

## Chapter 12

# Using Chromosome Kits to Assemble Human Karyotypes

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## Introduction

Since the mid-1950s there has been a dramatic increase in our knowledge of human cytogenetics. In 1956, it was discovered that the diploid chromosome number in man is 46 and not 48 as was previously believed. In 1959, the cause of Down syndrome was discovered to be an extra chromosome. This was quickly followed by the discovery of a number of other chromosome abnormalities.

These discoveries were made possible by techniques which were developed for human chromatin study. These methods permitted an accurate description of individual chromosomes and a normal karyotype. The techniques include:

1. The use of phytohemagglutinin to stimulate human lymphocytes to divide in culture.
2. The use of colchicine to stop all cell divisions at metaphase, when the chromosomes are spread apart and easily identified.
3. The discovery of staining techniques that show distinctive banding patterns in individual chromosomes.

Before looking at types of chromosomal abnormalities, it is important to note some of the basic differences between simple disorders, such as phenylketonuria, and chromosomal disorders, such as Down syndrome. One of the major functions of the genes is to supply the proper amino acid sequence for assembling proteins. A useful analogy is to think of the chromosomes as serving as a dictionary for the cell. When we want to assemble the letters of the alphabet into a particular word, we look up the correct sequence in the dictionary. Similarly, each gene tells the cell the proper sequence in which to assemble the amino acids in order to make a particular protein molecule. Each normal human somatic cell contains 23 pairs of chromosomes, one member of each pair is from the father and the other from the mother. In essence, the diploid cell has two similar dictionaries, one from each parent. A single recessive gene which could result in a disorder may be compared to having a misprint in one word of the dictionary. For such a disorder to appear, both parents must have at least one allele which carries the wrong instructions. If both parents are carriers of the disease (i.e., one allele carries the correct instructions, *P*, for a certain protein and the other allele carries the wrong instructions, *p*), there is one chance in four that the zygote will receive both incorrect instructions for the protein and will therefore develop the disease.

In some cases the amount of a particular protein produced appears to be related to the number of “normal” genes present. Thus, the person with two sets of correct instructions (*PP*) might have twice as much of the protein as the carrier (*Pp*). Generally, the lesser amount of the protein found in the carrier does not result in any clinical symptoms, although it can often be detected with special chemical tests. Chromosomal disorders differ from single gene disorders in that the parents rarely are carriers of any defect and the basic problem is too few or too many genes rather than an abnormal gene.

There are several types of chromosomal abnormalities. The most common is the presence of an extra chromosome. About 45% of all miscarriages are caused by the presence of an extra chromosome. Having this condition is like having an extra page in our dictionary. The additional instructions (genes) supplied by the extra chromosome would be expected to cause abnormally high production of some proteins. Extra chromosomes are caused by *nondisjunction* (the failure of a pair of homologous chromosomes to separate during meiosis). Nondisjunction results in a gamete with two homologous chromosomes. When the gamete is joined by a normal gamete, the resulting zygote has three of the chromosomes. This condition is known as trisomy.

When chromosome 21 is involved in nondisjunction, the resulting condition (trisomy 21) results in the most common type of Down syndrome (named for the English physician Langdon Down). Trisomy 21 is responsible for about 96% of those with Down syndrome and the condition is not considered to be hereditary.

Other trisomic combinations are known. Trisomy 13 produces a severely retarded individual with a cleft palate and lip, an extra finger on each hand, malformations of the eyes and ears, a small head, and other abnormalities. Trisomy 18 produces an individual with mental retardation and defects in the hands and head (including the eyes and ears). Usually, trisomy of the larger chromosomes, which presumably carry more genes, is not compatible with life. The sex chromosomes are an exception. The male sex chromosome (Y) apparently carries few genes and extra female sex chromosomes (X) are effectively removed (see below).

