

Supplemental Table 1

Probes for in situ hybridization

gene	size	references
<i>Col2a1</i>	405 bp	Biochem Biophys Acta 1089: 241-243, 1991
<i>Col10a1</i>	0.55 kb	Cell 108: 17-29, 2002
<i>lhh</i>	1.8 kb	Cell 75: 1417-1430, 1993
<i>Osteopontin</i>	1.3 kb	Biochem Biophys Res Commun 148: 1129-1136, 1987
<i>Mmp13</i>	801 bp	Mol Biol Cell 6: 1287-1303, 1995
<i>Vegf</i>	451 bp	Mech Dev 100: 245-250, 2001
<i>Runx2</i>	0.8 kb	J Biol Chem 235: 8695-8702, 2000
<i>Sox9</i>	255 bp	Mol Cell Biol 17: 2336-2346, 1997

For in situ hybridization, digoxigenin-labeled RNA probes listed above were prepared by in vitro transcription using MAXIscript T3/T7 Kit (Invitrogen). Mouse tissues were fixed overnight in 10% formalin, dehydrated, and embedded in paraffin. Seven-micrometer sections were cut by Leica RM2255 (Leica Microsystems Inc., Buffalo Grove, IL, USA). Slides were deparaffinized, rehydrated, then digested with proteinase K (10 µg/ml) for 10 min at 37 °C, and acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine hydrochloride. After washing with 2× SSC for 10 min, sections were hybridized with digoxigenin-labeled RNA probes in hybridization buffer (50% deionized formamide, 5× SSC, 1×Denhardt's , 0.1 mg/ml Salmon Sperm DNA, 0.1 mg/ml yeast tRNA) in a humidified chamber at 55 °C overnight. After hybridization, several washing steps followed: 10 min with 2× SSC at 50 °C, 20 min with 5× SSC at room temperature, 40 min with 20% formamide, 0.5× SSC at 60 °C, 15 min with 1×NTE (0.5 M NaCl, 10 mM Tris-HCl, pH 8.0, 5 mM EDTA) at room temperature, 30 min with 10 µg/ml RNase A in NTE at 37 °C, 30 min with 20% formamide, 0.5× SSC at 60 °C, and 30 min with 2× SSC at room temperature. Blocking was performed with a 10% blocking solution (Roche Applied Science). Antibody incubation and detection using anti-digoxigenin-alkaline phosphatase and NBT/BCIP were performed according to the manufacturer's instructions (Roche Applied Science). Sections were counterstained with Contrast Red (KPL Inc., MD, USA), and mounted with Clearmount (Invitrogen), and photographed with a Leica DC500 digital camera.