Advanced Human Chromosome Analysis

Background

Normal humans have a total of 46 chromosomes, a complete set consisting of 22 pairs of autosomal chromosomes, or autosomes, and 1 pair of sex chromosomes. For reproduction, human gametes (sperm and egg) are created through meiotic division, which produces gametes carrying 23 chromosomes, one chromosome from each homologous pair. During fertilization, the gametes combine, restoring the number of chromosomes to 46. In this way, half of an individual's chromosomes are inherited from the mother (the maternal component) and the other half from the father (the paternal component).
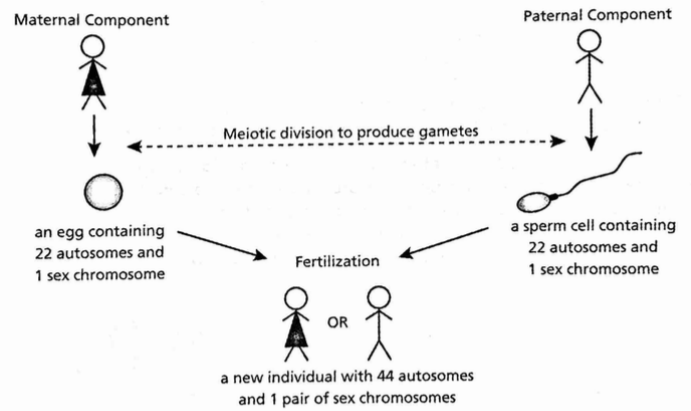

Figure 1. Chromosomal inheritance

The study of chromosomes, chromosome abnormalities, and related diseases is called cytogenetics. Many cytogeneticists use karyotypes to examine the chromosomes. A karyotype is a visual, ordered display of all the chromosomes from a somatic cell, a body cell that is not a gamete. Karyotypes are created using a photograph of the condensed, duplicated chromosomes that have been arrested, frozen in metaphase using a drug that inhibits mitosis so that they can be easily visualized. This step is necessary because in non-dividing cells, chromosomes take the form of long, stringy fibers that bunch together in the nucleus like a cotton ball. During metaphase of mitosis, however, the chromosomes are condensed and visible as separate entities.
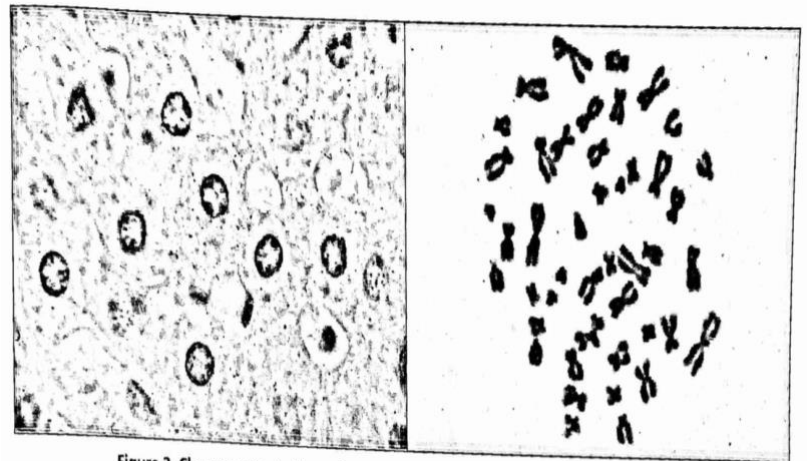

Figure 2. Chromosomes in the nucleus (left); chromosomes in a metaphase spread (right)

After the chromosomes have been arrested, a dye/stain is added. When stained with Giemsa stain, different chromosomes have different banding patterns. These patterns of dark and light bands uniquely identify each pair of chromosomes. The bands do not indicate genes—for some chromosomal regions, hundreds of genes may be present in one band, while in other regions, there may be relatively few genes per band.

