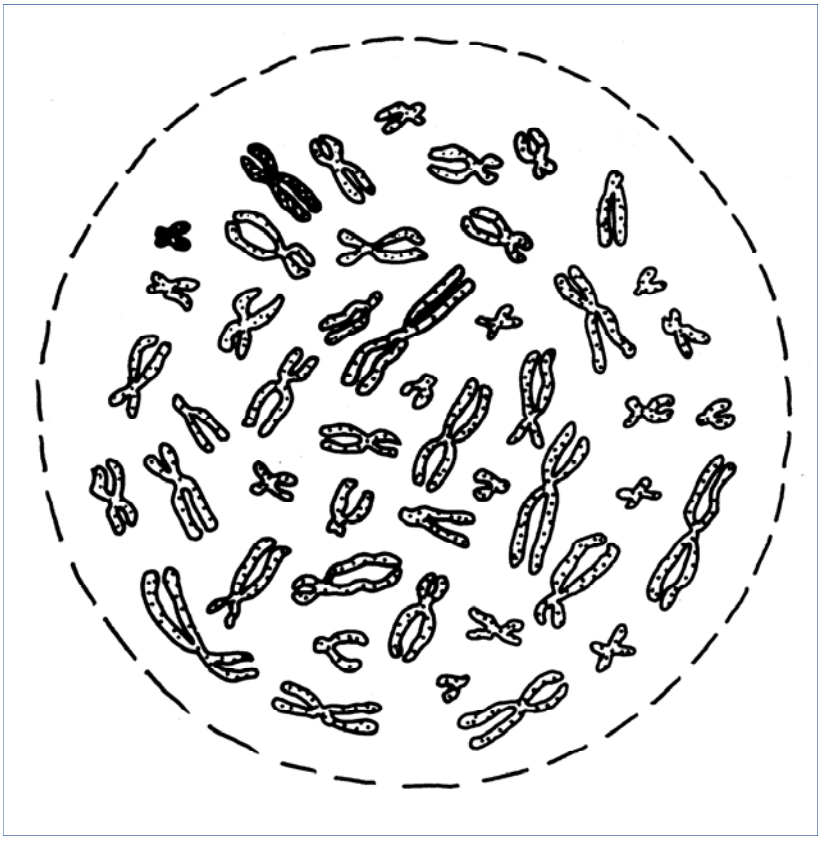
6900 HUMAN KARYOTYPE KIT
Teacher Guide

Purpose:

This kit will enable senior high school biological science students to see the differences in human chromosomes, and especially some of the chromosomal abnormalities which may occur in the human population. The materials contained in this kit will allow students to work singularly or in small groups. One to two class periods will be required to complete this investigation.



HUMAN KARYOTYPE KIT

(Teacher Manual)

INTRODUCTION

The total number of cells in the average adult is somewhere in the realm of 100 trillion, and the vast majority of these cells contain 46 chromosomes per nucleus. The study of structural and numerical characteristics of the chromosomes is known as *cytogenetics.* Any defect in either the structure or number of chromosomes creates abnormalities. The observation of normal and abnormal chromosomes is achieved through a process known as *Katyotyping,* derived from the greek word karyon which means nucleus. The viewing of the nucleus and its contents is accomplished through microscopic photography.

BACKGROUND INFORMATION

Chromosomes are composed of genes (segments of DNA) and are present in nearly every cell. They are the structures which determine heredity. The chromosomes of certain body cells can be stained, magnified and photographed to determine if a particular chromosome is normal or abnormal. The tens of thousands of different genes present in human chromosomes are presently being studied through an international enterprise called the Human Genome Project. The aim of this international project is to map all human chromosomes, and by doing so, scientists hope to isolate the genes behind many, if not all, human diseases.

Most every cell in the human body has 46 chromosomes, including 22 pairs of chromosomes called *autosomes.* Each autosome usually appears identical to its partner, but each pair is different in genetic makeup and frequently in appearance from all other pairs. Any defect on one specific chromosome would have an entirely different effect on an individual, than a defect on some other chromosome. In addition to these 22 matching pairs of chromosomes there are two chromosomes which are the sex chromosomes. All normal males have one Y chromosome and one X chromosome. The normal female has two X chromosomes and no Y chromosome. This genetic difference is responsible for male and female differences.

The cells in the body which do not have 46 chromosomes are the gametes (sex cells) and the reproductive cells from which they develop. These cells each have 23 chromosomes. When the sperm (male gamete) and the ova (female gamete) fuse together at conception, they form the beginnings of a new individual who will have 46 chromosomes in all cells except for the reproductive cells. Each mature reproductive cell contains only one of each pair of autosomes and either an X or Y chromosome. Since the normal cells of a woman's body carry two X chromosomes, it is obvious that all ova (egg cells) carry 22 autosomes and only one X chromosome. The sperm cells have 22 autosomes and *either* an X or a Y chromosome. When a Y carrying sperm fertilizes an ovum a male child is conceived. When a X carrying sperm fertilizes an ovum a female child is conceived.

