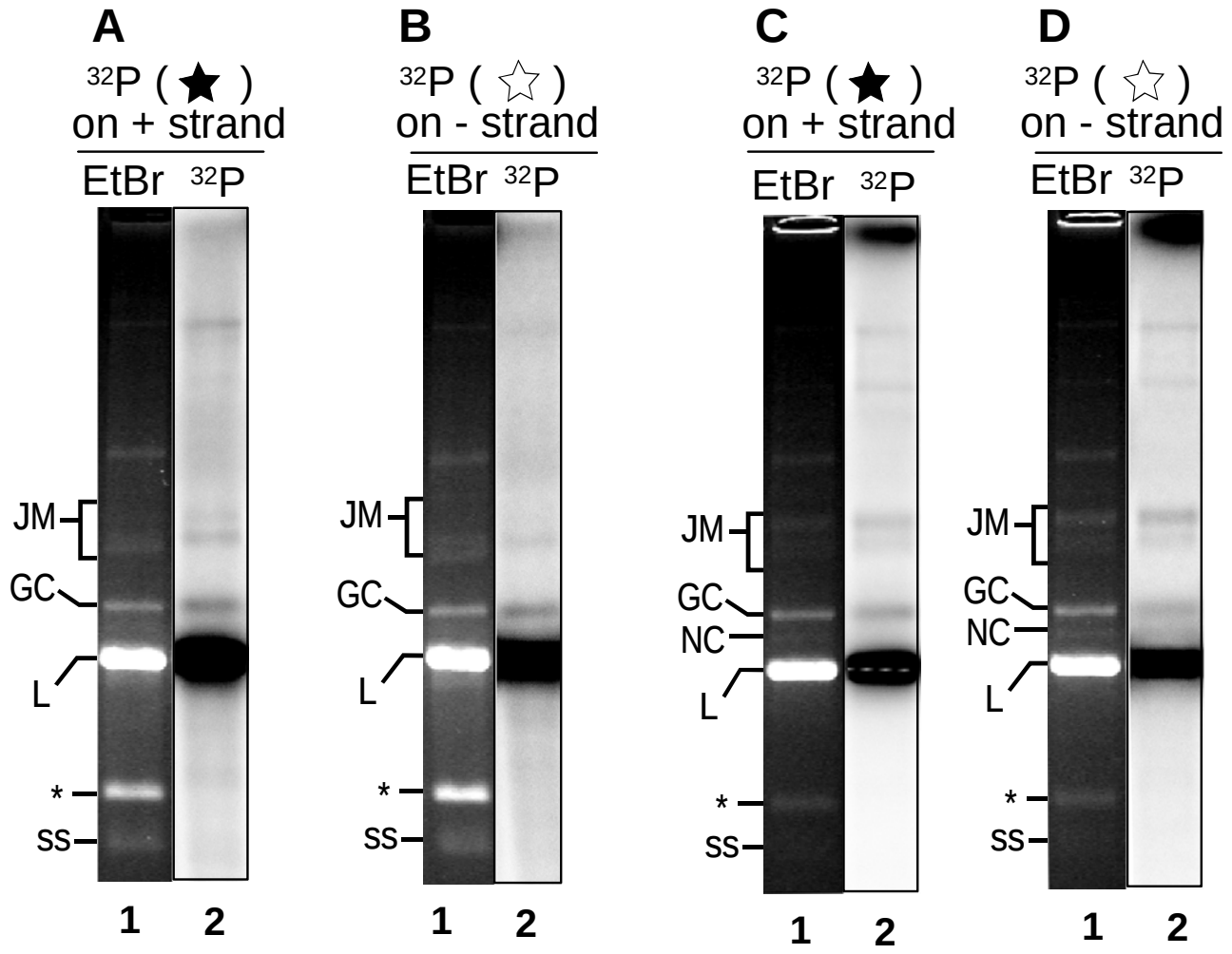

Supplementary Figure S2. Two-step DNA strand exchange and annealing was analyzed using the labeled substrates as shown in Figure 3, except that the second step was mediated by RecO and SSB under the same conditions as Figure 6 lane 7 **(A** and **B)**, or by Rad52 without RPA **(C** and **D)**.

RecO-mediated reaction produced only gapped circle DNA (A and B, lanes 1), and phosphorimaging (lanes 2) equally visualized the gapped circle regardless the ^{32}P is on plus- or minus-strand of the substrate dsDNA. When RPA was omitted from the second step (C and D), reaction produced gapped circle DNA and very low amount of nicked circle DNA (lanes 1). The gapped circle was visualized by ^{32}P on either DNA strand (C and D, lanes 2), but only the label at the minus-strand (D) visualized the nicked circle. These results support the idea that the annealing reaction by RecO and SSB, and by Rad52 without RPA cannot efficiently produce plasmid-sized dsDNA that is required for nicked circle production.