



S5 Fig. Fluorescent signal of dye moiety located outside of duplex region. Partially-complementary ECHO (labelled base indicated as red-colored T^E) and DNA oligonucleotide shown in (A) were prepared. These two oligonucleotides form a hybrid where the labelled thymine is 7 nucleotides away from the double-stranded region. For fluorescence melting curve assay, ECHO and DNA oligonucleotides were dissolved in a buffer containing 1.4mM dNTP, 20mM Tris-HCl, 10mM (NH₄)₂SO₄, 8mM MgSO₄, 0.1% Tween-20, 10mM KCl at a concentration of 1 μM. Fluorescence melting curves were measured by the same method as described in the main text. The negative first derivative of the melting curve of this ECHO/DNA duplex (B) showed a melting temperature around 53 °C, which is a little higher than predicted melting temperature of 50°C for the DNA/DNA duplex of the underlined sequence.