FISH Protocol for BACs and Paints

FISH / ZOO-FISH

1. Age slides for either: 1 hr at 70^{o}C, overnight at 37^{o}C or for 24 hrs at RT

1. Ethanol series 70%-80%-100% for 5 minutes each

2. Air dry

3. RNase treatment to remove background; 2.5µl 4mg/ml RNase A (Promega A7973) + 97.5µl 2xSSC per slide

4. Add 100µl RNase mix per slide and cover with a cover slip (22x50) and incubate 37^{o}C for 1 hr

5. During RNase treatment probes are prepared (3µl Hybridisation mixture and 1µl probe (for 13mm diameter coverslip)) and at 40 mins into RNase are denatured at 75^{o}C for 5 minutes

Coverslip	Final probe volume (ul)
7mm dia	2
13mm dia	4
18x18	6
22x22	10

6. Probes then incubated at 37^{o}C for 30-45 minutes to preanneal (do not exceed otherwise probes will reanneal too much)

7. When slides come out of RNase, wash twice in RT 2xSSC for 5 min each

8. Put slides through an EtOH series (70, 80 100%) 2 mins each and air dry

9. Slides denatured at 70^{o}C in 70% FA / 2xSSC for 1minute 30 seconds (slides will cool FA by ~1^{o}C per slide, so you may need to adjust initial temperature accordingly)

10. Quench slides in 70% ice cold ethanol for 2 minutes then put through 80% and 100% ethanol also for 2 minutes each and air dry

11. Once slides are dry add probe and coverslips and seal with rubber cement. Incubate at 37^{o}C in a moist chamber for 24 hrs (chicken on chicken) or longer for ZOO-FISH (times vary; e.g. chicken probes on turkey slides 48 hours, chicken on duck at least 72 hours; there is no harm in leaving slides longer)

Treatment B – Second Day

1. Remove rubber cement and wash in 2x SSC to remove coverslips

2. Wash off unbound probes in 50% FA / 2x SSC at 37^{o}C for 20 minutes (with ZOO-FISH the stringency may need to be lowered – either [FA], temperature or both)

3. Wash in 2x SSC + 0.1% igepal for 1 minute at RT

4. Storage buffer 4x SSC, 0.05% igepal for 15 minutes – 3 days at RT

5. Block non-specific antibody binding sites in 4x SSC, 0.05% igepal, 2-3 % BSA for 25 minutes at RT

6. During blocking make the detection mix; this is 100ul per slide; 4x SSC, 0.05% igepal, 1.5% BSA (ie 50ul storage buffer plus 50ul block buffer) plus antibody. Cy3-streptavidin and FITC-anti-dig we use at 1:200. Incubate the detection mix at RT in the dark for 25 mins, then spin down at 13,000rpm 5mins to remove aggregates.

7. Add detection mix to slides, cover with a 22x50 coverslip and incubate at 37^{o}C for 35 minutes

8. Wash in storage buffer for 2x 5 minutes in the dark on a shaker to remove unbound antibody

9. Rinse twice in ddH_2O

10. Air dry in the dark

11. Add two drops of DAPI to a coverslip then put coverslip on slide. We use Vectashield with anti-fade (Vector, H1200), adding it coverslip first helps avoid air bubbles. Press out excess DAPI on tissue and store at 4^{o}C for ~20mins before taking to microscope.