Laboratorio 3.

Chromosome Analysis (Karyotyping)

Chromosome Analysis (Karyotyping)

Karyotyping technique allows us to isolate the chromosomes from the nucleus. Chromosomes can be isolated from cells of live tissues, including blood lymphocytes, skin fibroblasts, amniocytes, placenta, bone marrow, and tumor specimens.

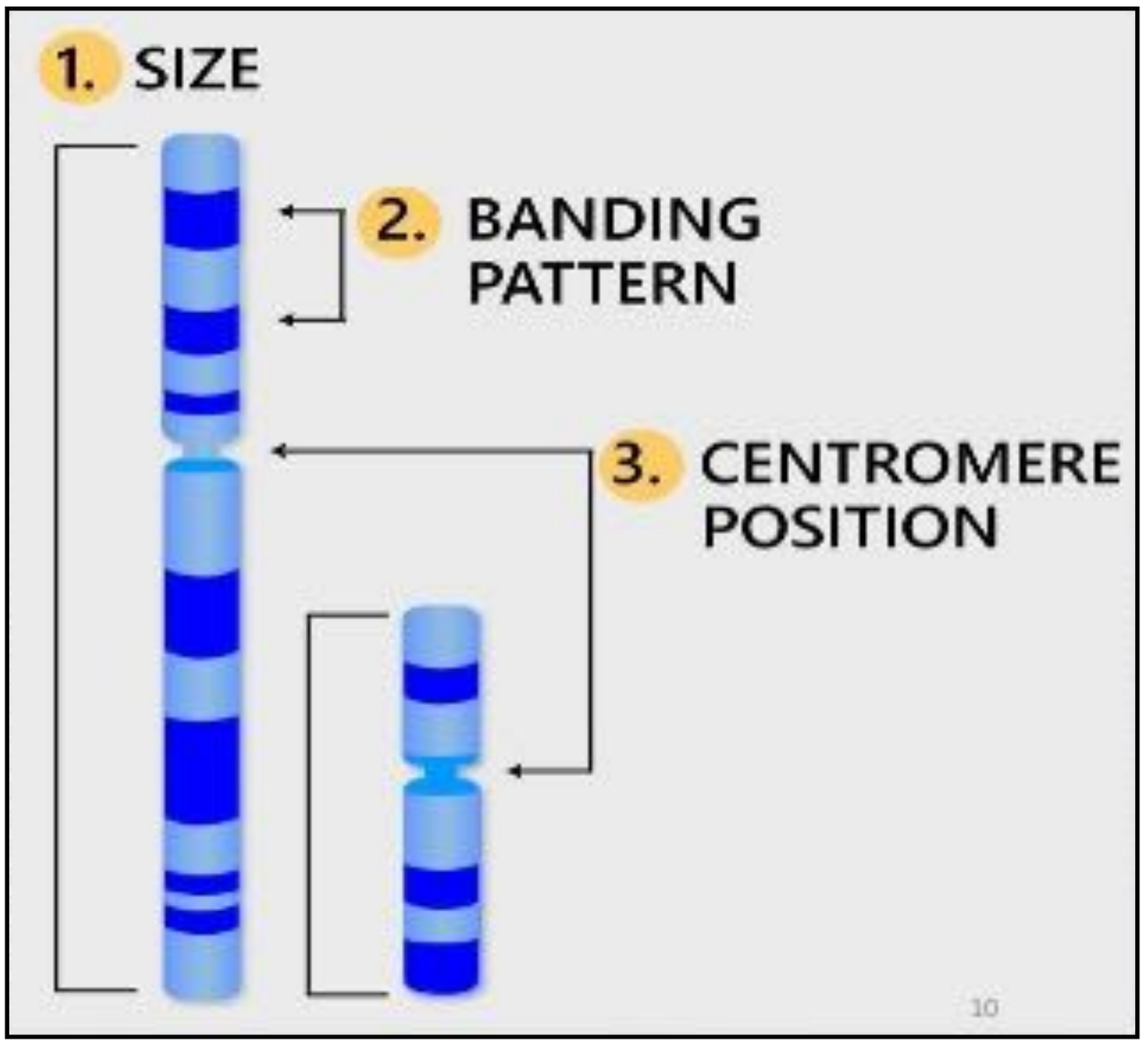
The main porpose of the karyotyping is to locate or visualize the changes in the **number of the chromosomes** and **abnormality in the structure**.

Thanks to **the karyotyping** we can obtained the following information about the cell lines:

- Species identification
- Index of genome stability
- Validation of normal diploid karyotype
- Numerical chromosomes abnormalities
- Structural abnormalities: deletion, duplication, translocation, Inversion

Basically karyotyping is based on three patterns:

1. **SIZE** of chromatids
2. **BANDING** pattern
3. **CENTROMERE** positions



CHROMOSOME SIZE

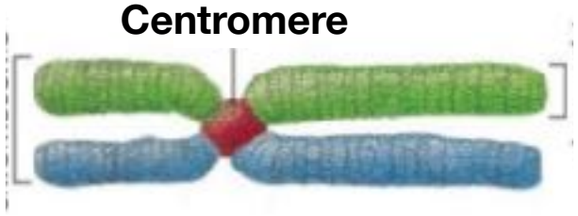
The size of chromosomes shows a remarkable variation depending upon the stages of cell division

- **INTERPHASE**: chromosome are longest and thinnest
- **PROPHASE**: there is a progessive decrease in their length accompanied with an increase in thinkness
- - **ANAPHASE**: chromosomes are smallest
- **METAPHASE:** chromosomes are the most easily observed and studied during the metaphase when they are very thick, quite short and well spread in the cell.

