**Chromosomes: background information**

The 46 chromosomes located in each cell of the human body except egg and sperm 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 I 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 their 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 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 and *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:

I. 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.

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 defects 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 have constructed a genetic map of the human genome. It is now the goal of researchers to discover the function of individual genes within a few years. 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. Recall your lab on diffusion and osmosis. The hypotonic solution is then replaced with 8

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 ideogram and is pictured on the following page. 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, a type of blood cell which unlike red blood cells does have a nucleus with DNA. As with fetal cells these cells are grown in culture, treated with hypotonic solution, and fixed prior to performing a karyotype.

* Procedure

1. The slides used can be either,

    a. dry and at room temperature

    b. placed in room temperature water prior (0 use

2. Place the wet or dry slide vertically at $45^0$ angle. See the diagram on the next page before you do steps 3(a) and 3(b)

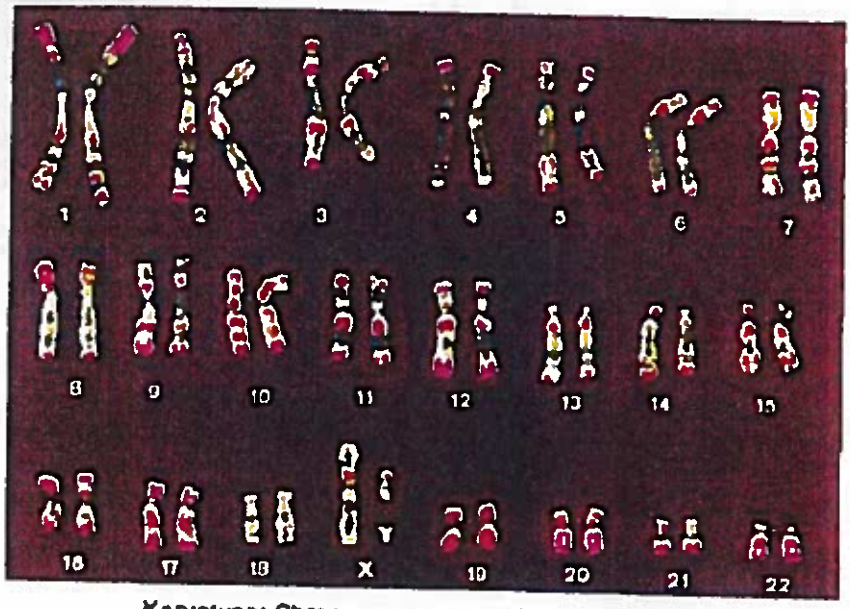
3a. With a pipette, gently resuspend the cells in the tube provided.

3b. 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 ¾ inch from the upper end and tumble down the slide.

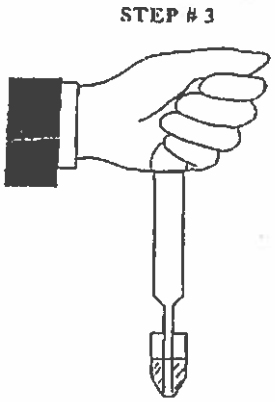
Carefully apply 6-8 more drops from various heights, one drop at a time, onto the same region of the slide. This procedure breaks open the swollen cell and releases the chromosomes.

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.

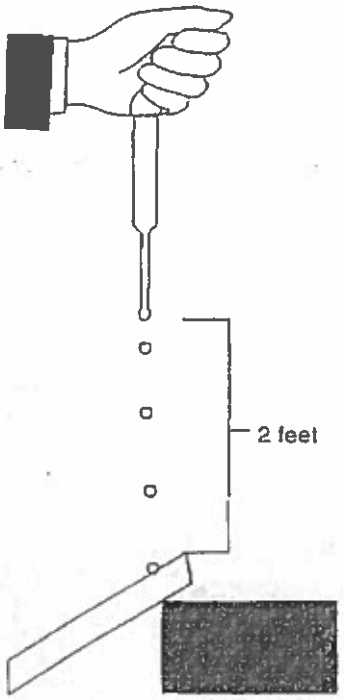



Karyotype: Chromosomes are paired by matching banding and are arranged by size and shape.




**Gently resuspend the cell mixture by pipeting up and down with the pipet.**

(a)




**Dropwise, apply 6 - 8 drops of the cell suspension onto the slide.**

(b)

4. Allow the cells to AIR DRY COMPLETELY

5. 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.

6. Drain off stain and dip the slide into the tube containing STAIN #2 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 the slide with a paper towel before transferring)

7. Remove slide from stain and thoroughly rinse with distilled water.

**STAIN #1**

**Dip slide into Stain #1 for 1 second. Remove and dip again for 1 second. Remove and dip a third time for 1 second.**

Step (5)

**STAIN #2**

**Dip slide into Stain #2 for 1 second. Remove and dip again for 1 second. Remove and dip a third time for 1 second.**

Step (6)

**WATER**

**Thoroughly rinse the slide in water.**

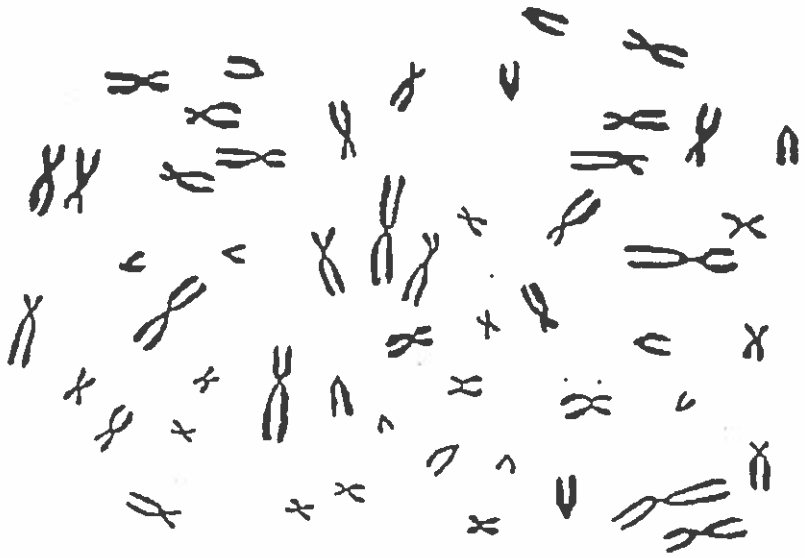
Step (7).

8. Allow the 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.

9. Low and high dry observations can be immediately. Under low power scan your spread for cells which appear to have ruptured and released their chromosomes. Allow your instructor to help find the chromosome spreads. Shift to high power (400X) to examine your spread more carefully. An ideal chromosome spread will contain chromosomes which appear distinct, do not overlap with adjacent chromosomes, and whose sister chromatids are separate and distinct (see figure in Results section below). This exercise requires careful observation so take your time when viewing. After a few minutes allow your instructor to place immersion oil on your slide and examine it at 1000x. Do not try to do this yourself if you haven't been shown how to before.

10. You may want to count the number of chromosomes in one of your good spreads. Don't worry if there are not 46. In addition, try to identify and locate the three characteristic chromosomes based on the location of the centromere.

**RESULTS**



Many of these cells will show aneuploidy; a condition that exists in a cancerous cell, which means that it has more than the usual complement of 46 chromosomes, or the diploid number. Note the typical chromosomes structure with the centromere evident in each chromosome. The sister chromatids are also evident. The position of the centromere is used to classify chromosomes as; metacentric, submetacentric or acrocentric. Close examination will show the presence of all three types in this photograph.