جامعة الملك سعود
King Saud University

HUMAN GENETICS

تمت المراجعة
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LECTURE 1

Human chromosomes:

Genotype / Phenotype

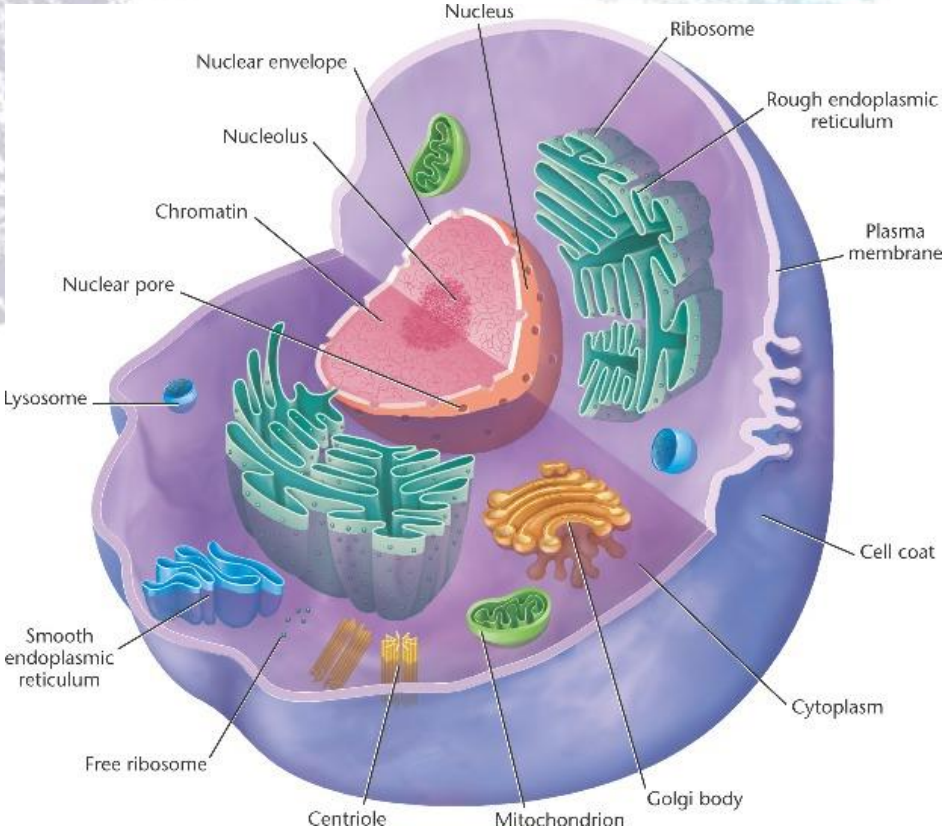
EDITION FILE

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



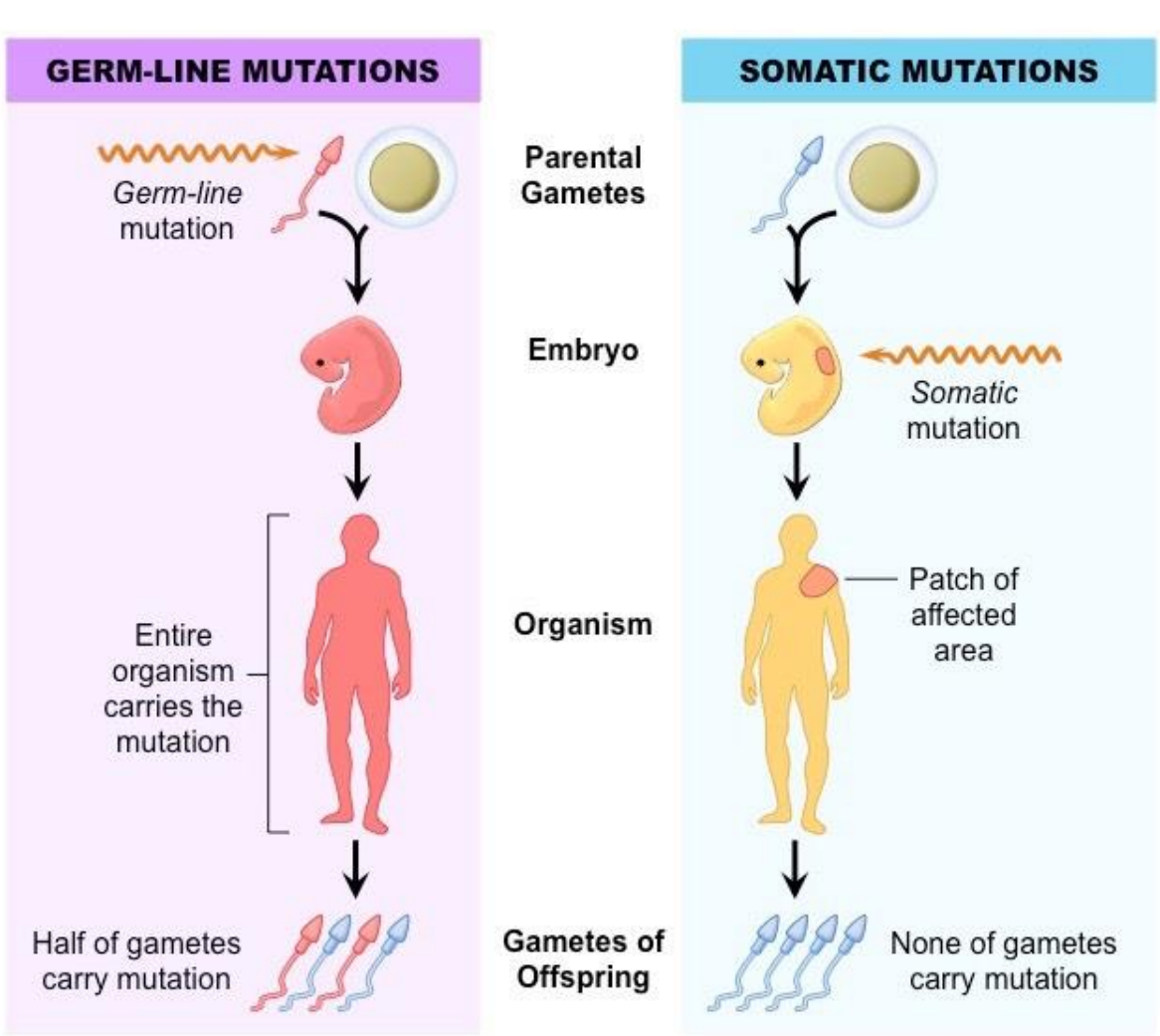
GENETICS

- **Cytogenetics:** (Chromatin)

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

- **Molecular genetics:** (a Single gene, i.e. A,G,C,T.)

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



*Only to differentiate between Somatic and Germ-Line division

CYTOGENETICS

Human Cytogenetics:

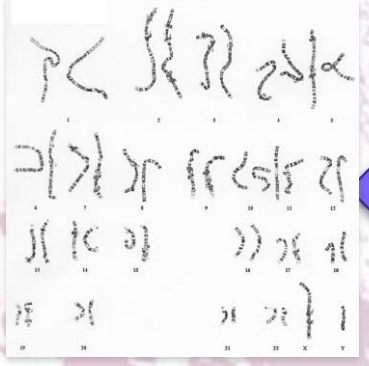
the study of human chromosomes in health and disease.

