Supporting Information

Yao et al. 10.1073/pnas.0906157106

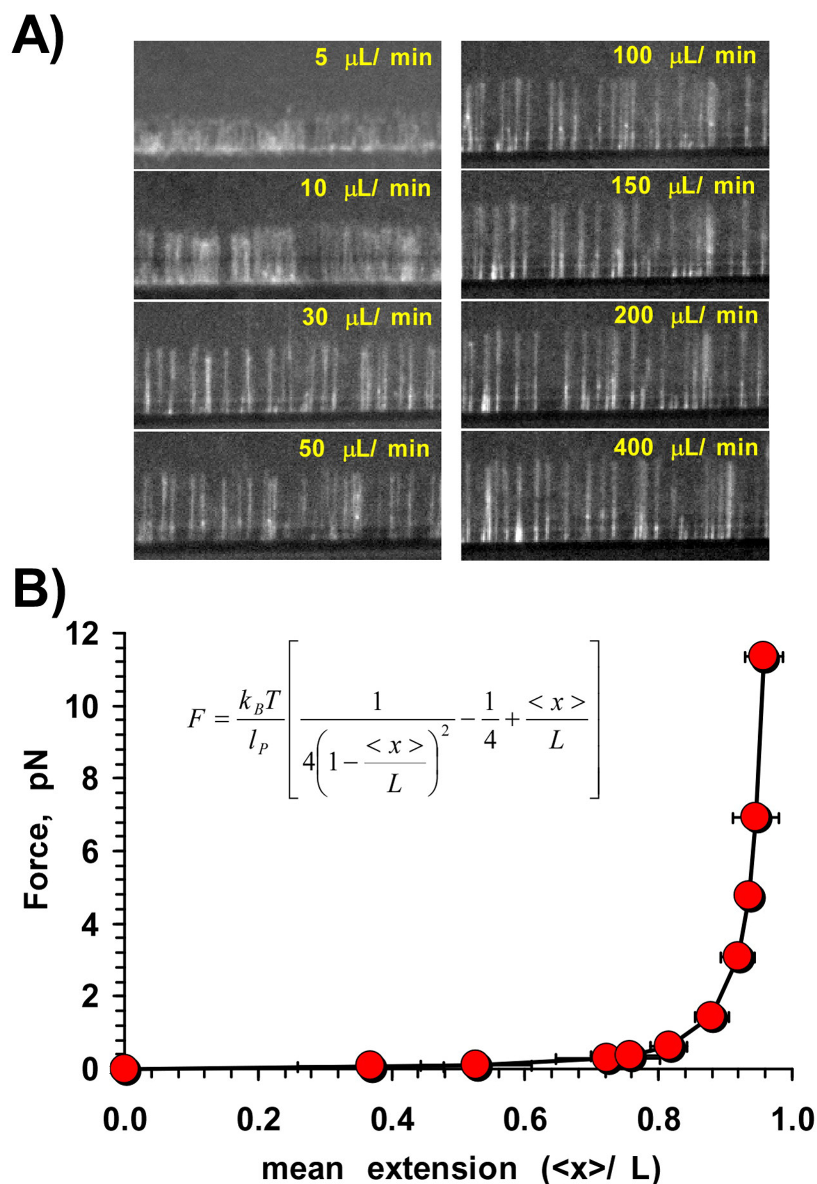


Fig. S1. Extension of immobilized lambda-DNA at different flow rates. (*A*) Series of images of a DNA curtain of lambda DNA taken at the specified flow rates. When corrected for the dimensions of the flow-cell chamber (0.25 × 0.0047 cm, W × H), these values correlate to flow velocities of 0.07, 0.14, 0.43, 0.71, 1.42, 2.13, 2.84, and 5.67 cm/s. (*B*) Relative mean extension (<x>/L) is plotted as a function of calculated force. The experimental data points are shown as red circles with corresponding standard deviations; the solid line is a fit of the data points to the equation shown, describing the worm-like chain model for DNA (*Inset*), where F is force (in pN), k_B is Boltzmann's constant, T is temperature (295 K), and lp is the persistence length of DNA (50 nm).

