

Supplementary Tables

Supplementary Table 1: MAGE oligonucleotides.

Name	Sequence	Strategy
acpP.high.1.NY	t*g*cttaacgcccagctgtccgcgataatcttctaacgcgttccatagatgctcactacttaaafttctctcaaaactcgtttc	Tolerant
acpP.low.1.NY	a*t*ttctcagcttcttcgctcgggaatcagatcatcaaaccttctcacagaccattaccagctcaacgggttcaagagaatccgcgccc	Tolerant
acpP.med.1.NY	g*t*aacttctctgcttaacgcccagctgtccgcgataatcttctaacgcgttctctgatagctcactactttaaaittctata	Tolerant
aspS.high.1.NY	a*g*tgagcggatatttcagacgcgcttcttcggttgagctgttctagtaacggcagaaacatctcgcggttgatgatagtcagcagcag	Tolerant
aspS.low.1.NY	g*c*aaagctcggctctcagctatcagacacgctgcgccgctgtctacggaaagcgcgataacgctcagatattgctggtccgctcagc	Tolerant
aspS.med.1.NY	t*a*tttcagacgcgcttctcgggttgagctgtttagagctcaagctacagaaacatctcgcggttgatgatagtcagcagcagcagcagc	Tolerant
can.high.1.NY	a*t*agctcattgctcggcgaatgcccaagagcgcctctctgattagtgtgtaactgaacgctatggaacaggtgtataacctggccc	Tolerant
can.low.1.NY	g*t*accgtcagcggatctcaacctcaagctgaaacacgccaactgaaataaaatgccatgccggatgcaacacatcggcgaacttca	Tolerant
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cysS.high.1.NY	g*g*ttctcctccagcagcccaatacagcggaaagttaacgacagctaaagatgccattgccctcggcctccatctctctgcttctaga	Tolerant
cysS.low.1.NY	c*a*atagcgaattacttaccagcggcagctgtccctcggcgccctattccagcagatccccatctcgttaagacgatcagcgcgcca	Tolerant
cysS.med.1.NY	c*c*ttggcagcagtttctcagtgagttcaataatctcgcgctagctcgcgcgcgctccatctccggcgcagatgttcaaaa	Tolerant
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era.high.1.NY	t*c*ttccggagagtgatgagctcctcagctagatgcttaccgcaactcgcgcaatagctcaacattcaaccgggttccgagagatt	Tolerant
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glnS.low.1.NY	a*c*cggttttcccgctgaaatggcggctatcagcagcaagactattaccgctcaactcggaaatgcttaccggcaaccgactttc	Tolerant
glnS.med.1.NY	t*c*cggttaccggtgttttaccgggtgctcagggctcggcgttctcggatctgctcggcctcagctcaacgtacgcccag	Tolerant
groS.high.1.NY	c*c*atgcttgaataacagatgctcgaacttcaatcagctcagcggctcactcgcctttcaagatacggcattccgacagccagc	Tolerant
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fmt.N.1TAG	a*c*tggatcgtctgaaagcccgggcttaaggataaagaactacgtgtagtcagaatcactactgattatftttcgggtacacctgacttt	Amino-terminal
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rpsE.N.1TAG	c*a*gcttttctcagcttcggcagcagcttttttcgatgtgagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsE.N.2TAG	c*a*gcttttctcagcttcggcagcagcttttttcgatgtgagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsE.N.3TAG	g*c*t*tttctcagcttcggcagcagcttttttcgatgtgagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsN.N.1TAG	g*t*ttactctcgtctcttctgattgcttagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsN.N.2TAG	g*t*ttactctcgtctcttctgattgcttagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsN.N.3TAG	t*t*ttactctcgtctcttctgattgcttagcctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplN.N.1TAG	a*c*cggagttgtcggcagcttcagcatagctcttcttgatctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplN.N.2TAG	a*c*cggagttgtcggcagcttcagcatagctcttcttgatctactactctactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplN.N.3TAG	c*c*ggagttgtcggcagcttcagcatagctcttcttgatctactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsQ.N.1TAG	a*a*caacgcagcttcgagagtagcagcttttatcggctactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsQ.N.2TAG	a*a*caacgcagcttcgagagtagcagcttttatcggctactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpsQ.N.3TAG	c*a*acgcagcttcgagagtagcagcttttatcggctactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplD.N.1TAG	g*g*aaacagtcagcgcgctctcgcgcttctcaactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplD.N.2TAG	g*g*aaacagtcagcgcgctctcgcgcttctcaactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplD.N.3TAG	a*a*acagtcagcgcgctctcgcgcttctcaactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplC.N.1TAG	g*a*agatacgggtcatacccactttttaccgactaaacactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplC.N.2TAG	g*a*agatacgggtcatacccactttttaccgactaaacactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rplC.N.3TAG	a*g*atagcgggtcatacccactttttaccgactaaacactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
fusA.N.1TAG	g*a*taccgatgttacgtagcgtcgcgatgggtgtgtacgagcctactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
fusA.N.2TAG	g*a*taccgatgttacgtagcgtcgcgatgggtgtgtacgagcctactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
fusA.N.3TAG	t*a*ccgatgttacgtagcgtcgcgatgggtgtgtacgagcctactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpoC.N.1TAG	t*t*cgggttttagtctcgcgtttcagaacttataaacttctactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpoC.N.2TAG	t*t*cgggttttagtctcgcgtttcagaacttataaacttctactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
rpoC.N.3TAG	c*g*gttttagtctcgcgtttcagaacttataaacttctactactactactctcctcaacttaccatgaaggaaaccgct	Amino-terminal
ppa.N.1TAG	t*a*ctcggcactgtttgcccacatattttaaaggaaacagacatgtagagcttactcaactcctcgggtaaaagatctgccggaagac	Amino-terminal
ppa.N.2TAG	t*c*ggcactgtttgcccacatattttaaaggaaacagacatgtagagcttactcaactcctcgggtaaaagatctgccggaagac	Amino-terminal
ppa.N.3TAG	c*g*gcactgtttgcccacatattttaaaggaaacagacatgtagagcttactcaactcctcgggtaaaagatctgccggaagac	Amino-terminal
dnaA.W6	t*t*ctgtgctgtaactactctcgaactcagcagctgtcgtctaaagcgaagtgacacggcggactcactcgaacaaagtcga	Functional
fusA.F32	a*g*cgcctcactcttctcgaatgattttatggtttacacggctgtactacagaatgcttcgtagtagtgttttaccggcgtcgtatgtg	Functional
glyQ.Y226	c*g*gcagcggatttccagcggcagcagctcgcgcttctctctactcgtcgaagcaggtgaacaggaagccacatccgctattc	Functional
Int.Y388	c*c*gcctcctcctcgaatgattttgacttactcgcgctatttctgtaggagatcattctcggcagcaagtcgcgataactccgccggat	Functional
murG.F243	a*a*cgacgacatccgccacgcatacggccgccatcatatcaatctattccgctacttattgctcgggttcccccttcggcatacgc	Functional
rpoA.Y152	t*g*ggcctcactcttctcgaatgattttatggtttacacggctgtactacagaatgcttcgtagtagtgttttaccggcgtcgtatgtg	Functional
serS.F213	g*t*ctgcttcttccagcggacgagatgtagaagcagcggcagcttactctcctcctcaacttaccatgaaggaaaccgct	Functional
thrS.Y313	g*c*agttaccgcttccagaagatgtgtgaacattgcatcttctggttcccagtgaccggtttttccacagacacggtccat	Functional

Asterisk (*) signifies a phosphorothioate bond. Oligonucleotides are grouped by strategy (Tolerant, Amino-terminal, and Functional).

Supplementary Table 2: MAGE pools for the introduction of TAG codons in essential genes.

