## Supplemental Materials and Methods

*Proteins and reagents*

Proteins were purified as described previously: RecA, RecQ, and SSB proteins (Harmon and Kowalczykowski 1998); RecF protein (Morimatsu and Kowalczykowski 2003); RecO and RecR proteins (Kantake et al. 2002); RecJ (Lovett and Kolodner 1989; Han et al. 2006); and RecA730 protein (Handa and Kowalczykowski 2007). Restriction enzymes, Klenow fragment and T4 polynucleotide kinase were products of New England Biolabs (NEB).

Chemical reagents, ATP, phosphoenolpyruvate, pyruvate kinase were purchased from Sigma. Shrimp alkaline phosphatase was purchased from United States Biochemical Corp. Proteinase K was product of Roche Molecular Biochemicals. $\alpha^{32}$P-dATP and $\gamma^{32}$P-ATP were purchased from Perkin-Elmer Corp.

*DNA substrates*

Homologous plasmid DNA substrates, pUC19 (2.7 kb) and pUC1950 (4.6 kb) (Harmon and Kowalczykowski 1998), were purified by alkaline lysis followed by CsCl centrifugation. Linear pUC19 DNA was prepared by digestion with EcoRI or HindIII, and then 3'-end labeled with $\alpha^{32}$P-dATP by using Klenow fragment (NEB) (Arnold et

al. 1998) or 5'-end labeled with $\gamma^{32}$P-ATP by using T4 polynucleotide kinase after shrimp alkaline phosphatase treatment.

*Joint molecule assays*

Aliquots were taken, mixed with stop solution (40 mM EDTA, 0.8% SDS, 1.5 μg/μl Proteinase K and 0.09% bromophenol blue), and placed on ice. When all samples were collected, they were incubated at room temperature for 10 min and analyzed by electrophoresis in 0.8% agarose with 1x TAE run at approximately 1.5 V/ cm for 15 hrs. After the electrophoresis, the gels were dried and analyzed on a Molecular Dynamics Storm 860 PhosphorImager using Image-QuaNT software to quantify the products.

*Two-dimensional electrophoresis of joint molecule products*

Two-dimensional electrophoresis was performed as described previously (Nimonkar and Boehmer 2003), with some modifications. Each reaction was run in duplicate in the first dimension through 0.8 % agarose in TEA at 1 V/cm for 17 hours. One of the lanes was used as a reference to visualize the products resolved in the first dimension (shown as the top lane in the figure). The other lane was excised, soaked in alkaline buffer (50 mM NaOH and 1 mM EDTA), embedded in a second gel consisting

of 1% agarose in the alkaline buffer, and electrophoresis was performed at 0.5 V/cm for 23 hours. Markers used for second dimension were prepared by the digestion of lambda DNA with HindIII, followed by labeling with [α-$^{32}$P]dATP using the Klenow fragment of DNA polymerase. After drying, the 1D and 2D gels were manually aligned and then imaged using a Molecular Dynamics Storm 860 PhosphorImager.

## References


Arnold, D.A., Bianco, P.R., and Kowalczykowski, S.C. 1998. The reduced levels of χ recognition exhibited by the RecBC$^{1004}$D enzyme reflect its recombination defect *in vivo*. *J. Biol. Chem.* **273**: 16476-16486.

Han, E.S., Cooper, D.L., Persky, N.S., Sutera, V.A., Jr., Whitaker, R.D., Montello, M.L., and Lovett, S.T. 2006. RecJ exonuclease: substrates, products and interaction with SSB. *Nucleic Acids Res.* **34**: 1084-1091.

Handa, N. and Kowalczykowski, S.C. 2007. A RecA mutant, RecA(730), suppresses the recombination deficiency of the RecBC(1004)D-chi* interaction in vitro and in vivo. *J. Mol. Biol.* **365**: 1314-1325.

Harmon, F.G. and Kowalczykowski, S.C. 1998. RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. *Genes Dev.* **12**:

1134-1144.

