Course Name- Human Cytogenetics

Paper code: (MZO-508)

Unit 1: Introduction of Human Cytogenetics

Unit 2: Chromosomes and Karyotyping

Dr. Mukta Joshi
Department of Zoology
Uttarakhand Open University,
Haldwani

Introduction

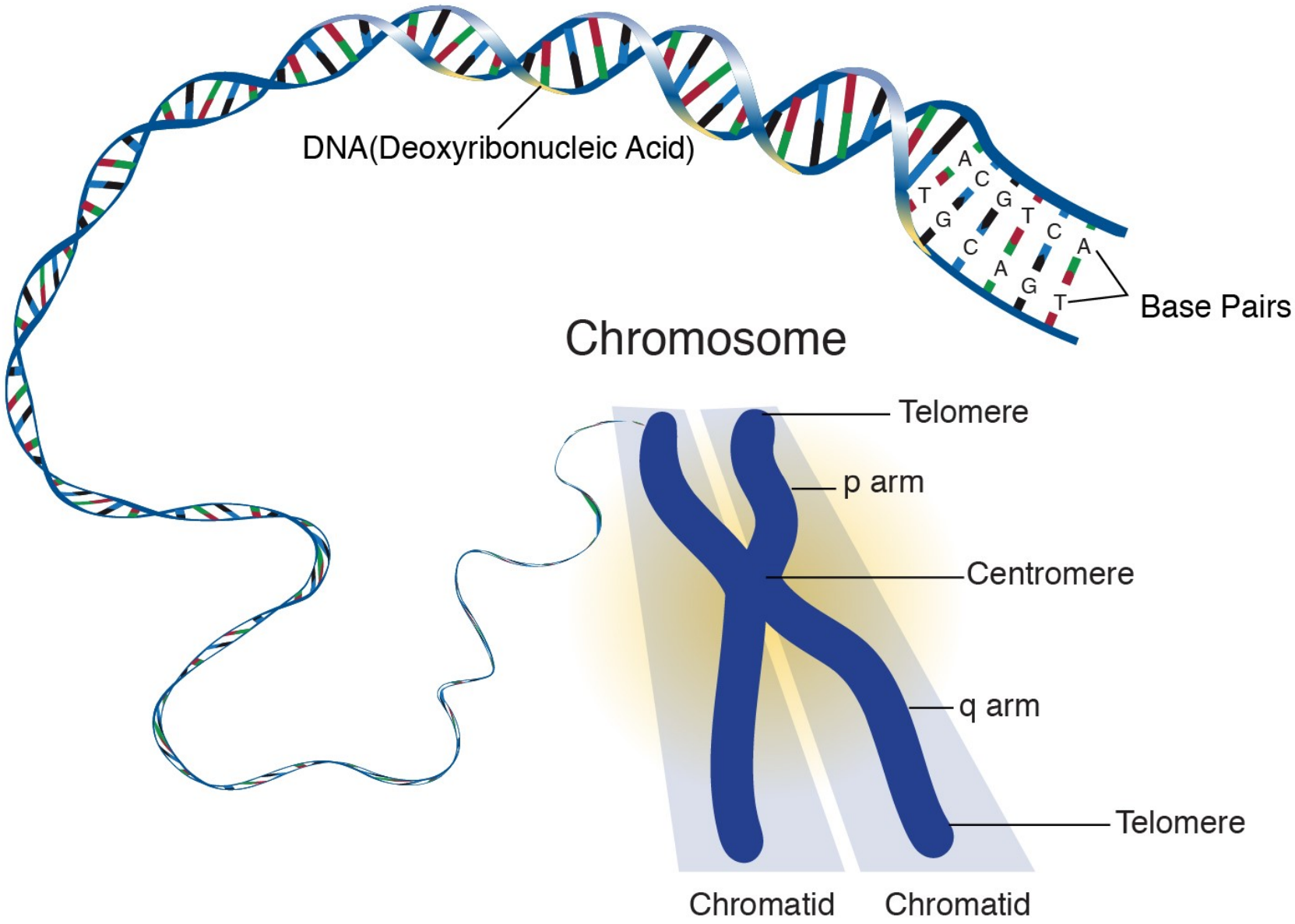
- Cytogenetics is essentially a branch of genetics, but is also a part of cell biology/ cytology (a subdivision of human anatomy), that is concerned with how the chromosomes relate to cell behavior, particularly to their behaviour during mitosis and meiosis.
- Techniques used include karyotyping analysis of G-band chromosomes, as well as molecular cytogenetics such as fluorescent in situ hybridization (CGH).
- Cytogenetic approaches to studying chromosomes and their relationship to human disease have improved greatly over the past several decades.