The stained chromosomes are then photographed under a microscope. The resulting array of chromosomes is called a metaphase spread. The chromosomes are then matched into pairs by size, banding pattern, and centromere position, and then examined for abnormalities. Chromosomes are classified as one of three types on the basis of the position of the centromere. Chromosomes with the centromere near the center are metacentric. Those in which the centromere lies off-center between the middle and tip of the chromosome are classified as submetacentric, and chromosomes in which the centromere lies near the tip are classified as acrocentric. Chromosomes are usually positioned in a karyotype with the shorter arm upwards; this shorter arm is designated p. The longer arm is designated q.
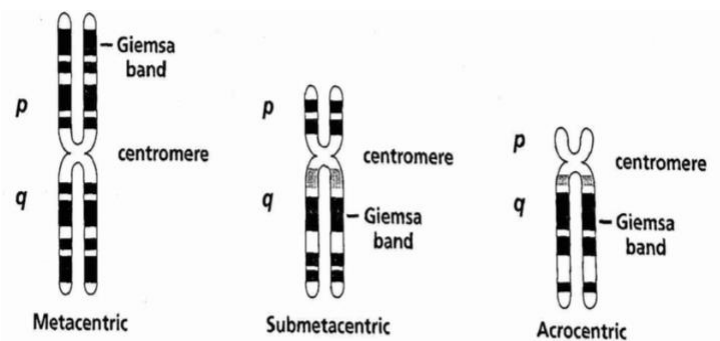

Figure 3. Chromosome characteristics

Normal homologous chromosomes have the same banding pattern, centromere position, arm length, and size. By careful analysis of chromosome morphology, cytogeneticists match pairs of homologous maternal and paternal chromosomes from the metaphase spread. In a karyotype, chromosomes are displayed in homologous pairs approximately from largest to smallest (1-22). Chromosomes

Chromosome Group	Chromosomes	Centromere Position
Group A	1 to 3	metacentric
Group B	4 and 5	acrocentric
Group C	6 to 12 and X	submetacentric
Group D	13 to 15	acrocentric
Group E	16 to 18	submetacentric
Group F	19 and 20	metacentric
Group G	21, 22, and Y	acrocentric

are also arranged into groups (A through G) according to common physical features, i.e., size and centromere position. Typically, the groups are separated slightly when laid out in a karyotype display. The sex chromosomes are traditionally located in the lower, right-hand corner of the karyotype. The table shows how chromosomes are typically grouped based on centromere position within size order.

Occasionally during meiosis, gametes are created with an improper number of chromosomes (a numerical abnormality) or with an irregular chromosome structure (a structural abnormality). Embryos with irregular gametes typically are non-viable or short-lived, or they result in an individual with a disorder. Numerical abnormalities result from a failure of homologous chromosomes to separate during Meiosis I or when sister chromatids (duplicated chromosome copies joined at the centromere) fail to separate during Meiosis II This failure to separate is called non-disjunction, and it can occur with any chromosome pair, including the sex chromosomes.
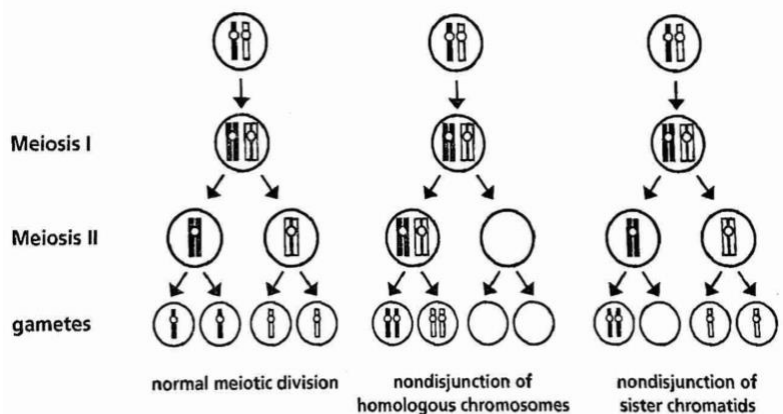

