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APPLICATIONS OF GENETICS IN MEDICINE

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Chapter I

GLOSSARY OF GENETIC TERMS

Alleles: Alternative forms of a genetic locus; a single allele for each locus is inherited separately from each parent (e.g., at a locus for eye color the allele might result in blue or brown eyes).

Amino acid: Any of a class of 20 molecules that are combined to form proteins in living things. The sequence of amino acids in a protein and hence protein function is determined by the genetic code.

Aneuploid: the abnormal number of chromosomes, which may be higher=hyperploidy (e.g. 2n+1) or lower=hypoploidy (e.g. 2n-1).

Autosome: A chromosome not involved in sex determination.

Chromosomes: The self- replicating genetic structures of cells containing the cellular DNA that bears in its nucleotide sequence the linear array of genes. In eukaryotes there is a number of chromosomes, whose DNA is associated with different kinds of proteins.

Congenital defect: a defect that is present at birth.

Diploid: A full set of genetic material, consisting of paired chromosomes, one chromosome from each parental set (2n). The diploid human genome consists of 46 chromosomes, 22 pairs of autosomes and 1 pair of sex chromosomes (XX or XY chromosomes).

Disease susceptibility/ predisposition gene: gene that tends to favor the development of a disease, in association with other genes and/or various environmental factors. For example, numerous cancer predisposition genes are known.

DNA (deoxyribonucleic acid): The molecule that encodes genetic information. DNA is a double- stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases: adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, base pairs form only between A and T and between G and C; thus the base sequence of each single strand can be deduced from that of its partner.

Dominant: a gene is expressed in heterozygosity and in homozygosity.

Eukaryote: Cell or organism with membrane-bound, structurally discrete nucleus and other well-developed subcellular compartments. Eukaryotes include all organisms except viruses, bacteria, and blue-green algae.

Euploid: the normal number of chromosomes that is characteristic for a cell.

Expressivity: qualitative aspect of the dominant gene, referring to the degree of severity of a disease determined by this gene/genes. ***Variable expressivity*** refers to the variable manifestation of this disease in different patients, even within the same family.

Gene: The fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (i.e., a protein or RNA molecule).

Genetic disorder: a disorder caused by alteration of the genetic material.

Genetics: The study of the patterns of inheritance of specific traits or diseases.

Genome: an organism's complete set of DNA, including all of its genes.

Genotype: The combination of particular alleles at specified loci (on homologous chromosomes) that determines a specific trait.

Haploid: A single set of chromosomes (half the full set of genetic material), present in the egg and sperm cells (n). Humans have 23 chromosomes in their reproductive cells.

Haplotype: a group of closely linked genetic markers or DNA variations located on a single chromosome that are typically inherited together as a unit

Hereditary disorder: a disorder caused by alteration of the genetic material inherited from the parents.

Heredity: Transmission of genetic characteristics from parents to offspring.

Hemizygosity: the genomic situation in which a gene is present in a single copy in a diploid individual, most commonly found in the case of genes located on the X chromosome in males, where there is no corresponding homologue on the Y chromosome.

Heterozygosity: The presence of different alleles at one or more loci on homologous chromosomes.

Homologous chromosomes: A pair of chromosomes containing the same linear gene sequences each derived from one parent.

Homozygosity: The presence of identical alleles at one or more loci on homologous chromosomes.

Karyotype: A photomicrograph of an individual's chromosomes, arranged in a standard format, showing the number, size, and shape of each chromosome type.

Locus (pl. loci): The position on a chromosome of a gene or other chromosome marker; also, the DNA at that position. The use of locus is sometimes restricted to mean regions of DNA that are expressed.

Messenger RNA (mRNA): RNA that serves as a template for protein synthesis.

Mosaicism: the presence of two or more cell lines within the same organism.

Nucleic acid: A large molecule composed of nucleotide subunits.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Thousands of nucleotides are linked to form a DNA or RNA molecule.

Oncogene: a gene that, when mutated or abnormally expressed, has the potential to cause normal cells to become cancerous.

Penetrance: the proportion of individuals with a specific genotype who actually express the associated phenotype.

Phenotype: All the traits of an individual (morphological, physiological, psychic).

Polymorphism: a genetic variant that occurs commonly in the population (typically in more than 1% of individuals) and is usually considered a normal variation rather than a cause of disease.

Recessive: a gene that is expressed only in homozygosity.

Ribonucleic acid (RNA): A chemical found in the nucleus and cytoplasm of cells; it plays an important role in protein synthesis and other chemical activities of the cell. The structure of RNA is similar to that of DNA. There are several classes of RNA molecules, including messenger RNA, transfer RNA, ribosomal RNA, and other small RNAs, each serving a different purpose.

Ribosomal RNA (rRNA): A class of RNA found in the ribosomes of cells.

Transfer RNA (tRNA): A class of RNA having structures with triplet nucleotide sequences that are complementary to the triplet nucleotide coding sequences of mRNA. The role of tRNAs in protein synthesis is to bond with amino acids and transfer them to the ribosomes, where proteins are assembled according to the genetic code carried by mRNA.

Variant or Mutation: Any heritable change in DNA sequence compared to a reference sequence; it can affect a single nucleotide, a larger segment of DNA (deletions, duplications, insertions), or chromosomal structure, with the potential to influence gene expression and the function of the encoded proteins.

Chapter II

MOLECULAR LEVEL OF HEREDITY

MOLECULAR METHODOLOGY AND GENETIC ENGINEERING

Genetic engineering is represented by the methods and technologies to manipulate an organism's DNA; it is used in order to generate new genes having a desired structure and serving either in the treatment of genetic diseases by gene therapy, or for the treatment of other disorders, by hormones, growth factors, insulin or other proteins synthesis.

Today, one of the foremost aims of medical genetics is to understand the basis for mutations that lead to genetic diseases, at a molecular level. This information can be used to improve the methods of diagnosis and treatment for many diseases.

In the past years, a number of techniques that allow the detailed analysis of normal and abnormal genes, were developed and used. They are useful not only in understanding the molecular processes, but also in providing the basis for many laboratory procedures for the detection and diagnosis of genetic disorders.

Molecular testing enables early and precise diagnosis of genetic diseases, guiding personalized treatment and informed family planning.

Future advances in molecular diagnostics promise broader accessibility and integration with AI, revolutionizing preventive care and therapeutic targeting.

Recombinant DNA and Gene Cloning

One of the key advances in the development of molecular analysis was the discovery of the **restriction enzymes**, also called restriction endonucleases, in bacteria. These enzymes are able to recognize specific sequences in DNA and then to cleave the DNA at the recognition site (fig. 1).

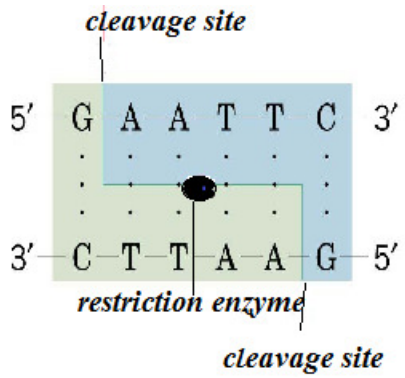


Fig. 1: Restriction enzymes cut within DNA molecule

Several hundred such enzymes are now known. They can digest the DNA molecules wherever those sequences are found in the genome. This property of the restriction enzymes has important implications due to their role in isolating genes and in recombinant DNA technology (fig. 2).

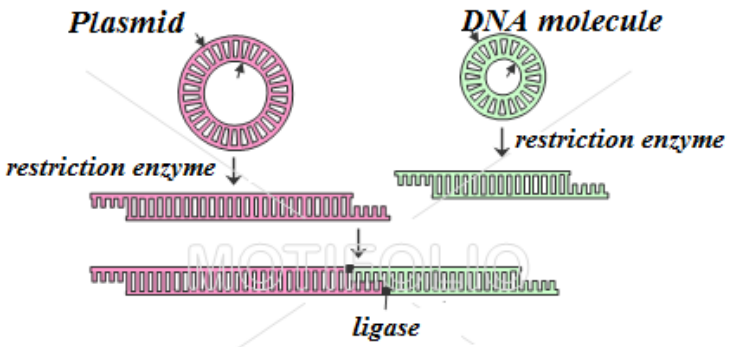


Fig. 2: Construction of recombinant DNA

Cloning refers to the process by which a DNA molecule is joined to another DNA molecule (termed as a vector) that can replicate autonomously in a special host, usually a bacterium. Various procedures for gene cloning are now in use.

The important steps to be made in all cases are:

- incorporation of the segment of DNA of interest into a vector
- multiplication, in order to produce many identical copies.

A **vector** is a DNA molecule that can replicate autonomously in a host. Usually, bacterial cells can be grown indefinitely in the laboratory and because replicating vectors can achieve a high number of copies per cell, a great number of DNA sequences of interest can be obtained. Examples of commonly used vectors, in molecular cloning are (fig. 3):

- **plasmids, or**
- **bacteriophages lambda**

Plasmids are naked circular double-stranded DNA molecules that replicate extrachromosomally, usually in bacteria and are useful for cloning smaller DNA fragments. The plasmids have been isolated from cells by density gradient procedures and then visualized with the electron microscope. They can be maintained in a moderate number of copies, so they are considered ideal for cloning the DNA sequences of interest.

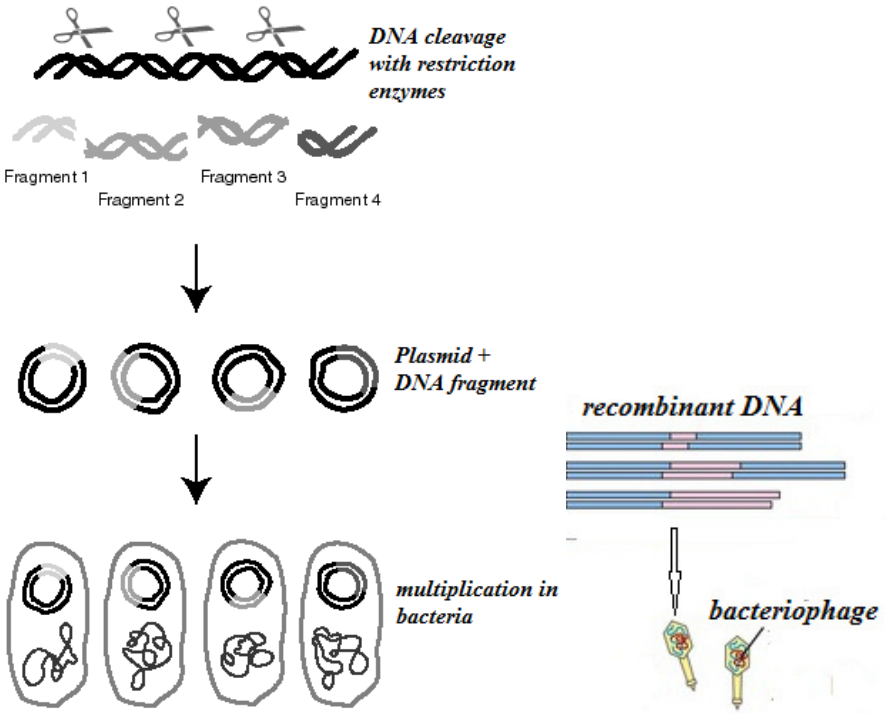


Fig. 3: Cloning using plasmids and bacteriophages

Bacteriophage lambda is a bacterial virus with a relatively large DNA molecule. It grows in E.coli and produces a huge number of infectious viruses, eventually killing the bacterial cells. Almost one third of the bacteriophage lambda is nonessential and can be replaced by other DNA sequences, thus being suitable for cloning fragments of human DNA.

Complementary DNA

Another tool used in human molecular genetics is obtaining complementary DNA (cDNAs) produced from purified mRNAs. The method is based on in vitro synthesis of a complementary DNA (cDNA) using a purified mRNA as a template. A single-stranded complementary DNA is initially synthesized by the enzyme reverse transcriptase (which catalyzes the reaction that is the reverse of transcription), and this single-stranded cDNA is then converted to a double-stranded molecule.

Construction of Libraries

Isolation of a particular DNA sequence is followed by its multiplication, usually by cloning the sequence of interest. The collection of clones is called a library. At the moment, there are two types of libraries:

a. **genomic libraries**, which contain millions of fragments from the entire genomic DNA, stored for the future isolation of the genes (or other DNA fragments of interest).
b. **complementary DNA libraries**, which represent copies of cDNA. These are often preferable to the first ones, because the obtained clone is a direct representation of the coding sequences (exons) without the noncoding sequences (introns) found in the genomic DNA.

Methods of Nucleic Acid Analysis

Nucleic acid analysis involves various techniques to isolate, quantify, and characterize DNA and RNA. Key methods include: extraction and purification, quantification (spectrophotometry, fluorometry, qPCR), amplification (PCR), separation (electrophoresis), sequencing (Sanger, NGS), and hybridization techniques (blotting, microarrays).

Nucleic Acid Extraction and Purification (fig.4):

This initial step isolates DNA or RNA from a biological sample, removing contaminants that could interfere with downstream analysis.

Methods include:

- Alkaline extraction: Specifically for plasmid DNA.
- Commercial kits: Offer standardized protocols for DNA/RNA extraction.

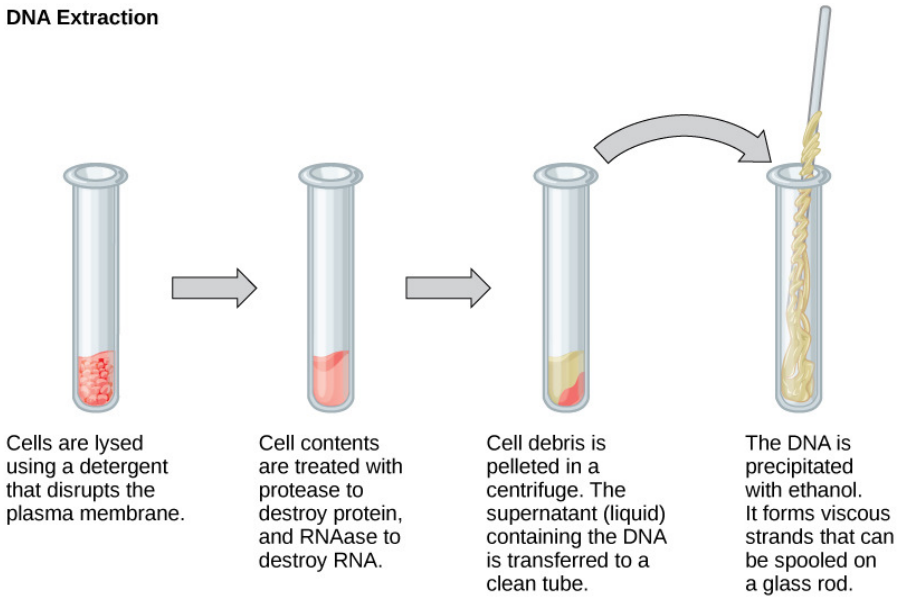


Fig. 4: Nucleic acid extraction

Nucleic Acid Quantification:

Nucleic Acid Quantification determines the concentration and purity of nucleic acids.

Common methods:

- Spectrophotometry: Measures absorbance at specific wavelengths to assess DNA/RNA concentration and purity.
- Fluorometry: Uses fluorescent dyes that bind to DNA or RNA, providing a more sensitive quantification.
- Real-time PCR (qPCR): Amplifies and quantifies specific DNA or RNA sequences.

Nucleic Acid Amplification:

- Polymerase Chain Reaction (PCR): Amplifies specific DNA sequences exponentially, enabling detection and analysis of rare DNA.
- Real-time PCR (qPCR): Allows for the quantification of DNA during the amplification process.
- Isothermal amplification methods: Amplify DNA at a constant temperature, offering advantages in certain applications.

