- 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 (Cancers)	20-80+%

Research Uses for Cytogenetic Evaluation

- **Localization of DNA onto a chromosome(s)** to detect abnormalities or identify missing genes.

- **Determination of genomic complement**

- **Characterization of genetic change(s)**

- **Recognition of chromosomal changes following treatment(s) or *in vitro* culturing**

Tissues for Chromosome Studies

(It depends on the disease to be studied)

✓ **Chromosomal testing is easier to perform in certain tissues than in others.**

- **Peripheral blood (lymphocytes)** – the **easiest** tissue to use for DNA sequencing or chromosomal studies because it is the **least invasive** specimen.
  - Lymphocytes are used because they contain DNA.

- **Bone marrow** – in certain tumors
  - Bone marrow samples require aspirate or biopsy procedures, which are painful and performed under anesthesia.

- **Chorionic villi biopsy** – during pregnancy

- **Amniotic fluid cells** – during pregnancy

- **Skin or organ biopsy**

  - During pregnancy, there may be a suspicion of increased **nuchal translucency**. If screening tests are positive, this indicates a possible abnormality. To rule out certain chromosomal anomalies such as Down syndrome, testing should be based on the **gestational age**: in *early stages, chorionic villi* are used; in *later stages, amniotic fluid* is used. Both contain non-maternal cells, and the procedures are invasive, requiring tissue collection.

- 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.

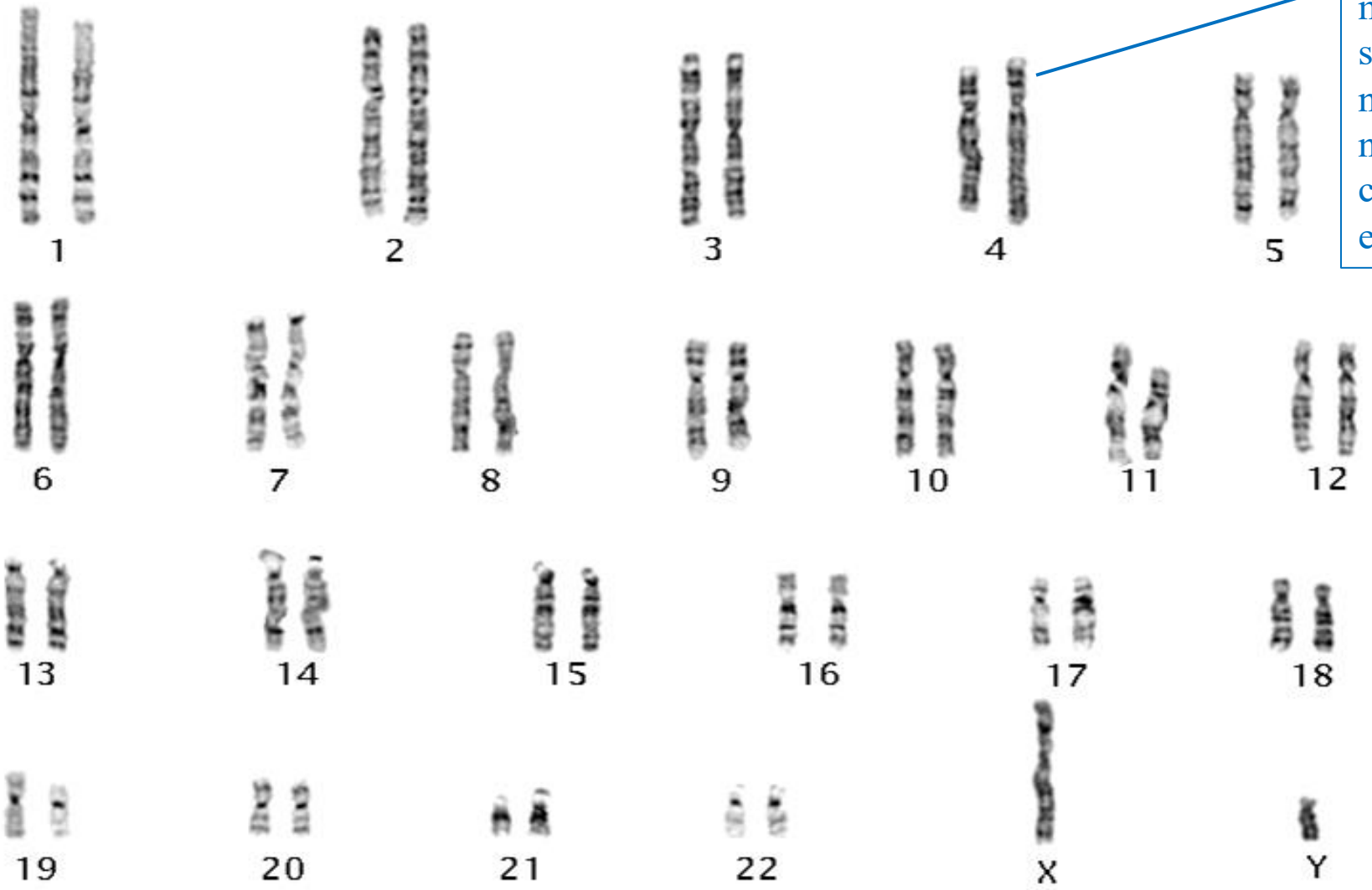
- The normal diploid chromosome number for humans is 46.

  - Chromosomes can be distinguished by their **shape, centromere location, size, and banding pattern**; for example, G-banding produces characteristic dark and light bands that help identify each chromosome and detect abnormalities such as deletions, duplications, or translocations.

Karyogram is also called Karyotype

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

1–22: autosomes
23: sex chromosomes



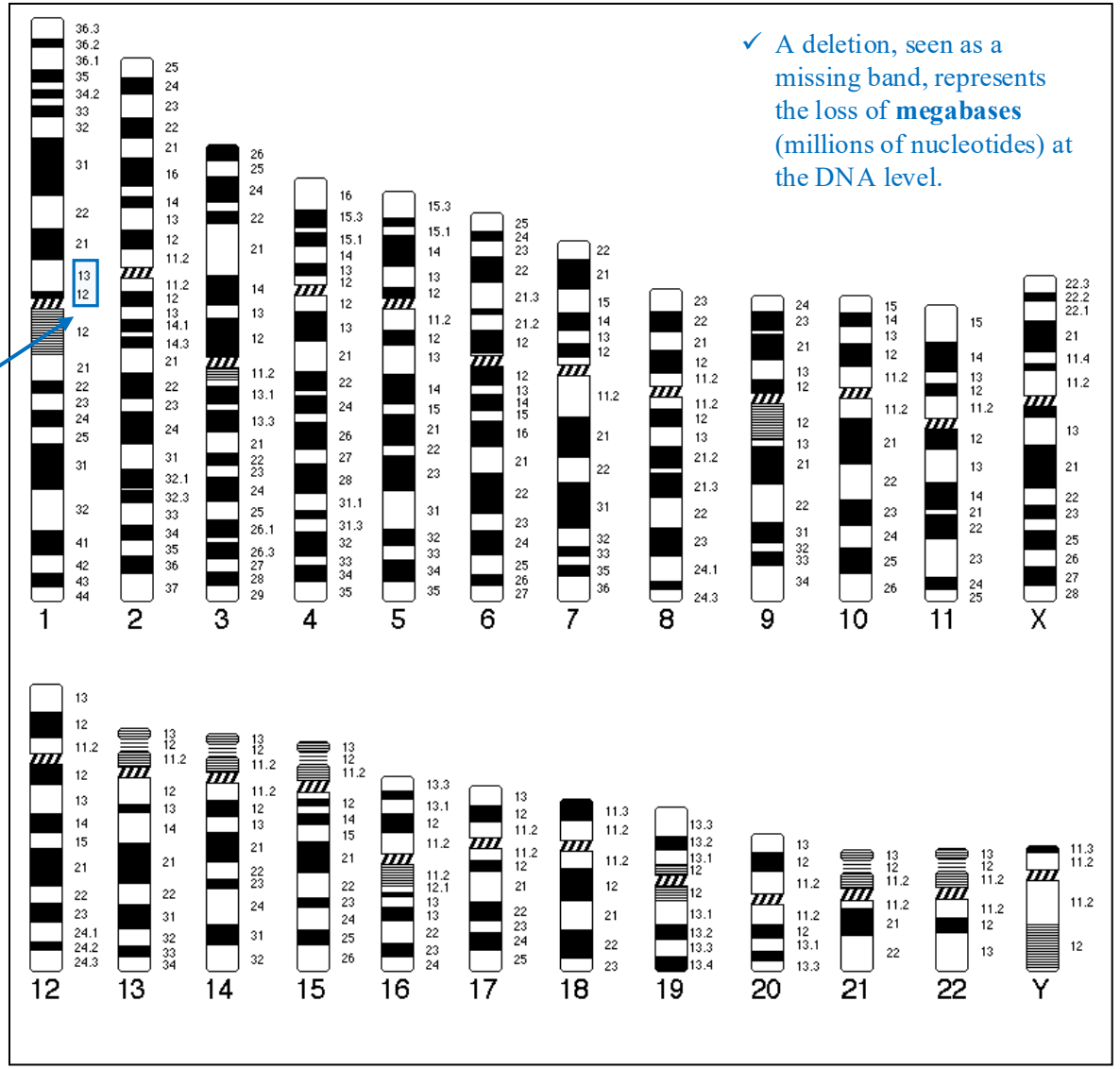
Chromosome pairs may vary slightly in size, but both can be normal; distinguishing normal from abnormal chromosomes requires experience.