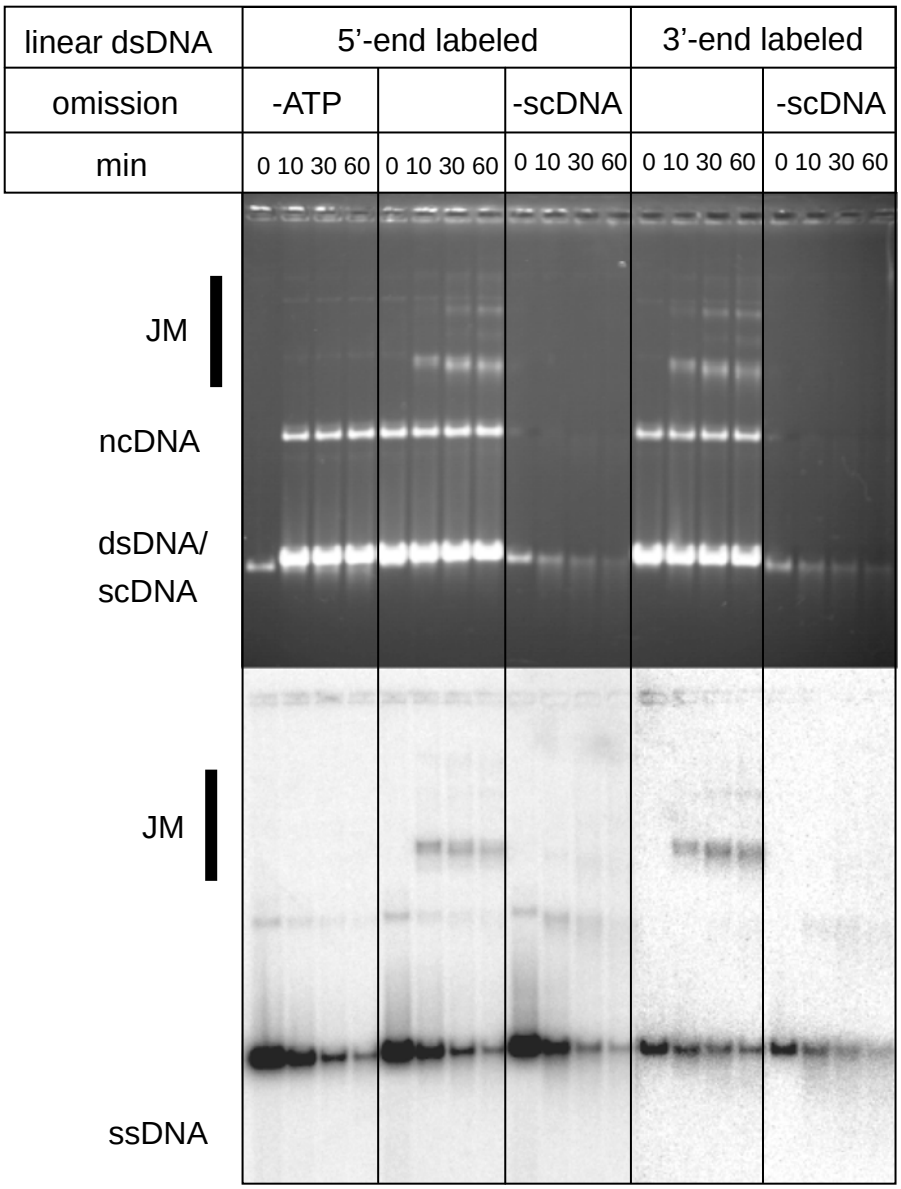
Kantake, N., Madiraju, M.V., Sugiyama, T., and Kowalczykowski, S.C. 2002. Escherichia coli RecO protein anneals ssDNA complexed with its cognate ssDNA-binding protein: A common step in genetic recombination. *Proc. Natl. Acad. Sci. U. S. A.* **99**: 15327-15332.

Lovett, S.T. and Kolodner, R.D. 1989. Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the recJ gene of Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* **86**: 2627-2631.

Morimatsu, K. and Kowalczykowski, S.C. 2003. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. *Mol. Cell* **11**: 1337-1347.

