High School Classroom Activities

Preparation of Human Chromosome Spreads

Introduction

Reprint from: "Preparation of Human Chromosome Spreads- Kit 4," by CellServ. Also visit www.cellservkits.com

Each somatic cell in the human body contains 23 pairs of chromosomes. During the interphase stage of the cell cycle each of these chromosomes is duplicated and consists of two chromatids joined by a common centromere (spindle attachment region). During mitosis the **chromatids** separate and become independent chromosomes which move to opposite ends of the cell. The subsequent division of the cytoplasm results in the formation of two new daughter cells each containing the same **diploid** number of chromosomes as the parent cell. Cells grown in tissue culture are used for most human chromosome studies. For this exercise, a human tumor cell line, HeLa, (which is highly **aneuploid**), is grown in culture and subsequently treated so as to allow for the microscopic examination of the chromosomes. **Colchicine**, a plant alkaloid, has the unique property of arresting cells in metaphase of the mitotic cycle by interfering with formation of the mitotic spindle which is needed for the movement of chromosome during the metaphase to anaphase progression. This blockage increases the frequency of metaphase cells. Metaphase chromosomes are most readily observed with the light microscope and various chromosomal features such as sister **chromatids** and **centromeres** are evident. In summary, the procedure for chromosome visualization (and karyotyping) entails arresting a fraction of a log phase population in metaphase, treating the cells with a **hypotonic** saline solution to swell the cells and increase their fragility, fixation with acetic acid-methanol, splattering onto slides, and staining. This is followed by a search for ideal chromosome spreads for the study of chromosome number and structure.

Materials

This kit contains the following materials:

- 15 tubes each containing 1 ml of metaphase-blocked cell suspension, fixed in acetic acid-methanol fixative.
- Stain #1 and Stain #2. (Be careful! Avoid contact with skin and clothes.)
- Permount (mounting medium). (Keep in closed container. Avoid prolonged contact with air.)
- Keep in a tightly sealed tube.

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- Printed background and procedural information as well as a Glossary of Terms, References, & Further Reading.
- **If your kit is not complete, contact us immediately.**

Background Information

The 46 chromosomes and mitochondria located in each somatic cell of the human body contain the entire human genetic complement (genome). Located within the nucleus, these 23 pairs of **homologous chromosomes** are comprised of 22 pairs of **autosomes** (non-sex chromosomes) and 1 pair of **sex chromosomes** (XX or XY). The genetic material, or **DNA (deoxyribonucleic acid)**, exists within the chromosomes and contains the entire genetic blueprint for development of an individual. It exists in a highly coiled and condensed state, due in part to the action of a class of DNA binding proteins called histones. All normal human cells contain identical numbers and types of chromosomes. Aberrations in the chromosomal number and/or structure will most likely result in some type of genetic defect. The analysis of human chromosomes has allowed researchers to identify specific genetic diseases and abnormalities which are attributed to this disruption in the normal complement and structure of the chromosomes. Each chromosome pair contains unique physical attributes which distinguishes them from all others. The three main criteria used to distinguish and identify individual chromosomes are:

- length of the chromosome
- position of the centromere (the primary constriction)
- staining/banding pattern of a chromosome when exposed to certain chemical conditions

Using these criteria, cytogeneticists (individuals who analyze and research chromosome structure and function) have set up a classification system for chromosomes which labels each chromosome with a number, or for the sex chromosomes, as **X** or **Y**. This system of standardization allows for accurate communication among scientists.

Many genetic diseases have been associated with a specific change or abnormality within the chromosomes. These abnormalities can include: an increase or decrease in the amount of; chromosome material or the translocation of one piece of a chromosome to another chromosome. Several kinds of cancer are associated with chromosomal abnormalities. Some examples of genetic diseases and their respective chromosomal aberrations are:

1. **Down's Syndrome** - characterized by an extra chromosome #21 (trisomy 21).
2. **Cri du Chat** - characterized by a deletion of the short arm of chromosome #5.