ANALYSIS OF HUMAN CHROMOSOMES

Human chromosomes are analyzed through the process of Karyotyping. Various human cells can be cultured in the laboratory and when a cell reaches the metaphase stage of cell division it is arrested. These cells are then microscopically photographed. These photographs are then enlarged to a workable size and a metaphase plate is formed. These plates can then be used to prepare a karyotype by cutting out the photographed chromosomes and arranging them in a particular order as described.

In forming a karyotype, chromosomes are paired and classified into groups according to length and the

location of the *centromere,* which is the region on the chromosome where the two replicated chromosome arms are attached. If the centromere is located away from the midline, one arm of each chromosome appears longer than the other. In some chromosome pairs, the centromere is located near one end, and the chromosomes form a "wishbone". Autosomal pairs are arranged in seven lettered groups (A-G) in descending order of length. Since the chromosome pairs with a lettered group are very similar in size and outward appearance, it is usually necessary to use biochemical information to be certain of a chromosome pair number within a lettered group. The two sex chromosomes are also unnumbered (Fig. 1). The female chromosome is described as 46XX and the male as 46XY.

The actual procedure for karyotyping usually involves the culturing of white blood cells from an adult, but may also be done as early as 16 weeks on a human fetus. The fluid from the amniotic sac usually contains cells from the developing fetus and through amniocentesis some of these cells can be removed and studied: thus abnormalities can be detected before birth.

A procedure known as the Buccal Smear is used to determine the number of X chromosomes a person has. The cheek cells of normal females have an area which stains very darkly, that is called a *Barr Body.* A Barr Body is an X chromosome that has condensed and become genetically inactive at some very early stage of development. The cells of normal females that are examined subsequent to this period no longer contain two X sex chromosomes; instead they have one X chromosome and one Barr Body. In normal males this condensing does not occur, and thus male cells retain both X and Y sex chromosomes.

From time to time abnormal numbers of chromosomes or improperly formed chromosomes cause abnormal development. It is possible for cells of a female embryo to contain three X chromosomes (XXX) and sometimes even four X chromosomes (XXXX). It is possible for cells of a male to have two to four X chromosomes (XXY, XXXY, XXXXY). If one of these situations occurs the total number of chromosomes is increased. In both males and females all but one of the X chromosomes condense to form Barr Bodies. The improper number of Barr Bodies may indicate abnormality in an individual.

One common type of cytogenetic effect is know as Mongolism or Down's Syndrome and most specifically Trisomy 21. Trisomy refers to a condition in which there are three, instead of two, of a particular chromosome. In Down's, there are three chromosomes of pair number 21. This condition may occur during the failure of chromosomes to separate during the formation of sex cells, known as *Meiotic Nondisjunction.* It results in one sex cell containing two chromosomes of the pair while the other contains none. If the sex cell with an extra chromosome unites with a normal sex cell during fertilization, the resulting individual will have 47 chromosomes with three of that particular set of chromosomes rather than the usual two, and is said to be Trisomic for that chromosome. If the cell with the missing chromosome unites with a normal one, the individual will only have 45 chromosomes and is called Monosomy. These two conditions occur most frequently in children of mothers over 35 years of age. The age of the father does not seem to be related.

The effects of Trisomy 21 are mental retardation, poor muscle tone, a large tongue, and slanted eyes, which originally gave the syndrome it name of Mongolism. These individuals also have abnormal hand and foot prints as well as a high rate of heart defects. There are similar nondisjunctions of sex chromosomes which may result in Monosomy X, also know as XO or Turner's Syndrome. This condition produces females who are abnormally short, have immature sex structures, do not menstruate and who are sterile.

The Trisomy X condition resembles Turner's Syndrome, but sometimes individuals may appear normal. These females have a high incidence of mental retardation. If nondisjunction occurs in the production of a male embryo, either the fetus will die if it does not contain an X chromosome, or may result in Kleinfelter's

Syndrome (XXY). This condition results in normal appearance but individuals are sterile and have small testes. These individuals are usually very tall, thin and may be extremely aggressive.

This kit should allow your students to prepare various karyotypes and better understand the value of karyotype and genetic counseling and education.

IDENTIFICATION OF KARYOTYPES

Plate 1	Normal Male Karyotype	46 chromosomes (XY)
Plate 2	Kleinfelter's Syndrome	47 chromosomes (XXY)
Plate 3	Turner's Syndrome	45 chromosomes (XO)

Time Allocation:

To prepare this product for an experimental trial should take less than ten minutes. Actual experiments will vary with needs of students and the method of instruction. One or two class periods will be required to complete this investigation.

Feedback:

If you have a question, a comment, or a suggestion that would improve this product, you may call our toll free number.