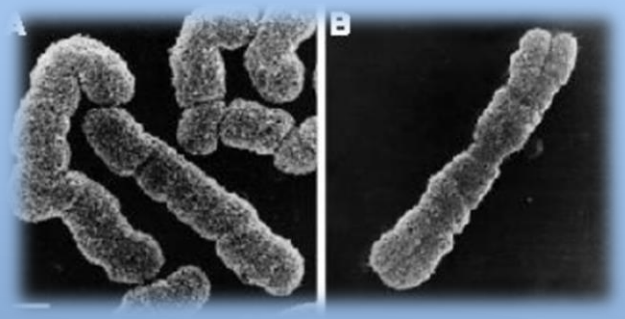


CYTOGENETICS '2'

Karyotyping, cell preparation and staining

Chromosomes

- **1970 Banding techniques**
  - identification of individual chromosomes
- **Karyotype and FISH**

- types of abnormalities;

. Extra copy of chromosome

. Missing copy of chromosome

. Structural abnormalities

Chromosomes

- **Centromere**
  - movement during cell division
  - divides the chromosomes into short (p) and long (q) arms

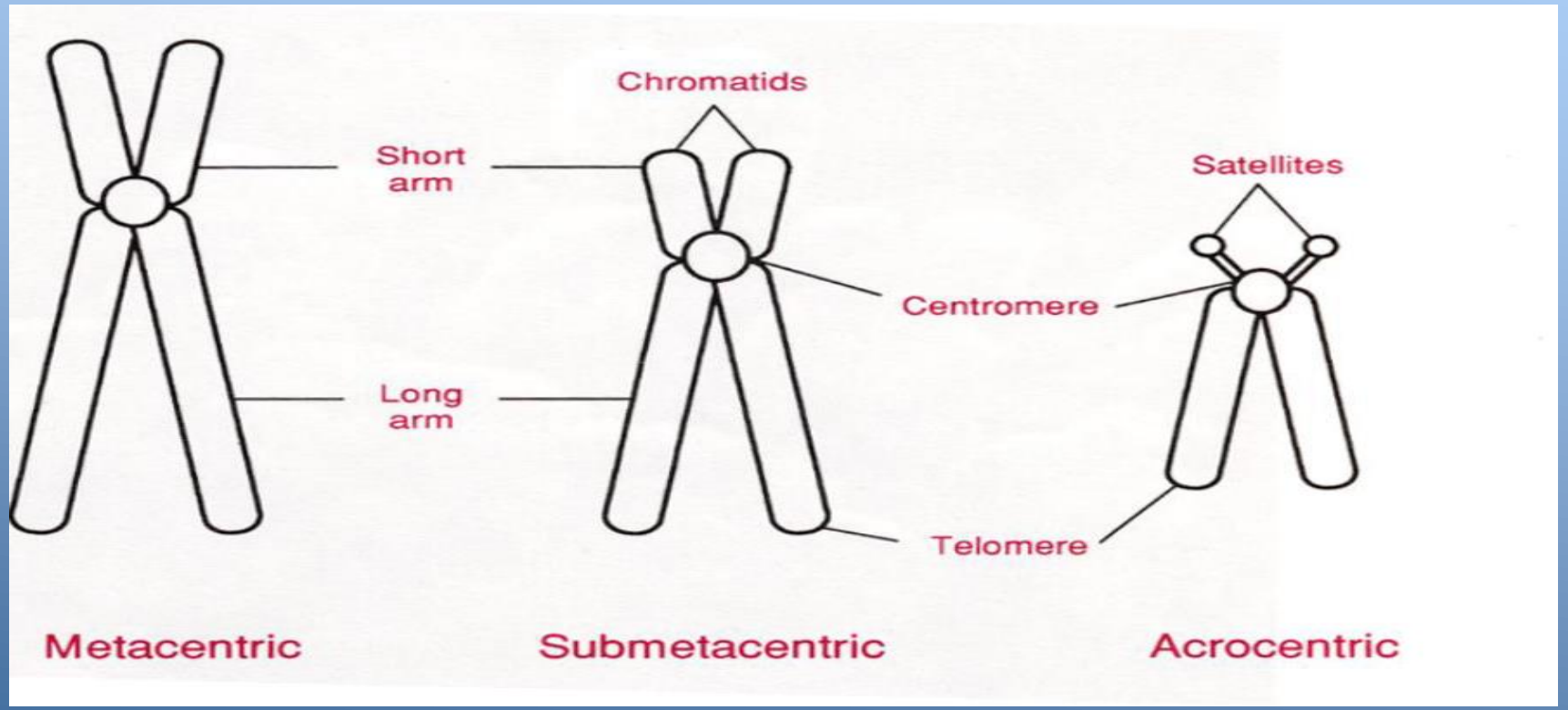
- **Telomere**
  - Tip of each chromosome
  - seal chromosomes and retain chromosome integrity
  - consists of tandem repeats **TTAAGGG**
  - maintained by enzyme; **telomerase**
  - telomerase & in tandem repeats imp in aging & cell death

Chromosomes

Classified according to position of centromere ;

- **Central centromere; metacentric**
- **Sub-terminal centromere ; acrocentric**
  - have satellites which contain multiple copies of genes for ribosomal RNA
- **Intermediate centromere; submetacentric**

