Supporting Information

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SI Materials and Methods

Oligonucleotides and chemicals were respectively purchased from IDT and Sigma unless otherwise noted.

Determination of Mutation Rates. JS200 cells harboring each mutant Pol I variant (on pECpolI-3'exo–) were transformed with reporter plasmid pLA230 (1) containing an active kanamycin resistance marker and a β-lactamase gene inactivated my a premature ochre stop codon. Cells were grown in LB at 30°C to an OD_{600} of approximately 0.3 at which time approximately 10^6 cells were plated on LB agar with 50 μg/mL carbenicillin, 50 μg/mL kanamycin and 30 μg/mL chloramphenicol to ensure that no preexisting carbenicillin-resistant cells were present in culture. Approximately 700 cells from the same culture were transferred to 5mL of 2XYT with 30 μg/mL chloramphenicol and 50 μg/mL kanamycin and grown for 16 hours at 37°C. To determine the reversion frequency, cells were plated on LB agar (30 μg/mL chloramphenicol and 50 μg/mL kanamycin) with and without 50μg/mL carbenicillin at dilutions that yielded 100 to 500 colonies per plate. At least fourteen separate cultures of each mutant were grown and plated for each calculation. Mutation rate was calculated according to the method of the median.

Growth Rate and Culture Density Measurements. A starter culture of bacteria was grown to OD_{600} of approximately 0.3, at which time 10μL of a 1/1000th dilution of the culture was used to inoculate 5mL of LB. The culture was grown until it reached OD_{600} of 0.3. OD_{600} and measurements at different times in culture were used to calculate the doubling time for the culture based on an exponential growth curve. Maximum culture density was measured after 24 hours of growth. Cultures and measurements were made in triplicate.

Library Competition of Pol I Mutants. Independent cultures of each mutant were initially grown to OD_{600} of 0.3, at which time, 1mL of each were combined. 1mL of this mixture was then used to inoculate 4mL of LB to establish input for the competition. Subsequent passages, performed daily, involved transferring 2 μL of culture to 5mL of fresh LB. At the end of 31 passages, the culture was diluted and plated on LB agar with 30 μg/mL chloramphenicol. Colonies were picked and cultured independently. Identification of the mutant plasmids in each colony was performed by DNA sequencing. Competition of Pol I mutants in M9 minimal media was established using a nearly identical procedure on a different set of transformed isolates of each Pol I strain. Differences included using M9 media supplemented with glycerol 0.2% and tryptophan 0.01% instead of LB, daily transfer of 4 μL instead of 2 μL to fresh media and competition for 33 rather than 31 days.

Competition of Winners and Ancestors with Wild-type. Independent cultures of each mutant and wild-type were initially grown to OD_{600} of 0.3, at which time they were mixed in a 50:50 ratio, which was also verified by plating. Each mixture was diluted 5000-fold and used as the started culture. For competitions under continuous growth conditions, cultures were grown in 5mL of LB and repeatedly passaged at an approximate OD_{600} of 0.3 for 96 generations. Competitions grown under the periodically saturating conditions of the whole library competition were carried out for 15 days. For both types of cultures, samples from various time points were frozen at -80°C and later sequenced simultaneously. Additional samples consisting of known mixtures of mutants and wild-type were sequenced to construct a standard curve of relative frequency for each allele. The ratio of mutant to wild-type bacteria was determined by relative chromatogram peak heights at mutant bases.

Pair-wise Competitions. Independent cultures of five mutant and wild-type isolates were initially grown to an OD_{600} of 0.3, at which time they were mixed in a 50:50 ratio, which was also verified by plating. Approximately 10^7 cells of each mixture was used to seed 12 identical 1 mL LB cultures. Competitions were carried out in deep 96 well plates with 3mm glass beads to assist with mixing. Daily passage of approximately 4×10^6 cells to fresh media was accomplished using a sterilized 96 prong innoculator. Identification of winning strains on day 18 was performed by DNA sequencing of bulk culture plasmid.

Protein Purification and Gapped Plasmid Assay. Mutant and wild-type Pol I Klenow fragments, encompassing the inactivated 3'-5' exonuclease domain and the active polymerase domain, were subcloned into the pLEX vector (Invitrogen, Carlsbad, CA) expressed, and purified as previously described (2, 3) Polymerase and 3'-5' exonuclease activity were assessed using previously reported templates and methods (2). The M13 gapped plasmid assay was performed as previously described (4). The complete fill-in of the M13 gapped substrate was confirmed by gel and by the constancy of mutation frequency after incubation with increased amounts of the DNA polymerase. A five-fold increase the amount of enzyme used for fill-in did not alter the mutation frequency significantly. Additionally, statistical analysis of error distribution showed a constant ratio of mutations throughout the gapped substrate.

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2. Loh E, Choe J, Loeb LA (2007) Highly tolerated amino acid substitutions increase the fidelity of Escherichia coli DNA polymerase I. *J Biol Chem* 282:12201–12209.
3. Shinkai A, Patel PH, Loeb LA (2001) The conserved active site motif A of Escherichia coli DNA polymerase I is highly mutable. *J Biol Chem* 276:18836–18842.
4. Bebenek K, Kunkel TA (1995) Analyzing fidelity of DNA polymerases. *Meth Enzymol* 262:217–232.