Hybridization (SKY)

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Reagents

dH_2O
Ethanol, absolute (70%, 90%, and 100%)
Formamide, deionized
Ambion, Cat. No. 9342
HCl, 1N
Rubber Cement
SSC, 20X

Preparation

70% Formamide/2X SSC

20X SSC	10 ml
dH_2O	20 ml
deionized formamide	70 ml

Adjust to pH 7.25 with 1N HCL
Aliquot and store at –20°C.

Pre-cool 70% ethanol to 0°C

Procedure

1. If SKY-Kit was stored at -20°C, prewarm at 37°C for 5-10 min shaking; vortex, spin briefly. If stored at 4-5°C, pre-warm 5 min; vortex, spin briefly.

2. Denature SKY-Kit at 80°C for 5 min in a thermomixer or waterbath.

3. Preanneal at 37°C for 1 hr.

4. For slide denaturation apply 120 µl of 70% deionized formamide/2X SSC to a 24 mm x 60 mm coverslip. Touch the slide to the coverslip (see note 1).

5. Denature slide at 80°C on a hot plate for 1 min, 30 sec (see note 2).

6. Immediately let coverslip slide off and place slide in 70% ethanol (0°C) for 3 min, followed by 90% ethanol (RT) and 100% ethanol (RT) for 3 min each.

7. Let slide air dry.

8. After pre-annealing add SKY-Kit (10 µl) to the denatured slide and cover with 18 mm^2 coverslip.

9. Seal coverslip with rubber cement, being sure that all edges are covered (see note 3).

10. Hybridize at 37°C in a humidified hybridization chamber for 48 hr (see note 4).

Notes

1. The slide should be pretreated prior to the denaturation step. See pretreatment protocol.

2. The denaturation time and temperature depends on the age of the slide, the species, and cell type. For example, you may need to reduce time and temperature for mouse chromosome preparations.

3. After applying the SKY-Kit and before sealing the coverslip with rubber cement all air bubbles should be removed by gently applying pressure to the coverslip (e.g. with forceps). In order to prevent the probe from drying out during the 48 hr hybridization time, it is important that the coverslip is completely sealed with rubber cement, and that the chamber is sufficiently moist but not over-saturated.

4. Hybridize mouse chromosome preparations for 48-96 hr.