Lecture 6.
The substructure of chromosomes and the phenomenon of differential staining

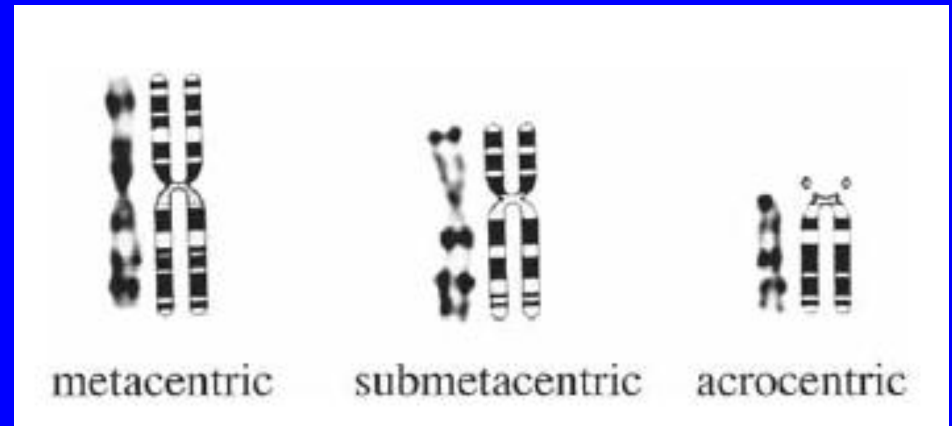
Lovinskaya Anna Vladimirovna,

PhD, Departure of Molecular Biology and Genetics

Chromosome Identification

Until the advent of certain specialized staining techniques, arbitrary identification of individual chromosome pairs was based on the size and position of the centromere. Variability in the centromere position of different chromosomes allowed them to be classified into three basic categories. A chromosome with its centromere in the middle is ***metacentric***, one with the centromere closer to one end is ***sub-metacentric***, and one with the centromere almost at one end is ***acrocentric***.

Based on decreasing relative size and centromere position, a karyotype comprised of seven groups labeled A through G was devised. The X chromosome belonged to the third or "C" group, whereas the Y was often placed separately. Although still used occasionally, these letter group names are now considered obsolete.



Chromosome Banding and Identification

Unequivocal identification of individual chromosomes and chromosome regions became possible with the technical developments of the late 1960s.

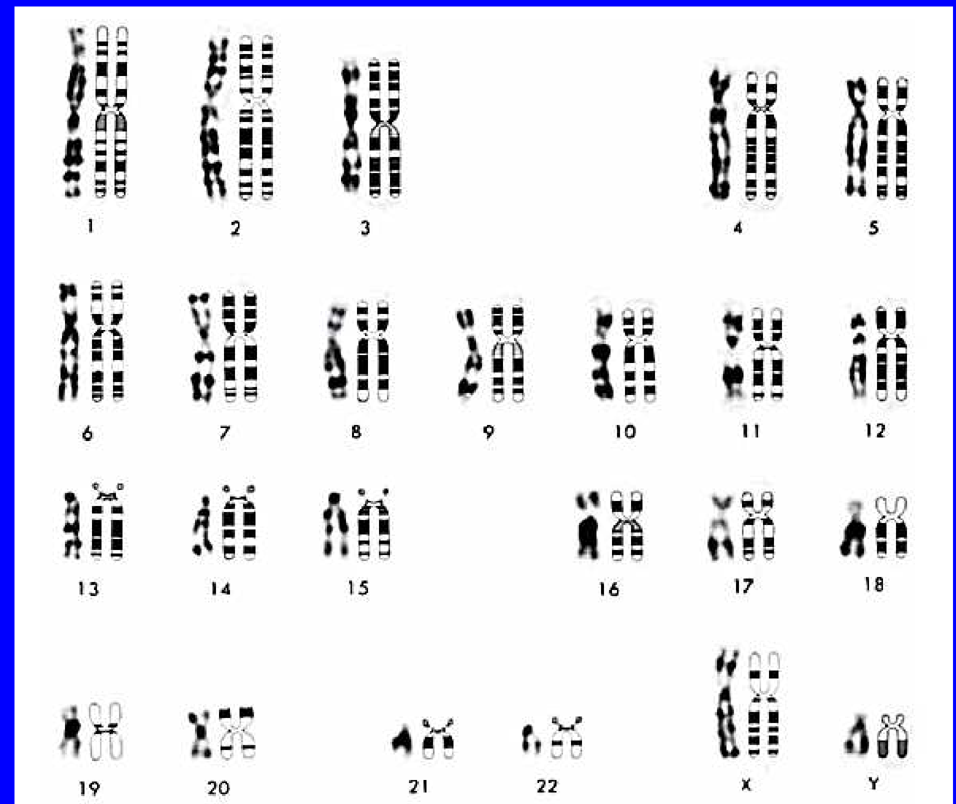
When chromosome preparations are treated with dilute solutions of proteolytic enzymes (trypsin, pepsin, etc.) or salt solutions (2X SSC) and treated with a chromatin stain such as Giemsa, alternating dark and light stained demarcations called ***bands*** appear along the length of each chromosome.

