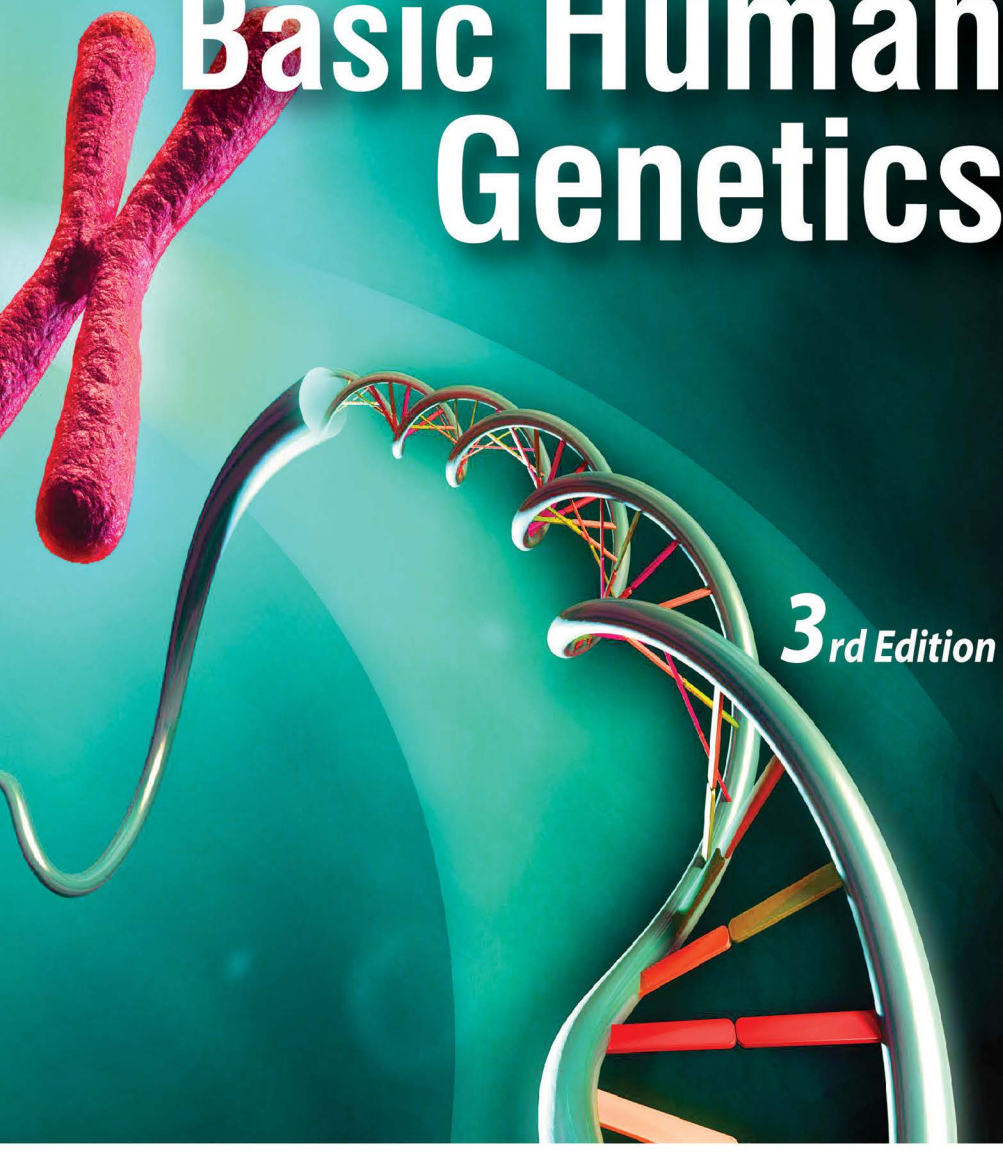
Kapur & Suri's

Basic Human Genetics



3rd Edition

Revised & Edited by

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Chromosomes

Greek: Chromos = colored, Soma = body

INTRODUCTION

Chromosomes are the vehicles of heredity. During interphase of the cell cycle, they are coiled in the form of chromatin threads. However, during cell division, they become highly condensed and are then visible as dark distinct rod-like basophilic structures.

The term 'chromosome' was introduced into the scientific vocabulary by Waldeyer in 1888.