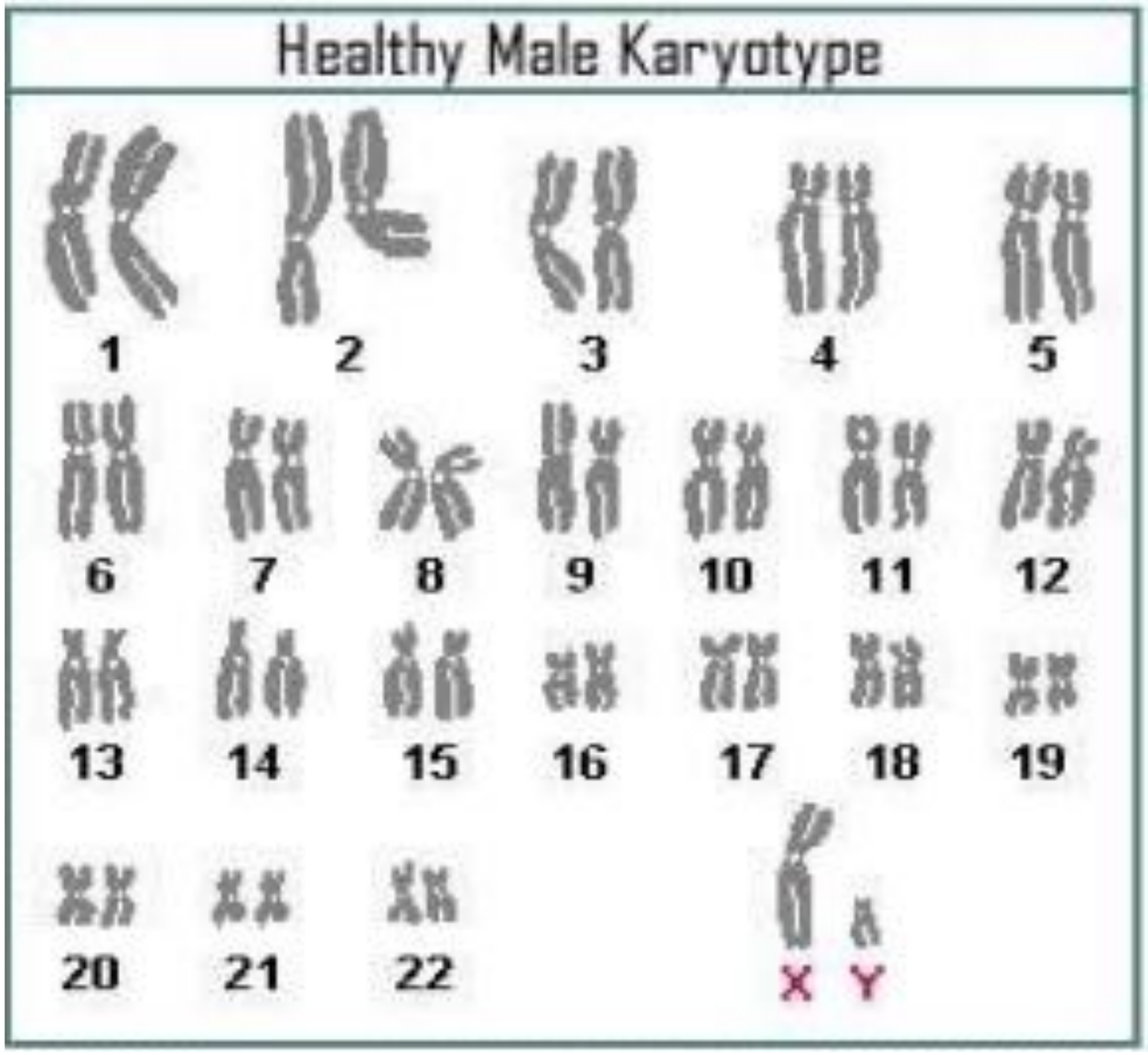
Therefore, chromosomes measurements are generally