The banding patterns produced are specific for each chromosome pair, thus enabling the identification not only of individual chromosomes but also of regions within each chromosome.

Chromosome Regions and Band Designations

The chromosomal details revealed by the new banding techniques necessitated the introduction of additional terminology and modifications of certain existing ones. This task was accomplished by a standing committee appointed at the Fourth International Congress of Human Genetics in Paris. The recommendations of the committee were published as Paris Conference (1971): Standardization in Human Cytogenetics.

Through a diagrammatic representation of banding pattern, the document elucidated the typical band morphology for each chromosome. The Paris Conference (1971) introduced a numbering system helpful in designating specific bands and regions.



Chromosome Regions and Band Designations

The centromere “cen” divides a chromosome into a short or “p” arm (from the French «petit») and a long or “q” arm («queue»). For descriptive purposes, the centromere is composed of two portions.

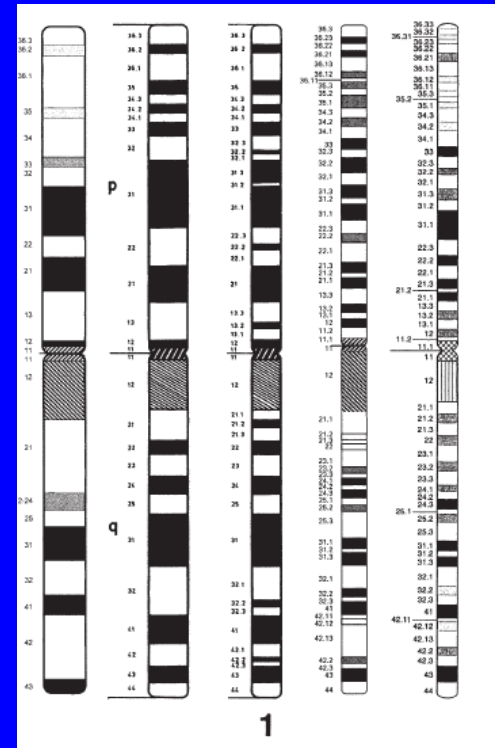
The portion of the centromere lying between its middle and the first band on the short arm is designated as “p10.” Similarly, the portion of the centromere lying between its middle and the first band on the long arm is designated as “q10.” The designations p10 and q10 allow us to describe accurately the nature and organization of centromeres in isochromosomes, whole-arm translocations, and Robertsonian translocations.

Each arm ends in a terminus (“ter,” thus “pter” and “qter”), where telomeres are present to prevent the chromosomes from having “sticky ends.”

Chromosome Regions and Band Designations

Each chromosome arm is divided into regions. This division is based on certain landmarks present on each chromosome. A ***landmark*** is "a consistent and distinct morphologic area of a chromosome that aids in the identification of that chromosome." A ***region*** is an area that lies between two landmarks. The two regions immediately adjacent to the centromere are designated as "1" (p1 and q1), the next distal as "2," and so on. Regions are divided into bands and the bands into subbands.

A ***band*** is that part of a chromosome that is distinctly different from the adjacent area by virtue of being lighter or darker in staining intensity. Sequential numbering of chromosome arms and bands helps make the designation of specific bands easy. For example, the terminal band on the long arm of chromosome 2 can be written as 2q37 to mean chromosome 2, long arm, region 3, band 7 and is referred to as "two q three-seven," not "two q thirty-seven".



