Harvest and Staining Protocols

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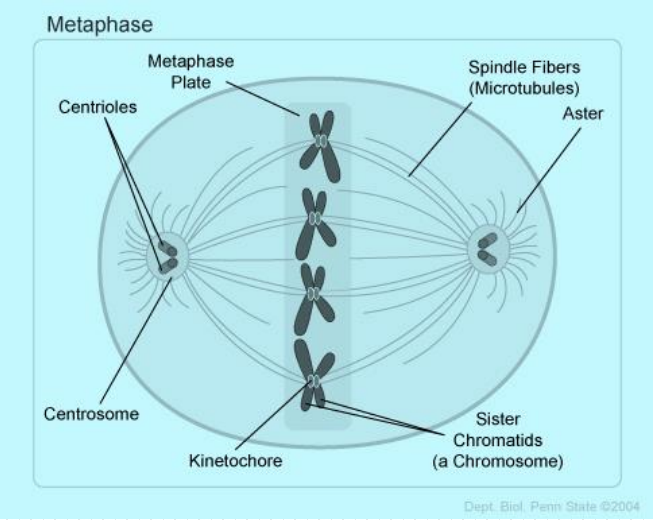
Harvest Protocols:

- Arresting cells at metaphase stage .
- Hypotonic treatment.
- Cells fixation.

metaphase stage:

chromosomes are lined at the equatorial plate before sister chromatides are pulled to opposite poles by spindle fibers for incorporation into the two daughter cells.



Arresting cells at metaphase stage .

Done by adding mitotic arrestant such as Colcemid.

Mechanism of Colcemid action:

preventing spindle fibers formation and thereby preventing pulling the two sister chromatides to the daughter cells.

Hypotonic treatment:

Using hypotonic solution results in :

- ✓ Swelling of the cells :

Thinning cytoplasm & enhance chromosome spreading

- ✓ Lysis of RBCs.

Cells fixation:

- Fixative is added to kill and preserve the cells.
- It removes the water from the cell (dehydrate them).
- hardens membranes and chromatin.

Cells fixation:

- 1st few drops of fixative stop the action of hypotonic solution and prepare cells for next higher fixative concentrations
- The brown supernatant is the color of the methemoglobin.(formed after the addition of fixative)
- Multiple fixative steps should be carried out. (until a clear supernatant is obtained)

Procedure:

- after incubation , add 50 µl of colcemid.
- Incubate for 15 mins at 37 **℃** .
- Centrifuge the tube for 8 mins at 1200 rpm speed .
- Discard the supernatant without touching the buffy coat (because cells are there)
- resuspend pellets :Mix thoroughly using the your palm hand , continue until the buffy coat disappears.
- Add 2 ml of pre-warmed hypotonic solution(0.075 M KCl) drop by drop while mixing using vortex .
- Add 8 ml of hypotonic solution without mixing .
- Incubate at 37 **℃** for 15 mins.
- 1st wash : Add 1 ml of fixative(3:1 methanol: acetic acid) dropwise
- Centrifuge for 8 min at 1200 rpm.

Procedure:

- Remove the supernatant but not completely .
- Mix thoroughly as the cells are so sticky
- Add 2 ml of fixative drop by drop using vortex .
- Add more fixative (up to 10 ml) without using vortex.
- Centrifuge for 8 min at 1200 rpm.
- Now the 2nd wash: starts after removing the supernatant.
- Mix to detach the cells from the tube wall
- Add 10 ml of fixative
- Centrifuge for 8 min at 1200 rpm.
- Don't remove the supernatant now.
- Keep at 4 **°C** until you can do the dropping and staining

Dropping Protocol:

Procedure:

1. Replace the fixative with a new one. Using a fine glass pasture pipette make a gentle mix without making air bubbles, as this will cause cells to clump.

2. Clean the slides with methanol, that will help to get a uniformity in spreading.

3. Carry the slide in 45 degrees and drop the sample from about one meter apart.

4. As a test slide drop about 4 drops on the slide, leave to air dry completely and observe the mitotic index and metaphases compactness.

Aging: Put the slides in the oven at 60C over night or at 90C for 90 minutes, that will driving off water and get better banding pattern.

