G-banding using Wright's stain

Equipment and reagents

- Coplin jars
- Staining slide rack
- Slide warmer
- Wright's stain (Sigma). To make a stain stock solution (2.5 mg/ ml methanol) swirl 100 ml absolute methanol in a flask. Add 250 mg of powdered stain to swirling methanol. Continue to swirl 30 min at moderate rate. Filter through Whatman #1 filter paper and aliquot into aluminum foil covered 10 ml bottles. Store away from heat and light.
- 2XSSC
- Sorensen's buffer pH 7.2. Prepare the buffer by mixing 49 ml of 0.06 M Na_2HPO_4 (8.52 g/liter) with 51 ml of KH_2PO_4 0.06 M (8.16 g/liter)
- Mounting medium (Molecular Probes)

Method

1. Bake the chromosome preparations in an oven or slide warmer at 60 °C overnight.
2. Place the slides in a coplin jar and fill it with 2XSSC. Incubate the slides at 60 °C in a water bath for 2-3 h. Discard the saline solution and rinse thoroughly at least 10 times with tap water.
3. Let the slides air dry at room temperature for 12-16 h.
4. Place the slide on a staining rack and cover the preparation with Sorensen's buffer for 45-50 sec[a].
5. Discard the phosphate solution and cover the slide with Wright's stain solution for 60-90 sec (0.5 ml Wright's stain in 1.5 ml buffer)[b].
6. Rinse slides gently with tap water[c], let air dry and mount if desired.
7. Observe under transmitted light microscope at 1000X magnification.

[a]Staining time may need to be optimized. Always stain one slide first and check banding quality before staining additional slides.

[b]Adjust staining time if necessary, but it should be always less than 2 min. Otherwise it is recommended to prepare a new stain stock solution. Wright's stain will form a precipitate when added to buffer. Therefore, the two should not be mixed until just prior to flooding the slide.

[c]Be careful not to over-rinse slides since excessive rinsing will fade stain.