

Simultaneous RNA/DNA FISH with Oligopaints

Adapted from Lanzuolo C, Roure V, Dekker J, Bantignies F, Orlando V (2007). Polycomb response elements mediate the formation of chromosome higher-order structures in the bithorax complex. Nat Cell Biol 9(10):1167-74 by Frederic Bantignies

1. Allow slides to warm to room temperature (RT) (~15 mins)
2. Add 30 μ l of 1X PBT (1X PBS + 0.1% v/v Tween 20) +/- RNase A to a 22x30cm coverslip. Invert a slide onto the coverslip and seal with rubber cement
3. Incubate at RT for 1 hour in a humidified chamber
4. Remove the coverslip and incubate for 2' in 1X PBS at RT
5. Incubate for 10' in 1X PBS + 0.5% v/v Triton X-100 at RT
6. Incubate for 2' in 1X PBS at RT
7. Incubate for 30' in 1X PBS + 20% v/v glycerol
8. Immerse the slide in liquid nitrogen using forceps and allow to freeze completely (~15 seconds)
9. Allow the slide to thaw face-up on a paper towel
10. Repeat steps 8 and 9 two additional times
11. Incubate for 1' in 1X PBS at RT
12. Incubate for 5' in 1X PBS at RT
13. Incubate for 5' in 1X PBS at RT
14. Incubate for 5' in 0.1 M HCl at RT
15. Incubate for 1' in 2X SSC at RT
16. Incubate for 1' in 2X SSC at RT
17. Incubate for 1' in 2X SSC at RT
18. Incubate for 35' in 2X SSC + 50% v/v formamide at RT
19. Tap slides dry on a paper towel (never allowing the face of the slide containing the cells to come into contact with the paper towel)
20. Create a hybridization cocktail consisting of 12.5 μ l of 2X hyb. mix (4X SSCT + 20% w/v dextran sulfate), 12.5 μ l of formamide, and 20-40 pmole of each Oligopaints probe set per slide.

21. Add the hybridization cocktail (typically 26-28 μ l after adding the probe sets) to a 22x22 cm coverslip, invert the slide onto the coverslip, and seal with rubber cement
22. Allow the rubber cement to dry for 5' at RT
23. Denature the slides for 3' at 78°C on a water-immersed heat block
24. Hybridize overnight at 42°C in a humidified chamber
25. The next day, remove the coverslip and wash for 15' in 2X SSCT (2X SSC + 0.1% v/v Tween 20) at 60°C
26. Incubate in 2X SSCT for 10' at RT
27. Incubate in 0.2X SSC for 10' at RT
28. Add 14 μ l of mounting media (e.g. SlowFade Gold + DAPI) to a 22x30 cm coverslip, invert the slide onto the coverslip, and seal with nail polish
29. Wait at least 30 minutes for the nail polish to dry before imaging