Rapid Hybridization Buffer - Protocol

Hybridization setup:

1. Take buffer from fridge and pre-warm to 37°C.
2. Mix buffer by tapping/vortexing.
3. Place slides on a warm plate (45°C) for 20min
4. Dehydrate the slides in ethanol series 70%, 85% and 100%, 1min each. And let dry.
5. Add 10µl probe mixture to slide (2ul probe + 8ul buffer) (Use a pipette tip with the end cut)
6. Apply clean 22 x 22 coverslip to slide. Seal edges with rubber cement
7. Co-denature chromosomes/probe on a hotplate at 72°C/2min
8. Place slide in a sealed humidified slide chamber
9. Incubate at 43°C/1hr -2h

Post-hybridization washes:

1. Pre-warm for 1h WS1 (0.4xSSC/0.3% NP-40) to 73°C
2. Carefully remove the rubber cement around the coverslip.
3. Keep the slide in WS2 (2xSSC/0.1% NP-40) at room temp.
4. Agitate gentle until the coverslip slips off.
5. Continue agitating slide for additional 2-3 min
6. Place slide in WS1 and let stand for exactly 2min
7. Transfer to WS2 (2xSSC/0.1% NP-40) at room temp/1min
8. Let dry in dark.
9. Apply 10µl DAPI and 22 x 22 coverslip
10. Wait 15-30 minutes then visualize under microscope using appropriate filter sets

Empire Genomics LLC
700 Michigan Avenue • Suite 200 • Buffalo NY 14203
Phone 716.856.3873 • Fax 716.856.3857