

FILE S2. SUPPLEMENTAL TABLES

Supplemental Table S1. Strains of *E. coli* used in this study

Strain	Relevant genotype	Donor	Recipient	Target gene	Method	Reference
NR11257	<i>dnaE925 mutD5</i>					(FIJALKOWSKA AND SCHAAPER 1996)
FC231	<i>lexA3</i>					(CAIRNS AND FOSTER 1991)
PFM2	MG1655 <i>rph</i> ⁺					(LEE <i>et al.</i> 2012)
PFM12	$\Delta proA::Kn^R$	JW0233	PFM2	<i>proA</i>	A	This Study
PFM50	$\Delta umuDC^{Sc}$					(FOSTER <i>et al.</i> 2015)
PFM106	<i>metA::Kn</i>	JW3973	PFM2	<i>metA</i>	A	This Study
PFM110	$\Delta proA^{Sc}$		PFM12	<i>proA::Kn^R</i>	B	This Study
PFM111	<i>metA</i> ⁺ <i>lexA3</i>	FC231	PFM106	<i>lexA</i>	C	This Study
PFM139	$\Delta proA^{Sc} \Delta mutL^{Sc}$	JW4128	PFM110	<i>mutL</i>	D	This Study
PFM132	$\Delta rnhA::cat-ISceI$		PFM2	<i>rnhA</i>	E	This Study
PFM149	<i>dnaE</i> ⁺ <i>mutD5</i>	NR11257	PFM132	<i>dnaQ</i>	F	This Study
PFM150	<i>dnaE925 mutD5</i>	NR11257	PFM132	<i>dnaQ</i>	F	This Study
PFM163	<i>dnaE</i> ⁺ <i>mutD5 proA</i> ⁺	PFM149	PFM110	<i>dnaQ</i>	C	This Study
PFM165	<i>dnaE</i> ⁺ <i>mutD5 proA</i> ⁺ $\Delta mutL^{Sc}$	PFM149	PFM139	<i>dnaQ</i>	C	This Study
PFM174	$\Delta proA::Kn lexA3$	JW0233	PFM111	<i>proA</i>	A	This Study
PFM397/399	<i>dnaE</i> ⁺ <i>mutD5</i> $\Delta mutL^{Sc}$	JW4128	PFM163	<i>mutL</i>	D	This Study
PFM459	<i>dnaE925 mutD5</i> $\Delta dinB^{Sc}$	JW0221	PFM150	<i>dinB</i>	G	This Study
PFM479	<i>dnaE</i> ⁺ <i>mutD5</i> $\Delta dinB^{Sc} proA+$	PFM459	PFM12	<i>dnaE</i>	C	This Study
PFM492	$\Delta proA^{Sc} \Delta umuDC^{Sc}$	JW0233	PFM50	<i>proA</i>	D	
PFM515/517	<i>dnaE</i> ⁺ <i>mutD5</i> $\Delta dinB^{Sc} proA+ \Delta umuDC^{Sc}$	PFM459	PFM492	<i>dinB dnaQ</i>	C	This Study
PFM686	<i>dnaE</i> ⁺ <i>mutD5 proA</i> ⁺ <i>lexA3</i>	PFM149	PFM174	<i>dnaQ</i>	C	This Study

Methods: **A.** P1 phage transduction (MILLER 1992) from donor selecting for *Kn*^R. **B.** FLP recombination to remove the *Kn*^R element. **C.** P1 phage transduction from donor selecting for prototrophy on minimal glucose medium. **D.** P1 phage transduction from donor selecting for *Kn*^R, followed by

FLP recombination to remove the Kn^{R} element (DATSENKO AND WANNER 2001); this procedure leaves an in-frame scar sequence, which is indicated above by superscript Sc. **E:** Intermediate in scarless gene deletion using a *cat-I-SceI* cassette (BLANK *et al.* 2011). **F:** P1 phage transduction from donor containing a *cat-I-SceI* cassette, selecting on LB plus anhydrotetracycline and carb, followed by gene replacement of the *cat-I-SceI* site cassette. **G:** Gene replacement by a Kn^{R} cassette, followed by FLP recombination to remove the Kn^{R} element; Kn^{R} element obtained from donor strain by PCR (BABA *et al.* 2006). JW strains are from the Keio collection of gene knockouts (BABA *et al.* 2006).

