Knowledge of chromosomes is important in many areas of clinical medicine and research.

In humans, approximately 0.6-1% of all liveborns have a chromosomal abnormality.

chromosomal aberrations are noted in:

(1) 20%-27% of individuals having sex reversal or pubertal anomalies;

(2) 33% to 67% of spontaneous miscarriages;

(3) 2% to 5% of couples having a history of multiple miscarriages;

(4) the majority of cells from leukemia samples or solid tumors.

Why Study Human Chromosomes?

Morbidity/Mortality	Estimate of Cases with Cytogenetic Abnormality
Early embryonic death in unrecognized pregnancies	?? 33-67%
Recognized embryonic and fetal deaths (≥ 5 weeks)	About 30% total; rate varies from 50% at 8-11 weeks to 5% in stillbirths (≥ 28 weeks)
Infant and childhood deaths	5-7%
Birth defects	4-8%
Congenital heart defects	13%
Sex reversal/pubertal anomalies	20-27%
Multiple miscarriages in couples	2-5%
Neoplasms	20-80+%

Research Uses for Cytogenetic Evaluation

- **Localization of DNA onto a chromosome(s)**
- **Determination of genomic complement**
- **Characterization of genetic change(s)**
- **Recognition of chromosomal changes following treatment(s) or *in vitro* culturing**

Tissues for Chromosome Studies

- Peripheral blood (lymphocytes)
- Bone marrow
- Chorionic villi biopsy
- Amniotic fluid cells
- Skin or organ biopsy

- A karyogram is photograph ora diagram of an ordered arrangement of chromosomes from cells that are placed in a standard order (generally by length; chromosome 1 is longest and 22 shortest).

- Once a computer image of the chromosomes from a dividing cell is obtained, the chromosomes are arranged as homologous pairs.

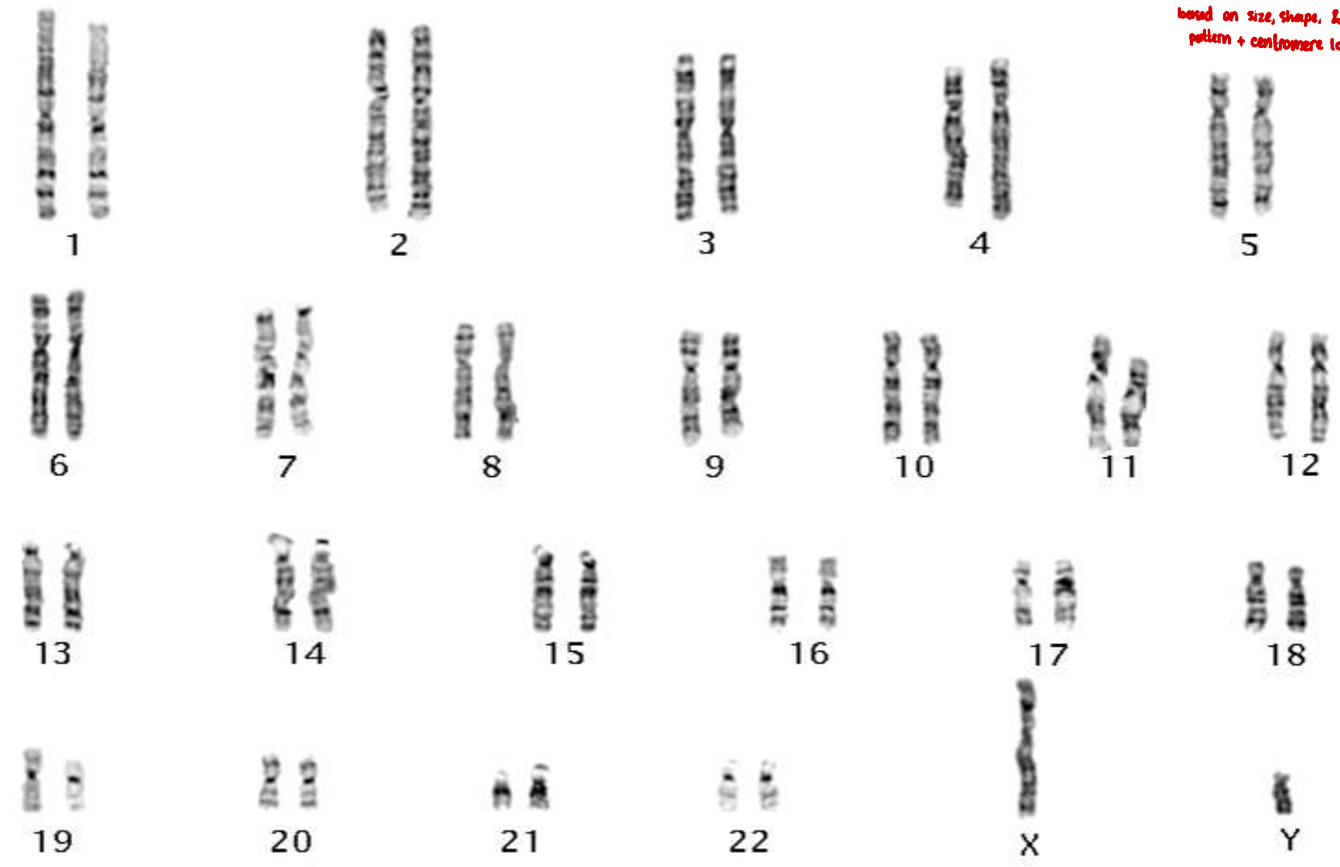
- Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome.

- The normal diploid chromosome number for humans is 46.

