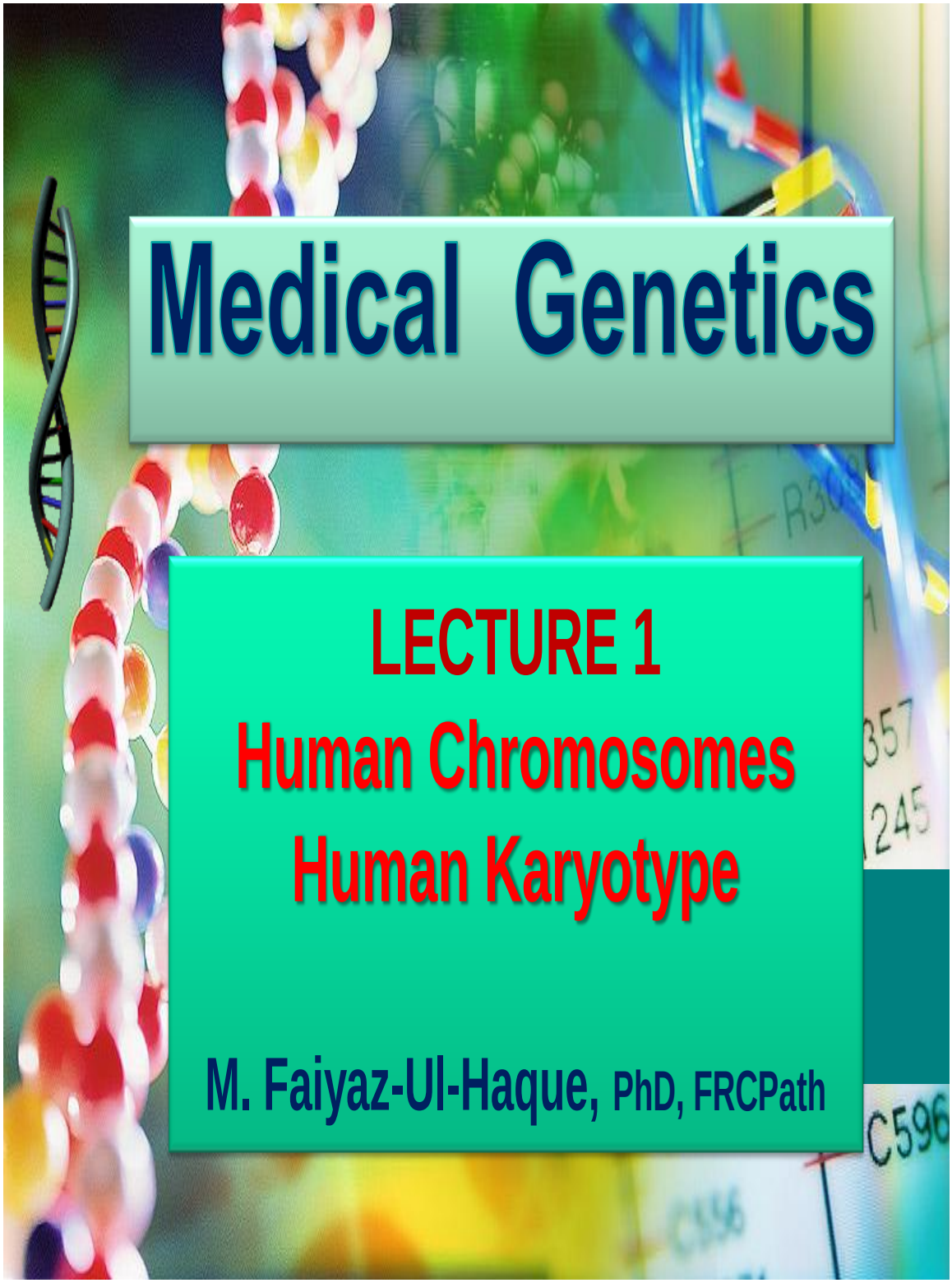

Medical Genetics

LECTURE 1
Human Chromosomes
Human Karyotype

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Lecture Objectives:

By the end of this lecture, the students should be able to:

- Describe the number, structure, and classification of human chromosomes.
- Explain what a Karyotype is and how it is obtained.
- Describe chromosomal banding and explain its use.

