Chapter 3

Human Chromosome Analysis

Charles C. Tseng

Department of Biological Sciences
Purdue University-Calumet
Hammond, Indiana 46323-2094
(219) 989-2403

Charles received his B.S. from Taiwan Normal University, M.S. from National Taiwan University, and the Ph.D. from the University of California, Los Angeles. He is professor of biology at Purdue University Calumet, where he is in charge of biotechnology and graduate programs. He teaches cell biology, genetics, and molecular biology. His research is in cell and molecular biology. Currently, he is working on an NSF-supported project to develop a computer-based image analysis program for the life sciences.

Reprinted from: Tseng, C. C. 1995. Human Chromosome Analysis. Pages 33-56, *in* Tested studies for laboratory teaching, Volume 16 (C. A. Goldman, Editor). Proceedings of the 16th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 273 pages.

Contents

Introduction

The Human Chromosome Analysis Program is designed to provide students with a basic knowledge of human cytogenetics and a hands-on experience in chromosome analysis and karyotyping. The materials included in the package are sufficient for 16 students.

The program is divided into two main parts. Part 1 (Background), which covers basic human cytogenetics, should be reviewed with students prior to the laboratory exercise. An understanding of such key terms as homologous chromosomes; centromere; p arm and q arm; metacentric, submetacentric, very submetacentric, and acrocentric chromosomes; chromosome banding; and satellite is essential to the success of the laboratory.

Part 2 (Laboratory) consists of two sessions, each of which requires 2–3 hours. No blood is required, since the instructor will be provided with unstained metaphase slides prepared from cell culture. The laboratory is suitable for undergraduate students at all levels. Two options are available in Session 1. In option 1, students learn the standard Giemsa staining technique. They compare the banded and unbanded chromosomes under the microscope and learn to identify a few of the G-banded chromosomes. In option 2, students learn a G-banding technique commonly performed in clinical and research laboratories. In Session 2, students practice karyotyping using a dichotomous key to individual chromosomes. This key was developed specifically for teaching purposes. Six worksheets are provided for the laboratory exercises.

For advanced undergraduate students and graduate students who wish to do independent research projects, suggestions and protocols of specialized techniques, including various banding methods, the study of DNA replication and sister chromatid exchanges, and fluorescence *in situ* hybridization (FISH), are available upon request.

Background Information

Development of Human Cytogenetics

Cytogenetics, the study of chromosomes, originated more than a century ago. However, not until the last 30 years or so have human chromosome studies become a major field in the biomedical sciences. Chromosome banding methods, for example, are today's vital tools in clinical genetics and evolutionary studies. Furthermore, human cytogenetics coupled with molecular techniques has revolutionized the field of molecular genetics, including gene mapping and recombinant DNA technology.

Prior to 1956 the number of human chromosomes was estimated to be between 37 and 48. The difficulty of determining the exact chromosome number was due to the lack of proper techniques for cell culturing and chromosome spreading for microscope observation. In the 1950s two major breakthroughs led to rapid advances in human cytogenetics: (1) the use of phytohemagglutinin (PHA), a substance from plants, to stimulate cell division of lymphocytes (white blood cells) so that the number of cells in metaphase can be obtained, and (2) the use of hypotonic (low salt) solution to cause cells to absorb water and swell, resulting in the spreading and separating of the individual chromosomes to make them readily distinguishable. In 1956 researchers in Sweden systematically counted 46 chromosomes in human cells. Three years later, the discovery of an extra chromosome 21 in patients with Down syndrome marked the beginning of clinical application of chromosome studies. The identification of other chromosome disorders soon followed.

Conventional dyes such as aceto-orcein used for direct chromosome staining could only distinguish chromosomes according to their sizes and centromere positions. On the basis of these two criteria, human chromosomes were classified into seven groups: A through G. Accurate chromosome identification started in the late 1960s when quinocrine mustard (QM) was used to stain human chromosomes. With this technique chromosomes were differentiated under the fluorescence microscope into bright and dark regions called Q-bands. Since then, numerous banding techniques have been developed, of which G-banding is the most widely used technique for chromosome analysis. Banding techniques are extremely useful for the detection of structural changes. Clinical applications include the association of chromosome disorders with numerous diseases, including cancers.

Today, chromosome analysis of cultured amniotic fluid cells is routinely performed in medical genetics laboratories for prenatal diagnosis. For structural changes too small to be detected by routine cytogenetic techniques, other approaches, such as the use of a known DNA sequence (DNA probe) to hybridize the homologous DNA of the chromosome (*in situ* hybridization), are available. The integration of cytogenetics with molecular technology has thus opened up an exciting field in modern biology. For further reading see Barch (1991) and Verma and Babu (1989).

Chromosome Morphology

The Cell Cycle

Chromosomes are not visible under the light microscope in non-dividing (interphase) cells. As the cell begins to divide, the threads of chromatin (DNA-protein complex) in the nucleus begin to condense into multiple levels of coiled structures recognizable as chromosomes. There are two modes of cell division: mitosis and meiosis. Mitosis is responsible for the proliferation of body (somatic) cells, whereas meiosis is responsible for the production of gametes. Because mitotic cells are easy to obtain, morphological studies are generally based on mitotic metaphase chromosomes.

The transition of interphase to cell division (mitosis) and back to interphase is called the cell cycle. Interphase of a dividing cell is divided into three stages: G_1, S, and G_2, with G meaning gap. During the G_1 stage, cytoplasmic components such as membranes, organelles, and ribosomes begin to proliferate. G_1 is followed by the S stage during which DNA synthesis occurs and the amount of DNA per chromosome is doubled, resulting in two sister-chromatids visible during prophase and metaphase. S is followed by a second period of growth, the G_2 stage. At the end of G_2, the cell starts to divide, and enters the M (mitosis) stage. When the cell is not cycling, interphase is said to be in G_0. The cell cycle for human cells averages about 20 hours (8–10 hours for G_1, 6–8 hours for S, 2–4 hours for G_2, and 1 hour for M; see Figure 3.1).

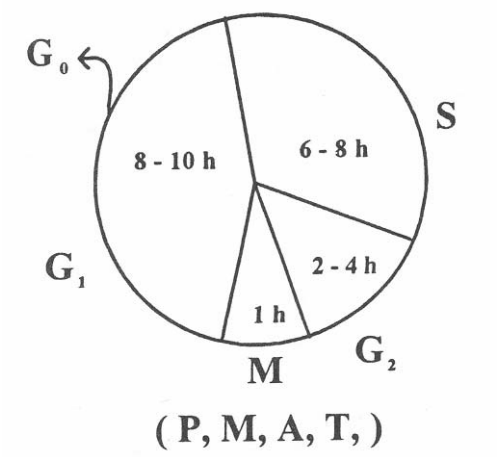


Figure 3.1. The cell cycle. Interphase is divided into G_1, S, and G_2 stages. Mitosis (M) phase is divided into prophase (P), metaphase (M), anaphase (A), and telophase (T).

