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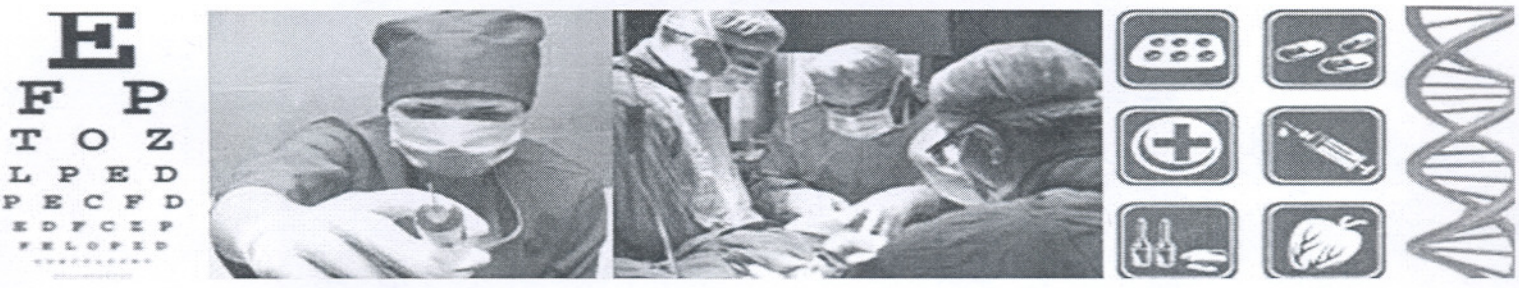
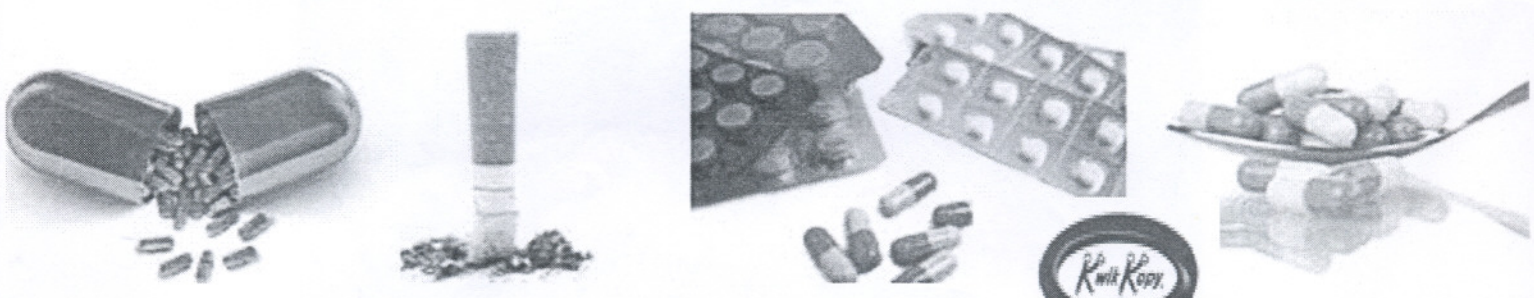


Genetics

Genetic diagnostic techniques



GENETCS: Lecture 1

Genetic diagnostic techniques(karyotype & FISH)

Overall objectives:

Students should understand:

- Describe the number, structure, and classification of human chromosomes.
- Explain what a karyotype is and how its obtained.
- Describe chromosomal banding and explain its use.
- Describe the process of in situ hybridization and recount the information it provides.

Syllabuses

- genetic diagnostic techniques overview
- Common molecular and cytogenetic diagnostic techniques and how they are applied to genetic disorders;
- Interphase, metaphase chromosomes
- structure of chromosomes
- Human chromosome
    - chromosome preparations, samples /procedures
    - number and Chromosome Morphology
    - Classification
- karyotype
    - Non banded karyotype
    - Nomenclture of non banded karyotype
    - Banding techniques
    - Banded karyotype
    - Nomenclture of banded karyotype
- FISH techniques

LECTURE 1 Human Chromosomes & Human Karyotype

Cytogenetics involves the study of human chromosomes in health and disease. Chromosome studies are an important laboratory diagnostic procedure

Chromosomes? Chromosomes are complex structures located in the cell nucleus, they are composed of DNA, histone and non-histone proteins, RNA , and polysaccharides. They are basically the "packages" that contain the DNA.