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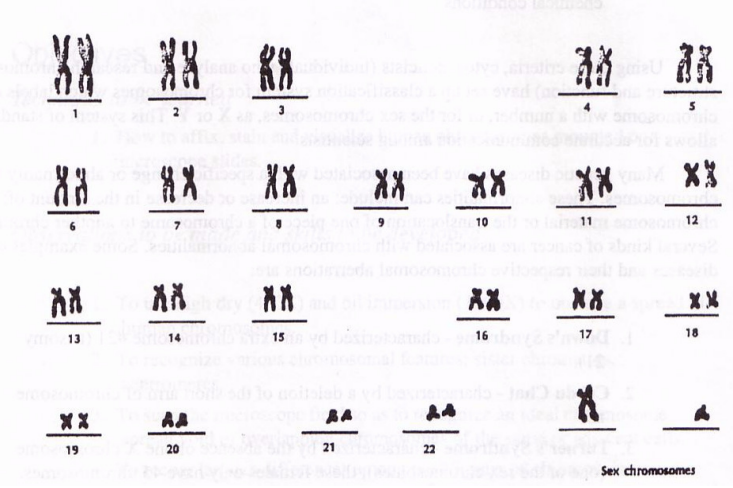
3. **Turner's Syndrome** - characterized by the absence of one X chromosome (one of the sex chromosomes); these females only have 45 chromosomes.

On the other hand, there are many genetic diseases which result from a defect within a particular gene. The abnormal genotype may result in an abnormal phenotype. Such detects may be more subtle and more difficult to analyze. Recent advances in recombinant DNA technology and genetics, however, have allowed researchers to identify specific locations of genes on chromosomes. This information is useful for researchers from around the world who are constructing a genetic map of the human genome. Continued advancement in this field may ultimately lead to the eradication of diseases such as diabetes, muscular dystrophy, cystic fibrosis, and hundreds more.

In order to analyze an individual's chromosomes, or prepare a karyotype, the chromosomes must be in a state in which they can be easily observed. This is accomplished by treating the cells with a chemical called colchicine. The action of colchicine causes the arrest of mitosis in the metaphase stage of the mitotic cycle. It is during this stage that the chromosomes are in their most condensed state and the most visible with the light microscope. Once the cells have been "arrested" in metaphase, the cells are placed in a hypotonic solution. Since the osmotic pressure is greater inside the cell as compared to the outside, water will enter the cell until a state of equilibrium between the cell and its environment has been reached. Movement of water into the cells causes the cells to swell in size. The hypotonic solution is then replaced with a fixative which preserves the existing cell architecture. The cells are now ready to be "splatted" onto microscope slides, stained and observed. When preparing a karyotype the investigator will take a photograph of a chromosome spread which shows clear and distinct chromosomes. The photograph is enlarged and the individual chromosomes are cut out and arranged based on the physical criteria stated earlier (i.e., size. centromere location, banding patterns). This representation of an individual's chromosomes is called an idiogram and is pictured here.



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One practical application of karyotype analysis is in the early detection of genetic defects through amniocentesis. In this process some of the amniotic fluid surrounding the fetus is removed by a physician. This fluid contains fetal cells which will propagate under very specific laboratory conditions. Once the cells have increased in number a karyotype can be performed on these fetal cells. The results of the karyotype analysis may alert the physician to potential problems or abnormalities of the fetus. In karyotyping not involving fetuses the cell type most often used for analysis are lymphocytes. As with fetal cells these cells are grown in culture, treated with hypotonic solution, and fixed prior to performing a karyotype.

In this exercise, the human tumor cell line, HeLa, is used for karyotyping. The HeLa cell line originated in the early-1950's from the cancerous cervical cells of a woman named Henrietta Lacks. Because the cells are of tumor origin they have continued to divide and multiply over the last 50 years and will continue to do so for an indefinite period of time. Furthermore, since these cells are of tumor origin, they will not contain the normal diploid number of chromosomes characteristic of human beings (46). Instead, these cells are considered aneuploid and will possess a chromosome number greater than the diploid number, with three, four, five, or more copies of a particular chromosome being present in some cells. When you make a chromosome count of your spreads you may find chromosome numbers of 50 to 60/cell, or more even greater than 100!

Note: See Appendix 1 for a complete description of the methods used to prepare the cells.

