

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 ( $\geq 5$ weeks)	About 30% total; rate varies from 50% at 8-11 weeks to 5% in stillbirths ( $\geq 28$ 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**

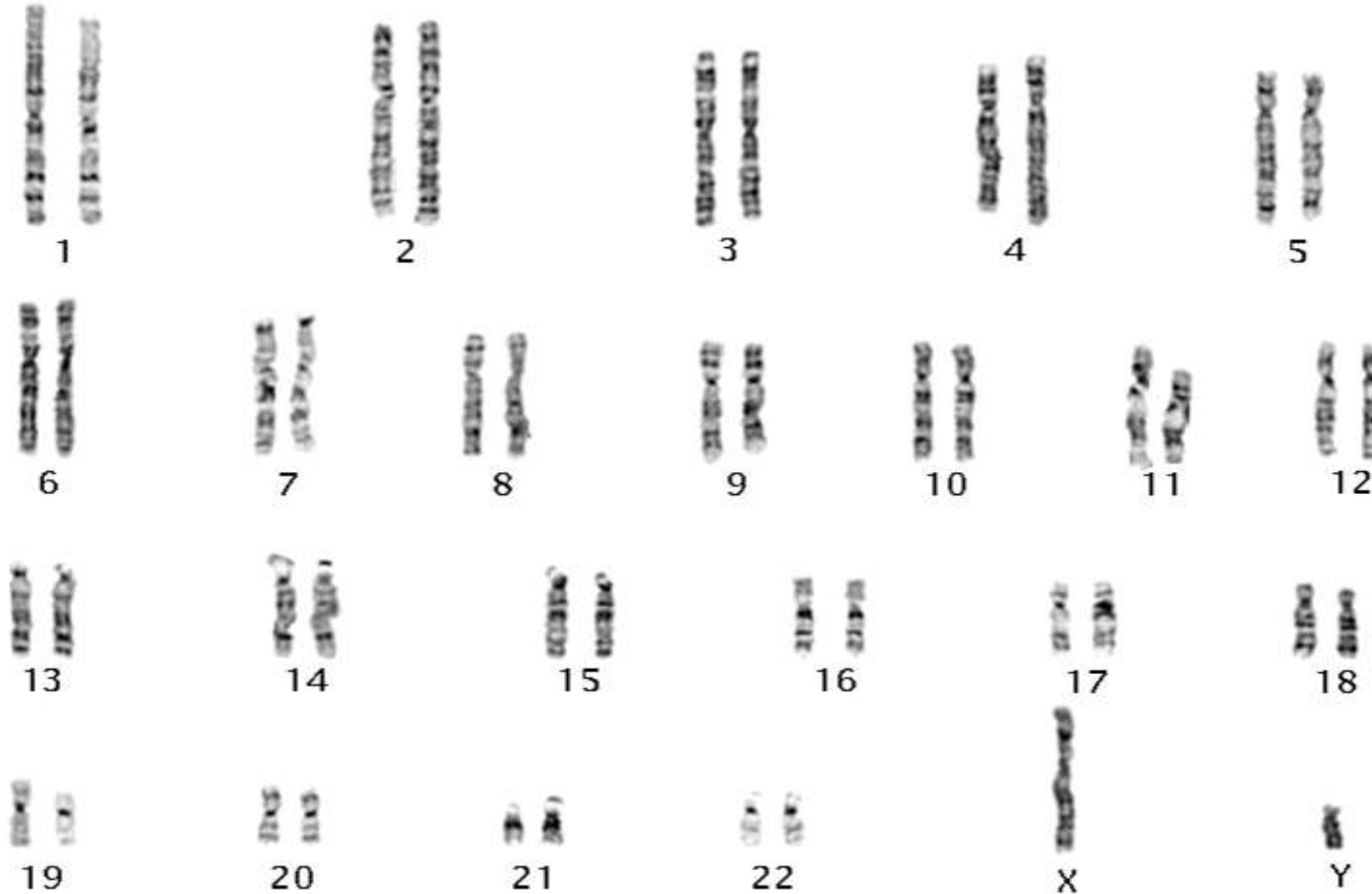
# **Tissues for Chromosome Studies**

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

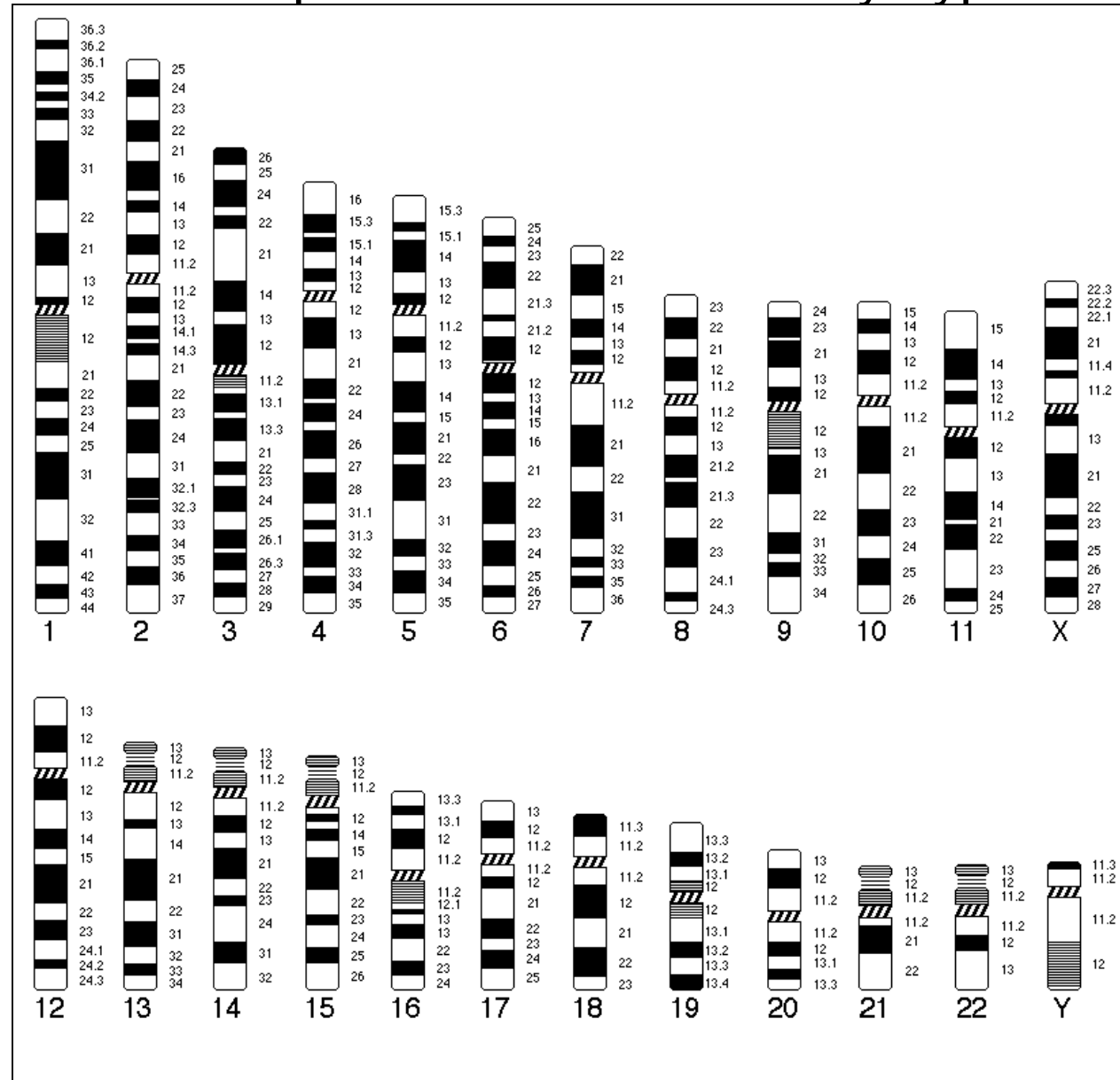
- A karyogram is photograph or a diagram of an ordered arrangement of chromosomes from cells that are placed in a standard order (generally by length; chromosome 1 is longest and 22 shortest).
- Once a computer image of the chromosomes from a dividing cell is obtained, the chromosomes are arranged as 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

