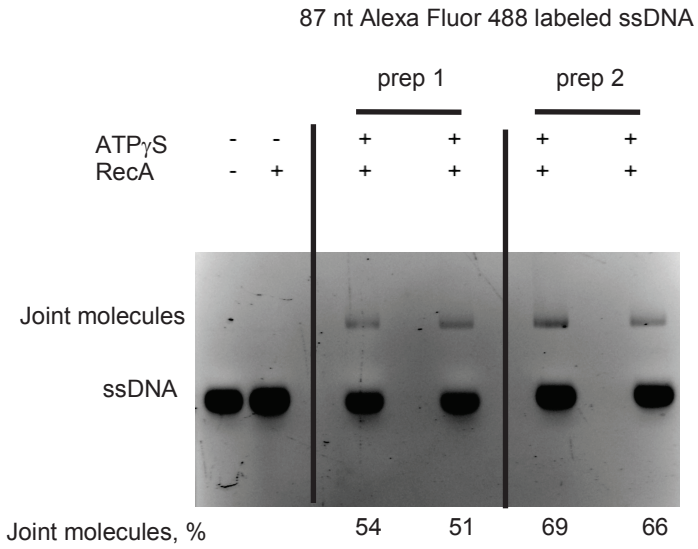
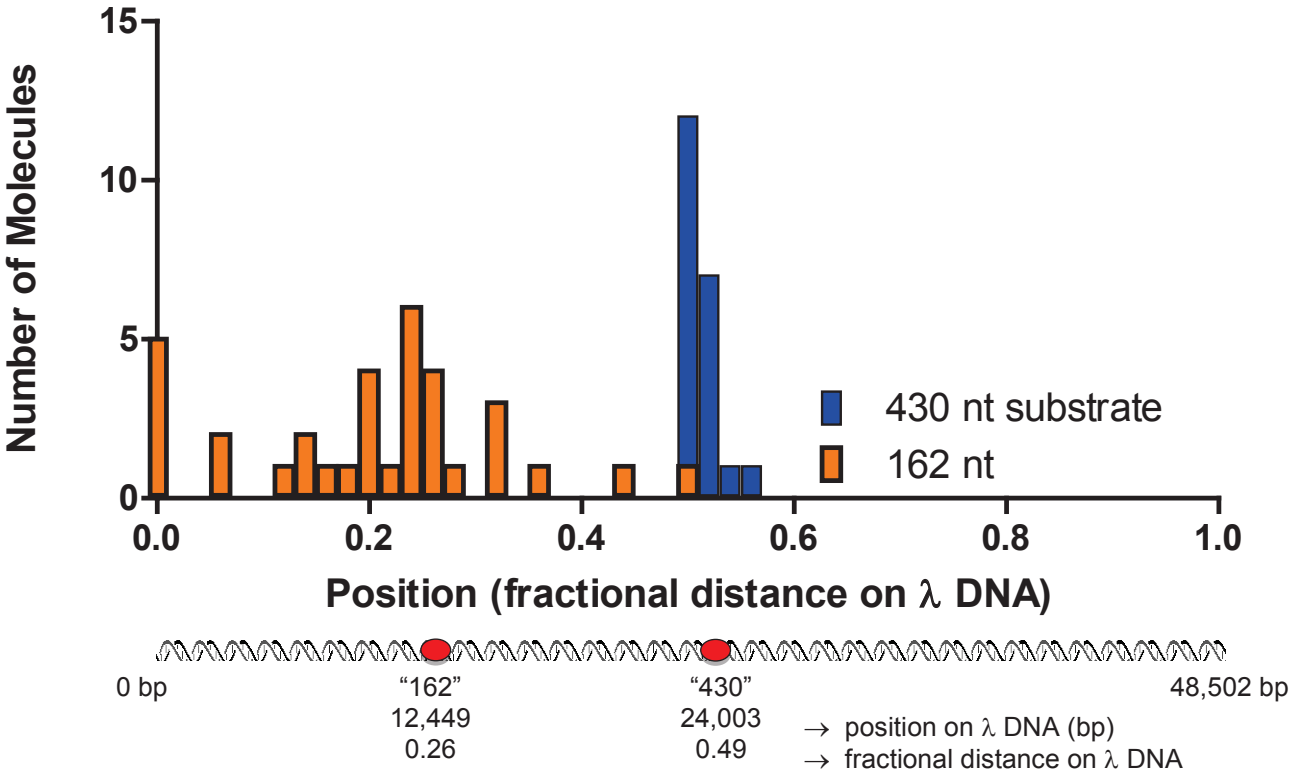
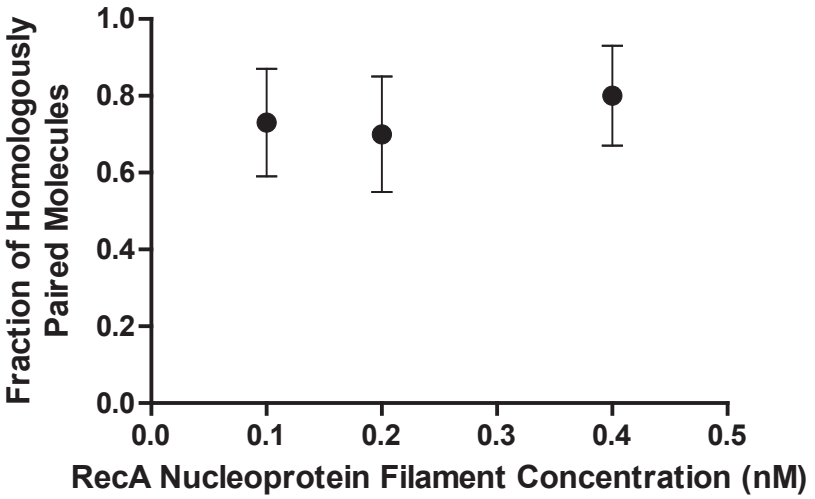
doi:10.1038/nature10782



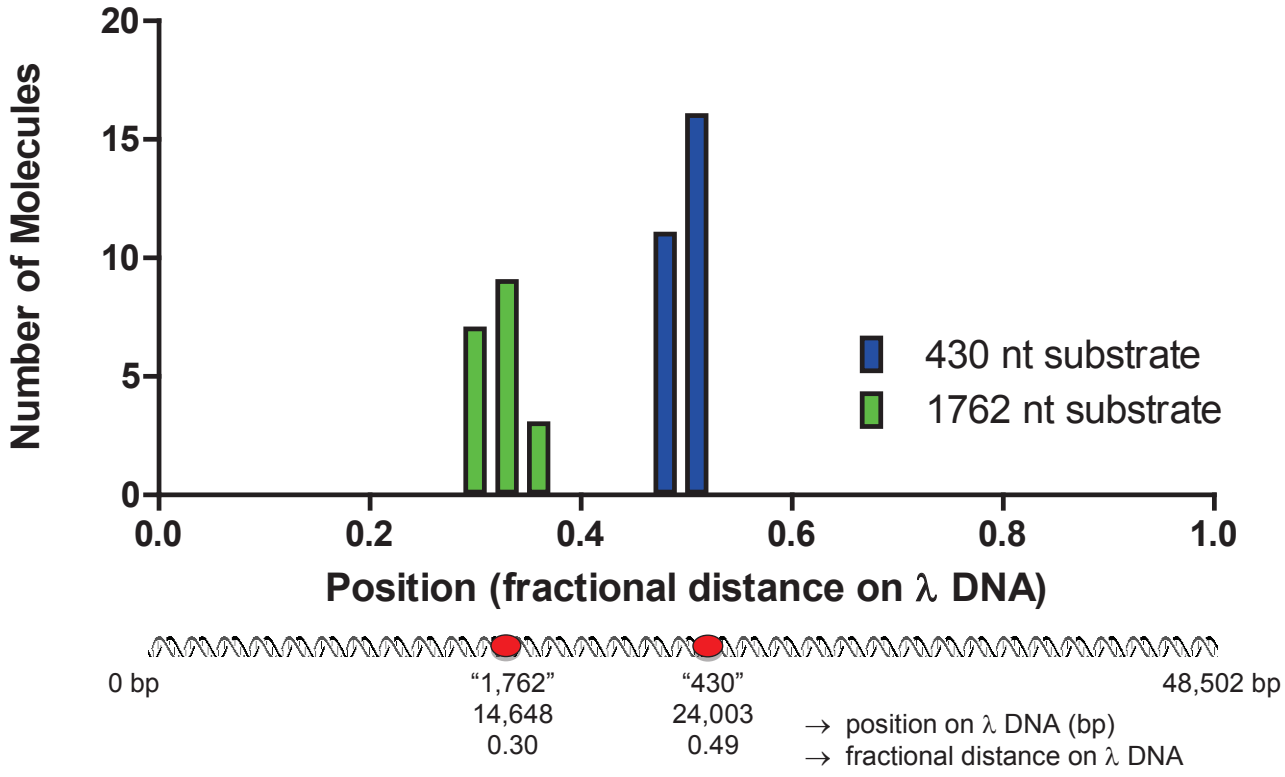
Supplementary Figure 1 – Fluorescently modified ssDNA is a substrate for RecA-mediated joint molecule formation. D-loop assays were performed using two independent preparations of fluorescently labeled 87 nt ssDNA and supercoiled pUC19**.** The degree of labeling for preps 1 and 2 was 1.4 and 4.6 dyes/ssDNA molecule, respectively. Lanes and 1 and 2 are control reactions: lane 1, DNA substrates alone and lane 2, reaction with RecA lacking ATPγS. 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 λ DNA. Ensemble reactions containing RecA nucleoprotein filaments and λ 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 λ DNA to the observed nucleoprotein filament divided by total length of λ DNA: the mean observed position for the 162 nt substrate (orange) was 0.20 ±0.12 (standard deviation; n=34) and for the 430 nt substrate (blue), it was 0.51±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 homologously 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 μm 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±0.01 (standard deviation; n=27) and for the 1,762 nt substrate (green) , it is 0.32±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 μm 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 λ 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 λ DNA that is observed as a sudden movement (jump) of the homologously-bound filament (right) to the right.