Supplemental Table S2. Oligonucleotides used in this study.

Relevant genotype	Name	Sequence	Reference
<i>ΔrnhA::cat-I/SceI</i>	mutD5_rightpWRG100	5' -GCTATCGCGGACGCGAGAAAACCTTTAGCGCTGGCTACACCCGCGACCACTTAGACTATATTACCTGTT-3'	
	mutD5_leftpWRG100	5' -CAGGCGGTTGGAGCCACCCGGCAATGTCGTAAAC CACAGGCTTAACTTCCGCCTTACGCCCCGCCCTGC-3'	
<i>dnaQ</i>	mutD5 Rv	5' -GCAAGTAAGTTACGCGTTG-3'	
	yafSdnaQ FW	5' -CCCGTCCAGGTACAGGCGGA-3'	
	mutD5 RV2	5' -CAACGCGTAACTTACTTGC-3'	
	yafSdnaQ RV	5' -CGCACCAATCTGGTTCATACCGGTGG-3'	
	yafSmutD5 RV	5' -CGCACCAATCTGGTTCATACCGGTGA-3'	
	dnaQ FW	5' -CGCCAGATCGTTCTCGATAACCGAAAC-3'	
	dnaQmutD5 FW	5' -CGCCAGATCGTTCTCGATAACCGAAAT-3'	
<i>dnaE</i>	rnhB_6_fw	5' -TTCCGTGAACTGCATCAGCA-3'	
	dnaEFW044	5' -CAAACGCGCACTGGGACTTG-3'	
	dnaEFW494	5' -CTATTTTCTCGAGCTGATCC-3'	
	dnaEFW965	5' -GGTTATCAACCAGATGGGCTTC-3'	
	dnaERV1749	5' -GTTTCAGTCTCCAGACGTTTCG-3'	
	dnaERV2207	5' -CATCGCCAGTTCAGCGTTG-3'	
	dnaERV2962	5' -GTGATGACTTTACCACGTTCTG-3'	
	dnaERV3503	5' -GTTCAAAATCAAGGAAATTC-3'	
<i>mutL</i>			(LEE <i>et al.</i> 2012)
<i>umuDC</i>			(FOSTER <i>et al.</i> 2015)
<i>dinB</i>			(FOSTER <i>et al.</i> 2015)
<i>lexA</i>	lexAF0079	5' -GAAAGGTTTACGCGCTGCATGGAT-3'	
	lexAF0121	5' -TCAGGAAGGCGTAGCGGTATTGTT-3'	
	lexAF0703	5' -TGTTGCAGGAAGAGGAAGAAGGGT-3'	
	lexAR0726	5' -ACCTTCTTCTCTTCTCTGCAACA-3'	
	lexAR0949	5' -TCAATACGTGCGACAACGACCTGA-3'	
	lexAR1598	5' -TGCGCTTAACCAGCGGATTTC AAG-3'	

Supplemental Table S3: Experimental parameters and base-pair substitution (BPS) results.

