

Supplemental Information

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1 Supplemental Experimental Procedures

1.1 Strain Construction

Landing Pad MutH F and Landing Pad MutH R were the primers used to amplify the landing pad that was used for the translational fusion of *mCherry* to *mutH* on the chromosome. mCherry::MutH F and mCherry::MutH R were the primers used to amplify the *mCherry* gene off a plasmid and add homologies to the chromosomal regions around the N-terminus of *mutH* so that after ligation into a plasmid it could be recombined into the chromosome from the plasmid pTKIP-*mCherry-mutH* into the chromosome. MutH verification F and MutH verification R were used to verify proper insertion of *mCherry* in the translational fusion with the native copy of *MutH* on the chromosome.

lacI-RBS4chswap F and RBS4swap R were used as primers with pUC57(amp)-*P_{tet}-mCherry-mutH-P_{tet}-tetR* as the template in order to make swap the consensus ribosomal binding site in *P_{tet}-mCherry-mutH-P_{tet}-tetR* for the lacI RBS and give it restriction sites matching the multiple cloning site of pAH144 as well as *IsceI* sites. This way it could be ligated into various plasmids. The lacI RBS primer was used to verify that the correct ribosomal binding site was in the construct.

Table S1: **Primers**

Name	sequence
Landing Pad MutH F	AAAAAGCAATATAAATCAGAGAATTTAGGGATAACAGGGTAATATT TACGTTGACACCACCTTTTCGCG
Landing Pad MutH R	GTAACCTTTTTCCACATGCGTTGTTTATTACCCTGTTATCCCTACTAA GCACTTGTCTCCTGTTTACTC
mCherry::MutH F	TAGGGATAACAGGGTAATAAAAAGCAATATAAATCAGAGAATTGAA CAACGCATGTGGAAAAAGTTACACTGCGAATATTCGGGCACATAATT GCTGTTTGTTTTTTAATCAAGGTATCATGACATGGTGAGCAAGGGC GAGGAGGATA
mCherry::MutH R	ATTACCCTGTTATCCCTACGTAACCAGCCGACAAGTGCCGCCAGTT CTCCCAATGTATAACCAGAAAGTTGCTGTGCTTGCGCTAACAACCTGT TCTTCAGTTTTCGGGAGGAGAGAGCAGTGGGCGAGGTTGGGACATGC CGCCGCCGCCGCCCTTGTACAGCTCGTCCATGCCG
MutH verification F	TTGCGCAACTCGATTACCGGCAACC
MutH verification R	CTTTATCGCGTTTTAAATTCTCTGG
_SalI-HindIII-tetO F	TAAGCAGTCGACAAGCTTTCCCTATCAGTGATAGAGA
tetR(C)-AflII-KpnI-	TGCTTAGGTACCCTTAAGTTAAGACCCACTTTTCACAT
lacI RBS	GAAGAGAGTCAATTCAGGGTGGTGAAT
lacI-RBS4chswap F	TAGGGATAACAGGGTAATTACGGCCCCAAGGTCCAAACGGTGAGTC GACTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAG ATACTGAGCACATCAGCAGGACGCACTGACCGAAGAGAGTCAATTC AGGGTGGTGAATATGGTGAGCAAGGGCGAGGAGGATA
RBS4swap R	ATTACCCTGTTATCCCTATTGGCTTCAGGGATGAGGCGCCATCGGT ACCTTAAGACCCACTTTCACATTTAAGT

Table S2: **Plasmids**

Name	Purpose	source
pTKRED	Landing pad recombineering	[4]
pTKIP- <i>neo</i>	Landing pad recombineering	[4]
pTKIP- <i>neo-P_{tet}-tetR</i>	Suppressing expression off <i>P_{tet}</i>	—
pTKIP- <i>mCherry-mutH</i>	Landing pad recombineering	This work
pCP20	Removal of FRT-flanked <i>cat</i> gene	[1]
pAH69	CRIM recombineering	[3]
pAH144	CRIM recombineering	[3]
pAH144- <i>P_{tet}-mCherry-mutH-P_{tet}-tetR</i>	CRIM recombineering	This work
pAH144- <i>P_{tet}-mCherry-mutH(lacIRBS)-P_{tet}-tetR</i>	CRIM recombineering	This work
pUC57(amp)- <i>P_{tet}-mCherry-mutH-P_{tet}-tetR</i>	tunable mutation rate construct	This work

