

Resource Summary Report

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In situ hybridization

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Protocol Information

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Summary: 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 Na₃C₆H₅O₇, 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).

Associated Publications: Tsuru M, Ono A, Umeyama H, Takeuchi M, Nagata K (2018) Ubiquitin-dependent proteolysis of CXCL7 leads to posterior longitudinal ligament ossification. PLoS ONE 13(5): e0196204. doi: [10.1371/journal.pone.0196204](https://doi.org/10.1371/journal.pone.0196204)

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