Figure 4. Numerical abnormalities in meiosis

When a gamete with an extra chromosome combines with a normal gamete during fertilization, the result is a zygote possessing three copies of the chromosome. This is known as a trisomy. When a gamete lacking a copy of a chromosome combines with a normal gamete during fertilization, the result is a zygote with only one copy of the chromosome. This is known as a monosomy.
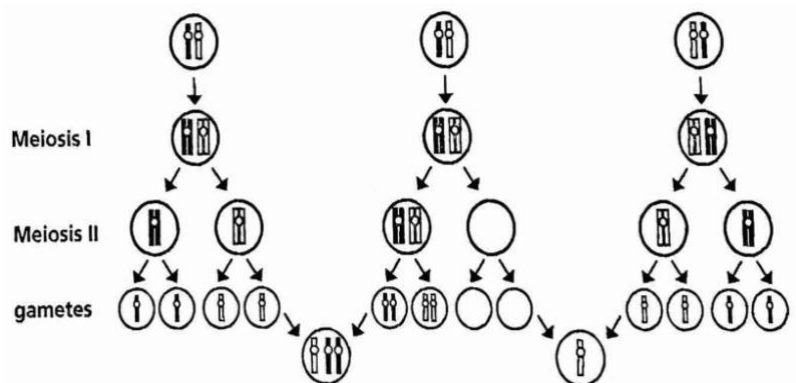

Figure 5. Formation of a trisomy (left) and a monosomy (right)

Another means through which abnormal karyotypes form is via translocation. There are two main types of translocation. In reciprocal translocation, parts of two nonhomologous chromosomes are exchanged. If the translocation is balanced and there is no loss of genetic material, then the individual with the translocation will be normal. However, the carrier of a reciprocal translocation may produce offspring with generally abnormal chromosomes. The other main type of translocation, known as Robertsonian translocation, occurs in acrocentric chromosomes. In such cases, the long arms of the chromosomes fuse to form one large chromosome, and the short arms fuse to form a smaller chromosome. The smaller chromosome typically is lost during cell division; however, the genetic information on the short arms of acrocentric chromosomes is often repeated elsewhere. Therefore, individuals with Robertsonian translocations usually are normal. Their offspring, however, are at increased risk of a trisomy or a monosomy. Deletion also produces abnormal karyotypes. Deletion occurs when a portion of a chromosome is lost. Generally, the size of the piece that is lost determines the severity of the genetic disorder. The loss of a larger piece of a chromosome has a greater effect than the loss of a short piece.

Cells may be obtained from various sources for karyotype analysis, including:
-blood.
-skin or other tissues.
-chorionic viii; (part of the placenta). Chorionic villus sampling (CVS) involves removing some of the chorionic villi so the cells can be analyzed. This test, which can be conducted at 10-13 weeks' gestation, carries a 41-2% risk of miscarriage.
-amniotic fluid. Amniotic fluid surrounds the fetus and contains fetal cells that have been shed. The process of withdrawing this fluid using a hollow needle is called amniocentesis. It is conducted at 14-20 weeks' gestation and carries a 1% or less risk of miscarriage.

Often a pregnant woman is offered a blood test to help identify certain chromosome disorders. Although the word "screening" is often used loosely as a synonym for "testing," the two are not identical and the maternal blood test is not used to create a karyotype for the fetus. The screening tests measure specific proteins found in the blood of pregnant women to identify who should be offered more extensive (and often more invasive and expensive) testing, such as amniocentesis to obtain fetal cells for karyotyping. The screening test is not 100% sensitive and often women who are carrying a normal fetus have an abnormal screening test and must deal with the stress of deciding whether or not to undergo amniocentesis. At the same time, in a small number of cases, truly abnormal pregnancies go undetected by the maternal blood screen.

Examples of Findings Commonly Identified by Karyotyping

Common Karyotype Findings	Associated Clinical Symptom
9/22 translocation	chronic myelogenous leukemia
5p deletion	cri du chat
22q11.2 deletion	22q11.2 deletion syndrome
trisomy 21	Down syndrome
trisomy 18	Edwards syndrome
XXY	Klinefelter syndrome
46 chromosomes (XX)	typical female
46 chromosomes (XY)	typical male
3p25q21 inversion (1 normal chr. 3, 1 inverted chr. 3)	no clinical symptoms present
9p11q12 inversion (1 normal chr. 9, 1 inverted chr. 9)	no clinical symptoms present
trisomy 13	Patau syndrome
14/21 translocation (1 normal chr 14, 1 chr. 14/21 translocation, 2 normal chr. 21)	Robertsonian translocation Down syndrome
monosomy X	Turner syndrome
trisomy X	XXX syndrome
XYY	47, XXY male