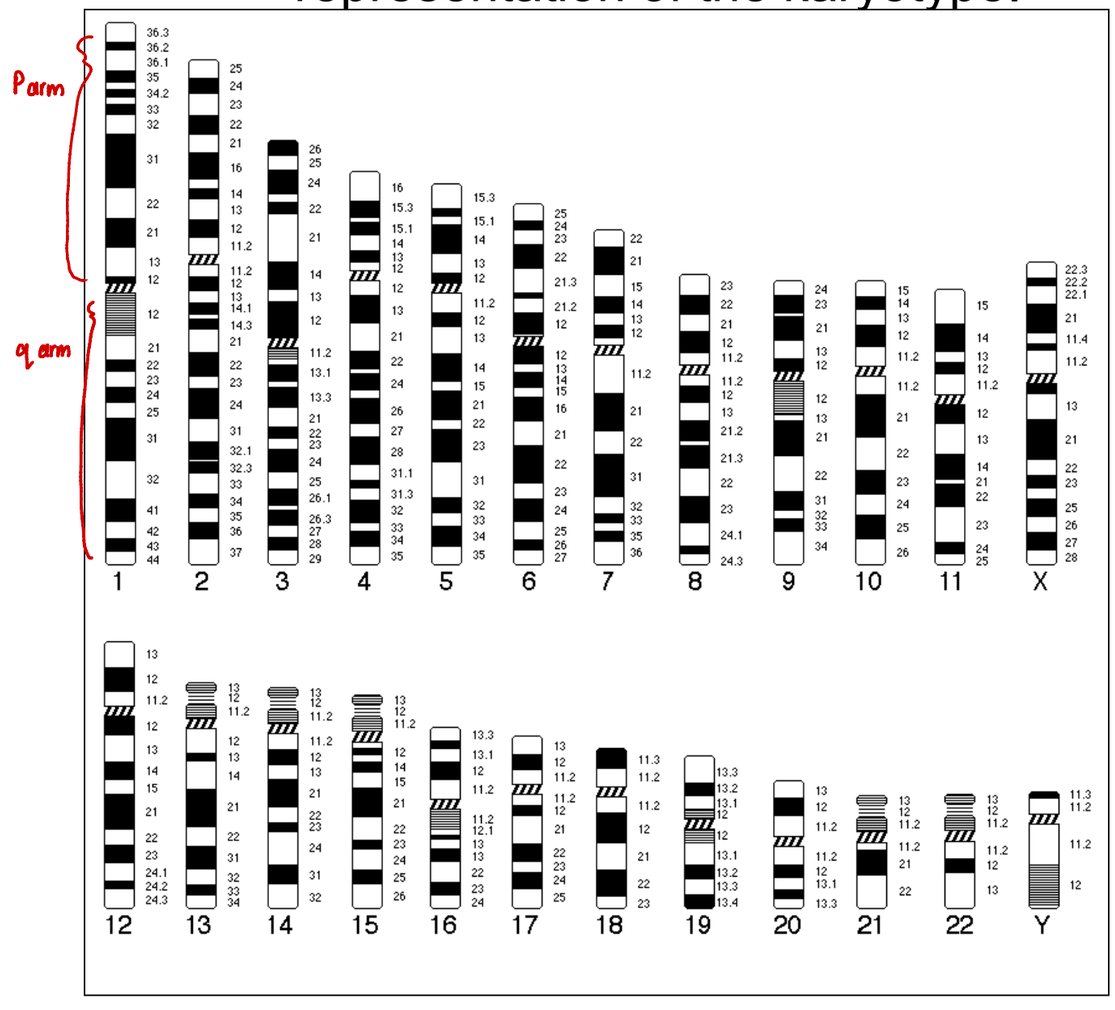
Karyogram is also called Karyotype

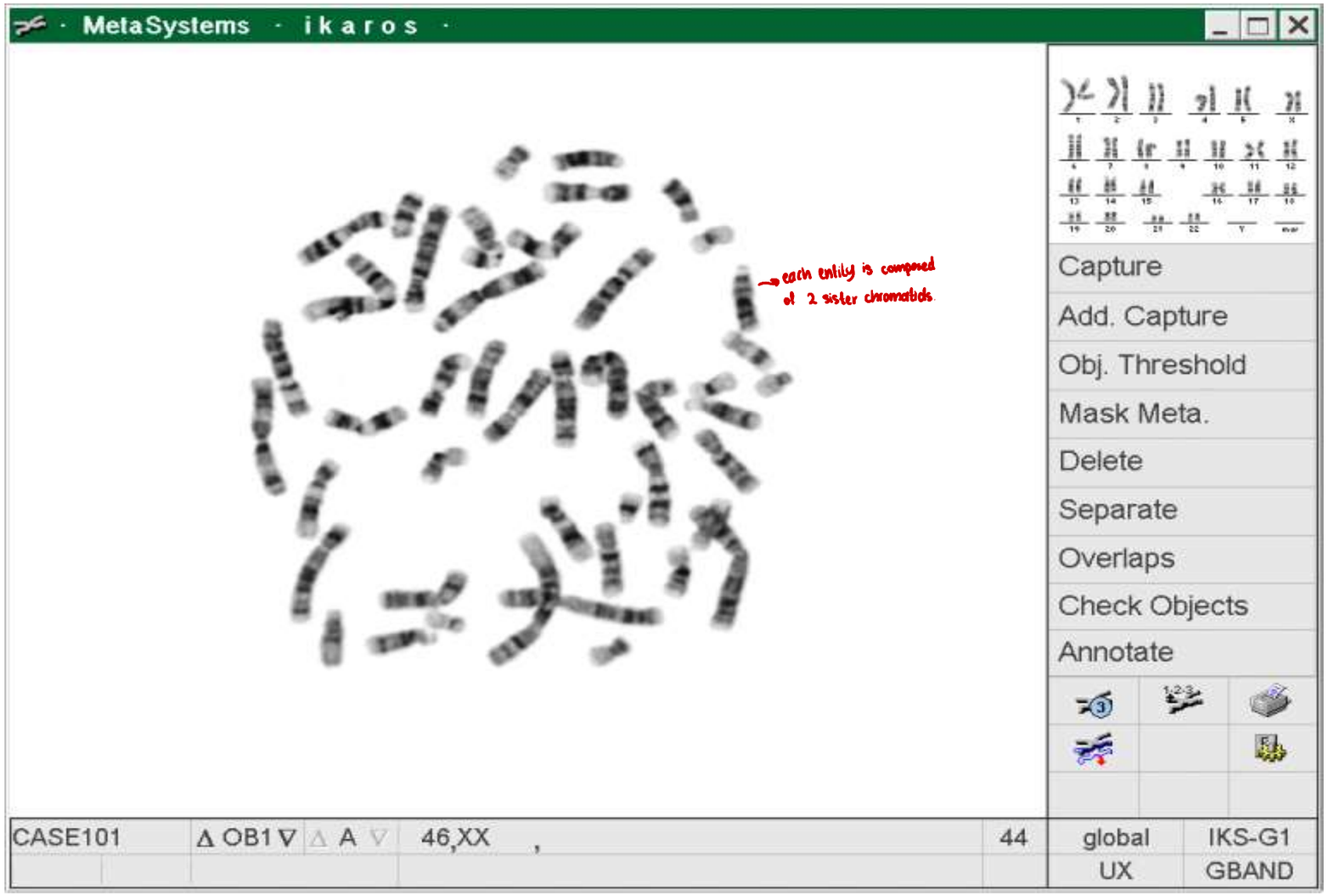
<u>Karyogram</u> – An ordered arrangement of the chromosomes from a cell placed in a standard sequence (generally by length).

