GENETICS

Sheet no. 5

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(navy blue: slides, black: notes by the doctor)

- 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.

- We study human chromosomes to early detect cytogenic abnormalities that may contribute to many morbidities and mortalities; here are some instances and the estimate of cases with cytogenic abnormality:
  - Early embryonic death in unrecognized pregnancies; (??) 33-67%.
  - Recognized embryonic and fetal deaths ($\underline{\geq}$ 5 weeks); About 30% total; rate varies from 50% at 8-11 weeks to 5% in stillbirths ($\underline{\geq}$ 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+%.
- While mentioning *Cytogenics* it is defined as 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; in 15th week of gestation, it's preferred to take a sample from amniotic fluid rather than CSF
  - Fetal tissue for products of conception in miscarriages
  - Skin or organ biopsy
  - Tumor biopsies
- It is referred for cytogenic analyses for many reasons, we're going to discuss the main ones:

1) **Prenatal** – Abnormal maternal serum screening (first or second trimester)
   - It is recommended to perform cytogenic analyses prenatally in any of these cases:
     - During fetal cell death some of the DNA shed into maternal blood, then we can **screen** fetal karyotype by performing cell-free DNA testing (cfDNA) to detect any abnormality such as down syndrome (trisomy 21), and if we find any, we further investigate by non-invasive prenatal testing (NIPT).
     - Abnormal ultrasound findings: cystic hygromas/hydrops, cardiac defects, other malformations, IUGR (intrauterine growth restriction; poor growth of the fetus in the womb), etc.
     - Advanced maternal age (AMA), generally ≥ 35 yrs; increased maternal age raises the possibility of chromosomal abnormalities.
     - Parental or familial chromosome/genomic abnormality.
     - Fetal or neonatal demise (products of conception, POC).
   - Type of samples: chorionic villi biopsy, amniotic fluid cells, products of conception.

2) Postnatal

- Childhood growth and development could be an indicator for chromosomal abnormality.
  - Perinatal/newborn: Birth defects, malformations, etc.
  - Growth: failure to thrive, etc.
  - Developmental delay (fine and gross motor (spatial-motor delay), speech); the first stitch is chromosomal analysis.
  - Cognitive
  - Neurological
  - Behavioral; related to neurology such as an autistic behavior.
- Type of samples: peripheral blood.

3) Adolescent

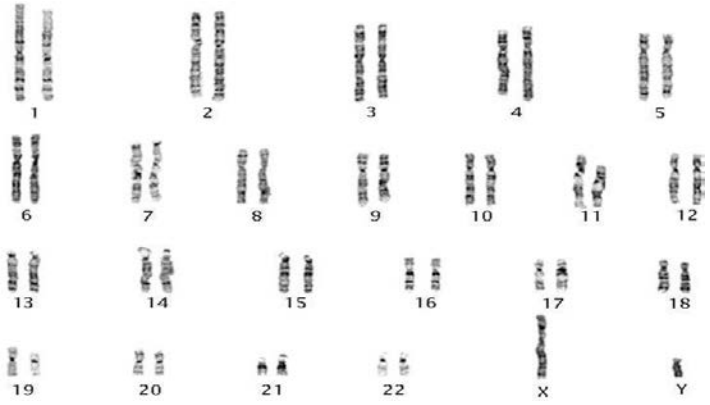
- Adult sexual development and fertility
  - Amenorrhea, primary or secondary ovarian failure, premature menopause.
  - Azoospermia, oligospermia, hypogonadism.
  - History of infertility or spontaneous abortions or having a child with chromosomal abnormality.
- In cases of infertility, it is firstly recommended to perform cytogenic analyses especially in the presence of family history, if it appears negative, the genetic counsellor will guide the patient for next steps.
- Type of samples: peripheral blood.