Nucleic Acid Separation:

- Gel Electrophoresis: Separates DNA or RNA fragments based on size and charge, allowing for visualization and analysis.
- Capillary Electrophoresis: Provides higher resolution and automation compared to gel electrophoresis.

Nucleic Acid Sequencing:

- Sanger Sequencing: A classic method for determining the sequence of DNA.
- Next-Generation Sequencing (NGS): High-throughput sequencing technologies that enable the rapid sequencing of large amounts of DNA or RNA.

Nucleic Acid Hybridization:

- Southern Blotting: Detects specific DNA sequences using labeled probes.
- Northern Blotting: Detects specific RNA sequences using labeled probes.
- Microarray Technology: Allows for the simultaneous analysis of many DNA or RNA sequences.

Other Methods:

- Restriction Fragment Length Polymorphism (RFLP): Detects variations in DNA sequences by analyzing fragment sizes after restriction enzyme digestion.
- In situ hybridization: Localizes specific DNA or RNA sequences within cells or tissues.

The Polymerase Chain Reaction

The polymerase chain reaction (PCR) has the potential to amplify a single molecule of DNA or RNA several millionfold in a few hours. PCR has revolutionized the detection of RNA and DNA. Using this method, the detection and analyze of specific gene sequences in a patient′s sample is now possible. Analyses can be performed on even a single cell. PCR is based on the enzymatic amplification of a fragment of DNA, amplification that occurs exponentially. With the use of the so-called "PCR machines", a round of amplification takes only 10 minutes, so that in 1 or 2 hours, many millions of copies can be done (fig. 5).

The amplification process is mediated by single-stranded oligonucleotide primers, which have complementary sequences to the regions flanking the interest sequence; it takes place through several cycles (20 – 40) of:

- **Denaturation** = increasing the temperature (90-95C) and breaking the hydrogen bonds between the 2 strands of the sequence of interest;
- **Annealing** = binding of the primers to the complementary sequences of the molecule of interest;
- **Amplification in vitro** with the aid of heat-resistant DNA polymerases (e.g. Taq polymerases, 72C).

This technique is used to detect single base change mutations in a gene and can also be applied to the analysis of small samples of RNA, and facilitates cloning of specific genes for the analysis of mutations. PCR technologies are of special interest in prenatal diagnosis, where limited amounts of fetal DNA is usually available and in establishing someone's identity (in forensic medicine).

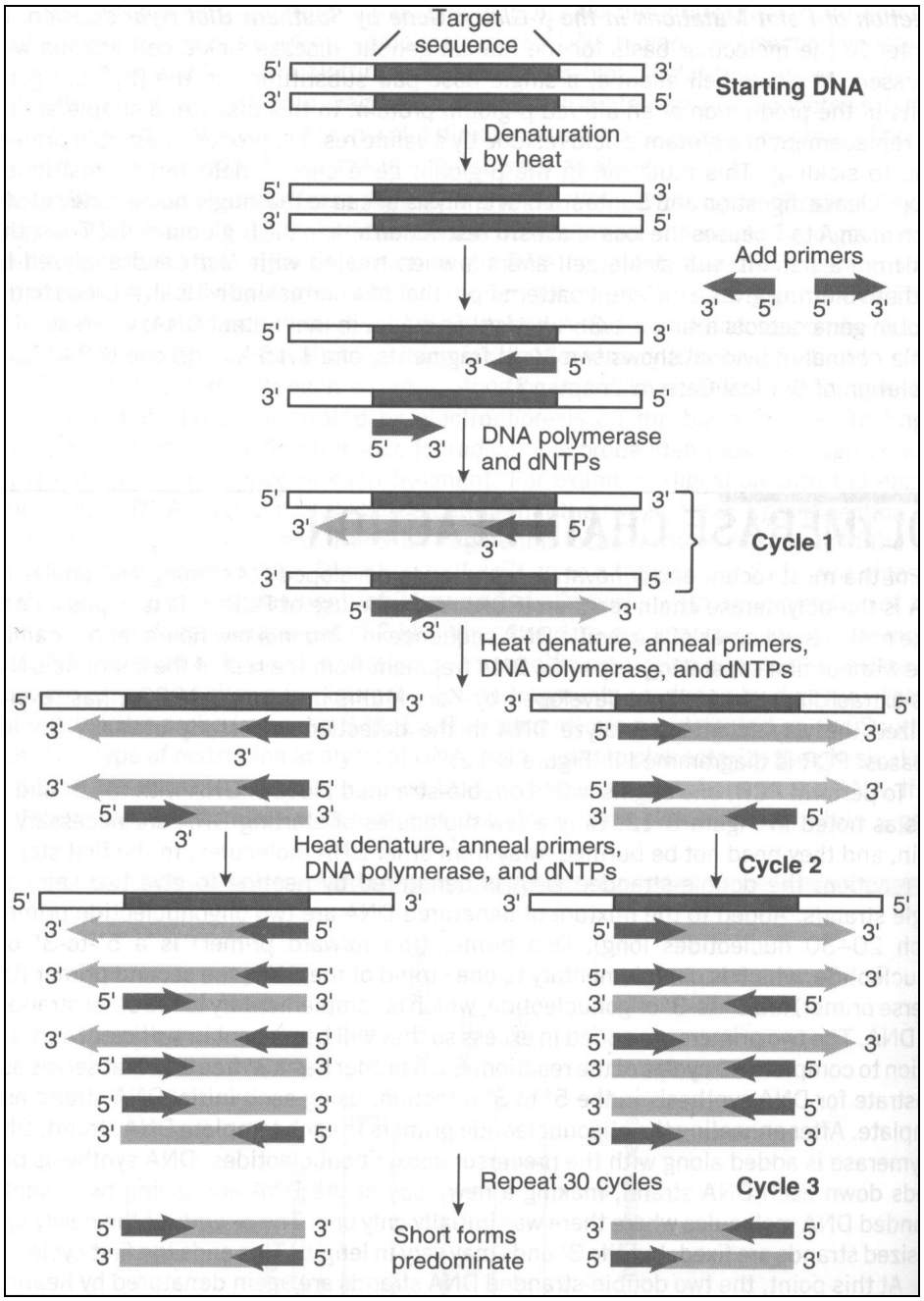


Fig. 5: PCR method of DNA fragments multiplication

Real-time PCR

While traditional PCR detects products at the end-point of the reaction real-time PCR will detect them while the reaction is occurring. It uses the same principle of amplification, but instead of studying the obtained bands on a gel at the end of the reaction, this process is monitored in "real-time".

There are different techniques that can be used to monitor the progress of the PCR reaction, all having in common that they link the amplification to the generation of fluorescence during each PCR cycle.

Real-time detection of PCR products is enabled by the inclusion of a fluorescent reporter molecule in each reaction, that yields increased fluorescence with an increasing amount of product DNA.

Specialized thermal cyclers equipped with fluorescence detection modules are used to monitor the fluorescence signal as amplification occurs. The measured fluorescence is proportional to the total amount of amplicon; the change in fluorescence over time is used to calculate the amount of amplicon produced in each cycle.

QF PCR analysis

Rapid testing for the common aneuploidies in prenatal and postnatal samples, and in the investigation of recurrent miscarriage, is routinely undertaken by a rapid polymerase chain reaction (PCR)-based method.

It includes amplification, detection and analysis of chromosome-specific DNA sequences known as genetic markers or small tandem repeats (STRs).

Fluorescently labeled marker specific primers are used for PCR amplification of individual markers and the copy number of each marker is indicative of the copy number of the chromosome. The resulting PCR products may be analyzed and quantified using an automated genetic analyzer.

The genetic markers/STRs may vary in length between individual chromosomes and subjects, depending on the number of repeated STRs. The relative copy number of each allele is determined by calculating the ratio of the peak areas or peak heights detected for each marker (fig.6).

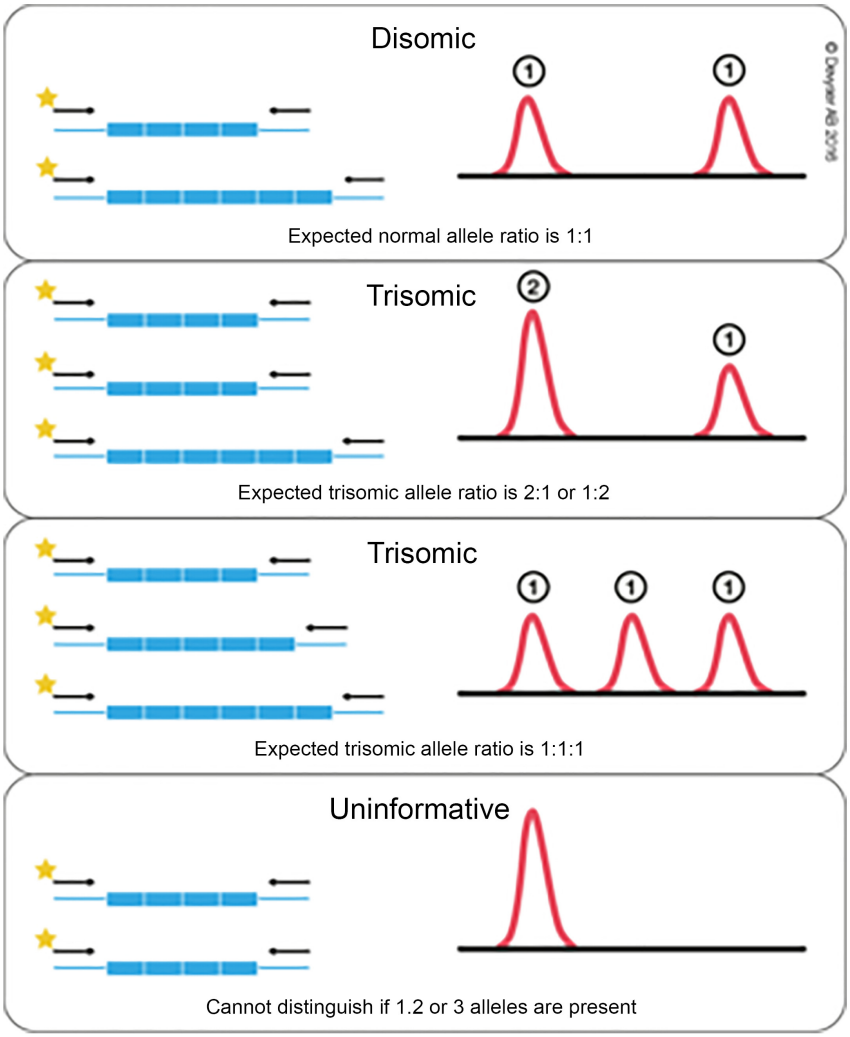


Fig. 6: The principle of rapid aneuploidy analysis.

A normal diploid sample has the contribution of two of each of the investigated chromosomes. Two alleles of a chromosome specific marker are detected as two peaks in a 1:1 ratio when the marker is heterozygous and as one peak when the marker is homozygous (have alleles of same length). The detection of an additional allele as three peaks in a 1:1:1 ratio or as two peaks in a 2:1/1:2 ratio indicates the presence of an additional marker copy possibly corresponding to an additional chromosome, as in the case of trisomy. Subjects who are homozygous or monosomic for a specific marker will display only one peak. https://devyser.com/blog/principles-of-qf-pcr

Sequencing methods

In this method, the entire gene is sequenced in order to identify the presence of any mutation. Complete gene sequencing is considered as a gold standard method because it can identify any type of mutation.

DNA sequencing (Sanger method)

The simplest method of sequencing DNA was invented by Dr. Fred Sanger for which he was awarded his second Nobel Prize. The Sanger method is also known as the "dideoxy chain termination method" (fig. 7).

- The DNA must be obtained in pure form, either by cloning or by PCR.
- Separation of the DNA strands.
- A primer is allowed to anneal to known sequence at one end of the target sequence.
- Deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP) and DNA polymerase are added and the primer sequence is thus elongated along the target "template" DNA.

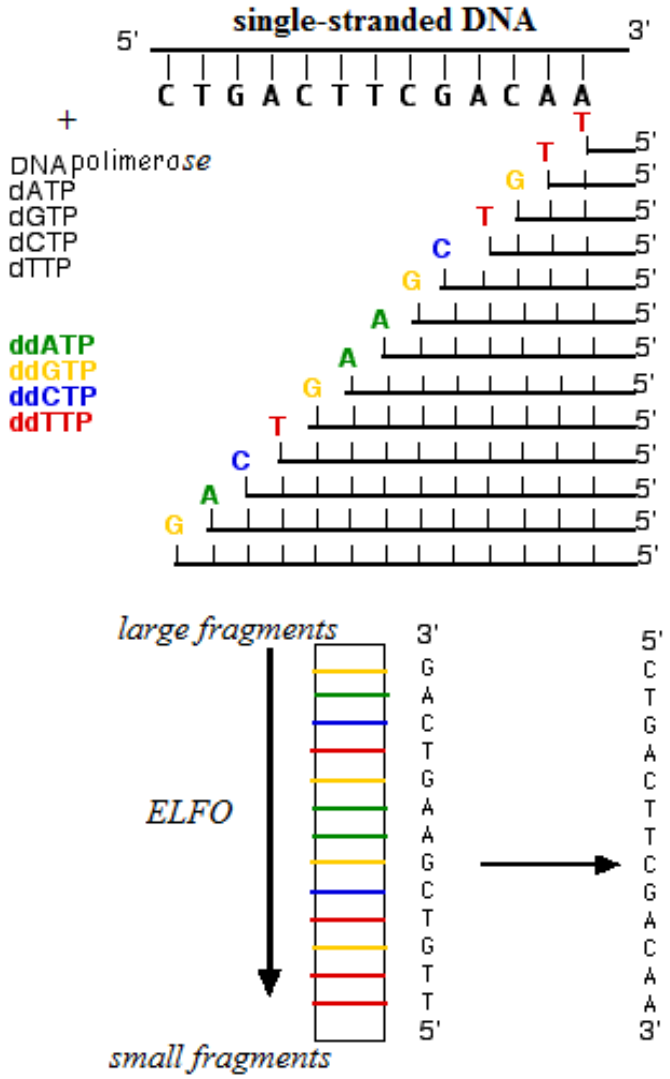


Fig. 7: DNA sequencing using modified (dideoxi-) labelled nucleotides

- Adding small amounts of the four dideoxynucleotide triphosphates (ddNTPs), each tagged with a different coloured fluorescent dye. When a molecule of ddNTP is incorporated into an elongating DNA strand it prevents further chain elongation because it lacks the necessary terminal 3' OH group.
- The products of synthesis are then separated according to size by electrophoresis on a polyacrylamide gel.
- As the products pass a point on the gel one by one, the coloured dye tags can be read by a laser scanner and computer.

Next generation sequencing

Next generation methods of DNA sequencing have three general steps (fig. 8):

- ❖ Library preparation: libraries are created using random fragmentation of DNA, followed by ligation with custom linkers
- ❖ Amplification: the library is amplified using clonal amplification methods and PCR
- ❖ Sequencing: DNA is sequenced using one of several different approaches

These technologies rely on PCR to grow clusters of a given DNA template, attaching the clusters of DNA templates to a solid surface that is then imaged as the clusters are sequenced by synthesis in a phased approach.

The new generation of sequencing technologies interrogate single molecules of DNA, such that no synchronization is required (a limitation), thereby overcoming issues related to the biases introduced by PCR amplification and dephasing.

More importantly, these technologies have the potential to increase read length (from tens of bases to tens of thousands of bases per read) and time to result (from days to hours or minutes) (generate over 10,000 bp reads or map over 100,000 bp molecules).

