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Supplementary Table 1 | DNA oligos used in construction of synthetic auxotrophs

DNA name	Sequence	Purpose
adk.Oligo1	a*t*gaactgagcctgagtccctttccccgcgcccggagcgccNACcagNGCgatacgcattgcgaaaatccccttaaaatgtgtcgaattt	MAGE oligo for adk CoS-MAGE
adk.Oligo2-1	t*c*agttcgtccggtacgtcgaattccagNRCgtaatcNRCattgatgcccgcttctttCATcgcgtctgcctgcggaatggtacgc	MAGE oligo for adk CoS-MAGE
adk.Oligo2-2	t*c*agttcgtccggtacgtcgaattccagNRCgtaatcNRCattgatgcccgcttctttNAGcgcgtctgcctgcggaatggtacgc	MAGE oligo for adk CoS-MAGE
adk.Oligo3-1	c*g*tcaactttcgcgtatttNRTattacccgcttctgcttctttggaNRCgtagccgatCTAcggtgcNGTcatctgatggtattcaacca	MAGE oligo for adk CoS-MAGE
adk.Oligo3-2	c*g*tcaactttcgcgtatttYRTattacccgcttctgcttctttggaNRCgtagccgatCTAcggtgcRAAcatctgatggtattcaacca	MAGE oligo for adk CoS-MAGE
alaS_TTC268GCN	g*t*ttgaaatagtcgccgaagctgaagttgcccagcatttcgaaNGCggtatggtgacgcgcggtgtaaccgacgttttccaggtcgttgt	MAGE oligo for alaS CoS-MAGE
alaS_TTC877GCN	c*c*acggttttcattggacggcattacgccatccgcgatcagNGCcgcacaagaacgaatgtggtcagcgattacgcgcagcgatttat	MAGE oligo for alaS CoS-MAGE
alaS_TAG_only	g*c*ctgctggcgtttcaggtcttcacccgcagagcccataacgtcgatCTAcggaccaaccagtttgtagaagaaggttt	MAGE oligo for alaS CoS-MAGE
alaS_TAG_M_div	g*c*ctgctggcgtttNRGgtcttcacccgcagagcccataacgtcgatCTAcggaccaaccagtttgtagaagaaggttt	MAGE oligo for alaS CoS-MAGE
alaS_TAG_A_div	g*c*ctgctggcgtttNRGgtcttcacccgcagagccNGCaacgtcgatCTAcggaccaaccagtttgtagaagaaggttt	MAGE oligo for alaS CoS-MAGE
alaS_TAG_M_div_redo	g*c*ctgctggcgtttNRCgtcttcacccgcagagcccataacgtcgatCTAcggaccaaccagtttgtagaagaaggttt	MAGE oligo for alaS CoS-MAGE
alaS_TAG_A_div_redo	g*c*ctgctggcgtttNRCgtcttcacccgcagagccNGCaacgtcgatCTAcggaccaaccagtttgtagaagaaggttt	MAGE oligo for alaS CoS-MAGE
alaS_TAGstart1	a*a*gtcgagaaacgcctgacggatctcagcggtgctcttgctCTAcataattatcctgaaatcaagctaacgaaatatcgccacca	MAGE oligo for alaS CoS-MAGE
alaS_TAGstart2	a*a*gtcgagaaacgcctgacggatctcagcggtgctcttgctCTACTAcataattatcctgaaatcaagctaacgaaatatcgccacca	MAGE oligo for alaS CoS-MAGE
holB.oligo1	g*g*cgaaccggcgcttaagcgcaaCTAggcaagtaatgcatcctgtgacattgtcacttcgcgtgaaagccaggtcacggcgtactgttcc	MAGE oligo for holB CoS-MAGE
holB.oligo2-1	g*c*acgctatatgccaacgcctgacacaatgtTTCacgagcctgNSCgttatctccctgNRCcaacgccagtgccgcgcc	MAGE oligo for holB CoS-MAGE
holB.oligo2-2	g*c*acgctatatgccaacgcctgacacaatgtNAGacgagcctgNSCgttatctccctgNRCcaacgccagtgccgcgcc	MAGE oligo for holB CoS-MAGE
metG.shell1	c*a*tcaacctggcncgcgtgctgatgacttacctgaagccggtactgccgaaactgaccgagcgtgcagaagcattctagaatacggaact	MAGE oligo for metG CoS-MAGE
metG.shell1+2	c*a*tcaacctggcncgcgtgctggcnacttacctgaagccggtactgccgaaactgaccgagcgtgcagaagcagbntagaatacggaact	MAGE oligo for metG CoS-MAGE
pgk.oligo1-1+2	c*c*agaacggtcagtttggtagatactttagaaccaccaacNRCagcNRCcatcgggcgagcaggttctttcagtgctttacccagcgcgt	MAGE oligo for pgk CoS-MAGE
pgk.oligo2-1+2	t*g*gccttgtgccgcgataaaggtgttagcgataccaccaccaacNSCcagctggtcagcgattttagacagggagtccagaacggtcagt	MAGE oligo for pgk CoS-MAGE
pgk.oligo1-3	c*c*agaacggtcagtttggtagatactttagaaccaccaacNRCagcCACcatcgggcgagcaggttctttcagtgctttacccagcgcgt	MAGE oligo for pgk CoS-MAGE
pgk.oligo3-1	t*c*gggaattcgaacacgccNRCcggaccgttccacagAATggttttcgcattcttCTAgatttcagcCAGttcctgagcggaagcatcac	MAGE oligo for pgk CoS-MAGE
pgk.oligo3-2	t*c*gggaattcgaacacgccNRCcggaccgttccacagNRCggttttcgcattcttCTAgatttcagcNGCttcctgagcggaagcatcac	MAGE oligo for pgk CoS-MAGE
pgk.oligo3-3	t*c*gggaattcgaacacgccNRCcggaccgttccacagAATggttttcgcattcttCTAgatttcagcNGCttcctgagcggaagcatcac	MAGE oligo for pgk CoS-MAGE
pgk.oligo4-1	a*t*tgctgccagagtgtcgccgccgccagcgatggagaacgcttcgctgtctgcGATagcgttagcNGCgatttcagtacctttgcggaag	MAGE oligo for pgk CoS-MAGE
pgk.oligo4-2	a*t*tgctgccagagtgtcgccgccgccagcgatggagaacgcttcgctgtctgcNRCagcgttagcCACgatttcagtacctttgcggaag	MAGE oligo for pgk CoS-MAGE
313000.T.lacZ.coMAGE-f	tgcttctcatgaacgataacacaacttgttcatgaattaaccattccggattgaggcacattaacgcc	tolC cassette primer for adk CoS-MAGE
313001.T.lacZ.coMAGE-r	acggaaaccagccagttcctttcgatgcctgaatttgatcccatagtttatctagggcggcggatt	tolC cassette primer for adk CoS-MAGE
