DNA Precipitation and Hybridization (FISH)

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National Institutes of Health

Reagents

Dextran Sulfate (50%)
Intergen, Cat. S4030
Ethyl Alcohol, absolute (ethanol)
Formamide, deionized
Ambion, Cat. 9342
HCl, 1N
Human Cot-1 ^{TM} DNA (1mg/ml)
Invitrogen Corp., Cat. 15279-011, 500 µg
Rubber Cement
Salmon Testes DNA (stock ~10 mg/ml)
SIGMA Molecular Biology, Cat. D-7657, 1ml
Sodium acetate, **3M (NaAcetate)**
20X SSC

Preparation

Master mix		final conc.
Dextran sulfate, 50%	40 ml	20%
20X SSC	20 ml	4X SSC
Sterile dH_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		final conc.
20X SSC	10 ml	2X SSC
dH_20	20 ml	
Deionized formamide	70 ml	70%
Total	**100 ml**	

Adjust to pH 7 with 1N HCL
Aliquot and store at –20˚C.

Procedure: Precipitation

1. Add to an eppendorf tube:
   Probe DNA (200 ng -1μg DNA)
   Human Cot-1 DNA (note 1) 10 μl
   Salmon sperm DNA 1 μl

2. Add Na-Acetate, 1/10 of the total volume of probe DNA mixture (above).

3. Add 100% ethanol, i.e., 2.5 x total volume of mixture of DNA + NaAcetate.

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

5. Centrifuge (14,000 rpm) to pellet DNA at 4°C for 30 min.

6. Carefully pour off supernatant. Place in a speed vac for 10 min (medium heat) until pellet is dry.

7. Add 500 μl 70% ethanol and spin at 14,000 rpm to wash off the salts.

8. Pipet off all ethanol or invert on Kimwipe to remove all ethanol.

9. Add 5 μl deionized pre-warmed (37°C) formamide (pH 7.0) and incubate tube at 37°C in a thermomixer, shaking, for 30 min; vortex several times during the incubation.

10. Add 5 μl pre-warmed (37°C) Master Mix, and incubate at 37°C in a thermo-mixer 15-30 min, the longer the better.

11. Denature probe DNA at 80°C for 5 min, centrifuge briefly.

12. Pre-anneal probe at 37°C for 1 hr.

Procedure: Slide Denaturation and Hybridization

1. Apply 120 μl 70% deionized formamide/2X SSC to a 24 mm x 60 mm coverslip. Touch the slide to the coverslip.

2. Denature slide at 80°C on a hot plate for 1.5 min (see note 2).

3. Quickly and carefully remove the coverslip and immediately place the slide in ice cold 70% ethanol, followed by 90% ethanol and 100% ethanol (for 3 min each).

4. Allow slides to air dry.

5 Add pre-annealed probe DNA to the denatured slide and cover with an 18 mm^2 coverslip, and completely seal the edges of the coverslip with rubber cement.

6 Hybridize at 37°C in hybridization chamber (use a light tight chamber if the probe is directly labeled) for 48 hr.

Notes

1. cDNA probes do not require Cot-1 DNA.

2. The denaturation time depends on the age of the slide. For slides older then 30 days a denaturation time of 2 min is recommended.