DNA Precipitation and Hybridization (CGH)

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

Reagents

Dextran sulfate (50%)
Intergen, Cat. S4030
Ethanol, absolute
Formamide, deionized
Mouse Cot-1™ DNA, Cat. 18440-016, 500 mg
Human Cot-1™ DNA, 1 mg/ml
Invitrogen Corp., Cat. 15279-011, 500 µg
Salmon testes DNA, 9.7 mg/ml
SIGMA Molecular Biology, Cat. D-7657, 1 ml
SSC, 20X
Sodium acetate (Na-Acetate), 3M
Water, sterile

Preparation

Master Mix

Dextran sulfate, 50%	40 ml
20X SSC	20 ml
Sterile H_2O	40 ml
Total	**100 ml**

Vortex solution and place tube on a shaking platform overnight to insure proper mixing.
Aliquot, and store at -20°C

70% Formamide/2X SSC

Deionized formamide	70 µl
20X SSC	10 µl
Sterile water	20 µl
Total	**100 µl**

Adjust to pH 7.0

Procedure

1. Add to an eppendorf tube:
   10-25 µl nick-translated test or tumor probe DNA (500-1000 ng DNA)
   Equal amount of nick-translated control whole genomic DNA as probe DNA
   (Note: can use 1-2 µg DNA if tumor DNA is isolated from paraffin material)
   30-60 µl human Cot-1 DNA (1 mg/ml)
   1 µl salmon sperm DNA (10 mg/ml)

   Note: Usually the test DNA is nick translated with Biotin-16-dUTP and the control DNA is nick translated with Digoxigenin-11-dUTP.

2. Add 1/10 volume Na-Acetate (3M).

3. Add 2.5-3.0 x total volume of absolute ethanol.

4. Vortex, store at -20°C overnight, or at -80°C for at least 15-30 min.

5. Centrifuge (14000 rpm) precipitated DNA at 4°C for 30 min.

6. Pour off supernatant and speed vac for 5-10 min to dry pellet.

7. Add 5 µl pre-warmed deionized formamide (pH 7.5), incubate at 37°C for 30 min, shaking; vortex a few times during the 30 min incubation.

8. Add 5 µl pre-warmed Master Mix, vortex, and centrifuge briefly. Incubate at 37°C 15-30 min.

9. Denature probe DNA at 80°C for 5 min and centrifuge briefly. Probe can be kept at 37°C until ready to denature.

10. Preanneal at 37°C for 1-2 hr.

11. For slide denaturation apply 120 µl 70% formamide/2X SSC to a 24 x 60 mm coverslip and place inverted slide onto coverslip.

12. Incubate slides at 75°C for 1.5 min on a slide warmer.

13. Remove coverslip and immediately place slide in ice cold 70% ethanol for 3 min, followed by 90% ethanol and 100% ethanol for 3 min each; air dry.

14. Add pre-annealed probe DNA to denaturated slides and cover with 18 mm^2 or 22 mm^2 coverslips; seal coverslips with rubber cement.

15. Hybridize at 37°C in a humidified chamber for 72 hr.