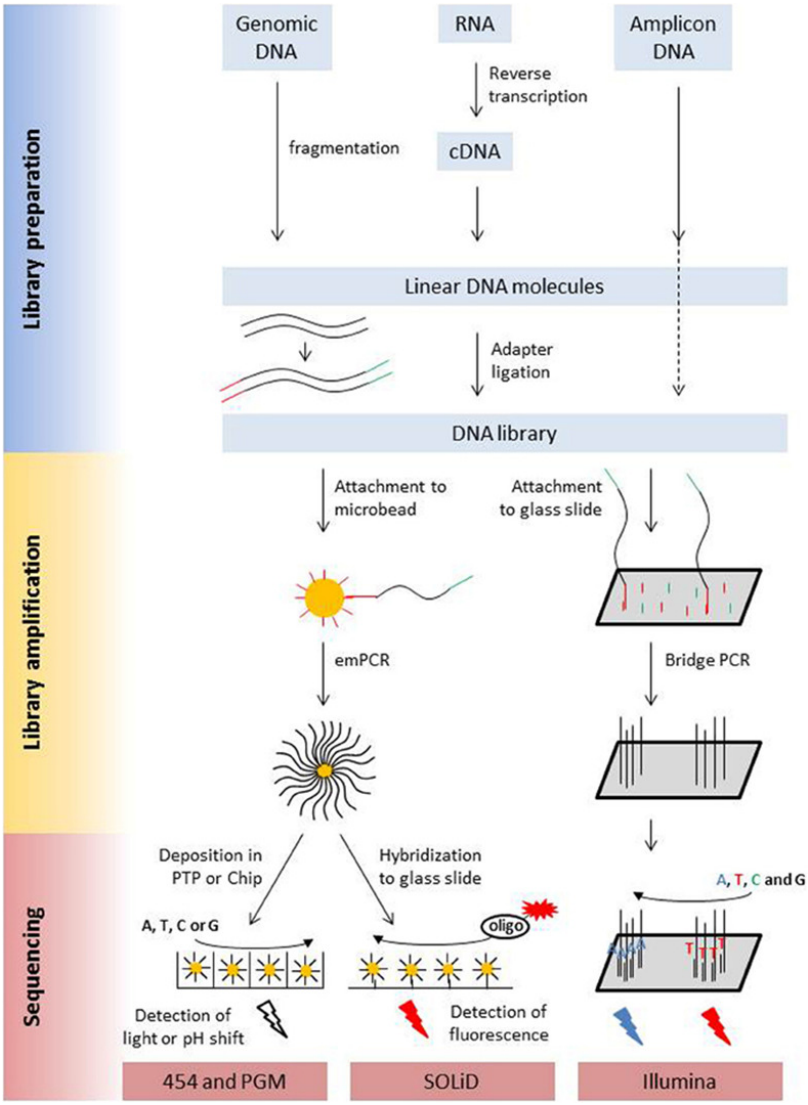


Fig. 8: DNA sequencing using next generation techniques

Whole genome sequencing

Whole genome sequencing (WGS) is a method that identifies the complete DNA sequence of an organism (fig. 9).

It sequences the entire genome including both coding and non-coding regions using different automated DNA sequencing techniques and bioinformatic tools.

WGS provides a comprehensive view of the entire genome. Patient´s DNA is fragmented and sequencing data are generated for the entire genome. Data analysis may then be restricted to a subset of genes relevant to the patient's features using a virtual panel. All data are stored.

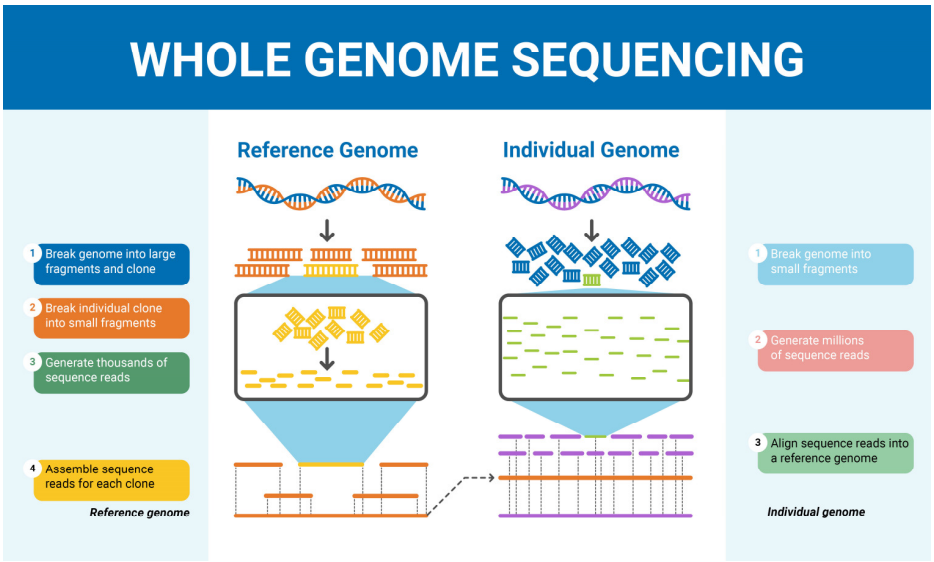


Fig. 9: Whole genome sequencing (https://sequencing.com/education-center/whole-genome-sequencing?srsltid=AfmBOooBc1AAKv5_twtat6Ww2VIdMaiPO72BFYIWR1HM_eLInawzr0_4)

Whole exome sequencing

Whole exome sequencing (WES) seeks genetic mutations tied to protein function variations. The basic principle encompasses (fig. 10):

- DNA Capture and Enrichment: DNA or RNA probes specific to exon regions are first used to capture and enrich DNA sequences. Typically, this employs the principles of base pairing to hybridize biotin-labelled RNA probes with DNA libraries with adapter sequences, and then enriches the DNA of the target area through magnetic bead binding.
- High-Throughput Sequencing: The enriched DNA sequences are then sequenced via high-throughput sequencing technology. Sequencing is the process of discovering all deoxyribonucleotide arrangements in the exome, which may help us understand potential pathophysiological changes in certain diseases.
- Data Analysis: The sequencing results are subjected to bioinformatic analysis to pinpoint genetic mutations associated with protein function variations.

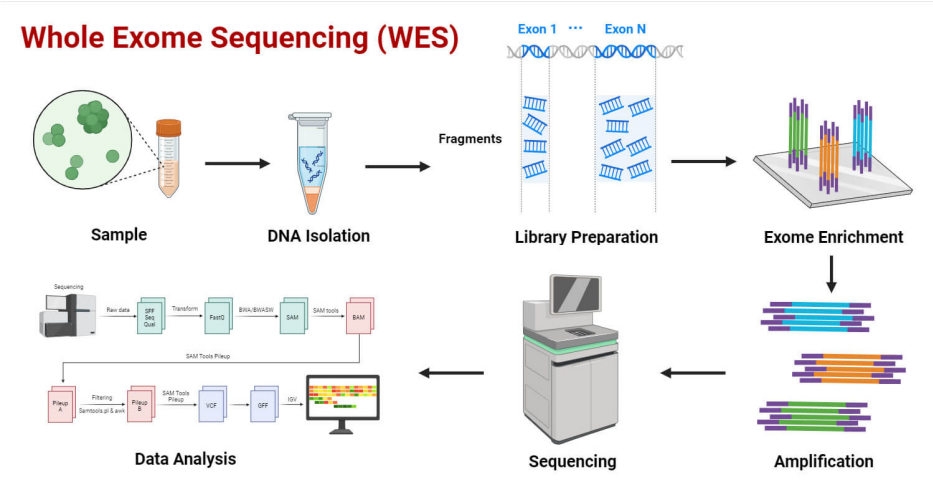


Fig. 10: Whole exome sequencing (https://microbenotes.com/whole-exome-sequencing/)

Advantages (fig. 11):

- generates a smaller and more manageable dataset, making analysis quicker and easier compared to whole-genome sequencing, reducing processing time and data storage requirements.
- can analyze all coding regions of the genome simultaneously.
- allows large-scale screening, useful for discovering mutations.
- is cost-effective compared to whole-genome sequencing.

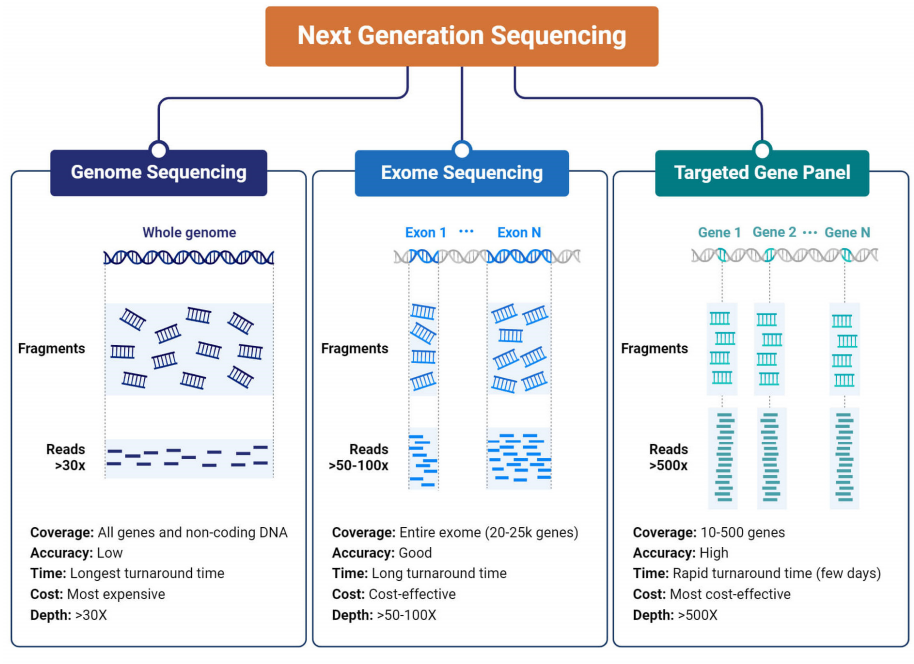


Fig. 11: Next generation sequencing (https://microbenotes.com/whole-exome-sequencing/)

Nucleic acid hybridization techniques

In order to find and analyze a specific DNA fragment of interest, among millions of DNA fragments found in the genomic DNA, methods based on **nucleic acid hybridization** can be used (fig. 12).

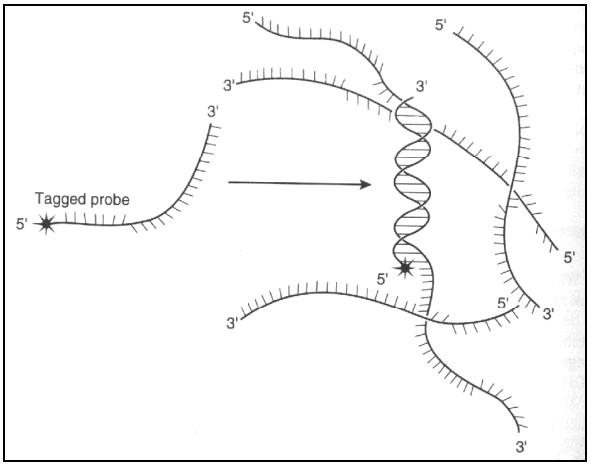


Fig. 12: Molecular hybridization

Principle: The two complementary strands of DNA are denatured, usually at high temperature. Single-stranded nucleic acids, which may be DNA or RNA, are mixed with a specific probe and tagged by a radioactive or fluorescent tracer, to allow its subsequent detection. Under appropriate conditions that favor formation of renatured double-stranded DNA or of DNA-RNA molecule, the probe hybridizes only to its complementary sequence, ignoring all the other sequences that have a different nucleotide sequence.

A probe is represented by nucleic acid molecules (usually DNA) that are used in the hybridization reactions. For example, a probe for the β-globin gene might be used to analyze a sample of a patient′s DNA for a particular mutation thought to be responsible for a sickle cell anemia. As seen, probes are essential in identification of gene structure and for specific mutation detection.

Southern blotting

Southern blotting technique is another method used for analyzing the structure of DNA cleaved by restriction enzymes.

The first step to be made is the isolation of genomic DNA, usually from lymphocytes obtained by routine venipuncture.

The DNA sample is then cleaved by restriction enzymes in many smaller fragments.

These fragments are separated on the basis of size by agarose gel electrophoresis, in which small fragments move rapidly, in contrast to the larger ones, which move slowly.

Double-stranded DNA fragments are then denatured and single-stranded DNA, molecules are transferred from the gel to a nitrocellulose or nylon filter by blotting and capillarity.

To identify the DNA fragment of interest, a specific labeled probe is used.

This probe will hybridize only to its complementary strand on the filter.

The unbound probe is removed (by washing the filter) and the filter is exposed to X-ray film to reveal the position of the DNA fragments to which the labeled probe hybridized (fig. 13).

Specific radioactive bands show up on the X-ray film (fig. 14, 15).

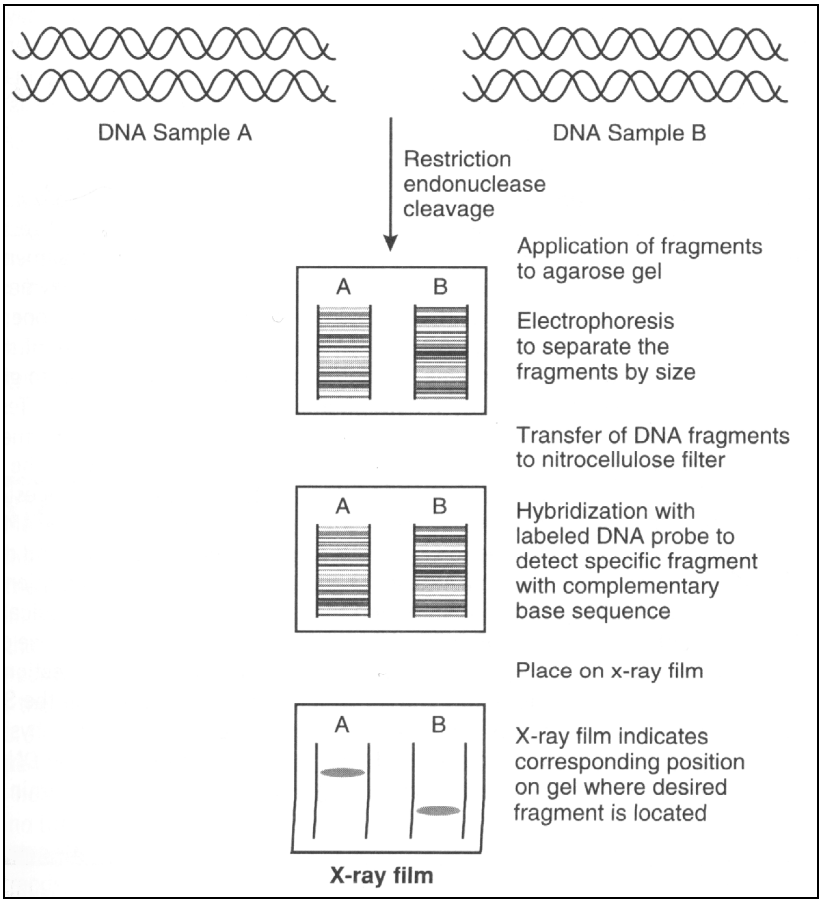


Fig. 13: Southern blotting-steps

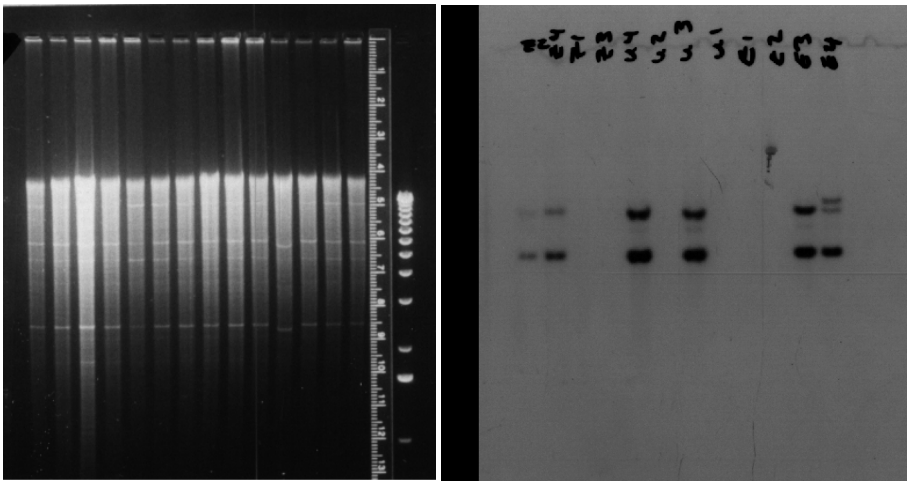


Fig. 14: Southern blotting

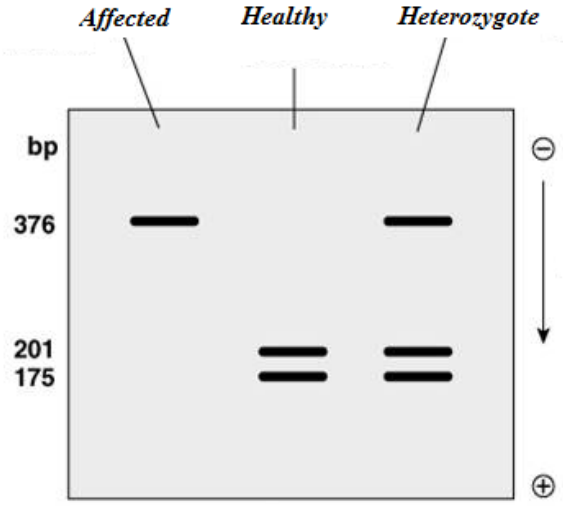


Fig. 15: Southern blotting method that highlights differences in migration between fragments of DNA from a patient with sickle cell anemia, a healthy person and a heterozygote

DNA microarray technology

This method is also based on base-pairing or complementarity. The potential applications of microarray technology are gene expression profiling and identification of gene sequences.

