Aim: Preparation of normal human karyotype from metaphasic chromosomal spread picture (normal male and female)

Cytogenetics is a branch of genetics dedicated to the study of chromosomes. Chromosome instability and chromosome rearrangements in the genome are used to diagnose and define several genetic diseases and even some cancers. [1, 2] Researchers use a variety of techniques to identify the structure of chromosomes, including fluorescence in situ hybridization (FISH), karyotyping, flow cytometry, and comparative genomic hybridization (CGH). karyotype is the number and appearance of chromosomes, and includes their length, banding pattern, and centromere position. 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. The term karyotype is also used for the complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number. Karyotypes can be used for many purposes; such as to study chromosomal aberrations, cellular function, taxonomic relationships, medicine and to gather information about past evolutionary events. Karyotype is a set of characteristics that isendtifies and describes particular set of chromosomes. These cahasteriscitcs are described by number of chromosomes, relative size of different chromosomes, position of centromere and length of chromosomal arms, presence of secondary constriction and satellite, banding pattern of chromosome and feature of sex chromosome.

Human karyotype

human karyotype : The typical human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes (allosomes). The most common karyotypes for females contain two X chromosomes and are denoted 46,XX; males usually have both an X and a Y chromosome denoted 46,XY. Approximately 1.7% percent of humans are intersex, sometimes due to variations in sex chromosomes.

The process of preparation of karyotype of a species is called karyotyping. Karyotypered by microphotographs of metaphase chromosomes.

Procedure:

Chromosome harvesting from whole blood: Phytohemagglutinin (PHA), a lectin derived from the red kidney bean, is a powerful mitogen for human T-cells16. 72 hr after the addition of PHA to the culture, about 45% of cells are in S phase. This represents the peak mitotic activity, and is the

optimum point at which to harvest for chromosome studies. Other mitogens such as pokeweed (1-10 μg/ml) may be used when analyzing B cells.

- Collect at least 1 ml of whole blood in a green top sodium heparin tube. Use within 3 days after collection. Store blood at RT until ready to use.
- Aliquot 0.25 ml of whole blood in 10 ml of complete RPMI media containing L-glutamine (20% fetal bovine serum, 1% Penicillin/streptomycin, 1% fungizone, and 1% PHA). Culture at 37 °C with 5% CO2

Slide Preparation and Staining: A rapid evaluation of one representative slide will provide information on the quality of the harvest. Slides are best prepared when the humidity is approximately 50% and the temperature ambient (20-25 °C).

- Centrifuge the cells at 200 x g for 5 min at 25 °C. Remove the supernatant until only 0.3-0.5 ml remains.
- After gently resuspending the pellet, pipette three drops of the cell suspension from a distance of about 2 in onto a slide which is tilted at an angle of about 45° and allow the suspension to roll across the slide. Add one large drop of fresh Carnoy's Fixative to the slide.
- Dry the back of the slide on a paper towel and then sit the slide out to dry for at least 10 min. The slide should be completely dry.
- Prepare fresh Giemsa Staining Solution (3:1 ratio of Gurr Buffer and Giemsa Stain). Place the slides on a staining rack. Cover the entire slide in the Giemsa staining solution. Let the slides remain in the staining solution for 5 min. Rinse slides with distilled water, drain, and allow to air dry.
- Add 4 drops of Permount and a cover slip to the slide. Make sure there are no bubbles under the coverslip. The excess Permount can be removed with a paper towel.
- Analyze cells with a light microscope under 10X and 100X magnification. If the metaphase cells are abundant and well spread take the photograph.

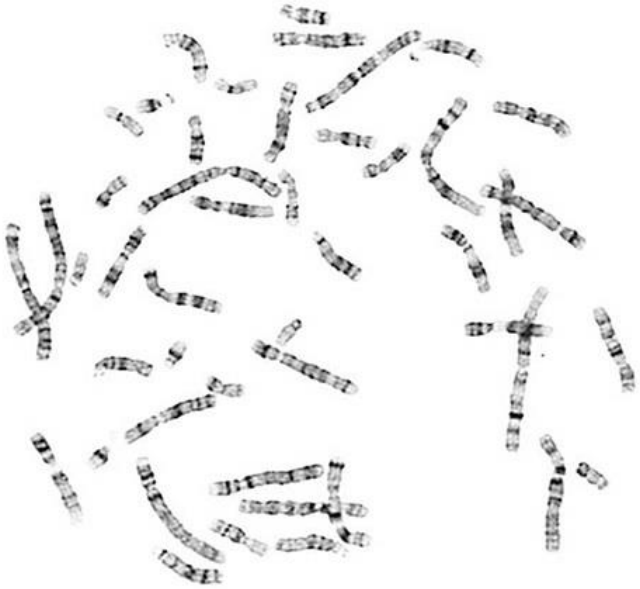
Sorting Chromosomes: After determining the number of chromosomes, the cytogeneticist will start sorting the chromosomes. To sort the chromosomes, a cytogeneticist will compare chromosome length, the placement of centromeres, and the location and sizes of G-bands.

