

**Course Objectives:** Genetic laboratory course to introduce the students to learn about prokaryotic and eukaryotic genetic system using modern techniques.

**Course Outcomes:** This course will provide to these students about the mechanics of experimentation methods of genetics.

### Practical

1. Permanent and temporary mount of mitosis.
2. Permanent and temporary mount of meiosis.
3. Mendelian deviations in dihybrid crosses
4. Demonstration of - Barr Body - *Rhoeo* translocation.
5. Karyotyping with the help of photographs
6. Pedigree charts of some common characters like blood group, color blindness and PTC tasting.
7. Study of polyploidy in onion root tip by colchicine treatment.

### References

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2. Klug, W.S., Cummings, M.R., & Spencer, C.A. (2009). *Concepts of Genetics* (9th ed.). Benjamin Cummings.
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**KARPAGAM ACADEMY OF HIGHER EDUCATION***(Deemed to be University Established Under Section 3 of UGC Act 1956)***Coimbatore - 641 021.****LECTURE PLAN  
DEPARTMENT OF BIOTECHNOLOGY****STAFF NAME: D. JEYAPRAGASH****SUBJECT NAME: Genetic Practical****SEMESTER: II****SUB.CODE:19BTU211****CLASS: I B.Sc (BT)**

<b>S.No</b>	<b>Lecture Duration Period</b>	<b>List of Practical's</b>
1	3	Permanent and temporary mount of mitosis
2	3	Permanent and temporary mount of meiosis.
3	3	Mendelian deviation in dihybrid crosses
4	3	Demonstration of Barr Body
5	3	Karyotyping with help of photographs
6	3	Pedigree charts
7	3	Study of polyploidy in onion root tip by Colchicine treatment

Ex. No. : 01

**Permanent and temporary mount of mitosis**

Date :

Aim:

To prepare a slide of onion root tip and identify the different mitotic stages.

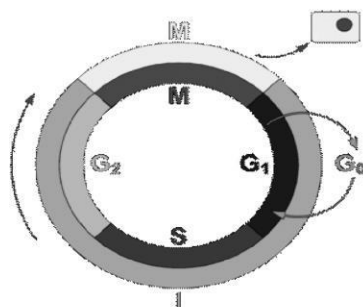
Introduction:

The cell cycle:

The cell cycle, or cell-division cycle, is the series of events that take place in a **eukaryotic cell** between its formation and the moment it replicates itself. These events can be divided in two main parts: **interphase** (*in between divisions* phase grouping **G<sub>1</sub> phase**, **S phase**, **G<sub>2</sub> phase**), during which the cell is forming and carries on with its normal metabolic functions; the mitotic phase (**M mitosis**), during which the cell is replicating itself (Figure 7). M phase is itself composed of two tightly coupled processes: **mitosis**, in which the cell's **chromosomes** are divided between the two daughter cells (karyokinesis), and **cytokinesis**, in which the cell's **cytoplasm** physically divides. Mitosis is also referred to as maintenance division. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of **quiescence** called **G<sub>0</sub> phase**, while cells that have permanently stopped dividing due to age or accumulated **DNA damage** are said to be **senescent**. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle. There are two key classes of regulatory molecules that determine a cell's progress through the cell cycle: **cyclins** and **cyclin-dependent kinases**.

*Interphase*

The mitotic phase is a relatively short period of the **cell cycle**. It alternates with the much longer **interphase**, where the cell prepares itself for cell division. Interphase is divided into three phases, **G<sub>1</sub>** (first gap), **S** (synthesis), and **G<sub>2</sub>** (second gap). During all three phases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are replicated only during the S phase.