2837380.T.24.25b-f	tttgccgattgtccttgcacaatcggcgggaaaaatattcaggtgaccggttgaggcacattaacgcc	tolC cassette primer for alaS CoS-MAGE
2837381.T.24.25b-r	gtcactaaataagcaagagagtgaattgttcattttttatatttgtgaaatctagggcggcggatt	tolC cassette primer for alaS CoS-MAGE
1124600.T.11C-f	tcccctcccgggaaataaaagatgagcactttctattctgcattgccgctttgaggcacattaacgcc	tolC cassette primer for holB CoS-MAGE
1124601.T.11C-r	gcgacgttttgataaaaaaatattgtccccgaagtgatgggaaaagtctttctagggcggcggatt	tolC cassette primer for holB CoS-MAGE
T.20.21yehQ-f	ctttgcgaacaattattccccatgcttcagcaacaaggatgaaatagtgcttgaggcacattaacgcc	tolC cassette primer for metG CoS-MAGE
T.20.21yehQ-r	aataccgtggcgtgagcagtccggaactggagtagaactcttagtaaaaatctagggcggcggatt	tolC cassette primer for metG CoS-MAGE
3176034.T.27.28h-f	actgccgtttgagcagtcatgtgttaaattgaggcacattaacgccctattgaggcacattaacgcc	tolC cassette primer for pgk CoS-MAGE
3176035.T.27.28h-r	ttattgcggattctagcagaagccgctaccgcaaaaggttggcgttacgtgccatctagggcggcggatt	tolC cassette primer for pgk CoS-MAGE
312869.seq-f	gaacttgcactacccatcg	tolC screening primer for adk CoS-MAGE
313126.seq-r	agtgacgggttaattatctgaaag	tolC screening primer for adk CoS-MAGE
2837231.Seq-f	gaaacggtcgcttttgaaac	tolC screening primer for alaS CoS-MAGE
2837530.Seq-r	tatcagtagggtagatgtagttgttatc	tolC screening primer for alaS CoS-MAGE
1124450.Seq-f	agatcagcgggaagacaac	tolC screening primer for holB CoS-MAGE
1124750.Seq-r	acgctgtaagtattactatacctgc	tolC screening primer for holB CoS-MAGE
2208971.Seq-f	tggcgaatgacctcacc	tolC screening primer for metG CoS-MAGE
2209291.Seq-r	aacagatgcaacaacgagg	tolC screening primer for metG CoS-MAGE
3175926.seq-f	gggcaggttgtctggct	tolC screening primer for pgk CoS-MAGE
3176137.seq-r	aagaatggggagcaatttcttcatt	tolC screening primer for pgk CoS-MAGE
tyrS-1-f	aaaagtcgtgtaccggcaaag	Primer for constructing fragment 1 of tyrS dependence variant pool
tyrS-1-r1	ggcccgcctgctggaagcgtttcaggcataacaatggaacnawatgccccasatgcaagctgtcagcg	Primer for constructing fragment 1 of tyrS dependence variant pool
tyrS-1-r2	ggcccgcctgctggaagcgtttcaggcataacaatggaacnawatgcccngcatgcaagctgtcagcg	Primer for constructing fragment 1 of tyrS dependence variant pool
tyrS-2-f	gttccattgttatgcctgaaac	Primer for constructing fragment 2 of tyrS dependence variant pool
tyrS-2-r1	tttgtacgggctggttttcttcggatccaaccagactgcgccgccttcagttttacccattttggtgccatctgc	Primer for constructing fragment 2 of tyrS dependence variant pool
tyrS-2-r2	tttgtacgggctggttttcttcggatccaaccagactgcgccgccttcagttttaccngctttggtgccatctgc	Primer for constructing fragment 2 of tyrS dependence variant pool
tyrS-2-r3	tttgtacgggctggttttcttc	Primer for constructing fragment 2 of tyrS dependence variant pool
tyrS-3-fA1	gaagaaaaccagcccgtacaaattctaccagttcgcnatcaacgcngcggatgccgacgtttaccgc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-fA2	gaagaaaaccagcccgtacaaattctaccagttcgsnatcaacactgcggatgccgacgtttaccgc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-fA3	gaagaaaaccagcccgtacaaattctaccagttcttyatcaacactgcggatgccgacgtttaccgc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-fA4	gaagaaaaccagcccgtacaaattctaccagttcttyatcaacgtngcggatgccgacgtttaccgc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-rA1	cttcttcttccagggcgttgatctcttcaatgctcataaaggtnccgaacttcaggaagcggtaaacgtcggcatc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-rA2	cttcttcttccagggcgttgatctcttcaatgctcataaaggtnccgaacttcagnaggcggtaaacgtcggcatc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-rA3	cttcttcttccagggcgttgatctcttcaatgctcataaaggtnccgaacttcagccagcggtaaacgtcggcatc	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-fB	cgcagtctggttggatccgaagaaaaccagcccgtac	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-3-rB	actgggcgcgcggtgctttaccgctgtttttatcttcttcttccagggcgttg	Primer for constructing fragment 3 of tyrS dependence variant pool
tyrS-4-f1	gaagaagataaaaacagcggtaaagcaccgcgcgcccagtatgtataggcggagcagatgactcgtctggttcacggtg	Primer for constructing fragment 4 of tyrS dependence variant pool
