

Figure S1: Steady state emission spectrum of fluorescein (Donor) attached to the 5' terminus of U6 snRNA as the only dye (black line), and with Cy3 labeled at 5' of U6-U2 chimeric RNA (blue line) in construct WT Δ L (Figure 1A) following excitation at 495 nm. Samples were 150 nM RNA in Tris (30 mM)-HEPES (60 mM), pH 7.6, 30 mM NaCl, 1 mM EDTA (no added Mg²⁺). The emission of an RNA sample with Cy3 (the acceptor) only following excitation at 495 nm (not shown to avoid distortion of the figure due to high emission peak of Cy3 alone) was subtracted from the FRET trace to eliminate contribution of direct excitation of Cy3 at this wavelength. The FRET efficiency is 13.7%, calculated from decrease in emission of the donor only, corresponds to a mean distance of ~ 76 Å between the dyes representing the mean distance of the four-helix junction conformer and the three-helix junction conformer in the wild-type.