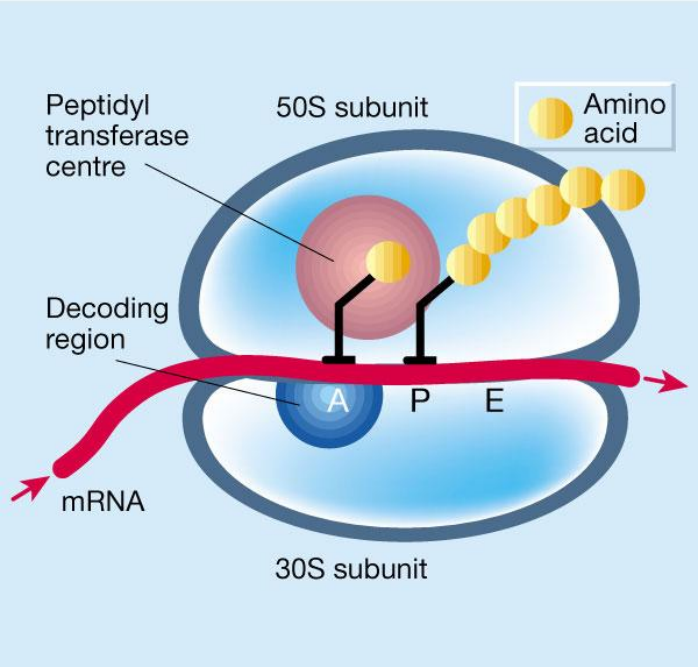
Describe the process of in situ hybridization and the information it provides

What do you know about Genetics?

Transfer of hereditary material from one generation to another

Gene Expression (from gene to protein)

DNA creates mRNA, then mRNA moves from the nucleus to the cytoplasm where the ribosome binds to mRNA (The ribosomal RNAs form two subunits, the large subunit and small subunit. mRNA is sandwiched between the small and large subunits.) Ribosomes begins trandslating it. It sequentially adds amino acids to a growing polypeptide chain until a protein is formed.



Definitions:

Cytogenetics:

The study of the structure and function of chromosomes and chromosome behaviour during somatic and germline division

Molecular genetics:

The study of the structure and function of genes at a molecular level and how the genes are transferred from generation to generation.

Cytogenesis

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

Chromosome studies are an important laboratory diagnostic procedure in

1) **prenatal diagnosis : diagnosis before birth in order to determine whether the fetus has a genetic abnormality. It can be done by by studying the chromosome of a sample.**
2) **certain patients with mental retardation and multiple birth defects : it caused of abnormal chromosome e.g. Huntington's disease**
3) **patients with abnormal sexual development : caused by abnormal sexual chromosome e.g. Klinefelter's syndrome.**
4) **some cases of infertility or multiple miscarriages**
5) **in the study and treatment of patients with malignancies & hematologic disorders e.g. Leukemia**

New techniques allow for increased resolution.

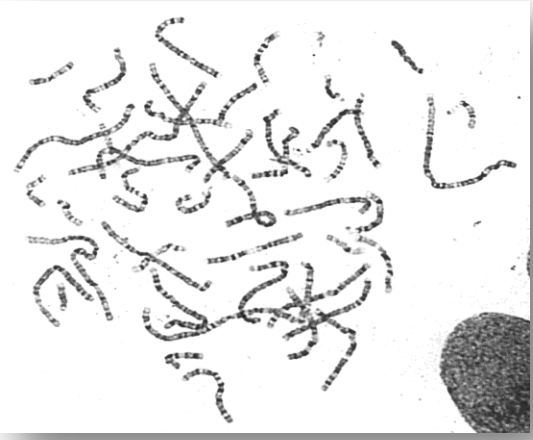
Karyotype

The study of structure and function of chromosomes.

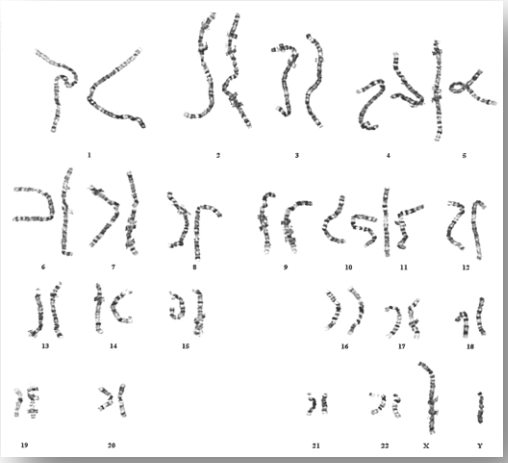
How? By a procedure in which you stain/ fix the chromosome in a slide and observe it under the microscope.

Chromosomes are observed according to the size, shape, position of centromere.