- The human cytogenetics was initiated in 1956, with the fundamental, but empowering, discovery that normal human cells contain 46 chromosomes.
- As a mature enterprise, cytogenetics now inform human genomics, disease and cancer genetics, chromosomes evolution and the relationship of nuclear structure to function.
- Cytogenetics involves testing samples of tissue, blood or bone marrow in a laboratory to look for changes in chromosomes including broken, missing, rearranged or extra chromosome.
- Cytogenetics may used to help diagnose a disease or condition, plan treatment or find out how well treatment is working.

Chromosomes

- Chromosomes are the rod shaped, dark-stained bodies seen during metaphase stage of mitosis.
- The chromosomes are the nuclear components of special organization, individuality and function.
- They are capable of self reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.
- Chromosomes are thread like structure located inside the nucleus.
- Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure.

- The number of chromosomes is constant for a particular species. Therefore, these are of great importance in the determination of the phylogeny and taxonomy of the species.
- The number of chromosomes varies from species to species but the number remains constant in a species. But sometime, due to certain accidents or irregularities at the time of cell division, crossing over or fertilization some alteration in morphology and number of chromosome takes place.
- Humans have 23 pairs of chromosomes-22 pairs of numbered chromosome, called autosomes, and one pair of sex chromosome, X and Y. Each parent contributes one chromosomes to each pair so that offspring get half of their chromosomes from mother and half from their father.


DNA(Deoxyribonucleic Acid)
A
C
T
G
G
T
C
C
A
A
G
T
Base Pairs
Chromosome
Telomere
p arm
Centromere
q arm
Telomere
Chromatid
Chromatid

Morphology

Size:

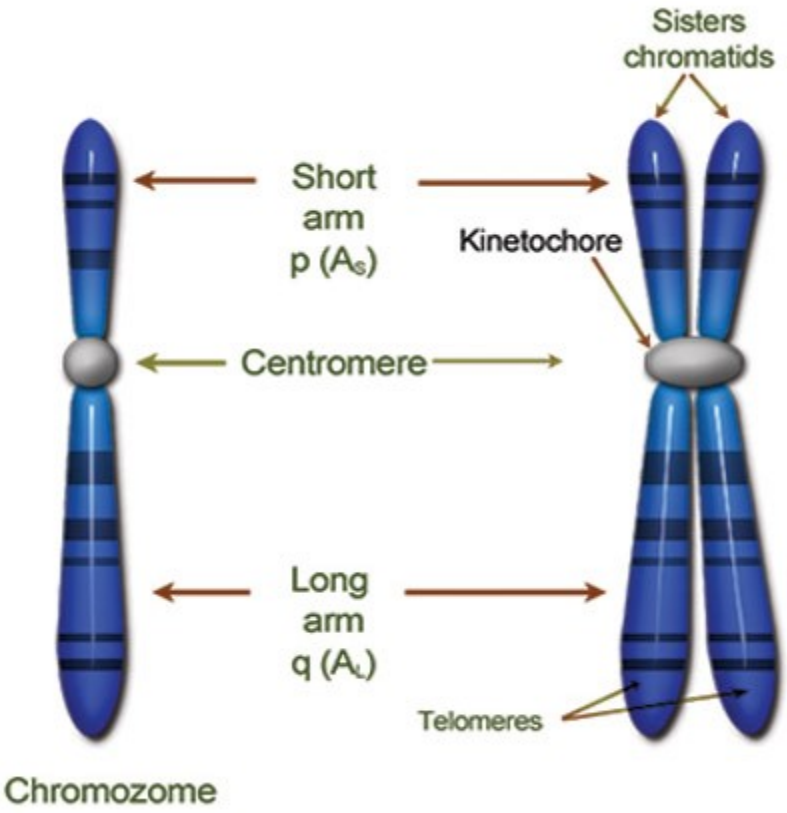
- The size of the chromosomes varies from species to species and relatively remains constant for a particular species.
- The length of the chromosomes may vary from 0.2 to 50µm. The diameter of the chromosome may be from 0.2 to 20µm.
- The human chromosomes are up to 6µm in length . Moreover the organism with less number of chromosomes contain compararatively large sized chromosomes than the chromosomes of the organisms having many chromosomes.
- The largest chromosome are lambrush chromosomes of certain vertebrate oocyte and polytene chromosomes of certain dipteran insects.