The normal chromosome make up of a male is 46,XY and that of a female is 46,XX. However, either a male or female may be born with too many, or too few sex chromosomes. Individuals having a Y chromosome will always appear to be male, even though there may also be one, two, three, or four X chromosomes. Males with Klinefelter syndrome have an extra X chromosome (47,XXY). These males are taller than normal, may be below normal in intelligence, and most are sterile. Klinefelter males often have a hard time adjusting to society.

Males may also be born with an extra Y chromosome. These males (47,XYY) are larger than normal. Many characteristics which have been associated with this condition (subjects are prone to mental illness, are extremely aggressive, and show dangerous antisocial behavior) have come from studies done on criminals with XYY. Recent studies, however, show that XYY individuals may be normal in all respects, may have borderline intelligence, or may have mild to severe behavioral problems.

An individual with Turner syndrome has one X chromosome and no Y (45,X). Those with Turner syndrome are females. They are usually under five feet tall, have webbing of the neck, do not develop under arm or pubic hair, and have underdeveloped ovaries. Turner syndrome accounts for about 20% of all miscarriages.

One, two, or more extra X chromosomes have much less effect than the presence of an extra autosomal chromosome. In 1961, geneticist Mary F. Lyon suggested an explanation for this phenomenon. Her hypothesis suggests that:

1. All but one X chromosome is inactivated in each somatic cell.
2. The inactivation is random for each cell.
3. Inactivation occurs early in embryonic development (about 16 days after conception).
4. Once inactivation occurs in an embryonic cell, the same X chromosome will be inactivated in all cells descending from that embryonic cell.

What happens to the inactivated X chromosome? There are masses that stain very dark in the nuclei of somatic cells from females. These dark bodies, called Barr bodies, are the inactive X chromosome. Cells from normal females (46,XX) have one Barr body. Cells from 47,XXX females

have two Barr bodies, and cells from Klinefelter males (47,XXY) have one Barr body. Note that the Lyon hypothesis also suggests a basis for the variable expression of X-linked disorders in female carriers. Female carriers of such disorders as hemophilia and muscular dystrophy may show no symptoms or symptoms almost as severe as those of a male with the disorder.

Another type of sex chromosome abnormality is the fragile X syndrome. This condition is demonstrated in opaque stained chromosome spreads. It appears as if the end of the long arm (q) of the chromosome is loose or has broken off. Fragile X syndrome occurs mostly in males, affecting about one out of every 1000. Individuals with fragile X syndrome show varying degrees of mental retardation, are shorter than average, have large heads with long, narrow faces, and large prominent ears. About one out of three female carriers shows some degree of mental retardation.

*Translocation* is a type of chromosomal disorder in which parts of two nonhomologous chromosomes are joined. If there is no loss of genetic material when a translocation occurs (i.e., balanced chromosome exchange), the individual will be normal. However, the individual becomes a carrier of this translocation, and may pass it on to succeeding generations. The hereditary forms of Down syndrome are caused by translocations. The most common translocation Down syndrome involves chromosomes 14 and 21. It appears that almost the entire 21st chromosome is attached to the short arm (p) of the 14th chromosome. The other form of translocation Down syndrome is caused by a translocation between the two 21st chromosomes. The parent who carries one of these translocations is normal, but has a high risk of having a Down child. If the mother is the carrier, she has a 10–20% chance of having a Down child. If the father is the carrier, his chance of having a Down child is about 4%. About 4% of Down syndrome cases are caused by the 14/21 translocation.

Another type of chromosome disorder is *deletion*. Deletion occurs when a piece of a chromosome simply breaks off and is lost. The severity of the defects is related to the size of the lost piece. Chromosomes may be broken with no loss of genetic materials. This breakage may be caused by irradiation, infections, drugs, or other environmental agents. The disorder known as Cri-du-chat syndrome (cat cry syndrome) is caused by a deletion of part of the short arm (p) of chromosome 5. This disorder is named because of the unusual cry of infants with the deletion. The infant's cry sounds like a kitten's meow.

