G-banding for analysing mouse chromosomal rearrangements

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Equipment and reagents

- 2 × SSC (8.8 g NaCl, 4.4 g trisodium citrate, in 500 ml distilled water)
- 0.9% NaCl
- Gurr buffer (Gurr pH 6.8 phosphate buffer plus an equal volume of distilled water)
- Coplin staining jars
- Trypsin-Giemsa solution (1.0 ml Gurr's Improved Giemsa R66 (Bio/medical Specialities), 45 ml Gurr pH 6.8 phosphate buffer (Bio/medical Specialities), four drops of 0.0125% trypsin (Sigma)

Method

1. Make air-dried mitotic preparations by dropping small droplets of cell suspension onto slides and blowing dry (Mitotic chromosomes from mouse peripheral blood). Bands are sharper if slides are aged 7–10 days at room temperature, but this is not essential.
2. Incubate the slides in Coplin jars (five or six per jar) in 2 × SSC at 60–65 °C for 1.5 h.
3. Transfer all slides to 0.9% NaCl at room temperature, then rinse each slide in fresh NaCl and drain. Thorough rinsing is critical.
4. Stain slides for 5–7 min in the trypsin-Giemsa solution. Remove the metallic film which forms on the stain surface with a cotton ball before placing the slides in the Coplin jar or float the film off with running water before removing the slides.
5. Transfer all the slides in the jar to fresh phosphate buffer.
6. Rinse slides individually in two changes of buffer. Thorough rinsing is critical. Shake off excess liquid and blow dry with an air jet (see Mitotic chromosomes from mouse peripheral blood, step D3).