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MEDICAL FACULTY
Department of Histology, Embryology, Cytology

INTERNATIONAL HIGHER SCHOOL OF MEDICINE
Natural Sciences Disciplines Department

INTRODUCTION TO HUMAN CYTOGENETICS

Teaching Manual (Basal reader) for senior students, PhD students, medical residents and interns

Compiled by Kalugina O.P., Kostritsyna T.V.

Dedicated to the 30th Anniversary of the Medical Faculty
of the Kyrgyz-Russian Slavic University
named after B.N. Yeltsin

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This publication on Human Cytogenetics has been compiled as a general brief review (Study guide /basal reader) of modern educational literature and Open Educational Resources. It reviews important concepts and developments in cytogenetics and highlights their applications in the medical practice.

Human Cytogenetics plays a key role in the detection of chromosomal abnormalities associated with malignancies, as well as the characterization of new alterations that allow more research and increase knowledge about the genetic aspects of these diseases. The publication provides also a general review of molecular cytogenetics methods, including standard FISH preparations and techniques for Spectral Karyotyping, molecular cytogenetics of human diseases.

Study guide (Basal reader) is aimed at senior students, PhD students, medical residents and interns.

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INTRODUCTION

Medicine has incorporated wave after wave of new scientific discoveries and technologies in recent years. Human Cytogenetics is one of these technologies and shows a dramatic example of the impact of advances in basic science. Advances in Human Cytogenetics have revolutionized daily clinical practice, particularly in developed countries. Practitioners who received medical education decades ago have to adapt to this new discipline. The cytogenetic method includes the preparation of chromosomal micro slides and their analysis. Karyotyping (karyotype study) allows determining the number and chromosome structure by using various methods of different staining. The revealed abnormalities in the number and structure of chromosomes can cause infertility, miscarriage, or chromosomal disease in the unborn child. This research method also makes it possible to evaluate and investigate the human body's mutagenic effects (chemical, radiation).

Over the past five decades, innovative technological advances in Cancer Cytogenetics have greatly enhanced the detection ability of chromosomal alterations. They have facilitated the research and diagnostic potential of chromosomal studies in neoplasms. These developments notwithstanding, chromosome analysis of a single cell is still the easiest way to delineate and understand the relationship between clonal evolution and disease progression of cancer cells. The use of advanced fluorescence in situ hybridization (FISH) techniques allows for further identification of unresolved chromosomal alterations by the karyotyping method.

This publication on Human Cytogenetics has been compiled as a general brief review (basal reader) of modern educational literature and Open Educational Resources. Basal readers are textbooks used to teach reading and associated skills to students. Commonly called "reading books" or "readers," they are usually published as anthologies that combine previously published short stories, excerpts of longer narratives, and original works. A standard basal series comes

with individual identical books for students, a Teacher's Edition of the book, and a collection of workbooks, assessments, and activities.

Open Educational Resources

OER refers to any educational resources such as curriculum maps, course materials, textbooks, streaming videos, multimedia applications, podcasts, and other materials designed for teaching and learning. They are openly available for use by educators and students without an accompanying need to pay royalties or license fees (Butcher, 2011). OER can exist as smaller, stand-alone resources (reusable learning objects) that can be mixed and combined to form larger pieces of content or as larger course modules or complete courses. OER can include simulations, labs, collections, journals, and tools. These materials are considered open if released under a license such as a Creative Commons license. Various initiatives focus on the provision, development and adaptation of OER. For example, UNESCO IITE Policy Brief 2014 4 has launched the OER Platform, which has resources available in 13 languages under a Creative Commons license (see http://www.oerplatform.org). It allows communities of practice, including teachers, learners, and education professionals, to copy freely, adapt, and share their resources. This sets an important example for UNESCO member nations to release publicly funded resources under open licenses.

Openness fosters a more democratic and competitive higher education system, potentially improving access to education, developing and localizing open educational services to suit local contexts, and enhancing education integration into everyday lives as part of lifelong learning. It facilitates informal, individualized, flexible, and lifelong learning and freedom for anyone worldwide (with internet access). Viewing these materials gives them an extraordinary spread of access, with the potential to reach and serve hundreds of thousands of learners who would otherwise not have access to education (Stacey, 2013). It is clear that openness is here to stay and is changing the nature of higher education. Therefore it is essential for institutions to engage with transparency as a potential core organizational value if they wish to remain relevant and contribute to the positive advancement of the field of higher education (Butcher, 2014).

HISTORY OF CYTOGENETICS

Cytogenetics is the branch of genetics that is concerned primarily in cellular components, especially chromosomes, in relation to heredity, genetic anomalies, and pathologic conditions. Cytogenetics is a combination of cytology and genetics. Cytology deals with the study of cells, their origin, structure, function and pathology. It makes use of light or electron microscopic methods for the study of morphology, and as a means to diagnose or test for cancer and other diseases. Genetics deals primarily with the patterns of inheritance of specific traits. It aims to explain how traits are passed down from patent to offspring. It therefore studies heredity, genetic structure, gene function, and genetic variation. Cytogenetics is a branch of genetics. Other branches of genetics include molecular genetics, population genetics, medical genetics, developmental genetics, etc. Cytogenetics, in particular, studies the structure, number, and function of chromosomes in the cell in connection with heredity, genetic anomalies, and pathologic conditions. It is the branch of genetics that deals at the cellular level. The most common methods used are cytogenetic banding techniques, karyotyping, fluorescent in situ hybridization, and comparative genomic hybridization. The person that specializes in cytogenetics is called cytogeneticist.

CYTOGENETICS IS THE STUDY OF CHROMOSOMES AND THEIR ROLE IN HEREDITY

The goal of Cytogenetics:

1. Diagnosis of chromosomal abnormalities.
2. Localization of any (often abnormal) chromosomal region/ DNA sequence.

Basic cytogenetic terms

Chromatin: non condensed DNA with proteins attached (interphase of the cell cycle)

Chromosome: condensed DNA with proteins attached (M phase of the cell cycle)

A karyotype is the number and appearance of chromosomes in the nucleus of a eukaryotic cell. The term is also used for the complete set of chromosomes in a species, or an individual organism.

The chromosomes are depicted (by rearranging a microphotograph) in a standard format known as a karyogram or idiogram: in pairs, ordered by size and position of centromere for chromosomes of the same size.

The Cell Cycle and the detection of the chromosomes

Human chromosomes are examined in dividing cells (bone marrow/placental cells, lymphocytes).

THE HISTORY OF HUMAN CHROMOSOME IDENTIFICATION

1879. Arnold: First visualization of human chromosomes.

1888. Waldeyer: The word chromosome (chroma: color, soma: body)

1882. Walther Flemming: 20-28 chromosomes in cells of cornea

1921. T.S. Painter: 48 human chromosomes, X & Y chromosomes (Science)

1956. Jo Hin Tijo es Albert Levan: 46 human chromosomes (Hereditas)

1959. Lejeune: trisomy 21=Down syndrome

KARIOTYPING CONFERENCES

1960. Denver: chromosomes numbered (1-22) based on their size

1963. London: chromosome grouping (A-G)

1966. Chicago: big chromosome syndromes

1971. Paris, 1976. Mexico, 1978. Stockholm: chromosome banding

1995. ISCN: International System for Human Cytogenetic Nomenclature

CELL

A eukaryotic cell contains a nucleus so the process of cell division involves nuclear division, called mitosis, and division of the cytoplasm, called cytokinesis. In mitosis, the daughter (or progeny) cells contain the same genetic information as the parent cell.

MITOSIS

Mitosis is the process by which the contents of the eukaryotic nucleus are separated into 2 genetically identical packages. Chromosomes replicate prior to the beginning of mitosis. As mitosis begins they condense and become visible under a light microscope. They appear as sister chromatids joined at the centromere. Mitosis is divided into 4 stages. During prophase, the nuclear envelope disintegrates and a spindle of microtubules forms. Centrioles may help organize the spindle as in this animal cell. During metaphase: the chromosomes line up neatly end-to-end along the center (equator) of the cell. The centrioles are now at opposite poles of the cell with the mitotic spindle fibers extending from them. The mitotic spindle fibers attach to each kinetochore of the sister chromatids. During anaphase: the sister chromatids are then pulled apart by the mitotic spindle which pulls one chromatid to one pole and the other chromatid to the opposite pole. During the final stage, telophase, a nuclear envelope is being formed around each set of chromosomes, the spindle disappears and the chromosomes decondense. The result is 2 nuclei, each with an identical set of chromosomes (Figure 1).

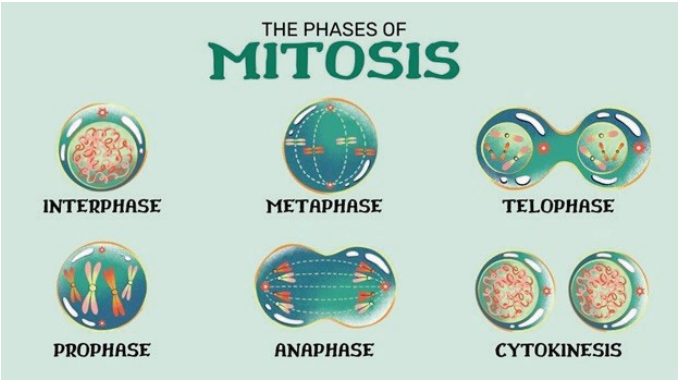


Figure 1. MITOSIS. https://earthhow.com/mitosis-phases/

THE KEY ROLES OF CELL DIVISION

The ability of organisms to reproduce their kind is the one characteristic that best distinguishes living things from nonliving matter. The continuity of life is based on the reproduction of cells, or cell division.

Cell division functions in reproduction, growth, and repair.

The division of a unicellular organism reproduces an entire organism, increasing the population. Cell division on a larger scale can produce progeny for some multicellular organisms. This includes organisms that can grow by cuttings. Cell division enables a multicellular organism to develop from a single fertilized egg or zygote.

All multicellular organisms use cell division for growth and the maintenance and repair of cells and tissues. Single-celled organisms use cell division as their method of reproduction. Somatic cells divide regularly; all human cells (except for the cells that produce eggs and sperm) are somatic cells. Cell division is part of the cell cycle, the life of a cell from its origin in the division of a parent cell until its own division into two.

Cell division results in genetically identical daughter cells

Cell division requires the distribution of identical genetic material—DNA—to two daughter cells. What is remarkable is the fidelity with which DNA is passed along, without dilution, from one generation to the next. A dividing cell duplicates its DNA, allocates the two copies to opposite ends of the cell, and then splits into two daughter cells. A cell's genetic information, packaged as DNA, is called its **genome**. In prokaryotes, the genome is often a single long DNA molecule. In eukaryotes, the genome consists of several DNA molecules.

A human cell must duplicate about 2 m of DNA and separate the two copies such that each daughter cell ends up with a complete genome. DNA molecules are packaged into **chromosomes**.

Every eukaryotic species has a characteristic number of chromosomes in each cell nucleus.

Human somatic cells have 46 chromosomes: 22 pairs and 2 sex chromosomes that may or may not form a pair. This is the **2n** or diploid condition. Human gametes have 23 chromosomes, one each of 23 unique chromosomes, one of which is a sex chromosome. This is the **n** or haploid condition.

Eukaryotic chromosomes are made of chromatin, a complex of DNA and associated proteins.

Each single chromosome contains one long, linear DNA molecule carrying hundreds or thousands of genes, the units that specify an organism's inherited traits. The associated proteins maintain the structure of the chromosome and help control gene activity. When a cell is not dividing, each chromosome is in the form of a long, thin chromatin fiber. Before cell division, chromatin condenses, coiling and folding to make a smaller package.

Each duplicated chromosome consists of two sister chromatids, which contain identical copies of the chromosome's DNA. The chromatids are initially attached by adhesive proteins along their lengths. As the chromosomes condense, the region where the chromatids connect shrinks to a narrow area, the **centromere.**

Later in cell division, the sister chromatids are pulled apart and repackaged into two new nuclei at opposite ends of the parent cell. Once the sister chromatids separate, they are considered individual chromosomes.

Mitosis, the formation of the two daughter nuclei, is usually followed by division of the cytoplasm, **cytokinesis.** These processes start with one cell and produce two cells that are genetically identical to the original parent cell.

Each of us inherited 23 chromosomes from each parent: one set in an egg and one set in sperm. The fertilized egg, or zygote, underwent cycles of mitosis and cytokinesis to produce a fully developed multicellular human made up of 200 trillion somatic cells.

These processes continue every day to replace dead and damaged cells. Essentially, these processes produce **clones**—cells with identical genetic information.

In contrast, gametes (eggs or sperm) are produced only in gonads (ovaries or testes) by a variation of cell division called meiosis.

