CHROMOSOME SPREADS AND KARYOTYPING

Criterium for injection:

At least 15 metaphase spreads should be counted and at least 80% of the spreads must have the right karyotype (40 chromosomes).

The following units are sufficient for a 4-well multidish, but can be changed for larger areas. The optimal area is 1.8 cm^2 (4-well) with ± 50% confluent cells. It is essential to have the cells growing in the exponential state for getting enough metaphases.

- Incubate the cells for 1-1.5 hours in 0.5 ml medium with 0.05 µg/ml colcemid (stock solution is 10µg/ml, Gibco 152120-012).
- Pipet the medium off and retain in an eppendorf tube.
- Wash the cells with PBS and retain this in the same eppendorf tube.
- Trypsinize the cells and neutralize the cell suspension, put the whole suspension also in the same eppendorf tube.
- Centrifuge the tube 5 min at 2000 rpm in an eppendorf centrifuge.
- Discard the supernatant in the blue container (colcemid is poisonous); resuspend the pellet in a little medium that is left in the tube; add carefully 1 ml of 0.075 M KCl prewarmed at 37 °C while shaking constantly. Leave at 37 °C for 10 min.
- Add a few drops of methanol:acetic acid (use dried methanol : glacial acetic acid = 3:1, prepared freshly).
- Centrifuge 5 min at 2000 rpm.
- Discard the supernatant; resuspend the pellet and add carefully 1 ml of methanol:acetic acid while shaking constantly. Leave at RT for 20 min.
- Centrifuge 5 min at 2000 rpm.
- Repeat fixation procedure two times.
- After the last centrifuge step add ± 0.7 ml of methanol : acetic acid (volume is depending on the pellet size). Slides can be made and the solution can be stored at -20 °C.
- Clean microscope slides with ethanol : ether = 1:1, wipe them with a dust-free tissue and place them on a flat table.
- Let a few drops of the cell suspension fall on the slide from ± 30 cm hight. Let the slide dry.
- Let the slides dry again and cover them with 25µl DABCO/PI or DABCO/DAPI and a coverslip 25x24x50. These slides can be stored at 4 °C in the dark.
- ALT: spreads can be counted not stained under a phase contrast microscope.

DABCO solution :

2 g DABCO in 90 ml glycerol, 15-30 min at 60 °C
Add 10 ml Tris HCl 1M, pH=8.0
Let it cool down to RT
Add 100 µl 20% thimerosal
Add 50 µl propidiumiodide (1 mg/ml) or 7.5 µl Dapi (1 mg/ml)
Store at 4 °C in the dark