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



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



MetaSystems · i k a r o s

The software interface displays a circular arrangement of 44 chromosomes in metaphase. The chromosomes are arranged in a circle, with some overlapping. The interface includes a toolbar on the right with the following options:

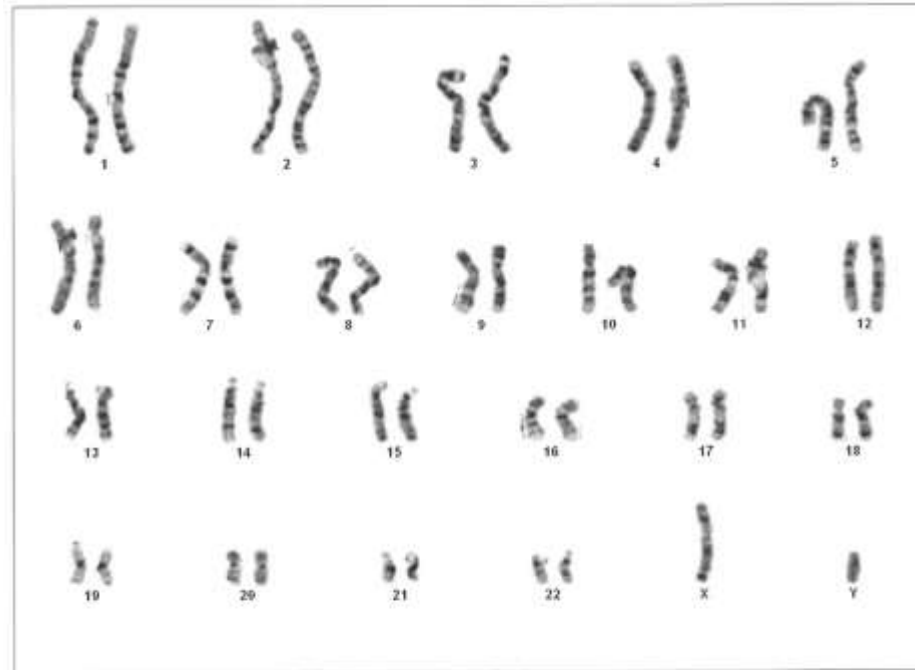
- Capture
- Add. Capture
- Obj. Threshold
- Mask Meta.
- Delete
- Separate
- Overlaps
- Check Objects
- Annotate

The status bar at the bottom contains the following information:

CASE101	Δ OB1 ▾	Δ A ▾	46,XX ,	44	global	IKS-G1
					UX	GBAND

**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.



Case: 12-Azab Slide: 13 Azab\_3 Cell: K32/3\_cell 95



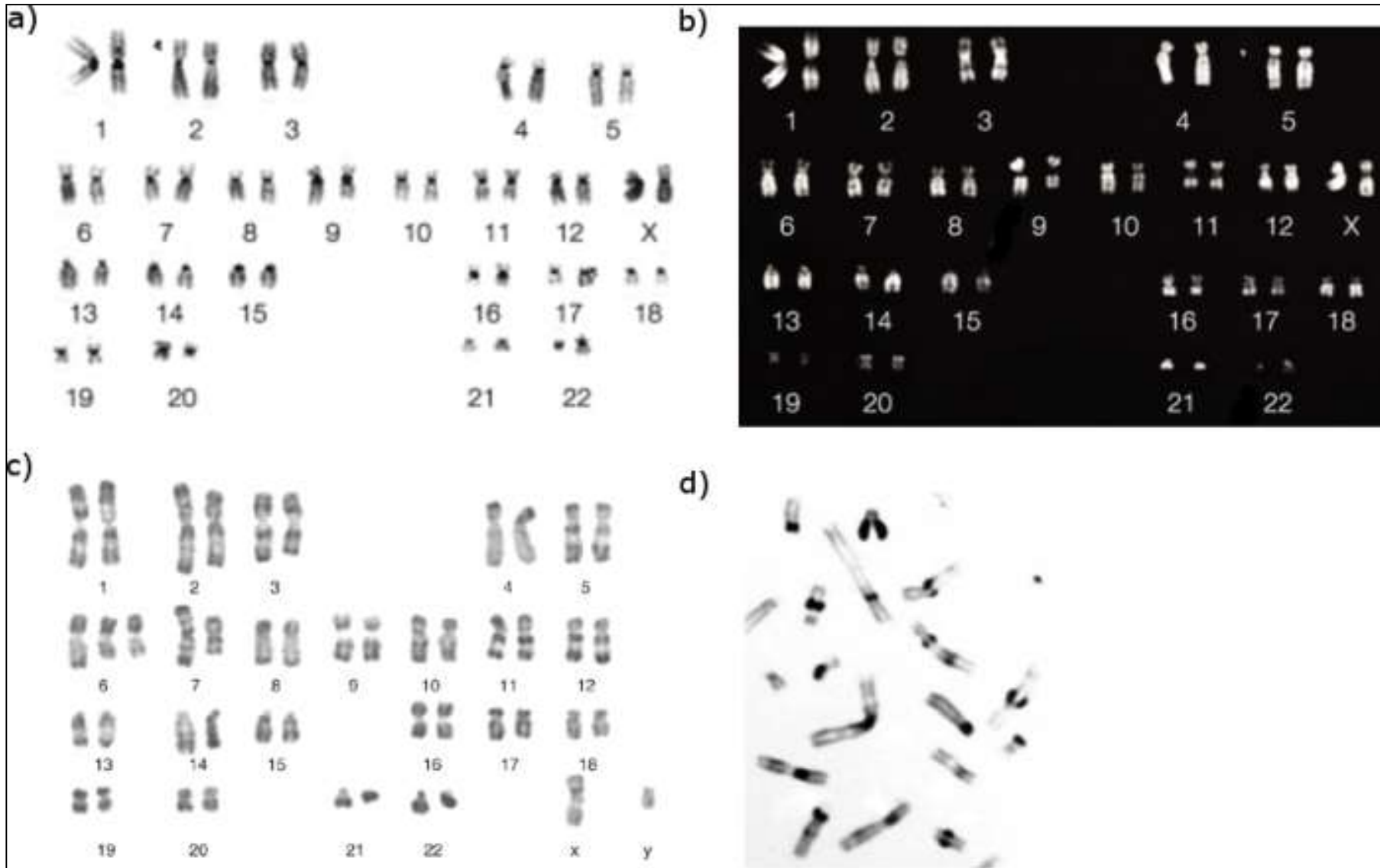
Case: 12-Azab Slide: 13 Azab\_3 Cell: K32/3\_cell 95a