Meiosis yields four non-identical daughter cells, each with half the chromosomes of the parent.

In humans, meiosis reduces the number of chromosomes from 46 to 23. Fertilization fuses two gametes together and doubles the number of chromosomes to 46 again.

THE MITOTIC PHASE ALTERNATES WITH INTERPHASE IN THE CELL CYCLE

The mitotic (M) phase of the cell cycle alternates with the much longer interphase. The M phase includes mitosis and cytokinesis. Interphase accounts for 90% of the cell cycle. During interphase, the cell grows by producing proteins and cytoplasmic organelles, copies its chromosomes, and prepares for cell division. Interphase has three subphases: the G1 phase ("first gap"), the S phase ("synthesis"), and the G2 phase ("second gap").

During all three subphases, the cell grows by producing proteins and cytoplasmic organelles such as mitochondria and endoplasmic reticulum. However, chromosomes are duplicated only during the S phase. The daughter cells may then repeat the cycle.

A typical human cell might divide once every 24 hours. Of this time, the M phase would last less than an hour, while the S phase might take 10–12 hours, or half the cycle. The rest of the time would be divided between the G1 and G2 phases. The G1 phase varies most in length from cell to cell.

Mitosis is a continuum of changes. For convenience, mitosis is usually broken into four subphases: prophase, metaphase, anaphase, and telophase. In S phase of interphase, the chromosomes have been duplicated but are not condensed. A nuclear membrane bounds the nucleus, which contains one or more nucleoli. The centrosome has replicated to form two centrosomes. In animal cells, each centrosome features two centrioles. In prophase, the chromosomes are tightly coiled, with sister chromatids joined together. The nucleoli disappear. The mitotic spindle begins to form. It is composed of centrosomes and the microtubules that extend from them. The radial arrays of shorter microtubules that extend from the centrosomes are called asters. The

centrosomes move away from each other, apparently propelled by lengthening microtubules. During late prophase (or prometaphase), the nuclear envelope fragments, and microtubules from the spindle interact with the condensed chromosomes. Each of the two chromatids of a chromosome has a kinetochore, a specialized protein structure located on surface of the chromatid. Kinetochore microtubules from each pole attach to one of two kinetochores. Non-kinetochore microtubules interact with those from opposite ends of the spindle. The spindle fibers push the sister chromatids until they are all arranged at the metaphase plate, an imaginary plane equidistant from the poles, defining metaphase. At anaphase, the centromeres divide, separating the sister chromatids. Each is now pulled toward the pole to which it is attached by spindle fibers. By the end, the two poles have equivalent numbers of chromosomes. At telophase, daughter nuclei begin to form at the two poles. Nuclear envelopes arise from the fragments of the parent cell's nuclear envelope and other portions of the endomembrane system. The chromosomes become less tightly coiled. Cytokinesis, division of the cytoplasm, is usually well underway by late telophase. In animal cells, cytokinesis involves the formation of a cleavage furrow, which pinches the cell in two. In plant cells, vesicles derived from the Golgi apparatus produce a cell plate at the middle of the cell.

THE MITOTIC SPINDLE DISTRIBUTES CHROMOSOMES TO DAUGHTER CELLS: A CLOSER LOOK

The mitotic spindle, fibers composed of microtubules and associated proteins, is a major driving force in mitosis. As the spindle assembles during prophase, the elements come from partial disassembly of the cytoskeleton. The spindle fibers elongate by incorporating more subunits of the protein tubulin. Assembly of the spindle microtubules starts in the centrosome. The centrosome (microtubule-organizing center) is a non-membranous organelle that organizes the cell's microtubules. In animal cells, the centrosome has a pair of centrioles at the center, but the centrioles are not essential for cell division. During interphase, the single centrosome replicates to form two centrosomes. As mitosis starts, the two centrosomes (or centrioles) are located near the nucleus. As the spindle

microtubules grow from them, the centrioles are pushed apart. By the end of prometaphase, they are at opposite ends of the cell. An aster, a radial array of short microtubules, extends from each centrosome (or centriole). The spindle includes the centrosomes, the spindle microtubules, and the asters. Each sister chromatid has a kinetochore of proteins and chromosomal DNA at the centromere. The kinetochores of the joined sister chromatids face in opposite directions. During prometaphase, some spindle microtubules (called kinetochore microtubules) attach to the kinetochores. When a chromosome's kinetochore is "captured" by microtubules, the chromosome moves toward the pole from which those microtubules come. When microtubules attach to the other pole, this movement stops and a tug-of-war ensues. Eventually, the chromosome settles midway between the two poles of the cell, on the metaphase plate. Non-kinetochore microtubules from opposite poles overlap and interact with each other. By metaphase, the microtubules of the asters have grown and are in contact with the plasma membrane. The spindle is now complete. Anaphase commences when the proteins holding the sister chromatids together are inactivated. Once the chromosomes are separate, full-fledged chromosomes, they move toward opposite poles of the cell. How do the kinetochore microtubules function into the poleward movement of chromosomes? One hypothesis is that the chromosomes are "reeled in" by the shortening of microtubules at the spindle poles. Experimental evidence supports the hypothesis that motor proteins on the kinetochore "walk" the attached chromosome along the microtubule toward the nearest pole. Meanwhile, the excess microtubule sections depolymerize at their kinetochore ends.

What is the function of the non-kinetochore microtubules? Non-kinetochore microtubules are responsible for lengthening the cell along the axis defined by the poles. These microtubules interdigitate and overlap across the metaphase plate.

During anaphase, the area of overlap is reduced as motor proteins attached to the microtubules walk them away from one another, using energy from ATP. As microtubules push apart, the microtubules lengthen by the addition of new tubulin monomers to their overlapping ends, allowing continued overlap.

Cytokinesis Divides the Cytoplasm: a Closer Look

Cytokinesis, division of the cytoplasm, typically follows mitosis. In animal cells, cytokinesis occurs by a process called cleavage. The first sign of cleavage is the appearance of a cleavage furrow in the cell surface near the old metaphase plate. On the cytoplasmic side of the cleavage furrow is a contractile ring of actin microfilaments associated with molecules of the motor protein myosin. Contraction of the ring pinches the cell in two. Cytokinesis in plants, which have cell walls, involves a completely different mechanism. During telophase, vesicles from the Golgi apparatus at the metaphase plate, forming a cell plate. The plate enlarges until its membranes fuse with the plasma membrane at the perimeter. The contents of the vesicles form new cell wall material between the daughter cells.

REGULATION OF CELL CYCLE

THE CELL CYCLE IS REGULATED BY A MOLECULAR CONTROL SYSTEM

The timing and rates of cell division in different parts of an animal or plant are crucial for normal growth, development, and maintenance.

The frequency of cell division varies with cell type.

- Some human cells divide frequently throughout life (skin cells).
- Others have the ability to divide, but keep it in reserve (liver cells).
- Mature nerve and muscle cells do not appear to divide at all after maturity.

Investigation of the molecular mechanisms regulating these differences provide important insights into the operation of normal cells, and may also explain cancer cells escape controls.

Cytoplasmic signals drive the Cell Cycle.

The cell cycle appears to be driven by specific chemical signals present in the cytoplasm.

Some of the initial evidence for this hypothesis came from experiments in which cultured mammalian cells at different phases of the cell cycle were fused to form a single cell with two nuclei.

- Fusion of an S phase cell and a G1 phase cell induces the G1 nucleus to start S phase.
- This suggests that chemicals present in the S phase nucleus stimulated the fused cell.
- Fusion of a cell in mitosis (M phase) with one in interphase (even G1 phase) induces the second cell to enter mitosis.

The Sequential Events of the Cell Cycle are Directed by a Distinct Cell Cycle Control System

Cyclically operating molecules trigger and coordinate key events in the cell cycle. The control cycle has a built-in clock, but it is also regulated by external adjustments and internal controls.

A checkpoint in the cell cycle is a critical control point where stop and go-ahead signals regulate the cycle. The signals are transmitted within the cell by signal transduction pathways. Animal cells generally have built-in stop signals that halt the cell cycle at checkpoints until overridden by go-ahead signals. Many signals registered at checkpoints come from cellular surveillance mechanisms. These indicate whether key cellular processes have been completed correctly. Checkpoints also register signals from outside the cell.

Three major checkpoints are found in the G1, G2, and M phases.

For many cells, the G1 checkpoint, the "restriction point" in mammalian cells, is the most important.

If the cell receives a go-ahead signal at the G1 checkpoint, it usually completes the cell cycle and divides. If it does not receive a go-ahead signal, the cell exits the cycle and switches to a nondividing state, the G0 phase. Most cells in the human body are in this phase. Liver cells can be "called back" to the cell cycle by external cues, such as growth factors released during injury. Highly specialized nerve and muscle cells never divide.

Rhythmic fluctuations in the abundance and activity of cell cycle control molecules pace the events of the cell cycle. These regulatory molecules include **protein kinases** that activate or deactivate other proteins by phosphorylating them. These kinases are present in constant amounts but require attachment of a second protein, **a cyclin**, to become activated. Levels of cyclin proteins fluctuate cyclically. Because of the requirement for binding of a cyclin, the kinases are called **cyclin-dependent kinases, or Cdks.**

Cyclin levels rise sharply throughout interphase, and then fall abruptly during mitosis. Peaks in the activity of one cyclin-Cdk complex, **MPF**, correspond to peaks in cyclin concentration. **MPF ("maturation-promoting factor" or "M-phase-promoting-factor")** triggers the cell's passage past the G2 checkpoint to the M phase. MPF promotes mitosis by phosphorylating a variety of other protein kinases. MPF stimulates fragmentation of the nuclear envelope by phosphorylation of various proteins of the nuclear lamina. It also triggers the breakdown of cyclin, dropping cyclin and MPF levels during mitosis and inactivating MPF.

The noncyclin part of MPF, the Cdk, persists in the cell in inactive form until it associates with new cyclin molecules synthesized during the S and G2 phases of the next round of the cycle.

At least three Cdk proteins and several cyclins regulate the key G1 checkpoint. Similar mechanisms are also involved in driving the cell cycle past the M phase checkpoint (Figure 2).

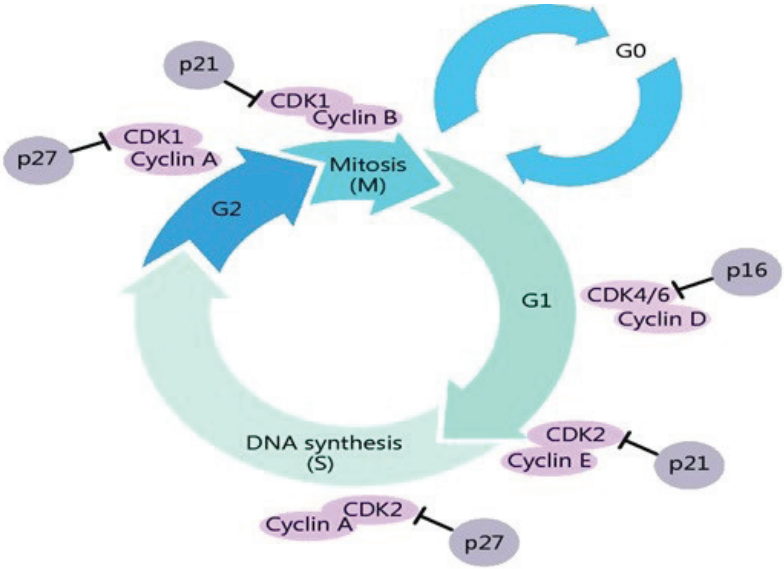


Figure 2. Regulation of Cell Cycle
https://www.researchgate.net/figure/A-schematic-view-of-the-cell-cycle-Each-phase-in-the-cell-cycle-progression-is-regulated_fig2_321798926

Internal and External Cues Help Regulate the Cell Cycle

While research scientists know that active Cdks function by phosphorylating proteins, the identity of all these proteins is still under investigation. Scientists do not yet know what Cdks actually do in most cases.

Some steps in the signaling pathways that regulate the cell cycle are clear.

Some signals originate inside the cell, others outside.

The M phase checkpoint ensures that all the chromosomes are properly attached to the spindle at the metaphase plate before anaphase. This ensures that daughter cells do not end up with missing or extra chromosomes. A signal to delay anaphase originates at kinetochores that have not yet attached to spindle microtubules.

This keeps the anaphase-promoting complex (APC) in an inactive state. When all kinetochores are attached, the APC activates, triggering breakdown of cyclin and inactivation of proteins holding sister chromatids together.

