Table S1: The bacterial strains used in this report

Strain	Relevant genotype	Donor	Recipient	Target gene	Method	Reference
PFM2	MG1665 rph^+					(LEE et al. 2012)
PFM5	$\Delta mutL^{Sc}$					(LEE et al. 2012)
PFM6	$\Delta mutY^{Sc}$					(FOSTER et al. 2015)
PFM91	Δnif^{Sc}					(FOSTER et al. 2015)
PFM101	$\Delta umuDC^{Sc} \Delta dinB$					(FOSTER et al. 2015)
PFM118	$\Delta mutL^{Sc} \Delta umuDC^{Sc} \Delta dinB$	JW4128	PFM101	$\Delta mutL::Kn^R$	B	This Study
PFM137	$\Delta mutL^{Sc} \Delta mutY^{Sc}$	JW4128	PFM6	$\Delta mutL::Kn^R$	B	This Study
PFM144	$\Delta mutL^{Sc}$	JW4128	PFM2	$\Delta mutL::Kn^R$	B	This Study
PFM191	$\Delta uvrD^{Sc}$	JW3786	PFM2	$\Delta uvrD::Kn^R$	B	This Study
PFM197	$\Delta mutH^{Sc}$	JW2799	PFM2	$\Delta mutH::Kn^R$	B	This Study
PFM244	$\Delta mutL$		PFM2	$\Delta mutL::cat-I-SceI$	C	This Study
PFM282	$\Delta purA::Kn^R$	JW4135	PFM2	$\Delta purA::Kn^R$	D	This Study
PFM286	Δmfd^{Sc}	JW1100	PFM2	$\Delta mfd::Kn^R$	B	This Study
PFM288	$\Delta mutL$	PFM244	PFM282	$\Delta mutL$	A	This Study
PFM292	$\Delta mfd^{Sc} \Delta purA::Kn^R$	JW4135	PFM286	$\Delta purA::Kn^R$	D	This Study
PFM294	$\Delta mutL \Delta mfd^{Sc}$	PFM244	PFM292	$\Delta mutL$	A	This Study
PFM304	$\Delta mutL \Delta mutS^{Sc}$	JW2703	PFM288	$\Delta mutS::Kn^R$	B	This Study
*PFM326/327	$\Delta srlD::Kn^R$	JW2674	PFM2	$\Delta srlD::Kn^R$	D	This Study
PFM332	$\Delta mutS$		PFM2	$\Delta mutS::cat-I-SceI$	C	This Study
PFM342	$\Delta mutS$	PFM332	PFM326	$\Delta mutS$	E	This Study
PFM343	$\Delta mutS$	PFM332	PFM327	$\Delta mutS$	E	This Study
PF1716	$srlD::Tn10$	RH4695	FC36	$srlD::Tn10$	D	(FOSTER AND CAIRNS 1992; MAENHAUT-MICHEL et al. 1992)
PFM366	$\Delta mfd^{Sc} srlD::Tn10$	PF1716	PFM286	$srlD::Tn10$	D	This Study
PFM368	$\Delta mutS \Delta mfd^{Sc}$	PFM332	PFM366	$\Delta mutS$	E	This Study
PFM550	$\Delta mutS \Delta mutH^{Sc}$	JW2799	PFM342	$\Delta mutH::Kn^R$	B	This Study
*PFM555/556	$\Delta mutS^{Sc}$ $\Delta mutS \Delta mutH^{Sc}$	JW2703	PFM2	$\Delta mutS::Kn^R$	B	This Study
PFM557	$\Delta purA::Kn^R$	JW4135	PFM550	$\Delta purA::Kn^R$	D	This Study
*PFM567/568	$\Delta mutS \Delta mutL \Delta mutH^{Sc}$	PFM244	PFM557	$\Delta mutL$	A	This Study
PFM627	Δndk^{Sc}	JW2502	PFM2	$\Delta ndk::Kn^R$	B	This Study
PFM666	$\Delta mutL^{Sc} \Delta ndk^{Sc}$	JW4128	PFM627	$\Delta mutL::Kn^R$	B	This Study
PFM794	$\Delta nif^{Sc} \Delta mutL$	PFM244	PFM91	$\Delta purA::Kn^R$	A	This Study
PFM798	$\Delta rdgB^{Sc}$	JW2921	PFM2	$\Delta rdgB::Kn^R$	B	This Study
PFM800	$\Delta rdgB^{Sc}\Delta mut$	PFM244	$\Delta purA::Kn^R$	$\Delta purA::Kn^R$	A	This Study

* Two isolates from the same transduction.
A. P1 phage transduction (MILLER 1992) from donor selecting for prototrophy on minimal glucose medium; **B**. P1 phage transduction from donor selecting for Kn^R, followed by FLP recombination to remove the Kn^R element, which leaves an in-frame scar sequence (DATSENKO AND WANNER 2001), which is indicated by superscript Sc; **C.** Scarless gene deletion using a *cat*-I-SceI cassette (BLANK et al. 2011); **D.** P1 phage transduction from donor selecting for drug resistance; **E.** P1 phage transduction from donor selecting for ability to utilize sorbitol as a carbon source. JW strains are from the Keio collection of gene knockouts (BABA et al. 2006).