Mitosis can be divided into four phases: prophase, metaphase, anaphase, and telophase. During prophase, chromosomes, consisting of two chromatids each, condense and undergo progressive coiling. In early prophase they appear as thin threads. During the middle of late prophase the chromosomes can be seen as discrete structures, each with two chromatids and a constricted area called the centromere where the two chromatids are joined. The transitional period from late prophase to metaphase is called prometaphase, during which the nuclear envelope breaks down and the chromosomes move toward the equatorial plane (metaphase plate) of the cell.

Chromosome Structure

At metaphase the chromosomes are at their most condensed state, with spindle fibers attaching to the area of the centromere called the kinetochore, forming pole-chromosome fibers. The fibers which bypass the centromeres are called pole-pole fibers. Anaphase begins with the division of the centromere and the separation of chromatids. Once separated, each chromatid is known as a chromosome. Telophase marks the final stage of mitosis, during which the cell undergoes a reversal of prophase, including uncoiling of the chromosomes, disappearance of the spindle fibers, and reappearance of the nuclear envelope and nucleolus. At the same time the cytoplasm is divided (cytokinesis) into two parts, forming two daughter cells.

The best mitotic stage for chromosome analysis is prometaphase or metaphase. A typical metaphase chromosome consists of two arms separated by a primary constriction or centromere. Each of the two sister-chromatids contains a highly coiled double helix of DNA. Often the sister-

chromatids are so close to each other that the whole chromosome appears as a single rod-like structure (see Worksheets 2 and 3). A chromosome may be characterized by its total length and the position of its centromere (Figure 3.2). A chromosome with the centromere at or near the middle is called metacentric. A submetacentric chromosome has a centromere somewhat displaced from the middle point. If the centromere is obviously off center (e.g., halfway between the middle and the tip of the chromosome), the term very submetacentric may be used. Acrocentric chromosomes have their centromeres very near one end. Telocentric chromosomes, which are absent in human cells, have their centromeres at the very tip of one end. The short chromosome arm is designated p (petite) and the long arm q (one letter after p). Certain human chromosomes may also contain a secondary constriction, which appears as an unstained gap (also called a satellite stalk) near the tip. The chromosomal segment distal to this gap appears as a satellite. Chromosome numbers 13–15 (D group) and 21–22 (G group) have satellites (Figure 3.2).

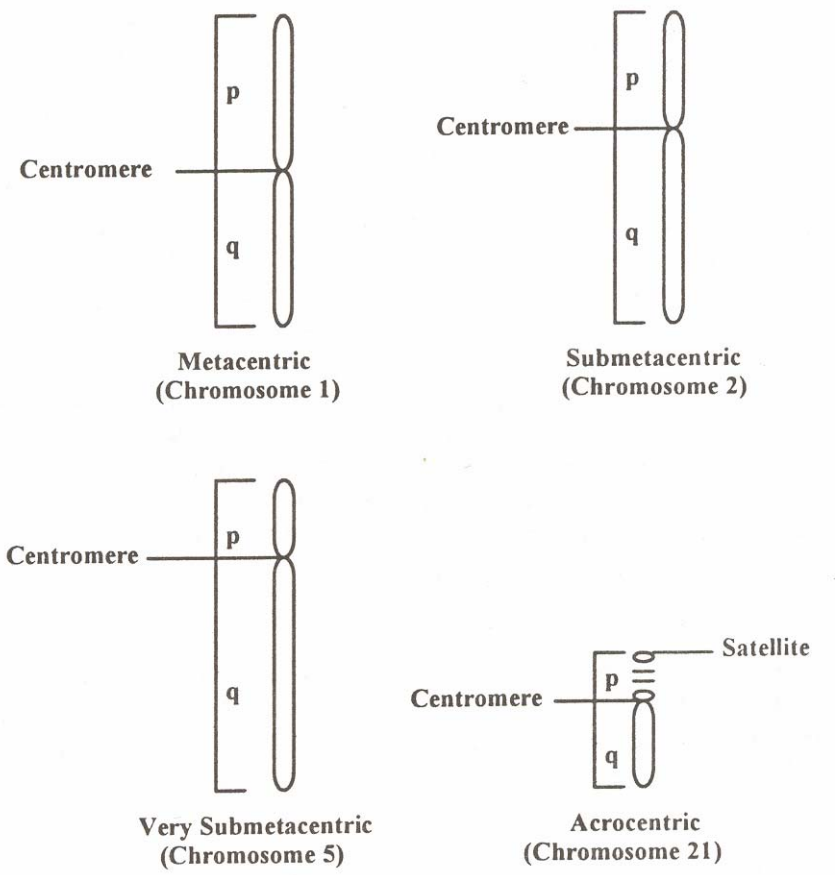


Figure 3.2. Chromosome morphology and terminology.

Chromosome Number

The diploid chromosome number is the number of chromosomes in the somatic cell and is designated by the symbol 2N. The gametes, which have one-half the diploid number, have the haploid number N. In humans the diploid number is 46, with 23 inherited from each parent through the sperm or egg. Same (homologous) chromosomes form a pair with one member from each parent. Thus, there are 23 pairs of chromosomes in human cells. Of these, 22 pairs are not directly involved in sex determination, and are known as autosomes. The remaining chromosome pair

consists of the sex chromosomes, and is directly involved in sex determination. In females the two sex chromosomes are identical (XX), whereas in males the two sex chromosomes are not identical (XY). The Y chromosome is smaller than the X chromosome. A gene which is responsible for switching the embryo to male characteristics was recently discovered on the Y chromosome.

Giemsa Banding

The purpose of learning banding techniques is to uniquely identify chromosomes or portions of chromosomes, both normal and abnormal. Most laboratories in North America use a form of Giemsa banding (G-banding) as a general-purpose banding method. Giemsa reagent is a DNA stain that consists of a mixture of dyes including the basic aminophenothiazine dyes (azure A, azure B, azure C, thionin, and methylene blue) and the acidic dye eosin. Prior to staining, some form of protease (an enzyme which digests proteins) treatment is needed. In this laboratory exercise students examine G-banded chromosomes by a standard pancreatin-Giemsa procedure. This technique produces patterns of light-staining (G-light) regions and dark-staining (G-dark) regions. The pattern is unique to each chromosome, and therefore serves as a landmark for chromosome identification.

