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# 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% NaCI
- 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

- Make air-dried mitotic preparations by dropping small droplets of cell suspension onto slides and blowing dry (<u>Mitotic chromosomes from mouse peripheral blood</u>). 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 <u>Mitotic chromosomes</u> <u>from mouse peripheral blood</u>, step D3).

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