Q-FISH TELOMERE LENGTH MEASSUREMENT PROTOCOL

A) Day I: METAPHASE OBTENTION

1. Add **Colcemid** (100µl stock 100X to 10ml of complete media) to cells in culture. Incubate 2-4h culture cells at 37C (2h=lymphocytes, immortalized cells; 4h=fibroblasts, primary cells).
2. Collect 10ml media from cells and transfer to a Falcon tube.
3. Wash cells with 5ml PBS, and combine with 10ml media.
   Cells in mitosis do not attach to the plate very well. It is important to keep the media and the PBS wash, since it might contain mitotic cells.
4. Add trypsin (2.5ml)
5. Incubate 10min. at 37C
6. Inactivate trypsin with the medium in the Falcon tube. Collect cells.
7. Centrifuge 8 min at 800rpm.
8. Aspirate supernatant until only 1mL remains.
9. Resuspend cells by mixing with fingertip.
10. Add dropwise (while gently vortexing) 9mL of **hypotonic** 0.03 M Na-citrate preheated at 37C (alternatively, 0.56% KCl).
11. Keep in water bath at 37C fot 25min (10 min if using 0.56% KCl).
12. Add 3 drops of fresh **Fixing solution** (MetOH/Acetic acid 3:1) at 4C. Cover tube label with transparent tape. Fixative is made fresh.
13. Centrifuge 8min at 800rpm.
14. Aspirate supernatant until only 1mL remains.
15. Add 2mL Fixing solution while gently vortexing. Add 9mL more. If there are too many tubes, add 2mL to all of them first, and then add the rest of the solution. If only a few tubes add 2+9mL to each tube.
16. Repeat steps 13-15.
17. Keep samples at -20C until preparation of metaphases (at least ON).
18. Centrifuge 8 min at 800rpm.
19. Aspirate supernatant until only 1mL remains.
20. Resuspend cells and add 10mL fresh **Fixing Solution** while vortexing.

21. Centrifuge 8 min at 800rpm.
22. Aspirate supernatant until only 1mL or less remains, depending on cell density.
23. Aspirate cells with a Pasteur pipette where a capillary end has been created.
24. Wet a glass slide in 45% Acetic acid and drain. Let some drops of the cell solution fall on the slide from the maximum height possible.
25. Let slides dry overnight. Check metaphases at the microscope.

B) Day II: METAPHASE HYBRIDATION

1. Prepare acidified pepsin and incubate 15min. at 37C

Pepsine	200mg
H_2O	200mL
HCl conc.	168µL

2. Wash slides in PBS (Ca/Mg free) 15min in shaker.
3. Fix cells in 4% formaldehyde in PBS for 2min.

	400mL	**800mL**
Formaldehyde 35%	47.2mL	94.4mL
PBS	352.8mL	705.6mL

4. Wash slides in PBS 15min in shaker.(three times)
5. Digest with preheated pepsin 10min at 37C in waterbath.
6. Wash slides in PBS 15min in shaker. Two times
7. Fix in cells in PBS/4% formaldehyde for 2min
8. Wash slides in PBS 15min in shaker.(three times)
9. Dehydrate in EtOH 70% -- 90% -- 100%. 5min each.
10. Air dry 5-20min.
11. Prepare probe mix:

Stock	**10 slides**	**20 slides**
Tris 1M pH7.4	2.5µL	5.0µL
Buffer $MgCl_2$	21.4µL	42.8µL
Deionized formamyde	175.0µL	350.0µL
Probe 25µg/mL (tel-probe)	5.0µL	10.0µL
BM 10% (Blocking reagent)	12.5µL	25.0µL
H_2O destilled	33.6µL	67.2µL

BM 10%: 10g blocking reagent (Boehringer) in 100mL Maleic Acid buffer pH7.5 (adjusted with NaOH). Dissolve on a heating block or microwave. Aliquot and store at -20C .

Maleic Acid Buffer: 100mM Maleic Acid, 150mM NaCl pH7.5 (20C). Adjust with NaOH.

2g Maleic Acid in 12.5mL H_2O (1M) → 20mL
5M NaCl → 6mL
Bring to 200mL with water.

Buffer $MgCl_2$:	**(For 100mL)**
25mM $MgCl_2$	1M $MgCl_2$---------- 2.5mL
9mM Citric Acid	0.1M Citric Acid----- 9.0mL
82mM Na_2HPO_4	1M Na_2HPO_4--------- 8.2mL
Adjust pH to 7.0	H_2O----------80.3mL

12. Add 2 drops (10-15µL) to a long cover slide. Turn slide upside down onto the cover, so that the probe extends by diffusion. Care not to make bubbles.
13. Denature at 80C 3min (exact time).
14. Make a wet chamber by covering the walls of a big cylinder with wet paper towels. Put slides into the chamber with covers facing down. Seal cylinder with saran wrap. Incubate in dark for 2 hours at RT.
15. Wash twice 15min while vortexing (If covers do not separate from slides after 5 min use tweezers.

Stock	**For 400mL**	**For 800mL**
Formamide (standard)	280mL	560mL
Tris 1M pH7.2	4mL	8mL
BSA 10% (in H_2O)	4mL	8mL
H_2O	112mL	224mL

16. Wash 5min with TBS-Tween 20 (Three times)

Stock 10X TBS
Tris pH 7.0-7.5 1M
1.5M NaCl

Dilute stock 10X TBS 1:10 in H_2O and add Tween-20 (0.08%). 800µL for 1L or 480µL for 600mL.

17. Dehydrate in EtOH 70%--90%--100%. 5min each.
18. Air dry slides.
19. Add 2drops (10-15µL) to a long cover slide of Antifade and DAPI (1:3 dilution of DAPI-labeled vectashield). Let dry for 5 min.
20. Seal cover to slides with nail polish.
21. Keep samples at 4C in dark