Tissues for Chromosome Studies

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

Medical Genetics Course

Dr. Bilal Azab
The University of Jordan
School of Medicine
Department of Pathology, Microbiology and Forensic Medicine
Email: b.azab@ju.edu.jo

Lecture link: https://youtu.be/7c0QI9w7aLc

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**

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.

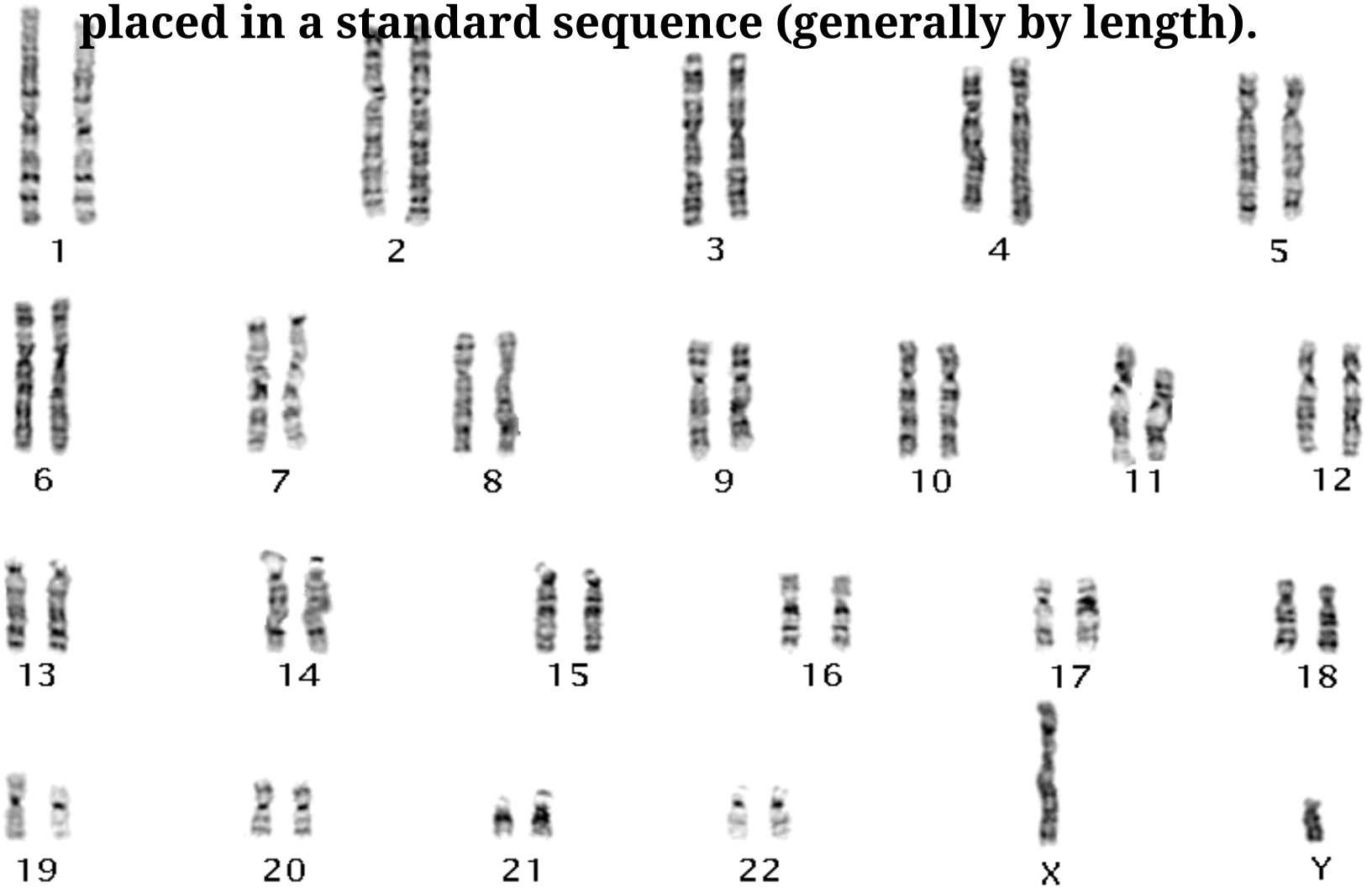
- Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome.

- The normal diploid chromosome number for humans is 46.

