Observation of chromosome

Materials:

cell

Colcemid

PBS

0.02%EDTA/0.25%tripsin

0.075MKCl

Acetic acid

Methanol

Glass slide

Hair dryer

Hoechst 33258(0.05μg/ml)

Fluorescence microscope

MilliQ

Procedure:

1. Add colcemid into cells during the logarithmic growth phase at a concentarion of 0.1μg/ml, incubate it for 3 hours.
2. Transfer the supernatant of the medium into a centrifuge tube.
3. Add 2ml of PBS into the dish, wash the cell layer, and transfer into the centrifuge tube of procedure 2.
4. Add 1ml of 0.02%EDTA/0.25%tripsin into the dish.
5. Transfer the cell suspension into the centrifuge tube of procedure2.
6. Centrifuge it at 1000 rpm, for 5min.
7. Discard the supernatant.
8. Add 3ml of 0.075MKCl into the pellet, pipetting it , incubate it at 37℃ for 20 min.
9. Instill 6ml of fixative solution(acetate: MeOH=1:3) into the cell suspension slowly, mixing it slowly, centrifuge it at 1200 rpm for 5 min, discard the supernatant. Ditto the procedure twice.
10. Add 1ml of fixative solution, mixing it slowly, put a drop of the cell suspension on a glass slide to cover it.
11. Breathe on the glass slide to humidify it. Seal it two-handed.
12. Dry the cell suspension part of glass slide with a hair dryer(cool mode) holding left hand above the slide.
13. Add Hoechst 33258(0.05μg/ml) on the glass slide, place it for 30 min blocking out light.
14. Wash the glass slide with MilliQ, remove the Hoechst 33258(0.05μg/ml), mount it.
15. Observe it with fluorescence microscope.