tyrS-4-f2	gaagaagataaaaacagcggtaaagcaccgcgcgcccagtatgtataggcggagcaggsnactcgtctggttcacggtg	Primer for constructing fragment 4 of tyrS dependence variant pool
tyrS-4-f3	gaagaagataaaaacagcggtaaagcaccgcgcgcccagtatgtataggcggagcagstnactcgtctggttcacggtg	Primer for constructing fragment 4 of tyrS dependence variant pool
tyrS-4-r	tgcattatttccagcaaatcagacag	Primer for constructing fragment 4 of tyrS dependence variant pool
tyrS_des7_ala_variant-Nr	cgtgaaccagacgagttgcctgctccgcctatacatactg	Primer for introducing V307A mutation to convert tyrS.d7 into tyrS.d8
tyrS_des7_ala_variant-Cf	cagtatgtataggcggagcaggcaactcgtctggttcacg	Primer for introducing V307A mutation to convert tyrS.d7 into tyrS.d8
adk.TAG.wt-f_correct1	ccatcagatgacngcaccgct	ascPCR primer to screen for UAG codon introduction into adk variants
adk.TAG.wt-f_correct2	ccatcagatgttygcaccgct	ascPCR primer to screen for UAG codon introduction into adk variants
adk.TAG.mut-f_correct1	ccatcagatgacngcaccgta	ascPCR primer to screen for UAG codon introduction into adk variants
adk.TAG.mut-f_correct2	ccatcagatgttygcaccgta	ascPCR primer to screen for UAG codon introduction into adk variants
alaS.TAG.wt-f	tacaaactggttggtccgct	ascPCR primer to screen for UAG codon introduction into alaS variants
alaS.TAG.mut-f	tctacaaactggttggtccgta	ascPCR primer to screen for UAG codon introduction into alaS variants
alaS_TAGstart_500_mut-f	gttagcttgatttcaggataattatgagc	ascPCR primer to screen for startUAG codon introduction into alaS variants
alaS_TAGstart_500_mut-f	cgttagcttgatttcaggataattatgtag	ascPCR primer to screen for startUAG codon introduction into alaS variants
alaS_TAGstart_500-r	gccgatgcgaataatacgttcg	ascPCR primer to screen for startUAG codon introduction into alaS variants
tyrS.TAG.wt-f	gcgcgcccagtatgtact	ascPCR primer to screen for UAG codon introduction into tyrS variants
tyrS.TAG.mut-f	cgcgcgcccagtatgtata	ascPCR primer to screen for UAG codon introduction into tyrS variants
tyrS-des7-V307A-V-f	gtatgtataggcggagcaggt	ascPCR primer to screen for V307A mutation to convert tyrS.d7 into tyrS.d8
tyrS-des7-V307A-A-f	tatgtataggcggagcaggc	ascPCR primer to screen for V307A mutation to convert tyrS.d7 into tyrS.d8
adk.DEP.seq-f	cttttcatcatctgcactttcc	Sequencing primer to confirm desired mutations in redesigned adk
adk.DEP.seq-r	ggaatagcgcagattgagg	Sequencing primer to confirm desired mutations in redesigned adk
alaS.DEP.seq-f	taccacttcccaacgctg	Sequencing primer to confirm desired mutations in redesigned alaS
alaS.DEP.seq-r	cgctccagagtacgagc	Sequencing primer to confirm desired mutations in redesigned alaS
holB_DEP.seq-f	gagcctgaacgtttactgg	Sequencing primer to confirm desired mutations in redesigned holB
holB_DEP.seq-r	atcaacattggtcacctgc	Sequencing primer to confirm desired mutations in redesigned holB
metG_DEP.seq-f	cgctatgtcgatgaacagg	Sequencing primer to confirm desired mutations in redesigned metG
metG_DEP.seq-r	gtgcttcaacctgcctc	Sequencing primer to confirm desired mutations in redesigned metG
pgk_DEP.seq-f	ctcacggtatcggtaaattcg	Sequencing primer to confirm desired mutations in redesigned pgk
pgk_DEP.seq-r	gccagtggagatgtaggag	Sequencing primer to confirm desired mutations in redesigned pgk
tyrS.DEP.seq-f	gaggaagcgttagcagag	Sequencing primer to confirm desired mutations in redesigned tyrS
tyrS.DEP.seq-r	tcaaagaaccgctgaacagg	Sequencing primer to confirm desired mutations in redesigned tyrS
tyrS_full_seq-f	gccttgaacagattgagttct	Sequencing primer flanking the complete tyrS ORF
tyrS_full_seq-r	cgtgagactggatagcgag	Sequencing primer flanking the complete tyrS ORF
tdk.90.del*	c*t*gccgagaagggtatatagcccggaagaagtgcgtaaaacgaaccagtatgcacagaagtgggtatatgcgttctcccttacgaagcct	MAGE oligo for deleting the endogenous copy of tdk
tdk.seq-f	cgcttataacaagcgaggc	tdk locus screening primer
tdk.seq-r	acgtcggtgcttaagagc	tdk locus screening primer
tyrS(tdk)-Nf	gcccgatcgcgctctattg	Primer for constructing N-terminal tyrS portion of the recoded tyrS+tdk cassette