Chromosome Morphology : Under the microscope chromosomes appear as thin, thread-like structures. They all have a short arm and long arm separated by a primary constriction called the **centromere**. The short arm is designated as **p** and the long arm as **q**. The centromere is the location of spindle attachment and is an integral part of the chromosome. It is essential for the normal movement and segregation of chromosomes during cell division. Human metaphase chromosomes come in *three basic shapes* and can be categorized according to the length of the short and long arms and also the centromere location. ***Metacentric*** chromosomes have short and long arms of roughly equal length with the centromere in the middle. ***Submetacentric*** chromosomes have short and long arms of unequal length with the centromere more towards one end. ***Acrocentric*** chromosomes have a centromere very near to one end and have very small short arms.

Normally chromosomes can't be seen with a light microscope but during cell division they become condensed enough to be easily analyzed at 1000X. To collect cells with their chromosomes in this condensed state they are exposed to a mitotic inhibitor which blocks formation of the spindle and arrests cell division at the metaphase stage.

A variety of *tissue types* can be used to obtain chromosome preparations. Some examples include peripheral blood, bone marrow, amniotic fluid, and products of conception. Although specific techniques differ according to the type of tissue used, the basic method for obtaining chromosome preparations is as follows:

- Sample log-in and initial setup.
- Tissue culture (feeding and maintaining cell cultures).
- Addition of a mitotic inhibitor to arrest cells at metaphase.
- Harvest cells. This step is very important in obtaining high quality preparations. It involves exposing the cells to a hypotonic solution followed by a series of fixative solutions. This causes the cells to expand so the chromosomes will spread out and can be individually examined.
- Stain chromosome preparations to detect possible numerical and structural changes.

Non-banded karyotype

Normal human somatic cells have 46 chromosomes: 22 pairs, or homologs, of autosomes (chromosomes 1-22) and two sex chromosomes. This is called the diploid number. Females carry two X chromosomes (46,XX) while males have an X and a Y (46,XY).

When microscopes were improved to the point that the human karyotype could be reliably discerned (in the 1950s) the chromosomes could be grouped on the basis of their relative sizes and the relative lengths of their two arms, i.e. the positions of their centromeres. Now, banding techniques make it possible to identify each chromosome.

Banded karyotype

Banding

Chromosomes in metaphase can be identified using certain staining techniques, so called banding(If chromosomes are treated briefly with proteinase before staining then each chromosome has a characteristic banding pattern.)

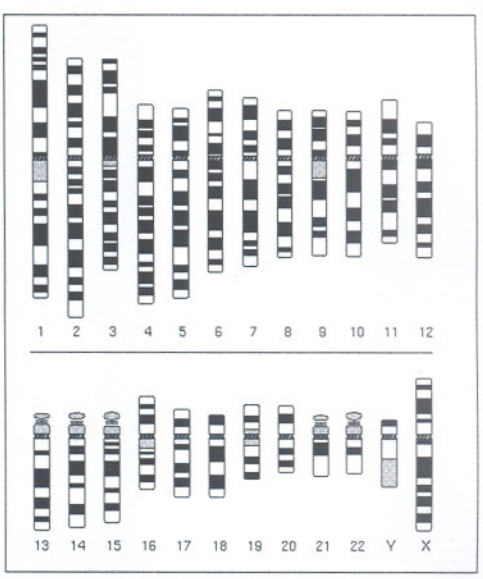
The banding techniques those resulting in bands distributed along the length of the whole chromosome, such as G-, Q- and R-bands

- G-bands are most commonly used. They take their name from the Giemsa dye, but can be produced with other dyes.
- R-bands are approximately the reverse of G-bands
- Q-bands are like fluorescent G-bands

A band is defined as that part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or brighter with one or more banding techniques. The chromosomes are visualized as consisting of a continuous series of bright and dark bands.