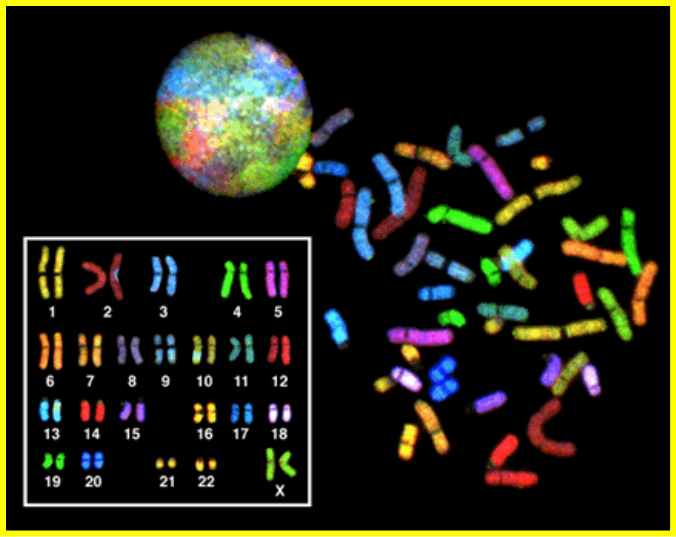
Chromosomes in our body



Fixed chromosomes by karyotype.



Spectral Karyotype: each chromosome painted by a special color. (more convenient and precise than the normal karyotype).



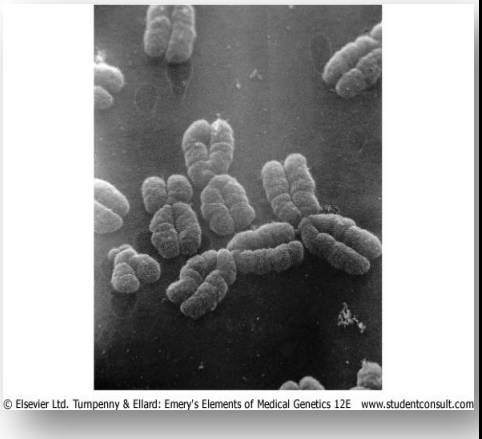
CHROMOSOMES:

■ **carry 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.**

■ **only seen by E.M. (Electronic Microscope)**



Structure of Chromosomes:

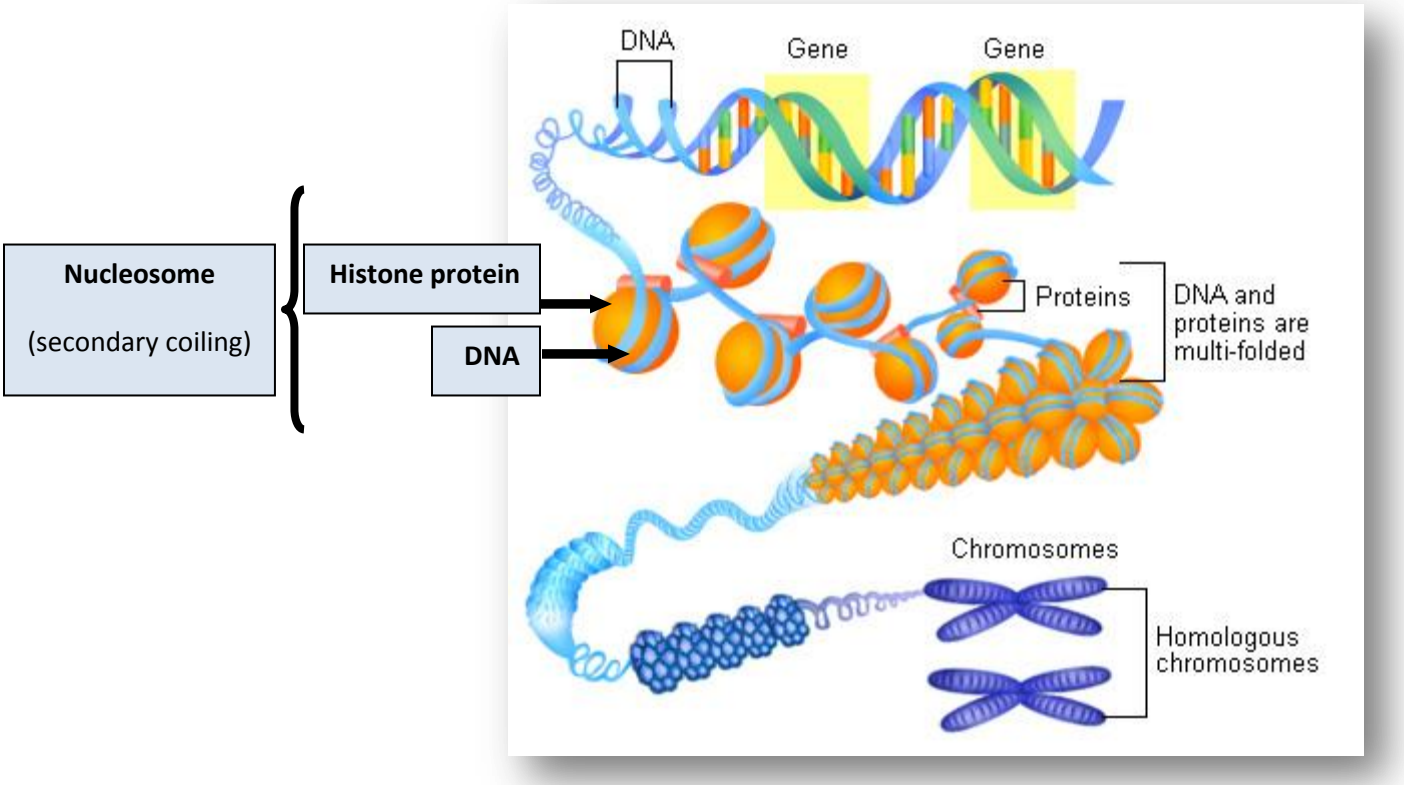
it is always colling and folding.