Chromosome studies are an important laboratory diagnostic procedure in;

I. prenatal diagnosis
II. certain patients with mental retardation and multiple birth defects
III. patients with abnormal sexual development
IV. some cases of infertility or multiple miscarriages
V. in the study and treatment of patients with malignancies & hematologic disorders.

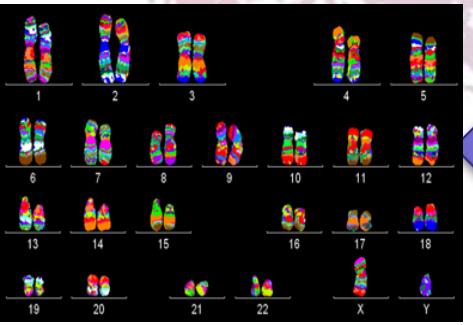
CYTOGENETICS

Non-Banded Karyotype



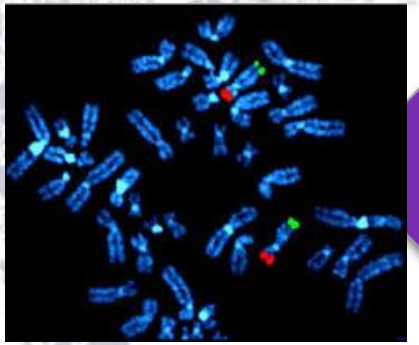
Banded Karyotype

Special dyes gives the chromosome signature



High Resolution Karyotype

MOLECULAR CYTOGENETICS



Fluorescent In Situ Hybridization (FISH)

Technology that is used to examine a specific gene by (Primers) that are made in the lab

