SUPPLEMENTAL INFORMATION

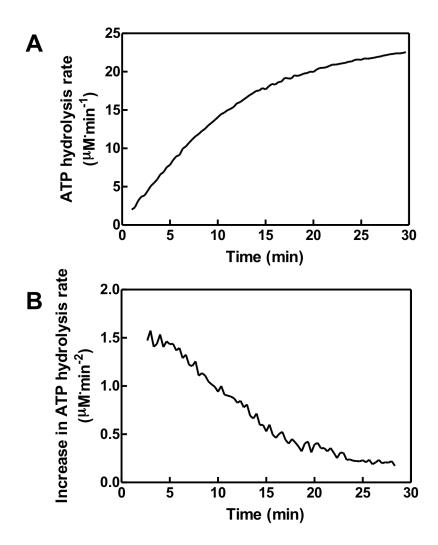
Supplemental Table 1. Oligonucleotides used.

Sequences are shown in 5' to 3' direction.

KAT-32	TCACCAATGAAACCATCGATAGCAGCACCGTAATC
KAT-33	AAAATATCTTTAGGTGCACTAACAACTAATAGATT
KAT-38	AATCTATTAGTTGTTAGTGCACCTAAAGATATTTT
KAT-71	GGAAGCGATAAAACTCTGCAGGTTGGATACGCCAA
KAT-72	AGGCATCCACGGCGCTTTAAAATAGTTGTTATAGA
KAT-73	CGACCATTCAAAGGATAAACATCATAGGCAGTCGG
KAT-74	CTGCTTATGGAAGCCAAGCATTGGGGATTGAGAAA
KAT-76	TTGGCGTATCCAACCTGCAGAGTTTTATCGCTTCC
фх-117F	GGACTGCTGGCGGAAAATGAGAAAATTCGACCTATCCTTGCGCAGCTCGA
φX-166R	TCGAGCTGCGCAAGGATAGGTCGAATTTTCTCATTTTCCGCCAGCAGTCC

DNA	gDNA 100 (5'-RNA)		gDNA 100 (5'-DNA)		gDNA 100 (5'-RNA)	
RNaseA	_		-		+	
Klenow	ı	+	ı	+	ı	+
	1	2	3	4	5	6

Supplemental Figure 1. The RNA in gDNA is intact during the RecA-loading reaction. A portion of sample that was used for the ATPase assay shown in Figure 6B (black line, at 10 min) was used to verify that the RNA in the gDNA substrate remained intact after the assay. To this sample, $[\alpha^{-32}P]dATP$, dTTP, dCTP and ddGTP were added to final concentrations of 0.1, 20, 20, and 10 μM, respectively. Klenow fragment (3' \rightarrow 5' exo. New England Biolabs) was added at final concentration of 50 Units/ml. After 0 (lanes 1, 3, and 5) and 2 min (lanes 2, 4, and 6) incubation, aliquots was taken and mixed with equal amount of stop buffer containing 82% formamide and 0.08% xylene cyanol. The samples were incubated at 100 °C for 10 minutes and analyzed by sequencing gel electrophoresis (6% polyacrylamide with 8 M urea) and autoradiography. RNA-containing gDNA-100 sample after the ATPase assay (lanes 1-2); controls, showing the conventional gDNA-100 lanes 3-4), and RNA-containing gDNA treated with RNaseA (lanes 5-6). RNaseA-treated sample was prepared by adding 0.2 μg RNaseA to 10 μl gDNA stock solution (500 μM nts) followed by 30 min incubation at 37 °C.



Supplemental Figure 2. The rate of elongation for a RecA filament on gDNA is ~60-80 RecA molecules per minute. A) The same experiment shown as black line in Figure 6A was repeated 8 times and the data was averaged to improve signal to noise. B) Graph showing the increase in ATP hydrolysis rate per minute (first derivative of panel A). The initial rate (data at less than 5 min) is $1.5 \, \mu \text{M} \cdot \text{min}^2$. From the k_{cat} of RecA ATPase activity (20-25 min⁻¹), this value corresponds to a 60-75 nM increase of concentration of DNA-bound RecA protein in sample per minute. Since the molecule concentration of gDNA in sample is 0.93 nM, this corresponds to the binding of 60-80 RecA molecules to a gDNA molecule per minute.