Medical Genetics Course

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

In humans, approximately 0.6-1% of all live born infants 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+%

Cytogenetics: The study of chromosomes and genomic structure, function, and variation and their role in human disease and heredity

Tissues for Chromosome Studies

- Peripheral blood (lymphocytes)
- Bone marrow
- Chorionic villi biopsy
- Amniotic fluid cells
- Fetal tissue for products of conception
- Skin or organ biopsy
- Tumor biopsies

The main referral reasons for cytogenetic analyses are:

1- Prenatal – Abnormal maternal serum screening (first or second trimester)

- Abnormal cell-free DNA testing (cfDNA), non-invasive prenatal testing (NIPT)
- Abnormal ultrasound findings: cystic hygromas/hydrops, cardiac defects, other malformations, IUGR, etc.
- Advanced maternal age (AMA), generally ≥ 35 yrs
- Parental or familial chromosome/genomic abnormality
- Fetal or neonatal demise (products of conception, POC)

Type of samples: ?

The main referral reasons for cytogenetic analyses are:

2- Postnatal, childhood growth and development

- Perinatal/newborn: Birth defects, malformations, etc
- Growth: failure to thrive, etc
- Developmental delay (fine and gross motor, speech)
- Cognitive
- Neurological
- Behavioral

Type of samples: ?

The main referral reasons for cytogenetic analyses are:

2- Adolescent, adult sexual development and fertility

- Amenorrhea, primary or secondary ovarian failure, premature menopause
- Azoospermia, oligospermia, hypogonadism
- History of infertility or spontaneous abortions or having a child with chromosomal abnormality

Type of samples: ?

The main referral reasons for cytogenetic analyses are:

4- Cancer

- Hematologic oncology
  - Myeloid diseases: AML, CML, MDS, MPNs
  - Lymphoid diseases: ALL, CLL, NHL, PCNs/MM
- Bone marrow transplant
- Other areas of oncology (solid tumors)

Type of samples: ?

- 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).

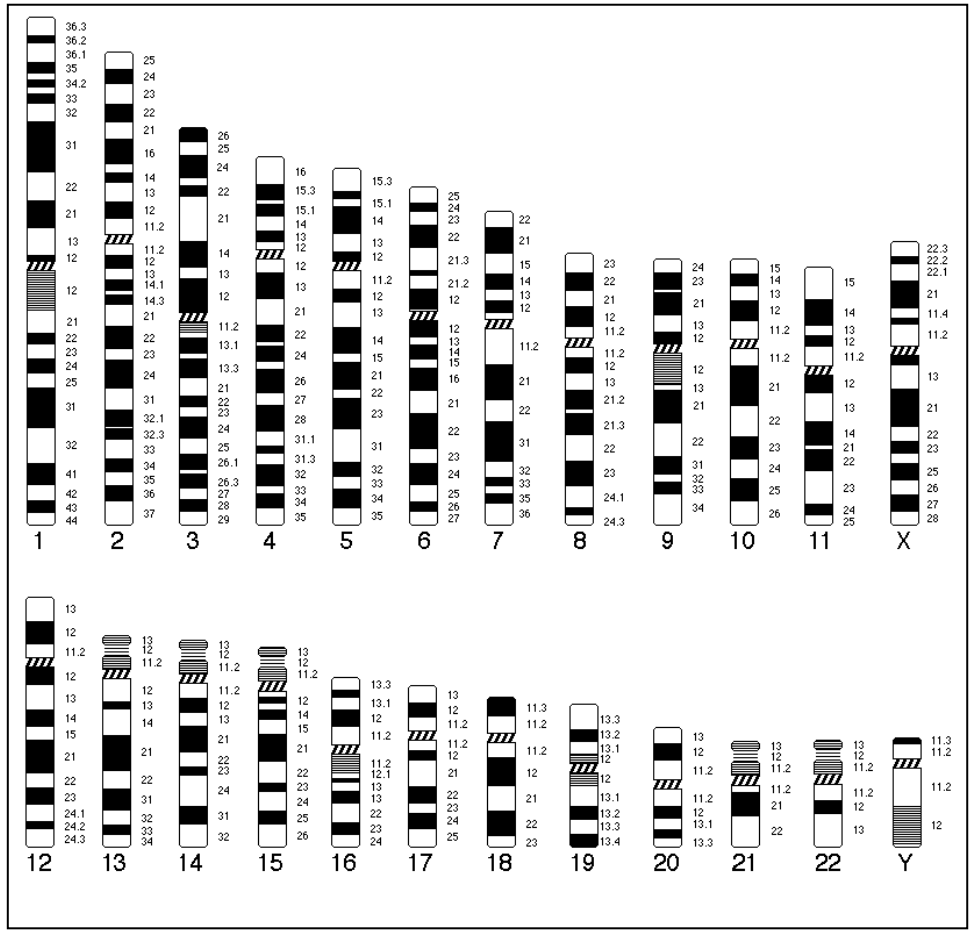
- Once a computer image of the chromosomes from a dividing cell is obtained, the chromosomes are arranged as homologous pairs.