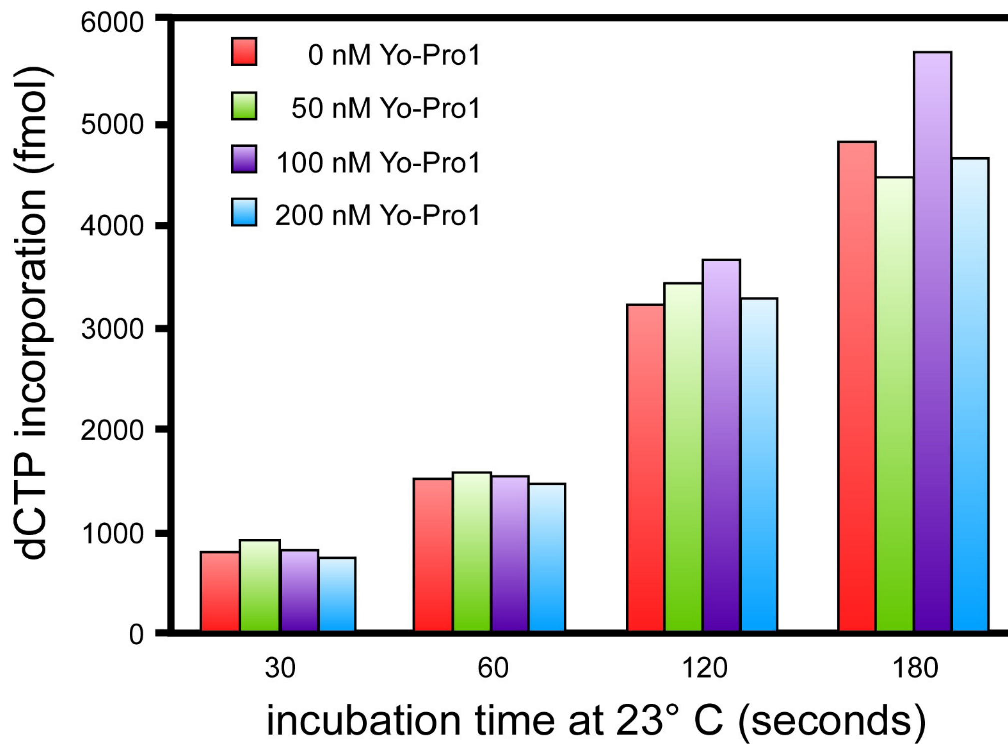
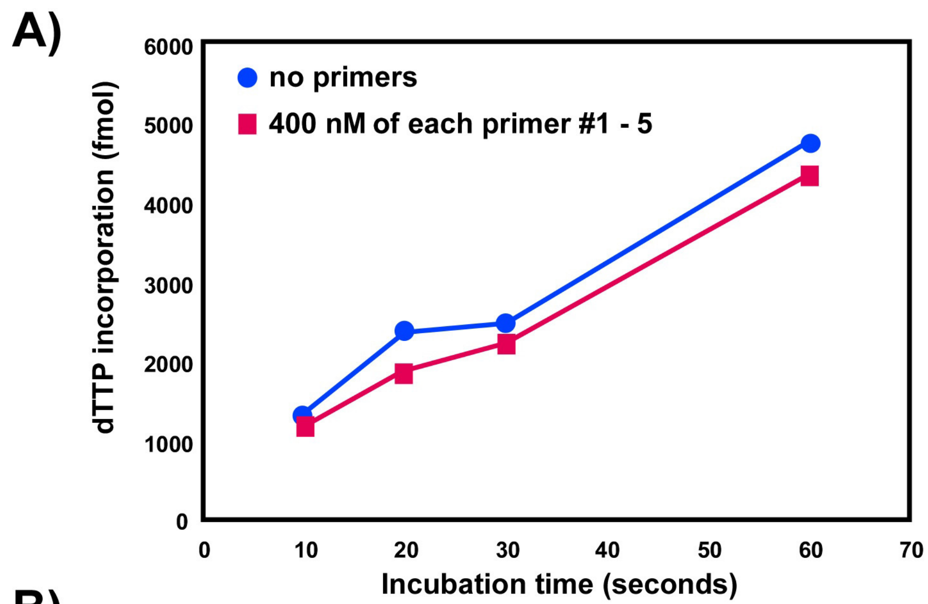


Fig. S2. Yo-Pro-1 does not inhibit leading and lagging strand rolling circle replication. Leading and lagging strand ensemble replication reactions in the presence of 0, 50, 100, and 200 nM Yo-Pro-1. Replication was performed at 23 °C for the indicated times and monitored by incorporation of ^{32}P-dCTP in both leading and lagging strand products.