Table S3: ***E. coli* strains**

Name	Purpose/Genotype	Plasmids	Source	Ancestral Strain
MG1655 $\Delta motA$	$\Delta motA$	None	This work	MG1655
MG1655 <i>mCherry-mutH</i>	<i>mCherry-mutH</i> translational fusion (native locus) $\Delta motA$	None	This work	MG1655 $\Delta motA$
ME121	<i>mutL218::Tn10</i> , $\Delta lacZ$, P_{lac} - <i>yfp-mutL-cat</i> , <i>mutH::kan</i>	None	[2]	See [2]
NS001	as ME121, <i>P_{tet}-mCherry- mutH(lacIRBS)- P_{tet}-tetR</i> at HK022 site	pTKIP- <i>neo-P_{tet}-tetR</i>	This work	ME121
NS001 Δcat	as NS001 but without <i>cat</i> gene	pTKIP- <i>neo-P_{tet}-tetR</i>	This work	NS001 (sans plasmid)
BW23474	CRIM plasmid host	varied	[3]	See [3]
DH5 α	plasmid cloning	varied	Douglas Hanahan	—

1.2 Mutation Rate Plating Assays

1.2.1 Plating assays for cells grown in shaker

The day before cells were to be grown for a mutation rate plating assay, overnight cultures of any strains to be tested were grown in supplemented M9 plus any needed antibiotics at 37° C in a shaker at 220 rpm. The next day the optical density of each overnight culture was measured and for each condition to be plated (a condition is determined by the strain of *E. coli*, the growth medium, the temperature, the concentrations of inducers and antibiotics, etc.) cells were diluted to an optical density of 10^{-4} and grown in that condition for 10 doublings to acclimate them to the condition. When the cells reach an optical density of 10^{-1} , they were diluted by a factor of 10^{-6} into fresh medium plus antibiotics and inducers and then this volume was divided into 250 microliter aliquots in 5 mL plastic tubes. One 500 microliter aliquot was also made to take measurements of optical density when it was close to time to plate the cultures. These tubes were numbered to keep track of any different strains or conditions grown the same night and placed in the shaker to grow for 20 doubling times. The next day when the optical density was $\approx .1$ in the aliquot set aside for measuring optical density, the aliquots were removed from the shaker. One μl of each 250 μl aliquot was taken and diluted by 10^{-6} in PBS buffer and plated on an LB plate. The remaining 249 μl was plated on a rifampicin plate (50 $\mu\text{l}/\text{ml}$). Plates were numbered by their aliquot. All plates were placed in a 37° C incubator for 24 hours. Then they were removed and colonies were counted on each plate.

1.2.2 Plating assays for cells grown in platereader

Plating assays for growth in the platereader were the same except cells were grown at 30° C in the shaker during acclimation, aliquots were 500 microliters and placed into the rows of a 48 well plate instead of into separate plastic tubes, and aliquots of cells were grown until they reached an optical density of $\approx .2$ in the platereader. From growth curves measured in the platereader in the same conditions, this is roughly the tail end of exponential growth. Because the platereader measures optical density over time, an extra aliquot to measure optical density did not need to be used for these experiments.

