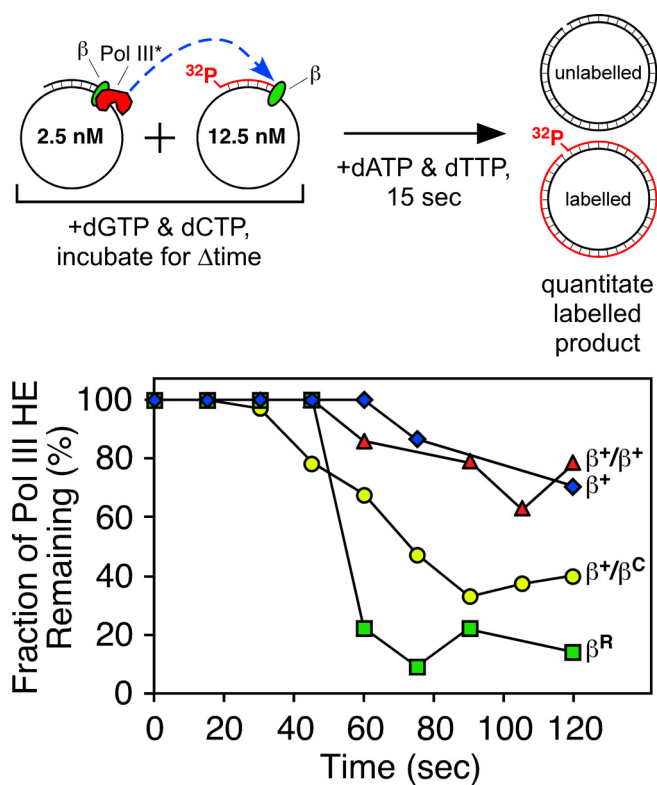# Supporting Information

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Fig. S1. Ability of wild-type and mutant β clamps to stabilize Pol III* on a primed DNA template in vitro. The cartoon above summarizes the method. Stability of Pol III* on the primed DNA template (fraction remaining) was calculated by measuring the level of replication products by densitometry (Molecular Analyst; Bio-Rad Laboratories) at each time point, and dividing the level observed at t = t_0 by that for t = t_n (n = 15–120 s), and is expressed as percent.
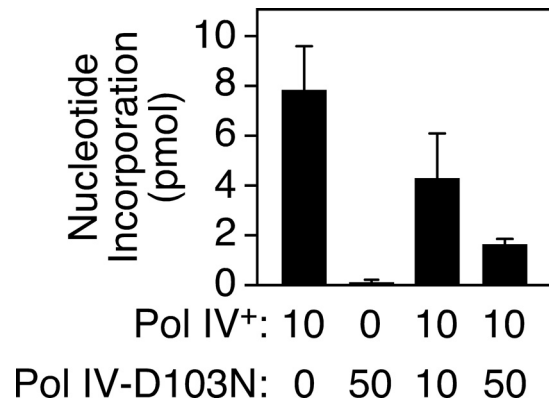
Fig. S2. Replication activity of Pol IV-D103N mutant. Replication activity was measured as described in *Materials and Methods*. Indicated levels (0, 10, or 50 nM) of Pol IV+ and Pol IV-D103N were mixed before addition of template and dNTPs. Results represent the average of triplicates. Error bars represent the standard deviation.
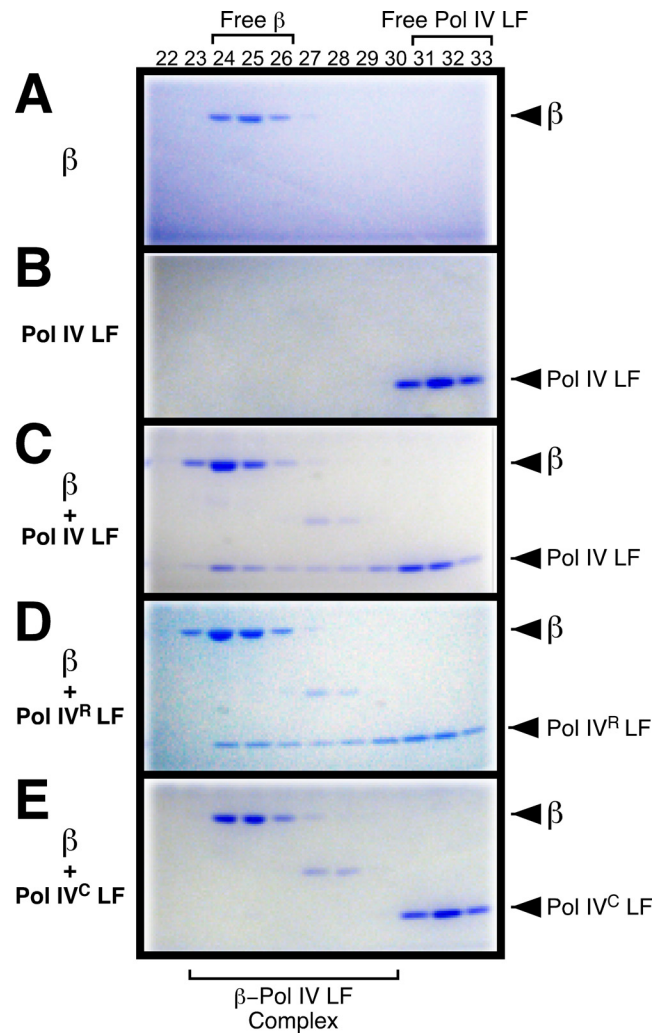
Fig. S3. Interactions of β clamp with different mutant Pol IV little finger proteins. Interactions of the wild-type Pol IV little finger domain (Pol IV LF), Pol IV^R LF, and Pol IV^C LF were measured by Superose-12 gel filtration chromatography (GE Healthcare) as described in *Materials and Methods*. Pol IV LF complexes were purified as described in *Materials