# Types of banding

- G-banding
- R-banding
- C-banding
- Q-banding
- T-banding
- Silver staining

# G-banding

# Q-banding



# R-banding

# C-banding

# G-banding (GTG)

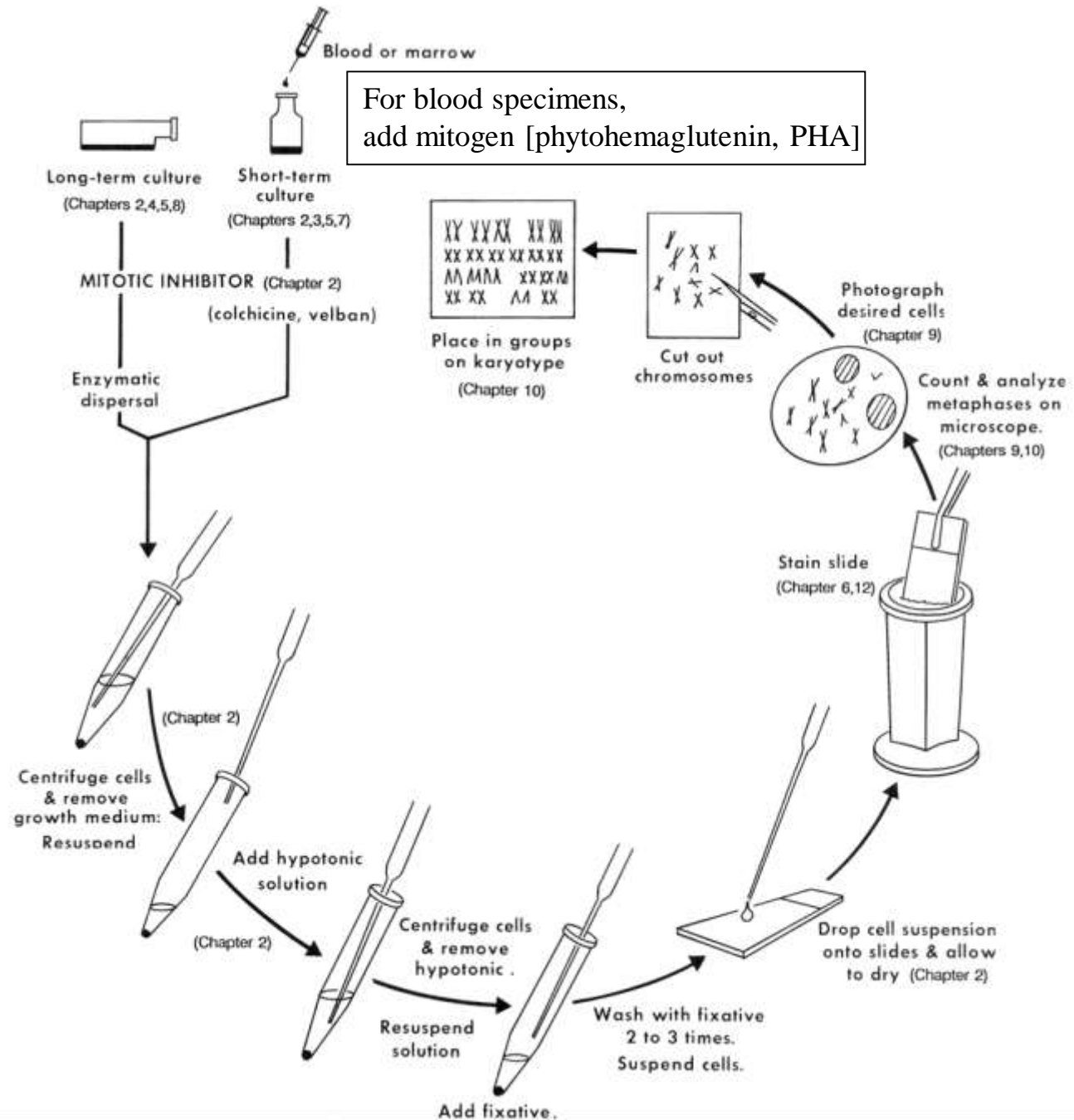
- heterochromatic regions, which tend to be AT-rich DNA and relatively gene-poor, stain more darkly The **light** regions tend to be **euchromatic, GC rich**.
- less condensed chromatin—which tends to be GC-rich and more transcriptionally active—incorporates less Giemsa stain, and these regions appear 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.