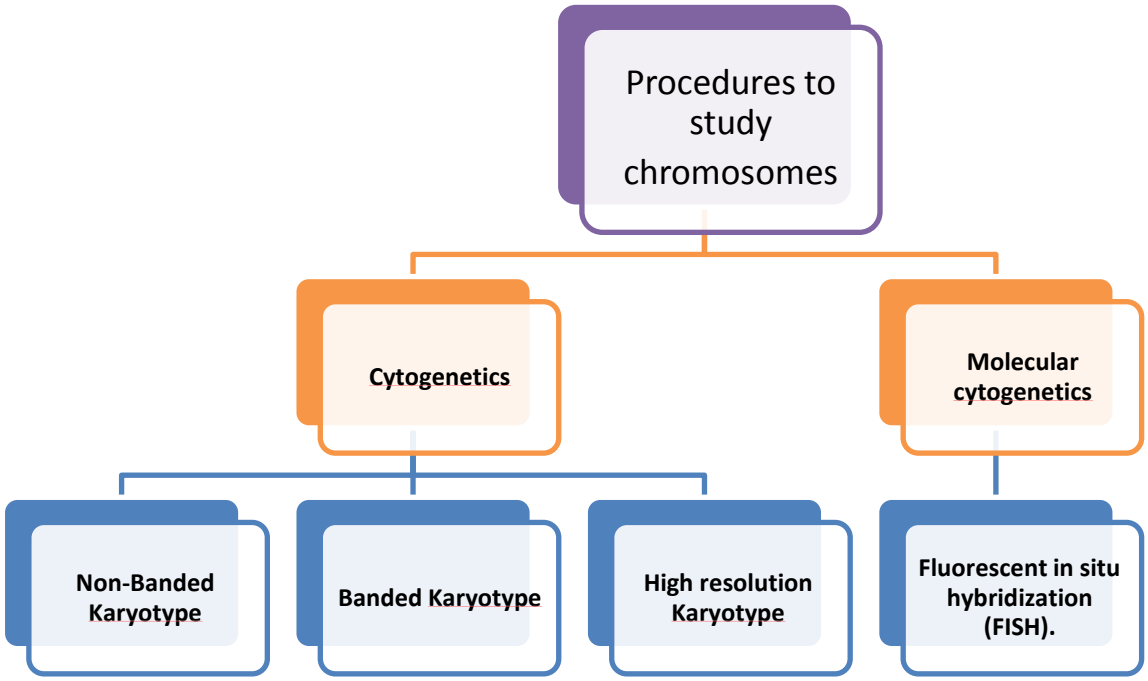
Primary coiling: DNA double helix

Secondary coiling: around histones (basic proteins)→nucleosomes. That means DNA + histones = nucleosomes

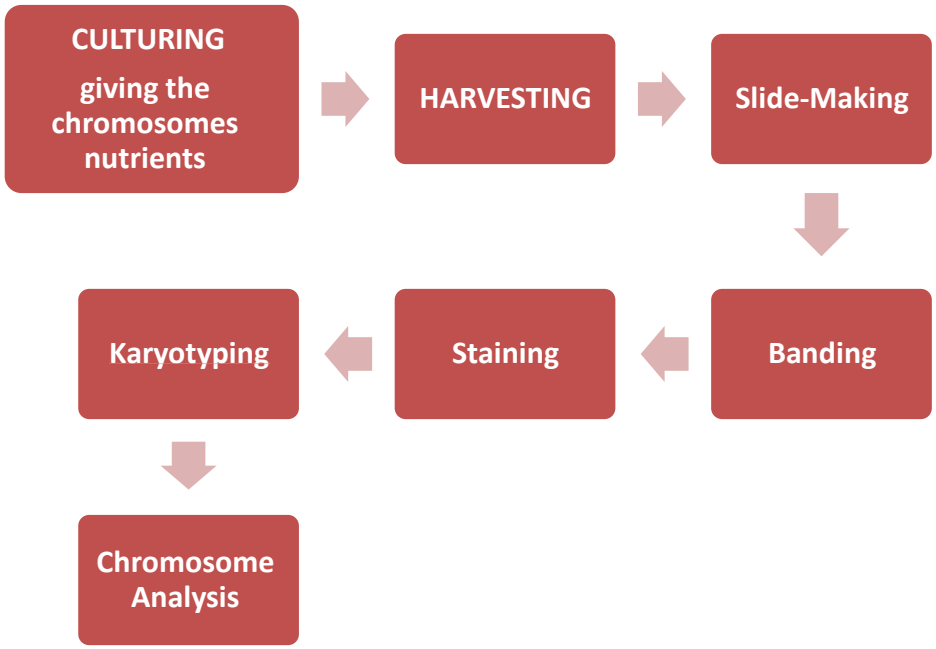
Tertiary coiling → chromatin fiber. That means many nucleosomes form chromatin fiber.