A variety of external chemical and physical factors can influence cell division. For example, cells fail to divide if an essential nutrient is left out of the culture medium. Particularly important for mammalian cells are growth factors, proteins released by one group of cells that stimulate other cells to divide.

For example, **platelet-derived growth factors (PDGF),** produced by platelet blood cells, bind to tyrosine-kinase receptors of fibroblasts, a type of connective tissue cell. This triggers a signal-transduction pathway that allows cells to pass the G1 checkpoint and divide.

Each cell type probably responds specifically to a certain growth factor or combination of factors. The role of PDGF is easily seen in cell culture. Fibroblasts in culture will only divide in the presence of a medium that also contains PDGF.

In a living organism, platelets release PDGF in the vicinity of an injury. The resulting proliferation of fibroblasts helps heal the wound. At least 50 different growth factors can trigger specific cells to divide.

The effect of an external physical factor on cell division can be seen in density-dependent inhibition of cell division. Cultured cells normally divide until they form a single layer on the inner surface of the culture container. If a gap is created, the cells will grow to fill the gap. At high densities, the amount of growth factors and nutrients is insufficient to allow continued cell growth. Most animal cells also exhibit anchorage dependence for cell division. To divide, they must be anchored to a substratum, typically the extracellular matrix of a tissue. Control appears to be mediated by pathways involving plasma membrane proteins and elements of the cytoskeleton linked to them. Cancer cells exhibit neither density-dependent inhibition nor anchorage dependence.

Cancer cells have escaped from Cell Cycle controls

Cancer cells divide excessively and invade other tissues because they are free of the body's control mechanisms. Cancer cells do not

stop dividing when growth factors are depleted. This is either because a cancer cell manufactures its own growth factors, has an abnormality in the signaling pathway, or has an abnormal cell cycle control system. If and when cancer cells stop dividing, they do so at random points, not at the normal checkpoints in the cell cycle. Cancer cells may divide indefinitely if they have a continual supply of nutrients. In contrast, nearly all mammalian cells divide 20 to 50 times under culture conditions before they stop, age, and die.

Cancer cells may be "immortal." HeLa cells from a tumor removed from a woman (Henrietta Lacks) in 1951 are still reproducing in culture. The abnormal behavior of cancer cells begins when a single cell in a tissue undergoes a transformation that converts it from a normal cell to a cancer cell. Normally, the immune system recognizes and destroys transformed cells. However, cells that evade destruction proliferate to form a tumor, a mass of abnormal cells. If the abnormal cells remain at the originating site, the lump is called a benign tumor. Most do not cause serious problems and can be fully removed by surgery.

In a malignant tumor, the cells become invasive enough to impair the functions of one or more organs. In addition to chromosomal and metabolic abnormalities, cancer cells often lose attachment to nearby cells, are carried by the blood and lymph system to other tissues, and start more tumors in an event called metastasis.

Cancer cells are abnormal in many ways. They may have an unusual number of chromosomes, their metabolism may be disabled, and they may cease to function in any constructive way. Cancer cells may secrete signal molecules that cause blood vessels to grow toward the tumor.

Treatments for metastasizing cancers include high-energy radiation and chemotherapy with toxic drugs. These treatments target actively dividing cells. Chemotherapeutic drugs interfere with specific steps in the cell cycle. For example, Taxol prevents mitotic depolymerization, preventing cells from proceeding past metaphase. The side effects of chemotherapy are due to the drug's effects on normal cells.

Researchers are beginning to understand how a normal cell is transformed into a cancer cell. The causes are diverse, but cellular transformation always involves the alteration of genes that influence the cell cycle control system.

CHROMOSOME STRUCTURE

What is a chromosome? Chromosomes are bundles of tightly coiled DNA located within the nucleus of almost every cell in our body. Humans have 23 pairs of chromosomes.

In plant and animal cells, DNA is tightly packaged into thread-like structures called chromosomes. This is in contrast to bacteria where DNA floats freely around the cell.

A single length of DNA is wrapped many times around lots of proteins called histones, to form structures called **nucleosomes.** These nucleosomes then coil up tightly to create chromatin loops. The chromatin loops are then wrapped around each other to make a full chromosome. Each chromosome has two short arms (p arms), two longer arms (q arms), and a centromere holding it all together at the center.

Humans have 23 pairs of chromosomes (46 in total): one set comes from your mother and one set comes from your father. Of these 23 pairs, one pair are sex chromosomes so differ depending on whether you are male or female (XX for female or XY for male). The other 22 pairs are autosomes (non-sex chromosomes) and look the same for both males and females.

The DNA making up each of our chromosomes contains thousands of genes. At the ends of each of our chromosomes are sections of DNA called **telomeres.** Telomeres protect the ends of the chromosomes during DNA replication by forming a cap, much like the plastic tip on a shoelace (Figure 3).

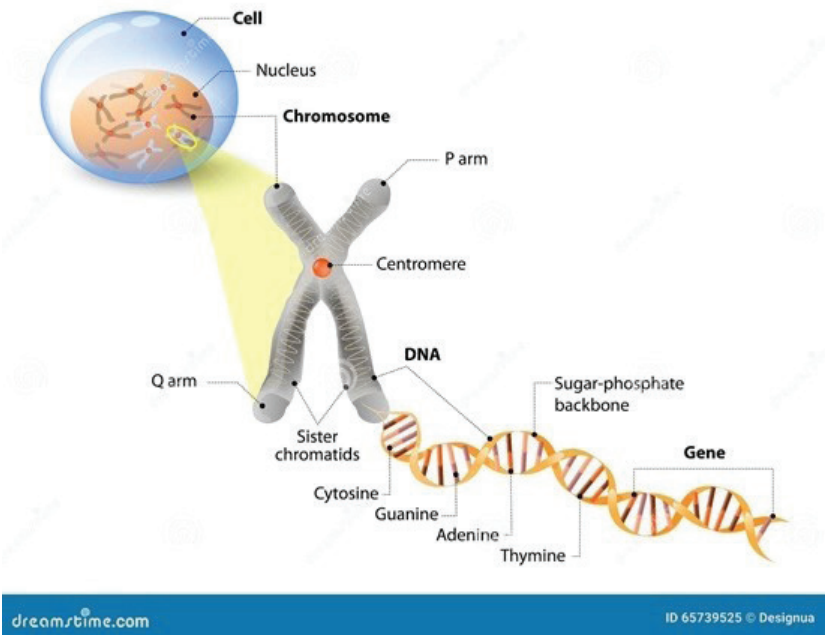


Figure 3. Chromosome structure. https://thumbs.dreamstime.com/z/cell-chromosome-dna-gene-structure-molecule-double-helix-length-codes-specific-65739525.jpg?ct=jpeg

CHROMOSOME CLASSIFICATION

Chromosomes are packaged and condensed into a complicated structure with proteins called chromatin. Chromosomes are best seen during cell division when they are most condensed, especially during metaphase. They are seen by light microscope or by special stains. The centromere is the point where the two chromatids touch, and where the microtubules attach therefore helping in the movement of chromosomes at cell division. It divides it into short (p; Figure 4).) and long arms (q; on Figure 4). The telomere is the tip of each end.

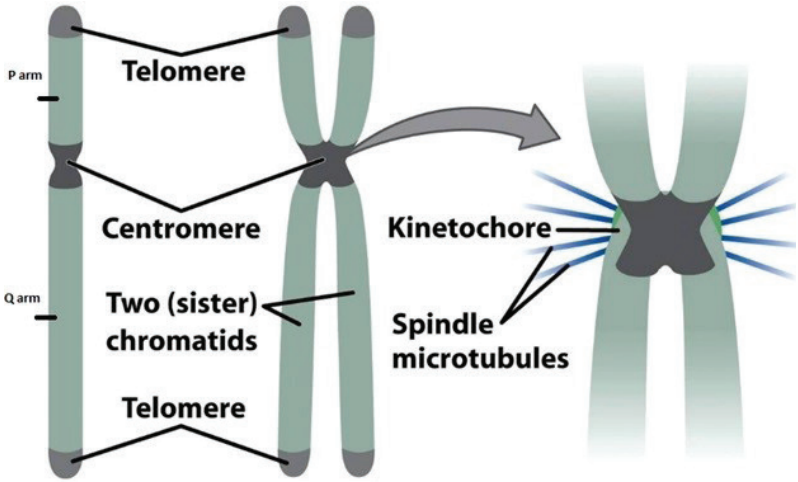


Figure 4. Chromosome Morphology. (https://www.toppr.com/ask/question/explain-the-structure-of-chromosome-with-diagram/)

Size & shape classification: this is based on the length of chromosomes, i.e. large, medium, or short chromosomes.

Morphological classification: this is based on the position of the centromere (Figure 5). Chromosomes can be:

- **Metacentric:** when the two chromosome arms are equal in length.
- **Submetacentric:** when the two chromosome arms are unequal in length, e.g. shorter p arms and longer q arms.
- **Acrocentric:** when the p (short) arm is too short to observe but is still present. There are 5 acrocentric chromosomes in the human genome: 13, 14, 15, 21, and 22.
- **Telocentric:** when the centromere is located at the end of the chromosome. There are no telocentric chromosomes in the human genome.
- **Holocentric:** when the centromere makes up the entire length of the chromosome. There are no holocentric chromosomes in the human genome.

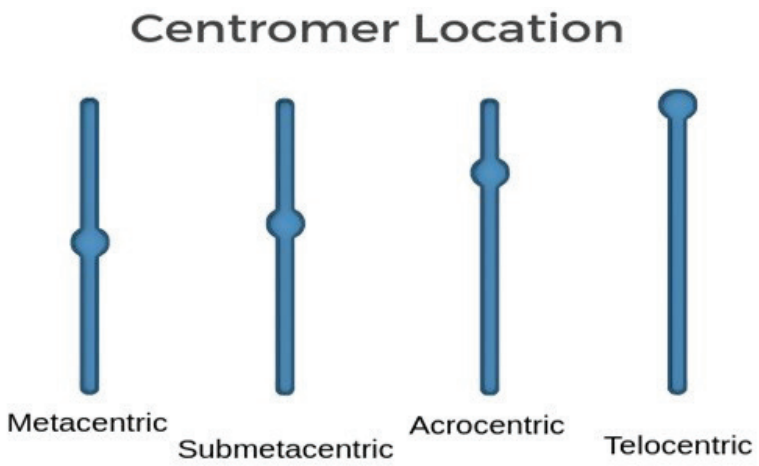


Figure 5. Centromere location (https://theory.labster.com/chromosome-structure/)

CHROMATIN STRUCTURE

In eukaryotic cells, the DNA molecule is found in the form of a nucleoprotein complex named chromatin. The basic unit of the chromatin is the nucleosome, which comprises 147 base pairs of DNA wrapped around an octamer of core histones (made of two molecules of each H2A, H2B, H3, and H4 histones). Each nucleosome is linked to the next by small segments of linker DNA. Most chromatin is further condensated by winding in a polynucleosome fibre, which may be stabilized through the binding of histone H1 to each nucleosome and to the linker DNA. The modulation of the structure of the chromatin fibre is critical for the regulation of gene expression since it determines the accessibility and the sequential recruitment of regulatory factors to the underlying DNA. Depending on the different transcriptional states, the structure of the chromatin may be altered in its constituents (e.g. the presence of repressors, activators, chromatin remodelling complexes, and/or incorporation of histone variants), and in covalent modifications of its constituents (such as DNA methylation at cytosine residues, and posttranslational modifications of histone tails).

HETEROCHROMATIN AND EUCHROMATIN

Eukaryotes are characterized by the extensive packaging of their genomes, initially in a nucleosomal array, and further into higher order domains. Differential packaging is used as a mechanism of gene regulation, with stable silencing of large domains achieved by packaging the deoxyribonucleic acid (DNA) into a heterochromatic structure. Chromosome rearrangements and transgene insertions that misplace euchromatic genes near or within the heterochromatin result in silencing of the euchromatic genes, testifying to a distinct heterochromatin assembly that can antagonize transcription. Most heterochromatic regions are rich in repetitious sequences, frequently derived from transposable elements, and such packaging helps to silence such elements. Domains of heterochromatin and euchromatin are defined by specific covalent modifications of histones and, in some cases, DNA, as well as by associations with a specific subset of nonhistone chromosomal proteins. Chromosomal domains may be targeted for heterochromatin formation by specific noncoding ribonucleic acids (RNAs).

Key Concepts:

- The chromatin of eukaryotes is differentially packaged into domains of euchromatin and heterochromatin.
- Displacing a euchromatic gene to near or within the heterochromatin often results in silencing of the euchromatic gene in some of the cells in which it should be active, resulting in a variegating phenotype.
- Euchromatin and heterochromatin are distinguishable biochemically by different covalent modifications of histones (and in some cases DNA) and by distinct nonhistone proteins.
- Members of the HP1a chromo domain protein family bind methylated histone H3 and interact with the H3K9 histone methyltransferase to organise transcriptionally repressive heterochromatin.
- The piRNA pathway is implicated in targeting transposon silencing through local heterochromatin formation. (https://www.ncbi.nlm.nih.gov/pubmed/16836980).