Cell cycle

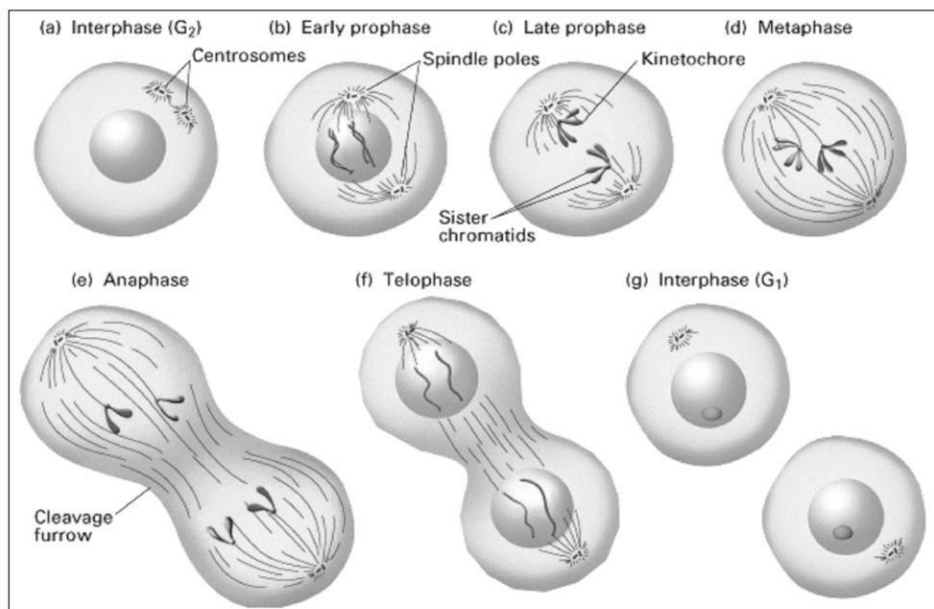


Figure 7. Different stages of mitotic cell division

**Pre-prophase:**

In plant cells only, prophase is preceded by a pre-prophase stage and followed by a post-prophase stage. In plant cells that are highly vacuolated and somewhat amorphous, the nucleus has to migrate into the center of the cell before mitosis can begin. This is achieved through the formation of a phragmosome, a transverse sheet of cytoplasm that bisects the cell along the future plane of cell division. In addition to phragmosome formation, pre-prophase is characterized by the formation of a ring of microtubules and actin filaments (called pre-prophase band) underneath the plasma membrane around the equatorial plane of the future mitotic spindle and predicting the position of cell plate fusion during telophase. The cells of higher plants (such as the flowering plants) lack centrioles. Instead, spindle microtubules aggregate on the surface of the nuclear envelope during prophase. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase.

**Prophase:**

Normally, the genetic material in the nucleus is in a loosely bundled coil called chromatin. At the onset of prophase, chromatin condenses together into a highly ordered structure called a chromosome. Since the genetic material has already been duplicated earlier in S phase, the replicated chromosomes have two sister chromatids, bound together at the centromere by the cohesion complex. Chromosomes are visible at high magnification through a light microscope. Close to the nucleus are two centrosomes. Each centrosome, which was replicated earlier independent of mitosis, acts as a coordinating center for the cell's microtubules. The two centrosomes nucleate microtubules (or microfilaments) (which may be thought of as cellular ropes) by polymerizing soluble tubulin present in the cytoplasm. Molecular motor proteins create repulsive forces that will push the centrosomes to opposite side of the nucleus. The centrosomes are only present in animals. In plants the microtubules form independently. Some centrosomes contain a pair of centrioles that may help organize microtubule assembly, but they are not essential to formation of the mitotic spindle.

**Prometaphase:**

The nuclear envelope disassembles and microtubules invade the nuclear space. This is called open mitosis, and it occurs in most multicellular organisms. These microtubules can attach to

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kinetochores or they can interact with opposing microtubules. The kinetochore is the protein structure in eukaryotes which assembles on the centromere and links the chromosome to microtubule polymers from the mitotic spindle during mitosis and meiosis.

#### Metaphase:

As microtubules (spindle fibres) find and attach to kinetochores in prometaphase, the centromeres of the chromosomes convene along the metaphase plate or equatorial plane, an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores.

