Human Genetics

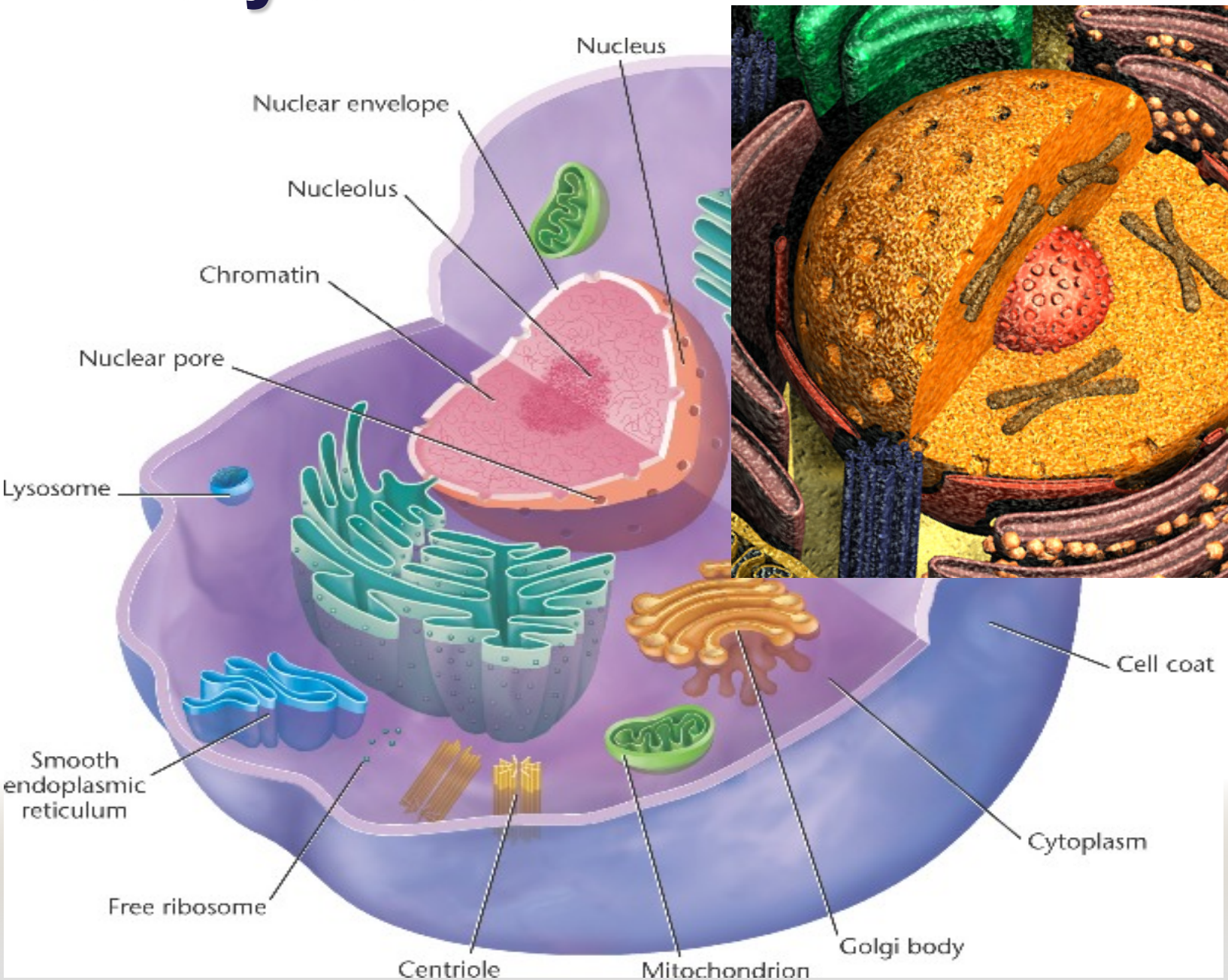
LECTURE 1
Human Chromosomes: Genotypes/Phenotypes

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.

Eukaryotic cell



GENETICS :

- **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.

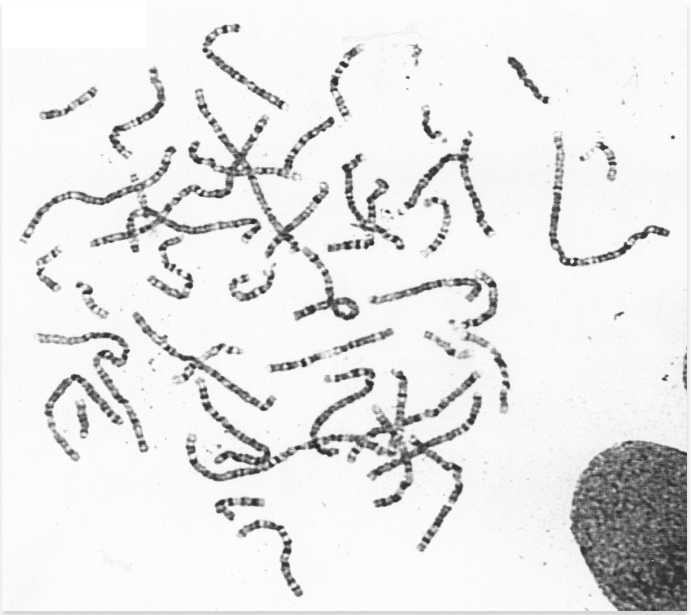
Cytogenetics:

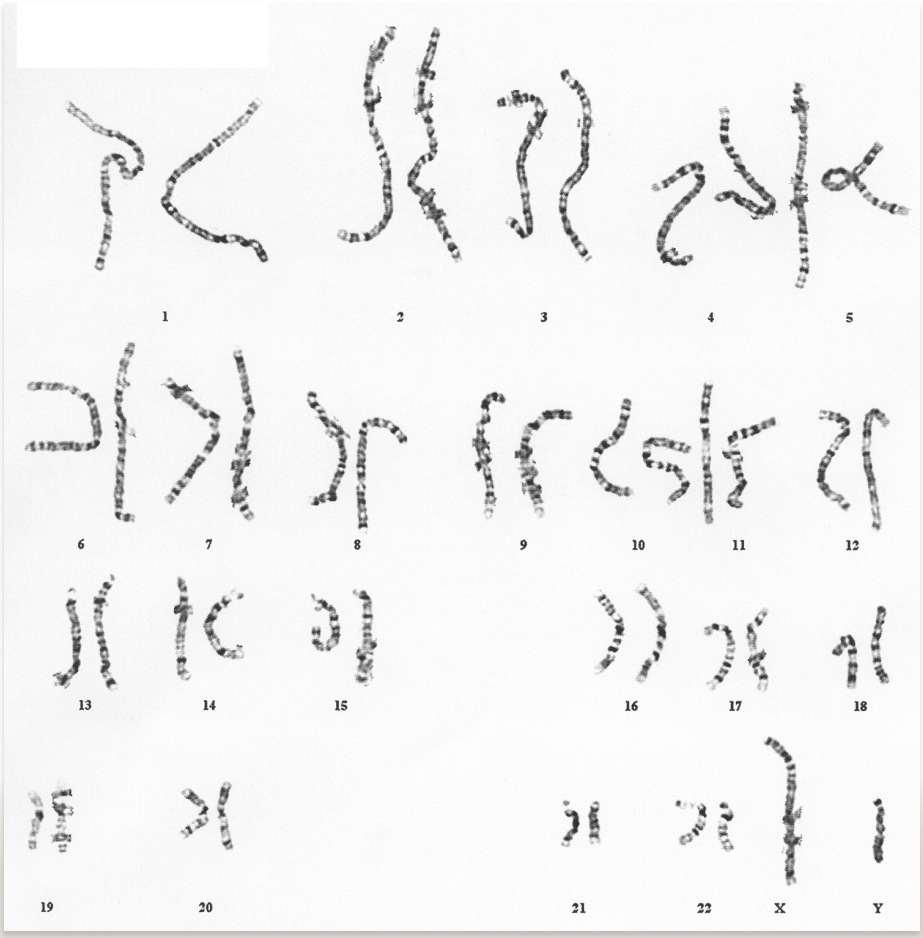
Human Cytogenetics:
the study of human chromosomes in health and disease.

Chromosome studies are an important laboratory diagnostic procedure in;

1) **prenatal diagnosis**
2) **certain patients with mental retardation and multiple birth defects**
3) **patients with abnormal sexual development**
4) **some cases of infertility or multiple miscarriages**
5) **in the study and treatment of patients with malignancies & hematologic disorders.**

KARYOTYPE



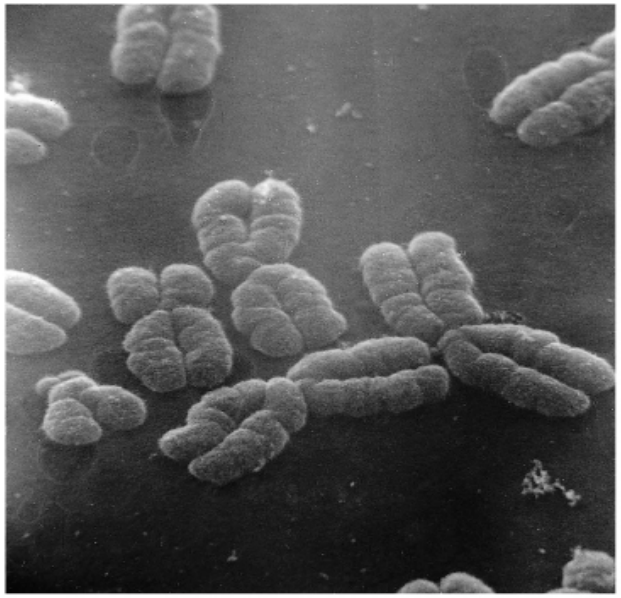


CHROMOSOMES:

- carry genetic material

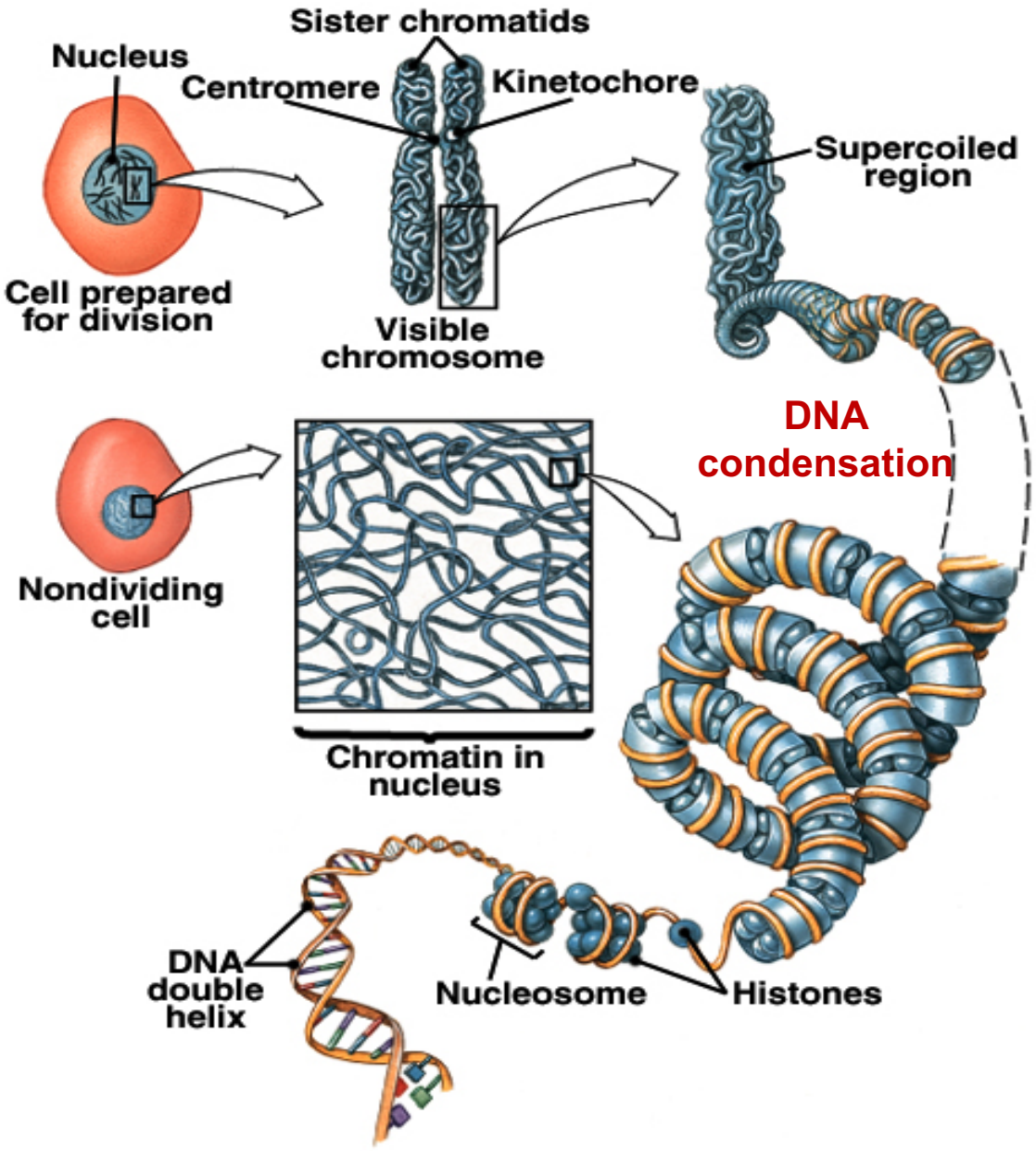
- heredity: each pair of homologues consists of one paternal and one maternal chromosome

- The intact set is passed to each daughter cell at every mitosis.



EM of human chromosomes

Structure of Chromosomes



Orders of DNA coiling and folding:

- Primary coiling: DNA double helix
- Secondary coiling: around histones (basic proteins)→ nucleosomes
- Tertiary coiling → chromatin fiber
- Chromatin fibers form long loops on non-histone proteins → tighter coils → chromosome

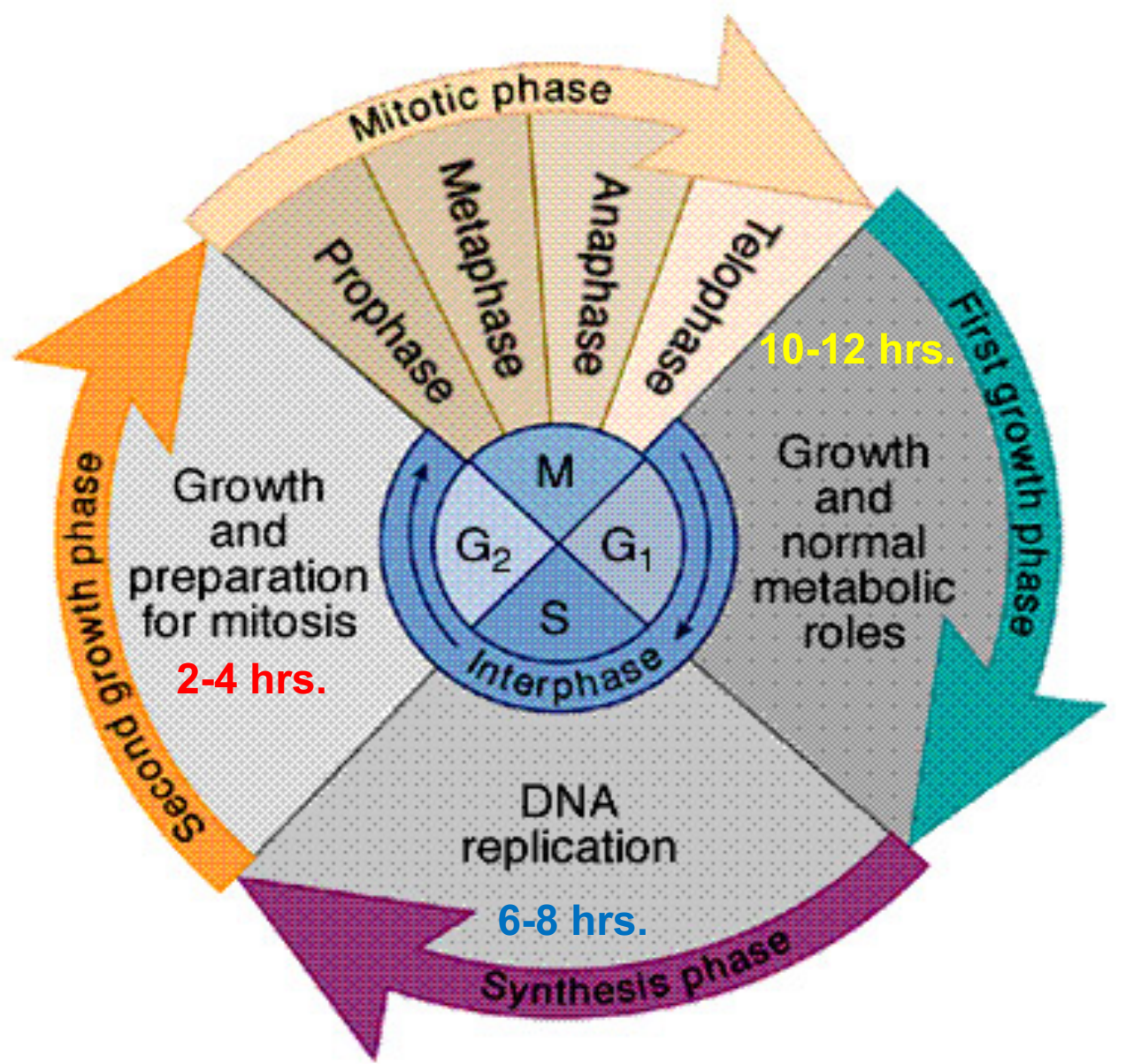
Cytogenetics:

- Non-Banded Karyotype
- Banded Karyotype
- High resolution Karyotype

Molecular cytogenetics:

- Fluorescent in situ hybridization (FISH)

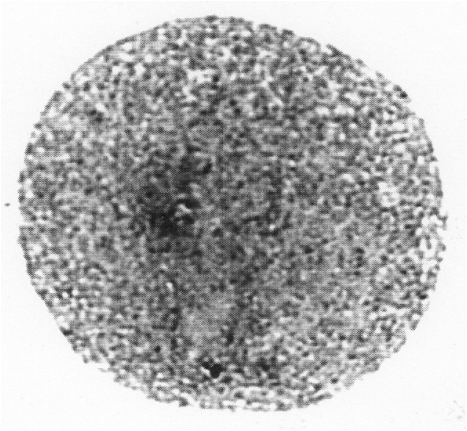
Mitotic cell cycle

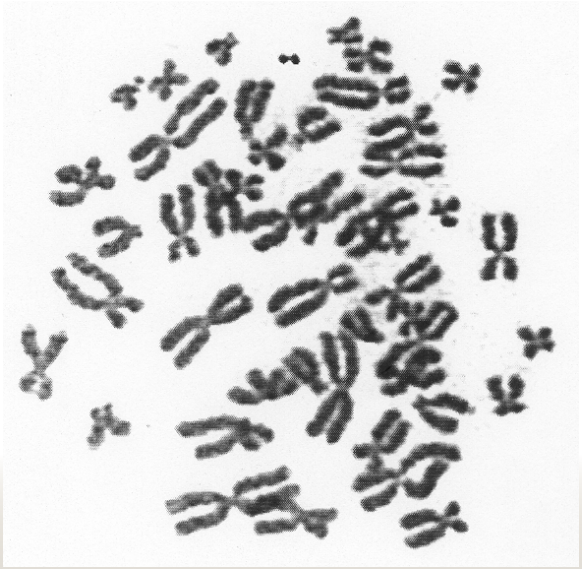


Karyotype

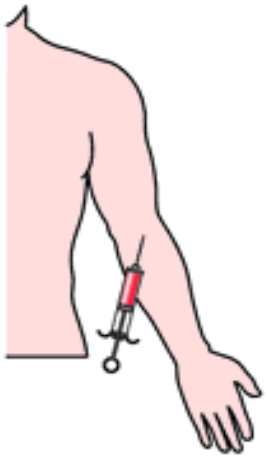
steps involved :

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





Chromosome Preparation from Peripheral Blood



5 mL venous blood

Add phytohemagglutinin and culture medium

Culture media contains Phytohemagglutinin to stimulate T lymphocytes to divide

Culture at 37°C for 3 days

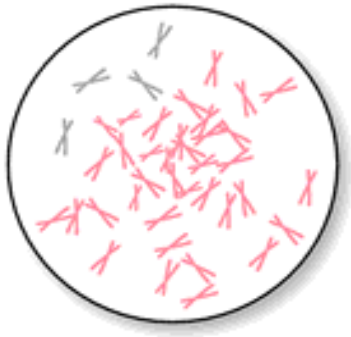
Prevents formation of the spindle → arrest cell division during metaphase

Add colchicine and hypotonic saline

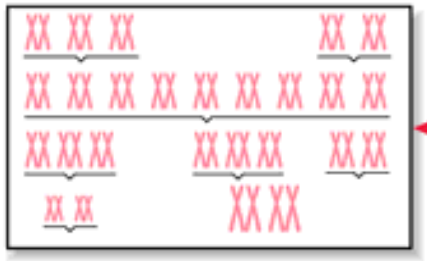
Cells fixed

Spread cells onto slide by dropping

Digest with trypsin and stain with Giemsa

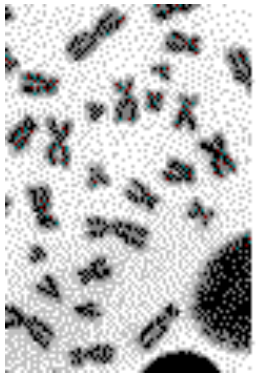


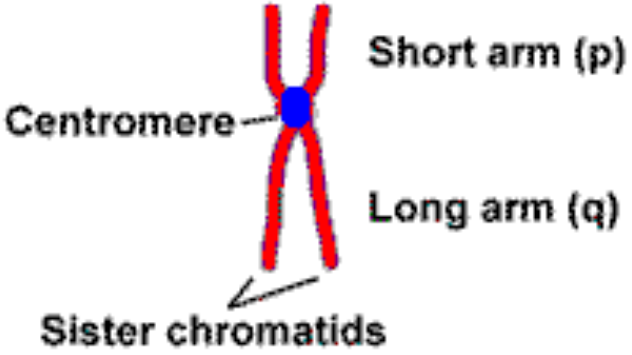
Analyze "metaphase spread"



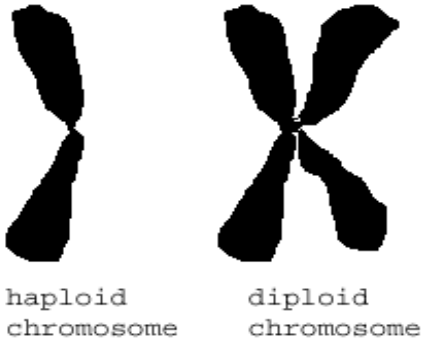
Karyotype

Metaphase chromosomes:



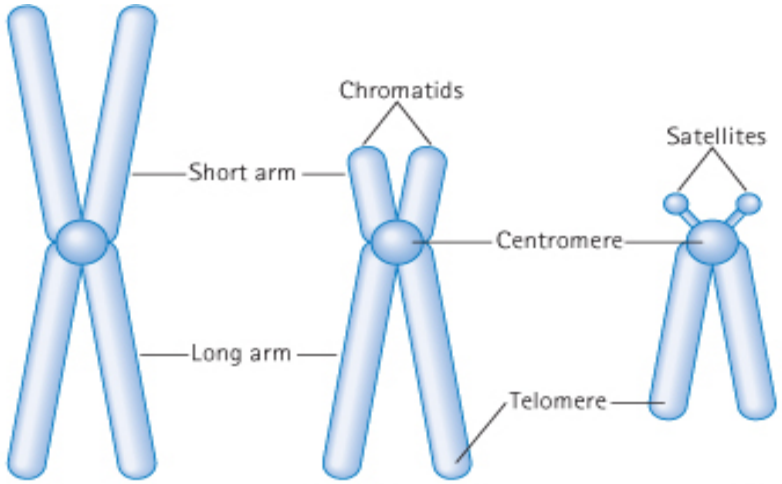


A single complete set of chromosomes. (N=23 for humans)



- 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:



The ratio of the lengths of the two arms is constant for each chromosome.

This ratio is an important parameter for chromosome identification and allows classification of chromosomes into several basic morphologic types:

i-*metacentric* ii-*sub-metacentric* iii-*acrocentric*

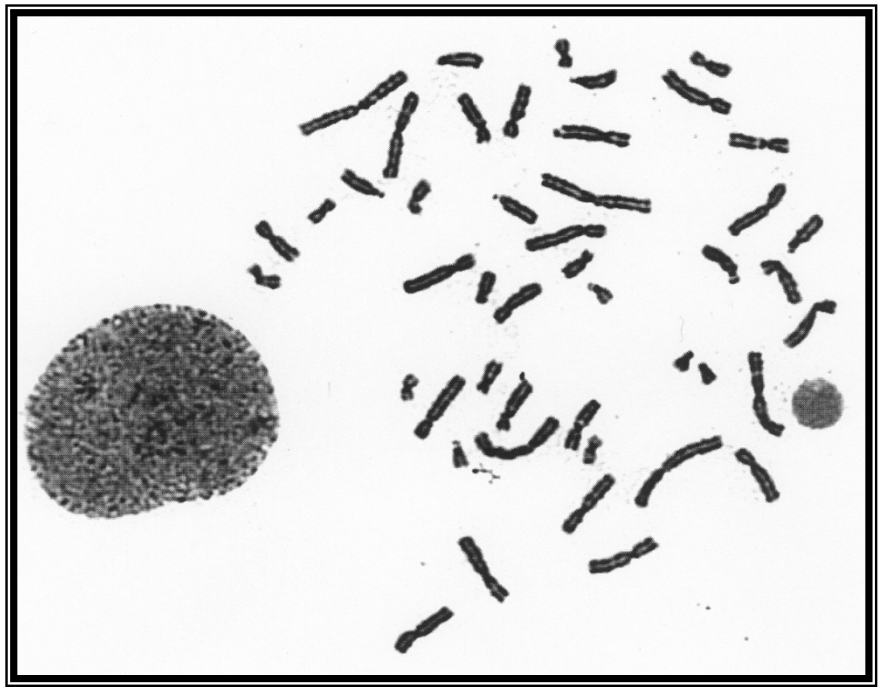
In the human karyotype chromosome pairs 13, 14, 15, 21, 22 are *acrocentric*

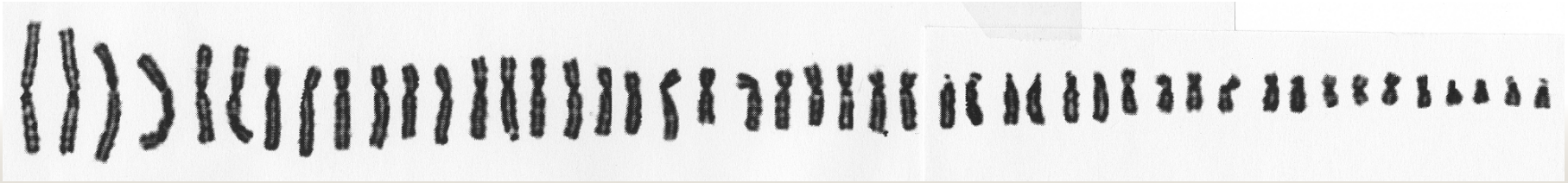
Chromosomal classification

- 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.