Number

The number of chromosomes in each cell is fixed for a particular species. In human beings, it is 46. This is called Diploid Number (2n). However, in spermatozoa and ova, the number of chromosomes is only half the diploid number, i.e. 23. This is called Haploid Number (n). These 46 chromosomes are arranged in the form of 23 pairs.

Types

One member of each pair of chromosomes is derived from the mother and the other from the father. Twenty-two out of these 23 pairs are identical in both the sexes and are known as Autosomes. The chromosomes in the remaining pair are called sex chromosomes. In females, both the sex chromosomes are identical and are called X-chromosomes. However, in males, the two sex chromosomes are not identical and are called X and Y chromosomes. Therefore, female germ cell always has X chromosome, whereas a male germ cell may either have an X or Y chromosome.

It is evident from Table 2.1 that if an ovum is fertilized by a sperm carrying X chromosome, it will result in a female child and, if fertilized by a sperm carrying Y chromosome, it will result in a male child. So, it is worth noticing that the sex of the child is determined by the father.

Table 2.1: Determination of sex

	Female Gamete		*Male Gamete*	*Fertilized Ovum*	*Child*
Sex chromosome	X	+	Y	= XY	Male
Sex chromosome	X	+	X	= XX	Female

Structure (Fig. 2.1)

Each chromosome is made up of two rod-shaped structures called chromatids. These chromatids are identical and lie parallel to each other. The two chromatids are united with each other at a pale-staining area called the centromere (Primary Constriction). The centromere divides each chromatid into two arms and is associated with the formation of spindles and chromosomal movements during cell division (Fig. 2.1). The free ends of the chromatids are known as telomere, which when intact, do not permit fusion with the adjacent chromosomes.

In certain chromosomes, another narrowing known as secondary constriction exists near one end of each chromatid. The part of chromatid beyond the secondary constriction looks like satellites. So, the chromosomes with such satellite bodies are known as SAT-chromosomes. These constrictions are said to be concerned with the formation of nucleoli. This is why they are also known as Nucleolar Organizers.

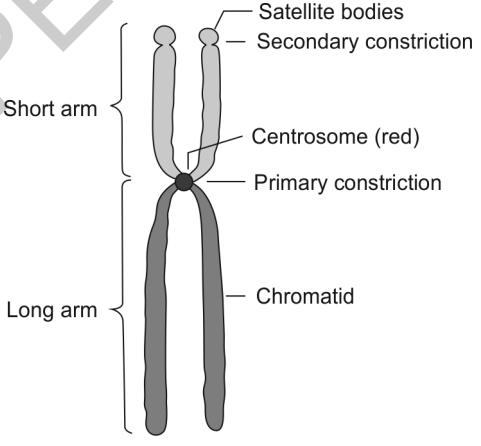


Fig. 2.1: Structure of a chromosome

Chromosomes may assume various shapes like twisted, spiral, curved or rod-like. Most of the chromosomes in human beings vary from 4 to 6 microns in length. They are the shortest during the metaphase of cell division.

Chemical Composition

The chemical constituents of chromosomes are:

- *Deoxyribonucleic acid (DNA)*: DNA is the most essential and stable molecular constituent of chromosomes. It is made up of deoxyribose sugar molecule and nucleotides. Each chromosome contains a single continuous double-stranded DNA molecule.
- *Ribose nucleic acid (RNA)*: Single-stranded structure having ribose as a sugar molecule.
- *Histones*: They are the basic proteins rich in arginine and lysine. They are aggregated along the DNA strand, which is coiled around each particle to form a complex body known as nucleosomes having 4 histones.
- *Acidic proteins*: They are nonhistone proteins and form many enzymes, e.g. DNA polymerase and RNA polymerase.

Significance

- Each cell of the body inherits from the fertilized ovum all the instructions necessary for proper organization and working of the various tissues and organs. Each chromosome bears on itself a large number of structures called genes; which guide the performance of particular cellular functions. This encyclopedia of information is stored within the chromosomes of each cell. Thus, each complete diploid set of chromosomes contain the cell's hereditary instruction or genome. The chromosomal threads bearing these instructions are known as Chromonemata or Genonemata.
- Cell activity is controlled by chromosomes which act by deciding the types of proteins synthesized within the cells.

Classification

Chromosomes can be classified on the basis of:

- Position of centromere
- Number of centromere
- Depending on function
- According to Denver system.