# 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

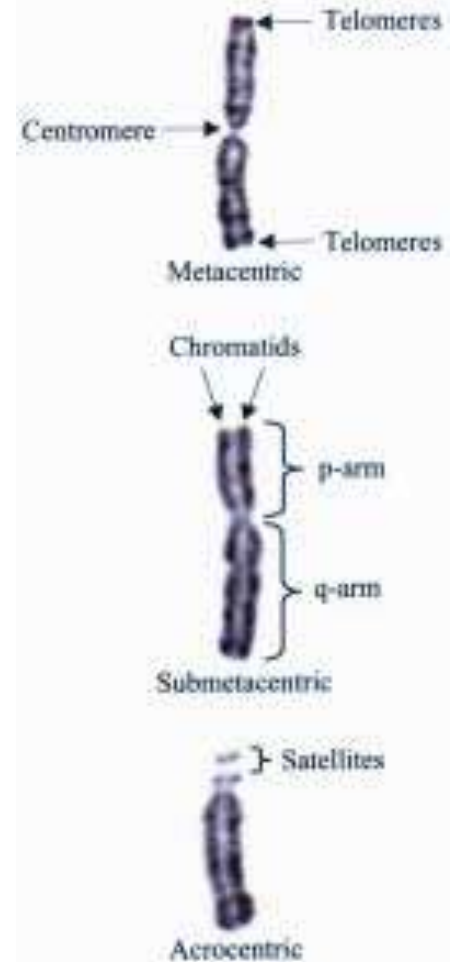
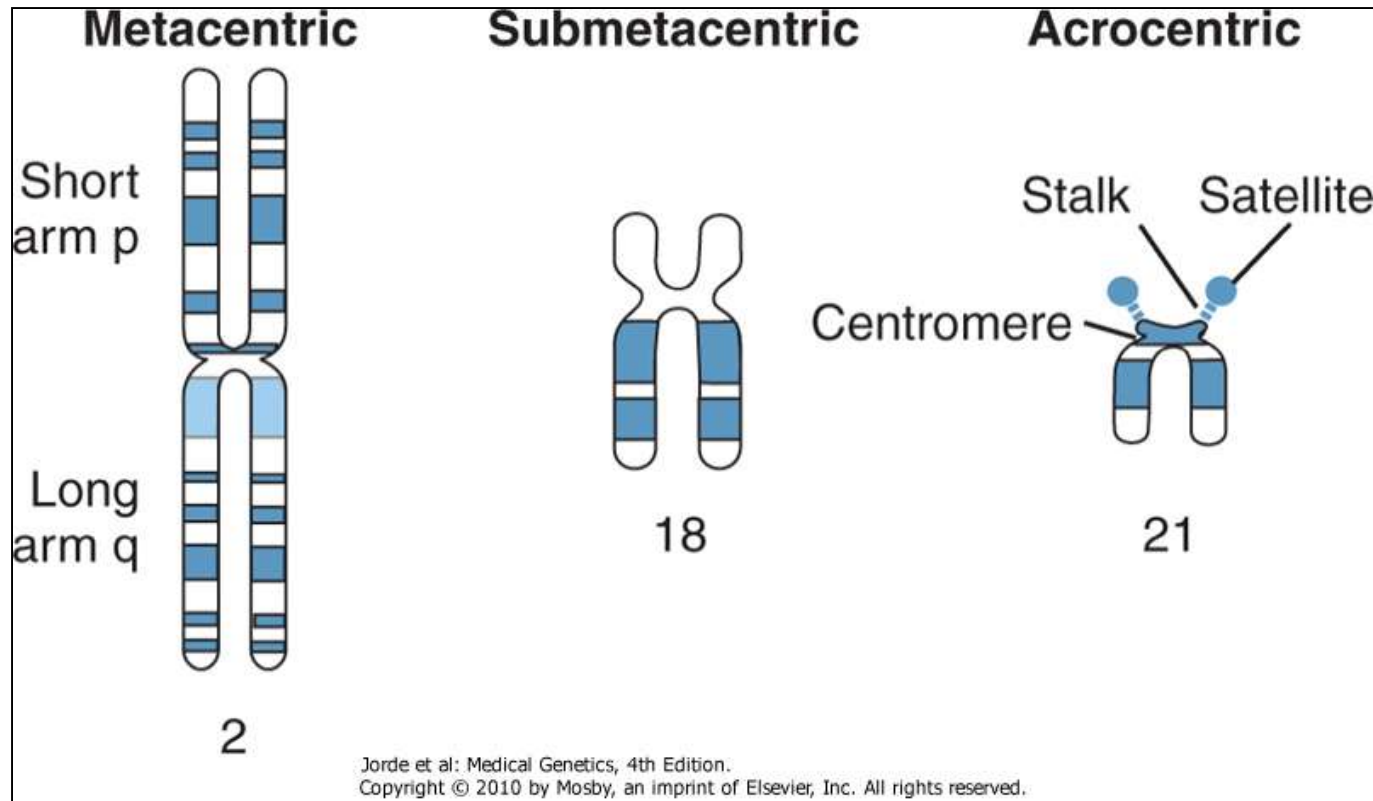


# Chromosome Shape

**Metacentric-** centromere is located in the middle of chromosome

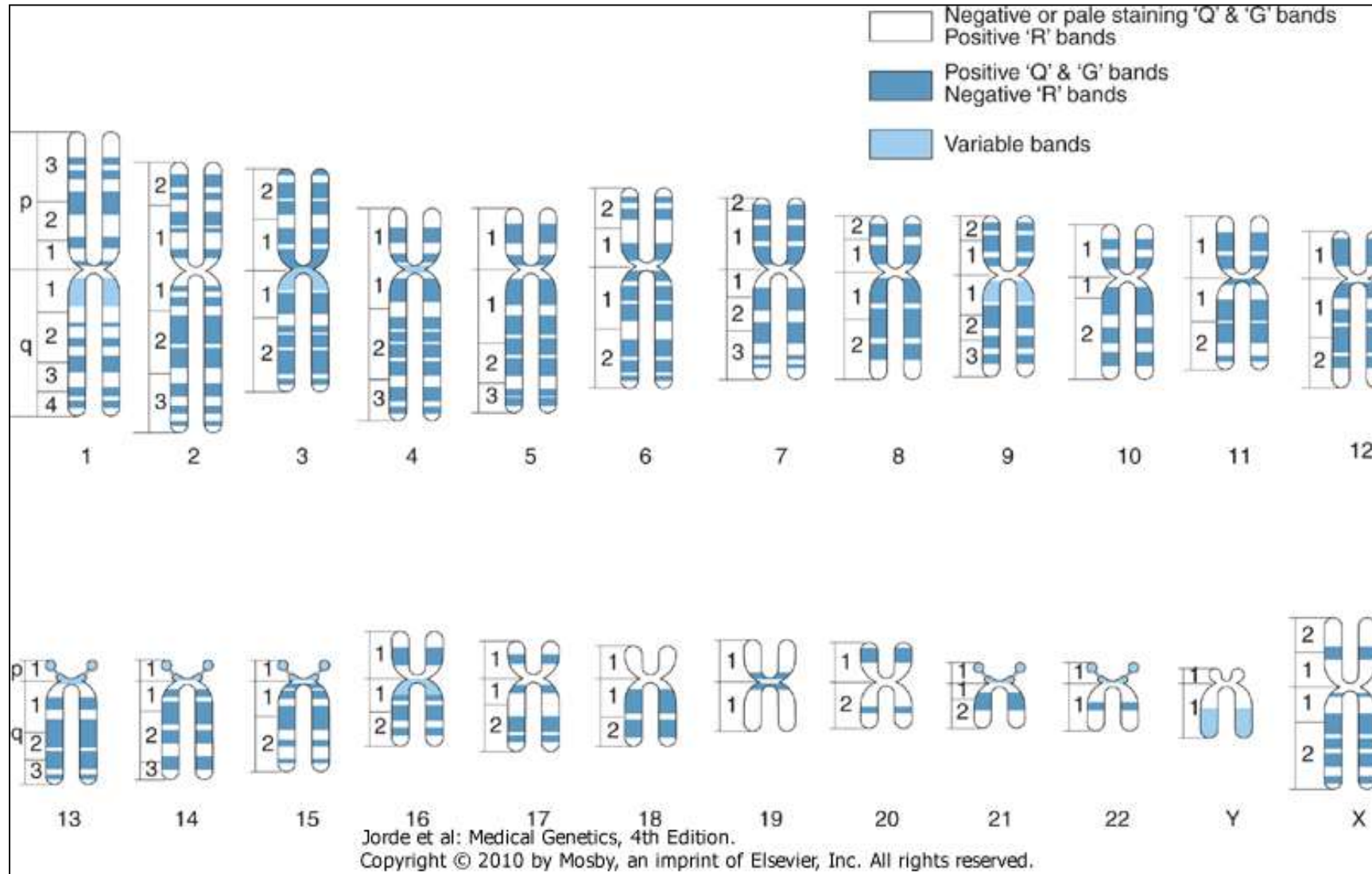
**Submetacentric-** centromere is displaced from the center

