Title: Karyotype Analysis

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Appropriate Level: Life Science, High School, Honors, or Advanced Placement Biology

Abstract: In this laboratory exercise, students stain a fixed preparation of human cancer cells that were cultured in the laboratory. The stained cells are microscopically observed; students will observe cells containing the classical “X” shaped chromatids at metaphase. Students gain microscope practice and collect interesting data about chromosome number and chromosome type. A karyotype of several well-spread cells is prepared and compared to a karyotype of normal human cells. This activity can serve as a springboard to class discussions about genetic defects that can be diagnosed by the use of karyotyping.

Time Required: One class period each of two days: Day 1 - stain the cells and prepare a permanent slide. Day 2 - observe the karyotypes and sketch the results.

Special Notes: Fixed cells, stains, etc. are available as a kit through CELLSERV. A classroom set of microscopes with low and high power magnification is needed. Oil immersion lenses and photographic accessories for the microscopes are also desirable.
Additional Teacher Information

Objectives

Students will:

• prepare and observe chromosome spreads that look as clear and vivid as any textbook micrograph.

• have the opportunity to develop their microscope skills, collect data, and discuss conclusions.

• recognize chromosomal features that are used in their identification, i.e., size and centromere position.

Information with Which the Students Must Be Familiar

1. All cells have their genetic information arranged on chromosomes.

2. Eukaryotic chromosomes have centromeres, which are the “sites” where replicated chromosomes are joined until they separate during anaphase.

3. Each organism has a characteristic chromosome number, and each chromosome has a characteristic centromere position that determines the shape of the condensed chromatid pair.

4. A karyotype is a presentation of chromosomes that makes it possible to analyze the number, size, and shape.

5. Each species of organism has its own characteristic karyotype pattern.

6. Cells in tissue culture, like cancerous cells, have lost many aspects of cellular control that normal cells rigidly exhibit. For this reason, cultured HeLa cells display an abnormal karyotype.

Time Required

In class:  Day 1 - Stain the cells and prepare a permanent slide.  Day 2 - Observe the karyotypes and sketch the results.

Before class: Several minutes are needed to set out materials and microscopes.

Materials

per team of students:

• Pasteur pipette and bulb or a disposable pipette

• tape or marker to label slides

• microscope
for the whole class:

- cold methanol
- several chilled microscope slides, #1 glass coverslips, xylene to clean up excess Permount
- The karyotyping kit can be ordered from The Catholic University of America (1994-5). The kit contains tubes of cells ready to stain, containers of Stain #1, Stain #2, and Permount. Be sure to order the kit several months ahead of time. The kits are prepared just before you need them and must be used within a week or two. When they arrive, the cells must be stored in the refrigerator. The address is:

  CELLSERV
  FAES/NIH
  Building 60, Rm 237
  1 Cloister Court
  Bethesda, MD 20814-1460
  (301) 496-8290; fax (301) 402-6292

Comments

The written materials that come with the karyotyping kit are lengthy. Teachers may want to pay particular attention to Appendix 1 as it describes the preparation of the fixed cells.

Answers to Questions

1. Draw the chromosomes from one cell.

   Student drawings will be different as the tissue culture cells may have differing numbers of chromosomes.

2. Count the number of chromosomes in 10 different cells. What is the average number of chromosomes in these 10 cells?

   This number will be different as the cells may have differing numbers of chromosomes.

3. On your drawing of the HeLa chromosomes circle and label one chromosome that represents each of the types of chromosome classification.

   Students should have circled and labeled the following types of chromosomes: metacentric, submetacentric, and acrocentric.

4. Why is it necessary to place cells in a hypotonic solution when preparing them for karyotyping?

   The cells used have been allowed to undergo early mitosis. The chemical colchicine has arrested mitosis at the metaphase stage. When the hypotonic solution is introduced, it causes the cells to swell. As the cells “splat” onto the slide the hypotonic solution helps to push the chromosomes apart allowing visualization of the chromosomes after staining.
5. How does the chromosome number in cultured cancer cells differ from the chromosome number in
diploid human cells?

