Supplemental Information

Table S1 **DNA Oligonucleotides used in this study.**

An asterisk (*) indicates use of a phosphorothioate bond to protect against exonuclease activity (1).

Name	**Used for**	**Sequence**
ygaR	Set 1.850	g*c*gaagatcagtaaagatatagaaggtggtatccctggctattaAcaaggtcaggttttgattccattcattaaagatccagtaacaa*a*a
yqaC	Set 1.700	a*t*taaaaattatgatgggtccacgcgtgtcggcggtgaggcgtaActtaataaaggttgctctacctatcagcagctctacaatgaat*t*c
gabT	Set 1.600	t*c*accattgaagacgctcagatccgtcagggtctggagatcatcagccagtgttttgatgaggcgaagcagtaAcgccgctcctatgc*c*g
ygaU	Set 1.500	t*g*acgccaattcccattatccagcaggcgatggctggcaattaaTtactcttccggaatacgcaacacttgccccggataaattttat*c*c
ygaM	Set 1.400	g*t*aggtatttttatcggcgcactgttaagcatgcgcaaatcgtaAtgcaaaaatgataataaatacgcgtctttgaccccgaagcctg*t*c
luxS	Set 1.300	t*t*tgaactggctttttcaattaattgtgaagatagtttactgaTtagatgtgcagttcctgcaacttctctttcggcagtgccagtt*c*t
mltB	Set 1.250	a*a*ttttacgaggaggattcagaaaaaagctgattagccagagggaagctcacgccccccctcttgtaaatagTtactgtactcgcgcca*g*c
srlE	Set 1.200	a*c*tgtactgatcgcctggtttgtctccggttttatctatcaataAaggctgaaacatgaccgttatttatcagaccaccatcacccgt*a*t
norW	Set 1.150	a*t*cggatgaaagaggcatttggattgttgaaaacattgccgatgtaAgtgggctactgtgcctaaaatgtcggatgcgacgctggcgc*g*t
ascB	Set 1.100	a*t*cattctggtggtataaaaaaagtgattgccagtaatggggaagatttagagtaAgtaacagtgccggatgcggcgtgaacgccttat*c*c
bioD	Set 2.850	t*c*gaagacgcgatctcgctcgcaatttaaccaaatacagaatggTtacaacaaggcaaggtttatgtactttccggttgccgcatttt*c*t
moaE	Set 2.700	c*g*taaacgtatgtactgagcggtgaaattgccggacgcagcggtgccttatccggctaacaaaaaaTtaccagcgttttgccgcctgc*t*g
ybhM	Set 2.600	g*c*gatgtgaagtttagttaagttctttagtatgtgcatttacggTtaatgaaaaaaacgcgtatgcctttgccagacaagcgttatag*c*t
ybhS	Set 2.500	t*t*tatcggcctgacgtggctgaaaaccaaacgtcggctggattaAggagaagagcatgtttcatcgcttatggacgttaatccgcaaa*g*a
ybiH	Set 2.400	c*a*tatcgacctgattttgcaaggattatcgcaaaggagtttgtaAtgatgaaaaaacctgtcgtgatcggattggcggtagtggtact*t*g
ybiR	Set 2.300	t*c*tgaattaatcttcaaaacttaaagcaaaaggcggactcataatccgccttttttatttgccagaccTtagttggccgggagtataa*c*t
yliD	Set 2.250	t*t*tcctgtgaggtgattaccctttcaagcaatattcaaacgtaaTtatcctttaattttcggatccagcgcatcgcgtaaaccatcgc*c*c
yliE	Set 2.200	g*a*ctgactgtaagtacgaacttattgattctggacatacgtaaaTtactcttttactaattttccacttttatcccaggcggagaatg*g*c
ybjK	Set 2.150	t*c*ggttcaaggttgatgggtttttgttatctaaaacttatctaTtaccctgcaaccctctcaaccatcctcaaaatctcctcgcgcg*a*t