Table S2: The oligonucleotides used in this study

Relevant genotype	Name	Sequence, 5´ to 3´	Reference
ΔmutL::cat-ISceI	mutLreadthru	GGTGACGACGCCAGATCGCACGCTGCCAAACTAAGGACGATTGATGCCAAT TGATGAGTGATATCAGTAAGGCGAGCCTGCCTAAGGCGATTTTTTTGATGGGG	
	mutLpWGR100dwnstrm	CCCCATCAAAAAAATCGCCTTAGGCAGGCTCGCCTTACTGATATCACTCATCC TAGACTATATTACCCTGTT	
	mutLpWGR100upstrm	GGTGACGACGCCAGATCGCACGCTGCCAAACTAAGGACGATTGATGCCAAT TCGCCTTACGCCCCGCCCTGC	
ΔmutS::cat-ISceI	mutSreadthru2	AATAAAAACCATCACACCCCATTTAATATCAGGGAACCGGACATAACCCCATG TAACAATTCCCGATAGTCTTTTGCTATCGGGAATATTAACGACAACTGAC	
	mutSright_pWGR100	AGCAAAAGACTATCGGGAATTGTTATTACACCAGGCTCTTCAAGCGATACTA GACTATATTACCCTGTT	
	mutSleft_pWGR100	AATAAAAACCATCACACCCCATTTAATATCAGGGAACCGGACATAACCCCCG CCTTACGCCCCGCCCTGC	
mutL	mutL Fw	GGCCTGCGCAATTACTTCCTTG	(Lee *et al.* 2012)
	mutL Rv	CGCAGCTCAATGGCTAACGC	(Lee *et al.* 2012)
mutS	mutSfw	CATCAAGAACTCGCCGTTCG	
	mutSrv	TAGCGTGTAGATGGCATGGTT	
mutH	mutH Fw	CGGGTTGGCGAAAGATCAAC	
	mutH Rv	GCGCCGAGGAATGATGAAAC	
uvrD	uvrD Fw	AGGCTTGTTGGATCAGACCG	
	uvrD Rv	ATGGGAACGCCGTGTTTTTG	
umuDC	umuC downstream rv	CGATGGCGCCGAGCGTC	(Foster *et al.* 2015)
	umuD upstream fwd	GCCGGGTGATATTTTTC	(Foster *et al.* 2015)
dinB	dinB del 201–3 F	GGGCAACCGGCGCATTGAGA	(Foster *et al.* 2015)
	dinB del 201-3 R	GGAAGCGCGCCTTTATGGGCT	
mutY	mutY Fw	GCAAGCATGATAAGGCCGTG	
	mutY Rv	TCAGCATGGTTTGCTTGTGC	
mfd	mfd FW	GTGTTGGCTTTTGCCGGTAG	
	mfd Rv	AGCGATCTGTTGTTCTCCCC	
ndk	ndk FW(2)	ATGGATGACGTGGGGCAAAT	
	ndk RV(2)	CGGTTGAGATCCAGCAGGTT	

Table S3: Results from the mutation accumulation experiments presented in this report.

Strain	Defect	MA lines	Genera-tions	Basepair Substitutions						
				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										
*Wild type	—	341	2015066	1933	388	664	162	302	307	110
#PFM5	mutL	34	12750	1625	1141	447	14	10	10	3
PFM144	mutL	25	14367	1350	1011	283	15	9	20	12
PFM288	mutL	38	22382	2709	2130	491	29	16	30	13
PFM304	mutL mutS	43	25270	2845	2314	447	30	13	25	16
PFM342	mutS	29	17041	2410	1915	442	20	9	18	6
PFM343	mutS	43	21922	2797	2128	593	30	14	20	12
†PFM555/556	mutS	41	24148	2764	2122	591	19	5	17	10
PFM197	mutH	37	99960	10896	7483	3093	122	66	93	39
PFM567	mutLSH	23	14752	1546	1088	417	4	17	11	9
PFM568	mutLSH	21	12367	1119	737	361	8	6	4	3
PFM191	uvrD	42	115746	6938	4414	2225	83	75	89	52
PFM118	mutL umuDC dinB	23	12078	1110	798	282	10	7	7	6
PFM137	mutL mutY	19	11019	1477	1104	253	14	94	7	5
PFM294	mutL mfd	32	19554	2126	1704	372	18	9	16	7
PFM368	mutS mfd	45	22942	3218	2487	652	30	13	26	10
PFM666	mutL ndk	40	22067	13542	12283	599	69	47	529	15
MA experiments on minimal medium										
‡PFM2	Wild type	50	308230	277	39	128	24	44	30	12
PFM5	mutL	48	28197	1435	663	740	5	13	4	10
PFM343	mutS	46	26280	1608	764	804	10	13	3	14
PFM137	mutL mutY	24	14205	1431	408	487	7	517	2	10
Buffered LB										
PFM144	mutL	24	13389	1291	863	387	13	9	11	8
MA experiments on diluted LB										
PFM343	mutS	40	22495	3546	2205	1256	30	21	20	14
MA experiments on supplemented minimal medium										
PFM343	mutS	39	23017	2476	1276	1130	16	28	15	11
MA experiments on LB at low temperature (28°C)										
PFM342	mutS	44	24875	1259	742	496	3	6	4	8

*Eight experiments reported in references (LEE *et al.* 2012) and (FOSTER *et al.* 2015) are combined; the strains are PFM2, wild type (two data sets, 3K and 6K generations), PFM35, *uvrA*, PFM40, *alkA tagA*, PFM88, *ada ogt*, PFM91, *nfi*, PFM101, *umuDC dinB*, PFM133, *umuDC dinB polB*.

#Data are from (LEE *et al.* 2012)

†The MA experiment used two isolates from the same transduction as founders.