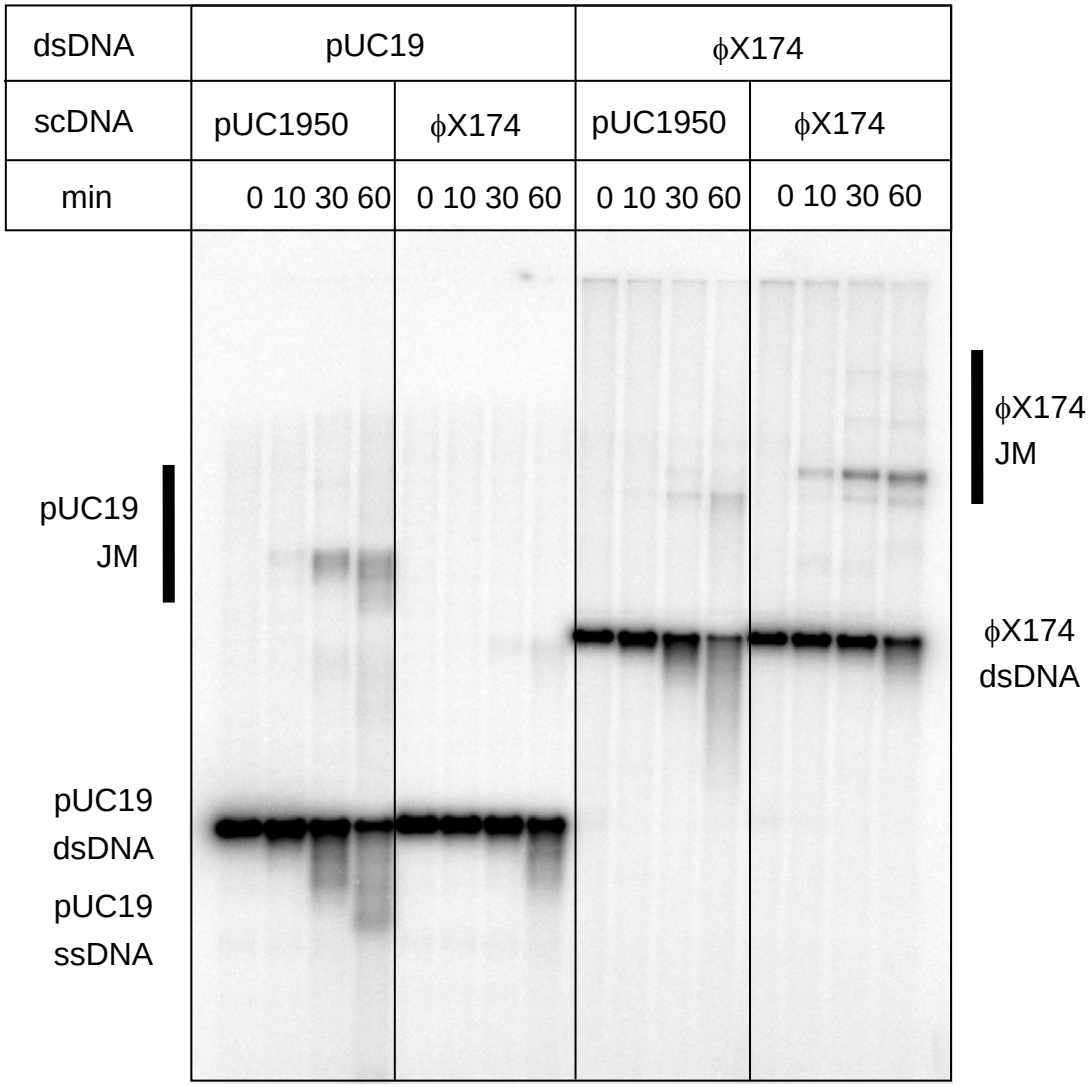
Nimonkar, A.V. and Boehmer, P.E. 2003. Reconstitution of recombination-dependent DNA synthesis in herpes simplex virus 1. *Proc. Natl. Acad. Sci. U. S. A.* **100**: 10201-10206.

Nimonkar, A.V., Ozsoy, A.Z., Genschel, J., Modrich, P., and Kowalczykowski, S.C. 2008. Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 16906-16911.