4) Cancer

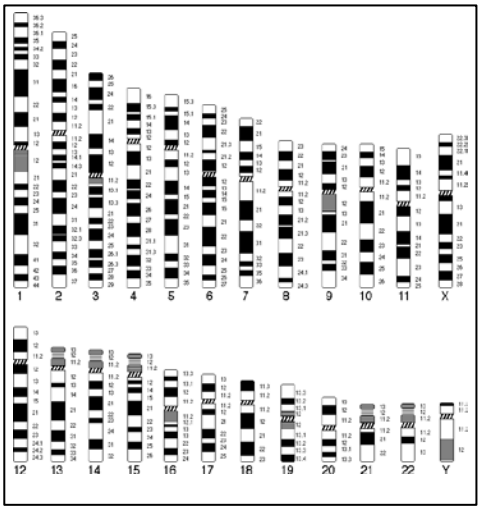
- Hematologic oncology
  - Myeloid diseases: AML, CML, MDS, MPNs
  - Lymphoid diseases: ALL, CLL, NHL, PCNs/MM
- Bone marrow transplant
- Other areas of oncology (solid tumors)
- Types of samples: tissue biopsy, blood samples in hematologic tumors, bone marrow sample in leukemias; it depends on cancer type.
- Chromosomal analyses are used in screening, diagnosis and monitoring the progression or deterioration of cancer.

- A *karyogram* aka *karyotype* 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 in addition to X,Y chromosomes).
- Once a computer image of the chromosomes from a dividing cell is obtained, the chromosomes are arranged as homologous pairs.
- The normal diploid chromosome number for humans is 46.

- This is a typical karyogram.
- Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome.



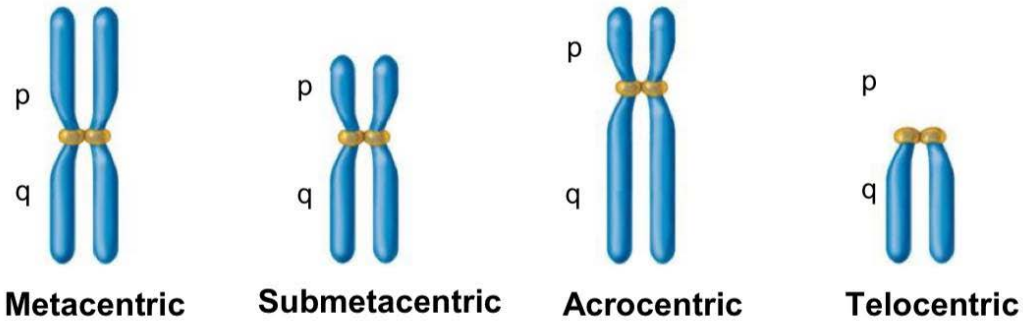
- This is an ideogram of a chromosomal complement, which is a diagrammatic representation of the karyotype.
- Patterns and bands can be well-detected in the ideogram.
- We can notice that chromosome 1 is the longest, having its unique pattern of banding (banding is another way of chromosome classification).



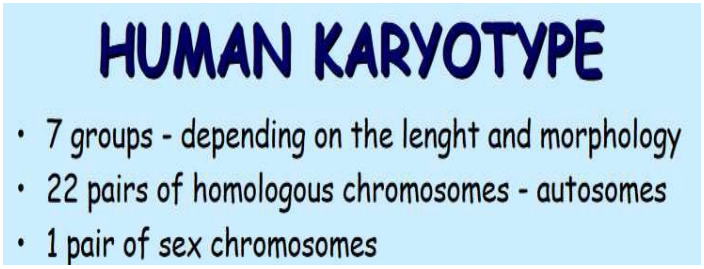
➢ Chromosomes can be classified in terms of many patterns; we've mentioned the length and banding, now we'll discuss *centromere positioning*.

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 rather in rodents).

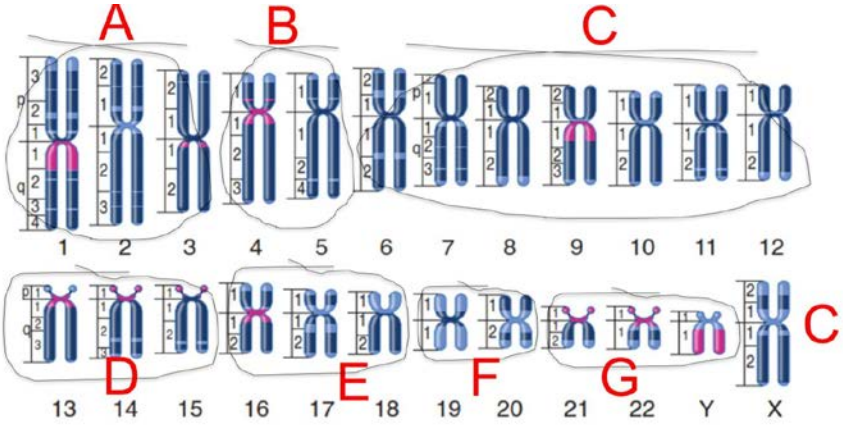


- An international classification of chromosomes classified them into 7 groups according to centromere position.
- You need to know the main points of this classification such as the number of groups.