tyrS(tdk)-Nr	ctggagtttatcataattcgtcagtttttcagctca	Primer for constructing N-terminal tyrS portion of the recoded tyrS+tdk cassette
(tyrS)tdk-Cf	ctgttgagctgaaaaactgac	Primer for constructing C-terminal tdk portion of the recoded tyrS+tdk cassette
(tyrS)tdk-Cr	tataaaaaccagggtgattgatttctcactcccccctttccacttaatgcactgccgagaagggtatatagc	Primer for constructing C-terminal tdk portion of the recoded tyrS+tdk cassette
adk(tdk)-Nf	atcgctttttcaaaaaattcgacacattttaaggg	Primer for constructing N-terminal adk portion of the recoded adk+tdk cassette
adk(tdk)-Nr	ctggagtttatcataattcgtcagtttttcagctca	Primer for constructing N-terminal adk portion of the recoded adk+tdk cassette
(adk)tdk-Cf	ctgttgagctgaaaaactgac	Primer for constructing C-terminal tdk portion of the recoded adk+tdk cassette
(adk)tdk-Cr	ctaaccctctccccgagggtgcgaggggactgtccgtgcgcgctttcgaactgccgagaagggtatatagc	Primer for constructing C-terminal tdk portion of the recoded adk+tdk cassette
adk_wt.seq-f	gggaaagggactcaggc	Primer to screen for the presence of wt adk sequence
adk_wt.seq-r309	ttcgtccggtacgtcga	Primer to screen for the presence of wt adk sequence
adk_recode.seq-f	ggtaagggcacgcaagc	Primer to screen for the presence of recoded adk sequence
adk_recode.seq-r309	ctcatctggaacatcaaactcaag	Primer to screen for the presence of recoded adk sequence

tyrS_wt.seq-f	cattgttatgcctgaaacgc	Primer to screen for the presence of wt tyrS sequence
tyrS_wt.seq-r500	cacctgattctgatgcagac	Primer to screen for the presence of wt tyrS sequence
tyrS_recode.seq-f	cgcttttgtgtctcaagagg	Primer to screen for the presence of recoded tyrS sequence
tyrS_recode.seq-r501	agacttggttttggtggag	Primer to screen for the presence of recoded tyrS sequence
Dasd::specR_v2_f	tggtgaaagatgtgccaagaggagaccggcacatttatacagcacacatccagccaggacagaaatgc	specR primers for Dasd::specR cassette to delete asd
Dasd::specR_v2_r	acatctgcgcttactcctgtattacgcactaacaggggcggcatcgcgcctgcagaaataaaaaggcctgc	specR primers for Dasd::specR cassette to delete asd
Dasd::kanR_v2_f	tggtgaaagatgtgccaagaggagaccggcacatttatacagcacacatccctgtgacggaagatcacttcg	kanR primers for Dasd::kanR cassette to delete asd
Dasd::kanR_v2_r	acatctgcgcttactcctgtattacgcactaacaggggcggcatcgcgccaaccagcaatagacataagcgg	kanR primers for Dasd::kanR cassette to delete asd
DthyA::cmR_f	tggtctgggcatatcgtcgcagcccacagcaacacgtttcctgaggaaccccctgtgacggaagatcacttcg	cmR primers for DthyA::cmR cassette to delete thyA
DthyA::cmR_r	gtaaaaaaaccgacgcacactggcgtcggctctggcaggatgtttcgtaaaaccagcaatagacataagcgg	cmR primers for DthyA::cmR cassette to delete thyA
asd_ver2-f	gaatatgggaccagagttcctgt	Primer to screen for the presence of wt asd sequence
Dasd::kanR_ver2-f	gataccaggatcttgccatcctatg	Primer to screen for the presence of Dasd::kanR sequence
asd_ver2-r	tgctctatttaactcccggtaaatca	Primer to screen for the presence of wt or Dasd::kanR sequence
thyA_ver2-f	caaacgtaaacccgaatccatctt	Primer to screen for the presence of wt thyA sequence
DthyA::cmR_ver2-f	tgggcaaatattatacgcaaggc	Primer to screen for the presence of DthyA::kanR sequence
thyA_ver2-f	gcaggacgttgcaaaatttcg	Primer to screen for the presence of wt or DthyA::kanR sequence
adk-A4+A1-TAG178NNN	c*t*tctttggaAACgtagccgatNNNcggtgcWGTcatctgatggtattcaaccagacgtttacgtacggtctcttcctgatcatctttac	MAGE oligo to convert adk TAG178NNN
alaS-A5+D8-TAG338NNN	g*c*ctgctggcgtttKGGgtcttcacccgcagagccWGCaacgtcgatNNNcggaccaaccagtttgtagaagaaggttt	MAGE oligo to convert alaS TAG338NNN
metG-A2-TAG507NNN	c*t*gaagccggtactgccgaaactgaccgagcgtgcagaagcaGGANNNaatacggaactgacctgggatggtatccagcaaccgct	MAGE oligo to convert metG TAG507NNN
pgk_1-1-1_TAG298NNN	a*a*cacgccaaccggaccgttccacagaatggttttcgcattcttNNNgatttcagccagttcctgagcggaagcatcaccgatatccagg	MAGE oligo to convert pgk TAG298NNN
tyrS-A1-TAG303NNN	t*g*taaaccttcttcaccgtgaaccagacgagtTGCctgctccgcNNNtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d6 and tyrS.d8 TAG303NNN
tyrS-des7-TAG303NNN	t*g*taaaccttcttcaccgtgaaccagacgagtTACctgctccgcNNNtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d7 TAG303NNN