rimK	Set 2.100	c*g*caaaaagcgcaggcaaaaccatgatcagtaatgtgattgcgaTtaaccacccgttttcaggcaatattctgtcgtagcgtggcgtt*c*g
ygfJ	Set 3.850	c*c*ggacgactttattacagcgaaggaaaggtatactgaaatttaAaaaacgtagttaaacgattgcgttcaaatatttaatccttccg*g*c
recJ	Set 3.700	g*g*gattgtacccaatccacgctctttttatagagaagatgacgTtaaattggccagatattgtcgatgataatttgcaggctgcggt*t*g
argO	Set 3.600	c*t*ctggaggcaagcttagcgcctctgttttatttttccatcagatagcgcTtaactgaacaaggcttgtgcatgagcaataccgtctc*t*c
yggU	Set 3.500	a*a*tccgcaacaaatcccgccagaaatcgcggcgttaattaattaAgtatcctatgcaaaaagttgtcctcgcaaccggcaatgtcggt*a*a
mutY	Set 3.400	g*t*ggagcgtttgttacagcagttacgcactggcgcgccggtttaAcgcgtgagtcgataaagaggatgatttatgagcagaacgattt*t*t
glcC	Set 3.300	g*c*caccatttgattcgctcggcggtgccgctggagatgaacctgagttaActggtattaaatctgcttttcatacaatcggtaacgct*t*g
yghQ	Set 3.250	a*c*tgagtcagccgagaagaatttccccgcttattcgcaccttccTtaaatcaggtcatacgcttcgagatacttaacgccaaacacca*g*c
yghT	Set 3.200	t*g*gttgatgcagaaaaagcgattacggattttatgaccgcgcgtggttatcactaAtcaaaaatggaaatgcccgatcgccaggaccg*g*g
ygiZ	Set 3.150	t*t*ctctgtctatgagagccgttaaaacgactctcatagattttaTtaatagcaaaatataaaccgtccccaaaaaagccaccaaccac*a*a
yqiB	Set 3.100	a*g*ggttaacaggctttccaaatggtgtccttaggtttcacgacgTtaataaaccggaatcgccatcgctccatgtgctaaacagtatc*g*c
ygfJ_AGR	Set 3X.850	c*c*actatgtcagccatcgactgtataattaccgctgccggattatcatcaAGGatggggcaatggaaaatgatgttaccctgggaaca*g*g
ygfT_AGR	Set 3X.700	g*a*tgccttcgtatcaaacagagttaacatatcgcgcgccgcctgTCTtcctgcggccattgcagtgacaaccagatccgcgccatgaa*c*t
ubiH_AGR	Set 3X.600	g*t*gcagagtttgcgccgcattgcccaccagcacggtacgatgggtaatagaCCTggcggcgtgggttaacgccagcggataagcactg*c*g
argO_AGR	Set 3X.500	g*g*attcagccaggtcactgccaacatggtggcgataattttccaCCTgccttgcttcatgacttcggcgctggctaactcaatattac*t*g
yqgC_AGR	Set 3X.400	g*a*atcctgagaagcgccgagatgggtataacatcggcaggtatgcaaagcAGGgatgcagagtgcggggaacgaatcttcaccagaac*g*g
trmI_AGR	Set 3X.300	t*t*ttttacgcagacgacggctacggttctttgccattatttcacTCTctcgaacattaagtcccatactccgtgaccaagacgatgac*c*a
glcC_AGR	Set 3X.250	a*c*gatctgctcgacgttcgcgcattactggagggcgaatcggcaAGActggcggcaacgctgggaacgcaggctgattttgttgtgat*a*a
yghT_AGR	Set 3X.200	g*t*gaacatcttattaccgttgtcgaaaaatatggtgctgccgaaAGGgttcatttaggaaaacaggccggaaatgtcggtcgtgcagt*g*a
ygiZ_AGR	Set 3X.150	a*a*tacatatacccaaaactcgaacatttcccgcataaagagtttCCTtaagataagaataataagtggcgtaagaagaaaaaatgctg*c*a
cpdA_AGR	Set 3X.100	c*t*tcgtgcttttgtgcaaacaggtgagtgtcggtaatttgtaaaatcctgacCCTggcctcaccagccagaggaagggttaacaggct*t*t
lacZ_KO1	Set lacZ jackpot +61	T*C*ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTtGaGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA*G*C

lacZ_KO2	Set lacZ jackpot +264	G*C*TGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAtAaTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAAC*G*T
lacZ_KO3	Set lacZ jackpot +420	C*A*CATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGtaAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGC*A*A
lacZ_KO4	Set lacZ jackpot +602	T*G*ATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGtAgATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCT*G*C
lacZ_KO5	Set lacZ jackpot +693	T*A*AACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTaaAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAG*A*T
lacZ_KO6	Set lacZ jackpot +1258	T*A*CGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACtGaATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTAC*C*G
lacZ_KO7	Set lacZ jackpot +1420	G*G*GAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATtGaTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAG*G*C
lacZ_KO8	Set lacZ jackpot +1599	G*T*CCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGtaGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGC*G*G
lacZ_KO9	Set lacZ jackpot +1710	G*T*TTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTaaGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCG*T*G
lacZ_KO10	Set lacZ jackpot +1890	A*G*CGCTGACGGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCtGaTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTC*C*G
ygfJ_2*:2*_lead	Set 3.850_lead oligo	G*C*CGGAAGGATTAAATATTTGAACGCAATCGTTTAACTACGTTTTTTAAATTTCAGTATACCTTTCCTTCGCTGTAATAAAGTCGTCC*G*G
recJ_2*:2*_lead	Set 3.700_lead oligo	C*A*ACCGCAGCCTGCAAATTATCATCGACAATATCTGGCCAATTTAACGTCATCTTCTCTATAAAAAAGAGCGTGGATTGGGTACAATC*C*C
argO_2*:2*_lead	Set 3.600_lead oligo	G*A*GAGACGGTATTGCTCATGCACAAGCCTTGTTCAGTTAAGCGCTATCTGATGGAAAAATAAAACAGAGGCGCTAAGCTTGCCTCCAG*A*G
yggU_2*:2*_lead	Set 3.500_lead oligo	T*T*ACCGACATTGCCGGTTGCGAGGACAACTTTTTGCATAGGATACTTAATTAATTAACGCCGCGATTTCTGGCGGGATTTGTTGCGGA*T*T
mutY_2*:2*_lead	Set 3.400_lead oligo	A*A*AAATCGTTCTGCTCATAAATCATCCTCTTTATCGACTCACGCGTTAAACCGGCGCGCCAGTGCGTAACTGCTGTAACAAACGCTCC*A*C
glcC_2*:2*_lead	Set 3.300_lead oligo	C*A*AGCGTTACCGATTGTATGAAAAGCAGATTTAATACCAGTTAACTCAGGTTCATCTCCAGCGGCACCGCCGAGCGAATCAAATGGTG*G*C
yghQ_2*:2*_	Set 3.250_lead oligo	G*C*TGGTGTTTGGCGTTAAGTATCTCGAAGCG

