Genetics
& Cell biology

◉ Sheet ○ Slides

Number

2

Done by:

Abdullah AlZibdeh

Corrected by:

Manar Alafeshat

Doctor

Bilal Azab

Introduction

Everything in the slides is included in the sheet, but you can study this sheet with the doctor's slides to see the figures in bigger sizes. Do not hesitate to report any corrections or edits.

The material is divided into cytogenesis and chromosomes, molecular genetics and population risk assessment.

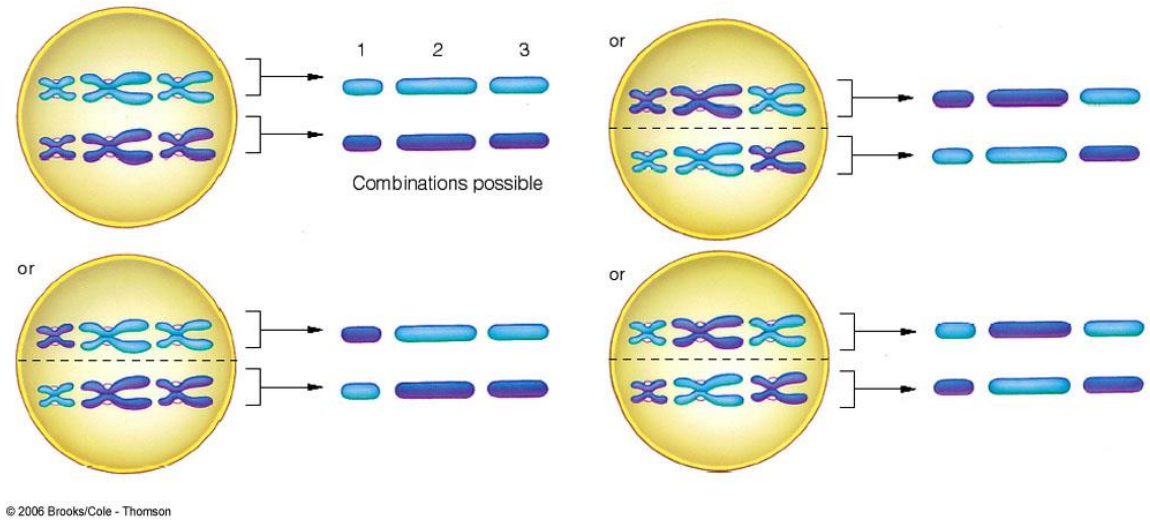
Mendel's laws (*review and summarization*)

1- Law of segmentation:

Homologous chromosomes carry the same genes but not necessarily the same version of the gene, which are called *alleles* (the gene of blood type has different versions, such as A, B or O. such versions are called alleles).

After meiosis, each gamete produced carries only one allele of each gene; because the homologous chromosomes have undergone separation, or *segregation*, into different cells. So, there is one allele per one gene in each gamete.

2- Law of independent assortment:



Notice this figure. Suppose that the number of chromosomes equals 3. Each cell has 3 chromosomes from the father, and their homologous 3 from the mother. When undergoing meiosis, there is no law that draws a specific scheme for the chromosomes to arrange in a specific manner before separation. In other words, there is no law which states that paternal chromosomes go together, or maternal

chromosomes go together. It is a random process, and so, the possibilities are very numerous (equal 2^n; where n = number of chromosomes). 8,388,608 possibilities exist for the outcomes of meiosis in our cells (at the level of meiosis I; i.e. without calculating the diversity results from crossing-over process). What is more astonishing is that possibilities increase more and more when putting crossing-over and the random sperm-egg meeting into account!

Notes:

- Single chromosomes do not carry more than one allele for each gene
- Crossing-over and diversity: when 2 gametes result with both having chromosome #2 from the father, crossing-over allows the chromosome of the same source to have diverse versions. So, even if the both gametes have the paternal version of chromosome 2, this version is not identical in the two gametes.

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Why do we study chromosomes?

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. (In multiple miscarriages cases, expect the presence of chromosomal abnormalities.)

(4) The majority of cells from leukemia samples or solid tumors.

Note: these numbers are not for memorization; appreciate the medical outcomes of the chromosomal abnormalities.

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+%

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Research Uses for Cytogenetic Evaluation

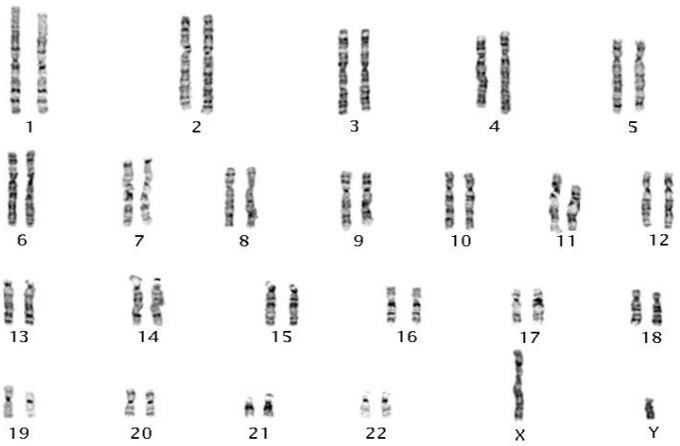
- Localization of DNA onto a chromosome(s).
- Determination of genomic complement.
- Characterization of genetic change(s).
- Recognition of chromosomal changes following treatment(s) or in vitro culturing.