STRUCTURE OF CHROMOSOMES

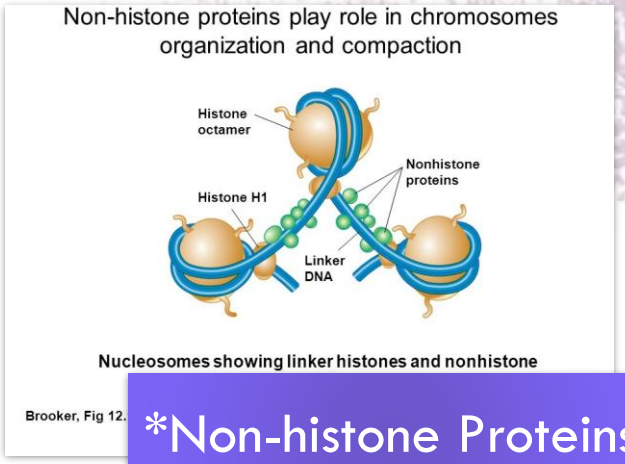
Orders of DNA coiling and folding

Primary coiling:
DNA double helix

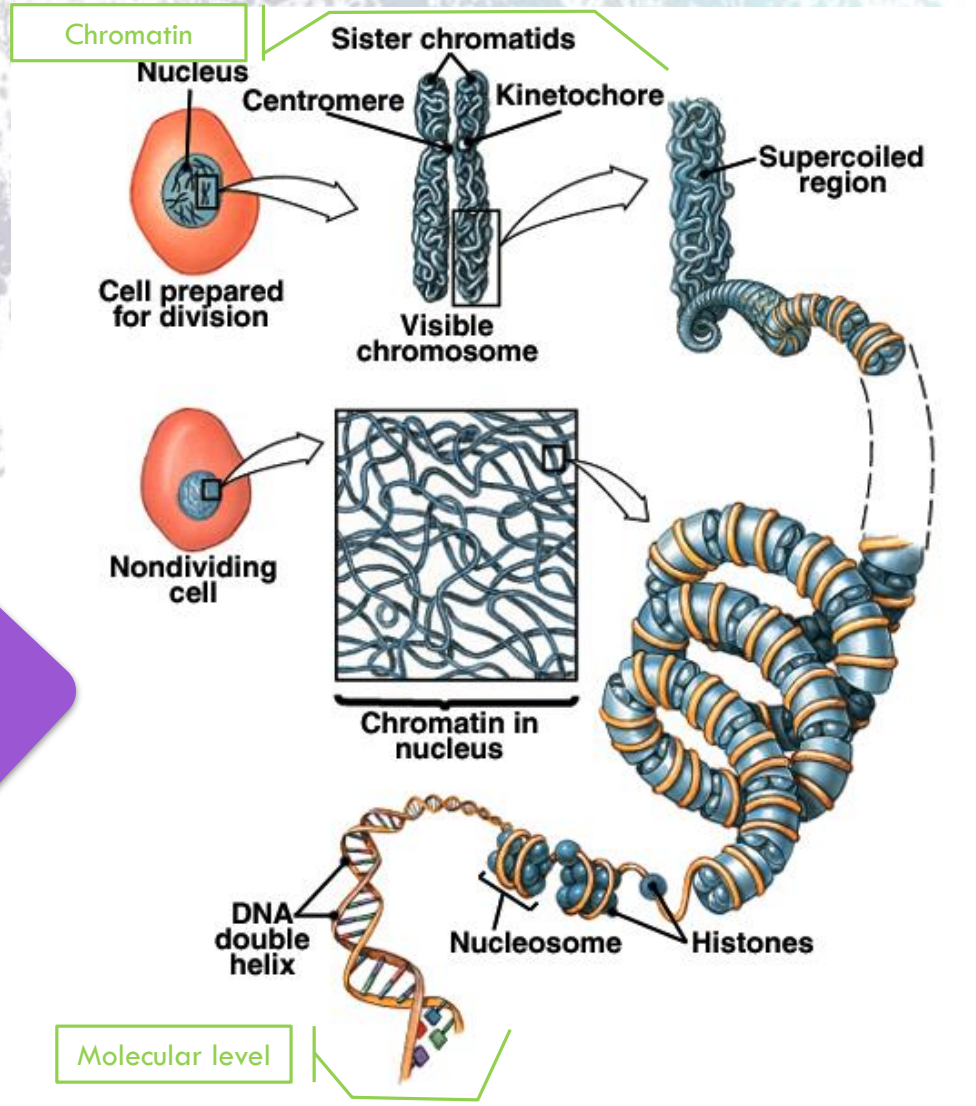
Secondary coiling:
around histones (basic proteins)
↳ nucleosomes

Tertiary coiling:
Chromatin fiber

Chromatin fibers form long loops on non-histone proteins*
↳ tighter coils
↳ chromosome



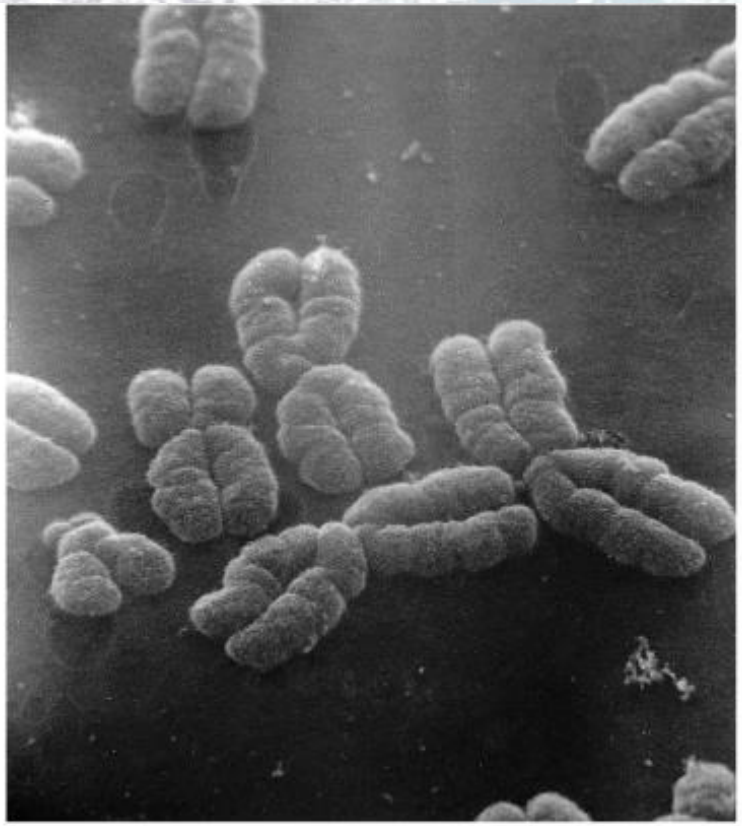
*Non-histone Proteins



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.

ELECTRON MICROSCOPE OF HUMAN CHROMOSOMES



*So there's no particular job for the chromosome itself, but the genetic information hold on this particular preserver are the one will affect and cause the disease if there's a malformation of this chromosome.