The G-light bands are thought to be relatively GC-rich (rich in the DNA bases guanine and cytosine), and the G-dark bands relatively AT-rich (rich in the DNA bases adenine and thymine). Furthermore, the light bands represent the regions which are relatively open and which contain most of the genes, including housekeeping genes (genes active in every cell type). On the other hand, the G-dark bands represent regions which are relatively compact and contain few genes. The genes in the dark regions are mainly tissue-specific.

It should be noted that the pattern and number of light and dark bands changes with the stage of mitosis, ranging from 300 bands or fewer in late metaphase to more than 1000 bands in early prophase. As mitosis progresses, bands coalesce, accounting for the reduction of number of visible bands. Most routine lab work is done at the 350–400 band level. For critical analysis, special techniques, know as high resolution analysis, must be employed to produce 550 or more bands.

Karyotype

The term karyotype refers to a display of the chromosomes of a cell by lining them up, beginning with the largest and with the short arm oriented toward the top of the karyotyping sheet. In humans seven (A–G) groups of autosomes are recognized. Sex chromosomes (X,Y) are placed last. A diagram of the karyotype based on chromosome measurements in many cells is called an idiogram.

Nomenclature

Human chromosome nomenclature systems were developed during six international conferences on chromosome nomenclature. To write a karyotype description, work from left to right and leave no spaces. Separate each item with a comma unless otherwise specified.

The karyotype formula begins with the total number of chromosomes in the cell, followed by the notation of the sex chromosomes (first the Xs and then the Ys). For example, the formula for a normal male is "46,XY" and for a normal female "46,XX". An extra or a missing chromosome is designated with a "+" or "–" sign, respectively, before the number of chromosome. Thus, a male with trisomy (three chromosomes) for chromosome 18 is 47,XY,+18, and a female with a monosomy (one chromosome) for 22 is 45,XX,–22. Addition or deletion of a chromosome segment is denoted with a plus "+" or minus "–" sign after the symbol of the chromosome arm, respectively. For example, the formula for a female with the cri du chat syndrome (resemblance of the cry of an affected newborn to cat mewing) is 46,XX,5p– (missing a piece of the short arm of chromosome 5). The formula for a male with a translocation (exchange of chromosome segments) between chromosome 14 and 21 is 46,XY,t(14;21).

The following is a partial list of symbols for chromosome nomenclature:

A–G	The chromosome groups
1–22	The autosome numbers
X,Y	The sex chromosomes
del	Deletion
dup	Duplication
inv	Inversion
p	Short arm of chromosome
q	Long arm of chromosome
s	Satellite
t	Translocation
+/–	Placed before the chromosome number, it indicates addition (+) or deletion (–) of the whole chromosome (e.g., +21 denotes an extra chromosome 21, as in Down syndrome). Placed after the chromosome number, the symbol indicates addition or deletion of the chromosome part (e.g., 5p– indicates loss of part of the short arm of chromosome 5, as in the cri du chat syndrome).

Materials

The laboratory consists of two sessions of 2–3 hours per session. The following list is for 16 students working in pairs.

Unstained human chromosome slides (unmounted) (8)
G-banded human chromosome slides (mounted) (2)
Worksheet 1: Representative Field With Metaphase Spreads (16)
Worksheet 2: Metaphase Spread (16)
Worksheet 3: Karyotype (16)
Worksheet 4: Description of Human Chromosomes (16)
Worksheet 5: Key to Human Chromosomes (16)
Worksheet 6: Karyotype Sheet (16)
Microscopes (10)
Waterbath (37°C) (1)
Scissors (8)
Glue sticks (16)
Coplin jars (3)
Beakers, 500 ml (2)

Beakers, 200 ml (1)
Graduate cylinders, 100 ml (2)
Pasteur pipets with bulbs (2)
Forceps (8)

Giemsa stain (Fisher SG28-100) (2 ml)
Wright stain (Sigma WS-16) (4 ml)
Gurr Phosphate Buffer tablet, pH 6.8 (Bio/Medical Specialties, 33193) (1)
Hanks Balanced Salt solution (10X) (Gibco-BRL 14180-012) (4.5 ml)
Sodium bicarbonate, 7.5% (Sigma S-8875) (1 ml)
Pancreatin (Sigma P-1750) (50 mg)

Notes for the Instructor

Solution Preparation (for entire class)

1. Phosphate buffer (pH 6.8): Dissolve one Gurr Phosphate Buffer tablet in 100 ml of distilled water in a 200-ml beaker.
2. Working Staining Solution: Add 2 ml of Giemsa stain and 4 ml of Wright stain to 100 ml of phosphate buffer solution and mix. Pour 45 ml of this Working Staining Solution into each of the two coplin jars.
3. Pancreatin Solution for G-Banding (for Option 2 exercise only): Preset waterbath at 37°C. To make 1X Hanks solution, place 5 ml of Hanks solution (10X) and 40.5 ml of distilled water in a coplin jar. Stir to mix. Heat this solution to 37°C by placing the coplin jar in the waterbath (about 20 minutes). Add 50 mg pancreatin to 2 ml distilled water and mix. Add the pacreatin solution to the 1X Hanks solution and mix. Then, using a Pasteur pipet, carefully add 1 or more drops of 7.5% sodium bicarbonate solution, mixing after each drop to achieve orange-red color (not pink). The pH of this solution is critical. Keep this coplin jar in the waterbath at 37°C.

Setting Up Demonstration Slides

Prior to the start of the laboratory session, the instructor sets up two microscope stations, each with a good metaphase spread of a G-banded slide under the 100X (oil) objective.

Procedure

Two options are available for the class exercise. Option I involves the conventional Giemsa staining technique, and Option II involves the G-banding technique. Since the materials given above are sufficient for one option only, the instructor selects one of the two options for the class exercise.

Student Outline
Giemsa Staining or G-Banding
(Session 1)

Objectives

1. To obtain hands-on experience in chromosome staining or banding.
2. To study basic chromosome morphology microscopically.

