Detection (CGH)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Antibodies:

Avidin-FITC
Vector, Cat. A-2011
Biotinylated anti-avidin
Vector, Cat. BA0300
Goat anti rabbit TRITC
Sigma, Cat. T-5268
Mouse anti-DIG
Sigma, Cat. D-8156
Rabbit anti mouse TRITC
Sigma, Cat. T-2402

Antifade (1,4-phenylene-diamine)
Bovine Serum Albumin (BSA)
DAPI
Formamide
HCl, 1 M
20X SSC
Tween 20
Water, sterile

Preparation

FA/SSC

20X SSC	30 ml
dH_2O	120 ml
Formamide	150 ml

Adjust pH to 7-7.5 with 1M HCl
Prewarm to 45°C

4X SSC/Tween 20

20X SSC	100 ml
dH_2O	400 ml
Tween 20	0.5 ml

Prewarm to 45°C

0.1X SSC

20X SSC	2.5 ml
Add H_2O to	500 ml

Prewarm to 60˚C

Note: For the above wash solutions you need 70 ml for glass coplin jars and 50 ml for plastic jars per step**.**

Blocking Solution (3% BSA)

Bovine serum albumin (powder)	0.3 g
Pre-warmed (37°C) 4X SSC/Tween 20	10 ml

Keep at 37˚C to dissolve

DAPI (80 ng/ml DAPI in 2X SCC)

Stock solution:		final conc.
DAPI	2 mg	0.2 mg/ml
Sterile water	10 ml	
Working solution:		
Stock solution	40 µl	80 ng/ml
2X SSC	100 ml	

Antibodies

Layer 1 avidin-FITC (1:200) + mouse-anti-DIG (1:200)
Layer 2 biotinylated anti-avidin (1:200) + rabbit anti mouse TRITC (1:200)
Layer 3 avidin-FITC (1:200) + goat anti rabbit TRITC (1:200)

NOTE: MAKE SURE SOLUTIONS ARE AT THE CORRECT TEMPERATURES BEFORE USING: CHECK WITH A THERMOMETER.

Procedure

1. Remove rubber cement and coverslips from hybridized slides.

2. First dip the slides into FA/SCC to remove coverslips; wash slides in FA/SSC (use coplin jars), 3 x 5 min, shaking;.

3. Wash slides in 0.1X SSC, 3 x 5 min, shaking.

4. Dip slides in 4X SSC/Tween 20; do not let them dry.

5. Add 120 µl of blocking solution to 24 x 60 mm coverslips, touch slides to coverslips, and incubate in hybridization chamber at 37°C for about 30 min.

6. Dip slides in 4X SSC/Tween 20; do not let them dry.

   Note: Spin all fluorescent dyes for 3 min at 14,000 rpm before use.

7. Antibodies: Add 100 µl of prepared antibody solution (antibodies should be diluted in 1% BSA) to coverslip (use 24 X 60 mm), touch slide to coverslip, and incubate in hybridization chamber for 45-60 min at 37°C.

8. Wash slides in 4X SSC/Tween 20, 3 x 5 min, shaking.

9. Apply second and third antibody layers by repeating steps 7 and 8.

10. DAPI staining: stain for 2-5 min in foil-covered coplin jar.

11. Wash in 2X SSC for 2 x 5 min, shaking.

12. Shake off excess buffer and apply 35 µl antifade, cover with 24 X 60 mm coverslip, and store in the dark at 4˚C.