‡Data are from (FOSTER *et al.* 2015)

Table S4A: BPS rates and spectra of the MMR defective strains on LB medium at 37°C

Strain	Genotype	Mutations/Generation/Nt x 10^8 ± 95% CL						
		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
*PFM5	mutL	2.75 ± 0.22	3.92 ± 0.38	1.49 ± 0.17	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
PFM144	mutL	2.03 ± 0.17	3.08 ± 0.31	0.84 ± 0.11	0.05 ± 0.03	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.02
PFM288	mutL	2.61 ± 0.18	4.17 ± 0.33	0.93 ± 0.10	0.06 ± 0.02	0.03 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
PFM342	mutS	3.05 ± 0.21	4.92 ± 0.44	1.10 ± 0.10	0.05 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.01 ± 0.01
PFM343	mutS	2.75 ± 0.14	4.25 ± 0.25	1.15 ± 0.12	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
PFM555/556	mutS	2.47 ± 0.16	3.85 ± 0.31	1.04 ± 0.08	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
PFM304	mutL mutS	2.43 ± 0.14	4.01 ± 0.26	0.75 ± 0.08	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
PFM197	mutH	2.35 ± 0.28	3.28 ± 0.35	1.31 ± 0.17	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.004
PFM567	mutL mutS mutH	2.26 ± 0.20	3.23 ± 0.34	1.20 ± 0.14	0.01 ± 0.01	0.05 ± 0.03	0.03 ± 0.02	0.03 ± 0.02
PFM568	mutL mutS mutH	1.95 ± 0.15	2.61 ± 0.26	1.24 ± 0.21	0.03 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.02
PFM191	uvrD	1.29 ± 0.13	1.67 ± 0.18	0.82 ± 0.11	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.004

Table S4B: Fractions of BPS of the MMR defective strains on LB medium at 37°C

Strain	Genotype	BPS Rate/Total Rate ± 95% CL					
		A:T>G:C	G:C>A:T	A:T>T:A	G:C>T:A	A:T>C:G	G:C>C:G
*PFM5	mutL	0.70 ± 0.09	0.28 ± 0.04	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.002 ± 0.002
PFM144	mutL	0.75 ± 0.10	0.21 ± 0.03	0.01 ± 0.01	0.01 ± 0.004	0.01 ± 0.01	0.01 ± 0.005
PFM288	mutL	0.79 ± 0.08	0.18 ± 0.02	0.01 ± 0.004	0.01 ± 0.003	0.01 ± 0.005	0.005 ± 0.002
PFM342	mutS	0.80 ± 0.09	0.18 ± 0.02	0.01 ± 0.004	0.004 ± 0.002	0.01 ± 0.004	0.002 ± 0.002
PFM343	mutS	0.76 ± 0.06	0.21 ± 0.02	0.01 ± 0.005	0.01 ± 0.002	0.01 ± 0.003	0.004 ± 0.002
PFM555/556	mutS	0.77 ± 0.08	0.21 ± 0.02	0.01 ± 0.003	0.002 ± 0.002	0.01 ± 0.003	0.004 ± 0.002
PFM304	mutL mutS	0.81 ± 0.07	0.16 ± 0.02	0.01 ± 0.005	0.005 ± 0.003	0.01 ± 0.003	0.01 ± 0.003
PFM197	mutH	0.69 ± 0.11	0.28 ± 0.05	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.003	0.004 ± 0.001
PFM567	mutL mutS mutH	0.70 ± 0.09	0.27 ± 0.04	0.003 ± 0.002	0.01 ± 0.01	0.01 ± 0.004	0.01 ± 0.004
PFM568	mutL mutS mutH	0.66 ± 0.08	0.32 ± 0.06	0.01 ± 0.01	0.01 ± 0.005	0.004 ± 0.003	0.003 ± 0.004
PFM191	uvrD	0.64 ± 0.09	0.32 ± 0.05	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002

The data shown here except for those for PFM191 were combined for the results given as "MMR" in the Tables and Figures; see Materials and Methods for the analysis.

* Data from (LEE *et al.* 2012)

Table 5A. Total BPSs/Generation/Triplet of the MMR defective strains on LB medium at 37°C