<u>But, how to extract the chromosomes?</u>

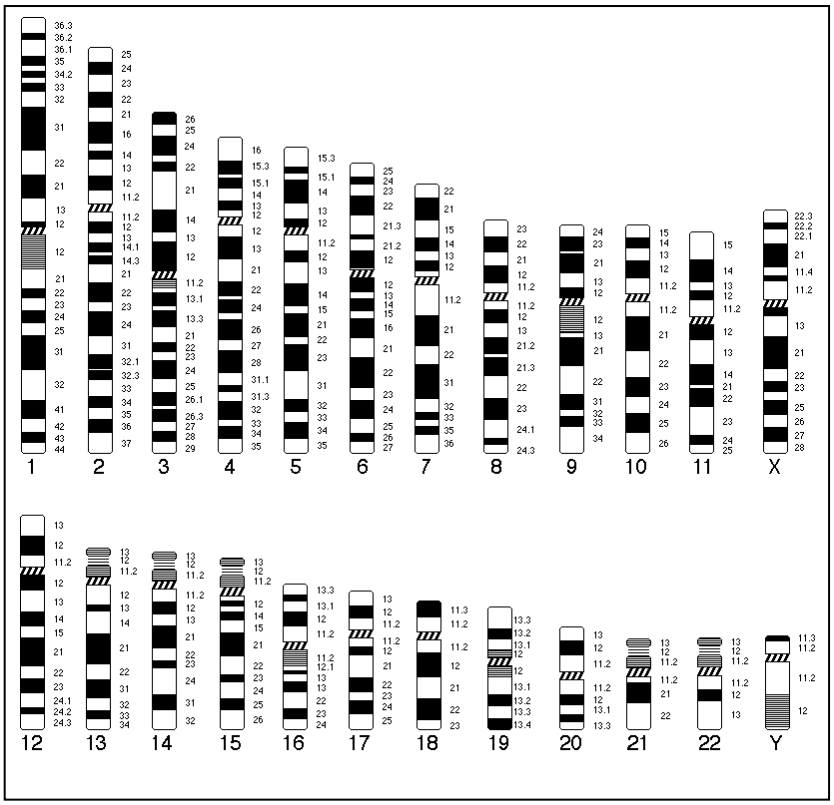
The common tissues for Chromosome Studies include: peripheral blood (lymphocytes), Bone marrow, Chorionic villi biopsy, Amniotic fluid cells and Skin or organ biopsy. Peripheral blood samples are the easiest and the typical tissues for studies, though sometimes samples must be taken from other tissues. For example, to extract fetal chromosomes, an invasive procedure is used to take a sample from the amniotic fluid and chorionic villi, by using intraperitoneal or intravaginal needle, guided by ultrasound. Another example is bone marrow samples leukemia patients.

A *karyogram* (or *karyotype*) is a 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

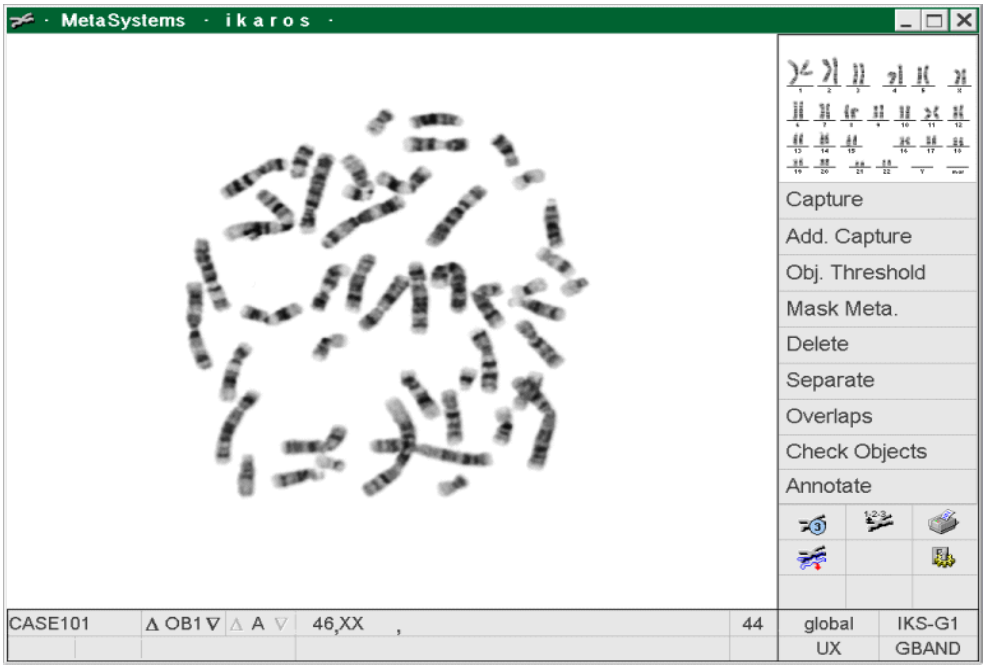
homologous pairs. Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome. The normal diploid chromosome number for humans is 46.