Human Chromosome

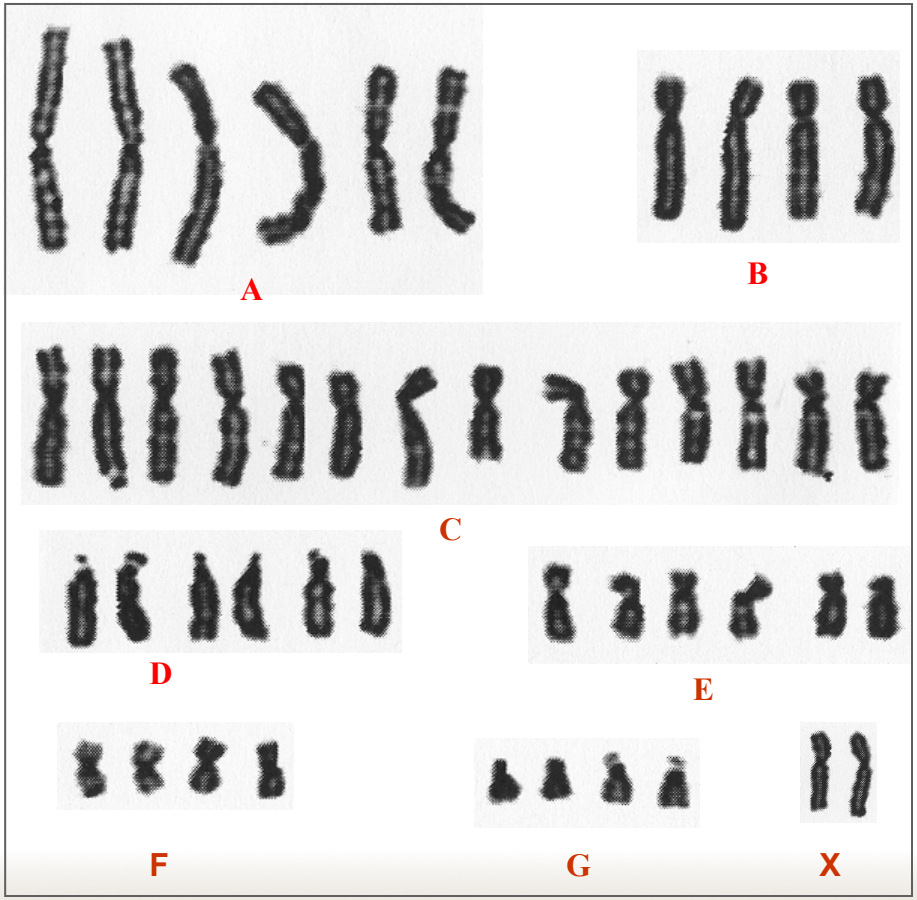




Karyotyping

Based on:

1. the length
2. the position of the centromere
3. the presence or absence of satellites



Items in the Description Of Karyotype:

- **Normal Karyotypes**

  46, XY
  46, XX

- **Abnormal Karyotypes**

  47,XY,+ 21 Down Syndrome

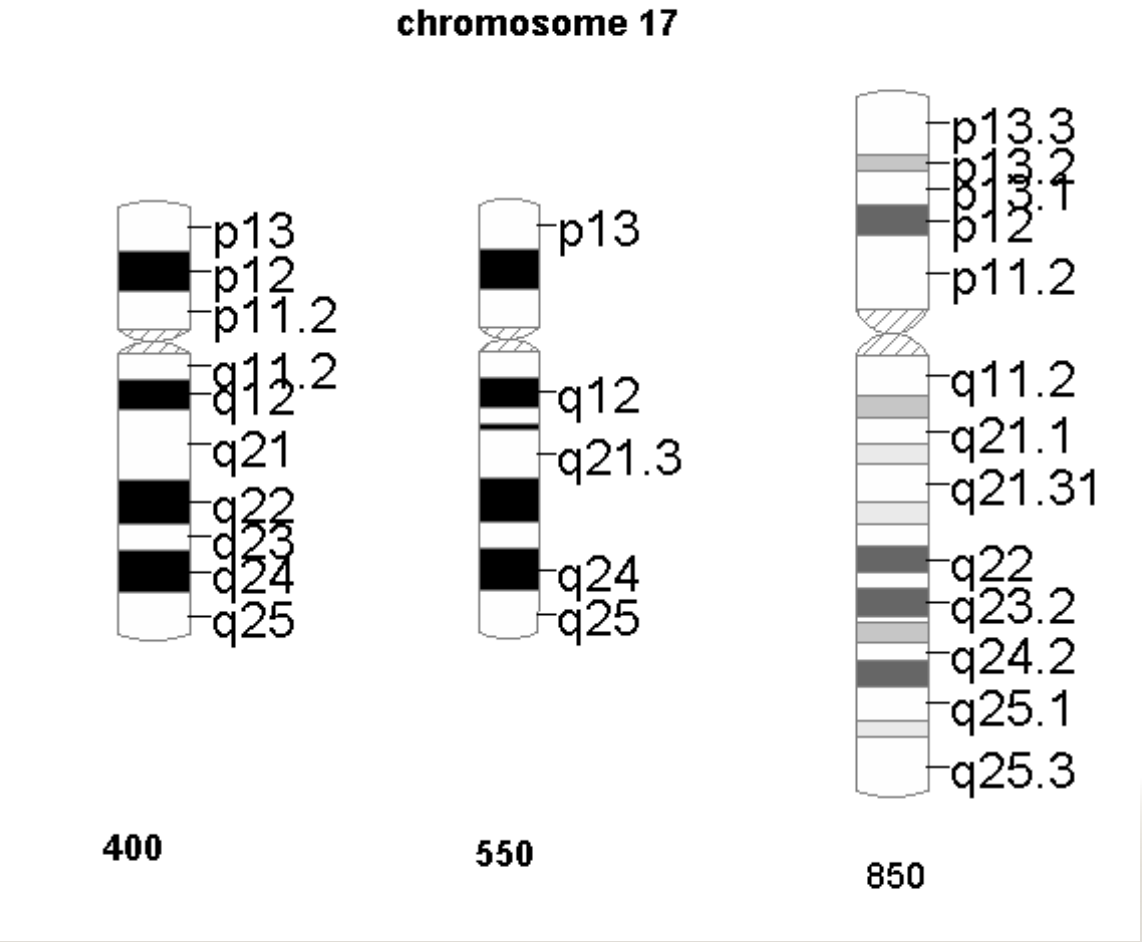
  45,X Turner Syndrome

Banding

- 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 accurate identification and longitudinal mapping for locating gene positions and characterising structural changes.
- Patterns, and the nomenclature for defining positional mapping have been standardised

Chromosome Banding

- Band resolution = estimate of number of light + dark bands per haploid set of chromosomes
- 400 $\rightarrow$ 850+



G Banding:

Treat with trypsin and then with Geimsa Stain.

