

Supporting Information

Dalia et al. 10.1073/pnas.1406478111

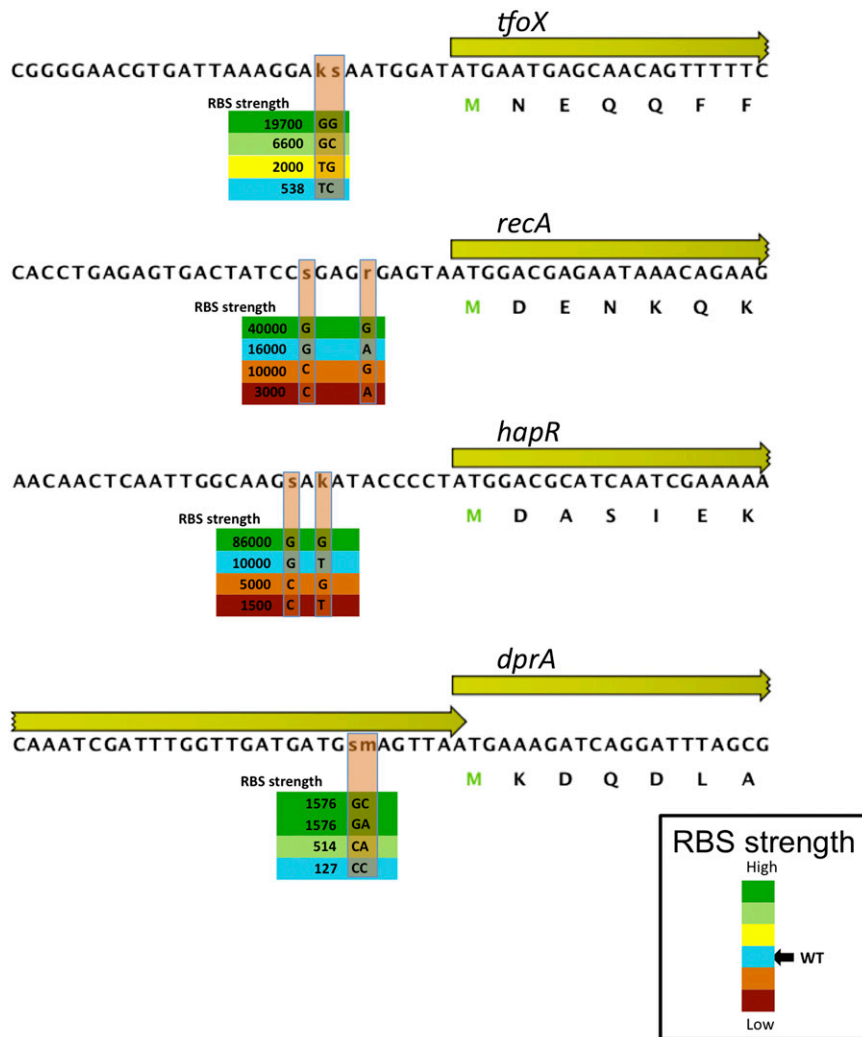


Fig. S1. The design for ribosome-binding site (RBS) optimization at *tfoX*, *recA*, *hapR*, and *dprA* showing the bases that were randomized. RBS strengths shown are from the ribosome binding site calculator (1) and are based on an arbitrary scale from 0–100,000.

1. Salis HM (2011) The ribosome binding site calculator. *Methods Enzymol* 498:19–42.

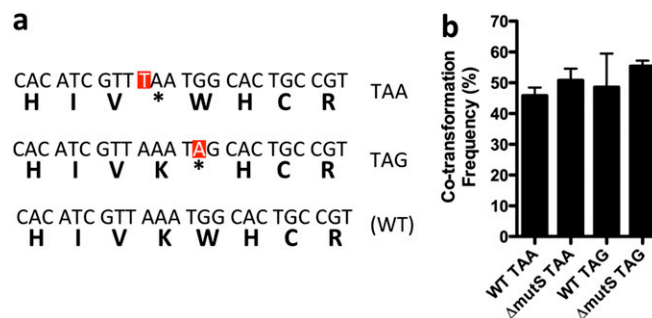


Fig. S2. Lack of mismatch repair (MMR) activity during natural competence of *Vibrio cholerae* on chitin. (A) Design of transversion (TAA) and transition (TAG) mutations in *lacZ*, which result in premature stop codons. Transition mutations are repaired by MMR more efficiently than are transversion mutations. (B) Cotransformation frequency for these mutations in WT and the MMR-deficient *mutS* deletion strain, demonstrating MMR has that little to no effect on co-transformation in *V. cholerae*. Data are from two biological replicates and are shown as the mean \pm SD.