lead		TATGACCTGATTTAAGGAAGGTGCGAATAAGCGGGGAAATTCTTCTCGGCTGACTCA*G*T
yghT_2*:2*_lead	Set 3.200_lead oligo	C*C*CGGTCCTGGCGATCGGGCATTTCCATTTTTGATTAGTGATAACCACGCGCGGTCATAAAATCCGTAATCGCTTTTTCTGCATCAAC*C*A
ygiZ_2*:2*_lead	Set 3.150_lead oligo	T*T*GTGGTTGGTGGCTTTTTTGGGGACGGTTTATATTTTGCTATTAATAAAATCTATGAGAGTCGTTTTAACGGCTCTCATAGACAGAG*A*A
yqiB_2*:2*_lead	Set 3.100_lead oligo	G*C*GATACTGTTTAGCACATGGAGCGATGGCGATTCCGGTTTATTAACGTCGTGAAACCTAAGGACACCATTTGGAAAGCCTGTTAACC*C*T
exoX.KO*	exoX KO oligo	t*t*c*g*gcctggagcatgccatgttgcgcattatcgatacagaaacTGAtgcggtttgcagggagggatcgttgagattgcctctgttgatg
xseA.KO*	xseA KO oligo	g*a*a*t*ttgatctcgctcacatgttaccttctcaatcccctgcaatTGAtttaccgttagtcgcctgaatcaaacggttcgtctgctgcttg
recJ.KO*	recJ KO oligo	g*g*a*g*gcaattcagcgggcaagtctgccgtttcatcgacttcacgTCAcgacgaagttgtatctgttgtttcacgcgaattatttaccgct
xonA.KO*	xonA KO oligo	a*a*t*a*acggatttaacctaatgatgaatgacggtaagcaacaatcTGAacctttttgtttcacgattacgaaacctttggcacgcaccccg
Lexo.KO.MM*	Lambda exo KO oligo	t*g*a*a*acagaaagccgcagagcagaaggtggcagcatgacaccgtaacattatcctgcagcgtaccgggatcgatgtgagagctgtcgaac
dnaG_Q576A	Oligo to make dnaG Q576A mutation	gcacgcatggtttaagcaacgaagaacgcctggagctctggacattaaacGCggaActggcgaaaaagtgatttaacggcttaagtgccg
dnaG_K580A	Oligo to make dnaG K580A mutation	cgcacgcatggtttaagcaacgaagaacgcctggagctctggacattaaaccaggaActggcgGCaaagtgatttaacggcttaagtgcc
tolC.90.del	Oligo that deletes endogenous tolC	gaatttcagcgacgtttgactgccgtttgagcagtcatgtgttaaagcttcggccccgtctgaacgtaaggcaacgtaaagatacgggttat
galK_KO1.100	Oligo to delete 100 bp including a portion of galK	C*G*CGCAGTCAGCGATATCCATTTTCGCGAATCCGGAGTGTAAGAAAACACACCGACTACAACGACGGTTTCGTTCTGCCCTGCGCGAT*T*G
galK_KO1.1149	Oligo to delete 1149 bp including a portion of galK	C*G*CGCAGTCAGCGATATCCATTTTCGCGAATCCGGAGTGTAAGAAACGAAACTCCCGCACTGGCACCCGATGGTCAGCCGTACCGACT*G*T
galK_KO1.7895	Oligo to delete 7895 bp including a portion of galK, galM, gpmA, aroG, ybgS, zitB, pnuC, and nadA	C*G*CGCAGTCAGCGATATCCATTTTCGCGAATCCGGAGTGTAAGAACTTACCATCTCGTTTTACAGGCTTAACGTTAAAACCGACATTA*G*C
ygaR_wt-f	Set 1.850_wt-f mascPCR	AAGGTGGTATCCCTGGCTATTAG
yqaC_wt-f	Set 1.700_wt-f mascPCR	CGGCGGTGAGGCGTAG
gabT_wt-f	Set 1.600_wt-f mascPCR	TTTTGATGAGGCGAAGCAGTAG
ygaU_wt-f	Set 1.500_wt-f mascPCR	GTTGCGTATTCCGGAAGAGTAG

ygaM_wt-f	Set 1.400_wt-f mascPCR	GTTAAGCATGCGCAAATCGTAG
luxS_wt-f	Set 1.300_wt-f mascPCR	GTTGCAGGAACTGCACATCTAG
mltB_wt-f	Set 1.250_wt-f mascPCR	GCTGGCGCGAGTACAGTAG
srlE_wt-f	Set 1.200_wt-f mascPCR	GGTTTGTCTCCGGTTTTATCTATCAATAG
norW_wt-f	Set 1.150_wt-f mascPCR	GATTGTTGAAAACATTGCCGATGTAG
ascB_wt-f	Set 1.100_wt-f mascPCR	CCAGTAATGGGGAAGATTTAGAGTAG
bioD_wt-f	Set 2.850_wt-f mascPCR	AGTACATAAACCTTGCCTTGTTGTAG
moaE_wt-f	Set 2.700_wt-f mascPCR	GCGGCAAAACGCTGGTAG
ybhM_wt-f	Set 2.600_wt-f mascPCR	AAGGCATACGCGTTTTTTTCATTAG
ybhS_wt-f	Set 2.500_wt-f mascPCR	CCAAACGTCGGCTGGATTAG
ybiH_wt-f	Set 2.400_wt-f mascPCR	AAGGATTATCGCAAAGGAGTTTGTAG
ybiR_wt-f	Set 2.300_wt-f mascPCR	TTAGTTATACTCCCGGCCAACTAG
yliD_wt-f	Set 2.250_wt-f mascPCR	CGCTGGATCCGAAAATTAAAGGATAG
yliE_wt-f	Set 2.200_wt-f mascPCR	TGGGATAAAAGTGGAAAATTAGTAAAAGAGTAG
ybjK_wt-f	Set 2.150_wt-f mascPCR	TTGAGAGGGTTGCAGGGTAG
rimK_wt-f	Set 2.100_wt-f mascPCR	GCCTGAAAACGGGTGGTTAG
ygfJ_wt-f	Set 3.850_wt-f mascPCR	AGCGAAGGAAAGGTATACTGAAATTTAG
recJ_wt-f	Set 3.700_wt-f mascPCR	TCATCGACAATATCTGGCCAATTTAG
argO_wt-f	Set 3.600_wt-f mascPCR	TGCACAAGCCTTGTTCAGTTAG
yggU_wt-f	Set 3.500_wt-f mascPCR	CAGAAATCGCGGCGTTAATTAATTAG
mutY_wt-f	Set 3.400_wt-f mascPCR	GGCGCGCCGGTTTAG
glcC_wt-f	Set 3.300_wt-f mascPCR	GCTGGAGATGAACCTGAGTTAG
yghQ_wt-f	Set 3.250_wt-f mascPCR	CTCGAAGCGTATGACCTGATTTAG