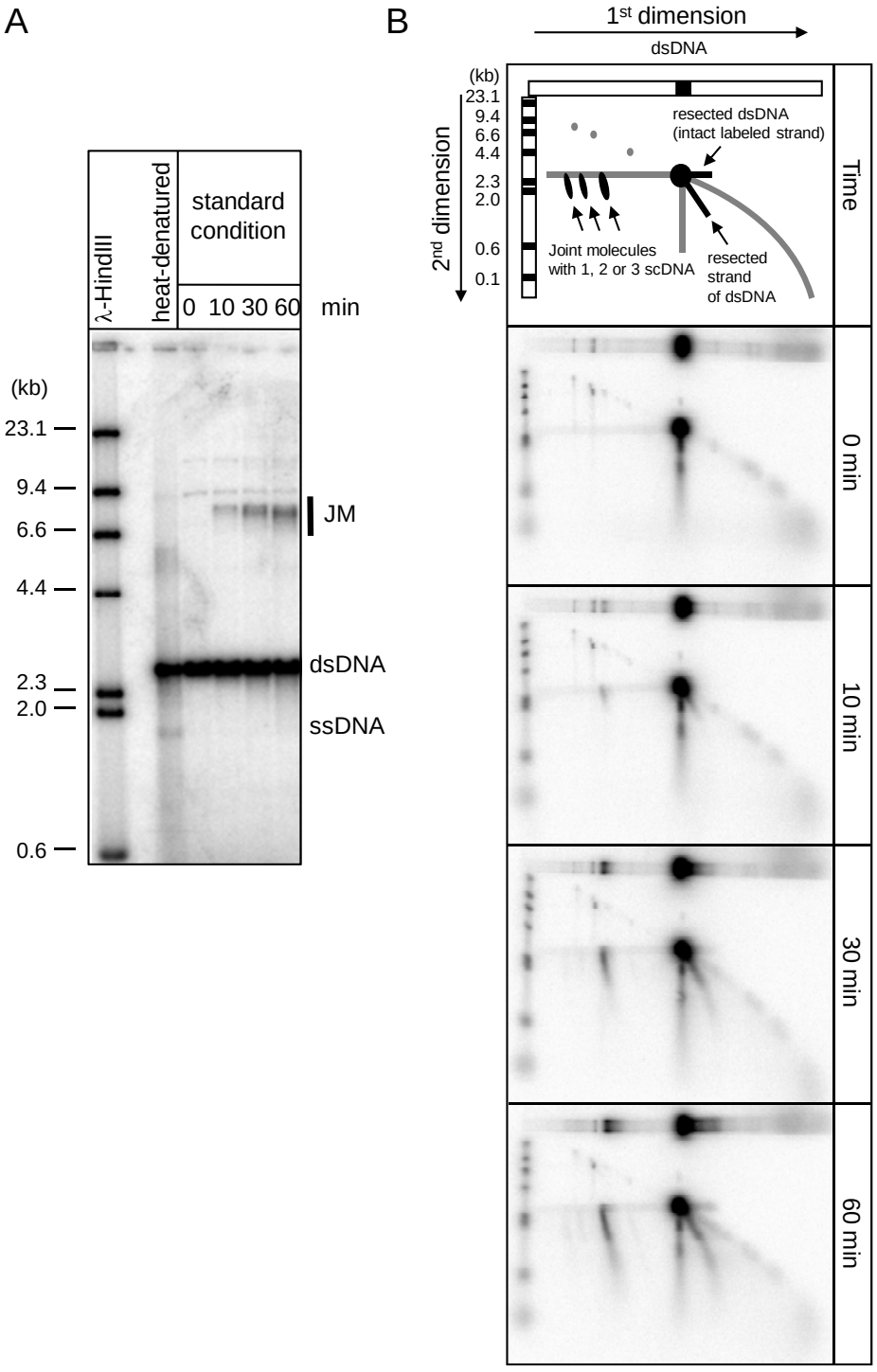



**Supplemental Figure 1**: Joint molecule formation by the coordinated action of RecA, RecF, RecO, RecR, RecQ, RecJ, and SSB proteins requires ATP and a supercoiled DNA target. Standard reaction conditions with pUC1950 as the supercoiled DNA were used, except that the indicated component (ATP or supercoiled DNA) was omitted. Note that supercoiled pUC1950 ("scDNA") runs in the same position as the linear pUC19 DNA ("dsDNA"); this is evident in the first lane, where the supercoiled DNA was