Chromosome Banding and Identification

Frequently Used Banding Methods and Their Abbreviations

Banding Method	*Abbreviation*
Q-bands	Q
Q-bands by quinacrine derivatives and fluorescence microscopy	QFQ
G-bands	G
G-bands by trypsin and Giemsa	GTG
C-bands	C
C-bands by barium hydroxide and Giemsa	CBG
R-bands	R
R-bands by acridine orange and fluorescence microscopy	RFA
R-bands by BrdU and Giemsa	RBG
Telomere bands or T-bands	T

CHROMOSOME STAINING AND BANDING

Techniques That Create Bands Along the Length of the Chromosomes - create unique patterns for each individual chromosome pair. This property allows for the positive identification of the individual chromosome pairs and permits characterization of structural abnormalities. These banding techniques answer many questions by facilitating the numerical and structural examination of the entire karyotype.

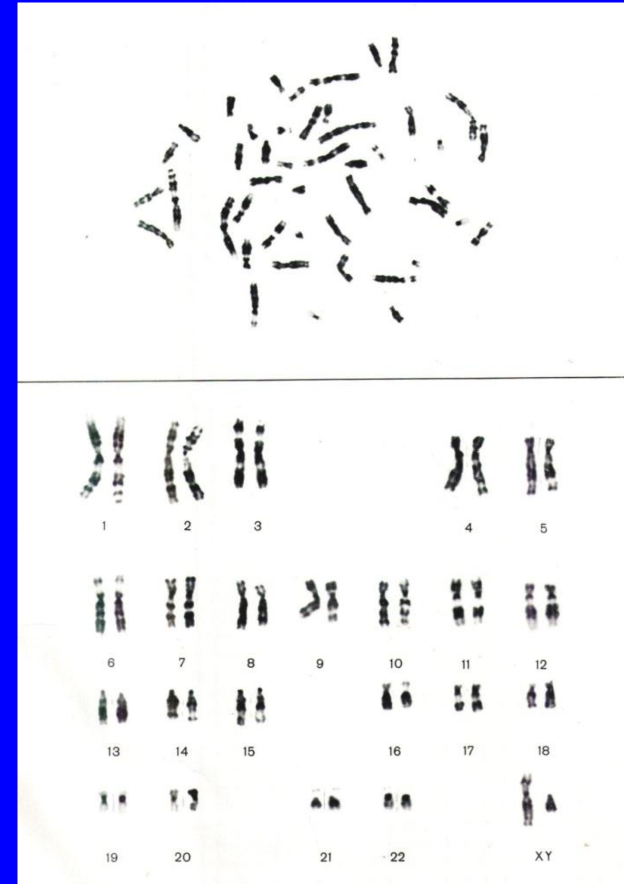
- ✓ G-banding;
- ✓ Q-banding;
- ✓ R-banding

Techniques that Stain Selective Chromosome Regions are used in special circumstances when a particular piece of information cannot be answered using a routine banding method. These special stains are typically utilized to obtain such specific data.

- ✓ C-banding;
- ✓ T-banding;
- ✓ Cd staining;
- ✓ NOR Staining;
- ✓ Fluorescence In Situ Hybridization (FISH)

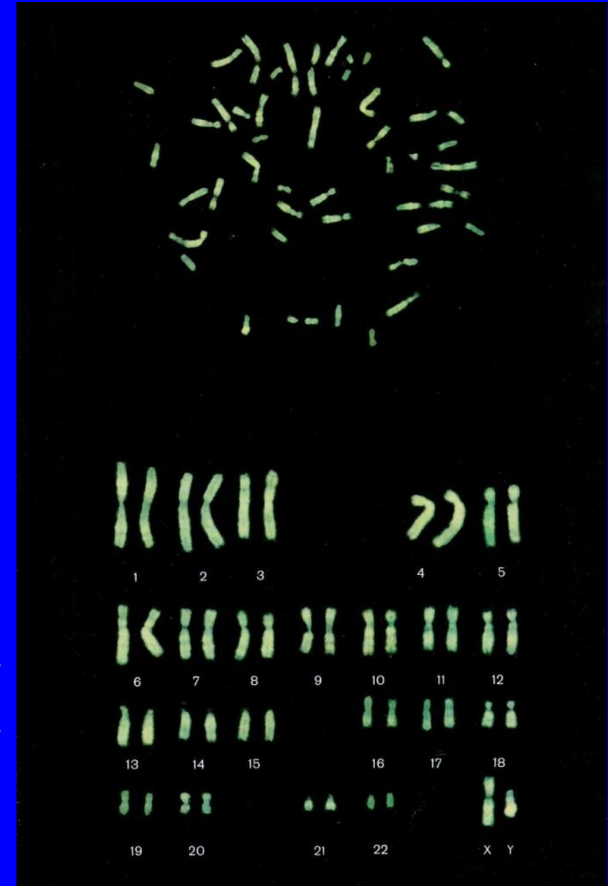
G-Banding (Giemsa Banding)

