FISH for BAC mapping/chromosome paints

FISH- Probe preparation and denaturation

· Prepare slides of the appropriate cell suspension
· Age slides (this can be done at 55-65°C for 2-3 hours, 1 hour at 70°C or at room temperature (RT) for 24 hours)
· Once aged prepare the probe, (use 1-2ml of the probe and 3-4ml of the hybridisation mix provided to create the probe mix)
o for a 9mm circular coverslip use a total of 2ml of the probe mix
o for a 13mm circular coverslip use a total of 4ml of probe mix
o for 18x18mm coverslip use a total of 10ml of the probe mix
o for 22x22mm coverslip use 13-15ml of the probe mix
· Apply probe mix to slide and place coverslip on top
· Seal the coverslip to the slide with rubber cement to prevent evaporation of the probe and allow to dry at RT for 3-5 minutes
· Place slides on a pre-warmed hotplate at 68.5°C for 5 minutes for denaturation
· Immediately after denaturation place in a sealed humidified chamber and incubate overnight (at least for 12 hours) at 37°C.

Post hybridisation washes

· Remove slides from 37°C and carefully remove the rubber cement
· Place slides into a coplin jar containing 2xSSC/0.1% IGEPAL (also known as tergitol/NP40), incubate slides for 5 minutes at RT allowing the coverslip to float off
· Once the coverslip has floated off incubate slides for a further 5 minutes with gentle agitation in the same solution (2xSSC/0.1% IGEPAL)
· Following this place slides in a coplin jar containing 0.4xSSC/0.3% IGEPAL, pre-warmed to 73°C for 2 minutes, initially gently agitate slides 3 times
· Following this 2 minute incubation remove slides carefully (with no agitation) and place slides into 2xSSC/0.1% IGEPAL for 1 minute at RT with no agitation
· Subsequently transfer slides into 4xSSC/0.05% TWEEN 20 for 15 minutes at RT (at this point slides can be left for up to 24 hours in this wash buffer if required)
· Once incubated slides are blocked using 4xSSC/0.05% TWEEN 20/2-3%BSA, slides are incubated for 25 minutes at RT.
· During the blocking incubation prepare the detection solution of 4xSSC/0.05% TWEEN 20/1.5%BSA to this add the secondary antibody (in the case of biotin use Gy3 streptavidin, dilute according to manufacturers guidelines).
· Remove slides from blocking solution, stand vertically for a few seconds to let excess solution drain off (DO NOT let slide dry!).
· Place slide in humidified chamber add 100ml of the detection solution (containing secondary antibody) and place a glass/plastic cover slip on top that covers the slide. Seal chamber and place at 37°C for 35 minutes.
· Place slide vertically and allow cover slip to fall off.

· Following detection remove slides and stand slide in 4xSSC/0.05% TWEEN 20, in the dark for 2 x 5 minute incubations at RT with occasional gentle agitation.
· Rinse with dd H2O twice.
· Dry in dark
· Mount slide with appropriate counter-stain
· Place on cover slip.