Those chromosomes are observed under a light microscope at ***1000***× magnification

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

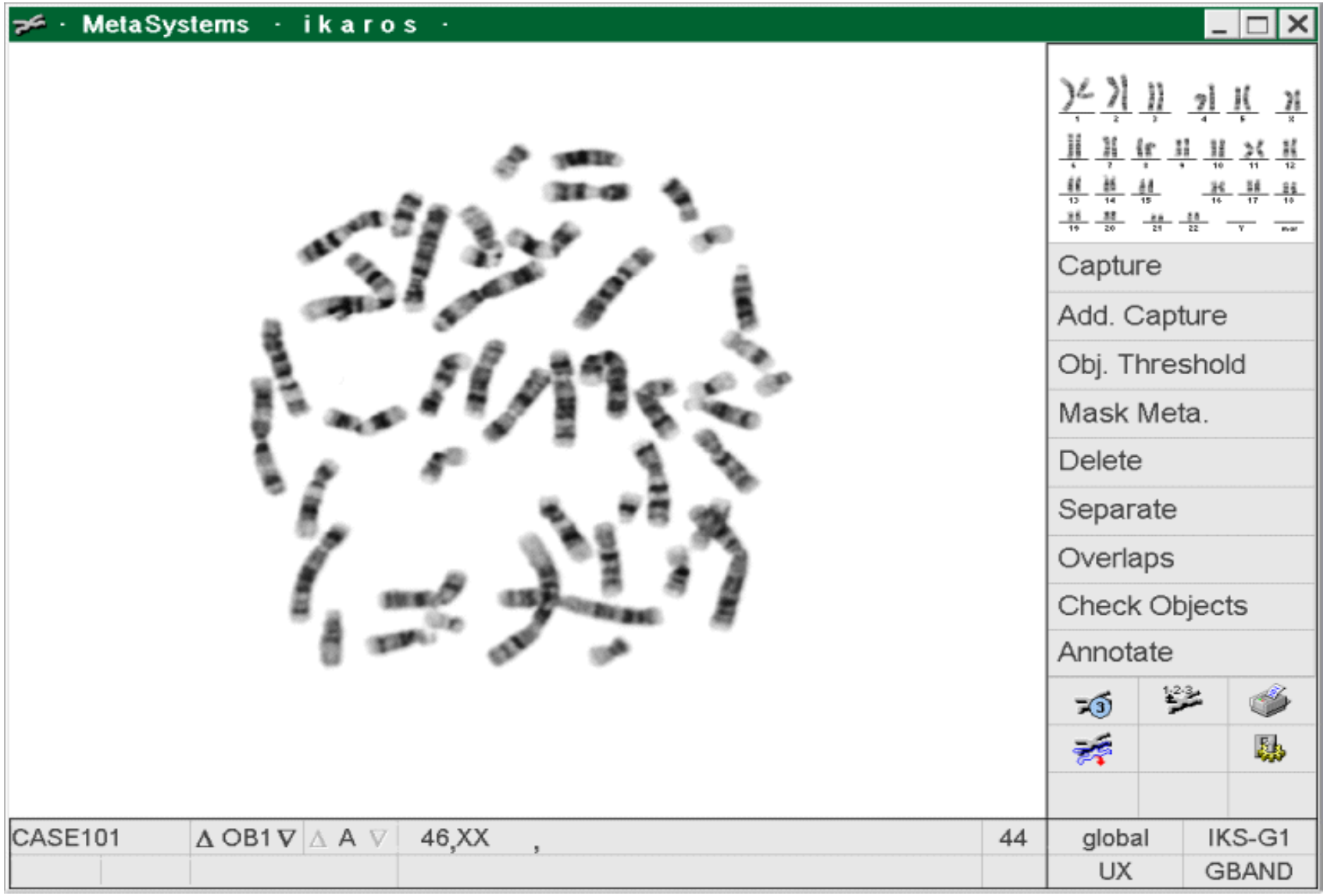
- Chromosomes are stained to produce characteristic **banding patterns**. Each chromosome consists of a short arm (p) and a long arm (q). Each arm is subdivided into regions. The numbering of regions increases progressively with distance from the centromere toward the telomere. For example, 12 and 13 refer to region 1, subregions 2 and 3, not the numbers twelve and thirteen.

- In highly condensed chromosomes, **fewer** bands are visible due to **reduced resolution**.



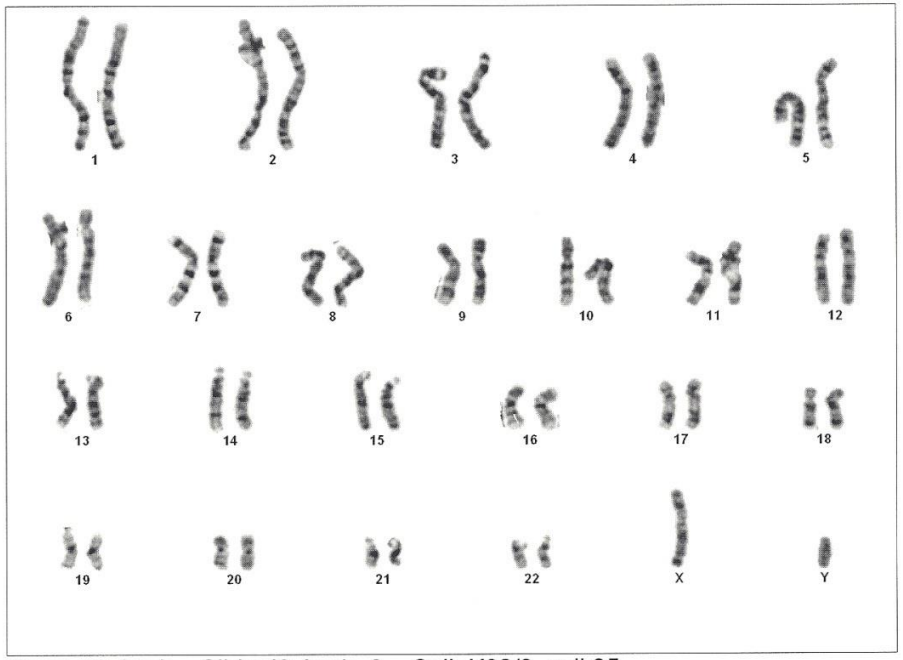
24 different chromosomes = 22 autosomes + X + Y