In this method, a large number of DNA fragments are placed on a glass slide. The fragments are allowed to bind with labeled DNA fragments (probes), which hybridize with the DNA on the glass slide. The amount of hybridization is then analyzed in each spot on the slide. The genes are given a color where the hybridized ones are coloured red and the genes that are hybridised least are colored green (fig.16).

When microarrays are used to compare mRNA expression in healthy and diseased cells, the following conventions are used:

- cDNA from healthy cells are typically tagged with green fluorescence, while cDNA from diseased cells are tagged with red
- This enables identification of genes present only in healthy cells (green), diseased cells (red), both (yellow) or neither (none)

It has been reported that microarray technlogy could be used in monitoring chromosome gains and losses, tumour classification, drug discovery and development.

Also the technique comes handy in detecting mutations and further investigating the mechanism of tumour development.

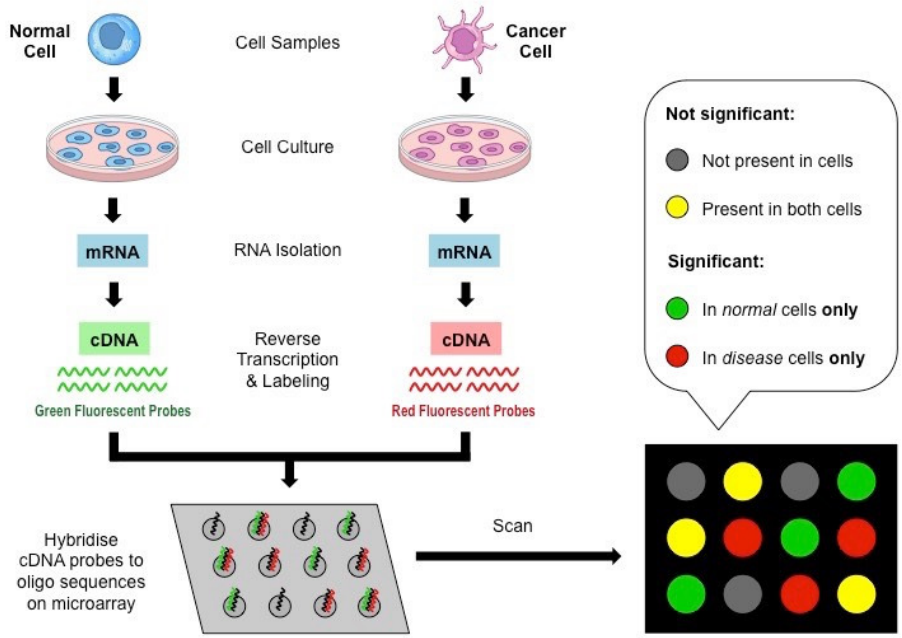


Fig. 16: DNA microarray overview (https://old-ib.bioninja.com.au/options/untitled/b4-medicine/dna-microarrays.html)

MUTATION/VARIANT DETECTION

CLASSIFICATION OF METHODS

The methods used for mutation/variant detection can be classified into:

Known Mutation	**Unknown Mutation**
- direct test - quick and easy - diagnostic - cheap - 100% effective	- used for screening - long and laborious - time scale for results not known - problems with polymorphisms - robotics needed - variability in effectiveness

Methods for detection of known mutations

- PCR and size separation eg. Duchenne muscular dystrophy
- Oligonucleotide ligation assay (OLA) eg. Cystic fibrosis
- Allele specific amplification (ASA), e.g. Cystic fibrosis
- Allele specific oligonucleotide hybridisation (ASO): Cystic fibrosis
- DNA sequencing

Methods for detection of unknown mutations

Gross Mutations

- Cytogenetic or molecular cytogenetic methods
- Southern blotting eg. 21 hydroxylase gene
- Pulsed Field Gel Electrophoresis eg. Facioscapulohumeral muscular dystrophy

Small Mutations

1. Physical methods
    - Denaturing gradient gel electrophoresis
    - Single stranded conformation polymorphism analysis
    - Heteroduplex analysis
2. Mismatch Cleavage
    - Chemical Cleavage Mismatch
3. Ribonuclease Cleavage

Chapter III

THE CHROMOSOMAL BASIS OF HEREDITY

Chromosome Morphology

Chromosomes are the vehicles of heredity. They contain almost all the DNA of the cell, with the exception of the tiny amount of DNA from the mitochondria.

The term chromosome (chroma=color, soma=body) is used because these organelles stain deeply with certain dyes.

Each species has a characteristic chromosome number and morphology. The normal human somatic cells have 46 chromosomes. This is called the diploid number of chromosomes (or 2n chromosome complement). They are grouped into homologous pairs. The members of a homologous pair match with respect to the genetic information carried by each one. One member of the homologous pair is inherited from the mother and the other one from the father. On the other side, one member of the homologous pair is transmitted to the child.

Each somatic cell contains 44 autosomes, which are alike in males and females and two sex chromosomes. These differ in males and females. Thus, the males have XY and the females have XX.

The gametes have only 23 chromosomes. This is called the haploid number of chromosomes (or n chromosome complement) and only one member of each chromosome pair is found.

Chromosomes can be seen with the light-microscope only during division. The best time to study their morphology is the phase called metaphase

The chromosomes are not seen in the nondividing nucleus, but they are nevertheless present in the nucleus. They are invisible at this time because the chromatin fiber of each chromosome is in highly elongated state (not condensed).

When describing chromosome morphology, we customary refer to the metaphasic chromosome because at metaphase they are in a compact state that can be studied easily with the light microscope.

Each chromosome is composed of (fig.17):

1. Two parallel subunits called sister chromatids, which are identical, being resulted from the replication of DNA.
2. A centromere, a narrow region common to both chromatids. This is also called primary constriction or kinetochore.
   The location of the centromere is a constant feature of each chromosome. There are three types of human chromosomes, classified by centromere position:
   a. metacentric, when the centromere is central:
   b. submetacentric, if the centromere is off-center
   c. acrocentric, when the centromere is near the end.
   The centromere divides each chromatid into two arms. The arms that are above the centromere are called "p"arms and those that are under the centromere are called "q" arms.
   In the metacentric chromosomes, "p" arms are equal to the "q" arms; in submetacentric chromosomes, the "p" arms are shorter than the "q" arms and in the acrocentric ones, the "p" arms are very, very short.
3. Some chromosomes have a secondary constriction, which is represented by another narrow region. It is especially found in chromosomes 1, 9, 16 and also in the acrocentric ones.
4. The acrocentric chromosomes may have satellites. These are small masses of chromatin, attached to the short arms by the secondary constrictions.
5. The end of each chromatid is called telomere. It stabilizes the chromosomes. If the telomeres are lost (bitelomeric deletions), the remained parts of the chromosome tend to get a ring shape. (ring-chromosome).

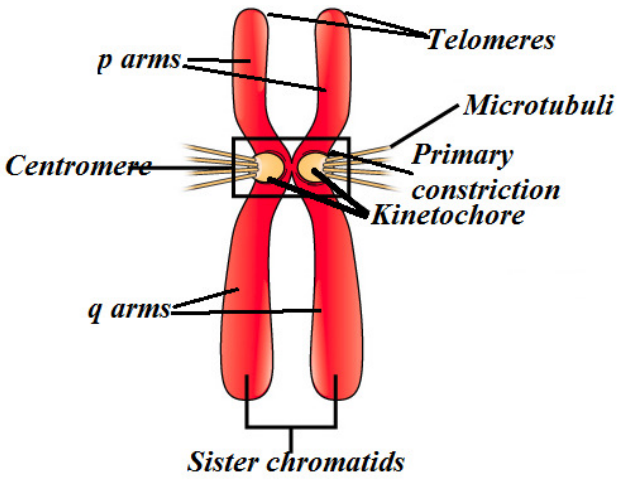


Fig. 17: Elements of chromosome morphology

Chromosome Polymorphisms

Chromosome polymorphisms represent variations from the normal morphology of the chromosomes, in healthy individuals. There can be variations in the amount of highly repetitive DNA sequences, which are detectable either by C-banding, or using Q or R-banding.

Enlarged satellites on the acrocentric chromosomes are frequently found; sometimes even a double-pair of satellites appears. Other morphological variations can concern the length of the homologous chromosomes, the centromere's elongation in chromosomes 1, 9, 16, or variations in the size of the secondary constrictions.

The Y-chromosome also shows variations in the amount of its heterochromatin, and thus, in its length. Using quinacrine-staining, a bright body is visible in interphase nuclei and it is called the Y-body.

Usually, polymorphisms are transmitted unchanged from parent to child and they have an autosomal dominant inheritance. Thus, they represent chromosome markers, useful in family studies or for determination of the parental origin of an abnormal gamete, in chromosomal abnormalities. The study of the chromosome polymorphisms can also be used as an auxiliary method to determine the twin zygosity or the paternity.

PRINCIPLES AND TECHNOLOGY FOR CYTOGENETIC ANALYSIS

Indications for cytogenetic analysis

- Confirmation or exclusion of the diagnosis for known chromosomal syndromes.
- Unexplained psychomotor retardation with or without dysmorphic features.
- Abnormalities of sexual differentiation and development.
- Infertility, sterility.
- Recurrent miscarriage.
- Pregnancies shown to be at risk of fetal aneuploidy.
- Monogenic disorders associated with dysmorphic features, which may indicate the presence of some microdeletion (along the chromosomes).
- Hematological malignancies, for the identification of specific chromosomal aberrations, which may be valuable in diagnosis and management.

Tissue samples for cytogenetic analysis

Chromosome preparations for analysis are made from dividing cells:

- either directly from tissue samples, such as:
  - neoplasic tissue,
  - chorionic villi,
  - testis
- or following cell culture, as for:
  - peripheral blood,
  - bone marrow,
  - biopsy of skin,
  - amniotic fluid cells,
  - fetal tissue

Direct Chromosome Preparation

In order to perform cytogenetic studies by the direct method, bone marrow is frequently used.

Procedure:

- 1-2 ml bone marrow is aseptically obtained into a heparinized syringe.
- 5 to 10 drops of marrow are added to 10 ml growth medium.
- Mitosis is arrested with colcemid, to which the cells are exposed for 30 minutes, at 37°C. Following colcemid treatment the culture is harvested. The most important next steps are:
- The culture tubes are centrifuged (8 minutes at 800 rpm) and the supernatant is discarded.
- The remained cell button is resuspended in a hypotonic solution (0,075 M KCl) and is incubated at 37°C, for about 17-18 minutes.
- Fresh made fixative is added and then the culture tubes are centrifuged.
- The supernatant is discarded and the cells are resuspended in fixative (5-10 ml).
- The above step is repeated for 3 times. The culture tubes are let to stay with the fixative for 5, 15 and 10 minutes. These periods can be modified, but it is essential that every lab establishes a standard protocol and sticks to the same regimen.
- After the final centrifugation, the cells are suspended in a small volume of fixative and chromosome slide preparations are made.
- The chromosome preparations are then stained using one of the banding methods, usually G-banding (Giemsa-banding).
- The obtained G-banded metaphases are analyzed at the light microscope and photographed.
- The last step to be made is the karyotype.

The bone marrow samples offer the advantage of immediate harvest due to continuous proliferation. On the other hand, there are some disadvantages too. The chromosomes tend to be more condensed, so they appear short and stubby and they are very sensitive to the pretreatment used for banding. Using bone- marrow, karyotypes can also be performed by a 24 hours culture.

Bone marrow short term culture

Bone marrow can be obtained by the relatively invasive, aseptically procedure of marrow biopsy. From 1-1,5 ml. bone marrow obtained into a heparinized syringe, about 0,5 ml marrow is introduced in a culture tube with 10 ml growth medium and incubated at 37°C, for 24 hours and then are treated with colcemid. The next steps, namely harvest the culture and preparation of slides, are the same as for the direct method.

Chromosome Analysis of Peripheral Blood

Chromosome preparations of peripheral blood are obtained from cell cultures. Samples should be placed in the sterile container, heparinized. The most frequent used samples for routine analysis are samples of peripheral blood from which lymphocytes chromosomes are prepared. Usually, 2-5 ml sample of heparinized venous blood is necessary. Cell culture takes 3 days. The cells are incubated at 37°C in special medium, with fetal serum and mitotic stimulants (e.g. Phytohemagglutinin).

Setting up the culture is followed by harvesting the lymphocyte cultures:

Colcemid is added in order to arrest mitosis during metaphase (this is the phase when chromosomes can be seen in their characteristic form, with the two chromatids). After 45 minutes of colcemid treatment, it is removed by discarding the supernatant after the culture tubes were centrifuged (for 8 minutes at 800rpm).

Hypotonic solution (KCl) is added gently (usually prewarmed at 37°C) and is incubated at 37°C, for 17 minutes.

A few drops of freshly made fixative are added and the culture tubes are again centrifuged. The supernatant is discarded and the cells are suspended in fixative. The fixative is then changed 2 times, using the protocol described at the direct method.

Slide making follows harvesting. Pre-cleaned slides are used. A few drops of suspension are dropped along the slide. Cell concentration and chromosome spreading is checked. The chromosome preparations are stained usually by Giemsa banding. Other banding techniques are used when appropriate.

The obtained metaphases are analyzed using the light-microscope and some of them are photographed and then karyotyped. Thus, the normal or abnormal chromosome number and structure can be precisely found out.