#### Anaphase:

When every kinetochore is attached to a cluster of microtubules and the chromosomes have lined up along the metaphase plate, the cell proceeds to anaphase. The proteins that bind sister chromatids together are cleaved, allowing them to separate. These sister chromatids turned sister chromosomes are pulled apart by shortening kinetochore microtubules and toward the respective centrosomes to which they are attached. This is followed by the elongation of the nonkinetochore microtubules, which pushes the centrosomes (and the set of chromosomes to which they are attached) apart to opposite ends of the cell.

#### Telophase:

Telophase is a reversal of prophase and prometaphase events. At telophase, the nonkinetochore microtubules continue to lengthen, elongating the cell even more. Corresponding sister chromosomes attach at opposite ends of the cell. A new nuclear envelope, using fragments of the parent cell's nuclear membrane, forms around each set of separated sister chromosomes. Both sets of chromosomes, now surrounded by new nuclei, unfold back into chromatin. Mitosis is complete, but cell division is not yet complete.

#### Cytokinesis:

In animal cells, a cleavage furrow (pinch) containing a contractile ring develops where the metaphase plate used to be, pinching off the separated nuclei. In both animal and plant cells, cell division is also driven by vesicles derived from the Golgi apparatus, which move along microtubules to

the middle of the cell. In plants this structure coalesces into a cell plate at the center of the phragmoplast and develops into a cell wall, separating the two nuclei. The phragmoplast is a microtubule structure typical for higher plants, whereas some green algae use a phycoplast microtubule array during cytokinesis. Each daughter cell has a complete copy of the genome of its parent cell. The end of cytokinesis marks the end of the M-phase.

Principle:

John Belling (1866-1933) born in Aldershot, England, was a cytogenetist who developed the iron-acetocarmine staining technique which is used in the study of chromosomes. Belling used plants such as lilies and hyacinths to demonstrate that segments between non-homogenous chromosomes can interchange. He was able to make accurate estimates of chromosome numbers and proposed that chromomeres, the small condensations along the chromosome, were individual genes. Acetocarmine is a basic dye used in staining chromosomes and is utilized in viewing the different stages of mitosis in fixed cells. Carmine is a basic dye that is prepared from the insect *Coccus cacti*.

Materials required:

Onion root tips, Carnoy's fixative, forceps, needles, slide, acetocarmine stain, 1N HCl, cover slip, microscope, etc.

Carnoy's fixative: Absolute alcohol 60ml, chloroform 30ml and glacial acetic acid 10 ml.

Procedure:

1. The 1cm long grown root tips of the onion plant was washed properly with excess amount of distilled water to remove soil attached to the roots.
2. The root tips were fixed in carnoys fixative for 24 hrs (can be stored in 95% alcohol for future use).
3. The root tips were placed in a small beaker containing 1N HCl which is necessary to hydrolyze the tissue.
4. The beaker was warmed to 60°C in a water bath for 10 – 15 minutes.
5. The HCl was rinsed out using fresh water.
6. The meristematic region (1mm) of the root tips were cut and placed on a slide.

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COURSE NAME: GENETIC PRACTICAL

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LAB MANUAL

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7. A drop of acetocarmine was added to the root tip and the tips were squashed well using a clean glass rod and covered with a cover slip.
8. The squashed, stained preparation was then viewed under the microscope.
9. The meristematic region was located with the 10X objective, and then the 40X objective to study individual cells.

**Note:** Make sure the specimen is a root tip. Avoid excess staining in order to have clear visibility of nuclear materials.

Observation

Result



Ex. No. : 02

**Permanent and temporary mount of meiosis**

Date :

Aim:

To observe the meiotic stages in the anthers of *Rheo discolor* and *Datura* buds.

Introduction:

Meiosis is the process that allows one diploid cell to divide in a special way to generate haploid cells. It is essential for sexual reproduction. It therefore occurs in most eukaryotes, including single-celled organisms. A few eukaryotes, notably the *Bdelloid rotifers*, have lost the ability to carry out meiosis and acquired the ability to reproduce by parthenogenesis. Meiosis does not occur in prokaryotes, which reproduce by asexual cell division processes.

