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STUDENT VERSION

EDVOTEK & PLTW Experiment #485

Clues in the Chromosomes

(PBS 2.2.4)

Experiment Objective:

The experimental objective is for students to develop an understanding of metaphase chromosome spreads. This important method is widely used for karyotyping to analyze numerical and structural changes in chromosomes.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: www.edvotek.com/Safety-Data-Sheets

Experiment Components

Component	Storage	Check √
• Karyotyping cells	-20°C Freezer	❑
• Giemsa stain	Room Temp.	❑

Supplies

• Slides	❑
• Transfer pipets	❑
• Snap top microcentrifuge tubes	❑

This experiment is designed for 10 groups.

Requirements *(NOT included with this experiment)*

- Microscopes (400X total magnification recommended)
- Water bath
- Distilled water
- Paper towels
- Gloves
- Slide coverslips (optional)
- Mounting medium (optional)

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Understanding Human Chromosomes

CHROMOSOMES AND KARYOTYPING TECHNIQUES

Chromosomes are thread-like structures composed of double-stranded DNA associated with specific proteins (Figure 1). Each chromosome contains the genetic information required to make each unique plant and animal cell. For example, the nuclei of normal human somatic cells each contain 23 pairs of chromosomes – one of each pair derived from either the mother or the father.

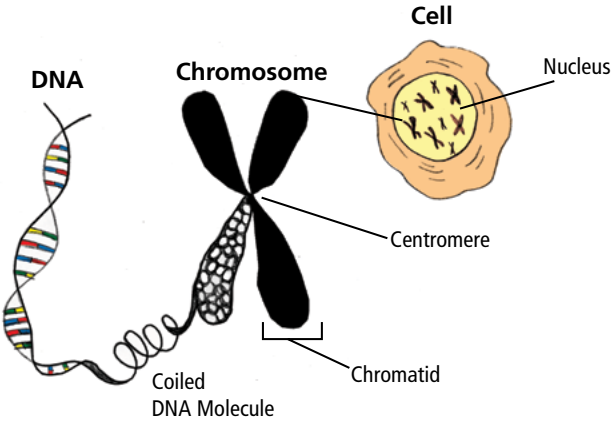


Figure 1

As a cell progresses through the cell cycle the chromosomes will take on different conformations. For example, during the interphase portion of the cell cycle, chromosomes are found in a relaxed position, loosely wrapped around histones and other proteins. This allows the chromosomes to maintain an organized structure within the cells. During metaphase, the chromosomes will fully condense in preparation of cell division. At this point the chromosomes are packaged tightly enough to be visible under a standard microscope during a process known as karyotyping.

The size and staining patterns of specific chromosomes can allow scientists to identify chromosomes in the nucleus of cells, and to identify potential abnormalities. The autosomes, or non-sex chromosomes, are numbered from 1 to 22 approximating the decreasing size order. The 23rd pair is the sex chromosome, X and Y; female humans contain two X chromosomes per nucleus and males contain one X and one Y chromosome. Variations in the normal karyotype, or compliment of chromosomes, can be used to diagnose prenatal abnormalities, certain types of cancer, and other diseases. Cells can be collected by amniocentesis or chorionic villus sampling (during prenatal testing) or biopsied. The samples are then treated with colcemid, which disrupts spindle fiber formation and arrests the cells in metaphase, and then karyotyped.

There are several modern techniques used for karyotype analysis, including spectral karyotype (SKY) analysis and comparative genomic hybridization (CGH). In the first technique, purified DNA samples are isolated from each of the normal chromosomes. Each of the 23 samples is labeled with a unique dye and then hybridized to the metaphase spread to be analyzed. For normal metaphase spreads each chromosome pair can then be observed as a different fluorescent color, and subjected to computer analysis. In this way, chromosome duplications, deletions and translocations can be recognized.

The second technique, CGH, utilizes similar technology in which the pooled DNA is derived from a combination of all the chromosomes to be analyzed. This DNA is then labeled, mixed with DNA from normal chromosomes, and then observed. The hybridization of the control and experimental DNA can then be analyzed by computer using fluorescent colors, and chromosome duplications and deletions determined along the length of each chromosome. While these techniques are mainly used currently in research they eventually will become an important aid to cytogeneticists.