Pre-laboratory Questions

1. During the process of creating a karyotype, why are cells arrested in metaphase of mitosis?

2. How is the centromere positioned on Chromosome 1, Chromosome 9, and Chromosome 14?

3. If an individual has a trisomy of chromosome 18, how many chromosomes total does the person have?

4. If an individual has a monosomy of chromosome 12, how many chromosomes total does the person have?

5. Often a pregnant woman is offered a blood test to help identify certain chromosome disorders. Is this a karyotype? Explain.

6. If a human gamete possessing one extra chromosome participates in fertilization with a normal human gamete, what will be the result? How many chromosomes will the zygote have?

7. If a human gamete that is missing a chromosome participates in fertilization with a normal human gamete, what will be the result? How many chromosomes will the zygote have?

8. If nondisjunction occurs in humans for one pair of homologous chromosomes during meiosis I, will any normal gametes result? How many chromosomes will each gamete have?

9. If nondisjunction occurs in humans for sister chromatids of one chromosome during meiosis II, will any normal gametes result? What chromosome number would each gamete have?

10. Many genetic disorders are caused by errors (mutations) in the genes on our chromosomes. Usually, in disorders caused by chromosomal abnormalities, the genes themselves are not mutated; rather, there are too many or too few of them. Why do you think that having too many or too few normal genes create disorders?

LT: I can observe the anatomy of a human chromosome in order to construct a simulated karyotype on the basis of size, banding patterns, and centromere position.

Activity 1: Actual Karyotype Procedure
1. Record the letter printed on the top of the sheet, in the Data Table for Activity 1.
2. Cut out each rectangle that contains an image of a chromosome.
3. As you cut out each rectangle, note the banding pattern, size, and centromere position of the chromosome depicted.
4. Pair the chromosomes, matching those that have the same banding pattern, size, and centromere position.
5. Arrange the chromosome pairs by size from largest to smallest.
6. Place each chromosome in its correct location on the Karyotyping Mat. Remember, this is based on chromosome size and centromere position. Refer to the table in the Background section to help you identify chromosome groups A through G.
7. When you are certain you have determined the correct position for each of the chromosomes, glue or tape them to the Karyotype Mat.
8. Examine the karyotype that you created. In the Data Table, record the gender (male or female) the karyotype represents.
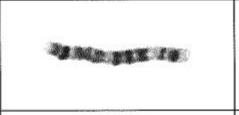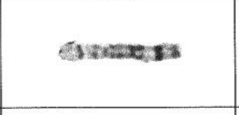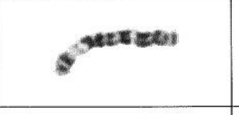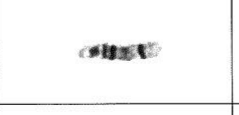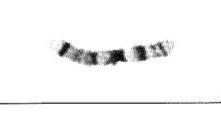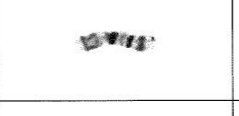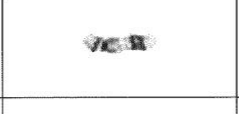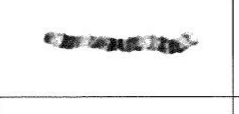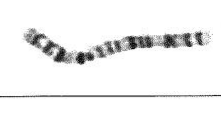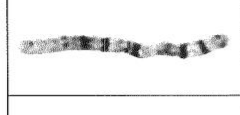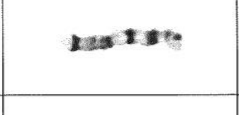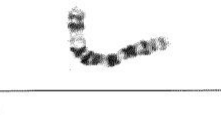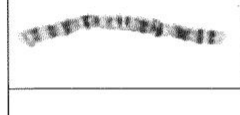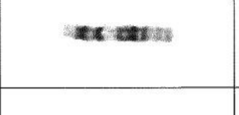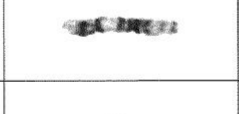
Activity 1	
Letter	
Number of Chromosomes	
Gender	
Is the karyotype normal?	
Disorder	

Karyotyping Mat

A

1 2 3 4 5

B

6 7 8 9 10 11 12

C

13 14 15 16 17 18

D

E

19 20 21 22

F

G

Sex Chromosomes

Sheet B

Chromosome images ©2013 Greenwood Genetic Center

LT: I can construct and analyze a simulated karyotype in order to diagnose patient disorders.