Y	NY	N	C1	C2	F
acpP.low.3	acpP.high.1.NY	ftsL.N.1TAG	dnaX.high.20	dnaX.high.20	dnaA.W6
can.high.20.a	acpP.low.1.NY	ftsI.N.1TAG	dnaX.low.5	lspA.high.20	fusA.F32
can.high.20.b	acpP.med.1.NY	murD.N.1TAG	dnaX.med.16	lspA.med.15	glyQ.Y226
can.low.2	aspS.high.1.NY	ftsA.N.1TAG	dxs.high.20	secY.low.1	Int.Y388
dnaX.high.20	aspS.low.1.NY	ftsZ.N.1TAG	dxs.low.6	secY.med.18	murG.F243
dnaX.low.5	aspS.med.1.NY	secA.N.1TAG	dxs.med.11	topA.med.14	rpoA.Y152
dnaX.med.16	can.high.1.NY	frr.N.1TAG	eno.high.20	dxs.N.1TAG	thrS.Y313
dxs.high.20	can.low.1.NY	accA.N.1TAG	rplB.high.20	ftsI.N.1TAG	
dxs.low.6	can.med.1.NY	dxs.N.1TAG	rplB.low.1.a	murD.N.1TAG	serS.F213 was included in this pool
dxs.med.11	cysS.high.1.NY	glnS.N.1TAG	secY.high.20		
eno.high.20	cysS.low.1.NY	prsA.N.1TAG	secY.low.1		
eno.low.2.a	cysS.med.1.NY	nadE.N.1TAG	secY.med.18		
eno.low.2.b	eno.high.1.NY	pgsA.N.1TAG	trmD.low.2		
fabG.high.20	eno.low.1.NY	nrdB.N.1TAG	trmD.med.16		
fabG.low.1	eno.med.1.NY	gltX.N.1TAG	tyrS.med.18		
fabG.med.18	era.high.1.NY	lepB.N.1TAG	dxs.N.1TAG		
ispH.low.1	era.low.1.NY	trmD.N.1TAG	dxs.N.2TAG		
ispH.med.10	era.med.1.NY	grpE.N.1TAG	dxs.N.3TAG		
ispH.med.11	fabG.high.1.NY	metK.N.1TAG	grpE.N.1TAG		
lspA.high.20	fabG.low.1.NY	infB.N.1TAG	grpE.N.2TAG		
lspA.low.3	fabG.med.1.NY	fnt.N.1TAG	grpE.N.3TAG		
lspA.med.15	glnS.high.1.NY	secY.N.1TAG	rplO.N.1TAG		
pheT.high.20	glnS.low.1.NY	rplO.N.1TAG	rplO.N.2TAG		
pheT.low.2	glnS.med.1.NY	rpsE.N.1TAG	rplO.N.3TAG		
pheT.med.14	groS.high.1.NY	rpsN.N.1TAG	secY.N.1TAG		
rplB.high.20	groS.low.1.NY	rplN.N.1TAG	secY.N.2TAG		
rplB.low.1.a	groS.med.1.NY	rpsO.N.1TAG	secY.N.3TAG		
rplB.low.1.b	ispH.high.1.NY	rplD.N.1TAG	trmD.N.1TAG		
rplJ.low.4	ispH.low.1.NY	rplC.N.1TAG	trmD.N.2TAG		
rplJ.med.10	ispH.med.1.NY	fusA.N.1TAG	trmD.N.3TAG		
rplJ.med.13	lepB.high.1.NY	rpoC.N.1TAG			
rplM.low.2	lepB.low.1.NY	ppa.N.1TAG			
rplM.low.4	lepB.med.1.NY				
rplM.low.5	murD.high.1.NY				
rplS.low.1	murD.low.1.NY				
rplS.low.2	murD.med.1.NY				
rpmB.low.4	rplJ.high.1.NY				
rpsD.low.1	rplJ.high.2.NY				
rpsD.low.5	rplJ.low.1.NY				
rpsK.low.8	rplJ.low.2.NY				
rpsL.low.1	rplJ.med.1.NY				
rpsL.low.2	rplJ.med.2.NY				
rpsP.low.2	rplM.high.1.NY				
secY.high.20	rplM.low.1.NY				
secY.low.1	rplM.med.1.NY				
secY.med.18	rplS.high.1.NY				
topA.high.20	rplS.low.1.NY				
topA.low.5	rplS.med.1.NY				
topA.med.14	rpmD.high.1.NY				
trmD.low.2	rpmD.low.1.NY				
trmD.low.4	rpmD.med.1.NY				
trmD.med.16	rpoA.high.1.NY				
tyrS.low.1	rpoA.low.1.NY				
tyrS.med.18	rpoA.med.1.NY				
	rpsB.high.1.NY				

	rpsB.low.1.NY				
	rpsB.med.1.NY				
	rpsD.high.1.NY				
	rpsD.low.1.NY				
	rpsD.med.1.NY				
	thrS.high.1.NY				
	thrS.low.1.NY				
	thrS.med.1.NY				
	trmD.high.1.NY				
	trmD.high.2.NY				
	trmD.low.1.NY				
	trmD.low.2.NY				
	trmD.med.1.NY				
	trmD.med.2.NY				

Oligonucleotides within each pool were designed by a single or combination ("complex" pools) of strategies. Pool names are abbreviated as follows: Tolerant tyrosine (Y), tolerant non-tyrosine (NY), amino-terminal (N), complex 1 (C1), complex 2 (C2), and functional (F) pools.

Supplementary Table 3: Analysis of sAA-dependence screens.

sAA	aaRS	Pool ^a	MAGE cycles	Total screened	Total dependent	Unique dependent	Unique 1TAG	Unique 2TAG	Unique 3TAG	Unique 4TAG
pAcF	pAcF-RS	Y	4	88	3	3	2	1	0	0
pAcF	pAcF-RS	NY	4	88	0	0	n/a ^b	n/a ^b	n/a ^b	n/a ^b
pAcF	pAcF-RS	N	4	88	2	2	2	0	0	0
pAcF	pAcF-RS	C1	4	93	3	3	3	0	0	0
pAcF	pAcF-RS	F	8	86	0	0	n/a ^b	n/a ^b	n/a ^b	n/a ^b
pAzF	pAzF-RS	C2	3	255	0	0	n/a ^b	n/a ^b	n/a ^b	n/a ^b
pAzF	pCNF-RS	Y	4	130	13	8	4	3	0	1
pAzF	pCNF-RS	N	4	84	8	4	3	1	0	0
pAzF	pCNF-RS	C2	4	124	8	6	3	1	2	0
pAzF	pCNF-RS	F	8	344	57	14	6	8	0	0
pIF	pIF-RS	C2	3	172	6	3	2	1	0	0
pIF	pIF-RS	F	8	62	0	0	n/a ^b	n/a ^b	n/a ^b	n/a ^b

^aPool names are abbreviated as follows: Tolerant tyrosine (Y), tolerant non-tyrosine (NY), amino-terminal (N), complex 1 (C1), complex 2 (C2), and functional (F) pools.

^bWe only quantitatively assess strains that were sAA-dependent and cannot report all successful TAG incorporations that generated viable or nonviable non-contained clones.

Supplementary Table 4: Conversion of aaRS specificity.

Residue	TyrRS (WT)	pAcF-RS	pIF-RS	pAzF-RS	pCNF-RS
32	Y	L	L	T	L
107	E	E	S	N	D ^a
108	F	F	F	F	W
109	Q	Q	Q	Q	M
158	D	G	P	P	G
159	I	C	L	L	A
162	L	R	E	Q	L
286	D	R	R	R	R

^aThe published pCNF-RS contains E107 but we pursued a D107 variant.

>*Methanocaldococcus jannaschii* TyrRS

MDEFEMIKRNTSEIIISEEELREVLKKDEKSA**Y**IGFEPGKIHHLGHYLQIKKMIDLQNA
 GFDIIILLADLHAYLNQKGELEIRKIGDYNKKVFEAMGLKAKYVYGS**EFQ**LDKDYTL
 NVYRLALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVND**I**H**Y**L**G**VDVAVGGM**E**Q**R**
 KIHMLARELLPKKVVCIHNPVLTGLDGEGKMSSSKGNFIAVDDSP**E**IRAKIKKAYCP
 AGVVEGNPIMEIAKYFLEYP**L**TIKRPEKFGGDLTVNSYEELES**L**FKNKELHP**M**DLKNA
 VAEELIKILEPIRKRL

Supplementary Table 5: Summary of 22 essential genes containing TAG codons in sAA-dependent strains.