According to Position of Centromere (Fig 2.2)

- *Metacentric*: It is a chromosome with a centromere located in the middle of a chromosome. As a result of this, the two arms are almost equal.
- *Submetacentric*: It is a chromosome, with a centromere located slightly away from the mid-point. Consequently, the two arms are unequal. The longer arm is known as 'q' arm and the shorter arm is known as 'p' arm.
- *Acrocentric*: In this type of chromosomes, the centromere occupies subterminal position. One arm is very long and the other is short.
- *Telocentric*: It is a chromosome with a terminal centromere. Each chromatid, therefore, has one arm only.

According to Number of Centromere

- *Monocentric*: Having one centromere only, which is usual and normal.

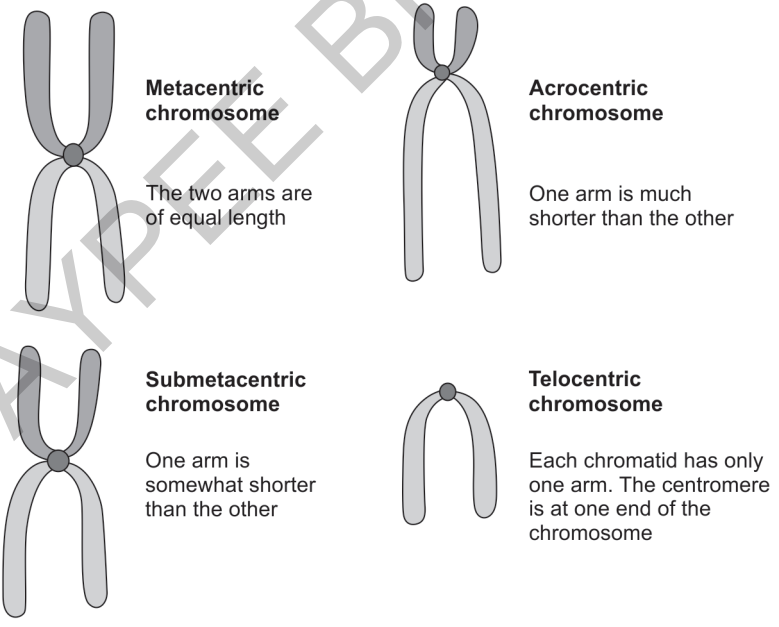


Fig. 2.2: Types of chromosomes

- *Dicentric*: Having two centromeres, which is found in some species of wheat.
- *Polycentric*: Having more than two centromeres seen in some forms of roundworms.
- *A-centric*: It represents only a fragment of chromosome having no centromere. It is not viable.

Depending on Function

- *Autosomes*: In man, there are 22 pairs of autosomes which are responsible for the determination of body parts and their functions.
- *Sex chromosomes*: There is one pair of sex chromosome in each sex. In males, it is XY and in females, it is XX. The sex chromosomes are responsible for the determination of sexes and their functions.

According to Denver System

In 1960, at a conference of Geneticists at Denver, human chromosomes including sex chromosomes are arranged into seven groups (from I to VII) depending on the size of chromosomes, which is popularly known as Denver system. According to modern convention, chromosomes are subdivided into Group-A to Group-G depending on the position of the centromere. This grouping is known as Patau's modification of Denver method. (Details of each group will be dealt with karyotyping).

Methods of Study

- To study the complete chromosome complement of an individual.
  - Karyotype preparation
  - FISH (Fluorescent *in situ* hybridization)
- To study sex chromosome constitution of a person, the following methods are available:
  - Study of Sex Chromatin or Barr Body
  - Study of Fluorescent Bodies in Buccal Smear
  - Study of Drumsticks in Polymorphonuclear Leukocytes

To study the complete chromosome complement:

KARYOTYPE

Karyotype is a complete chromosome set of a somatic cell. It also refers to a photomicrograph of an individual's chromosomes arranged in a standard manner.

Karyotyping

It is a process by which a karyotype is obtained.

Specimen

Chromosomes can be studied from the different tissues of the body. The basic principle involving cytogenetic preparations remain same for all tissues with slight modifications.

- Peripheral blood—most commonly used
- Skin fibroblast
- Bone marrow
- Chorionic villi
- Amniotic fluid cells } For prenatal diagnosis
- Fetal blood.

Chromosome analysis requires the provision of a large number of cells which are actively dividing. This is because it is only during critical stages of mitotic or meiotic cycle that chromosomes are in a suitable state to study.