DIFFERENCE BETWEEN HETEROCHROMATIN AND EUCHROMATIN

When the non-dividing cells of the nucleus were observed under the light microscope, it exhibited the two regions, on the basis of concentration or intensity of staining. The dark stained areas are said as heterochromatin and light stained areas are said as euchromatin.

Around 90% of the total human genome is euchromatin. They are the parts of chromatin and participate in the protection of DNA in the genome present inside the nucleus. Emil Heitz in the year 1928, coined the term Heterochromatin and Euchromatin.

DEFINITION OF HETEROCHROMATIN

The area of the chromosomes which are intensely stained with DNA-specific strains and are relatively condensed is known as heterochromatin. They are the tightly packed form of DNA in the nucleus.

The organization of heterochromatin is so highly compact in the way that these are inaccessible to the protein which is engaged in gene expression. Even the chromosomal crossing over is not possible due to the above reason. Resulting them to be transcriptionally as well as genetically inactive. The major difference between heterochromatin and euchromatin is that heterochromatin is such part of the chromosomes, which is a firmly packed form and are genetically inactive, while euchromatin is an uncoiled (loosely) packed form of chromatin and are genetically active.

When the non-dividing cells of the nucleus were observed under the light microscope, it exhibited the two regions, on the basis of concentration or intensity of staining. The dark stained areas are said as heterochromatin and light stained areas are said as euchromatin.

Heterochromatin is of two types: **Facultative heterochromatin** and **constitutive heterochromatin.** The genes which get silenced through the process of Histone methylation or siRNA through RNAi are called as facultative heterochromatin. Hence, they contain inactive genes and is not a permanent character of every nucleus of the cells.

While the repetitive and structurally functional genes like telomeres or centromeres are called as Constitutive heterochromatin. These are the continuing nature of the cell's nucleus and contains no gene in the genome. This structure is retainable during the interphase of the cell.

The main function of the heterochromatin is to protect the DNA from the endonuclease damage; it is due to its compact nature. It also prevents the DNA regions to get accessed to proteins during gene expression.

DEFINITION OF EUCHROMATIN

That part of chromosomes, which are rich in gene concentrations and are loosely packed form of chromatin is called as euchromatin. They are active during transcription.

Euchromatin covers the maximum part of the dynamic genome to the inner of the nucleus and is said that euchromatin contains about 90% of the entire human genome.

To allow the transcription, some parts of the genome containing active genes are loosely packed. The wrapping of DNA is so loose that DNA can become readily available. The structure of euchromatin resembles the nucleosomes, which consist of histones proteins having around 147 base pairs of DNA wrapped around them.

Euchromatin actively participates in transcription from DNA to RNA. The gene regulating mechanism is the process of transforming euchromatin into heterochromatin or vice versa.

The active genes present in euchromatin gets transcribed to make mRNA whereby further encoding the functional proteins is the main function of euchromatin. Hence they are considered as genetically and transcriptionally active. Housekeeping genes are one of the forms of euchromatin.

By focussing on the few more points, we will be able to understand the difference between both types of chromatin. Given below is the comparison chart along with them (Table 1).

Table 1. **HETEROCHROMATIN VS EUCHROMATIN**
(https://thebiologynotes.com/heterochromatin-vs-euchromatin/)

BASIS FOR COMPARISON	HETEROCHROMATIN	EUCHROMATIN
Meaning	The tightly packed form of DNA in the chromosome is called as heterochromatin	The loosely packed form of DNA in the chromosome is called as euchromatin
DNA density	High DNA density	Low DNA density
Kind of staining	Stained dark	Lightly stained
Where they are present	These are found at the periphery of the nucleus in eukaryotic cells only	These are found in the inner body of the nucleus of prokaryotic as well as in eukaryotic cells
Transcriptional activity	They show little or no transcriptional activity	They actively participate in the process of transcription
Other features	They are compactly coiled	They are loosely coiled
	They are late replicative	They are early replicative
	Regions of heterochromatin are sticky	Regions of euchromatin are non-sticky
	Genetically inactive	Genetically active
	Phenotype remains unchanged of an organism	Variation may be seen, due to the affect in DNA during the genetic process
	It permits the gene expression regulation and also maintains the structural integrity of the cell	It results in genetic variations and permits the genetic transcription

TELOMERES AND TELOMERASE IN AGEING AND CANCER

Telomeres are protective structures that cap the ends of linear chromosomes. In humans, they are made of a repetitive DNA (deoxyribonucleic acid) sequence, (TTAGGG), and associated proteins. Because DNA polymerases are unable to completely replicate the ends of linear chromosomes, telomeres shorten each time cells divide. This gradual shortening of the telomeres limits their cellular lifespan and contributes to the ageing process. During cancer development, this obstacle to immortality is almost always bypassed by the overexpression of telomerase, a specialized reverse transcriptase that functions to add back telomeric repeats to telomeres. In humans, its expression is limited to rare stem cells of renewal tissues, but a notable exception has been its overexpression in the great majority of cancers. Accordingly, telomerase is being developed as both a novel marker for the detection of cancer cells and a novel target for the treatment of cancers. Herein, we discuss the role of telomeres and telomerase in cancer and ageing, with the emphasis on potential future applications in the treatments of cancers and age-related diseases.

Key Concepts

- Telomeres are protective structures that cap the ends of chromosomes.
- Human telomeres are made of TTAGGG DNA repeats and associated proteins.
- Because of 'end replication problems', telomeres shorten each time cells divide, and this shortening limits cellular lifespan and contributes to the ageing process.
- Telomerase is a specialized reverse transcriptase that functions to add back telomeric repeats to the ends of telomeres, thereby extending cellular lifespan.
- Telomerase expression in humans is limited to rare stem cells of renewal tissues (gastrointestinal track, blood and skin).

- Telomerase is almost always aberrantly overexpressed in cancer. This overexpression is detected in 85% of all cancers, irrespective of the tumor type.
- Telomerase is being developed as a marker to allow the detection of cancer cells in otherwise telomerase-negative normal tissues.
- Inhibitors of telomerase are being developed to limit tumor growth and reduce the incidence of recurrences following conventional cancer therapy.
- Strategies for transient telomerase reactivation are also being developed for the treatment of degenerative diseases and age-related diseases.

HUMAN KARYOTYPE: IDENTIFICATION OF CHROMOSOMES

The isolation and microscopic observation of chromosomes forms the basis of Cytogenetics and is the primary method by which clinicians detect chromosomal abnormalities in humans. A karyotype is the number and appearance of chromosomes. To obtain a view of an individual's karyotype, cytologists photograph the chromosomes and then cut and paste each chromosome into a chart, or karyogram, also known as an ideogram.

Chromosomes are packaged and condensed into a complicated structure with proteins called chromatin. Chromosomes are best seen during cell division when they are most condensed, especially during metaphase. They are seen by light microscope or by special stains (Figure 6).

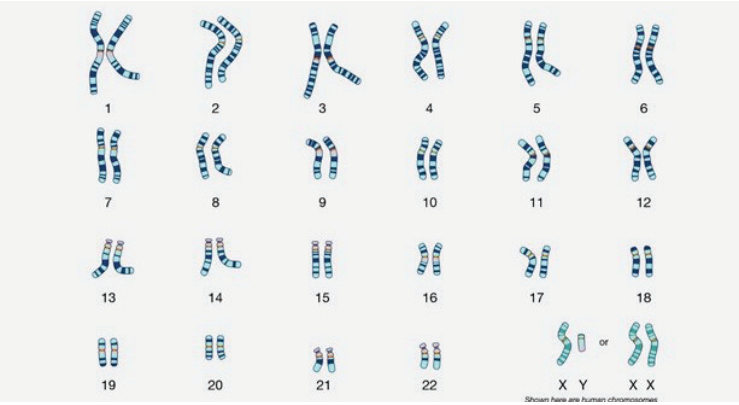


Figure 6. A HUMAN KARYOTYPE: This karyotype is of a male human. Notice that homologous chromosomes are the same size, and have the same centromere positions and banding patterns. A human female would have an XX chromosome pair instead of the XY pair shown. https://www.genome.gov/genetics-glossary/Karyotype

CHROMOSOME ABNORMALITIES

1. **Constitutional:** exist at birth. These are usually present in all tissues, if present only in some tissues, it is called mosaicism and it means that the abnormality occurred in the mitotic divisions that follow zygote formation

2. **Acquired:** occur during the life of a healthy individual and are confined to one tissue as seen in tumor cells.

Frequencies of Chromosome Abnormalities

- 2% of sperms have Chromosomal abnormalities
- l20% of ova have Chromosomal abnormalities
- So among 100 conceptions, there are 25% chromosome abnormalities
- In every 100 pregnancies, there occurs 15 spontaneous miscarriages, 50% of which have chromosome abnormalities
- Among 160 births, one baby is born with a chromosome abnormality

We recognize two types of chromosomal abnormalities:

- numerical;
- structural.

We are able to find the disorders due to karyotype testing. The cytogeneticists get the samples (blood, amnionic fluid), then stain the chromosomes and arrange them in the right order by their length. Then they can see some changes in pattern (banding). The most typical method is Giemsa stain, so it is usually called "G banding". This is important for structural abnormalities. Numeral abnormalities can be identified even easier.

Other classification of chromosomal abnormalities depends on fact which type of chromosome is affected – autosomes (Down syndrome, DiGeorge syndrome) or sex chromosomes (Klinefelter syndrome, Turner syndrome).

NUMERICAL CHROMOSOMAL ABNORMALITIES

Autosomal Chromosomal Abnormalities

Condition: TRISOMY 13 (PATAU SYNDROME)

Inheritance: Chromosomal.

Genetic etiology: Trisomy for chromosome 13 due to nondisjunction.

Frequency: Approximately 1:12000 live births.

Clinical features: Infants with trisomy 13 are born with low birth weight and have multiple congenital anomalies. Most notable are facial anomalies, including hypotelorism, and, in many cases, cleft lip and palate. There may be areas of deficient skin in the scalp and "rocker bottom feet". Anomalies of internal organs, including the heart, brain, and kidneys, are common.

Management: Most affected children die in infancy or childhood. Those who survive tend to have severe developmental impairment.

Genetic counseling: Couples who have had an affected child are usually counseled that recurrence risk is around 1%. Trisomy 13 can be detected by prenatal chromosomal analysis (Figure 7).

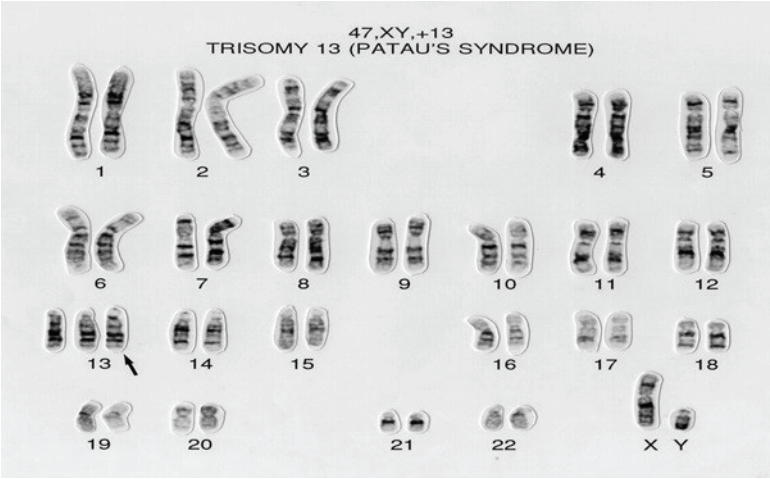


Figure 7. PATAU-SYNDROME (https://iiif.wellcomecollection.org/image/B0000251/full/full/0/default.jpg)

CONDITION: TRISOMY 18 (EDWARDS SYNDROME)

Inheritance: Chromosomal.

Genetic etiology: Trisomy for chromosome 18 due to nondisjunction.

Frequency: Approximately 1:6,000 live births.

Clinical features: Infants with trisomy 18 are born with multiple congenital anomalies. Most notable are low birth weight, prominent occiput, and tightly clenched fingers. Internal organ malformations, especially of the heart and brain, are common.

Management: Most affected children die in infancy or childhood. Those who survive tend to have severe developmental delay.

