Non-radioactive *in situ* hybridization on paraffin sections using DIG-labeled miRCURY™ detection probes

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Deparaffinize the sections

- Xylene 3x 5min
- Ethanol 100% 2x 5min
- Ethanol 70% 5min
- Ethanol 50% 5min
- Ethanol 25% 5min
- DEPC 1x 1min

Deproteinate the sections

- 2 x 5 min PBS
- 5 min Prot.K at 10 µg/mL at 37ºC (add Prot.K 20mg/mL to warm Prot.K buffer 20 min before incubation)
- 30 sec 0.2% Glycine in PBS
- 2 x 30 sec PBS
- Fix sections for 10 min in 4% PFA*
- Rinse slides 2 x in PBS

Prehybridization

- 2h-in hybridization buffer (50%Formamide, 5xSSC, 0.1%Tween, 9.2 mM citric acid for adjustment to pH6, 50 µg/mL heparin, 500 µg/mL yeast RNA) in a humidified chamber (50% formamide, 5xSSC). Use DAKO Pen.

Hybridization (hybridization temperature=Tm probe –21ºC)

- Dilute probe to 20 nM in hybridization buffer
- Add 200 µL hybridization mix per slide
- Hybridize slides overnight covered with Nescofilm in a humidified chamber

Stringency Wash

- Rinse in 2 x SCC at hybridization temperature
- 3 times 30 min in 50% formamide, 2 x SSC at hybridization temperature
- 5 x 5 min in PBST at RT

Immunological Detection

- 1 h block in blocking buffer (2% sheep serum, 2mg/mL BSA in PBST) at RT
- o/n antibody incubation (1:2000 anti-DIG-AP Fab fragments in blockingbuffer) in a humidified chamber at 4ºC
- Wash 5-7 times 5 min in PBST
- Wash 3 times 5 min in AP buffer

Colour reaction (RT, darkness)

- light sensitive colour reaction (NBT/BCIP) 1h-48h (400 µL/slide) in a humidified chamber
- Wash slides 3x 5 min in PBST
- Mount in aqeous mounting medium (glycerol) or dehydrate and mount in Entellan.

Buffers

Colour solution (Light sensitive)

45 µL 75vmg/mL NBT (in 70% dimethylformamide)
35 µL 50vmg/mL BCIP-phosphate (in 100% dimethylformamide)
2.4 mg Levamisole in 10 mL AP buffer.

AP buffer (100 mM Tris HCl pH9.5, 50 mM $MgCl_2$, 100 mM NaCl, 0.1% Tween 20)

100 mL Tris (100mM) 12.1g/L
20 mL 5M NaCl (100mM) 5.84g/L
5 mL 1M $MgCl_2$ (5mM)
700 mL sterile H_2O, pH 9.5 and fill up to 1L

*Please note: For optimal fixation it may be critical to use fresh formaldehyde solutions. Fresh 4% solutions can be made from 16%, methanol free, formaldehyde or from solid paraformaldehyde (4% w/v).

For preparation of buffers please refer to :
Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. --
3rd ed. -- New York: Cold Spring Harbor Laboratory, 2001.