Metaphase chromosomes



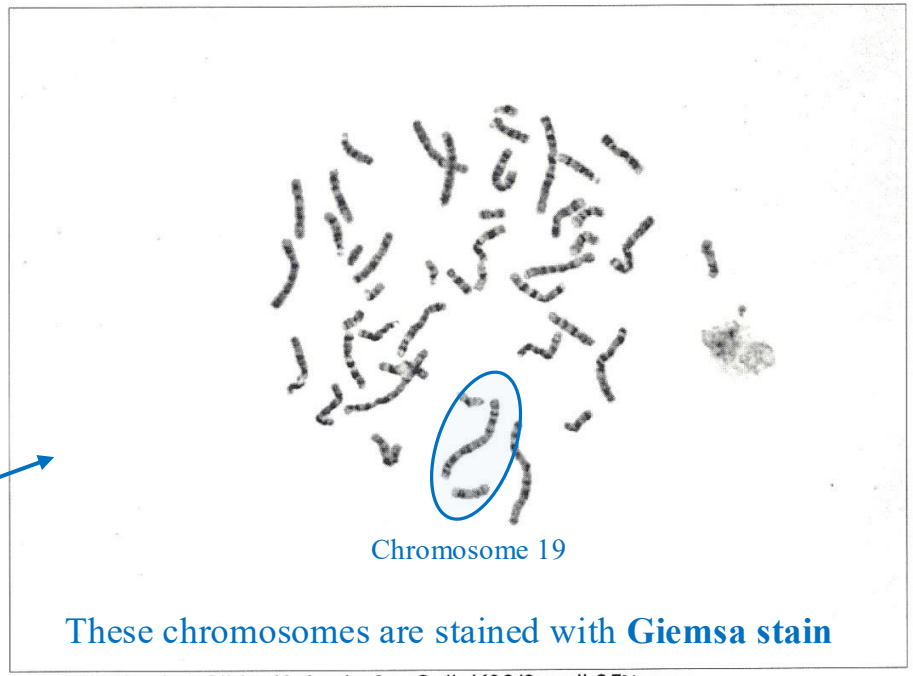
✓ Chromosomes are visualized in **metaphase of M phase** because they are highly **condensed**, aligned at the metaphase plate, and easily distinguishable for banding and karyotyping.

- 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.



Case: 12-Azab Slide: I3 Azab_3 Cell: K32/3_cell 95

- This is not the actual appearance of chromosomes under the microscope. In the past, images were printed, and scissors were used to cut out each chromosome and manually arrange them. Nowadays, software programs are used to outline each chromosome and arrange them digitally by dragging and dropping them into their correct positions. However, no software can yet distinguish all chromosomes from each other with complete accuracy.


Chromosome 19

These chromosomes are stained with **Giemsa stain**

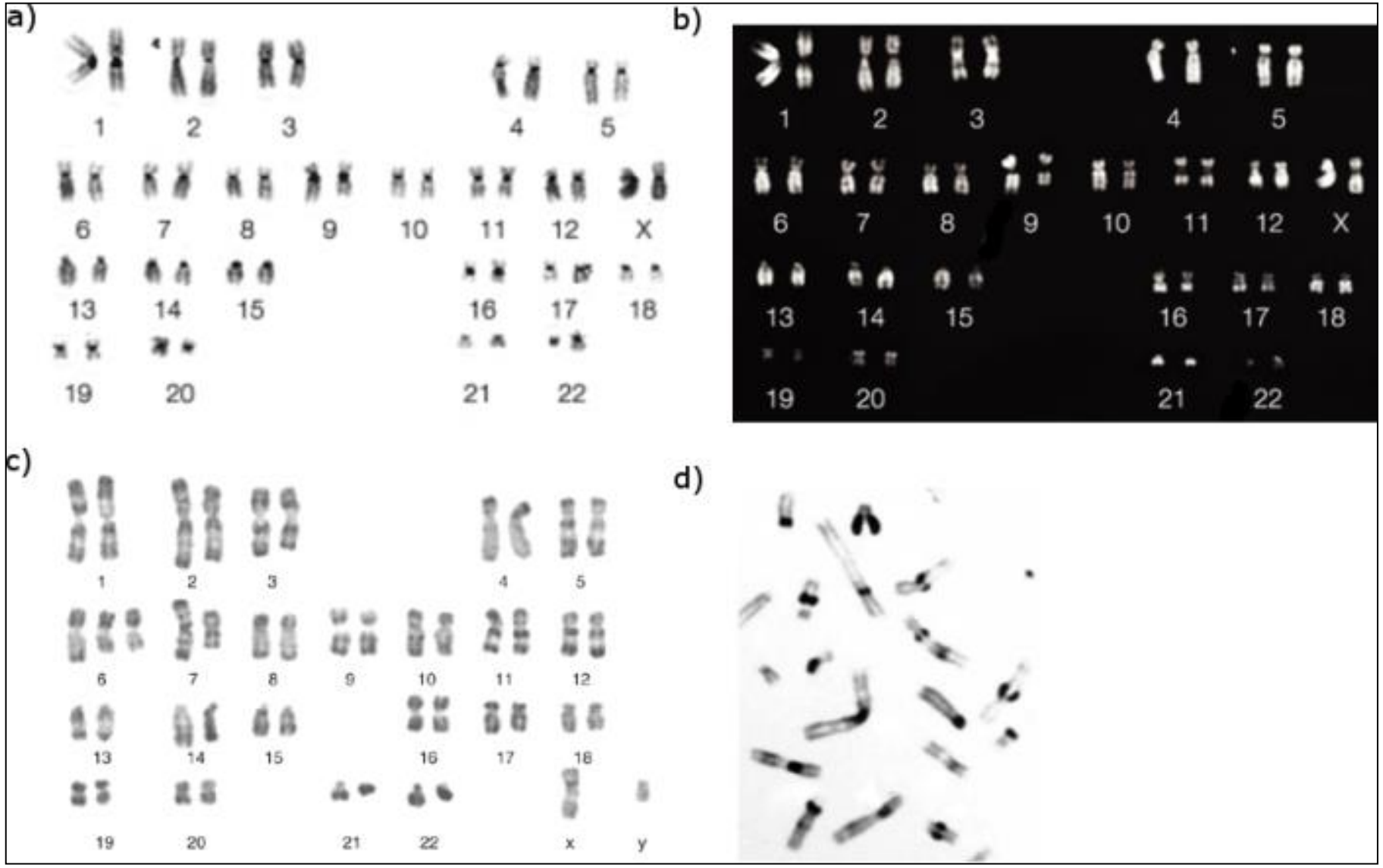
Case: 12-Azab Slide: I3 Azab_3 Cell: K32/3_cell 95

Types of banding – staining

- G-banding – most common (**G: Giemsa**)
- R-banding
- C-banding
- Q-banding – Quinacrine
- T-banding
- Silver staining

G-banding

Q-banding



R-banding

C-banding

✓ Heterochromatin is highly condensed DNA
✓ Euchromatin is less condensed DNA

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**.
- 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.