Genetic counseling: Couples who have had an affected child are usually counseled that recurrence risk is around 1%. Trisomy 18 can be detected by prenatal chromosomal analysis (Figure 8).

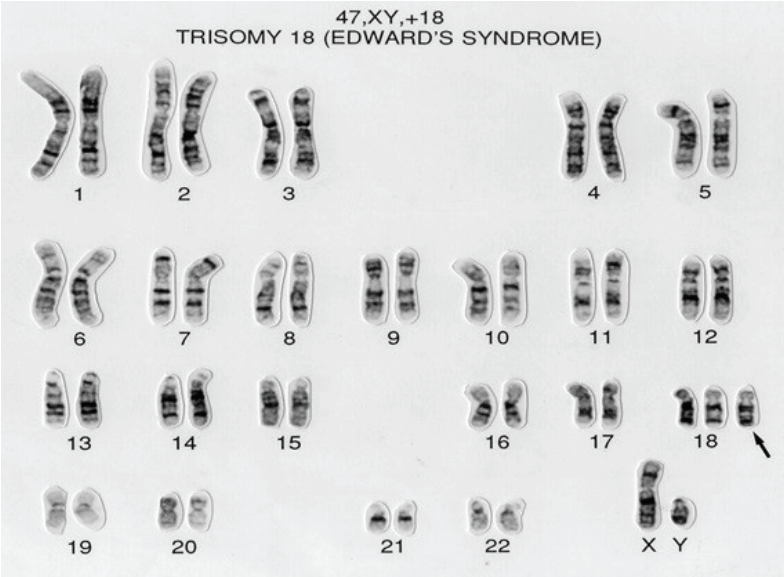


Figure 8. TRISOMY 18 (EDWARDS SYNDROME)
(https://iiif.wellcomecollection.org/image/B0000250/full/full/0/default.jpg)

CONDITION: DOWN SYNDROME (TRISOMY 21)

Inheritance: Chromosomal.

Genetic etiology: Presence of three copies (trisomy) of chromosome 21 material. Most often this is due to having 47 chromosomes, with three copies of №21, due to nondisjunction. In about 5% of cases, it is due to translocation between chromosome 21 and another chromosome, usually 14. Approximately 2% of affected individuals are mosaic for a trisomy 21 cell line.

Frequency: Approximately 1:800 live births.

Clinical features: Children are hypotonic at birth and may have congenital anomalies, especially cardiac and gastrointestinal. Facial features include flat occiput, up-slanting palpebral fissures, furrowed tongue, short fingers and toes, incurved fifth finger (clinodactyly), and a wide space between the first and second toes. Cognitive development tends to be delayed, though is variable. There is an increased risk of respiratory infection during childhood and a higher risk of leukemia and

transient leukemoid reactions in infancy than the general population. Adults are at risk of early onset Alzheimer disease.

Management: Although there is no definitive treatment, affected individuals benefit from surveillance for treatable complications and participation in early intervention and educational programs to stimulate development. Congenital anomalies, when they occur, usually can be treated surgically.

Genetic counseling: Recurrence risk to a couple with an affected child is approximately 1%. Instances where a parent carries a balanced Robertsonian translocation are associated with an increased recurrence risk. Pregnancies can be screened for carrying fetuses with Down syndrome by biochemical testing and ultrasound, and trisomy 21 can be readily detected by prenatal chromosomal analysis. Advanced maternal age is the best documented risk factor other than having a previously affected child (Figure 9).

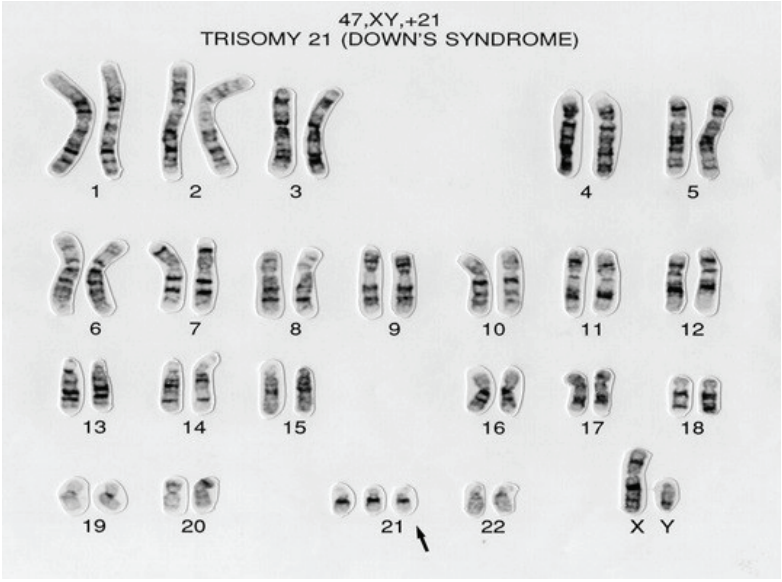


Figure 9. DOWN SYNDROME (TRISOMY 21)
(https://iiif.wellcomecollection.org/image/B0000249/full/full/0/default.jpg)

SEX CHROMOSOME ABNORMALITIES

CONDITION: 45, X (TURNER SYNDROME)

Inheritance: Chromosomal.

Genetic etiology: 45, X karyotype due to nondisjunction or loss of a structurally abnormal X or Y chromosome. Many are mosaics, with a cell line containing 46 chromosomes with a structurally abnormal X or Y.

Frequency: Approximately 1/3,000 female live births.

Clinical features: Individuals with Turner syndrome have a female phenotype, but may fail to develop normal secondary sex characteristics, such as pubic and axillary hair. They tend to have short stature, primary amenorrhea and usually are infertile. Coarctation of the aorta may occur, and some have renal anomalies. Lymphedema is common at birth, and redundant nuchal skin may lead to "webbing" of the neck. Learning disabilities, especially visual-spatial perceptual problems, are common.

Management: Treatment with hormones to promote secondary sexual development; surgical correction of congenital heart defects; anticipatory guidance and early intervention to overcome learning disabilities.

Genetic counseling: Recurrence is rare and can be detected by prenatal chromosomal analysis (Figure 10).

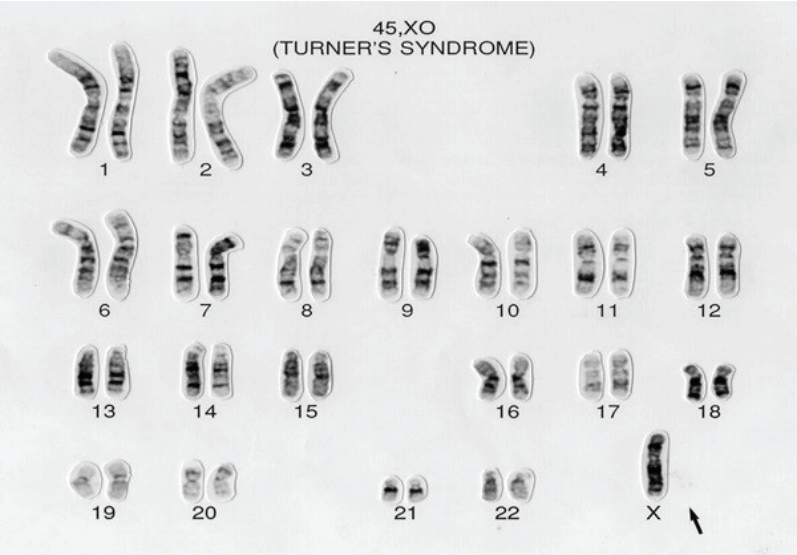


Figure 10. TURNER SYNDROME (https://iiif.wellcomecollection.org/image/B0000252/full/full/0/default.jpg)

CONDITION: XXY (KLINEFELTER SYNDROME)

Inheritance: Chromosomal.

Genetic etiology: 47, XXY karyotype due to nondisjunction; some have additional X chromosomes.

Frequency: 1/500 1/1,000 male live births.

Clinical features: Individuals with Klinefelter syndrome have a male phenotype, but may fail to develop normal secondary sex characteristics, such as pubic and axillary hair. They tend to have small testes and usually produce few or no sperm. Some degree of breast development may occur. Learning disabilities, especially involving language skills, are commonly seen.

Management: Treatment with testosterone to promote male secondary sexual development; counseling regarding probable infertility; early intervention to manage learning disabilities.

Genetic counseling: Recurrence is rare and can be detected by prenatal chromosomal analysis (Figure 11).

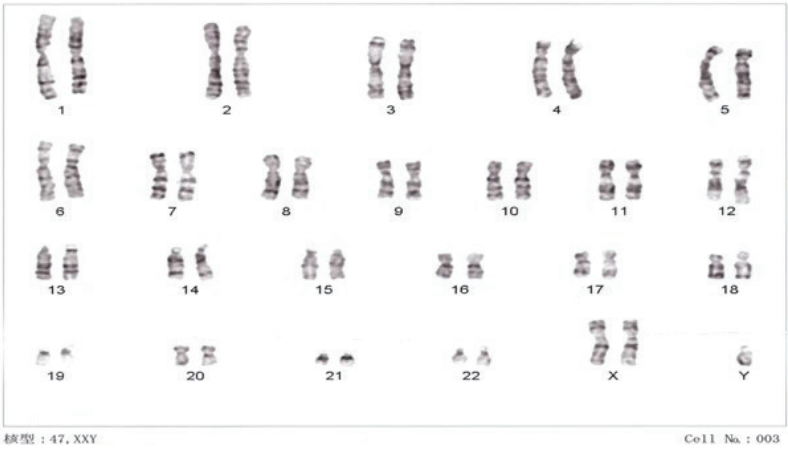


Figure 11. XXY (KLINEFELTER SYNDROME) (https://en.wikipedia.org/wiki/Klinefelter_syndrome#/media/File:Human_chromosomesXXY01.png)

CONDITION: 47, XYY (JACOB'S SYNDROME OR XYY, YY SYNDROME)

Inheritance: Chromosomal.

Genetic etiology: 47, XYY karyotype due to nondisjunction.

Frequency: Approximately 1:1,000 live born males.

Clinical features: Individuals with XYY have a male phenotype and are fertile. They tend to have learning disabilities and behavioral problems. Many have relatively tall stature.

Management: Anticipatory guidance and support for learning disabilities.

Genetic counseling: Recurrence is rare and can be detected by prenatal chromosomal analysis. Although males with XYY are fertile, transmission of XXY or XYY is rare (Figure 12).

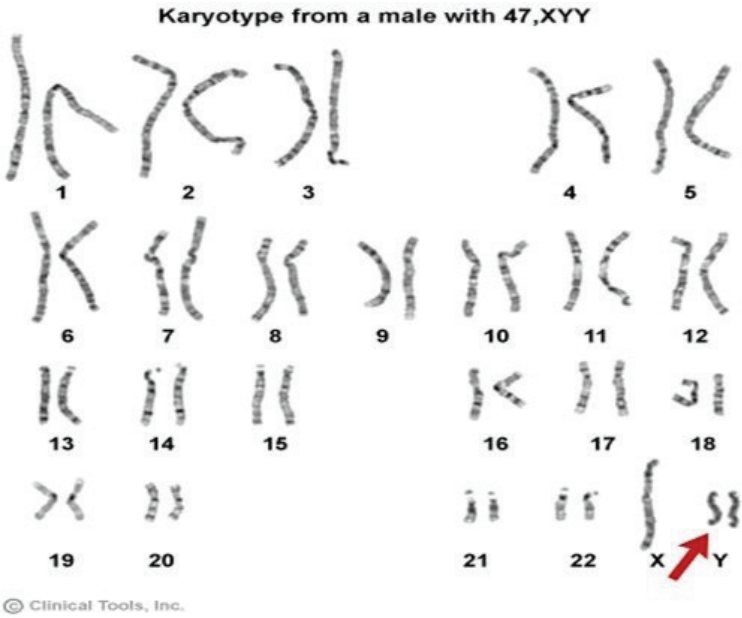


Figure 12. 47, XYY or JACOB'S SYNDROME. (https://en.wikipedia.org/wiki/File:XYY_Syndrome_DNA.jpg)

CONDITION: 47, XXX (TRIPLE X SYNDROME OR 47, XXX)

Inheritance: Chromosomal.

Genetic etiology: 47, XXX karyotype due to nondisjunction; some have more than 3 X chromosomes.

Frequency: Approximately 1/1,000 live born females.

Clinical features: Individuals with XXX syndrome have a female phenotype and tend to be fertile. They tend have cognitive impairment but usually do not have major congenital anomalies. People with more than one extra copy of the X chromosome (48, XXXX or 49, XXXXX) have been identified, but these chromosomal changes are rare. As the number of extra sex chromosomes increases, so does the risk of learning problems, intellectual disability, birth defects, and other health issues.

