Figure SII. 15. Autoradiograms of 20% denaturing PAGE, showing the cleavage kinetics of target RNA in AON:RNA hydrides by *E. coli* RNase H1.

Conditions of cleavage reactions: Target 0.1 µM RNA (specific activity 80 000 cpm) and AON (2 µM) were incubated in a buffer containing 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM DTT at 21 ˚C in the presence of 0.08 U *E. coli* RNase H (obtained from USB corporation, Cleveland, Ohio USA). Prior to the addition of the enzyme, reaction components were preannealed in the reaction buffer by heating at 80 ˚C for 5 min followed by slow cooling to 21˚C and 30 min equilibration at this temperature. Total reaction volume was 30 µL. Aliquots of 3 µL were removed after 5, 10, 15, 30, and 60 min, and the reactions were terminated by mixing with stop solution [containing 0.05 M EDTA, 0.05% (w/v) bromophenol blue, and 0.05% (w/v) xylene cyanole in 80% formamide]. The samples were subjected to 20% 7 M urea PAGE and visualized by autoradiography. Pseudo-first order reaction rates could be obtained by fitting the digestion curves to single-exponential decay functions which were shown on the right of the PAGE picture.