adk_P5_alt_prep_4N-f	caggagttgtctaggcgatcnnnncgtctggttgaataccatcagatg	Primer to add P5_alt Illumina adapter for adk TAG303NNN sequencing with 4N spacer
adk_P5_alt_prep_5N-f	caggagttgtctaggcgatcnnnnncgtctggttgaataccatcagatg	Primer to add P5_alt Illumina adapter for adk TAG303NNN sequencing with 5N spacer
adk_P5_alt_prep_6N-f	caggagttgtctaggcgatcnnnnnncgtctggttgaataccatcagatg	Primer to add P5_alt Illumina adapter for adk TAG303NNN sequencing with 6N spacer
adk_P7_prep_4N-r	gacgtgtgctcttccgatctnnnngatcagcgcgaacttcagc	Primer to add P7 Illumina adapter for adk TAG303NNN sequencing with 4N spacer
adk_P7_prep_5N-r	gacgtgtgctcttccgatctnnnnngatcagcgcgaacttcagc	Primer to add P7 Illumina adapter for adk TAG303NNN sequencing with 5N spacer
adk_P7_prep_6N-r	gacgtgtgctcttccgatctnnnnnngatcagcgcgaacttcagc	Primer to add P7 Illumina adapter for adk TAG303NNN sequencing with 6N spacer
alaS_P5_alt_prep_4N-f	caggagttgtctaggcgatcnnnncttctacaaactggttggtcc	Primer to add P5_alt Illumina adapter for alaS TAG303NNN sequencing with 4N spacer
alaS_P5_alt_prep_5N-f	caggagttgtctaggcgatcnnnnncttctacaaactggttggtcc	Primer to add P5_alt Illumina adapter for alaS TAG303NNN sequencing with 5N spacer
alaS_P5_alt_prep_6N-f	caggagttgtctaggcgatcnnnnnncttctacaaactggttggtcc	Primer to add P5_alt Illumina adapter for alaS TAG303NNN sequencing with 6N spacer
alaS_P7_prep-4N-r	gacgtgtgctcttccgatctnnnncgctccagagtacgagc	Primer to add P7 Illumina adapter for alaS TAG303NNN sequencing with 4N spacer
alaS_P7_prep-5N-r	gacgtgtgctcttccgatctnnnnncgctccagagtacgagc	Primer to add P7 Illumina adapter for alaS TAG303NNN sequencing with 5N spacer
alaS_P7_prep-6N-r	gacgtgtgctcttccgatctnnnnnncgctccagagtacgagc	Primer to add P7 Illumina adapter for alaS TAG303NNN sequencing with 6N spacer
metG_P5_alt_prep_4N-f	caggagttgtctaggcgatcnnnngaccgagcgtgcagaagc	Primer to add P5_alt Illumina adapter for metG TAG303NNN sequencing with 4N spacer
metG_P5_alt_prep_5N-f	caggagttgtctaggcgatcnnnnngaccgagcgtgcagaagc	Primer to add P5_alt Illumina adapter for metG TAG303NNN sequencing with 5N spacer
metG_P5_alt_prep_6N-f	caggagttgtctaggcgatcnnnnnngaccgagcgtgcagaagc	Primer to add P5_alt Illumina adapter for metG TAG303NNN sequencing with 6N spacer
metG_P7_prep_4N-r	gacgtgtgctcttccgatctnnnncagtgcttcaacctgcctc	Primer to add P7 Illumina adapter for metG TAG303NNN sequencing with 4N spacer
metG_P7_prep_5N-r	gacgtgtgctcttccgatctnnnnncagtgcttcaacctgcctc	Primer to add P7 Illumina adapter for metG TAG303NNN sequencing with 5N spacer
metG_P7_prep_6N-r	gacgtgtgctcttccgatctnnnnnncagtgcttcaacctgcctc	Primer to add P7 Illumina adapter for metG TAG303NNN sequencing with 6N spacer
pgk_P5_alt_prep_4N-f	caggagttgtctaggcgatcnnnngtgatgcttccgctcagg	Primer to add P5_alt Illumina adapter for pgk TAG303NNN sequencing with 4N spacer
pgk_P5_alt_prep_5N-f	caggagttgtctaggcgatcnnnnngtgatgcttccgctcagg	Primer to add P5_alt Illumina adapter for pgk TAG303NNN sequencing with 5N spacer
pgk_P5_alt_prep_6N-f	caggagttgtctaggcgatcnnnnnngtgatgcttccgctcagg	Primer to add P5_alt Illumina adapter for pgk TAG303NNN sequencing with 6N spacer
pgk_P7_prep_4N-r	gacgtgtgctcttccgatctnnnngaacgcttcgctgtctgc	Primer to add P7 Illumina adapter for pgk TAG303NNN sequencing with 4N spacer
pgk_P7_prep_5N-r	gacgtgtgctcttccgatctnnnnngaacgcttcgctgtctgc	Primer to add P7 Illumina adapter for pgk TAG303NNN sequencing with 5N spacer
pgk_P7_prep_6N-r	gacgtgtgctcttccgatctnnnnnngaacgcttcgctgtctgc	Primer to add P7 Illumina adapter for pgk TAG303NNN sequencing with 6N spacer
tyrS_P5_alt_prep_4N-f	caggagttgtctaggcgatcnnnngcaccgcgcgcccagtatgt	Primer to add P5_alt Illumina adapter for tyrS TAG303NNN sequencing with 4N spacer
tyrS_P5_alt_prep_5N-f	caggagttgtctaggcgatcnnnnngcaccgcgcgcccagtatgt	Primer to add P5_alt Illumina adapter for tyrS TAG303NNN sequencing with 5N spacer
tyrS_P5_alt_prep_6N-f	caggagttgtctaggcgatcnnnnnngcaccgcgcgcccagtatgt	Primer to add P5_alt Illumina adapter for tyrS TAG303NNN sequencing with 6N spacer
tyrS_P7_prep_4N-r	gacgtgtgctcttccgatctnnnncttcactcagcgcactcaaag	Primer to add P7 Illumina adapter for tyrS TAG303NNN sequencing with 4N spacer
