Manual Hybridization

Required Reagents & Equipment (Not Supplied)

70%, 85%, and 100% EtOH
22x22 mm dimension coverglass
22x50 mm dimension coverglass
Rubber cement
Hot Plate
Humidified chamber
Incubator capable of maintaining 37°C
Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC @ 73°C
Wash Solution 2 (WS2) – 0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC
DAPI with Antifade

Prep: Heat slide warmer to 73°C for peripheral blood/ bone marrow preparations, and 75°C for FFPE tissue slides. Ensure incubator is at 37°C.

Manual Hybridization Procedure:

1. Dehydrate slides:
    a. Place slides in 70% EtOH for 2 minutes.
    b. Transfer slides in 85% EtOH for 2 minutes.
    c. Transfer slides in 100% EtOH for 2 minutes.
    d. Air dry slides.
2. While the slides dry, prepare a probe mixture to apply to slides (2µL of probe and 8µL of buffer for a total of 10µL per cellular area is the recommended mixture).
3. Pipette 10µL of the probe mixture onto each cellular area and place 22x22 mm coverglass over the area, taking care that there are no air bubbles.
4. Seal the coverglass with rubber cement. Be sure that all edges of coverglass have been sealed with the rubber cement so the probe does not dry out under the coverslip.
5. Denature the slides using the hot plate:
    a. For peripheral blood/ bone marrow preparations: place the slide on the warmer at 73°C and cover to keep it from direct light. Keep the slide on the hot plate for 2 minutes.
    b. For FFPE tissue slide preparations: place the slide on the hot plate at 75°C and cover to keep it from direct light. Keep the slide on the hot plate for 7 minutes.
6. Remove the slide from the hot plate and place it into the humidified chamber. Place the humidified chamber in the incubator and allow to incubate at 37°C for at least 16 hours.
7. After incubation period, remove the slides from incubator and humidified chamber, carefully remove the rubber cement and coverglass, and move on to the wash process.
8. Wash Slides:
    a. Place slides in WS1 at 73°C for 2 minutes; agitate slides for the first 10-15 seconds.
    b. Transfer slides into WS2 at room temperature for 2 minutes; agitate slides for the first 10-15 seconds.
    c. Remove the slides and wipe the backside to dry. Allow the rest of the slide to dry in a dark place.
9. Once the slide is completely dry, apply 10µL of DAPI with Antifade to the cellular area and a 22x50 mm coverslip. Store slide in a dark place and wait 15-20 minutes before viewing it under the microscope.

Empire Genomics Corp. 1000 Youngs Road Suite 207 Williamsville NY USA 14221 T 716.856.3873 F 716.431.3440 www.empiregenomics.com