During meiosis, the genome of a diploid germ cell, undergoes DNA replication followed by two rounds of division, resulting in haploid cells called gametes. Each gamete contains one complete set of chromosomes, or half of the genetic content of the original cell. These resultant haploid cells can fuse with other haploid cells of the opposite gender or mating type during fertilization to create a new diploid cell, or zygote. Because the chromosomes of each parent undergo genetic recombination during meiosis, each gamete, and thus each zygote, will have a unique genetic *blueprint* encoded in its DNA. In other words, meiosis is the process that produces genetic variation. The different stages in meiosis include:

*First meiotic division – Meiosis I***Prophase I**

In the *prophase* stage, the cell's genetic material, which is normally in a loosely arranged pile known as chromatin, condenses into visible threadlike structures called chromosomes.

Leptotene: The first stage of Prophase I (Figure 10) is the leptotene stage, during which individual chromosomes begin to condense into long strands within the nucleus.

Zygotene: The *zygotene* stage then occurs as the homologous chromosomes pair up with each other. The combined homologous chromosomes are said to be *bivalent*. They may also be referred to as a *tetrad*, a reference to the four sister chromatids.

Pachytene: The *pachytene* stage heralds crossing over. Non-sister chromatids of homologous chromosomes randomly exchange segments of genetic information. Because the chromosomes cannot be distinguished in the synaptonemal complex, the actual act of crossing over is not perceivable through the microscope.

Diplotene: During the *diplotene* stage, the synaptonemal complex degrades. Homologous chromosomes fall apart and begin to repel each other. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. They are held together by the chiasmata.

Diakinesis: Chromosomes recondense during the *diakinesis* stage. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. The nucleolus disappears and the nuclear membrane disintegrates into vesicles.

During these stages, the centrioles, duplicated during interphase, migrate to the two poles of the cell. Centrioles sprout microtubules, which invade the nuclear membrane after it disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centriole. There are two kinetochores on each tetrad, one for each centrosome. Prophase I is the longest phase in meiosis.

### Metaphase I

As kinetochore microtubules from both centrioles attach to their respective kinetochores, the homologous chromosomes align equidistant above and below the equatorial plane, due to counterbalancing forces exerted by the two kinetochores of the bivalent.

### Anaphase I

Kinetochore microtubules shorten, pulling homologous chromosomes apart. Since each chromosome has only one kinetochore, whole chromosomes are pulled toward opposing poles, forming two haploid sets. Each chromosome still contains a pair of sister chromatids.