tyrS_P7_prep_5N-r	gacgtgtgctcttccgatctnnnnncttcactcagcgcactcaaag	Primer to add P7 Illumina adapter for tyrS TAG303NNN sequencing with 5N spacer
tyrS_P7_prep_6N-r	gacgtgtgctcttccgatctnnnnnncttcactcagcgcactcaaag	Primer to add P7 Illumina adapter for tyrS TAG303NNN sequencing with 6N spacer
Sol-P5_alt-PCR	aatgatacggcgaccaccgagatctacactctttccctcaggagttgtctaggcgatc	Primer to amplify NNN Illumina library and custom sequencing primer for P5 read
p7_A01	caagcagaagacggcatacgagatcaggttgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 1)
p7_A02	caagcagaagacggcatacgagattcacaagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 2)
p7_A03	caagcagaagacggcatacgagatacatcagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 3)
p7_A04	caagcagaagacggcatacgagatagcgcagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 4)
p7_A05	caagcagaagacggcatacgagatcatcaagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 5)
p7_A06	caagcagaagacggcatacgagatgctattgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 6)
p7_A07	caagcagaagacggcatacgagattagatcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 7)
p7_A08	caagcagaagacggcatacgagatcatgacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 8)
p7_A09	caagcagaagacggcatacgagatgaatcggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 9)
p7_A10	caagcagaagacggcatacgagattcttctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 10)
p7_A11	caagcagaagacggcatacgagatattccggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 11)
p7_A12	caagcagaagacggcatacgagatggaattgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 12)
p7_B01	caagcagaagacggcatacgagatacggtggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 13)
p7_B02	caagcagaagacggcatacgagatctcagcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 14)
p7_B03	caagcagaagacggcatacgagattccggtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 15)
p7_B04	caagcagaagacggcatacgagattgcagtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 16)
p7_B05	caagcagaagacggcatacgagatttcatagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 17)
p7_B06	caagcagaagacggcatacgagatatacacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 18)
p7_B07	caagcagaagacggcatacgagatcgttatgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 19)
p7_B08	caagcagaagacggcatacgagatctcggagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 20)
p7_B09	caagcagaagacggcatacgagattgtgtggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 21)
p7_B10	caagcagaagacggcatacgagataccgcggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 22)
p7_B11	caagcagaagacggcatacgagatgatcgggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 23)
p7_B12	caagcagaagacggcatacgagattcacgggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 24)
p7_C01	caagcagaagacggcatacgagatattactgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 25)
p7_C02	caagcagaagacggcatacgagatcttagagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 26)
p7_C03	caagcagaagacggcatacgagatgcagctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 27)
p7_C04	caagcagaagacggcatacgagattcctccgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 28)
p7_C05	caagcagaagacggcatacgagatgaactagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 29)
p7_C06	caagcagaagacggcatacgagatacaaccgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 30)
p7_C07	caagcagaagacggcatacgagatggtaacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 31)
p7_C08	caagcagaagacggcatacgagatgtggtcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 32)
p7_C09	caagcagaagacggcatacgagatccgcgtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 33)
p7_C10	caagcagaagacggcatacgagatctgacagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 34)
p7_C11	caagcagaagacggcatacgagatccgaatgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 35)
p7_C12	caagcagaagacggcatacgagatagccgcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 36)