intentionally omitted, and in the reactions without supercoiled DNA ("-scDNA"). The scDNA contained nicked circular DNA ("ncDNA") as a contaminant, which is evident in the ethidium bromide-stained gel. The faint band above the ncDNA is linear pUC19 dimer. The DNA species with a lower mobility than the linear dsDNA that are seen in the absence of scDNA result from annealing of the ssDNA-tailed dsDNA that has been resected beyond the midpoint (Nimonkar et al. 2008). The multiple joint molecule ("JM") products are indicated. The 5'-end labeled DNA was HindIII-digested pUC19 labeled using T4 polynucleotide kinase and [γ-P32] ATP after shrimp alkaline phosphatase treatment; the 3'-end labeled DNA was EcoRI-digested pUC19 labeled using T4 polynucleotide kinase and [α-P32] dATP. Top panels: ethidium bromide staining; bottom panels: radioisotopic imaging. Due to loss of the 5'-end label by RecJ exonuclease, joint molecule yield based on radioisotopic quantification is an underestimate. The comparison of joint molecule yield is therefore based on the gels stained with ethidium bromide; the yield of joint molecules at 60 minutes in each reaction set is (from left to right): 0%, 17%, 0%, 23%, and 0%.

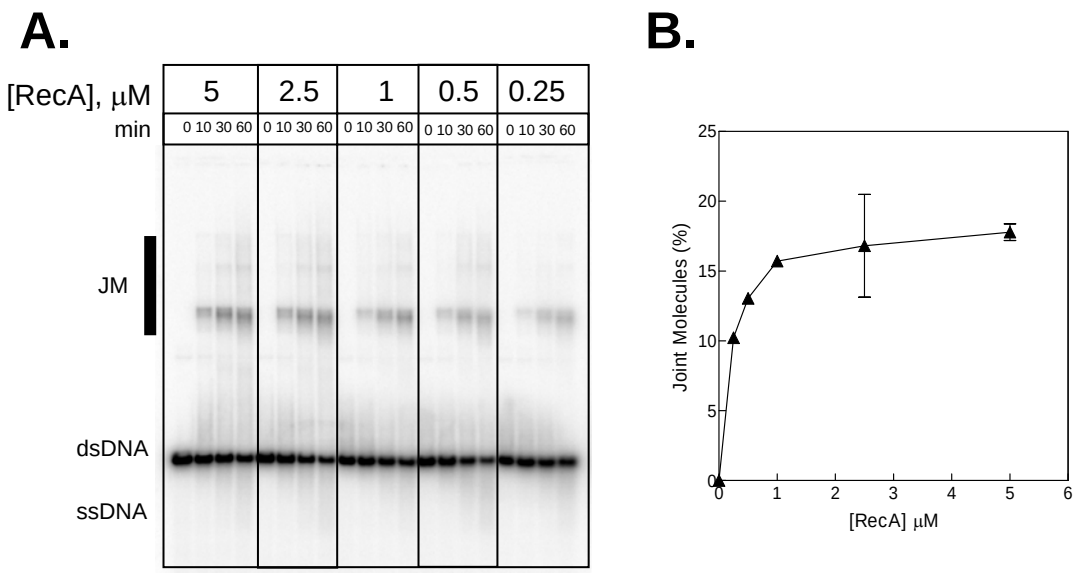



**Supplemental Figure 2**: Joint molecule formation requires homologous DNA partners. Standard joint molecule reaction conditions were used except that the DNA pairs (pUC19 or ϕX174 (New England Biolabs)) were 10 μM for the 3'-end labeled linear dsDNA (EcoRI-digested pUC19 or MfeI-digested ϕX174) and 20 μM for the scDNA. The apparent DNA pairing product in the heterologous reactions at the longer times is due to annealing of the highly resected linear dsDNA upon deproteinization. The yield of joint molecules at 60 minutes is (from left to right): 9%, 0%, 0.4%, and 10%.