CHROMOSOMAL ABNORMALITIES IN PREGNANCY

For prenatal diagnosis, a number of diseases have been shown to be associated with specific chromosomal abnormalities. About 0.5% of all live births are associated with a chromosomal abnormality. The most common of these is Down's syndrome (0.125% of live births) which arises as the result of three copies of a gene located on chromosome 21 (Figure 2). This syndrome can be diagnosed cytogenetically by observing if part or all of chromosome 21 (trisomy 21), containing this specific region, has been duplicated. Trisomy for all of chromosomes 13 (Patau syndrome) and 18 (Edward syndrome) in live births are less common. Trisomy 9 and 22 have also been reported in live births, but are rare. Trisomies for every other autosome have been documented in miscarried fetuses. Deletions of entire autosomes are usually not tolerated and result in spontaneously aborted pregnancies, although single copies (monosomy) for chromosomes 21 and 22 have been reported in live births.

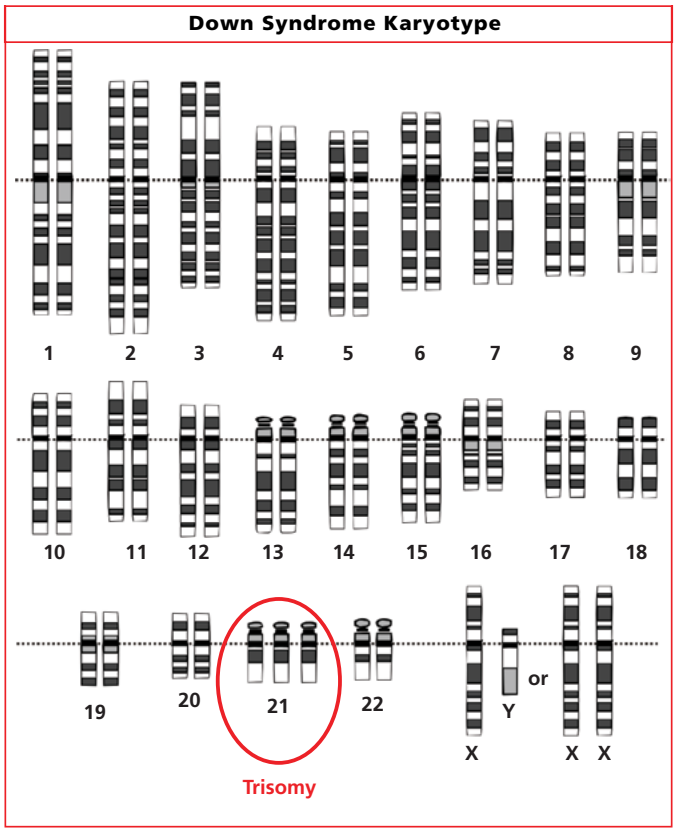
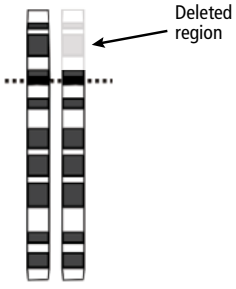


Figure 2

OTHER MUTATIONS AND ALTERATIONS IN CHROMOSOMES

Other diseases that are commonly detected by karyotypic analysis include partial deletions of chromosomes, including Cri du chat, which entails deletion of the short or "p" arm of chromosome (5p-). It is a French-derived term that refers to the characteristic cat-like cry of affected children (Figure 3). In contrast to autosomes, deletions or duplications of sex chromosomes are better tolerated in developing fetuses due to the fact that all but one of the X chromosomes are inactivated, as well as the limited number of genes on the Y chromosome. Thus, monosomy X (Turner syndrome), as well as XXX, XXY, XYY and even XXXXXX karyotypes have been reported in live individuals. Certain "abnormal" karyotypes can give rise to normal healthy individuals. For example, balanced translocations, in which reciprocal regions of different chromosomes have been switched, are usually not deleterious, although the offspring of these individuals run the risk of inheriting trisomic chromosome regions, or partial deletions that may be detrimental (Figure 4).