1.3 Transfer Culture Platereader Evolution Experiments

Every three days or at the end of the experiment, every well from a plate was mixed with an equal volume of 80% glycerol in a cryogenic freezer vial, labeled, and stored at -80° C. This both provided a backup in case of an accident and allowed us to resume the experiment after a pause since the platereader was shared by multiple users. There were two times the experiment was temporarily suspended. Cells frozen on days 9 and 26, were revived for days 10 and 27. To revive cells we used a flamed inoculating loop to pick up a small amount of cells from the corresponding vial for each well into fresh medium in a 48-well plate. Because our control of the initial population size on this day is inexact and it takes time for cells to adjust to the culture conditions, we have not included these days or the very first day cells were grown in the platereader in our data analysis.

Table S4: Mutation rates of Evolved *E. coli* compared to ancestor

Replicate	Mutation (end)	Rate	95% confidence in- terval	Mutation (start)	Rate	95% confidence in- terval
High 1	1.4×10^{-7}		$(1.1, 1.7) \times 10^{-7}$	2.2×10^{-7}		$(1.6, 2.9) \times 10^{-7}$
HiMid 1	6.0×10^{-8}		$(4.7, 7.5) \times 10^{-8}$	4.1×10^{-8}		$(2.6, 5.9) \times 10^{-8}$
Mid 1	$.78 \times 10^{-8}$		$(.43, 1.2) \times 10^{-8}$	1.4×10^{-8}		$(.64, 2.5) \times 10^{-8}$
LoMid 1	Rif resistant		Rif resistant	3.8×10^{-9}		$(1.2, 7.4) \times 10^{-9}$
LoMid 5	2.3×10^{-9}		$(.77, 4.3) \times 10^{-9}$	3.8×10^{-9}		$(1.2, 7.4) \times 10^{-9}$
Low 1	$.61 \times 10^{-9}$		$(.07, 1.5) \times 10^{-9}$	1.7×10^{-9}		$(.41, 3.6) \times 10^{-7}$
Low 5	1.1×10^{-9}		$(.16, 2.7) \times 10^{-9}$	1.7×10^{-9}		$(.41, 3.6) \times 10^{-7}$