The figure shows a karyotype. Each homologous (maternal and paternal) chromosomes are arranged next to each other. Each of them has 2 sister chromatids, which are not obviously seen. Notice the difference between the autosomal chromosomes and the sex chromosomes.

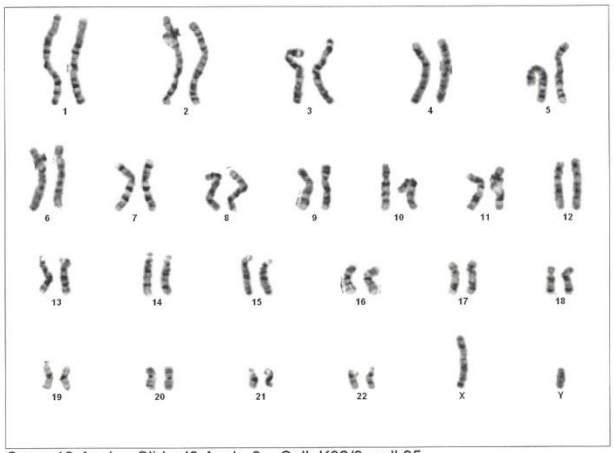


The ideogram of a chromosomal complement is a diagrammatic representation of the karyotype.

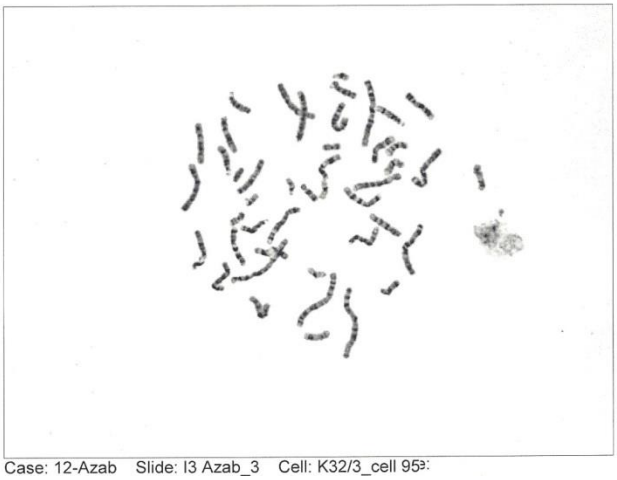


Metaphase chromosomes.

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.



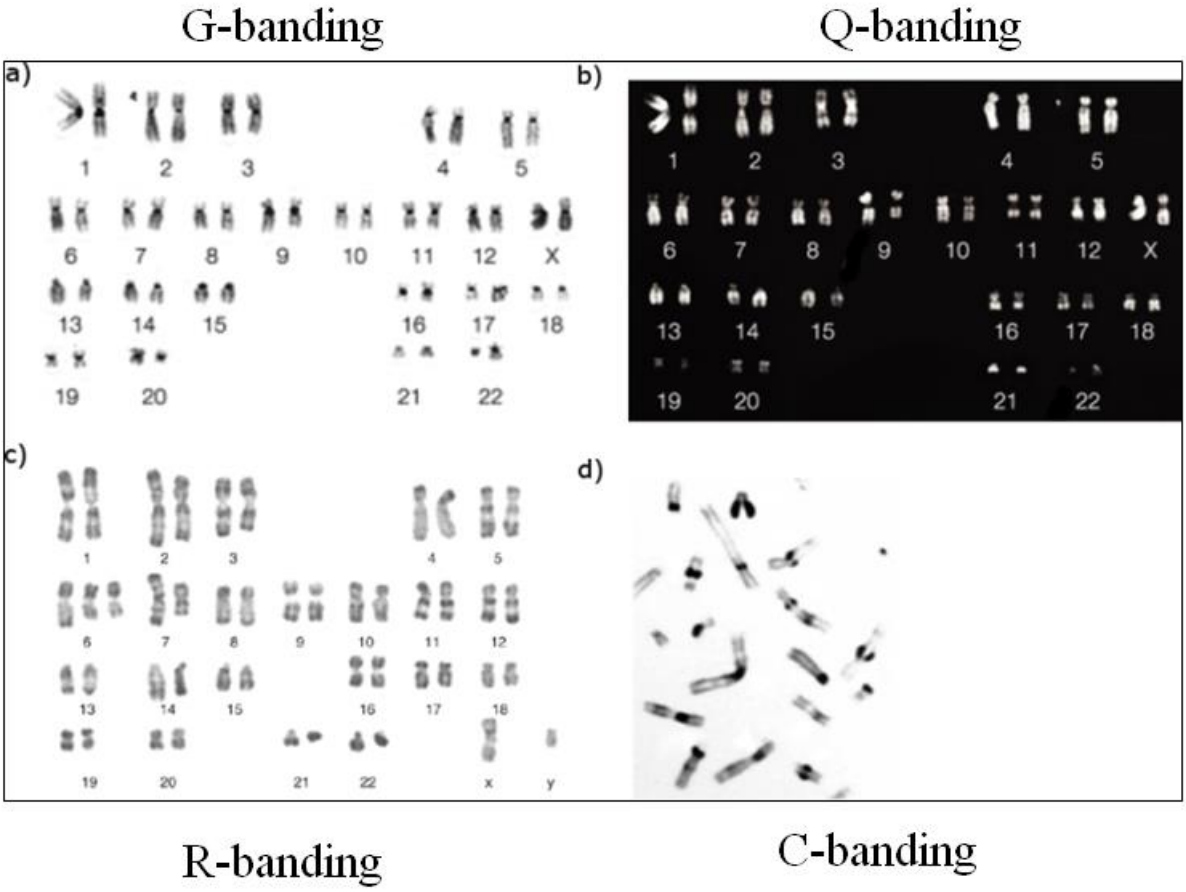
Case: 12-Azab Slide: I3 Azab_3 Cell: K32/3_cell 95



