

Mitotic chromosomes from mouse peripheral blood

Ellen C. Akesson

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.

Muriel T. Davisson

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.

Equipment and reagents

- ◆ RPMI 1640 medium containing glutamine and Hepes (Gibco-BRL); supplement each 100 ml of medium with 1 ml of gentamicin solution (10 mg/ml, Sigma)
- ◆ PHA (Murex); for optimum results construct a dose-response curve with each new lot of PHA to assay the mitotic index and strain response. We have found that 7.5 µg/ml of purified PHA with mitogenic units in the same range gives the best results regardless of the mitogenic units marked on the vial or the strain used.
- ◆ 750 µg/ml LPS (Sigma, from *Escherichia coli* serotype 0111:B4)
- ◆ 500 USP units/ml of sterile sodium heparin (Sigma), diluted in RPMI 1640 medium)
- ◆ fetal calf serum (FCS; Sigma)
- ◆ 50 µg/ml colchicine (Sigma) in saline
- ◆ 0.56% (0.075 M) KCl
- ◆ 70% ethanol
- ◆ Carnoy's fixative (3:1 methanol:glacial acetic acid)
- ◆ Pressurized air (e.g. Dust Off Plus, Polysciences, or from most photographic suppliers)
- ◆ Cleaned microscope slides
- ◆ Razor blade
- ◆ Haematocrit tubes
- ◆ Snap-cap tubes
- ◆ Conical glass centrifuge tubes
- ◆ Bench-top centrifuge
- ◆ 16 &mul; 125 mm Corning culture tubes

A. Culture procedure

- 1 Use aseptic technique throughout until the harvest procedure (part C).
- 2 For each mouse, set up one 16 &mul; 125 mm Corning culture tube containing:
 - 0.95 ml of RPMI 1640 medium with gentamicin
 - 0.1 ml of PHA (concentration as determined by dose-response curve, see Section 3)

- 0.1 ml of 750 µg/ml LPS
 - 0.15 ml of FCS
- 3 Refrigerate the tubes until they are needed.
 - 4 Obtain two tubes of blood per mouse from the retro-orbital sinus using heparinized 70 µl micro-haematocrit capillary tubes. Those unskilled in this procedure may obtain blood from the tail vein (Part B). In the UK, it is easier to obtain a Home Office licence for the tail-vein method.
 - 5 Inoculate the two 70 µl haematocrit tubes of blood immediately into a 12 × 75 mm Fisher snap-cap tube containing 0.1 ml of heparin.
 - 6 Cap the tubes tightly and swirl gently to mix the blood and heparin. Blood should be held at room temperature (for not more than 2 h) until inoculation into the culture medium.
 - 7 Add 0.2 ml of blood and heparin mixture to each prepared culture tube. Cap the tubes tightly and swirl gently to mix the cells and medium.
 - 8 Incubate the culture tubes at an angle of ~45 ° angle in a shaking water bath at 37 °C. Set the shaking speed so that the basket moves back and forth about 32–35 times/minute. If an incubator is used to incubate the cultures, then tilt the rack of tubes at an angle by resting it on two 1/2 to 5/8 inch high Petri dishes to allow maximum surface area exposure. Hand-shake the tubes three times daily to resuspend the cells.

B. Collecting blood by the tail-vein method

1. Pre-warm the mouse in a large jar placed beneath a desk lamp for 1–2 min. The mouse is warm enough when it rubs its nose or shows excessive activity.
Caution: overheated mice can go into shock and die.
2. Place the mouse in a restraint so that the tail is free. Wash the tail with 70% ethanol and wipe it twice with a clean tissue.
3. Gently cut across the vein on the side of the tail about one inch from the base of the tail with a razor blade wiped with 70% ethanol.
4. Collect at least 0.15 ml of blood (five drops), letting it run down the side of the tube containing 0.1 ml of heparin. Be careful not to touch the tail to the mouth of the tube.
5. Follow Part A, steps 6–8.

C. Harvest procedure

1. At 41–43 h add 0.15 ml of colchicine solution (50 µg/ml) to each culture tube and incubate the tubes at 37 °C for a further 15–20 min.

2. Transfer each culture to a 5 ml conical glass centrifuge tube and centrifuge at 400*g* for 10 min at room temperature in a clinical bench-top centrifuge.
3. Remove the supernatant and gently add 2–3 ml of warm (37 °C) 0.075 M (0.56%) KCl. Gently suspend the cells by pipetting with a Pasteur pipette.
4. Incubate the tubes at 37 °C for 15 min.
5. Centrifuge at 500*g* for 10 min.
6. Remove the supernatant without disturbing the pellet, being careful not to remove the buffy coat (white layer) on top of the pellet. Gently add 3–4 ml of Carnoy's fixative down the side of the tube. Pipette gently but rapidly to prevent the cells from clumping.
7. Stopper the tubes and allow them to sit for at least 30 min at room temperature. (Refrigerate if tubes are to be held for >30 min.) The procedure can be interrupted at this point for 1–2 h.
8. After 30 min, centrifuge the cells at 400*g*, remove the fixative and resuspend the cells in fresh fixative.
9. Repeat step 8 twice more.
10. Centrifuge the cells at 400*g* and resuspend the cells in about 0.3–0.5 ml of fresh fixative. Slides are prepared from this cell suspension by placing the entire sample on one slide. If slides are not made immediately, save this last wash until just before making slides. Slides can be made the next day but wash the cells at least twice before leaving overnight.

D. Slide making

1. Immerse pre-cleaned slides in fixative at least 15 min prior to use. Wipe slides dry with a lint-free tissue.
2. Drop small drops of cell suspension onto the slide surface and allow it to spread. If too much sample has been used, it will bubble at the edges.
3. As soon as the drop begins to contract and Newton's rings (rainbow colours around the edge of the drop) are visible, blow on the slide surface to accelerate drying. Slides may also be dried by using tubing connected to an air supply or by lightly spraying with pressurized air. Rapid drying is critical to obtaining well-spread metaphases.
4. The 'bomb' method of dropping one drop of fixative onto the cell suspension once it has started to dry may be used to increase the spreading of the chromosomes. Use a very small drop of fixative and keep the slide in a flat position while adding the fixative.
5. Repeat steps 2 and 3 until all of the sample is on the slide. Monitor cell concentration by phase microscopy.