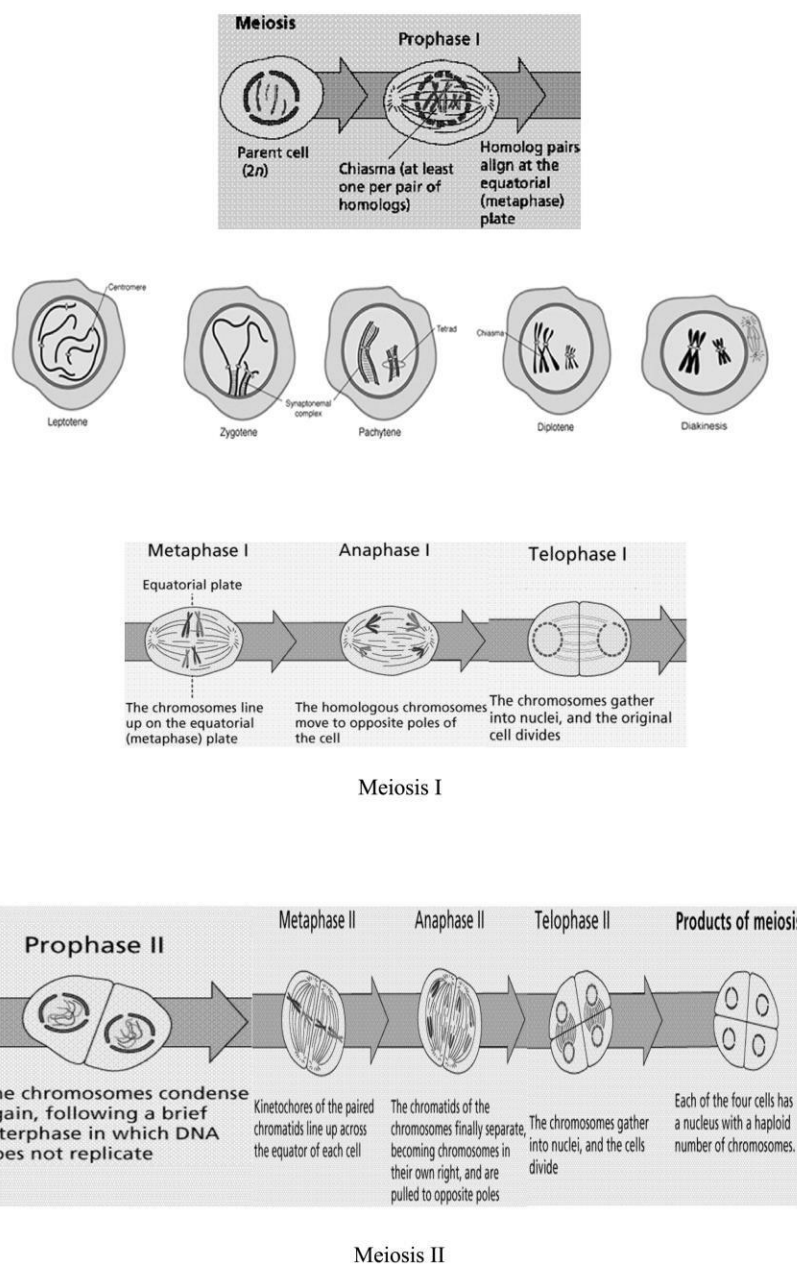


Figure 10. Different stages of meiosis

### *Telophase I*

The first meiotic division ends when the centromeres arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules disappear, and a new nuclear membrane is formed. The chromosomes uncoil back into chromatin. Cytokinesis occurs, completing the creation of two daughter cells.

### *Second meiotic division - Meiosis II*

#### *Prophase II*

The nucleoli and the nuclear envelope disappear once again and the chromatids thicken. Centrioles move to the poles and the spindle fibres appear. The new equatorial plane is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plane.

#### *Metaphase II*

The centromeres contain two kinetochores, organizing fibers from the centrosomes on each side.

#### *Anaphase II*

Metaphase II is followed by anaphase II, where the centromeres are cleaved, allowing the kinetochores to pull the sister chromatids apart. The sister chromatids are now called daughter chromosomes, and they are pulled toward opposing poles.

#### *Telophase II*

The process ends with *telophase II*, which is similar to telophase I, marked by uncoiling, lengthening, and disappearance of the chromosomes occur as the disappearance of the microtubules. Nuclear envelopes reform; cell wall formation eventually produces a total of four daughter cells, each with an haploid set of chromosomes.

#### Materials required:

Immature anthers, forceps, needles, glass slide, cover slip, 1N HCl, acetocarmine, carnoy's fixative, coverslip, microscope, etc.,

**Procedure:**

1. Immature flower buds were collected and the anthers were removed.
2. The anthers were fixed using carnoy's fixative for 24 hrs.
3. The fixed anther was hydrolysed using 1N HCl for few minutes.
4. The anther was mounted on a slide. A drop of acetocarmine was added and a squash was made.
5. A cover slip was placed.
6. Excess stain was removed with the help of soft tissue paper.
7. The slide was viewed first under 10X and later under 40X to see the different meiotic stages.

**Note:** Locate the pollen grains and do the acidification. Add minimum stain to have clear visibility of nuclear materials.

**Observation**

**Result**

Ex. No. : 04

**Study of Barr Bodies in Buccal Epithelial Cells**

Date :

**Aim:**

To identify the inactivated X chromosome (Barr body) in the human buccal epithelial cells.