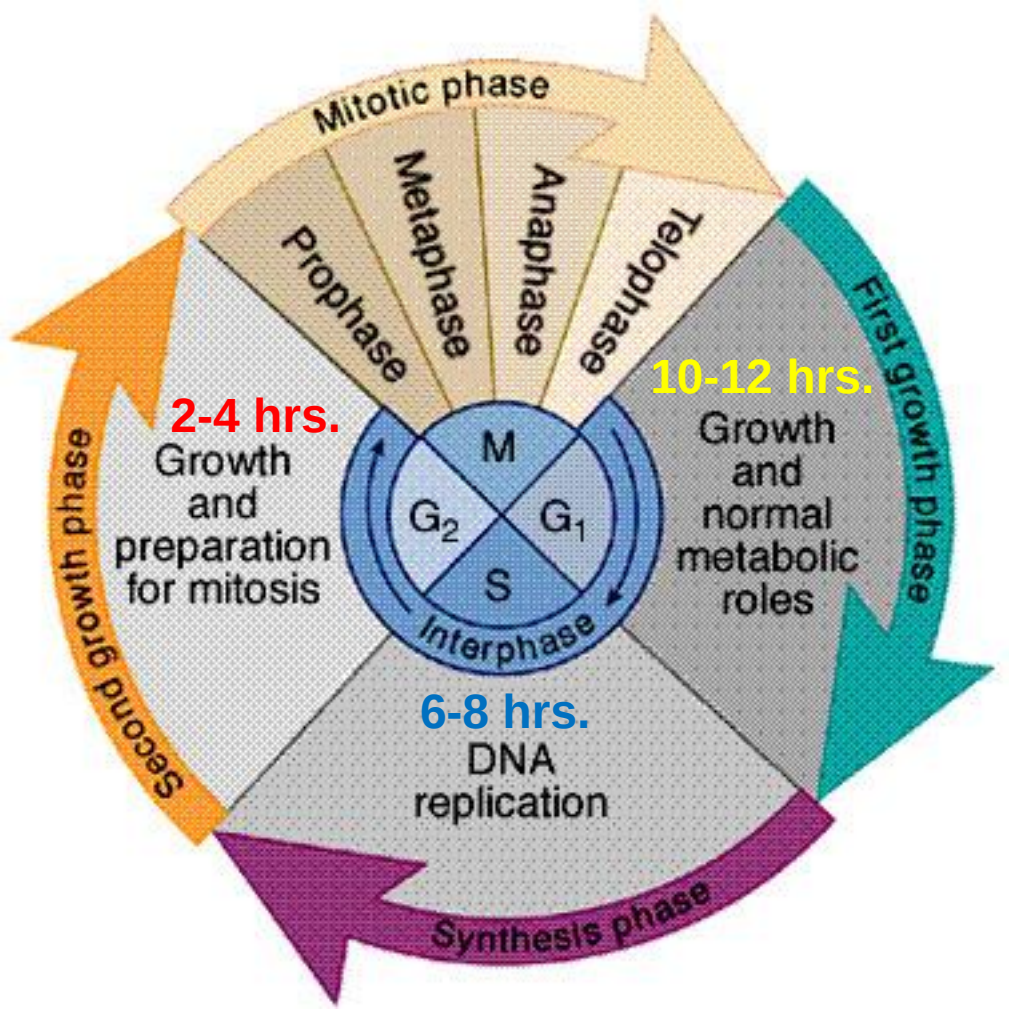
A couple of **homologous chromosomes, or homologs, are a set of one maternal and one paternal chromosome that pair up with each other inside a cell during meiosis. Homologs have the same genes in the same loci where they provide points along each chromosome which enable a pair of chromosomes to align correctly with each other before separating during meiosis

MITOTIC CELL CYCLE

The best phase to study chromosomes is the **late Prophase (Prometaphase)**;

Because it would give us a better resolution of the chromosome.

why we need a better resolution to study the chromosomes?
Because the chromosome is segmented as parts: black and white.
Therefore we will know the inactive areas and the active ones.