Management: Anticipatory guidance and support for developmental impairment.

Genetic counseling: Recurrence is rare and can be detected by prenatal chromosomal analysis (Figure 13).

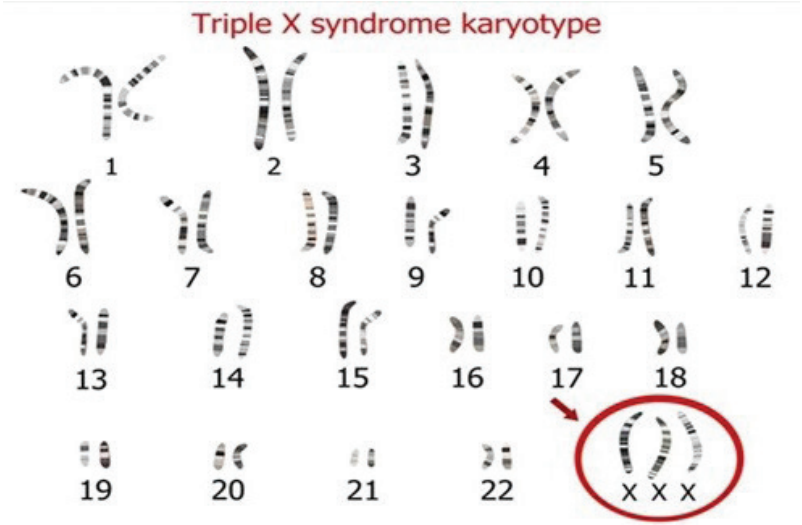


Figure 13. 47, XXX (Triple X syndrome karyotype – superfemale. Image Credit: Zuzanae / Shutterstock)

STRUCTURAL HUMAN CHROMOSOMAL ABNORMALITIES

Structural abnormalities arise from chromosome breakage and fusion/ reconstitution of chromosomes in an aberrant manner. Such chromosomal rearrangements can be balanced or unbalanced, depending on how they arise. For instance, in unbalanced rearrangements, the individuals may be missing chromosome material or may have gained additional chromosomal material.

In summary, chromosome abnormalities can be inherited from a parent (such as a translocation) or can be *de novo* (new to the individual). Chromosomal abnormalities could arise as a result of an abnormality produced by specific chromosomal mechanisms, such

as misrepair of broken chromosomes or improper recombination events, or by incorrect segregation of chromosomes during mitosis or meiosis. There are certain other factors that can increase the risk of chromosomal abnormalities, such as maternal age.

When the chromosome's structure is altered, this can take several forms:

- **Deletions**: A portion of the chromosome is missing or has been deleted. Known disorders in humans include Wolf–Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder.
- **Duplications:** A portion of the chromosome has been duplicated, resulting in extra genetic material. Known human disorders include Charcot–Marie–Tooth disease type 1A, which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.
- **Inversions:** A portion of the chromosome has broken off, turned upside down, and reattached, therefore the genetic material is inverted.
- **Insertions:** A portion of one chromosome has been deleted from its normal place and inserted into another chromosome.
- **Translocations:** A portion of one chromosome has been transferred to another chromosome. There are two main types of translocations:
  - **Reciprocal translocation:** Segments from two different chromosomes have been exchanged.
  - **Robertsonian translocation:** An entire chromosome has attached to another at the centromere - in humans, these only occur with chromosomes 13, 14, 15, 21, and 22.
- **Rings:** A portion of a chromosome has broken off and formed a circle or ring. This happens with or without the loss of genetic material.
- **Isochromosome:** Formed by the mirror image copy of a chromosome segment including the centromere.

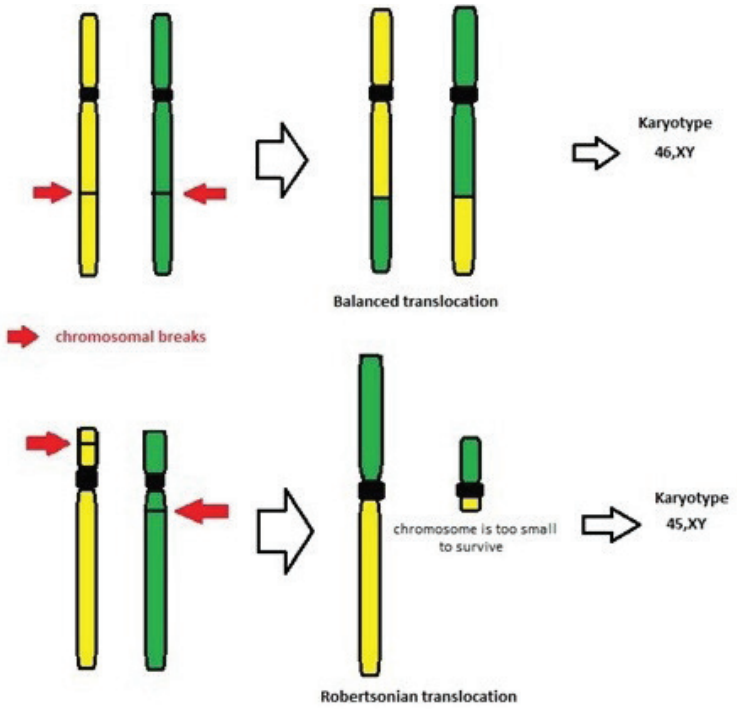


Figure 14. Types of Translocations https://www.wikilectures.eu/sites/www.wikilectures.eu/images/e/ee/Translocation.jpg

Chromosome instability syndromes are a group of disorders characterized by chromosomal instability and breakage. They often lead to an increased tendency to develop certain types of malignancies (Figures 14–17).

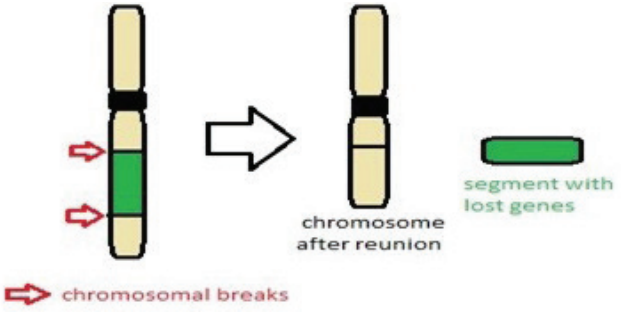


Figure 15. Deletion. https://www.wikilectures.eu/sites/www.wikilectures.eu/images/1/1b/Deletion.jpg

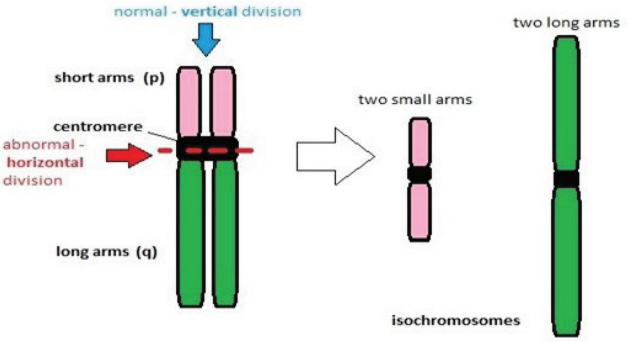


Figure16. Isochomosomes. https://www.wikilectures.eu/w/Chromosomal_Abnormalities#/media/File:Isochromosomes.jpg

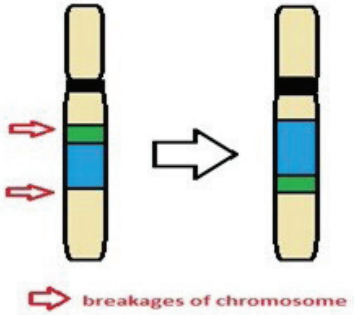


Figure17. Inversion. https://www.wikilectures.eu/w/Chromosomal_Abnormalities#/media/File:Invesion_chromosome.jpg

Chromosomal deletions can occur in many different sizes anywhere within the genome. Certain deletions in specific regions of chromosomes lead to specific syndromes that have been well characterized.

PRADER-WILLI SYNDROME AND ANGELMAN SYNDROME

Cause: Both diseases are due to an identical deletion on chromosome 15q and demonstrate imprinting, a phenomenon in which the same mutation results in different phenotypes depending on whether the mutated chromosome was of maternal or paternal origin.

PRADER-WILLI SYNDROME develops when the deletion is on the paternally derived chromosome, whereas Angelman syndrome develops when the deletion is on the maternally derived chromosome.

Characteristics: Prader-Willi syndrome: Mental retardation; hypogonadism; hypotonia; obesity leading to diabetes.

ANGELMAN SYNDROME: "Happy puppet" with ataxic gait and inappropriate laughter; mental retardation; seizures. Clinically, affected individuals develop an uncontrollable compulsion to eat starting at about five years of age. This leads to obesity and related health problems such as diabetes. Other symptoms include mental retardation, poor sexual development, and behavioral problems. The occurrence of this condition is estimated to be approximately 1 in 10,000 to 1 in 25,000 people. There is a greater preponderance of males affected with this condition.

Lymphocyte and fibroblast cell cultures from individuals with Prader-Willi syndrome are available from several different cell culture repositories. Detection of the aberrant karyotype can be conducted by using a specific fluorescent probe available through several different companies.

The following protocol for detection of Prader-Willi syndrome using FISH is based on the D15S11 (dig-labeled) DNA probe available from ONCOR, Inc. (3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate)

CRI DU CHAT SYNDROME

Cause: Caused by partial deletion of chromosome 5p.

Characteristics: Patients exhibit a high-pitched cat-like cry at birth, which is usually due to structural abnormalities in the larynx; patients also have severe developmental delay and cognitive deficits and distinctive facial abnormalities (round face, low-set ears, microcephaly, and a hypoplastic nasal bridge).

Lab findings: Cytogenetic studies reveal a deletion of chromosome 5p.

ETIOLOGY OF CHROMOSOME ABNORMALITIES

Etiology of chromosome abnormalities is pretty variable. The most often reason is mistake which occurs during the cell division. It is connected with wrong development of the sperm or ovum (female reproduction cell). Another causes are **the maternal age** and the influence of the **environmental**.

Chromosome abnormalities are usually fatal. Each second first-trimester abortion is caused by them. Children who survive and get born suffer from very serious mental and physical problems. The screening for chromosome abnormalities is very important. The cytogeneticists use the karyotype testing.

Age of Parents

It is widely known about the strong influence of **the maternal age** (especially in Down syndrome). **The paternal age** is less important, but still has its importance. The *difference is in cell division* of reproduction cells. The number of eggs (female´s cells) is done by our birth. So eggs underway meiosis many times. The later age of delivery means the higher risk of some abnormalities. It takes just 72 hours to the development of sperm cells. It is less probably to make a mistake during this period.

To the older women is recommended to visit some **genetic counseling** center. “The older women” means more than 35 years. The prenatal diagnosis is the best way to find some affected children. The method of prenatal diagnosis as an amniocentesis.

Influence of the Environment

It is very hard to tell how the environmental is important. Doctors can´t find any significant differences between parents with child with a chromosome abnormality and parents with healthy child. They have usually very similar lifestyle or habits. But there are still some **dangerous influences** – *X-rays*, *medication* or *food*. Most of them have a cumulative character.

Because doctors are not sure about the origin of abnormalities, it is hard to recommend any prevention. Sometimes it is said that the folic acid has a positive role in prevention of congenital abnormalities. Pregnant women should also get vitamins to reduce risks.

METHODS OF MOLECULAR CYTOGENETICS FLUORESCENCE IN SITU HYBRIDIZATION

Fluorescence in situ hybridization (FISH), a newer method for analyzing chromosomes, uses fluorescent molecules, called dyes, to "paint" genes on a chromosome. This technique is particularly useful for gene mapping and for detecting various chromosomal abnormalities. In this procedure, short sequences of DNA complementary to the sequence of interest, called probes, are hybridized to the sample DNA. Because the probes are labeled with fluorescent tags, a researcher can see the exact location of the DNA sequence of interest on a chromosome. An additional advantage of FISH is that it can be performed on nondividing cells, making it much more versatile than traditional karyotyping.

Scientists can actually create three types of FISH probes, each of which has a different application. Locus-specific probes hybridize to a particular region of a chromosome and are useful for detecting the location of a gene on a chromosome. Alphoid, or centromeric repeat probes, are generated from repetitive sequences found at the centromeres of chromosomes. Because each chromosome can be painted a different color, researchers use these probes to determine whether an individual has the correct number of chromosomes. Whole chromosome probes are actually collections of smaller probes, called libraries, that each hybridize to a different sequence along the same chromosome. Using these libraries, researchers can paint an entire chromosome with various colors, generating what is called a spectral karyotype. These types of probes are useful for examining both large- and small-scale chromosomal abnormalities.