yghT_wt-f	Set 3.200_wt-f mascPCR	CGCGCGTGGTTATCACTAG
ygiZ_wt-f	Set 3.150_wt-f mascPCR	TGGGGACGGTTTATATTTTGCTATTAG
yqiB_wt-f	Set 3.100_wt-f mascPCR	CGATGGCGATTCCGGTTTATTAG
ygfJ_WT	Set 3X.850_wt-f mascPCR	GCTGCCGGATTATCATCAAGA
ygfT_WT	Set 3X.700_wt-f mascPCR	GCAATGGCCGCAGGAAGG
ubiH_WT	Set 3X.600_wt-f mascPCR	GCACGGTACGATGGGTAATAGAT
argO_WT	Set 3X.500_wt-f mascPCR	GAAGTCATGAAGCAAGGCAGA
yqgC_WT	Set 3X.400_wt-f mascPCR	CGGCAGGTATGCAAAGCAGA
trmI_WT	Set 3X.300_wt-f mascPCR	AGTATGGGACTTAATGTTCGAGAGG
glcC_WT	Set 3X.250_wt-f mascPCR	AGGGCGAATCGGCAAGG
yghT_WT	Set 3X.200_wt-f mascPCR	GAAAAATATGGTGCTGCCGAAAGA
ygiZ_WT	Set 3X.150_wt-f mascPCR	CTTCTTACGCCACTTATTATTCTTATCTTAAGA
cpdA_WT	Set 3X.100_wt-f mascPCR	TGGCTGGTGAGGCCAGA
exoX.KO*-wt-f	exoX wt-f mascPCR primer	GCGCATTATCGATACAGAAACCT
xseA.KO*-wt-f	xseA wt-f mascPCR primer	CTTCTCAATCCCCTGCAATTTTTACC
recJ.KO*-wt-f	recJ wt-f mascPCR primer	CAACAGATACAACTTCGTCGCC
xonA.KO*-wt-f	xonA wt-f mascPCR primer	GAATGACGGTAAGCAACAATCTACC
Lexo_WT-f	Lambda exo KO wt-f mascPCR primer	GGCAGCATGACACCGGA
dnaG_Q576A_wt-f	dnaG_Q576A wt-f mascPCR primer	TGGAGCTCTGGACATTAAACCA
dnaG_K580A_wt-f	dnaG_K580A wt-f mascPCR primer	CATTAAACCAGGAACTGGCGAA
ygaR_mut-f	Set 1.850_mut-f mascPCR	AAGGTGGTATCCCTGGCTATTAA
yqaC_mut-f	Set 1.700_mut-f mascPCR	CGGCGGTGAGGCGTAA
gabT_mut-f	Set 1.600_mut-f mascPCR	TTTTGATGAGGCGAAGCAGTAA

ygaU_mut-f	Set 1.500_mut-f mascPCR	GTTGCGTATTCCGGAAGAGTAA
ygaM_mut-f	Set 1.400_mut-f mascPCR	GTTAAGCATGCGCAAATCGTAA
luxS_mut-f	Set 1.300_mut-f mascPCR	GTTGCAGGAACTGCACATCTAA
mltB_mut-f	Set 1.250_mut-f mascPCR	GCTGGCGCGAGTACAGTAA
srlE_mut-f	Set 1.200_mut-f mascPCR	GGTTTGTCTCCGGTTTTATCTATCAATAA
norW_mut-f	Set 1.150_mut-f mascPCR	GATTGTTGAAAACATTGCCGATGTAA
ascB_mut-f	Set 1.100_mut-f mascPCR	CCAGTAATGGGGAAGATTTAGAGTAA
bioD_mut-f	Set 2.850_mut-f mascPCR	AGTACATAAACCTTGCCTTGTTGTAA
moaE_mut-f	Set 2.700_mut-f mascPCR	GCGGCAAAACGCTGGTAA
ybhM_mut-f	Set 2.600_mut-f mascPCR	AAGGCATACGCGTTTTTTTCATTAA
ybhS_mut-f	Set 2.500_mut-f mascPCR	CCAAACGTCGGCTGGATTAA
ybiH_mut-f	Set 2.400_mut-f mascPCR	AAGGATTATCGCAAAGGAGTTTGTAA
ybiR_mut-f	Set 2.300_mut-f mascPCR	TTAGTTATACTCCCGGCCAACTAA
yliD_mut-f	Set 2.250_mut-f mascPCR	CGCTGGATCCGAAAATTAAAGGATAA
yliE_mut-f	Set 2.200_mut-f mascPCR	TGGGATAAAAGTGGAAAATTAGTAAAAGAGTAA
ybjK_mut-f	Set 2.150_mut-f mascPCR	TTGAGAGGGTTGCAGGGTAA
rimK_mut-f	Set 2.100_mut-f mascPCR	GCCTGAAAACGGGTGGTTAA
ygfJ_mut-f	Set 3.850_mut-f mascPCR	AGCGAAGGAAAGGTATACTGAAATTTAA
recJ_mut-f	Set 3.700_mut-f mascPCR	TCATCGACAATATCTGGCCAATTTAA
argO_mut-f	Set 3.600_mut-f mascPCR	TGCACAAGCCTTGTTCAGTTAA
yggU_mut-f	Set 3.500_mut-f mascPCR	CAGAAATCGCGGCGTTAATTAATTAA
mutY_mut-f	Set 3.400_mut-f mascPCR	GGCGCGCCGGTTTAA
glcC_mut-f	Set 3.300_mut-f mascPCR	GCTGGAGATGAACCTGAGTTAA

