Fluorescent In-Situ Hybridization using Pinpoint FISH™ Probes Protocol

MATERIALS:

Reagents: (preparation and storage methods provided where indicated)

- RNase A in 1X PBS (Qiagen catalog: 19101) Final concentration 0.5mg/mL. ***Note**: this can be reused up to 5 times if stored at 4°C in between uses*
- FISH Hybridization Buffer (provided)
- De-ionized water (dH_2O)
- Series-diluted ethanol (75%, 85%, 100%), prepared in separate Coplin jars and stored at -20°C in between uses. Prepare using molecular grade ethanol (200 proof) and dH2O. Note: The ethanol series used after RNase treatment can be used up to 5X over 1 month if stored at -20°C in between uses.
- VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories catalog: H-1200)
- 0.4X SSC/ 0.3% NP-40 solution (preparation method provided upon request)
- 2xSSC/0.3% NP-40 solution (preparation method provided upon request)

Equipment:

- In-situ thermocycler instrument (Thermobrite or Cytobrite)
- 37°C Incubator (cell culture incubator, or oven)
- Water Bath
- Temperature Probe
- Humidity chamber (an airtight container that holds slides and water such that slides will lay flat and not come into direct contact with the water)
- Container with ice

Supplies:

- 24x50 Coverslips
- 0.6 mL Microcentrifuge tubes or 0.2 mL PCR tubes
- Rubber Cement
- 8-12 50 mL 5 well upright glass Coplin Jars with screw caps
- Forceps for slide handling
- Kimwipes

METHOD:

Hybridization

1. Place slides in Coplin jar containing 50 mL of RNase A solution at 37 C and incubate for 1 hr.
2. Move slides to Coplin jar containing dH2O for 5 min.
3. Air dry slides in an upright position for 1-2 min.
4. Begin chilled ethanol series:
   Note: Keep Coplin jars at -20 C until use.
   a. Place slides in Coplin jar with 50 mL of 75% ethanol for 2 minutes.
   b. Transfer slides to Coplin jar with 50 mL of 85% ethanol for 2 minutes.
   c. Transfer slides to Coplin jar with 50 mL of 100% ethanol for 2 minutes.
5. Allow slides to airdry completely.
6. Prepare hybridization mix for one full slide (30 µL).
   Note: Keep probe products on ice and away from full light if not in use, preferably in the dark.
   a. In a PCR tube add 18 µL of FISH Hybridization Buffer
   b. Add 12µL of probe solution
   c. Vortex the tube contents three times, for 5 seconds each time
   d. Centrifuge for 10 seconds
7. Apply 30µL of hybridization mix to each slide in one even drop, using a micropipetter. Use care not to introduce air bubbles.
8. Apply cover slip.
9. If an air bubble is present underneath the coverslip, it can be forced out with gentle, even pressure to the middle of the coverslip.
   Note: Pressing on coverslip can physically damage cells if not done with great care.
10. Seal with rubber cement applied on periphery of coverslip.
    Note: Take great care that no rubber cement gets underneath the slides, it can obstruct slide
11. Place slides on Thermobrite or Cytobrite and raise slides to 75ºC for 5 minutes.
12. After program ends, carefully move slides into humidity chamber.
13. Incubate slides in humidity chamber at 37ºC for 4-6 hours, or overnight.
    Hybridization time should not exceed 12 hours.

Postwash

1. Prepare Wash 1: Place 50 mL of 0.4X SSC/ 0.3% NP-40 solution in a Coplin jar and heat using a water bath set to 60.0C.
2. Prepare Wash 2: Place 50 mL of 2xSSC/0.3% NP-40 solution in a Coplin jar and leave at room temperature.
3. Gently roll off the dry rubber cement seal, and remove coverslip by submersion in 2X SSC in a Coplin jar at room temp. Gently agitate, if necessary, to dislodge coverslip.
4. Ensure temperature of 0.4X SSC/ 0.3% NP-40 solution (Wash 1) inside the Coplin jar is 60.0 C, then add slides and wait 1 min.
5. Move slides to Wash 2 (2xSSC/0.3% NP-40 solution at room temperature).
6. After 1 to 2 minutes, remove slides from Wash 2 and wipe back of slides with a clean kimwipe.
7. Airdry slides upright in the dark.
8. Add 18 µL Vectashield with DAPI and apply a coverslip. Use care not to introduce air bubbles.
   Note: Nail polish can be applied around the edges of the coverslip to seal it in place.
9. Let slides sit for 15 minutes.
10. 10. Image slides.