**Acrocentric** – centromere is placed near the end



# Human Chromosome Ideogram

Ideogram- A diagrammatic representation of a karyotype



## **Chromosome 3**

**p: 2 regions**

**q: 2 regions**

## **Chromosome 7**

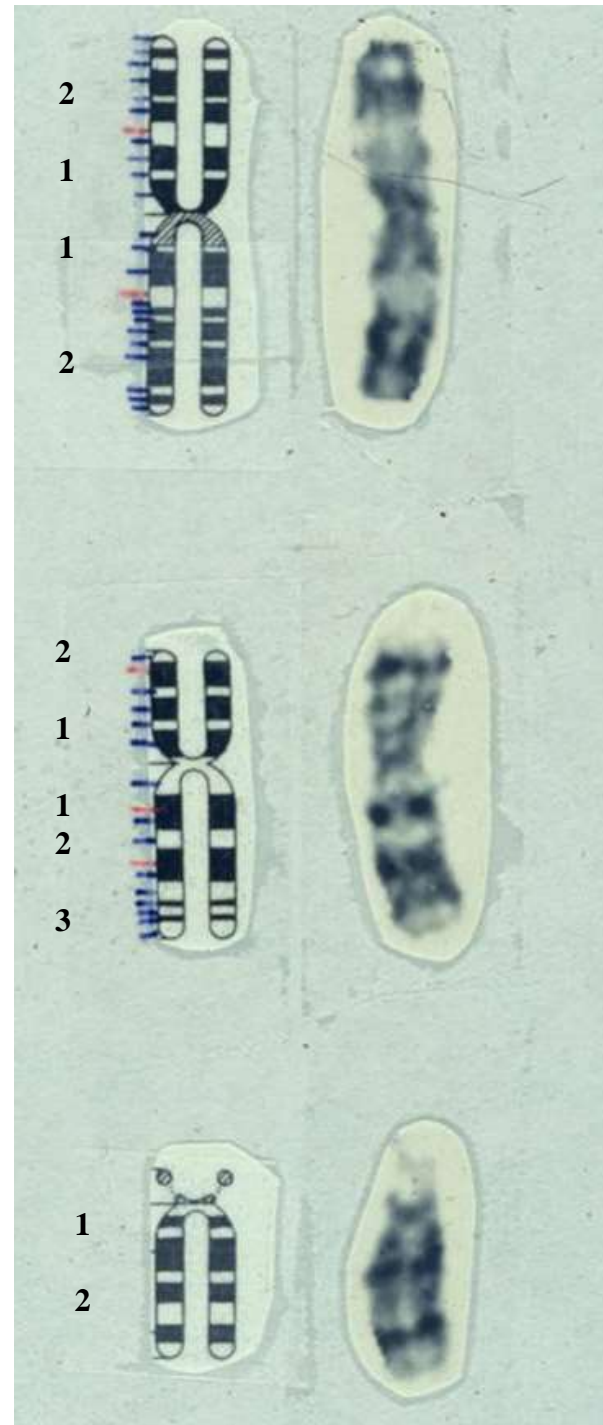
**p: 2 regions**

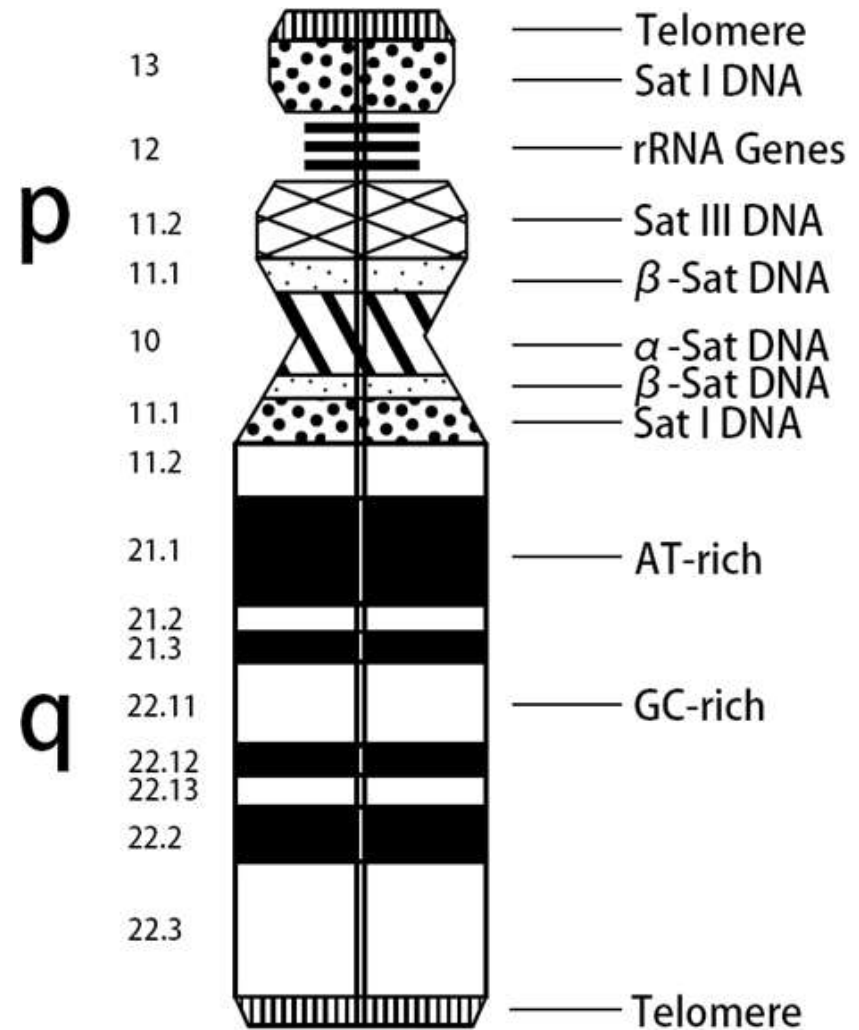
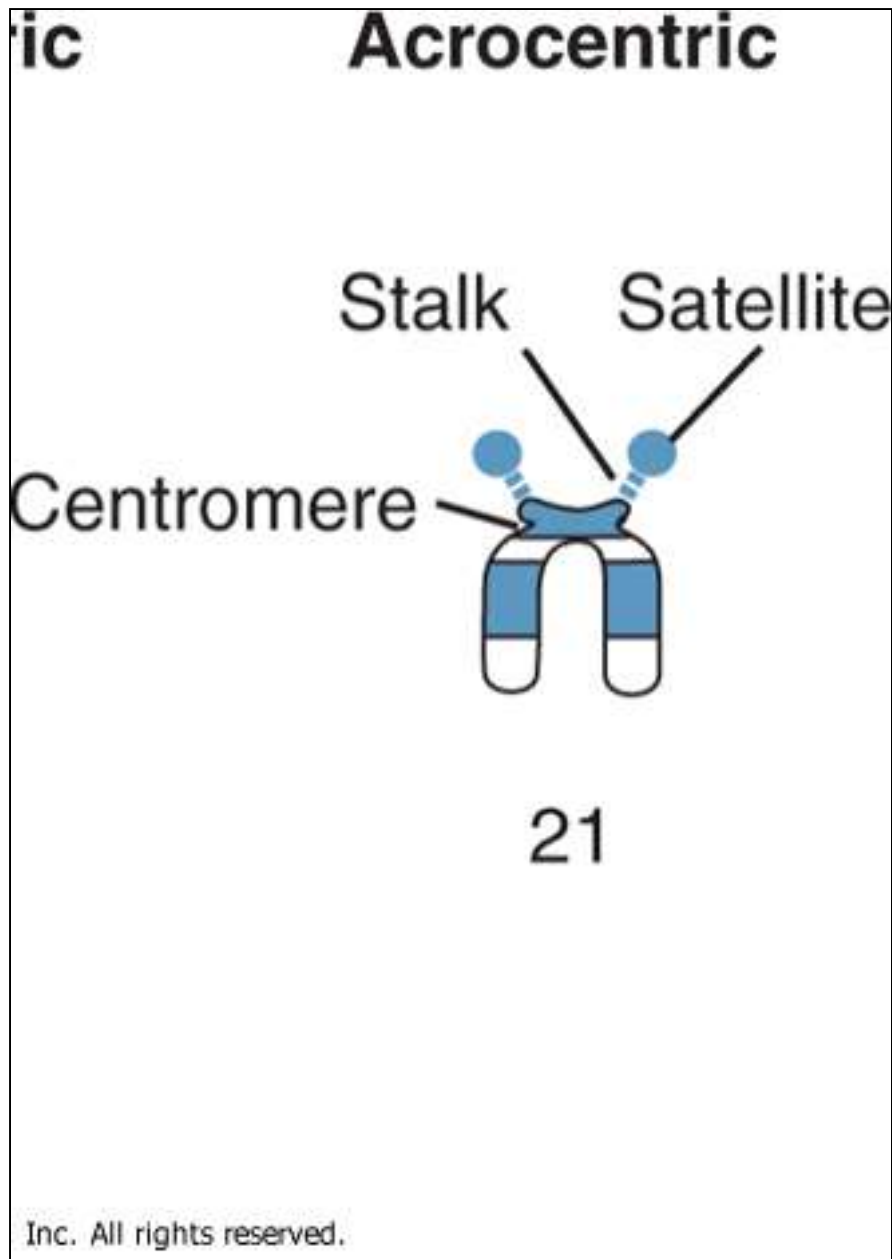
**q: 3 regions**