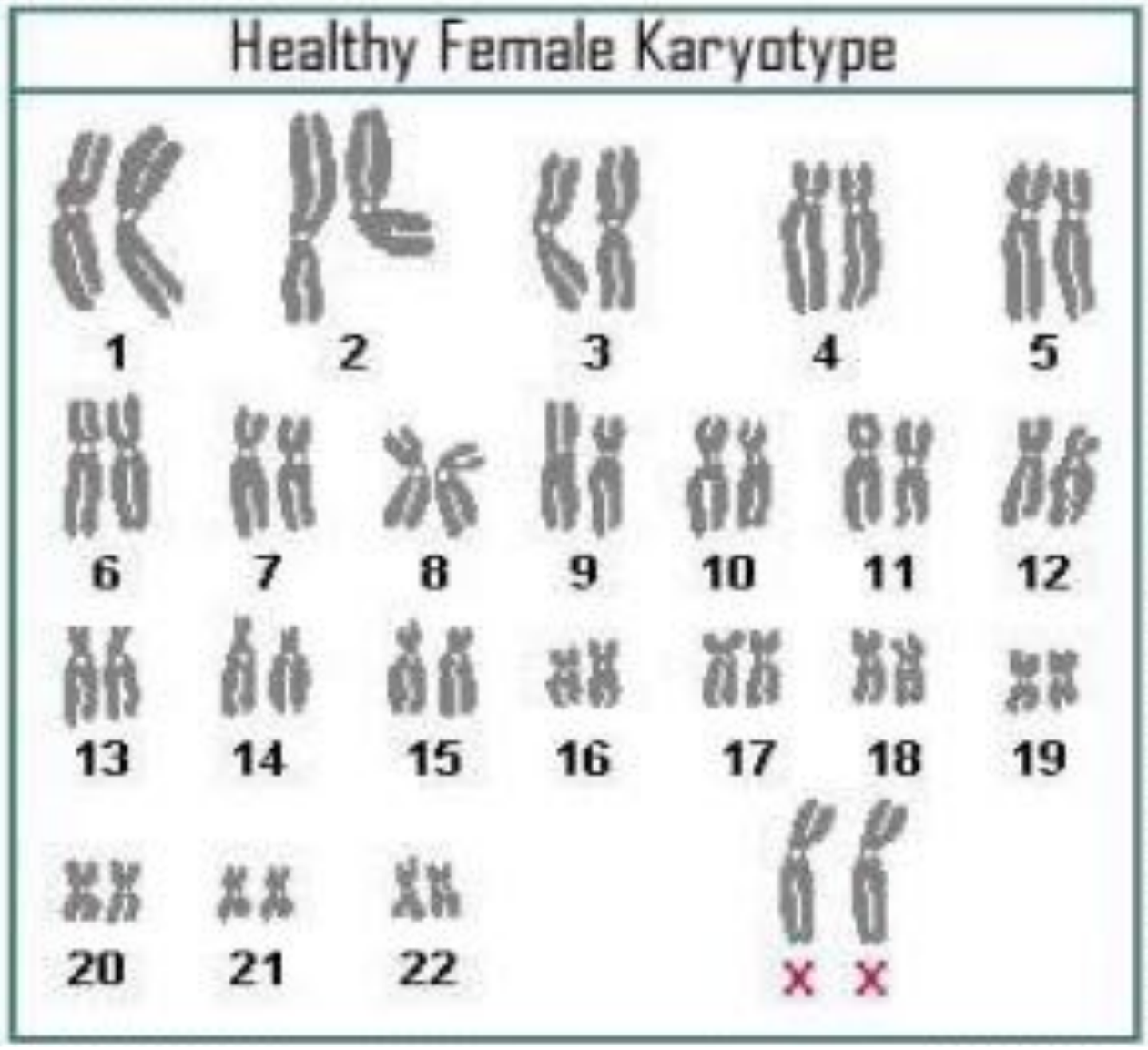
taken **during mitotic metaphase.**

CHROMOSOME PARTS



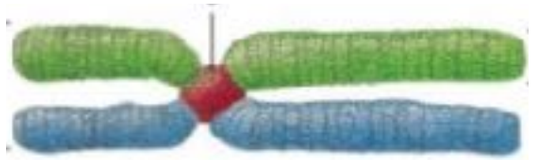
1. On Basis of SIZE





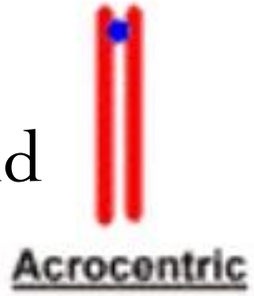
According to the possition of centromere

The chromatides tend to separate but are held together by a specific region called the **centromere**

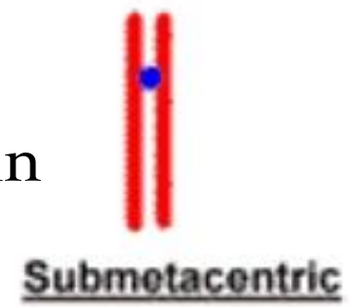


1. **Telocentric** - no long arm, centromere is on end

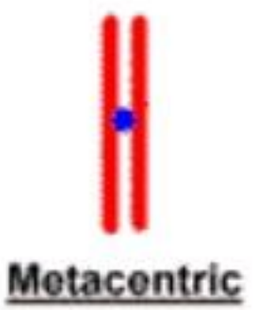
2. **Acrocentric** - vert small p arm, centromere is very near end

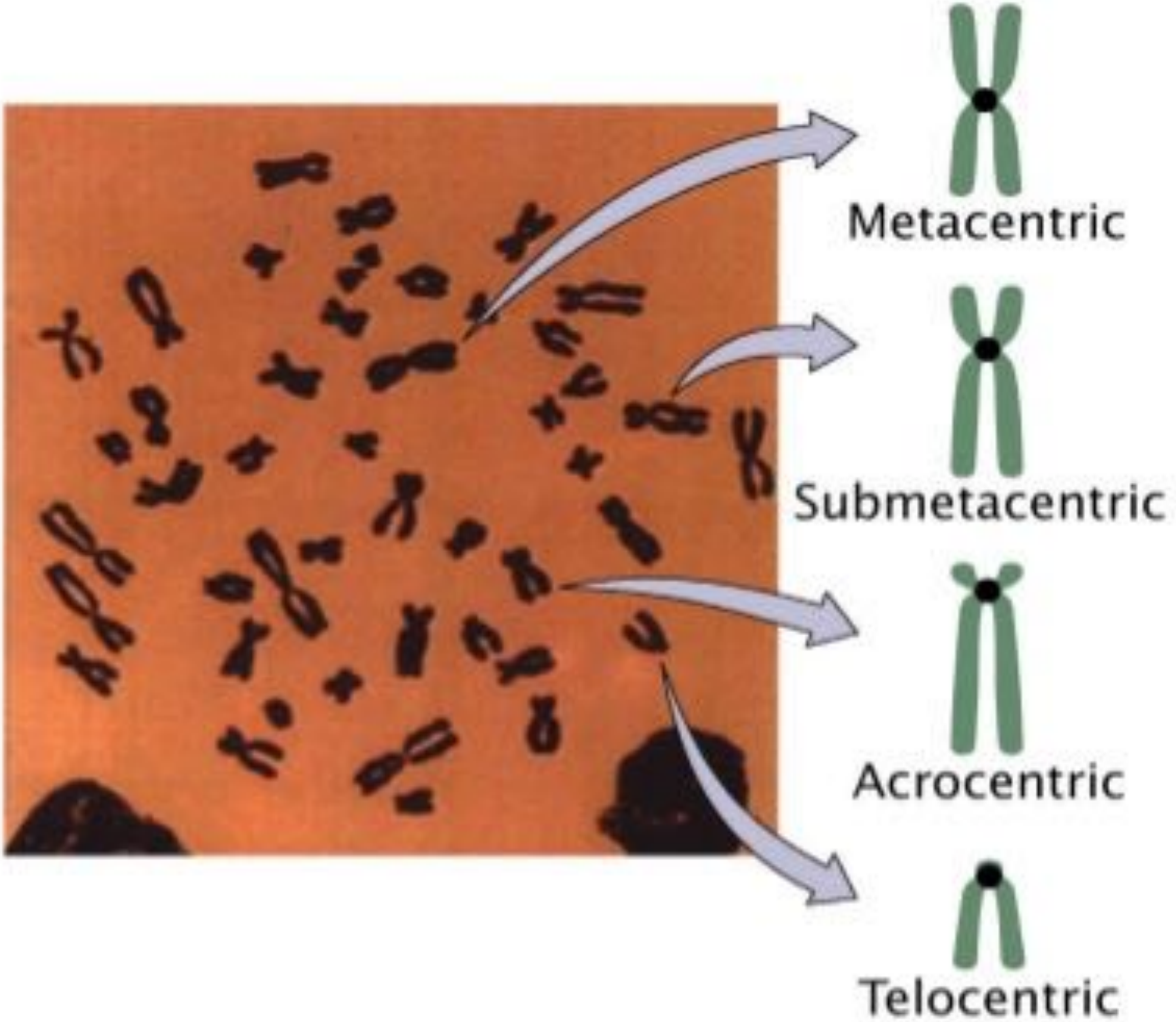


3. **Submetacentric** - p arm just little smaller than q arm, centromere in middle

