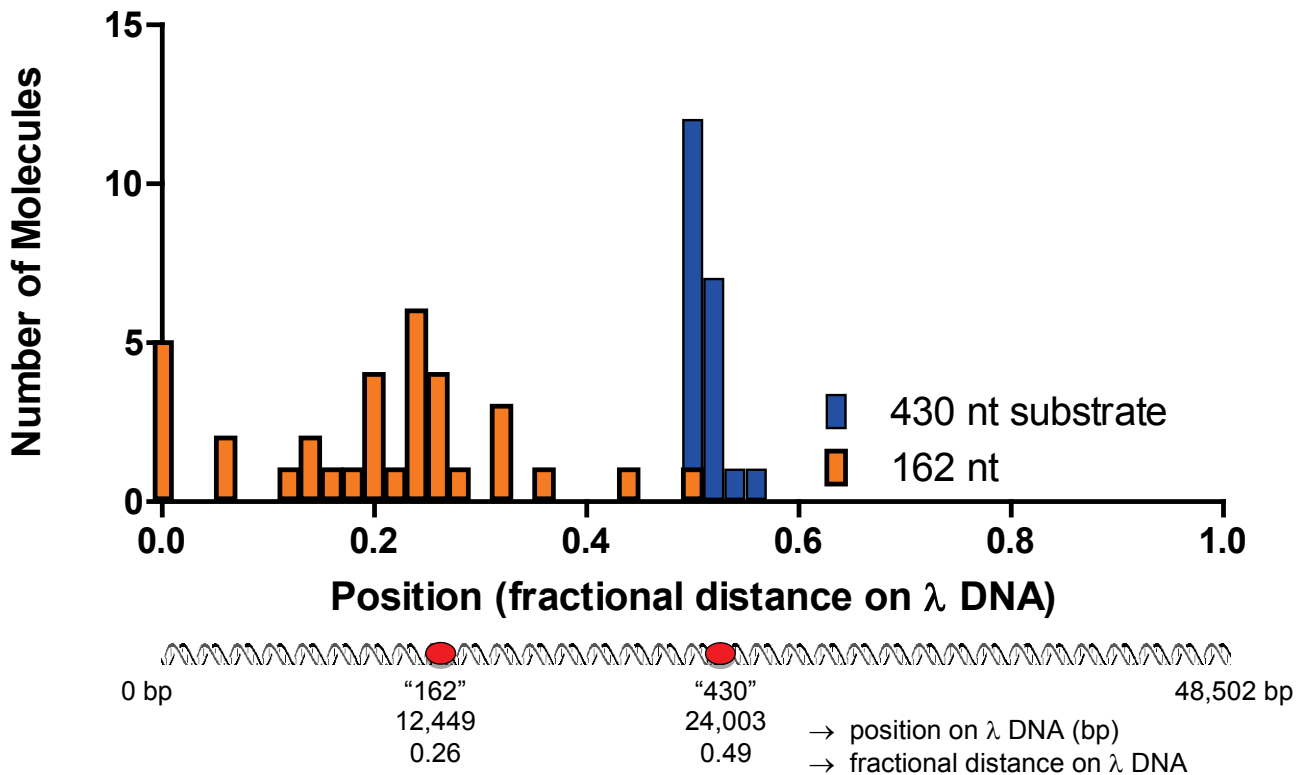
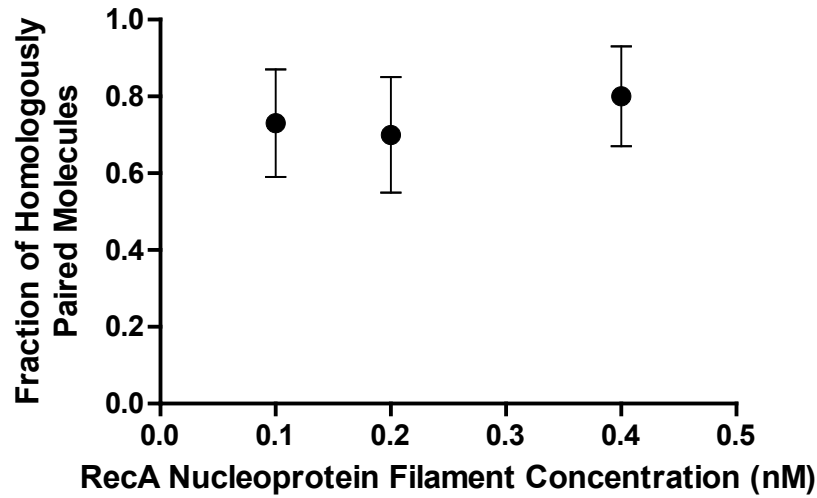