Triplet	PFM5 $\Delta mutL^{Sc}$	PFM144 $\Delta mutL^{Sc}$	PFM288 $\Delta mutL$	PFM304 $\Delta mutL$ $\Delta mutS^{Sc}$	PFM342 $\Delta mutS$	PFM343 $\Delta mutS$	PFM555/556 $\Delta mutS^{Sc}$	PFM197 $\Delta mutH^{Sc}$	PFM567 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	PFM568 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	Mean	SD
AAA+TTT	0.36	0.45	0.29	0.25	0.40	0.31	0.36	0.37	0.31	0.18	0.33	0.08
AAC+GTT	11.11	9.35	13.15	11.76	13.96	11.88	11.63	9.58	9.07	7.73	10.92	1.95
AAG+CTT	1.23	1.32	1.27	1.12	1.11	1.72	1.66	1.31	1.07	1.78	1.36	0.27
AAT+ATT	0.71	0.67	0.70	0.50	0.71	0.60	0.37	0.47	0.49	0.24	0.55	0.16
CAA+TTG	0.41	0.41	0.41	0.36	0.53	0.50	0.54	0.39	0.53	0.26	0.43	0.09
CAC+GTG	12.34	10.79	12.91	14.59	15.59	14.04	12.06	10.39	10.10	8.52	12.13	2.22
CAG+CTG	2.11	1.14	1.96	1.35	2.09	1.67	1.91	1.75	1.01	1.09	1.61	0.43
CAT+ATG	0.56	0.86	0.79	0.70	1.26	0.51	0.46	0.58	0.71	0.69	0.71	0.23
GAA+TTC	1.27	0.79	1.44	1.21	1.23	1.34	0.84	1.11	0.89	0.72	1.08	0.25
GAC+GTC	16.56	12.65	16.93	15.87	21.33	17.46	15.24	11.73	12.07	8.91	14.87	3.57
GAG+CTC	1.84	0.82	1.63	2.18	2.48	3.21	1.94	2.44	2.23	2.37	2.11	0.63
GAT+ATC	2.04	0.97	1.76	1.17	1.73	1.87	1.27	1.58	1.41	1.31	1.51	0.34
TAA+TTA	0.85	0.40	0.58	0.69	1.07	0.73	0.78	0.69	0.74	0.23	0.68	0.23
TAC+GTA	16.84	14.22	19.69	20.49	23.69	19.50	18.69	15.18	15.91	11.14	17.53	3.61
TAG+CTA	2.61	2.06	2.56	2.27	3.59	3.04	2.91	2.57	2.26	2.10	2.60	0.48
TAT+ATA	1.30	0.66	1.13	0.59	1.20	1.40	0.68	0.98	1.23	1.02	1.02	0.29
AGA+TCT	0.49	0.12	0.08	0.11	0.16	0.12	0.04	0.25	0.30	0.36	0.20	0.14
AGC+GCT	1.22	0.60	0.72	0.59	0.76	0.62	0.69	0.83	0.97	0.85	0.79	0.19
AGG+CCT	0.39	0.14	0.27	0.39	0.17	0.27	0.45	0.35	0.34	0.24	0.30	0.10
AGT+ACT	0.16	0.14	0.18	0.12	0.29	0.14	0.12	0.26	0.07	0.16	0.16	0.07
CGA+TCG	1.37	0.68	0.66	0.58	0.78	1.06	0.96	1.10	0.71	1.30	0.92	0.28
CGC+GCG	2.89	1.45	1.78	1.34	1.89	2.00	2.19	2.73	2.44	2.18	2.09	0.51
CGG+CCG	1.89	0.96	1.21	0.73	1.15	1.39	1.19	1.50	1.29	1.95	1.33	0.38
CGT+ACG	0.96	0.81	0.88	0.46	0.96	0.81	0.71	1.26	0.93	0.88	0.87	0.20
GGA+TCC	1.82	1.24	0.80	0.95	1.88	1.71	1.03	1.22	1.27	0.94	1.29	0.39
GGC+GCC	4.36	2.56	3.04	2.50	3.20	3.10	2.51	3.65	3.55	3.01	3.15	0.59
GGG+CCC	1.56	1.32	1.08	1.08	1.72	1.87	1.70	1.37	1.71	2.04	1.54	0.32
GGT+ACC	1.16	0.70	0.90	0.66	1.26	1.10	1.25	1.21	1.23	1.14	1.06	0.23
TGA+TCA	0.89	0.58	0.35	0.35	0.70	0.65	0.57	0.66	0.45	0.82	0.60	0.18

Triplet	PFM5	PFM144	PFM288	PFM304	PFM342	PFM343	PFM555/556	PFM197	PFM567	PFM568	Mean	SD
TGC+GCA	1.35	1.06	1.24	0.93	1.17	1.55	1.13	1.73	1.81	1.14	1.31	0.29
TGG+CCA	0.59	0.28	0.47	0.35	0.34	0.64	0.53	0.51	0.40	0.61	0.47	0.13
TGT+ACA	1.07	0.59	0.38	0.61	0.45	0.62	0.50	0.80	0.98	1.11	0.71	0.26
All A:T	4.00	3.19	4.28	4.11	5.02	4.35	3.91	3.37	3.27	2.65	3.82	0.69
All G:C	1.53	0.90	0.99	0.80	1.14	1.20	1.07	1.36	1.27	1.27	1.15	0.22
All sites	2.75	2.03	2.61	2.43	3.05	2.75	2.47	2.35	2.26	1.95	2.46	0.34

Table S5B. BPSs/Generation/Triplet of the MMR defective strains on LB medium at 37°C with the purine on the lagging strand template

