Telomere FISH

Metaphase Spreads

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

FISH Slide Staining

1. Rehydrate slides in 1X PBS for 15 min at room temperature. Prewarm pepsin solution (43.1uL 12.1N HCl in 50mL dH20 + 25 uL 10% Pepsin) at 37 ºC.
2. Incubate slides in prewarmed pepsin solution at 37 ºC for 7.5 min, shake frequently.
3. Wash slides in 1X PBS for 5 min at room temperature.
4. Dehydrate slides through cold ethanol series (70%, 85%, 100%) by shaking slides in each.
5. Quickly air dry slides.
6. Add 10 µL of CY3-telomere PNA (amino-Cy3-CCCTAACCCTAACCCTAA-carboxyl) under coverslip.
7. Denature slides for 7 min at 70 ºC.in oven on metal block.
8. Incubate slides in moist chamber at room temperature for 1 hr. *Prewarm 1X PBS + 0.1% Tween-20 at 57 ºC.*
9. Dip slides in 1X PBS + 0.1% Tween-20 to remove coverslips.
10. Incubate slides in pre-warmed 1X PBS + 0.1% Tween-20 for 20 min at 57 ºC.
11. Rinse slide in 2X SSC + 0.1% Tween-20 for 1 min at room temperature.
12. Stain slides with 1 µg/mL DAPI in 2X SSC + 0.1% Tween-20 for 5 min.
13. Rinse slides briefly in 2X SSC + 0.1% Tween-20
14. Mount and coverslip slides.
15. Dry at room temperature and seal with nail polish.
16. Store slides in dark at 4 ºC.