Chromosomal Karyotyping
Karyology

- **Karyotyping** - process of pairing and ordering all chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosomes.

- **Karyotypes** describe chromosome count of an organism and what these chromosomes look like (length, position of centromeres, banding pattern, differences between sex chromosomes, and other physical characteristics) under a light microscope.

- Preparation and study of karyotypes is part of **Cytogenetics**.

- Chromosomes are depicted (by rearranging a photomicrograph) in a standard format (in pairs, ordered by size and position of centromere for chromosomes of the same size) known as a **Karyogram** or **Idiogram**.

- **Clinical Cytogeneticists** analyze human karyotypes to detect gross genetic changes (anomalies involving several megabases or more of DNA).
Karyotypes

Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome.

Male (44XY)  Female (44XX)
Sampling

- Peripheral Blood
- Placental Villi (Second Trimester)
- Amniotic Fluid
- Chorionic Villus
- Skin Biopsy
- Tumor Biopsies
- Bone Marrow

Complication of Sampling Methods
- Rare
- Amniocentesis carries a very minimal risk of miscarriage
- Slight risk of bleeding and infection in bone marrow biopsy
- Chemotherapy can cause breaks in chromosomes leading to skewed results
Making a Karyotype

1. Draw 3 to 5 ml blood
2. Add a few drops of blood
3. Add phytohemagglutinin to stimulate mitosis
4. Incubate at 37°C for 3 days
5. Centrifuge to concentrate cells. Add low-salt solution to eliminate RBC and swell lymphocytes
6. Transfer cells to tube
7. Add colchicine to culture for 1 to 2 hours to stop mitosis in metaphase
8. Transfer to tube containing fixative
9. Drop cells onto microscope slide
10. Stain slide with Giemsa
11. Examine under microscope
12. Digitized chromosome images processed to make karyotype
Preparing Karyotypes from Mitotic Cells

- Karyotypes are prepared from mitotic cells arrested in *metaphase* or *prometaphase* portion of cell cycle, when chromosomes assume most condensed conformations.

- Begins with the *short-term culture* of cells derived from specimen
- Allow a period of *cell growth and multiplication*
- Dividing cells are *arrested in metaphase* by addition of colchicine, which poisons the mitotic spindle
- Cells are next treated with hypotonic solution that causes their *nuclei to swell and cells to burst*
- Nuclei are then treated with a chemical *fixative*, dropped on a glass slide, and treated with various *stains* that reveal structural features of chromosomes

Banding Patterns Reveal Structural Details of Chromosomes

• **Q-banding** - first banding technique (1970)
  – Involves use of the fluorescent dye quinacrine, which alkylates DNA and is subject to quenching over time.

• **G-banding/Giemsa banding** – Giemsa dye offers better resolution of individual bands, produces more stable preparation, & can be analyzed with ordinary bright-field microscopy
  – Metaphase chromosomes are first treated briefly with trypsin that degrades proteins before staining with Giemsa.
  – Trypsin relaxes chromatin structure and allows Giemsa dye access to the DNA.
  – Heterochromatic regions, AT-rich & relatively gene-poor stain more darkly
  – Less condensed chromatin, GC-rich & more transcriptionally active, stain lightly
  – Giemsa stain produces ~400-800 bands distributed among 23 pairs of chromosomes thus representing several million to 10 million base pairs DNA, containing hundreds of genes.

• **R-banding** - also involves Giemsa stain, but generates reverse pattern from G-banding
  – Before Giemsa staining, heat treatment melts DNA helix in AT-rich regions that bind Giemsa stain most strongly, leaving only GC-rich regions to take up the stain.
  – Often used to provide critical details about gene-rich regions located near telomeres.

• **C-banding** - used to specifically stain constitutive heterochromatin, or genetically inactive DNA, but it is rarely used for diagnostic purposes these days.

Chromosome Banding Revealed by Different Staining Techniques

a) G-/Giemsa banding

b) Q-banding

c) R-banding

d) C-banding

Organizing Chromosomes in Karyograms for Review

• According to international conventions, human autosomes are numbered from 1 to 22, in descending order by size, with exceptions of chromosomes 21 and 22, the former actually being smallest autosome.

• Sex chromosomes are placed at end of a karyogram.

• Short p (petite) arms are at top & long q (queue) arms are at bottom.

• Chromosomes are aligned along a horizontal axis shared by their centromeres.

• Centromere placement can also be used to identify the gross morphology, or shape, of chromosomes. Eg.
  – Metacentric chromosomes 1, 3, and 16 have p and q arms of nearly equal lengths
  – Submetacentric chromosomes 2, 6, and 10 have centromeres slightly displaced from center
  – Acrocentric chromosomes 13, 14, 15, 21 and 22 have centromeres located near their ends

• Arranging chromosomes into a karyogram can simplify the identification of abnormalities.

Resolution of chromosomal changes detectable by karyotyping is typically a few megabases

**Chromosomal Disorders Detected by Karyotyping** –

- Aneuploidy, which is often caused by absence or addition of a chromosome.
  - Down syndrome (Trisomy 21) caused by an extra chromosome 21
  - Edwards syndrome (Trisomy 18) caused by an extra chromosome 18
  - Patau syndrome (Trisomy 13) caused by an extra chromosome 13
- Subtle structural changes, such as chromosomal Deletions (Cri du chat syndrome, Angelman syndrome, Prader-Willi syndrome), Insertions, Duplications, Translocations, or Inversions.
- Genetic diseases (eg. Premature Ovarian Failure)
- Some birth defects
  - Klinefelter syndrome caused by an extra X chromosome (most common sex chromosome abnormality in males)
  - Turner syndrome caused by missing one X chromosome in females
- Certain haematologic and lymphoid disorders (e.g., Leukaemia, Lymphoma, Myeloma, Refractory Anaemia)
When to Get Tested?

- When pregnancy screening tests are abnormal
- Bad obstetric history
- Recurrent pregnancy loss
- Infertility
- Primary amenorrhea
- Dismorphic features of newborn
- When signs of a chromosomal abnormality or associated disorder are present
- When a specific abnormality has been detected in a family member
- When a person has leukaemia, lymphoma, myeloma, myelodysplasia or another cancer and an acquired chromosome abnormality is suspected
Genetic Tests For Non-obstructive Azoospermia/Severe Oligospermia

Karyotype is recommended by the American Urological Association (AUA) and the European Academy of Andrology (EAA) guidelines in all men with a total motile sperm count below 5 million who are thought to have non-obstructive azoospermia.

## Tests Done in SRL

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Thank You