Essential gene(s)	GO Annotation (Biological process)	GO Annotation (Molecular function)
<i>dcx</i>	GO:0006744 (ubiquinone biosynthetic process) GO:0008615 (pyridoxine biosynthetic process) GO:0009228 (thiamine biosynthetic process) GO:0008152 (metabolic process) GO:0008299 (isoprenoid biosynthetic process) GO:0016114 (terpenoid biosynthetic process) GO:0052865 (1-deoxy-D-xylulose 5-phosphate biosynthetic process)	GO:0008661 (1-deoxy-D-xylulose-5-phosphate synthase activity) GO:0030976 (thiamine pyrophosphate binding) GO:0000287 (magnesium ion binding) GO:0003824 (catalytic activity) GO:0016740 (transferase activity) GO:0046872 (metal ion binding)
<i>ftsI</i>	GO:0042493 (response to drug) GO:0051301 (cell division) GO:0007049 (cell cycle) GO:0008152 (metabolic process) GO:0008360 (regulation of cell shape) GO:0009252 (peptidoglycan biosynthetic process) GO:0046677 (response to antibiotic)	GO:0005515 (protein binding) GO:0008658 (penicillin binding) GO:0008955 (peptidoglycan glycosyltransferase activity) GO:0003824 (catalytic activity) GO:0016740 (transferase activity) GO:0016757 (transferase activity, transferring glycosyl groups)
<i>metK</i>	GO:0006556 (S-adenosylmethionine biosynthetic process) GO:0006730 (one-carbon metabolic process)	GO:0000287 (magnesium ion binding) GO:0004478 (methionine adenosyltransferase activity) GO:0005515 (protein binding) GO:0030955 (potassium ion binding) GO:0042802 (identical protein binding) GO:0000166 (nucleotide binding) GO:0005524 (ATP binding) GO:0016740 (transferase activity) GO:0046872 (metal ion binding)
<i>murD</i>	GO:0007049 (cell cycle) GO:0008360 (regulation of cell shape) GO:0009058 (biosynthetic process) GO:0009252 (peptidoglycan biosynthetic process) GO:0051301 (cell division)	GO:0008764 (UDP-N-acetylmuramoylalanine-D-glutamate ligase activity) GO:0000166 (nucleotide binding) GO:0005524 (ATP binding) GO:0016874 (ligase activity)
<i>pgsA</i>	GO:0006629 (lipid metabolic process) GO:0006655 (phosphatidylglycerol biosynthetic process) GO:0008654 (phospholipid biosynthetic process)	GO:0008444 (CDP-diacylglycerol-glycerol-3-phosphatidyltransferase activity) GO:0016740 (transferase activity) GO:0016780 (phosphotransferase activity, for other substituted phosphate groups) GO:0004089 (carbonate dehydratase activity)
<i>can</i>	GO:0008152 (metabolic process) GO:0015976 (carbon utilization)	GO:0008270 (zinc ion binding) GO:0016829 (lyase activity) GO:0046872 (metal ion binding)
<i>dnaX</i>	not found	not found
<i>fabG</i>	GO:0006633 (fatty acid biosynthetic process) GO:0008610 (lipid biosynthetic process) GO:0009102 (biotin biosynthetic process) GO:0030497 (fatty acid elongation) GO:0006629 (lipid metabolic process) GO:0006631 (fatty acid metabolic process) GO:0008152 (metabolic process) GO:0051114 (oxidation-reduction process)	GO:0004316 (3-oxoacyl-[acyl-carrier-protein] reductase (NADPH) activity) GO:0042802 (identical protein binding) GO:0050661 (NADP binding) GO:0016491 (oxidoreductase activity) GO:0046872 (metal ion binding) GO:0051536 (iron-sulfur cluster binding) GO:0051538 (3 iron, 4 sulfur cluster binding)
<i>ispH</i>	GO:0006744 (ubiquinone biosynthetic process) GO:0046677 (response to antibiotic) GO:0008299 (isoprenoid biosynthetic process) GO:0016114 (terpenoid biosynthetic process) GO:0019288 (isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway) GO:0050992 (dimethylallyl diphosphate biosynthetic process) GO:0051114 (oxidation-reduction process) GO:0010033 (response to organic substance)	GO:0042380 (hydroxymethylbutenyl pyrophosphate reductase activity) GO:0016491 (oxidoreductase activity) GO:0046872 (metal ion binding) GO:0051536 (iron-sulfur cluster binding) GO:0051538 (3 iron, 4 sulfur cluster binding) GO:0051745 (4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase activity)
<i>lptD</i>	GO:0015920 (lipopolysaccharide transport) GO:0043165 (Gram-negative-bacterium-type cell outer membrane assembly) GO:0061024 (membrane organization)	GO:0005515 (protein binding)
<i>lspA</i>	GO:0006465 (signal peptide processing) GO:0006508 (proteolysis)	GO:0008233 (peptidase activity) GO:0004190 (serpin-type endopeptidase activity) GO:0016787 (hydrolase activity) GO:0005515 (protein binding)
<i>pheT</i>	GO:0006412 (translation) GO:0006432 (phenylalanyl-tRNA aminoacylation) GO:0008033 (tRNA processing)	GO:0000049 (tRNA binding) GO:0000166 (nucleotide binding) GO:0000287 (magnesium ion binding) GO:0003723 (RNA binding) GO:0004812 (aminoacyl-tRNA ligase activity) GO:0004826 (phenylalanine-tRNA ligase activity) GO:0005524 (ATP binding) GO:0016874 (ligase activity) GO:0046872 (metal ion binding) GO:0005515 (protein binding)
<i>rpoA</i>	GO:0006351 (transcription, DNA-templated)	GO:0008270 (zinc ion binding) GO:0003677 (DNA binding) GO:0003899 (DNA-directed RNA polymerase activity) GO:0016740 (transferase activity) GO:0016779 (nucleotidyltransferase activity) GO:0046983 (protein dimerization activity)
<i>secY</i>	GO:0006886 (intracellular protein transport) GO:0043952 (protein transport by the Sec complex) GO:0065002 (intracellular protein transmembrane transport) GO:0006605 (protein targeting) GO:0006810 (transport) GO:0015031 (protein transport)	GO:0005515 (protein binding)
<i>serS</i>	GO:0006434 (seryl-tRNA aminoacylation) GO:0016260 (selenocysteine biosynthetic process) GO:0006412 (translation) GO:0006418 (tRNA aminoacylation for protein translation) GO:0097056 (selenocysteinyl-tRNA(Sec) biosynthetic process)	GO:0000287 (magnesium ion binding) GO:0004828 (serine-tRNA ligase activity) GO:0042802 (identical protein binding) GO:0000166 (nucleotide binding) GO:0004812 (aminoacyl-tRNA ligase activity) GO:0005524 (ATP binding) GO:0016874 (ligase activity)
<i>topA</i>	GO:0006265 (DNA topological change) GO:002616 (DNA strand elongation) GO:0006200 (ATP catabolic process)	GO:0003917 (DNA topoisomerase type I activity) GO:0000166 (nucleotide binding) GO:0000287 (magnesium ion binding) GO:0003677 (DNA binding) GO:0003918 (DNA topoisomerase type II (ATP-hydrolyzing) activity) GO:0005524 (ATP binding) GO:0016853 (isomerase activity) GO:0046872 (metal ion binding) GO:0000166 (nucleotide binding)
<i>tyrS</i>	GO:0006412 (translation) GO:0006418 (tRNA aminoacylation for protein translation) GO:0006437 (tyrosyl-tRNA aminoacylation)	GO:0003723 (RNA binding) GO:0004812 (aminoacyl-tRNA ligase activity) GO:0004831 (tyrosine-tRNA ligase activity) GO:0005524 (ATP binding) GO:0016874 (ligase activity)

<i>dnaA</i>	<p>GO:0006260 (DNA replication) GO:0006275 (regulation of DNA replication) GO:0006270 (DNA replication initiation) GO:0006351 (transcription, DNA-templated) GO:0006355 (regulation of transcription, DNA-templated) GO:0008156 (negative regulation of DNA replication)</p>	<p>GO:0003677 (DNA binding) GO:0003688 (DNA replication origin binding) GO:0005515 (protein binding) GO:0043565 (sequence-specific DNA binding) GO:0000166 (nucleotide binding) GO:0005524 (ATP binding) GO:0017111 (nucleoside-triphosphatase activity) GO:0000166 (nucleotide binding)</p>
<i>fusA</i>	<p>GO:0006184 (GTP catabolic process) GO:0006412 (translation) GO:0006414 (translational elongation)</p>	<p>GO:0003746 (translation elongation factor activity) GO:0003924 (GTPase activity) GO:0005525 (GTP binding) GO:0000166 (nucleotide binding)</p>
<i>gylQ</i>	<p>GO:0006412 (translation) GO:0006426 (glycyl-tRNA aminoacylation)</p>	<p>GO:0000166 (nucleotide binding) GO:0004812 (aminoacyl-tRNA ligase activity) GO:0004820 (glycine-tRNA ligase activity) GO:0005524 (ATP binding) GO:0016874 (ligase activity)</p>
<i>Int</i>	<p>GO:0042158 (lipoprotein biosynthetic process) GO:0006807 (nitrogen compound metabolic process)</p>	<p>GO:0016410 (N-acyltransferase activity) GO:0016740 (transferase activity) GO:0016746 (transferase activity, transferring acyl groups) GO:0016787 (hydrolase activity)</p>
<i>murG</i>	<p>GO:0009252 (peptidoglycan biosynthetic process) GO:0007049 (cell cycle) GO:0008360 (regulation of cell shape) GO:0019277 (UDP-N-acetylgalactosamine biosynthetic process) GO:0030259 (lipid glycosylation) GO:0051301 (cell division)</p>	<p>GO:0016810 (hydrolase activity, acting on carbon-nitrogen [but not peptide] bonds) GO:0050511 (undecaprenyl diphosphate-muramoyl pentapeptide-beta-N-acetylglucosaminyl transferase activity) GO:0016740 (transferase activity) GO:0016757 (transferase activity, transferring glycosyl groups) GO:0016758 (transferase activity, transferring hexosyl groups) GO:0030246 (carbohydrate binding) GO:0051991 (UDP-N-acetyl-D-glucosamine:N-acetylmuramoyl-L-alanyl-D-glutamyl-meso-2,6-diaminonimetyl-D-alanyl-D-alanine-diphosphoundecaprenol-4-beta-N-acetylglucosaminyl transferase activity)</p>

Supplementary Table 6: Summary of escape frequencies and doubling times for 60 sAA-dependent strains.