Table S1. Parent strains used in this study

Strain name in article	Genotype and antibiotic resistance(s)	Description
WT <i>V. cholerae</i>	E7946 Sm ^R	Sm ^R derivative of WT <i>V. cholerae</i> O1 El Tor (1), which was used to optimize cotransformation in <i>V. cholerae</i> .
Parent strain for <i>V. cholerae</i> multiplex genome editing by natural transformation (MuGENT)	E7946 SmR ΔVC1807 <i>lacI^q::lacZ</i> Kan ^R	Contains <i>lacI^q</i> in the <i>lacZ</i> gene, which regulates the P _{tac} promoter used in MuGENT for natural transformation in <i>V. cholerae</i> . Generated by cotransformation with a selected product that replaces VC1807 with the kanamycin-resistance marker.
Δ <i>mutS</i> <i>V. cholerae</i>	E7946 SmR ΔVC0535 (<i>mutS</i>) Spec ^R	<i>mutS</i> gene replaced by Spec ^R marker, used to study the effect of MMR on cotransformation in <i>V. cholerae</i> .
WT <i>Streptococcus pneumoniae</i>	TIGR4	WT virulent serotype 4 pneumococcal isolate (2), used for pneumococcal histidine triad (<i>pht</i>) MuGENT
MMR-deficient <i>S. pneumoniae</i>	TIGR4 Sm ^R magellan2::SP_2076 (<i>hexA</i>)	Mariner transposon insertion in <i>hexA</i> , a critical component of MMR in <i>S. pneumoniae</i> (3), used for <i>pht</i> MuGENT in an MMR-deficient strain

Kan^R, kanamycin-resistance marker; Sm^R, streptomycin resistance; Spec^R, spectinomycin-resistance marker.

- Levine MM, et al. (1982) The pathogenicity of nonenterotoxigenic *Vibrio cholerae* serogroup O1 biotype El Tor isolated from sewage water in Brazil. *J Infect Dis* 145(3):296–299.
- Tettelin H, et al. (2001) Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. *Science* 293(5529):498–506.
- Hava DL, Camilli A (2002) Large-scale identification of serotype 4 *Streptococcus pneumoniae* virulence factors. *Mol Microbiol* 45(5):1389–1406.