(or GTG-banding; where the first G= G-banding striped pattern; the T=trypsin, which is how the bands were obtained; and the last G= the stain used (in this case Giemsa; if a different stain is used this letter could be different).

R-banding

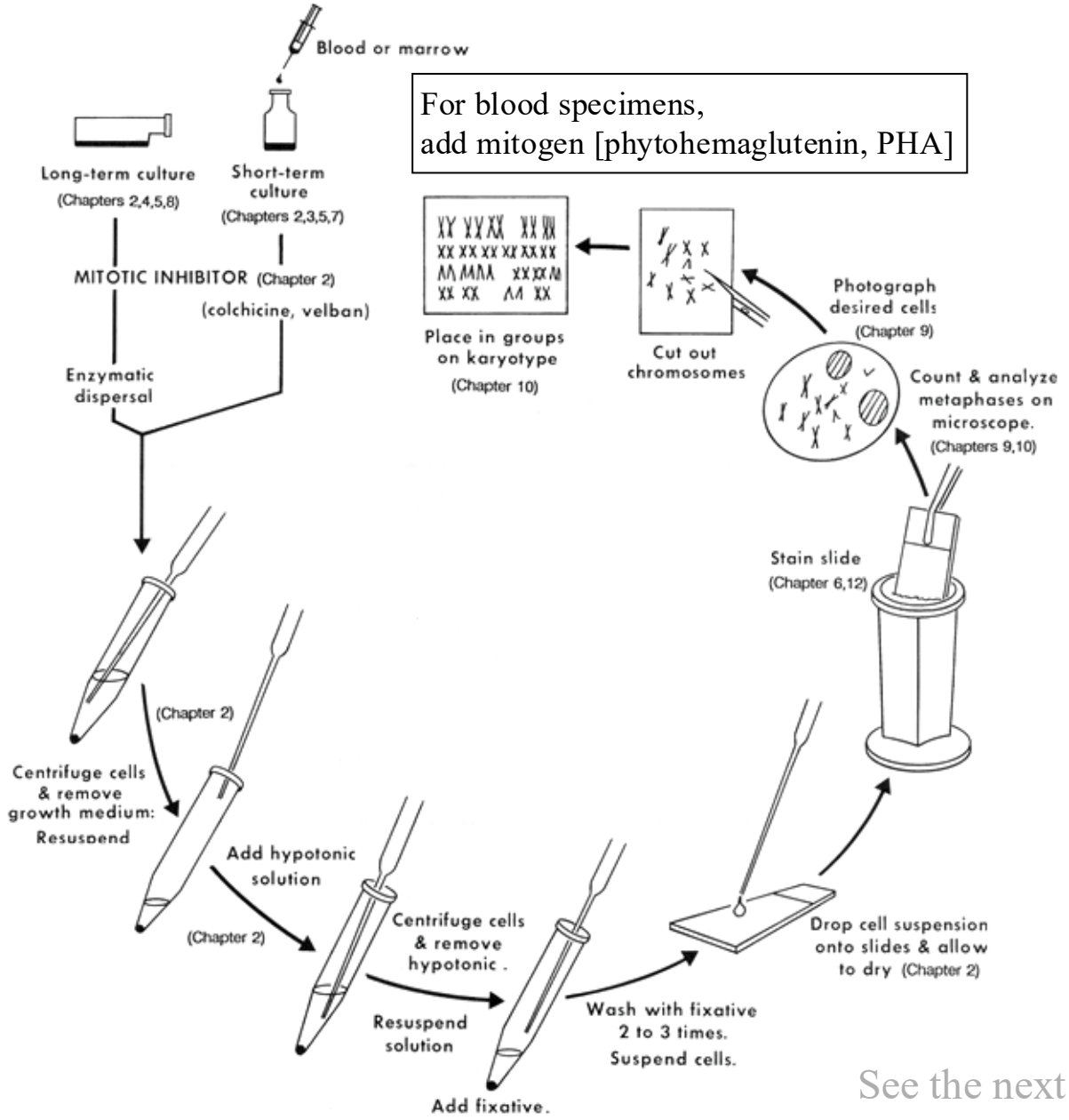
- is the reverse of G-banding (the R stands for "reverse"). The dark regions are euchromatic (guanine-cytosine rich regions). The bright regions are heterochromatic (thymine-adenine rich regions)

- 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**



See the next slide

*A **mitogen** is a chemical substance that encourages a cell to commence cell division, triggering mitosis.*

Primary Steps for Culture Establishment and Harvest of Specimens

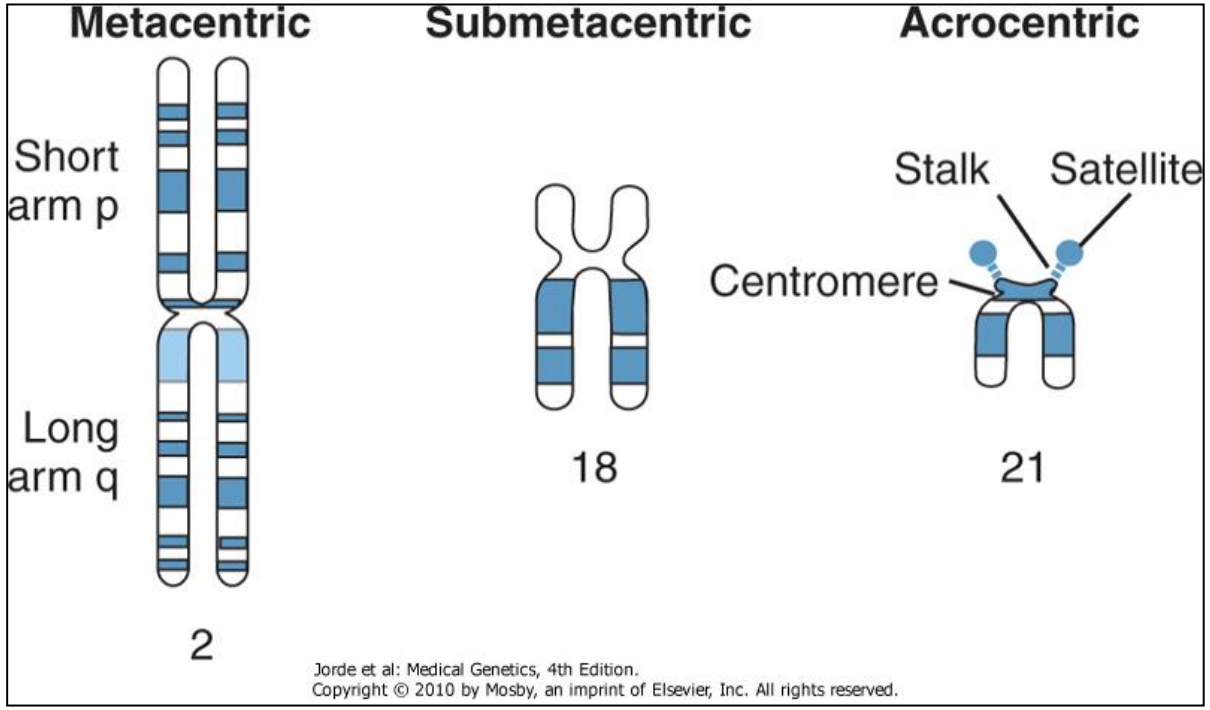
- First, a **tissue sample** is collected (e.g., blood or bone marrow).
- Then, cells are stimulated to enter the cell cycle, as many differentiated cells are arrested in interphase (e.g., G1). *Mitogens* such as phytohemaglutenin (**PHA**) are used to induce cell division.
- After some time, the cells are treated with a *mitotic inhibitor* (e.g., **colchicine**) to arrest them in **metaphase**.
- Next, the cells are centrifuged to form a pellet, while the supernatant remains as the liquid.
- A **hypotonic** solution is then added, causing the cells to **swell and become fragile** due to water influx.
- After that, a **fixative** is added, and the cells are **washed** with **water or PBS**.
- The swollen cells are then dropped onto a glass slide from a height, causing them to burst open.
- These lysed cells allow the chromosomes to spread out (become scattered), and the slide is stained with **Giemsa stain**.
- Finally, the chromosomes become **stained and visible** under the microscope.