* chromosomes can be distinguished based on size, shape, & banding pattern + centromere location.



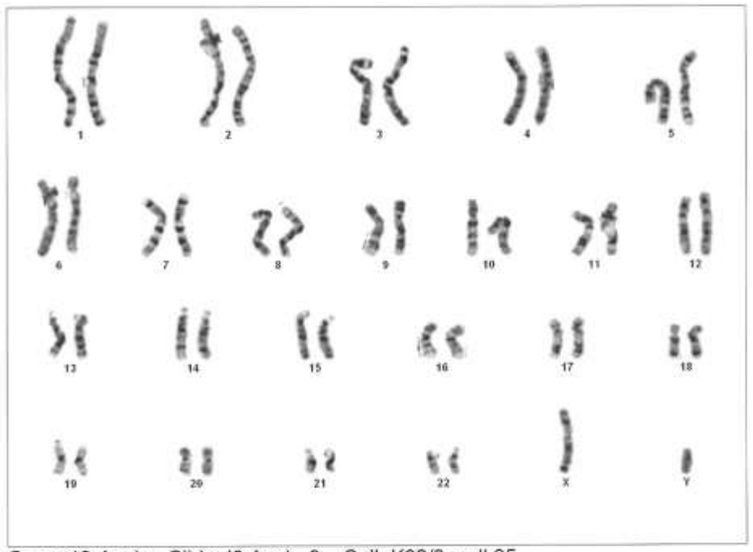
The ideogram of a chromosomal complement is a diagrammatic representation of the karyotype.



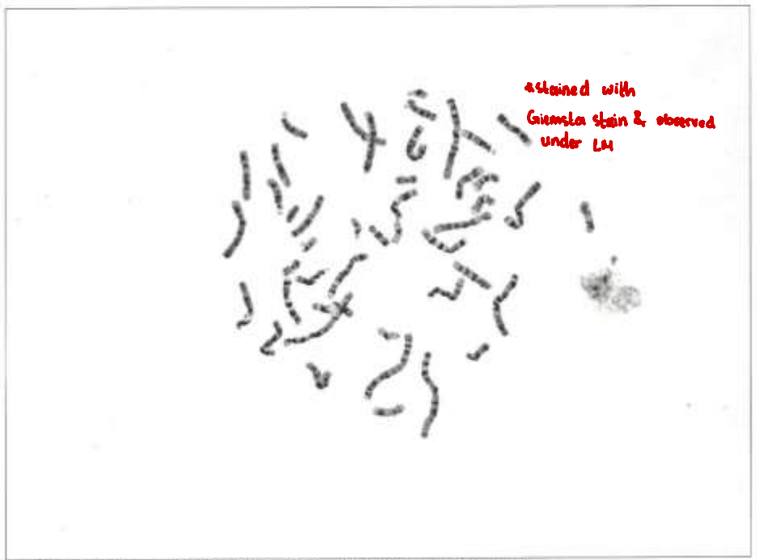


Metaphase chromosomes

- A karyotype is the number and appearance of chromosomes in the nucleus.
- The chromosomal complement for a normal female is indicated as : 46,XX
- The chromosomal complement for a normal male is indicated as : 46,XY



- To be examined by chromosome analysis for clinical purposes, cells must be capable of proliferation in culture. The most accessible cells that meet this requirement are white blood cells, specifically T lymphocytes.

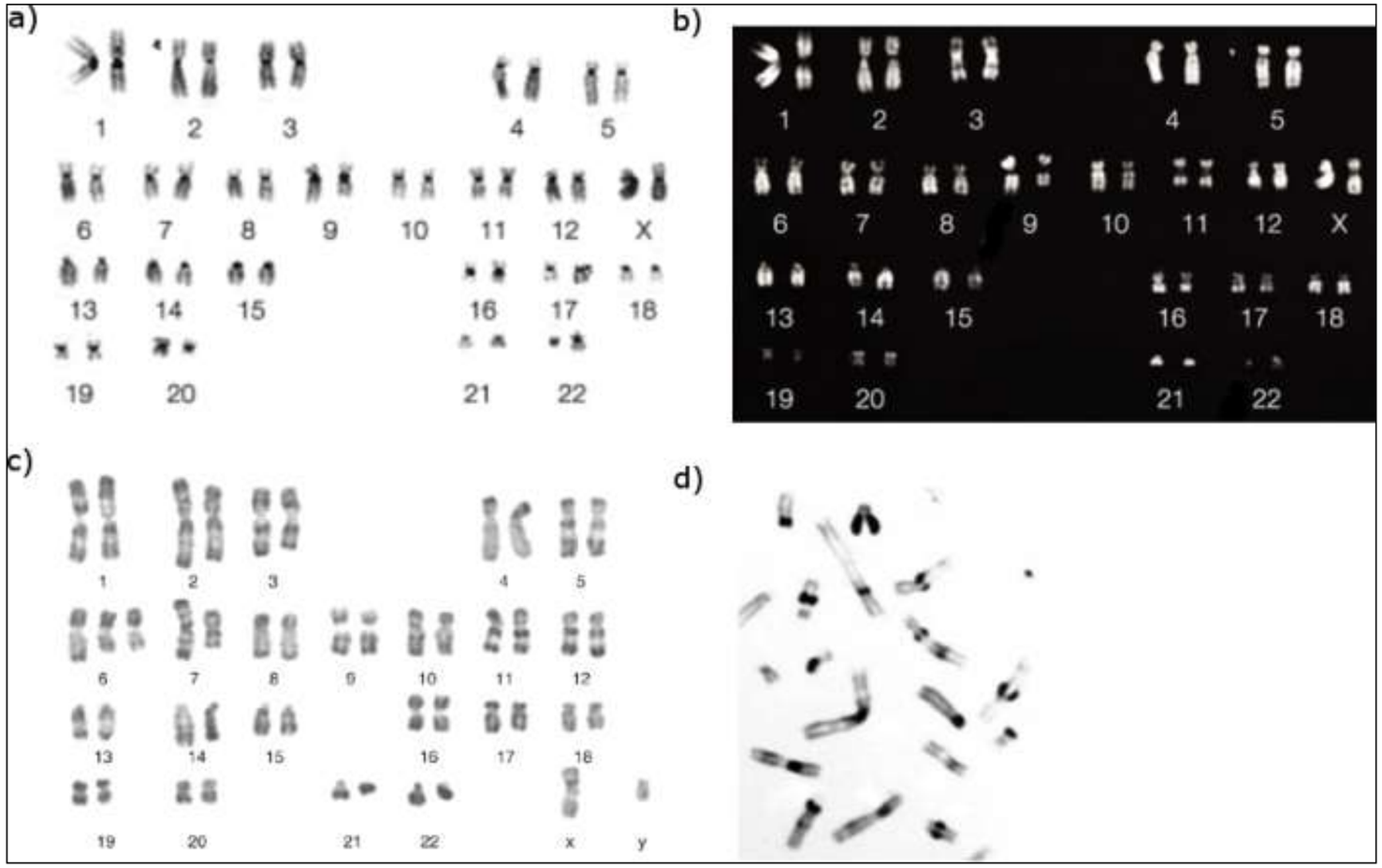


Types of banding

- G-banding → + most common + Giemsa.
- R-banding
- C-banding
- Q-banding
- T-banding
- Silver staining

