

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 and hybridization.**
2. Place slides in a jar of 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 dH₂O onto the absorbent pad for each Hybex hybridization chamber.
6. Prepare hybridization mixture:
 - To make 15 ul hybridization mixture per slide, add:
 - o 14 ul of hybridization buffer
 - o 0.5 ul of **10 uM** Cy3-Telomere probe (TTAGGG)₃
 - o 0.5 ul of **2 uM** FITC-17cen probe (optional)
 - o 0.5 ul of **1 uM** FITC-9 sat III probe (optional)
7. Place mixture on a thermomixer at 45°C/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. Screw the chamber handle into the top of the assembled chamber. Take out water bath and cool down with tap water. Lift the chamber, carefully keep it level, and place the chambers into Hybex heating unit. Remove the handles and set the temperature to 75 °C. Temperature would drop to around 33~40°C and Increase to 75 °C in about 9 minutes.
11. Incubate the slide in the hybridization chamber for 5 minutes when the temperature reached 75°C. 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. 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 cool down quickly.

The heating unit cools down to below 40°C in about 5 minutes.

12. Exchange water bath with chamber when the heating unit cools down to below 40°C. Leave the chamber for hybridization for 3 hours. Temperature will fluctuate above 35 °C when the chamber are placed, but will reach 30°C in 25 minutes. This temperature fluctuation does not affect the hybridization.
13. Prepare SSC series at 45 °C to wash the slides. After hybridization, remove and disassemble the chamber. Soak slides in 2xSSC at 45 °C water bath for 5 minutes to remove the coverslips.
14. At 45 °C water bath, wash slides in 2xSSC for 10 mins, 1x SSC for 10 minutes, 0.5x SSC for 10 minutes, and 0.1xSSC for 10 minutes separately.
15. Remove the slides from the 0.1XSSC and drain excess liquid. Mount the slide with 15 ul of mounting medium containing 250 ng/ml DAPI under a coverslip. The slide can be analyzed next day.
16. After use, leave the chamber open and the chamber cover face up to allow the moisture to evaporate.

Solutions and Reagents

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°C). Autoclave and store the aliquots at -20°C.

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

Prepare hybridization Buffer:

To make 20 ml hybridization mixture, add:

- 10 ml of 100% formamide (50% formamide)
- 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 50x Denhardt's solution (1x Denhardt's solution)

Make 1 ml aliquots and store at -20°C. The buffer works well for six months.