Triplet	PFM5 $\Delta mutL^{Sc}$	PFM144 $\Delta mutL^{Sc}$	PFM288 $\Delta mutL$	PFM304 $\Delta mutL$ $\Delta mutS^{Sc}$	PFM342 $\Delta mutS$	PFM343 $\Delta mutS$	PFM555/556 $\Delta mutS^{Sc}$	PFM197 $\Delta mutH^{Sc}$	PFM567 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	PFM568 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	Mean	SD
AAA+TTT	0.50	0.38	0.37	0.29	0.48	0.34	0.42	0.47	0.31	0.15	0.37	0.11
AAC+GTT	16.53	14.58	20.56	17.06	20.89	18.17	16.95	14.54	13.79	11.16	16.42	3.03
AAG+CTT	1.36	1.87	2.12	1.69	1.67	2.66	2.03	1.89	1.39	2.55	1.92	0.44
AAT+ATT	0.95	0.75	1.13	0.76	0.64	0.93	0.45	0.52	0.65	0.29	0.71	0.25
CAA+TTG	0.61	0.27	0.29	0.46	0.84	0.54	0.49	0.46	0.53	0.11	0.46	0.20
CAC+GTG	15.98	13.24	17.20	19.39	20.22	17.84	15.32	13.61	12.80	10.30	15.59	3.14
CAG+CTG	2.25	1.26	2.56	1.47	2.74	2.18	2.37	2.24	1.23	1.23	1.95	0.59
CAT+ATG	1.02	1.18	1.04	0.72	1.22	0.65	0.59	0.64	0.97	0.74	0.88	0.24
GAA+TTC	1.50	1.17	2.09	1.52	1.69	1.37	1.04	1.32	1.14	1.26	1.41	0.31
GAC+GTC	22.93	17.93	25.63	23.50	29.91	25.17	21.49	17.29	16.47	12.56	21.29	5.19
GAG+CTC	3.12	1.30	2.20	3.24	3.43	4.59	2.62	3.58	2.38	4.16	3.06	0.97
GAT+ATC	2.00	1.13	2.22	1.24	1.83	2.06	1.39	1.99	2.19	1.59	1.76	0.40
TAA+TTA	1.14	0.30	0.78	1.03	1.53	0.86	1.08	0.80	0.89	0.23	0.87	0.38
TAC+GTA	23.53	20.22	28.50	29.53	35.32	28.15	27.91	22.60	21.36	17.96	25.51	5.26
TAG+CTA	3.81	2.86	4.17	3.70	5.48	4.26	4.18	3.55	4.05	2.72	3.88	0.78
TAT+ATA	1.48	0.76	1.33	0.87	1.75	1.93	0.78	1.32	1.38	1.14	1.28	0.40
AGA+TCT	0.42	0.00	0.08	0.21	0.00	0.08	0.00	0.18	0.12	0.15	0.12	0.13
AGC+GCT	1.07	0.69	0.67	0.79	1.10	0.51	0.36	0.70	0.68	1.01	0.76	0.24
AGG+CCT	0.31	0.14	0.35	0.71	0.23	0.45	0.57	0.36	0.40	0.00	0.35	0.20
AGT+ACT	0.16	0.14	0.18	0.08	0.24	0.09	0.08	0.26	0.00	0.16	0.14	0.08
CGA+TCG	1.31	0.39	0.31	0.44	0.57	0.64	0.58	0.59	0.47	0.45	0.57	0.28
CGC+GCG	1.78	0.85	1.13	0.83	1.23	1.23	1.70	1.44	1.30	1.34	1.28	0.31

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Triplet	PFM5	PFM144	PFM288	PFM304	PFM342	PFM343	PFM555/556	PFM197	PFM567	PFM568	Mean	SD
CGG+CCG	1.17	0.88	0.57	0.50	0.74	1.00	0.67	0.97	0.31	1.21	0.80	0.30
CGT+ACG	0.32	0.29	0.67	0.22	0.24	0.37	0.34	0.68	0.46	0.44	0.40	0.16
GGA+TCC	0.70	0.75	0.40	0.49	1.05	1.30	0.59	0.66	1.09	0.72	0.78	0.28
GGC+GCC	2.55	1.51	1.55	1.16	2.29	1.68	1.53	2.01	1.84	1.58	1.77	0.41
GGG+CCC	1.32	1.32	0.38	0.83	1.36	1.54	0.87	0.99	0.86	1.02	1.05	0.34
GGT+ACC	0.74	0.66	0.60	0.32	0.63	0.68	0.78	0.55	0.36	0.65	0.60	0.15
TGA+TCA	0.56	0.58	0.16	0.14	0.21	0.38	0.35	0.40	0.32	0.39	0.35	0.15
TGC+GCA	0.74	0.58	0.80	0.62	0.55	0.91	0.74	0.97	1.28	0.25	0.75	0.28
TGG+CCA	0.64	0.41	0.26	0.09	0.41	0.59	0.58	0.33	0.32	0.28	0.39	0.17
TGT+ACA	0.54	0.60	0.31	0.54	0.30	0.39	0.21	0.67	0.70	0.69	0.49	0.18
All A:T	5.44	4.37	6.21	5.77	7.04	6.07	5.40	4.77	4.45	3.69	5.32	1.01
All G:C	0.98	0.65	0.59	0.52	0.75	0.78	0.69	0.80	0.72	0.70	0.72	0.13
All sites	3.18	2.48	3.36	3.11	3.85	3.39	3.01	2.76	2.56	2.18	2.99	0.50

Table S5C. BPSs/Generation/Triplet of the MMR defective strains on LB medium at 37°C with the purine on the leading strand template

