SINGLE PAINT HYBRIDIZATION & DETECTION PROTOCOL

For Research Use Only - Not for use in Clinical Diagnosis

Reagents Required/ Not Supplied:

- 20XSSC
- Distilled water
- Formamide
- 70% Ethanol stored in -20°C
- 80%, 100% Ethanol stored at room temperature
- Tween 20

Reagents preparation:

DAY 1

Ethanol series	Prepare 70%, 80% and 100% ethanol and place the 70% at -20°C and the 80% and 100% at room temp.
Denaturation solution (70% formamide /2SSC)	Add 35 ml formamide, 10 ml 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 1 ml 20X SSC 49 ml distilled water **Total: 50 ml** Mix well and heat to 74°C.
Washing solution II (4 X SSC/0.1%Tween 20)	Add 100 ml 20X SSC 400 ml distilled water 0.5ml Tween 20 **Total: 500 ml**

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 100µl 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 10µl 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 10µl from the denature probe mixture to the denaturized chromosome preparation.
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 10µl 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

D) 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 20µl of antifade solution with DAPI place a cover glass (24X60mm^2) over the surface. Try to remove any air bubbles that may have formed