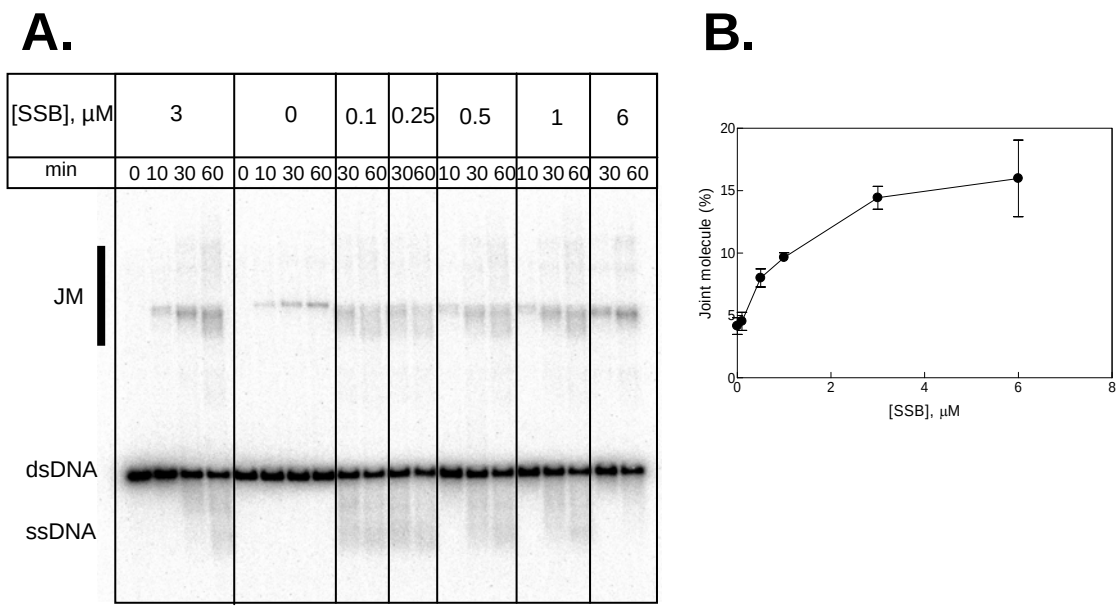



**Supplemental Figure 3**: One and two-dimensional gel electrophoresis of reaction products. A) Standard joint molecule assay conducted for 0, 10, 30, and 60 min

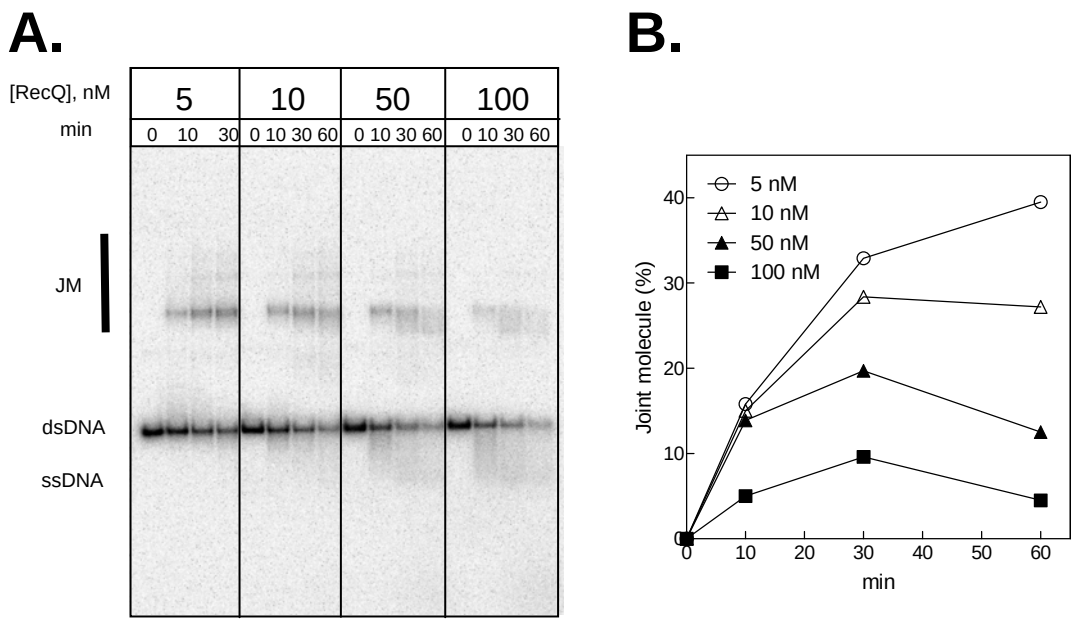
analyzed by one-dimensional electrophoresis. B) Two-dimensional electrophoresis: DNA was analyzed by native gel electrophoresis in the first dimension; one of two replicate lanes is shown at the top. The other gel slice was subjected to electrophoresis in a second dimension using denaturing conditions. The illustration at the top defines each of the reaction products; the 0 and 60 minute time points are the same results as in Figure 1C. The markers used for the second electrophoresis (shown at the left) were HindIII-digested lambda DNA, as in panel A. The reaction products are indicated by arrows; the other spots are nonspecific background spots which are present at time zero. The yield of joint molecule products at 60 minutes is (from right to left): 7%, 0.5%, and 0.2%.