Table S2. Primers used in this study

Primer name	Sequence (5' → 3')	Description
Mutant construct primers		
ABD346	GTAGAATAAGTGGCGGTGAGCC	F1 oligo for 3 kb UP arm Δ VC1807 SOE
ABD344	GATTAGCAACGATTCTAGCGCAGGAG	F1 oligo for 2-kb UP arm Δ VC1807 SOE
ABD342	ATTTTTCAGTTGGCCTACAATGCTTTCC	F1 oligo for 1-kb UP arm Δ VC1807 SOE
ABD340	gtcgacggatccccgggaatACGTTTCATTAGTCACCTCTATT-GTTAACTTGTTCC	R1 oligo for UP arm Δ VC1807 SOE
ABD341	gaagcagctccagcctacaTAGTCGAAAAATAAAAAAAGAGG-CTCGCCTC	F2 oligo for DOWN arm Δ VC1807 SOE
ABD343	CGATGAGGATAAAAAACAGTACAGGCC	R2 oligo for 1-kb DOWN arm Δ VC1807 SOE
ABD345	CTTGCTAACCGTTGGTGTACCAGC	R2 oligo for 2-kb DOWN arm Δ VC1807 SOE
ABD347	GCGCCCAATGTGTCCCTTTGATG	R2 oligo for 3-kb DOWN arm Δ VC1807 SOE
ABD123	ATCCGGGGATCCGTCGAC	F oligo for Kan ^R , Amp ^R , and Spec ^R markers in <i>V. cholerae</i>
ABD124	TGTAGGCTGGAGCTGCTTC	R oligo for Kan ^R , Amp ^R , and Spec ^R markers in <i>V. cholerae</i>
ABD357	GCGCTGCCAAGTAGGTGTAACG	F1 oligo for UP arm of Δ mutS SOE for <i>V. cholerae</i>
ABD358	gtcgacggatccccgggaatCATAATCTTATGTCGCTGCTTAT-CATCATCTG	R1 oligo for UP arm of Δ mutS SOE for <i>V. cholerae</i>
ABD359	gaagcagctccagcctacaTAGTTATTGCCATATCTCAAGC-ATGGAATC	F2 oligo for UP arm of Δ mutS SOE for <i>V. cholerae</i>
ABD360	AGATCTTGCCCTGATGACGCTTTACTC	R2 oligo for UP arm of Δ mutS SOE for <i>V. cholerae</i>
ABD336	ACCCTAAGCGGTTCAATTTTGTGCGATG	F1 oligo for 3 kb <i>lacZ</i> UP arm
ABD334	AGTGCTCCGGACTCTTTGCTCTG	F1 oligo for 2 kb <i>lacZ</i> UP arm
ABD332	GGCTGAACGTGGTGTGCGAAAAATGAC	F1 oligo for 1 kb <i>lacZ</i> UP arm
ABD333	ATCACATGCCCAATTTCGGGATG	R2 oligo for 1 kb <i>lacZ</i> DOWN arm
ABD335	CACTGCTCACTAGCGATGCAGTG	R2 oligo for 2 kb <i>lacZ</i> DOWN arm
ABD337	TGATCCGATGATCTTTTCGCCACC	R2 oligo for 3 kb <i>lacZ</i> DOWN arm
ABD329	GAACATGGGGTGTACGGCAGTGCCATTaAACGATGTGCGGGT-TTTGCCAATCTTG	R1 oligo for <i>lacZ</i> TAA transversion mutation
ABD328	CAAGATTGGCAAAACCCGCACATCGTTtAATGGCACTGCCGT-ACACCCCATGTTT	F2 oligo for <i>lacZ</i> TAA transversion mutation
ABD331	GAACATGGGGTGTACGGCAGTGCTATTTAACGATGTGCGGGT-TTTGCCAATCTTG	R1 oligo for <i>lacZ</i> TAG transversion mutation