Triplet	PFM5 $\Delta mutL^{Sc}$	PFM144 $\Delta mutL^{Sc}$	PFM288 $\Delta mutL$	PFM304 $\Delta mutL$ $\Delta mutS^{Sc}$	PFM342 $\Delta mutS$	PFM343 $\Delta mutS$	PFM555/556 $\Delta mutS^{Sc}$	PFM197 $\Delta mutH^{Sc}$	PFM567 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	PFM568 $\Delta mutS$ $\Delta mutL$ $\Delta mutH^{Sc}$	Mean	SD
AAA+TTT	0.21	0.51	0.20	0.22	0.32	0.29	0.30	0.26	0.31	0.22	0.28	0.09
AAC+GTT	5.70	4.13	5.73	6.47	7.03	5.58	6.32	4.61	4.35	4.31	5.42	1.02
AAG+CTT	1.11	0.77	0.42	0.56	0.55	0.79	1.30	0.72	0.75	1.02	0.80	0.27
AAT+ATT	0.47	0.58	0.27	0.24	0.77	0.27	0.30	0.42	0.33	0.19	0.38	0.18
CAA+TTG	0.20	0.54	0.52	0.26	0.23	0.47	0.59	0.32	0.53	0.42	0.41	0.14
CAC+GTG	8.66	8.32	8.58	9.76	10.92	10.21	8.77	7.14	7.38	6.73	8.65	1.35
CAG+CTG	1.98	1.01	1.35	1.23	1.43	1.15	1.45	1.24	0.79	0.94	1.26	0.33
CAT+ATG	0.10	0.55	0.53	0.67	1.31	0.36	0.33	0.52	0.44	0.64	0.55	0.32
GAA+TTC	1.03	0.42	0.80	0.90	0.77	1.31	0.64	0.91	0.65	0.19	0.76	0.31
GAC+GTC	10.13	7.32	8.16	8.17	12.66	9.68	8.94	6.13	7.63	5.22	8.40	2.11
GAG+CTC	0.55	0.33	1.05	1.12	1.52	1.83	1.27	1.30	2.08	0.57	1.16	0.56
GAT+ATC	2.08	0.80	1.29	1.10	1.63	1.69	1.15	1.17	0.63	1.03	1.26	0.44
TAA+TTA	0.57	0.51	0.39	0.34	0.60	0.60	0.48	0.58	0.59	0.23	0.49	0.13
TAC+GTA	10.14	8.21	10.87	11.44	12.05	10.84	9.45	7.74	10.44	4.31	9.55	2.28
TAG+CTA	1.44	1.28	0.98	0.87	1.72	1.84	1.67	1.62	0.50	1.48	1.34	0.43

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TAT+ATA	1.12	0.55	0.92	0.31	0.65	0.86	0.59	0.65	1.07	0.89	0.76	0.25
AGA+TCT	0.55	0.25	0.08	0.00	0.31	0.16	0.07	0.32	0.48	0.57	0.28	0.21
AGC+GCT	1.36	0.52	0.77	0.39	0.44	0.73	1.02	0.95	1.26	0.70	0.81	0.33
AGG+CCT	0.46	0.14	0.18	0.08	0.12	0.09	0.33	0.34	0.27	0.48	0.25	0.15
AGT+ACT	0.16	0.14	0.18	0.16	0.35	0.18	0.17	0.26	0.14	0.16	0.19	0.07
CGA+TCG	1.44	0.98	1.01	0.73	0.99	1.48	1.34	1.62	0.96	2.17	1.27	0.43
CGC+GCG	4.00	2.05	2.43	1.85	2.54	2.76	2.68	4.00	3.57	3.01	2.89	0.76
CGG+CCG	2.61	1.04	1.85	0.95	1.55	1.78	1.71	2.03	2.26	2.69	1.85	0.58
CGT+ACG	1.61	1.33	1.10	0.70	1.68	1.25	1.07	1.83	1.39	1.32	1.33	0.33
GGA+TCC	2.93	1.73	1.19	1.41	2.71	2.11	1.47	1.78	1.45	1.15	1.79	0.61
GGC+GCC	6.16	3.59	4.52	3.83	4.10	4.51	3.47	5.28	5.25	4.44	4.52	0.84
GGG+CCC	1.81	1.31	1.78	1.33	2.09	2.20	2.51	1.74	2.55	3.05	2.04	0.56
GGT+ACC	1.57	0.74	1.19	1.00	1.88	1.52	1.71	1.87	2.08	1.62	1.52	0.42
TGA+TCA	1.21	0.58	0.53	0.56	1.19	0.92	0.79	0.93	0.56	1.25	0.85	0.29
TGC+GCA	1.96	1.52	1.67	1.24	1.77	2.19	1.51	2.49	2.33	2.02	1.87	0.40
TGG+CCA	0.54	0.16	0.67	0.60	0.27	0.69	0.48	0.68	0.47	0.94	0.55	0.22
TGT+ACA	1.61	0.59	0.46	0.67	0.60	0.86	0.78	0.92	1.27	1.52	0.93	0.40
All A:T	2.56	2.00	2.35	2.44	3.00	2.63	2.42	1.97	2.10	1.61	2.31	0.40
All G:C	2.08	1.15	1.38	1.08	1.52	1.61	1.44	1.91	1.83	1.83	1.58	0.33
All sites	2.32	1.57	1.86	1.75	2.25	2.11	1.92	1.94	1.96	1.72	1.94	0.23

The data shown here were combined for the results given as "MMR" in the tables and figures; see Materials and Methods for the analysis.

Table S6. Colony sizes of the Δ*mutS* mutant strain at different times and temperatures

Colony:	Colony diameter in mm, 24 hrs after streaking on LB agar medium				Colony diameter in mm, 48 hrs after streaking on LB agar medium			
	Temperature				Temperature			
	37°C	30°C	28°C	26°C	37°C	30°C	28°C	26°C
1	2.9	1.9	1.2	1.1	5	3	3	2.5
2	3	2	1.1	1	4	3	2	2.5
3	3	2	1	0.8	4	2.5	1.5	2
4	2.9	1.6	1.2	0.9	4	2.5	2	2
5	2.2*	1.3	1	1	2.5*	2.5	2	2
6	2.1*	1	1.2	1	2.5*	2	1.5	2
Average	2.68	1.63	1.12	0.97	3.67	2.58	2	2.17
SD	0.42	0.41	0.1	0.1	0.98	0.38	0.55	0.26
Compared to 24 hrs at 37°C	1	0.61	0.42	0.36	1.37	0.96	0.75	0.81

Δ*mutS* mutant strain PFM342 was streaked from frozen onto 4 LB agar plates that were then incubated at the indicated temperature. Diameters of 6 colonies were measured at 24 and 48 hrs.
* These small colonies were close together

