The protocols of in situ hybridization

Method 1: In situ RNA localization using digoxigenin-labeled riboprobes for paraffin embedded section

Day 1

1.1 Deparaffinization.

1.2 Rinse array slide in 2 changes of 100% ethyl alcohol (ETOH) for 3 min.

1.3 Rinse twice with 95% ETOH for 3 min.

1.4 Wash in phosphate-buffered saline (PBS) for 3 min.

1.5 Dip in 2% DEPC-H2O for 10 min at room temperature.

1.6 Wash array slide in PBS for 10 min.

1.7 Incubate array slide in pepsin (25μg/ml) at 37°C for 15 min.

1.8 Wash array slide twice with PBS for 3 min.

1.9 Incubate array slide in 0.2N HCl for 30min.

1.10 Wash array slide twice with PBS for 3min.

1.11 Incubate in 0.25% acetic anhydride in 0.1M triethanolamine for 10 min.

1.12 Wash array slide twice with PBS for 5 min each.

1.13 Incubate array slide in prehybridization buffer for 30 min.

1.14 Prepare riboprobe mix.

1.15 Hybridization.

Day 2

1.16 Dip array slide twice SSC to remove cover slips for 5 min.

1.17 Wash array slide in PBS for 3 min.

1.18 Incubate array slide in RNAse A solution (0.1-1ng/ml in PBS) at 37°C for 30 min.

1.19 Wash array slide in PBS for 5 min.

1.20 Wash array slide in 2XSSC at room temperature for 10 min.

1.21 Wash array slide in 1XSSC at 37°C for 10 min.

1.22 Wash array slide in 0.5XSSC at 37°C for 10 min.

1.23 Incubate array slide in buffer A for 10 min.

1.24 Incubate array slide in buffer A with 1% normal sheep serum and 0.03% Triton X-100 for 30 min.

1.25 Add anti-Dig antibody and incubate array slide at 37°C for 3 h.

1.26 Wash array slide twice with buffer A for 10 min each.

1.27 Wash array slide twice with buffer B for 5 min each.
1.28 Develop in NBT/BCIP in the dark for 30-60 min, monitoring under the microscope. If background is good, a longer time of visualization is allowed.
1.29 Stop the reaction in buffer B and wash briefly in H2O.
1.30 Counter stain and cover slip.

Method 2: In situ RNA localization using digoxigenin-labeled oligonucleotide probes for paraffin section

Day 1

1.1 Deparaffinize.
1.2 Rinse array slide twice with 100% ETOH for 5 min each.
1.3 Rinse array slide twice with 95% ETOH for 5 min.
1.4 Wash array slide in PBS for 5 min.
1.5 Dip in 2% DEPC-H2O for 10 min at room temperature.
1.6 Wash array slide in PBS for 5 min.
1.7 Incubate array slide in pepsin at 37°C for 10 min.
1.8 Wash array slide in 2 changes of PBS for 5 min.
1.9 Incubate array slide in 0.2N HCl for 30 min.
1.10 Wash array slide twice with PBS for 5 min.
1.11 Incubate array slide in 0.25% acetic anhydride in 0.1M triethanolamine for 10 min.
1.12 Wash array slide in PBS for 5 min.
1.13 Incubate array slide in prehybridization buffer for 30 min.
1.14 Prepare oligo-probe mix.
1.15 Hybridization.

Day 2

1.16 Dip array slide in 2XSSC to remove cover slips.
1.17 Wash array slide in 2XSSC at room temperature for 10 min.
1.18 Wash in 1XSSC at 37°C for 10 min.
1.19 Wash in 0.5XSSC at room temperature for 10 min.
1.20 Incubate array slide in buffer A for 10 min.
1.21 Incubate array slide in buffer A with 1% normal sheep serum and 0.03% Triton X-100 for 30 min.
1.22 Add anti-Dig antibody and incubate array slide at 37°C for 3 h.
1.23 Wash array slide in 2 changes of buffer A for 5 min.

1.24 Wash array slide in 2 changes of buffer B for 5 min.

1.25 Develop in NBT/BCIP in the dark for 30-60 min, monitoring under the microscope. If background is good, a longer time of visualization is allowed (up to 16 h).

1.26 Stop the reaction in buffer B and wash briefly in H_2O.

1.27 Counterstain and cover slip.