Staining :

Staining Protocols:

- G banding (Giemsa)
- Q banding (Quinacrine,fluorescent stain)
- R banding (Reverse)
- C banding (Centromeric(heterochromatin)
- Ag-Nor stain (Nucleolar Organizing Regions)

G banding :

- The most common .
- Typsin hydrolyses the protein component of the chromatin (Wang and Federoff) :

  allowing the Giemsa dye to react with the exposed DNA.

G banding :

Giemsa stain is a complex mixture of dyes:

The basic dye: aminophenothiazin dyes azure A,azureB, azure C, thionin, and mythelen blue.

The acidic dye: eosin.

G banding :

Immersing slides in fetal calf serum to stop the activity of the trypsin since the serum contains α1-antitrypsin

G banding :

- Appropriately stained chromosomes

  neither too dark nor too pale.
- Under-trypsinized chromosomes

  have indistinct bands and little contrast. i.e.fuzzy in appearance.

G banding :

- Over-trypsinized chromosomes

  have sharp bands and often appear frazzled at the ends.
- Eventually overtrypsinized chromosomes

  are very pale after staining and may appear ghost-like and very swollen.

G banding :

- Each chromosomes characterized by dark & light bands
- 400 bands in the haploid genome
- Light bands are genetically active sites
- Dark bands are gene poor rich in AT pairs.

G banding :

- Chromosomes 13,18,21gene poor (very dark chromosomes)
- Chromosome 21 is smaller than Chromosome 22
- Chromosome 21(200 genes) is half as many as Chromosome22 (400 genes)

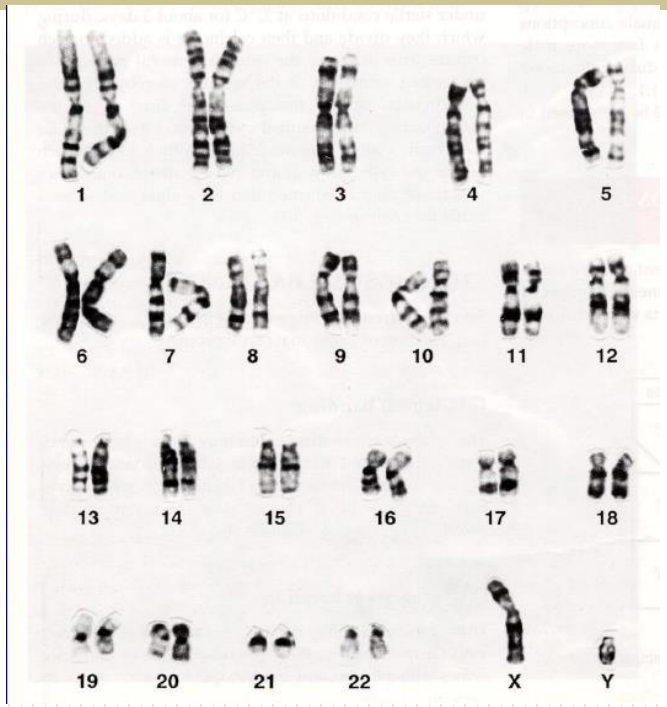
Procedure :

- Slides must cool down to RT
- Immerse in trypsin solution 10-15 sec
- Wash in phosphate buffer or serum (to end trypsin activity)
- Transfer to Gimsa stain 4 mins.
- All the staining solution must be kept at 37 **℃** water bath during staining procedure.
- Raines in D-water
- Leave to dry completely
- Examine under microscope

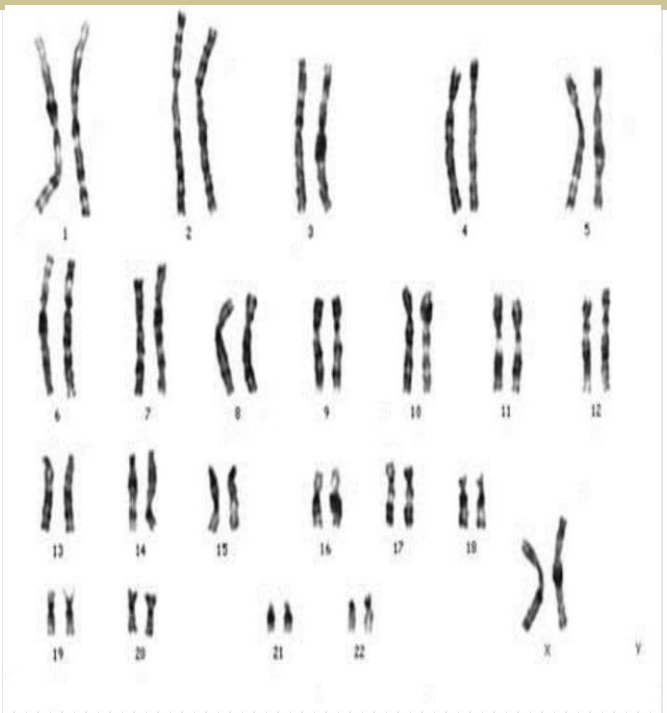
Analysis:

- Count chromosomes in 10-15 metaphases
- Count 30 if mosaicism suspected
- Detailed analysis of 3-5metaphase

G banding :



Normal Male Karyotype



Normal Female Karyotype

Q banding

- Used for Y chromosome abnormalities or mosaicism
- Similar to G band (but It can detect polymorphism)
- Examined under fluorescent microscope

R banding:

- Used to identify X chromosome abnormality
- Heat chromosomes before treating with Gimsa
- Light and dark bands are reversed

C banding

To identify centromere/ heterochromatin

Heterochromatic region:

-contains highly repetitive DNA sequence

-highly condense chromatin fibres

-genetically inactive (structural elements)

•Treated chromosomes:

-Acid

-Alkaline

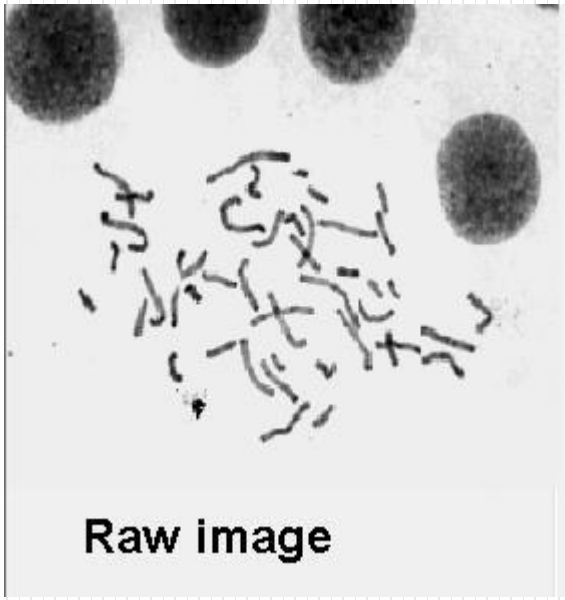
-Then G band

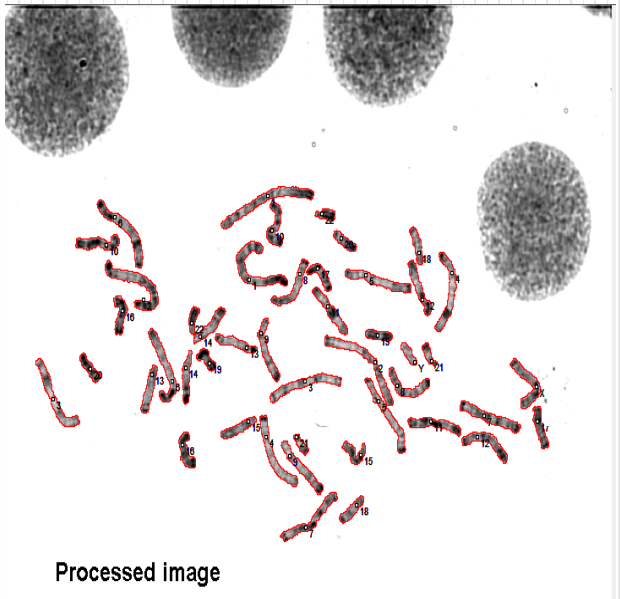
Karyotyping:

Organization of the chromosomes of an individual, lined up using computer image processing according to:

- Size, the largest to smallest
- Location of centromere
- Banding pattern

Karyotyping:





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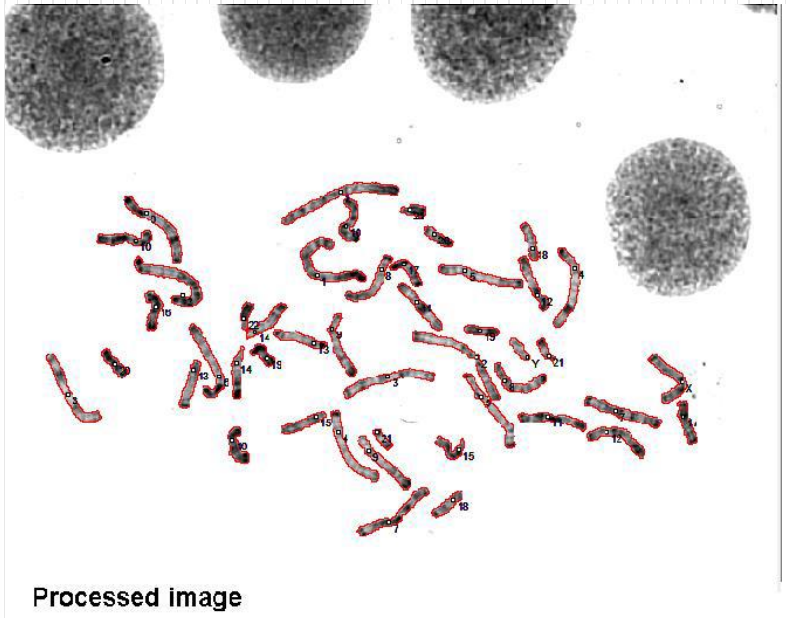
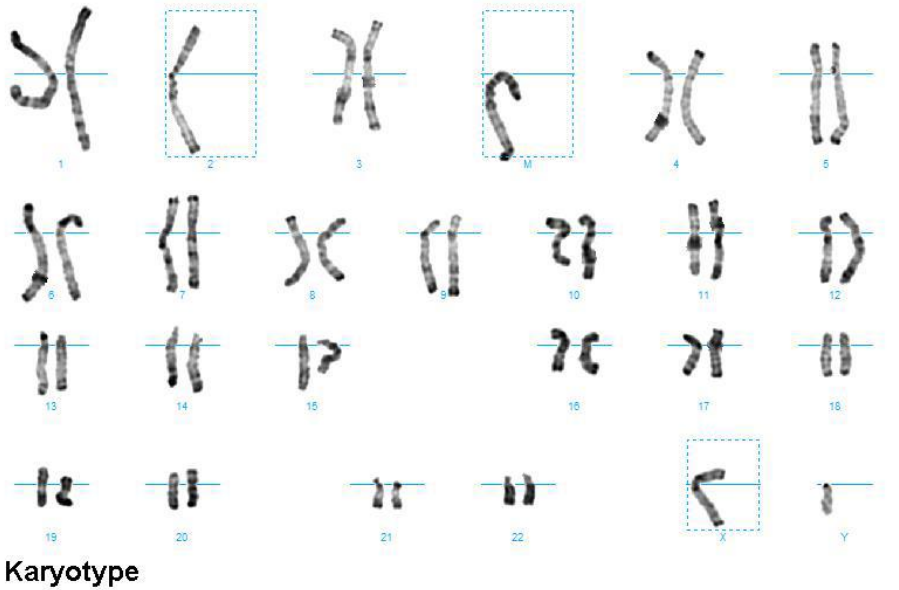
Karyotyping:



Processed image



Karyotype

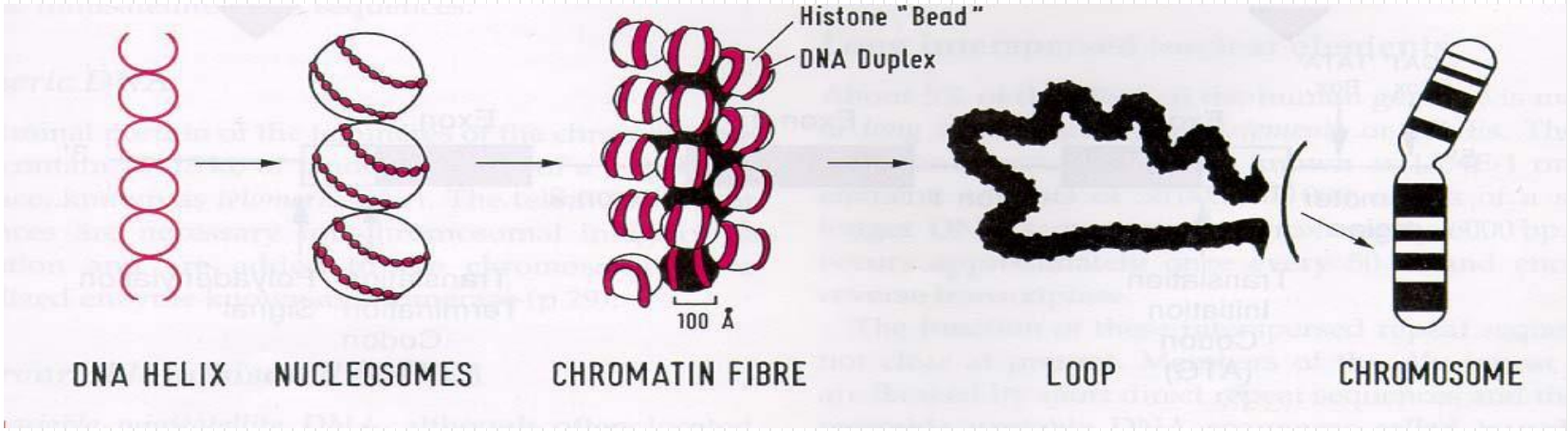
Natureprotocols.com

Banding pattern:

A ‘band’ is defined as that part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or lighter with one or more banding techniques.

Chromosome condensation:

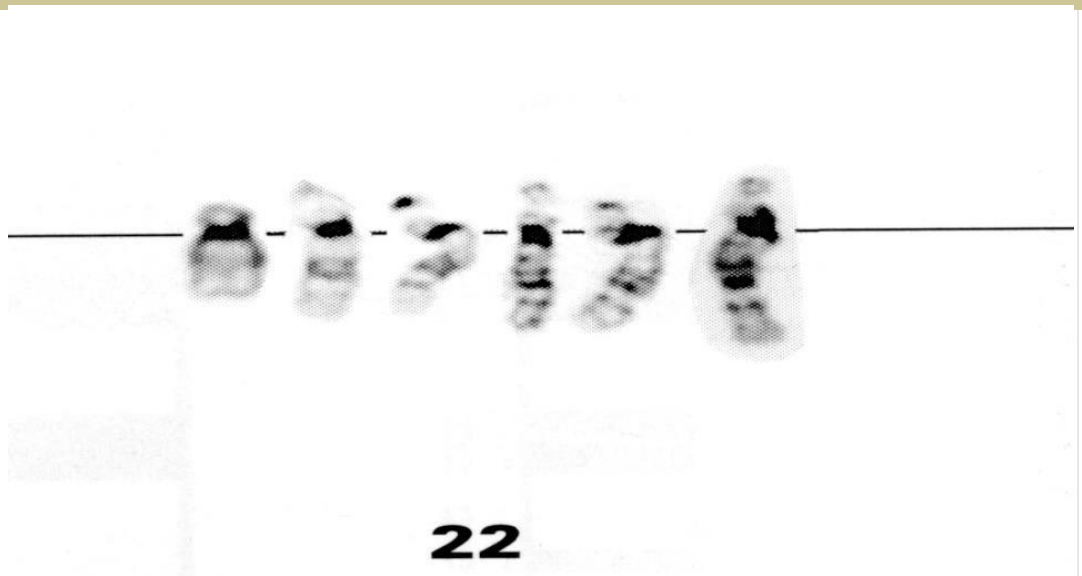
Various stages in condensation of DNA and Chromatin to form a metaphase chromosome



High resolution banding:

- prometaphase and prophase chromosomes
- treatment of the cultures with chemical agent to produce cell synchrony such as (Methotrexate).
- then adding a release agent to prevent contraction such as (Thymidine).

High resolution banding:



Resolution G-banding: G-banded chromosome 22 at various levels from 400-850 band level. Illustrate how a prophase and prometaphase bands coalesce to form metaphase bands.