G-banding is the most widely used routine banding method. GTG banding (G bands produced with trypsin and Giemsa) is one of several G-band techniques. With this method, prepared slides are treated with the enzyme trypsin and then stained with Giemsa. This produces a series of light and dark bands that allows for the positive identification of each chromosome. The dark bands are A-T-rich, late replicating, heterochromatic regions of the chromosomes, whereas the light bands are G-C-rich, early replicating, euchromatic regions. The G-light bands are biologically more significant, because they represent the active regions of the chromosomes, whereas the G-dark bands contain relatively few active genes.



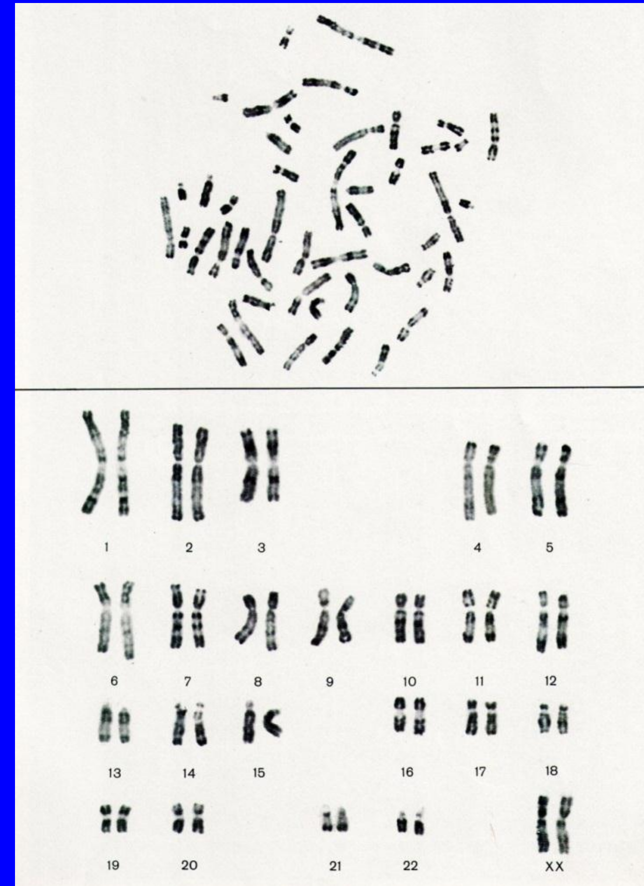
Q-Banding (Quinacrine Banding)

Q-banding is a fluorescent technique and was the first banding method developed for human chromosomes. Certain fluorochromes, such as quinacrine dihydrochloride, will bind to DNA and produce distinct banding patterns of bright and dull fluorescence when excited with the proper wavelength of light. Because adjacent A-T pairs are necessary to create binding sites, the brightly fluorescing regions are A-T rich. The Q-banding pattern is similar to the G-banding pattern with some notable exceptions. In particular, the large polymorphic pericentromeric regions of chromosomes 1 and 16, and the distal long arm of the Y fluoresce brightly; the distal long arm of the Y chromosome is the most fluorescent site in the human genome. Q-banding is, therefore, useful to confirm the presence of Y material or when studying the cited polymorphic regions.