Nickname	Synthetic amino acid	Essential protein(s)	TAG location(s)	Escape frequency (EF) (Day 1)	Doubling time (minutes) ^a	
					1mM sAA	5mM sAA
rEc.α	pAcF, α	n/a	n/a	n/a	54.25 ± 0.19	data not collected
DnaX.Y113α	pAcF, α	DnaX	Y113	6.73E-07 ± 1.89E-07	54.51 ± 0.60	data not collected
DnaX.Y113α'	pAcF, α	DnaX	Y113	1.95E-07 ± 5.09E-08	64.97 ± 0.73	data not collected
LspA.Y54α	pAcF, α	LspA	Y54	8.39E-06 ± 1.16E-06	52.90 ± 0.11	data not collected
SecY.Y122α	pAcF, α	SecY	Y122	2.05E-05 ± 8.38E-06	58.90 ± 0.27	data not collected
SecY.Y309α	pAcF, α	SecY	Y309	4.97E-04 ± 3.15E-04	52.79 ± 0.39	data not collected
Dxs.αN	pAcF, α	Dxs	N-term	1.85E-06 ± 5.65E-07	61.77 ± 0.15	data not collected
FtsI.αN	pAcF, α	FtsI	N-term	1.07E-05 ± 6.27E-06	57.72 ± 0.03	data not collected
MurD.αN	pAcF, α	MurD	N-term	2.97E-05 ± 2.88E-06	55.28 ± 0.41	data not collected
rEc.α.dB.8	pAcF, α	TopA LspA	Y728 Y28	6.54E-04 ± 4.45E-04	51.47 ± 0.37	data not collected
rEc.β	pIF, β	n/a	n/a	n/a	60.21 ± 0.46	data not collected
DnaX.Y113β	pIF, β	DnaX	Y113	8.71E-07 ± 5.31E-07	60.29 ± 0.68	data not collected
LspA.Y54β	pIF, β	LspA	Y54	1.86E-05 ± 5.57E-06	61.68 ± 1.52	data not collected
SecY.Y122β	pIF, β	SecY	Y122	2.62E-06 ± 6.47E-07	62.57 ± 0.36	data not collected
rEc.β.dB.9	pIF, β	DnaX LspA	Y113 Y54	1.31E-08 ± 9.52E-09	59.63 ± 0.29	data not collected
rEc.β.dB.9'	pIF, β	DnaX LspA	Y113 Y54	4.92E-09 ± 1.62E-09	60.58 ± 0.28	data not collected
rEc.β.dC.11	pIF, β	DnaX LspA Dxs	Y113 Y54 N-term	5.01E-09 ± 3.61E-09	61.62 ± 0.19	data not collected
rEc.β.dC.11'	pIF, β	DnaX LspA Dxs	Y113 Y54 N-term	3.31E-09 ± 4.90E-10	61.97 ± 0.22	data not collected
rEc.β.dC.12	pIF, β	DnaX LspA SecY	Y113 Y54 Y122	4.68E-09 ± 1.99E-09	60.04 ± 0.29	data not collected
rEc.β.dC.12'	pIF, β	DnaX LspA SecY	Y113 Y54 Y122	2.17E-09 ± 3.69E-10	61.81 ± 0.65	data not collected
rEc.β.dC.12'.ΔtY	pIF, β	DnaX LspA SecY	Y113 Y54 Y122	< 4.85E-12	60.99 ± 0.70	data not collected
rEc.γ	pAzF, γ	n/a	n/a	n/a	58.63 ± 0.87	59.41 ± 0.13
Can.Y83γ	pAzF, γ	Can	Y83	2.39E-06 ± 7.90E-07	57.03 ± 0.27	data not collected
Can.Y99γ	pAzF, γ	Can	Y99	1.05E-05 ± 3.84E-06	58.34 ± 0.28	data not collected
DnaX.Y113γ	pAzF, γ	DnaX	Y113	1.37E-06 ± 2.08E-07	58.79 ± 0.36	data not collected
FabG.Y242γ	pAzF, γ	FabG	Y242	1.16E-04 ± 6.96E-05	57.86 ± 0.14	data not collected
LptD.Y212γ	pAzF, γ	LptD	Y212	data not collected	data not collected	data not collected
LptD.Y354γ	pAzF, γ	LptD	Y354	data not collected	data not collected	data not collected
SecY.Y122γ	pAzF, γ	SecY	Y122	3.38E-06 ± 5.28E-07	59.82 ± 0.66	data not collected
TyrS.Y127γ	pAzF, γ	TyrS	Y127	2.88E-06 ± 1.75E-06	58.45 ± 0.27	data not collected
Dxs.γN	pAzF, γ	Dxs	N-term	3.41E-06 ± 2.10E-06	62.25 ± 0.54	data not collected
FtsI.γN	pAzF, γ	FtsI	N-term	1.53E-05 ± 1.15E-05	58.67 ± 0.13	data not collected
MurD.γN	pAzF, γ	MurD	N-term	4.24E-05 ± 2.57E-05	59.01 ± 0.54	data not collected
PgsA.γN	pAzF, γ	PgsA	N-term	1.36E-05 ± 3.22E-06	68.74 ± 1.34	data not collected
DnaA.W6γ	pAzF, γ	DnaA	W6	5.14E-07 ± 1.69E-07	65.20 ± 0.89	66.23 ± 0.28
FusA.F32γ	pAzF, γ	FusA	F32	5.00E-03 ± 4.14E-03	73.76 ± 0.17	62.93 ± 0.28

GlyQ.Y226 γ	pAzF, γ	GlyQ	Y226	1.01E-05 \pm 3.10E-06	65.32 \pm 1.22	60.66 \pm 1.19
Lint.Y388 γ	pAzF, γ	Lint	Y388	6.80E-06 \pm 3.19E-06	60.98 \pm 0.26	62.27 \pm 0.06
MurG.F243 γ	pAzF, γ	MurG	F243	8.05E-07 \pm 4.00E-07	59.16 \pm 0.15	60.41 \pm 0.52
SerS.F213 γ	pAzF, γ	SerS	F213	1.29E-06 \pm 8.75E-07	58.68 \pm 0.19	59.52 \pm 0.36
rEc. γ .dB.16	pAzF, γ	PgsA MetK	N-term	9.87E-07 \pm 4.24E-07	61.51 \pm 1.02	data not collected
rEc. γ .dB.17	pAzF, γ	SecY	Y122 Y309	1.77E-06 \pm 1.14E-06	64.02 \pm 1.13	data not collected
rEc. γ .dC.18	pAzF, γ	TopA SecY122 Dxs	Y728 Y122 N-term	3.38E-05 \pm 2.65E-05	61.15 \pm 0.88	data not collected
rEc. γ .dD.20	pAzF, γ	LspA LspA PheT IspH	Y28 Y50 Y615 Y142	1.02E-05 \pm 5.70E-06	62.61 \pm 0.32	data not collected
rEc. γ .dB.22	pAzF, γ	SecY PheT	Y309 Y615	2.93E-05 \pm 1.53E-05	59.97 \pm 0.57	data not collected
rEc. γ .dB.23	pAzF, γ	PheT	Y558 Y601	5.48E-06 \pm 3.73E-06	58.22 \pm 0.30	data not collected
rEc. γ .dC.24	pAzF, γ	TopA LspA Dxs	Y728 Y28 N-term	2.85E-06 \pm 2.10E-06	62.12 \pm 0.83	data not collected
rEc. γ .dB.26	pAzF, γ	DnaX	Y113 Y328	1.42E-07 \pm 8.69E-08	60.49 \pm 0.15	data not collected
rEc. γ .dB.28	pAzF, γ	LptD	Y212 Y354	data not collected	data not collected	data not collected
rEc. γ .dB.29	pAzF, γ	LptD	Y458 Y514	data not collected	data not collected	data not collected
rEc. γ .dB.31	pAzF, γ	LptD	Y212 Y244	data not collected	data not collected	data not collected
rEc. γ .dB.38	pAzF, γ	FusA RpoA	F32 Y152	data not collected	data not collected	data not collected
rEc. γ .dB.39	pAzF, γ	FusA MurG	F32 F243	data not collected	data not collected	data not collected
rEc. γ .dB.40	pAzF, γ	DnaA GlyQ	W6 Y226	data not collected	data not collected	data not collected
rEc. γ .dB.41	pAzF, γ	DnaA MurG	W6 F243	1.56E-09 \pm 1.02E-09	63.78 \pm 0.26	62.62 \pm 0.04
rEc. γ .dB.41'	pAzF, γ	DnaA MurG	W6 F243	5.98E-10	62.22 \pm 0.31	60.90 \pm 0.21
rEc. γ .dB.42	pAzF, γ	DnaA FusA	W6 F32	data not collected	data not collected	data not collected
rEc. γ .dB.43	pAzF, γ	DnaA SerS	W6 F213	2.32E-09 \pm 6.53E-10	75.89 \pm 2.79	59.85 \pm 0.10
rEc. γ .dB.44	pAzF, γ	DnaA RpoA	W6 Y152	data not collected	data not collected	data not collected
rEc. γ .dB.45	pAzF, γ	Lint MurG	Y388 F243	data not collected	data not collected	data not collected
rEc. γ .dC.46	pAzF, γ	DnaA MurG SerS	W6 F243 F213	< 7.88E-11	71.01 \pm 0.36	63.42 \pm 0.30
rEc. γ .dC.46'	pAzF, γ	DnaA MurG SerS	W6 F243 F213	< 4.36E-11	71.86 \pm 0.59	64.27 \pm 0.23
rEc. γ .dC.46' Δ tY	pAzF, γ	DnaA MurG SerS	W6 F243 F213	< 6.3E-12	64.91 \pm 0.39	61.41 \pm 0.18

^aAverage doubling times are shown for growth in permissive media; n=3 technical replicates; error is \pm s.d.