Case: 12-Azab Slide: I3 Azab_3 Cell: K32/3_cell 95³:

Types of banding

We can identify the chromosomes of the epiGRAPH result according to their size, centromere site. Banding also helps us to identify the chromosomes according to the arrangement of the bands on them. Types of banding include:



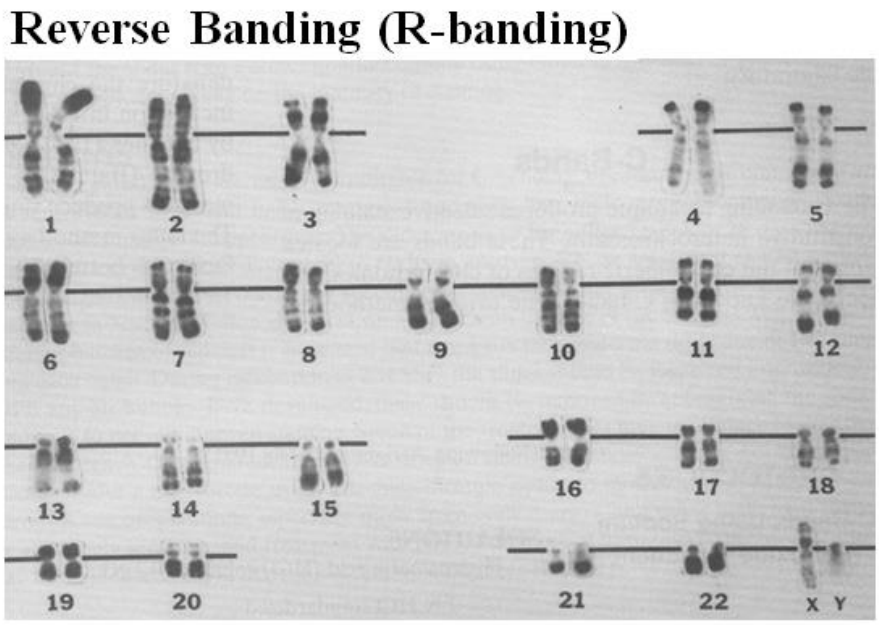
1- G banding (GTG)

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. Giemsa stain binds AT-rich DNA, which has double bonds, more easily than CG-rich DNA, which contains more closely packed triple bonds.

This method will normally produce 300-400 bands among the 23 pairs of human chromosomes, and the longer the chromosome the more the bands it contains. 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. This method is the typical and the most used method.

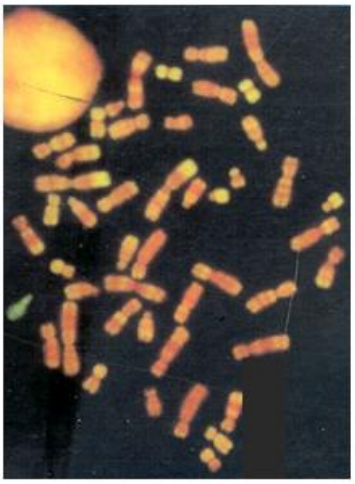
2- R-banding

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). This method is used when G-banding results are not so obvious. It provides critical details about gene-rich regions that are located near the telomeres. It is 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.



