

CO-FISH Protocol

Metaphase Spreads for CO-FISH

1. Grow cells in 10 μ M BrdU/BrdC (3:1 ratio 7.5 μ M : 2.5 μ M) for 24 hrs
2. Add Colcemid for last 4 hrs at 200 ng/mL (10,000X of 2 mg/mL stock, ie 1.0 μ L per 10 mL)
3. Harvest cells **floating** and adherent by trypsinization
4. Spin cells at 180 xg for 5 min
5. Resuspend cell pellet in 5 mL prewarmed hypotonic solution (75 mM KCl)
6. Incubate 16 min at 37 °C
7. Add 4 drops of fix solution (3:1 MeOH:Acetic Acid). Spin at 180 xg for 5 min. Aspirate hypotonic solution, leaving behind 0.2mL
8. Add 5 mL fix, dropwise for first mL while gently vortexing. Let sit at room temperature for 15 min
9. Spin at 180 xg for 5 min
10. Wash again, repeat steps 8 and 9
11. Drop cells on cold slides
12. Air dry in dark

CO-FISH Slide Staining

1. Rehydrate slide for 15 min at room temperature in 1X PBS.
2. Treat slides with RNase A (0.5mg/mL) 10min at 37 °C under coverslip.
3. Stain slides with Hoescht 33258 (10 μ g/mL, 1:1000) in 2X SSC for 15 min at RT.
4. Expose slides to 365 nm UV light with Stratalinker for 30 min in 2X SSC under coverslip.
5. Incubate slides in ExoIII (1.6% ExoIII in 1X ExoIII buffer) for 10 min at RT with 100 μ L under coverslip. *Prewarm pepsin solution (43.1 μ L 12.1N HCl in 50mL dH2O + 25 μ L 10% Pepsin) at 37 °C.*
6. Incubate slides in prewarmed pepsin solution at 37 °C for 7.5 min, shake frequently.
7. Wash slides in 1X PBS for 5 min at room temperature.
8. Dehydrate slides through cold ethanol series (70%, 85%, 100%) by shaking slides in each.
9. Quickly air dry slides.
10. Add 10 μ L of FITC-G-rich-telomere PNA under coverslip.
11. Incubate slides in moist chamber at room temperature for 1 hr.
12. Dip slides in 1X PBS + 0.02% Tween-20 to remove coverslips.
13. Rinse slides in 1X PBS + 0.02% Tween-20 for 1 min at room temperature.
14. Add 10 μ L of CY3-C-rich-telomere PNA under coverslip.
15. Incubate slides in moist chamber at room temperature for 1 hr. *Prewarm 1X PBS + 0.02% Tween-20 at 57 °C.*
16. Dip slides in 1X PBS + 0.02% Tween-20 to remove coverslips.
17. Incubate slides in pre-warmed 1X PBS + 0.02% Tween-20 for 20 min at 57 °C.
18. Rinse slide in 2X SSC + 0.02% Tween-20 for 1 min at room temperature.
19. Stain slides with 1 μ g/mL DAPI in 2X SSC + 0.02% Tween-20 for 5 min.
20. Rinse slides briefly in 2X SSC + 0.02% Tween-20
21. Mount and coverslip slides.
22. Dry at room temperature and seal with nail polish.
23. Store slides in dark at 4 °C.