G-banding

Q-banding



R-banding

C-banding

*Reminder:
heterochromatin = condensed DNA
euchromatin = loose DNA

heterochromatin → dark stain
euchromatin → light stain

G-banding (GTG)

- heterochromatic regions, which tend to be AT-rich DNA and relatively gene-poor, stain more darkly The **light** regions tend to be **euchromatic**, **GC rich**. → also gene-rich; gene's promoters are GC rich
- less condensed chromatin—which tends to be GC-rich and more transcriptionally active—incorporates less Giemsa stain, and these regions appear as light bands
- This method will normally produce 300-400 bands among the 23 pairs of human chromosomes.
- Measured in DNA terms, a G-band represents several million to 10 million base pairs of DNA, a stretch long enough to contain hundreds of genes.
- metaphase chromosomes are first treated briefly with trypsin, an enzyme that degrades proteins, before the chromosomes are stained with Giemsa. Trypsin partially digests some of the chromosomal proteins, thereby relaxing the chromatin structure and allowing the Giemsa dye access to the DNA.

R-banding

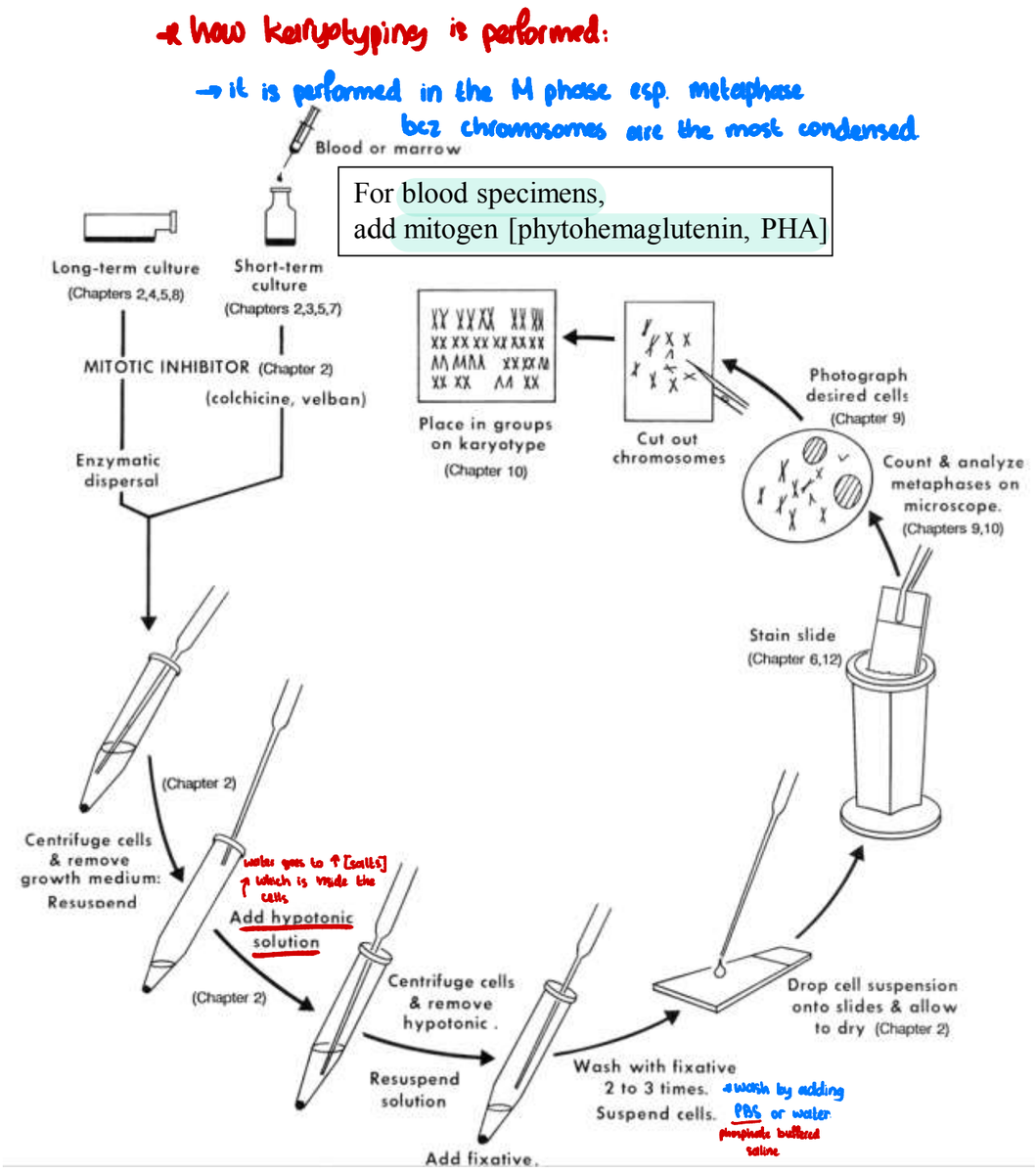
- is the reverse of G-banding (the R stands for "reverse"). The dark regions are euchromatic (guanine-cytosine rich regions). The bright regions are heterochromatic (thymine-adenine rich regions)

- provide critical details about gene-rich regions that are located near the telomeres
- often used together with G-banding on human karyotype to determine whether there are deletions.