3. To practice the use of landmarks (banding patterns) for identification of selected chromosomes under the microscope.

Conventional Giemsa Staining (Option I)

Staining of Human Chromosomes

1. Fill a 500 ml beaker with tap water for the entire class.
2. Students are divided into eight teams of two each. Each team receives one unstained slide. This slide is unmounted; do not touch the chromosome side of the slide!
3. Label with a pencil the group number (e.g. 1, 2) on the frosted end of the slide.
4. Place the unstained slide with forceps in the Giemsa staining solution in the coplin jar (four slides per jar; face the chromosome side of all slides in the same direction) for eight minutes at room temperature.
5. Take the slide out of the staining solution and wash it by dipping it several times in and out of the tap water in a 500 ml beaker.
6. Shake and drain the excess water with a paper towel on the back side of the slide. After air-drying, it is ready for microscope examination. (If a slide warmer or a hot oven is available, warm the slide for about one minute at 40–60°C to speed up the drying process.)

Examination Under the Microscope

1. Examine the slide with Giemsa stain: Observe the slide just prepared with a low power (10X) objective. Move the slide around until you find a representative field containing metaphase spreads. To become familiar with metaphase chromosomes under the 10X objective, use Worksheet 1 as a guide. Note that metaphase chromosomes are extremely small and occur with numerous interphase nuclei. Center the best metaphase spread and add a drop of immersion oil on the top of the slide. Observe with a high power (100X) objective. If the metaphase spread is not ideal for examination (e.g., individual chromosomes are not well spread and overlap with one another, or chromosomes are too long or too short), return to the low power objective to find a better field. Under oil immersion, examine a few long chromosomes and identify the location of the centromere of each.
2. Examine the demonstration slide of G-banded chromosomes: Take turns observing the light and dark bands along the chromosomes under a 100X objective on either of the two mounted slides set up by the instructor. Also identify the centromeres of a few of the long chromosomes.

Exercise

1. Diagram the longest chromosome you observed in the metaphase spread with Giemsa staining and label the following structures: centromere, p (short) arm, and q (long) arm.
2. Diagram the longest chromosome you observed in the demonstration slide of G-banded chromosomes. Your diagram should include all the light and dark bands along the chromosome. Again, identify and label: centromere, p arm, and q arm.
3. Compare the diagrams of the unbanded and G-banded chromosomes. Are they morphologically similar?

4. Using Worksheets 3 (Karyotype) and 4 (Description of Human Chromosomes) as references, identify your diagram of the longest G-banded chromosome. Is it chromosome 1 or 2 according to the banding landmarks? If it is neither, you have not selected the longest chromosome. Try again until you correctly identify chromosome 1 or 2.
5. Draw the smallest chromosome from the G-band slide. Using Worksheets 3 and 4 as references, identify the chromosome you just drew. It may be chromosome 21 or 22. Both of these have satellites at the p terminals. However, the satellite may not always be visible, depending on staining conditions. For a male, the smallest chromosome could be the Y chromosome which, however, does not have a satellite.
6. If time permits, you may wish to identify and draw a few more chromosomes for practice.

Giemsa Banding (Option II)

Pancreatin-Giemsa Staining and Microscope Examination

1. Students are divided into eight groups. Each group will receive an unstained chromosome slide. Do not touch the chromosome side of the slide, since it is unmounted!
   Note: Because pancreatin digestion time is critical to the success of G-banding, it is advisable to have one group of students to go through the following procedure steps (2–8) first. The result will be evaluated for quality of G-banding. If necessary, the pancreatin incubation time will be adjusted for the remaining seven groups according to the suggestions in procedure step 9 below. While one group is trying out the protocol, the others may take turns observing the demonstration slides with G-banded chromosomes. By this time the instructor will have set up the demonstration slides with good metaphase spreads under the high power (oil) objective.
2. Use a pencil to label the group number (e.g., 1, 2) on the frosted end of the unstained slide.
3. Take turns placing slides in a coplin jar with previously prepared pancreatin solution in the waterbath at 37°C. Incubate for 12 seconds.
4. Rinse the slide immediately in a 500 ml beaker of tap water by dipping the slide in and out of water several times.
5. Shake and drain the excess water and place the slide in the Giemsa stain solution (3 or 4 slides per coplin jar, facing the chromosome side in the same direction). Stain the chromosomes for 8–10 minutes.
6. Rinse the slide thoroughly in a new 500 ml beaker of tap water by dipping it in and out of the water several times.
7. Shake and drain the slide with a paper towel on the back side of the slide.
8. After air-drying or heating the slide on a slide warmer (40–60°C) for 1 minute, observe the slide with a low power (10X) objective. Scan the slide until you find a representative field containing good metaphase spreads.

   Using Worksheet 1 as a guide, center the best metaphase spread and add a drop of immersion oil on the top of the slide. Observe with a high power (100X) objective. If the metaphase is not ideal for examination (e.g., individual chromosomes are not well spread and overlap extensively, or chromosomes are too long or too short), return to the lower power objective to find a better field. Under oil immersion, evaluate the quality of G-banding (see step 9 below).

9. Fuzzy chromosomes indicate that the time of pancreatin incubation was too long (over-digestion). Chromosomes with few bands indicate that the incubation time was too short. It is important to examine several metaphase spreads and to select the best one for analysis.

   If all or most of the chromosomes are over-digested, reduce the pancreatin time to 10 seconds or less for the next slides. If few bands are found in all or most of the chromosomes, increase the pancreatin incubation time to 20 seconds or more (certain slides may require an incubation period of 40 seconds or more). Use the demonstration slide and Worksheets 2 and 3 as references for high quality banding.

10. Take turns repeating the same procedure above for the remaining slides with the adjusted pancreatin incubation time. No adjustment is necessary if the first slide shows good quality G-banding.

Exercise

1. Diagram the longest chromosome from the slide you prepared (or from the demonstration slide if your slide is unsuitable). Your diagram should include all the light and dark bands along the chromosome. Also, label the following: centromere, p arm, and q arm.
2. Using Worksheet 3 (Karyotype) in conjunction with Worksheet 4 (Description of Human Chromosomes) as references, identify your diagram of the G-banded chromosome. Is it chromosome 1 or 2 according to the banding landmarks? If it is neither, you have not selected the longest chromosome. Try again until you correctly identify chromosome 1 or 2.
3. Draw the smallest chromosome from the G-banded slide. Again using Worksheets 3 and 4 as references, identify the chromosome you just drew. It may be chromosome 21 or 22. Both of these have satellites at the p terminals. However, the visibility of the satellite depends on staining conditions. For a male the smallest chromosome could be the Y chromosome which, however, lacks a satellite.
4. If time permits, you may draw and identify a few more chromosomes for practice.