## **Chromosome 14**

**p: 1 region**

**q: 2 regions**

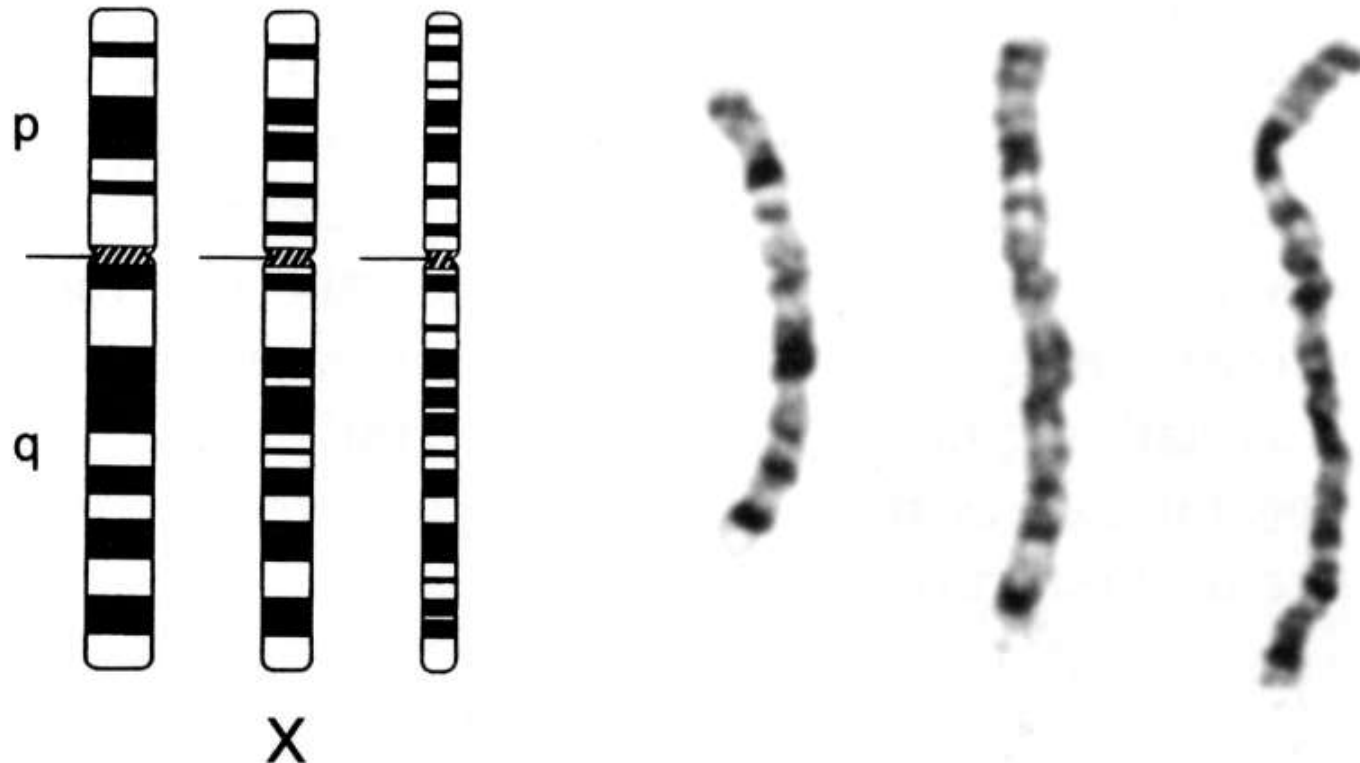




# High Resolution Banding

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.