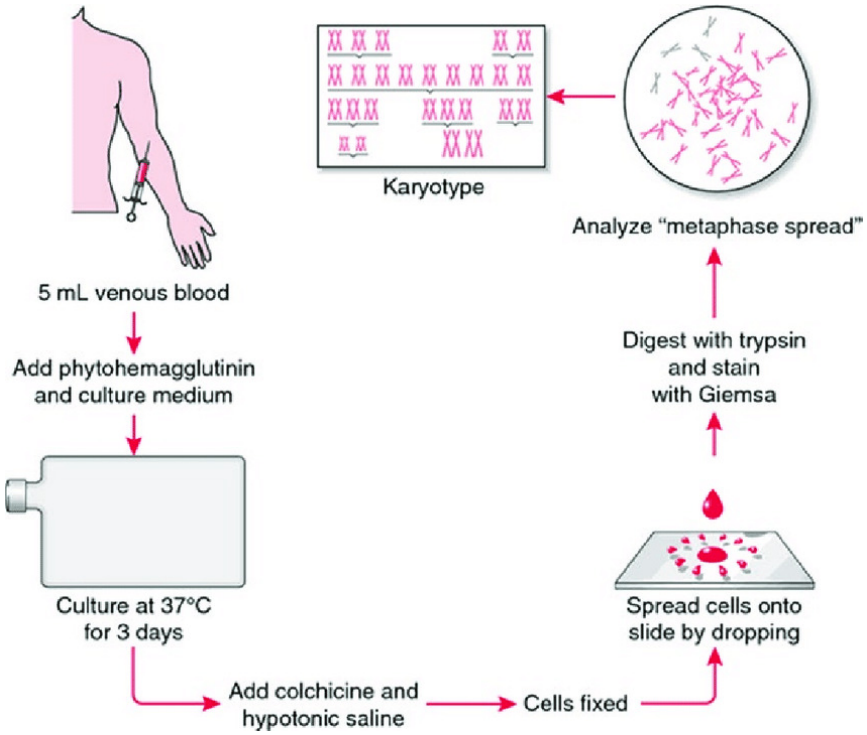


Fig. 18. Chromosome analysis of peripheral blood

Chromosome Analysis of Amniotic Fluid

15-20 ml of amniotic fluid are obtained in sterile tubes. These are spun at 800 rpm for 8 minutes. The supernatant is removed. It might be used for alpha-fetoprotein analysis. For chromosome analysis, the cell button is necessary (fig.19).

Amniotic cells cultures are prepared using a complete culture medium. They are incubated in CO_2 atmosphere at 37°C. setting up amniotic cells cultures requires a prolonged period of time (7-10 days). Colcemid is used to arrest mitosis, but this treatment takes 2-3 hours. A treatment with trypsin-EDTA is then necessary. Hypotonization and the following steps during which the fixative is added are the same as for peripheral blood, but prolonged periods are required.

Slides are then made and the chromosome preparations are G-banded.

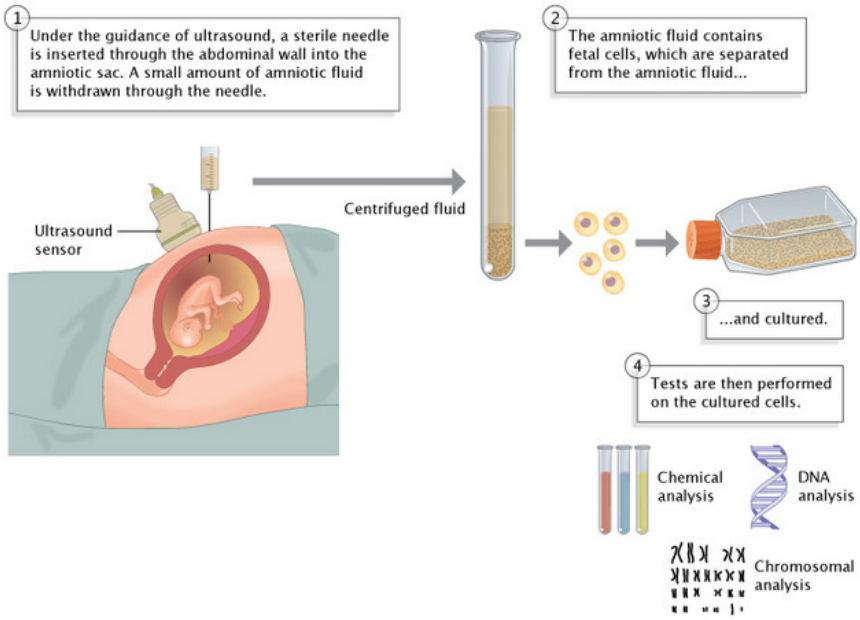


Fig. 19. Obtaining Fetal DNA through amniocentesis

Chromosome Study of Chorionic Villus Sample

Chorionic villi are composed of an outer layer of trophoblastic cells and an inner mesenchimal core that contains cellular elements found in an abundant intercellular substance. The fibroblasts that exist in the villous core are capable of rapid proliferation in vitro and they represent an appropriate material for diagnostic purposes.

The typical sample of chorionic villi consists of 10 to 40 mg of chorionic villus tissue. This sample is divided in two parts that are necessary for direct preparations and for culture, respectively.

In order to initiate the preparations, it is necessary to disaggregate the villi and release the cells. This can be realized by removing the trophoblastic layer and then by breaking down the intercellular substance of the core.

Direct preparation

The clean free of decidua villi are added to a culture medium and then they are incubated in CO_2 atmosphere at 37°C for several hours. The next important steps are:

- arresting mitosis during metaphase
- adding the hypotonic solution
- adding fixative
- slide making and banding

Each step must be performed carefully, following the existing standards.

Culture method

The clean villi are incubated for 1 hour at 37°C with a collagenase solution and for another hour with Trypsin-EDTA. The cell clumps are broken up. A special culture medium is required. Usually, cells are ready for harvest in 5-7 days. The other steps are the same as those used for amniotic fluid.

Chromosome analysis of solid tissues

Sometimes, the standard method of chromosome analysis using whole blood must be completed with the chromosome study of skin fibroblasts. One of its indications is when the standard test has shown evidence of a mosaic condition.

Chromosome analysis of fetal tissue is performed when a chromosome abnormality in a stillborn or aborted fetus is suspected. Strict aseptic conditions are necessary. A minimum of two cultures per tissue type must be setup. The tissue is first dissociated, then cultured in special media, a prolonged period, until there are sufficient actively dividing cells for harvest. The other steps are similar to those mentioned above.

Chapter IV

HUMAN CHROMOSOME CLASSIFICATION

The human chromosome classification is called karyotyping and the obtained picture is called a karyotype. The karyotype shows all the chromosomes from one metaphase. They are arranged by pairs of homologous. The autosome pairs are numbered from 1 to 22. There can be distinguished seven major groups of chromosomes and they are generally referred to as groups A-G. The criteria used to classify the chromosomes into these seven groups were defined at "The Denver Conference of Cytogenetics" in 1960, refined at the Chicago Conference in 1966 and in Paris in 1971.

These criteria were:

- the total length of the chromosome, and
- the centromere position

With the advanced methods of staining, chromosomes appeared banded and the position of the euchromatic and heterochromatic bands was used as another criteria.

The pairs of homologous chromosomes were classified into seven groups (from A to G) on the basis of their overall length and the position of the centromere. With techniques in common use, all the chromosomes can be individually identified.

Group A: contains three pairs of chromosomes, the pairs numbered with 1-3. They are large chromosomes; pairs 1 and 3 are metacentric, but pair no. 2 is submetacentric.

Group B: contains two pairs of chromosomes, numbered with 4 and 5. They are also large (though they are smaller than the A-ones) and submetacentric.

Group C: contains seven pairs of chromosomes, numbered from 6 to 12. They have a medium size and they are submetacentric. Group C also contains the X chromosome (or 2 X-chromosomes in females).

Group D: contains three pairs of chromosomes, numbered 13, 14 and 15. These are of a medium size and are acrocentric. They may have satellites.

Group E: contains the next three pairs of chromosomes, namely pairs no.16, 17 and 18. The chromosomes from pair no.16 have a medium size and they are metacentric, but the others are small and submetacentric.

Group F: contains two pairs of chromosomes, numbered with 19 and 20. They are small and metacentric.

Group G: contains the pairs number 21 and 22. They are small and acrocentric. They may have satellites. Group G also includes the Y-chromosome, in males. It never has satellites and its long arms are parallel.

Normal male and female karyotypes are shown in fig. 20 and fig. 21, respectively.

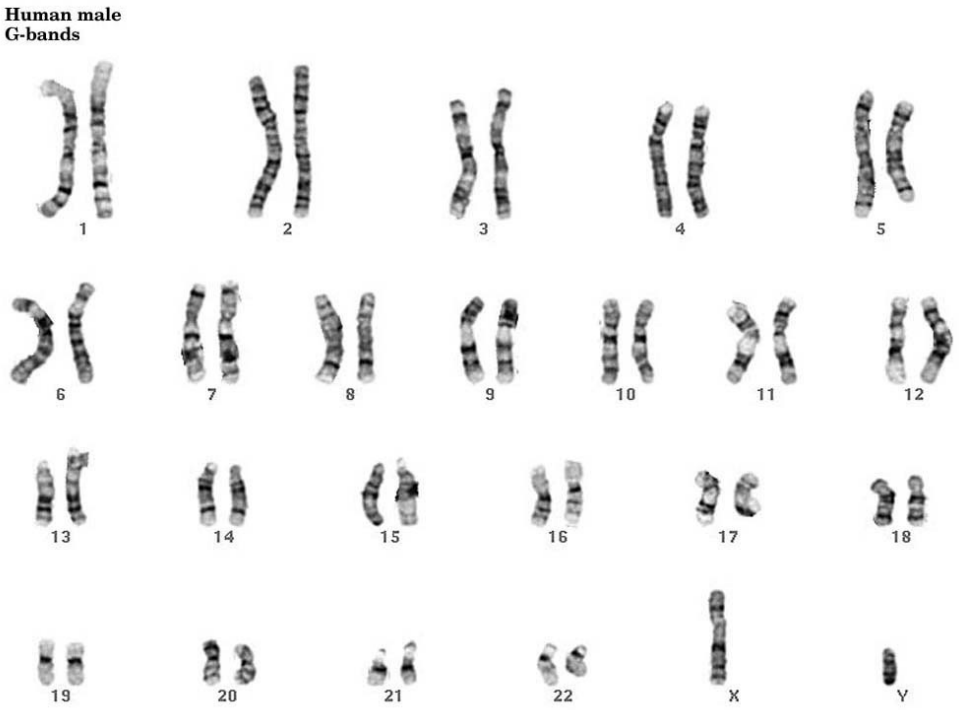


Fig. 20: Normal male karyotype: 46,XY

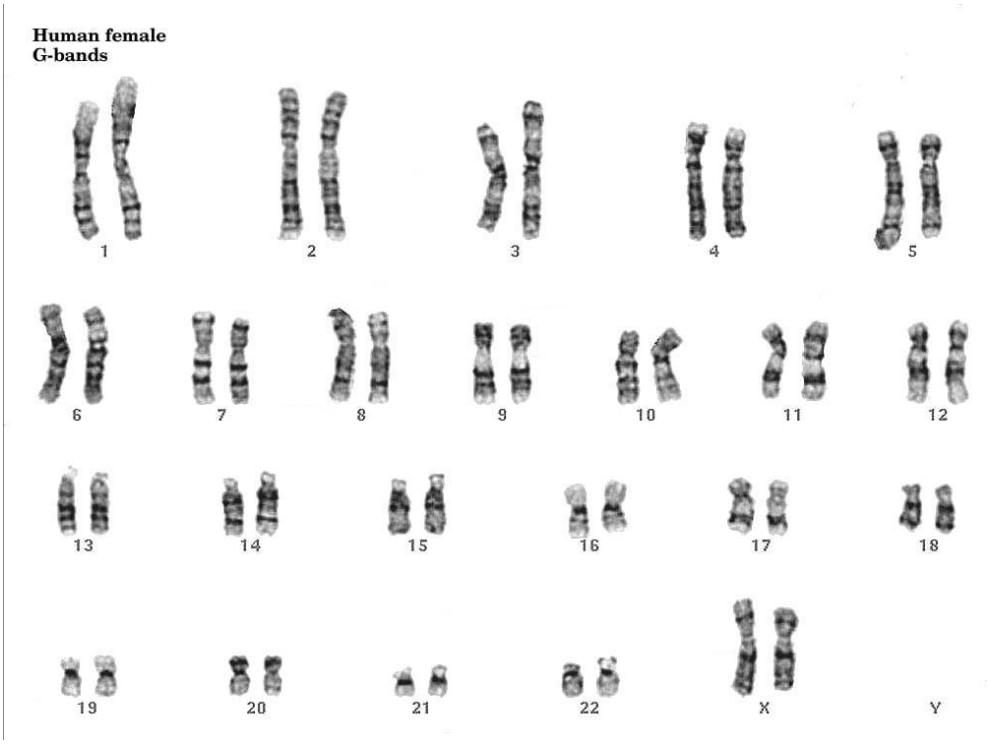


Fig. 21: Normal female karyotype: 46,XX

Chromosome banding

The discovery of a special staining of the chromosomes, when using quinacrine staining under ultraviolet light represented the beginning of a new era in chromosome identification. Caspersson, in 1977 noticed that each chromosome presented a unique banding pattern, by quinacrine staining.

Banding allows exact identification of each chromosome, pairing of homologous chromosomes and the establishment of chromosomal rearrangements, such as: inversions, translocations, deletions, and duplications.

A band is defined as a chromosomal region, which can be distinguished from its neighboring regions, on the basis of the differences in stain intensity. A certain number of bands form the chromosomal regions (fig.22).

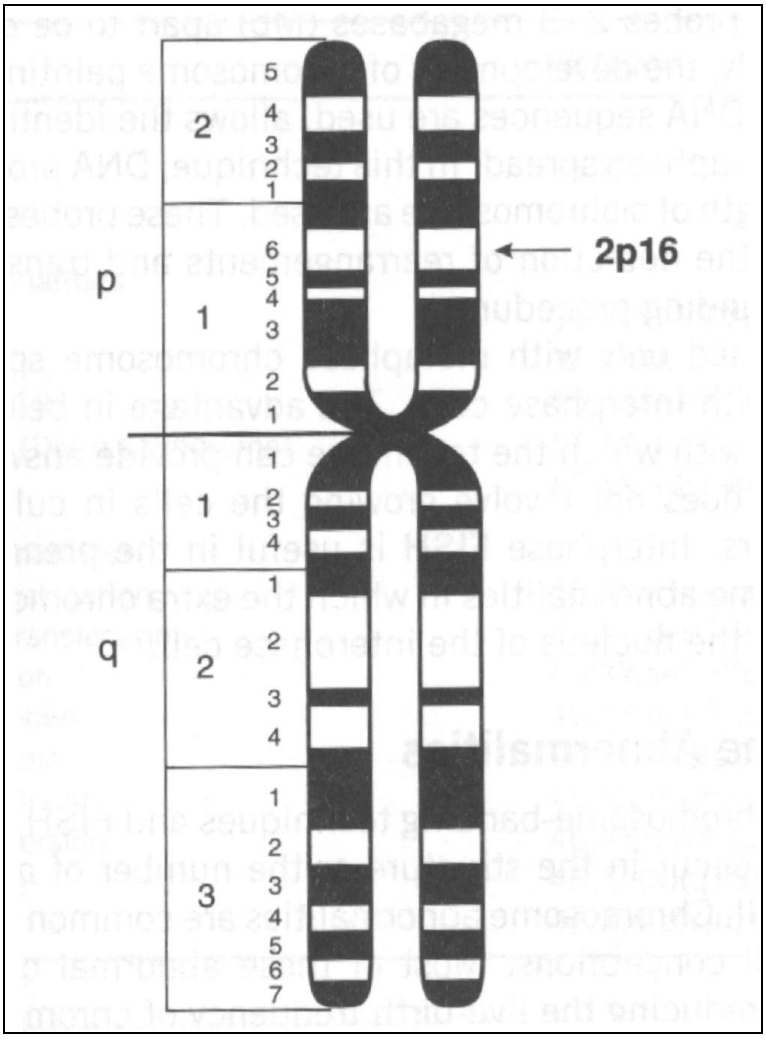


Fig. 22: Chromosome nomenclature

Several techniques have been developed for staining chromosomes in banded patterns. It is important that each method would be correct applied, in order to maintain the specific banding pattern (fig. 23, 24).