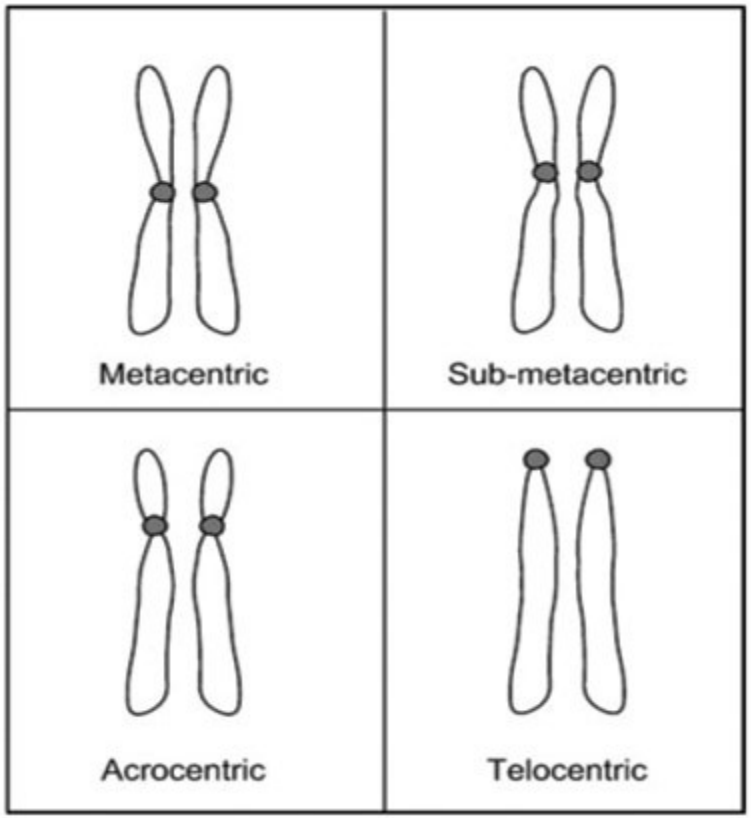
Shape:

- The shape of the chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division.
- In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin coiled, elastic and contractile, thread- like stainable structures, the chromatin threads.
- The metaphase and the anaphase, the chromosomes become thick and filamentous. Each chromosome contains a clear zone, known as centromere, along their length.
- The centromere divides the chromosomes into two parts, each part is called chromosome arms.

- The position of centromere very from chromosome to chromosome and it provides different shapes :

1. **Telocentric:** The rod-like chromosomes which have the centromere on the proximal end known as the telocentric chromosomes.
2. **Acrocentric:** The acrocentric chromosomes are also rod- like in shape but these have the centromere at one end and thus giving a very short arm and an exceptionally long arm. The locusts have the acrocentric chromosomes.
3. **Submetacentric:** The submetacentric chromosomes are J- or L-shaped. In these, the centromere occurs near the centre or at medium portion of the chromosomes and thus forming two unequal arms.


Sisters chromatids
Short arm p (A_S)
Kinetochore
Centromere
Long arm q (A_L)
Telomeres
Chromozome


Metacentric
Sub-metacentric
Acrocentric
Telocentric

4. Metacentric: The metacentric chromosomes are V- shaped and in these chromosomes the centromere occurs in the centre and forming two equal arms. The amphibians have metacentric chromosomes.