Supplementary Table 7: Escape mechanisms of MutS-deficient strains with one essential TAG codon.

Escape mutant	Essential gene	Causative SNP ^a	Causative SNP location ^b	Relative fitness in media without sAA ^c
SecY.Y122 α .E1	<i>secY</i>	cag→cGg	RpsD.Q54R	0.68 ± 0.035
SecY.Y122 α .E2	<i>secY</i>	tag→Cag	SecY. α 122Q	0.97 ± 0.006
SecY.Y122 α .E3	<i>secY</i>	ctg→ctA	<i>glnV</i> → <i>supE</i>	1.00 ± 0.028
DnaX.Y113 α .E1	<i>dnaX</i>	tag→tGg	DnaX. α 113W	0.97 ± 0.011
DnaX.Y113 α .E2	<i>dnaX</i>	tag→tGg	DnaX. α 113W	0.95 ± 0.015
DnaX.Y113 α .E3	<i>dnaX</i>	tag→tGg	DnaX. α 113W	0.94 ± 0.014

^aCausative SNP is capitalized within the codon or anticodon sequence, from 5' to 3', for protein and tRNA-encoded genes, respectively.

^bLocation of the amino acid change is shown for protein-encoded genes and names conferred by anticodon mutations for tRNA-encoded genes.

^cAverage ratio of doubling times are shown for contained ancestor to escape mutant grown in permissive and nonpermissive media, respectively; n=3 technical replicates; error is \pm s.d.

Supplementary Table 8: Whole genome sequencing of MutS-deficient strains with one essential TAG codon.

Strain	Feature	Start	End	WT	Mut	Gene	Consequence	AA change	Product	GO Annotation (Biological process)
DnaX.Y113a.E1	indel	2	2	-	TCA	<i>thrL</i>	UPSTREAM (+188)	-	the operon leader peptide	GO:008652 (cellular amino acid biosynthetic process) GO:009088 (threonine biosynthetic process) GO:001555 (transcriptional attenuation) GO:001556 (transcriptional attenuation by ribosome)
DnaX.Y113a.E1	snp	491478	491478	A	G	<i>dnaX</i>	NON_SYNONYMOUS_CODING	αW	DNA polymerase III/DNA elongation factor III, τ and γ subunits	GO:0006200 (ATP catabolic process) GO:0006260 (DNA replication) GO:0008555 (drug transmembrane transport) GO:0030641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:0055085 (transmembrane transport) GO:0006810 (transport)
DnaX.Y113a.E1	snp	890780	890780	T	G	<i>mdfA</i>	START_REMOVED	M*	multidrug efflux system protein	GO:0008555 (drug transmembrane transport) GO:0030641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:0055085 (transmembrane transport) GO:0006810 (transport)
DnaX.Y113a.E1	snp	890782	890782	C	T	<i>mdfA</i>	STOP_GAINED	Q*	multidrug efflux system protein	GO:0008555 (drug transmembrane transport) GO:0030641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:0055085 (transmembrane transport) GO:0006810 (transport)
DnaX.Y113a.E1	snp	892572	892572	C	T	<i>yhlJ</i>	NON_SYNONYMOUS_CODING	M1	FMN and erythrose-4-P phosphatase	GO:0016311 (dephosphorylation) GO:0023663 (FMN catabolic process) GO:0008152 (metabolic process) GO:0006810 (transport)
DnaX.Y113a.E1	snp	1052407	1052407	C	T	<i>glcE</i>	SYNONYMOUS_CODING	A	lipoprotein required for formation of O-antigen capsule, predicted exopolysaccharide export protein	GO:0006810 (transport) GO:0006811 (ion transport) GO:0015774 (polysaccharide transport) GO:0006810 (transport)
DnaX.Y113a.E1	snp	1214889	1214889	C	G	<i>stfP</i>	NON_SYNONYMOUS_CODING	L/V	uncharacterized protein from lambdaoid prophage e14 region	Not found
DnaX.Y113a.E1	snp	1639395	1639395	C	T	<i>ffaQ_ydD</i>	NON_SYNONYMOUS_CODING, NON_SYNONYMOUS_CODING	P, G/R	Qin prophage; predicted tail fiber assembly protein (TfaQ), predicted transporter (YdD)	Not found ydlD GO:0006813 (potassium ion transport) GO:0006810 (transport) GO:0005508 (transmembrane transport) GO:0005514 (oxidation-reduction process)
DnaX.Y113a.E1	snp	2043895	2043895	A	G	<i>yeoY</i>	NON_SYNONYMOUS_CODING	D/G	membrane-anchored, periplasmic TMAO, DMSO reductase	GO:0006810 (transport)
DnaX.Y113a.E1	indel	2769807	2769807	G	GC	<i>yfM</i>	UPSTREAM (+11)	-	CP4-57 prophage, predicted protein	Not found
DnaX.Y113a.E1	snp	4603891	4603891	C	T	<i>yjB</i>	SYNONYMOUS_CODING	A	conserved inner membrane protein	Not found
DnaX.Y113a.E1	snp	4643550	4643553	TTT	CAG	<i>thrL</i>	UPSTREAM (+194)	-	the operon leader peptide	GO:008652 (cellular amino acid biosynthetic process) GO:009088 (threonine biosynthetic process) GO:001555 (transcriptional attenuation) GO:001556 (transcriptional attenuation by ribosome)
DnaX.Y113a.E2	indel	3	3	CT	TATTTTCAGCT	<i>thrL</i>	UPSTREAM (+187)	-	the operon leader peptide	GO:008652 (cellular amino acid biosynthetic process) GO:009088 (threonine biosynthetic process) GO:001555 (transcriptional attenuation) GO:001556 (transcriptional attenuation by ribosome)
DnaX.Y113a.E2	snp	491478	491478	A	G	<i>dnaX</i>	NON_SYNONYMOUS_CODING	αW	DNA polymerase III/DNA elongation factor III, τ and γ subunits	GO:0006200 (ATP catabolic process) GO:0006260 (DNA replication) GO:0016311 (dephosphorylation) GO:0023663 (FMN catabolic process) GO:0008152 (metabolic process)
DnaX.Y113a.E2	snp	892572	892572	C	T	<i>yhlJ</i>	NON_SYNONYMOUS_CODING	M1	FMN and erythrose-4-P phosphatase	GO:0016311 (dephosphorylation) GO:0023663 (FMN catabolic process) GO:0008152 (metabolic process)
DnaX.Y113a.E2	snp	1214889	1214889	C	G	<i>stfP</i>	NON_SYNONYMOUS_CODING	L/V	uncharacterized protein from lambdaoid prophage e14 region	Not found
DnaX.Y113a.E2	snp	1216718	1216718	C	G	<i>stfE</i>	WITHIN_NON_CODING_GENE	-	pseudogene	GO:0019048 (modulation by virus of host morphology or physiology) GO:0046718 (viral entry into host cell) GO:0019062 (viral attachment to host cell)
DnaX.Y113a.E2	indel	2769807	2769807	G	GC	<i>yfM</i>	UPSTREAM (+11)	-	CP4-57 prophage, predicted protein	Not found
DnaX.Y113a.E2	snp	3074740	3074740	A	G	<i>pgk</i>	SYNONYMOUS_CODING	R	phosphoglycerate kinase	GO:0000796 (glycolysis) GO:0016310 (phosphorylation) GO:0008152 (metabolic process) GO:0045918 (negative regulation of cytolysis) GO:0009252 (peptidoglycan biosynthetic process) GO:0008360 (regulation of cell shape) GO:0046677 (response to antibiotic)
DnaX.Y113a.E2	snp	3524039	3524039	G	A	<i>mrcA</i>	SYNONYMOUS_CODING	E	fused penicillin-binding protein 1a: murein transglycosylase/murein transpeptidase	Not found
DnaX.Y113a.E2	snp	4603891	4603891	C	T	<i>yjB</i>	SYNONYMOUS_CODING	A	conserved inner membrane protein	Not found
DnaX.Y113a.E3	indel	2	2	GC	TATTTTCAGC	<i>thrL</i>	UPSTREAM (+188)	-	the operon leader peptide	GO:008652 (cellular amino acid biosynthetic process) GO:009088 (threonine biosynthetic process) GO:001555 (transcriptional attenuation) GO:001556 (transcriptional attenuation by ribosome)
DnaX.Y113a.E3	snp	491478	491478	A	G	<i>dnaX</i>	NON_SYNONYMOUS_CODING	αW	DNA polymerase III/DNA elongation factor III, τ and γ subunits	GO:0006200 (ATP catabolic process) GO:0006260 (DNA replication) GO:0016311 (dephosphorylation) GO:0023663 (FMN catabolic process) GO:0008152 (metabolic process)
DnaX.Y113a.E3	snp	892572	892572	C	T	<i>yhlJ</i>	NON_SYNONYMOUS_CODING	M1	FMN and erythrose-4-P phosphatase	GO:0016311 (dephosphorylation) GO:0023663 (FMN catabolic process) GO:0008152 (metabolic process)
DnaX.Y113a.E3	snp	1214889	1214889	C	G	<i>stfP</i>	NON_SYNONYMOUS_CODING	L/V	uncharacterized protein from lambdaoid prophage e14 region	Not found
DnaX.Y113a.E3	snp	1961000	1961000	G	A	<i>tarZ</i>	SYNONYMOUS_CODING	G	trimethylamine N-oxide reductase system III, catalytic subunit	GO:0045333 (cellular respiration) GO:0005114 (oxidation-reduction process) GO:0006635 (fatty acid beta-oxidation) GO:0006631 (fatty acid metabolic process) GO:0016042 (lipid catabolic process) GO:0006429 (lipid metabolic process) GO:0008152 (metabolic process) GO:0055114 (oxidation-reduction process)
DnaX.Y113a.E3	snp	2461771	2461771	G	A	<i>fadL</i>	NON_SYNONYMOUS_CODING	T1	fused enoyl-CoA hydratase and epimerase and isomerase/3-hydroxyacyl-CoA dehydrogenase	GO:0042773 (ATP synthesis coupled electron transport) GO:0055114 (oxidation-reduction process)
DnaX.Y113a.E3	snp	2609236	2609236	T	C	<i>hydD</i>	NON_SYNONYMOUS_CODING	L/P	hydrogenase 4, membrane subunit	Not found
DnaX.Y113a.E3	indel	2769807	2769807	G	GC	<i>yfM</i>	UPSTREAM (+11)	-	CP4-57 prophage, predicted protein	Not found
DnaX.Y113a.E3	snp	4464463	4464463	C	T	<i>mrdD</i>	NON_SYNONYMOUS_CODING	A/T	anaerobic ribonucleoside-triphosphate reductase	GO:0006260 (DNA replication) GO:0008152 (metabolic process) GO:0015949 (nucleoside-containing small molecule interconversion) GO:0055114 (oxidation-reduction process)
DnaX.Y113a.E3	snp	4603891	4603891	C	T	<i>yjB</i>	SYNONYMOUS_CODING	A	conserved inner membrane protein	Not found
SeeY.Y122a.E1	snp	270639	270639	T	C	<i>insN_insI</i>	NON_SYNONYMOUS_CODING, SYNONYMOUS_CODING	Y/H, N	pseudogene (InsN), IS30 transposase (InsI)	Not found insN GO:0006310 (DNA recombination) GO:0006313 (transposition, DNA-mediated) GO:0032196 (transposition) insI GO:0006316 (transposition, DNA-mediated) GO:0006310 (DNA recombination) GO:0019074 (DNA integration) GO:0032196 (transposition) GO:0005978 (copper ion homeostasis) GO:0010273 (detoxification of copper ion) GO:0046688 (response to copper ion) GO:0010272 (response to silver ion) GO:0010043 (response to zinc ion)
SeeY.Y122a.E1	snp	596378	596378	C	T	<i>catF</i>	NON_SYNONYMOUS_CODING	P/L	periplasmic copper- and silver-binding protein	GO:0005978 (copper ion homeostasis) GO:0010273 (detoxification of copper ion) GO:0046688 (response to copper ion) GO:0010272 (response to silver ion) GO:0010043 (response to zinc ion)
SeeY.Y122a.E1	indel	732441	732441	-	G	<i>rhcC</i>	FRAMESHIFT_CODING	-	rhcC element core protein RhcC	Not found
SeeY.Y122a.E1	snp	945441	945441	G	A	<i>rarA</i>	NON_SYNONYMOUS_CODING	V/I	DNA-dependent ATPase involved in processing recombination intermediates at replication forks	GO:0006310 (DNA recombination) GO:0006261 (DNA-dependent DNA replication)
SeeY.Y122a.E1	snp	961133	961133	G	A	<i>foxA</i>	SYNONYMOUS_CODING	L	formate channel	GO:0015724 (formate transport) GO:0006810 (transport)
SeeY.Y122a.E1	snp	2139525	2139525	G	A	<i>wcc</i>	NON_SYNONYMOUS_CODING	P/S	protein-tyrosine kinase	GO:0005222 (capsule polysaccharide biosynthetic process) GO:0009242 (sulfonic acid biosynthetic process) GO:0009103 (lipopolysaccharide biosynthetic process) GO:0038083 (peptidyl-tyrosine autophosphorylation) GO:0018108 (peptidyl-tyrosine phosphorylation) GO:0016310 (phosphorylation) GO:0000271 (polysaccharide biosynthetic process) GO:0050790 (regulation of catalytic activity) GO:0005334 (amino acid transmembrane transport) GO:0006865 (amino acid transport) GO:0006835 (dicarboxylic acid transport) GO:0032329 (serine transport) GO:0015826 (threonine transport) GO:0006810 (transport)
SeeY.Y122a.E1	indel	3241660	3241660	G	-	<i>satT</i>	FRAMESHIFT_CODING	-	pseudogene	GO:0008299 (isoprenoid biosynthetic process) GO:0045828 (positive regulation of isoprenoid metabolic process)
SeeY.Y122a.E1	snp	3350931	3350931	C	T	<i>edbB</i>	NON_SYNONYMOUS_CODING	R/H	isoprenoid biosynthesis protein with amidotransferase-like domain	GO:0008299 (isoprenoid biosynthetic process) GO:0045828 (positive regulation of isoprenoid metabolic process)