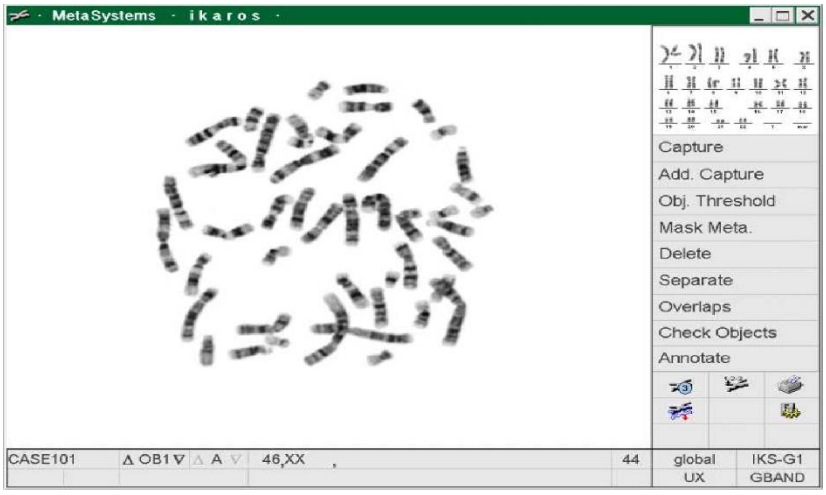


Grouping	Number Of Chromosome	Description chromosome
Group A	Chromosome 1-3	Metacentric chromosomes are large and easily distinguished from the others because of its size and location of the centromere
Group B	Chromosome 4-5	has two large-sized chromosome sub metacentric
Group C	Chromosome 6-12, X	Metacentric 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

- This figure shows the exact groups of this classification.
- You may notice that both D & G groups are acrocentric, however, D is the medium-sized acrocentrics while G is the small-sized ones.



- This is a karyotype of metaphase chromosomes imaging by using metasystems.
- The captured chromosomes are counted, arranged and any abnormality is detected (rearrangements, dislocations, etc).



- You can notice that this is a karyotype of 46 chromosomes, XX (female).
- Remember that chromosomes can be seen in M phase of cell division, where there are the highest compaction and individual arrangement.
- 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.

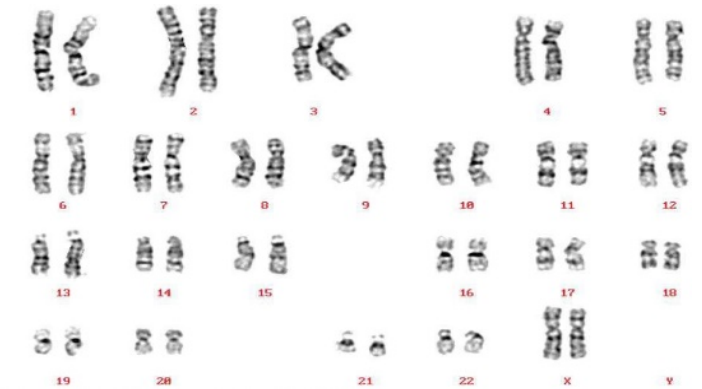


Fig. 2. A karyotype of a normal female (46,XX)