Activity 2: Simulated Karyotype Procedure

You and a partner will take on the role of a cytogeneticist working in a hospital. A case study will be given to you for review, along with a set of patient chromosomes. You and your partner will arrange the chromosomes into a completed karyotype on a prepared board. You will analyze the karyotype and diagnose your patient. Your patient may have one of the many types of recognized chromosomal abnormalities, though normal karyotypes are also represented. Be careful and use your observation skills - things are not always as simple as they seem.

1. You will receive a Chromoscan board containing a case study and set of patient chromosomes. Each case study has a Case ID (letters A-O) and a unique color. The color of the patient chromosomes matches the color that is printed around the case study section of the Chromoscan board. Confirm that the colors of the chromosomes and the board match.

2. Read the case study found on the left side of the board.

3. On the Cytogenetics Report, record patient information, including name, case ID, reason for referral, patient age, and source of cells.

4. Select a chromosome decal from the cryostorage area of the board and sketch it on your Cytogenetics Report, noting/labeling the centromere, telomere, and p and q arms. Note the centromere position and identify the chromosome as metacentric, submetacentric, or acrocentric.

5. To make the process of karyotype assembly less complex, one of each of the homologous chromosomes is already illustrated on the board. Identify the other homolog and place it on the board in the proper position.

6. Once the karyotype is completed, analyze it for chromosomal anomalies, paying particular attention to chromosome number and structure.

7. Record chromosome number, gender, and chromosomal findings on the Cytogenetics Report.

8. Determine the suggested diagnosis by looking at the table "Examples of Findings Commonly Identified by Karyotyping."

9. Complete the Cytogenetics Report on your patient.

10. At the end of the activity, return the chromosome decals to the cryostorage region of the Chromoscan board. Check carefully around your desks and lab tables to make sure all the chromosomes have been collected and returned to the board.
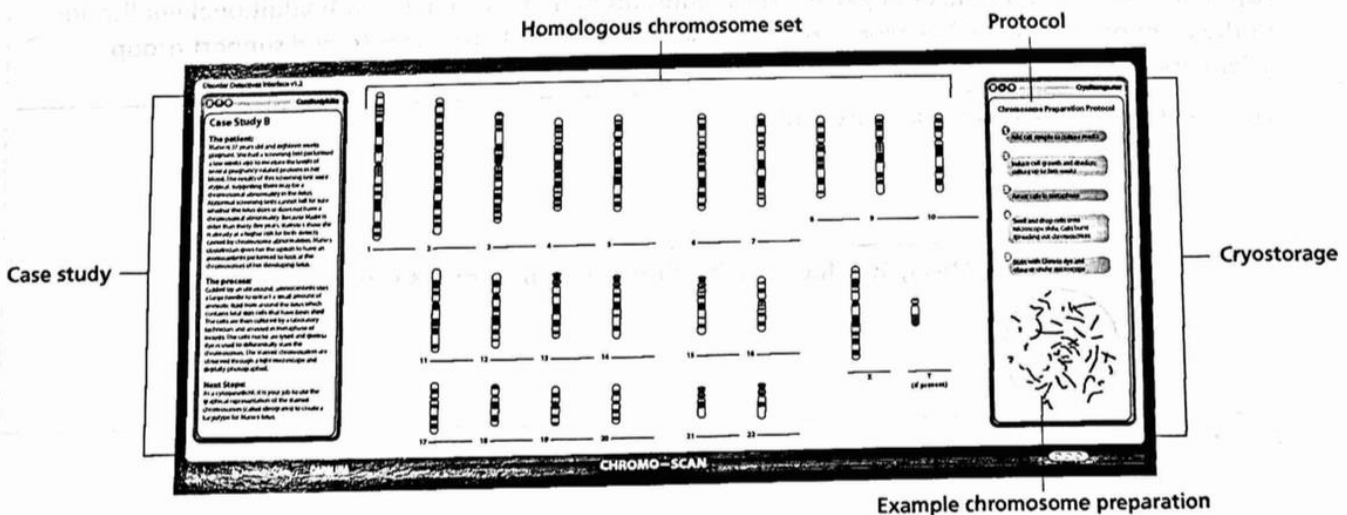
Cytogenetics Report for G-Banded Karyotype