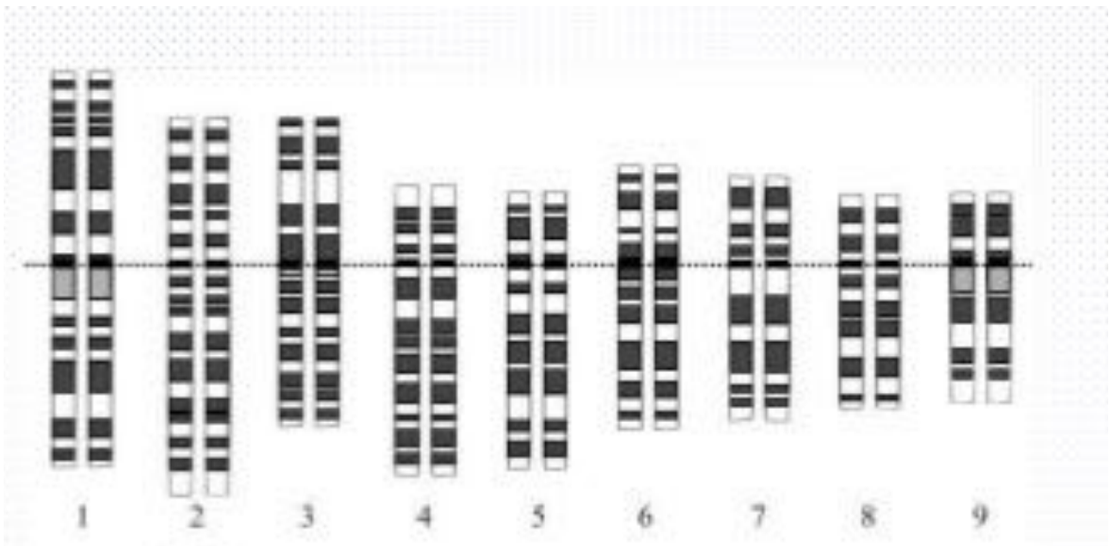


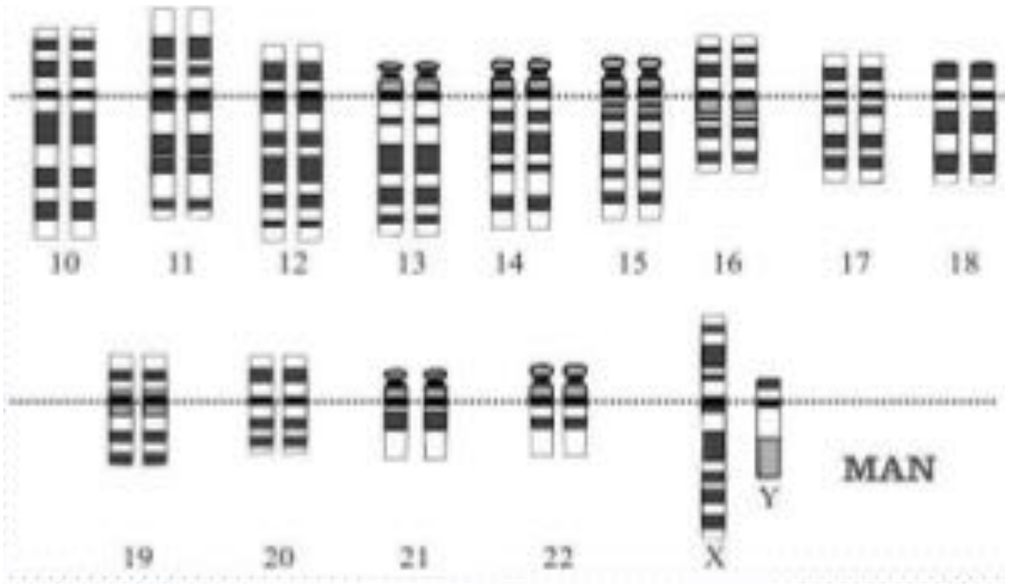
4. **Metacentric** - p and q arms are exactly the same lenght, sentromere in exact middle of chromosome




Metacentric
Submetacentric
Acrocentric
Telocentric

3. Basing on the banding





HETEROCHROMATINE:

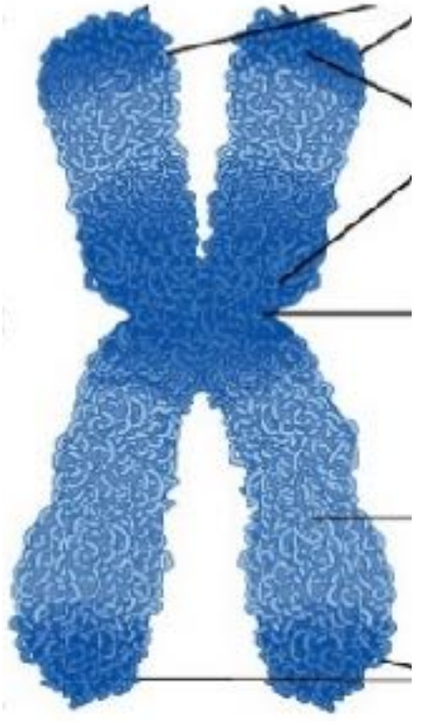
Deep stain

- More condensed
- Silenced gene (methylated)
- Gene poor (high content)
- **Stained darker**

EUROCHROMATINE:

Light stain

- Less condensed
- Gene expressing
- Gene rich (higher GC content)
- **Stains lighter**

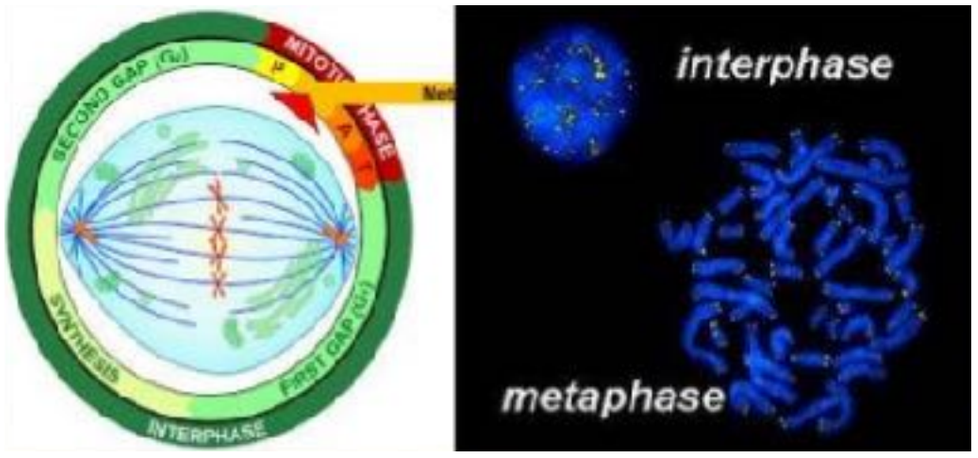


To do the analysis, the cell cycle needs to be blocked in **metaphase** (where **chromosomes are condensed** and appear well distinguishable under the microscope).
This is possible by the addition of ***Colchicine*** **(or *colcemid*)** to the culture medium.