p7_D01	caagcagaagacggcatacgagattagcgcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 37)
p7_D02	caagcagaagacggcatacgagattgacctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 38)
p7_D03	caagcagaagacggcatacgagatcttatcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 39)
p7_D04	caagcagaagacggcatacgagatgtagccgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 40)
p7_D05	caagcagaagacggcatacgagatccataggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 41)
p7_D06	caagcagaagacggcatacgagatgaggcagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 42)
p7_D07	caagcagaagacggcatacgagataattgagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 43)
p7_D08	caagcagaagacggcatacgagatactcacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 44)
p7_D09	caagcagaagacggcatacgagataagttggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 45)
p7_D10	caagcagaagacggcatacgagattacgatgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 46)
p7_D11	caagcagaagacggcatacgagatcaccacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 47)
p7_D12	caagcagaagacggcatacgagatgcattcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 48)
p7_E01	caagcagaagacggcatacgagatttccatgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 49)
p7_E02	caagcagaagacggcatacgagataggcgagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 50)
p7_E03	caagcagaagacggcatacgagatctctcggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 51)
p7_E04	caagcagaagacggcatacgagatgccttggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 52)
p7_E05	caagcagaagacggcatacgagataggttcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 53)
p7_E06	caagcagaagacggcatacgagattagcaggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 54)
p7_E07	caagcagaagacggcatacgagatcactgcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 55)
p7_E08	caagcagaagacggcatacgagatagtggcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 56)
p7_E09	caagcagaagacggcatacgagatatgtaggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 57)
p7_E10	caagcagaagacggcatacgagatggtacggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 58)
p7_E11	caagcagaagacggcatacgagattaaggtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 59)
p7_E12	caagcagaagacggcatacgagatcctgtagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 60)
p7_F01	caagcagaagacggcatacgagatcctccagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 61)
p7_F02	caagcagaagacggcatacgagatacaatagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 62)

p7_F03	caagcagaagacggcatacgagatactgatgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 63)
p7_F04	caagcagaagacggcatacgagatatgtgtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 64)
p7_F05	caagcagaagacggcatacgagatgtgtccgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 65)
p7_F06	caagcagaagacggcatacgagattagagtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 66)
p7_F07	caagcagaagacggcatacgagatgctaaggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 67)
p7_F08	caagcagaagacggcatacgagattggactgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 68)
p7_F09	caagcagaagacggcatacgagatcacttagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 69)
p7_F10	caagcagaagacggcatacgagatctattggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 70)
p7_F11	caagcagaagacggcatacgagataatacagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 71)
p7_F12	caagcagaagacggcatacgagatattcttgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 72)
p7_G01	caagcagaagacggcatacgagatggcatcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 73)
p7_G02	caagcagaagacggcatacgagatggttgggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 74)
p7_G03	caagcagaagacggcatacgagatttggcggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 75)
p7_G04	caagcagaagacggcatacgagatctgctggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 76)
p7_G05	caagcagaagacggcatacgagattgagtagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 77)
p7_G06	caagcagaagacggcatacgagatctaagtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 78)