Kind of chromosomes :

- The eukaryotic chromosomes have been classified into autosomes and sex chromosomes.
- The autosomes have nothing to do with the determination of sex and exceed in number than sex chromosomes.
- The sex chromosomes also knows as accessory chromosomes or hetero-chromosomes, determine the sex of their bearer. They are usually two in number and are two kinds-

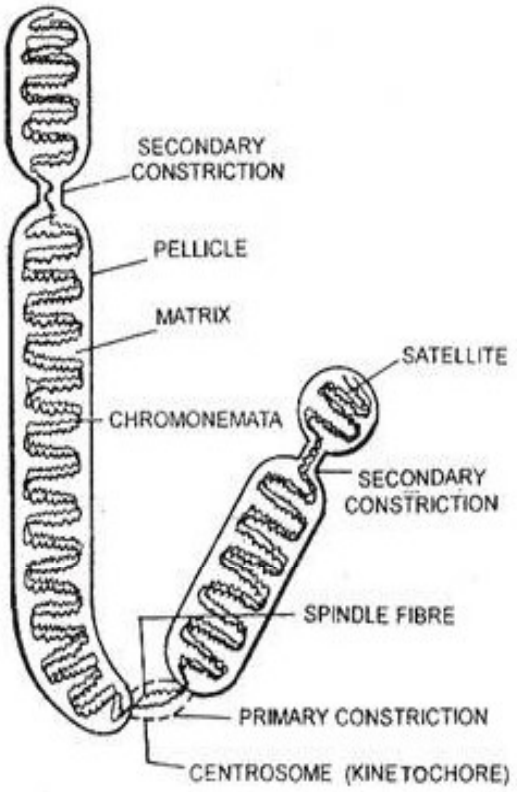
X- chromosomes and Y- chromosomes.

Structure

According to cytology the chromosome are composed of the chromonema and the centromeres.

Chromonema:

- In less compact chromosomes, under the optical microscope, a coiled filament is seen in the chromosome.
- The chromonema may be composed of two, four or more fibres according to the species.
- Further, the number of threads in the chromonema may depend on the different phase because at one phase the chromonema may contain one thread and other phase it may contain three or four threads.
- The thread or fibrils of the chromonema remain coiled with each other.



Structure of chromosome and its parts

Chromomeres:

- The chromomeres of thin chromosomes of mitotic and meiotic prophase have been found to contain alternating thick and thin regions and thus giving the appearance of a necklace in which several bends occur on a string.
- The thick or bead like structures of the chromonema are known as the chromomeres and the thin region in between the chromosomes is termed as the inter-chromomeres.
- The position of the chromomeres in the chromonema is found to be constant for a given chromosome.

Centromere:

- The shape of chromosomes is determined by the primary constriction located at the point where the arms of a chromosome meet.
- Within the constriction is a clear zone containing a small granule, or spherule. This clear region is the so called centromere.

- The chromosomes of most organism contain only one centromere and are known as monocentric chromosomes.
- Certain chromosomes may have two or more centromeres and can be termed as dicentric and polycentric chromosomes.

Secondary constriction:

- The chromosomes besides having the primary constriction or the centromere possess secondary constriction at any point of the chromosomes.
- Certain secondary constrictions play a vital role in the formation of the nucleus, therefore, knows as the nucleolar zone or nucleolar organizers. Usually in a nucleus only two chromosomes possess such nucleolar zone and therefore, known as the nucleolar chromosomes.

Telomere:

- Each extremity of the chromosomes has a polarity and therefore, it prevents other chromosomal segments to be fused with it. The chromosomal ends are know as the telomeres.

Satellite:

- Sometimes the chromosomes bear round elongated or knob-like appendages known as satellites.
- The satellite remains connected with the rest the chromosome by a thin chromatin filament.
- The chromosomes with the satellite are designated as the sat-chromosomes.
- The shape and size of the satellite and the chromatin filament remain constant.

Heterochromatin and Euchromatin

- The composing material of the chromosomes is the chromatin substance (DNA+ nucleoproteins ,etc.) which may occur in form of euchromatin and heterochromatin.
- Heterochromatin as those regions of chromosome that remain condensed during interphase and early prophase and form the so-called chromocenters or false nucleoli.
- The rest of the chromosome, which remains in a non-condensed state, was called euchromatin. There occur a mass of heterochromatin in close contact with the nucleous, and correspond to the nucleolar organizer.
- During mitosis the heterochromatic regions appear localized at the centromeres, at the telomeres or are intercalated in other parts of the chromosomes.

- Such regions may stain more strongly or weakly than the euchromatic regions, showing what called a positive or a negative heteropyknosis.
- Heterochromatin and euchromatin are seen with the electron microscope to differ in their physical structure.
- Heterochromatin is composed of 250Å fibrils, whereas euchromatin contains mostly 30 to 80Å fibrils. So, heterochromatin represents a condensed inter coiled state of chromatin, containing two to three times more DNA than euchromatin.
- There are two types of heterochromatin, constitutive heterochromatin and Facultative heterochromatin.