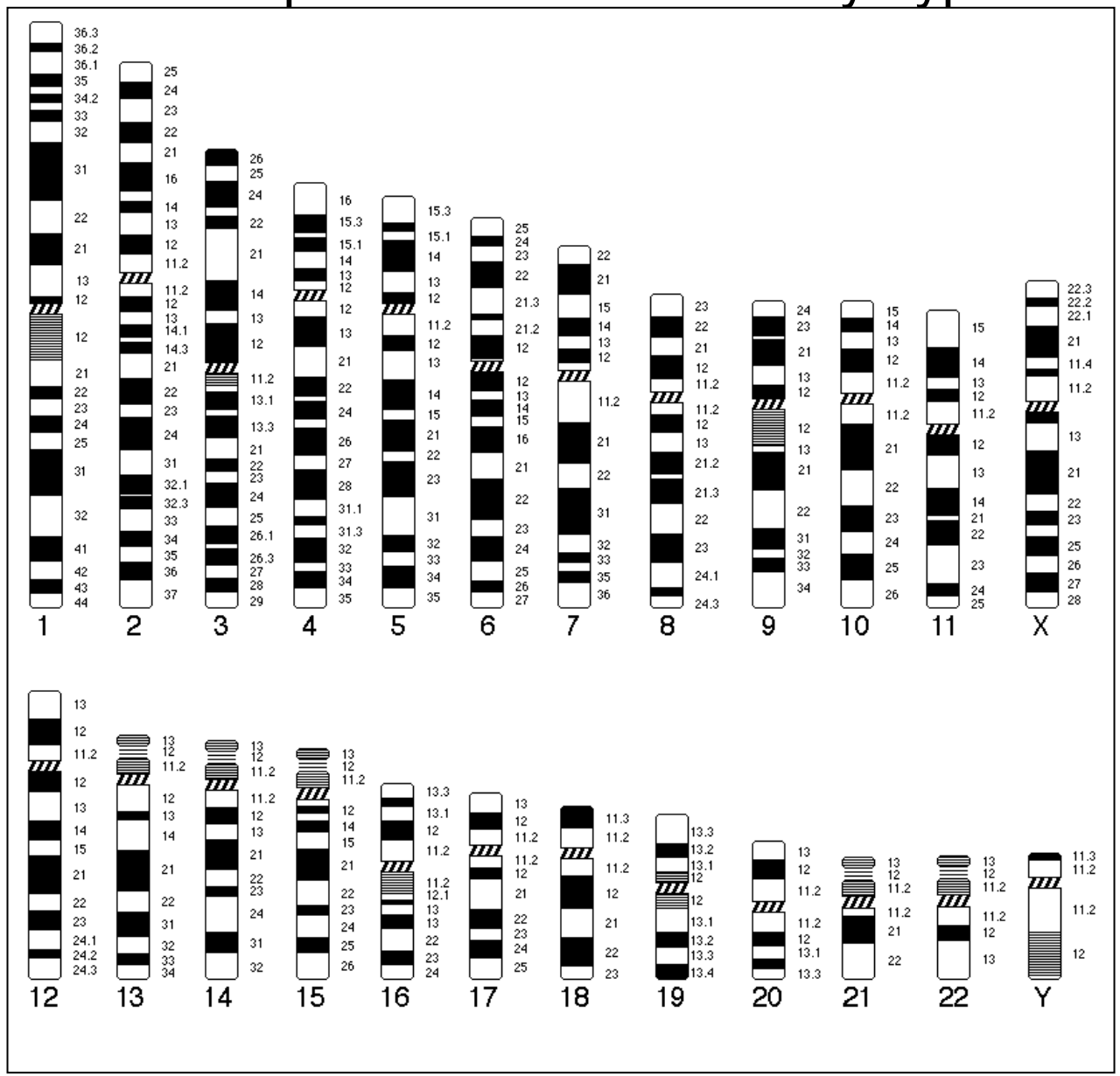
Karyogram is also called Karyotype

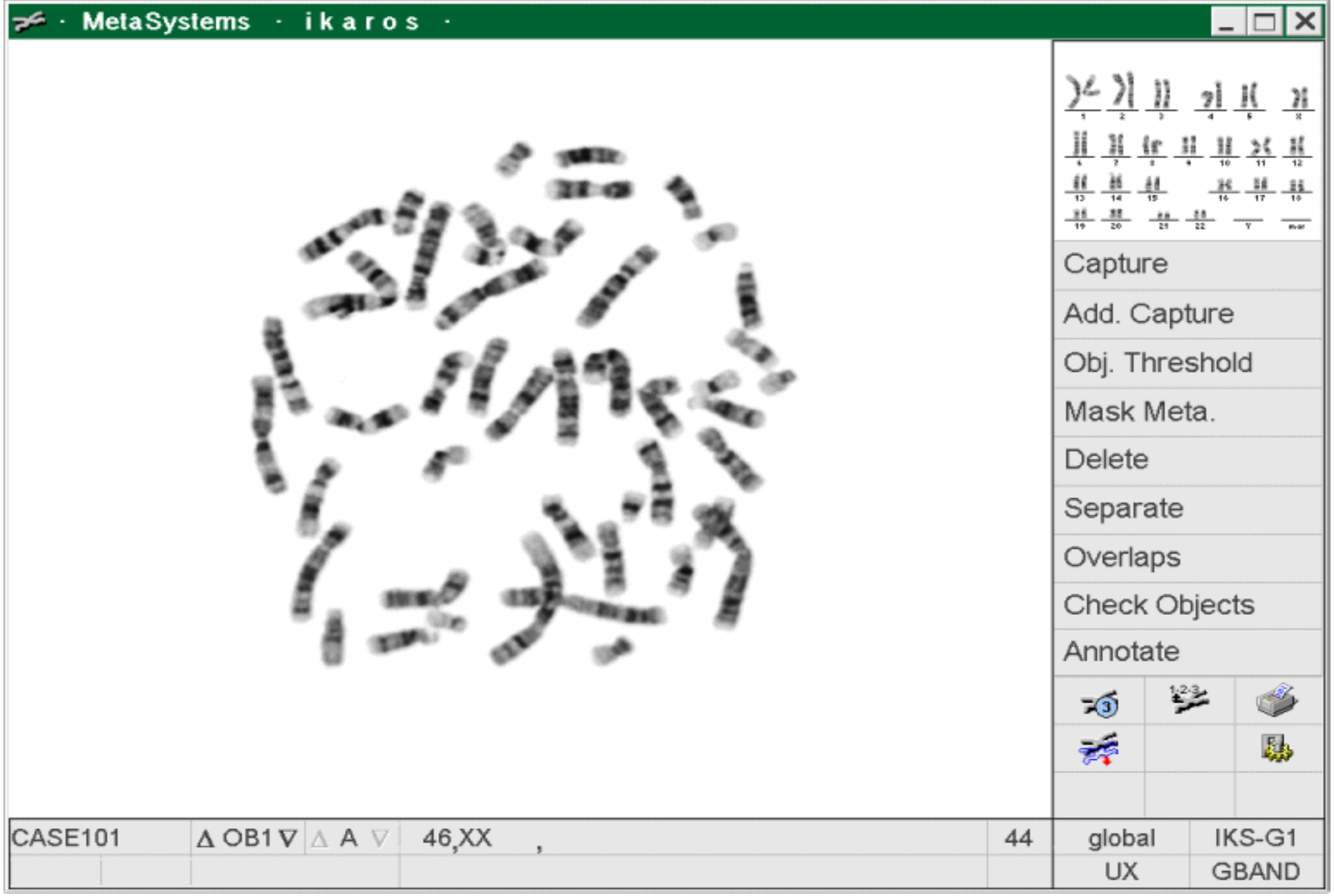
<u>Karyogram</u> – An ordered arrangement of the chromosomes from a cell

placed in a standard sequence (generally by length).



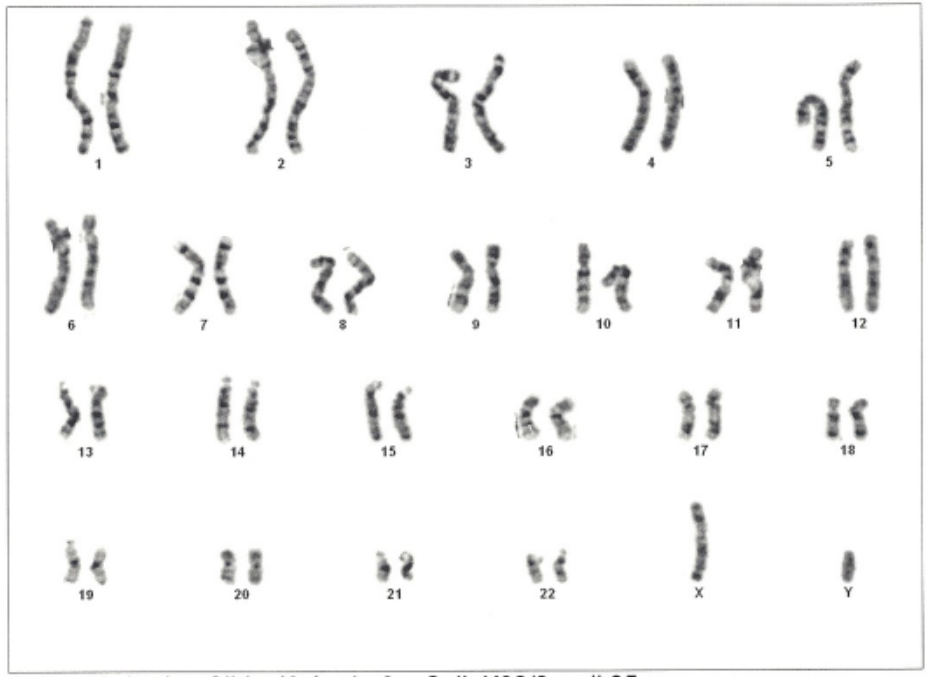
The ideogram of a chromosomal complement is a diagrammatic representation of the karyotype.



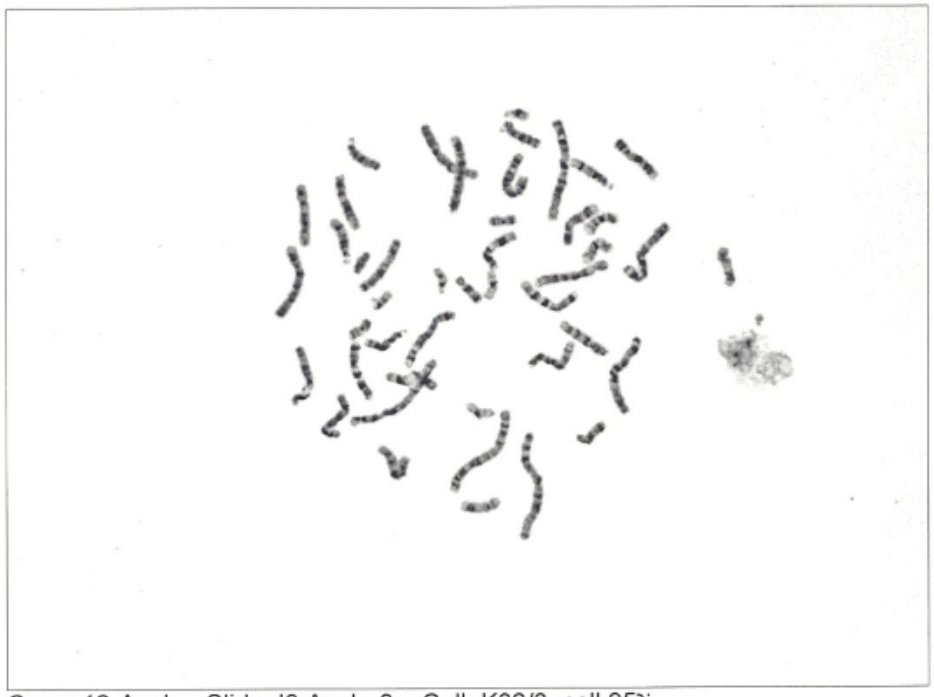

MetaSystems · ikaros ·
Capture
Add. Capture
Obj. Threshold
Mask Meta.
Delete
Separate
Overlaps
Check Objects
Annotate
CASE101
Δ OB1 ∇
Δ A ∇
46,XX ,
44
global
IKS-G1
UX
GBAND

Metaphase

- 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

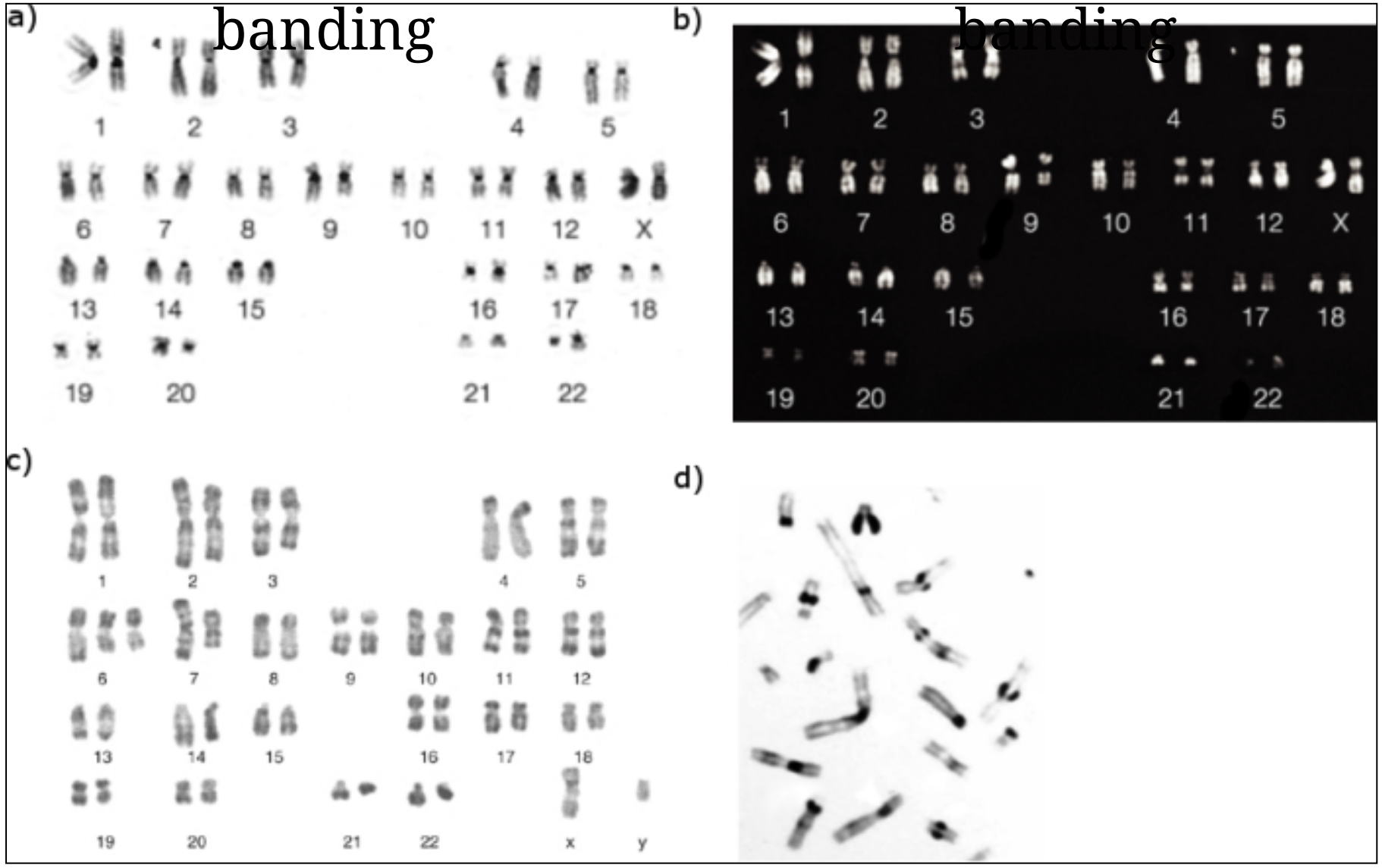

Case: 12-Azab Slide: I3 Azab_3 Cell: K32/3_cell 95

Types of banding

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

G-banding

Q-banding



R-

C-

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.