Introduction to Fluorescence In Situ Hybridization (FISH)

In situ hybridization is a technique that allows highly sensitive detection of specific nucleic acid sequences (DNA or RNA) in specimens fixed on a microscope slide. The specimen may be chromosome spreads, cells, or tissue sections. Until recently, in situ hybridization was performed using radioactive labeled probes.

The transition to using fluorescent labels has vastly broadened the applications and increased the usage of the technology. Use of fluorescent labels for in situ hybridization is referred to as FISH. The major advantages to fluorescent probes include: safety, increased spatial resolution, reduced turn around time for results, and the capability of simultaneous detection of multiple DNA regions of interest by using different combinations of fluorochrome labeled probes.

There are many different applications of FISH in mammalian cytogenetics.

The major categories of applications include:

- **mapping** of genes and other DNA segments in genome research
- **identification** of species-specific chromosomes in somatic cell hybrids
- **identification** of chromosome aberrations, numerical and structural
- **characterization** of unknown marker chromosomes

There are two main types of probe labeling systems for FISH: direct and indirect. In the direct method, the fluorochrome molecule is bound directly to the nucleotides of the probe. This allows visualization of the probe signal immediately following the hybridization reaction and wash steps. In the indirect labeling method, hapten molecules are attached to the nucleotides which are detected by a secondary molecule conjugated to a fluorochrome. Two of the most popular detection systems are the digoxygenin-antidigoxygenin system and the biotin-strepavidin system.

Digoxygenin (dig) is a steroid isolated from digitalis plants (*Digitalis purpurea and Digitalis lanata*). Since the blossoms and the leaves of these plants are the only naturally occurring sources of this compound, the anti-dig antibody is highly specific for its target. Background hybridization is minimal. The anti-dig antibody can be conjugated with a variety of different fluorochromes, such as fluorescein (FITC), Texas Red, and rhodamine. These molecules can emit a distinct visible color upon excitation by a fluorescence or confocal microscope.

Alternatively, biotin may be conjugated to the base of nucleotides. Strepavidin has a very high binding capacity for biotin. Strepavidin may be conjugated to a variety of different fluorochromes.

The probes can be prepared in various ways. Two of the most common methods are referred to as nick translation and polymerase chain reaction (PCR). For nick translation, several companies sell nick translation kits that contain all the necessary reagents. Briefly, the procedure involves treating the DNA source with DNAse I to generate single stranded nicks along the double stranded DNA molecule. The 5' to 3' exonuclease activity of DNA polymerase I enzyme removes additional nucleotides to generate gaps. The same enzyme replaces the excised nucleotides with "labeled" nucleotides using the other complementary intact strand as template. The "labeled" nucleotides may contain a digoxigenin or biotin conjugated base for the indirect labeling method, or a fluorochrome conjugate such as fluorescein, rhodamine, or Texas Red for direct labeling.

The second method of preparing the probe uses PCR. Specific primers must be available to amplify the DNA sequences of interest. The primer sequences can be synthesized to order through a variety of different vendors. The nucleotides added to the PCR reaction may contain a digoxigenin or biotin conjugated base for the indirect labeling method, or a fluorochrome conjugate for direct labeling.

Hybridization involves the reannealing (also called renaturation) of denatured single stranded DNA to their complementary sequences. In FISH, the probe must anneal with its complementary sequence on the chromosome before the original complementary strand does. There are a number of factors that effect this process. They include:

- **Concentration of formamide** - DNA typically requires very high temperatures for a prolonged period of time for denaturation. This process can cause deterioration of sample morphology. However, certain organic solvents, such as formamide, reduces the thermal stability of double stranded DNA and allows hybridization to occur at lower temperatures. The rate of renaturation decreases in the presence of formamide.
- **Salt concentration -** The electrostatic repulsion between the two strands of a DNA molecule, mainly at the negatively charged phosphate groups, are reduced in higher concentrations of monovalent cations, such as sodium ions. This results in increased stability of

the DNA. Thus higher salt concentrations decreases the stringency of the hybridization.

- **Temperature** – Temperature effects both the denaturation and renaturation process. Increasing the temperature will favor the denaturation process, while decreasing the temperature will favor renaturation. Therefore, higher temperatures will increase the stringency of the hybridization (Figure 18).

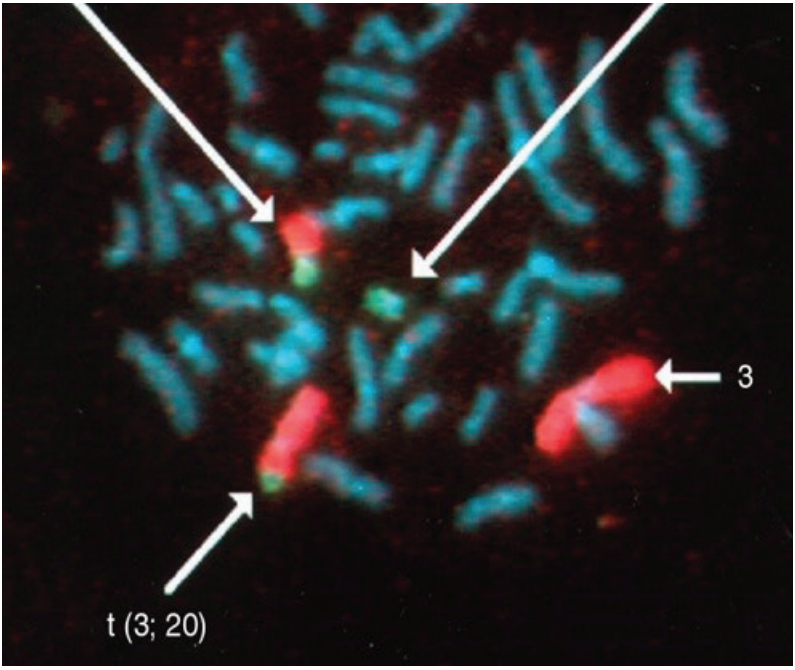


Figure 18. Chromosome painting showing a reciprocal translocation involving chromosomes 3 (red) and 20 (green) (Courtesy Catherine Delmege, Bristol Genetics Laboratory, Southmead Hospital, Bristol, UK)

The majority of FISH applications involve hybridization of one or two probes of interest as an adjunctive procedure to conventional chromosomal banding techniques. So, FISH can be utilized to identify specific chromosomes, characterize

de novo duplications or deletions, and clarify subtle chromosomal rearrangements. Its greatest utilization in constitutional analysis is in the detection of microdeletions.

In cancer cytogenetics, it is used extensively in the analysis of structural rearrangements .

The usually performed FISH is metaphase FISH. However**, interphase analysis** can be used to make a rapid diagnosis in instances when metaphase chromosome preparations are not yet available. Interphase analysis also increases the number of cells available for examination, allows for investigation of nuclear organization, and provides results when cells do not progress to metaphase.

The use of interphase FISH has increased recently, especially for analyses of amniocentesis samples and cancer cytogenetics .

FISH comparative genomic hybridization (CGH) is a method that can be used only when DNA is available from a specimen of interest. The entire DNA specimen from the sample of interest is labeled in one color (e.g., green), and the normal control DNA specimen is indicated by another color (e.g., red). These are mixed in equal amounts and hybridized to normal metaphase chromosomes. The red-to-green ratio is analyzed by a computer program that determines where the DNA of interest may have gains or losses of material (Figure 19).

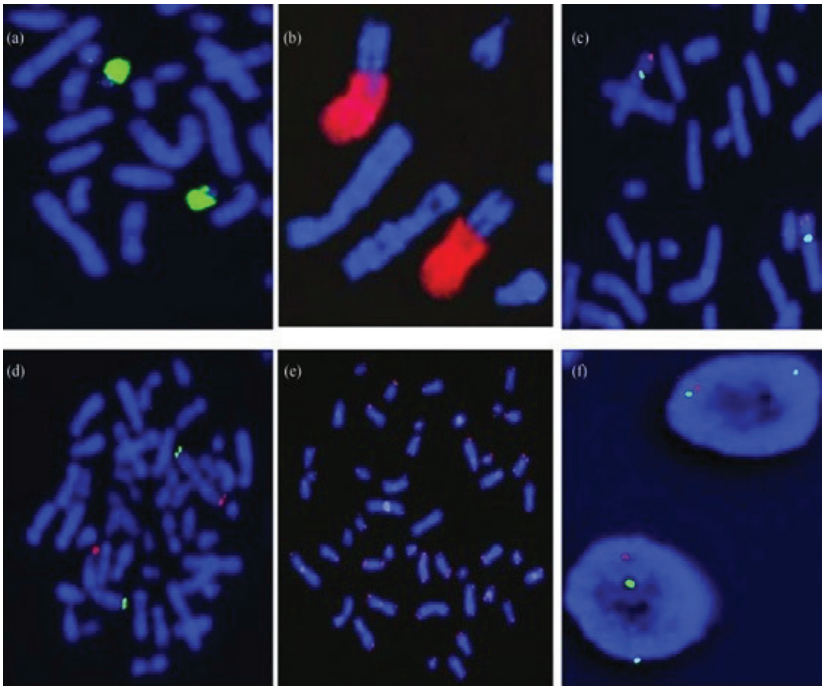


Figure 19. FISH with different types of probes and partial metaphases. (a) Whole chromosome 21 painting; (b) partial chromosome painting probe for the long arm of chromosome 9; (c) locus-specific probe for chromosome 4p16.3 (red) and Alfa satellite probe 4p11-q11(green); (d) subtelomeric probe for the short arm (red) and long arm (green) of chromosome 1; (e) human telomeric probes; and (f) Interphase-FISH with locus-specific SRY (sex-determining region Y) probe located in Yp11.31(red) and control probes for the X centromere (DXZ1) (blue) and for the heterochromatic block at Yq12 (green). https://www.researchgate.net/figure/FISH-with-different-types-of-probes-and-partial-metaphases-a-Whole-chromosome-21_fig2_261883314

SPECTRAL KARYOTYPING

A new karyotyping method, called spectral karyotyping, uses fluorescent dyes that bind to specific regions of chromosomes. By using a series of specific DNA probes, each with various amounts of the fluorescent dyes attached, different pairs of chromosomes

demonstrate unique spectral characteristics. A special feature of this technology is the use of a device called an interferometer, similar to the device used by astronomers for measuring light spectra emitted by stars. Slight variations in color, normally not visible to the human eye, can be detected using a computer program that then reassigns an easy-to-distinguish color to each pair of chromosomes. The result is a digital image in full color, rather than just a photograph. Pairing the chromosomes is now much simpler because homologous pairs are the same color. In addition, chromosomal aberrations are more easily recognizable (Figure 20).

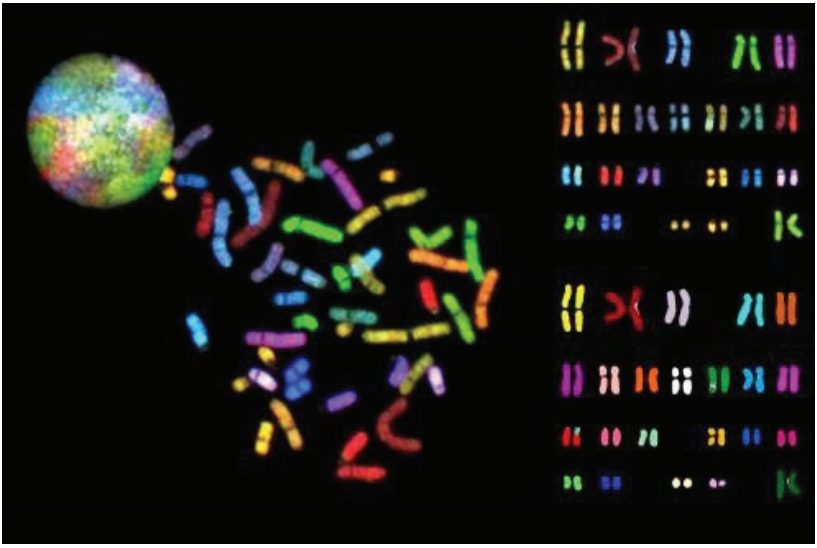


Figure 20. Spectral karyotyping of human chromosomes. The image shows a normal metaphase plate after the simultaneous hybridization of 24 differentially labeled chromosome painting probes. The image was acquired using spectral imaging (a combination of Fourier spectroscopy and CCD-imaging) through a custom-designed filter cube from Chroma Technology Corp, A classfication algorithm then allows the assignment for spectra-specific color to all chromosomes. SKY(TM) is a registered trademark of Applied Spectral Imaging. (http://www.cyto.purdue.edu/cdroms/micro1/7_spon/chroma/image4.htmIMAGE: Evelin Schröck, Stan du Manoir, Thomas Ried; FILTERS: Chroma Technology Corp.)