SecY122a.E1	snp	3442201	3442201	T	C	<i>rpsD</i>	NON_SYNONYMOUS_CODING	Q/R	30S ribosomal subunit protein S4	GO:0006353 (DNA-dependent transcription, termination) GO:004947 (negative regulation of translational initiation) GO:0006355 (regulation of transcription, DNA-dependent) GO:0006117 (regulation of translation) GO:0046677 (response to antibiotic) GO:001564 (transcription anti-termination) GO:0006351 (transcription, DNA-templated) GO:0006112 (translation)
SecY122a.E2	snp	270639	270639	T	C	<i>insN, insI</i>	NON_SYNONYMOUS_CODING, SYNONYMOUS_CODING	V/H, N	pseudogene (InsN), IS30 transposase (InsI)	insN: GO:0006310 (DNA recombination) GO:0006313 (transposition, DNA-mediated) GO:0032196 (transposition) insI: GO:0006316 (transposition, DNA-mediated) GO:0006310 (DNA recombination) GO:0015074 (DNA integration) GO:0032196 (transposition)
SecY122a.E2	snp	425116	425116	C	T	<i>queA</i>	NON_SYNONYMOUS_CODING	R/C	S-adenosylmethionine: tRNA ribosyltransferase-isomerase	GO:0008616 (quasione biosynthetic process) GO:0002999 (RNA wobble guanine modification)
SecY122a.E2	snp	647801	647801	G	A	<i>citF</i>	SYNONYMOUS_CODING	S	citrate lyase, citrate-ACP transferase (a) subunit	GO:0006884 (acetyl-CoA metabolic process)
SecY122a.E2	snp	945441	945441	G	A	<i>rarA</i>	NON_SYNONYMOUS_CODING	V/I	DNA-dependent ATPase involved in processing recombination intermediates at replication forks	GO:0006310 (DNA recombination) GO:0006261 (DNA-dependent DNA replication)
SecY122a.E2	snp	961133	961133	G	A	<i>focA</i>	SYNONYMOUS_CODING	L	formate channel	GO:0015724 (formate transport) GO:0006810 (transport)
SecY122a.E2	snp	2139525	2139525	G	A	<i>wzc</i>	NON_SYNONYMOUS_CODING	P/S	protein-tyrosine kinase	GO:0045227 (capsaicin polysaccharide biosynthetic process) GO:0009242 (colanic acid biosynthetic process) GO:0009103 (heparan sulfate biosynthetic process) GO:0038083 (peptidyl-tyrosine autophosphorylation) GO:0018108 (peptidyl-tyrosine phosphorylation) GO:0016310 (phosphorylation) GO:0002711 (polysaccharide biosynthetic process) GO:0007900 (regulation of catalytic activity)
SecY122a.E2	indel	2466909	2466909	-	C	<i>yfiP</i>	UPSTREAM (+131)	-	predicted protein	Not found
SecY122a.E2	indel	3241660	3241660	G	-	<i>sstI</i>	FRAMESHIFT_CODING	-	pseudogene	GO:0003333 (amino acid transmembrane transport) GO:0006865 (amino acid transport) GO:0006835 (dicarboxylic acid transport) GO:0013229 (serine transport) GO:0015826 (threonine transport)
SecY122a.E2	snp	3444418	3444418	A	G	<i>secY</i>	NON_SYNONYMOUS_CODING	o/Q	preprotein translocase membrane subunit	GO:0006810 (transport) GO:0005002 (intracellular protein transmembrane transport) GO:0006886 (intracellular protein transport) GO:0006605 (protein targeting) GO:0015031 (protein transport) GO:0043952 (protein transport by the Sec complex) GO:0006810 (transport)
SecY122a.E3	snp	158987	158987	C	T	<i>penB</i>	UPSTREAM (+30)	-	poly(A) polymerase	GO:0006378 (mRNA polyadenylation) GO:0006397 (mRNA processing) GO:0006276 (plasmid maintenance) GO:0009451 (RNA modification) GO:0006396 (RNA processing) GO:0006351 (transcription, DNA-templated)
SecY122a.E3	snp	270639	270639	T	C	<i>insN, insI</i>	NON_SYNONYMOUS_CODING, SYNONYMOUS_CODING	V/H, N	pseudogene (InsN), IS30 transposase (InsI)	insN: GO:0006310 (DNA recombination) GO:0006313 (transposition, DNA-mediated) GO:0032196 (transposition) insI: GO:0006316 (transposition, DNA-mediated) GO:0006310 (DNA recombination) GO:0015074 (DNA integration) GO:0032196 (transposition)
SecY122a.E3	snp	695629	695629	C	T	<i>glnI</i>	NON_SYNONYMOUS_CODING	n/a	tRNA-Gln	Not found
SecY122a.E3	snp	945441	945441	G	A	<i>rarA</i>	NON_SYNONYMOUS_CODING	V/I	DNA-dependent ATPase involved in processing recombination intermediates at replication forks	GO:0006310 (DNA recombination) GO:0006261 (DNA-dependent DNA replication)
SecY122a.E3	snp	961133	961133	G	A	<i>focA</i>	SYNONYMOUS_CODING	L	formate channel	GO:0015724 (formate transport) GO:0006810 (transport)
SecY122a.E3	snp	1082918	1082918	C	T	<i>putA</i>	SYNONYMOUS_CODING	P	fixed PutA DNA-binding transcriptional repressor/proline dehydrogenase-1-pyruvate-5-carboxylate dehydrogenase	GO:0006537 (glutamate biosynthetic process) GO:0008152 (metabolic process) GO:0055114 (oxidation-reduction process) GO:0006561 (proline biosynthetic process) GO:0006562 (proline catabolic process) GO:0010133 (proline catabolic process to glutamate) GO:0006560 (proline metabolic process) GO:0006355 (regulation of transcription, DNA-dependent) GO:0006351 (transcription, DNA-templated)
SecY122a.E3	snp	1656103	1656103	G	A	<i>yjJ, insD</i>	SYNONYMOUS_CODING, SYNONYMOUS_CODING	F, P	predicted transporter, IS2 element protein	yjJ: GO:0006813 (potassium ion transport) GO:0006810 (transport) insD: GO:0055085 (transmembrane transport) GO:0006310 (DNA recombination) GO:0015074 (DNA integration) GO:0032196 (transposition)
SecY122a.E3	snp	2139525	2139525	G	A	<i>wzc</i>	NON_SYNONYMOUS_CODING	P/S	protein-tyrosine kinase	GO:0045227 (capsaicin polysaccharide biosynthetic process) GO:0009242 (colanic acid biosynthetic process) GO:0009103 (heparan sulfate biosynthetic process) GO:0038083 (peptidyl-tyrosine autophosphorylation) GO:0018108 (peptidyl-tyrosine phosphorylation) GO:0016310 (phosphorylation) GO:0002711 (polysaccharide biosynthetic process) GO:0007900 (regulation of catalytic activity)
SecY122a.E3	snp	2886400	2886400	C	T	<i>casA</i>	NON_SYNONYMOUS_CODING	G/R	CRISPR RNA containing Cascade antiviral complex protein	GO:0007900 (regulation of catalytic activity)
SecY122a.E3	snp	3181496	3181496	A	G	<i>ygcC</i>	NON_SYNONYMOUS_CODING	Q/R	glutathionylspermidine synthase-like protein	GO:0051607 (defense response to virus)
SecY122a.E3	indel	3241660	3241660	G	-	<i>sstI</i>	FRAMESHIFT_CODING	-	pseudogene	GO:0003333 (amino acid transmembrane transport) GO:0006865 (amino acid transport) GO:0006835 (dicarboxylic acid transport) GO:0013229 (serine transport) GO:0015826 (threonine transport)
SecY122a.E3	snp	3331601	3331601	T	C	<i>obgE</i>	SYNONYMOUS_CODING	L	GTPase involved in cell partitioning and DNA repair	GO:0007059 (chromosome segregation) GO:0006154 (GTP catabolic process) GO:0042255 (ribosome assembly)