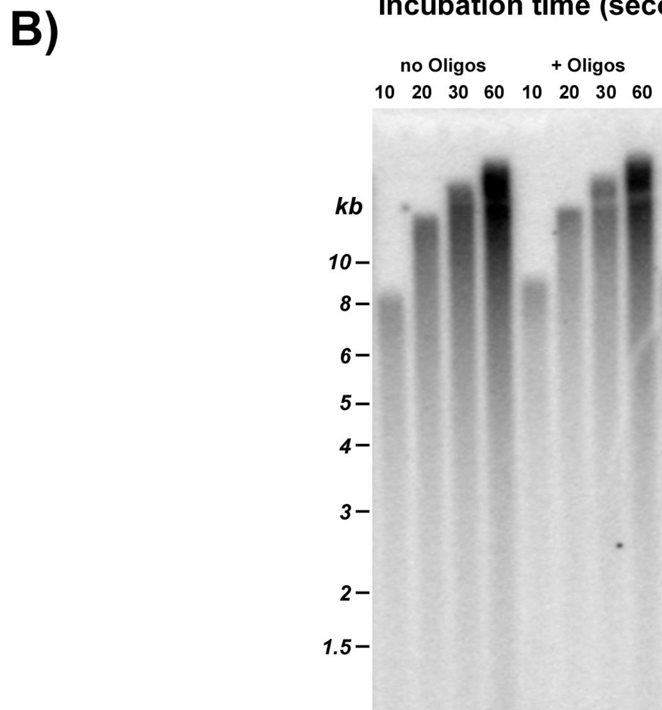


Fig. S3. DNA 20mers complementary to the leading strand product do not inhibit rolling circle replication. Ensemble experiment of leading strand replication reactions in the presence or absence of 400 nM each of 5 different ssDNA 20mers, that are complementary to the leading strand ssDNA product. Replication was performed at 23 °C for the indicated times and monitored by incorporation of ^{32}P-dTTP.