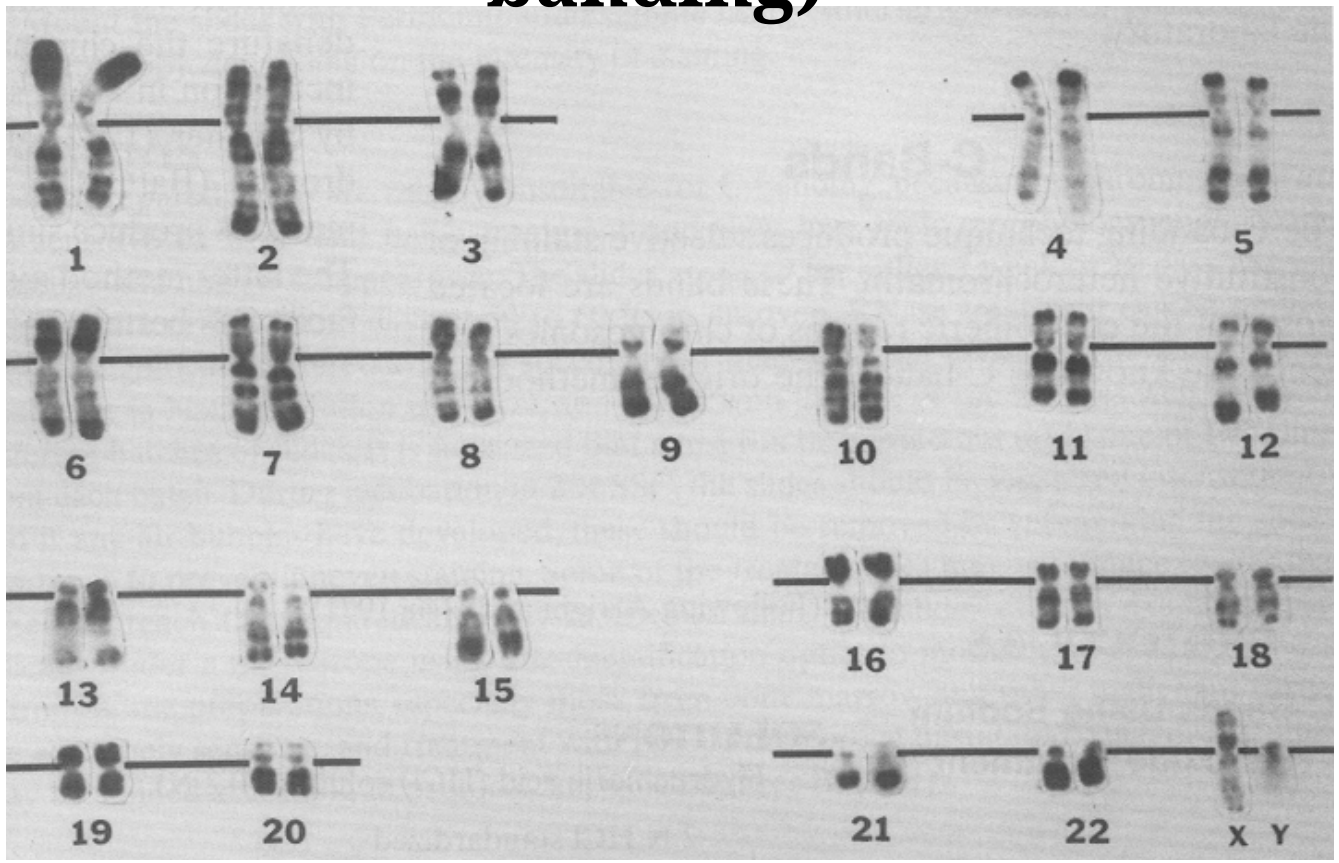
R-banding

- 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.

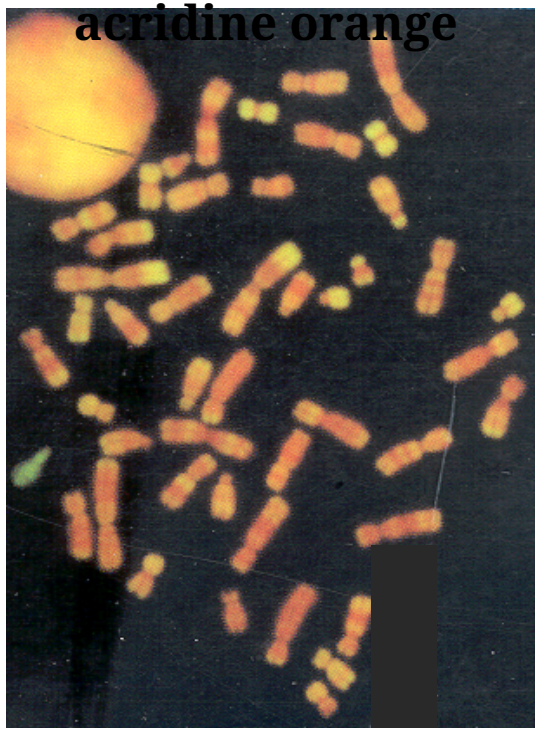
- the chromosomes are heated before Giemsa stain is applied. The heat treatment is thought to preferentially melt the DNA helix in the AT-rich regions that usually bind Giemsa stain most strongly, leaving only the comparatively GC-rich regions to take up the stain. R-banding

Reverse Banding (R-banding)



RHG (R-bands by heating using Giemsa)

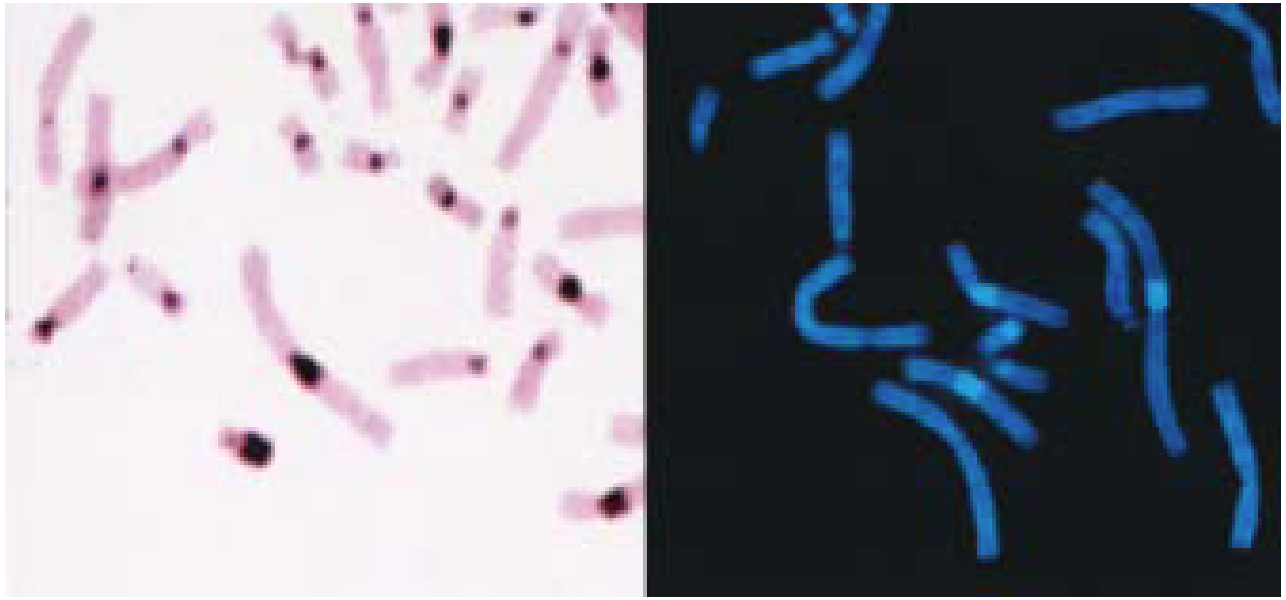
RFA (R-bands by fluorescence using acridine orange



C-banding

- Yet another method is C-banding which can be used to specifically stain constitutive heterochromatin , so it stains centromeres.

