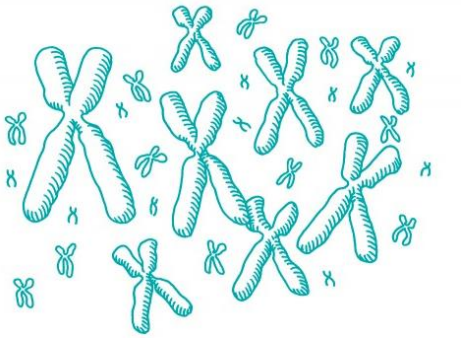
Chromosomal Analysis



Introduction: this technique allows the isolation of chromosomes from the nucleus.
It is useful to count the chromosomes per cell and, after proper staining of metaphases, it is possible also to evaluate the presence of some chromosomal mutation (i.e. duplication, translocation, deletion, insertion) to establish the presence of numerical or structural abnormalities.
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. This substance 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. After blocking the cycle, cells are "swollen" by the action of hypotonic solution (osmotic effect) and finally they are fixed. To observe the chromosomes, they need to be placed on a slide and stained with nuclear dye (i.e. Giemsa)

MATERIALS

- Cell culture in monolayer
- MEM + 10% FBS
- Trypsin
- 0.5 µg/ml demecolcine (to add to culture medium)
- 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

PROCEDURE

- Three hours prior to cell collection add 0.5 µg/ml *demecolcine* (*colcemid*) to culture medium;
- Trypsinize cells, collect and centrifuge 1200 rpm, 5min, to have cell pellet.
- Discard the supernatant.
- **Gently** re-suspended the pellet in 2ml of warm (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 warm hypotonic solution pipetting (bring to 6 ml)
- Add 1ml of cold fixative.
- Mix the tube gently for inversion, two times.
- Centrifuge 1200 rpm, 5 minutes.
- Discard the supernatant and re-suspend gently in 1ml of cold 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 cold 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 cold fixative and transfer into an Eppendorf.
- Store at -20'C.

PREPARE THE SLIDES

- Clean and degrease the slides with ethanol and write on them with a pencil.
- With a glass Pasteur pipette, take 50-100 µl of cells/fixative solution and spread 3-4 on slide from a distance of 10-15 cm. Try to cover the entire surface of the slide.
- Let it dry air.
- Immerse the slide into the Giemsa solution an leave for 10-12 minutes.
- Remove the excess dye by washing the slide under running water, for 30-40 seconds.
- Let it dry air.
- Mount the slide with cover glass and observe under inverted microscope

SETTING THE MICROSCOPE:

- Set the objective at 4X before starting to observe.
- Place the slide so that the border is exactly under the light (see the picture A)
- Move the micrometric roller in order to see a well delineate line of the border. The line appears lice unclear and blurry (see picture B).
- Move in both sense until you understand that you are on the right way 😊 (See picture C)
- When you find the focus (see picture C), move on the center of the slide and search for nuclei/metaphases: you can find both of course... see the last pictures.
- Enjoy!