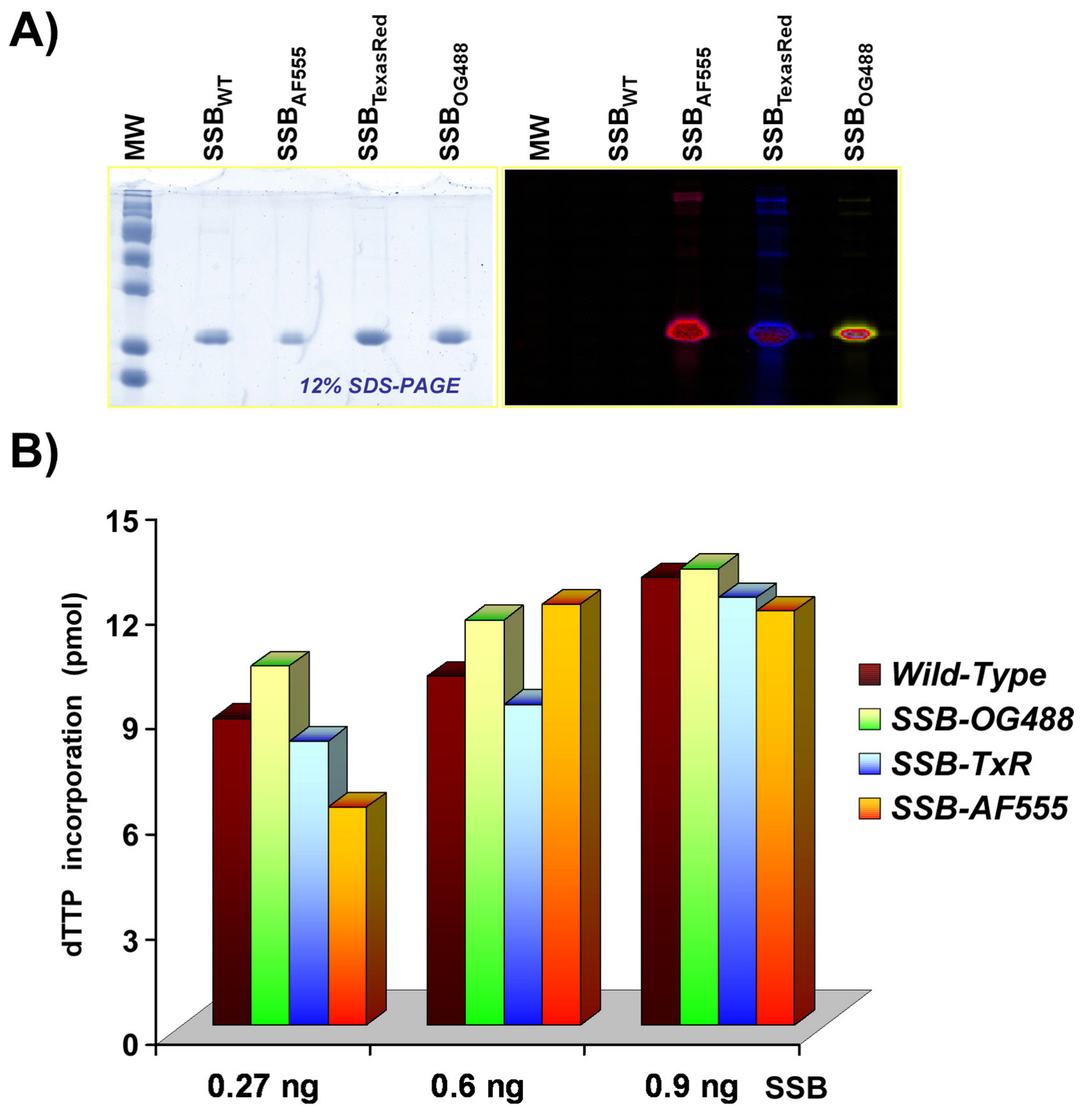
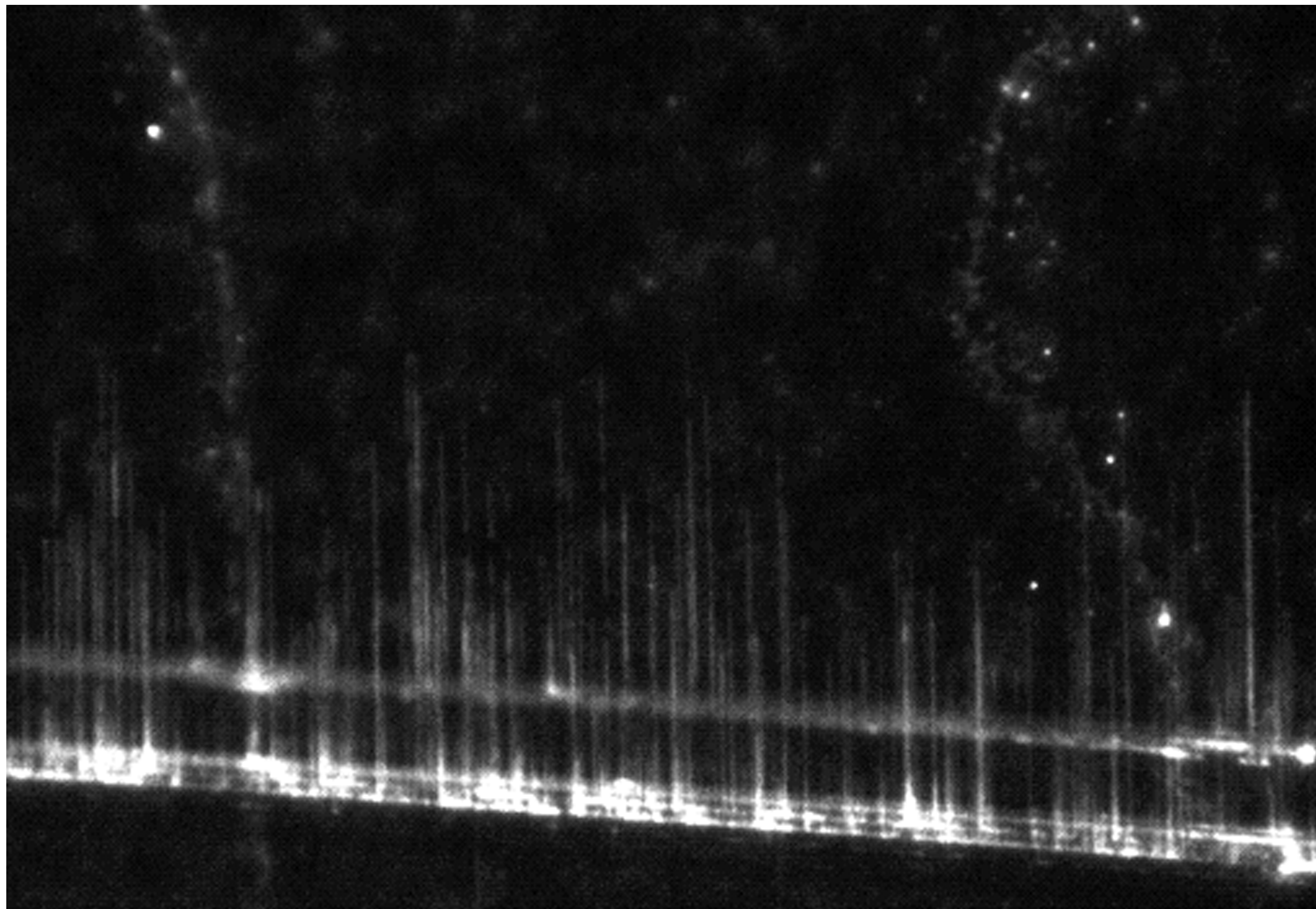