Procedures

a) Place a dry slide vertically at a 45˚angle.

b) With a pipette, ***Gently Resuspend*** the cells in the tube provided. Remove a small sample of cell suspension with a pipette and hold the pipette 2 feet above the slide. Allow one drop of cell suspension to "splat" onto the slide about 3/4 inch from the upper end and tumble down the slide. Carefully apply 6-8 more drops from various heights, One drop at a time, onto the same region of the slide. It is important to release the cell suspension one drop-at-a-time. Do not expel all of your cell suspension in one squirt, or you will obtain poor results. Gently blow across the slide for 2-3 seconds. The drying will help "spread" the chromosomes.

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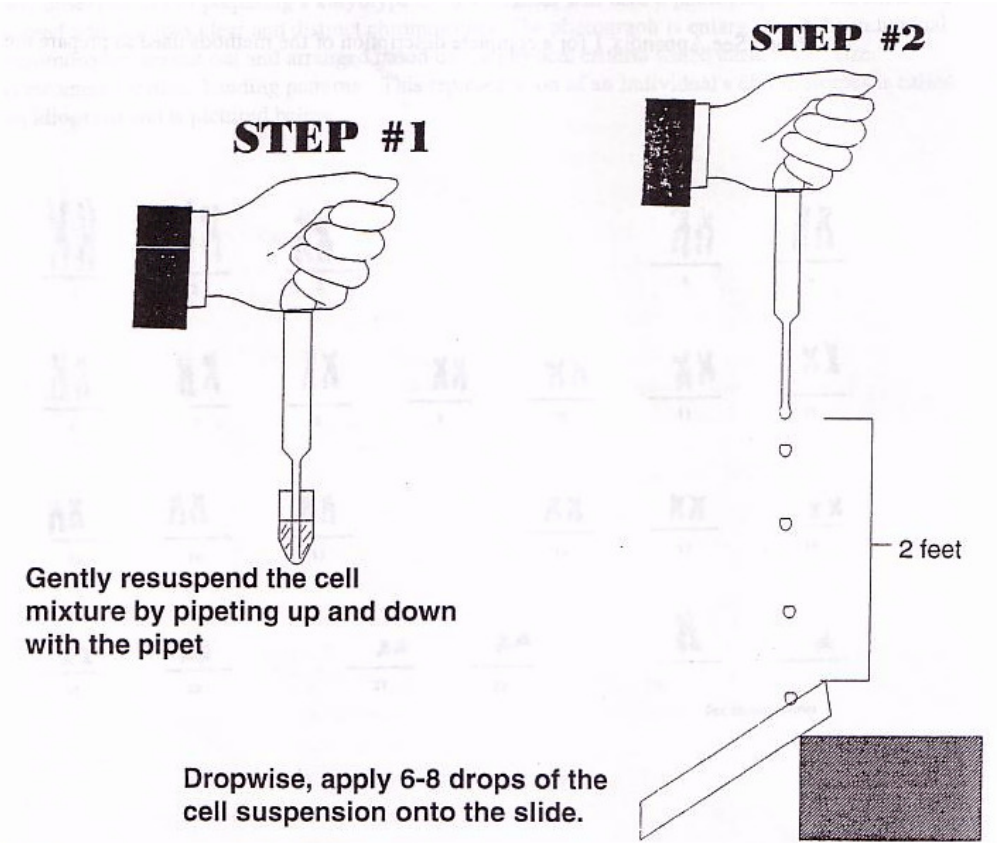

c) Allow the cells to AIR DRY COMPLETELY.

d) Dip the slide into the tube containing STAIN #1 for **1 second only**.

Remove the slide and dip into STAIN #1 again for **1 second only**.

Remove the slide and dip into STAIN #1 again for **1 second only**.

e) Drain off stain and dip the slide into tube containing STAIN #2 for **1 second only**. Remove the slide and dip into STAIN #2 again for **1 second only**. Remove the slide and dip into STAIN #2 again for **1 second only**.

Caution should be taken to avoid carryover of stains (wipe the bottom of slide with a paper towel before transferring).

f) Remove slide from stain and thoroughly rinse with distilled water.

g) Allow slide to AIR DRY COMPLETELY. A stream of warm air or blowing may help speed up the drying process. Incomplete drying will result in very poor resolution.

h) View your slides on your microscope. Scan on low power for cells that have been ruptured and released their chromosomes. Shift to high power (400x) to examine the chromosome spread more carefully.