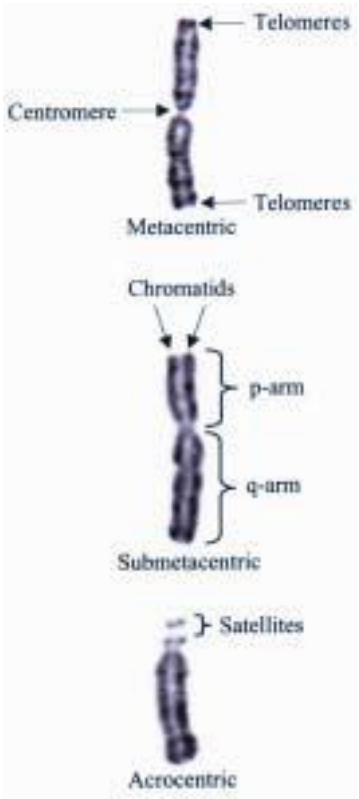
Sample collection (blood / bone marrow) → Cell stimulation (PHA) → Mitotic arrest (colchicine, metaphase) → Centrifugation (pellet) → Hypotonic treatment (swelling) → Fixation & washing → Drop on slide (lysis & spreading) → Giemsa staining → Visible chromosomes

Chromosome Shape

- Metacentric- centromere is located in the middle of chromosome
- Submetacentric- centromere is displaced from the center (p arm is shorter than the q arm)
- Acrocentric – centromere is placed near the end

- **Satellite**: Repetitive DNA sequences.
- **Stalk**: region of DNA containing ribosomal DNA genes (non-coding for protein) → transcribed into *rRNA*.
- Ribosomes: ribonucleoprotein complexes (rRNA + proteins translated from mRNA)
- **rRNA**: most **abundant** RNA in the cell
- mRNA: the only RNA that can be translated into a protein.

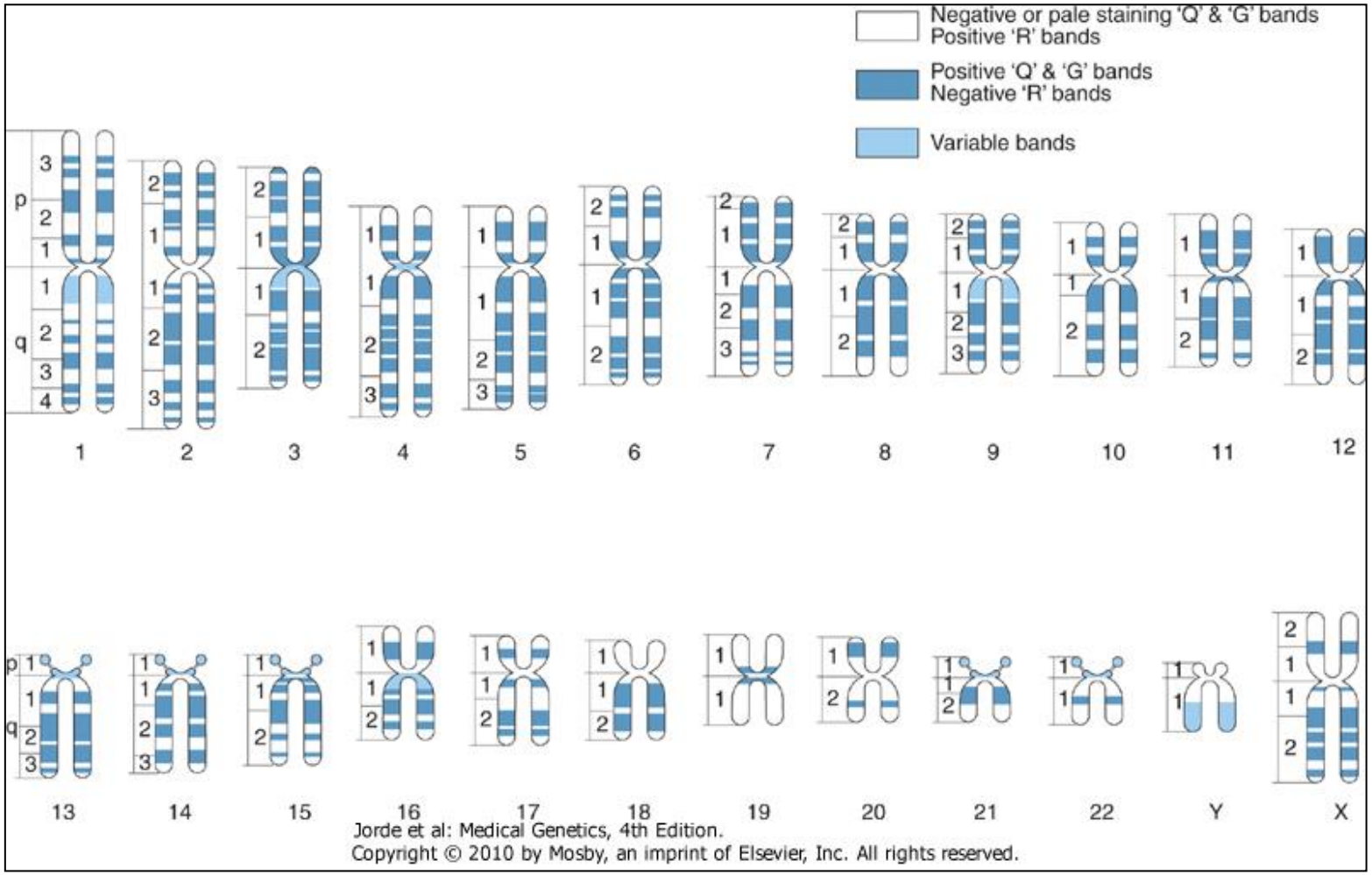




The stalks of these five chromosome pairs contain hundreds of copies of genes for ribosomal RNA