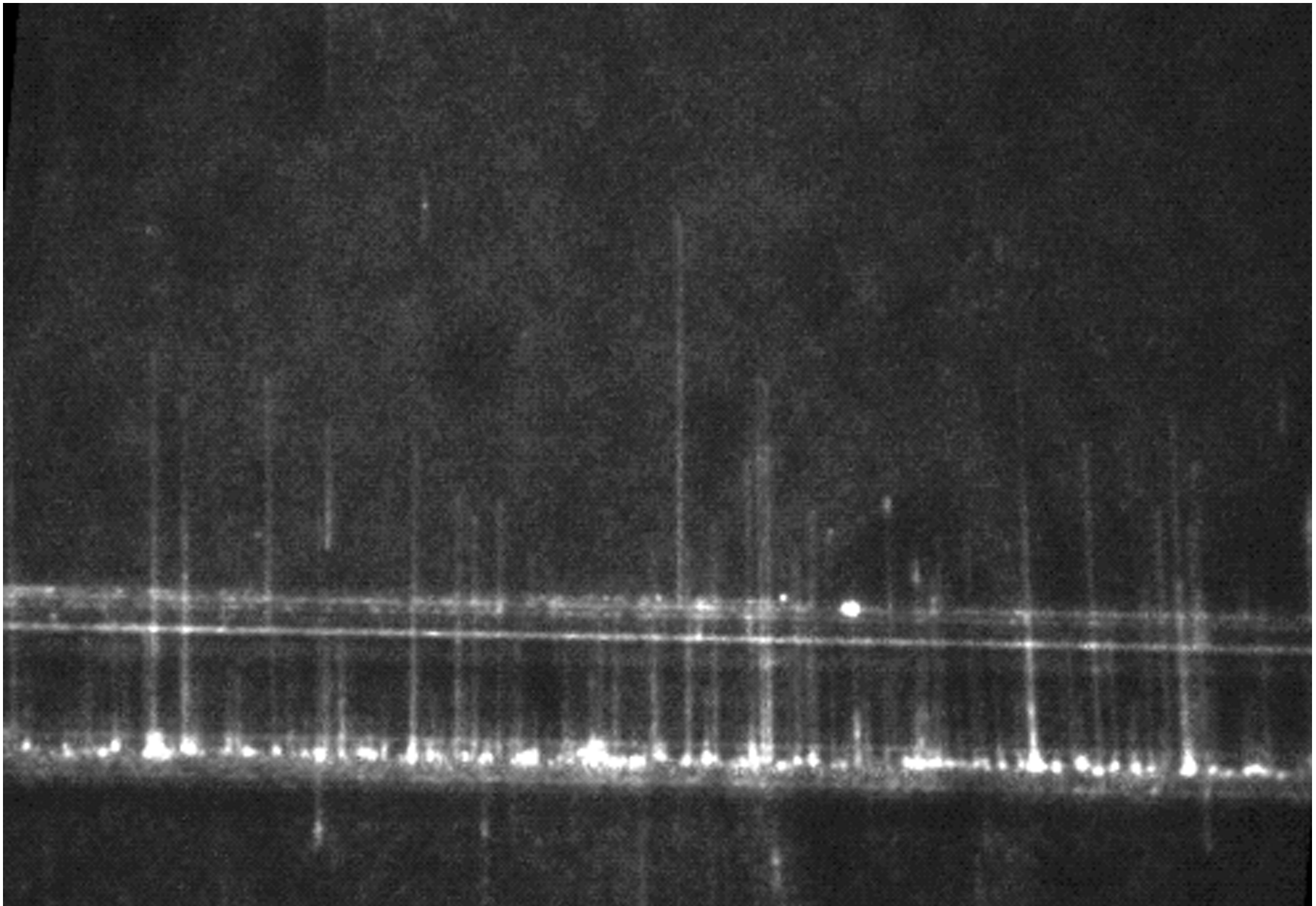