**Introduction:**

In those species (including humans) in which sex is determined by the presence of the Y or W chromosome rather than the diploidy of the X or Z, a Barr body is the inactive X chromosome in a female cell, or the inactive Z in a male (Lyon, 2003), rendered inactive in a process called Lyonization. The Lyon hypothesis states that in cells with multiple X chromosomes, all but one is inactivated during mammalian embryogenesis. Barr bodies are named after their discoverer, Murray Barr. The inactivation state of chromosomes is passed on to daughter cells during mitosis. Since random chromosomes are selected for inactivation early in embryonic development, this results in different regions of the adult body having different chromosomes inactivated. This can be significant if different alleles of a gene are present on the different chromosomes; in some regions of the body one allele will be active, and in other regions the other will. This is what results in the coloration pattern of female calico cats; pigmentation genes on the X chromosome are activated in different patches of skin based on which chromosome is condensed in those regions. The Barr body chromosome is generally considered to be inert, but in fact a small number of genes remain active and expressed in some species. These genes are generally those which are present on the other sex chromosome (Y or W) (Lyon, 2003).

**Principle:**

Normal male (XY) does not have any Barr body, whereas normal female (XX) has one Barr body (Figure 9). Abnormal males who do not have a Y chromosome (Turner's syndrome – XO) also do not have Barr body. But abnormal males who have an extra X chromosome (Klinefelter's syndrome – XXY) have one Barr body.

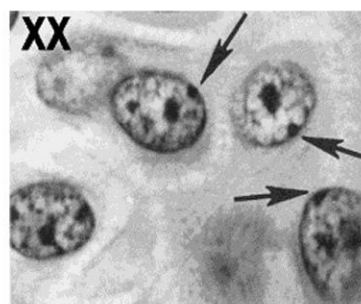
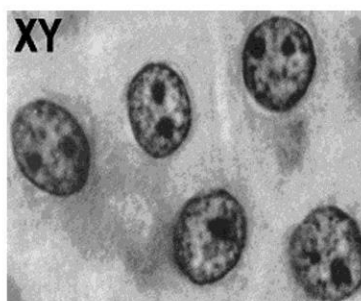


Figure 9. Barr bodies as observed in human buccal epithelial cells

Females who have an extra X chromosome (Super female – XXX) have two Barr bodies. This reveals that inactivation occurs only in the presence of two or more X chromosomes. The number of Barr bodies of a cell is one less than the number of X chromosomes in the original cell. Any one of the two X chromosomes has the chance of being inactivated. This inactivation occurs in order to compensate for the dosage of X chromosome genes in both the sexes.

Materials required: Buccal epithelial cells, slides, acetocarmine dye, microscope, *etc.*,

Procedure:

1. A sterile glass slide was taken.
2. It was gently slid over the buccal cavity and the fluid was collected in one corner.
3. A second slide was placed at an angle of  $45^{\circ}$  to the first slide and a smear was made.
4. It was air dried.
5. One drop of acetocarmine stain was added and left for five minutes.
6. A few drops of distilled water were added to remove the excess stain.
7. The preparation was air dried and observed under the microscope by using 4, 10, 40 & 100X magnifying lenses.

**Note:** Care should be taken while collecting the buccal fluid to avoid injuring oneself. A uniform smear gives better results.

Observation

Result



Ex. No. : 05 **Karyotyping with help of photographs**  
Date :

According to the position of centromere and size of the chromosomes, they are classified as following:

**Acrocentric:**

A Chromosome whose centromere is close to one end.

**Sub-metacentric:**

A Chromosome whose centromere is close to its centre, but is slightly off the centre.

**Metacentric:**

A Chromosome whose centromere is at its centre.

Chromosomes are divided into pairs 1 to 23 progressing from largest to smallest and placing the sex chromosome pair at the last.

**Chromosome no.1:**

- i. Metacentric-largest.
- ii. There is a secondary constriction (purely stained segment) near the centromere of one of the arms.

**Chromosome no.2:**

Largest sub-metacentric chromosome.

**Chromosome no.3:**

- i. Second largest metacentric.
- ii. Distinguished from pair one only by its slightly shorter length.