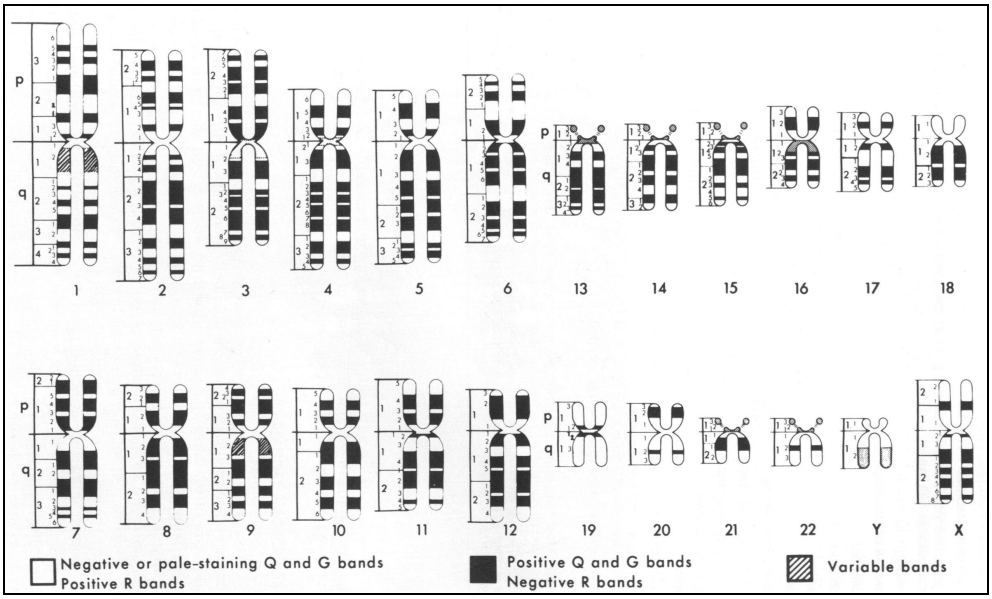


Fig. 23: Chromosome bands obtained by G, Q and R banding

Q-banding*:* is the first banding technique, used at the beginning of the years 1970. Caspersson used quinacrine mustard and noticed that each pair of chromosomes stained in a specific pattern of bands, when they were examined by fluorescence microscopy. These bands can appear bright or dim. They were called Q bands and were used as the reference bands for the standard classification.

Q banding requires special equipment, ultraviolet light microscope, thus is not commonly used. However, it is used to examine polymorphic heterochromatic chromosomal regions, which may be brightly fluorescent. This method is also useful to distinguish Y variants from other chromosomes and to examine the parental origin of chromosome abnormalities.

G-banding*:* This is a widely used technique. Chromosomes preheated at 90°C, are treated with trypsin, which denatures chromosomal proteins and then are stained with Giemsa stain. A special pattern of dark and light bands appears. The dark band correspond to the bright band resulted by Q-banding.

Giemsa banding technique

Setting up the cultures, harvesting and slide making are performed by the standard procedure, mentioned before.

The slides are heated at 80°C-90°C, one hour.

Then they are introduced in trypsin solution in phosphate buffer 1:250, for 1 minute.

Slides are dipped in 70% ethanol and then rinsed with demineralized water.

Slides are placed in a staining solution for approximately 5 minutes.

Test slides are done first, in order to check the appearance of the obtained bands. Dipping and staining time may, therefore, vary.

Slides are rinsed with demineralized water and dried before examination.

***R-banding**:* This technique is less widely used. The chromosomes are previously preheated and then stained. The resulting bands are the reverse of those produced by Q or G banding.

***C-banding**:* This method stains centromeric heterochromatin and other regions containing constitutive heterochromatin, such as the secondary constrictions of chromosomes 1, 9, 16, the short arm of chromosome 15 and the distal end of the Y-chromosome. This technique is useful for locating centromeres on ring chromosomes or small fragments, as well as the centromeres of the dicentric chromosomes.

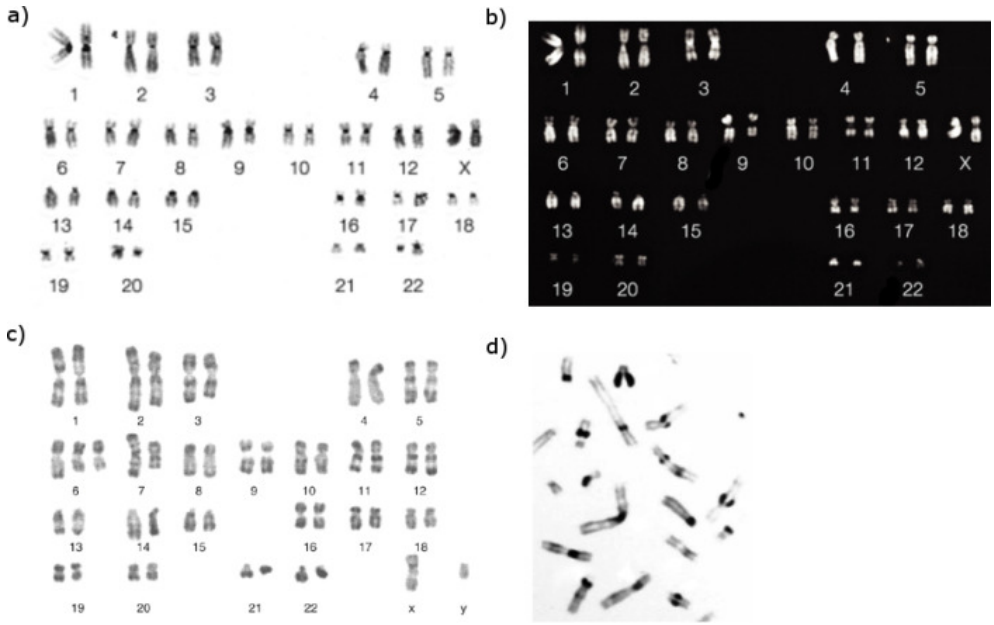


Fig. 24. Different chromosomal staining techniques reveal variations in chromosome structure. Cytogeneticists use these patterns to recognize the differences between chromosomes and enable them to link different disease phenotypes to chromosomal abnormalities. Giemsa banding (a), Q-banding (b), R-banding (c) and C-banding (d) are shown.

***High-resolution banding**:* In contrast to the routine banding techniques, which generally show only 200 bands, the high-resolution banding technique reveals a much larger number of bands (800 to 1400) (fig. 25). Prophase and prometaphase chromosomes are needed, because they are longer than during metaphase. This technique allows the detection of very small chromosomal changes and moreover, precise gene localization can be made.

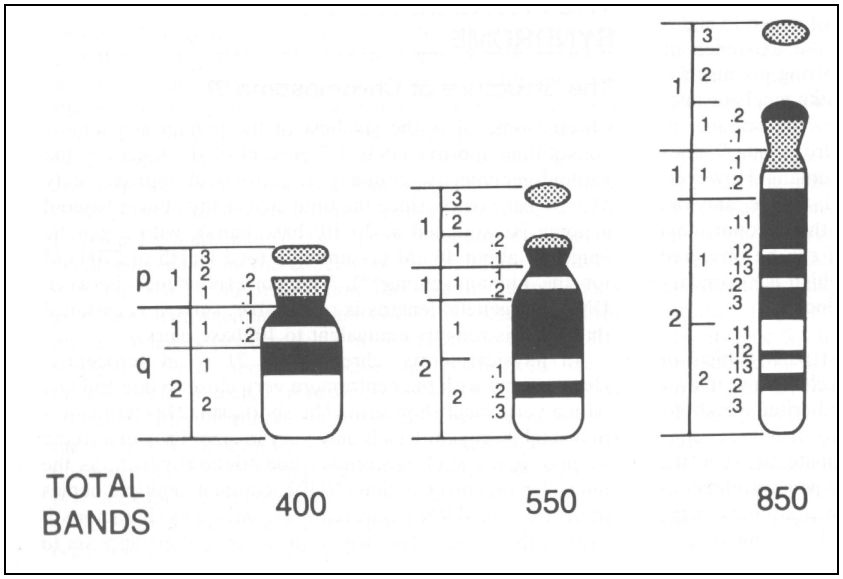


Fig. 25: High resolution banding for chromosome 21

Molecular cytogenetics

FISH technique

Cytogenetics entered the molecular era with the introduction of *in situ* hybridization. This allows locating the positions of specific DNA sequences on chromosomes. Most *in situ* hybridization procedures use probes labeled with fluorescent dyes to detect DNA sequences, and the method is called **fluorescence in situ hybridization** (FISH).

Steps (fig.26):

- probe is labeled by various means
- the probe and the target DNA are denatured
- annealing of complementary DNA sequences.

FISH is important in the clinical diagnosis of different chromosomal abnormalities, eg. deletions, duplications, translocations.

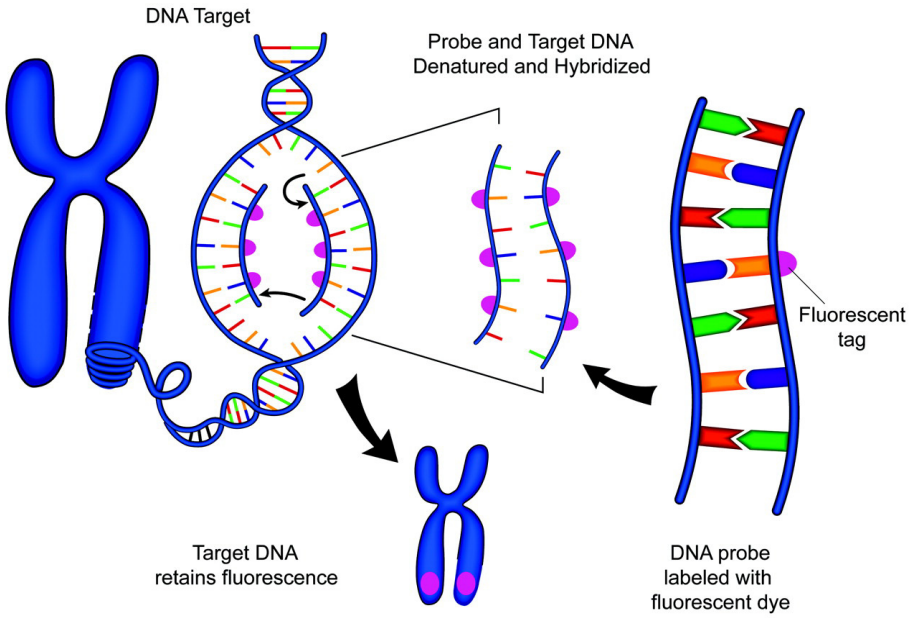


Fig. 26: FISH technique- steps

FISH may be used to analyze **interphase** as well as the **metaphase chromosomes,** offering the advantage that cells do not need to be cultured before chromosomes can be prepared for analysis. In addition, it can be used to analyze chromosomes from solid tumors, which do not divide frequently, but are of great clinical interest.

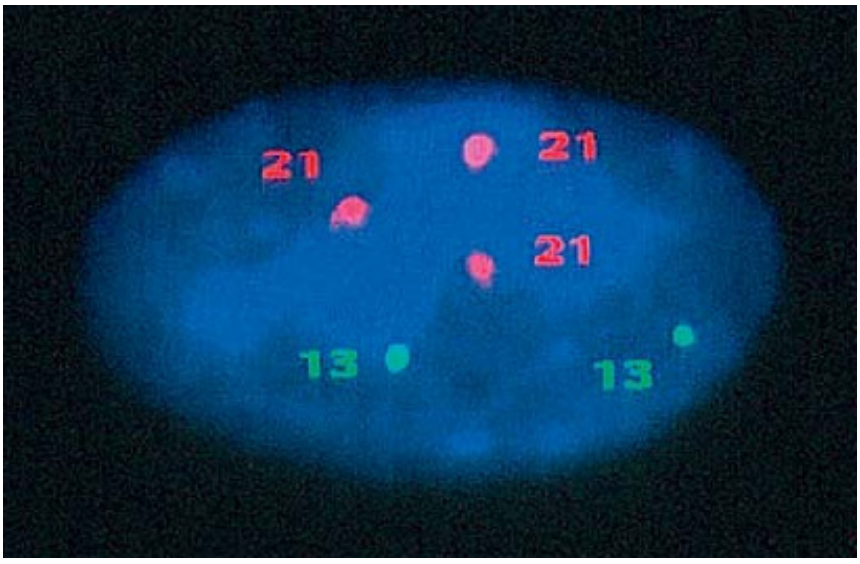


Fig. 27: Interphasic FISH showing a free trisomy 21 (red) with control on the chromosomes 13 (green)

There are also molecular cytogenetic techniques that allow simultaneous visualization of all human chromosomes in different colors. **Multifluor FISH or spectral karyotyping** will scan metaphase chromosomes to detect chromosome rearrangements. Multifluor FISH generates a karyotype in which each chromosome appears to be painted with a different color.

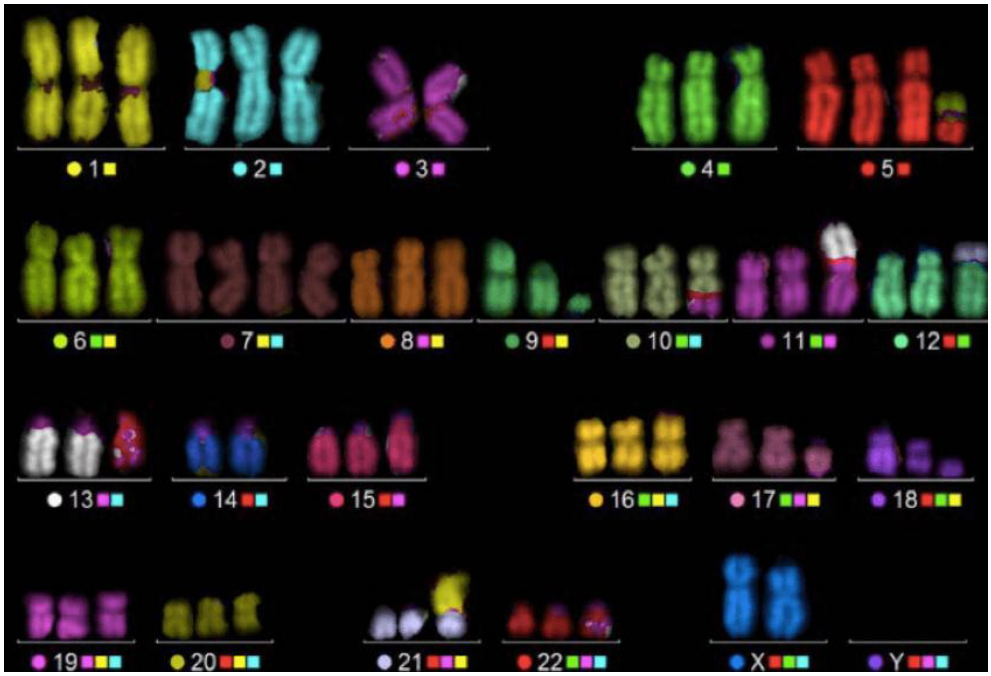


Fig. 28: Multifluor FISH in a tumor cell with multiple chromosome rearrangements and structural and numerical chromosomal anomalies

Comparative genome hybridization (CGH) is used to detect loss or gain of genetic information by performing a genome-wide scan. *The important steps* are: isolation and fragmentation of genomic DNA from a control and from the tested individual. The fragmented control DNA is labeled with a green fluorescent dye and the tested DNA is labeled with red fluorescence. The two DNA samples are used together as DNA probes in hybridization experiments with normal chromosomes. These green and red probes compete to bind to the chromosomes. *Interpretation:* the green and red probes bind equally leading to an orange colour if there are no chromosomal abnormalities. If there is a deletion in the tested DNA, the region will appear greener under the microscope. A duplication will lead to more red under the microscope.

