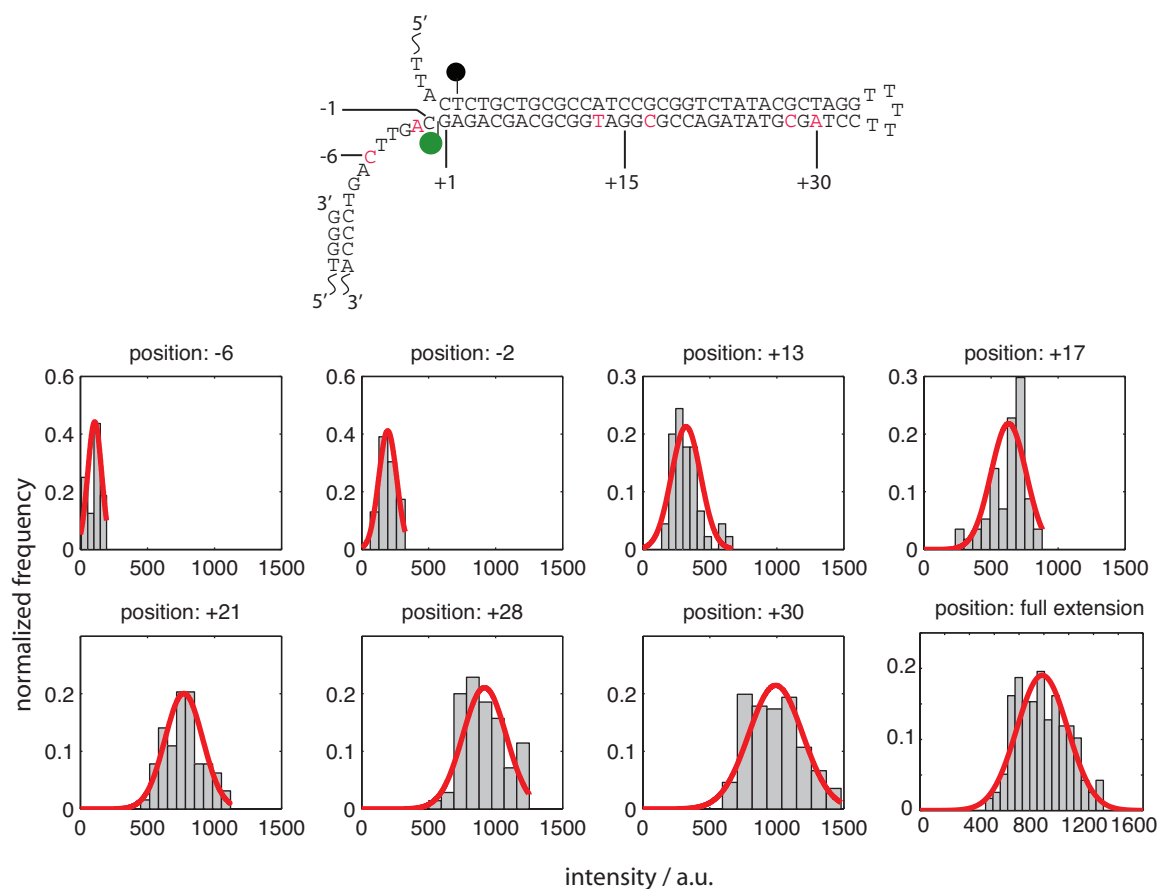


# Supporting Information

Schwartz and Quake 10.1073/pnas.0907404106



**Fig. S1.** Partially extended primers permit FRET distance calibration. (Top) Primed DNA hairpins were partially extended to known positions on the template by adding polymerase and only 3 of the 4 dNTPs. Primer stop positions (highlighted on the template in red) refer to the adjacent position awaiting nucleotide addition. The dNTP sets and the corresponding stop positions are shown in Table S1. Each set of intensities was fit to a Gaussian distribution to determine the mean intensities for the FRET calibration curve.

