LAB 7: Karyotyping & Chromosome Staining, Banding Techniques

1. Karyotyping : The characterization of the chromosomal complement of an individual or a species, including number, form, and size of the chromosomes in the nucleus of a eukaryotic cell . Staining techniques were developed to produce the chromosome bands characteristic of modern karyotypes. The normal human female karyotype 46, xx, while mail karyotype 46, xy.

- Chromosome: the rod like, deep staining bodies found within nucleus. They composed of DNA, protein AND there are two Types of chromosomes are
  - sex chromosome: X and Y chromosome which differ in male and female and responsible for sex determination (female xx, male xy)
  - autosomal chromosome: any chromosome other than sex chromosome.

The Classification of chromosome: according to

• A. number of centromeres	B. location of centromeres
1. Acrocentric: absence of centromere. 2. Monocentric: (only one on human being). 3. Diecentric: (two centromere). 4. Polycentric: (more than two centromere).	1. Metacentric: centromere is located in the middle of the chromosome. 2. Sub metacentric: centromere is located in the upper arm). 3. Acrocenric: centromere is located near one end of the chromosome, sub terminal in position. 4. Telocentric: centromere located on the one end of the chromosome .these are not found on human.

- C. size and length

1-Group A	(1,2 ,3)	large size , metacentric
2-Group B	(4,5).	Large size , Submetacentric
3-Group C	(6,7,8,9,10,11.12. X)	medium size , submetacentric
4- Group D	(13,14,15).	Medium size , acrocentric
5- Group E	(16,17,18)	small size , metacentric
6- Group F	(19,20).	small size metacentric
7- Group G	(21, 22, Y).	smallest size acrocentric

2, 3 subMeta

4, 7 acrocentric

Chromosome Staining, Banding Techniques

Early Karyotypes were useful in counting the number of chromosomes, but structural abnormalities were often undetected that cannot be detected in conventional Giemsa stain. Staining technique was developed to produce the chromosomal bands characteristic of modern karyotypes that facilitates the correct identification of individual chromosomes

1. Conventional **Staining:**	2-Banding staining:
Giemsa Staining Technique : Giemsa staining : provides a uniform, unbanded appearance to chromosomes , useful for studying chromosome breakage, gaps, deletions, and ring chromosomes. Materials: 1. Phosphate buffer (pH 6.8) 2. Giemsa stain: Method: 1. Stain the slide in Giemsa solution for 8 minutes. 2. Rinse the slide twice with deionized water. 3. Air dry the slide.	➢ Giemsa banding technique: The most usual methods to obtain this staining are to treat the slides with a protease such as trypsin or in hot saline – citrate. Trypsin – giemsa banding Materials: 1. Phosphate buffer saline (PBS) (pH=7) 2. Trypsin solution. 3. Giemsa stain: Methods: 1. Incubate the slide 20-40 second in trypsin solution in a coplin jar. 2. Rins the slide thoroughly with cold PBS. 3. Rins the slide in D.W and air dry. 4. Stain for 5 min in Giemsa solution.

The chromosomal banding techniques involved:

1. Quinacrine Banding (Q-banding):
   - Uses fluorochrome quinacrine.
   - Stains mostly heterochromatin regions.
2. Giemsa Banding (G-banding):
   - Suitable for animal cells but not plants.
   - Chromosomal proteins are partially digested by trypsin before staining.
3. **Reverse Banding (R-banding):**
   - Stains GC-rich regions typical of euchromatin.
4. Centromer Banding (C-banding):
   -Involves alkaline denaturation, leading to depurination of DNA before staining.
   - Targets centromeric or constitutive heterochromatin.
5. Hy-bandings:
   - Chromosomes are treated with hot HCl and stained with acetic acid carmine.
   - Shows a different pattern from C-banding, useful in prophase or early pro-metaphase.

Figure (copin JAR)