**Chromosome no.4:**

Large sub-metacentric chromosome with band in shoulder region.

**Chromosome no.5:**

Large sub-metacentric chromosome with a band in middle (belly) region.

**Chromosome no.6:**

- i. Sub-metacentric.

- ii. Largest 'C' pair usually.
- iii. It is less metacentric than X chromosome, but more metacentric than chromosome no.7 where the longest is not the more median.
- iv. Light band on p arm.

**Chromosome no.7:**

- i. Medium size sub-metacentric.
- ii. Chromosome with distinct 'V' shape, p arm and 3 bands in q arm.

**Chromosome no.8:**

- i. Sub-metacentric.
- ii. 'p' arm is not 'v' shaped, 2 bands are present.

**Chromosome no.9:**

Medium sized Sub-metacentric with 'p' arm is shorter and 'v' shaped with 2 bands.

**Chromosome no.10:**

Medium sized Sub-metacentric with up shaped 'p' arm.

**Chromosome no.11:**

Medium sized sub-metacentric with dark 'q' arm and jar like bottom.

**Chromosome no.12:**

Medium sized sub-metacentric with dark 'q' arm, bands close to centromere.

**X-chromosome:**

- i. Medium sized sub-metacentric.
- ii. Bands are in shoulder region, bands are seen in 'p' arm and 'q' arm.

**Chromosome no.13:**

Medium sized Acrocentric with satellite with band in 'q' arm away from centromere.

**Chromosome no.14:**

Medium sized Acrocentric with satellite with 2 prominent bands.

**Chromosome no.15:**

Medium sized Acrocentric with satellite with band in 'q' arm near centromere.

**Chromosome no.16:**

Small Metacentric with band near centromere.

**Chromosome no.17:**

Small Sub-metacentric with band near tail region of 'q' arm.

**Chromosome no.18:**

Small Sub-metacentric with square shaped 'q' arms within 2 hours.

**Chromosome no.19:**

Small Metacentric with band on centromere.

**chromosome no.20:**

Small Metacentric with band on 'p' arm.

**Chromosome no.21:**

Small Acrocentric with satellite showing broad band.

**Chromosome no.22:**

Small Acrocentric with satellite showing short band in centromere.

**Y-chromosome:**

- i. Small Acrocentric without satellite.
- ii. Denser staining arms are closer together.

Ex. No. : 06 **Pedigree analysis**

Date :

### **Basic principles**

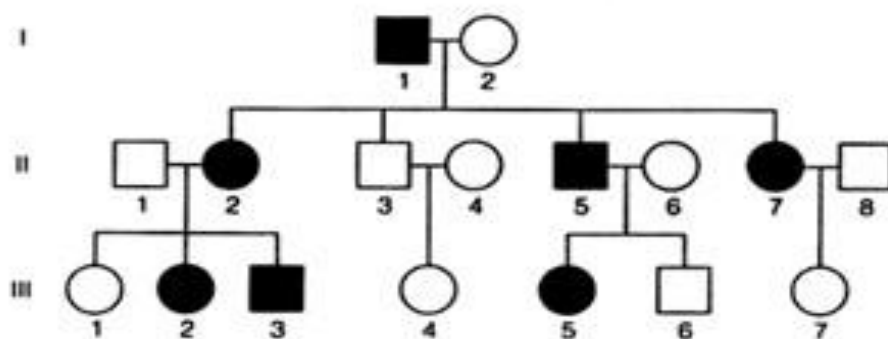
If more than one individual in a family is afflicted with a disease, it is a clue that the disease may be inherited. A doctor needs to look at the family history to determine whether the disease is indeed inherited and, if it is, to establish the mode of inheritance. This information can then be used to predict recurrence risk in future generations.

A basic method for determining the pattern of inheritance of any trait (which may be a physical attribute like eye color or a serious disease like Marfan syndrome) is to look at its occurrence in several individuals within a family, spanning as many generations as possible. For a disease trait, a doctor has to examine existing family members to determine who is affected and who is not. The same information may be difficult to obtain about more distant relatives, and is often incomplete.