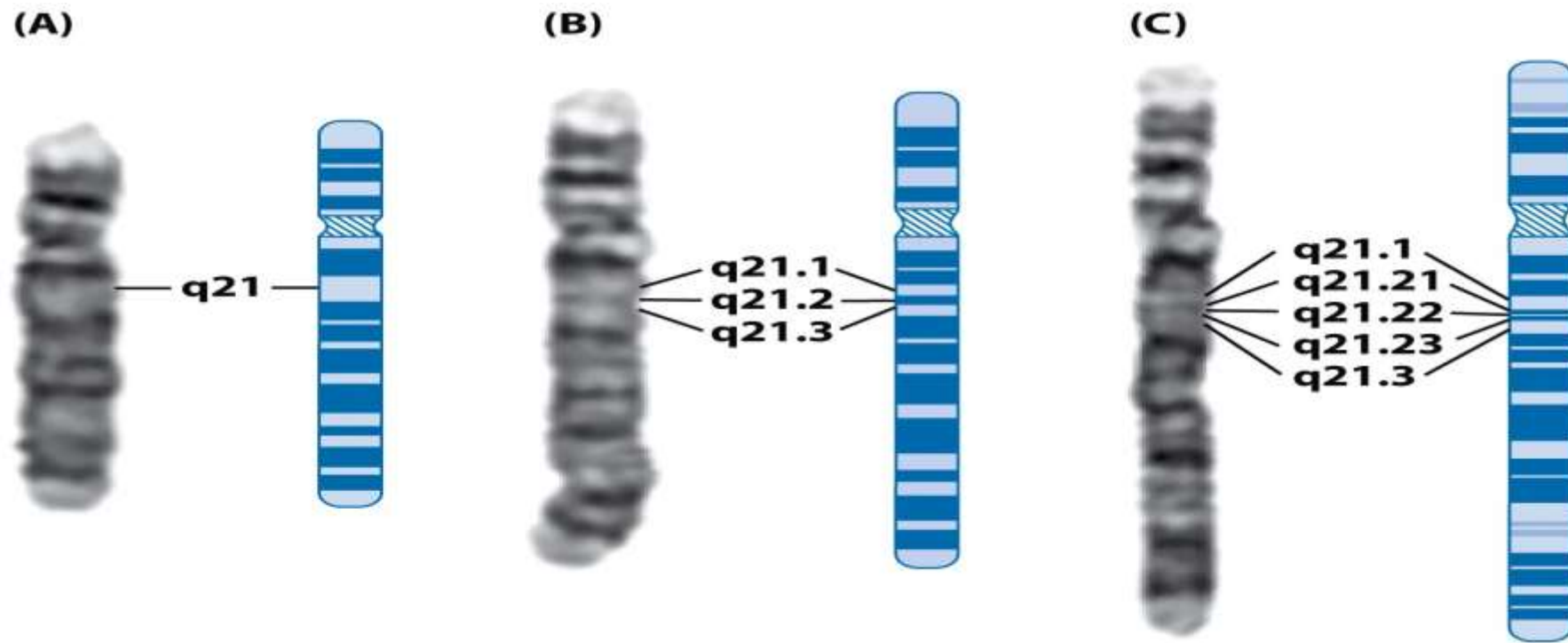


Figure 2.14 Human Molecular Genetics, 4ed. (© Garland Science)

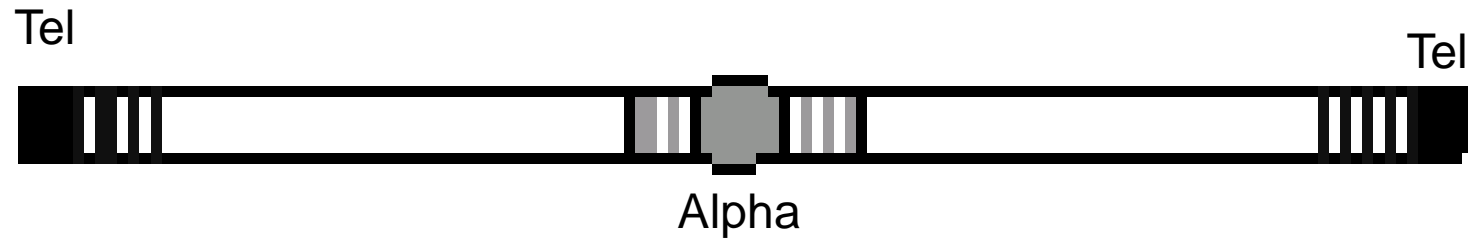
Figure 2.14 Different chromosome banding resolutions can resolve bands, sub-bands, and sub-sub-bands.






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



-  171-bp monomers
-  Pericentromeric region\*\*
-  Unique chromosome-specific DNA
-  (TTAGGG)<sub>n</sub>
-  Subtelomeric region\*

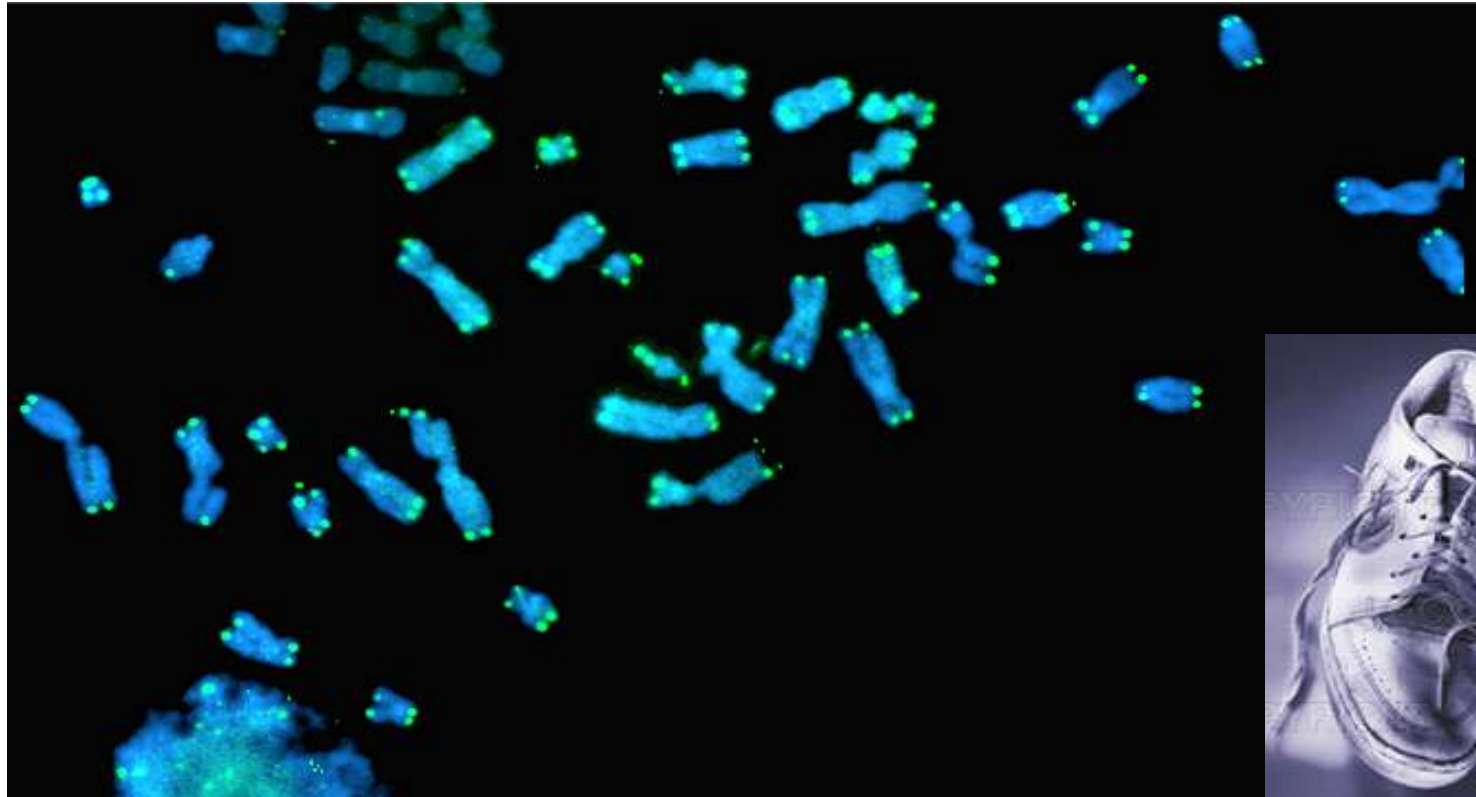
\*Highly polymorphic; implicated in location of “hotspots for structural chromosomal abnormalities

