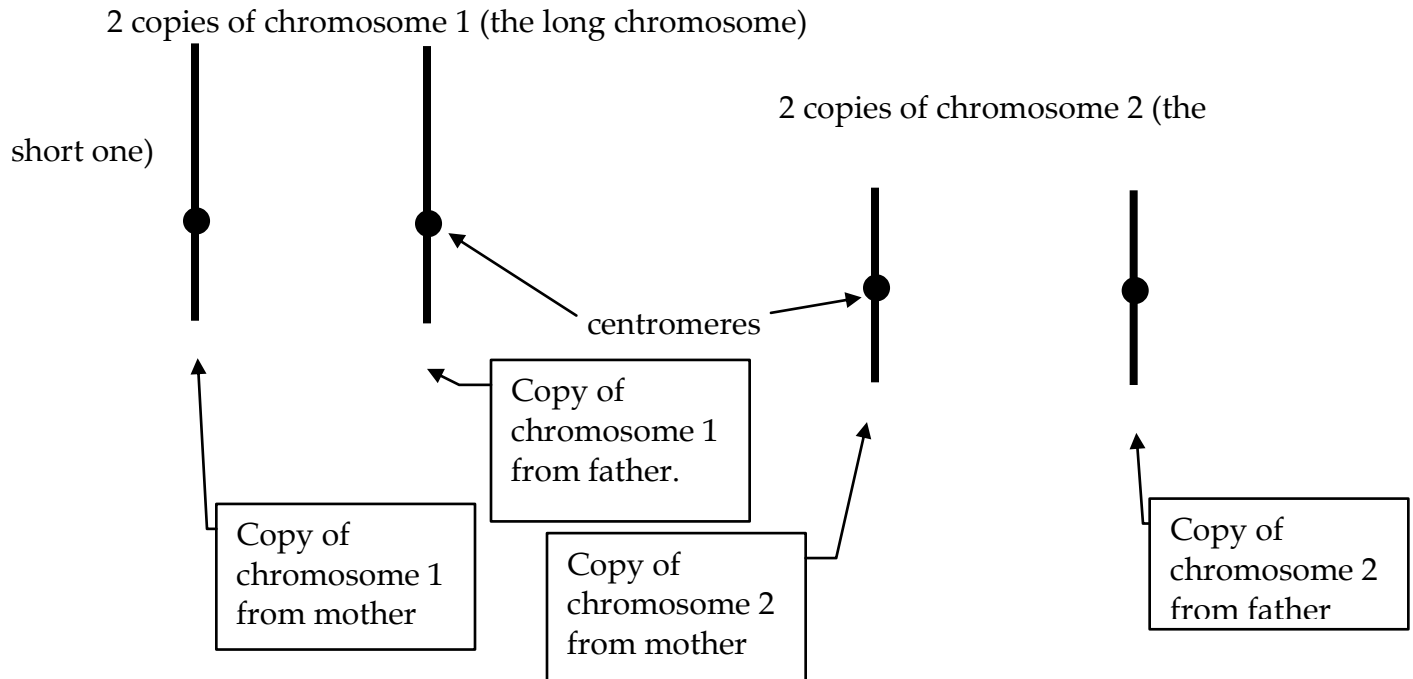


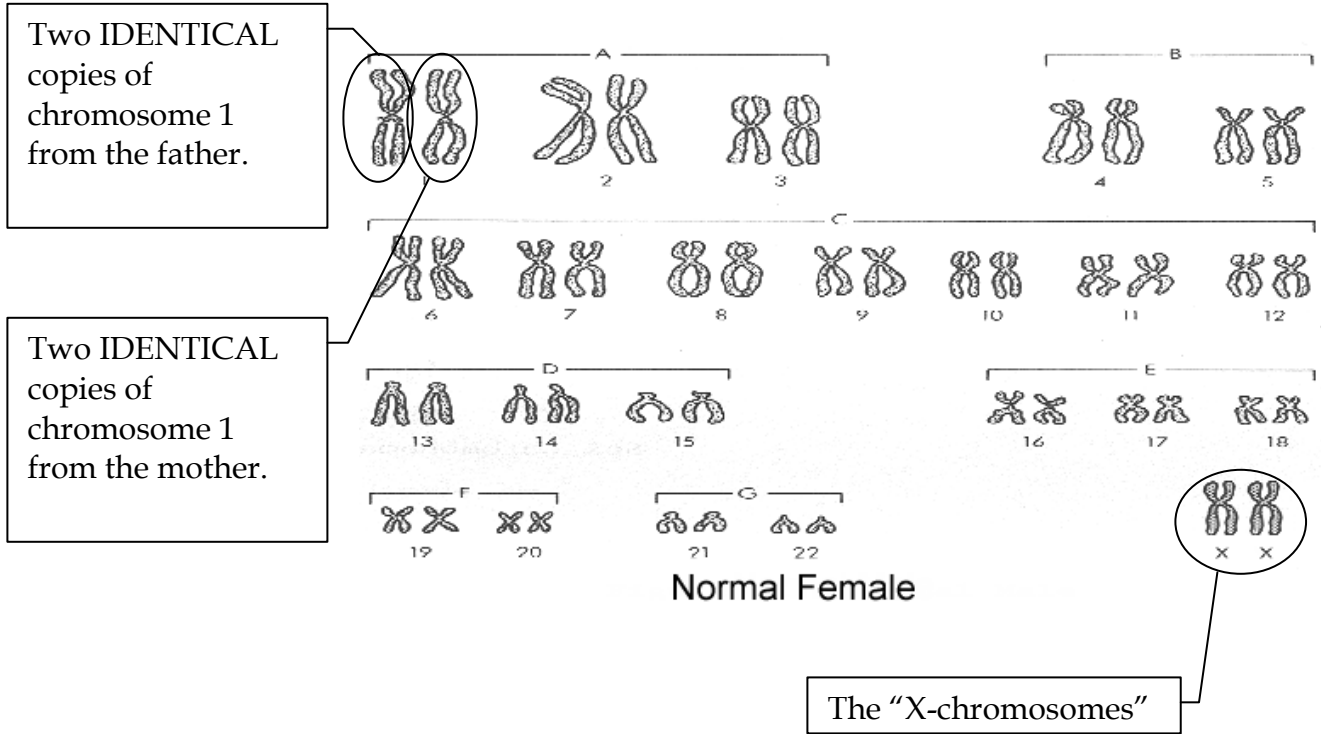
Figure 1

When we say “Furbies have two chromosomes”, we mean “two different types of chromosomes” – in this case, the two different chromosomes are the long chromosome (#1) and the short chromosome (#2). Thus $N = 2$. Therefore, a diploid cell, which is $2N$, will have two copies of each different chromosome for a total of 4 chromosomes (if $N = 2$, then $2N = 4$). A haploid cell (N) would have one copy of each type of chromosome (one long & one short) for a total of 2 chromosomes.

