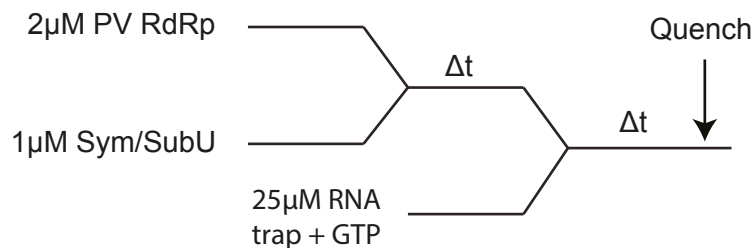


Figure S1

A



B

GMP misincorporation		
Enzyme	k_{pol} (s^{-1})	$K_{d,app}$ (μM)
WT	$1.5 \pm 0.1 \times 10^{-2}$	250 ± 10
K276R	$5.0 \pm 0.1 \times 10^{-3}$	250 ± 80
G64S-K276R	$2.0 \pm 0.1 \times 10^{-3}$	300 ± 50
G64S	$4.0 \pm 0.1 \times 10^{-3}$	300 ± 40

S1 Figure. In vitro assay of polymerase mediated single nucleotide incorporation. (A) Schematic of GTP misincorporation assay (G opposite the U). Primer-template (sym-subU) and polymerase are assembled in the absence of nucleotide. GTP and a 25-fold excess of unlabeled trap RNA are then added after an incubation period (Δt). Excess of RNA trap ensures that if the polymerase dissociates from primer-template it is taken up by the trap and cannot re-assemble. (B) k_{pol} and K_d for GMP misincorporation. Concentrations of GTP were used over time-points to calculate k_{pol} and K_d . Each GTP time-course was plotted to single exponential, then combined plot to hyperbola. Note that all polymerase variants have the I92T mutation.