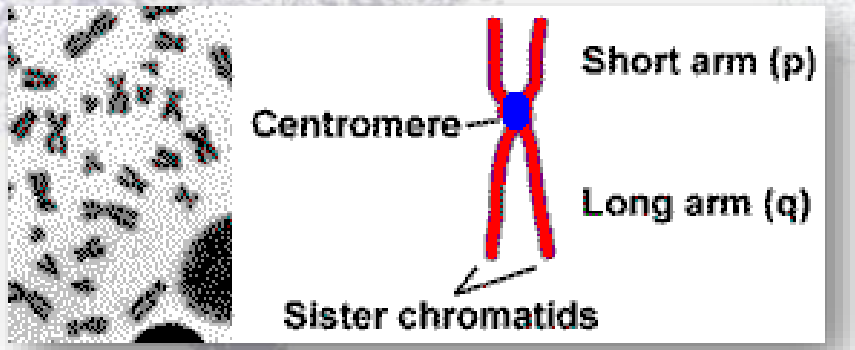


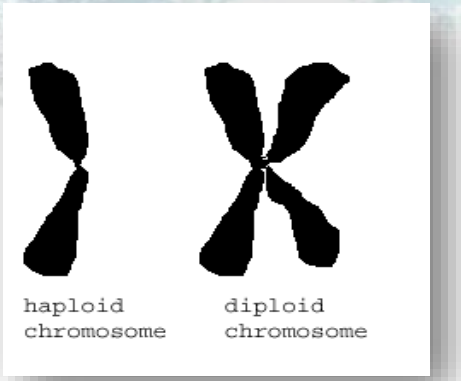
METAPHASE CHROMOSOMES

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

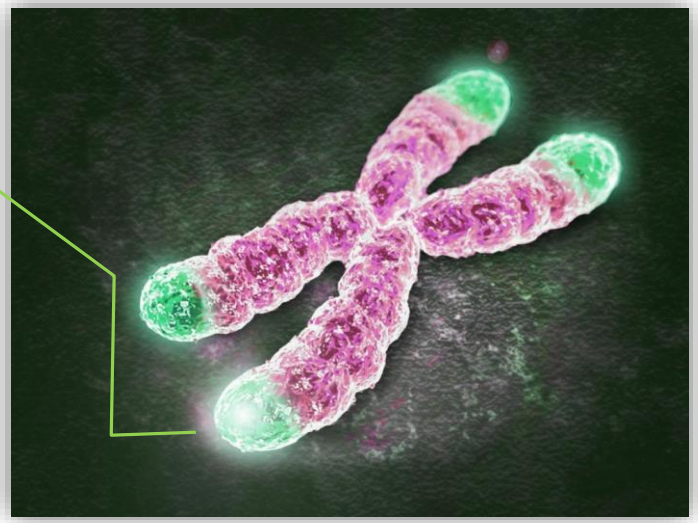
- The 2 sister-chromatids are principally held together at the centromeric region.
- Each chromosome has a centromere (CEN), region which contains the kinetochore.
- CEN divides the chromosome into two arms: the short arm (p arm) and the long arm (q arm).
- Each arm terminates in a telomere*.

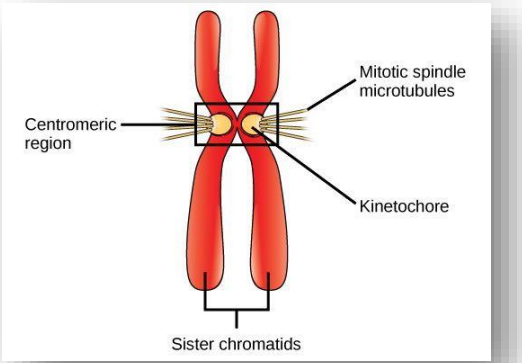
*which will protect the chromosome from degradation "الانحلال".





Telomeres





CENTROMERIC POSITION AND ARM LENGTH

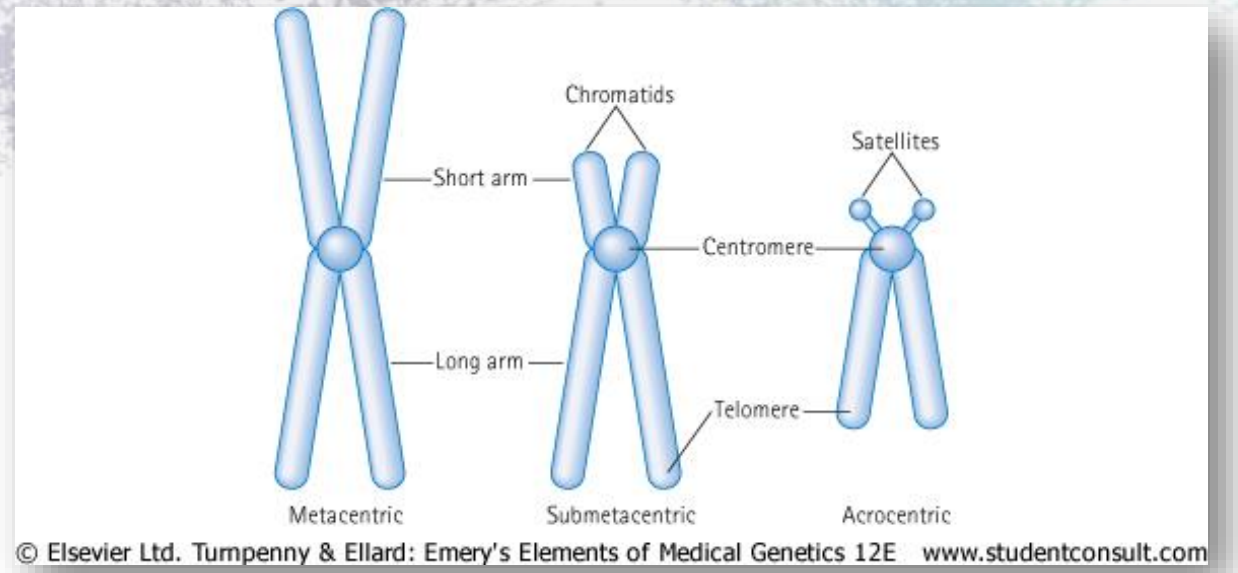
The ratio of the lengths of the two arms is constant for each chromosome.

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*.