R Banding:

Heat and then treat with Geimsa Stain.

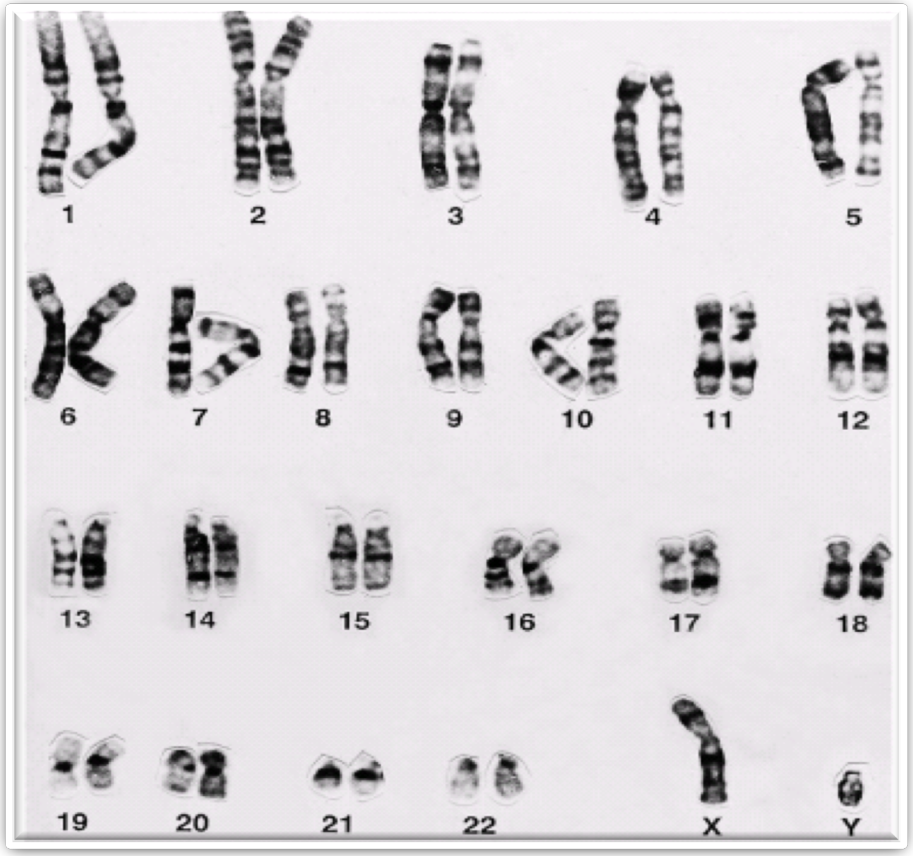
Q Banding:

Treat with Quinicrine dye giving rise to fluorescent bands. It requires an ultraviolet fluorescent microscope

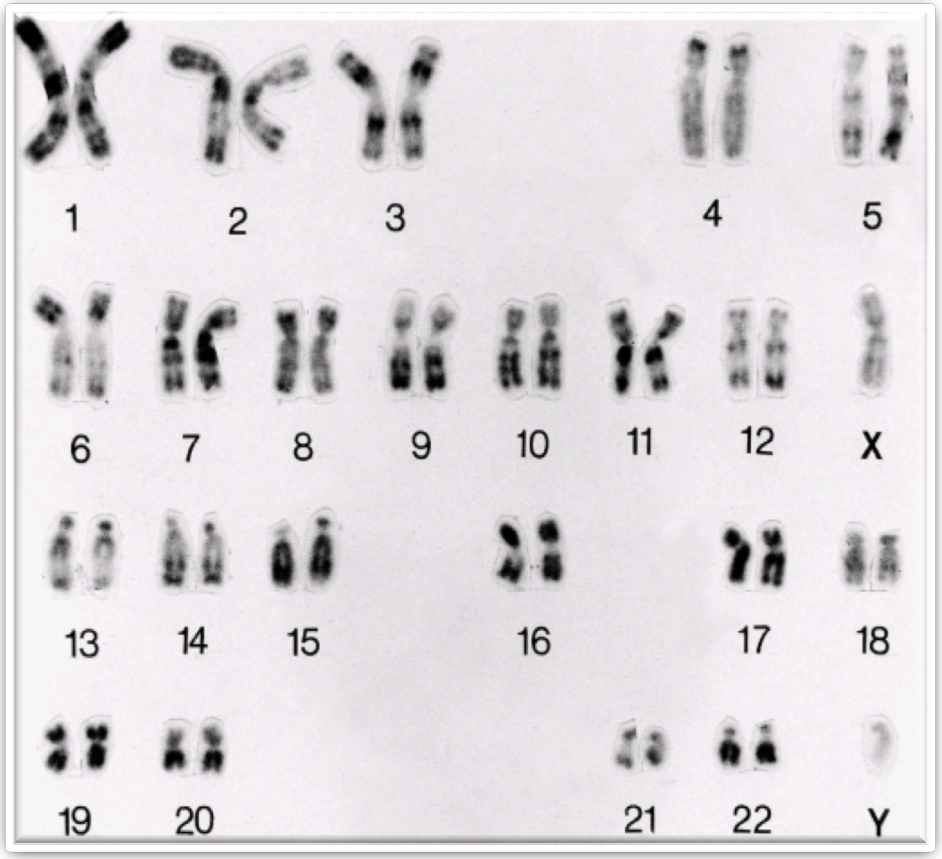
C Banding:

Staining of the Centromere. Treat with acid followed by alkali prior to G banding