Cri-du-chat Chromosome 5 pair

Figure 3

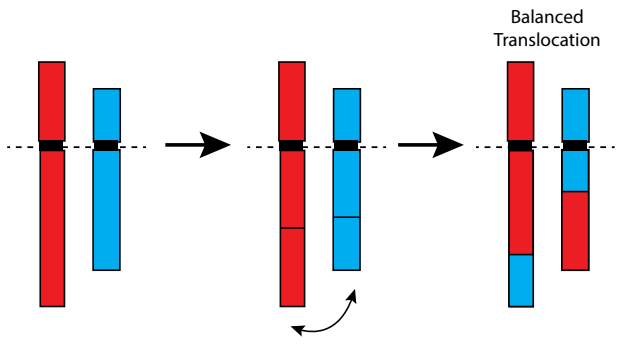


Figure 4

Cytogenetic analysis is also used to analyze certain types of cancers. Leukemia, lymphomas, and an increasing number of sarcomas are characterized by specific chromosomal translocations. These rearrangements lead to the activation of oncogenes or the formation of chimeric oncogene fusion proteins. The first specific abnormality described for a human cancer was the "Philadelphia" chromosome in chronic myelocytic leukemia in which a reciprocal translocation between chromosomes 9 and 22 results in the fusion of the c-abl proto-oncogene and the bcr gene (Figure 5). At least 16 other malignancies are the result of specific translocations generating chimeric oncogenes. In addition to translocations, karyotypic studies show that the majority of human cancers have gained or lost whole chromosomes as the result of increased genetic instability. The average cancer of the colon, breast, pancreas, or prostate typically loses one-fourth of its genetic alleles.

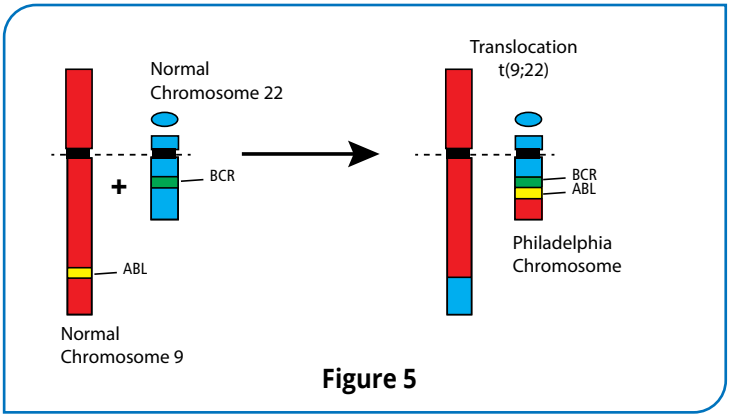


Figure 5

The cells that are utilized in this experiment were originally derived from a patient with Chronic Myelogenous Leukemia (CML). The cells feature the characteristic bcr:abl fusion gene, as well as additional chromosomal translocations. In addition, these cells have been grown in cell culture for a number of years, which further contributes to genomic instability. Thus, the karyotype you will observe is far from typical.

This experiment familiarizes students with the basic principles of microscopy and the study of cell chromosomes. Chromosome number and morphology are roughly determined by using a general stain for nuclei and chromosomes.

Experiment Overview

EXPERIMENT OBJECTIVE:

The experimental objective is for students to develop an understanding of metaphase chromosome spreads. This important method is widely used for karyotyping to analyze numerical and structural changes in chromosomes.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory.
2. Always exercise extreme caution when working in the laboratory.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