R-Banding (Reverse Banding)

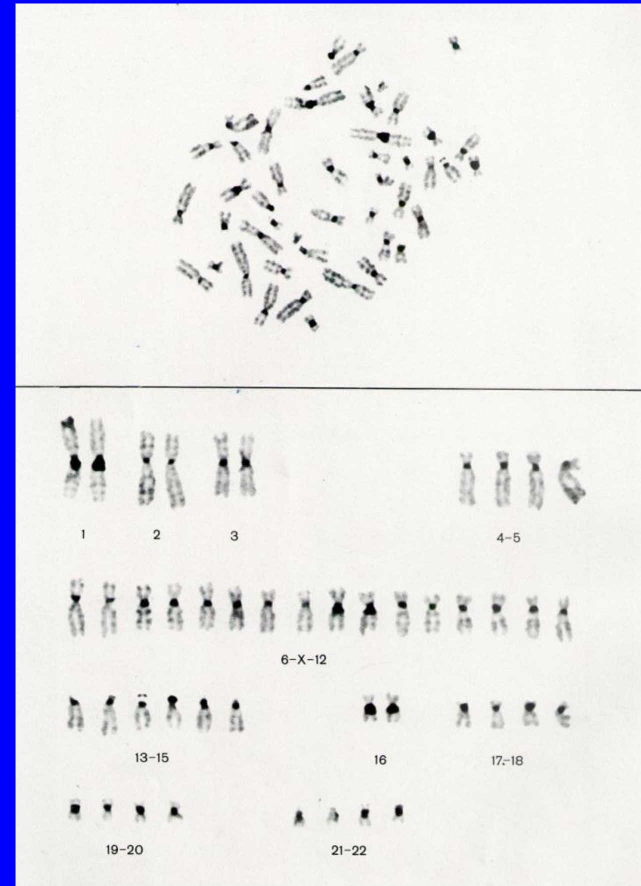
R-banding techniques produce a banding pattern that is the opposite or reverse of the G-banding pattern. There are fluorescent and nonfluorescent methods. The C-G-rich, euchromatic regions stain darkly or fluoresce brightly, whereas the A-T-rich, heterochromatic regions stain lightly or fluoresce dully. The euchromatic, R-band-positive regions are the more genetically active regions of the chromosomes. Many human chromosomes have euchromatic terminal ends that can be difficult to visualize with standard G-band techniques because the pale telomeres might fade into the background. R-banding is a useful technique for the evaluation of these telomeres. R-banding is typically used as an additional procedure in many countries, but is the standard method for routine banding in France.



C-Banding (Constitutive Heterochromatin Banding)

C-banding techniques selectively stain the constitutive heterochromatin around the centromeres, the areas of inherited polymorphisms present on chromosomes 1, 9, and 16, and the distal long arm of the Y chromosome. C-band-positive areas contain highly repetitive, late replicating sequences of α-satellite DNA.

C-banding is useful for determining the presence of dicentric and pseudodicentric chromosomes, and also for studying marker chromosomes and polymorphic variants.

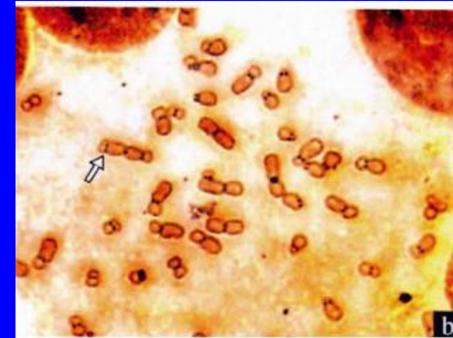


T-Banding (Telomere Banding)

T-banding is an offshoot of R-banding that results in only the terminal ends or telomeres of the chromosomes being stained. A harsher treatment of the chromosomes diminishes staining except at the heat-resistant telomeres. There are fluorescent and nonfluorescent T-banding techniques.

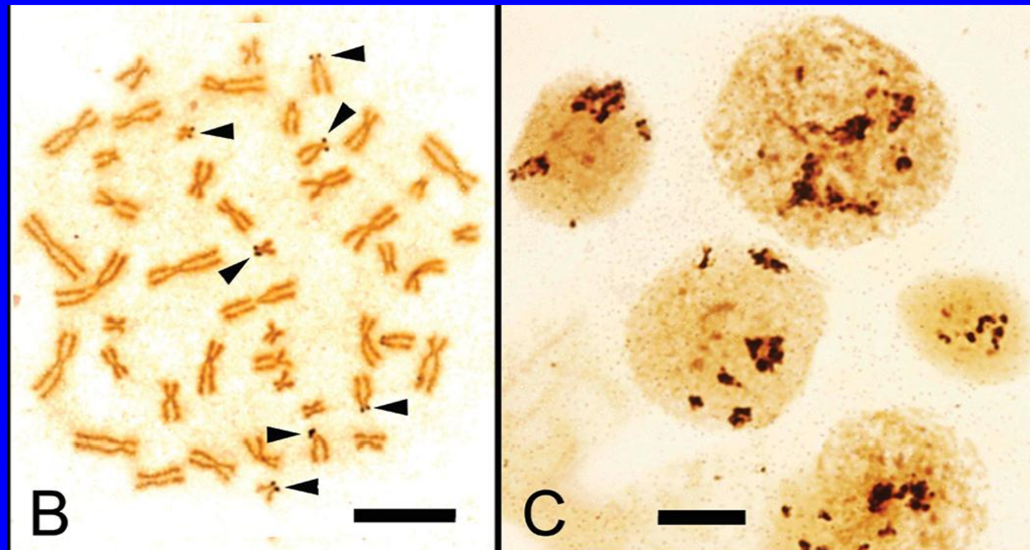
Cd Staining (Centromeric dot or Kinetochore Staining)

