In situ hybridization

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Abstract

We generated a miR-340 probe by tagging Has-miR-340 aatcaG(L)t5(L)aT(L)tG(L)cT(L)ttataa_N(6)_Y with Alexa Fluor 488. LNA-*in situ* hybridization (ISH) was performed according to the manufacturer's instructions (http://www.exiqon.com/mirna-ish-kit). Sections of FFPE tissues of human OPLL and null mice (4-µm-thick) were fixed with 4 % PFA in PBS for 20 min at room temperature, washed with PBS (3 × 5 min), treated with 0.5 % Triton X-100 (10 min at 4 °C), and briefly washed with PBS followed by two washes (10 min each) in saline-sodium citrate buffer (2× SSC, 0.3 M NaCl, 0.03 M $Na_3C_6H_5O_7$, pH 7.0). The sections were then digested with 15 µg/mL proteinase K (Exiqon, Vedbaek, Denmark) at 37 °C for 10 min and rinsed for 3 × 5 min in PBS. Hybridization was performed in a humid chamber for 18 h at 65 °C. *In situ* hybridization of miR-340 was conducted using a miRCURY LNA microRNA ISH Optimization kit (Exiqon, Vedbaek, Denmark). miR-340-Alexa Fluor 488 and bone morphogenetic protein 2 (Alexa Fluor 555) were detected in human OPLL tissue by *in situ* hybridization and immunocytochemistry, respectively. Nuclei were counterstained with DAPI. After staining, the tissues were observed by fluorescence microscopy (BZ-X700, Keyence, Osaka, Japan).

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