- the chromosomes are heated before Giemsa stain is applied. The heat treatment is thought to preferentially melt the DNA helix in the AT-rich regions that usually bind Giemsa stain most strongly, leaving only the comparatively GC-rich regions to take up the stain. R-banding

Primary Steps for Culture Establishment and Harvest of Specimens

- **Add Mitogen (when needed)**
- **Hypotonic Swelling**
- **Fixation**
- **Analysis**



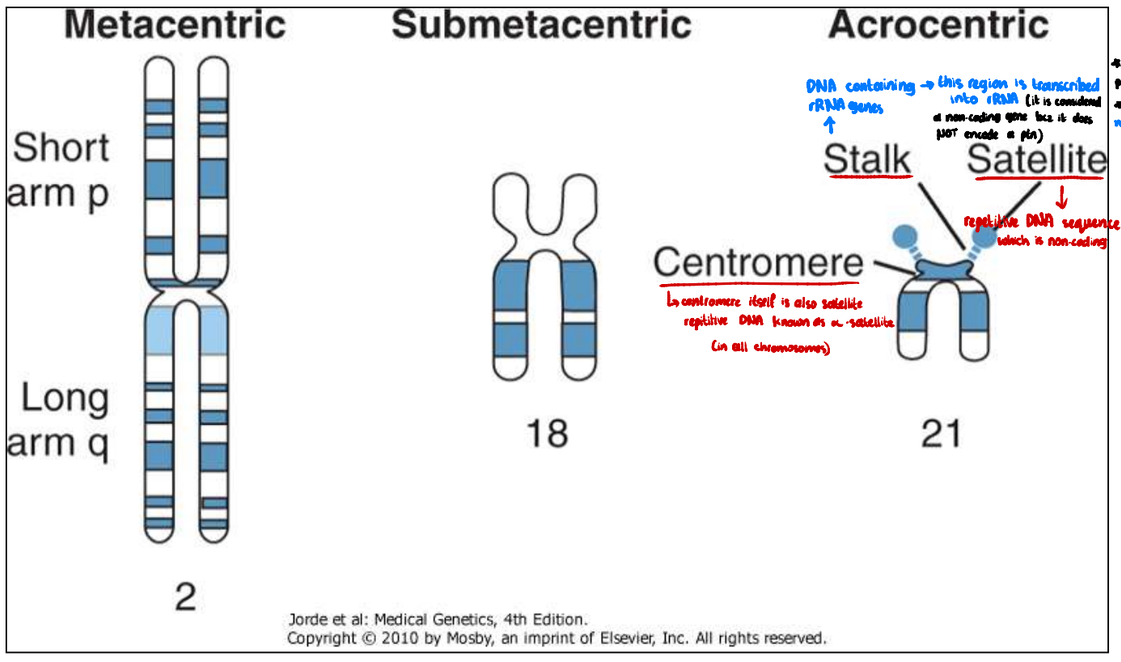
obtain tissue (i.e: blood) → stimulate cells to undergo cell cycle by adding a mitogen → after some time, collected cells are treated with a mitotic inhibitor such as colchicine → cells become arrested in the metaphase. → centrifuge → cells will percipitate (pellet) → add hypotonic solution (↓ [salt]) → cells become swollen & fragile. → add a fixative → wash → cells are dropped on a glass slide from a distance which will cause them to break open (lyse) → chromosomes become scattered → stained (Giemsa)

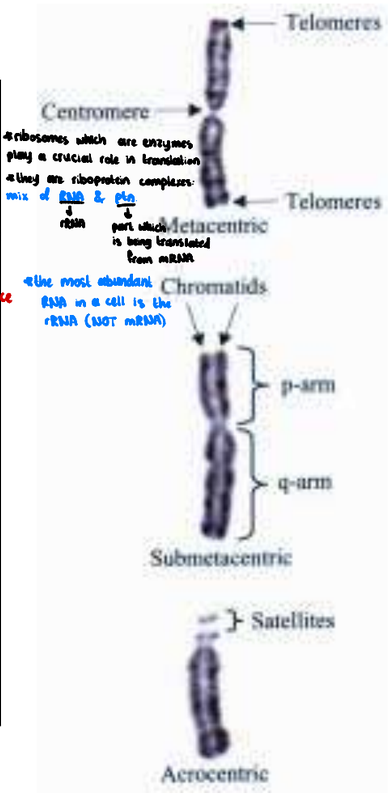
Chromosome Shape

Metacentric- centromere is located in the middle of chromosome ↱ harder to distinguish between p & q arms.

Submetacentric- centromere is displaced from the center

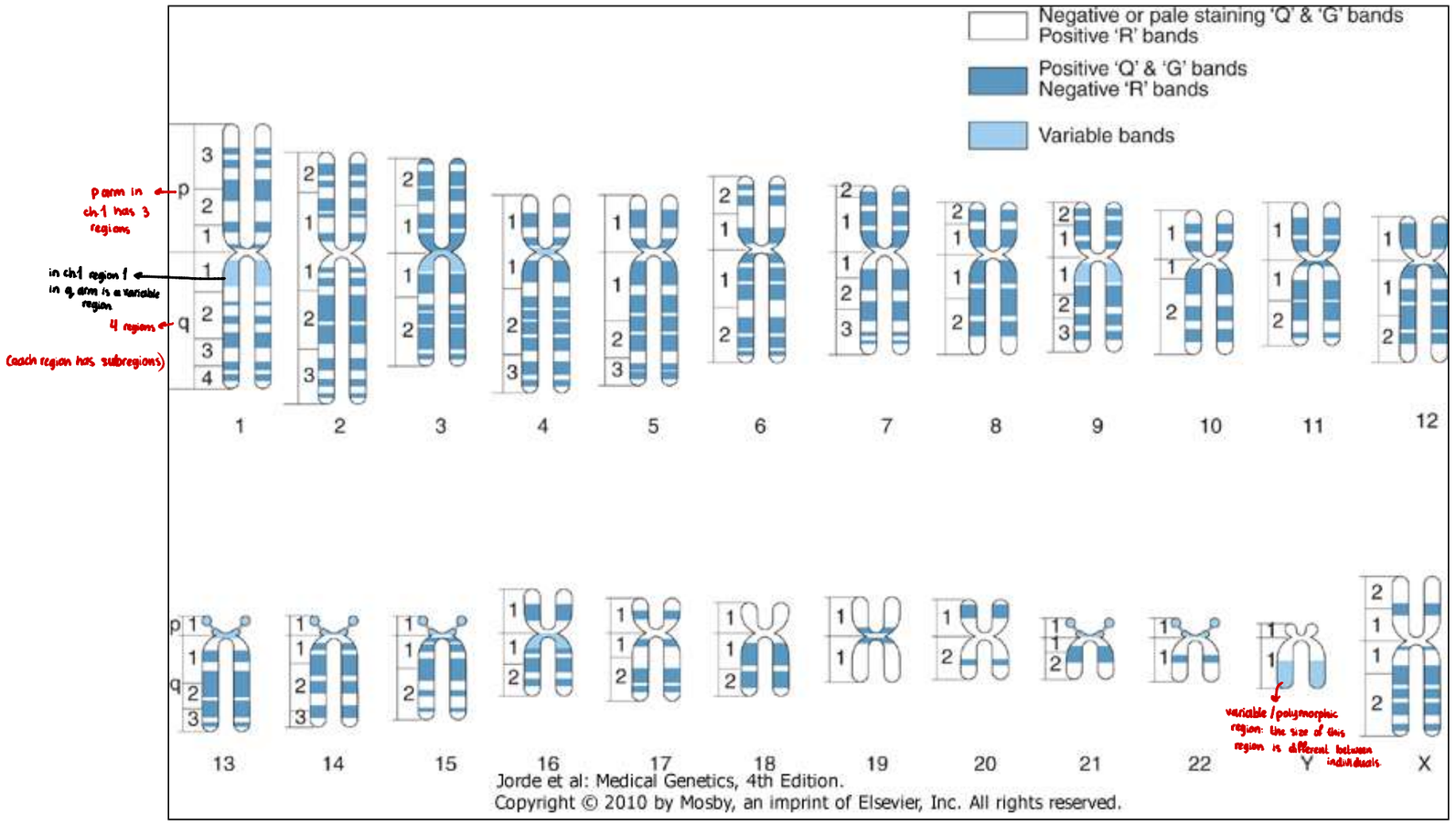
Acrocentric – centromere is placed near the end → p arm only contains stalk & satellite