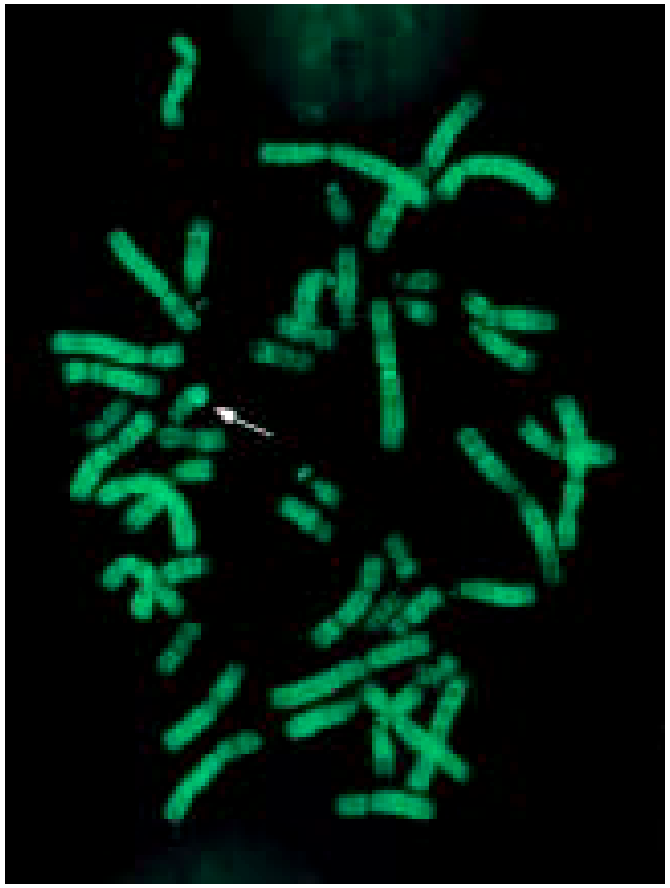
- it is rarely used for diagnostic purposes these days.
- C-banding is a specialized Giemsa technique that primarily stains chromosomes at the centromeres, which have large amounts of AT-rich satellite DNA.

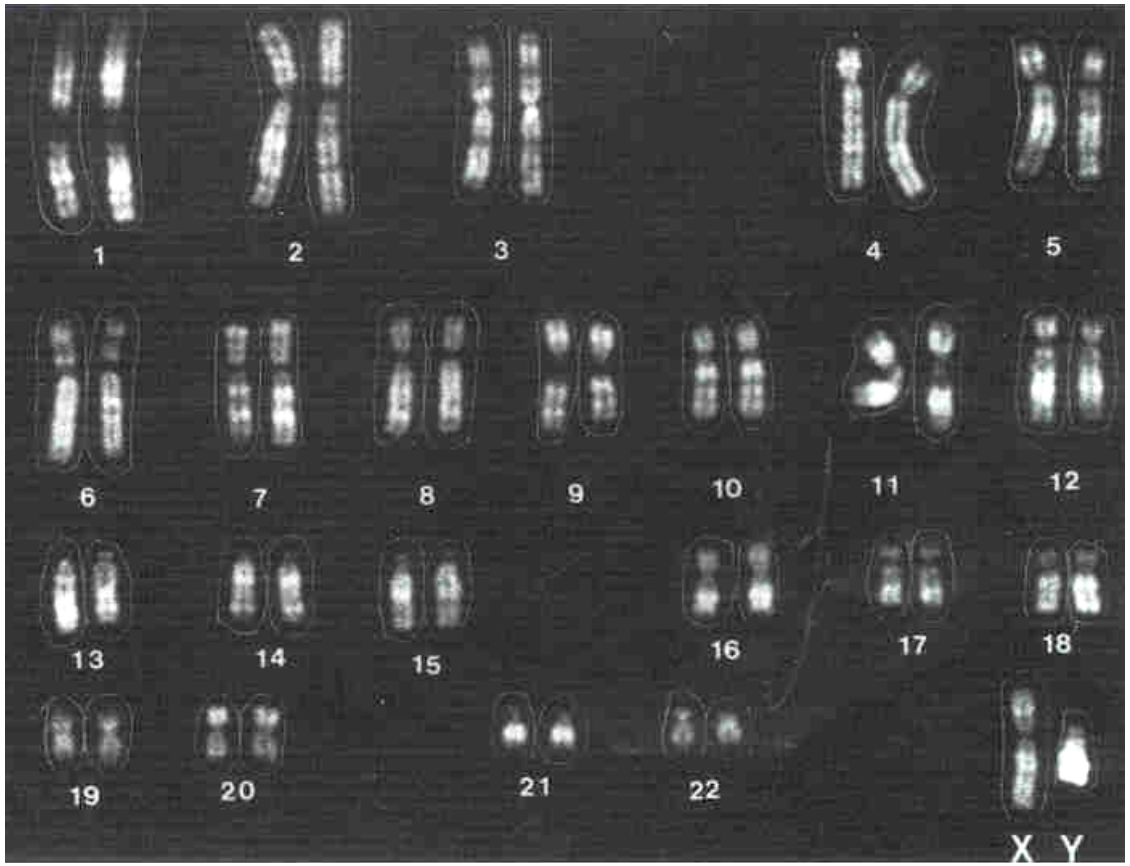


Q-banding

- The first method to be used to identify all 46 human chromosomes was
- Q-banding is a fluorescent pattern obtained using quinacrine for staining and examining them under UV light.
- The pattern of bands is very similar to that seen in G-banding.
- This method is most useful for examining chromosomal translocations, especially ones involving the Y chromosome

Quinacrine Banding (QFQ)

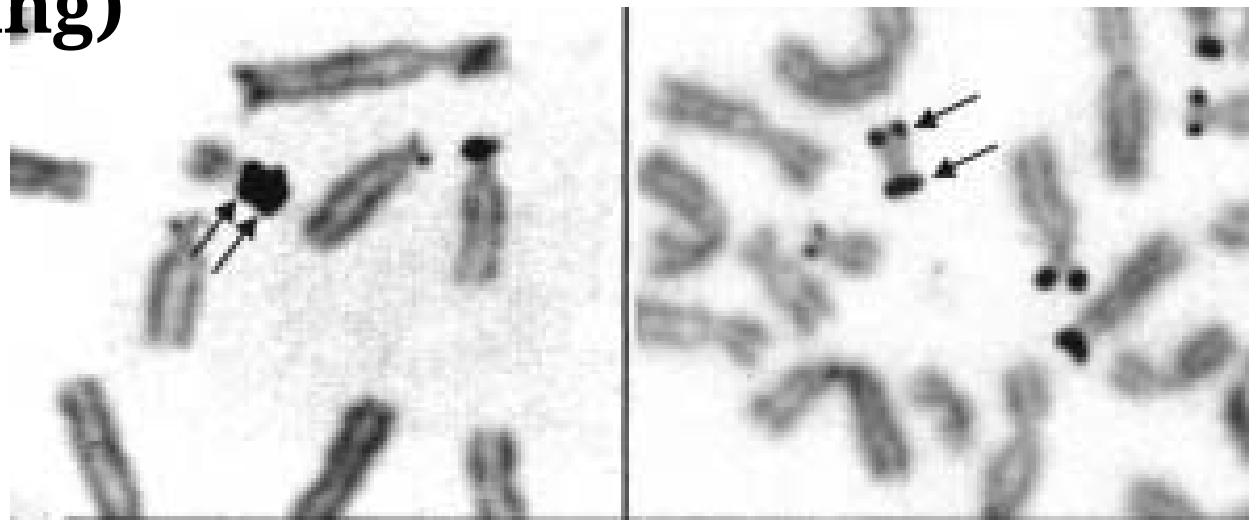




Silver staining

- Silver nitrate stains the nucleolar organization region-associated protein. This yields a dark region where the silver is deposited, denoting the activity of rRNA genes within the NOR.

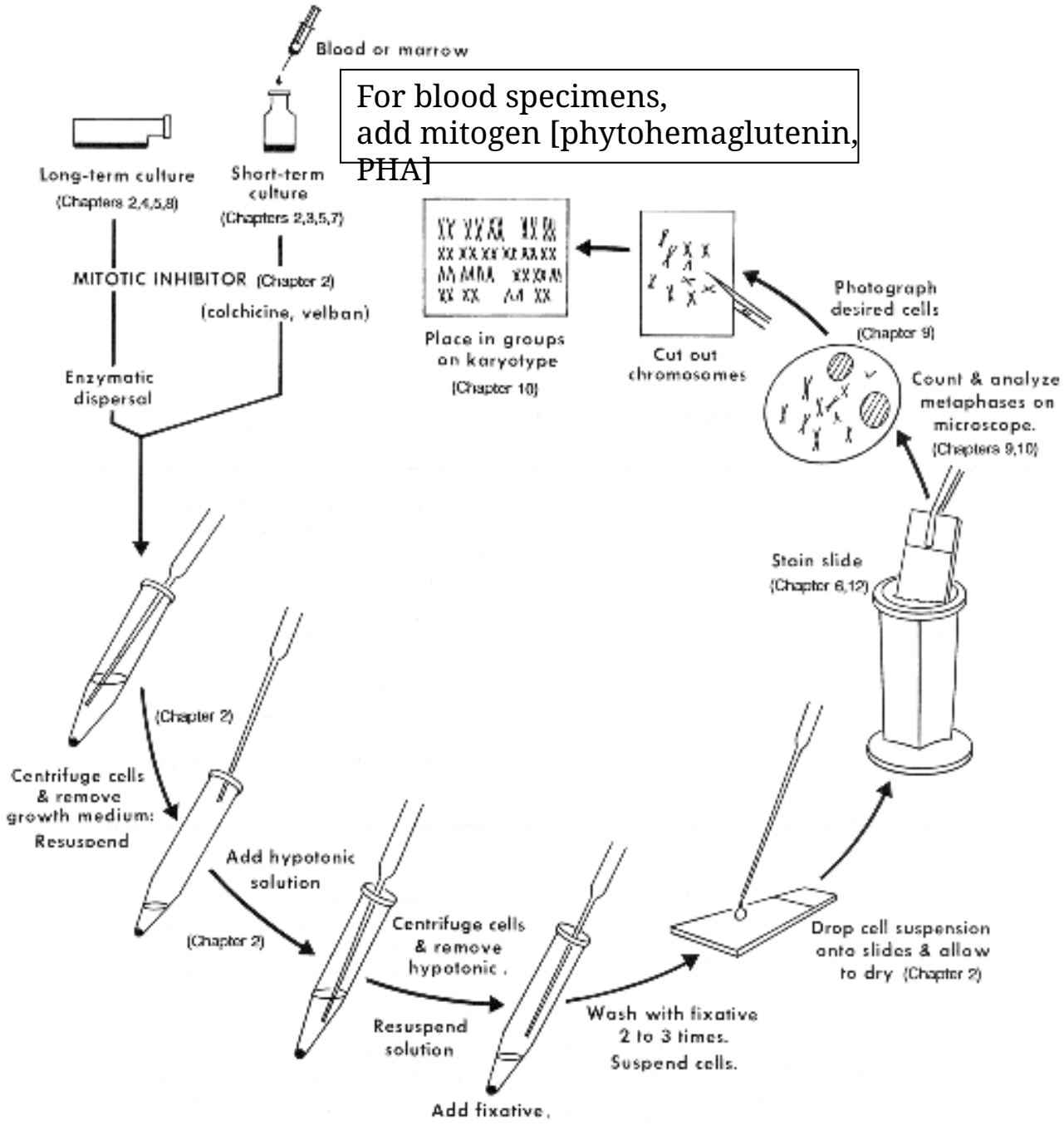
Nucleolar Organizer Regions (NORs – silver staining)