A. Experimental parameters and numbers of BPS

Strain	Defect	Experi- ments	MA lines	Genera- tions	BPSs							A:T sites	G:C sites
					Total	A:T>G:C	G:C>A:T	A:T>T:A	G:C>T:A	A:T>C:G	G:C>C:G		
MA experiments on LB medium													
*Wild type	—	8	341	2,015,066	1933	388	664	162	302	307	110	857	1,076
*MMR	<i>mmr⁻</i>	10	334	264,958	30,061	22,069	7,165	291	165	248	123	22,608	7,453
PFM163	<i>mutD5</i>	1	26	3,481	13,625	6,757	4,962	790	652	431	33	7,978	5,647
#PFM165/ 397/399	<i>mutD5 mutL</i>	3	75	7,012	40,686	14,185	23,985	1,638	635	166	77	15,989	24,697
PFM479	<i>mutD5 dinB</i>	1	34	3,748	12,505	6,101	4,904	693	547	239	21	7,033	5,472
[†] PFM515/ 517	<i>mutD5 dinB umuDC</i>	1	30	3,037	1,2696	5,958	5,146	694	571	289	38	6,941	5,755
PFM686	<i>mutD5 lexA3</i>	1	43	4,006	21,594	10,042	9,347	1,055	824	258	68	11,355	10,239
MA experiments on minimal medium													
PFM163	<i>mutD5</i>	1	41	6,720	4,595	1,710	2,023	461	264	121	16	2,292	2,303
PFM165	<i>mutD5 mutL</i>	1	16	2,202	8,570	3,487	4,812	156	79	19	17	3,662	4,908

B. Types and consequences of BPS

Strain	Defect	Type of BPS		Position of BPS		Amino acid changes			
		Transitions	Transversions	Non-Coding	Coding	Synonymous	Non-Synonymous	Conservative	Non-Conservative
<u>MA experiments on LB medium</u>									
*Wild type	—	1,052	881	448	1,368	395	1,090	535	555
*MMR	<i>mmr[−]</i>	29,234	827	3,970	26,091	8,759	17,332	11,295	6,037
PFM163	<i>mutD5</i>	11,719	1,906	2,106	11,519	3,816	7,703	4,400	11,719
#PFM165/ 397/399	<i>mutD5 mutL</i>	38,170	2,516	5,109	35,577	12,654	22,923	13,786	9,137

PFM479	<i>mutD5 dinB</i>	11,005	1,500	1,921	1,0584	3,584	7,000	3,953	3,047
[†] PFM515/ 517	<i>mutD5 dinB</i> <i>umuDC</i>	11,104	1,592	1,856	10,840	3,701	7139	4,051	3088
PFM686	<i>mutD5 lexA3</i>	19,389	2,205	3,210	18,384	6,286	12,098	7,009	5,089
<u>MA experiments on minimal medium</u>									
PFM163	<i>mutD5</i>	3,733	862	914	3,681	1,240	2,441	1,307	1,134
PFM165	<i>mutD5 mutL</i>	8,299	271	1,088	7,482	2,717	4,765	2,948	1,817

*Data for WT and MMR are from Foster *et al.*, accompanying paper

Two separate transductions of the *mutL*^{Sc} allele into the *mutD5* mutant strain, PFM163; PFM397 and PFM399 are two isolates from the same transduction.

[†]Two isolates from the same transduction

Supplemental Table S4. The numbers of indels accumulated in the MA experiments

Type of indel	<u>Numbers of Indels</u>							
	<u>MA experiments on LB medium</u>					<u>MA experiments on minimal medium</u>		
	<i>mutD5</i>	<i>mutD5 mutL</i>	<i>mutD5 dinB</i>	<i>mutD5 dinB umuDC</i>	<i>mutD5 lexA3</i>	<i>mutD5</i>	<i>mutD5 mutL</i>	
Total	1018	3184	851	921	1275	208	504	
+1 bp	459	1613	465	488	632	128	267	
-1 bp	556	1556	385	429	638	79	232	
+ > 1 bp	2	14	1	3	3	0	3	
- > 1 bp	1	1	0	1	2	1	2	
+1 A:T	242	1097	266	287	389	108	174	
+1 G:C	217	516	221	201	243	20	93	
-1 A:T	318	644	199	241	315	51	124	
-1 G:C	238	912	164	188	323	28	108	
In run	965	3100	691	861	1218	198	489	
Not in run	53	84	160	60	57	10	15	
Noncoding	275	895	250	238	355	80	168	
Coding	743	2289	601	683	920	128	336	

bp, base pair; a run is more than two of the same base pair or sequence.