

## Treatment of slides prior to FISH

### Lyndal Kearney

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

#### Equipment and reagents

- ◆ Slide containing metaphase chromosomes
- ◆ PBS/50 mM MgCl<sub>2</sub>: 50 ml 1 M MgCl<sub>2</sub> plus 950 ml phosphate-buffered saline (PBS)
- ◆ PBS/50 mM MgCl<sub>2</sub>/1% formaldehyde (make up fresh each time): 2.7 ml formaldehyde in 100 ml PBS/MgCl<sub>2</sub>
- ◆ PBS: 8 g NaCl, 0.2 g KCl, 1.44 g Na<sub>2</sub>HPO<sub>4</sub>, 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml H<sub>2</sub>O, pH to 7.4 with HCl; add H<sub>2</sub>O to 1 litre
- ◆ RNase A (10 mg/ml) (Sigma): boiled for 10 min to remove contaminating DNase
- ◆ 40% formaldehyde (w/v)

#### Method

- 1 Place 100 µl RNase (100 µg/ml) on slides under a 24 x 50 mm cover-slip and incubate at 37 °C for 30 min-1 h.
- 2 Wash twice (3 min each) in 2 x SSC (with agitation).
- 3 Place slides in PBS/50 mM MgCl<sub>2</sub> for 5 min.
- 4 Fix in PBS/50 mM MgCl<sub>2</sub>/1% formaldehyde for 10 min.
- 5 Wash in PBS for 5 min (with agitation).
- 6 Dehydrate slides through an alcohol series (70%, 95%, 100%) and allow to air dry. Slides can be stored desiccated at 4 °C for up to one month before use.