Most chromosomal abnormalities arise from errors occurring during cell division. The fact that Down syndrome occurs about once in every 600 births suggests that these errors are not extremely rare. A number of such errors probably occur in our bodies each day. However, it is only those which occur during gametogenesis that can be readily detected.

Through the use of a procedure called amniocentesis it is possible to determine the chromosomal make up of a fetus before birth. Amniotic fluid containing fetal cells is withdrawn from the uterus. The cells are then grown for 2–3 weeks to get enough cells to warrant definite conclusions. Examination of these cells will show whether the fetus has a detectable chromosome anomaly. Single gene disorders cannot be detected by looking at the chromosomes. However, some single gene disorders can be detected by chemically measuring the activity of certain enzymes in the fetal cells.

### Notes for the Instructor

Chromosomal preparations are most easily obtained from white blood cells. A few drops of blood are placed in a tube containing growth medium supplemented with phytohemagglutinin to stimulate lymphocyte division. The tube is capped and incubated at 7°C. After the lymphocytes increase, colchicine is added and incubation is continued for a short time. Colchicine arrests cell division at metaphase. At metaphase, each replicated chromosome consists of two strands joined at

the centromere. If cell division had continued, the strands would have pulled apart and one strand would have entered each of the daughter nuclei. Thus, the X-shaped units seen in the work sheets would each have become two chromosomes if they had been allowed to separate. The chromosomal preparation is centrifuged to separate the cells, which are then treated with a hypotonic solution to destroy the red cells and make the lymphocytes easier to rupture. The lymphocytes are fixed in a mixture of methanol and glacial acetic acid. The fixed cells are dropped on a cold slide and air dried to rupture the walls and spread the chromosomes.

Human Biophoto<sup>®</sup> Sheets can be purchased from Carolina Biological Supply Co. and are produced from high quality photographs of carefully prepared material. The human chromosome spreads have been stained with Geimsa stain to show the distinct banding patterns of the chromosomes. Pairing is accomplished by comparison of the distinct banding patterns for each pair of chromosomes.

Each pad of Biophoto<sup>®</sup> Sheets has 30 sheets punched for an 8.5 × 11" notebook, a chipboard back, and a card cover. For the instructor's convenience, a copy of the photomicrograph with the chromosomes labelled is printed on the back cover of each pad.

Human Chromosome Abnormalities BioKits<sup>®</sup> are available from Carolina Biological Supply Co. Each kit is designed for 30 students. Students assemble normal and abnormal karyotypes. Information concerning the mechanisms by which abnormalities occur and how they are inherited is included. Each kit contains Biophoto<sup>®</sup> Sheets of normal and abnormal banded chromosome spreads, karyotypes forms, 30 Student Guides (with sample karyotype), and a Teacher's Manual.

The Student Guide from the Down Syndrome Biokit<sup>®</sup> (Cat. #17-3826) is presented below, accompanied by a human karyotype form and Biophoto<sup>®</sup> Sheets of chromosomes from a normal female (46,XX) and a normal male (46,XY).

### **Student Guide from Down Syndrome BioKit<sup>®</sup>**

You and your partner will first prepare karyotypes of a normal human male and female, then you and your partner will have an opportunity to prepare five karyotypes that result in, or show carriers of, Down syndrome. You will be given two blank Karyotype Forms and two Biophoto<sup>®</sup> Sheets showing the chromosomes from a normal human male and female.

Do not do the male and female karyotypes at the same time. Carefully cut out the individual chromosomes. Using a sample karyotype as a guide, arrange each chromosome on the blank karyotype form. Do not fasten the chromosomes to the karyotype form until your instructor has checked it. Keep all paper scraps until you have identified each chromosome.