HUMAN KARYOTYPE NOMENCLATURE

The symbols, which designate certain features of the karyotype, were suggested in 1960 at the Denver Conference of Cytogenetics, but the 1971 Paris Conference established and numbered the bands and also made some changes and additions to the nomenclature.

Some of the more commonly used symbols in human cytogenetics are (fig. 29):

chr: chromosome;
A-G: groups of chromosomes;
1-22: number of the autosomal pairs of chromosomes;
X,Y: sex chromosomes; / : mosaicism;
ace: acentric fragment;
cen: centromere;
del: deletion;
dic: dicentric chromosome;
dup: duplication;
i: isochromosome;
inv: inversion;
mar: marker chromosome;
ns: nullisomy;
ph_1: chromosome philadelphia;
r: ring chromosome;
rob: robertsonian translocation;
s: satellite;
t: translocation;
tel: telomere;
ter: terminal end of a chromosome;
tet: tetrasomy;
ts: trisomy;
Updi: uniparental disomy.

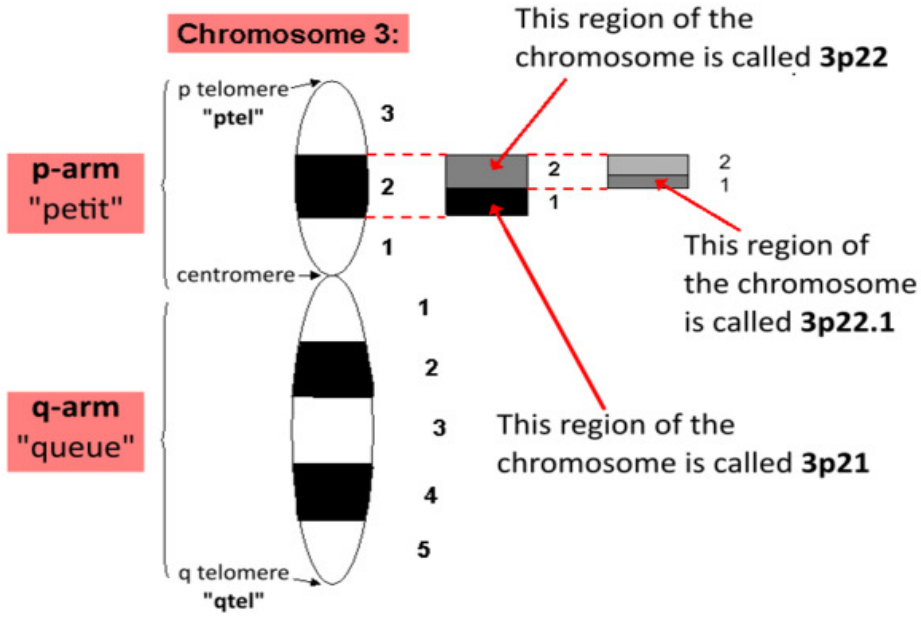


Fig. 29: Cytogenetic banding nomenclature (Cytogenetic banding)

The karyotype is described in three parts, which are separated by a comma: the total number of the chromosomes is followed by the sex chromosomes and the description of the chromosome abnormality, when necessary.

Autosomal aneuploidy is indicated by the sign (+) or (-) before the chromosome which is plus or minus (extra or missing).

Examples of autosomal aneuploidies are:

- ❖ autosomal trisomy:
  - 47,XX,+21 : there is a total of 47 chromosomes, with an extrachromosome 21; it is called trisomy 21
  - 47,XY,+18 : there is a total of 47 chromosomes, with an extrachromosome 18; it is called trisomy 1847,XY,+13 : there is a total of 47 chromosomes, with an extrachromosome 13; it is called trisomy 13
- ❖ autosomal monosomy: 45,XY,-3: there is a total of 45 chromosomes, one chromosome, namely no3, is missing.

Sex chromosome aneuploidy is described numerically, but also directly, writing all the chromosomes found in the studied metaphase.

Examples:

45,X: there are only 45 chromosomes and X- chromosome is missing
47,XXX: there are 47 chromosomes, with an extra X, in females
47,XXY: there are 47 chromosomes, with an extra X, in males
47,XYY: there are 47 chromosomes, with an extra Y- chromosome.

Structural abnormalities:

The description of the chromosomal anomaly includes: the number of the affected chromosome and the specific areas involved in the chromosome′s abnormality.

Examples:

- syndromes with deletions of various autosomes:
  46,XX, 4p-
  46,XX, 5p-
  46,XX, 18p- or
  46,XX, 18q-
- syndromes with duplications of the short or of the long arms of different autosome chromosomes:
  46,XX, 4p+
  46,XX, 9p+ or 46,XX, 9q+
- deletions of the short arm and of the long arm, respectively, of the X-chromosome:
  46,X (Xp-) or 46,X (Xq-)
- isochromosomes:

46,XX, i(18q): isochromosome 18, consisting of two long arms and no short arms

46,Xi(Xq): isochromosome X

- ring chromosomes:

46,XX, r(13): ring chromosome 13, where the ends of the chromosome break (both p and q arms), and join together to form a circular structure.

- translocations:
  - ❖ reciprocal translocations: when two non-homologous chromosomes exchange fragments

46,XY, t(2;8): translocation between two chromosomes, namely 2 and 8

46,XX,t(2;10)(q31;q22): translocation between chromosome 2 (band q31) and chromosome 10 (band q22)

- ❖ robertsonian translocations: a fusion of two acrocentric chromosomes at their centromeres, with loss of their short arms (which carry repetitive, non-essential genes like rRNA):

45,XX, rob(13;13)(q10;q10): robertsonian translocations of chromosomes 13 which have fused in the centromere region of the long arms (q10), resulting in a derivative chromosome rob(13;13), accounted as one in the cytogenetic formula (45 chromosomes instead of 46). Since the short arms are lost (which are non-essential), this can be balanced and asymptomatic, but it may affect reproductive outcomes:

Balanced carrier mother: 45,XX, rob(13;13)(q10;q10)

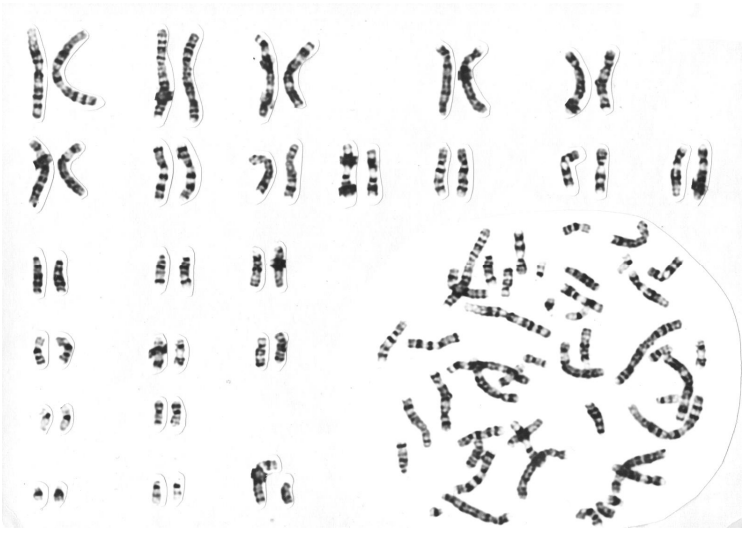
Affected child: 46,XY,+13, rob(13;13) (trisomy 13)

46,XX, +21, rob(14;21)(q10;q10): an individual with Robertsonian translocation Down syndrome due to a (14;21) translocation. The "+21" indicates the presence of a free (untranslocated) chromosome 21.

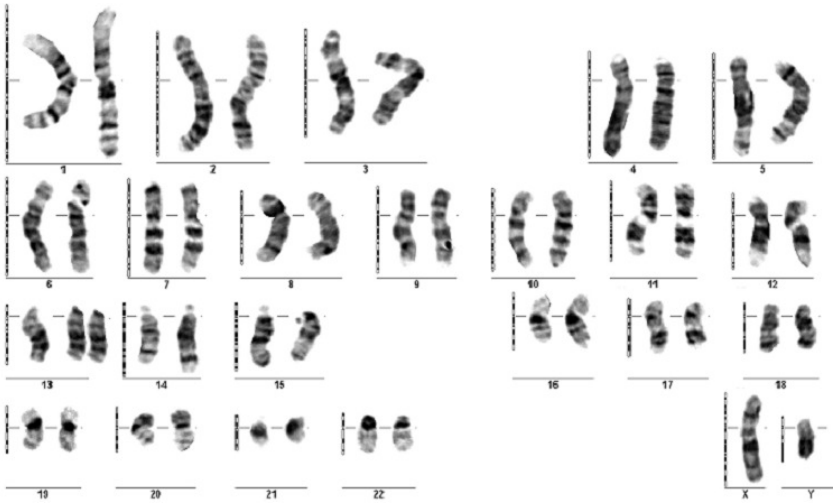
Chapter V

EXERCISES

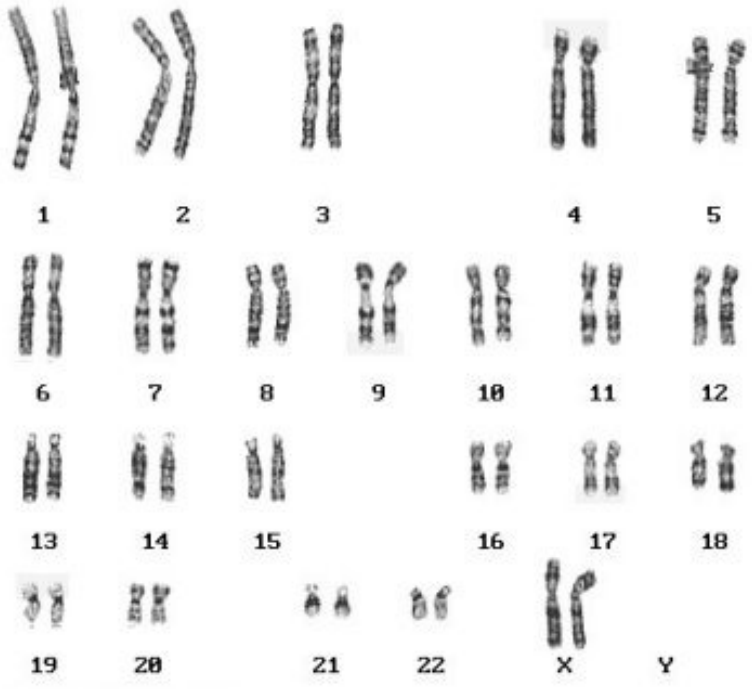
1. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



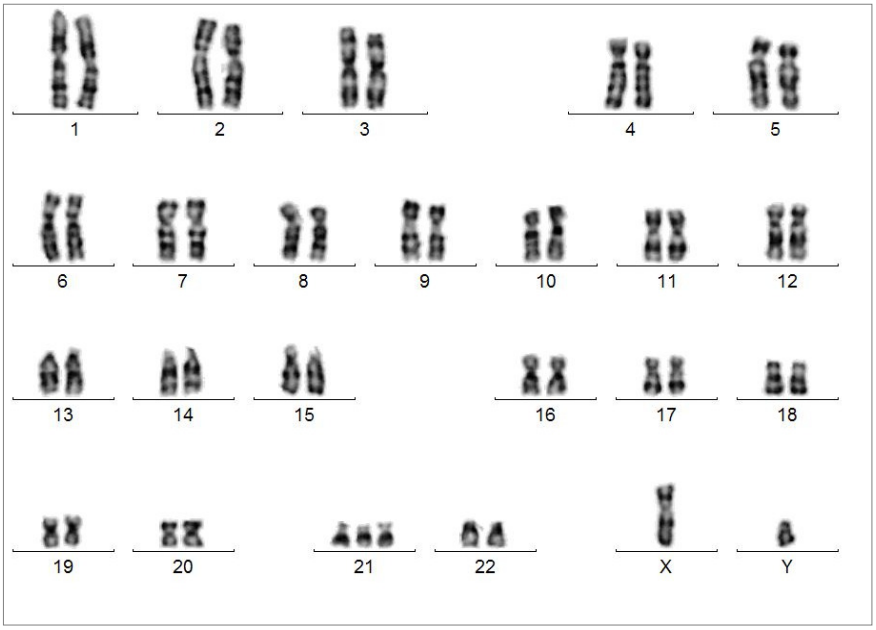
2. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



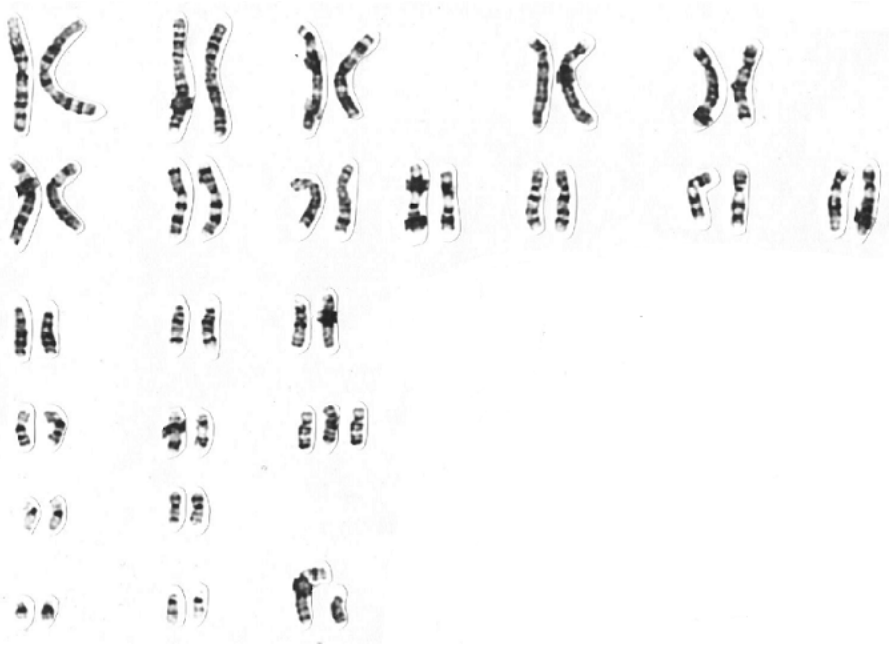
3. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



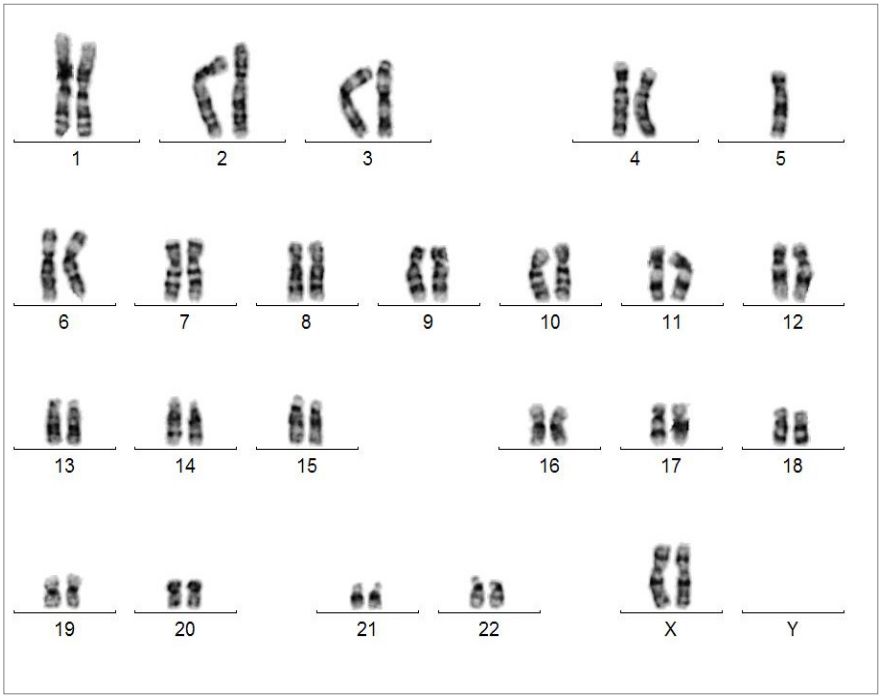
4. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