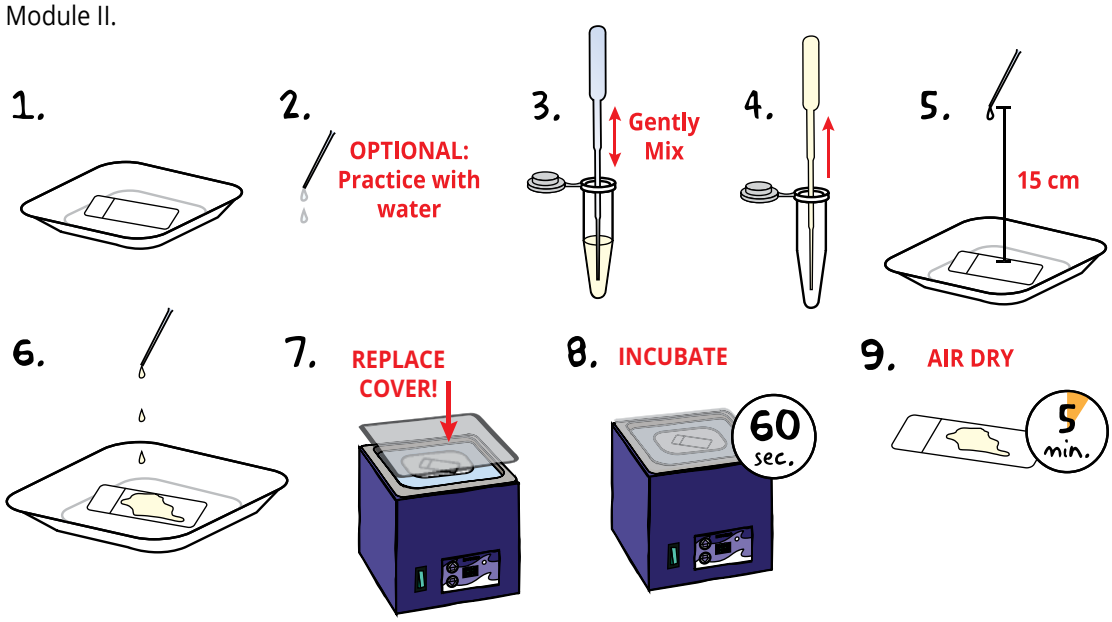
- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: Dropping the Cells

In this module, you will drop cells onto slides to prepare them for staining in Module II. The cells have been treated with colcemid to prepare them for the karyotyping assay and are ready for use. You will use a transfer pipet to drop the cells, one drop at a time, from different heights onto your slide. This should cause the cells to spread evenly across the slide, resulting in better results in the later modules. You will then immediately transfer the slides to a preheated water bath. The heat and humidity will help to burst the cells and allow you to view the chromosomes in Module II.

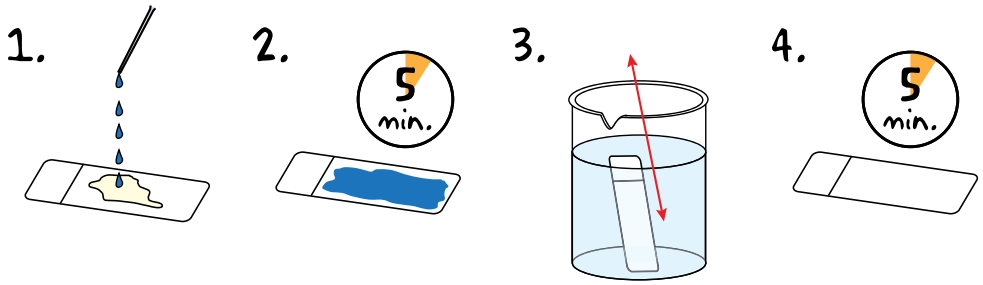


1. **PLACE** your slide flat in a plastic dish. Ensure that the dish will fit into the water bath and that it floats without allowing water to reach the slide.
2. **OPTIONAL** - If you would like to practice dropping beforehand, you can use water. Be sure to **DRY** the slide carefully before beginning the actual experiment.
3. Using a transfer pipet, gently **MIX** the suspension of cells by slowly pipetting up and down.
4. Using the same pipet as in Step 3, **REMOVE** the entire cell suspension.
5. Carefully **DROP** the cells onto the slide from a distance of approximately 15 cm.
6. Continue to **DROP** the remaining cells, one drop at a time, from various heights between 30 cm and 60 cm, onto the same region of the slide. You should have enough cells for 6-7 drops.
7. **Immediately PLACE** the tray containing your slide into the water bath and **REPLACE** the cover.
   NOTE: It is important for the water bath to be hot AND humid. Replace the cover immediately.
8. **INCUBATE** the slide for 60 seconds.
9. **REMOVE** the tray from the water bath and **AIR DRY** the slide for 5 minutes on your lab bench. Continue to Module II. ***Remember to replace the lid on the water bath!***

HINT:
Practice using water first to aim and judge the distance and location of the drop.

OPTIONAL STOPPING POINT:
Dried slides can be stored at room temperature for up to 2 weeks.