Human Chromosome Ideogram

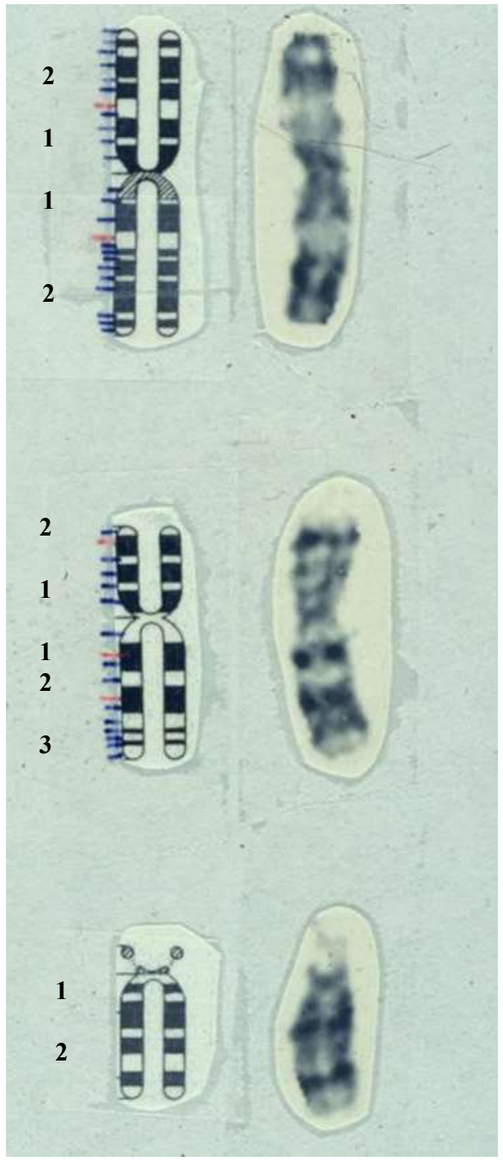
<u>Ideogram</u>- A diagrammatic representation of a karyotype