Karyotyping Using a Dichotomous Key (Session 2)

Objectives

1. To learn the human chromosome classification system.
2. To use G-banding landmarks to identify individual chromosomes.
3. To learn the karyotyping procedure.

Procedure

1. Review Worksheet 3 (Karyotype) in conjunction with Worksheet 4 (Description of Human Chromosomes). First, familiarize yourself with such terms as metacentric, submetacentric, very submetacentric, acrocentric; p arm, q arm; proximal and distal regions of a chromosome arm; and satellite (see also Figure 3.2). Then study the banding pattern of each chromosome as well as the grouping of chromosomes from A to G (based on size: X chromosomes belong to the C group and Y chromosomes belong to the G group). Note that groups D and G are the only two groups having acrocentric chromosomes with satellites.
2. Count the total number of chromosomes in Worksheet 2 (Metaphase Spread).
3. Use scissors to cut out each chromosome from the metaphase spread.
4. Arrange all individual chromosomes according to size, the longest one first, on a smooth surface such as a clean lab bench.
5. Group similar chromosomes as pairs if possible.
6. Read the first two paragraphs (instructions) of Worksheet 5 (Key to Human Chromosomes). Then key out each chromosome according to the instructions.
7. When some of the chromosomes are identified, place them on the Karyotype sheet (Worksheet 6). Check and double check for accuracy, using the prepared Karyotype (Worksheet 3) as reference. Critically identify the location of the centromeres for each chromosome and be sure that the p arm is up and the q arm is down; upside-down chromosomes are frequent sources of misidentification. Remove and replace misidentified chromosomes with the correct ones.
8. Check with your instructor for accuracy. When all the chromosomes are correctly identified, arrange them neatly in the same manner as on Worksheet 3. Use a glue stick to glue each chromosome cutout firmly on the Karyotype Sheet.
9. Write on the top of the Worksheet: 46,XX if it is a female, and 46,XY if it is a male.

Questions

mild. 1. Write a formula for:

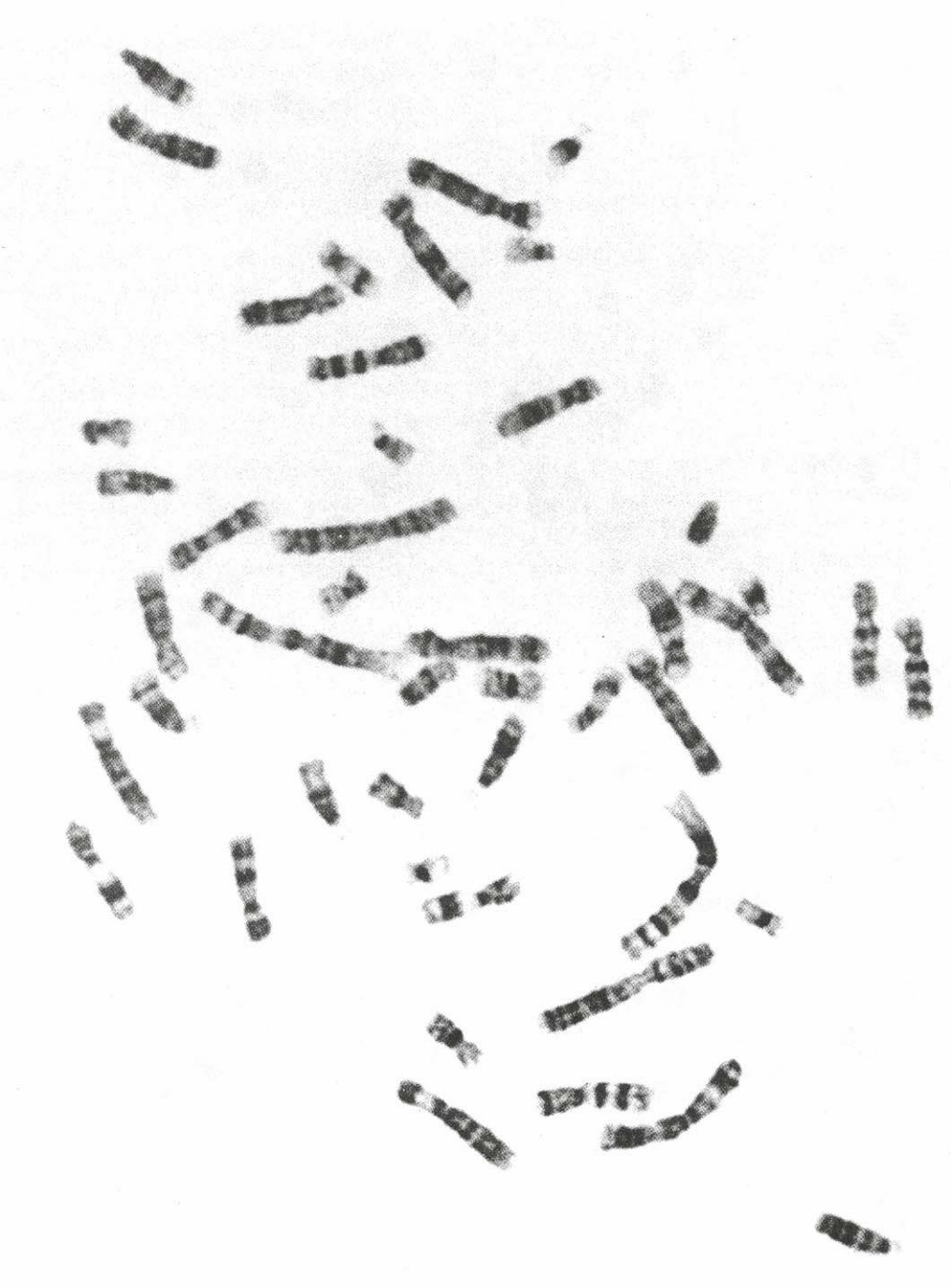
   (a) a male with Down syndrome (due to the presence of an extra chromosome 21),
   (b) a female with Turner Syndrome (only one X chromosome),
   (c) a female with cri du chat syndrome (deletion of a portion of the p arm of chromosome 5,
   (d) a male with Klinefelter Syndrome (two X chromosomes and one Y chromosome), and
   (e) a female with a Philadelphia chromosome (translocation between chromosomes 9 and 22). People suffering from a form of leukemia (chronic myeloid leukemia) frequently harbor cells containing the Philadelphia chromosome.