- Euchromatin is lightly packed form of chromatin that is rich in gene concentration.
- It is often under active transcription.
- Euchromatin comprises the most active portion of the genome within the nucleus, 92% of the human genome is euchromatic.
- The basic structure of euchromatin is an elongated, open 10nm micro fibril, as noted by electron microscopy.
- Euchromatin participates in the active transcription of DNA to mRNA products.

Functions of heterochromatin

- The heterochromatin has specific role in the biogenesis of ribosomes and t-RNA molecules.
- Centromeric heterochromatin may be involved in the separation of chromosomes during cell division.
- Heterochromatin has been associated with several functions, from gene regulation to the protection of chromosome integrity some of these role can be attributed to the dense packing of DNA, which makes it less accessible to protein factors that usually bind DNA or its associated factors.

Chromosome Banding

- Chromosome banding refers to alternating light and dark regions along the length of a chromosome, produced after staining with a dye.
- A band is defined as the part of a chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter with the use of one or more banding techniques.
- Two main categories of the chromosome banding patterns have been described:

1. Band of presumptive heterochromatin distributed along the arm of the chromosomes have been revealed by staining with fluorescent dyes such as quinacrine mustards (Q-band) or Giemsa and other non fluorescent stains(G-bands). A reverse band (R band) patterns occur when method is varied slightly, producing R- band that are reciprocal of Q-bands and G-bands.

Q-banding is found to result from interaction between quinacrine and regions of DNA that are rich in adenine and thymine. Guanine and cytosine quench fluorescence, so region rich in these bases would be present in the unstained interband.

2. C-band that distinguish constitutive heterochromatin regions, particularly around the centromere, are visualized by Giemsa staining after special pretreatments that include HCL and NaOH denaturing steps.

C-banding is found to occur because a greater concentration of DNA remain after specific pretreatments.

Thus, banding patterns provide much greater resolution of heterochromatic regions along each chromosome than either the study of replication time.

Chemical composition of chromosome

- Chemically chromosome are nucleoprotein in nature means are composed of DNA, RNA and protein.
- Generally chromosomes contains 30-40% DNA, 50-65% protein and 0.5-10% RNA.

1. **DNA-** The amount of DNA present in somatic cell is constant. DNA content of gametic cell is half of that of somatic cell. DNA of chromosome is of two types i) Unique DNA

   ii) Repetitive DNA

   Unique DNA:

- Unique DNA consists of those DNA sequence which are present in a single copy per genome and are unique in nature.
- Unique DNA is also known as non repetitive DNA. Codes for protein which requires in large quantity for cell e.g. storage protein.

- Repetitive DNA: Repetitive DNA consists of DNA nucleotide or base sequence, which are few to several hundred base pair long and are present to a million copies per genome. Human genome contains 30% repetitive DNA. Repetitive DNA is further divided into
- i) Highly repetitive DNA and

  ii) Moderately repetitive DNA

2. RNA- purified chromatin contain 10-15% RNA. RNA associated with chromosome is messenger RNA, transfer RNA and ribosomal RNA.

3. Proteins- Protein associated with chromosome is classified into two broad groups

i) Histone

ii) Non histone protein

- Non histone protein are acidic in nature and histone protein are basic in nature because of basic amino acid

i) Histone protein- histone constitutes about 80% of the total chromosomal protein. They are present in an almost 1:1 ratio with DNA.

ii) Non histone protein- non histone proteins make up to 20% of the total protein mass. Content of non histone protein is different from species to species. Non histone protein include many important enzyme like DNA and RNA polymerase.

Cytological Functions of Chromosomes

- The chromosomes are the most significant components of the cell.
- They control most of the cell biological and genetical activities of a species.
- They contain the genetical material, the DNA, which ultimately influences all the biological phenomena at molecular, physiological and gross morphological levels.

Karyotype

Definition:

- A karyotype is the number and appearance of chromosome in the nucleus of eukaryotic cell. The term is also used for the complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number.

History of karyotyping

Grygorii Levitsky (1931) seems to have been first person to define the karyotype as the "phenotypic appearance of the somatic chromosomes, in contrast to their genic contents".