Chromosomes

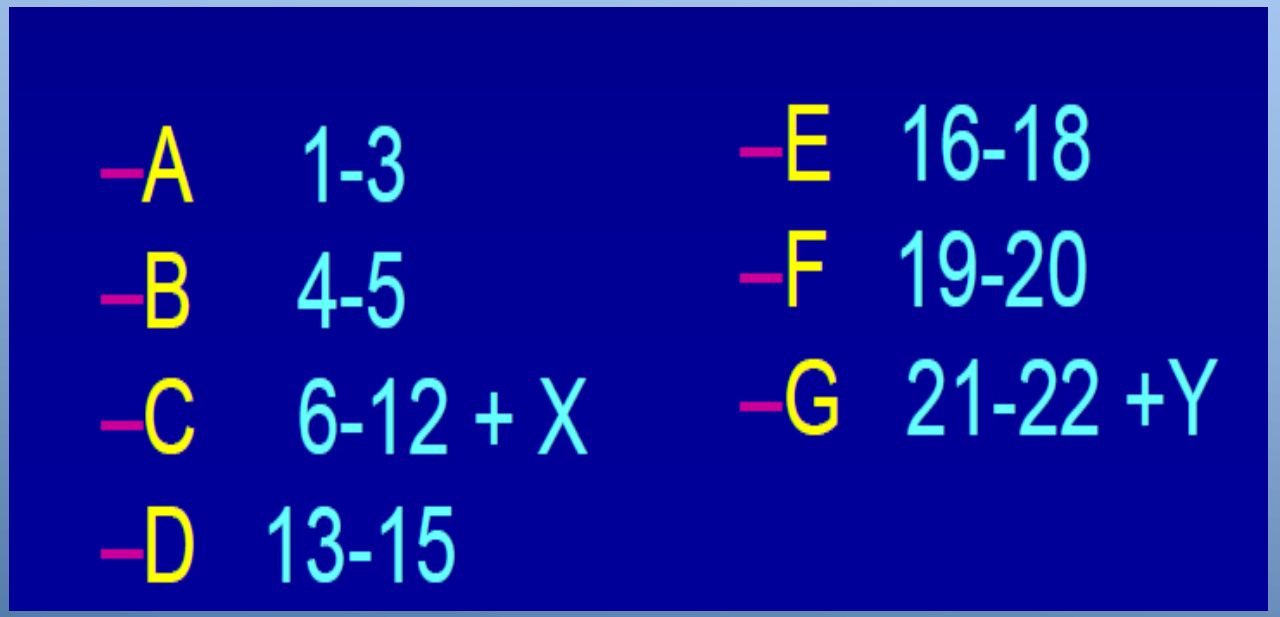


Chromosomes ideogram

Chromosomes

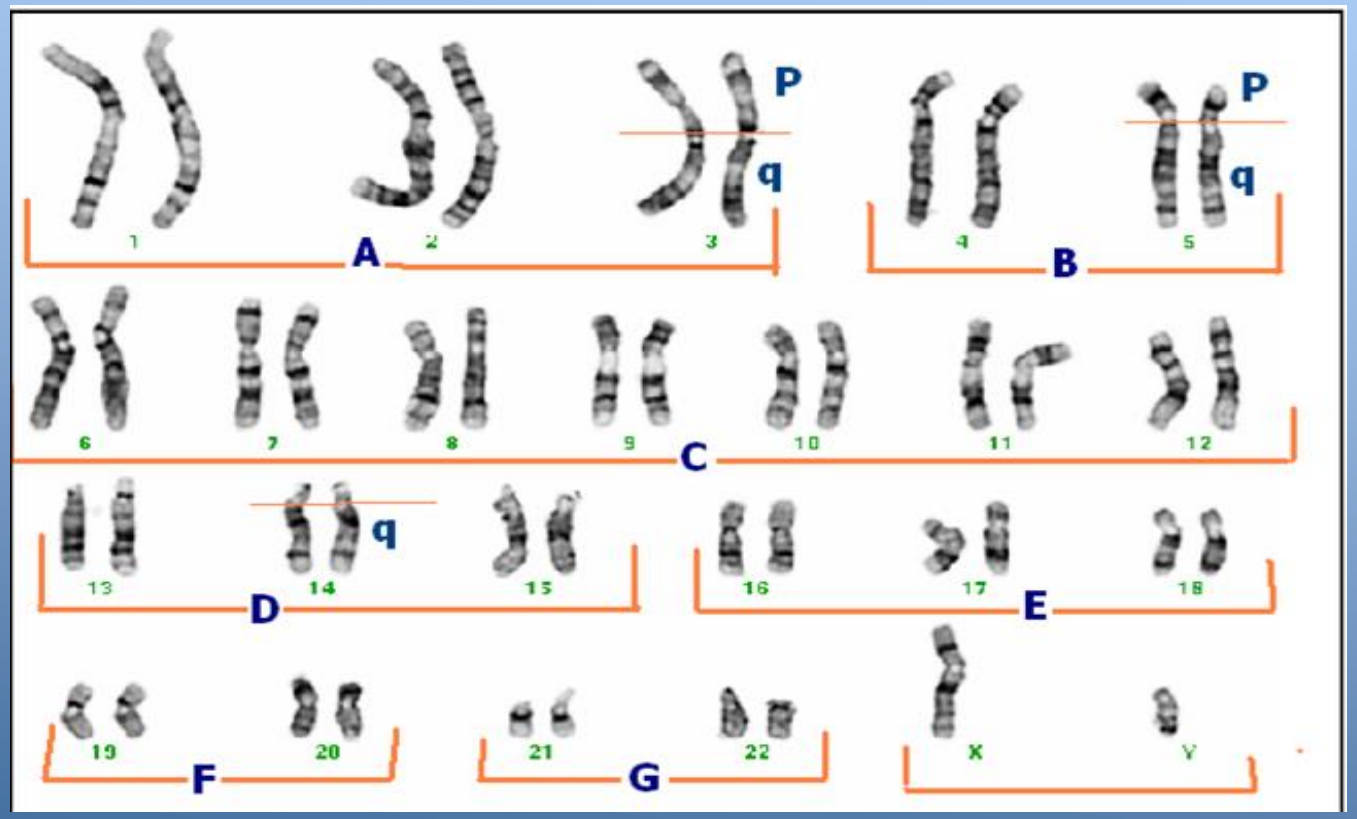
- **22 autosomal, and sex chromosomes in pairs**
- **Classified according to:**
  - Length
  - position of centromere
  - presence or absence of satellites
- **Chromosomes divided into groups labelled A-G**

Chromosomes



Chromosomes divided into groups labelled A-G

Chromosomes



Chromosomes divided into groups labelled A-G

Karyotyping

Staining method to identify chromosomes in a single cell

G banding .. Giemsa
Q banding .. Quinacrine, fluorescent stain (structural rearrangements)
R banding .. Reverse
C banding .. Centromeric (heterochromatin)

Ag-Nor stain .. Nucleolar Organizing Regions (active)

Cell preparation

Required cell stage; **metaphase**

1. Culture cells until sufficient mitotic activity

2. **Harvest protocol:**

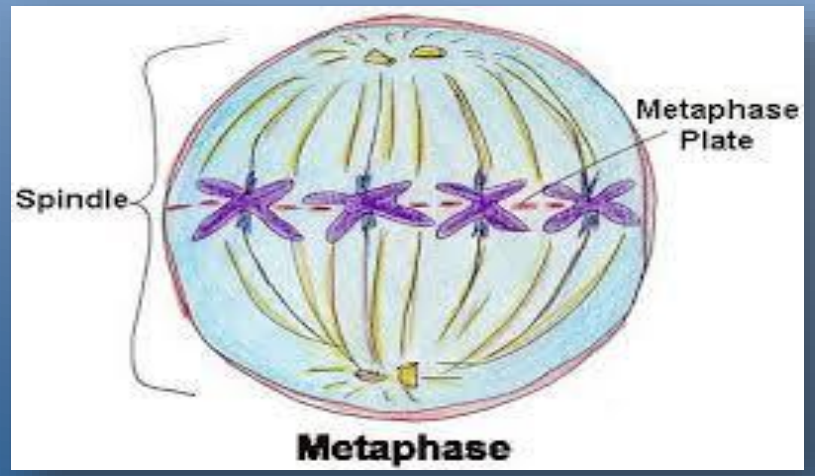
- Mitotic arrest
- Add hypotonic treatment
- Fixation with mix of methanol ; acetic acid