2. Abnormalities in chromosome number are detrimental and usually lethal. Speculate as to why the impact of abnormal numbers of chromosome 21 and sex chromosomes is relatively

WORKSHEET 1

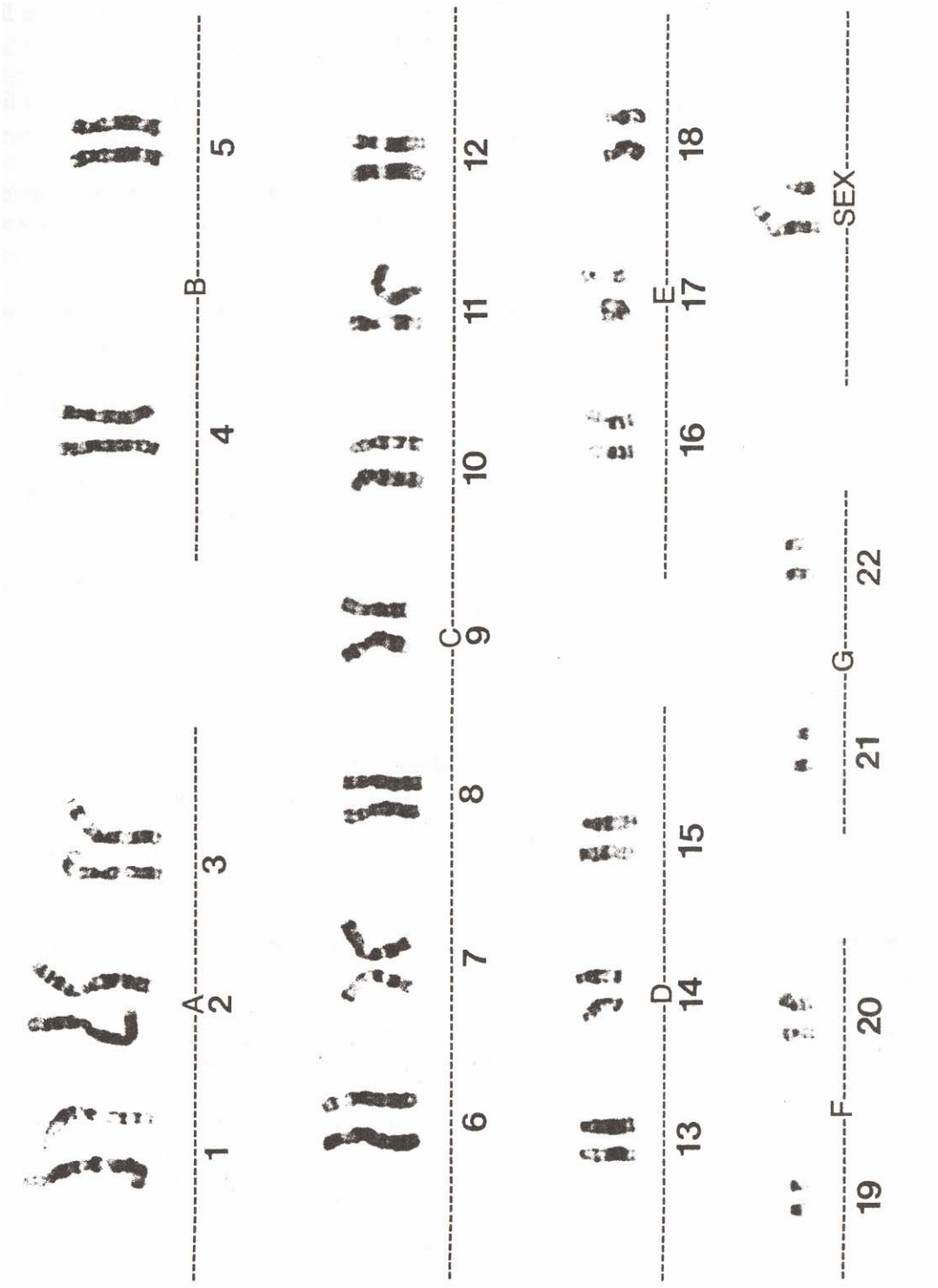
Representative Field with Metaphase Spreads

(Enlarged picture taken under 10X objective)



WORKSHEET 2
Metaphase Spread

WORKSHEET 3
Karyotype 46,XY



WORKSHEET 4
Description of Human Chromosomes

Chromosome 1 (Group A): The longest chromosome, metacentric; p arm fading at distal end, with at least two dark bands above centromere; q arm with a dark area just below centromere, a light band below, followed by four dark bands of which the middle two are often more intense.

Chromosome 2 (Group A): Almost as long as chromosome 1, submetacentric; p arm without light distal end, with four dark bands; q arm with more than four dark bands.

Chromosome 3 (Group A): Metacentric; both p and q arms with a large light band in the middle; dark area (consisting of two or three close dark bands) distal to the light band of q arm thicker than its counterpart in p arm.

Chromosome 4 (Group B): Very submetacentric; p arm with a thick dark band which may sometimes be resolved as double; q arm with a distinct, prominent dark band below centromere.

Chromosome 5 (Group B): Very submetacentric; p arm with a single dark band; q arm without a prominent dark band below centromere, with three close dark bands in the middle.

Chromosome 6 (Group C): Submetacentric; p arm with a distinct, large light band in the middle; q arm with several dark bands.

Chromosome 7 (Group C): Submetacentric; p arm with a prominent flat dark band at the top; q arm with two distinct dark bands followed by a much less intense dark band.

Chromosome 8 (Group C): Very submetacentric; p arm with two dark bands (sometimes unclear); q arm with two (sometimes three) dark bands, the distal dark band characteristically more intense.

Chromosome 9 (Group C): Varying from slightly submetacentric to very submetacentric; p arm with a broad (often square in shape) dark band (which may be resolved as double); q arm with a variable, proximal light area (from as long as the p arm to almost non-existent) followed by two distinct, equally intense broad dark bands with a light band between them; these three bands (two dark and one light) equal in thickness.

Chromosome 10 (Group C): Submetacentric; p arm with a dark band; q arm with three distinct dark bands, the proximal dark band most intense.

Chromosome 11 (Group C): Submetacentric; p arm with one or occasionally two dark bands; q arm with a very large light area followed by a dark band which may be resolved into two dark bands.

Chromosome 12 (Group C): Very submetacentric; p arm with a dark band; q arm with a proximal light band (which is relatively thinner than the counterpart of chromosome 11) followed by a dark area in the middle which may be resolved into two, or more often three, bands.

Chromosome 13 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with several dark bands.