Primary Steps for Culture Establishment

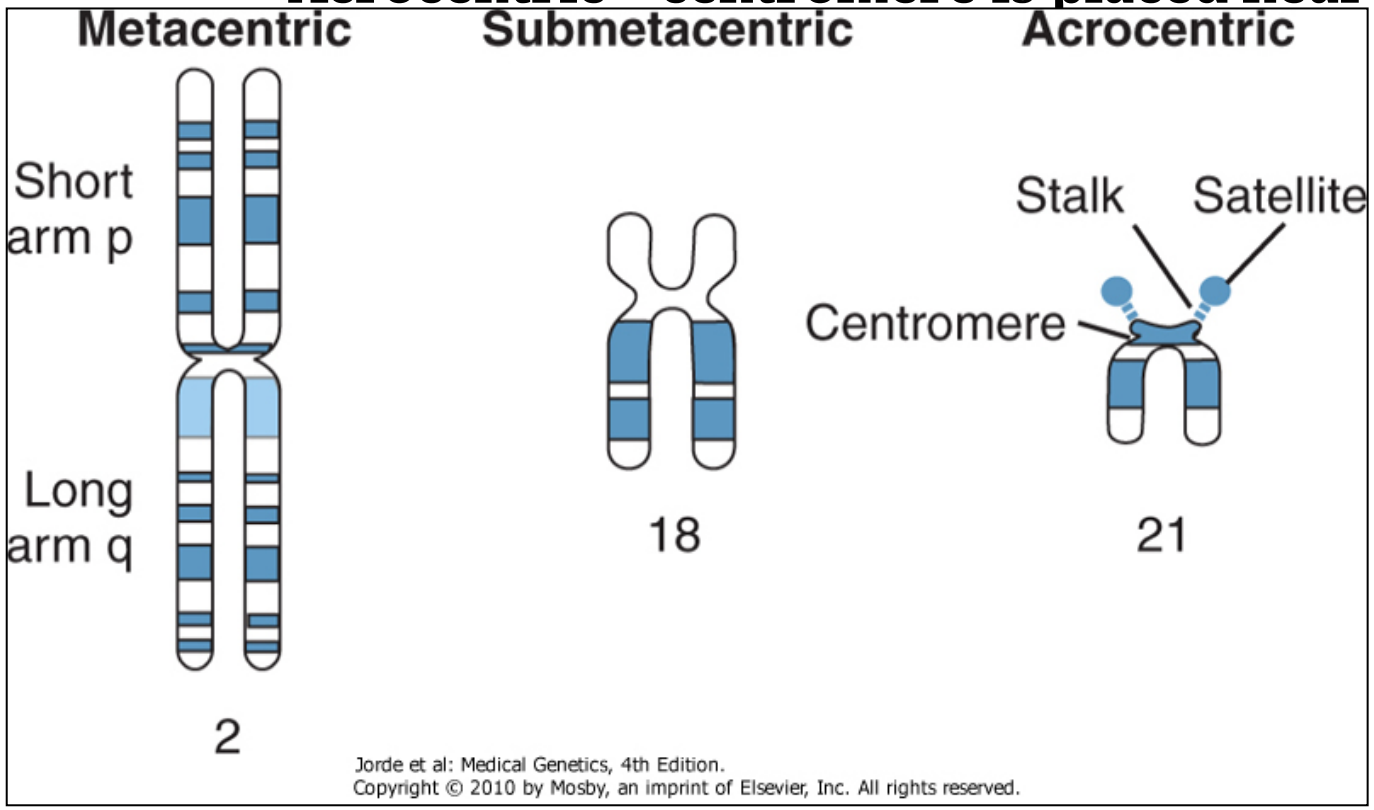
and Harvest of Specimens

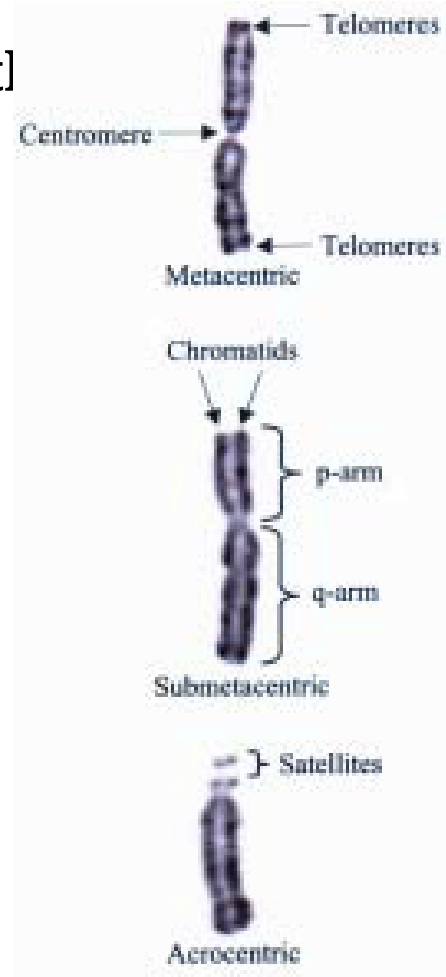
- Add Mitogen (when needed)
- Hypotonic Swelling
- Fixation
- Analysis



Chromosome Shape

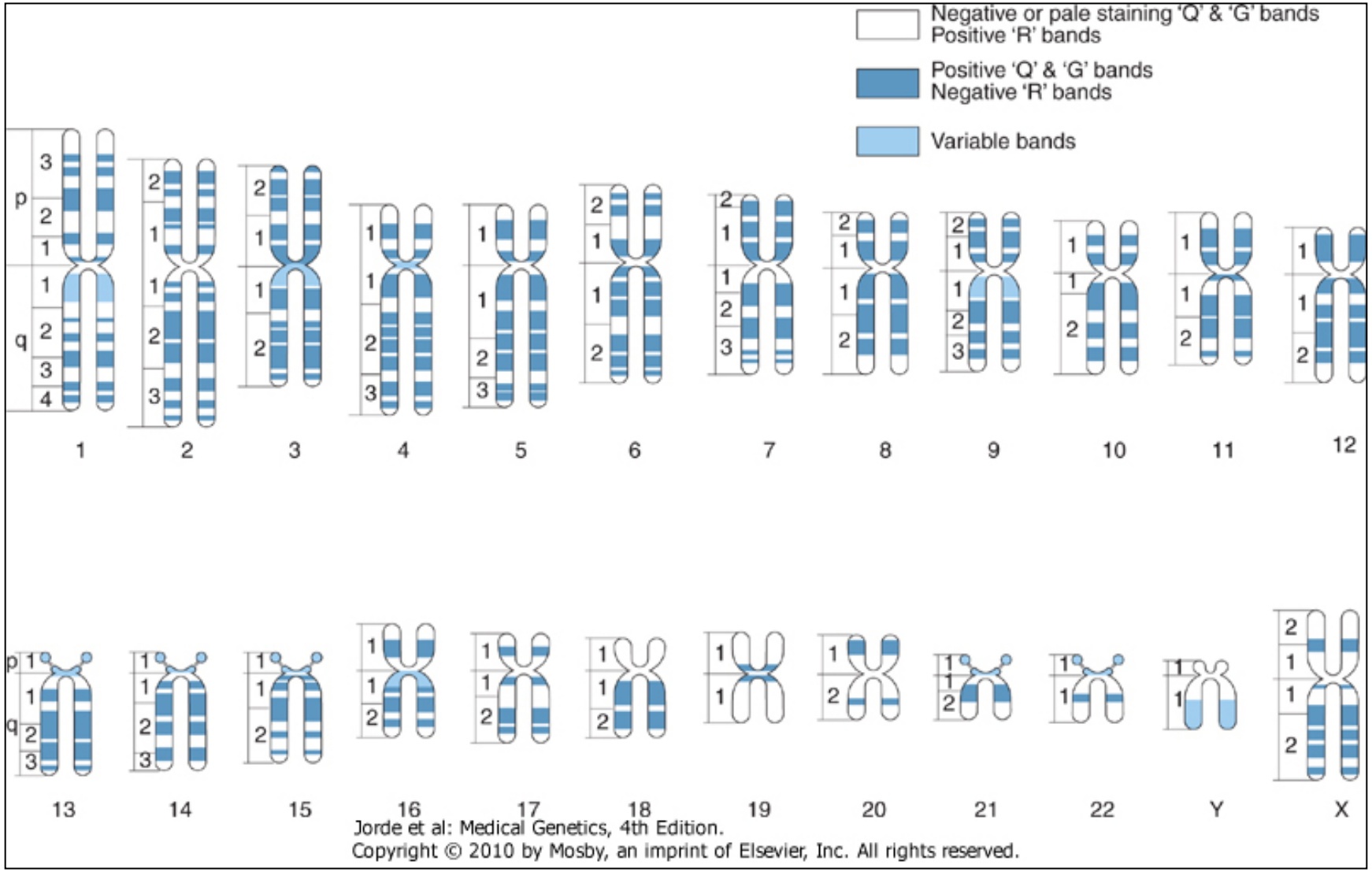
Metacentric- centromere is located in the middle of chromosome
Submetacentric- centromere is displaced from the center
Acrocentric – centromere is placed near t





