Human Chromosome Paint Probe

Needed But Not Provided

- **Tween 20**
- **Distilled water**
- **Formamide**
- **20× SSC**
- **80%, 100% ethanol (stored at room temperature)**
  Prepare by adding dH_2O to 800ml pure ethanol to a final volume of 1L.
- **70% Ethanol stored in -20°C**

Reagents preparation:

Day 1

Denaturation solution (70% formamide /2SSC)
Add 35ml formamide, 10ml distilled H2O, 5ml 20xSSC Adjust pH to 7.0 using HCL, heat to 72°C.

Day 2

Rapid wash (0.4 X SSC solutions)
Add 1ml 20X SSC, 49ml distilled water. Mix well and heat to 74°C.

Washing solution II (4 X SSC/0.1%Tween 20)
Add 100ml 20X SSC, 400ml distilled water, 0.5ml Tween 20.

Protocol

Day 1

A) Chromosome denaturation

1. Put the slides in 2XSSC at RT for 2 min and then dehydrate in Ethanol series: 70%, 80% and 100%, 2 min. each. Air dry.
2. Heat 40ml of denaturation solution to 70°C (±2°C) in a glass Coplin jar. Place slides in the solution for 1.5 minutes. DO NOT OVER DENATURE, some samples denature in 60 seconds. Hot plate can also be used for denaturation: put 100ul of the denaturation solution on the slide, cover with a cover glass and put on a slide warmer at 72°C (±2°C) for 1.5 minutes.
3. Immediately place slides in Cold 70%, and in 80% and 100% ethanol, 2 min. each. Air dry.

B) Probe denaturation and hybridization

1. Centrifuge briefly the content of the probe mixture, take 10ul for each slide and denature the probe by incubation at 80°C in a water bath for 7 minutes.
2. Put in a water bath at 37°C for 10 minutes.
3. Add 10ul from the denature probe mixture to the denaturized chromosome preparation.

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SUITE 115, 17 Ramsey Road, Shirley, NY 11967, USA
Tel: 1-631-626-9181 Fax: 1-631-614-7828
E-mail: info@creative-bioarray.com
www.creative-bioarray.com

4. Place an 18 x 18mm^2 cover slip over the probe mix, being careful not to trap air bubbles under the cover slip. Seal the edges with rubber cement. Transfer the slide to a humidified chamber or container and place in incubator or baking oven set at 37 °C for 12-16 hours.
   Alternatively: **Co-denaturation can be used: apply 10 ul from the probe, put a cover glass (18X18mm) and seal with rubber cement. Denature sample and probe together on a hot plate at 74 °C for 4 minutes. Place in an incubator or baking oven set at 37 °C for 12-16 hours.**

Day 2

Detection

Note: During the whole procedure the slides should remain wet and protected from direct light.

1. Remove slides from the humidified chamber and carefully remove the rubber cement.
2. Transfer the slides to a Coplin jar containing 0.4XSSC. Wash slides in 0.4XSSC at 74 °C (± 2°C) for 3-5 min. Dip slides in washing solution II (4XSSC/ 0.1%. Tween 20) for 2 minutes.
3. Put 20ul of antifade solution with DAPI place a cover glass (24X60mm^2) over the surface. Try to remove any air bubbles that may have formed.

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