Chromatin fibers form long loops on non-histone proteins →tighter coils → chromosome That means chromatin fiber+ colling on non-histone proteins= chromosome.

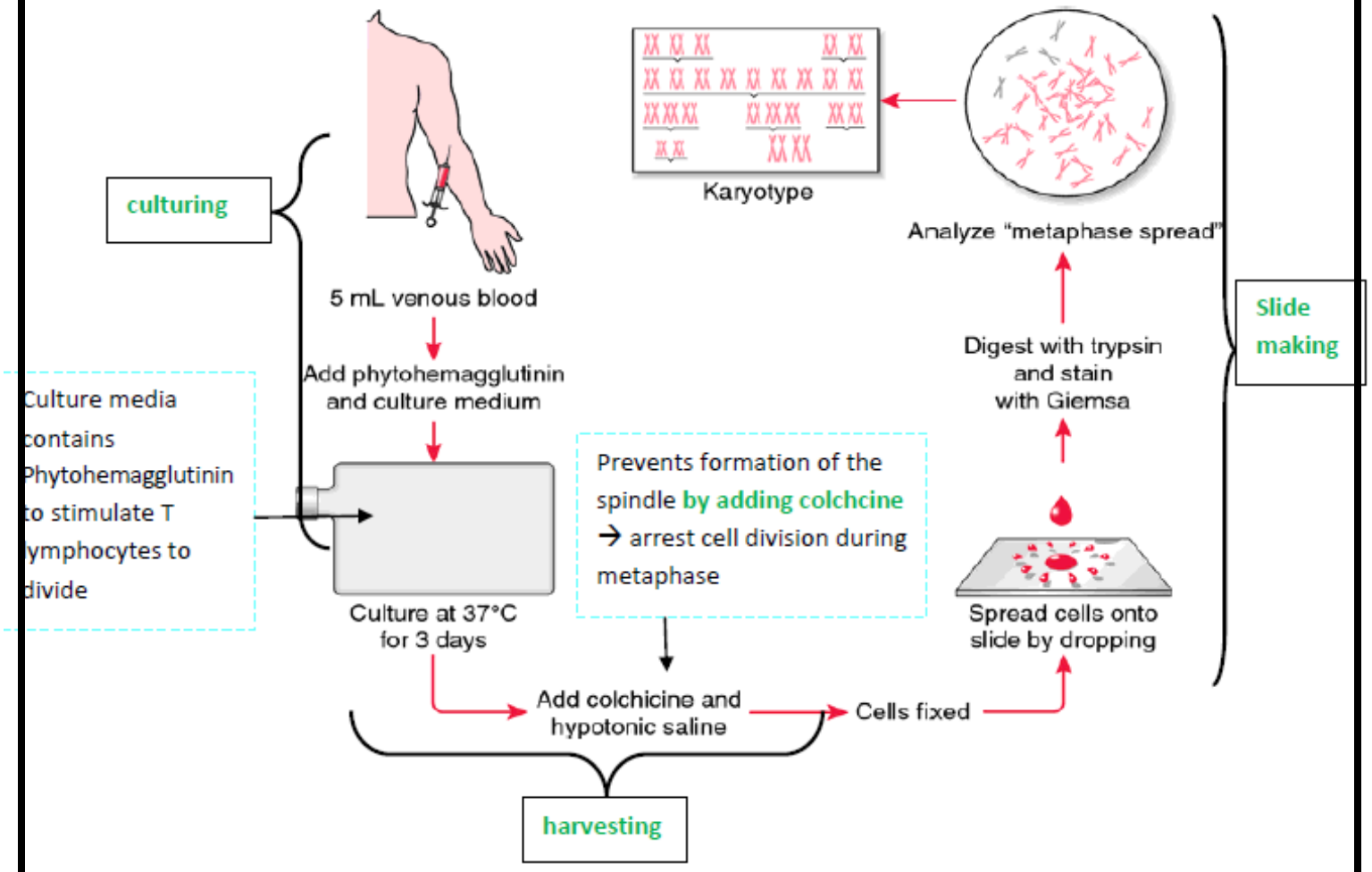




Karyotype: A series of steps involved:



Steps of Chromosome Preparation from Peripheral Blood



Why do we need T-lymphocytes ??? Because they are easier to culture in vivo and have better metaphase spread of chromosome

In what Phase do we study chromosomes? **Methaphase**

How do we stop the division at metaphase? **we use colchicine: to cut the bindles (spinal fibers) so that the division would stop.**

we can see DNA in White blood cells but not in red blood cells because RBCs don't have nucleus.

