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Supplemental Data

Division of Labor at the Eukaryotic Replication Fork

Stephanie A. Nick McElhinny, Dmitry A. Gordenin, Carrie M. Stith, Peter M.J. Burgers, and Thomas A. Kunkel

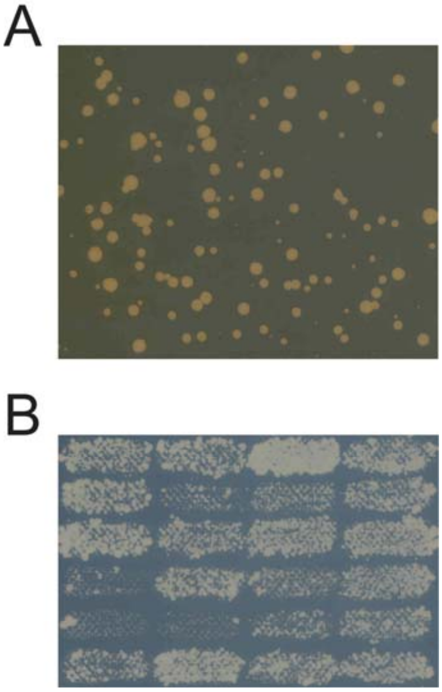

Figure S1. Variability of *pol3-L612M msh2*Δ Colonies
(A) Population of haploid *pol3-L612M msh2*Δ colonies demonstrating variability in colony size and shape.
(B) Variation in the mutability of *URA3* in haploid *pol3-L612M msh2*Δ colonies. Independent colonies from (A) were patched onto YPDA and replica plated to media containing 5-fluoro-orotic acid.

Table S1. Orientation Dependence of Mutagenesis in *pol3-L612M msh2*Δ Strains

Orientation	**OR1**			**OR2**		
Mutation Rate (x 10^{-7})	560			510		
95% Confidence Intervals	500-750			360-790		
Mutants Sequenced	174			166		
	#	**Mutation rate (x 10^{-7})**		#	**Mutation rate (x10^{-7})**	
Event on *URA3* coding strand		Total for all sites	Per detectable nucleotide		Total for all sites	Per detectable nucleotide
T-A to C-G Transitions						
T to C at 97	18	58	58	1	3.1	3.1
T to C – other sites (16)	18	58	3.6	0	≤3.1	≤0.19
G-C to A-T Transitions						
C to T at 310	0	≤3.2	≤3.2	32	98	98
C to T – other sites (16)	0	≤3.2	≤0.20	22	68	4.3
G to A at 764	16	51	51	0	≤3.1	≤3.1
G to A – other sites (34)	33	106	3.1	2	6.1	0.18
Deletion of T-A Base Pairs in Runs[a]						
ΔA at 174-178 (5)	1	3.2	0.64	20	61	12
ΔA – other sites (28)	0	≤3.2	≤0.11	30	92	3.3
ΔT at 201-205 (5)	25	80	16	1	3.1	0.62
ΔT at 255-260 (6)	28	90	15	4	12	2.0
ΔT – other sites (17)	5	16	0.94	0	≤3.1	≤0.18

[a] Homopolymeric runs of 3 or more nucleotides in length

For each mutational event listed, the number of events is given with the mutation rate for the event calculated both as the total mutation rate including all sites, as well as per detectable nucleotide, for which the rate for all sites was divided by the number of detectable nucleotides not including the hotspot nucleotides (shown in parentheses next to each event; for base substitution hotspots, the number of detectable sites is 1).

Table S2. In Vitro Error Bias of L612M Pol δ at the Three *URA3* Base Substitution Hotspots

Position	**Template•Mismatched dNTP**	**Bias (normalized[a])**	**(+1)/(-1) product ratio[b]**
97	**T•dGTP**	2.0	12
	A•dCTP		1.6
310	**G•dTTP**	4.4	6.4
	C•dATP		1.1
764	**G•dTTP**	3.6	22
	C•dATP		3.3

[a] Bias of L612M Pol δ in favor of the bold mispair for each position after first accounting for any inherent preference of L612M Pol δ for one substrate over the other (as measured by the ratio of correct insertion for each substrate).

[b] The abundance of (+1) misinsertion product relative to (-1) excision product reflects the efficiency of proofreading for each mispair. The higher the value, the less efficiently the mispair is corrected by L612M Pol δ.

Table S3. Mutagenesis in *pol3-L612M MSH2* Strains

	***ura3* Forward Mutation Rates**[a]			
Orientation	**OR1**		**OR2**	
Mutation Rate (x 10^{-7})	2.7		3.5	
95% Confidence Intervals	1.3 – 3.6		2.9 – 4.9	
Mutants Sequenced	65		83	
Event	**#**	**Mutation Rate**[a] **(x 10^{-7})**	**#**	**Mutation Rate**[a] **(x 10^{-7})**
T to C at 97	3	0.12	0	≤0.04
C to T at 310	3	0.12	22	0.92
G to A at 764	7	0.29	0	≤0.04
ΔA at 174 - 178	0	≤0.04	1	0.04
ΔT at 201 - 205	0	≤0.04	0	≤0.04
ΔT at 255 - 260	0	≤0.04	1	0.04

[a] Mutation rates are for haploid *pol3-L612M MSH2* strains

[b] Mutation rates for events are the total mutation rates including all *URA3* sites.