Colcemid acts on **microtubules blocking** the formation of the mitotic fuse; therefore, the chromosome remains condensed and blocked in the **metaphase stage** of the cell cycle.

This kind of block is reversible.

Excessively long incubations with Colcemid result in **overcondensed chromosomes** that band poorly and more- over some cell types, especially those from the mouse, eventually escape the Colcemid block and proceed through the cell cycle.



Hypotonic Solution

- Hypotonic solution having a **lower salt concentation** than in the cytoplasm of normal cell is used

- According to the rule of Osmosis there is a **movement of fluid from the hypotonic solution to the cell through the cell membrane**, leading to the swelling of the cells with subsequent dispersion of the chromosomes.

Fixatives

- Fixatives are used to **preserve**, the form of the cells and their contents as closely as possible to their living state and to render them resistant to further changes caused by subsequent processing

- The commonly used fixative is a mixture of **glacial acetic acid and methyl alcohol in the ratio of 1:3 proportions**.

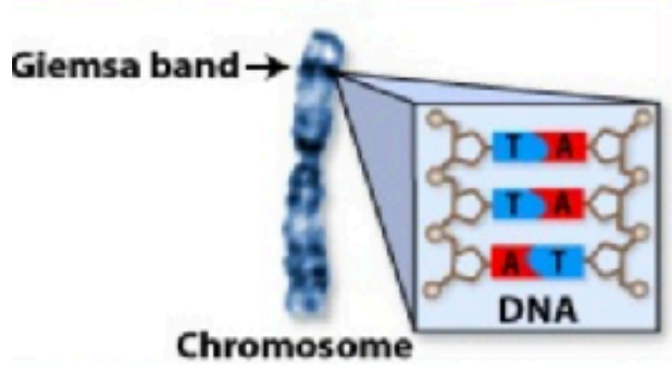
Staining

- Chromosomes are colorless. In order to be able to tell one chromosome from another, need to be stained.

- The most common, fast and cheap staining of the chromosomes is **8% Giemsa staining** (pH 7.2).

G-banding

Dye gives chromosomes a **striped appearance** because it stains the regions of DNA that are rich in adenine (A) and thymine (T) base pairs.



Regions that stain as dark G bands replicate late in S phase of the cell cycle and contain more condensed chromatin.

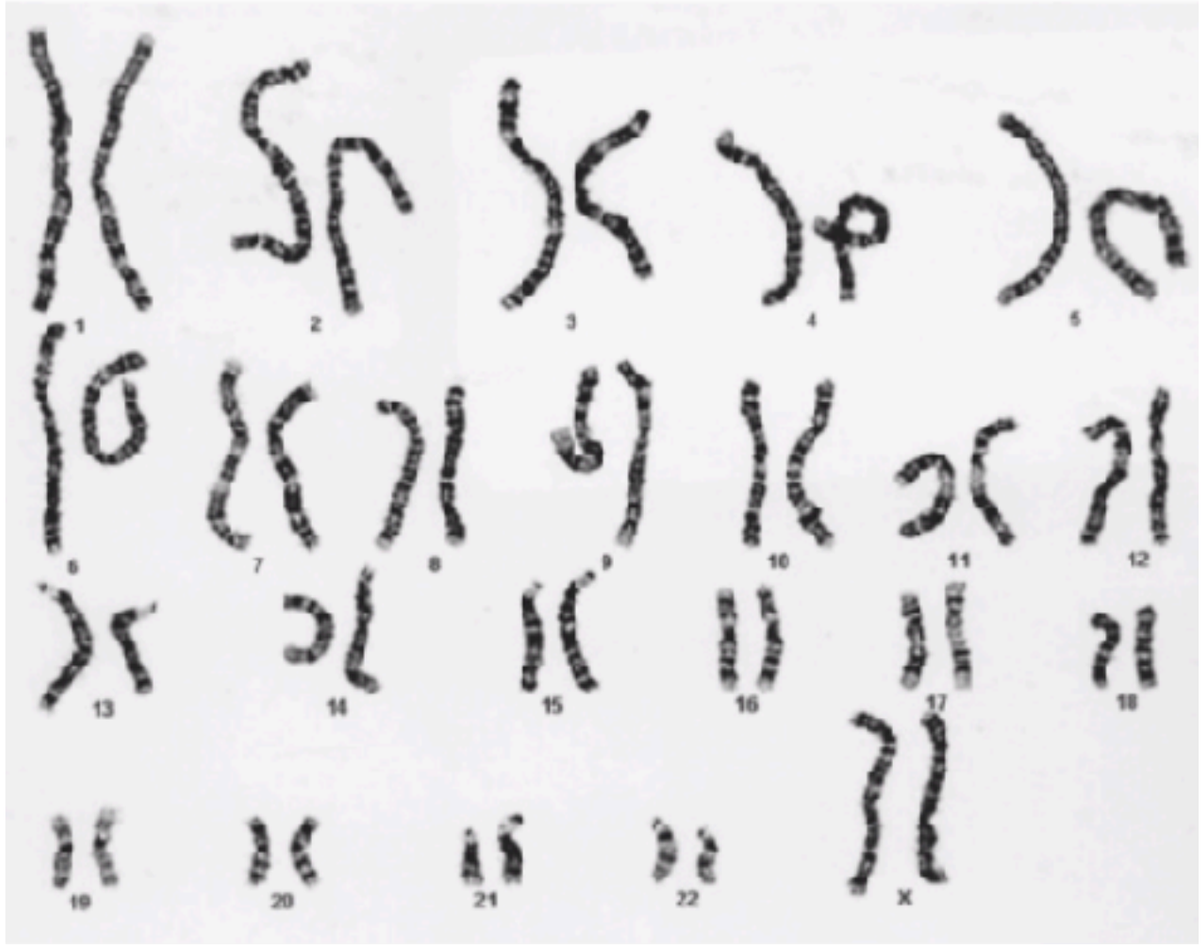
While ligh G bands generally replicate early in S phase, and have less condensed chromatin. Those regions are usually reach in guanin and cytosie

Preparation a Karyotype

The slides are scanned for metaphase spreads and usually 10 to 30 cells are analyzed under the microscope

When a good spread (minimun number of overlapping chromosomes) is found, a photograph is taken or the analysis is done by a computer

