TERRA FISH with Dig-labeled DNA probe + IF (Katharina Deeg, AG Rippe)

DAY 1

- cells are grown on cover slips and incubated with CSK buffer for 5 min on ice (CSK buffer either with Vanadyl Ribonucleoside Complex (1:20) or with RNase A (1:200))
- wash cells with 1X PBS
- fix cells with 4% PFA for 12 min (under hood)
- wash cells with 1X PBS
- make EtOH series: 70% → 85% → 100%, 3 min each
- air dry coverslips
- put drops of 2-5 µl of Dig-labeled TERRA (final concentration 50 ng/µl in 50% Formamide, 2x SSC + 10% Dextrane) on slides
- put coverslips slowly on it (cell side down)
- seal with fixogum and put slides in wet chamber
- hybridize at 37 °C overnight

CSK buffer:
100mM NaCl
300mM sucrose
3mM MgCl2
10mM PIPES
0.5% triton
10mM Vanadyl Ribonulceoside Complex (1:20)

20x SSC buffer:
3M Sodium Chloride
300 mM Sodium Citrate
pH 7.0

TERRA FISH with Dig-labeled DNA probe + IF - continued

DAY 2

- Remove fixogum carefully and transfer coverslips in 12-well plate for washing
- Wash with 2X SSC, 50% formamide, 2x15 min @ RT
- Wash with 0.2X SSC, 0.1% Tween, 1x10 min @ 40°C
- Wash with 2X SSC, 1x5 min @RT
- Wash cells with 1X PBS
- Permeabilize in 0.1% ice cold TritonX/PBS for 5 min
- Wash cells with 1X PBS
- Block in 10% goat serum in PBS for >15 min (30-50 µl for large coverslips; 15-20 µl for small ones)
- 1st Antibody: Mouse anti-DIG (Roche) 1:100 in 10% goat serum/PBS
    - on parafilm in wet chamber for >1 h at RT
- Wash 3x5 min with 0.002% NP40/PBS in well plate
- 2nd Antibody: Anti-mouse Alexa488 1:500 in PBS
    - on parafilm in wet chamber for >30 min at RT
- Wash 3x 5 min PBS, protect from light!
- Quickly in H20 (to remove salts)
- 1 min in 100% EtOH
- air dry coverslips
- Mount with Prolong (5-10 µl)