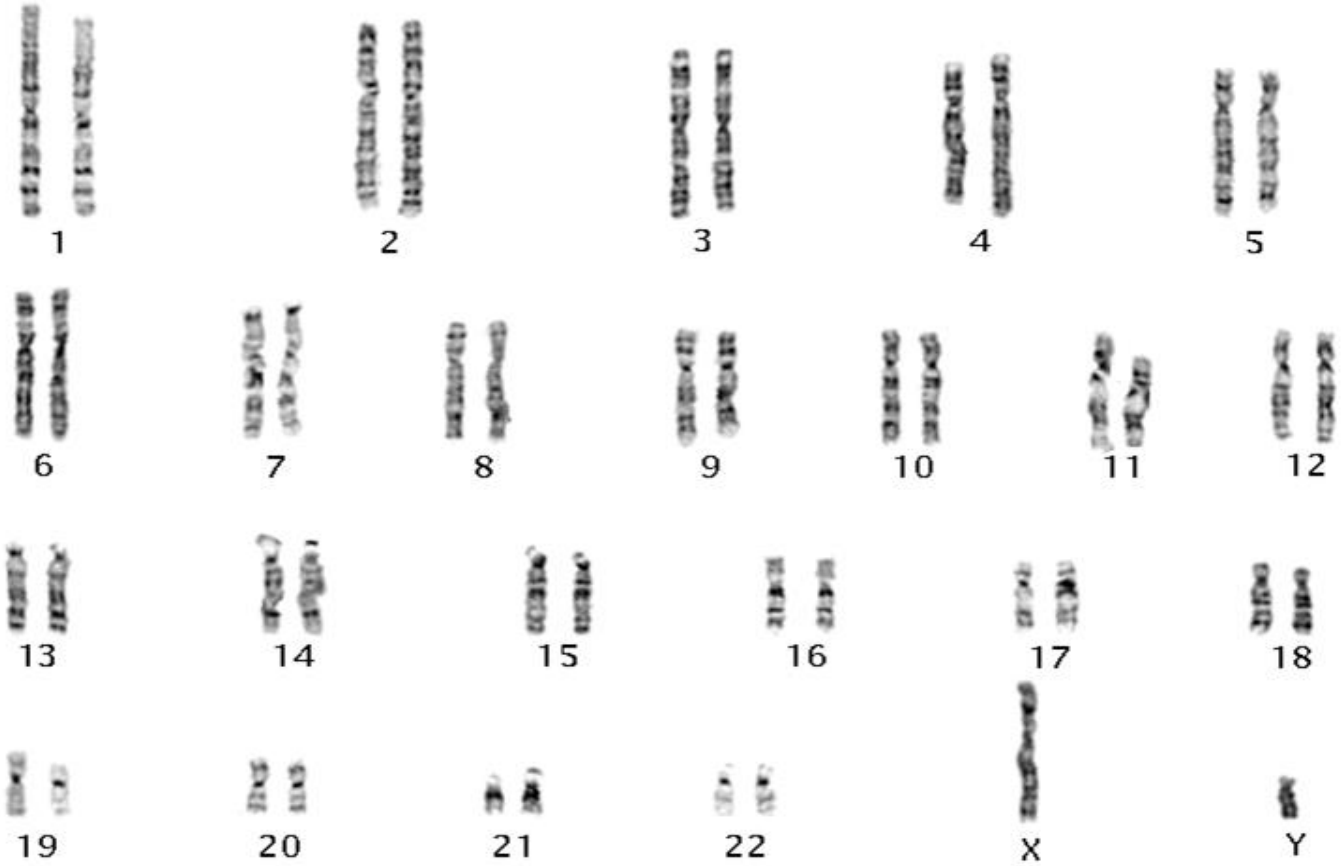
- Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome.

- The normal diploid chromosome number for humans is 46.

Karyogram is also called Karyotype

<u>Karyogram</u>: is a display of 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.

Centromere position

The ratio of lengths of the two arms allows classification of chromosomes into several basic morphologic types:

- **Metacentric:** p and q arms are exactly the same length; have the centromere near the middle
- **Submetacentric:** p arm just a little smaller than q arm; the centromere displaced toward one end
- **Acrocentric:** very small p arm; have the centromere far toward one end
- **Telocentric:** centromere is on end (not in human)