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 in a telomere.

Centromeric position and arm length:

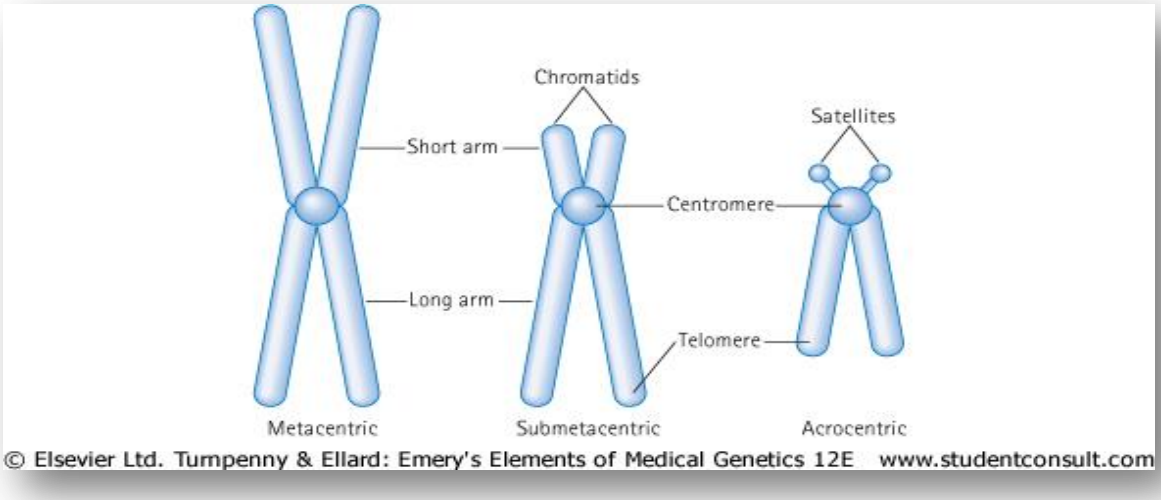
i-*metacentric* : a chromosome of two arms are equal in length

ii-sub-metacentric: **chromosome whose centromere lies between its middle and its end but closer to the middle.**

iii-acrocentric: **a chromosome in which the centromere is located quite near one end of the chromosome. (reaching the telomere→ almost no upper arm). The remaining of the upper arm is called Satellites.**

In the human karyotype chromosome pairs 13, 14, 15, 21, 22 are *acrocentric.*

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

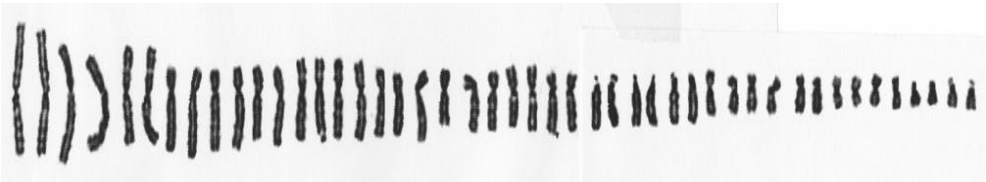


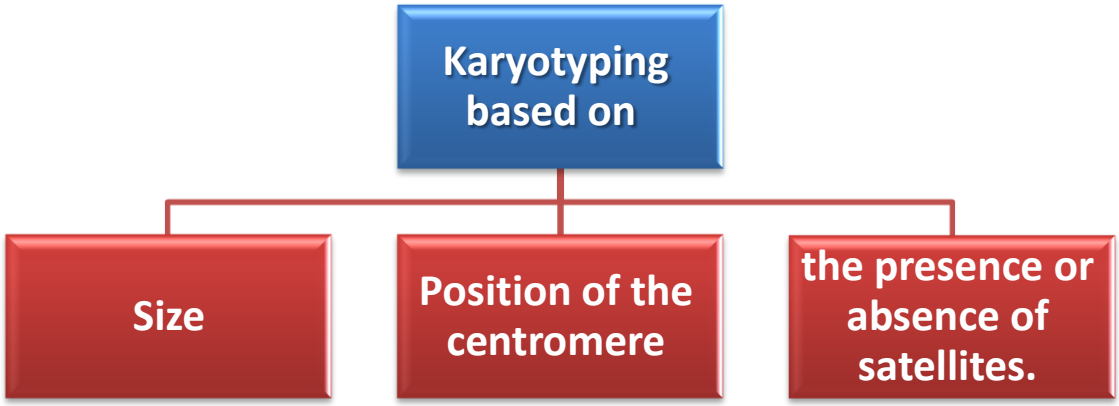
Chromosomal classification:

- **22 pairs of autosomes, numbered from 1 to 22 by order of decreasing length**
- **1 pair of sex chromosomes:**

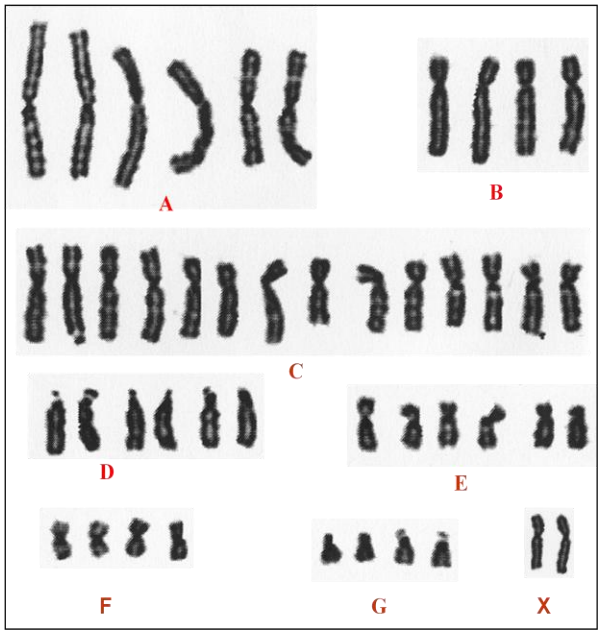
  XX in the female,

  XY in the male.





Chromosomes are divided into 7 groups (A, B , C, D, E, F, G, X) based on their size, position of centromere, the presence or absence of satellites



Normal Karyotypes

46,XY → male

46,XX → female

Abnormal Karyotypes

47, XY, + 21 : addition of one chromosome (down syndrome)

45, XY, t (D;G) : translocation between group G and D

(cut of the two centromers→ two chromosomes join together becoming one → the result is loss of a chromosome.) NO REDUCTION

Banding

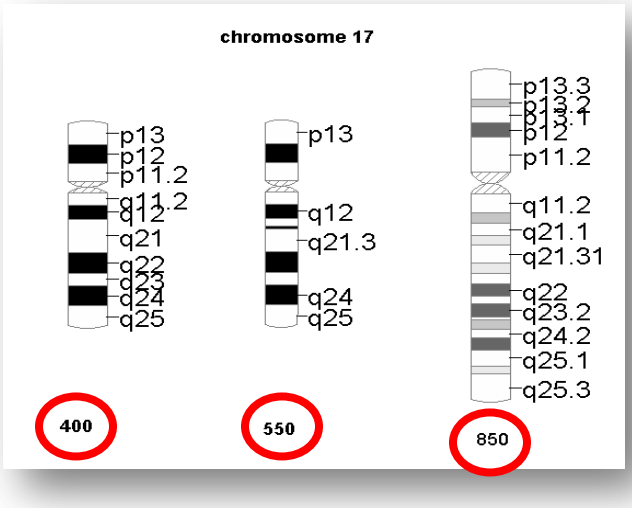
Chromosome banding: The treatment of chromosomes to reveal characteristic patterns of horizontal bands like bar codes.

Each banding pattern in a chromosome is different than all other chromosomes (banding in chromosome 1 is different than banding in chromosome 2,3,4…)

- Certain staining techniques cause the chromosomes to take on a banded appearance,
- Each arm presenting a sequence of dark and light bands . Each chromosome has a unique banding pattern of dark bands (stained regions) and light bands (unstained regions)
- Patterns are specific and repeatable for each chromosome,
- Allowing accurate identification and longitudinal mapping for locating gene positions and characterising structural changes.
- Patterns, and the nomenclature for defining positional

mapping have been standardised

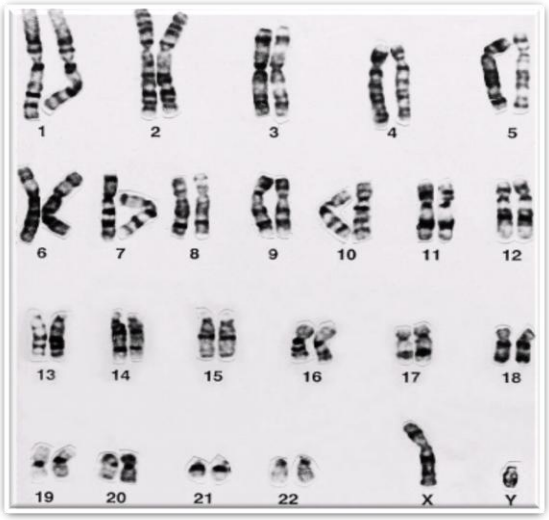
Band resolution = estimate of number of light + dark bands per haploid set of chromosomes