Human Chromosome Ideogram

Ideogram- A diagrammatic representation of a karyotype



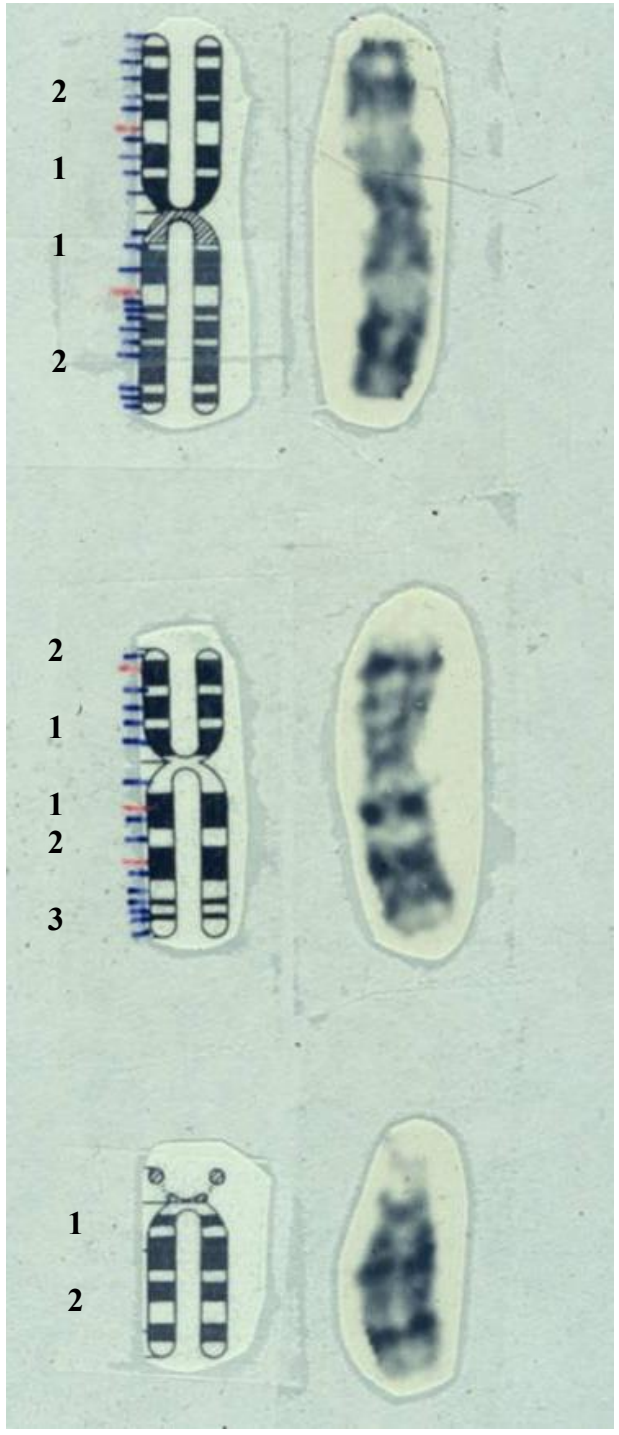
- **Variable region** (polymorphic): a region whose size **differs** between individuals in a population, *without* being disease-causing.

✓ Even if the p-arm of one acrocentric chromosome is deleted, there are **no clinical consequences** because the other acrocentric chromosomes have identical p-arms with the same genes, providing sufficient rRNA.