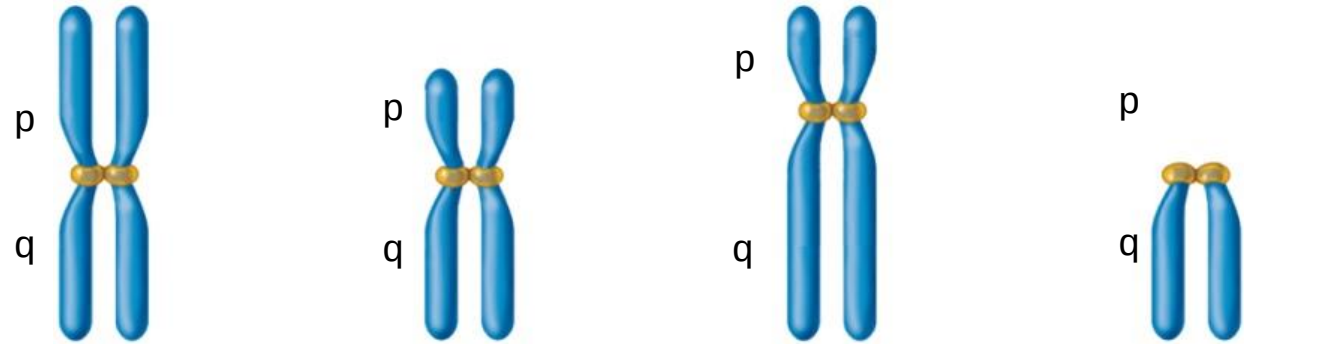


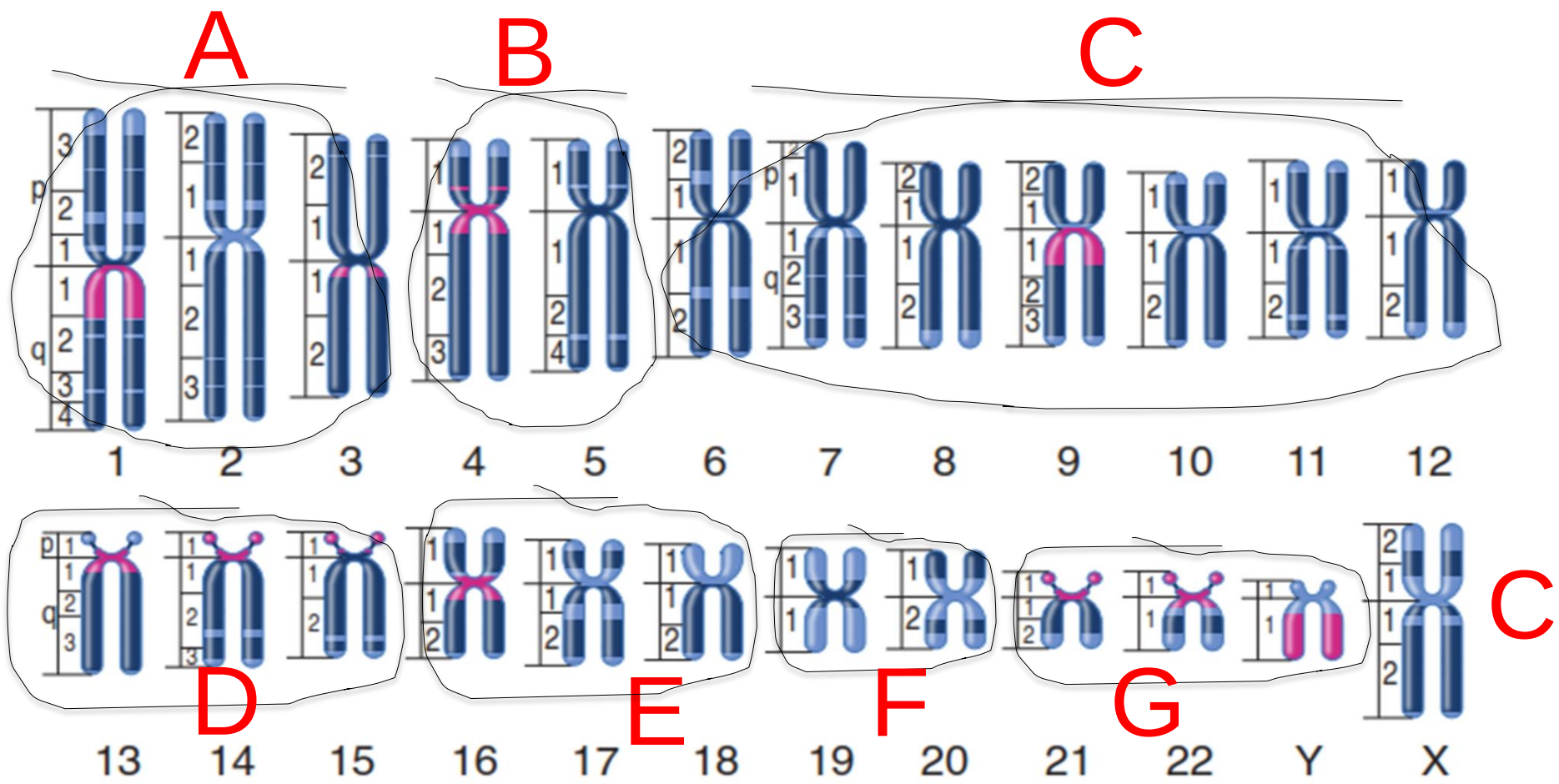
Figure 8.1

The classification of chromosomes

HUMAN KARYOTYPE

- 7 groups - depending on the lenght and morphology
- 22 pairs of homologous chromosomes - autosomes
- 1 pair of sex chromosomes

Grouping	Number Of Chromosome	Description chromosome
Group A	Chromosome 1-3	Metacentric chromosomes are large and easily distinguished from the others because of its size and location of the centromere
Group B	Chromosome 4-5	has two large-sized chromosome sub metacentric
Group C	Chromosome 6-12, X	Metacentric chromosomes and medium-sized sub metacentric
Group D	Chromosome 13-15	acrocentric chromosomes of medium size and has satellite
Group E	Chromosome 16-18	Metacentric chromosomes and small-sized sub metacentric
Group F	Chromosome 19-20	Very small mmetacentric chromosome
Group G	Chromosome 21-22, Y	Acrocentric chromosomes are very small and have satellites except for Y chromosome


A
B
C
1
2
3
4
5
6
7
8
9
10
11
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D
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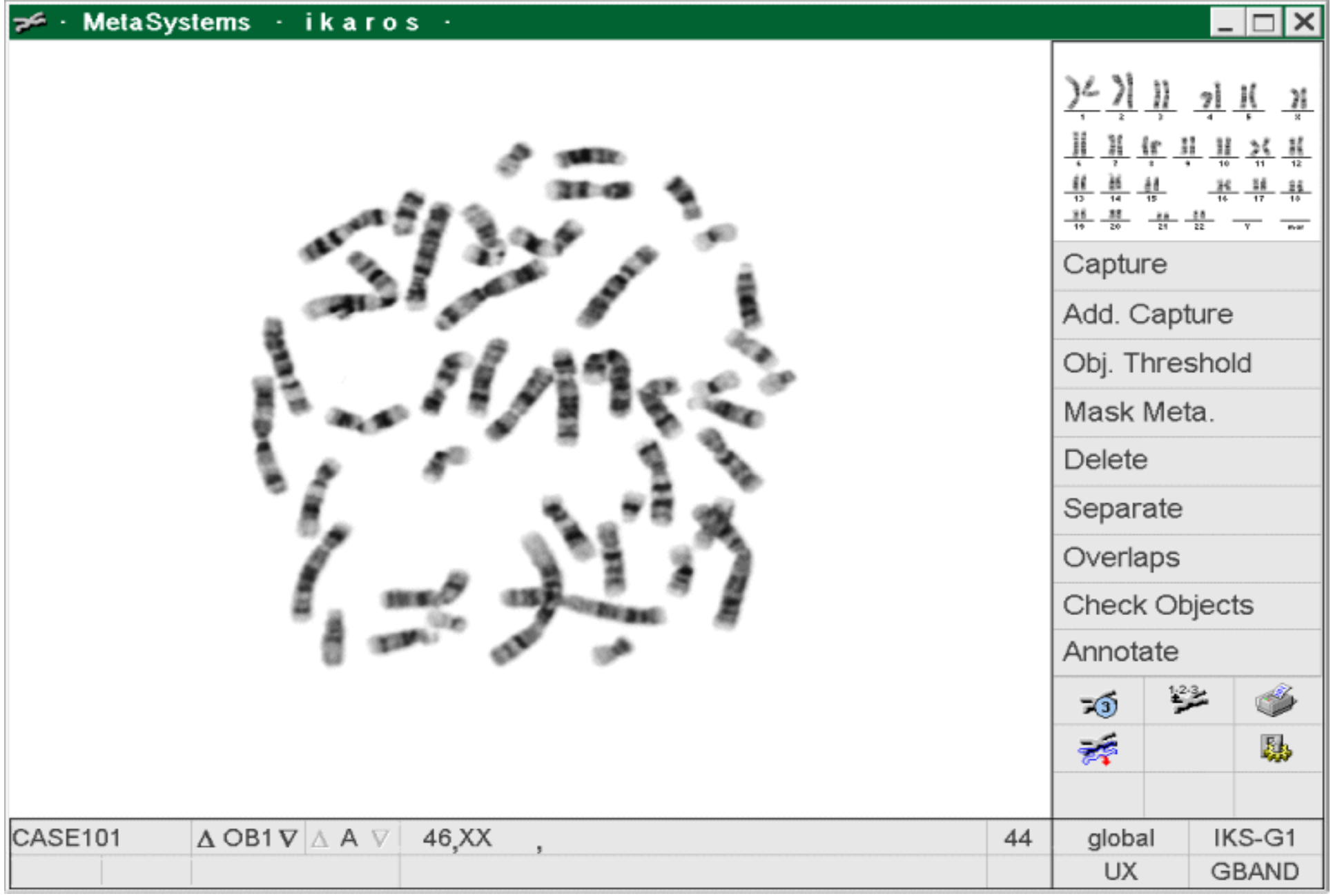
Y
X



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.