Procedure (Fig. 2.3)

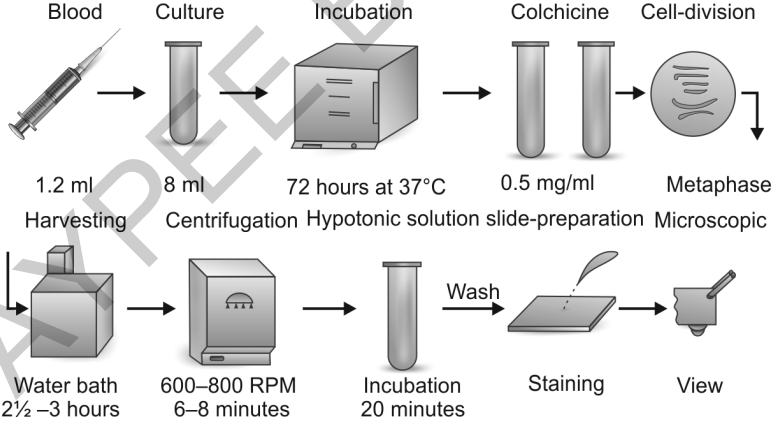


Fig. 2.3 : Procedure for karyotyping

Sample Collection

- Blood is collected from the peripheral vein under sterile conditions in Heparinized Vaccute.
- Planting should be done preferably within 4–5 hours of collection.

Planting

Under sterile conditions, blood sample is transferred to the culture tube containing the following constituents:

- *Culture medium*: Commonly used media are RPMI, TC 199, etc.
- *Neonatal calf serum/fetal bovine serum*: Added to nourish the culture cells.
- *Phytohemagglutinin*: A mitotic agent, added to increase the rate of mitosis of the cultured cells.
- *Antibiotics*: Penicillin and streptomycin combination is usually added to prevent the bacterial growth.

Incubation

The culture tube is kept in an incubator at 37° for 72 hours. During this period lymphocytes present in blood undergo mitosis.

Harvesting

- Around 69–70 hours after planting, colchicine is added to the culture tube to arrest the mitosis at metaphase by preventing the formation of spindle tubules.
- After 2 hours, cells are collected by centrifugation and then they are treated with hypotonic solution (0.56% KCL) so that the cells swell and chromosomes are dispersed.
- The hypotonic solution is discarded by centrifugation. Now the cells are treated with fixative solution containing acetic acid and methanol. Three such fixative washes are given to get cell suspension.

Slide Preparation

- Cell suspension is dropped from a height on chilled slides.
- Slides are allowed to dry at room temperature or on spirit lamp flame.

Staining

- For chromosome analysis, various banding techniques available are G-banding, Q-banding, R-banding, C-banding and NOR-banding. Commonly G-banding is practiced for study.
- *G-banding*: Slides with chromosome preparation are first treated with solution of trypsin. Trypsin denatures the chromosome protein.

Slides are then treated with Giemsa solution. The chromosomes show dark and light bands which can be observed under a microscope.

Microscopy

The stained slides are seen under the microscope. Good chromosome plates are identified and photographs are taken with the help of camera attached to microscope.

Preparation of Karyotype

It can be done as follows:

- Individual chromosomes are cut manually from the photograph, 22 autosomes including sex chromosomes are identified and arranged in pairs to prepare a Karyotype by using the following parameters:
  - Shape of the chromosome
  - Length of the chromosome
  - *Centromeric index:* This index is expressed in the form of ratio of the short arm length to the total chromosome length.