The chromosomes can be arranged in seven groups (A to G) according to length. Group A consists of the six longest chromosomes. The B group consists of four long chromosomes with the centromeres very close to one end. The C group consists of 14 medium length chromosomes with the centromeres slightly off center. The female sex chromosome (X chromosome) also falls in this group. Therefore, a male will have 15 C-length chromosomes and a female will have 16. The D group consists of six chromosomes slightly smaller than the Cs with the centromeres very near one end. The E group resembles the C group but the chromosomes are much smaller. The F group chromosomes are very small with the centromeres in the middle. The G group includes the four smallest chromosomes with the centromeres so close to the ends that it is difficult to see any short arms at all. The male sex chromosome (Y chromosome) falls into the G group. Therefore, a male will have five G length chromosomes and a female will have four.

It is possible to distinguish individual chromosomes. Using new staining techniques, each chromosome is stained to show the horizontal bands that are unique to that chromosome. Note the

banding patterns of the chromosomes, usually about 12 bands per chromosome. Each band represents regions covering several hundred genes.

When you have completed the normal karyotype, you will be given Biophoto<sup>®</sup> Sheets illustrating chromosomes of individuals with, or carriers of, Down syndrome and five blank karyotype forms. The Biophoto<sup>®</sup> Sheets include trisomy 21 Down syndrome; 14/21 translocation carrier; 14/21 translocation Down syndrome; 21/21 translocation carrier; and 21/21 translocation Down syndrome.

First, count the chromosomes on the Biophoto<sup>®</sup> Sheets; the normal number is 46. The trisomy 21 Down syndrome has 47 chromosomes. The extra chromosome is a 21st and is caused by nondisjunction (homologous chromosomes that do not separate during meiosis).

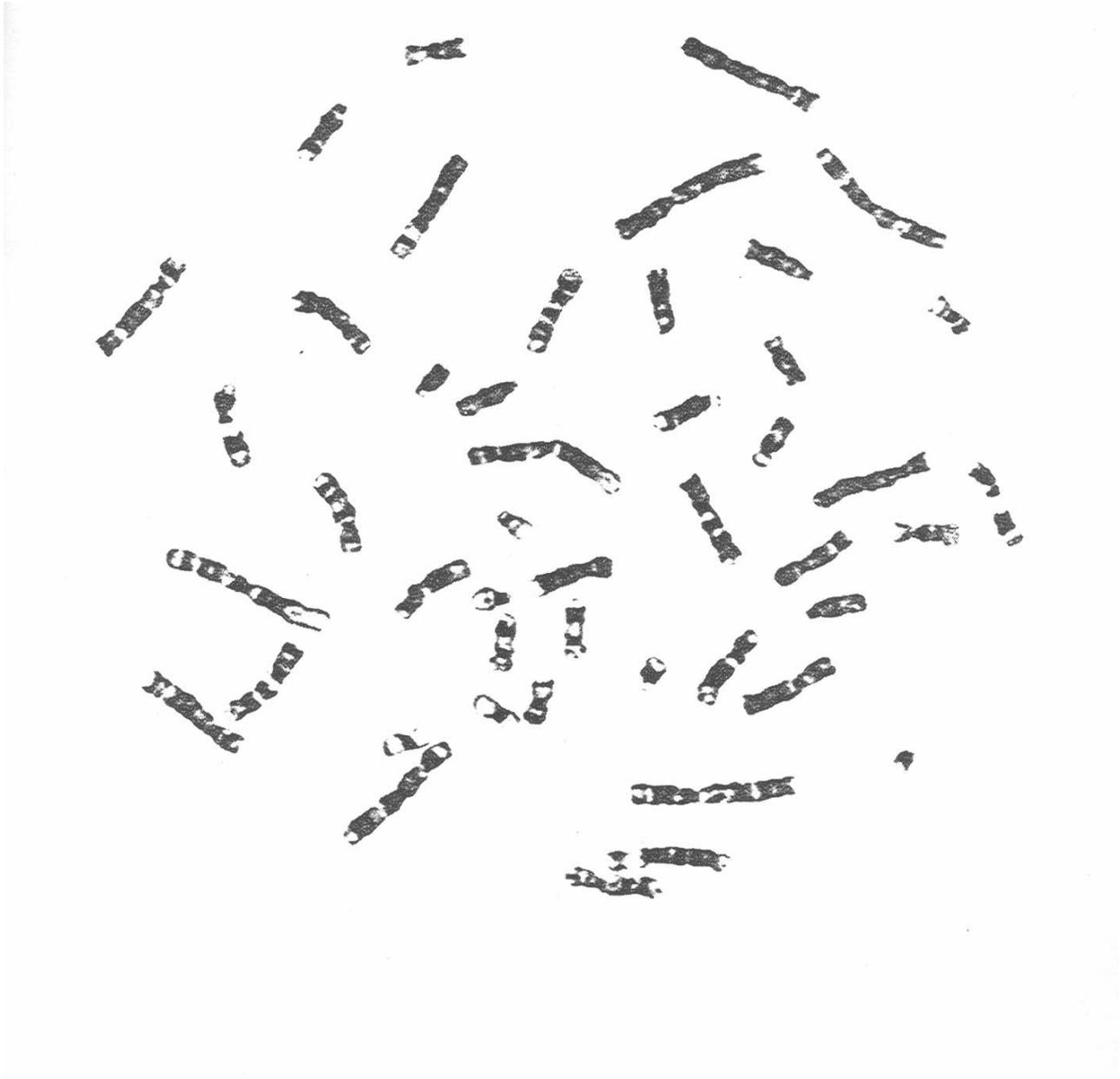
The 14/21 and 21/21 translocation carriers have only 45 chromosomes. When you prepare the 14/21 translocation carrier karyotype, you will be missing a 14 and a 21 chromosome, but you will have an extra chromosome similar to the C group. This C group chromosome is actually a 21 chromosome attached to the short arm of a 14 chromosome. Put this extra C type chromosome with pair number 14. The 21/21 translocation carrier has no individual 21 chromosomes, the 21 chromosomes are attached at their short arms. These attached chromosomes are placed at the 21 space on the karyotype form. The 14/21 and 21/21 translocation Down syndrome will have 46 chromosomes each. The 14/21 translocation Down has two 21 chromosomes, plus the 14/21 translocation chromosome that is paired with the 14th chromosome. The 21/21 translocation Down syndrome has one 21st chromosome and the 21/21 translocation chromosome.