Satellites: are small regions on the top of a chromosome exists to classify the chromosome



It's important to identify the exact place of the centromere

CHROMOSOMAL CLASSIFICATION

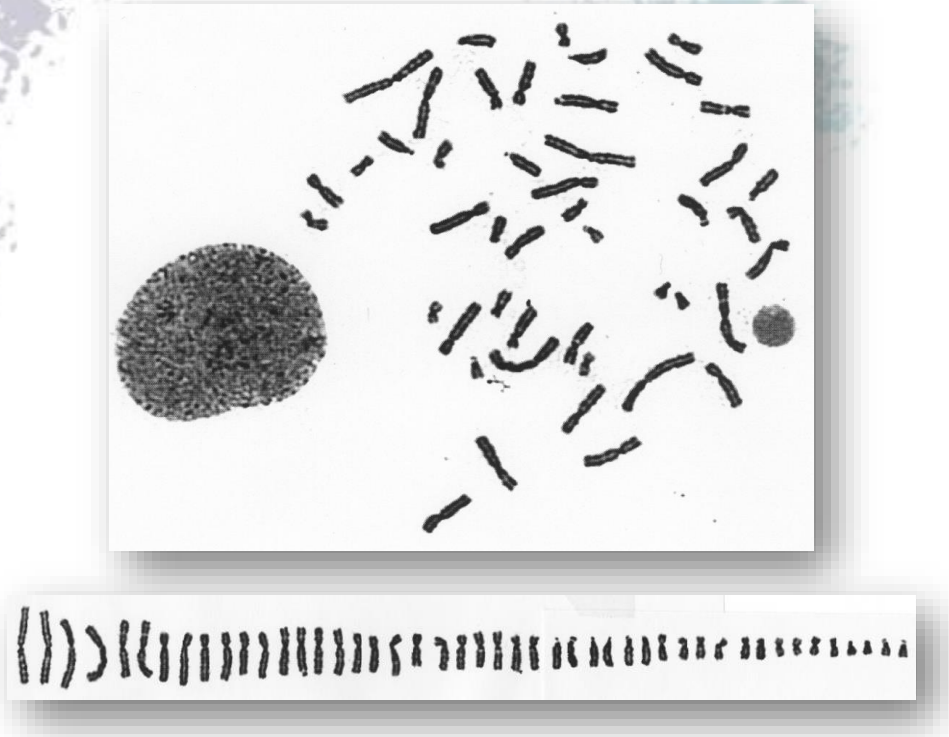
22 Pairs

- 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

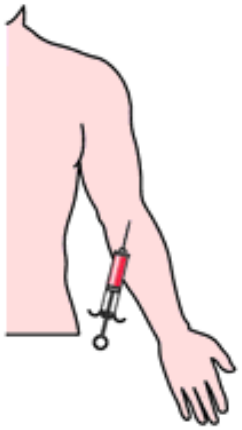


I M P O R T A N T

CHROMOSOME PREPERATION FROM PERIPHERAL BLOOD

You must know the added chemicals and in which they arrest the cell division
اعرفوا اسمائهم وفي أي مرحلة ينحطون

For extra information
(turn sound off please)



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

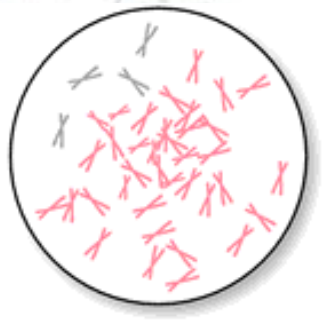
Add colchicine and hypotonic saline

Cells fixed

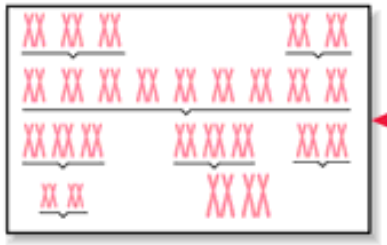
These drops are colorless actually

Spread cells onto slide by dropping

Digest with trypsin and stain with Giemsa



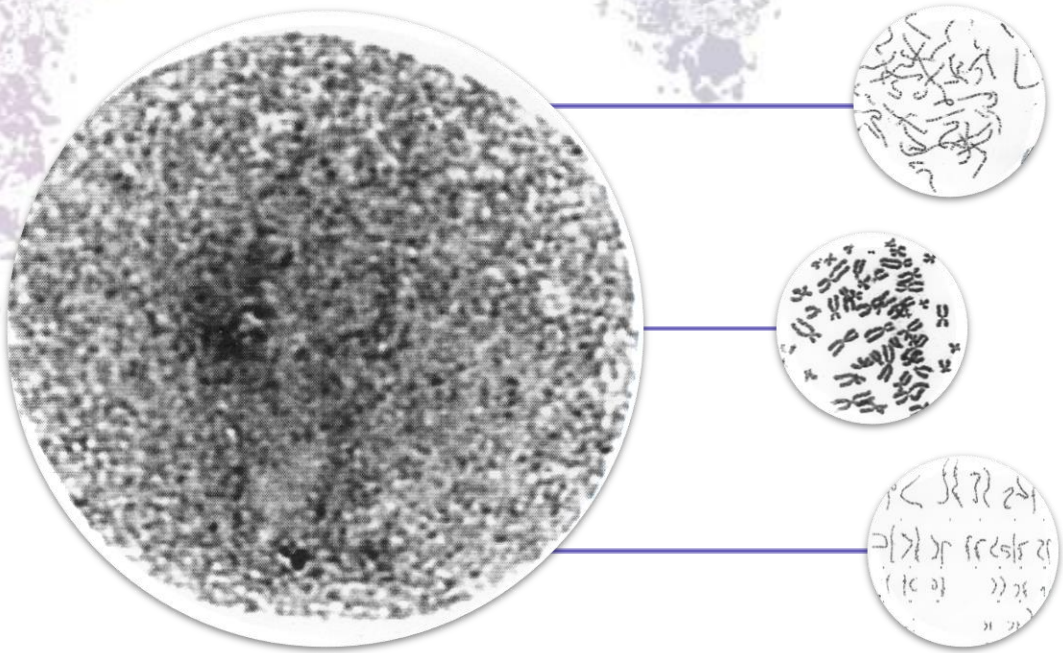
Analyze "metaphase spread"