Chromosome 14 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with a distal dark band which is more intense than other dark bands.

Chromosome 15 (Group D): Acrocentric; p arm with satellites (visible or invisible); q arm with dark proximal half and light distal half.

Chromosome 16 (Group E): Metacentric or occasionally submetacentric; p arm light or slightly dark; q arm with a prominent dark band just below centromere, followed by one or two lesser dark bands.

Chromosome 17 (Group E): Submetacentric or very submetacentric; p arm with a thin dark band (may be invisible); centromere dark followed by a distinct, large light band occupying a large part of q arm, q arm with one or two distinct dark bands near the terminal end.

Chromosome 18 (Group E): Submetacentric or very submetacentric; p arm often uniformly dark; q arm with two distinct dark bands, one proximal and one distal; the light band between these two dark bands slightly smaller than the counterpart of chromosome 17.

Chromosome 19 (Group F): Metacentric; p and q arms both light; centromere dark.

Chromosome 20 (Group F): Metacentric; p arm dark; q arm light, with two narrow, less intense dark bands.

Chromosome 21 (Group G): Acrocentric; p arm with satellites (visible or invisible); q arm with intense dark area proximally, fading out toward distal end.

Chromosome 22 (Group G): Acrocentric; p arm with satellites (visible or invisible); centromere dark; q arm with a narrow, less intense dark band.

X Chromosome (sex chromosome): Submetacentric; p arm with distinct, strong dark band at the middle; q arm with a proximal dark band about the same distance from the centromere as the p arm dark band, with two less intense dark bands near distal end.

Y Chromosome (sex chromosome): Very submetacentric; p arm very short or invisible; q arm dark throughout.

WORKSHEET 5
Key to Human Chromosomes

Prior to using the following key, review Figure 3.2 in to familiarize yourself with the basic chromosome morphology and terminology. Then cut out individual chromosomes from Worksheet 2 (Metaphase Spread) with a pair of scissors and arrange them according to size with number one being the largest (longest). Keying starts by searching for the largest chromosomes first and then proceeding to the smaller ones. The key is dichotomous. For keying every chromosome in each step, select one of the two choices (e.g., between A1 and A2, B1 and B2, etc.) until a particular chromosome number is reached.

The initial step is to divide all the chromosomes into two size classes. Class I consists of the first 12 chromosome pairs (or 24 individual chromosomes) plus the X chromosome(s). Thus, Class I may consist of 26 chromosomes if it is a female or 25 chromosomes if it is a male. Class II comprises the remaining small chromosomes (chromosomes 13–22) plus a Y chromosome (if a male). Although you do not know yet whether the metaphase spread is from a male or a female, you still can determine the exact number (25 or 26) of chromosomes in Class I by examining carefully the smallest chromosomes in this class. They should not be acrocentric. On the other hand, Class II begins with acrocentric chromosomes. In fact, the largest three chromosome pairs (13–15) in Class II are acrocentric.

Class I

A1. Longest two pairs, metacentric or submetacentric (Group A).

- B1. Metacentric; p arm fading at distal end; q arm with a dark area (sometimes triangular in shape) just below centromere, a light band below, followed by four distinct dark bands, of which the middle two bands are often more intense 1
- B2. Submetacentric; p arm without lighter distal end, with four dark bands; q arm with more than four dark bands 2

A2. Not the longest two pairs.

- B1. Second longest three pairs (chromosomes 3–5); metacentric or very submetacentric (Groups A and B); if metacentric, both p and q arms with a large light band in the middle; if very submetacentric, p arm never with a distinct, large light band in the middle.
  - C1. Metacentric; p arm with a large light band in the middle; q arm similar, also with a light band at the middle; the distal dark area is thicker than the distal dark area in p arm 3
  - C2. Very submetacentric.
    - D1. p arm with a thick dark band in the middle which may be resolved as double; q arm with a distinct, prominent dark band below centromere ... 4
    - D2. p arm with a dark band which appears single; q arm without a prominent dark band just below centromere, with three close dark bands in the middle 5

B2. Not one of the first longest five pairs (Group C); submetacentric or very submetacentric; the longest chromosome in this group having a distinct, large light band in the middle of p arm.

C1. p arm with a distinct, large light band in the middle 6

C2. p arm without a distinct, large light band in the middle.

D1. p arm with a prominent flat dark band at the top; q arm with two distinct dark bands followed by a less intense dark band at the distal end.....7

D2. p arm without a distinct flat dark band terminally.

E1. Two distinct, intense dark bands (one in p arm and the other in q arm) widely spaced and equally distant from the centromere area; q arm with two lesser dark bands near the distal end X

E2. Two distinct, intense dark bands (one in p arm, one in q arm) spaced equidistantly to the centromere are absent.

F1. Upper q arm with a distinct, large light band (proximal band).

G1. Lower q arm with two intense dark bands and a light band between them. These three bands (two dark and one light) equal in thickness.....9

G2. Lower q arm without the two intense dark bands and the sandwiched light band described in G1.

H1.q arm with a very broad proximal light band, followed by a central dark band which may be resolved as double 11

H2. The proximal light band of q arm narrower than that of chromosome 11, followed by a dark area which may be resolved into several parts..... 12

F2. q arm without a large light band proximally.

G1. q arm with 3 distinct dark bands, the proximal one may be slightly more intense than the other two 10

G2. q arm with two (occasionally three) dark bands, the proximal one not more intense.

H1.q arm with two (occasionally three) dark bands, the distal band more intense; p arm with a dark area which may be resolved into two bands with a light band between them..... 8

H2. q arm with two equally thick, dark bands of same intensity; p arm dark overall or with two close dark bands 9

Class II

A1. Largest three pairs in class II; acrocentric with satellites which may or may not be visible (Group D).

B1. q arm with a distal dark band which is more intense than other dark bands..... 14

B2. q arm without the most intense distal dark band.

C1. q arm with several dark bands 13

C2. q arm with dark bands on the upper half, the lower half lighter..... 15

A2. Not the largest three chromosome pairs in class II; significantly shorter than 1/2 the length of chromosome 1 or 2.

B1. Smallest two pairs of chromosomes (21 and 22); acrocentric, with satellite (Group G).

C1. q arm with an intense, broad dark band, the intensity gradually fading out to almost invisible light, distal region 21

C2. Centromere dark; q arm with a narrow, dark band 22

B2. Chromosomes without satellites, not truly acrocentric.

C1. Metacentric, or occasionally submetacentric (Groups E and F).