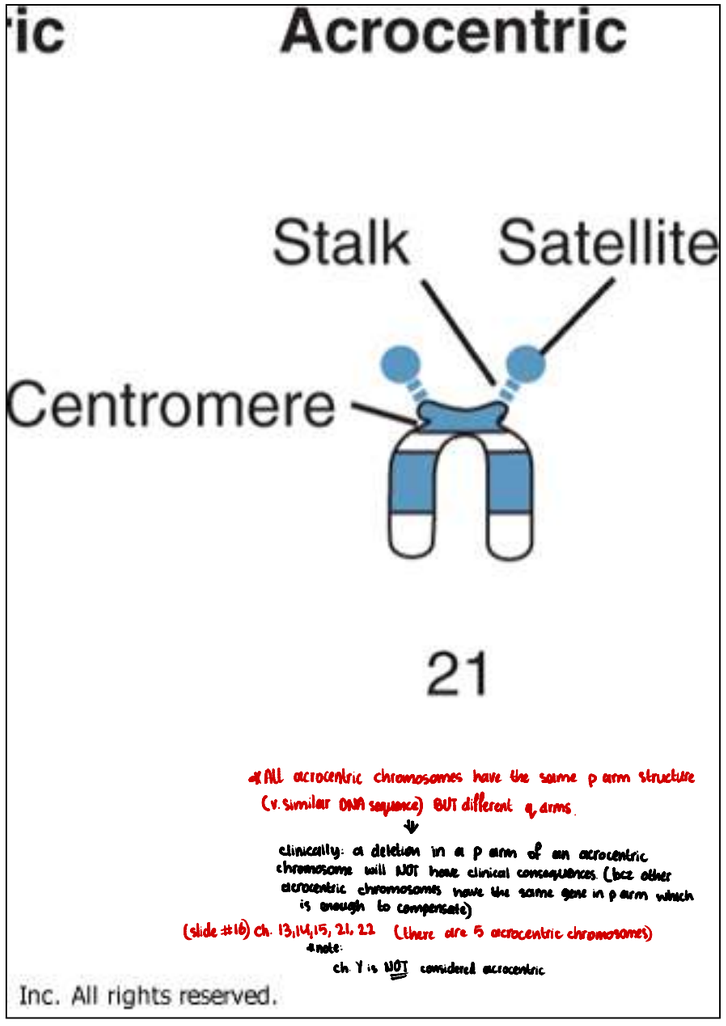


Chromosome 3
p: 2 regions
q: 2 regions

Chromosome 7
p: 2 regions
q: 3 regions

Chromosome 14
p: 1 region
q: 2 regions





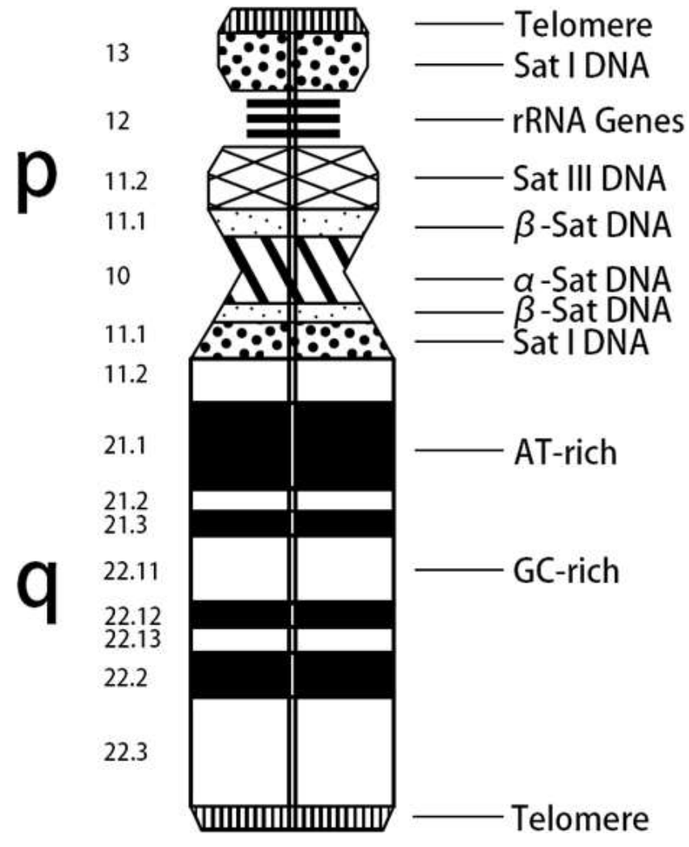
*ALL acrocentric chromosomes have the same p arm structure (v. similar DNA sequence) BUT different q arms

↓

clinically: a deletion in a p arm of an acrocentric chromosome will NOT have clinical consequences (bcz other acrocentric chromosomes have the same gene in p arm which is enough to compensate)

(slide #16) Ch. 13, 14, 15, 21, 22 (there are 5 acrocentric chromosomes)

*note:
ch. Y is NOT considered acrocentric



High Resolution Banding

High-resolution banding involves the staining of chromosomes during prophase or prometaphase, before they reach maximal condensation.
Because prophase and prometaphase chromosomes are more extended than metaphase chromosomes, the number of bands observable for all chromosomes increases from about 300 to 450 to as many as 800 per haploid set. This allows the detection of less obvious abnormalities usually not seen with conventional banding.

⇒ arresting the cell at a less condensed phase which provides higher resolution to look for abnormalities.

⇒ all of these are different resolutions of ch. X

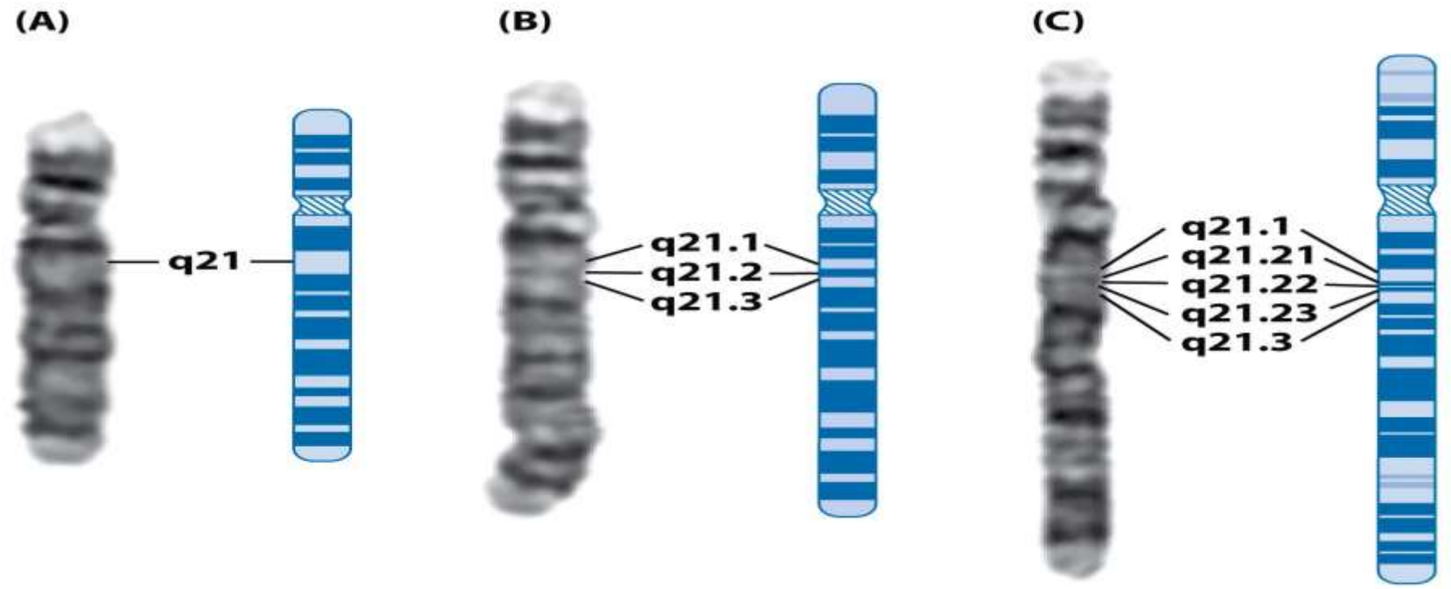


Figure 2.14 Human Molecular Genetics, 4ed. (© Garland Science)

Figure 2.14 Different chromosome banding resolutions can resolve bands, sub-bands, and sub-sub-bands.

G-banding patterns for human chromosome 4 (with accompanying ideogram at the right) are shown at increasing levels of resolution. The levels correspond approximately to (A) 400, (B) 550, and (C) 850 bands per haploid set, allowing the visual subdivision of bands into sub-bands and sub-subbands as the resolution increases. [Adapted from Cross & Wolstenholme (2001). Human Cytogenetics: Constitutional Analysis, 3rd ed. (DE Rooney, ed.). With permission of Oxford University Press.]

Components of Chromosomes: Centromeres, Telomeres/Sub-telomeres

Structures of chromosomes:

Centromere
Telomere
Sub-telomere

↳ telomeres at both ends they have the same sequence in 46 chromosomes.

171-bp monomers ↳ repeated many times next to each other which gives rise to α-satellite

centromere: α-satellite, repititive DNA (tandem repeats) → non-coding DNA therefore it is always heterochromatin

Pericentromeric region**

Unique chromosome-specific DNA

(TTAGGG)n ↳ telomeres get shorter & shorter with age ⇒ cancer cells lose this characteristic which explains why cancer cells are immortal ⇒ they can divide indefinately w/o shortening telomeres.

