IF-FISH

- Grow cells on cover slips to sub-confluence. Aspirate medium and wash in PBS.
- Fix the cells for 5 min @ RT in 2% paraformaldehyde or alternatively for 10 min at -20ºC in 100% cold methanol.
- Wash 3 x 5 min in PBS.

Cells can be stored at 4ºC at this point, add 1000x stock NaN_3 from 10% stock in H_2O. Use caution, NaN_3 is poisonous.

- Incubate cover slips for 30 min in **blocking solution**.
- Incubate 1 hour in primary antibody diluted in 50- 100 ul blocking solution.
- Wash 3 x 5 minutes in PBS.
- Incubate 30 min in secondary antibody* (1:250 dilutions, use red!) diluted in 100 ul blocking solution. Keep slides in the dark from now on!
- Wash 3x 5 minutes in PBS.
- Fix the cells on the cover slips for 2 min at RT in 1% paraformaldehyde. (this step can be skipped!)
- Wash 2x 5 minutes each in PBS.
- Dehydrate the cells in ethanol, consecutively 70%, 95%, 100% EtOH, 5 min each. Aspirate the ethanol completely and let the cover slips dry for a couple of minutes.
- Place coverslips upside down on a drop of **hybridizing solution** (50-80 ml). For this steps use a microscope slide or a glass plate, prevent liquid from being disperse using a liquid silicon pen.
- Denature with hybridizing solution for 5-10 min at 70-80ºC by placing slides on heat block. Keep slides in the dark!
- Incubate in the dark for 2 hours at RT or over night at 4ºC.
- Wash 2 x 15 min in washing solution.
- Wash 3 x 5 min in PBS; add DAPI to the second wash.
- Air dry at RT for 10 minutes.
- Embed and seal.

Solutions

Blocking solution
(in PBS, store at -20ºC in 10 ml aliquots) 1 mg/ml BSA 3% goat serum 0.1% Triton X100 1 mM EDTA pH 8.0

Blocking reagent (Roche 11096176001) Stock is 10%, dissolved in maleic acid buffer: 100 mM maliec acid 150 mM NaCl Adjust pH to 7.5 with NaOH. Store at 4ºC

Hybridizing solution
Make fresh. 70% formamide (from deionized stock) 0.5% blocking reagent (from 10% stock) 10 mM Tris-HCl pH 7.2 FITC-TelC PNA probe (1:1000)

PNA probes (Custom from BioSynthesis) FITC-TelC: FITC-OO-CCCTAACCCTAACCCTAA 3' Stock concentration: 111 mM.
Store at 4°C in the dark.

Washing solution
70% formamide 10 mM Tris-HCl
pH 7.2

3000x DAPI
Dissolve 0.5 mg/ml 4',6-diamino-2-phenylindole (Sigma D-9542) in H_2O. Stable for at least one year at 4°C.

Embedding medium
Dissolve 20 mg p-phenylene diamine (Sigma P-6001) in 2 ml 10xPBS by vortexing. Immediately add 18 ml glycerol, mix carefully without creating air bubbles. Do not vortex. Store in 1 ml aliquots at -70°C.