*The number of chromosomes in a human somatic cell is 46. The number of chromosomes found in
the HeLa cells may be more than 46 and even as high as 65-70. Cells in this state are called
aneuploid. Although aneuploidy is usually associated with meiosis and results in such
chromosomal disorders as Down’s and Turner’s Syndrome, it may also occur in mitosis. Non-
disjunction of one of the replicated chromosomes occurs, resulting in cells with one more or one
less chromosome. In HeLa cells this has occurred many times resulting in the high chromosome
number.*

6. During which stage of mitosis are the chromosomes in their most condensed state and best for
karyotyping?

*During metaphase of mitosis the chromosomes are most condensed. This is the stage at which
chromosomes are looked at in this lab.*

7. What are two sources of cells typically used for karyotype preparation?

*White Blood Cells are often used in karyotyping. WBCs don’t divide actively; therefore, they are
induced to divide. A karyotype can be made easily from cancer cells since they are actively
dividing. Among the cells grown in issue culture, connective tissue is the easiest to grow.
Karyotypes are often made from these cells. The techniques of chorionic villi testing and
amniocentesis also involve the growing of human cells and karyotypes may be made from these
cells.*

8. **Imagine:** you will soon be a new parent. The Doctor has just told you the results of an
amniocentesis test. Your child has a genetic abnormality that is caused by an abnormal number of
chromosomes. Obtain from your instructor the name of the disorder your child has.

*Students should be provided with a variety of textbooks to look up their child’s disorder. The
following chromosomal disorders may be copied and cut out for distribution to your students.*

1. **Down’s Syndrome** - an extra chromosome #21 (trisomy 21), characterized by mental
retardation, short stature, and often heart defects.

2. **Cri-du-Chat** - a deletion of part of chromosome #5, characterized by a catlike cry in the
babies, and severe mental retardation.

3. **Turner’s Syndrome** - only 45 chromosomes are present, with the normal number of autosomes
but only one X chromosome. Most Turner fetuses are spontaneously aborted. Those who
survive to be born alive are relatively normal although short, sexually undeveloped, and
infertile.

4. **Klinefelter Syndrome (XXY)** - characterized by tall stature, sterility, small testes, some female
breast development at puberty, and often some learning disabilities.

5. **Trisomy X (XXX)** - total of 47 chromosomes, limited fertility, some mental retardation.
From the karyotyping done after the amniocentesis it has been determined that your fetus has Down’s syndrome.

From the karyotyping done after the amniocentesis it has been determined that your fetus has Trisomy X.

From the karyotyping done after the amniocentesis it has been determined that your fetus has Klinefelter’s syndrome.

From the karyotyping done after the amniocentesis it has been determined that your fetus has Edward’s syndrome.

From the karyotyping done after the amniocentesis it has been determined that your fetus has Turner’s syndrome.

From the karyotyping done after the amniocentesis it has been determined that your fetus has Patau syndrome.

From the Karyotyping done after the amniocentesis it has been determined that your fetus has Cri-du-Chat syndrome.
Karyotyping Analysis Lab

Background Information

DNA carries the genetic information for each individual. In humans, as for all eukaryotes, most of the DNA is present in the nucleus. A small molecule of DNA is also present in each mitochondrion. The total genetic information for an individual is called the genome.

In the nucleus, DNA is combined with proteins in specific ways to make up chromosomes. Normal human somatic (body) cells usually have 46 chromosomes which come in pairs. One member of each pair was inherited from each parent. 22 of the pairs are called autosomes and the remaining 2 chromosomes are the sex chromosomes. Female mammals have 2 X chromosomes while males have one X and one Y chromosome. Most normal somatic cells contain identical numbers and types of chromosomes. Differences in the chromosomal number or structure observable under the light microscope usually result in some type of genetic defect. Therefore, an analysis of human chromosomes under the microscope allows researchers to identify those genetic disorders that are caused by obvious differences in chromosome number or structure.

The physical structure of chromosomes changes during the cell cycle. During metaphase of mitosis, the chromosomes are in their most condensed form and therefore are most easy to see and characterize. Using appropriate methods of staining, each human chromosome pair can be distinguished from the other 22 pairs. The three main criteria used in identifying individual chromosomes at metaphase are: 1. the length of the chromosome, 2. the position of the centromere, and 3. staining and banding pattern using particular stains. Scientists have established a classification system that allows identification of each chromosome. The sex chromosomes are named X and Y, while the autosomes are numbered in descending order, with chromosome 1 the largest, chromosome 2 the next largest, etc. (NOTE: it turns out that chromosome 21 is actually smaller than 22, but that wasn’t clear at the time the human chromosomes were named.) The entire set of chromosomes of an individual or a cell as seen under a microscope at metaphase is called a karyotype.

Some genetic diseases have been associated with a specific abnormal karyotype. The abnormalities include an increase or decrease in the total amount of chromosomal material or a rearrangement of chromosomes, for example, translocation of a piece of one chromosome to another. Some examples of unusual phenotypes and the chromosomal aberrations that cause them are:
1. Down’s Syndrome - an extra chromosome #21 (trisomy 21), characterized by mental retardation, short stature, and often heart defects.