- Want long chromosomes with non-overlapping

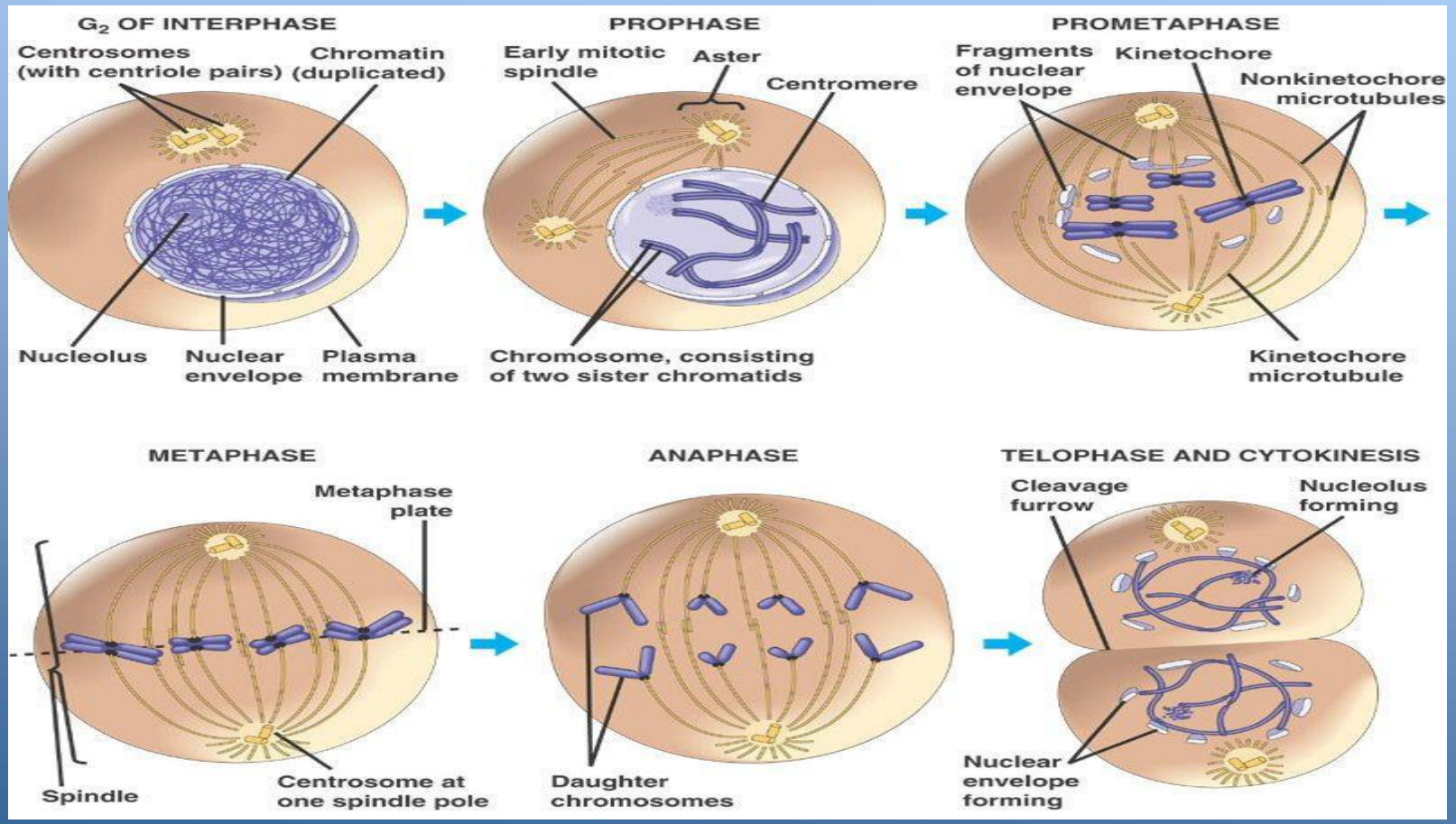
Harvest protocol

a. <u>Mitotic arrest:</u>

Colemid arrest cells at the equatorial plate by preventing spindle fibres formation



Mitosis



Studyblue.com

Harvest protocol

b. Hypotonic treatment:

- Cells swell (osmotic potential):
  reduce cytoplasm & allow chromosome spreading
- RBCs lysis

Harvest protocol

c. fixation:

- Prevent further swelling
- Maintain good morphology

Methamoglobin: brown supernatant formed after addition of fixative

Multiple fixative -- clear supernatant

Harvest protocol

Harvesting procedure:

1. Add 100µ colemid to the culture 20min at 37C
2. Spin; 8 min at 1000 RPM
3. Discard supernatant, add 10ml hypotonic solution
4. Incubate 20min at 73C
5. Add 5 drops fixative 3:1 methanol:acetic acid, spin
6. Discard supernatant & resuspend pellets
7. Add 10ml fixative 30min at RT
8. Repeat until clear solution
9. Preserve at fridge (-4C) not freezer

Dropping protocol

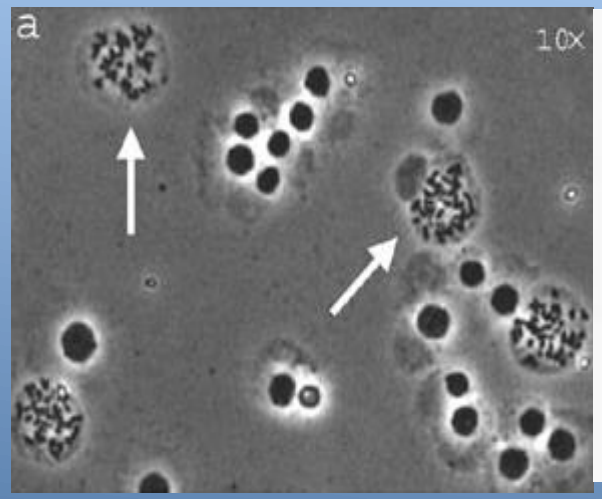
- 8 drops on slide
- dry in a controlled environmental evaporation chambers
- Observe mitotic index

- Aging: 60C over night Or 90C for 90min for better banding pattern

Dropping

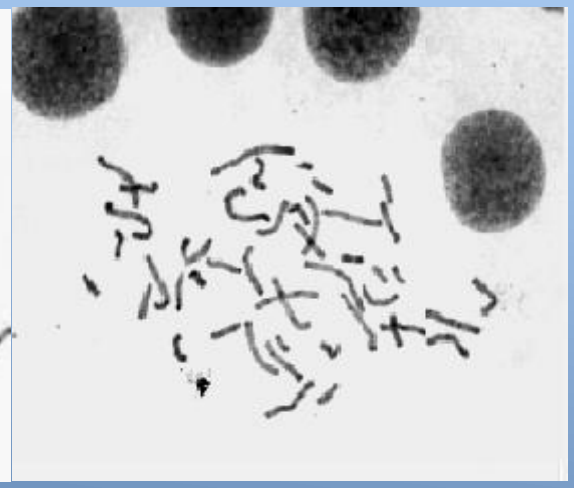
- Residual cytoplasm ↓ staining quality
- Dark chromosomes with sharp borders
- No overlapping, no burst