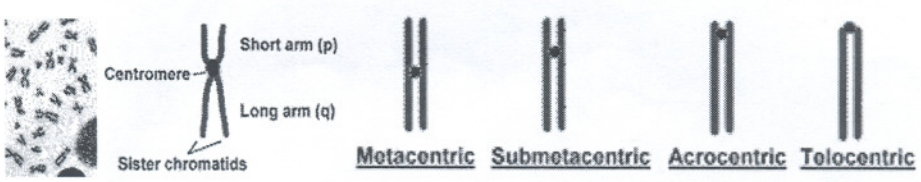
ISCN Nomenclature

The two chromosome arms are refered to as p and q (short and long respectively). Bands are numbered from the centromere. As microscopes improved the coarse banding patterns were refined by the addition of further levels of numbering so that band 9q34.1 means the 1st subband of the 4th band of the 3rd region of the long arm of chromosome 9



Fluorescent In Situ Hybridisation

Advances in the use of DNA probes have allowed cytogeneticists to hybridize these probes to chromosomes and determine if a specific DNA sequence is present on the target chromosome. This has been useful in detecting abnormalities beyond the resolution level of studying banded chromosomes at the microscope, and also in determining the location of specific genes on chromosomes.



Non-Banding Karyotype:

Group 1–3 (A)	Large metacentric chromosomes readily distinguished from each other by size and centromere position
Group 4–5 (B)	Large submetacentric chromosomes which are difficult to distinguish from each other
Group 6–12–X (C)	Medium-sized metacentric chromosomes. The X chromosome resembles the longer chromosomes in this group. This large group is the one which presents major difficulty in identification of individual chromosomes without the use of banding techniques.
Group 13–15 (D)	Medium-sized acrocentric chromosomes with satellites
Group 16–18 (E)	Relatively short metacentric chromosomes (No. 16) or submetacentric chromosomes (Nos. 17 and 18)
Group 19–20 (F)	Short metacentric chromosomes
Group 21–22–Y (G)	Short acrocentric chromosomes with satellites. The Y chromosome is similar to these chromosomes but bears no satellites.

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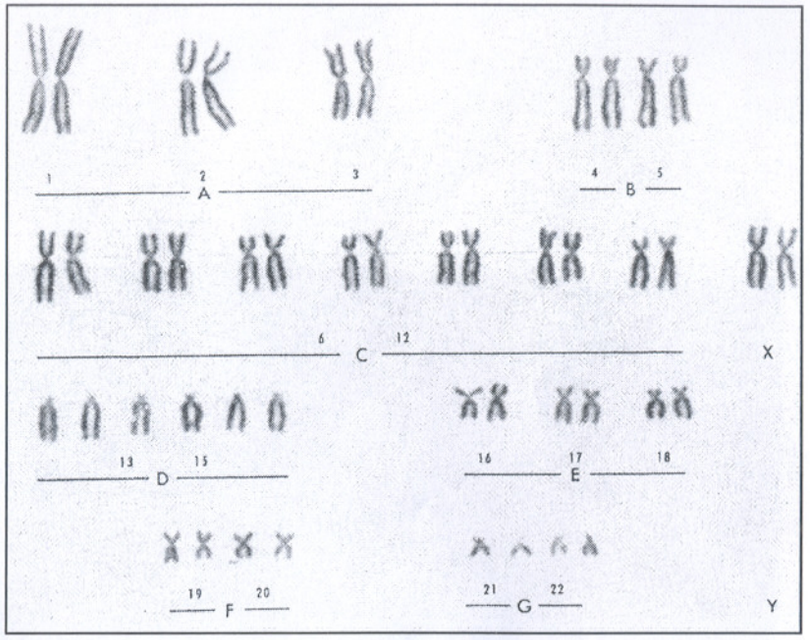
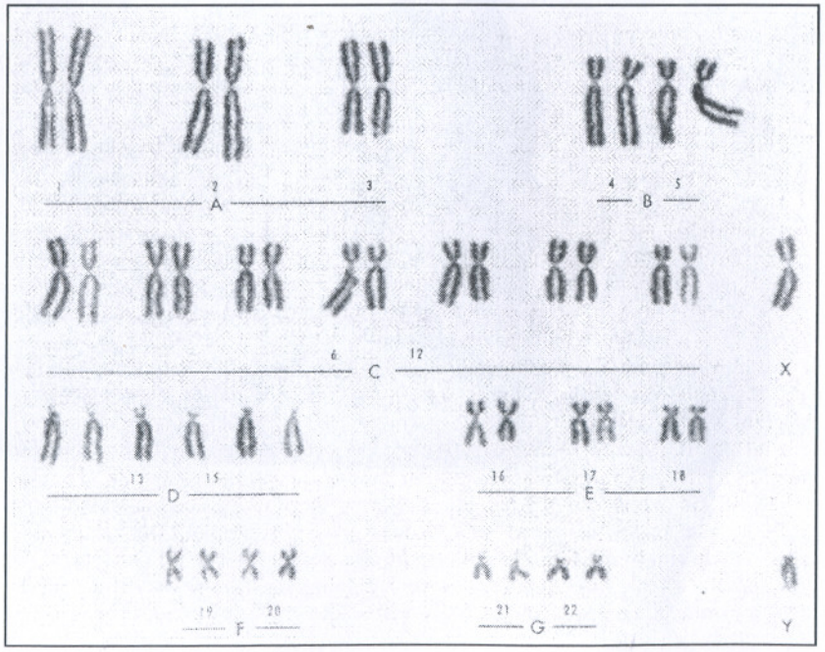