RHG (R-bands by heating using Giemsa)

RFA (R-bands by fluorescence using acridine orange



3- C-banding

4- Q-banding

5- T-banding

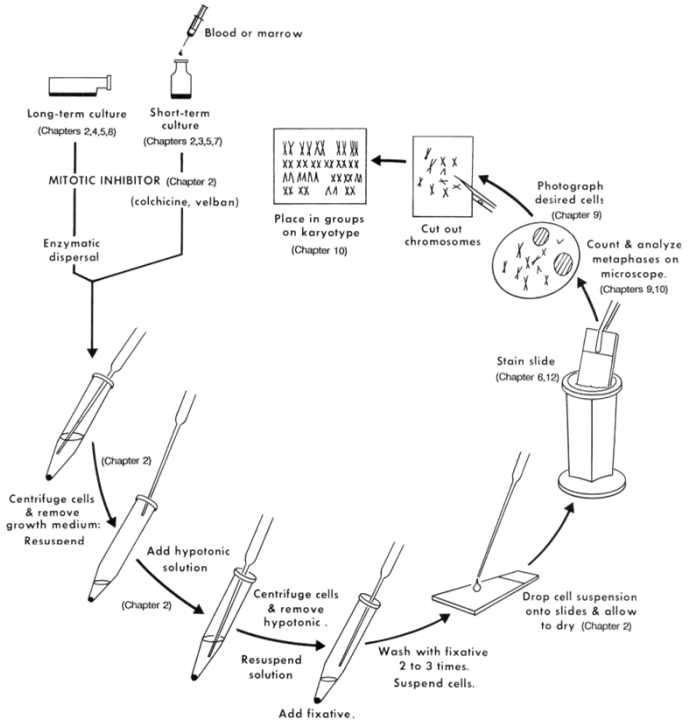
6- Silver staining

These methods are less used, and not for memorization.

Primary steps for culture establishment and harvest of specimens

Blood sample is isolated in examination dish. Chromosomes are most visible at the M-phase (specifically at the metaphase) of the cell cycle (at the interphase,

chromosomes are diffuse in the form of chromatin). The isolated cells are usually in the interphase (usually at G_1 phase) rather than the M-phase, because the interphase is longer in duration, so we add mitogen (when needed) to induce cell cycle progression. After that, we add colchicine, which prevents spindle fibers formation, arresting the cell at the metaphase.

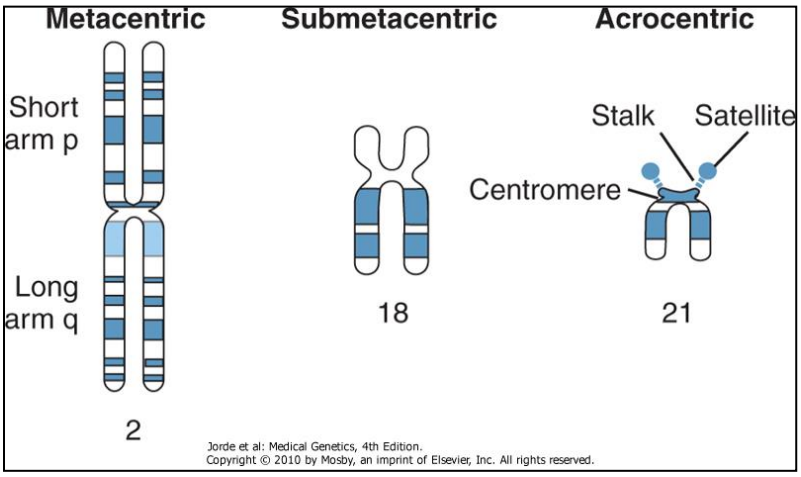


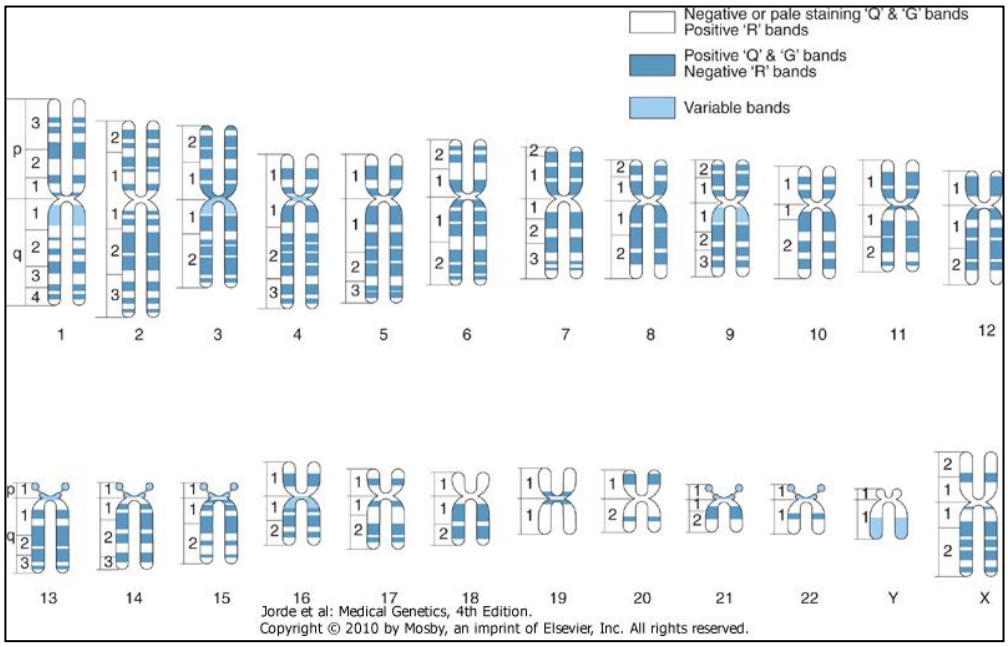
Next, we put the cells media into a tube, and apply low-speed centrifugation to separate the cells from the media. A hypotonic solution is added to the tube, resulting with cell swelling. Then, fixation is done.

