

Generation of Metaphase Spreads

Reagents required

- Colcemid
- Microscope slides soaked in ddH₂O at 4°C (pre-soak in 80% ETOH before spreading)
- Carnoy's Fix (3:1 methanol/acetic acid at 4°C)
- 75mM KCL

Harvesting of cells

- Treat cycling cells (at ~60-80% confluency) with 0.1µg/ml colcemid for 2h (depending on cell cycle: longer cycle = longer treatment.)
- Remove and collect growth medium and rinse cells with PBS .
- Trypsinize cells at 37°C.
- Re-add the collected growth medium to inactivate the trypsin and resuspend cells .
- Centrifuge cells at 1500RPM 5min.
- Aspirate the medium but leave a small amount of fluid and flick the tube to fully resuspend the cells

Addition of hypertonic solution

- Add 5ml of 75mM KCL drop wise to cells.
- Incubate at 37°C for 20min .
- Add 5 drops of Carnoy's to cells then centrifuge and aspirate supernatant.

Fixation

- Resuspend cells slowly in 2ml of Carnoy's.
- Incubate at 20°C for 1/2h.
- Spin down and resuspend twice more.

Slide preparation

- Spread cells drop-wise from a height onto slides.
- Stain with marker of choice.