Module II: Staining Procedure



1. Using a transfer pipet, **ADD** the entire volume of Giemsa stain to the area of the slide containing the metaphase spreads. Try to cover the slide without letting the stain overflow.
2. **INCUBATE** the slide for 5 minutes at room temperature.
3. **RINSE** the slide by briefly submerging in a beaker of distilled water. Gently **TAP** the slide on a paper towel to remove excess water. If residual stain remains, change the water in the beaker and repeat rinsing the slide until the water no longer turns blue.
4. **AIR DRY** the slide for 5 minutes.

OPTIONAL STOPPING POINT:
At this point, the stained slides can be stored at room temperature. If a coverslip is required by your microscope, one can be added by following the instructions in Appendix A.

Module III: Microscopic Observation

1. Using the low-powered objective, 10X or 20X typically, locate cells with good metaphase spreads. **LOOK** for a field that is nicely stained and contains well spread, non-overlapping chromosomes.

2. **MOVE** to the 40X or 100X objective and count the number of chromosomes in the field of view. Make note of distinguishing chromosomal features including the presence of centromeres or abnormal chromosomal structures, if the cell contained individual chromosomes or paired chromatid (X-shaped, replicated chromosomes), and other noteworthy observations. Record the data below or in your lab notebook.

3. **FOCUS** on a different field of cells and repeat the observation an additional four times.

4. **TABULATE** your data for the observed chromosomes in the grid provided.

Field	Total Chromosomes	Observations

Study Questions

1. What is the normal chromosome number for human cells? Is this a diploid or aneuploid line?

2. How does colcemid work?

3. Why do only some of the cells on your slide display chromosomes while others have normal-looking nuclei?

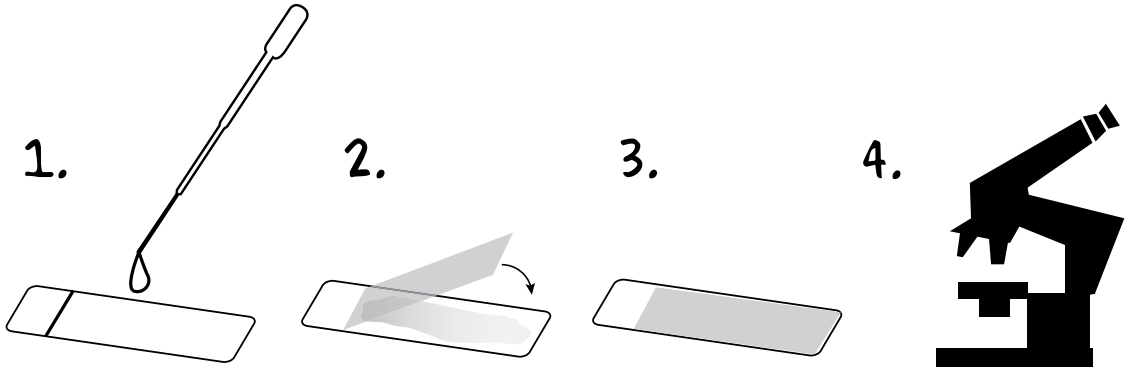
4. Using the tools that you have, can the chromosomes in your karyotypes be classified?

5. Why would karyotyping be useful to potential parents before deciding to have children?

Appendix A

Mounting Glass Coverslips (Optional)

NOTE: Mounting medium and slide coverslips are not included with this kit.



Glass coverslips may be required for some microscope objectives and can help to increase the visibility of nuclei and organelles on these microscopes. Occasionally, mounting media can cause lightly stained cells to fade. Because of this we recommend observing slides without a coverslip unless necessary. If time allows students can visualize slides before and after adding mounting media and coverslips.

Wear gloves and safety goggles

ADDING A COVERSLIP

1. Using a fresh transfer pipet, **ADD** 2 small drops of mounting medium to the middle of the slide.
2. Carefully **PLACE** a coverslip on top of the mounting medium to cover the slide. **HINT:** Avoid bubbles by placing the cover slip and at 45° angle to the slides and slowly lowering. If bubbles are seen, gently press on the coverslip to displace.
3. Gently **ADJUST** the coverslip so that it is centered on the slide.
4. **PROCEED** to Module III: Microscopic Observation.

OPTIONAL STOPPING POINT:
Once the coverslip has been placed, the stained slides can be stored at 4°C for up to 24 hours before moving to Module III.

