

Culture and harvesting of human metaphase chromosomes

Lyndal Kearney

MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK.

Equipment and reagents

- ◆ Pre-cleaned microscope slides (Superfrost, BDH)
- ◆ 5–10 ml peripheral blood in sodium heparin (20 U/ml) or preservative-free lithium heparin (10 U/ml)
- ◆ 15 mg/ml thymidine (Sigma, crystalline)
- ◆ 10 µg/ml colcemid (Gibco)
- ◆ Lymphocyte culture medium: RPMI 1640, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine, 20% fetal calf serum (FCS), 2% PHA (M form) (all from Gibco BRL)
- ◆ Hypotonic solution: 0.075 M KCl
- ◆ Fixative: 3:1 AnalaR methanol:glacial acetic acid, at 4 °C

Method

- 1 Add 0.2 ml whole blood to 5 ml of lymphocyte culture medium.^a
- 2 Incubate for 48 h or 72 h at 37 °C.^b
- 3 Add 0.1 ml thymidine (final concentration of 300 µg/ml) and incubate for a further 16–18 h.
- 4 Centrifuge at 200 *g* for 5 min. Wash twice in RPMI (no FCS) and resuspend in fresh complete medium (no PHA).
- 5 Culture for a further 5 h prior to harvesting.
- 6 10 min before harvesting add 0.1 ml colcemid (final concentration 0.2 µg/ml).
- 7 Centrifuge at 200 *g* for 5 min. Discard the supernatant and resuspend the pellet in hypotonic solution (pre-warmed to 37 °C). Incubate at 37 °C for 20 min.
- 8 Centrifuge, then discard the supernatant, and mix the pellet in the small volume of hypotonic solution remaining. Add freshly made fixative dropwise, with mixing. Add the first 1 ml of fixative slowly, then make up to 10 ml.
- 9 Leave in fixative for 30 min at 4 °C. Centrifuge at 200 *g* for 5 min, then wash in three to five changes of fixative before making slides.
- 10 Wipe Superfrost slides clean with absolute ethanol just before use.

- 11** Place a drop of cell suspension on each slide and air dry. Monitor the quality of chromosome spreading under phase contrast. Chromosomes should be well spread without visible cytoplasm and should appear dark grey under phase contrast (not black and refractile or light grey and almost invisible).

Notes

- a For infants under 1 year of age, use 0.1 ml whole blood per 5 ml culture.
- b For lymphoblastoid cell lines, grow until a healthy dividing population is obtained, with $0.5-1 \times 10^6$ cells/ml (24–48 h after a medium change). Omit the thymidine synchronization steps and follow this protocol from Step 6, with a colcemid exposure time of 50–60 minutes.