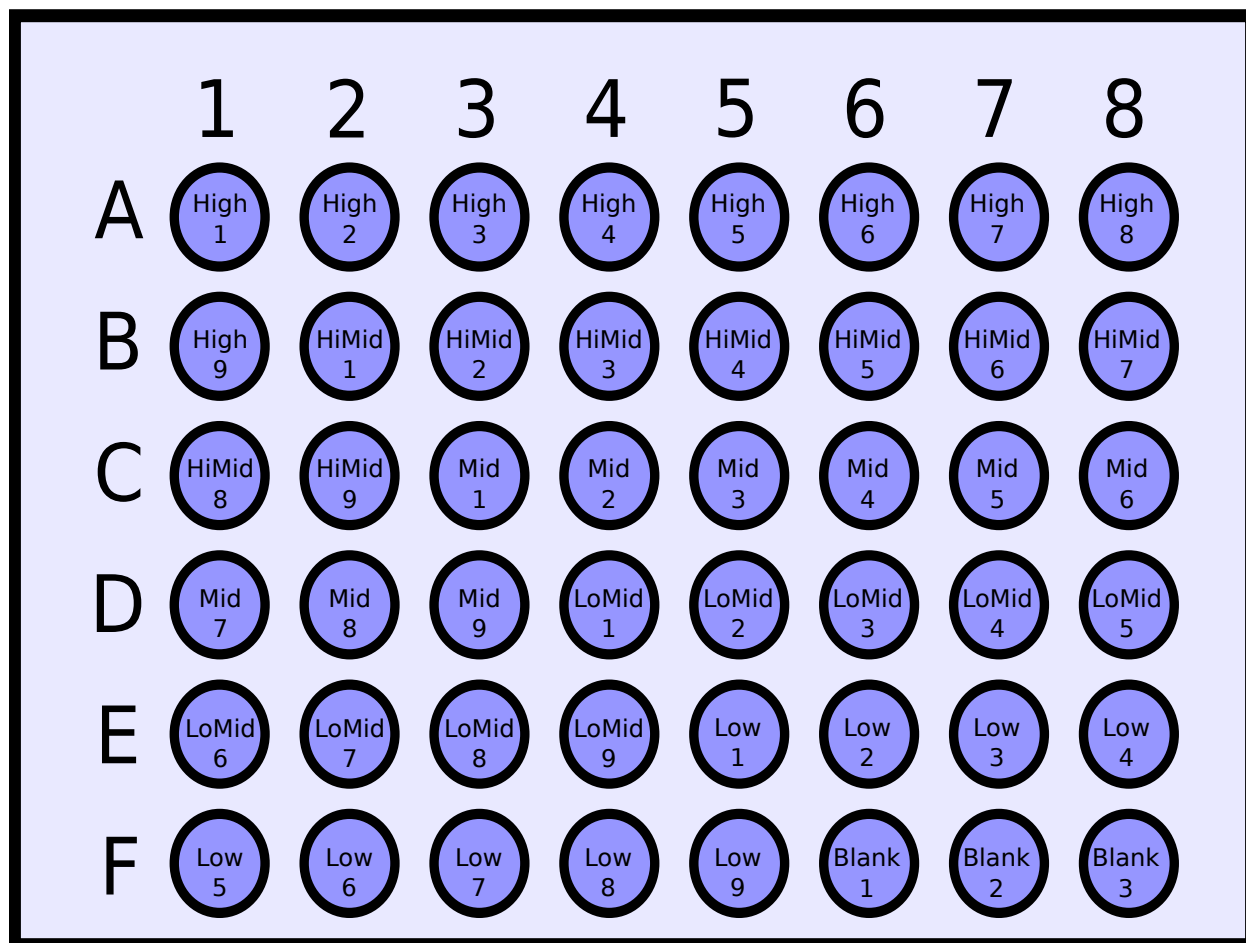


Figure S1: Layout of the conditions NS001 Δ *cat* was grown in for the evolution experiment

2 Supplemental Data Analysis

2.1 Mutation rate as a function of MutL induction

ME120, ME121, NS001, and NS001 all express yfp-mutL off of a lac promoter. So mutL expression is induced by IPTG. Although not designed to give a tunable mutation rate, we find that it is possible to hit intermediate mutation rates between no induction and full induction although the response is very steep. We measured the mutation rate with the same rifampicin plating methods used for measuring the mutation rate as a function of aTc induction. For all experiments measuring the mutation rate as a function of IPTG concentration the medium was supplemented M9, and we induced mutH to a saturating level by putting 100 ng/ml of aTc into the medium as well. We fit a hill function plus shift to the data as a phenomenological description and found a hill coefficient of 5.4 (figure S2).

References

- [1] K A Datsenko and B L Wanner. “One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products”. In: *Proceedings of the National Academy of Sciences of the United States of America* 97.12 (2000), pp. 6640–5.
- [2] Marina Elez, Miroslav Radman, and Ivan Matic. “Stoichiometry of MutS and MutL at unrepaired mismatches in vivo suggests a mechanism of repair”. In: *Nucleic Acids Research* 40.9 (Jan. 2012), pp. 3929–3938.
- [3] A Haldimann and B L Wanner. “Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies of bacteria”. In: *Journal of bacteriology* 183.21 (2001), pp. 6384–93.
- [4] Thomas E Kuhlman and Edward C Cox. “Site-specific chromosomal integration of large synthetic constructs”. In: *Nucleic acids research* 38.6 (2010), e92.