ABD330	CAAGATTGGCAAAACCCGCACATCGTTAAATaGCACTGCCGT-ACACCCCATGTTT	F2 oligo for <i>lacZ</i> TAG transversion mutation
ABD361	CCTCtctccctatagtgagtcgtattaatttcATTTAACGAT-GTGGGGTTTTGCCAATC	R1 oligo for Δ 50, Δ 100, Δ 464, and Δ 1166 bp in <i>lacZ</i>
ABD362	CGTTAAATgaaattaatacagactcactataggagaGAGGCT-CGTTTGGATGTTGGG	F2 oligo for Δ 50 bp in <i>lacZ</i>
ABD363	CGTTAAATgaaattaatacagactcactataggagaGaGGTT-TGCTCTGTTGAGAAGCC	F2 oligo for Δ 100 bp in <i>lacZ</i>
ABD364	ATCGTTAAATgaaattaatacagactcactataggagaGAGG-GTGA AACCTGTTGGTGG	F2 oligo for Δ 464 bp in <i>lacZ</i>
ABD365	CATCGTTAAATgaaattaatacagactcactataggagaGAG-GCCAATCTCGAAACCCAC	F2 oligo for Δ 1166 bp in <i>lacZ</i>
ABD288	GCCAATCGTGGAGTCGACCC	F1 oligo for a transversion point mutation upstream of VCA0063 that abolishes an MboI cleavage site
ABD069	AAATAAaATCAATTATCATTGTGTTATTTTAGAGC	R1 oligo for a transversion point mutation upstream of VCA0063 that abolishes an MboI cleavage site
ABD068	AAATGATAATTGATtTTATTTAGATTGTGTTAGAGTGGCGAC	F2 oligo for a transversion point mutation upstream of VCA0063 that abolishes an MboI cleavage site
ABD289	CGTCACCTGAAGATCCATCCGTC	R2 oligo for a transversion point mutation upstream of VCA0063 that abolishes an MboI cleavage site
ABD841	GGCACTGCCGTACACCCCATGTTCCGGTACCCGACCCATCG-AATG	F oligo to amplify <i>lacI^f</i> with overlaps to insert into the <i>lacZ</i> gene of <i>V. cholerae</i> .
ABD842	GGCTCTCTGGCTTATTGTGGGGGAAACCTGCTGCCAGCTGG	R oligo to amplify <i>lacI^f</i> with overlaps to insert into the <i>lacZ</i> gene of <i>V. cholerae</i> .
ABD840	TTAATTGCGTTGCGCTCACTGCCGACTCCCGTTCTGGATAA-TGTTTTTTGC	F oligo for P _{tac} and <i>rrnB</i> promoter construct (i.e., the MIDDLE for the MuGENT PCR products of <i>tfoX</i> , <i>hapR</i> , and <i>recA</i>)
ABD625	CTGATGAATCCCCTAATGATTTTGG	R oligo for P _{tac} and <i>rrnB</i> promoter construct (i.e., the MIDDLE for the MuGENT PCR products of <i>tfoX</i> , <i>hapR</i> , and <i>recA</i>)
ABD808	GTGTGGCTTGTGCGTCCGGCAAAGG	F1 oligo for UP arm of <i>tfoX</i> MuGENT SOE product
ABD809	CGGGCAGTGAGCGCAACGCAATTAATGCAATACTTTTGGCG-CAGATTATG	R1 oligo for UP arm of <i>tfoX</i> MuGENT SOE product

