Multi-color FISH for Chromosome Specific Telomere Length Measurement

1. Chromosome preparations are dropped onto clean glass slide and air dried. The slides are kept at RT for 7 days to age the slide for optimal hybridization. **Important: changing the slide aging time will affect the denaturation time and hybridization.**
2. Place slides in a jar of fixative (methanol: acetic acid=3:1) for 1 hour at RT and air dry slide for 15 minutes.
3. Dehydrate slides using ethanol series (70%, 80%, 90% and 100%), each for 2 minutes. (change fresh ethanol when needed), air dry slide for 15 minutes.
4. Turn on the Hybex with water bath inside (without water) and set up the temperature at 60°C.
5. Add 1 ml of dH_2O onto the absorbent pad for each Hybex hybridization chamber.
6. Prepare hybridization mixture:

   To make 15 ul hybridization mixture, add:

   - 14 ul of hybridization buffer
   - 0.5 ul of 10 uM Cy3-Telomere probe
   - 0.5 ul of centromere probe mixture
7. Place hybridization mixture on a thermomixer at 45℃/900 rpm for 10 minutes
8. Apply 15ul hybridization mixture to each slide and cover the area with a coverslip.
9. Insert slides into the rack and place the racks into the Hybex hybridization chamber at the correct orientation and tighten corner screws to seal the chamber.
10. Place the chamber into the Hybex heating unit, set the temperature to 75 °C (temperature will drop to around 33~40°C first and then increase to 75 °C in about 9 minutes).
11. When the temperature reached 75°C, incubate the slide in the hybridization chamber for 5 minutes. Take out the chamber, submerge the hybridization chamber in tap water for 30 seconds (this will cool down the chamber to < 40°C), and then place the chamber on a flat surface at RT.
12. Reset temperature of the Hybex heating unit at 30°C (Place the water bath insert with tap water into the Hybex heating unit to help cooling down quickly. The heating unit cools down to below 40°C in about 5 minutes).
13. When the heating unit cools down to below 40°C, place the hybridization chamber back

to the heating unit, then incubate at 30 ^{o}C for 3 hours (temperature will fluctuate above 30 °C when the chamber are placed, but will reach 30°C in 25 minutes. This temperature fluctuation does not affect the hybridization).

14. Prepare SSC series (2x, 1x, 0.5x, and 0.1x) pre-warm to 45 ^{0}C to wash the slides. After hybridization, remove and dissemble the chamber. Soak slides in 2×SSC at 45 ^{0}C water bath for 5 minutes to remove the coverslips.
15. Wash slides in 2xSSC for 10 mins, 1× SSC for 10 minutes, 0.5× SSC for 10 minutes, and 0.1×SSC for 10 minutes.
16. Remove the slides from the 0.1XSSC and drain excess liquid. Mount the slide with 15 ul of anti-fade mounting medium containing 250 ng/ml DAPI under a coverslip. The slide can be analyzed next day.

Solutions and Reagents

1. 10% blocking reagent solution: Dissolved blocking reagent (Roche Diagnostics, cat# 1 096 176) in maleic acid buffer to a final concentration of 10% (W/V) with shaking and heating (80^{o}C). Autoclave and store the aliquots at -20^{o}C.

2. Maleic acid buffer: 100 mM maleic acid, 150 mM NaCl, pH 7.5. Adjust the pH with NaOH solution.

3. Prepare hybridization Buffer:

   To make 20 ml hybridization buffer, add:

   - 10 ml of 100% formmamide (50% formmamide)
   - 1.0 ml of 200 mM Tris HCl (10mM Tris pH 7.5)
   - 10 ml of 10% block reagent (4.26% block reagent)
   - 400 ul of 50× Denhart's solution (1× Denhardt's solution)

Make 1 ml aliquots and store at -20^{o}C. The buffer works well for six months.

4. PNA Probe sequences

   Telomere probe: TTAGGG TTAGGG TTAGGG

   Chromosome 2 centromere probe: TGT CTA GCT TTG AGG ATT

   Chromosome 9 satellite III probe: TCC ACT CGG GTT GAT T

Chromosome 18 centromere probe: GTG TGT CCT CAA CTA AAG

Chromosome X centromere probe: CTG AAC ATT CGT TAT GAT

How to prepare centromere probe mixture

Centromere probe	Con. Of stock	volume
Water		150µl
cen_X-cy3	100µm	125µl
cen_ch2-FITC	50µm	50µl
cen_ch9-FITC	0.2µm	50µl
cen_ch18-FITC	40µm	125µl
Total		500µl

5. Note: PNA probes were ordered from PNAbio (order@pnabio.com). Formmamide and Denhart's were ordered from Fisher.