Metaphase compactness



Natureprotocols

IMSTARA.com



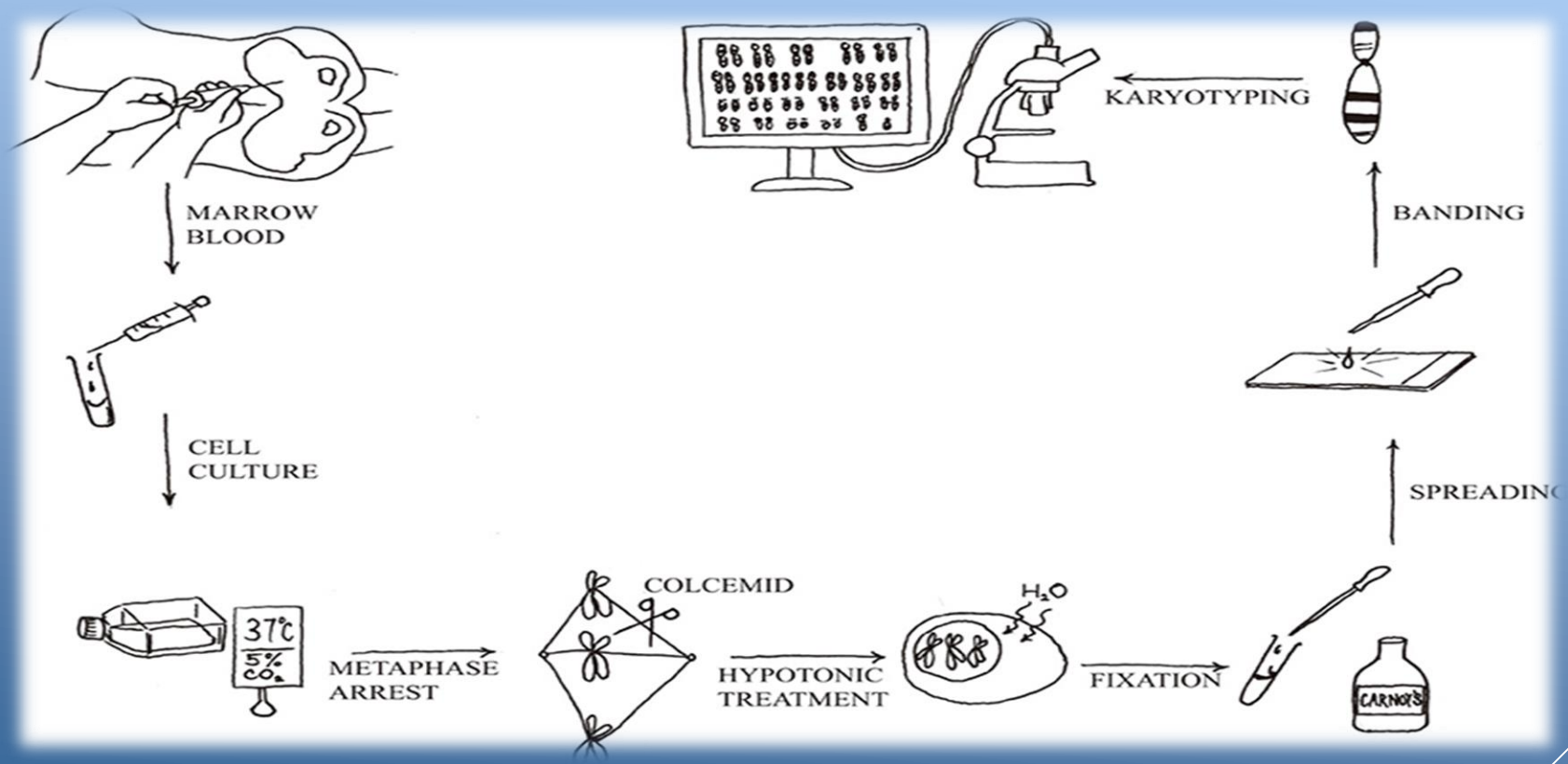
glown.com

Burst

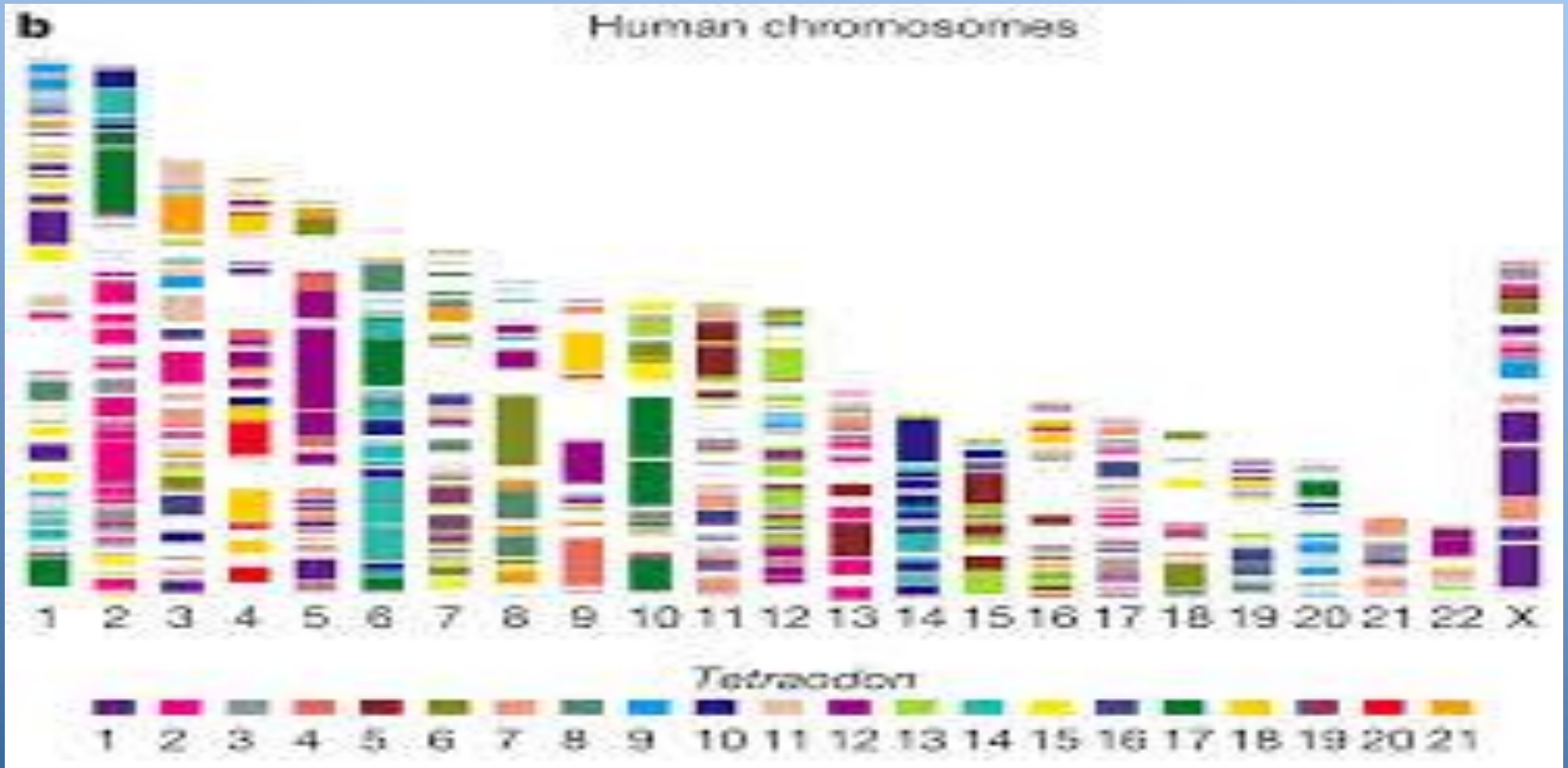
overlapped

good

Cell preparation



Banding (staining)