Table S7: The sequence context of repeat base-pair substitutions associated with runs

No. of occurrences	Nt Position	Type of BPS	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	Run length	Consistent with transient misalignment?
5	4604118	AT>GC	T	C	A	A	G	**T**	C	C	C	C	C	C	C	C	T	8	YES
5	2819446	GC>CG	G	G	A	G	G	**G**	C	C	C	C	C	C	C	A	A	7	YES
4	4604239	AT>GC	T	C	A	A	G	**T**	C	C	C	C	C	C	C	C	T	8	YES
4	3377281	AT>GC	T	C	G	T	T	**T**	C	C	C	C	C	C	C	G	C	7	YES
†4	284872	GC>CG	G	G	A	T	G	**G**	C	C	C	C	C	C	A	C	A	6	YES
†1	284872	GC>AT	G	G	A	T	G	**G**	C	C	C	C	C	C	A	C	A	6	NO
4	2730715	AT>GC	C	C	A	G	G	**T**	C	C	C	C	C	A	C	A	C	5	YES
3	4604354	AT>GC	T	C	A	A	G	**T**	C	C	C	C	C	C	C	C	T	8	YES
3	320789	AT>GC	A	T	G	T	T	**T**	C	C	C	C	C	C	C	A	C	7	YES
3	2953118	AT>GC	G	G	G	T	T	**T**	C	C	C	C	C	C	C	G	G	7	YES
3	2743627	AT>GC	A	G	C	T	G	**T**	C	C	C	C	A	T	T	C	C	4	YES
3	195808	AT>GC	A	G	C	T	G	**T**	C	C	C	C	A	T	T	C	C	4	YES
3	921950	AT>GC	C	C	T	T	G	**T**	C	C	C	C	T	G	T	C	A	4	YES
3	4521567	AT>GC	G	C	T	G	G	**T**	C	C	C	C	G	G	C	G	G	4	YES
3	865431	AT>GC	A	T	C	A	A	**A**	C	C	C	C	G	G	A	A	C	4	NO
2	149559	GC>AT	A	A	C	A	G	**G**	A	A	A	A	A	A	A	A	C	8	YES
2	1592272	AT>GC	C	A	A	T	T	**T**	C	C	C	C	C	C	C	C	A	8	YES
2	2680172	AT>GC	G	C	C	C	T	**T**	C	C	C	C	C	C	C	T	T	7	YES
2	2765522	AT>GC	A	A	A	T	T	**T**	C	C	C	C	C	C	C	T	T	7	YES
2	119745	AT>GC	C	A	G	G	T	**T**	C	C	C	C	C	C	G	C	G	6	YES
2	212815	AT>GC	C	C	A	G	T	**T**	C	C	C	C	C	C	T	T	G	6	YES
2	247672	AT>GC	C	A	T	G	T	**T**	C	C	C	C	C	C	T	G	A	6	YES
2	998926	AT>GC	T	T	A	T	G	**T**	C	C	C	C	C	C	G	T	T	6	YES
2	1207272	AT>GC	G	T	A	T	A	**T**	C	C	C	C	C	C	C	T	T	6	YES
2	1336066	AT>GC	C	G	T	C	A	**T**	C	C	C	C	C	C	T	T	C	6	YES
2	1594791	AT>GC	C	A	C	A	T	**T**	C	C	C	C	C	C	G	T	G	6	YES
2	2008710	AT>GC	C	A	G	T	C	**T**	C	C	C	C	C	C	G	A	A	6	YES
2	2208489	AT>GC	C	A	G	C	G	**T**	C	C	C	C	C	C	G	C	C	6	YES