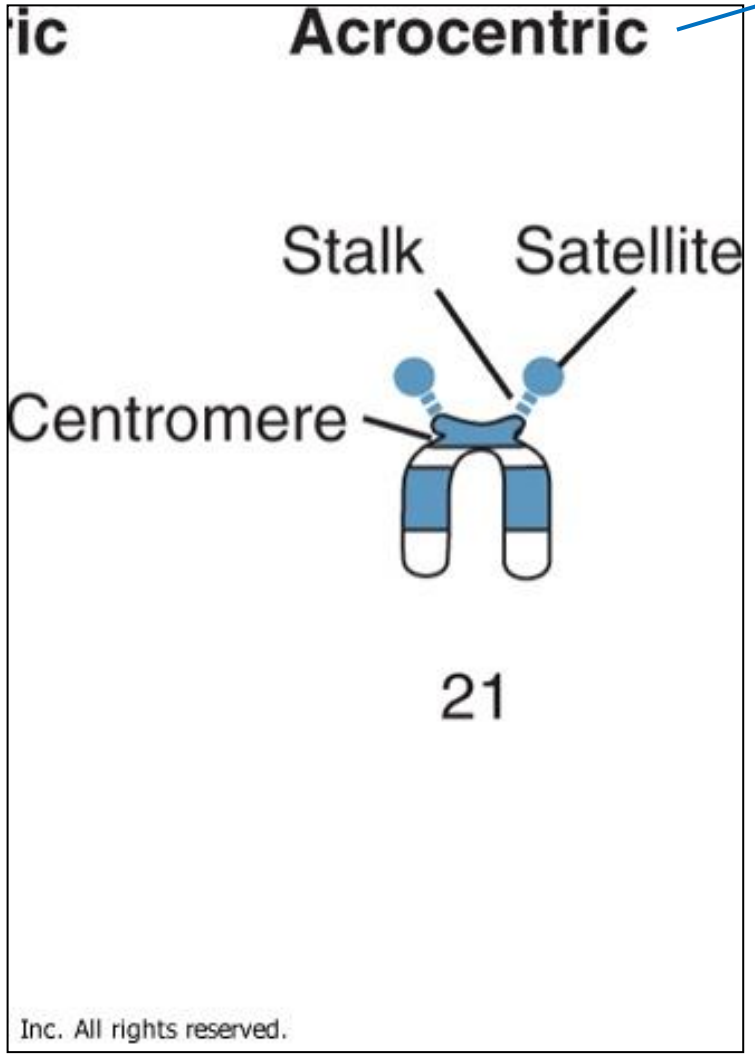
Chromosome 3
p: 2 regions
q: 2 regions

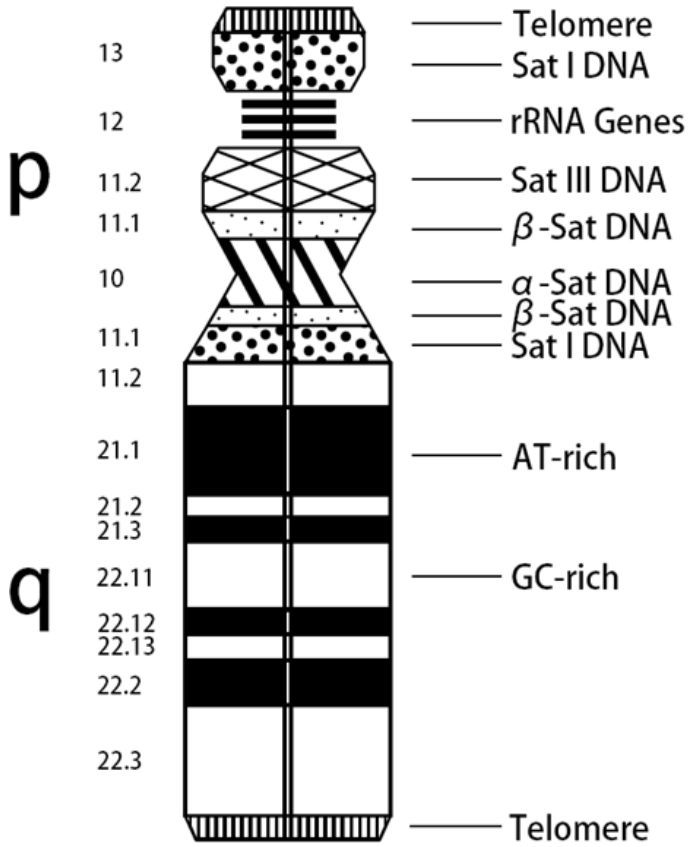
Chromosome 7
p: 2 regions
q: 3 regions

Chromosome 14
p: 1 region
q: 2 regions



13/14/15/21/22



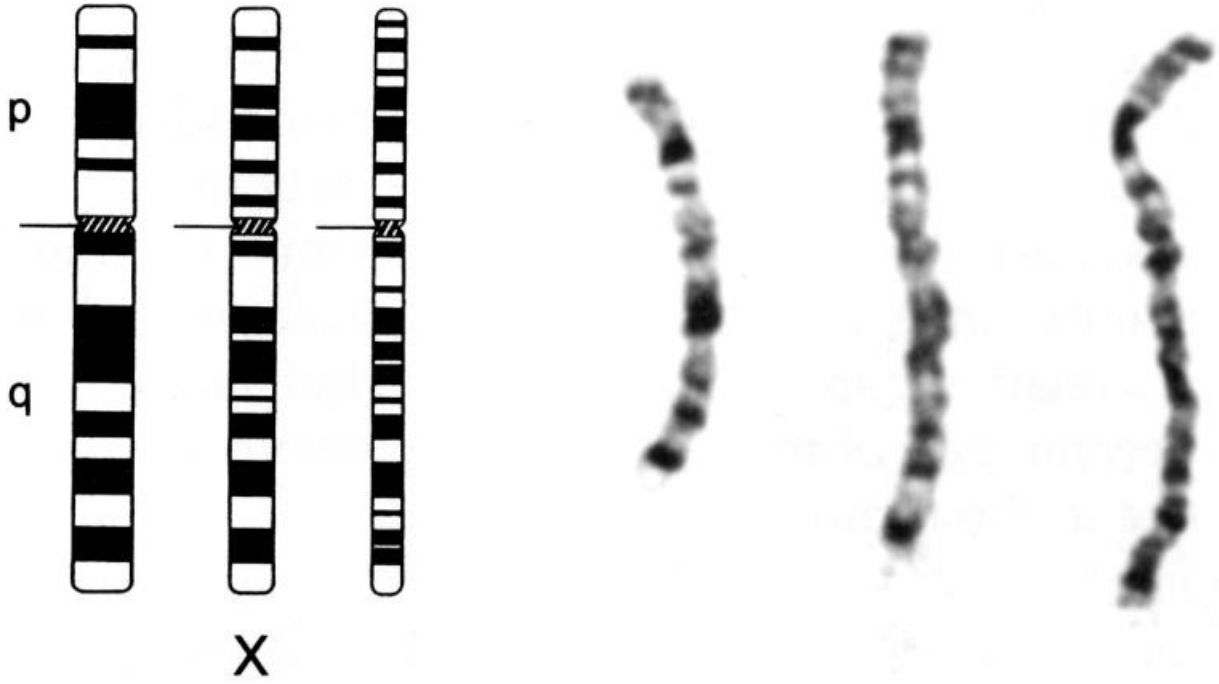


- All acrocentric chromosomes have the ***same unique p-arm*** structure with the same genes, but their q-arms differ.
- The centromere of all chromosomes is composed of satellite DNA, known as ***α-satellite*** DNA.

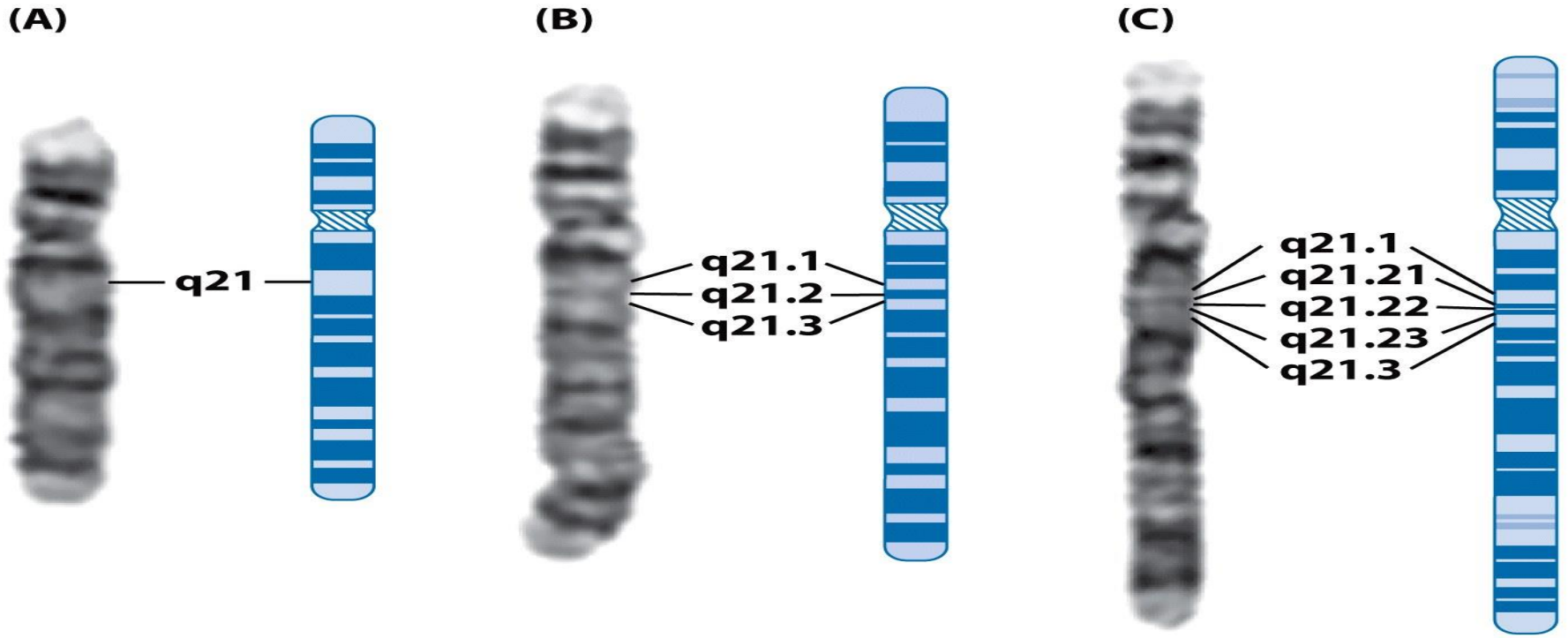
High Resolution Banding

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.

All of those are different resolutions of chromosome X



More bands: higher resolution banding



By arresting the cell at an earlier stage

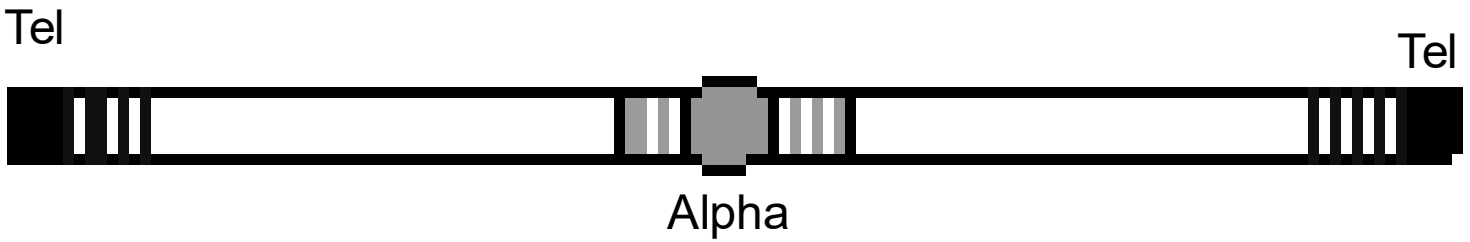
Figure 2.14 Different chromosome banding resolutions can resolve bands, sub-bands, and sub-sub-bands.

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



171-bp monomers

Pericentromeric region**

Unique chromosome-specific DNA

(TTAGGG)n

Subtelomeric region*

Alpha satellite DNA consists of tandemly repeated sequences arranged next to each other and is non-coding; therefore, it is present as **heterochromatin**.