The cromosomes are arranged in a standard presenation format of longest to shortest



MATERIALS

- Cell culture in monolayer
- MEM + 10% FBS
- Trypsin

To split the cells

- 0.5 µg/ml demecolcine (to add to culture medium) To block cells in metaphase stage
- Hypotonic solution: 0.9% Na Citrate (in H_2O) and 0.4% KCl (in H_2O), 1:1
- Cold fixative Methanol and Glacial Acetic Acid, 3:1
- Giemsa staining 8% in H_2O
- Inverted microscope

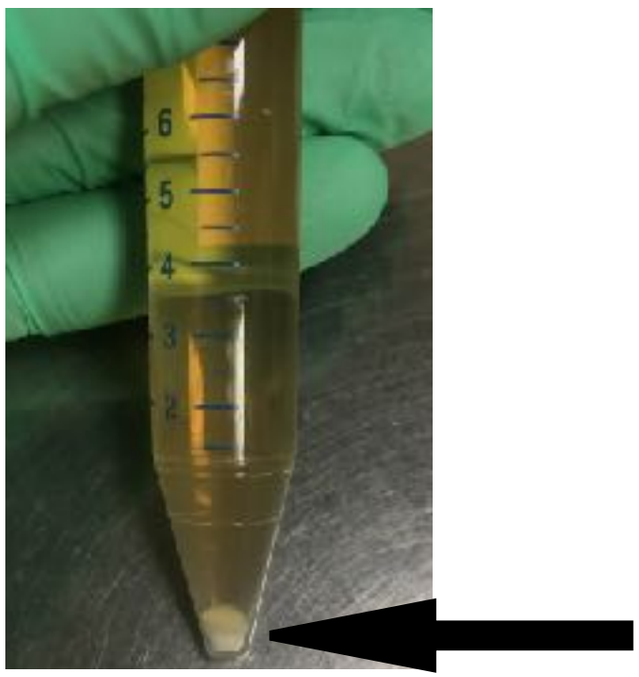
3 h before add 0,5 ug/ml of colcemid to the cell culture

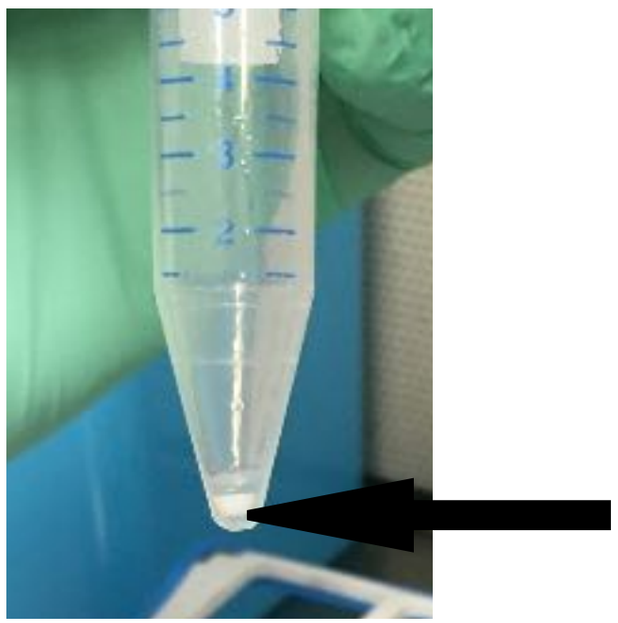
Remove spent media from the culture vessel.

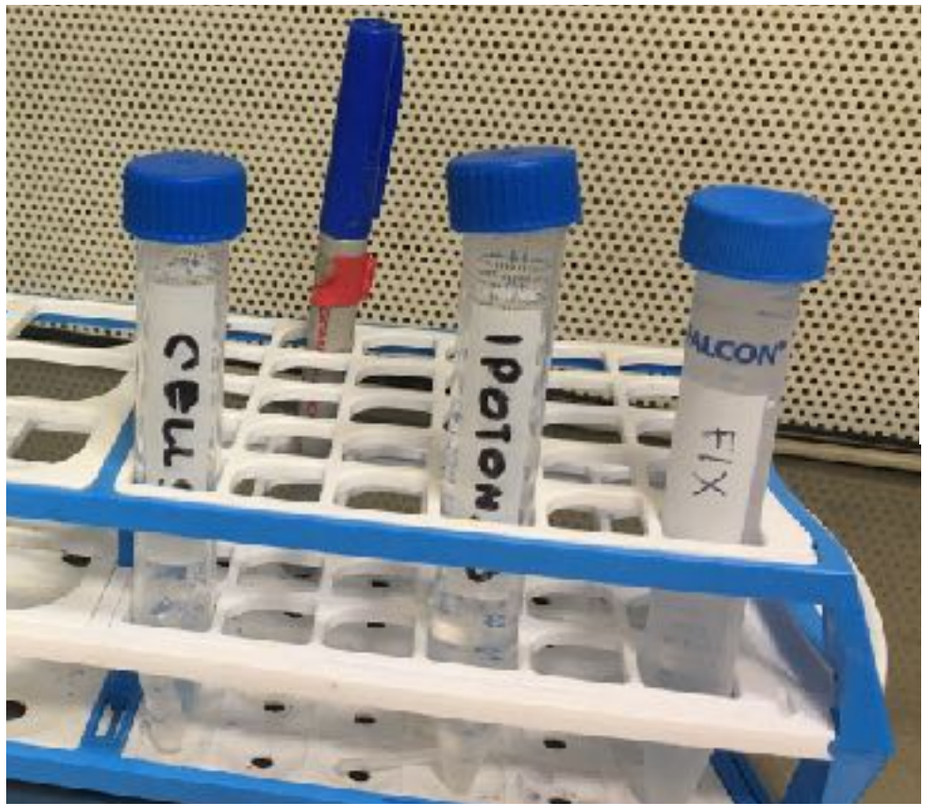
Add the pre-warmed dissociation reagent such as trypsin. Gently rock the container to get complete coverage of the cell layer.

Incubate the culture vessel at room temperature for approximately 2 minutes.