yghQ_mut-f	Set 3.250_mut-f mascPCR	CTCGAAGCGTATGACCTGATTTAA
yghT_mut-f	Set 3.200_mut-f mascPCR	CGCGCGTGGTTATCACTAA
ygiZ_mut-f	Set 3.150_mut-f mascPCR	TGGGGACGGTTTATATTTGCTATTAA
yqiB_mut-f	Set 3.100_mut-f mascPCR	CGATGGCGATTCCGGTTTATTAA
ygfJ_MUT	Set 3X.850_mut-f mascPCR	GCTGCCGGATTATCATCAAGG
ygfT_MUT	Set 3X.700_mut-f mascPCR	GCAATGGCCGCAGGAAGA
ubiH_MUT	Set 3X.600_mut-f mascPCR	GCACGGTACGATGGGTAATAGAC
argO_MUT	Set 3X.500_mut-f mascPCR	GAAGTCATGAAGCAAGGCAGG
yqgC_MUT	Set 3X.400_mut-f mascPCR	GGCAGGTATGCAAAGCAGG
trmI_MUT	Set 3X.300_mut-f mascPCR	GAGTATGGGACTTAATGTTCGAGAGA
glcC_MUT	Set 3X.250_mut-f mascPCR	GAGGGCGAATCGGCAAGA
yghT_MUT	Set 3X.200_mut-f mascPCR	AAAATATGGTGCTGCCGAAAGG
ygiZ_MUT	Set 3X.150_mut-f mascPCR	CTTCTTACGCCACTTATTATTCTTATCTTAAGG
cpdA_MUT	Set 3X.100_mut-f mascPCR	GGCTGGTGAGGCCAGG
exoX.KO*-mut-f	exoX mut-f mascPCR primer	GCGCATTATCGATACAGAAACTGA
xseA.KO*-mut-f	xseA mut-f mascPCR primer	CTTCTCAATCCCCTGCAATTGA
recJ.KO*-mut-f	recJ mut-f mascPCR primer	CAACAGATACAACTTCGTCGTGA
xonA.KO*-mut-f	xonA mut-f mascPCR primer	GAATGACGGTAAGCAACAATCTGA
Lexo_MUT-f	Lambda exo KO mut-f mascPCR primer	TGGCAGCATGACACCGTAA
dnaG_Q576A_mut-f	dnaG_Q576A mut-f mascPCR primer	GGAGCTCTGGACATTAAACGC
dnaG_K580A_mut-f	dnaG_K580A mut-f mascPCR primer	ACCAGGAACTGGCGGC
ygaR_rev	Set 1.850_rev mascPCR	TAGGTAGAGCAACCTTTATTAAGCTACG
yqaC_rev	Set 1.700_rev mascPCR	TAAAAATATCTACATTTCTGAAAAATGCGCA

gabT_rev	Set 1.600_rev mascPCR	GCGGCGATGTTGGCTT
ygaU_rev	Set 1.500_rev mascPCR	AGGGTATCGGGTGGCG
ygaM_rev	Set 1.400_rev mascPCR	CGCAACGCTTCTGCCG
luxS_rev	Set 1.300_rev mascPCR	ATGCCCAGGCGATGTACA
mltB_rev	Set 1.250_rev mascPCR	AGACTCGGCAGTTGTTACGG
srlE_rev	Set 1.200_rev mascPCR	GGATGGAGTGCACCTTTCAAC
norW_rev	Set 1.150_rev mascPCR	GTGTTGCATTTGGACACCATTG
ascB_rev	Set 1.100_rev mascPCR	CGCTTATCGGGCCTTCATG
bioD_rev	Set 2.850_rev mascPCR	CGGGAAGAACTCTTTCATTTCGC
moaE_rev	Set 2.700_rev mascPCR	CGTCAATCCGACAAAGACAATCA
ybhM_rev	Set 2.600_rev mascPCR	TTACTGGCAGGGATTATCTTTACCG
ybhS_rev	Set 2.500_rev mascPCR	CTGTTGTTAGGTTTCGGTTTTCCT
ybiH_rev	Set 2.400_rev mascPCR	GTCATAGGCGGCTTGCG
ybiR_rev	Set 2.300_rev mascPCR	ATGAGCCGGTAAAAGCGAC
yliD_rev	Set 2.250_rev mascPCR	AATAAAATTATCAGCCTTATCTTTATCTTTTCGTATAAA
yliE_rev	Set 2.200_rev mascPCR	CAGCAATATTTGCCACCGCA
ybjK_rev	Set 2.150_rev mascPCR	AACTTTTCCGCAGGGCATC
rimK_rev	Set 2.100_rev mascPCR	TACAACCTCTTTCGATAAAAAGACCG
ygfJ_rev	Set 3.850_rev mascPCR	GATGAACTGTTGCATCGGCG
recJ_rev	Set 3.700_rev mascPCR	CTGTACGCAGCCAGCC
argO_rev	Set 3.600_rev mascPCR	AATCGCTGCCTTACGCG
yggU_rev	Set 3.500_rev mascPCR	TAACCAAAGCCACCAGTGC
mutY_rev	Set 3.400_rev mascPCR	CGCGAGATATTTTTCATCATTCCG