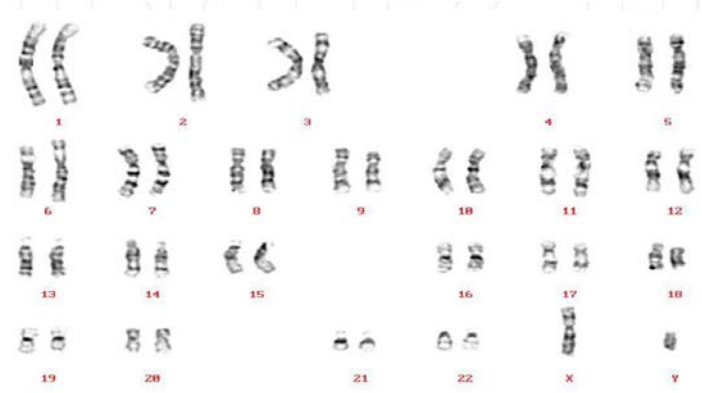
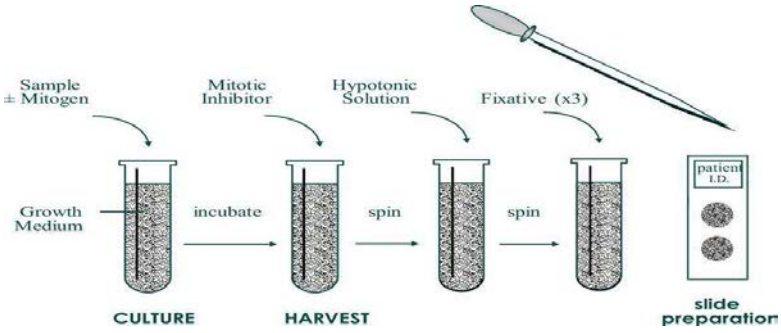
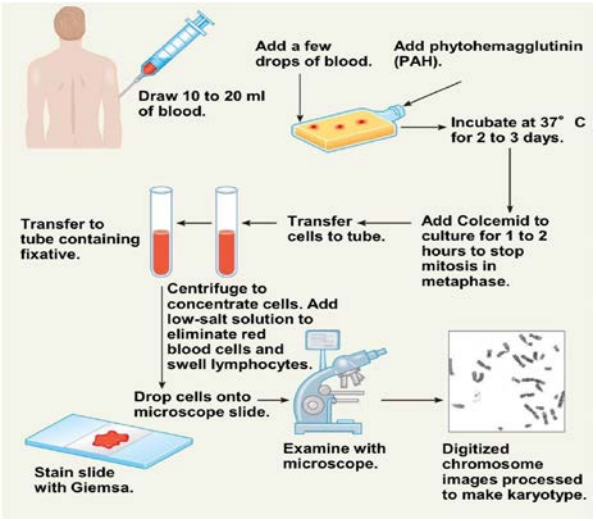


Fig. 1. A karyotype of a normal male (46,XY)

- The most convenient samplas that are frequently used in chromosomal analyses are from peripheral blood collected in green tubes (either faint; lithium heparin or dark; sodium heparin).
- Samples for DNA extraction and PCR are collected in EDTA tubes.

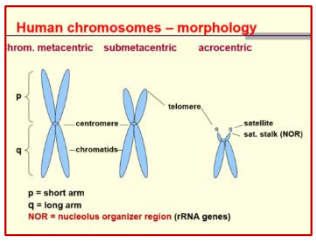
- **Making a karyotype:**



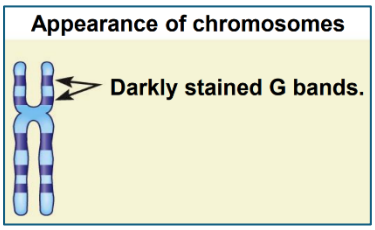


- Blood is collected from the patient in a heparinized tube.
- We let the sample grow in an appropriate media that has all the needed nutrients for lymphocyte growth, mitogen AKA phytohemagglutinin is added to enhance cell division.
- Cells are incubated for 2-3 days then colcemid or colchicine is added to prevent spindle fibers formation that chromosomes remain at the center of this cell; cells are arrested in metaphase and mitosis has stopped.
- Cells are then treated with a hypotonic solution that causes hemolysis.
- Thereafter, cells are dropped onto microscope slides that are placed 70cm far way to further enhance cell burst.
- Fixation and staining to the sample to visualize them under LM.

- **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, for instance, telomere, centromere or satellite or NOR of acrocentrics.
  - Nowadays, we mainly use the standardized stain for karyotyping which is Giemsa stain (G banding).



- **Banding technique** (G- is the standard karyotype in chromosome reports)
  1. Treat metaphase spreads with trypsin, an enzyme that digests part of chromosomal protein.
  2. Stain with Giemsa stain.
  3. Observe banding pattern with light microscope.