**Supplementary Figure 1 – Fluorescently modified ssDNA is a substrate for RecA-mediated joint molecule formation.** D-loop assays were performed using two independent preparations of fluorescently labeled 87 nt ssDNA and supercoiled pUC19. The degree of labeling for preps 1 and 2 was 1.4 and 4.6 dyes/ssDNA molecule, respectively. Lanes 1 and 2 are control reactions: lane 1, DNA substrates alone and lane 2, reaction with RecA lacking ATP<sub>γ</sub>S. Reactions with each ssDNA were performed in duplicate. Lanes 3 and 4 represent a complete reaction with fluorescent ssDNA prep 1 and lanes 5 and 6 with fluorescent DNA prep 2. The fluorescent signal from the Alexa Fluor 488 labeled ssDNA was imaged using a STORM scanner. Efficiency of joint molecule formation, expressed relative to the limiting dsDNA, is indicated below each lane.

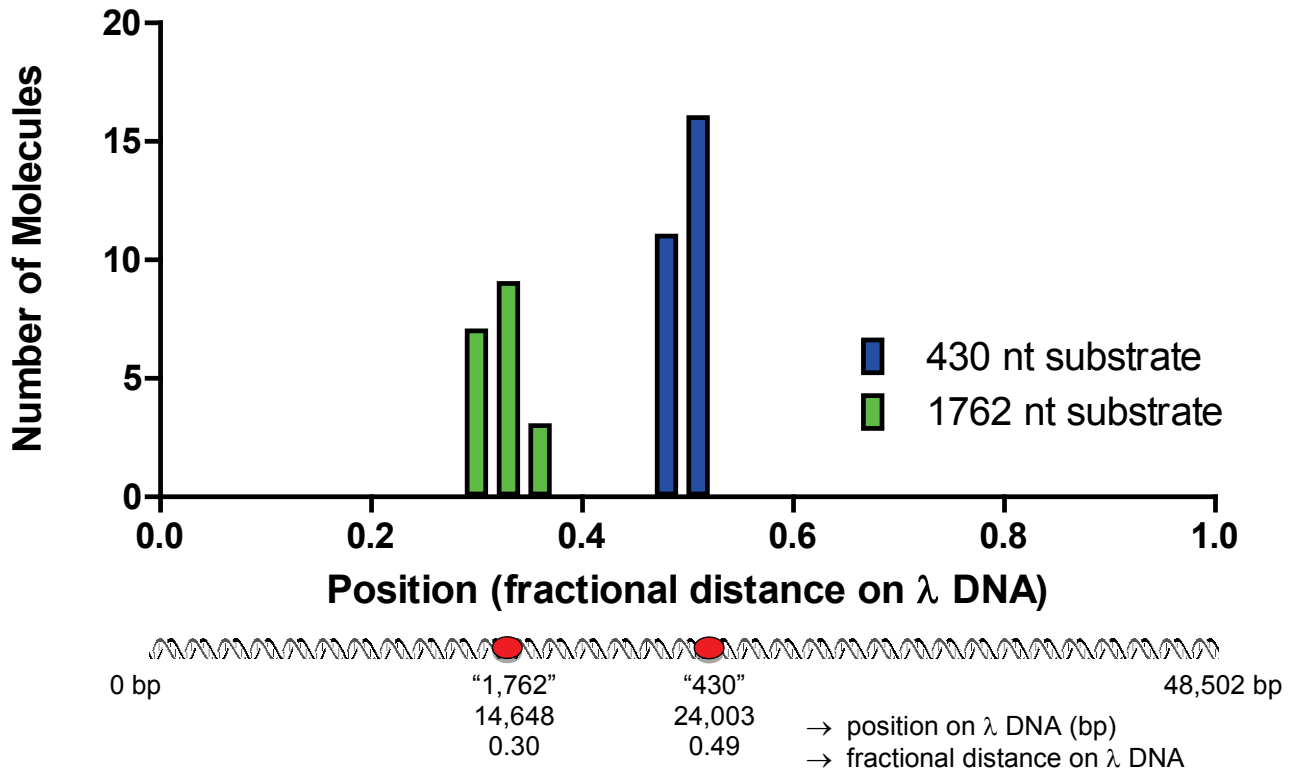


### Supplementary Figure 2 –RecA-mediated joint molecule formation in ensemble

**reactions, assayed by TIRFM.** Frequency distribution plot for the position of nucleoprotein filaments bound to surface tethered  $\lambda$  DNA. Ensemble reactions containing RecA nucleoprotein filaments and  $\lambda$  DNA were performed as described in the Methods Summary in microcentrifuge tubes. The products were then tethered to the surface of a flowcell, visualized by TIRFM, and analyzed with regard to position. The position is defined as the ratio of distance from the closest end of  $\lambda$  DNA to the observed nucleoprotein filament divided by total length of  $\lambda$  DNA: the mean observed position for the 162 nt substrate (orange) was  $0.20 \pm 0.12$  (standard deviation;  $n=34$ ) and for the 430 nt substrate (blue), it was  $0.51 \pm 0.02$  (standard deviation;  $n=21$ ). The predicted positions are 0.26 and 0.49 for the 162 nt substrate and 430 nt substrates, respectively.



**Supplementary Figure 3 – Varying the concentration of the RecA nucleoprotein filaments does not affect the yield of homologically paired products.** The DNA dumbbell pairing assay was performed using nucleoprotein filaments assembled on the 430 nt ssDNA at concentrations that were one-half and twice the standard reaction concentration (standard reaction is 0.2 nM). The bead separation was 2  $\mu\text{m}$  and the reaction was for 60 seconds. Error bars indicate SEM, with  $n \geq 10$  for each concentration tested.