Karyotype

KARYOTYPE

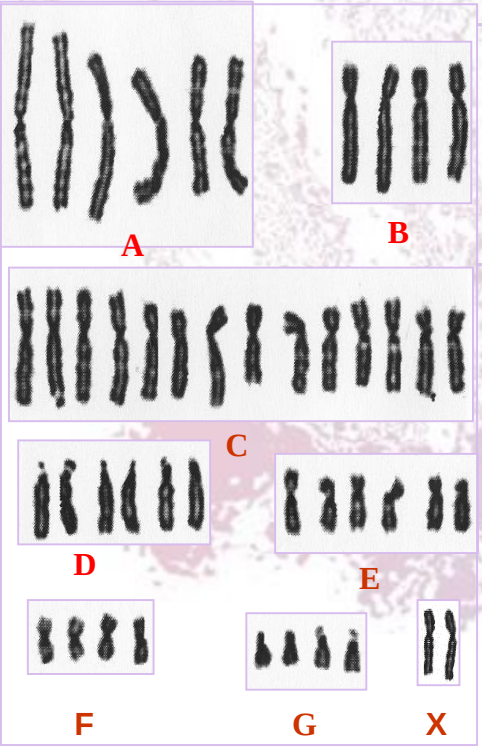
CULTURING → HARVESTING → SLIDE-MAKING → BANDING → STAINING → KARYOTYPING → CHROMOSOME ANALYSIS



KARYOTYPING

KARYOTYPING
Is based on:

- The Length
- The position of the centromere
- The presence or absence of satellites*



*chromosome segment that is separated from the main body of the chromosome, can be found in some chromosomes at metaphase. used as markers that identify particular chromosomes

Items in the Description of Karyotype

- **Normal Karyotypes**
  - 46, XY
  - 46, XX
- **Abnormal Karyotypes**
  - 47,XY,+ 21 Down Syndrome
  - 45,X Turner Syndrome

You must know and remember the right numbers and the right order of the Normal Karyotypes.

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 characterizing structural changes.
- Patterns, and the nomenclature for defining positional mapping have been standardized.

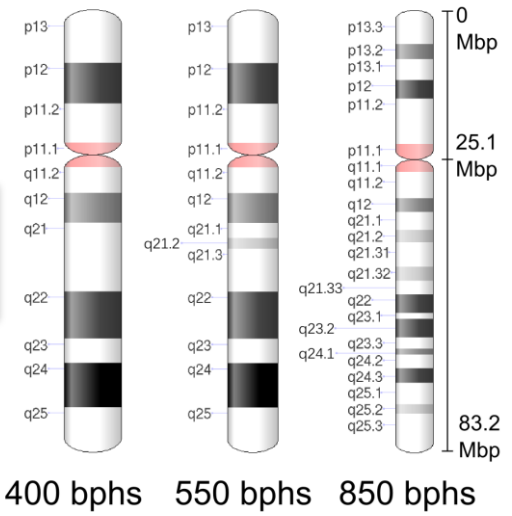
*So every chromosome has different dyed look.

CHROMOSOME BANDING

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

400 → 850+

Just like the fingerprint, every chromosome differs from another



BANDING

The most important bandings are: G & R Bandings

Sometimes we use 2 kinds of banding

R-Banding is important, and is used to locate the satellites at the distal region (because it's black thus easy to see)

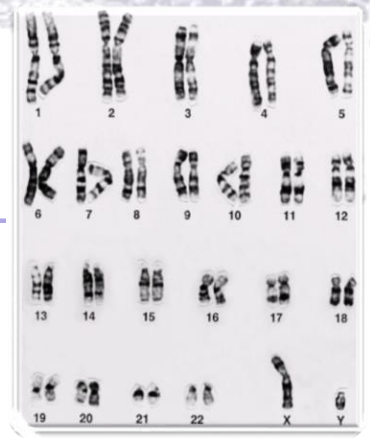
*Extra information

Active and inactive areas in:

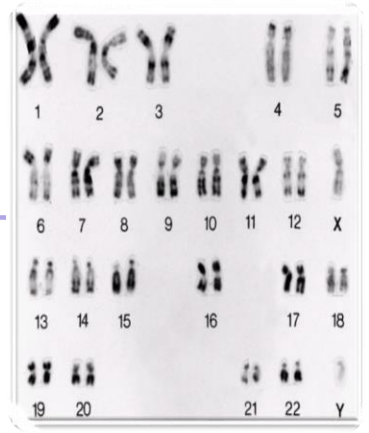
- G-Banding
  - Black: Inactive
  - White: Active
- R-Banding
  - Black: Active
  - White: Inactive

Banded Karyotype: Normal Banded Karyotypes

A normal G-banded male Karyotype



A normal R-banded male Karyotype



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.