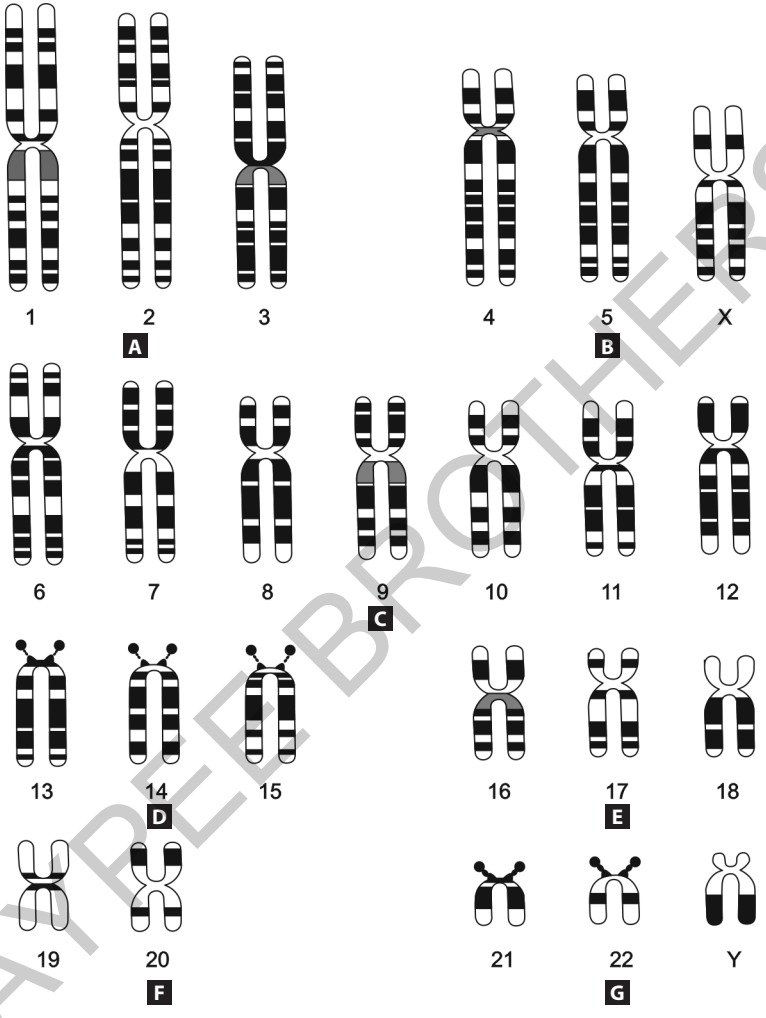
    $$\text{So, Centromeric Index} = \frac{\text{Short arm length}}{\text{Total chromosome length}}$$

    For example, in a metacentric chromosome, the centromeric index is 0.5.
  - *Proportion of the arms:* It is the ratio between the long and short arms of the chromosome. In a metacentric chromosome, this ratio is 1:1.
  - As per the location of bands along the length of chromosomes.

  Recently, automatic karyotype system is available in which pairing of chromosomes is done automatically to prepare the karyotype of the patient (Fig. 2.4).

The 22 pairs of chromosomes can be arranged in 7 groups as per Patau's modification of Denver system. These are as follows:

- A group : 1 to 3 pairs—Metacentric
- B group : 4 to 5 pairs—Submetacentric
- C group : 6 to 12 pairs—Submetacentric including X chromosome
- D group : 13 to 15 pairs—Acrocentric
- E group : 16 to 18 pairs—Submetacentric
- F group : 19 to 20 pairs—Metacentric
- G group : 21 to 22 pairs—Acrocentric including Y chromosome.



Figs 2.4A to G: Idiogram of a human male karyotype

Points to be Noted

- Groups A and F are metacentric
- Groups D and G are acrocentric
- Other groups are submetacentric

- In males, group G includes Y chromosome. Y chromosome is an acrocentric chromosome like others in the group but it shows two differences:
  1. It is usually the longest in group G.
  2. Its long arms are usually parallel to each other, which are divergent in the other members of group G.

The X chromosome is a member of group C and can be distinguished from other members of the group by banding techniques and by using special stains.

FLUORESCENT IN SITU HYBRIDIZATION (FISH) (FIG. 2.5)

We have seen that karyotyping is used to study the chromosomes of an individual. This helps to identify numerical as well as structural chromosomal abnormalities but it has limitations to identify very minute structural rearrangements or submicroscopic deletions.

FISH is based on the principle of DNA hybridization. In this technique, a labeled single-stranded DNA segment (probe) is exposed to denatured interphase or metaphase chromosomes. The probe undergoes complementary base pairing (hybridization) only with the complementary DNA sequence at a specific location on one of the denatured chromosomes. The site at which hybridization occurs on a particular chromosome can be visualized under a fluorescent microscope as the probe is labeled with fluorescent dye.

Fig. 2.5: Fluorescent *in situ* hybridization (FISH)

Uses of FISH

- To identify specific chromosome
- To identify missing or additional chromosomal material
- To know structural chromosomal defects especially microdeletions
- To assess the radiation effect or damage on a chromosome.

Types of FISH

Various types of chromosome-specific probes are available for FISH.