G banding

- Most common
- Chromosomes treated with **trypsin**;
  - denatures protein
  allow Gimsa react with exposed DNA (wang &Federoff)

G banding

Gimsa stain:

- Each chromosomes characterised by dark & light band
- 400 bands / haploid genome
- Dark bands are gene poor
- Appropriately stained chromosomes;
  neither too dark nor too pale

G banding

Under-trypsinized chromosomes:

Indistinct bands, little contrast and fuzzy

G banding

Over-trypsinized chromosomes:

- Sharp bands and frazzled at the end
- Eventually become very pale (ghost-like) and very swollen
- Fetal calf serum immersion is recommended (α1-antitrypsin)

Broz. J et al, Scielo (2000)

G banding

Staining procedure:

- Slides must cool down to RT
- Immerse in trypsin solution 10-15 sec
- Wash in phosphate buffer or serum (stop trypsin activity)
- Transfer to Gimsa stain 90-120 sec
- Raines in d-water
- Dry
- Examine under microscope

G banding

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

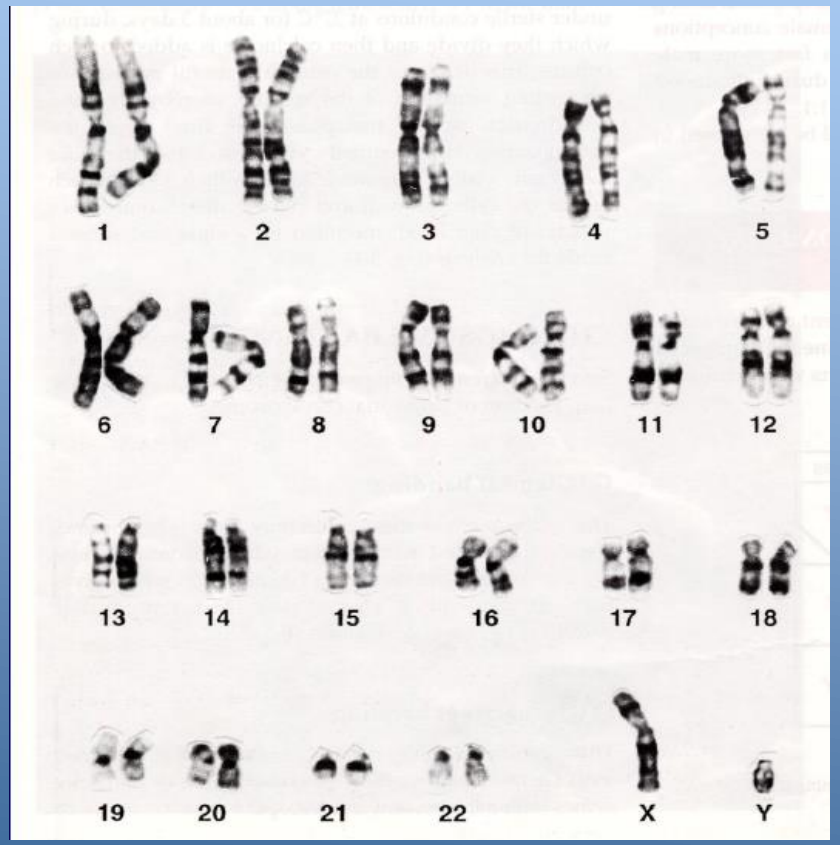
G banding

- 13,18,21 gene poor (**very dark** chromosomes)

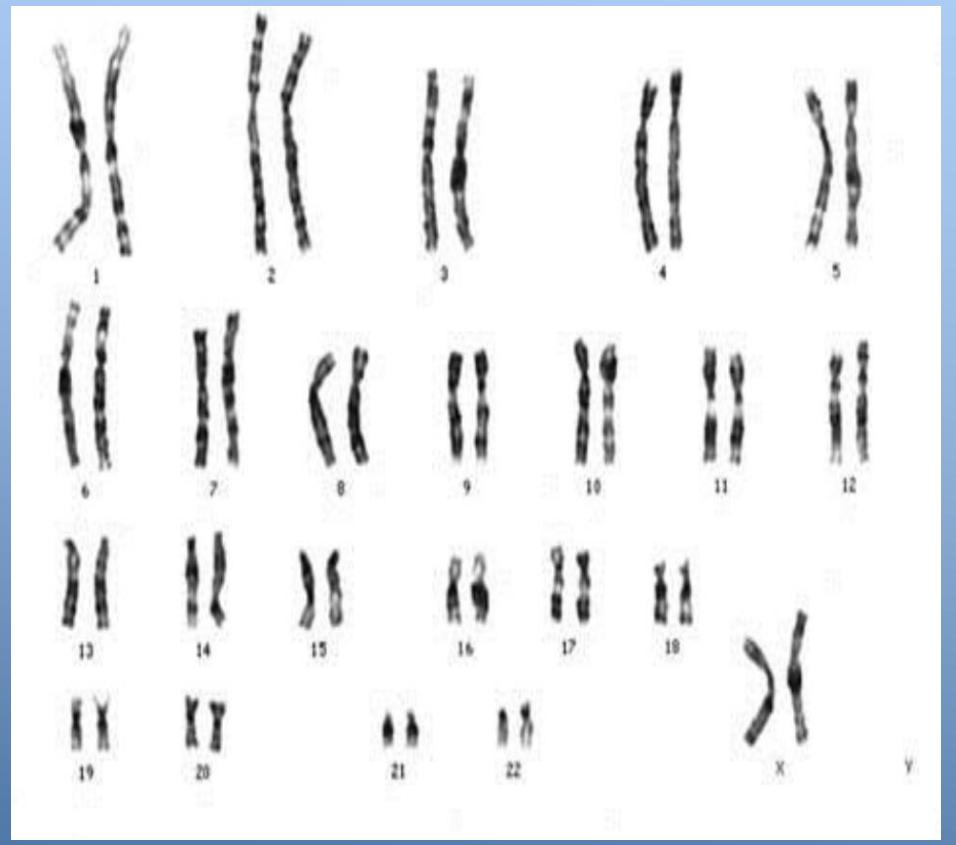
- 21 smaller than 22
- 21 (200 genes) twice as mane as 22 (400 genes)

- Bands stains darkly with Gimsa DNA rich in AT pairs (genes poor)
- Pale bands (gene active)