2. Cri-du-Chat - a deletion of part of chromosome #5, characterized by a catlike cry in the babies, and severe mental retardation.

3. Turner’s Syndrome - only 45 chromosomes are present, with the normal number of autosomes but only one X chromosome. Most Turner fetuses are spontaneously aborted, while those who survive to be born alive are relatively normal although short, sexually undeveloped, and infertile.

4. Klinefelter Syndrome - XXY, characterized by tall stature, sterility, small testes, some female breast development at puberty, and often some learning disabilities.

5. Several kinds of cancer are associated with chromosomal abnormalities.

There are also many genetic diseases, like muscular dystrophy and cystic fibrosis, which result from a defect within a particular gene. These small differences however, cannot be seen in a karyotype. Advances in recombinant DNA technology and genetics have allowed researchers to identify some of these mutations.

In order to analyze an individual’s chromosomes (that is, to prepare a karyotype) the chromosomes must be in a state where they can be easily observed. Since chromosomes are only visible during mitosis, cells must be actively dividing. If the cell sample is taken from blood cells (which are not ordinarily dividing) the cells must first be treated with a chemical which makes them divide. This can take a week or more. The dividing cells are then treated with colchicine, a chemical isolated from autumn crocus. Colchicine interrupts the microtubule network responsible for chromosome movement during mitosis. This causes the cells to “freeze” in the metaphase state of mitotic cell division so that the chromosomes remain arranged along the equator of the cell. The cells are placed in a hypotonic (low salt) solution which causes water to rush into the cells. This swells the cells including the nuclei and pushes the chromosomes apart. The hypotonic solution is then replaced with a fixative that allows the cells to be maintained in the swollen, arrested metaphase state for a long period of time. At this point, the cells are ready to be “splatted” onto microscope slides, stained, and observed.

When preparing a karyotype the researcher looking through the microscope locates a chromosome spread which shows clear and distinct chromosomes; she then takes a photograph through the microscope objective. This is done several times for each cell preparation. Each photograph is enlarged and the individual chromosomes are cut out and arranged based on the physical criteria described earlier. Some practical applications of karyotype analysis include the detection of chromosome abnormalities in children or fetuses.

In this exercise, karyotyping is done on HeLa cells grown in tissue culture. The HeLa cell line originated in the early 1950s from the cancerous cervical cells of a woman named Henrietta Lacks. Because the cells are cancer cells, they often do not contain the normal
A diploid number of chromosomes (46) characteristic of humans. There may be 3, 4, or 5 copies of a particular chromosome present in some cells. When you make a chromosome count of your spreads, you may find 50 to 60 per cell.

**Procedure**

1. Slides should be kept in cold methanol (40%) in a freezer or ice bath until ready for use.

2. Remove a slide from the cold methanol and orient it vertically at a 45° angle. The slide should remain moist with methanol.

3. With a pipette, gently resuspend the swollen and fixed cells in the tube provided. Remove a small sample of the cell suspension with a pipette and hold the pipette 6-12 inches above the moist slide. (It may be necessary to hold the pipette 2-6 feet above the slide.) **Allow one drop** of the cell suspension to “splat” onto the slide about 3/4 inch from the upper end and tumble down the slide. Carefully apply 3-4 more drops from various heights onto the same region of the slide. Gently blow across the slide for 2-3 seconds. The drying will help “spread” the chromosomes.

4. Allow the cells to **AIR DRY COMPLETELY**.

5. Dip the slide into the tube containing stain #1 for **1 SECOND**. **Repeat the staining with stain #1 one more time**. Drain off the excess stain. **Caution** should be taken to avoid carryover of stains. Wipe the bottom of slide with a paper towel before transferring it to Stain # 2. Dip the slide into a tube containing stain #2 for **1 SECOND**. **Repeat the staining with stain #2 one more time**.

6. Remove the slide from the stain and rinse it with distilled water.

7. Allow the slide to **AIR DRY COMPLETELY**.

8. If you wish to examine your slide now proceed to step #9. A permanent slide may be made, but it will take 72 hours to dry. If you wish to make a permanent slide, proceed. Place two drops of Permount on the stained area of your slide and place a #1 coverslip over the Permount. Apply gentle pressure to the coverslip to spread the Permount evenly under the coverslip. You may wish to place 2 coverslips side by side to allow viewing of the entire microscope slide. Once the Permount has dried the slide is ready for viewing.