Spin 5 min / 1200 rpm







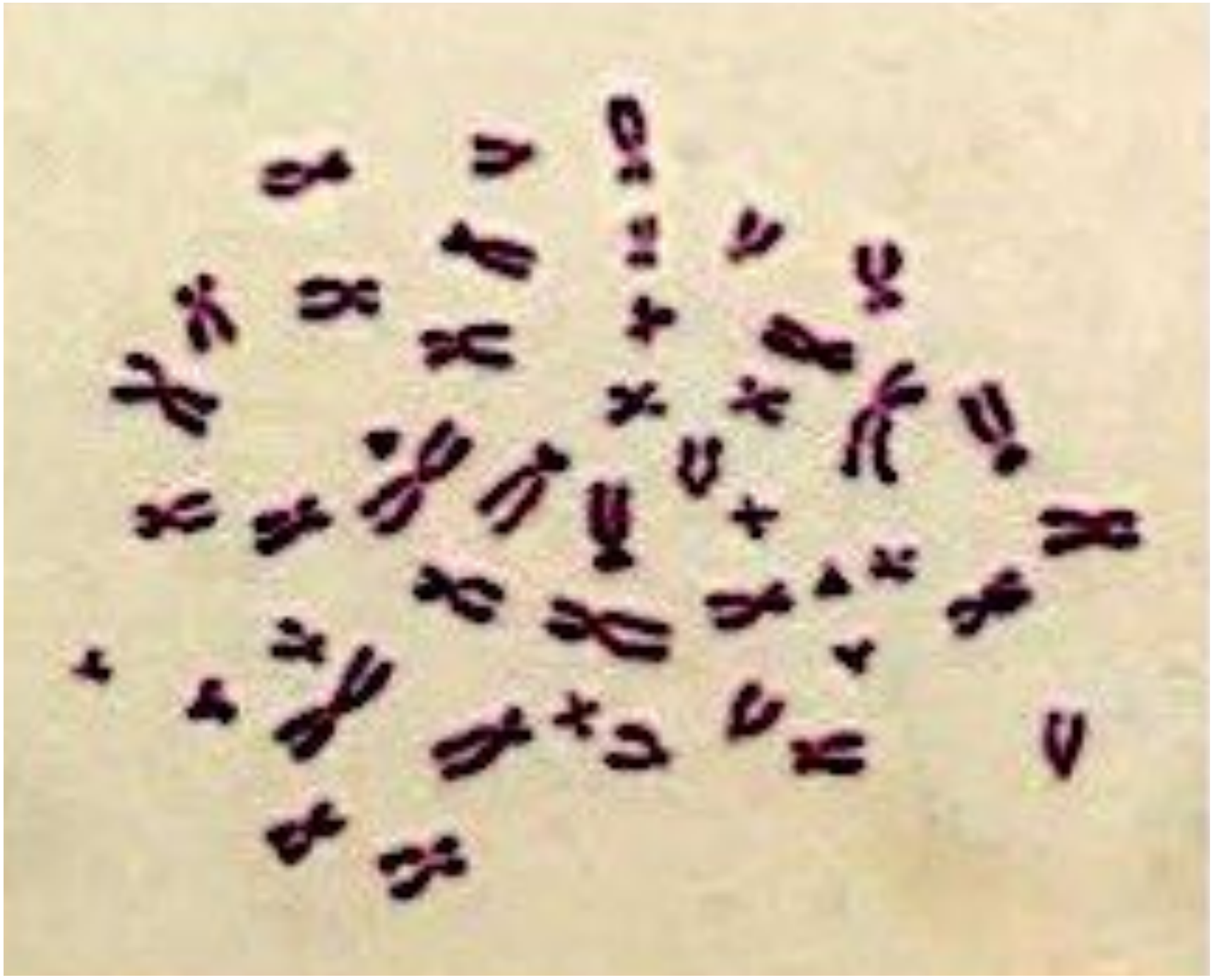
Hypotonic solution: 0.9% Na Citrate (in H_2O) and 0.4% KCl (in H_2O), 1:1
Cold fixative Methanol and Glacial Acetic Acid, 3:1

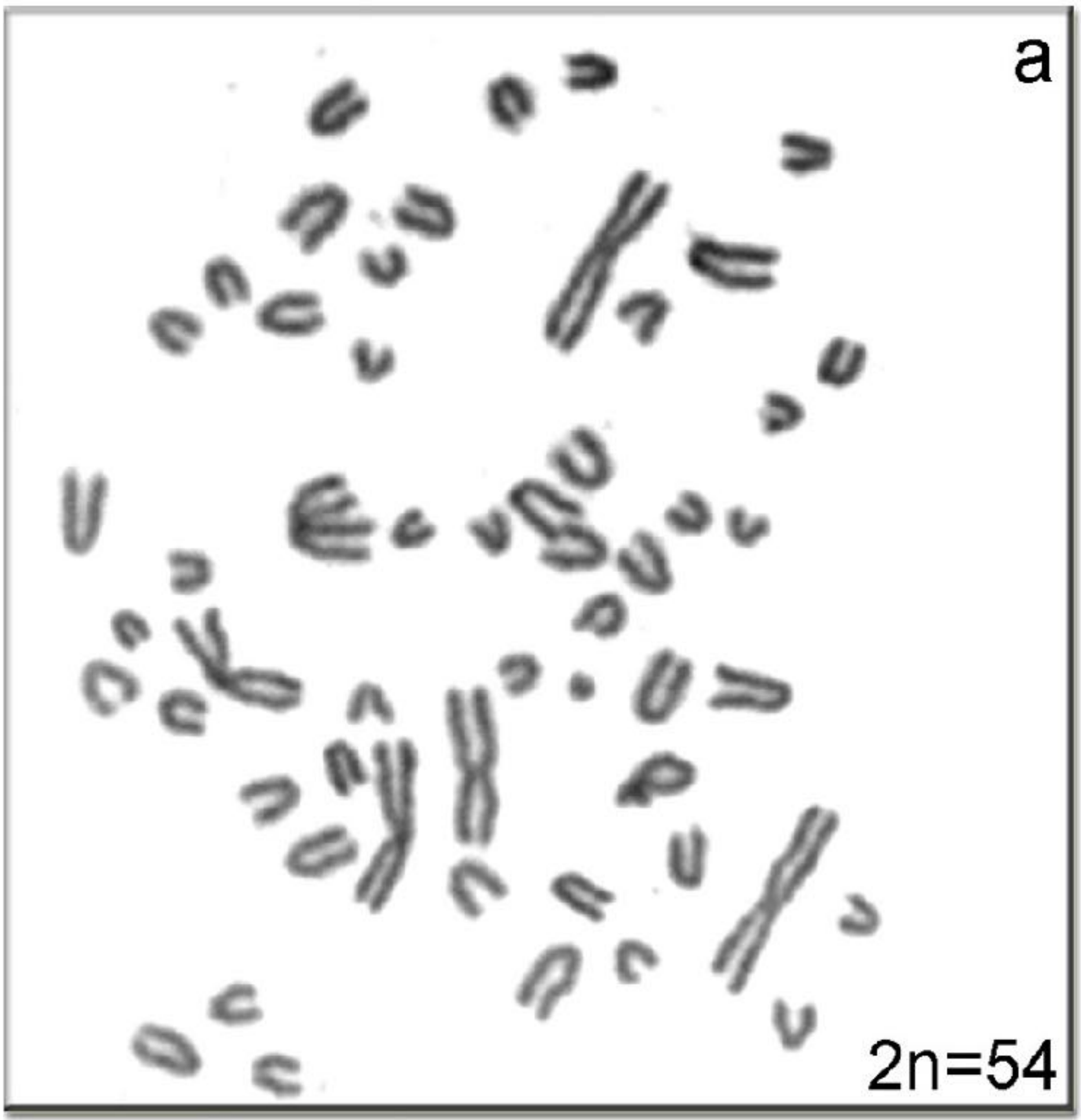
- **<u>Gently</u>** re-suspended the pellet in 2ml of <u>warm</u> (37°C) hypotonic solution.
  ➔ ***Be careful***, *it could lead to osmotic shock*! Leave the tube in the water bath, 37 'C, for 15 minutes. You will see the cells forming a pellet white and bigger.
- After 20 minutes, add 4 ml of new <u>warm</u> hypotonic solution pipetting (bring to 6 ml)
- Add 1ml of <u>cold</u> fixative.
- Mix the tube gently for inversion, two times.
- Centrifuge 1200 rpm, 5 minutes.
- Discard the supernatant and re-suspend gently in 1ml of <u>cold</u> fixative. Then, add 3 ml of fixative (bring to 4 ml) and leave at 4'C for 8-10 minutes.
- Take the tube from the 4°C and centrifuge, 1200 rpm, 5 minutes.
- Discard the supernatant and re-suspend again with 1ml of <u>cold</u> fixative. Then, add 3 ml of fixative (bring to 4 ml) and centrifuge 1200 rpm, 5 minutes.
- Discard the supernatant, re-suspend in 1 ml of <u>cold</u> fixative and transfer into an Eppendorf.