G banding



Normal Male Karyotype

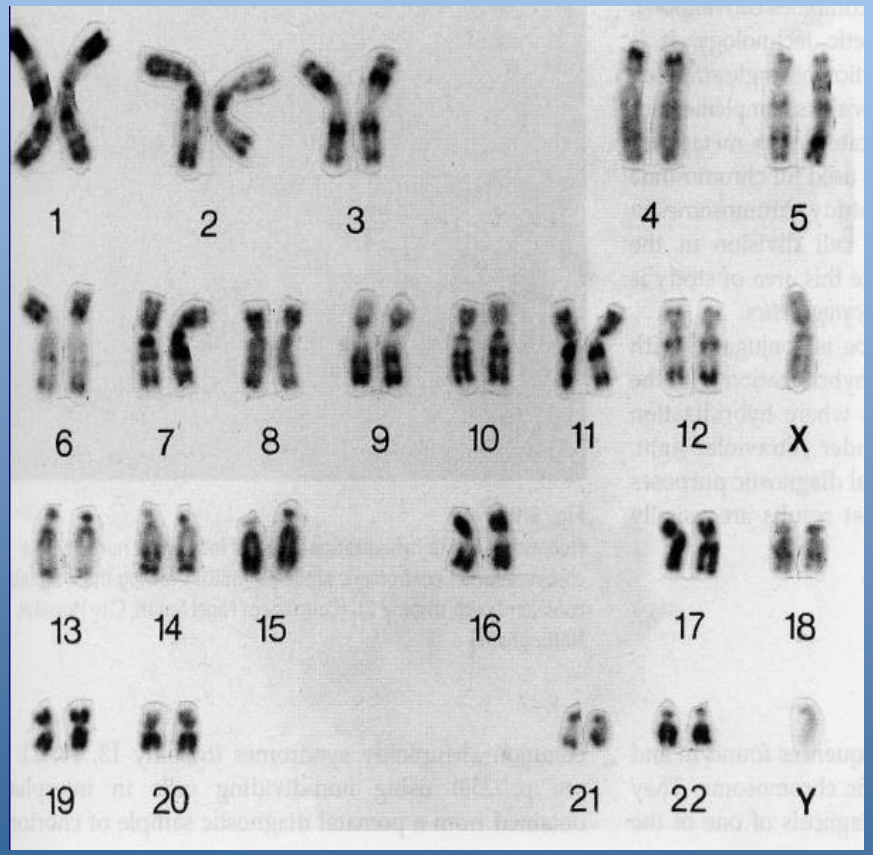


Normal Female Karyotype

Q banding

- Especially used for **Y chromosome** abnormalities or mosaicism
- similar to G band (exp. It can detect polymorphism)
- Need fluorescent microscope

R banding



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

C banding

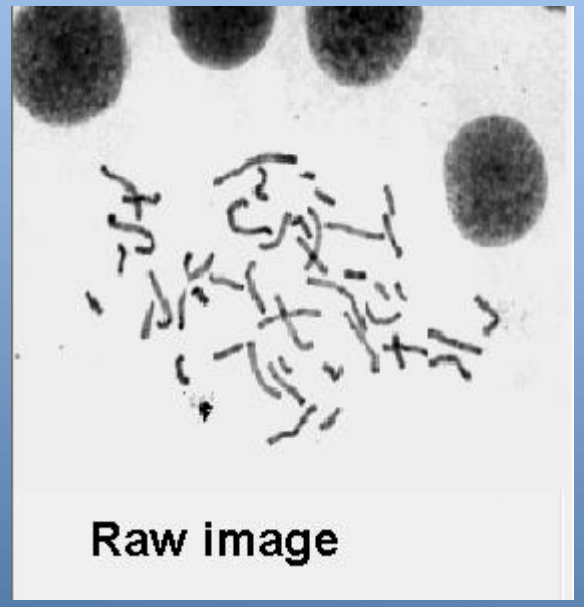
- To identify centromere/ heterochromatin
- **Heterochromatic region**:
  - contains highly repetitive DNA sequence
  - highly condense chromatin fibres
  - genetically inactive (structural elements)
- Treated chromosomes:
  - Acid
  - Alkaline
  - Then G band

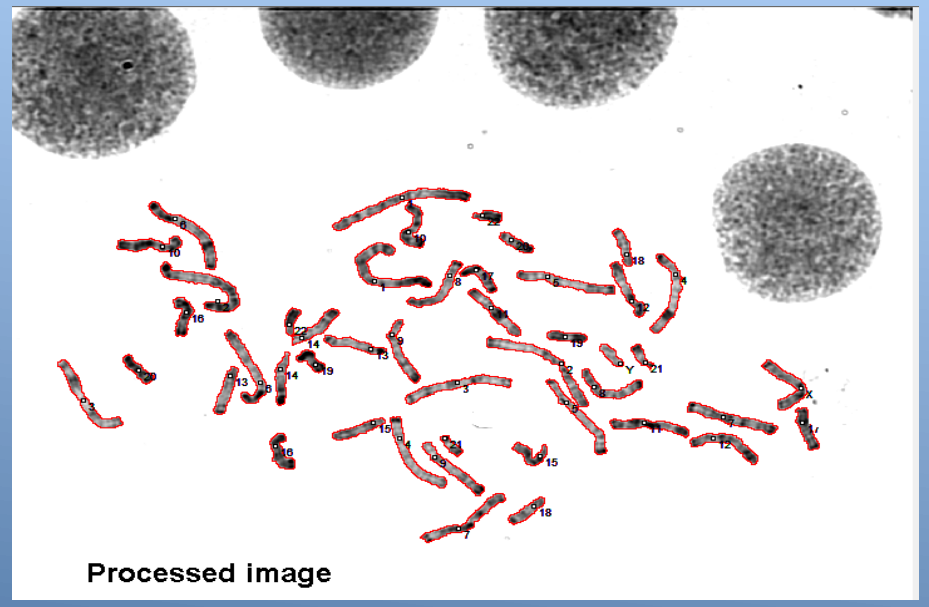
Karyotyping

Organization of the chromosomes, lined up according to:

- Size
- Location of centromere
- Banding pattern

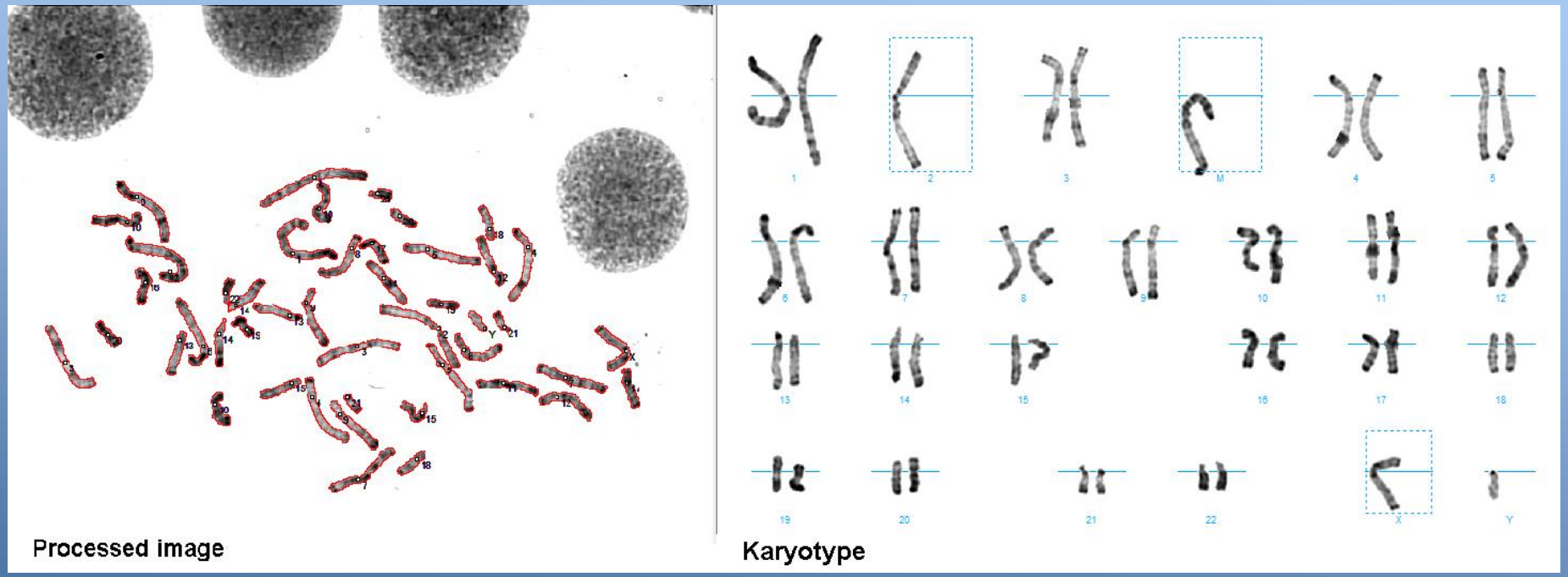
Karyotyping





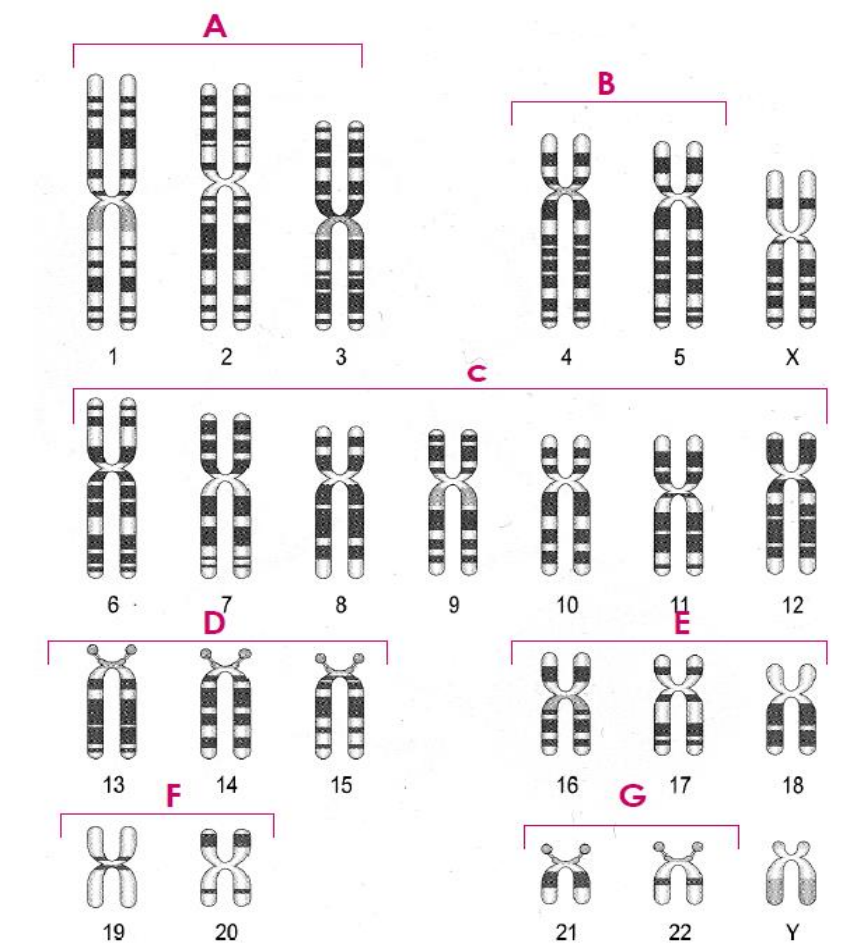
Natureprotocols.com

Karyotyping



Natureprotocols.com

Ideogram



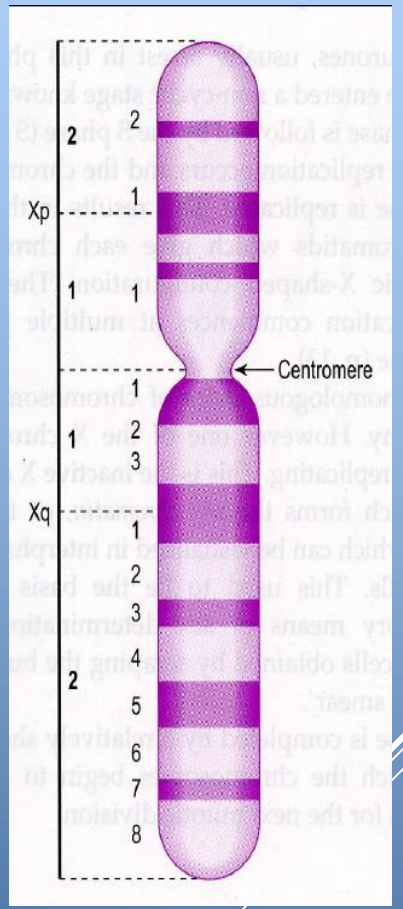
Is a schematic representation of chromosomes

Show relative size of chromosomes & their banding patterns

ISCN

International System for Human Cytogenetic Nomenclature

- Each area of chromosome given number
- Lowest number (proximal) to centromere
- Highest number (distal to centromere)



ISCN

- del - deletion
- dic - dicentric
- fra - fragile site
- i - isochromosome
- inv - inversion
- p - short arm
- r - ring
- der - derivative
- dup - duplication
- h - heterochromatin
- ins - insertion
- mat - maternal origin
- q - long arm
- t - translocation

ISCN

,

46,XX,del(5p)