D1. Centromere dark; p and q arms equal in length, very light; occasionally the distal end of p arm may not be visible, appearing much shorter than q arm 19

D2. p and q arms never both light.

E1. p arm with a dark terminal band; q arm with two less intense dark bands which may be inconspicuous 20

E2. Metacentric or occasionally submetacentric; longer than chromosomes 19 and 20 and close to chromosomes 13–15 in size, p arm light or slightly dark, q arm with a prominent dark band just below centromere, followed by one or two lesser dark bands 16

C2. Submetacentric to very submetacentric (Groups F and G); (The Y chromosome may occasionally be almost acrocentric).

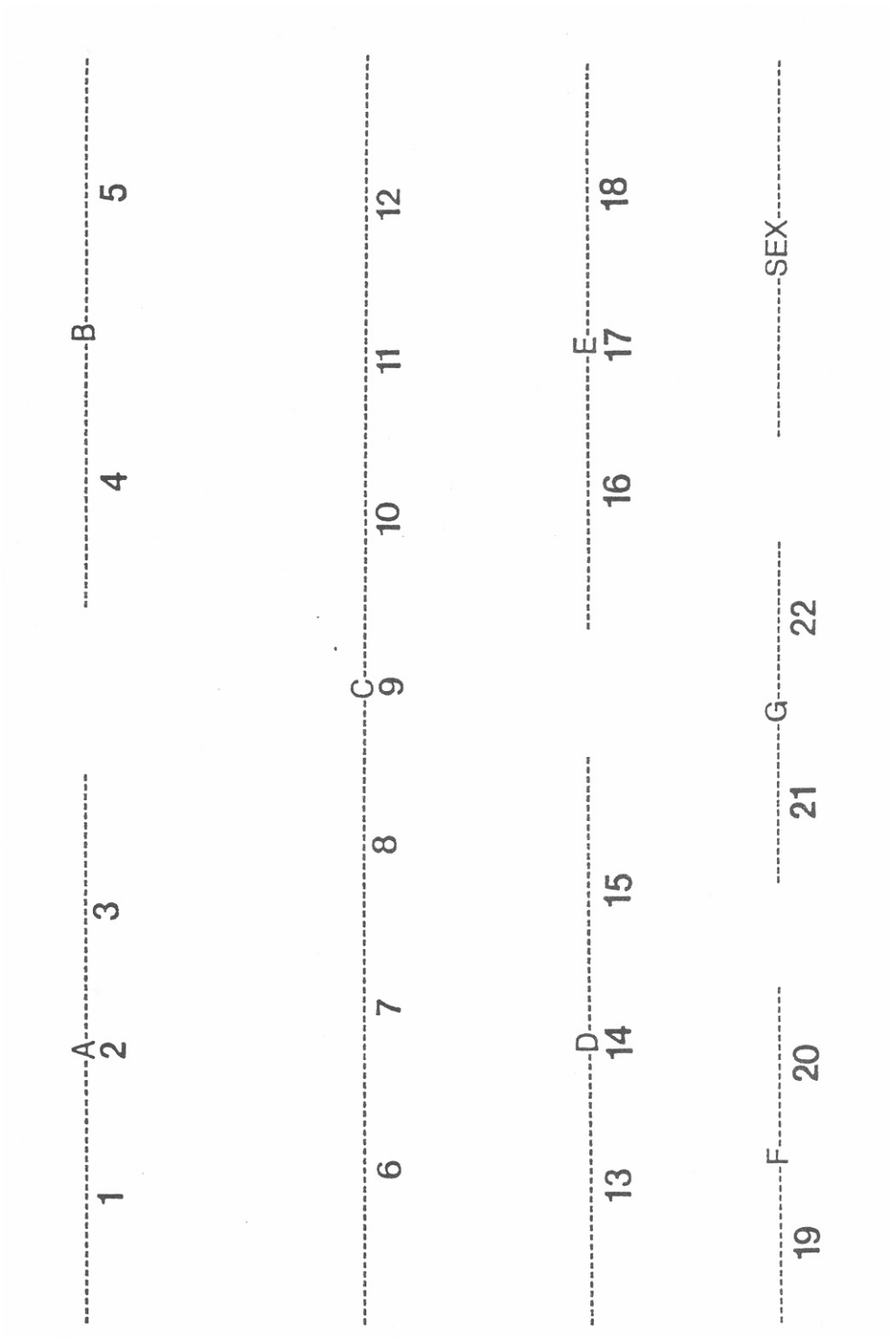
D1. Among the smallest chromosomes (close to chromosomes 21 and 22 in size); p arm short; q arm dark ..... Y

D2. Considerably larger than the smallest chromosomes (21 and 22); q arm with a distinct, light band.

E1. Centromere dark, followed by a large light band on q arm 17

E2. q arm with 2 dark bands, one just below the centromere and the other close to the q terminal, separated by a light band which is narrower than the light band of chromosome 17 18

WORKSHEET 6
Karyotype Sheet



Literature Cited

Barch, M. J. (Editor.) 1991. The ACT cytogenetics laboratory manual. Second edition. Raven Press, New York, 625 pages.

Verma, R. S., and A. Babu. 1989. Human chromosomes. Pergamon Press, Elmsford, 240 pages.

APPENDIX A
Answers to Questions

1. (a) 47,XY,+21
   (b) 45,X
   (c) 46,XX,5p−
   (d) 47,XXY
   (e) 46,XX,t (9;22)

2. Proteins are gene products. A cell can function properly only when the protein concentrations are within certain ranges. In the case of Down syndrome (trisomy 21), the increased protein levels due to the presence of an extra chromosome 21 are apparently tolerated better than those encoded by genes of other chromosomes. Furthermore, chromosome 21 is one of the smallest chromosomes, accounting for only 1.5% of total nuclear DNA and for a relatively small number of genes.

   Extra X chromosomes have milder effects because only one X chromosome is active per cell. The additional X chromosomes are genetically inactivated very early in embryonic development. Thus, regardless of the number of X chromosomes (e.g., X, XX, XXX), only one X is genetically active. Inactivation tends to minimize the phenotypic effects of extra chromosomes, but there are still some effects due to groups of genes near the tip of the short arm that are not inactivated. Since most genes on the X chromosomes encode somatic functions, the inactivation equalizes the number of active copies of X-linked genes in females and males, a phenomenon known as dosage compensation.

   The relatively mild effect of variation of Y chromosome number is due to the fact that genes on the Y chromosome do not encode somatic functions.

APPENDIX B
Numbered Chromosomes for Worksheet 2