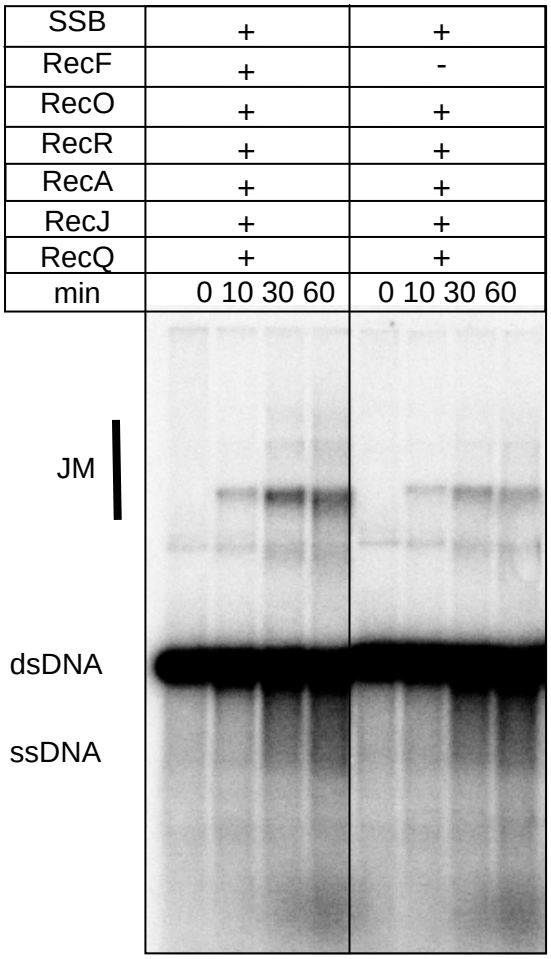

**Supplemental Figure 4**: The RecA protein concentration dependence of joint molecule formation in the RecAFORQJ reaction. The standard reaction conditions described in Materials and Methods were used, except that the RecA protein concentration was varied as indicated; 3'-end labeled EcoRI-digested linear pUC19 and supercoiled pUC1950 DNA were used. **A**. Agarose gel showing the time course of homologous pairing at the indicated RecA concentrations. **B**. Quantification of joint molecules formed at 60 min from panel "**A**". Error bars show standard error for replicate experiments; points without error bars were performed once, hence, error bars are not shown.