Human Chromosome Ideogram

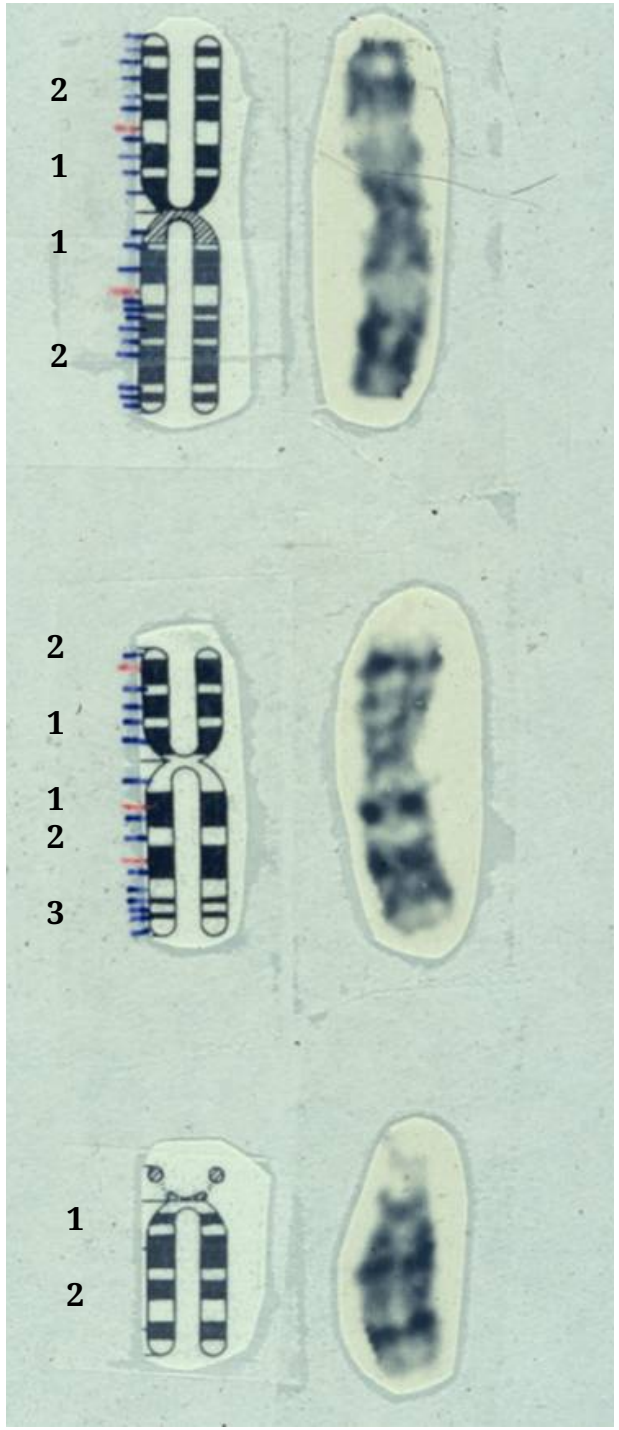
Ideogram - A diagrammatic representation of a karyotype