Medical Genetics

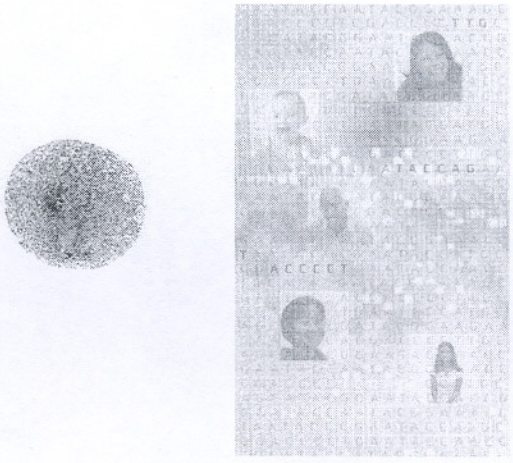
LECTURE 1•

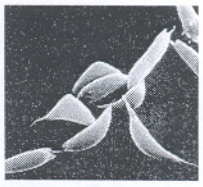
Human Chromosomes

Human Karyotype

GENETICS :

- **Cytogenetics:**
- **"Molecular genetics":**





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Cytogenetics:

Human Cytogenetics involves the study of human chromosomes in health and disease.

Chromosome studies are an important laboratory diagnostic procedure in
-prenatal diagnosis
-Certain patients with mental retardation and multiple birth defects
-patients with abnormal sexual development
-some cases of infertility or multiple miscarriages.
-also in the study and treatment of patients with malignancies and hematologic disorders.

New techniques allow for increased resolution.

In the future cytogenetic methods will become more and more linked to molecular techniques

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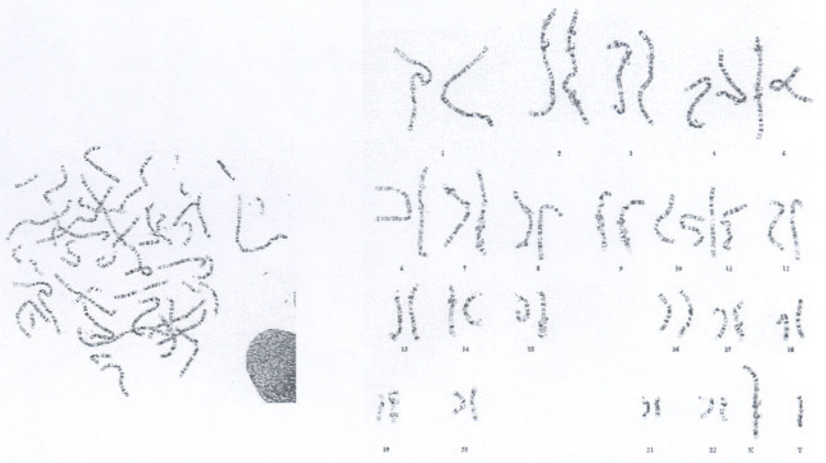
Karyotype



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CHROMOSOMES:

- carry most of the genetic material
- heredity: each pair of homologues consists of one paternal and one maternal chromosome-
.
- The intact set is passed to each daughter cell at every mitosis.
- cell life: will be perturbed if regular segregation fails