For extra information

Chromosomes Banding

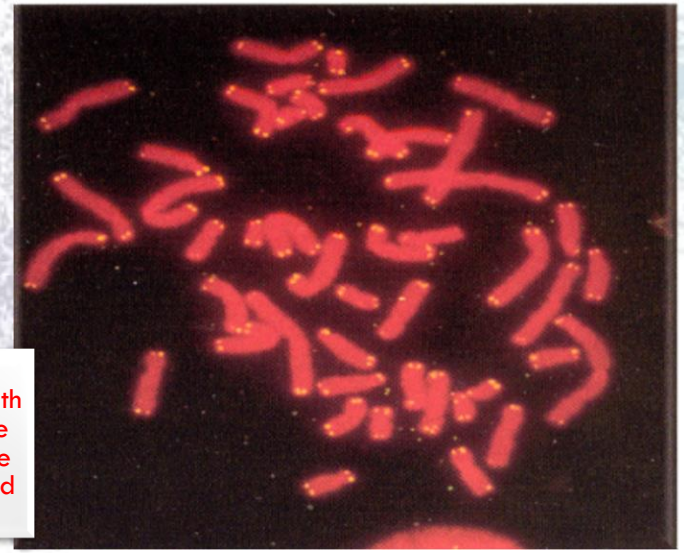
Type	Stain	Area Stained	Effect
Q-banding	Quinacrine	Chromosome arms; mostly repetitive AT-rich DNA	Under UV light, distinct fluorescent banded pattern for each chromosome.
G-banding	Giemsa	Chromosome arms; mostly repetitive AT-rich DNA	Distinct banded pattern for each chromosome; same as Q-banding pattern except single additional band near centromere of chromosomes 1 and 16
R-banding	Variety of techniques	Chromosome arms; mostly unique GC-rich DNA	Reverse banding pattern of that observed with Q- or G-banding
C-banding	Variety of techniques	Centromere region of each chromosome and distal portion of Y chromosome; highly repetitive, mostly AT-rich DNA	Largest bands usually on chromosomes 1, 9, 16, and Y; chromosomes 7, 10, and 15 have medium-sized bands; size of C-bands highly variable from person to person

https://www.slideshare.net/xulkifal/cytogenetics/1

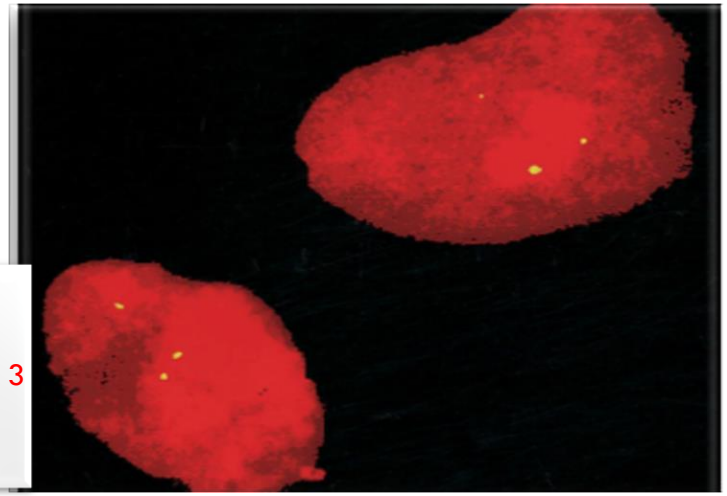
FLUORESCENCE IN-SITU HYBRIDIZATION (FISH)

FISH, an acronym "اختصار" for Fluorescent In-Situ Hybridization, is a method used to detect and visualize protein, RNA, and DNA structures in the cell. FISH analysis is a relatively fast method that provides great resolution as it incorporates "تدمج" fluorescent probes labeled for detection of specific regions, deletions, and translocations.

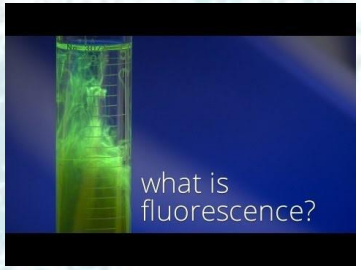
ببساطة: هذه التقنية تُستخدم لتحديد و إظهار بروتينات أو مواد جينية أخرى، و لكنّها محدودة لإظهار جزء مرغوب معيّن، و هذه من السلبيات حيث لا نستطيع اكتشاف مرض محدد إلا بعد استخدام التقنية المحددة لهذا المرض أو هذا الجزء المرغوب.
كيف؟ هذه العملية السريعة نسبياً و تكون عن طريق دمج أعواد مضيئة مصنّفة، مع شريط واحد من الدي إن إي مثلاً؛ لتحديد: مناطق معيّنة أو مناطق حذف أو تبديل
(سيتم شرح هذه المناطق في المحاضرة القادمة)



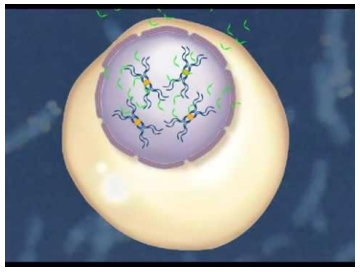
FISH of metaphase with a probe for telomere showing signals at the end of each chromatid



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



https://www.youtube.com/watch?v=Bsnyy5JAKUE



Some helping videos

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.
- 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.

MCQ

https://www.onlineexambuilder.com/human-genetics-1/exam-181773

ALTERNATIVE HYPERLINKS (IF DIDN'T WORK):

Chromosomes spread (turn sound off please): https://www.youtube.com/watch?v=BUWOgY0ohos

What is Fluorescence?: https://www.youtube.com/watch?v=WnWIt0iz00A

FISH Technique animation: https://www.youtube.com/watch?time_continue=1&v=w5I5SmKvS1o

تمّ بحمدِ الله

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