- 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 (2 H bonds between A and T, stain enters more easily) and stain more darkly. The light regions tend to be euchromatic, GC rich (3 H bonds). The G-light bands are biologically more significant because they represent the active regions (coding regions) of the chromosomes, while the G-dark bands contain relatively few active genes→ non-coding regions. G-banding techniques (G-light/ G-dark)

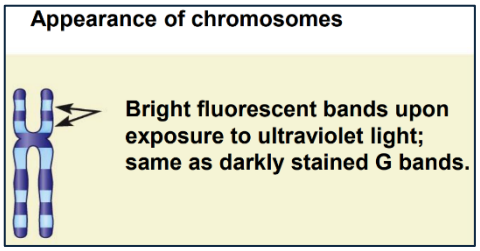
> - A common misconception is that bands represent single genes, but in fact the thinnest bands contain over a million base pairs and potentially hundreds of genes. For example, the size of one small band is about equal to the entire genetic information for one bacterium.
> - Standard G-band staining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

- **R-banding**

  Used when you're not able to diagnose a patient but you know there is something wrong, it's no longer used though.

  - 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.

- **Q-banding**
  1. Treat metaphase spreads with the chemical quinacrine mustard.
  2. Observe fluorescent banding pattern with a special ultraviolet light microscope.

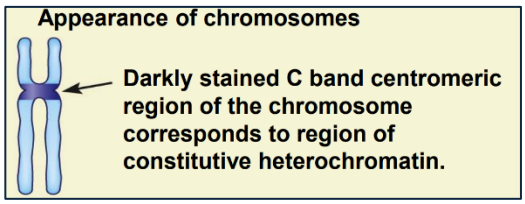


  - This method requires a fluorescence microscope (quinacrine fluoresces strongly in the ultraviolet) and is no longer as widely used as G-banding.
  - This method is most useful for examining chromosomal translocations, especially ones involving the Y chromosome.

- 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).

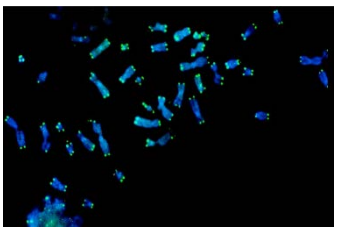
- Please note the differences between G and Q banding.
- The previous bands were under the category of alternative staining patterns along the length of the chromosome while C banding is for specific regions of the chromosome.

- **C-banding**
  1. Chemically treat metaphase spreads to extract DNA from the arms but not the centromeric regions of chromosomes.
  2. Stain with Giemsa stain and observe with light microscope.



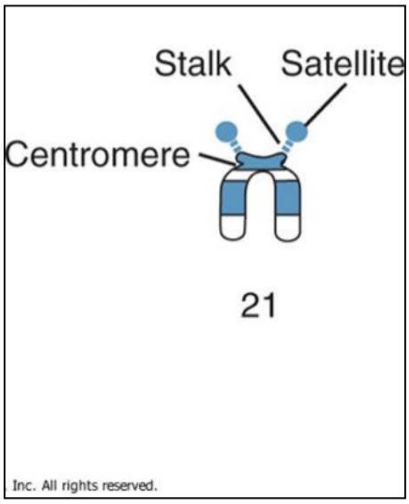
  - C banding only stains centromeres and is important to inform you if the centromere is single, dicentric or has polymorphic regions in it.

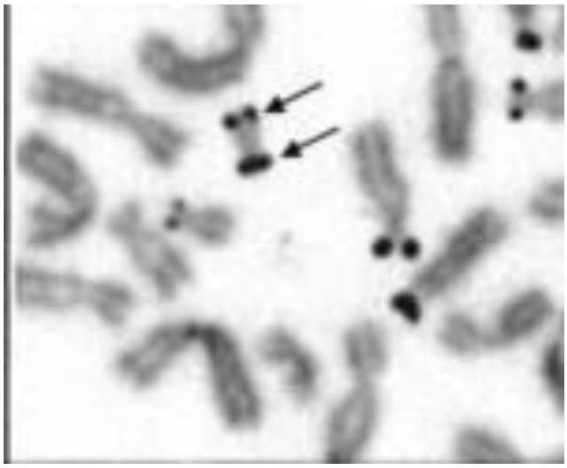
- **T-Banding (Telomere Banding)**



  - Notice: there are 4 dots per chromosome 1 on each end, these dots each represent a signal. This is fluorescence and T banding could be fluorescence and could be not.
  - 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.
  - In acrocentric chromosomes, we have qr, and pr divided into satellite and stalk. Satellites are repetitive regions of dna; these regions are tandems.