The members of each pair of chromosomes are said to be homologous - that is, on both maternal and paternal copies of chromosome 1, all the genes are in the same locations, although the alleles may differ.



Using the same terminology, we'd say “humans have 23 different types of chromosomes” or $N=23$. Thus, a diploid cell from a human (most of the cells in your body) will have 2 copies of each chromosome ($2N = 46$). The picture on the following page shows the chromosomes from a human cell after the chromosomes have replicated (prophase of mitosis), thus there are 4 copies of each chromosome ($4N=92$). Each “X” is

two identical copies of one chromosome. The "X"s are arranged in homologous pairs - the "X" from the father and the "X" from the mother. **Note** although all of the chromosomes here look like "X"s, they are not all "X-chromosomes" (the chromosomes involved in sex-linked traits).





The three genes we will study in the Furbies are as follows:



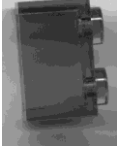
(1) The gene for Height

<u>allele</u>	<u>contribution to phenotype</u>	<u>Lego Piece</u>
H	tall (dominant)	<u>square</u>  yellow "one"
h	short (recessive)	<u>round</u>  yellow "one"

(2) The gene for Eye color

<u>allele</u>	<u>contribution to phenotype</u>	<u>Lego Piece</u>
B	black (dominant)	<u>black</u>  flat "two"
b	blue (recessive)	<u>blue</u>  flat "two"

(3) The gene for Blood-type

<u>allele</u>	<u>contribution to phenotype</u>	<u>Lego Piece</u>
Q ^x	type X blood (co-dominant)	<u>solid</u>  red "two"
Q ^y	type Y blood (co-dominant)	<u>clear</u>  red "two"
Q ^z	type Z blood (co-dominant)	<u>clear</u>  pink "two"

Note that the Lego pieces that represent different alleles of the same gene are *very similar* – for example, all the alleles of the blood-type gene are the same shape. This is deliberate – the different alleles of a particular gene are very similar; much more similar to each other than they are to alleles of a different gene.

The genes are located on the chromosomes as follows:

Note: that, no matter which alleles are present, each gene is *always* in the same place on its particular chromosome.

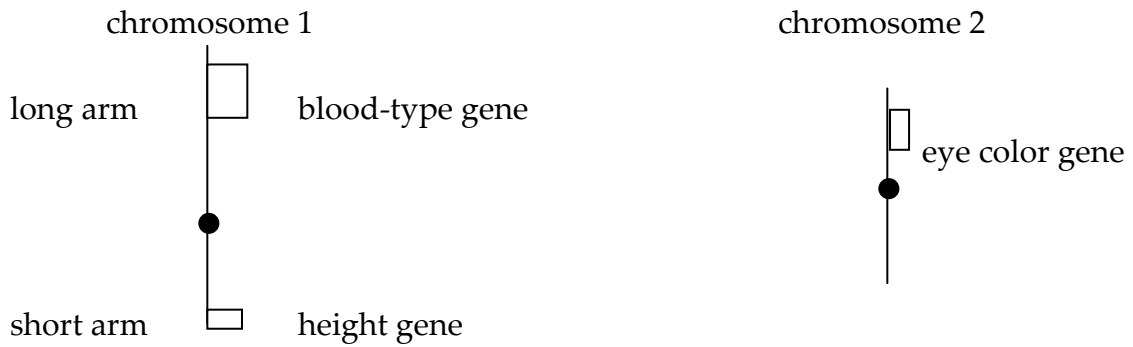
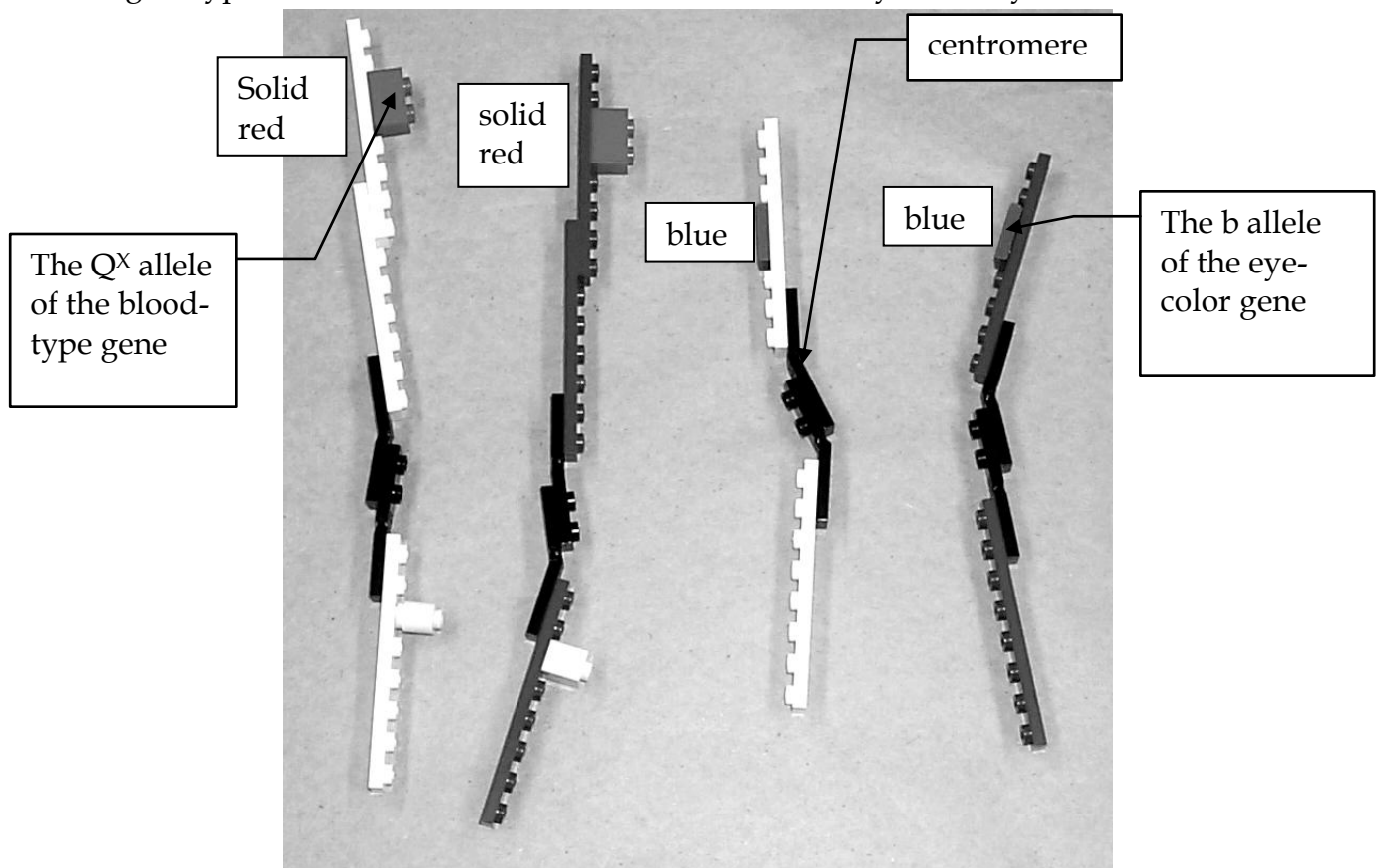