Karyotyping

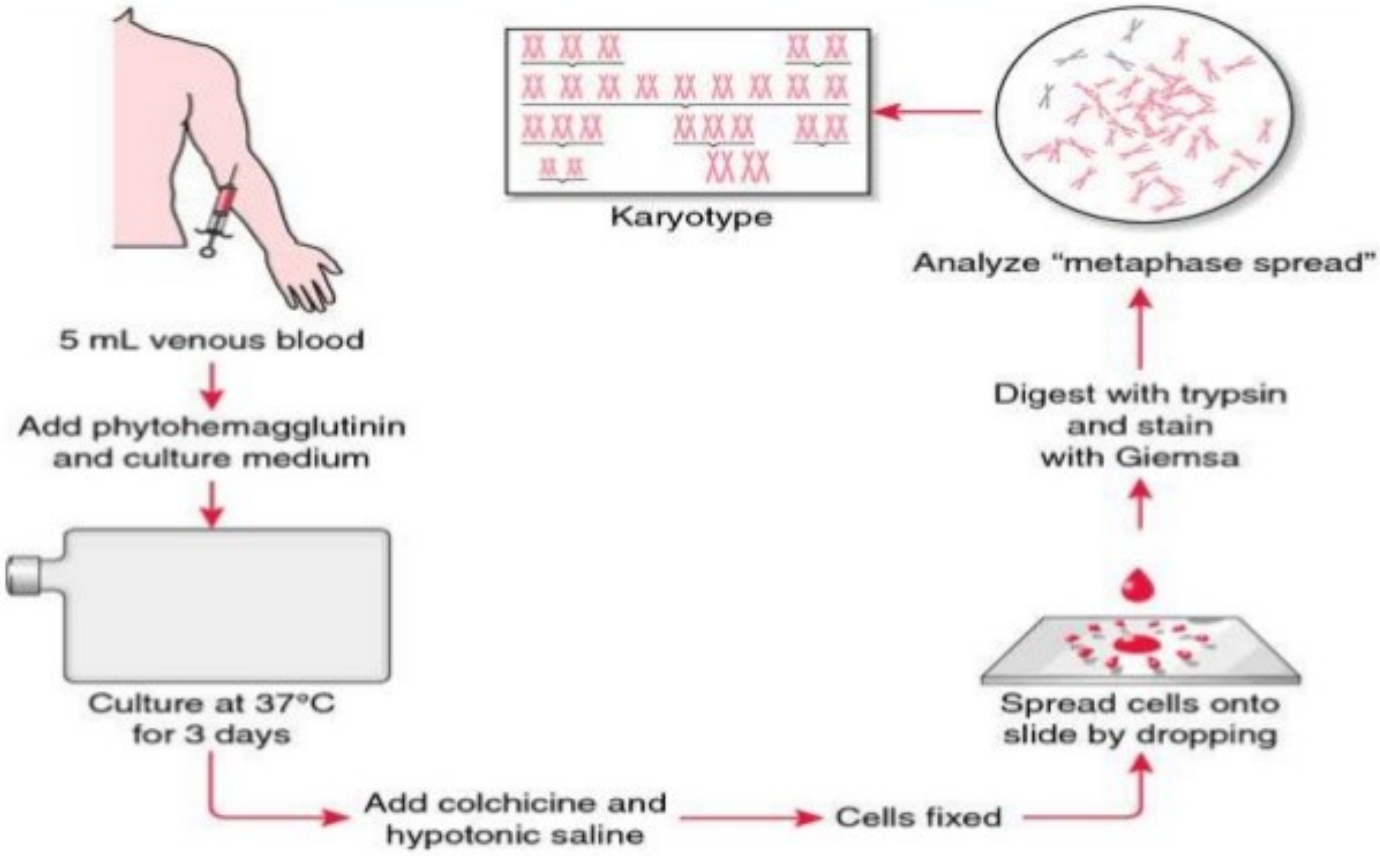
- Karyotyping is the process by which photographs of chromosomes are taken in order to determine the chromosome complement of an individual, including the number of chromosomes and any abnormalities.
- The term is also used for the complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number.
- Karyotyping is the technique that is use to examine chromosomes in sample of cells which can help identify genetic problems as the cause of disorder or a disease.
- Main purpose of karyotyping is to locate or visualize the changes in the number of chromosomes and abnormality in the structure.