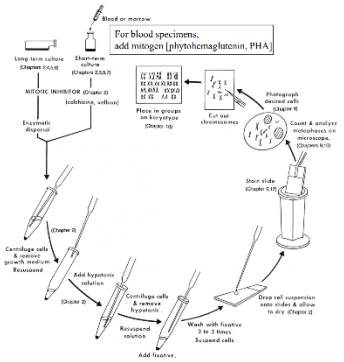
Example of tandem: every 100 bp repeated after each other. Stalk connects satellites to arms and has genes that encode for rRNA. All acrocentric chromosomes contain these two features: stalk and satellites. If you have a problem with the stalk in chromosome 21, nothing with happen because we have 4 more acrocentric chromosomes that contain genes that code rRNA and compensate. One of the reasons for down syndrome is acrocentric chromosomes; chromosome 14 sticks to chromosome 21. Most of the down syndrome cases occur due to having 3 copies of chromosome 21 though. Satellite is heterochromatic while stalk is euchromatic because it has coding genes.





Dr said ignore the following slide.

Primary Steps for Culture Establishment and Harvest of Specimens

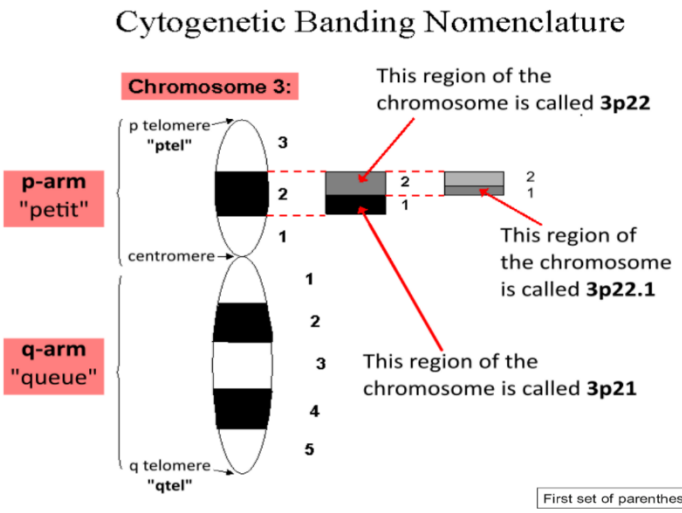


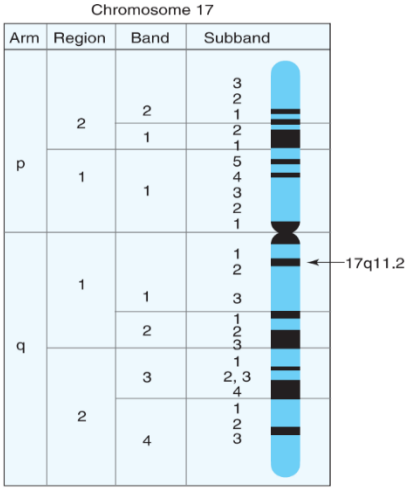
1. Add Mitogen (when needed)
2. Hypotonic Swelling
3. Fixation
4. Analysis

Chromosomes regions and bands

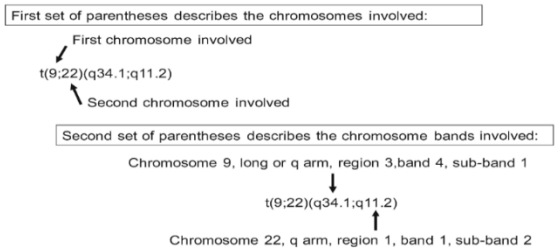
- regions and bands within a given chromosome are observed when banding techniques are used.
- Chromosome regions refer to those areas lying between two distinct landmarks and are divided into bands.
    - For example, the long arm of chromosome 7 has three regions: 7q1, 7q2, and 7q3 (Fig. 3.2).
    - 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.
- Notice in the blue diagram below, chromosome 17 has 2 regions in p and 2 regions in q. The numbering starts from the centromere towards the end. How do we read 17q11.2? 17 is the chromosome number, q is the arm, 1st 1 is the region, 2nd 1 is the band, 2 is the sub band. So, 11 is read as one one not eleven!