Figure 3

The picture below shows the Lego model of the chromosomes of a diploid cell with the genotype: $Q^X Q^X Hh bb$. It would be from a tall, blue-eyed, Furby with blood



type X.

Figure 4

The maternal copy of each chromosome (the copy this Furby got in the egg from its mother) is shown in white. Therefore, the egg had the genotype: $Q^x h b$.

The paternal copy of each chromosome (the copy this Furby got in the sperm from its father) is shown in purple. Therefore, the sperm had the genotype: $Q^x H b$.

Notes:

1. the chromosomes are different because of their different lengths and the different genes on them; the different colors are just to track the maternal and paternal copies.
2. while the exact position of the alleles along the chromosomes is not critical, they should be in similar positions.
3. the complete kit of parts is shown in the following pages:

Procedure

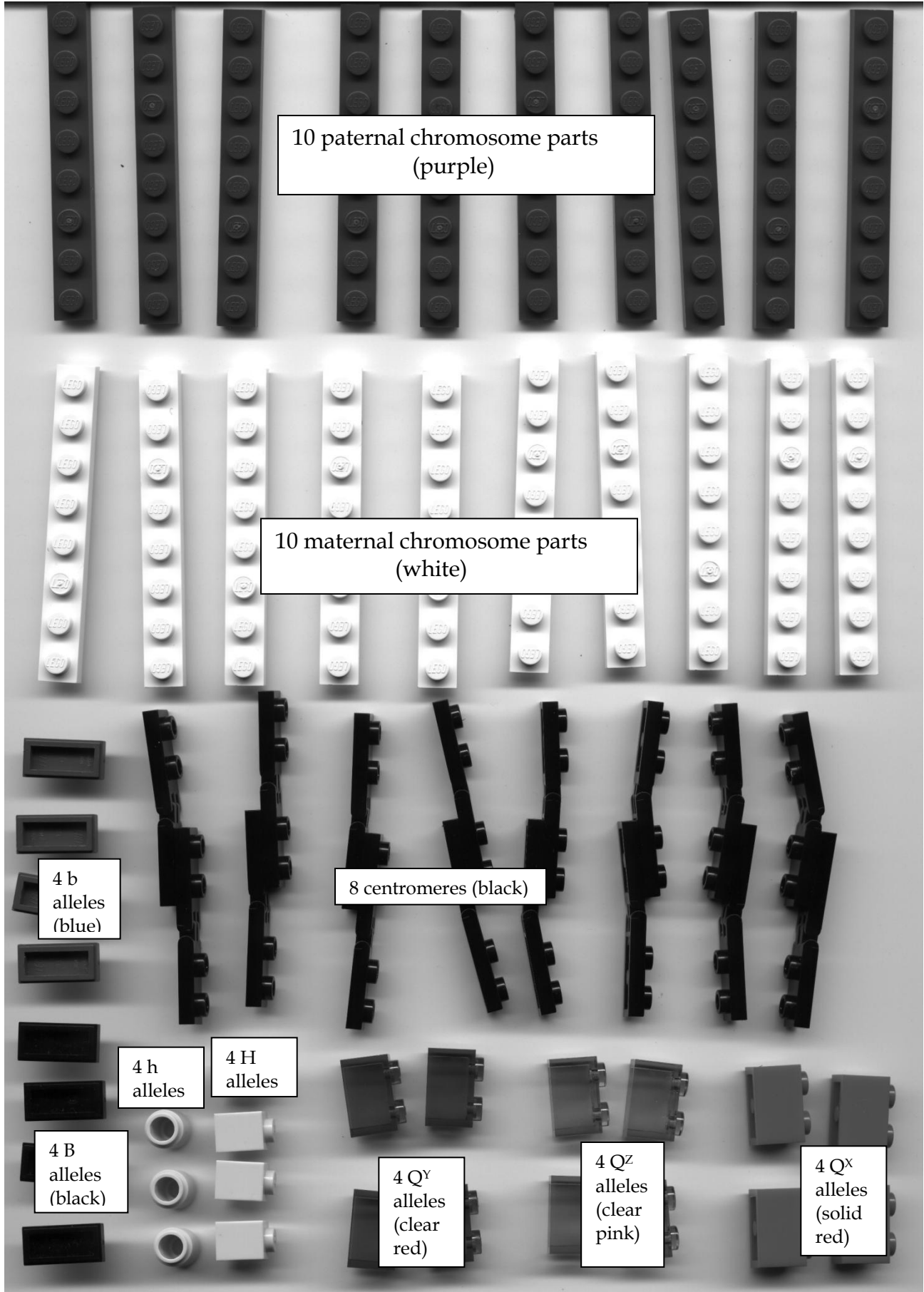
(1) Open up the container of Lego and place each piece on its part of the photos on the previous pages to be sure that you have all the pieces.