Cut out the chromosomes and prepare the karyotypes. Remember, a female has two X chromosomes and a male has one X and one Y chromosome. Finish each karyotype before starting another. On the bottom of each completed karyotype, list the number of chromosomes, the sex of the subject, and the abnormality (e.g., 47 chromosomes, female, trisomy 21 Down syndrome).

The trisomy Down syndrome is nonhereditary and is responsible for about 96% of all Down syndrome cases. The translocation Down syndromes are hereditary and are responsible for 4% of all Down syndrome cases. After studying the karyotypes, explain why the translocation Down syndromes are hereditary.

The following are presented (as 90% reductions of the originals) on the next three pages (reprinted with permission of Carolina Biological Supply Co.):

1. Human Chromosome 1: Normal Male (46,XY)
2. Human Chromosome 2: Normal Female (46,XX)
3. Human Karyotype Form



**HUMAN CHROMOSOMES 1.**  
**Normal Male 46,XY**



\*Photomicrograph courtesy of  
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Biophoto\* Sheet

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HUMAN CHROMOSOMES 2.  
Normal Female 46,XX



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# HUMAN KARYOTYPE FORM

_____ 1	_____ 2	_____ 3	_____ 4	_____ 5
	A		B	
_____ 6	_____ 7	_____ 8	_____ 9	_____ 10
			C	
_____ 13	_____ 14	_____ 15	_____ 16	_____ 17
	D		E	
_____ 19	_____ 20	_____ 21	_____ 22	_____ X
	F		G	
				_____ Y

Sex chromosomes

Number of Chromosomes \_\_\_\_\_ Sex of Subject \_\_\_\_\_ Type of Disorder \_\_\_\_\_



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