glcC_rev	Set 3.300_rev mascPCR	GGGCAAAATTGCTGTGGC
yghQ_rev	Set 3.250_rev mascPCR	ACCAACTGGCGATGTTATTCAC
yghT_rev	Set 3.200_rev mascPCR	GACGATGGTGGTGGACGG
ygiZ_rev	Set 3.150_rev mascPCR	ATCGCCAAATTGCATGGCA
yqiB_rev	Set 3.100_rev mascPCR	AAAATCCTGACTCTGGCCTCA
ygfJ_rev	Set 3X.850_rev mascPCR	TCTGTTTGCACTGCGGGTAC
ygfT_rev	Set 3X.700_rev mascPCR	TGGTTGGGCAATCTAATAGATTCTCC
ubiH_rev	Set 3X.600_rev mascPCR	atgAGCGTAATCATCGTCGGTG
argO_rev	Set 3X.500_rev mascPCR	CCGTCTCTCGCCAGCTG
yqgC_rev	Set 3X.400_rev mascPCR	AGCACACGACGTTTCTTTCG
trmI_rev	Set 3X.300_rev mascPCR	ATCTGTTCTTCCGATGTACCTTCC
glcC_rev	Set 3X.250_rev mascPCR	CTTCCAGCTCGATATCGTGGAG
yghT_rev	Set 3X.200_rev mascPCR	CACCACCAAAGGTTAACTGTGG
ygiZ_rev	Set 3X.150_rev mascPCR	CACAAACCAGACAAATACCGAGC
cpdA_rev	Set 3X.100_rev mascPCR	CGATGGTATCCAGCGTAAAGTTG
exoX.KO*-r	exoX rev mascPCR primer	GACCATGGCTTCGGTGATG
xseA.KO*-r	xseA rev mascPCR primer	GGTACGCTTAAGTTGATTTTCCAGC
recJ.KO*-r	recJ rev mascPCR primer	GGCCTGATCGACCACTTCC
xonA.KO*-r	xonA rev mascPCR primer	GAAATGTCTCCTGCCAAATCCAC
Lexo-r	Lambda exo KO rev mascPCR primer	CAAGGCCGTTGCCGTC
dnaG_seq-r	dnaG rev mascPCR primer for both Q576A and K580A	GCTCCATAAGACGGTATCCACA
Rx-P19	forward screening primer for wt tolC deletion	GTTTCTCGTGCAATAATTTCTACATC

Rx-P20	reverse screening primer for wt tolC deletion	CGTATGGATTTTGTCCGTTTCA
lacZ_jackpot_seq-f	forward sequencing primer for lacZ jackpot alleles	GAATTGTGAGCGGATAACAATTTC
lacZ_jackpot_seq-r	reverse sequencing primer for lacZ jackpot alleles	CCAGCGGCTTACCATCC
cat_mut*	cat inactivation oligo	G*C*ATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTTAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTA*A*A
cat_restore*	cat reactivation oligo	G*C*ATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTA*A*A
tolC-r_null_mut*	tolC inactivation oligo	A*G*CAAGCACGCCTTAGTAACCCGGAATTGCGTAAGTCTGCCGCTAAATCGTGATGCTGCCTTTGAAAAAATTAATGAAGCGCGCAGTCCA
tolC-r_null_revert*	tolC reactivation oligo	C*A*GCAAGCACGCCTTAGTAACCCGGAATTGCGTAAGTCTGCCGCCGATCGTGATGCTGCCTTTGAAAAAATTAATGAAGCGCGCAGTCCA
tolC_null_revert*	tolC reactivation oligo (leading targeting)	T*G*GACTGCGCGCTTCATTAATTTTTTCAAAGGCAGCATCACGATCGGCGGCAGACTTACGCAATTCCGGGTTACTAAGGCGTGCTTGCTG
bla_mut*	bla inactivation oligo	G*C*C*A*CATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTATTAGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG
bla_restore*	bla reactivation oligo	G*C*C*A*CATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG
313000.T.lacZ.coMAGE-f	Cassette primer for T.co-lacZ (lacZ coselection)	TGCTTCTCATGAACGATAACACAACTTGTTCATGAATTAACCATTCCGGATTGAGGCACATTAACGCC
313001.T.lacZ.coMAGE-r	Cassette primer for T.co-lacZ (lacZ coselection)	ACGGAAACCAGCCAGTTCCTTTCGATGCCTGAATTTGATCCCATAGTTTATCTAGGGCGGCGGATT
312869.seq-f	Screening primer for tolC (lacZ coselection)	GAACTTGCACTACCCATCG
313126.seq-r	Screening primer for tolC (lacZ coselection)	AGTGACGGGTTAATTATCTGAAAG
1255700.S.12.13b-f	Cassette primer for S.12.13b	TTTCATCTTGCCAGCATATTGGAGCGTGATCAATTTTGATCAGCTGTGAACAGCCAGGACAGAAATGC
1255701.S.12.13b -r	Cassette primer for S.12.13b	CATTAGCAGTGATATAACGTAAGTTTTTGTATCACTACACATCAGCCCCCTGCAGAAATAAAAAGGCCTGC