Part I: Mitosis

(2) Choose a genotype for your starting cell. Write this genotype here _____
_____.

(note that your genotype must include all three genes)

(3) Build a set of diploid chromosomes that models the genotype you picked in step (2). Use figure 4 as a model.



10 paternal chromosome parts
(purple)

10 maternal chromosome parts
(white)

4 b alleles
(blue)

8 centromeres (black)

4 h alleles

4 H alleles

4 B alleles
(black)

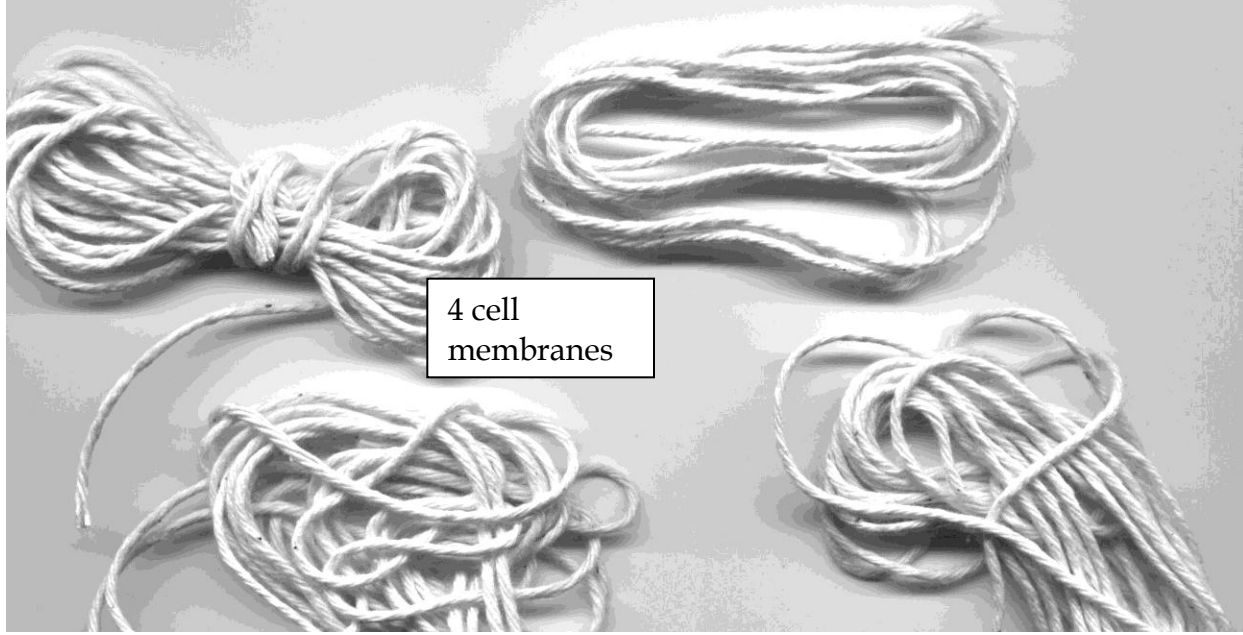
4 Q^Y alleles
(clear red)

