CHROMOSOME PREPARATION FOR MITOTIC SPREADING

(ZM modified - May 2011)

Solutions and Materials

Growth Media	M15 (DMEM with 15% FBS, P/S)
Colcemid solution	10µg/ml Gibco 15210-040
Hypotonic solution	0.075 M KCl
Giemsa stain	Sigma GS-500 dilute 1:20 in dH_20
DAPI	Stock: 1mg/ml (-20°C) Working solution: 200µl/100ml PBS
Fixative	3:1 Methanol:Acetic Acid mix, make fresh daily (methanol must be from glass storage bottles)
Glass Slides	Pre-clean in ethanol overnight, wipe, clean in diH_2O and drain slightly
Cover Slips	24x60 mm #1 or 1.5
Permount solution	

Preparation

1. Plating cells
   - Fast-growing cells, $1x10^6$ cells/15cm dish ($0.3x10^6$/10cm dish) incubate for ~40h;
   - Slow-growing cells, 2-3 x 10^6 cells/15cm dish ($1x10^6$/10cm dish) and incubate for ~40h;

2. Add Colcemid with fresh, pre-warmed media in final concentration of 0.02µg/ml (2µl/ml media) and incubate at 37°C for 90 minutes. NOTE: the longer the culture incubates in Colcemid, the more mitotic figures there will be, but the chromosomes will get progressively more contracted with time and be unsuitable for banding.

3. Collect all media and PBS used for rinsing cells and spin down (1000 RPM, 7 min) in order not to loose dislodged, mitotic cells; discard most supernatant leaving behind cell pellet and ~2 ml liquid.

4. Harvest cells by trypsinization, collect with 3 ml media, add cells from step 3, count and spin down as before.

5. Aspirate supernatant, leaving a only small quantity such as 0.3 ml of supernatant, resuspend cells in that volume by gently pipetting bubbles into the media**.**

6. Add 10ml of 37° hypotonic solution and gently pipette up and down once, incubate cells for 15-18 minutes at 37°. The cells will swell and become very fragile.

7. Add few drops of fixative

8. Collect cells by centrifugation (800 RPMs, 8 min)

9. Aspirate the supernatant leaving a small volume, flick tube gently to resuspend the cells thoroughly to avoid the formation of large clumps of cells when fixative is added.

10. Add 1ml of fixative drop by drop while agitating do this carefully, cells clump easily.

11. Incubate on ice for 30-60 minutes.

12. Spin cells at 500 RPM for 5 minutes, aspirate supernatant, add 1ml of fixative.

13. Prepare first spreads to check cell density and to determine appropriate final volume of fixative

14. Spin cells at 500 RPM for 5 minutes, aspirate supernatant, add fixative. Repeat this step 2-3 times.

15. Prepare mitotic spreads by dropping the fixed swollen cells onto glass microscope slides. Use about 20 µl. The spreading of the fixative layer and subsequent evaporation causes the cell membranes to rupture, releasing the chromosomes. (Dropping height of ~10 cm or less works). Immediately run the slides through the flame of a burner (bottom side of slide towards the flame), then place the slide on a slide warmer (~55-60°C) where they can be left for 1h or longer.

16. Excess cell solution can be stored at -20° in fixative. Stored preparations should be resuspended in fresh fixative before dropping new slides.

17. At this point, slides may be examined under phase contrast to determine if the spreads are suitable for staining and counting. Slides can be stored at -80°C in slide box.

18. Slides can be stained either by DAPI and rinse with PBS or by diluted Giemsa for 10-15 minutes followed by rinsing in Gurr's Buffer for 5 minutes and in distilled water for a few seconds.

19. After air-drying, add several drops of Permount to the slide surface and add a cover slip. Allow this to dry at least one hour. Chromosomes can then be examined by 100x oil immersion lens. DAPI stained slides can be observed under the fluorescent microscope and chromosomes counted from the image.