Subtelomeric region*

***Highly polymorphic; implicated in location of "hotspots for structural chromosomal abnormalities**

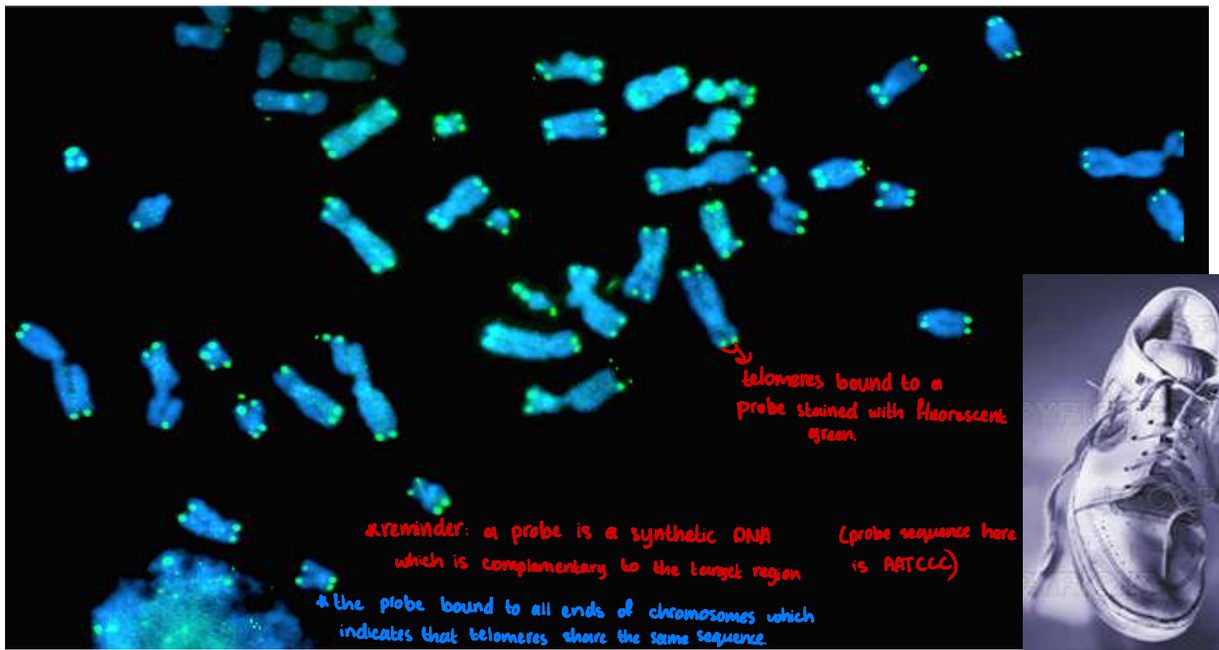
Centromere

The genetic locus required for chromosome segregation; contains DNA and proteins on which the kinetochore is formed.

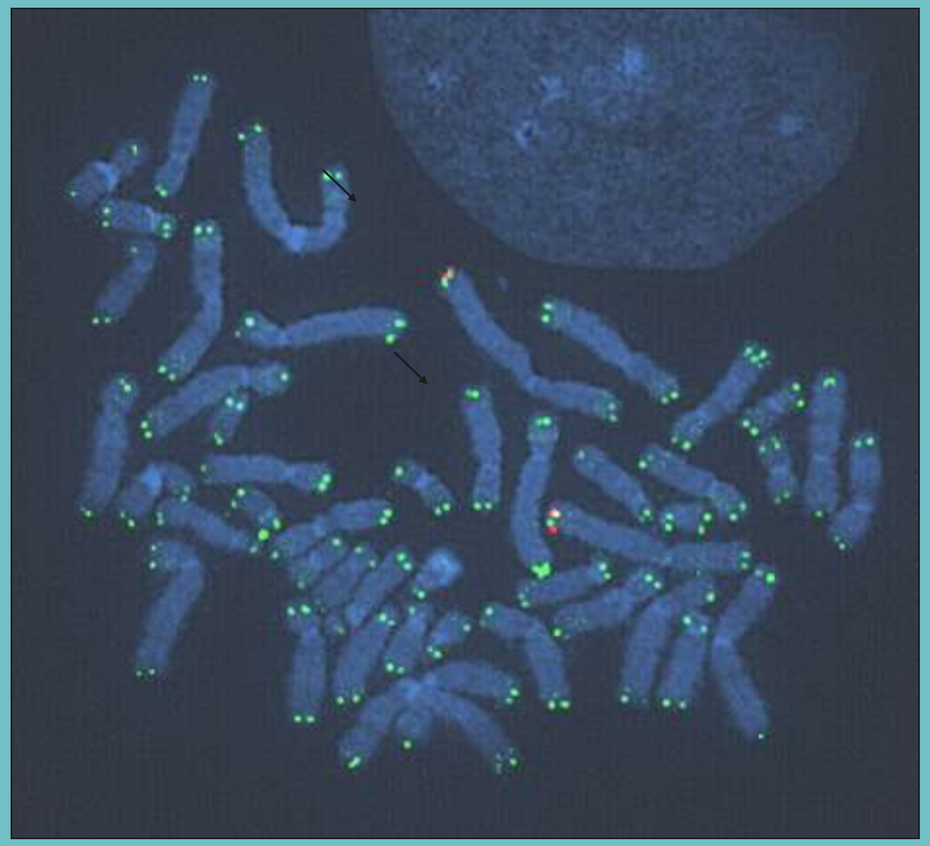
Telomere $(TTAGGG)_n$

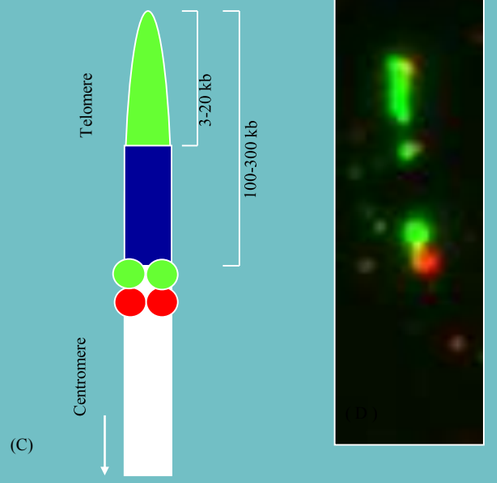
A specialized structure at the ends of eukaryotyic chromosomes. Function: Maintain chromosomal integrity by preventing end-to-end fusion of chromosomes.

⇒ if telomeres were removed → ends of chromosomes will be sticky.



Human Sub-telomeric Regions





(TTAGGG)n

Telomere Associated Repeats (TAR)

Degenerate (TTAGGG)n

Unique Subtelomere DNA Probe → stained red, seen in only some chromosomes which implies that chromosomes have different subtelomeric sequences.

Unique DNA → region which encodes for genes

There is some sequence homology between subtelomeres