At the end, suspensions of the isolated cells are taken by pipettes. These suspensions are dropped on a surface, resulting with collapsing the swelled cells and spreading of the chromosomes out of the cells. Computer analysis is used to produce the karyotype.

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.

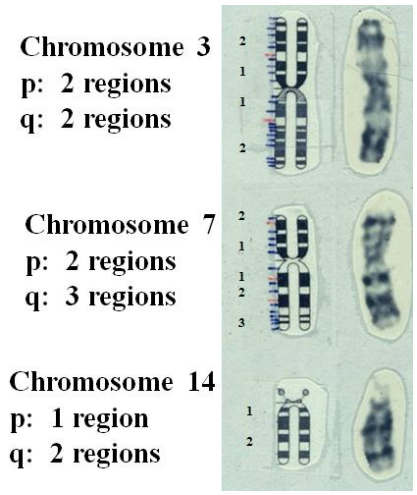




Human Chromosome Ideogram

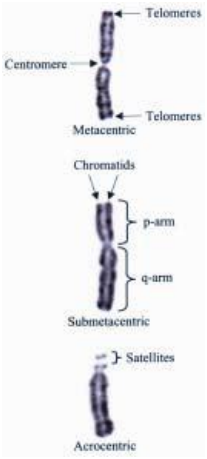
Ideogram- A diagrammatic representation of a karyotype

From the figure above, we can notice the five acrocentric chromosomes: 13; 14; 15; 21 and 22.



Structure of chromosomes

The upper arm of the chromosome is called p, and the lower is called q. in metacentric chromosomes, the lengths of the arms are the same, but with different arrangement of bands. The arms are divided into regions, according to the banding, starting from the centromeres and heading towards the telomeres.

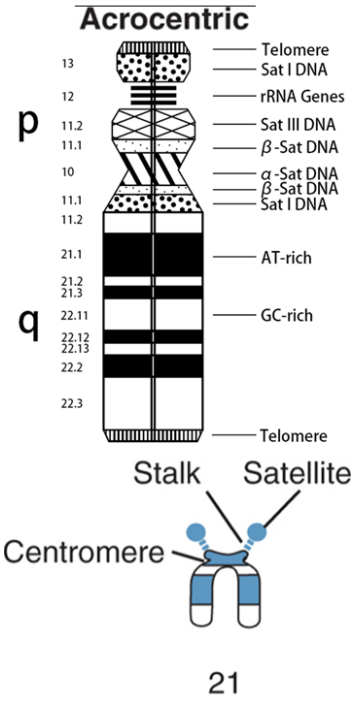


In acrocentric chromosome, the p arm contains 2 elements:

1- Satellite DNA: Noncoding DNA sequences.

2- Stalk: rRNA-coding region. rRNA is the most abundant RNA molecules in the cell. All acrocentric chromosomes carry this region. If any defects occur to one of the acrocentric chromosomes' p arm stalk, no clinical consequences will happen, since the genes carried are

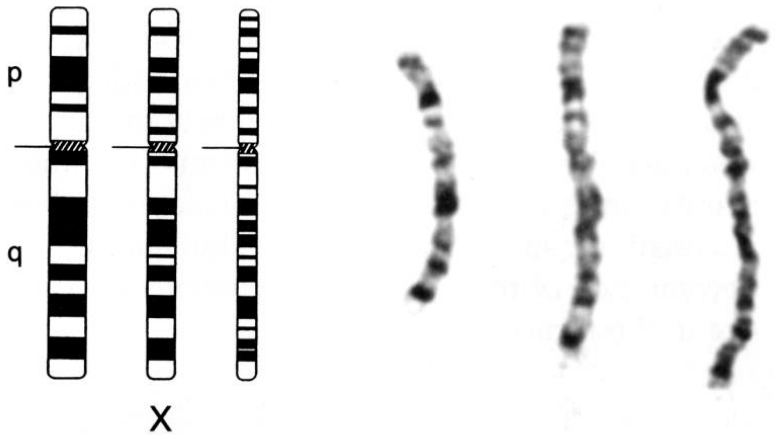
present on the other acrocentric chromosomes, which compensate for the loss.



