BAC Fluorescence *in situ* Hybridization Single Color

Hybridization

1. Prepare metaphase slides using standard procedures (** for simultaneous banding use BrDU synchronized cultures).
   Age slides overnight at room temp. (Better to age a week)

2. Apply 200ul RNAse (100ug/mL 2X SSC) to each slide and cover with a 24x60mm coverslip. Place slides in a moist 37°C chamber for 1 hour.
   a. 998ul 2XSSC + 2ul Rnase A (50mg/mL)
      - enough for 5 slides
      - use 3mL snap cap Falcon Tubes
   b. Lay out all coverslips
   c. Place slides in green tray with broken pipets and moistened paper towel at the bottom
   d. Cover the tray with aluminum foil and place in 37°C lower incubator.

3. Rinse slides in 4 changes of 2XSSC (pH 7) for 3min. each at room temp.
   a. remove coverslip with a flick of the wrist before washes

4. Dehydrate slides in an ethanol series (70, 85, and 100%), 2min. each at r.t., and dry with filtered air jet or let air dry.
   a. Use Coplin jars

5. Denature target DNA by immersing the slides in denaturing solution heated to 70°C for EXACTLY 2min.
   a. Denaturing solution consists of : 70% Formamide in 2XSSC (pH 7)
   b. Aggitate briefly to remove denaturing solution
   c. Continue dehydration in ice cold 70%, 85%, and 100% ethanol for 2min. each
   d. Air dry slides.

   NOTE: 1. 70% formamide MUST be AT LEAST 70°C at all times, therefore, do only 2 slides at a time!!
   2. Use Copelin jars with covers and a forceps to put slides in and take slides out of Formamide and EtOH.
   3. Cover water bath so don't lose heat.
   4. Keep all EtOH in the freezer until use and then bury it in ice
   5. Bury the Copelin jars in ice
   6. Put slides in the jars first and then add the EtOH.
   7. May reuse 70% and 85% EtOH but NOT 100% EtOH.

6. Warm slides to 37°C on a heating plate

7. Place 10ul denatured/preannealed probe onto each slide and cover with a 22 square coverslip. Seal edges of coerslip with rubber cement and place slides in a moist 37°C chamber overnight.
   a. Use a 5cc syringe filled with rubber cement to seal coverslip
   b. Lay coverslips out so they can be used easily

c. **Mark the slide with a SuperPermanent Sharpie Pen** -which BAC was used as a probe
d. Make sure all edges of the slide coverslip is sealed with rubber cement
e. When adding 10ul probe avoid bubbles

f. Use the green container with the broken pipets and moistened paper towel

g. Make sure the slides haven't moved when placed in 37°C incubator

Post Hybridization Washes

1. Carefully remove rubber cement and coverslip.
   a. Use the smallest forceps you can
   b. Do not move coverslip too much
   c. Peel a corner of glue away from the slide and then peel around the coverslip.
2. Wash the slides 3X for 5min. each in a 42°C solution containing: 55% Formamide 2XSSC (pH 7)
   a. Place Coplin jars in 42°C water bath for about 1 hour **BEFORE** washes
   b. All slides can be washed at once but leave enough space so they don't stick together.
   c. 55% Formamide/2XSSC can be reused

NOTE: NEVER LET SLIDES DRY AFTER STARTING WASHES

3. Wash slides in 0.1XSSC three times, 5min. each, at 60°C. (1XSSC for BACs)
4. Wash slides in 4X SSC for 5min. at room temp.
5. Prepare BLOCKING solution: 4XSSC / 3% BSA

   850uL 4XSSC
   150uL 20% BSA
   1 mL

   a. Double the amount so enough for 10 slides

   Prepare Ab Buffer: 4XSSC / 0.1% Tween / 1% BSA

   4.75mL 4xSSC/0.1%Tween
   250ul 20% BSA
   5mL

   Prepare 4XSSC / 0.1% Tween 20:

   750mL 4XSSC
   750ul Tween 20

   a. Place in 3 wash trays in a 45°C water bath.
6. To each slide add 200uL 4XSSC / 3%BSA Blocking buffer and incubate for 1 hour at 37°C.
   a. Add Blocking buffer in the center of the slide and then cover with a 24x60mm coverslip
   b. Place in green tray with moistened paper towel and cover with aluminum foil.
   c. Place tray in 37°C incubator for 1 hour

FOLLOWING STEPS PERFORMED IN THE DARK

7. Remove the cover slip and add 200uL Avidin (1ug/mL in 4XSSC/0.1% Tween/1%BSA)
    a. Incubate at 37°C for 30 min.
    b. Stock Avidin is 2mg/mL = 1:200 dilution (5uL Ab + 995uL Buffer for 6 slides)
    c. Ab in Dairy compartment of refrig.
    d. Place coverslip over slide and tap into place with pipet tip

8. Remove coverslip and wash slides in 4XSSC/0.1%Tween at 45°C three times, 5min. each.
    a. Cover waterbath top with aluminum foil.

9. Add 200uL Fluorescein labeled anti-Avidin solution (10ug/mL in 4X SSC/0.1% Tween/1%BSA) and incubate

    at 37°C for 30min.
    a. Stock anti-Avidin is 0.5mg/mL - 1:50 dilution
    b. Keep slides wet and do not let them dry out
    c. Add coverslip and place in 37°C incubator

10. Remove coverslip and wash slides in 4XSSC/0.1%Tween at 45°C three times, 5 min. each.

11. Add 20ul Vectashield and cover with 24x60mm coverslip.
    a. Allow Vectashield to warm to room temp. about 10min.

12. Place slides in a cardboard folder and place in 4°C refridg. overnight.