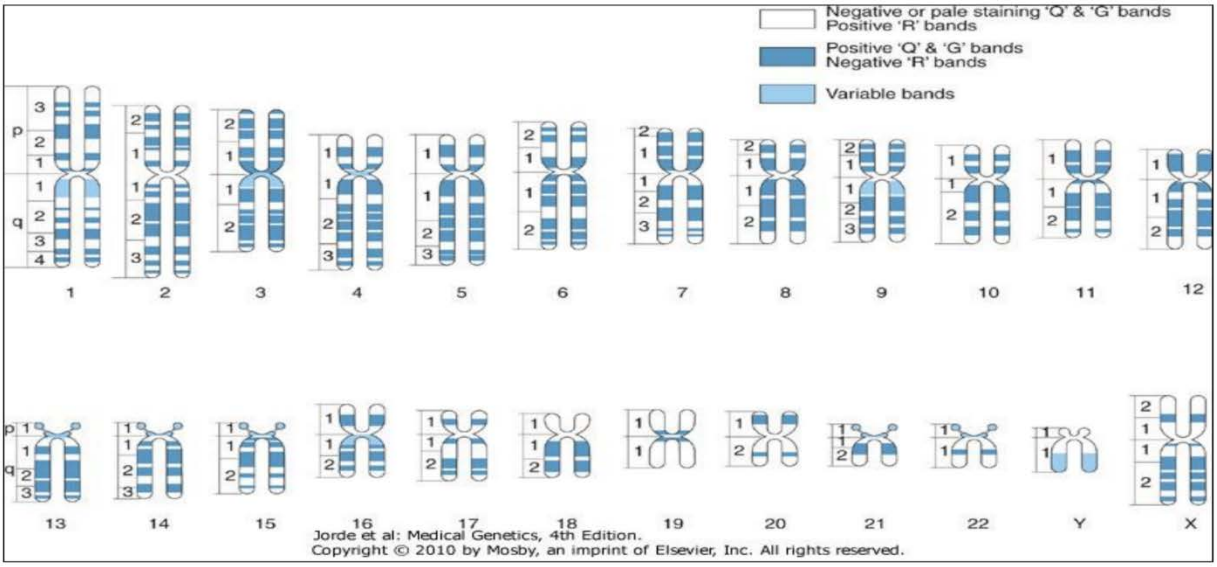


The centromere divides the chromosomes into the short or "p" and long or "q" arms. Each arm is divided into regions. **Each band within a region is numbered centromere to telomere. Bands may be subdivided into sub-bands**



Human Chromosome Ideogram

Ideogram- A diagrammatic representation of a karyotype

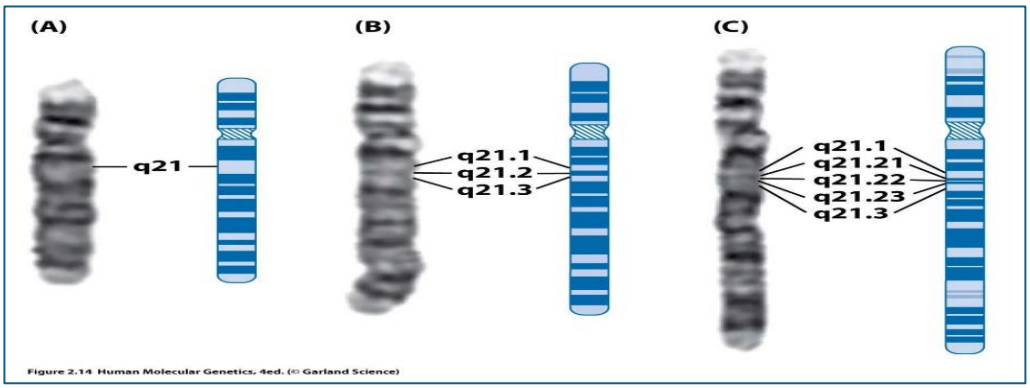


High-resolution banding

High-resolution banding involves the staining (stopping cell division during early stages in relaxation phases) 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.

- Notice starting from left to right: 1st chromosome is in metaphase, you can see the p region as one, as you go on to prometaphase you notice 2 bands, and if you move on to the 3rd chromosome in prophase you will see the bands in a higher resolution as they are more extended.

- In general, in karyotypes, we can only detect up to 5 mega base of nucleotide so if the size of defects in chromosomes is less than 4.5-5 mega base we cannot find it under the microscope. We will have to look for a deeper test such as fish or microarray.



- 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 subbands and sub-subbands as the resolution increases (more extension).

V2