(ONLINE SUPPLEMENTAL MATERIAL)

Supplement 1

Sections (8 μm) were cut and mounted on superfrost plus slides, deparaffinized, rehydrated, washed in PBS and treated with proteniase K (1 μg/ml) for 10 min at 37 C. Acetylated sections were washed with 2x SSC and air dried. Hybridization was performed overnight at 70 ^{0}C with digoxigenin (DIG)-labeled antisense riboprobes in 50% formamide, 5x SSC (pH 4.5), 50 μg/ml tRNA, 50 μg/ml heparin, and 1% SDS. Slides were subsequently washed in 5xSSC, 3 times for 15 minutes at 70°C hybridization temperature and 50% formamide, 2x SSC at 45 ^{0}C for 15 minutes. Non-hybridized probes were removed by RNase A digestion at 37 ^{0}C for 30 minutes. Hybridized probe signal was detected by immunochemistry using alkaline phosphatase conjugated antibody against digoxigenin (Roche). Briefly, sections were blocked with 10% sheep serum in 2% blocking reagent (Roche blocking reagent in 0.06M maleic acid, 100 mM NaCl, and 0.1% Tween-20, pH 7.5) for 1 hour and incubated with pre-absorbed antibody at 1:1000 dilution overnight at 4 ^{0}C. Sections were washed in NTMT (40mM Tris, 100mM NaCl, 40 mM MgCl, 0.1% Twen-20), 30 minutes for 10 times, followed by washing in NTMT containing 2mM levamisole to inactivate endogenous alkaline phosphatase. Color reaction was developed with BM-purple (Roche) until color development was observed, and the reaction was terminated with PBS wash. For counter-staining, slides were briefly rinsed in 90% ethanol and briefly dipped in 0.1% eosin, and mounted in Permount.