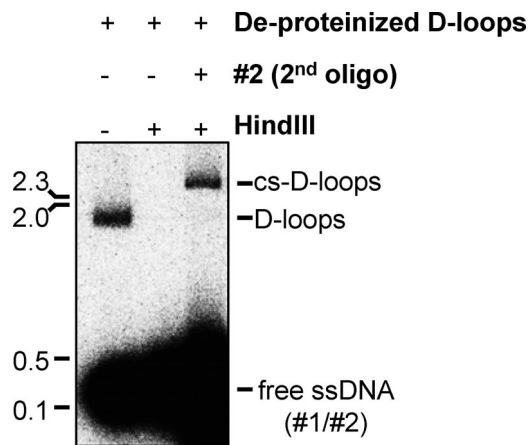
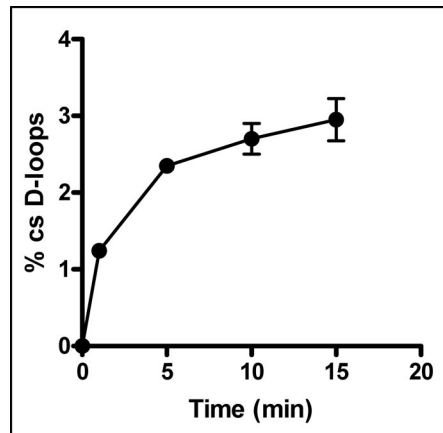


# Supporting Information

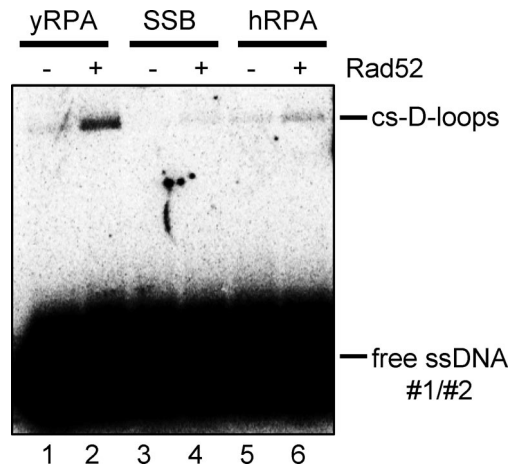
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**Fig. S1.** Rad51 and Rad54 are not required for the second step of complement-stabilized D-loop formation. Single D-loops with oligonucleotide 1 were formed as described in *Materials and Methods*. The D-loops were deproteinized by extraction using a Qiagen PCR cleanup kit. The purified D-loops were tested for cs-D-loop formation by using standard reaction conditions. Left lane, single D-loops; middle lane, single D-loops digested with HindIII; right lane, cs-D-loops treated with HindIII. The positions of D-loops, cs-D-loops, free oligonucleotides, and molecular size markers (kbp) are indicated.



**Fig. S2.** Time course of complement-stabilized D-loop formation. Standard reactions were performed as described. The concentrations of replication protein A (RPA) and Rad52 were 0.4 and 0.8  $\mu\text{M}$ , respectively. Aliquots were withdrawn at 0, 5, 10, and 15 min and terminated. The percentage of cs-D-loops was expressed with respect to the limiting supercoiled DNA concentration.



**Fig. S3.** Rad52 cannot form complement-stabilized D-loops in the presence of heterologous ssDNA-binding proteins (SSBs). Standard reactions were performed as described. Yeast RPA (yRPA), *Escherichia coli* SSB, or human RPA (hRPA) was present at 0.4  $\mu$ M. When included, the concentration of Rad52 was 0.8  $\mu$ M. Lanes 1 and 2, RPA in the absence and presence of Rad52, respectively; lanes 3 and 4, SSB in the absence and presence of Rad52, respectively; lanes 5 and 6, hRPA in the absence and presence of Rad52, respectively. The positions of D-loops, cs-D-loops, and free oligonucleotides are indicated.