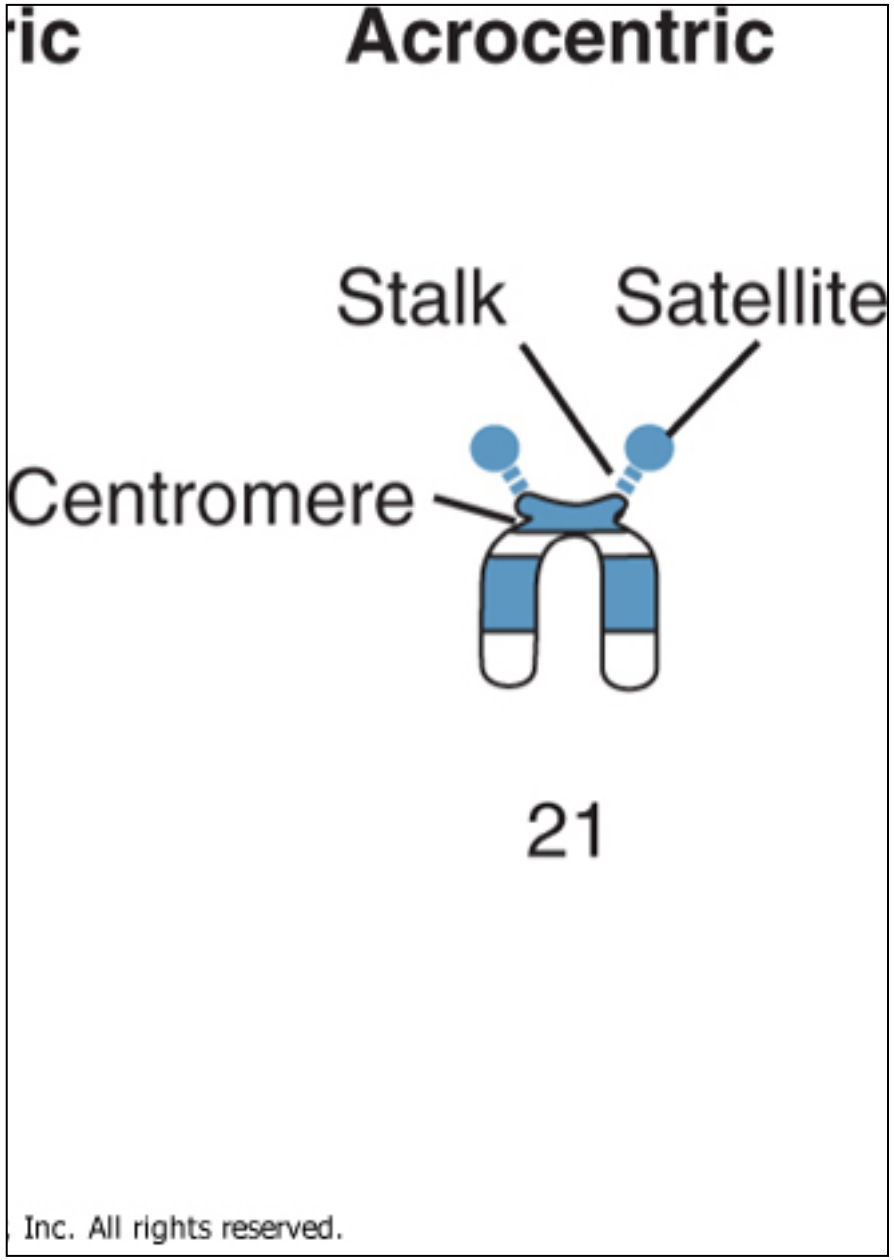


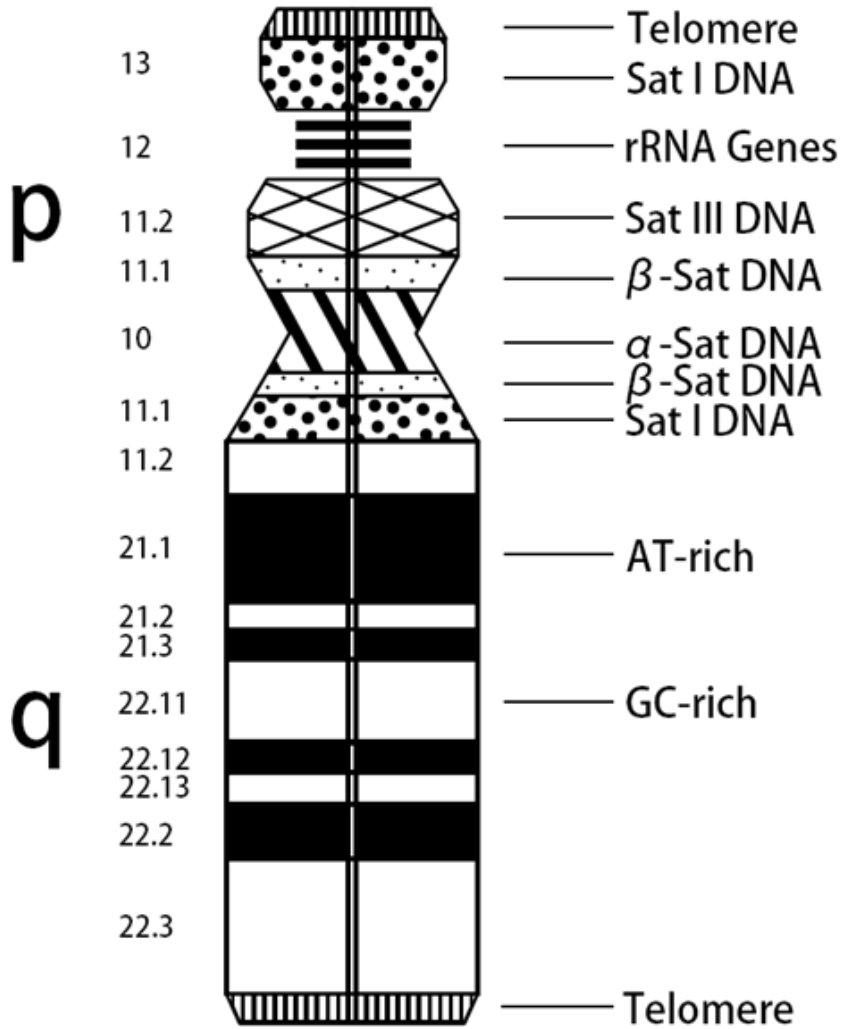
Chromosom e 3
p: 2 regions
q: 2 regions

Chromosom e 7
p: 2 regions
q: 3 regions

Chromosome 14
p: 1 region
q: 2 regions

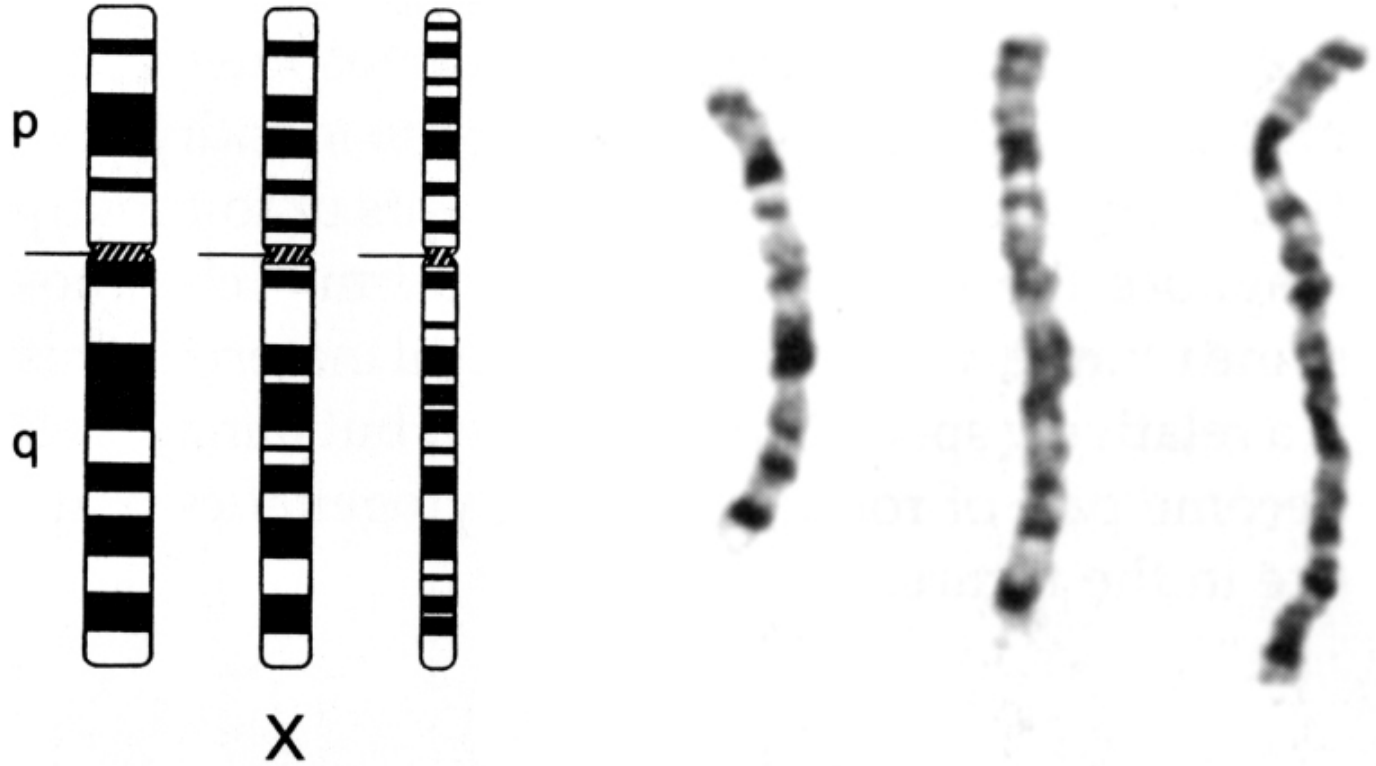



ic
Acrocentric
Stalk
Satellite
Centromere
21
Inc. All rights reserved.


p
q
13
12
11.2
11.1
10
11.1
11.2
21.1
21.2
21.3
22.11
22.12
22.13
22.2
22.3
Telomere
Sat I DNA
rRNA Genes
Sat III DNA
β-Sat DNA
α-Sat DNA
β-Sat DNA
Sat I DNA
AT-rich
GC-rich
Telomere

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.



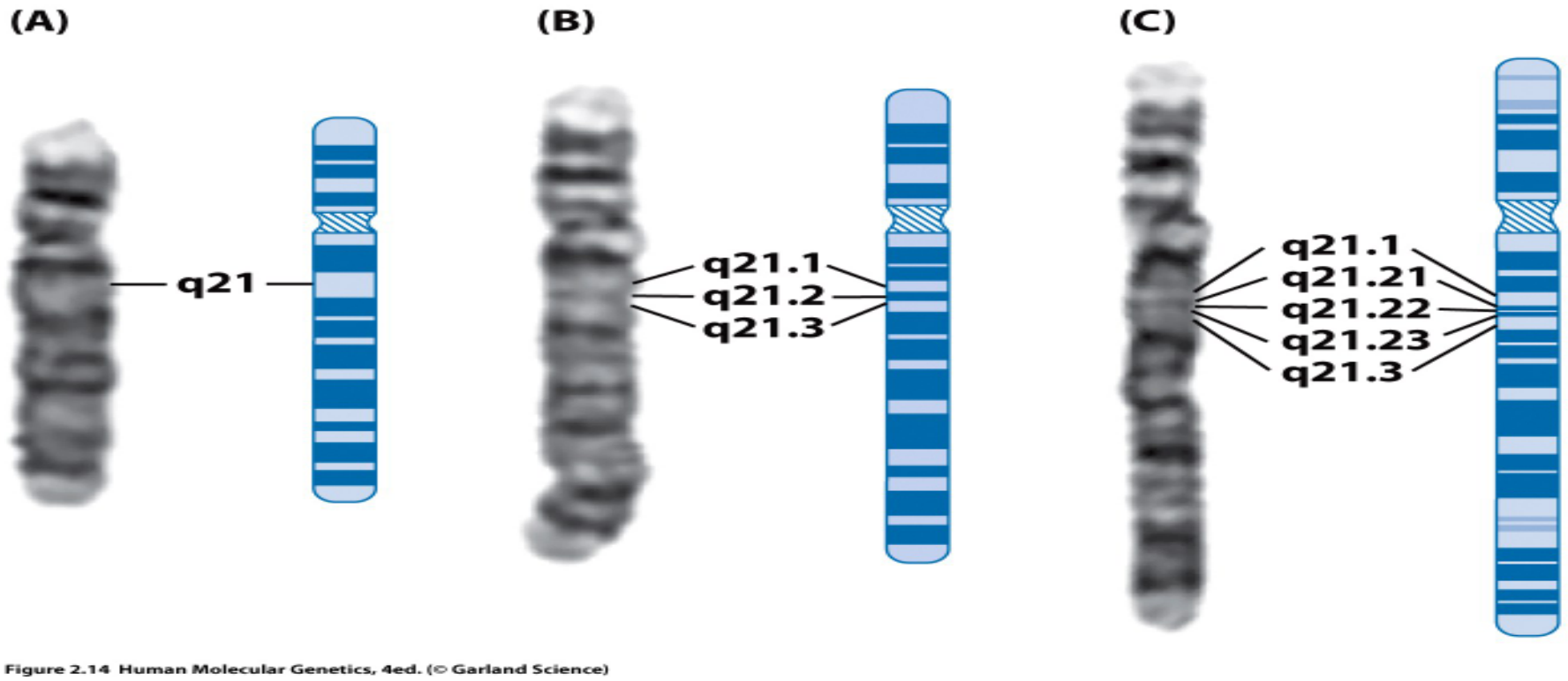


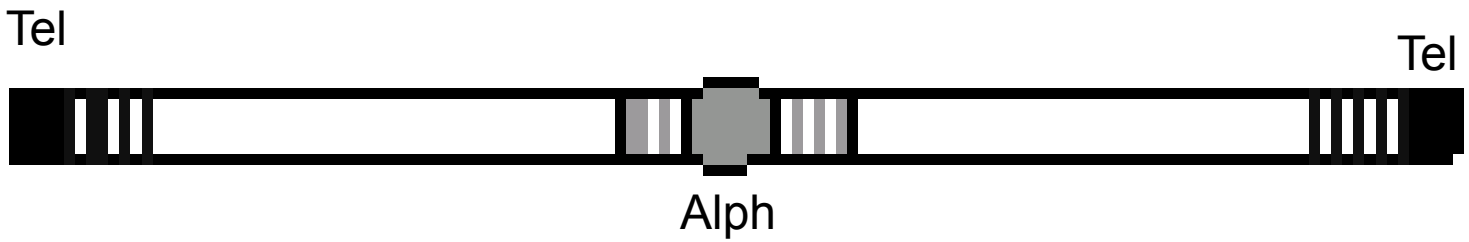
Figure 2.14 Different chromosome banding resolutions can resolve bands, sub-bands, and sub-sub-bands.