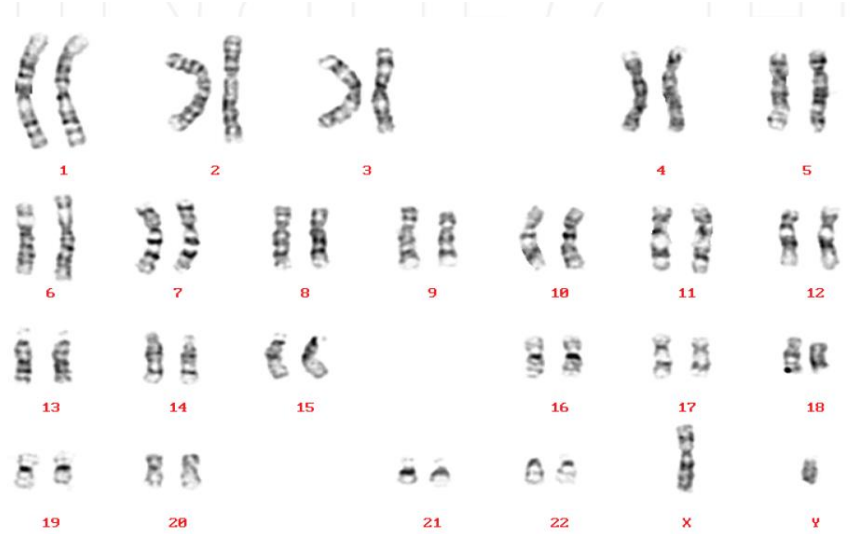


Fig. 1. A karyotype of a normal male (46,XY)

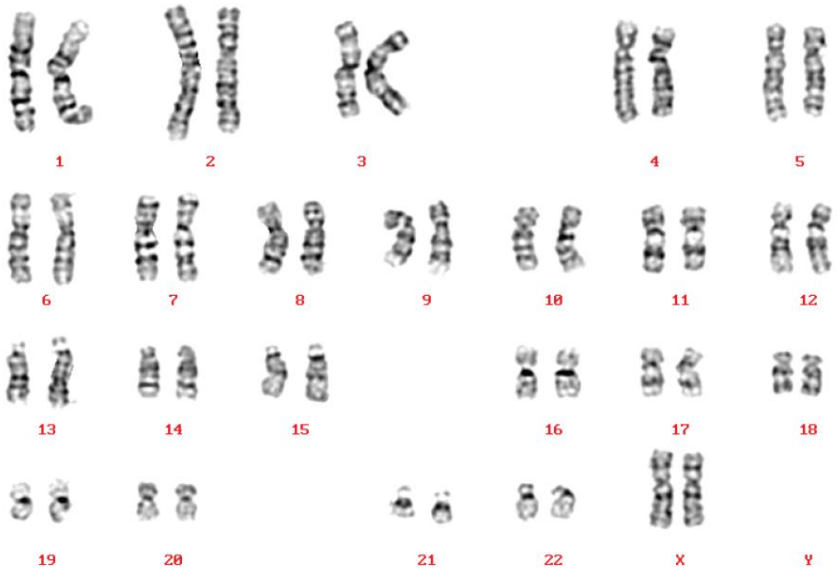
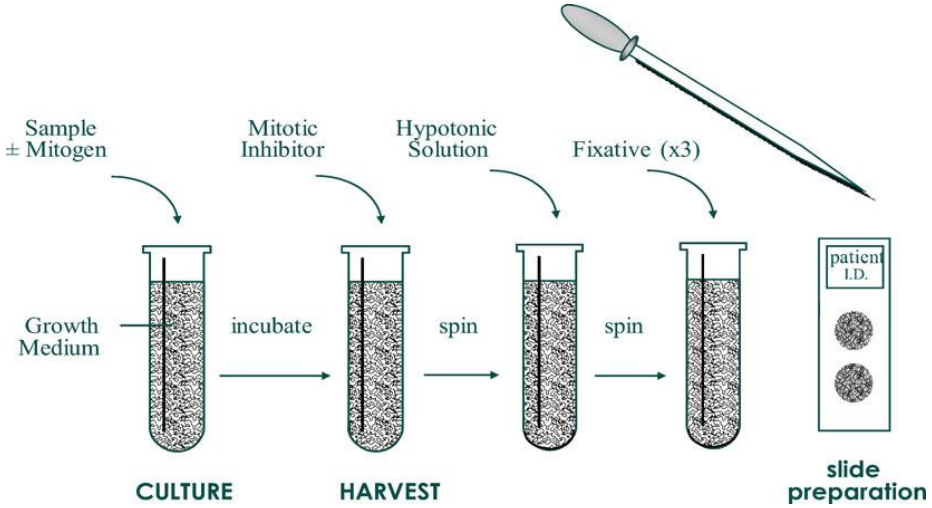
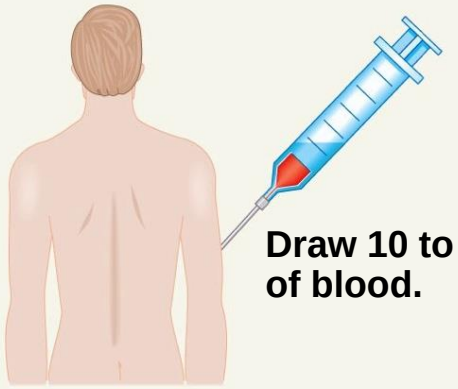


Fig. 2. A karyotype of a normal female (46,XX)

Making a Karyotype

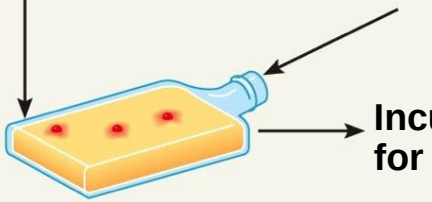




Draw 10 to 20 ml of blood.

Add a few drops of blood.

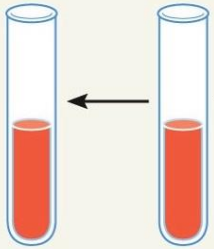
Add phytohemagglutinin (PAH).



Incubate at 37° C for 2 to 3 days.

Add Colcemid to culture for 1 to 2 hours to stop mitosis in metaphase.

Transfer cells to tube.

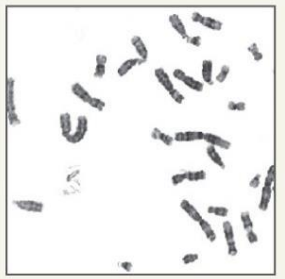


Transfer to tube containing fixative.

Centrifuge to concentrate cells. Add low-salt solution to eliminate red blood cells and swell lymphocytes.

Drop cells onto microscope slide.

Examine with microscope.



Digitized chromosome images processed to make karyotype.

Stain slide with Giemsa.