MOLECULAR CYTOGENETICS OF HUMAN DISEASES

One of the most effective tools for teaching biological concepts is to provide human examples to which all students can readily relate. In the field of genetics, this comes naturally due to the many human traits that have a genetic etiology. Many human genetic disorders have a chromosomal basis which can be demonstrated to students by using a technique called fluorescence in situ hybridization, or FISH. These chromosomal abnormalities include aneuploidies (trisomies, monosomies), deletions, duplications, translocations, and inversions. A vast selection of normal and chromosomally aberrant cell cultures are available through several different cell line repositories. Two of the major facilities are the National Institute of General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository, and the American Type Culture Collection (ATCC). The cell lines may be obtained as either fibroblast or lymphoblast cultures.

There are a number of different laboratory exercises that can be designed to illustrate the concept of the chromosomal basis of human genetic diseases. A few examples are provided below, all of which rely on the use of the FISH technology. Each exercise opens up the opportunity to expand the classroom discussion to include broader topics such as the Human Genome Project, prenatal diagnosis and reproductive technology, ethical and legal issues, and eugenic concerns (Figure 21).

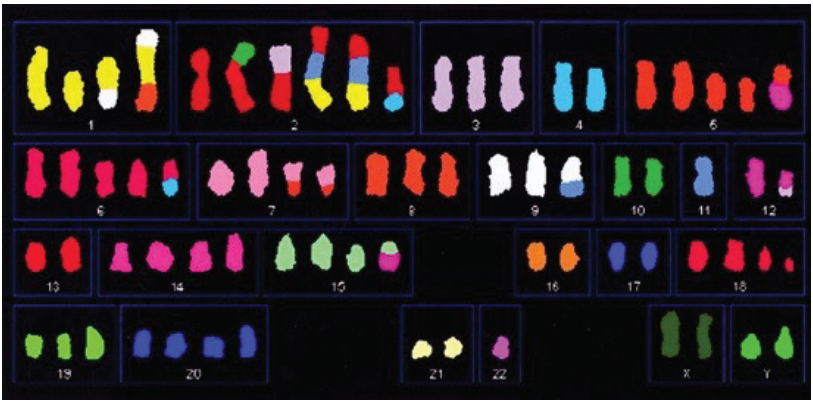


Figure 21. Classified image of spectral karyotype in a muscle-invasive, poorly differentiated TCC of the bladder (Intl Journal of Cancer, Volume: 92, Issue: 6, Pages: 824-831, First published: 03 May 2001, DOI: (10.1002/ijc.1267)

TYPES OF PRENATAL GENETIC TESTING

Prenatal genetic tests can be performed using DNA extracted from amniocytes (amniocentesis), chorionic villus sampling (CVS), or circulating fetal DNA (cfDNA). Genetic testing of cfDNA, unlike amniocentesis and CVS, is non-invasive since it is present in the mother's blood.

Karyotyping, the traditional prenatal genetic test, is a low-resolution method that examines a picture of stained chromosomes. Because of its low resolution, certain genetic disorders, such as those with tiny mutations, can be missed by karyotyping.

Chromosomal Microarray Analysis (CMA) is a microarray-based analysis that can analyze DNA of known genes from the entire human genome in one test. It is a high-resolution test useful in analyzing the entire genome or a subset of the genome for the detection of aberrations in multiple genes, changes in chromosomal structure, and chromosomal aneuploidy.

DNA sequencing of cfDNA, which is also referred to as non-invasive prenatal testing or NIPT, reduces the need for invasive

procedures and can be beneficial for women diagnosed with high-risk pregnancies. Most NIPT is a screening test, so positive results must be confirmed by another diagnostic methodology.

TYPES OF POSTNATAL GENETIC TESTING

BENEFITS OF POSTNATAL GENETIC TESTING

Typical postnatal genetic tests are performed by analyzing a blood sample from the child and the parents. DNA from the blood is extracted, amplified, processed, and analyzed using advanced software.

Chromosomal Microarray Analysis (CMA) is a microarray-based analysis that can analyze DNA of known genes from the entire human genome in one test. It is considered the first-line test for multiple congenital anomalies, developmental delays, intellectual disabilities, and autism spectrum disorders. It is a high-resolution test useful in analyzing the entire genome or a subset of the genome for the detection of aberrations in multiple genes, changes in chromosomal structure, and chromosomal aneuploidy.

As next-generation sequencing becomes more accessible for routine clinical use, whole genome sequencing and whole exome sequencing are becoming more prevalent for postnatal genetic testing. Whole genome sequencing (WGS) analyzes all the DNA sequences for an individual looking for known causative variants. Whole exome sequencing (WES) analyzes only the DNA sequences for all the genes coding for proteins (representing about 1% of one's entire genome).

Depending on the patient's case, clinicians will use the proper tests to help with finding and confirming a diagnosis.

The future of cytogenetics isn't in a single technique. Most of the methods developed in the late 20th century are still relevant in the 21st, whether in limited contexts or to augment the information provided by more modern techniques. Techniques developed for genomics, such as next-generation sequencing (NGS), also provide insight into the same sorts of questions that cytogenetics pursues. Future innovations in cytogenetics will combine what works about all

of the past's techniques to find newer, more efficient ways to collect cytogenetic information, as well as new tricks for answering questions that still vex modern practitioners.

CONCLUSION

Each new discovery in human molecular genetics and cell biology brings new challenges and raises new dilemmas for which there are often no easy answers. On a global scale it is essential that safeguards are in place to ensure that fundamental principles such as privacy, conidentiality, and respect for human life at all stages and ages are upheld. The medical genetics community can, and should, continue to play a pivotal role in trying to balance the needs of their patients and families with the ethical issues and tensions outlined here. This is an important advocacy role, and towards that end it is hoped that this chapter, and indeed the rest of this book, can make a positive contribution.

SELF-CONTROL TESTS

Checking your knowledge

1. **A replicated chromosome consists of two very long strands of identical chromosomal material called**

A) telomeres.
B) chromatids.
C) centromeres.
D) genes.
E) histones

2. **The field of genetics that studies the relationship between chromosome variations and specific traits is called _____.**

A) Mendelian genetics
B) cytogenetics
C) quantitative genetics
D) behavioral genetics
E) Morgan genetics

3. **DNA synthesis occurs during the ____ phase of the cell cycle.**

A) gap 1 (G1)
B) gap 2 (G2)
C) S
D) Mitosis
E) Meiosis

4. **Which factors do not influence cell division?**

A) crowding
B) growth hormones
C) cyclins
D) All of the above influence cell division.
E) Organelles Replication

5. **Karyotype is:**

A) Size, shape and number of chromosome

B) Gene packing

C) DNA assay

D) DNA Transcription

E) All of above

6. **What event during meiosis produces trisomies and monosomies?**

A) Independent assortment of chromosomes

B) Allele segregation

C) Non disjunction

D) Recombination

E) Replication

7. **Cdks bind with _____ , enabling the Cdks to function as enzymes.**

A) MPF

B) cyclins

C) histones

D) p53

E) nucleosomes

8. **The event(s) which does not occur during interphase, is/are**

A) Chromatin condenses

B) Protein Synthesis

C) Organelles Replication

D) DNA Replication

E) RNA Transcription

9. **What contains protein-encoding genes.**

A) Heterochromatin

B) **Euchromatin**

C) Pericentric inversions

D) Aneuploids

E) Cyclins

10. **An inversion that includes the centromere is called _____.**

A) pericentric
B) paracentric
C) Robertsonian
D) lethal
E) silent

11. **When a chromosome is broken in two places and reconnected so that a region is flipped from the normal order, this is called...**

A) inversion
B) duplication
C) deletion
D) simple translocation
E) reciprocal translocation

12. **Which of the following occurs when a piece of one chromosome breaks off and is attached to another chromosome?**

A) inversion
B) duplication
C) deletion
D) **simple translocation**
E) reciprocal translocation

13. **How many chromosomes does a person with Edward syndrome have?**

A) 45
B) 46
C) 47
D) 48
E) 49

14. **In nondisjunction, which parent leads to the sex chromosome aneuploid XYY?**

A) mother
B) father
C) either parent
D) both parents
E) none of parents

15. **Which of the following cell types is not used to examine chromosomes?**
A) white blood cells
B) bone marrow cells
C) red blood cells
D) All of the above can be used.

16. **Which of the following utilizes labeled DNA probes?**
A) amniocentesis
B) fetal cell sorting
C) chorionic villus sampling
D) fluorescence in situ hybridization
E) all of the above can be used.

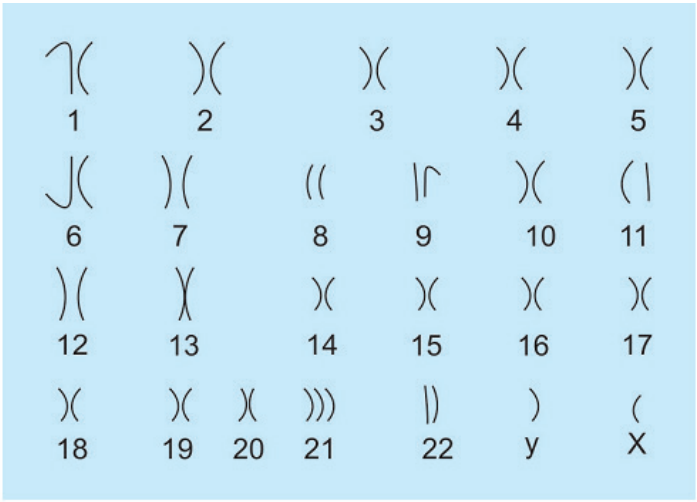
17. **Which of the following tests is used to differentiate the chromosome of normal and cancer cells?**
A) PCR
B) Comparative genomic hybridization
C) Western blotting
C) Karyotyping
E) Splicing

18. **Which of the following is a result of reciprocal translocation?**
A) Burkitt's lymphpma
B) Trychothiodystrophy
C) Thalassemia
D) Cockyne's syndrome
E) Turner syndrome

19. **Down's syndrome is associated with the clinical manifestation of mental retardation. Which of the following is not associated with Down's syndrome?**

A) Trisomy 21
B) Mosaic 21
C) Translocation t (14,21), t (21,21)
D) Deletion of 21
E) Trisomy 18

20. **A married middle aged female gives history of repeated abortions for the past 5 years. The prenatal karyogram of the conceptus is given below**



This karyogram suggests the following:

A) Klinefelter's syndrome
B) Turner's syndrome
C) Down's syndrome
D) Patau's syndrome
E) Edward's syndrom

21. **A tall man with gynecomastia and testicular atrophy has a testicular biopsy that shows sparse, completely hyalinized seminiferous tubules with a complete absence of germ cells and only rare Sertoli cells. Leydig cells are present in large clumps between the hyalinized tubules. Which of the following genetic disorders should be suspected?**

A) Testicular feminization syndrome
B) 47, + 18
C) 47, + 21
D) 47, XXY
E) Normal 46

22. **A nineteen year old female with short stature, wide spread nipples and primary amenorrhea most likely has a karyotype of:**

A) Normal 46
B) 47, XX + 18
C) 46, XXY
D) 47, XXY
E) **45 X**

23. **Which of the following techniques can be used to detect exact localisation of a genetic locus?**

A) Chromosome painting
B) **Fluorescence in situ hybridisation**
C) Comparative genomic hybridization
D) Western blot
E) Northern blot

24. **Which of the following cytogenetic techniques would be the most likely to identify a microdeletion?**

A) Northern blot
B) G banding
C) R banding
D) **Fluorescence in situ hybridisation**
E) inversion analysis

25. **You generate a fluorescent probe against a gene that has been deleted. You expose the DNA to the probe and observe it under fluorescent microscope. What will you see?**

A) Fluorescence will correspond to the gene of interest
B) There will be a number of regions that emit fluorescence
C) Most parts of the chromosome emits fluorescence
D) **Nothing is seen under fluorescence microscope**
E) all chromosomes emit fluorescence

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36. Emery's 15th edition Peter D. Turnpenny BSc MB ChB FRCP FRCPCH FRC Path FHEA// Consultant Clinical Geneticist, Royal Devon and Exeter NHS Foundation Trust, and Honorary Clinical Professor, University of Exeter Medical School, © 2017, Elsevier Limited. All rights reserved.

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CONTENT

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INTRODUCTION
TO HUMAN CYTOGENETICS

Teaching Manual (Basal reader) for senior students,
PhD students, medical residents and interns

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