Banded Karyotype: Normal Banded Karyotypes

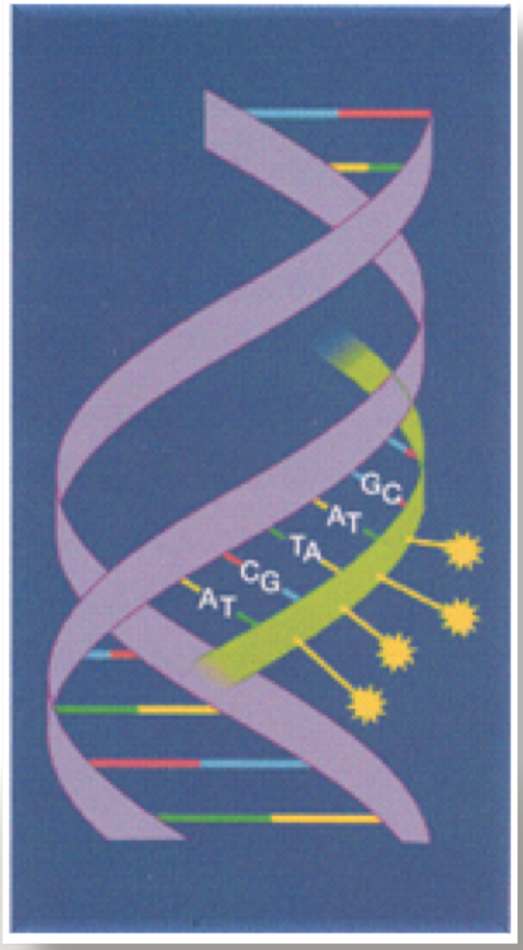


A normal G-banded male Karyotype

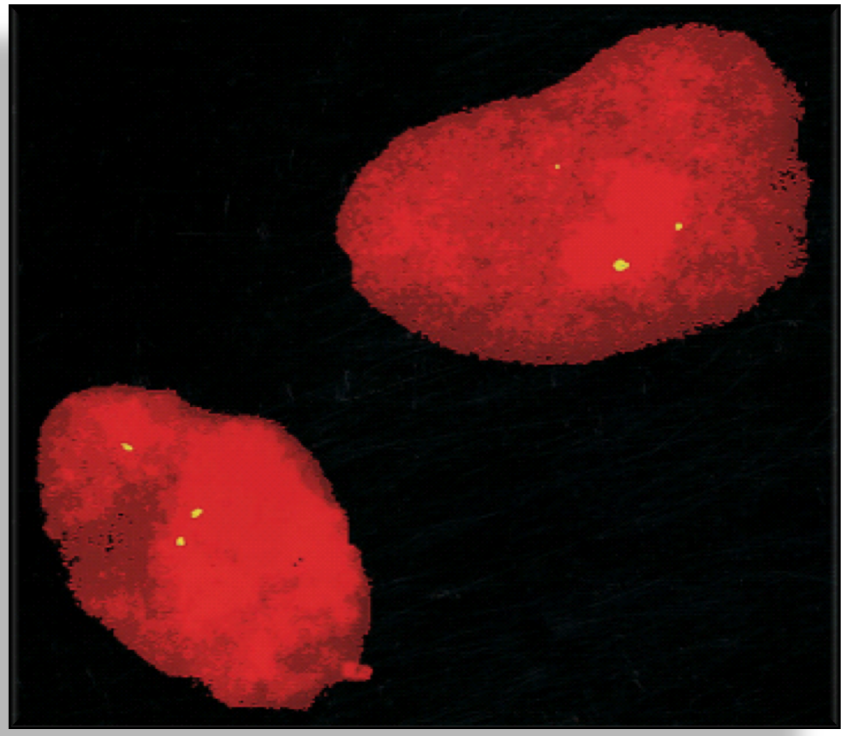


A normal R-banded male Karyotype

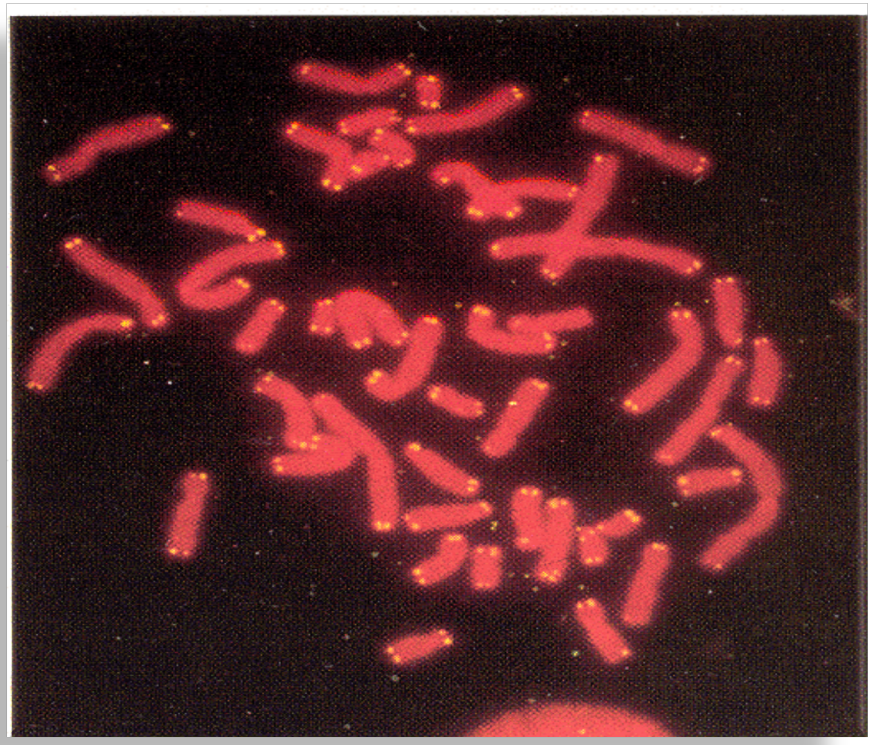
Fluorescence In-Situ Hybridization (FISH)



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

Take Home Message

- 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.
- FISH is based on the ability of a single-stranded DNA probe to anneal to its complementary target sequence. It can be used to identify and study genes on chromosomes in metaphase or interphase.

THANK YOU