Courtesy of Ifti Ahmed

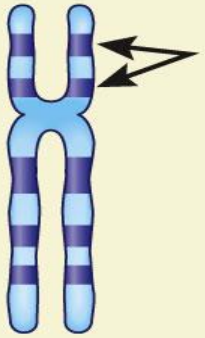
Chromosome Staining and Banding

A large number of banding and staining techniques have been developed.

These can be divided into two broad categories:

1- those that produce specific alternating bands along the length of each entire chromosome

2- and those that stain only a specific region of some or all chromosomes

Banding technique	Appearance of chromosomes
G-banding — Treat metaphase spreads with trypsin, an enzyme that digests part of chromosomal protein. Stain with Giemsa stain. Observe banding pattern with light microscope.	 **Darkly stained G bands.**

It yields a series of lightly and darkly stained bands - the dark regions tend to be heterochromatic regions, which tend to be AT-rich DNA and stain more darkly
The light regions tend to be euchromatic, GC rich.
The **G-light** bands are biologically more significant because they represent the active regions of the chromosomes, while the **G-dark** bands contain relatively few active genes. **G-banding techniques (G-light/ G-dark)**

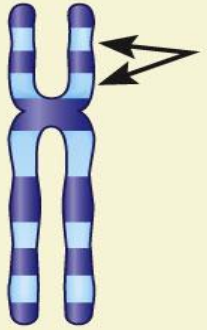
- A common misconception is that bands represent single genes, but in fact the thinnest bands contain over a million base pairs and potentially hundreds of genes. For example, the size of one small band is about equal to the entire genetic information for one bacterium.
- Standard G-band staining techniques allow between 400 and 600 bands to be seen on metaphase chromosomes.

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.

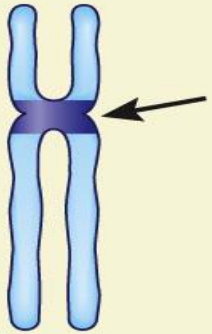
- the chromosomes are heated before Giemsa stain is applied. The heat treatment is thought to preferentially melt the DNA helix in the AT-rich regions that usually bind Giemsa stain most strongly, leaving only the comparatively GC-rich regions to take up the stain. R-banding

Banding technique	Appearance of chromosomes
Q-banding — Treat metaphase spreads with the chemical quinacrine mustard. Observe fluorescent banding pattern with a special ultraviolet light microscope.	 **Bright fluorescent bands upon exposure to ultraviolet light; same as darkly stained G bands.**

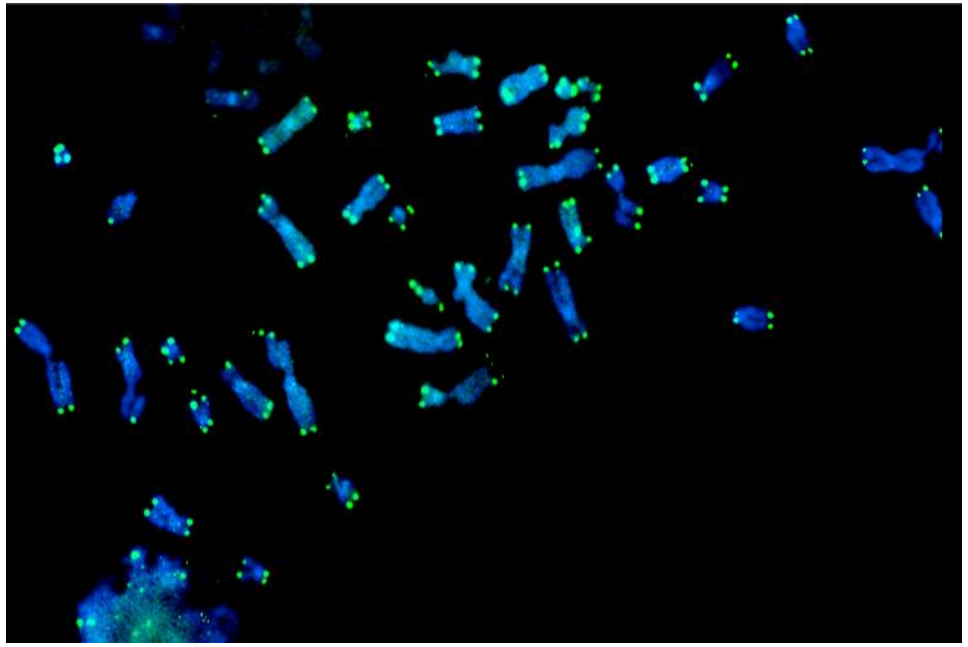
- This method requires a fluorescence microscope (quinacrine fluoresces strongly in the ultraviolet) and is no longer as widely used as G-banding.

- Quinacrine dihydrochloride was subsequently substituted for quinacrine mustard.
- The alternating bands of bright and dull fluorescence were called Q bands. Quinacrine-bright bands were composed primarily of DNA that was rich in the bases adenine (A) and thymine (T), and quinacrine-dull bands were composed of DNA that was rich in the bases guanine (G) and cytosine (C).

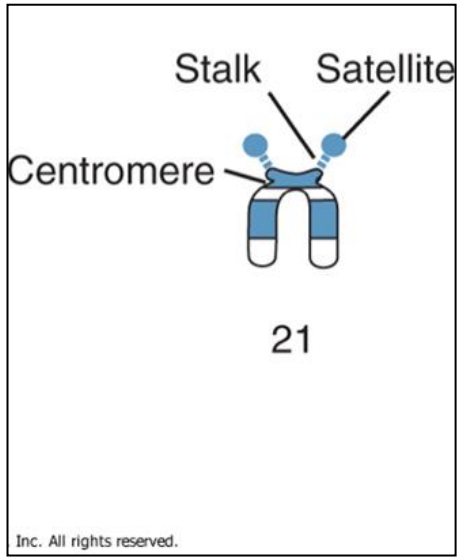
- This method is most useful for examining chromosomal translocations, especially ones involving the Y chromosome

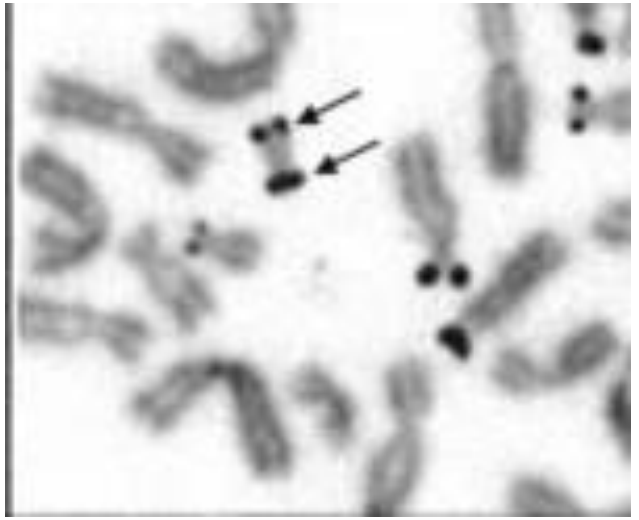
Banding technique	Appearance of chromosomes
C-banding — Chemically treat metaphase spreads to extract DNA from the arms but not the centromeric regions of chromosomes. Stain with Giemsa stain and observe with light microscope.	 **Darkly stained C band centromeric region of the chromosome corresponds to region of constitutive heterochromatin.**