1255550.Seq-f	Screening primer for S.12.13b	CATTTTTGCATTACTAATAAGAAAAAGCAAA
1255850.Seq-r	Screening primer for S.12.13b	GTCCTAATCATTCTTGTAACATCCTAC
1710450.Z.16.17b-f	Cassette primer for Z.16.17b	TCAGGTTAAAATCATTTAAATTTACACACGCAACAAATATTGACCTACAAGGTGTTGACAATTAATCATCGGC
1710451.Z.16.17b-r	Cassette primer for Z.16.17b	TTTTTACTAGTGAGATAGTCCAGTTTCTGAAAAATAGCCAGTGTAATGTTAGCTTGCAAATTAAAGCCTTCG
1710300.Seq-f	Screening primer for Z.16.17b	TCAGGTAATCCGTTTGCGG
1710600.Seq-r	Screening primer for Z.16.17b	AACGGCAGATTTTTTCACTGC
LacZ::KanR.full-f	Cassette primer for lacZ::kanR	TGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGCCTGTGACGGAAGATCACTTCG
LacZ::KanR.full-r	Cassette primer for lacZ::kanR	GTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTAACCAGCAATAGACATAAGCGG

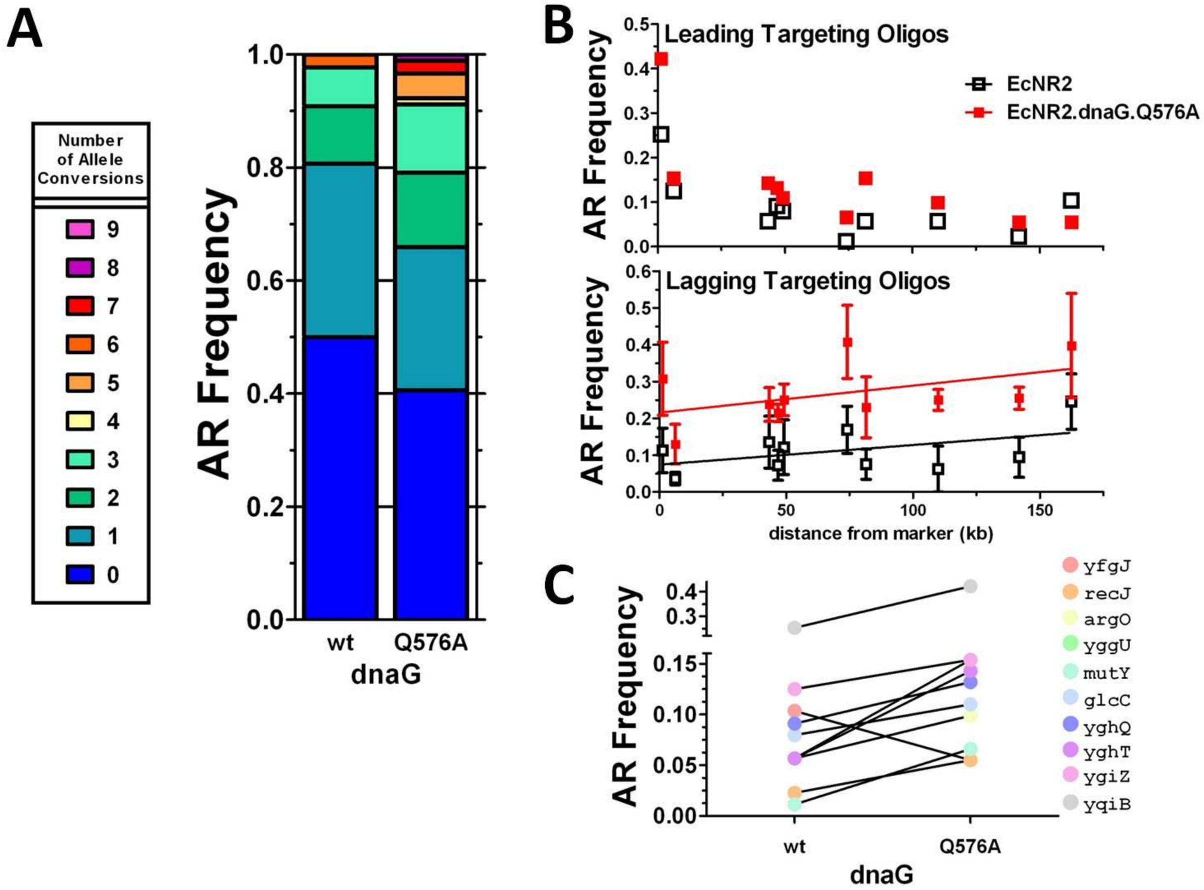
Table S2: Estimation of Okazaki fragment length in EcNR2.dnaG.K580A and EcNR2.dnaG.Q576A

[Primase] (nM)	WT dnaG Okazaki Frag (kb)	K580A Okazaki Frag (kb)	Q576A Okazaki Frag (kb)
80	2.5	5	23
160	1.5	2.5	18
320	1	1	8
640	0.8	nd	3
Average Fold effect compared to WT		**1.6**	**8.2**

Average Okazaki Fragment length was estimated based on *in vitro* results (gel images) from Tougu et al. (2) for the same DnaG primase variants, tabulated above. We compared the fold difference in OF sizes for the specified primase concentrations and determined the average fold difference (variant OF length/wt OF length). We estimate *in vivo* OF lengths of ~2.3-3.1 kb and ~12-16 kb for the K580A and Q576A mutants, respectively, based on the reported ~1.5-2 kb OF lengths in wt cells grown in rich media (3-5). However, these approximations may be imperfect since Tougu et al. (2) performed this analysis *in vitro* and did not use the same EcNR2.dnaG.K580A and EcNR2.dnaG.Q576A strains. Other conditions and/or host factors not accounted for *in vitro* may affect priming efficiency, thereby rendering these calculations inaccurate.

