FISH hybridization and subsequent washes

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Reagents

- Human *Cot-1* DNA (BRL Life Technologies)
- 3 M sodium acetate
- Denaturing solution: 70% (v/v) formamide, 2 x SSC, 0.1 mM EDTA pH 7
- Hybridization buffer: 50% (v/v) formamide, 10% (w/v) dextran sulfate, 1% (v/v) Triton X-100, 2 x SSC pH 7
- Formamide (Fluka)
- 50% dextran sulfate
- 20 x SSC: 3 M sodium chloride, 0.3 M sodium citrate pH 7
- Blocking solution: 3% (w/v) BSA in 4 x SSC, 0.05% (v/v) Triton X-100 (make up fresh)
- Wash solution: 4 x SSC, 0.05% (v/v) Triton X-100

Method

1 Dry down the appropriate concentration of probe and competitor[a,b] either in a vacuum desiccator (SpeedVac) or by ethanol precipitation; e.g. for cosmids:

- 100 ng labelled probe (usually 2 μl)
- 2.5 μg (2.5 μl) *Cot-1* DNA
- 0.1 vol. 3 M sodium acetate
- 2 vol. ice-cold ethanol

Allow to precipitate for 1–2 h at –70 °C.

2 Centrifuge and dry down the pellet as for labelled probes. Resuspend pellet in 11 μl hybridization buffer (warmed to room temperature).

3 Denature the probe mixture at 95 °C in a hot block for 10 min. Plunge the tubes on ice for a few minutes, then centrifuge briefly in a microcentrifuge.

4 Place the probe mixture at 37 °C for 15 min-2 h.

5 Just prior to hybridization, denature the chromosomal DNA as follows:

(a) Incubate slides in denaturing solution (in water-bath in a fume-hood) at 70 °C for 5 min.

(b) Wash slides in cold 2 x SSC, followed by two changes of 2 x SSC.

(c) Dehydrate through a cold alcohol series (70%, 90%, 100%).

6 Air dry the slides and place on a hot plate at approx. 42 °C.

7 Centrifuge the probe mixture quickly to get the liquid to bottom of tube. Place this mixture on the previously treated slide containing chromosomes and cover with 22 x 32 mm coverslip (do not let drop dry). Seal the coverslip with rubber solution and place the slides in a moist chamber at 37 °C for overnight-four days.

8 Remove rubber solution. Coverslips can then be removed either by soaking in 2 x SSC or gently tipping them off into the glass disposal bin (never pull them off!).

9 Carry out the following washes (in a water bath in fume hood):[c]

(d) Three washes (5 min each) in 50% formamide, 2 x SSC at 42 °C (with agitation).

(e) Three washes (5 min each) in 2 x SSC at 42 °C.

10 Wash slides in wash solution for 3 min.

11 Incubate slides in blocking solution for 10–20 min (room temperature).

12 Wash in wash solution for 3 min before carrying out the appropriate detection steps.

Notes

a The following is a guide: For cosmids, use 100 ng probe, 2.5 μg *Cot-1;* plasmids, 100-200 ng probe, no *Cot-1*; phage, 200 ng probe, 2.5 μg *Cot-1*; YACs (total yeast DNA), 400-1 μg probe, 5-7.5 μg *Cot-1*; PAC, P1, 200-400 ng probe, 3-5 μg *Cot-1*; repetitive centromere, 10 ng probe, no *Cot*-1; whole chromosome painting probes (PCR-derived), 100 ng probe, 6.25 μg *Cot-1*.

b With alphoid repetitive probes and unique small insert clones (< 1–3 kb), the whole probe contributes to the hybridization signal. The appropriate amount of labelled probe is dried and resuspended in 10 μl hybridization buffer. Denature the probe mixture at 75 °C for 5 min, then place on ice for 5 min. Follow step 5 as usual.

c To avoid the use of formamide, we have found that the following alternative washes give the same stringency: (i) three washes (3 min each) in 2 x SSC at room temperature (with agitation), (ii) two washes (20 min each) in 0.1 x SSC at 65 °C, and (iii) one 5 min wash in 0.1 x SSC at room temperature (with agitation).