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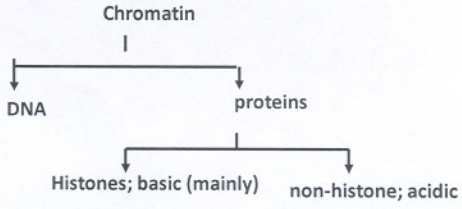
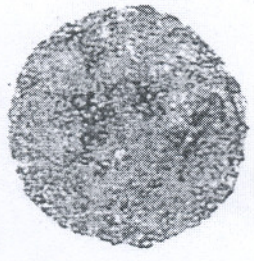
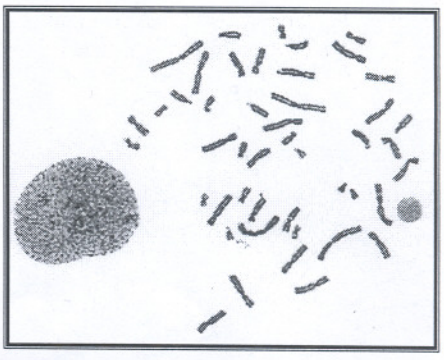
Composition: In **eukaryotes**, chromosomes consist of

- a **single molecule of DNA** associated with:
- many copies of 5 kinds of **histones**. Histones are proteins rich in lysine and arginine residues and thus positively-charged.
- a small number of copies of many different kinds of **non-histone**proteins.



Interphase, metaphase chromosomes





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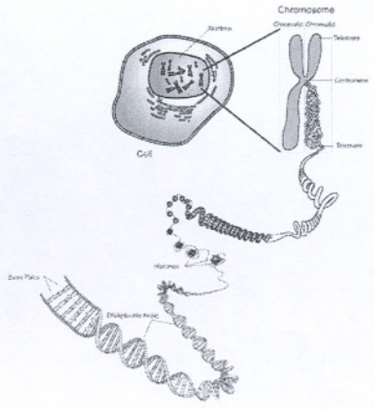
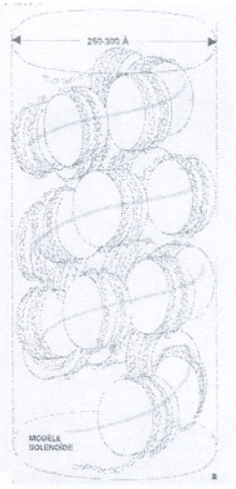
structure of hromosomes





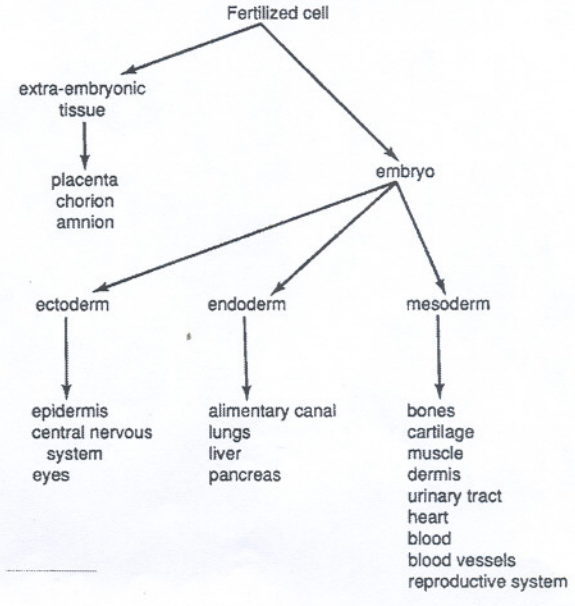
- **Cytogenetics:**
- ***Non-Banded Karyotype***
- ***Banded Karyotype***
- ***High resolution karyotype***