Supplementary Table 9: Escape mechanisms of higher-order, MutS-proficient strains with multiple essential TAG codons.

Escape mutant	Essential genes	Causative SNP ^a	Causative SNP location ^b	Suppressor type	Relative fitness in media without sAA ^c
rEc.β.dB.9'.E1	<i>dnaX, lspA</i>	Gta→Tta	<i>tyrV'</i> → <i>supC</i>	ochre (TAA)	0.83 ± 0.006
rEc.β.dB.9'.E2	<i>dnaX, lspA</i>	Gta→Tta	<i>tyrV'</i> → <i>supC</i>	ochre (TAA)	0.83 ± 0.009
rEc.β.dB.9'.E3	<i>dnaX, lspA</i>	Gta→Cta	<i>tyrT'</i> → <i>supF</i>	amber (TAG)	0.88 ± 0.010
rEc.β.dC.12'.E5	<i>dnaX, lspA, secY</i>	Gta→Cta	<i>tyrU'</i> → <i>supZ</i>	amber (TAG)	0.85 ± 0.019
rEc.β.dC.12'.E7	<i>dnaX, lspA, secY</i>	Gta→Tta	<i>tyrT'</i> → <i>supC</i>	ochre (TAA)	0.78 ± 0.012
rEc.β.dC.12'.E8	<i>dnaX, lspA, secY</i>	Gta→Tta	<i>tyrT'</i> → <i>supC</i>	ochre (TAA)	0.80 ± 0.012
rEc.γ.dB.41'.E1	<i>dnaA, murG</i>	Gta→Cta	<i>tyrT'</i> → <i>supF</i>	amber (TAG)	0.48 ± 0.008
rEc.γ.dB.41'.E2	<i>dnaA, murG</i>	Gta→Cta	<i>tyrV'</i> → <i>supF</i>	amber (TAG)	0.48 ± 0.063
rEc.γ.dB.41'.E3	<i>dnaA, murG</i>	Gta→Cta	<i>tyrU'</i> → <i>supZ</i>	amber (TAG)	0.50 ± 0.072
rEc.γ.dB.46'.E4	<i>dnaA, murG, serS</i>	Gta→Cta	<i>tyrV'</i> → <i>supF</i>	amber (TAG)	0.56 ± 0.093
rEc.γ.dB.46'.E5	<i>dnaA, murG, serS</i>	Gta→Cta	<i>tyrV'</i> → <i>supF</i>	amber (TAG)	0.62 ± 0.040
rEc.γ.dB.46'.E6	<i>dnaA, murG, serS</i>	Gta→Cta	<i>tyrV'</i> → <i>supF</i>	amber (TAG)	0.57 ± 0.036

^aCausative SNP is capitalized within the codon or anticodon sequence, from 5' to 3', for protein and tRNA-encoded genes, respectively.

^btRNA suppressor name conferred by the anticodon mutation is shown.

^cAverage ratio of doubling times are shown for contained ancestor grown to escape mutant grown in permissive and nonpermissive media, respectively; n=3 technical replicates; error is ±s.d.

Supplementary Table 10: Whole genome sequencing of higher-order MutS-proficient strains with multiple essential TAG codons.