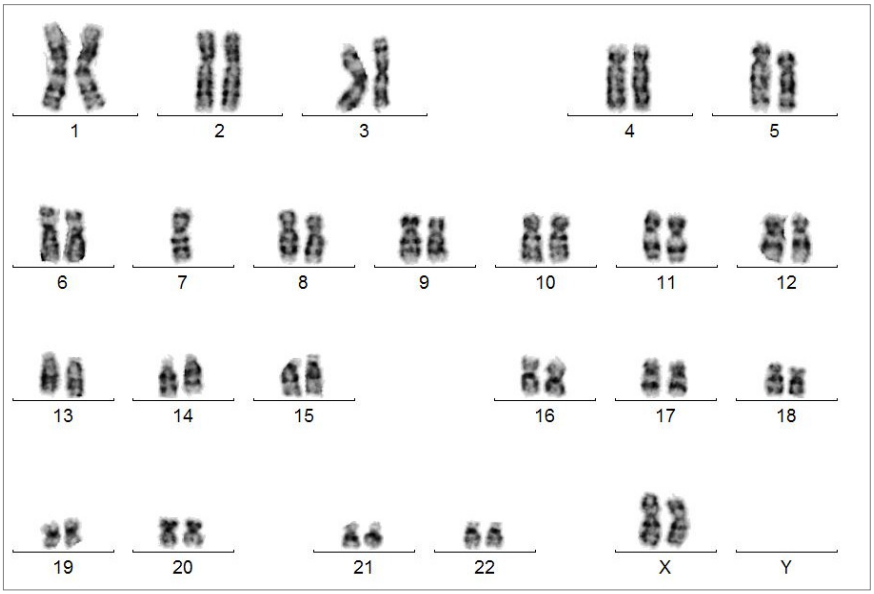
5. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



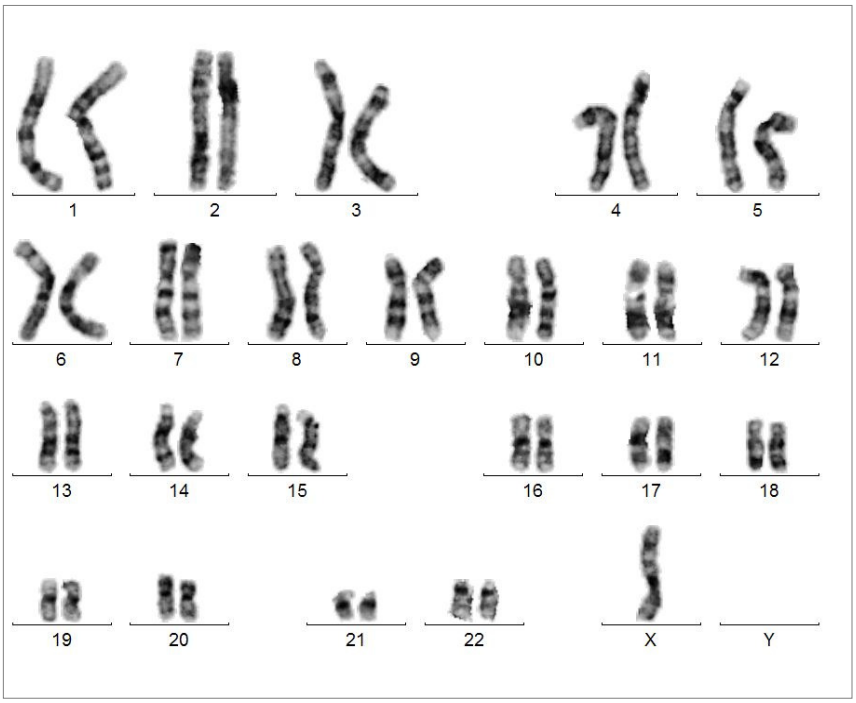
6. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



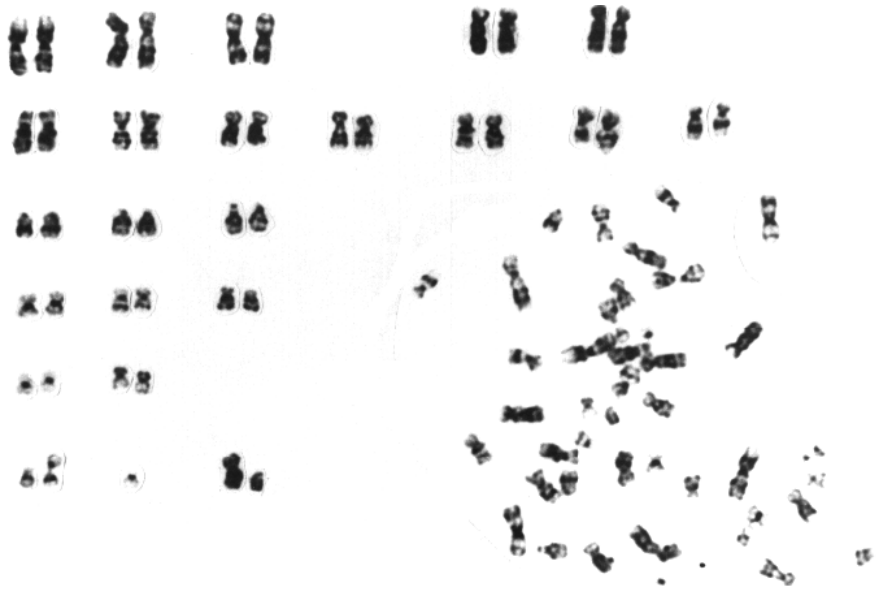
7. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



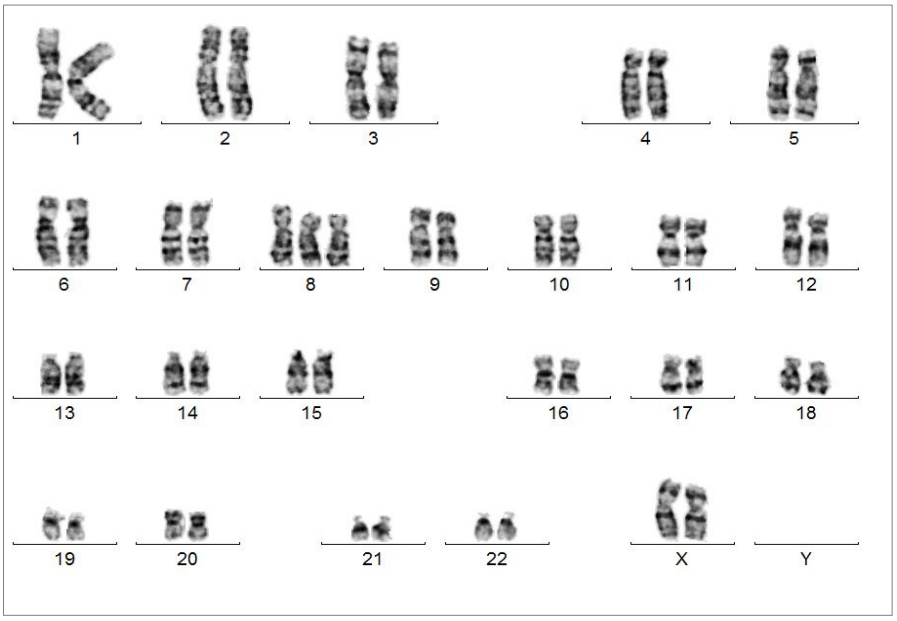
8. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



9. Specify the total number of chromosomes. Write the cytogenetic formula. Specify the type of banding. Identify chromosomes 21 and 22.



10. Specify the total number of chromosomes and the sex chromosomes. Write the cytogenetic formula. Specify the type of banding.



Chapter VI

INHERITANCE OF SOME PHYSIOLOGICAL TRAITS

A genetic system consists of the genes, the product of their activity and the relationship between them.

The physiological systems are classified into different phenotypes. Because of their single-gene pattern of inheritance and different frequencies in different populations, they represent useful genetic markers in family or population studies and in linkage analysis. Genetic systems analysis is also useful for parentage testing and for twin zygosity determination.

A genetic trait must fulfill some terms, in order to be useful as a genetic marker. Thus, it must have a simple and unequivocal pattern of inheritance, a relatively high frequency of each of the common alleles at the locus, absence of effect of environmental factors or other variables on the expression of a specific trait and an accurate identification of each phenotype.

Many genetic systems have been studied until now, such as:

- Blood group systems:
  - ABO system
  - Rh system
  - MN system
  - Lewis system
  - Duffy system
  - Lutheran system
  - Kell system
  - Xg system
- Plasma systems:
  - Haptoglobin system
  - Transferrin system
  - α_1-antitrysin system
  - Immunoglobulin system
- Secretion systems: saliva and others

Most of the systems mentioned above are determined by codominant alleles; thus, in heterozygotes the products of the two genes are detectable and the genotype can be inferred directly from the phenotype. On the other hand, most of these systems are determined by multiple allelism, so numerous polymorphisms exist in healthy individuals, in population.

BLOOD GROUPS

The blood groups are important in medicine, in blood transfusion, hemolytic disease in newborn and in tissue transplantation.The study of the blood group systems had important contributions to human genetics. Thus, the existence of multiple alleles at one locus was first demonstrated by the ABO blood group genes. The Xg blood group was useful in the investigations of X inactivation and sex chromosome aneuploidy.

The ABO system

The ABO blood groups were discovered by Karl Landsteiner, in 1900. He found that there are four types of human blood, according to the presence of two antigens, A and B, on the surface of the red blood cells and the two corresponding antibodies, anti-A and anti-B, in the plasma. The four major phenotypes in humans are: O, A, B and AB.The genes that determine these phenotypes are: A, B and O. They are alleles at a locus on chromosome 9. This is called multiple allelism, because more than two alternative alleles exist in the population.

Gene A has two variants: A_1 and A_2.
Gene O is recessive to genes A_1, A_2 and B.
Genes A_1 and A_2 are codominant with B.
Gene A_2 is recessive to A_1.

Genotypes	Phenotypes
OO	O
A_1 B	A_1 B
A_2 B	A_2 B
A_1 O A_1 A_2 A_1 A_1	A_1
A_2 O A $_2A_2$	A_2
B O B B	B

Knowing the phenotypes and the corresponding genotypes, parental determinations can be made. Examples are given bellow:

Parents blood group	The blood group possible in children	The blood group that is impossible in children
O+O	O	A; B; A_1 B; A_2 B
O+A_1	A_1 ; A_2 ; O	B; A_1 B; A_2 B
O+A_2	A_2 ; O	A_1 ;B; A_1 B; A_2 B
A_1 +A_1	A_1; A_2; O	B; A_1B; A_2B
B+B	O; B	A_1; A_2; A_1 B; A_2B
O+A_1 B	A_1; B	A_2 ; O; A_1 B; A_2 B
B+A_2 B	A_2 B; B; A_2	A_1; O; A_1 B
A_1 B+A_1B	A_1; B; A_1 B	O; A_2; A_2B

Problems:

1. A woman of blood group AB has an AB child. What are the possible blood groups of the father?
2. Four pairs of parents claim for their kidnapped children. Assign the following children to the right parents:

Children	Parents
O	AB+B
AB	AB+O
A	O+O
B	B+B

3. Is there possible that a woman of blood group A_1 and her husband of blood group O have three children, of blood groups A_1, A_2 and O, respectively?

The Rh system

The Rh system also has a clinical importance, because of its role in hemolytic disease of the fetus and in blood transfusions.

This system was discovered in 1940 by K. Landsteiner and A. Wiener. The name comes from the rhesus monkeys, which were used in the experiments.The populations are divided into two phenotypic groups:

- Rh-positive individuals, who have an antigen called D-antigen
- Rh-negative individuals, who lack the D-antigen

The Rh locus (or loci) is on chromosome 1. The alleles that determine this blood group are called D, C, E, d, c, and e.
Practically, D and d are most important.

Phenotype	Genotype
Rh+	DD Dd
Rh-	dd

As for the ABO system, knowing the Rh groups of the parents we can find out the possible blood groups of their children.

Blood group of the parents	Possible blood groups in children
Rh(-) + Rh(-)	Rh(-)
Rh(+)+Rh(+)	Rh(+); Rh(-)
Rh(+)+Rh(-)	Rh(+); Rh(-)

Rh hemolytic disease

During pregnancy small amounts of fetal blood cross the placental barrier and reach the maternal blood stream. If the mother is Rh negative (she doesn′t have D antigens) and her fetus is Rh positive (he has the D antigen from father), the fetal cells stimulate the formation of anti-Rh antibodies. Usually no ill effects are associated with exposure of the mother to the Rh(+) antigen, during the first pregnancy. These effects might appear only if the mother was previously exposed to the antigen, by transfusions with Rh(+) blood.

In subsequent pregnancies, children may be exposed to the antibodies produced by the mother, which are transferred to the fetal circulation, where they attach to the red cells membrane. The life span of these cells is thus, shortened. The fetus becomes anemic. Symptoms of hemolytic jaudice may appear. A great number of erythroblasts is released into the blood, accounting for the name erythroblastosis fetalis, used for this disease. Hyperbilirubinemia must be prevented; otherwise unconjugated bilirubin is stored in the brain, causing cerebral damage. The vast majority of such children die; the few ones that survive may have mental retardation, cerebral palsy or deafness.The severe complications of Rh- immunization could be avoided by appropriate blood transfusions to the Rh-negative women. In pregnant women, the risk of immunization can be minimized by giving injections of Rh immune globulin.

The MN system

In 1927, Landsteiner and Levine recognized other blood group antigens. They were called M and N. The MN groups are model of genetic simplicity. They depend upon a pair of alleles, which are codominant. These are called M and N and they produce 3 genotypes.

Genotype	Phenotype
MM	M
NN	N
MN	MN

About 20 years later, the Ss subdivisions of the MN group were discovered. Still, we refer to as the MN system, because these groups are inherited as units, as the genes are very closely linked.

The MN system has little importance in blood transfusion or in maternal-fetal incompatibility. It is important in medical genetics, in solving identification problems, or in paternity establishing.

Knowing the parents' blood groups in the MN system, the children's blood system can be found. Examples are given bellow:

Blood system of the parents	Possible blood system in children
M+M	M
N+N	N
M+N	MN
M+MN	M,MN
N+MN	N,MN
MN+MN	M,N,MN

Problem:
A woman of blood group B, N has a child of blood group O, MN. She states that the father of the child is a man of blood group A, M.
Can the man be excluded as the father on the blood group evidence?
What genes must the child have received from the father?

The Xg system

The Xg system is inherited as an X-linked trait. It has little clinical importance, but it is of great genetic interest, because its determining gene does not undergo X-inactivation.
The phenotypes are different in males and females. There are two alleles: Xg^{a} and Xg^{-}.

Genotypes	Phenotypes
$Xg^{a}Xg^{a}$ $Xg^{a}Xg^{-}$	Xg^{a}
$Xg^{-}Xg^{-}$	Xg^{-}
$Xg^{a}Y$	Xg^{a}
$Xg^{-}Y$	Xg^{-}

As seen, the female may be either homozygous or heterozygous for an X-linked gene. A male is said to be hemizygous. Thus, an X-linked recessive trait is expressed by all males who carry the gene. In this particular case, the males are Xg^{-}, though they carry only one recessive X-linked gene. The study of the Xg system is also useful in the investigation of the sex chromosome aneuploidy.

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