- **"Molecular cytogenetics":**
- ***Fluorescent in situ hybridization(FISH).***

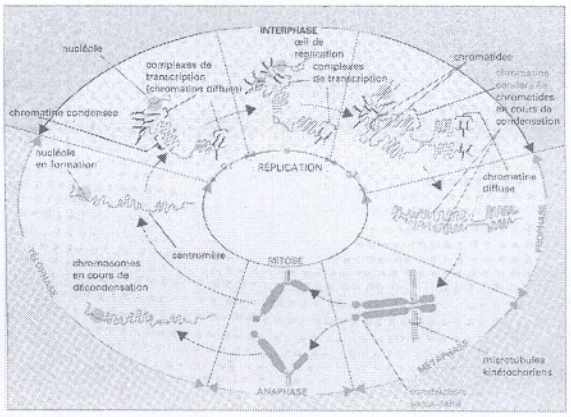
karyotype

- Specimen
- protocols

- Chromosome morphology
- Classification

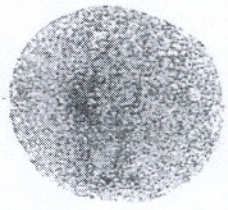


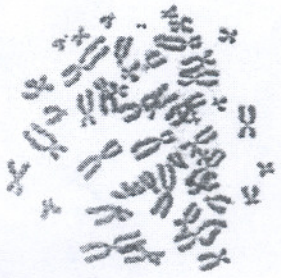
Development of Chromosome Morphology During The Cell Cycle



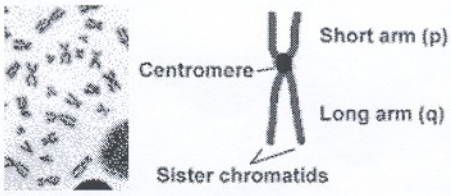
A series of steps involved :

- CULTURING
- HARVESTING
- Slide-Making
- Banding
- Staining
- Karyotyping
- Chromosome Analysis





Metaphase chromosomes:



- The 2 sister-chromatids are principally held together at the centromeric region.
- Each chromosome has a centromere (CEN), region which contains the kinetochore,
- CEN divides the chromosome into two arms: the short arm (p arm) and the long arm (q arm).
- Each arm terminates (pter, qter) in a telomere,

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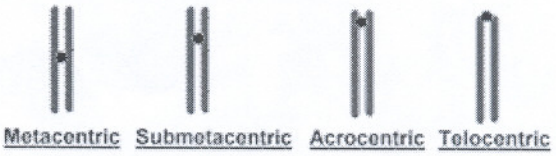
Centromeric position and arm length:



the relative position of the centromere is constant, the ratio of the lengths of the two arms is constant for each chromosome.

This ratio is an important parameter for chromosome identification, and also, allows classification of chromosomes into several basic morphologic types:
metacentric; *sub-metacentric*; *sub-telocentric*; *acrocentric.*

In the human karyotype,
chromosome pairs 13, 14, 15, 21, 22 are *acrocentric,*
and Y is *sub-telocentric.*

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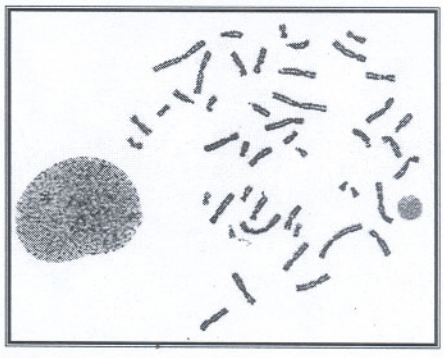
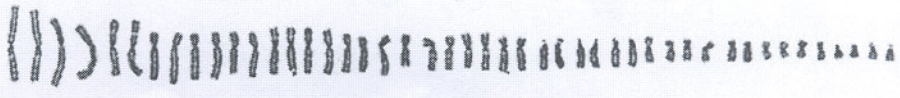
Chromosomal classification

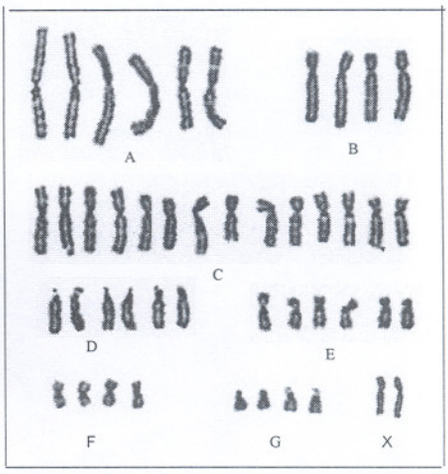
22 pairs of autosomes,-
numbered from 1 to 22
by order
of decreasing length

1 pair of gonosomes, -
or sex chromosomes:
XX in the female,
XY in the male.