T-Banding (Telomere Banding)



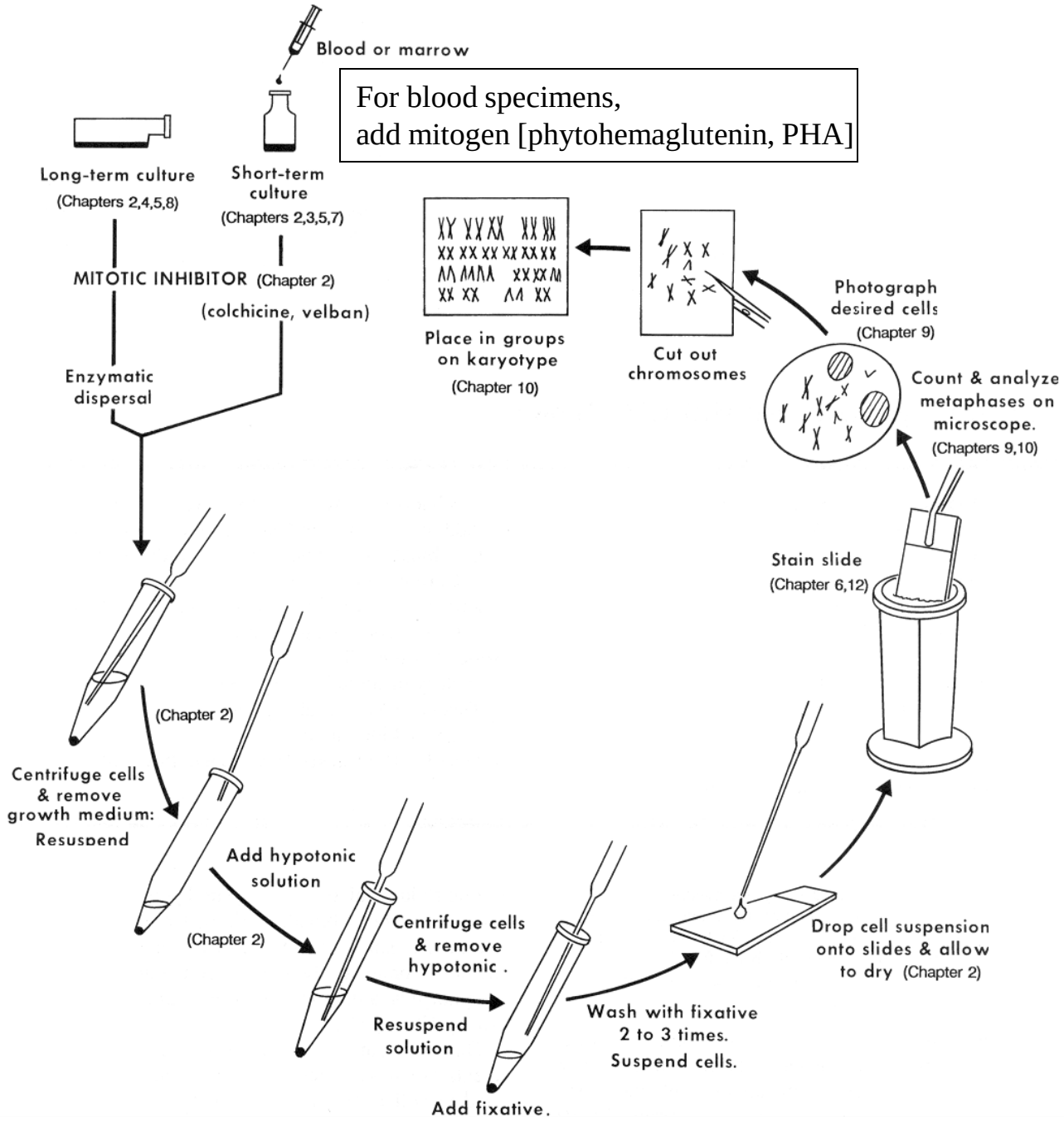
- **Silver nitrate stains the nucleolar organization regio**n-associated protein. This yields a dark region where the silver is deposited, denoting the activity of rRNA genes





Primary Steps for Culture Establishment and Harvest of Specimens

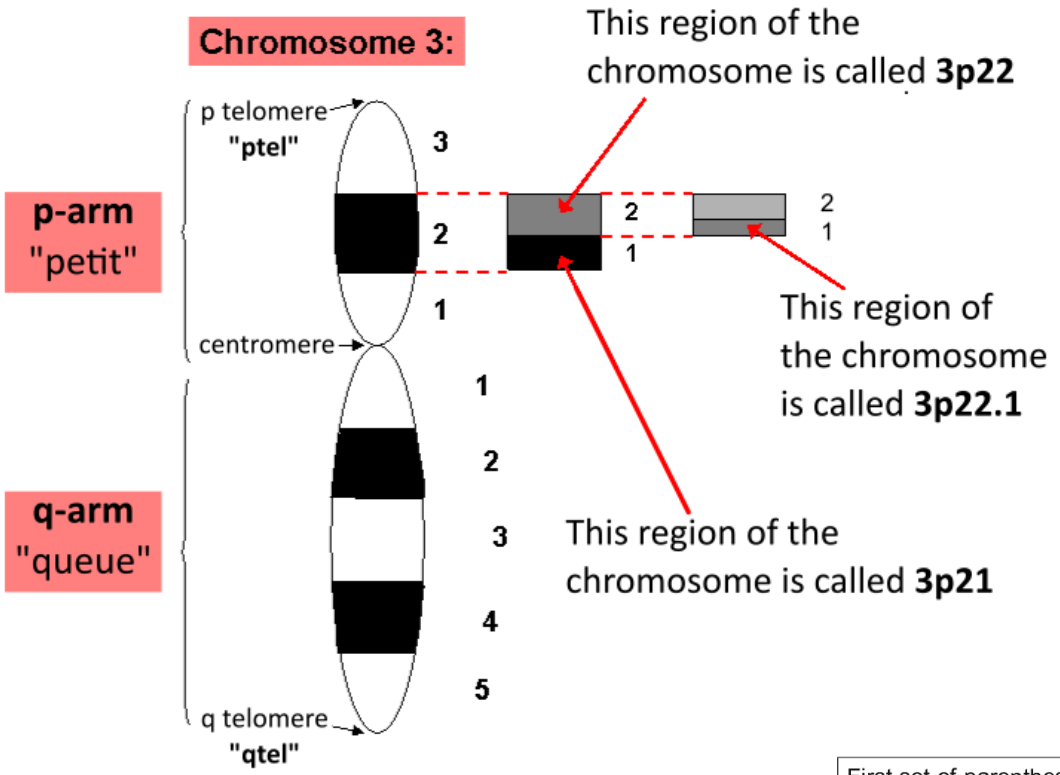
- **Add Mitogen (when needed)**
- **Hypotonic Swelling**
- **Fixation**
- **Analysis**