- The chromosomes pairs are numbered from largest (number 1) to smallest (number 22). There are 22 pairs of chromosomes, called autosomes, which match up exactly.

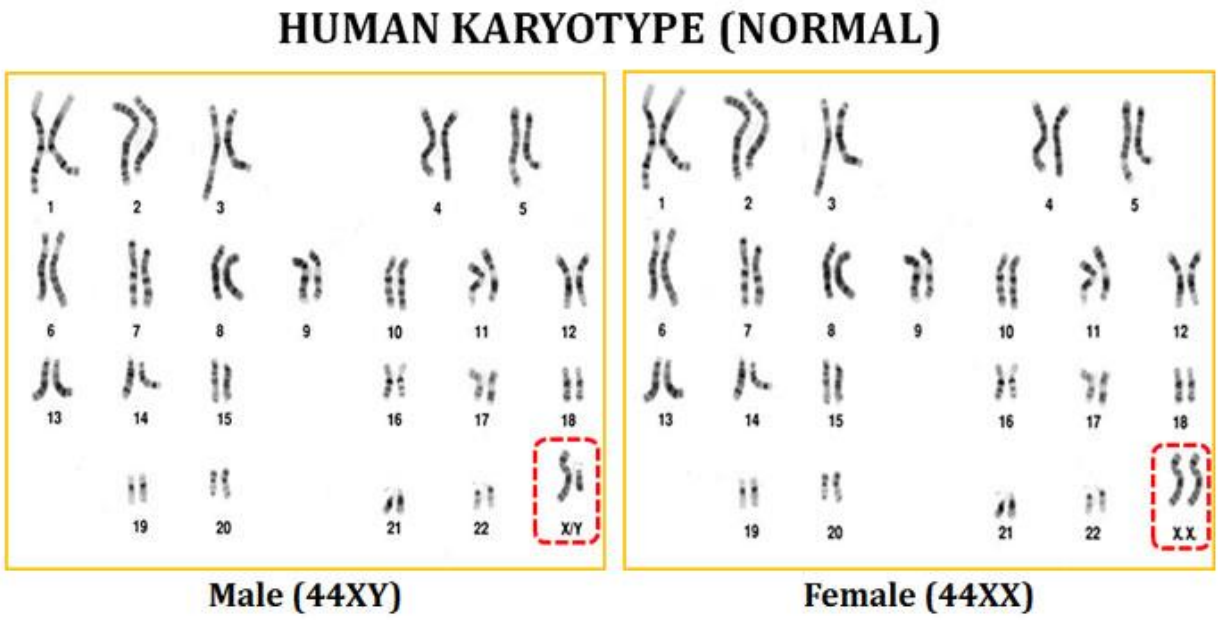
- There are also the sex chromosomes, females have two X chromosomes while males have an X and a Y.
- Rearranging the pictures of the chromosomes to match up pairs and arrange them by size, from largest to smallest, numbers 1 to 22, followed by the sex chromosomes as the 23rd pair.
- The pictures also allow the chromosomes to be vertically oriented.
- Each chromosome looks like a striped straw. It has two arms that differ in length (a short arm (p) and a long arm (q)), a pinched-in area between the arms called a centromere, and a series of light and dark horizontal bands.
- The length of the arms and the location of the bands help determine top from bottom.
- Once the chromosome photo arrangement is completed, a laboratory specialist evaluates the chromosome pairs and identifies any.
- Centrom ere index (Cl) is traditionally defined as the length of the short arm divided by the total length of the chromosome X100. It has been a valuable quantitative m easure for describing the shape of the chromosome, especially before the chromosome banding era

Karyotype photographs:

Metaphase spread of chromosome



Normal Human Karyotype



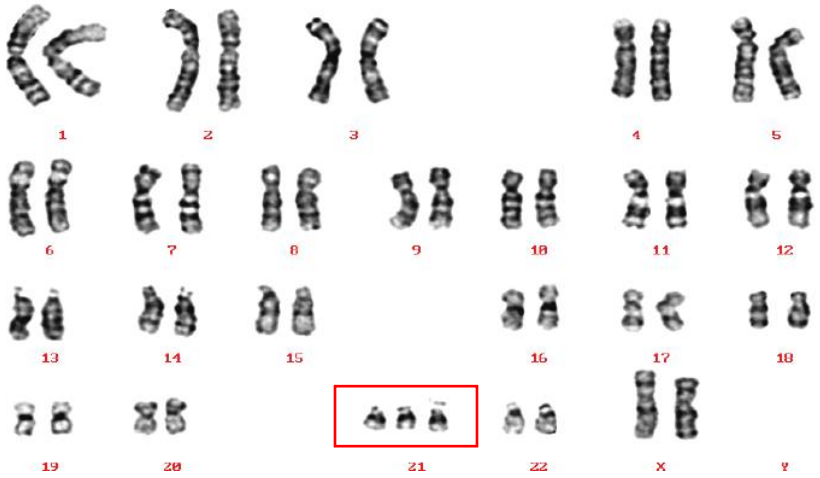
Study characters and karyotypes of syndromes : Down, Klinefelter and Turner

A syndrome is a set of medical signs and symptoms which are correlated with each other and often associated with a particular disease or disorder. Syndromes are majorityly associated with chromosomal abnormalities. There are many types of chromosome abnormalities. However, they can be organized into two basic groups: numerical abnormalities and structural abnormalities. Numerical Abnormalities: When an individual is missing one of the chromosomes from a pair, the condition is called monosomy. When an individual has more than two chromosomes instead of a pair, the condition is called trisomy. Structural Abnormalities: A chromosome's structure can be altered in several ways. These abnormalities can be identified by studying karyotype.

Downs Syndrome:

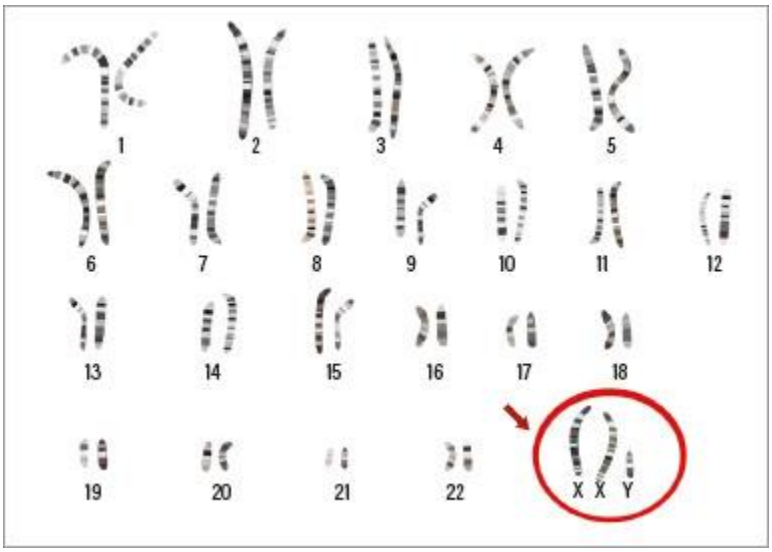
Down syndrome is a genetic disorder caused when abnormal cell division results in an extra full or partial copy of chromosome 21. Each person with Down syndrome is an individual intellectual and developmental problems may be mild, moderate or severe. Children and adults with Down syndrome have distinct facial features. Some of the more common features include Flattened face, Small head, Short neck, Protruding tongue, Upward slanting eye lids (palpebral fissures), Unusually shaped or small ears, Poor muscle tone, Broad, short hands with a single crease in the palm, Relatively short fingers and small hands and feet, Excessive flexibility, Tiny white spots on

the colored part (iris) of the eye called Brushfield's spots, Short height, Intellectual disabilities. Most children with Down syndrome have mild to moderate cognitive impairment. Language is delayed, and both short and long-term memory is affected.