# Centromere

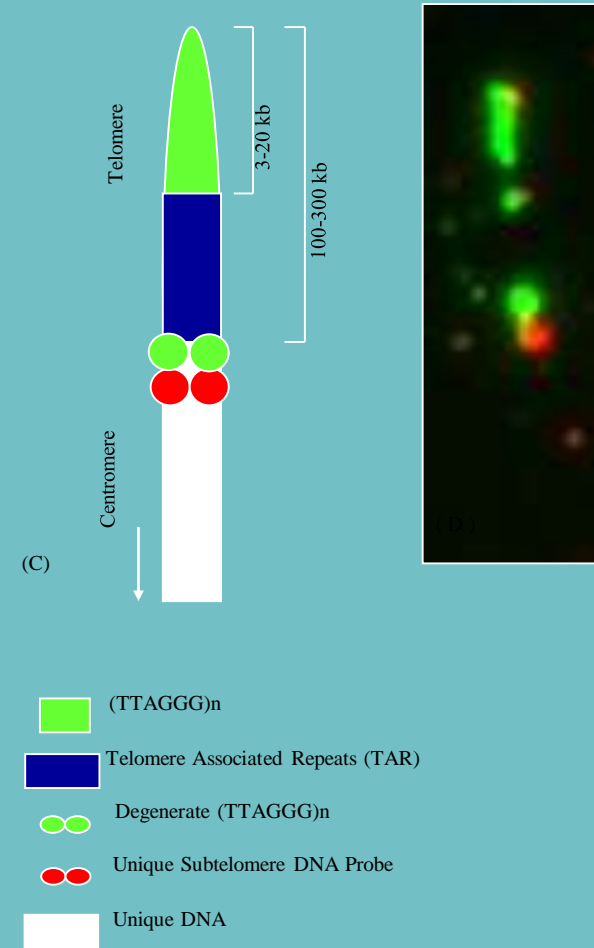
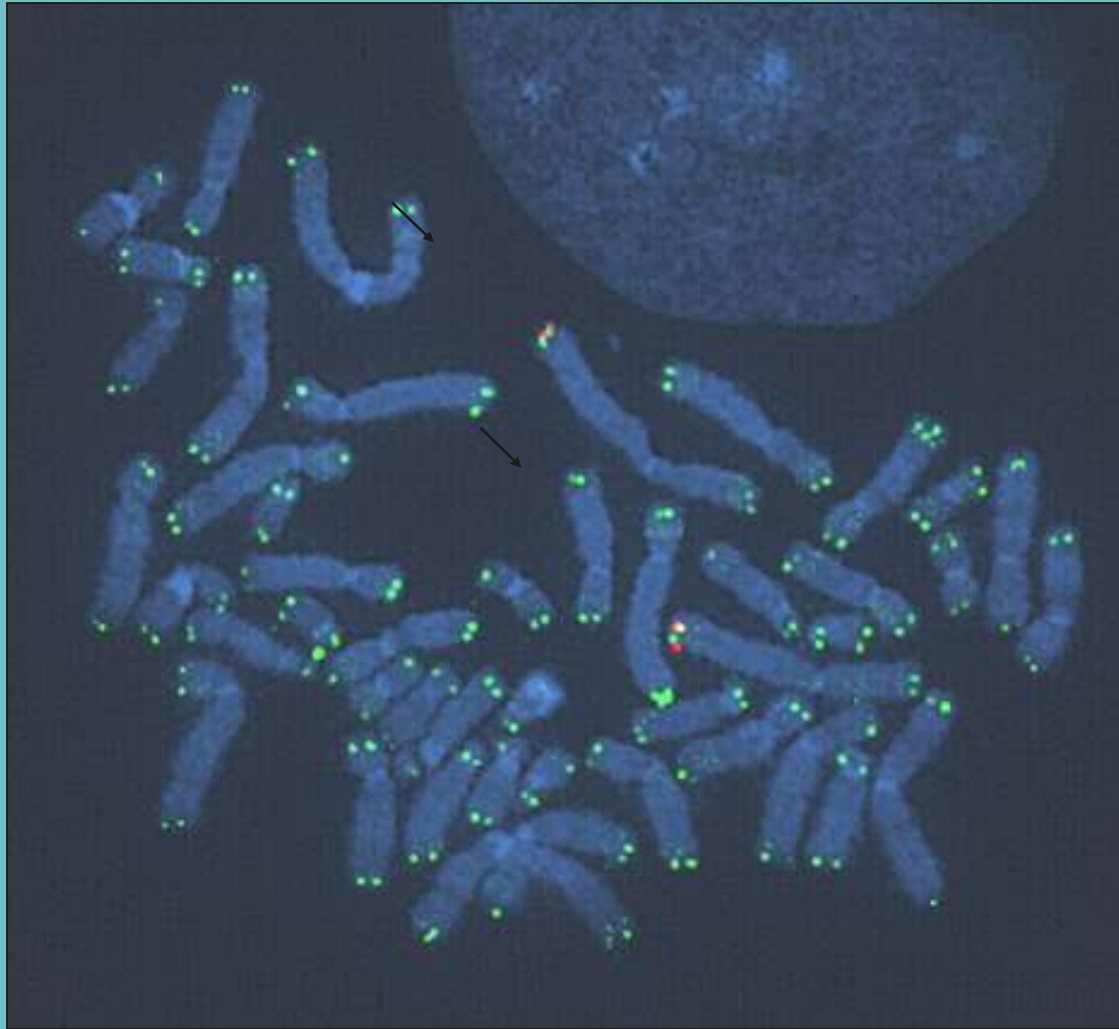
The genetic locus required for chromosome segregation; contains DNA and proteins on which the kinetochore is formed.

# Telomere (TTAGGG)<sub>n</sub>

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



# Human Sub-telomeric Regions





There is some  
sequence homology  
between subtelomeres