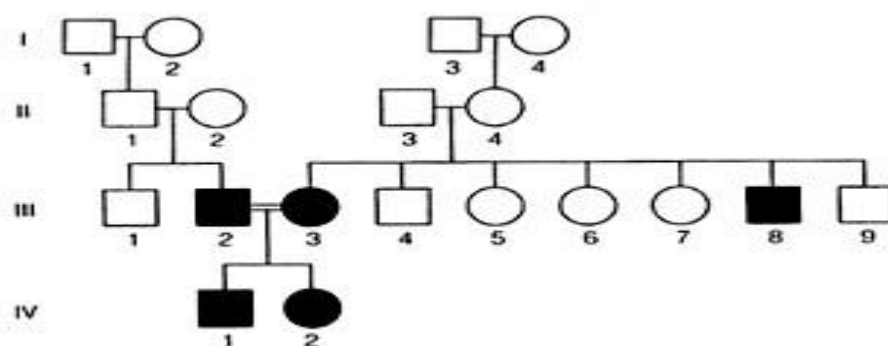
Once family history is determined, the doctor will draw up the information in the form of a special chart or family tree that uses a particular set of standardized symbols. This is referred to as a pedigree. In a pedigree, males are represented by squares □ and females by circles ○. An individual who exhibits the trait in question, for example, someone who suffers from Marfan syndrome, is represented by a filled symbol ■ or ●. A horizontal line between two symbols represents a mating □—○. The offspring are connected to each other by a horizontal line above the symbols and to the parents by vertical lines. Roman numerals (I, II, III, etc.) symbolize generations. Arabic numerals (1,2,3, etc.) symbolize birth order within each generation. In this way, any individual within the pedigree can be identified by the combination of two numbers (i.e., individual II3).

### **Dominant and recessive traits**

Using genetic principles, the information presented in a pedigree can be analyzed to determine whether a given physical trait is inherited or not and what the pattern of inheritance is. In simple terms, traits can be either dominant or recessive. A dominant trait is passed on to a son or daughter from only one parent. Characteristics of a dominant pedigree are: 1) Every affected individual has at least one affected parent; 2) Affected individuals who mate with unaffected individuals have a 50% chance of transmitting the trait to each child; and 3) Two affected individuals may have unaffected children.



Recessive traits are passed on to children from both parents, although the parents may seem perfectly "normal." Characteristics of recessive pedigrees are: 1) An individual who is affected may have parents who are not affected; 2) All the children of two affected individuals are affected; and 3) In pedigrees involving rare traits, the unaffected parents of an affected individual may be related to each other.



The reason for the two distinct patterns of inheritance has to do with the genes that predispose an individual to a given disease. Genes exist in different forms known as alleles, usually distinguished one from the other by the traits they specify. Individuals carrying identical alleles of a given gene are said to be homozygous for the gene in question. Similarly, when two different alleles are present in a gene pair, the individual is said to be heterozygous. Dominant traits are expressed in the heterozygous condition (in other words, you only need to inherit one disease-causing allele from one parent to have the disease). Recessive traits are only expressed in the homozygous condition (in other words, you need to inherit the same disease-causing allele from both parents to have the disease).

### **Penetrance and expressivity**

Penetrance is the probability that a disease will appear in an individual when a disease-allele is present. For example, if all the individuals who have the disease-causing allele for a dominant disorder have the disease, the allele is said to have 100% penetrance. If only a quarter of individuals carrying the disease-causing allele show symptoms of the disease, the penetrance is 25%. Expressivity, on the other hand, refers to the range of symptoms that are possible for a given disease. For example, an inherited disease like Marfan syndrome can have either severe or mild symptoms, making it difficult to diagnose.

### **Non-inherited traits**

Not all diseases that occur in families are inherited. Other factors that can cause diseases to cluster within a family are viral infections or exposure to disease-causing agents (for example, asbestos). The first clue that a disease is not inherited is that it does not show a pattern of inheritance that is consistent with genetic principles (in other words, it does not look anything like a dominant or recessive pedigree).