This technique produces a pair of dots (kinetochores or the chromatin associated with them) at each centromere, one on each chromatid. Only active or functional centromeres will stain with Cd staining, in contrast to C-banding, which will stain inactive and active centromeric regions. Cd staining can be used to differentiate functional from nonfunctional centromeres and to study Robertsonian translocations (centromere-to-centromere translocations of acrocentric chromosomes), ring chromosomes, and marker chromosomes.



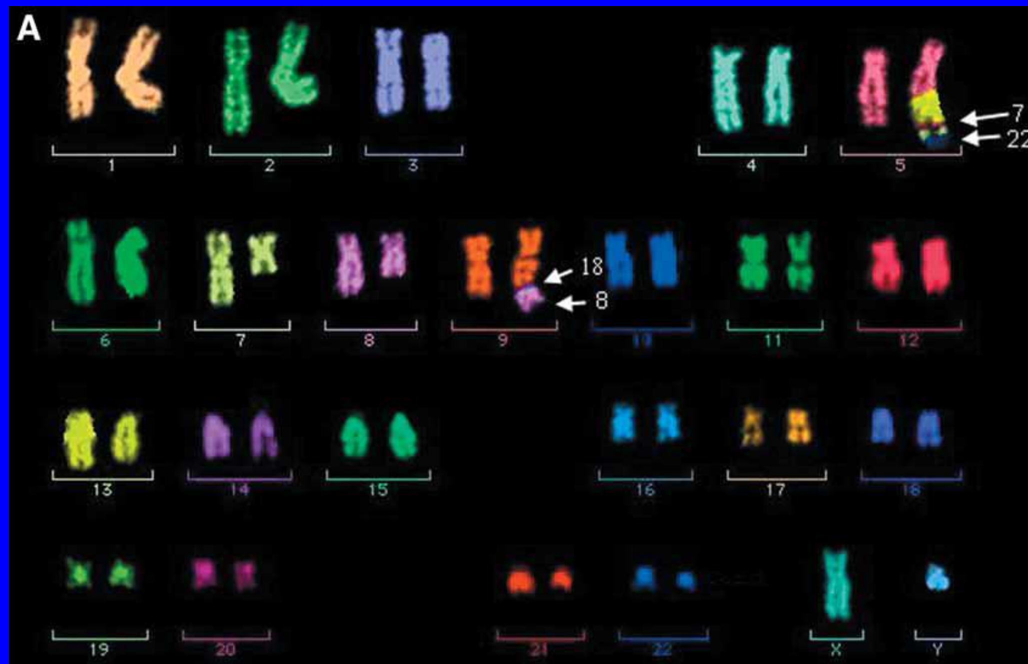
NOR Staining or Ag-staining (Silver Staining for Nucleolar Organizer Regions)

This technique selectively stains the nucleolar organizer regions (NORs) located on the satellite stalks of the acrocentric chromosomes. These regions contain the genes for ribosomal RNA and can be stained with silver nitrate. Theoretically, there are 10 NORs per cell, 1 for each acrocentric chromosome. However, not all will usually stain at any one time because the silver stains the activity, not presence, of rRNA genes. NOR staining is useful for the identification of marker chromosomes and rearrangements or polymorphisms involving the acrocentric chromosomes.



Fluorescence *in situ* hybridization (FISH)

FISH-method is a molecular cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. Current limitations of standard FISH protocols are probe availability and the limited number of spectrally nonoverlapping fluorochromes used for detection.



Thank you for attention!