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

Centromere

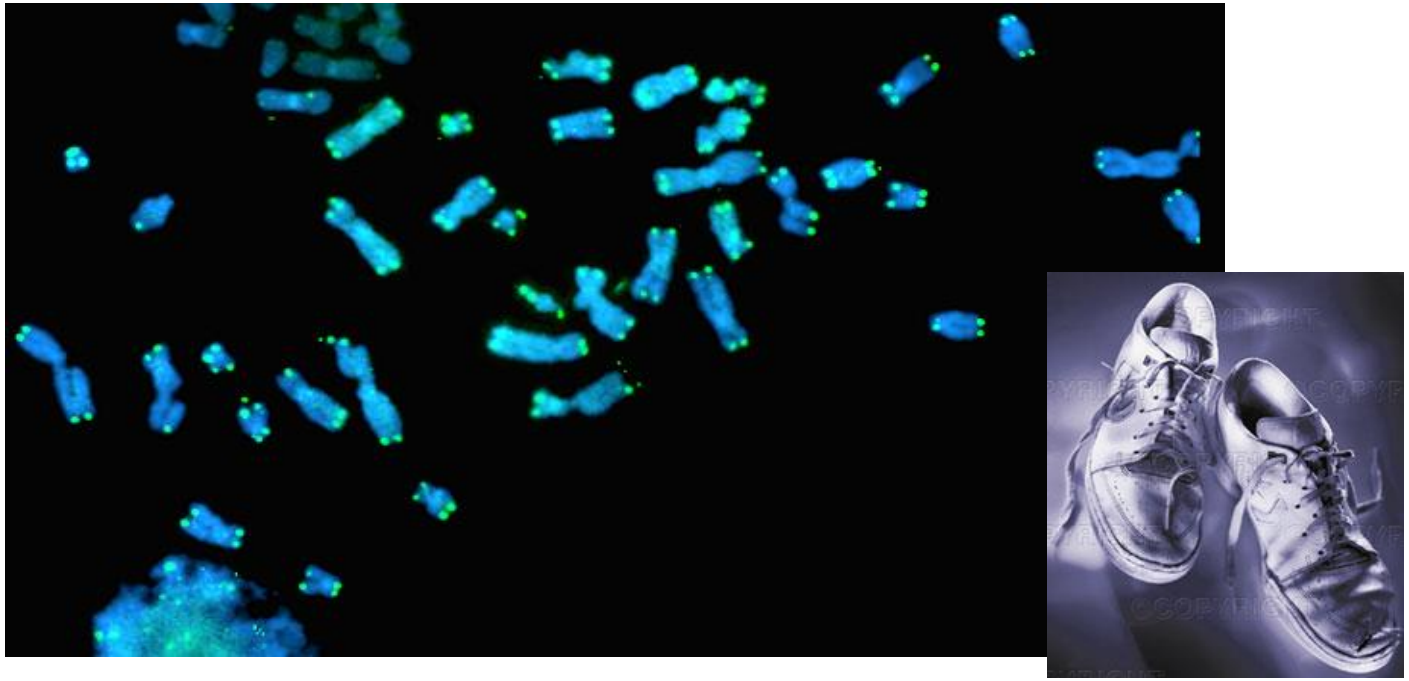
The genetic locus required for chromosome segregation; contains DNA and proteins on which the kinetochore is formed.

Protein complex

Telomere $(TTAGGG)_n$

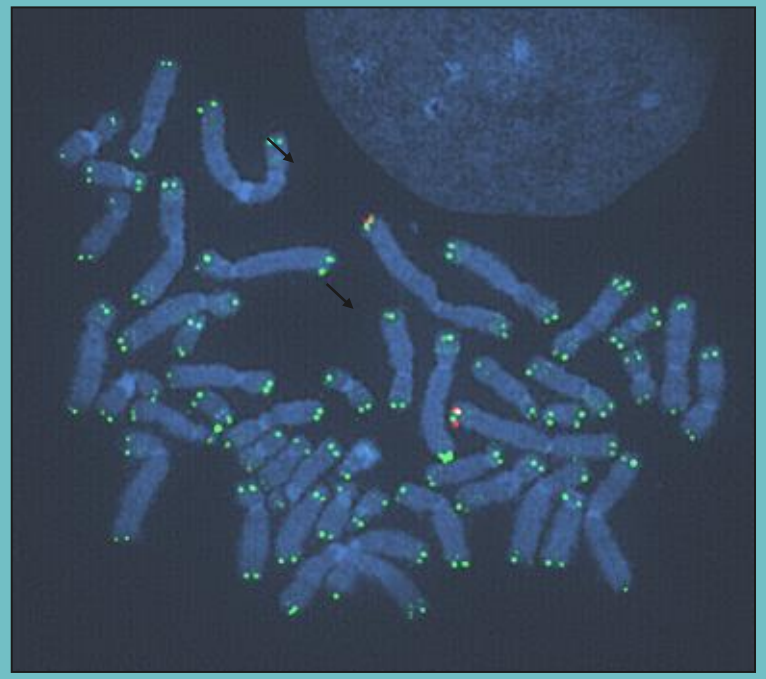
A specialized structure at the ends of eukaryotic chromosomes. Maintain chromosomal integrity by preventing end-to-end fusion of chromosomes. (If a telomere is deleted, the chromosome end becomes sticky and can abnormally fuse with other chromosomes)

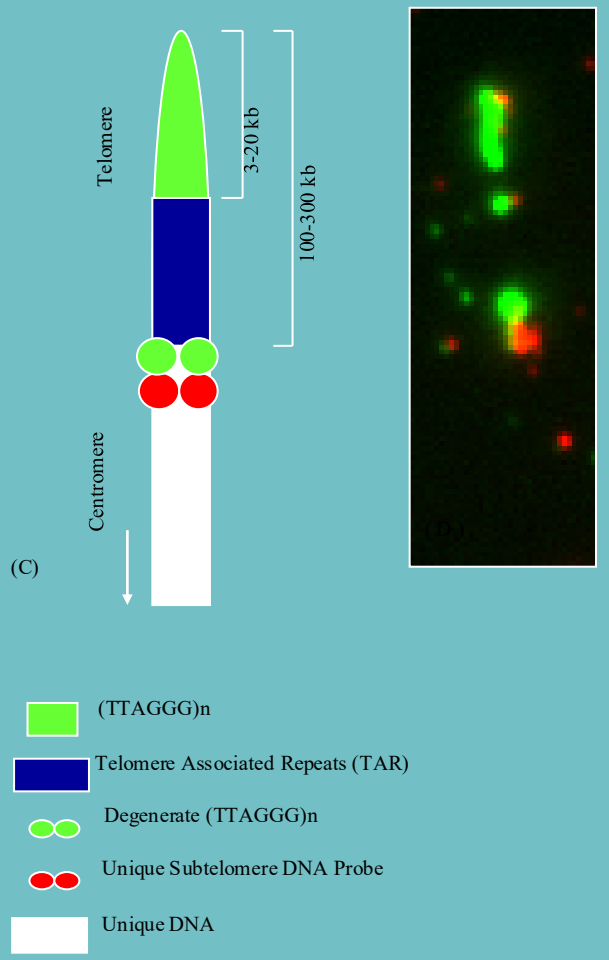
- Telomeres are repetitive DNA sequences located at both ends of chromosomes, and they have the **same sequence** in all 46 chromosomes.
- Telomeres **shorten** over time; as individuals age, their telomeres become *progressively shorter*.



✓ In these chromosomes, the telomeric sequence (**TTAGGG**) can be detected using a probe, which is a synthetic DNA sequence complementary to the target region (telomere). A complementary sequence (**AATCCC**) is synthesized, labeled with a fluorescent dye, and added to the chromosomes. This probe binds to the telomeres of all chromosomes, confirming that ***all chromosomes share the same telomeric sequence***.

Human Sub-telomeric Regions





- Immediately after the telomere: Telomere-Associated Repeat (**TAR**) region.
- Following TAR: subtelomeric region, which is unique for each chromosome.
- ✓ Subtelomeric regions can be visualized with staining (e.g., red), showing **chromosome-specific sequences**.
- In normal cells, telomeres shorten progressively as cells divide. Shortening eventually leads to loss of critical DNA, causing cell death.
- ✓ *Exception*: Cancer cell as they **maintain telomere length** despite repeated divisions. This prevents gene loss and contributes to cellular immortality.

There is some sequence homology between subtelomeres