**CAUTION:** All attempts should be made to keep Permount off of the objectives of the microscope. If Permount does get onto an objective it can be removed by wiping it with lens paper and xylene.
9. Low, high dry, and oil observations can be made immediately from any slide that is not made permanent. Observations of permanent slides should await drying of the Permount (72 hours). Under low power scan your spread for cells which appear to have ruptured and released their chromosomes. Shift to high power (400X) to examine your spread more carefully. An ideal spread will contain chromosomes that appear distinct (see Figure below). This exercise requires careful observation so take your time when viewing. Once you have found what appears to be a clear and distinct set of chromosomes, place a small drop of **immersion oil** (if available) on the coverslip over that area and switch to 1000X. **Note:** When using the oil immersion lens you will have to increase the amount of light passing through the specimen. This can be accomplished by increasing the light intensity (if available), and increasing the aperture of the iris diaphragm. **Caution:** Use immersion oil only on those areas of the slide containing a coverslip.

10. Count the number of chromosomes present in 10 different cells on the slide. Remember that this cell line is aneuploid and each cell will probably contain a different number of chromosomes, each greater than the diploid number (46). In addition, try to identify and locate the three characteristic chromosomes based on the location of the centromere.

![HeLa Chromosomes](image)

**HeLa Chromosomes**  
**Centromere Position**

HeLa cells, a human tumor cell line, show the typical aneuploid condition common to transformed cells. Normal human diploid cells contain 46 chromosomes but it is evident that this cell line contains more than this number. Note the typical chromosome structure with the centromere evident in each chromosome. The sister chromatids should also be evident. The position of the centromere is used to classify chromosomes as either: **metacentric** (1), **submetacentric** (2), **acrocentric** (3). A chromosome of a fourth category, **telocentric** (4) chromosome, has the centromere terminally situated. However, there are no human telocentric chromosomes. Close examination will show the presence of all three types in this photograph.
Questions (Answer all questions in complete sentences.)

1. Draw the chromosomes from one cell.

2. Count the number of chromosomes in 10 different cells. What is the average number of chromosomes in these 10 cells?

3. On your drawing of the HeLa chromosomes, circle and label one chromosome that represents each of the types of chromosome classification.

4. Why is it necessary to place cells in a hypotonic solution when preparing them for karyotyping?

5. How does the chromosome number in cultured cancer cells differ from the chromosome number in diploid human cells?

6. During which stage of mitosis are the chromosomes in their most condensed state and best for karyotyping?

7. What are two sources of cells typically used for karyotype preparation?
8. Imagine: you soon will be a new parent. The Doctor has just told you the results of an amniocentesis test. Your child has a genetic abnormality that is caused by an abnormal number of chromosomes. Obtain from your instructor the name of the disorder your child has.

Your fetus is fifteen weeks old. Research this condition. What is the prognosis for your child?

What are three possible choices you and your spouse could make concerning your child?

In order to make the best decision possible it is important to understand the viewpoints of the people concerned with your child. Describe the viewpoints of each of the following people will have.

Your spouse:

Your parents:

Your clergy:

What choice would you and your spouse make?

List the sources you have used in your research.
Karyotyping Analysis Lab
(The Quick Method)

Procedure

1. Dry slides will be used today.

2. Orient the slide at a 45° angle. This may be done by resting the end of the slide on a Styrofoam tray.

3. You will be dropping cells onto the slide from various heights. Practice with water first. Make sure you replace the wet slide with one or more dry slides. With a pipette, gently resuspend the swollen and fixed cells in the tube provided. Remove a small sample of the cell suspension with a pipette. Holding the pipette 6-12 inches above the dry slide, allow one drop of the cell suspension to “splat” onto the slide about 3/4 inch from the upper end and to tumble down the slide. Carefully apply 3-4 more drops from various heights (up to six feet) onto the same region of the slide. Gently blow across the slide for 2-3 seconds. The drying will help “spread” the chromosomes.

4. Allow the cells to AIR DRY COMPLETELY.

5. Dip the slide into the tube containing stain #1 for 1 SECOND. Repeat the staining with stain #1 one more time. Drain off the excess stain. Caution should be taken to avoid carryover of stains. Wipe the bottom of slide with a paper towel before transferring it to Stain # 2. Dip the slide into a tube containing stain #2 for 1 SECOND. Repeat the staining with stain #2 one more time.

6. Remove the slide from the stain and rinse it with distilled water.

7. Allow the slide to AIR DRY COMPLETELY.

8. Slides may be examined now or made permanent. Permanent slides may be viewed after 72 hours of drying. To make permanent place two drops of Permount on the stained area of your slide and place a #1 coverslip over the Permount. Apply gentle pressure to the coverslip to spread the Permount evenly under the coverslip. Once the Permount has dried the slide is ready for viewing.