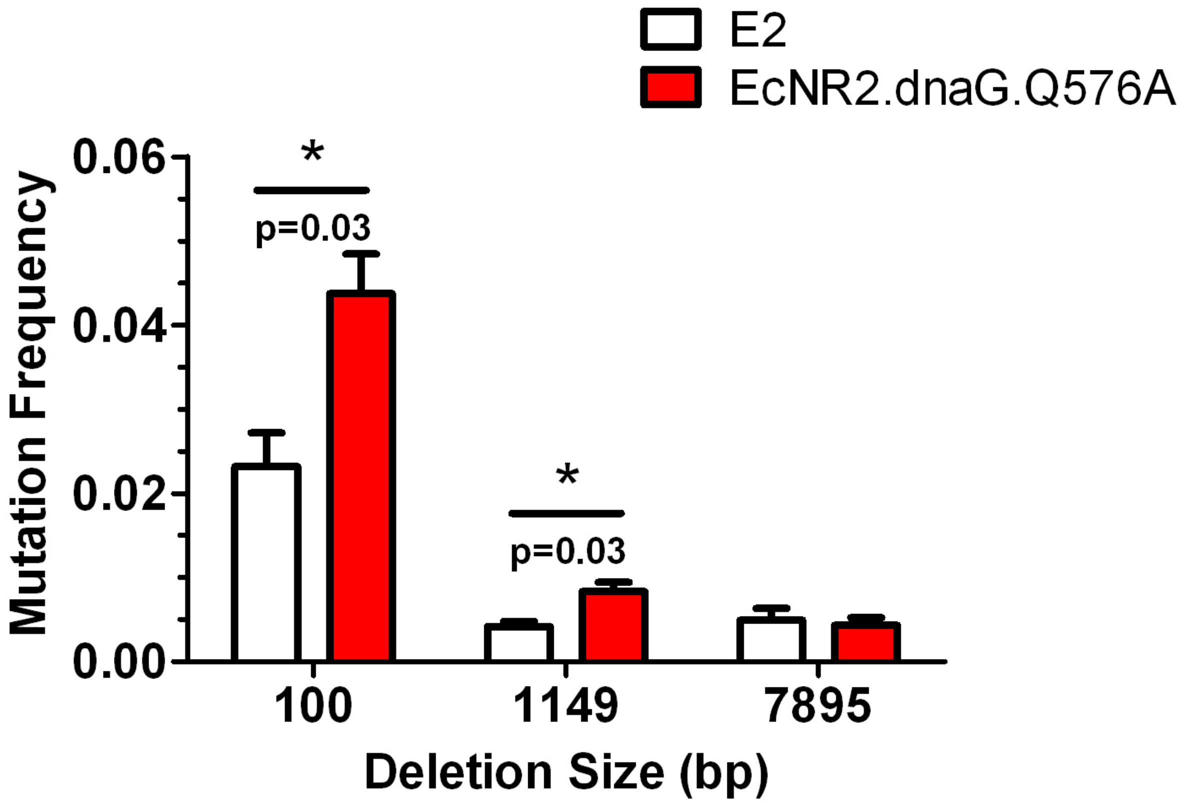
Supplemental Figures

Figure S1. **Effect of dnaG variants and co-selection on leading-targeting CoS-MAGE**



(A) EcNR2.dnaG.Q576A (n=91) outperformed EcNR2 (wt, n=88) in leading-targeting Set 3 CoS-MAGE, with a reduction in zero conversion events as well as a broadening of the distribution of total allele conversions per clone. **(B)** For leading-targeting Set 3 oligos, AR frequency decays rapidly with increasing distance from the selectable marker (top panel). In contrast, co-selection using the corresponding set of lagging targeting oligos (see also Figure 3C, right panel) provides robust co-selection spanning at least 0.162 Mb (bottom panel). For the lagging-targeting oligos (bottom panel), linear regression analyses (solid trendline) show that co-selection does not decrease with distance for either strain over this 0.162 Mb genomic region. **(C)** Individual CoS-MAGE AR frequency is plotted for each leading-targeting Set 3 oligo in EcNR2 (wt) and EcNR2.dnaG.Q576A (Q576A). AR frequency is improved for 9/10 alleles in EcNR2.dnaG.Q576A. Note that the most proximal allele to the selectable marker (*yqiB*) is separated from the other alleles with a broken axis, since its AR frequency was much higher than that of the others.

Figure S2. **Effect of dnaG attenuation on deletion frequency**



DnaG primase disruption enhances gene-sized deletion frequency. Oligos that deleted 100 bp, 1,149 bp, or 7,895 bp of the genome, including a portion of *galK*, were recombined into EcNR2 and EcNR2.dnaG.Q576A. The recombined populations were screened for the GalK- phenotype (white colonies) on MacConkey agar plates supplemented with galactose as a carbon source. EcNR2.dnaG.Q576A significantly outperformed EcNR2 for the 100 bp and 1,149 bp deletions, but there was no difference detected between the two strains for the 7,895 bp deletion.

Supplementary References

1. Wang, H.H., Isaacs, F.J., Carr, P.A., Sun, Z.Z., Xu, G., Forest, C.R. and Church, G.M. (2009) Programming cells by multiplex genome engineering and accelerated evolution. *Nature*, **460**, 894-898.
2. Tougu, K. and Marians, K.J. (1996) The Interaction between Helicase and Primase Sets the Replication Fork Clock. *Journal of Biological Chemistry*, **271**, 21398-21405.
3. Corn, J.E. and Berger, J.M. (2006) Regulation of bacterial priming and daughter strand synthesis through helicase-primase interactions. *Nucleic Acids Res.*, **34**, 4082-4088.
4. Lia, G., Michel, B. and Allemand, J.-F. (2012) Polymerase Exchange During Okazaki Fragment Synthesis Observed in Living Cells. *Science*, **335**, 328-331.
5. Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K. and Sugino, A. (1968) Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains. *Proceedings of the National Academy of Sciences*, **59**, 598-605.