4 Q^Z alleles
(clear pink)

4 Q^X alleles
(solid red)



8 kinetochore microtubules

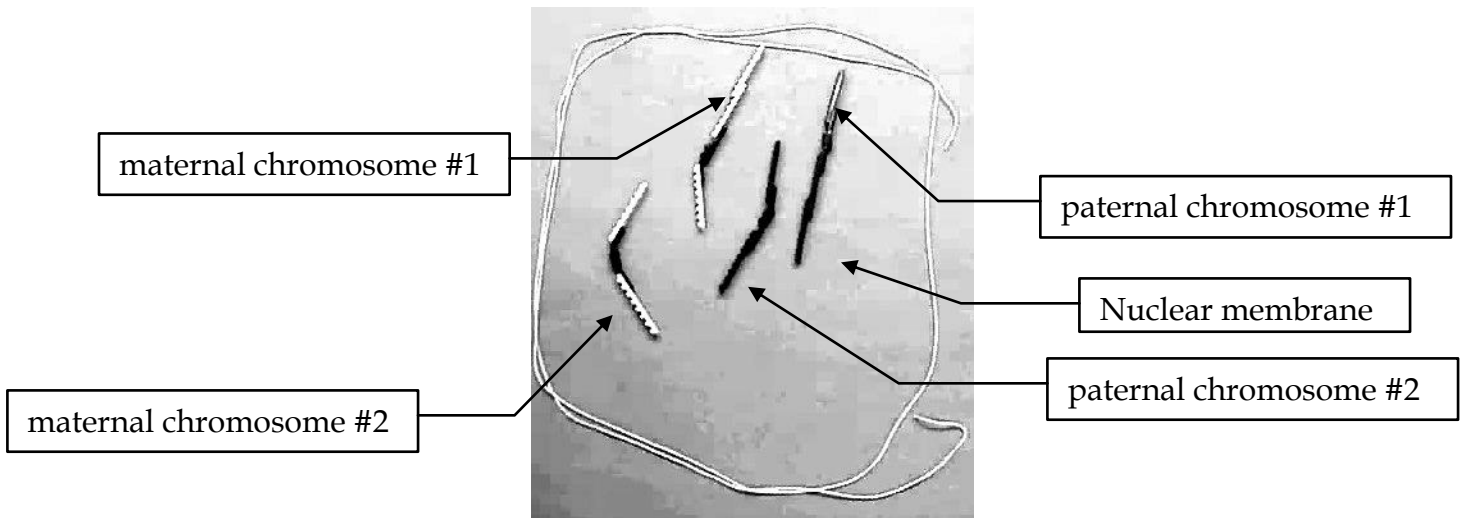


4 cell membranes

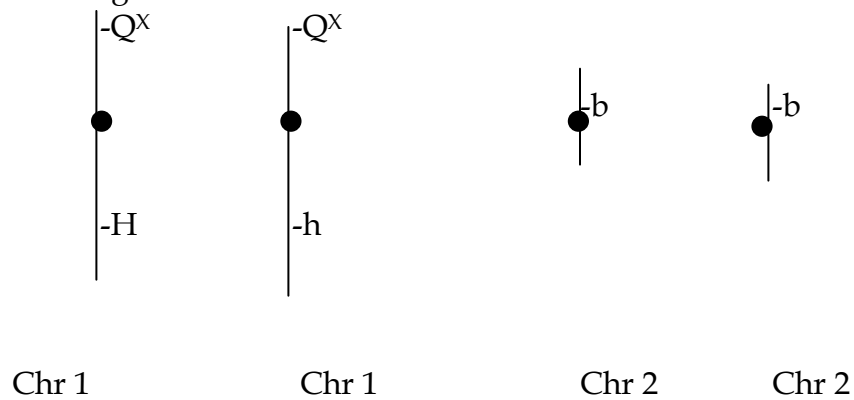
(4) Use the Lego to model the mitosis of a Furby cell with the genotype you picked in step (2). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

a) A resting cell (before the chromosomes have been replicated)

(Note the chromosomes are not visible in a real cell at this stage; we are showing them for the purposes of illustration only)

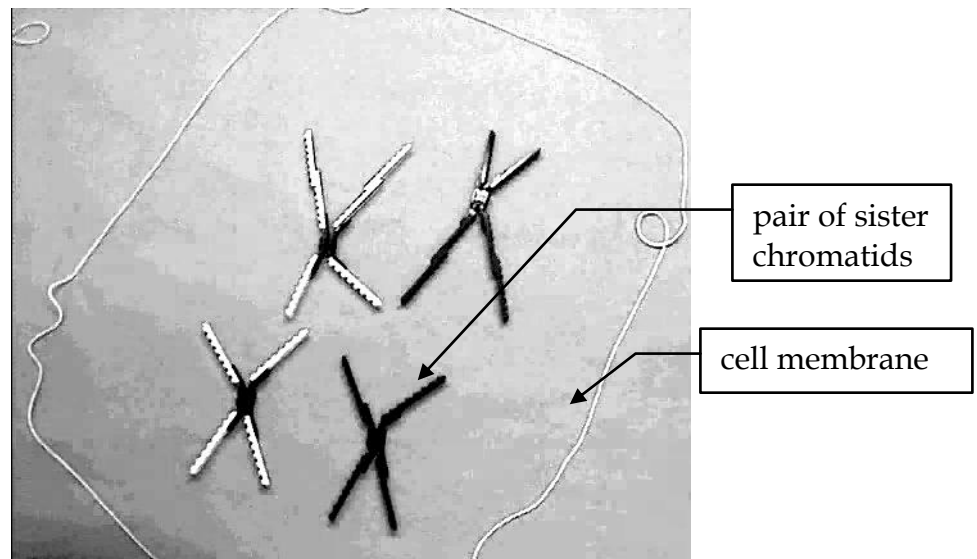


The drawing below shows a schematic of the chromosomes at this stage assuming a genotype of $Q^X Q^X H h b b$. Use this as a model for the drawings you will make of the later stages of mitosis and the stages of meiosis.



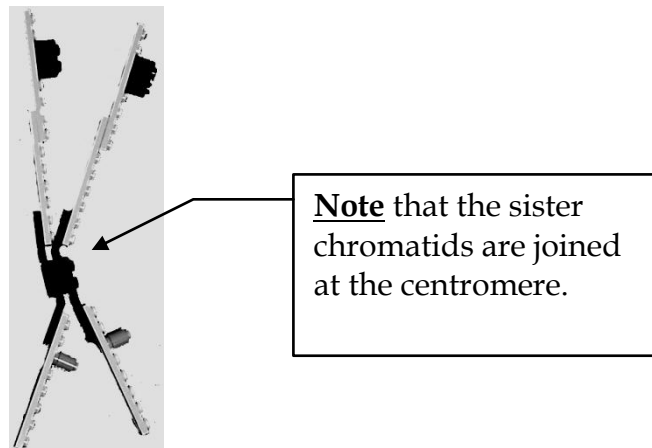
Draw a similar diagram for the genotype you chose in part (2) as they would appear in a resting cell:

b) Prophase (chromosomes have duplicated; the "X"s here correspond to the



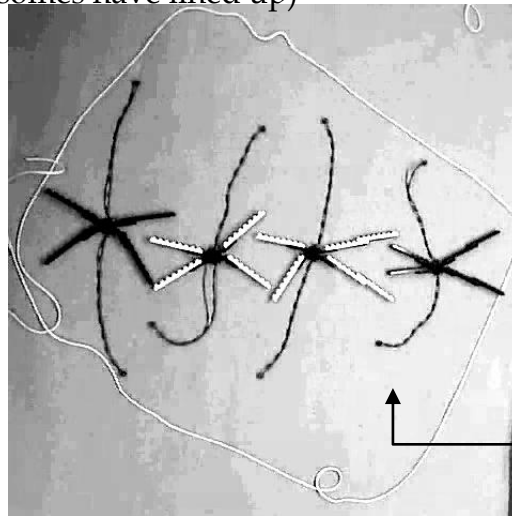
"X"s in the figure that shows human chromosomes at the start of this section of the lab manual)

Note that the sister chromatids are exact duplicates. Therefore the alleles should be identical for all genes in a given pair. This is shown below:



Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at prophase:

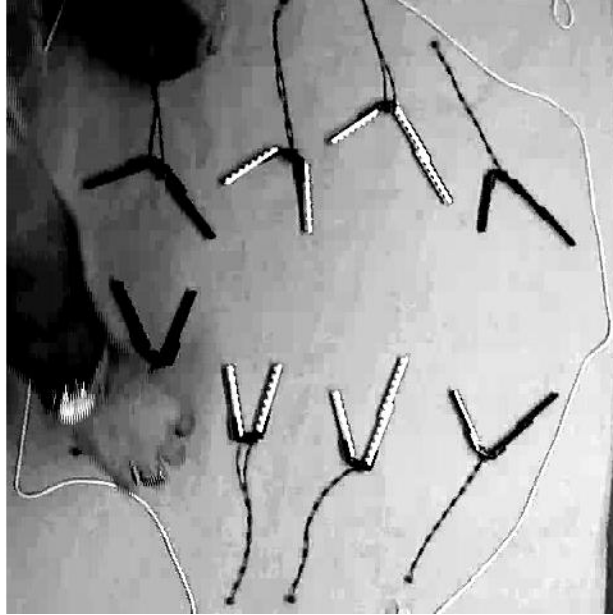
c) Metaphase (chromosomes have lined up)



kinetochore
microtubule

Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at metaphase:

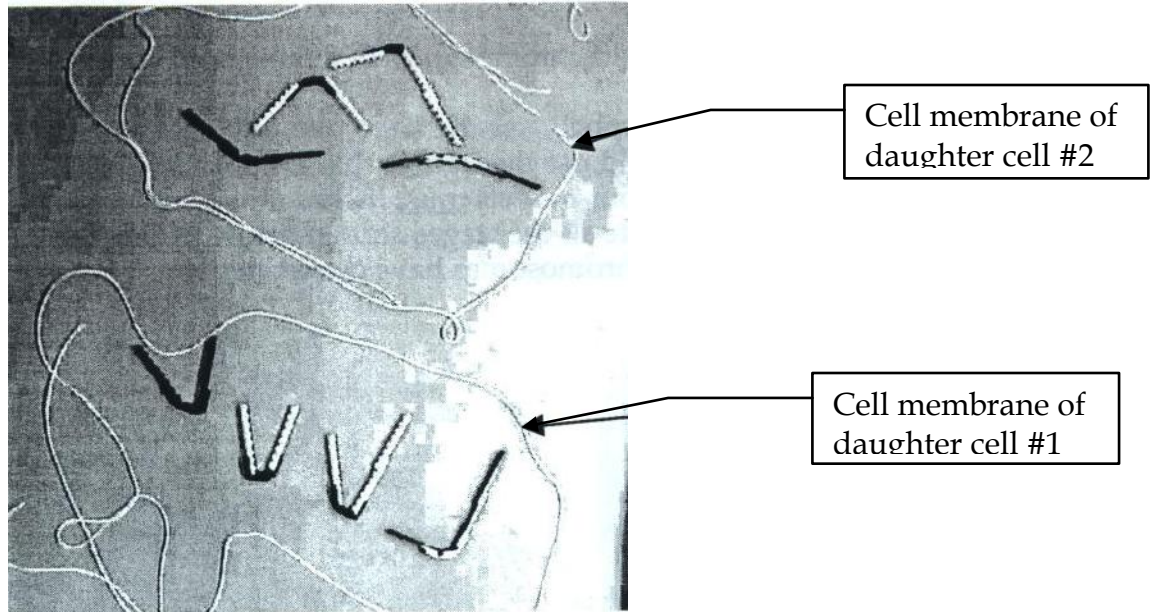
d) Anaphase (sister chromatids pulled apart by kinetochore microtubules)



Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at anaphase:

e) Telophase & Cytokinesis (cell divides)

Note that both cells now have the same genotype as the starting cell.



Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at the end of cytokinesis:

Week 1 Check-Off. You should try to complete the mitosis exercise for today's check-off credit. If time is short your TA may have you do a simplified version that tracks only chromosome, but not loci or alleles.

Part II: Meiosis

(5) Construct a model of the diploid chromosomes of a Furby cell with the genotype $Q^XQ^X Hh Bb$.

(6) Use the Lego to model the meiosis of a Furby cell with the genotype from step (5). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

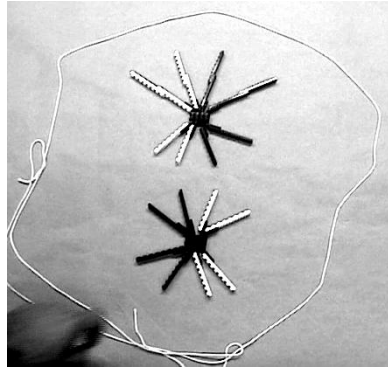
a) resting cell (before the chromosomes have duplicated)



Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a resting cell:

