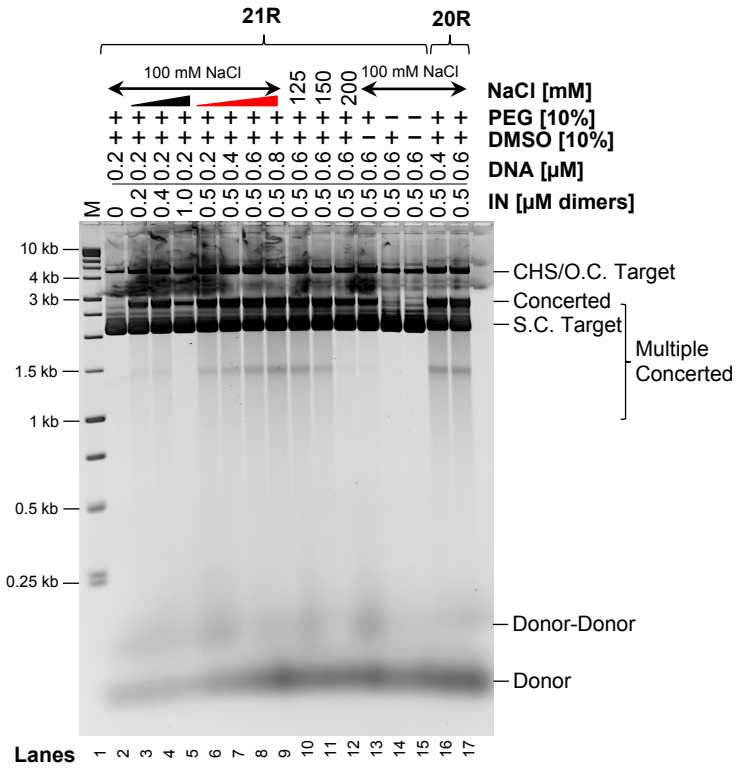
Supporting Information Figure S2



Supporting Information Figure S2. Optimized assay conditions for concerted integration by HIV IN with ODN substrates. Varying quantities of IN were preassembled with 21R (lanes 2 to 15) and 20R (lanes 16 and 17) at 14°C for 15 min followed by addition of target DNA. Strand transfer was for 1 h at 37°C. Reactions were stopped with EDTA to a final concentration of 25 mM, deproteinized, and the strand transfer products were analyzed on a 1.8% agarose gel. The gel was stained with SYBR GOLD and scanned on a Typhoon Trio Variable Mode Imager. The black triangle (lanes 3 to 5) have increasing concentrations of IN (0.2 μM to 1.0 μM as dimers) with a fixed quantity of 21R (0.2 μM). The red triangle (lanes 6 to 9) indicates a fixed concentration of IN (0.5 μM) with increasing quantities of 21R (0.2 to 0.8 μM). Lanes 10 to 12 had varying concentrations of NaCl at 125 mM, 150 mM and 200 mM, respectively, at a fixed IN (0.5 μM) to 21R (0.6 μM) ratio . All other reactions were carried out at 100 mM NaCl. Poly(ethylene glycol) (PEG) was omitted in the reactions carried out in lanes 14 and 15. DMSO was omitted from the reactions in lanes 13 and 15. Both 21R and 20R produce significant quantities of multiple concerted integration products, indicated on the right. Concerted and circular half-site (CHS) products are also marked on the right. O.C. and S.C. are open circle and supercoiled target, respectively. Lane 1 contains molecular weight markers.