- Karyotypes can reveal change in chromosome number associated with aneuploid conditions, such as trisomy 21

  (Down syndrome). Careful analysis of karyotypes can also reveal more subtle structure changes, such as chromosomal deletion, duplication, translocations or inversions.
- Karyotypes are prepared from mitotic cells that have been arrested in the metaphase or prometaphase portion of the cell cycle, when chromosome assume their most condensed conformations.
- The process of generating a karyotype begins with the short-term culture of cells derived from a specimen.
- Karyotype is test to identify and evaluate the size, shape and number of chromosomes in a sample of body cells.

- Extra or missing chromosomes, or abnormal position of chromosome pieces, can cause problems with a person's growth, development and body functions.
- Particular chromosome complement of an individual or a related group of individuals, as defined by the chromosome size. Morphology and number is know as a "Karyotype".
- Karyotype:
- Size of chromosome
- Position of centromere
- Presence of secondary constriction
- Size of satellite

Procedure of karyotyping

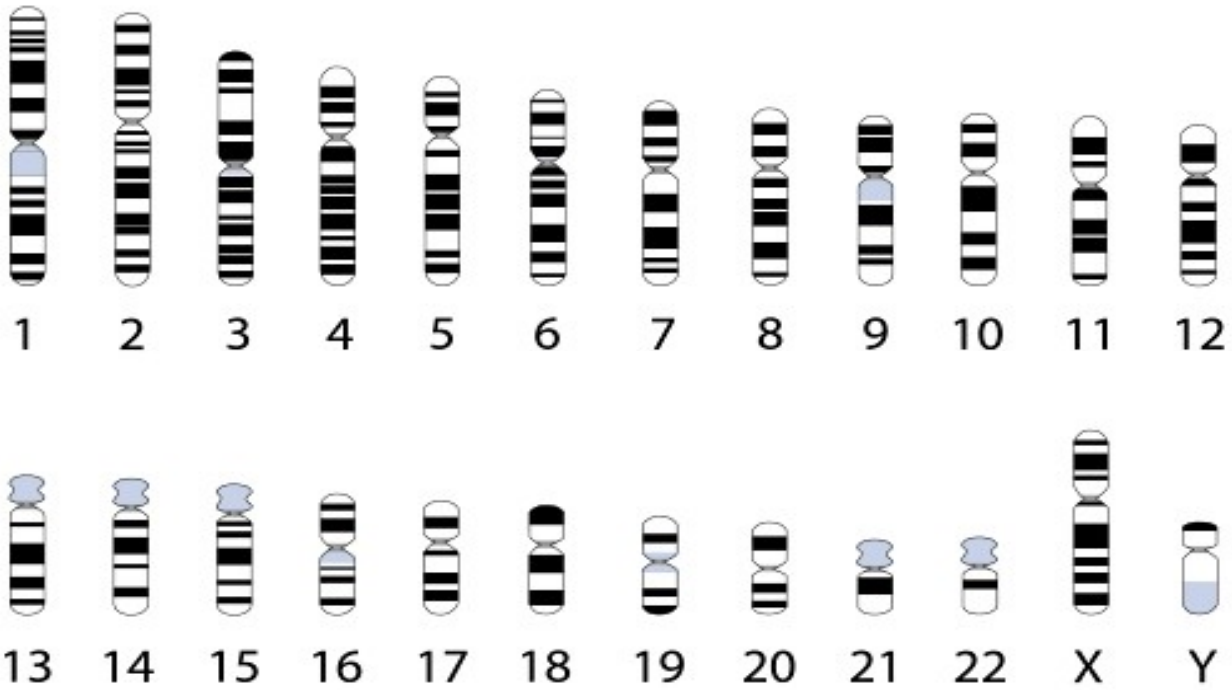


- Derived from Greek word “karyon” which means “nucleus”, karyotype is represented as Idiogram.

- When the haploid set of chromosomes of an organism are ordered in a series of decreasing size, it is said to be an idiogram.

- In other sense diagrammatic representation of a karyotype is an Idiogram.

Idiograms of Human Chromosomes



Advantage of Karyotyping

- Help in studying chromosome banding pattern.
- Helps in the identification of chromosomal aberrations.
- Diagnosis of prenatal genetic defects.
- Aids in studying evolutionary changes.
- Reveals structural feature of each chromosomes.

THANK YOU