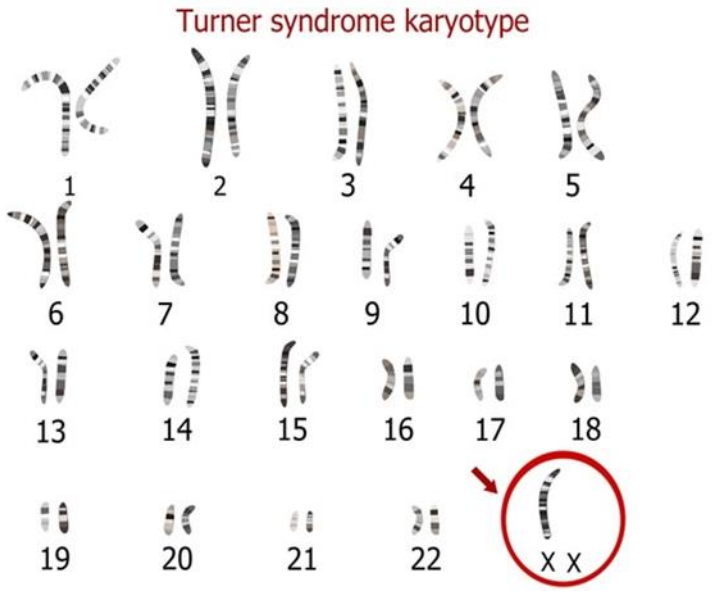


Klinefelter Syndrome: Klinefelter syndrome is a genetic condition that results when a boy is born with an extra copy of the X chromosome. Klinefelter syndrome is a genetic condition affecting males, and it often isn't diagnosed until adulthood. Klinefelter syndrome may adversely affect testicular growth, resulting in smaller than normal testicles, which can lead to lower production of testosterone. The syndrome may also cause reduced muscle mass, reduced body and facial hair, and enlarged breast tissue. The effects of Klinefelter syndrome vary, and not everyone has the same signs and symptoms. Most men with Klinefelter syndrome produce little or no sperm, but assisted reproductive procedures may make it possible for some men with Klinefelter syndrome to father children. Signs and symptoms of Klinefelter syndrome vary widely among males with the disorder. Signs and symptoms may include: In babies Weak muscles, Slow motor development — taking longer than average to sit up, crawl and walk, Delay in speaking, Problems at birth, such as testicles that haven't descended into the scrotum. In boys and teenagers symptoms include taller than average stature, Longer legs, shorter torso and broader hips compared with other boys, Absent, delayed or incomplete puberty. After puberty, less muscle and less facial and body hair compared with other teens, Small, firm testicles, Small penis, Enlarged breast tissue (gynecomastia), Weak bones, Low energy levels, Tendency to be shy and sensitive, Difficulty expressing thoughts and feelings or socializing, Problems with reading, writing, spelling or math and in men symptoms are Low sperm count or no sperm, Small testicles and penis, Low sex drive,

aller than average height, Weak bones, Decreased facial and body hair, Less muscular compared with other men, Enlarged breast tissue, Increased belly fat.



Turner syndrome: Turner syndrome, a condition that affects only females, results when one of the X chromosomes (sex chromosomes) is missing or partially missing. Turner syndrome can cause a variety of medical and developmental problems, including short height, failure of the ovaries to develop and heart defects. Turner syndrome may be diagnosed before birth (prenatally), during infancy or in early childhood. Occasionally, in females with mild signs and symptoms of Turner syndrome, the diagnosis is delayed until the teen or young adult years. Signs and symptoms of Turner syndrome may vary among girls and women with the disorder. Signs of Turner syndrome at birth or during infancy may include: Wide or weblike neck, Low-set ears, Broad chest with widely spaced nipples, High, narrow roof of the mouth (palate), Arms that turn outward at the elbows, Fingernails and toenails that are narrow and turned upward, Swelling of the hands and feet, especially at birth, Slightly smaller than average height at birth, Slowed growth, Cardiac defects, Low hairline at the back of the head, Receding or small lower jaw, Short fingers and toes. In childhood, teens and adulthood Slowed growth, No growth spurts at expected times in childhood, Adult height significantly less than might be expected for a female member of the family, Failure to begin sexual changes expected during puberty, Sexual development that "stalls" during teenage years, Early end to menstrual cycles not due to pregnancy, For most women with Turner syndrome, inability to conceive a child without fertility treatment


Turner syndrome karyotype
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