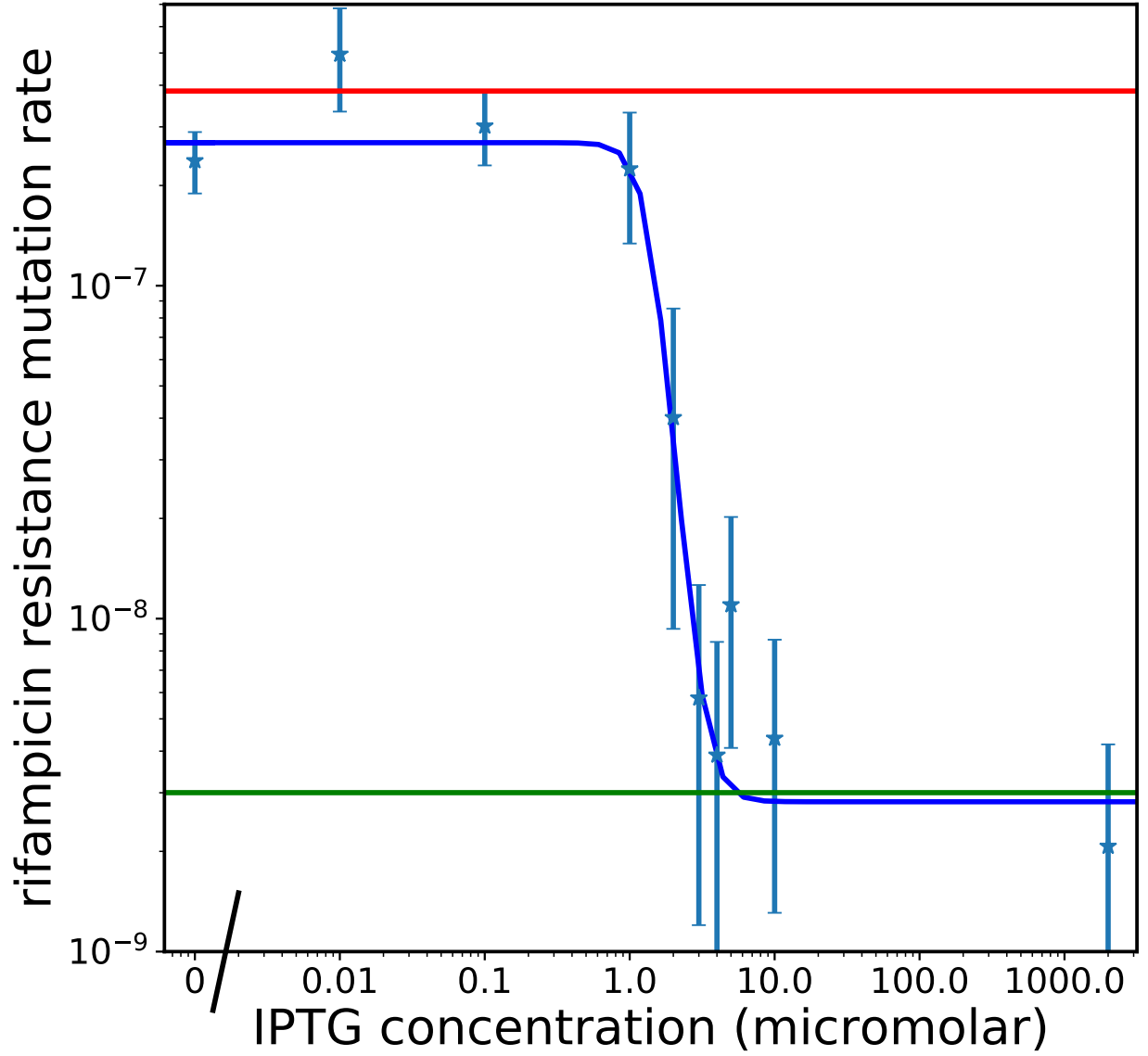


Figure S2: The mutation rate as a function of IPTG concentration. The red line is the mutation rate of ME121 which has no mismatch repair. The green line is the mutation rate of ME120 grown with 2000 micromolar IPTG. The light blue points are NS001 with 100 ng/ml aTc and varying concentrations of IPTG. The dark blue line is a hill function plus shift fit to the NS001 data $y = \frac{A}{(1+(\frac{k_a}{x})^n)} + C$. The parameters were $A = -2.66 \times 10^{-7}$, $k_a = 1.38$, $n = 5.4$, and $C = 2.69 \times 10^{-7}$.