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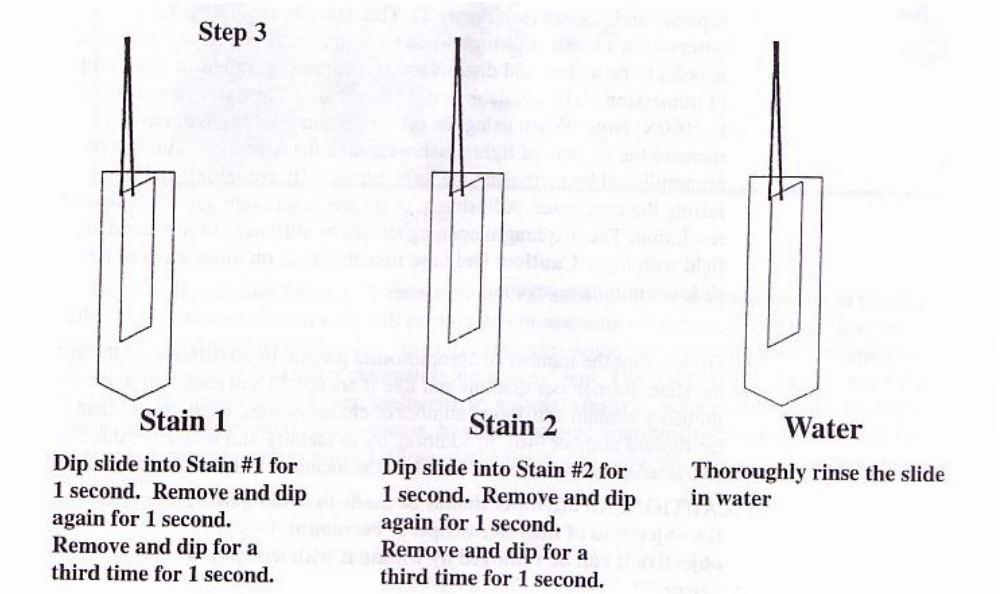
i) If you find a good spread, try to count the number of chromosomes present. Try and identify three characteristic chromosomes based on the location of their centromere.



Results

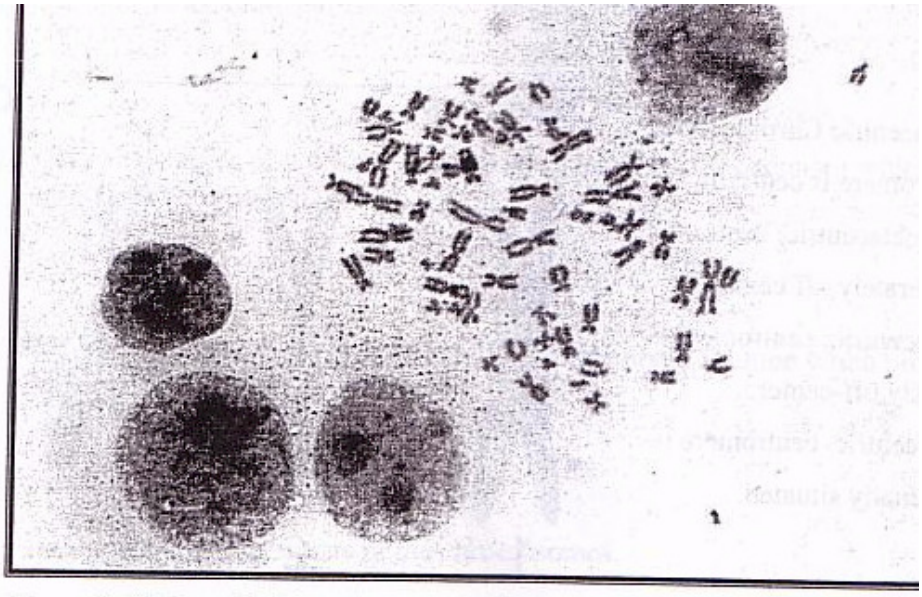


Figure 1. HeLa cell chromosome spread.

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