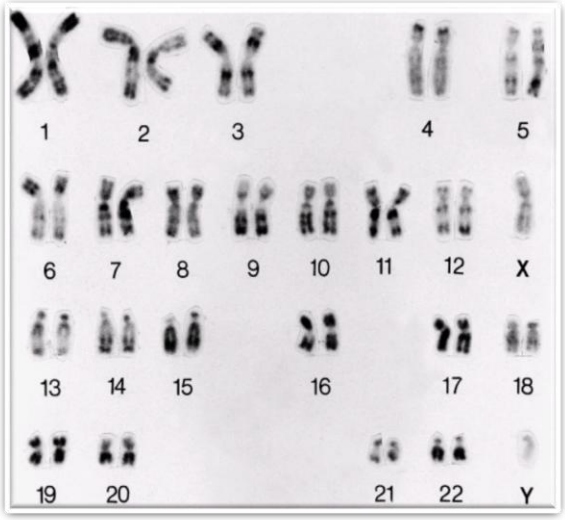
Types of Banding

The most popular

G Banding:	**Treat with trypsin and then with Geimsa Stain.** **Trypsin" which is a digestive fluid that digest the chromosomes.**
R Banding	**Heat and then treat with Geimsa Stain.** **Reverses G-banding , e.g. the dark band in G- banding is a light one in R- banding. It's made to confirm the banding pattern**
Q Banding:	**Treat with Quinicrine dye giving rise to fluorescent bands. It requires an ultraviolet fluorescent microscope**
C Banding:	**Staining of the Centromere. Treat with acid followed by alkali prior to G banding**



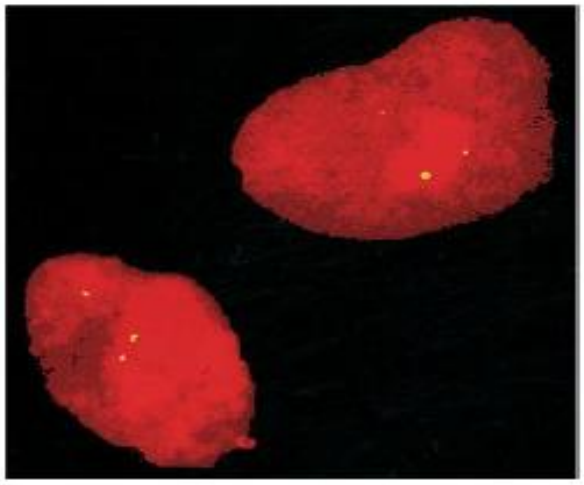
A normal G-banded male Karyotype



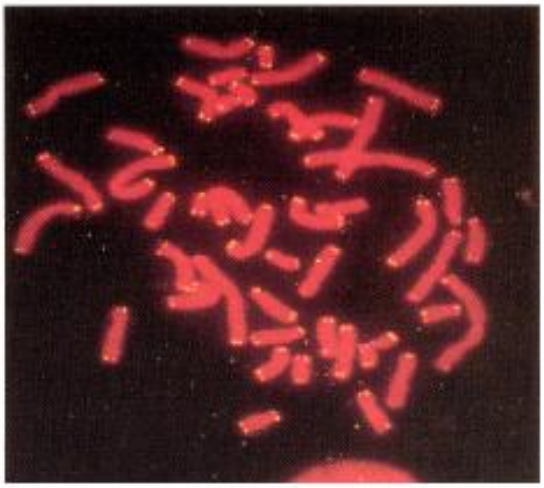
A normal R-banded male Karyotype

Molecular cytogenetics:

- Fluorescence In-Situ Hybridization (FISH)



FISH of interphase nuclei with a chromosome 21 centromeric probe showing 3 signals consistent with trisomy 21.



FISH of metaphase with a probe for telomere showing signals at the end of each chromatid .

In situ hybridization: The use of a DNA or RNA probe to detect complementary genetic material in cells or tissue. In situ hybridization involves hybridizing a labeled nucleic acid to suitably prepared cells or tissues on microscope slides to allow visualization in situ (in the normal location).

Take home messages:

- **The packaging of DNA into chromosomes involves several orders of DNA coiling and folding.**
- **The normal human karyotype is made up of 46 chromosomes consisting of 22 pairs of autosomes and a pair of sex chromosomes, XX in the female, and XY in the male.**
- **Each chromosome consists of a short (p) and a long (q) arm joined at the centromere.**
- **Chromosomes are analyzed using cultured cells and specific banding patterns can be identified using special staining techniques.**

Molecular cytogenetic techniques (e.g. FISH) are based on the ability of a single-stranded DNA probe to anneal with its complementary target sequence. They can be used to study chromosmes in metaphase or interphase