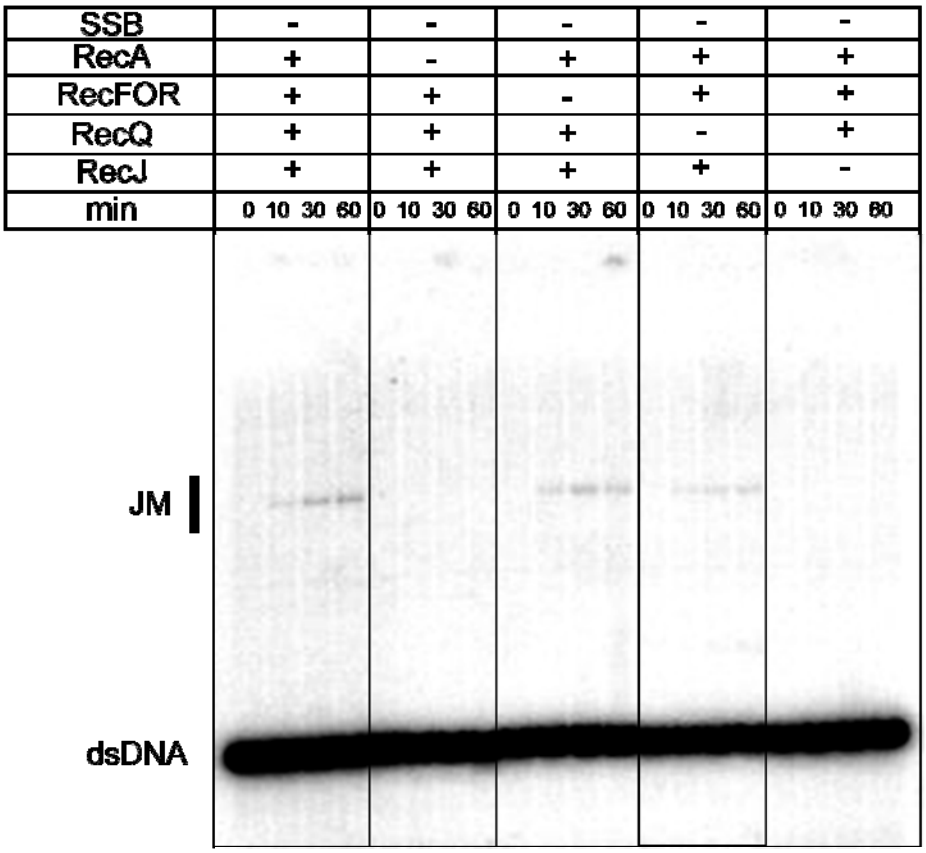

**Supplemental Figure 5**: The SSB protein concentration dependence of joint molecule formation in the RecAFORQJ reaction. The standard reaction conditions described in Materials and Methods were used, except that the SSB protein concentration was varied as indicated; 3'-end labeled EcoRI-digested linear pUC19 and supercoiled pUC1950 DNA were used. **A**. Agarose gel showing the time course of homologous pairing at the indicated SSB concentrations. **B**. Quantification of joint molecules formed at 60 min from panel "**A**"; error bars represent the standard error.




**Supplemental Figure 6**: RecQ stimulates DNA resection but inhibits joint molecule formation in the RecAFORQJ concerted reaction. The standard reaction conditions described in Materials and Methods were used, except that the RecQ helicase concentration was varied as indicated; 3'-end labeled EcoRI-digested linear pUC19 and supercoiled pUC1950 DNA were used. **A**. Agarose gel showing the time course of homologous pairing at the indicated RecQ concentrations. **B**. Quantification of the kinetics of joint molecules formation at each RecQ concentration from panel "**A**". Because this particular time-course was performed once, error bars are not shown; however, a similar experiment using 5'-end labeled linear pUC19 instead, yielded comparable trends.




**Supplemental Figure 7**: RecF protein stimulates the RecAFORQJ reaction at a low concentration of RecOR proteins. The standard reaction conditions described in Materials and Methods were used, except that RecO and RecR protein concentrations were 15 nM and 30 nM, respectively. The DNA was linear 3'-end labeled EcoRI-digested pUC19 and supercoiled pUC1950. The yield of joint molecules at 60 minutes is (from left to right): 4% and 1%.




**Supplemental Figure 8**: In the absence of SSB protein, joint molecule formation by RecA and RecJ does not require RecFOR proteins. Reactions were conducted as described in the Materials and Methods, except that both SSB and the indicated proteins were omitted. The yield of joint molecules at 60 minutes is (from left to right): 0.4%, 0%, 0.5%, 0.3%, and 0%.