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 (so, more bands can be noticed).



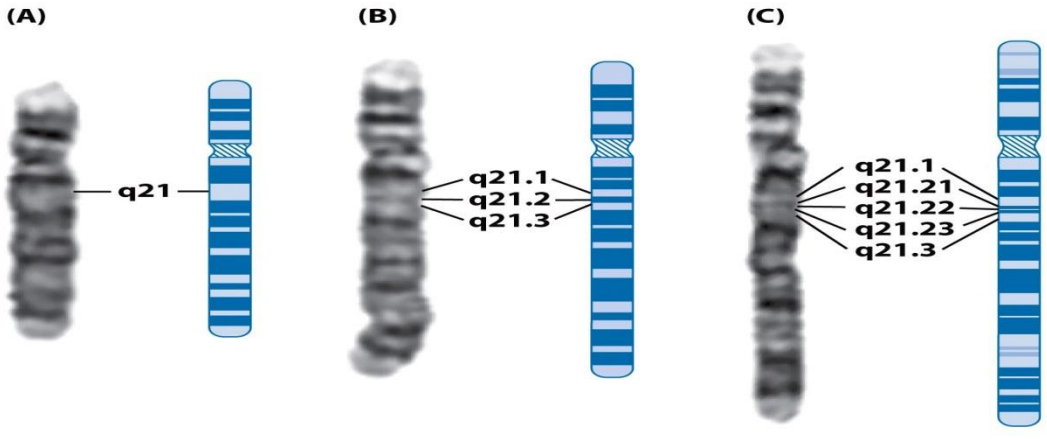
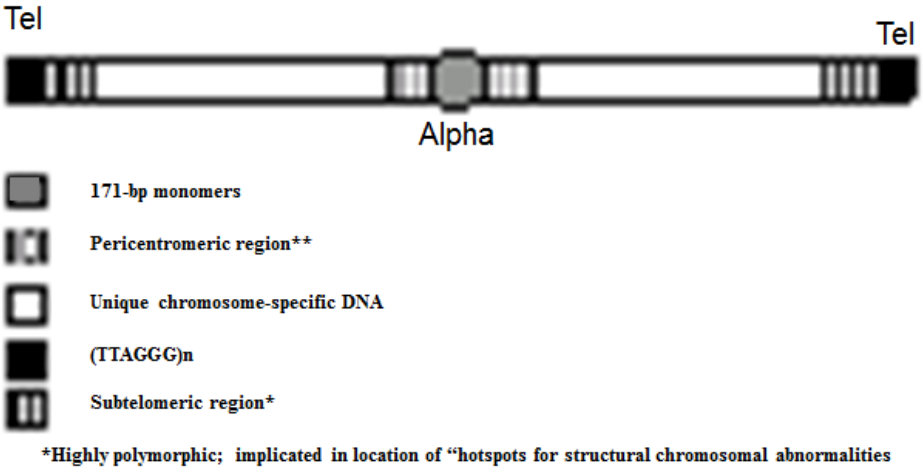


Figure 2.14 Human Molecular Genetics, 4ed. (© Garland Science)

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



Centromere

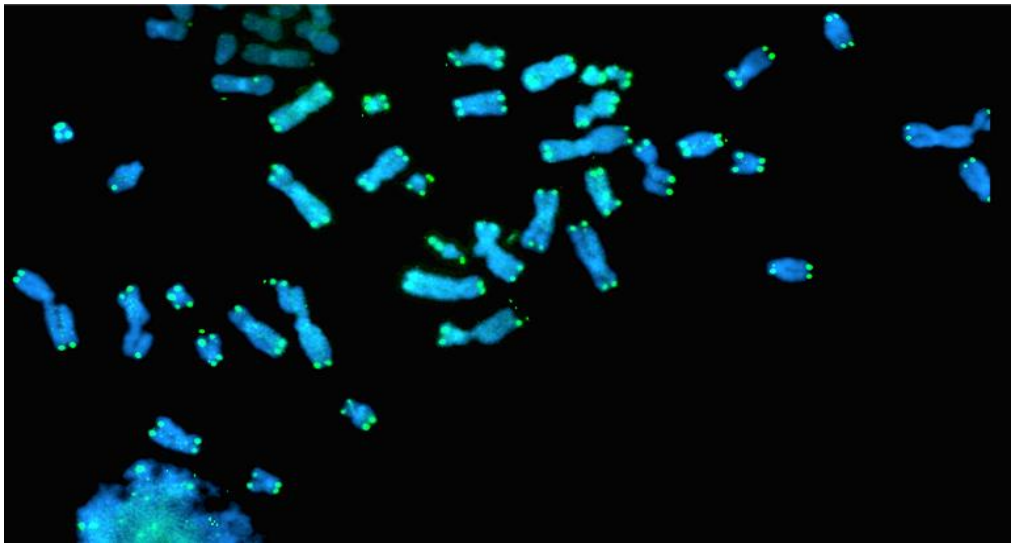
The genetic locus required for chromosome segregation. It contains DNA and proteins on which the kinetochore is formed.