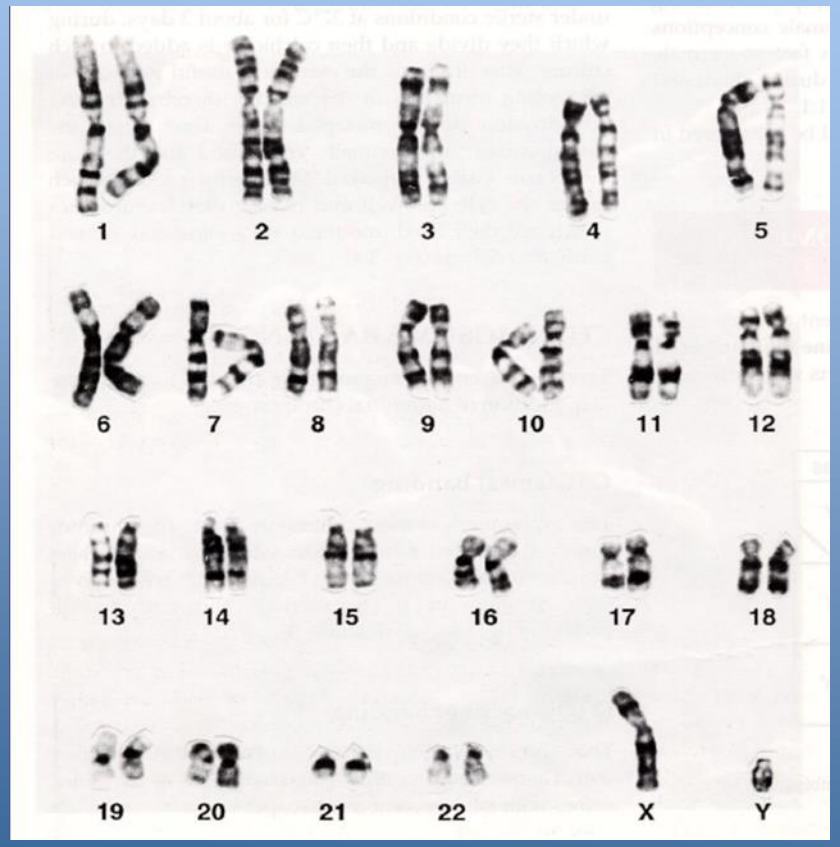
separates
- chromosome numbers
- Sex chromosomes
- Chromosome abnormalities

;

46,XX,t(2;4)(q21;q24)

Separates
- Altered chromosomes
- Break points structural rearrangement involving more than 1 chromosome

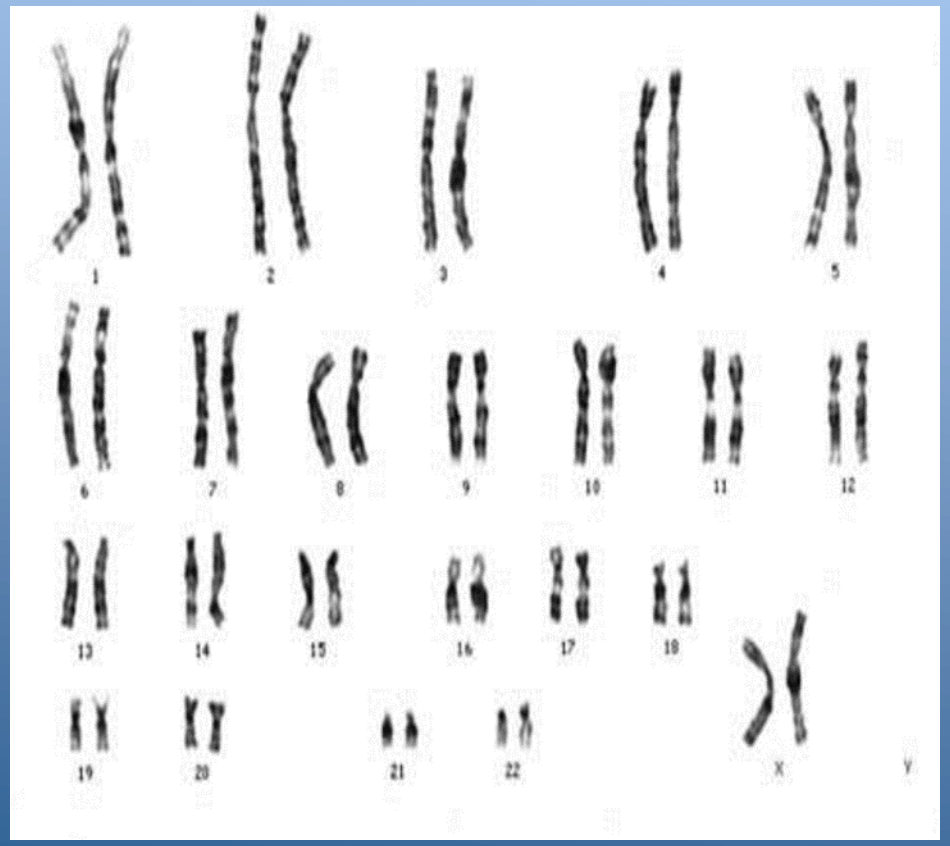
ICSN



Normal Male

46,XY

ICSN



Normal Female

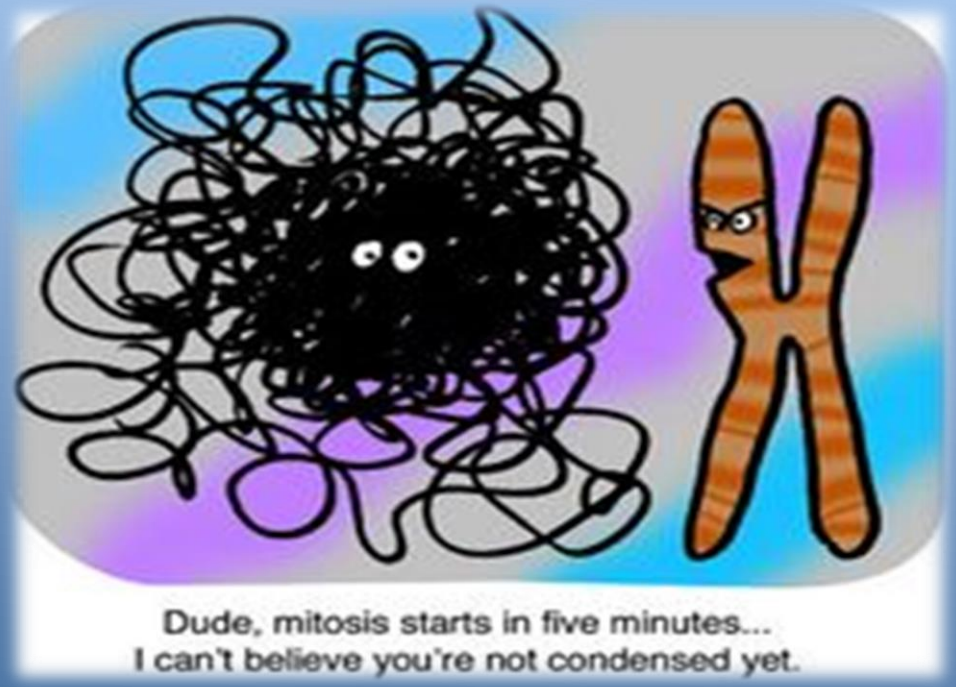
46,XX

Karyotyping activity

- Make a karyotype:

http://mrforde.blogspot.com/2009/01/karyotype-game.html

Have fun ;)


Dude, mitosis starts in five minutes...
I can't believe you're not condensed yet.

Thank You