2	2882109	AT>GC	G	A	C	T	T	**T**	C	C	C	C	C	C	G	T	T	6	YES
2	3012989	AT>GC	G	G	T	T	T	**T**	C	C	C	C	C	C	A	G	C	6	YES
2	3335117	AT>GC	G	C	C	A	G	**T**	C	C	C	C	C	C	G	T	A	6	YES
2	4561802	AT>GC	A	C	C	T	G	**T**	C	C	C	C	C	C	G	G	C	6	YES
2	1227842	AT>GC	G	A	G	G	G	**T**	A	A	A	A	A	A	C	A		6	NO
†2	756054	GC>CG	G	T	G	T	G	**G**	C	C	C	C	C	A	C	G	G	5	YES
†1	756054	GC>AT	G	T	G	T	G	**G**	C	C	C	C	C	A	C	G	G	5	NO
2	178096	GC>CG	A	A	C	T	G	**G**	C	C	C	C	C	T	G	G	A	5	YES
2	698617	GC>CG	A	T	T	T	G	**G**	C	C	C	C	C	G	T	A	A	5	YES
2	1513684	AT>GC	A	A	A	C	G	**T**	C	C	C	C	C	G	T	T	G	5	YES
2	2070147	AT>GC	T	C	C	G	G	**T**	C	C	C	C	C	T	T	C	C	5	YES
2	2071691	AT>GC	A	G	A	A	G	**T**	C	C	C	C	C	T	G	T	A	5	YES
2	2074259	AT>GC	G	G	A	A	G	**T**	C	C	C	C	C	G	T	G	A	5	YES
2	2563582	AT>GC	T	A	T	A	G	**T**	C	C	C	C	C	G	C	G	C	5	YES
2	2925702	AT>GC	T	C	C	G	C	**T**	C	C	C	C	C	G	G	T	G	5	YES
2	4528093	AT>GC	C	T	C	A	G	**T**	C	C	C	C	C	T	A	T	C	5	YES
2	1831229	GC>AT	A	T	C	A	C	**G**	C	C	C	C	C	G	T	C	T	5	NO
2	3370127	GC>AT	C	G	A	C	G	**G**	C	C	C	C	C	A	C	A	G	5	NO
2	3267978	AT>GC	A	A	A	A	G	**T**	A	A	A	A	A	C	C	C	G	5	NO
2	1476862	AT>GC	C	A	A	T	G	**T**	T	T	T	T	C	C	C	G	G	5	YES
2	8296	AT>GC	C	G	A	T	G	**T**	C	C	C	C	A	G	T	G	T	4	YES
2	164633	AT>GC	A	T	C	G	G	**T**	C	C	C	C	T	C	T	C	C	4	YES
2	280037	AT>GC	C	C	A	T	G	**T**	C	C	C	C	G	A	C	C	A	4	YES
2	285142	AT>GC	G	C	T	C	G	**T**	C	C	C	C	T	T	T	G	C	4	YES
2	604964	AT>GC	C	G	G	C	G	**T**	C	C	C	C	T	T	T	A	T	4	YES
2	732668	AT>GC	T	A	T	A	G	**T**	C	C	C	C	T	T	T	A	C	4	YES
2	1072271	AT>GC	T	A	A	C	G	**T**	C	C	C	C	A	T	A	T	T	4	YES
2	1114343	AT>GC	A	T	T	T	G	**T**	C	C	C	C	A	A	C	G	C	4	YES
2	1124625	AT>GC	T	A	T	T	G	**T**	C	C	C	C	G	A	A	G	T	4	YES
2	1179612	AT>GC	T	T	G	C	G	**T**	C	C	C	C	T	T	G	C	A	4	YES
2	1485764	AT>GC	G	A	T	T	G	**T**	C	C	C	C	A	G	T	C	A	4	YES
2	1801735	AT>GC	G	C	A	T	G	**T**	C	C	C	C	T	T	A	G	T	4	YES
2	1819069	AT>GC	A	C	C	C	C	**T**	C	C	C	C	A	A	A	G	C	4	YES
2	2292056	AT>GC	G	A	A	A	G	**T**	C	C	C	C	A	A	C	A	G	4	YES

2	2352672	AT>GC	A	G	A	A	G	**T**	C	C	C	C	G	T	C	T	G	4	YES
2	2769240	AT>GC	T	G	C	G	G	**T**	C	C	C	C	G	G	A	C	T	4	YES
2	3071457	AT>GC	A	T	C	G	C	**T**	C	C	C	C	T	G	G	C	G	4	YES
2	3169922	AT>GC	A	A	C	C	G	**T**	C	C	C	C	A	A	A	A	A	4	YES
2	3512084	AT>GC	A	A	A	C	G	**T**	C	C	C	C	T	C	T	T	C	4	YES
2	3917888	AT>GC	T	C	C	A	G	**T**	C	C	C	C	T	T	A	A	G	4	YES
2	4306386	AT>GC	C	T	T	T	G	**T**	C	C	C	C	A	A	T	C	T	4	YES
2	4382888	AT>GC	C	G	G	C	G	**T**	C	C	C	C	T	G	G	A	T	4	YES
2	303014	AT>GC	T	T	G	T	T	**A**	C	C	C	C	T	G	A	A	A	4	NO
2	1037031	AT>GC	T	T	T	G	T	**A**	C	C	C	C	T	T	A	C	C	4	NO
2	1386996	AT>GC	T	A	C	A	T	**A**	C	C	C	C	G	T	T	T	G	4	NO
2	1485713	AT>GC	T	C	A	G	G	**A**	C	C	C	C	A	C	C	A	T	4	NO
2	1820478	AT>GC	A	T	T	G	A	**A**	C	C	C	C	T	C	C	A	G	4	NO
2	2648824	AT>GC	A	G	A	A	A	**A**	C	C	C	C	G	C	T	A	A	4	NO
2	3173706	AT>GC	C	G	A	G	A	**A**	C	C	C	C	T	A	C	A	C	4	NO
2	1108783	AT>GC	T	G	A	A	G	**A**	C	C	C	C	A	T	T	C	A	4	NO
2	3191199	AT>GC	G	C	G	G	G	**T**	A	A	A	A	C	C	T	T	C	4	NO
2	111326	AT>GC	A	G	C	C	G	**T**	G	G	G	G	T	A	A	A	G	4	NO
2	337229	AT>GC	G	T	T	G	G	**T**	G	G	G	G	T	C	A	A	T	4	NO
2	4500425	AT>GC	A	G	C	T	G	**T**	G	G	G	G	T	G	T	C	T	4	NO
82																			63 YES
																			19 NO

*The sequences have been arranged so that the run is 3' to the mutated base.

†Two different types of BPSs occurred at this basepair, one that is consistent with transient misalignment and one that is not.

Table S8: Mutation rates to NalR of Δ*mutL* Δ*nfi* and Δ*mutL* Δ*rdgB* strains as determined by fluctuation test

	PFM244 Δ*mutL*	PFM794 Δ*mutL* Δ*nfi*	PFM800 Δ*mutL* Δ*rdgB*
Mutations/generation x 10^8 ± 95% CL	1.14 ± 0.12	1.20 ± 0.12	1.03 ± 0.11

The mutation rates were calculated from 30, 32, and 32 parallel cultures of PFM244, PFM794, and PFM800, respectively, plated on LB plus nalidixic acid agar. See Supplemental Materials and Methods for details.

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