At the α region, DNA noncoding sequences are tendemly repeated next each other. And since this region is noncoding and only has structural roles, it is found as heterochromatin (condensed) and not euchromatin.

Telomere

These are specialized structure at the ends of eukaryotyic chromosomes. They maintain chromosomal integrity by preventing end-to-end fusion of chromosomes.

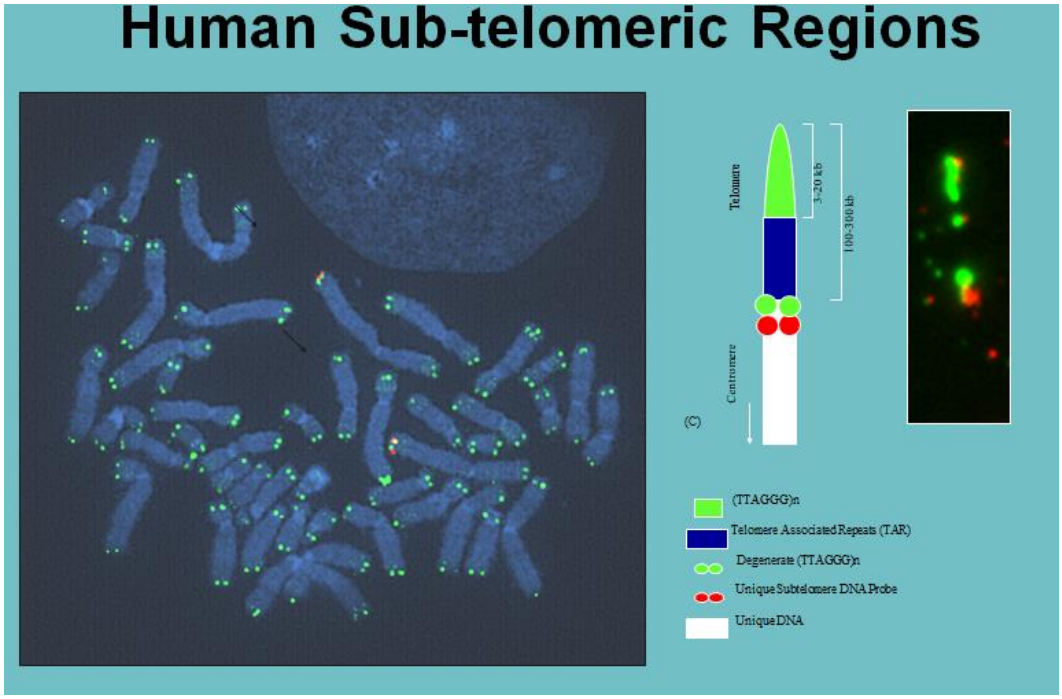
DNA polymerase cannot replicate the ends of the DNA strands, so successive replications would result with shortening of the DNA. So, noncoding telomeres protect the coding regions from being lost.



Telomerase is the enzyme that synthesizes the telomeres. It is active in children, and its activity deteriorates till adulthood.

Although cause ageing, telomeres have evolutionary protective functions against cancer, because they limit the adult cells from undergoing uncontrolled growth. In cancer cells (transformed cells), telomerase activity is activated again at a point in the carcinogenesis scheme, which renders the cancer cells immortal.

Structure of the telomeres is similar between all the chromosomes that we carry in our cells, although may differ in length. They are composed of tendemly repeated sequences of the hexanucleotide sequence (oligo sequence) TTAGGG repeated n times $(TTAGGG)_n$. Unlike telomeres, sub-telomere regions differ between chromosomes.



If we mix denatured (single stranded) chromosomes with a labeled probe (radioactive or fluorescence; green in the figure above) with a sequence which is complementary to the TTAGGG sequence, we will notice that all the chromosomes will have the label complemented at the same peripheral region (the telomere region), because it has the same sequence between all chromosomes. But for the sub-telomere regions, they are not identical in all chromosomes, and we will notice that a labeled probe (red in the figure above) will be complementary to some chromosomes and not others.

There is some sequence homology between sub-telomer

﴿إِنَّ في خلْقِ السَّمواتِ والأرضِ واختلافِ اللَّيلِ والنَّهارِ لآياتٍ لأولي الألباب ﴿الَّذين يَذْكُرونَ الله قياماً وقعوداً وعلى جُنوبهم ويتفَكَّرونَ في خلْقِ السَّمواتِ والأرضِ ربَّنا ما خلَقْت هذا باطلاً سبحانَك فَقِنَا عذابَ النَّار﴾

(آل عمران - 191.190)