Strain	Feature	Start	End	WT	Mut	Gene	Consequence	AA change	Product	GO Annotation (Biological process)
Ec:ΔC:9:E1	snp	74910	74910	C	T	<i>nibB</i>	SYNONYMOUS_CODING	L	thiamin ABC transporter and periplasmic binding protein	GO:000200 (ATP catabolic process) GO:000810 (transport)
Ec:ΔC:9:E1	snp	129310	129310	C	A	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	GO:001588 (thiamine transport) GO:005585 (transmembrane transport)
Ec:ΔC:9:E1	indel	286207	286207	ACTGACGA	CCTTTATTCTCG	<i>ppbB</i>	UPSTREAM (+61)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:9:E2	snp	223618	223618	G	G	<i>osfI</i>	NON_SYNONYMOUS_CODING	na	16S ribosomal RNA	GO:0006412 (translation)
Ec:ΔC:9:E2	indel	890767	890767	CGAA	CAGCATCACC	<i>mdf1</i>	UPSTREAM (-12)	-	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:9:E2	snp	890782	890782	C	T	<i>mdf1</i>	STOP_GAINED	Q*	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:9:E2	snp	129310	129310	C	A	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:9:E2	indel	243482	243482	GCATCAATAAAAATGGCCGTA	TCCTGAGTAGGACAAATCCCGCCCTAG	<i>cypI</i>	UPSTREAM (-90)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:9:E2	indel	286157	286157	GGGTG	GCAGTA	<i>mus5</i>	FRAMESHIFT_CODING	-	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:9:E2	indel	286163	286163	TG	CT	<i>mus5</i>	NON_SYNONYMOUS_CODING, SYNONYMOUS_CODING	V,D,Y	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:9:E2	snp	286204	286204	A	C	<i>ppbB</i>	UPSTREAM (-54)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:9:E3	snp	374183	374183	G	C	<i>nbpT</i>	UPSTREAM (-327)	-	3-hydroxyphenyl(propionate)-3-hydroxycinnamate-H ⁺ symporter	GO:001371 (3-hydroxyphenyl propionate transport) GO:004206 (3-hydroxyphenyl propionate acid transport) GO:000810 (transport)
Ec:ΔC:9:E3	snp	890782	890782	C	T	<i>mdf1</i>	STOP_GAINED	Q*	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:9:E3	snp	129304	129304	C	G	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:9:E3	indel	243490	243490	TGAAA	CGCCCTAG	<i>cypI</i>	UPSTREAM (-109)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:9:E3	snp	286154	286154	G	A	<i>mus5</i>	NON_SYNONYMOUS_CODING	M,I	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:9:E3	indel	417344	417344	GAACCGTAGGG	CAICATGAGT	<i>trpB</i>	NON_SYNONYMOUS_CODING	na	23S ribosomal RNA	GO:0006412 (translation)
Ec:ΔC:9:E3	indel	417360	417360	UCFACAAAGCGGAA	ACGGTCACAGCTTG	<i>trpB</i>	NON_SYNONYMOUS_CODING	na	23S ribosomal RNA	GO:0006412 (translation)
Ec:ΔC:12:E5	snp	890780	890780	T	G	<i>mdf1</i>	START_REMOVED	MR	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:12:E5	snp	890782	890782	C	T	<i>mdf1</i>	STOP_GAINED	Q*	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:12:E5	snp	243496	243496	A	G	<i>cypI</i>	UPSTREAM (-114)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:12:E5	indel	243498	243498	ATA	ACTT	<i>cypI</i>	UPSTREAM (-116)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:12:E5	snp	417325	417325	G	C	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:12:E5	snp	129304	129304	C	A	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:12:E5	snp	129304	129304	C	A	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:12:E5	snp	239091	239091	G	A	<i>yjgM</i>	UPSTREAM (-72)	-	predicted protein	Not found
Ec:ΔC:12:E5	snp	243496	243496	A	G	<i>cypI</i>	UPSTREAM (-114)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:12:E5	indel	243498	243498	ATA	ACTT	<i>cypI</i>	UPSTREAM (-116)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:12:E5	indel	286157	286157	GGGTG	GCAGTA	<i>mus5</i>	FRAMESHIFT_CODING	-	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:12:E5	indel	286207	286207	ACTGACGA	CCTTTATTCTCG	<i>ppbB</i>	UPSTREAM (-61)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:12:E5	indel	286207	286207	ACTGACGA	CCTTTATTCTCG	<i>ppbB</i>	UPSTREAM (-61)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:12:E5	snp	404245	404245	G	A	<i>trpI</i>	UPSTREAM (-11)	-	5S ribosomal RNA	Not found
Ec:ΔC:41:E1	indel	890767	890767	CGAA	CAGCATCACC	<i>mdf1</i>	UPSTREAM (-12)	-	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:41:E1	snp	129304	129304	C	G	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found
Ec:ΔC:41:E1	indel	243489	243489	GCTGAAA	CTCTAG	<i>cypI</i>	UPSTREAM (-107)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:41:E1	snp	271460	271460	A	C	<i>yjgM</i>	UPSTREAM (-273)	-	CP4-57 phosphatase, predicted GTP-binding protein	Not found
Ec:ΔC:41:E1	indel	286157	286157	GGGTGTT	GCAGTAICTCA	<i>mus5</i>	FRAMESHIFT_CODING	-	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:41:E1	indel	286207	286207	ACTGACGA	TTTTATTCTCG	<i>ppbB</i>	UPSTREAM (-61)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:41:E1	snp	404245	404245	G	A	<i>trpI</i>	UPSTREAM (-11)	-	5S ribosomal RNA	Not found
Ec:ΔC:41:E2	indel	890767	890767	CGAA	CAGCATCACC	<i>mdf1</i>	UPSTREAM (-12)	-	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:41:E2	snp	129310	129310	C	G	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	GO:0006412 (translation)
Ec:ΔC:41:E2	snp	239091	239091	C	T	<i>yjgM</i>	UPSTREAM (-51)	-	predicted protein	Not found
Ec:ΔC:41:E2	indel	271460	271460	A	AC	<i>yjgM</i>	UPSTREAM (-273)	-	CP4-57 phosphatase, predicted GTP-binding protein	Not found
Ec:ΔC:41:E2	indel	286157	286157	G	GC	<i>mus5</i>	FRAMESHIFT_CODING	-	DNA mismatch repair protein	GO:000018 (regulation of DNA recombination) GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:41:E2	snp	286158	286158	G	A	<i>mus5</i>	FRAMESHIFT_CODING	GS	DNA mismatch repair protein	GO:000200 (ATP catabolic process) GO:0006298 (mismatch repair) GO:0006974 (cellular response to DNA damage stimulus)
Ec:ΔC:41:E2	indel	286207	286207	ACTGACGA	TTTTATTCTCG	<i>ppbB</i>	UPSTREAM (-61)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process) GO:001611 (dephosphorylation)
Ec:ΔC:41:E2	snp	404245	404245	G	A	<i>trpI</i>	UPSTREAM (-11)	-	5S ribosomal RNA	Not found
Ec:ΔC:41:E3	snp	890780	890780	T	G	<i>mdf1</i>	START_REMOVED	MR	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:41:E3	snp	890782	890782	C	T	<i>mdf1</i>	STOP_GAINED	Q*	multidrug efflux system protein	GO:000855 (drug transmembrane transport) GO:003641 (regulation of cellular pH) GO:0046677 (response to antibiotic) GO:005585 (transmembrane transport) GO:000810 (transport)
Ec:ΔC:41:E3	snp	121489	121489	C	G	<i>trpI</i>	NON_SYNONYMOUS_CODING	L,V	uncharacterized protein from lambdaoid prophage	Not found
Ec:ΔC:41:E3	indel	243498	243498	ATA	ACTT	<i>cypI</i>	UPSTREAM (-116)	-	membrane protein required for colicin V production	GO:000403 (toxin biosynthetic process)
Ec:ΔC:41:E3	indel	271460	271460	A	AC	<i>yjgM</i>	UPSTREAM (-273)	-	CP4-57 phosphatase, predicted GTP-binding protein	Not found
Ec:ΔC:41:E3	indel	286204	286204	GACGA	YCTGC	<i>ppbB</i>	UPSTREAM (-58)	-	protein-tyrosine-phosphatase phosphoprotein phosphatase	GO:000812 (metabolic process)
Ec:ΔC:41:E3	snp	417325	417325	G	C	<i>trpI</i>	NON_SYNONYMOUS_CODING	na	tRNA-try	Not found

Supplementary Table 11: Frequencies of escape for sAA-dependent strains over time.

Genotype	EF Day 1	EF Day 2	EF Day 3	EF Day 4	EF Day 5	EF Day 7
DnaA.W6 γ	7.81E-08	9.79E-06	1.19E-05	1.42E-05	1.42E-05	1.46E-05
rEc. β .dC.12	3.31E-09	9.93E-09	1.48E-07	2.49E-07	4.51E-07	1.44E-06
rEc. β .dC.12'	3.79E-09	3.41E-08	1.52E-07	1.67E-07	2.02E-07	5.49E-07
rEc. β .dC.12'. Δ tY	< 4.85E-12	< 4.85E-12	< 4.85E-12	< 4.85E-12	< 4.85E-12	< 4.85E-12
rEc. γ .dB.41	2.17E-09	3.62E-09	5.07E-09	5.07E-09	5.80E-09	6.52E-09
rEc. γ .dB.41'	5.98E-10	1.56E-07	1.82E-07	2.08E-07	2.08E-07	2.08E-07
rEc. γ .dC.46	< 4.03E-11	5.05E-10	3.53E-09	4.50E-09	4.75E-09	5.13E-09
rEc. γ .dC.46'	< 4.36E-11	5.80E-09	9.02E-09	1.05E-08	1.12E-08	1.21E-08
rEc. γ .dC.46'. Δ tY	< 6.30E-12	< 6.30E-12	< 6.30E-12	< 6.30E-12	< 6.30E-12	< 6.30E-12

Representative EFs are shown (see methods).