- *Centromeric probe*: Each chromosome has specific DNA sequences around the centromere. These probes are used to identify a particular chromosome.
- *Locus-specific probe*: The DNA sequences of homologous chromosomes and specific gene locations can be identified by locus specific probes. These probes are used in tumors, prenatal and postnatal samples.
- *Whole chromosome paint probe (WCP)*: Entire length of an individual chromosome is visualized by using this probe.
- *Multicolor probe*: Multiple probes labeled with different fluorescent dyes are used to paint all the chromosomes simultaneously. It helps to identify numerical chromosomal abnormalities.

To study the complete chromosome complement of an individual:

- Study of sex chromatin or Barr body
- Study of fluorescent bodies in buccal smear
- Study of drumsticks in polymorphonuclear leukocytes.

Sex Chromatin or Barr Body

Discovery: *Barr* and Bertram, in 1949, described these bodies in nuclei of phrenic nerve cells in a female cat. During interphase, somatic cell of a normal female presents a heterochromatin planoconvex body beneath the nuclear membrane. This is known as sex chromatin or Barr body. Out of the two X chromosomes in a normal female, one of them is highly coiled and the other member highly uncoiled. The highly coiled genetically inactive X chromosome forms the Barr body. These bodies help in nuclear sexing of the tissues.

Method of study: The cell nuclei of all tissues in a human female contain a sex chromatin body or Barr body (Fig. 2.6). But for convenience, the most suitable cells for study are those of buccal mucosa. The inside of cheek is gently scraped with a spatula and the cells obtained are spread on to a glass slide. This is called buccal smear. These cells are then fixed

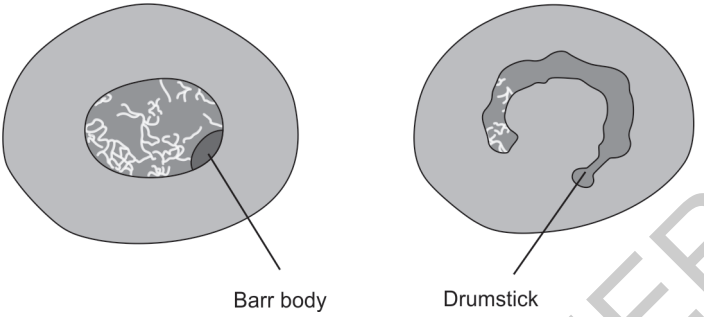


Fig. 2.6: Two different cells from a female showing Barr body in a squamous cell and a drumstick in a polymorphonuclear cell

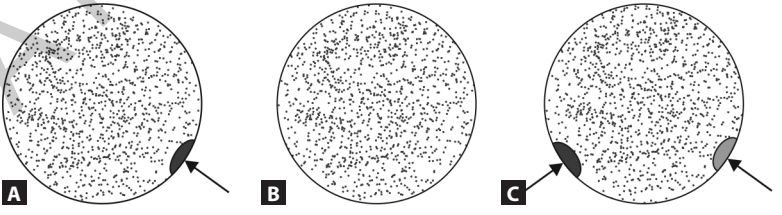
and stained, after which they can be examined for the presence of Barr bodies. These bodies are observed in 30 to 60% of nuclei of a normal female.

Time of appearance: It appears in body cells when a female embryo is 2 weeks old.

Number (Fig. 2.7)

The number of Barr bodies in a cell is equal to the total number of X chromosomes minus one.

- Normal female (XX)—One Barr body
- Normal male (XY)—No Barr body
- Klinefelter's syndrome (XXXY)—2 Barr bodies
- Turner's syndrome (XO)—No Barr body
- Triple X syndrome (XXX)—2 Barr bodies.



Figs 2.7A to C: Nucleus under different conditions; (A) Nucleus of a normal female (XX) showing single Barr body (marked by arrow); (B) Nucleus of a normal male (XY) does not show Barr body; (C) Nucleus of an individual with 2X chromosomes (e.g., Klinefelter's syndrome) showing 2 Barr Bodies (marked by arrow)

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Basic Human Genetics

Salient Features

- Provides the basic concepts of human genetics
- Concise presentation of text for easy learning and quick recapitulation during examination
- Includes new tables, flow charts and new improved diagrams
- Discusses gene, chromosomes with its abnormalities, inheritance, prenatal diagnosis, genetic counseling, etc.
- Gives an overview of Human Genome Project.

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