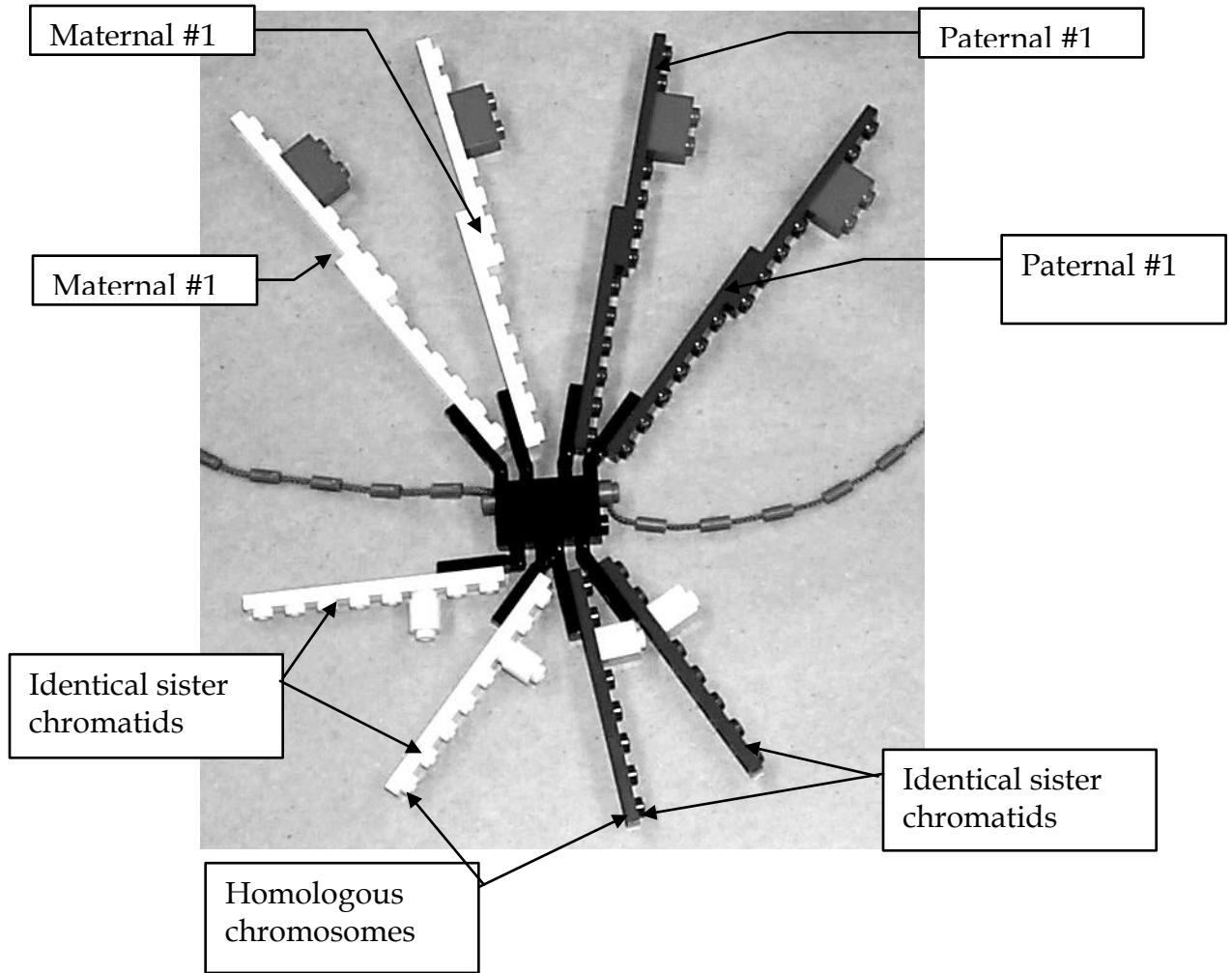
b) Prophase I (chromosomes have duplicated and homologs have paired to form tetrads)

(note that we will not worry about chiasmata)

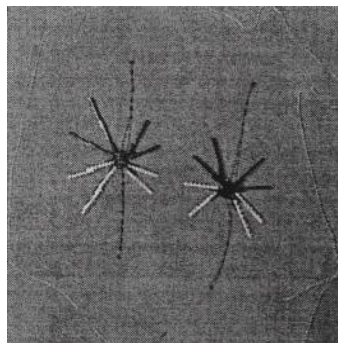


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at prophase I of meiosis (see photo on next page for hints):

close-up of a homologs pairing in a tetrad:



c) Metaphase I (tetrads have lined up) **Note** that there are two possible configurations here. You should work all the way through meiosis with one configuration and then go back and do the other.

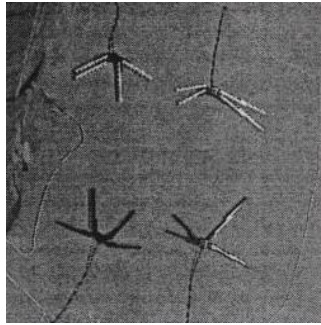


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at Metaphase I of meiosis:

configuration 1:

configuration 2:

d) Anaphase I (tetrads split between homologs - sisters remain attached)

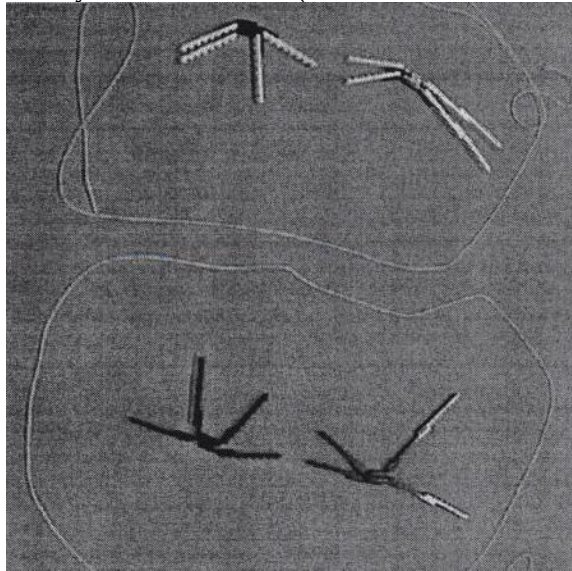


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at Anaphase I of meiosis:

configuration 1:

configuration 2:

e) Telophase I & Cytokinesis (cells have divided for the first time)

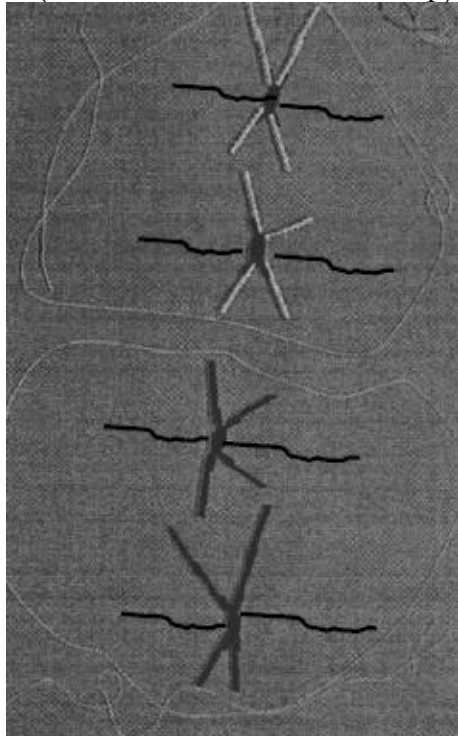


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at the end of Cytokinesis I of meiosis:

configuration 1:

configuration 2:

f) Metaphase II (chromosomes have lined up)