Chromosomes regions and bands

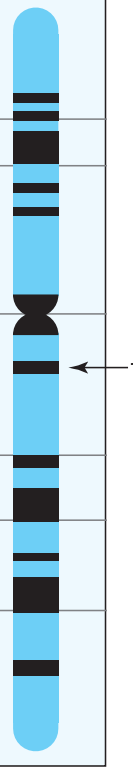
- regions and bands within a given chromosome are observed when banding techniques are used.
- Chromosome **regions** **refer to those areas lying between two distinct landmarks and are divided into bands.**
  - For example, the long arm of chromosome 7 has three regions: 7q1, 7q2, and 7q3 (Fig. 3.2).
  - These regions are further subdivided into bands.
  - A band **is defined as a part of the chromosome that is clearly distinguishable from its adjacent segments based on its staining properties.**

Cytogenetic Banding Nomenclature



Chromosome 17

Arm	Region	Band	Subband
p	2	2	3 2 1
		1	2 1
	1	1	5 4 3 2 1
q	1	1	1 2 3
		2	1 2 3
	2	3	1 2, 3 4
		4	1 2 3



The centromere divides the chromosomes into the short or “p” and long or “q” arms. Each arm is divided into regions. **Each band within a region is numbered centromere to telomere. Bands may be subdivided into sub-bands**

First set of parentheses describes the chromosomes involved:

First chromosome involved

t(9;22)(q34.1;q11.2)

Second chromosome involved

Second set of parentheses describes the chromosome bands involved:

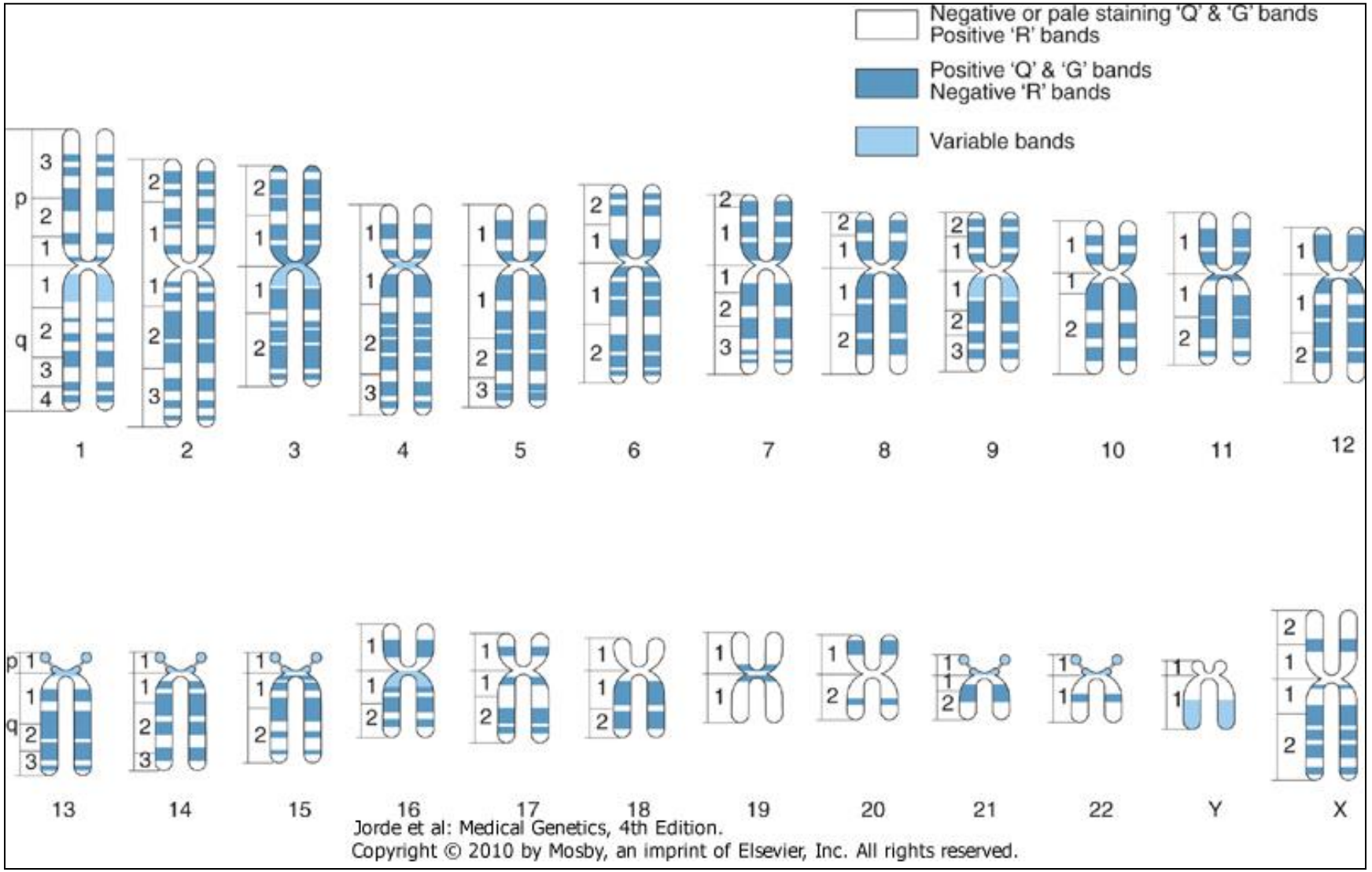
Chromosome 9, long or q arm, region 3,band 4, sub-band 1

t(9;22)(q34.1;q11.2)