**Supplementary Figure 4 – The stable products of DNA pairing with λ DNA are at the regions of homology.** Frequency distribution plot for the position of nucleoprotein filaments bound to λ DNA dumbbells. The position was defined as the ratio of distance from the closest end of λ DNA to the nucleoprotein filament divided by total length of λ DNA: the mean position for 430 nt substrate (blue) is  $0.50 \pm 0.01$  (standard deviation;  $n=27$ ) and for the 1,762 nt substrate (green), it is  $0.32 \pm 0.02$  (standard deviation;  $n=31$ ). The predicted positions are 0.49 and 0.30 for the 430 nt and 1,762 nt substrates, respectively

**Supplementary Movie 1 – Composite movie depicting the experimental procedure used to visualize DNA pairing on single DNA-dumbbell molecules by optical trapping.**

A DNA pairing reaction (2 min) was performed with the 430 nt substrate at a 2  $\mu\text{m}$  bead distance. Text and illustrations were inserted at appropriate places to facilitate description.

**Supplementary Movie 2 –** Movie showing RecA nucleoprotein filaments, both heterologously- and homologously-bound (left and right red spots, respectively) during the extension step (Fig. 2b, step 6) of a pairing assay performed using the 1,762 nt ssDNA. As the beads are separated, several loop-release events are observed involving the heterologously-bound filament (left) before its dissociation from  $\lambda$  DNA, whereas the homologously-bound RecA nucleoprotein filament (right) remains stably bound.

**Supplementary Movie 3 –** Movie showing RecA nucleoprotein filaments, both heterologously- and homologously-bound (left and right red spots, respectively) during the extension step (Fig. 2b, step 6) of a pairing assay performed using the 430 nt ssDNA. This is the molecule depicted in the kymograph of Figure 4a. As the beads are separated, the heterologously-bound filament (left) dissociates, releasing a loop of  $\lambda$  DNA that is observed as a sudden movement (jump) of the homologously-bound filament (right) to the right.