Karyotyping



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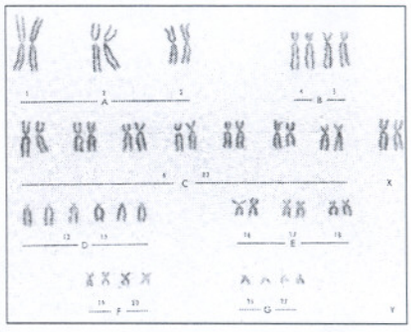

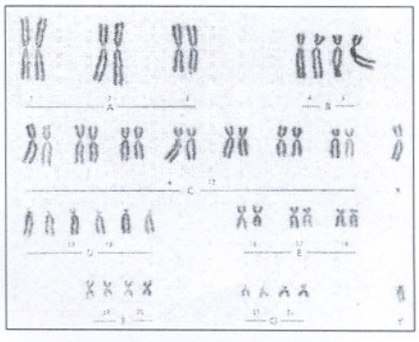

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Items in theDescription Of Karyotype:

- Normal Karyotypes

46 , XY.

47 , XY , + G.

45 , XY , t (D;G).

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- Certain staining techniques cause the chromosomes to take on a banded appearance,
- each arm presenting a sequence of dark and light bands .
- Patterns are specific and repeatable for each chromosome,
- allowing unambiguous identification and longitudinal mapping for locating gene positions and characterising structural changes.
- Patterns, and the nomenclature for defining positional mapping have been standardised

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Staining Methods for Cytogenetic Analysis

G Banding:

Treat with trypsin and then with Geimsa Stain.

R Banding:

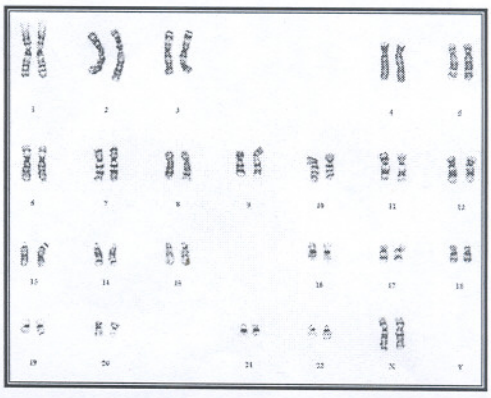
Heat and then treat with Geimsa Stain.

Q Banding:

Treat with Quinicrine dye giving rise to Fluorescent bands.

C Banding:

Staining of the Centromere.



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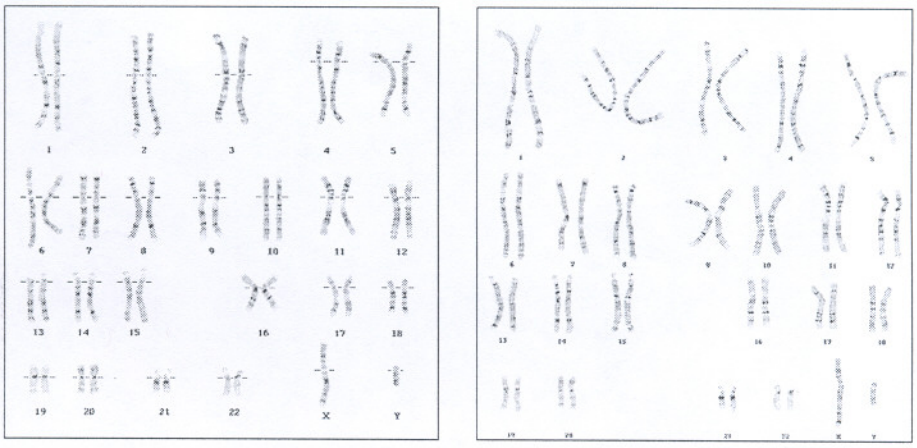
Normal Banded Karyotypes:



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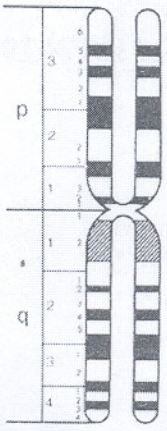

47 , XY , +21.

47 , XY , +3 , t (9;22)(q34;q11).

Fluorescence In-Situ Hybridization (FISH):

To determine if a specific DNA sequence is present on the target chromosome.