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*

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

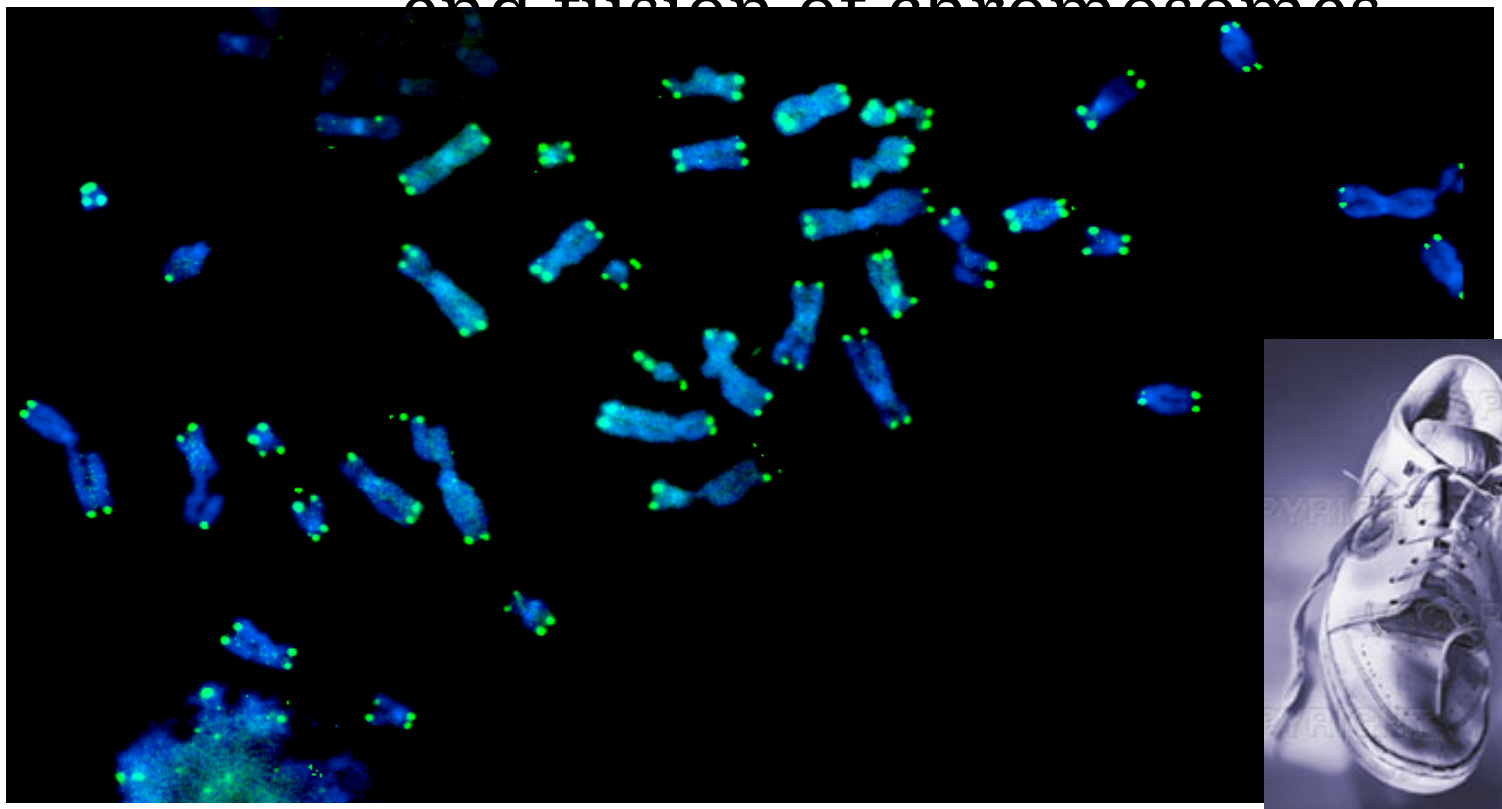
Centromere

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

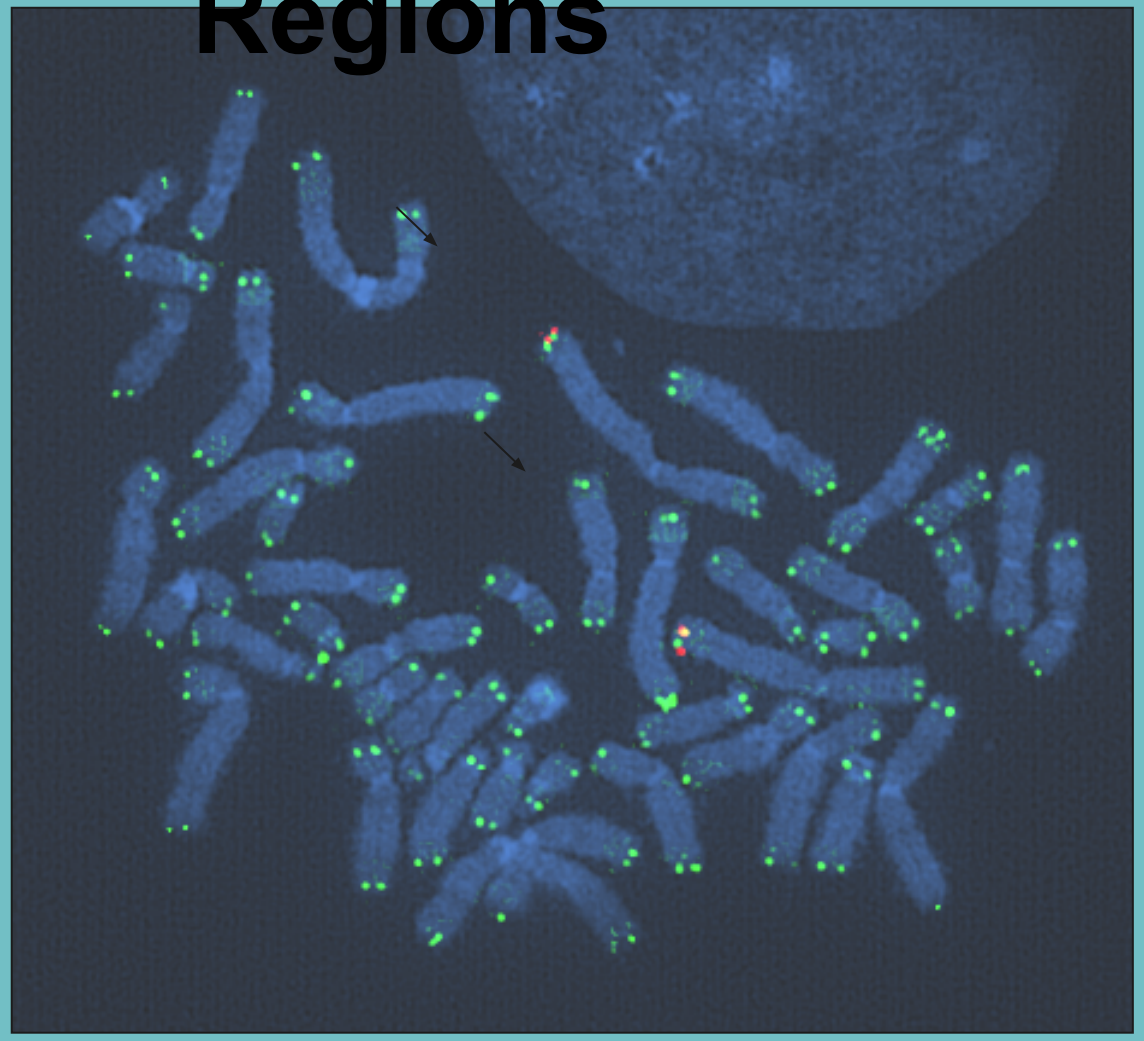
Telomere

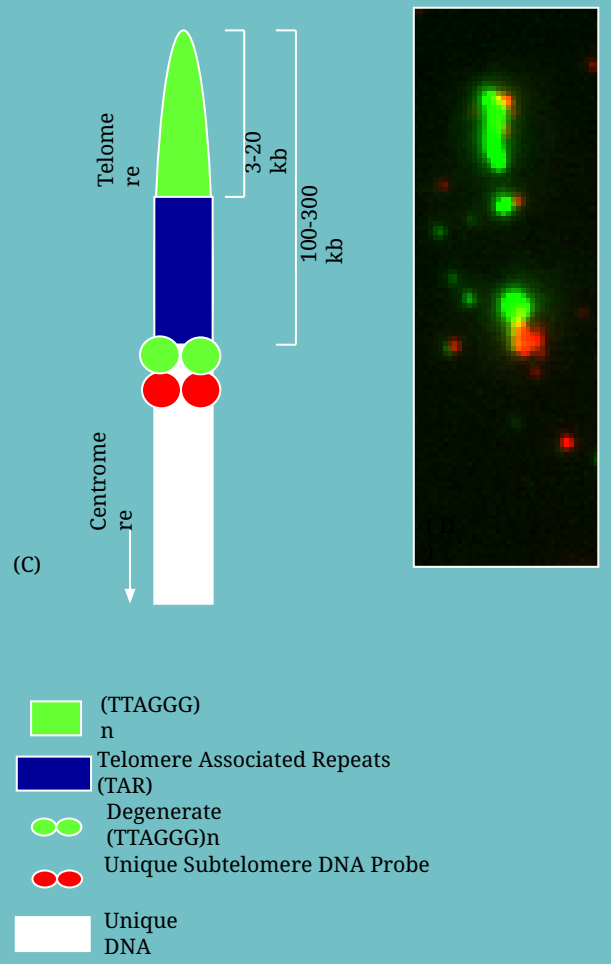
(TTAGGG)n

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



Human Sub-telomeric Regions





There is some sequence homology between subtelomeres