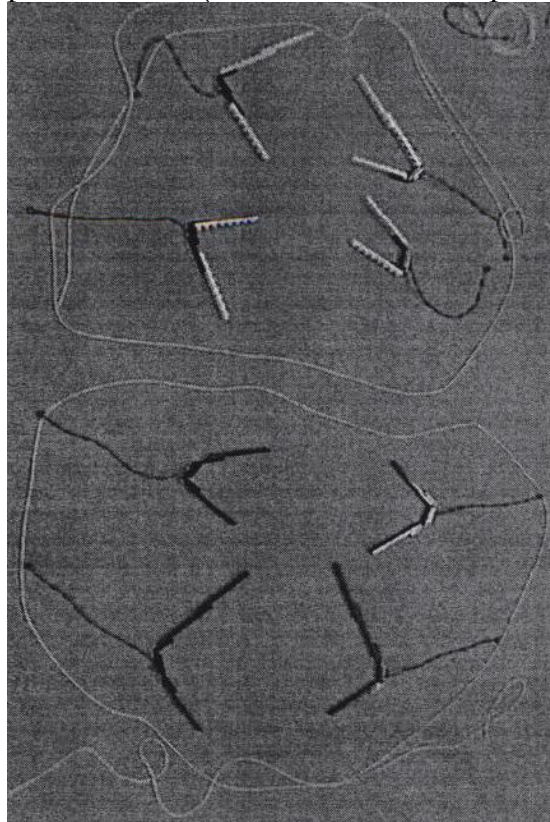


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at Metaphase II of meiosis:

configuration 1:

configuration 2:

g) Anaphase II (sister chromatids separate)

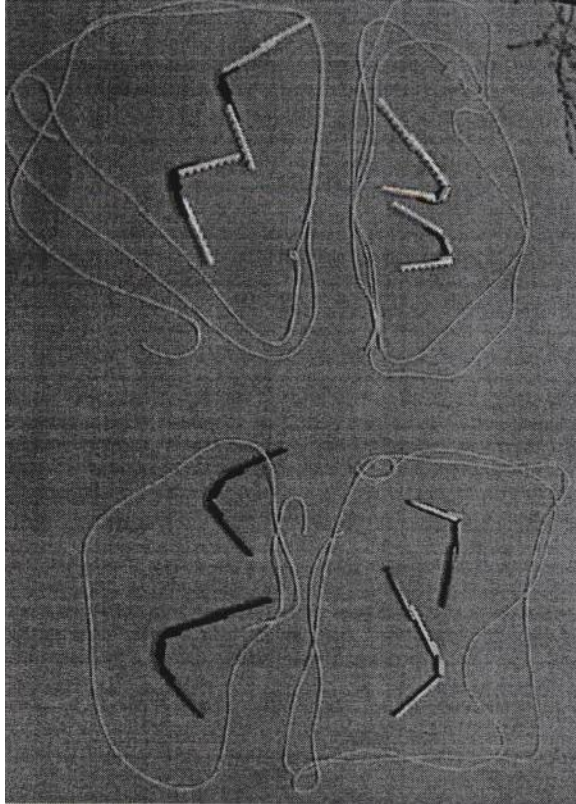


Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at Anaphase II of meiosis:

configuration 1:

configuration 2:

h) Telophase II (cells divide to give 4 gametes)



Draw a schematic diagram of the chromosomes for the genotype $Q^XQ^X Hh Bb$ as they would appear in a cell at the end of Cytokinesis II of meiosis:

configuration 1:

configuration 2:

Note that there are four possible gametes that a $Q^X Q^X Hh Bb$ cell can produce:

$Q^X H B$ $Q^X H b$ $Q^X h B$ $Q^X h b$

you must show how all four can be made in order to be checked off.

Fertilization

(6) Take one gamete that you made in step (5) and combine it with a gamete produced by another group. What is the genotype of the resulting offspring?

Finishing up

(7) Have your TA note that all the parts have been signed off by members of another group.

(8) Place all your Lego pieces on the photos at the beginning of this section and have your TA check off that you are returning all of them.

Lego Exercise II:

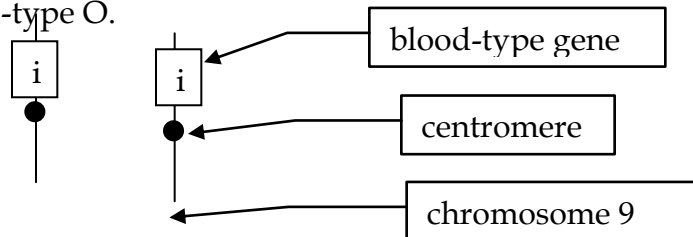
Note

Your answers will be based on the following scenario:

A **human** father, Fred, has blood-type AB. His wife, Wilma, has blood-type B. Their son, Alfred, has blood-type A. The gene for blood-type in humans is located on chromosome 9.

and should consist of the following :

(1) A diagram of Fred's copies of chromosome 9 as they would appear (if they were visible) in a resting cell. Here is a sample diagram of the copies of chromosome 9 carried by an individual with blood-type O.



(2) A diagram of Wilma's copies of chromosome 9 as they would appear (if they were visible) in a resting cell. Use symbols similar to those you used in question (1).

(3) A diagram of Fred's copies of chromosome 9 as they would appear as they went through the stages of meiosis you modeled in step (6) of the lab. You must have a drawing for each of the stages shown in the lab manual (6b through 6h). Note that there is only one possible configuration here.

(4) A diagram showing the copies of chromosome 9 contained in the egg and sperm that gave rise to Alfred. Draw egg and sperm as separate cells and label as 'egg' or 'sperm' as appropriate.

(5) A diagram of Alfred's copies of chromosome 9 as they would appear as they went through the stages of mitosis you modeled in step (4) of the lab. You must have a drawing for each of the stages shown in the lab manual (4a through 4e).

Pitfalls to avoid

- Use the right symbols. Blood types are A,B, AB, and O. The alleles are I^A , I^B , and i . People have blood types; alleles go on chromosomes.
- Humans are diploid so a human genotype **MUST** contain two alleles. The only exceptions are eggs and sperm which are haploid (only one allele).
- Each chromosome carries only **ONE** allele for a given gene.
- Label your diagrams **THOROUGHLY** - be sure you've marked all that the question calls for.
- Chromosomes duplicate only **ONCE** before the cell divides. Thus, in a resting cell there are 2 copies of each chromosome (homologs) and at metaphase there are 4 copies (2 copies of each homolog).