Patient Name	Case Study ID	Age

Why is the patient being referred for karyotyping?	Source of Cells for Karyotyping _____ Blood _____ Amniocytes _____ Chorionic Viiii _____ Other (specify) _____

Select a chromosome from the cryostorage area. Sketch the chromosome, labeling the p arm, q arm, centromere, and telomere.
Chromosome type: _____ metacentric _____ submetacentric _____ acrocentric

Total Number of Chromosomes Observed	Gender

Chromosomal Findings ___no observable chromosomal abnormalities ___monosomy (chromosome # _____) ___trisomy (chromosome # _____) ___deletion (chromosome # _____, arm _____) ___insertion (chromosome #, arm _____) ___translocation (chromosome #s ____ & _____) ___inversion (chromosome # _____, arm(s) _____)	Patient Diagnosis

Briefly explain how the karyotype was prepared.

On the back of this paper, describe notes for patient's caregiver with additional implications of the diagnosis, including life expectancy, complications, available treatments, and support group information.

Table of Chromosomal Disorders

The following table lists the chromosomal disorders, along with the prevalence and common characteristics of each disorder. Not every individual with a given structural or numerical abnormality will display all the characteristics listed, and some may display symptoms not listed here.

Disorder (Common Synonyms)	Abnormality	Incidence	Common Characteristics
Cri-du-chat (Cat Cry) Syndrome (5p deletion) (5p-)	A portion of the 5p arm of a chromosome is missing.	1 in 20,000 to 50,000 live births	-intellectual disability -delayed development -during infancy, a cry that sounds much like a kitten's mew -distinctive facial features such as wide set eyes, low set ears, and a rounded face
Down Syndrome (Trisomy 21) (14/21 Translocation)	An additional chromosome 21 is present.	1 in 700 live births	-intellectual disability -speech defects -a stocky body type with a thick neck -distinctive facial features such as a round face, almond-shaped eyes, and a flattened profile -increased susceptibility to infection -heart and other organ abnormalities
Edwards Syndrome (Trisomy 18)	An additional chromosome 18 is present.	1 in 5,000 live births	slow growth before birth -low birth weight -abnormal growth of organs -heart defects -small, abnormally shaped head -90-95% mortality rate during the first year
Fragile X	A sequence on the X chromosome is repeated, causing one end to appear loose or broken off.	1 in 4,000 male live births; 1 in 8,000 female live births	-increased likelihood of learning disabilities -males often have a long, narrow face with large ears, flexible fingers, and enlarged testicles after puberty
Klinefelter's Syndrome (XXY)	An additional X chromosome is present in a male, producing an individual that is XXY.	1 in 500 to 1,000 male live births	-taller than normal -increased likelihood of low IQ -speech and language difficulties -high rate of sterility
Patau Syndrome (Trisomy 13)	An additional chromosome 13 is present.	1 in 16,000 live births	-a small head -heart defects -severe intellectual disability -cleft palate and/or lip -additional fingers and sometimes toes -malformations of the eyes and ears -brain or spinal cord abnormalities -90-95% mortality rate during the first year
Triple X (XXX)	An additional X chromosome is present in a female.	1 in 1,000 female live births	-increased likelihood of learning disabilities -delayed speech and language development -normal sexual development
Turner's Syndrome (XO)	An X chromosome is missing in a female.	1 in 2,500 female live births	-shorter than normal height -webbing of the neck -no underarm or pubic hair development -underdeveloped ovaries
XYY	An additional Y chromosome is present in a male.	1 in 1,000 male live births	-increased likelihood of learning disabilities -delayed speech and language development -normal sexual development