Chromosome 22, q arm, region 1, band 1, sub-band 2

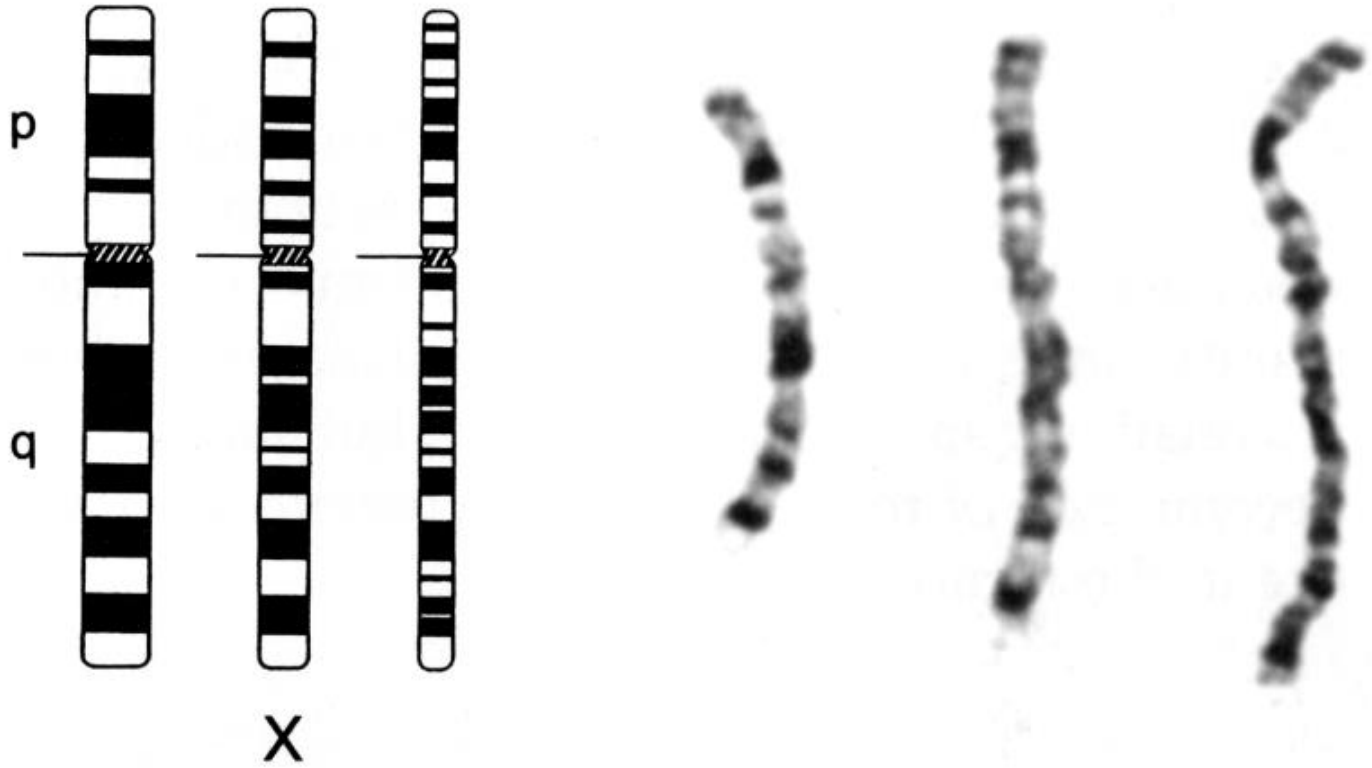
Human Chromosome Ideogram

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



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.



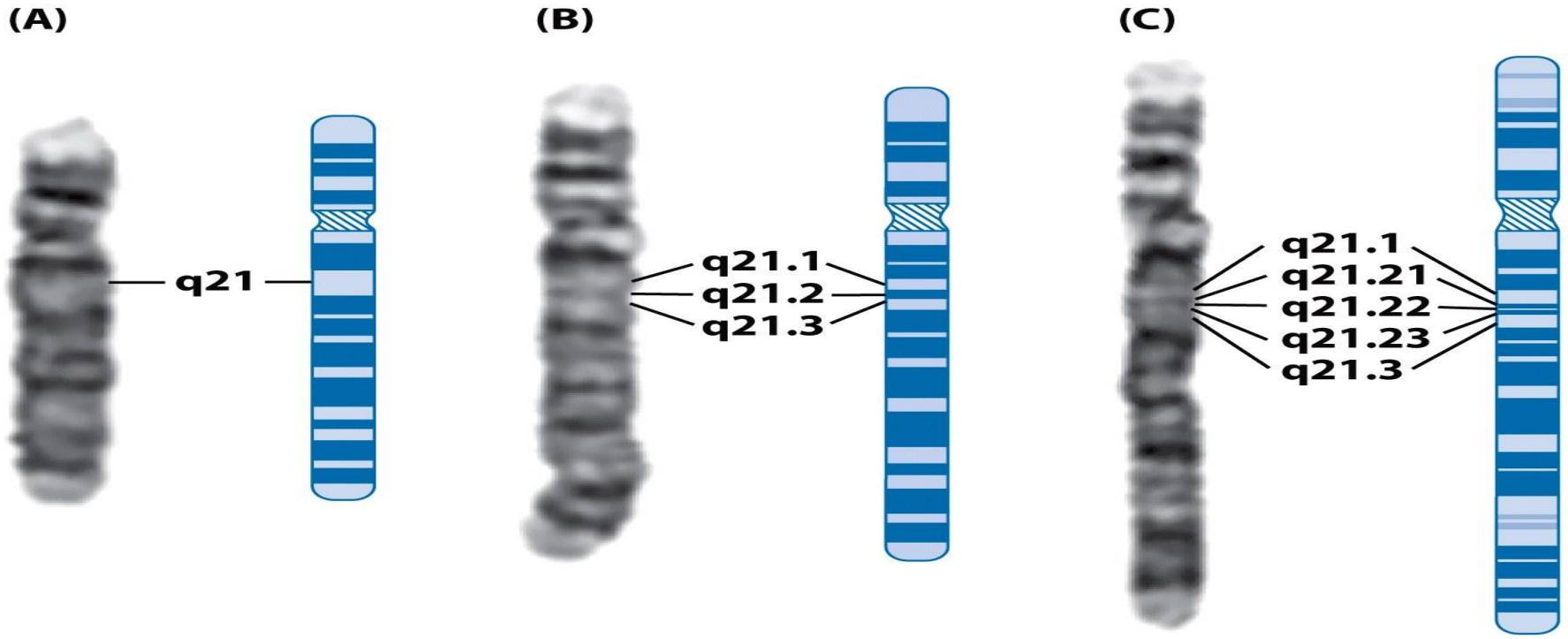


Figure 2.14 Human Molecular Genetics, 4ed. (© Garland Science)

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.]