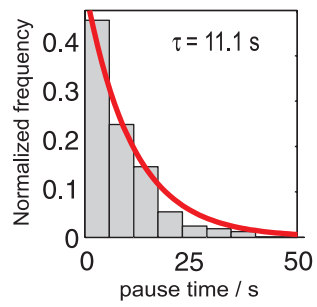
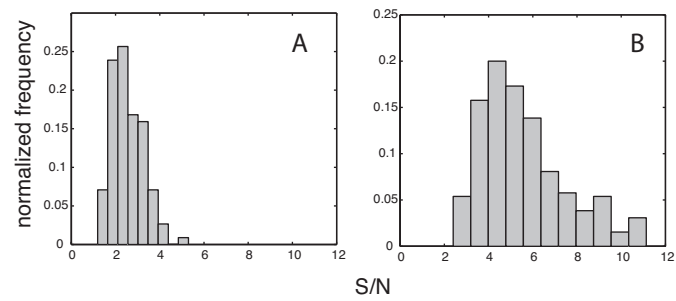
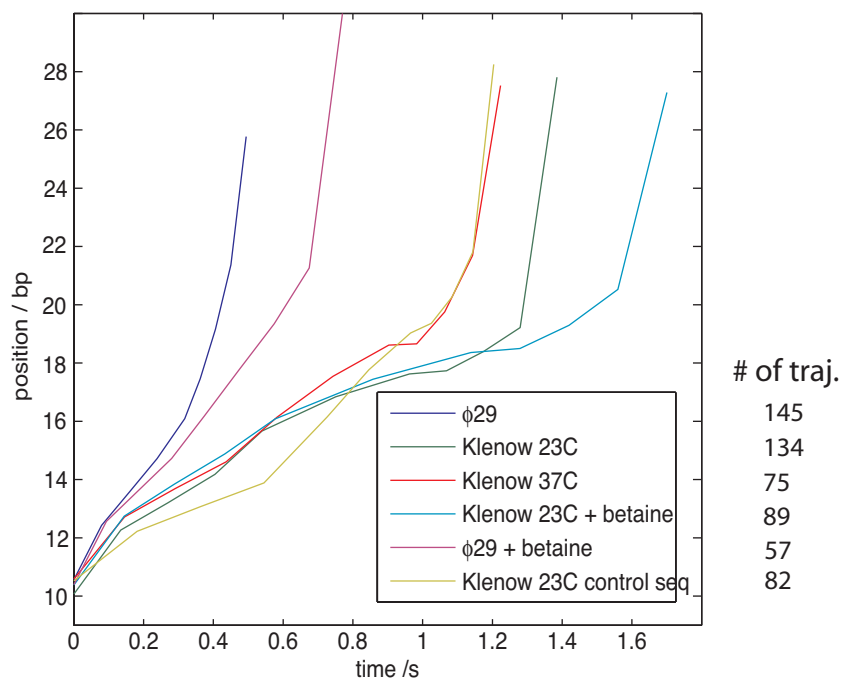


Fig. S2. Normalized frequency of pause times for Pol I(KF) at 37 °C. The histogram was fit to a single exponential as in Fig. 4.



**Fig. S3.** Histograms of S/N ratios for (A) trajectories during a pause and (B) after full extension. The trajectories for 96 pauses and 134 full extensions were analyzed and the mean S/N ratio for each was calculated by taking the net signal and dividing it by the RMS noise (SD of the signal). The mean values were calculated to be 2.6 for pause intensities and 5.6 for full extensions.



**Fig. 54.** Average trajectories for polymerase molecules that did not pause. Trajectory data were interpolated with a cubic spline on a 20X finer mesh to further improve our time resolution. The number of interpolated trajectories sampled to produce each “average” trajectory is shown to the right of the legend. Sampling was done by first identifying the start and finish times for each trajectory during which replication was observable. Each time interval was then divided equally into 8 subintervals and the intensity at each time point was recorded. The time and intensity values for each of these subintervals were then averaged to produce the “average” trajectory.

**Table S1. Nucleotide combinations used to pause the polymerase at known template positions**

Nucleotides used	Nucleotide missing	Stop position
dATP dCTP dTTP	dGTP	-6
dATP dCTP dGTP	dTTP	-2
dGTP dCTP dTTP	dATP	+13
dATP dCTP dTTP	dGTP	+17
dATP dCTP dGTP	dTTP	+21
dATP dCTP dTTP	dGTP	+28
dATP dCTP dGTP	dTTP	+30
dATP dCTP dGTP dTTP	n/a	n/a