Fig. S4. SSB does not affect the rate of leading strand synthesis during rolling circle replication. Ensemble leading-strand minicircle replication reactions were performed under the same conditions as in Fig. S3, except the ssDNA 20mers were absent, and 502 nM SSB was present during replication. Replication was performed at 23 °C for the indicated times and monitored by incorporation of ^{32}P-dTTP in the leading strand product. Alkaline agarose gel analysis is shown to the left, and dTTP incorporation is shown to the right.



Movie S1. An example of a movie depicting real-time observation of a leading/lagging strand replication of a minirolling circle template by *E. coli* Pol III*. The force of the hydrodynamic flow pushes the DNA-lipid complex to a diffusion barrier etched in the glass surface and concentrates numerous DNA molecules in the visual field shown here. Width of the visible area in the direction of the flow is 94 μ (equivalent to 313 kb) and the flow direction is from bottom to the top. Individual DNA molecules are fluorescently stained by means of the intercalating dye yo-pro1, stretched by flow (100 μL/min) and imaged through total internal reflection fluorescence (TIRF) microscopy. Movie contains approximately 8 min of experimental data rendered at 20 frames per second. (original data acquisition is 1 fps at 100-ms exposure).

Movie S1 (MOV)



Movie S2. An example of a movie depicting real-time observation of a leading strand only replication of a minirolling circle template by *E. coli* Pol III*. Primase, the β-clamp proteins are absent from the buffer flow; the leading single-strand product is visualized by converting it to dsDNA upon flowing a solution of buffer containing 400 nM of each of 5 DNA 20mers that hybridize end-to-end along the repeated 100mer sequence. Width of the visible area in the direction of the flow is 75 μm (equivalent to 250 kb) and the flow direction is from bottom to the top. The individual DNA molecules, stretched by flow (100 μL/min), are fluorescently stained by means of the intercalating dye yo-pro1 and imaged through TIRF microscopy. Movie contains approximately 6 min of experimental data rendered at 20 frames per second (original data acquisition is 1 fps at 100-ms exposure).

Movie S2 (MOV)