and Methods*. β clamp (250 μg) was mixed with 250 μg of the indicated Pol IV LF protein in 20 mM imidazole, 100 mM NaCl, 50 mM Tris-HCl, pH 7.0, and 10% glycerol. Samples were incubated for 30 min at room temperature prior gel filtration. After filtration, 10 μL of each indicated fraction (500 μL) were electrophoresed through 13% SDS/PAGE. Gels were stained with Coomassie brilliant blue R-250, and the fraction of the β clamp in complex with Pol IV LF was determined by densitometry using a BSA standard curve and the Molecular Analyst software (Bio-Rad Laboratories). Wild-type Pol IV LF (50%) was determined to be in complex with β clamp, while only 24% of the Pol IV^R LF was determined to be in complex with β clamp. Pol IV^C LF was unable to interact with the β clamp with this assay.

Table S1. Primers used for QuikChange site-directed mutagenesis

Protein	Mutation(s)	Primer sequence (5′→3′)
β^R	E93→K	CGTGCAGCTGAAAGGTGAACGGATGAAAGTACGCTCCGG
	L98→K	CCGGAGCGTACTTTCATCCGTTCACCTTTCAGCTGCACG
Pol IV-D103N	D103→N	GACATCGAGATAGGCCTCATTCAGTGACAACGG
		CCGTTGTCACTGAATGAGGCCTATCTCGATGTC
Pol IV^C	Δ346–351	GGAAAGACAATGAGTGCTGGGATTATG
		CATAATCCCAGCACTCATTGTCTTTCC
Pol IV^{LF}	Δ1–242	CAGCGAACGGTTGCGACATATGGTCGGCGTGGAACGC
		GCGTTCCACGCCGACCATATGTCGCAACCGTTCGCTG
Pol IV^R	^{303}VWP^{305}→^{303}AGA^{305}	GGAGCACGCCGGGGCGCGGCTGAATAAAGC
		GCTTTATTCAGCCGCGCCCCGGCGTGCTCC