Video 1 - Hypotonic solution and Fixation

Video 2 - Slides preparation

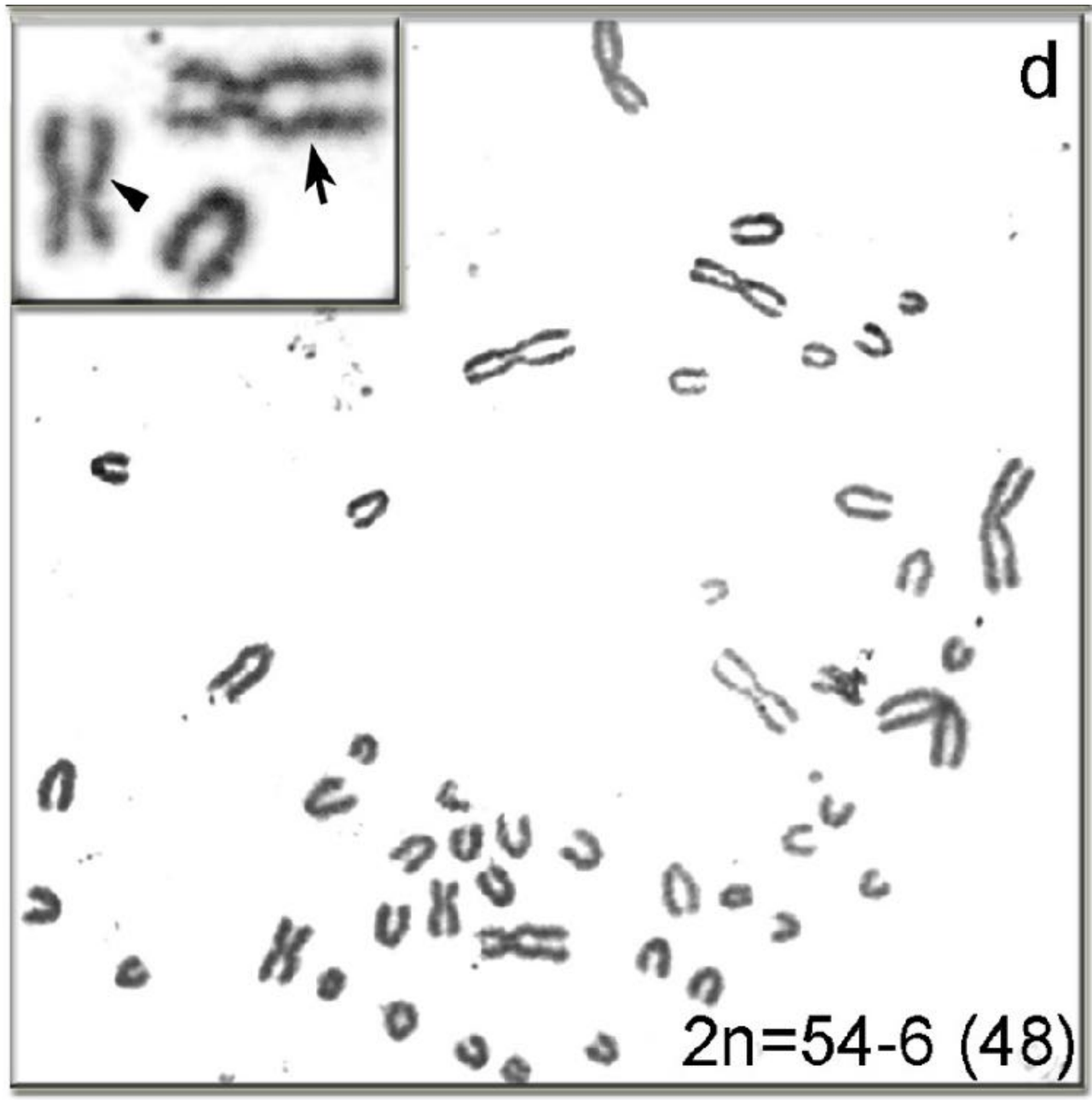
spread




a
2n=54


b
2n=54-2 (52)


c
2n=54-1 (53)


d
2n=54-6 (48)

How to analyse kariotype

https://www.youtube.com/watch?v=XuE_QUFC4ws

Karyotypes

With Ms. Lantz

https://www.youtube.com/watch?v=MP3mm04OrQg

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