Table S2. Cont.

Primer name	Sequence (5' → 3')	Description
ABD810	CAAAATCATTAGGGGATTCATCAGGGGAACGTGATTAAGG-AksAATGGATATGAATG	F2 oligo for DOWN arm of <i>tfoX</i> MuGENT SOE product
ABD811	GTTAGAAGAGCTTATCCATCACCG	R2 oligo for DOWN arm of <i>tfoX</i> MuGENT SOE product
ABD812	AAATGGAGTTTGATCGCATTGGC	F1 oligo for UP arm of <i>recA</i> MuGENT SOE product
ABD813	CGGGCAGTGAGCGCAACGCAATTAACCCCTGTATAGAAAAA-GTTTGGC	R1 oligo for UP arm of <i>recA</i> MuGENT SOE product
ABD814	CAAAATCATTAGGGGATTCATCAGGTGACTATCCSGAGRGAG-TAATGGACGAGAATAAAC	F2 oligo for DOWN arm of <i>recA</i> MuGENT SOE product
ABD815	TGATCAGCGTTTGGAAATACGTCG	R2 oligo for DOWN arm of <i>recA</i> MuGENT SOE product
ABD816	AAAGCACCAAATAATCCCGCGACC	F1 oligo for UP arm of <i>hapR</i> MuGENT SOE product
ABD817	CGGGCAGTGAGCGCAACGCAATTAACCTGTGGTGAAAAA-TGTGC	R1 oligo for UP arm of <i>hapR</i> MuGENT SOE product
ABD818	CAAAATCATTAGGGGATTCATCAGATTGGCAAGSAKATACCC-CTATGGACGCATCAATCG	F2 oligo for DOWN arm of <i>hapR</i> MuGENT SOE product
ABD819	GGCCTGCTTAGCAAATTTACCAG	R2 oligo for DOWN arm of <i>hapR</i> MuGENT SOE product
ABD820	CGCTCTTATCTGCTTGGATAATGG	F1 oligo for UP arm of <i>dprA</i> MuGENT SOE product
ABD821	GCCGCTAAATCCTGATCTTTCATTAACKSCATCATCAACCA-AATCGATTTGTTC	R1 oligo for UP arm of <i>dprA</i> MuGENT SOE product
ABD822	AGTTAATGAAAGATCAGGATTTAGCGGC	F2 oligo for DOWN arm of <i>dprA</i> MuGENT SOE product
ABD823	TGAAGTACAAGGCCAGTTACTGG	R2 oligo for DOWN arm of <i>dprA</i> MuGENT SOE product
ABD824	TTTAGCCCCATTGGCGAAGTGGG	F1 oligo for UP arm of <i>mutS</i> MuGENT SOE product
ABD825	GAGTATCTTTGACGTATTGGATCtcatattataactacATAAT-CTTATGTCGCTGCTTATC	R1 oligo for UP arm of <i>mutS</i> MuGENT SOE product
ABD826	GATAAGCAGCGACATAAGATTATGtagtataaatatgaGATCC-AATACGTCAAAGATACTC	F2 oligo for DOWN arm of <i>mutS</i> MuGENT SOE product
ABD360	AGATCTTGCCTGATGACGCTTTACTC	R2 oligo for DOWN arm of <i>mutS</i> MuGENT SOE product
Spn cat F	CGGTATCGATAAGCCTTGATG	F oligo for SpecR and CmR markers in <i>S. pneumoniae</i>
Spn cat R	TGGAGCTGTAATATAAAAAACCTTCTTC	R oligo for SpecR and CmR markers in <i>S. pneumoniae</i>
ABD528	GGAGAACTTAATGAATTTAGAGGACATG	F1 oligo for UP arm of Δ SP_1051 SOE
1051_R1	CATCAAGCTTATCGATACCGTTCATTAGGCCACCATC	R1 oligo for UP arm of Δ SP_1051 SPE
1051_F2	GAAGAAGGTTTTTATATTACAGCTCCAGGGGAAAAGAGACT-TAATG	F2 oligo for DOWN arm of Δ SP_1051 SOE
ABD529	CTATGTGTTTCAGCTGACTCCCATG	R2 oligo for DOWN arm of Δ SP_1051 SOE
ABD534	AAAAGTGCACGACAAATAGCCC	F1 oligo for <i>phtD</i> inactivation MuGENT SOE product
ABD535	GACAATGTCTGAATCCTaCAACTGcTAATTCGGATCTTTCAT-GAGGAGC	R1 oligo for <i>phtD</i> inactivation MuGENT SOE product
ABD536	GCTCCTCATGAAAGATCCGAATTAgCAGTTgTAGGATTCAGACATTGTC	F2 oligo for <i>phtD</i> inactivation MuGENT SOE product
ABD539	GACAATATCAGCGTCTTaAAGTTGcTAGTTTGGATCCTTCAT-CAAGAGTTCTTCAC	R2 oligo for <i>phtD</i> inactivation MuGENT SOE product
ABD536	GCTCCTCATGAAAGATCCGAATTAgCAGTTgTAGGATTCAGACATTGTC	F1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD539	GACAATATCAGCGTCTTaAAGTTGcTAGTTTGGATCCTTCAT-CAAGAGTTCTTCAC	R1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD540	GTGAAGAACTCTTGATGAAGGATCCAAACTAgCAACTTtAAG-ACCGTGATATTGTC	F2 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD541	AAAGGATAACAACAGATCCAGCTGC	R2 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD548	GTTAATGAGGTCAAGGGTGGATAgGTTATCtAGGTAGATGGA-AAATACTATG	F1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD543	GACAATGTCTGAATCCTaCAACTGcTAATTCGGATCTTTCAT-GAGGAGC	R1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD544	GCTCCTCATGAAAGATCCGAATTAgCAGTTgTAGGATTCAGACATTGTC	F2 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD545	TGCATAAAGCAATCCCTTTCTGATTT	R2 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD546	GAGACATTATTACTGTCCAAGAAACGC	F1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD547	CATAGTATTTCCATCTACCTaGATAACcTATCCACCCTTGA-CCTCATTAAC	R1 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD548	GTTAATGAGGTCAAGGGTGGATAgGTTATCtAGGTAGATGGA-AAATACTATG	F2 oligo for <i>phtE</i> inactivation MuGENT SOE product
ABD543	GACAATGTCTGAATCCTaCAACTGcTAATTCGGATCTTTCAT-GAGGAGC	R2 oligo for <i>phtE</i> inactivation MuGENT SOE product