p7_G07	caagcagaagacggcatacgagatagaagggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 79)
p7_G08	caagcagaagacggcatacgagattcggacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 80)
p7_G09	caagcagaagacggcatacgagattgttcagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 81)
p7_G10	caagcagaagacggcatacgagataaccttgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 82)
p7_G11	caagcagaagacggcatacgagatggtcgtgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 83)
p7_G12	caagcagaagacggcatacgagattatcctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 84)
p7_H01	caagcagaagacggcatacgagatggcgacgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 85)
p7_H02	caagcagaagacggcatacgagatgtgcctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 86)
p7_H03	caagcagaagacggcatacgagatgctgccgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 87)
p7_H04	caagcagaagacggcatacgagatccgacggtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 88)
p7_H05	caagcagaagacggcatacgagataccacagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 89)
p7_H06	caagcagaagacggcatacgagatctatgagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 90)
p7_H07	caagcagaagacggcatacgagattactctgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 91)
p7_H08	caagcagaagacggcatacgagatgacagagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 92)
p7_H09	caagcagaagacggcatacgagatgcgctagtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 93)
p7_H10	caagcagaagacggcatacgagatgaagtcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 94)
p7_H11	caagcagaagacggcatacgagatttgattgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 95)
p7_H12	caagcagaagacggcatacgagatcgactcgtgactggagttcagacgtgtgctcttccgatct	Primer to amplify NNN Illumina library and sequencing primer for P7 read (index 96)
ME-Plus9-kanoriT-f	/5Phos/ctgtctcttatacacatctcaaccatcacctgtgacggaagatcacttcg	Mosaic end primers for kanoriT amplification (EZ-Tn5 custom transposome prep)
ME-Plus9-kanoriT-r	/5Phos/ctgtctcttatacacatctcaaccctgaagcgcttttccgctgca	Mosaic end primers for kanoriT amplification (EZ-Tn5 custom transposome prep)
kanR.seqOUT-Nr1	catcgggcttcccatac	Nested PCR primer for enrichment of flanking genomic DNA around Tn5-kanR-oriT integration sites
kanR.seqOUT-Nr2	gaatttaatcgcggcctc	Nested PCR primer for enrichment of flanking genomic DNA around Tn5-kanR-oriT integration sites
kanR.seqOUT-Nr3	tttcccgttgaatatggctc	Nested PCR primer for enrichment of flanking genomic DNA around Tn5-kanR-oriT integration sites; sequencing primer for identifying flanking genomic DNA around Tn5-kanR-oriT integration sites
murF-TAG-f	ctttacaggagaatgggacatgttag	mascPCR primer to screen for conjugation-mediated UAG reversion
yaiS-TAG-f	cttgctgcattaaaaaaacataaaagctag	mascPCR primer to screen for conjugation-mediated UAG reversion
lon-TAG-f	tatgcaggttgtgactgcaaaatag	mascPCR primer to screen for conjugation-mediated UAG reversion
adk.TAG.wt-f_correct1	ccatcagatgacngcaccgct	mascPCR primer to screen for conjugation-mediated adk escape
ybhS-TAG-f	ccaaacgtcggctggattag	mascPCR primer to screen for conjugation-mediated UAG reversion
lolA-TAG-f	tcacggtagatgatcaacgtaagtag	mascPCR primer to screen for conjugation-mediated UAG reversion
ycaI-TAG-f	tgccagtggataacgggtag	mascPCR primer to screen for conjugation-mediated UAG reversion
fabH-TAG-f	cgcgctggttcgtttctag	mascPCR primer to screen for conjugation-mediated UAG reversion
hemA-TAG-f	gcctcgggctggagtag	mascPCR primer to screen for conjugation-mediated UAG reversion
global.prfA-f	gcgcagttaagtgatgtttcg	mascPCR primer to screen for conjugation-mediated reversion of wt prfA
pspF-TAG-f	cgttgttgaaaaagcaccagatttag	mascPCR primer to screen for conjugation-mediated UAG reversion
tyrS.TAG.wt-f	gcgcgcccagtatgtact	mascPCR primer to screen for conjugation-mediated tyrS escape
ydiE-TAG-f	cgcgctggttcgtttctag	mascPCR primer to screen for conjugation-mediated UAG reversion
rcsC-TAG-f	ggaaatcgcgggattcgtag	mascPCR primer to screen for conjugation-mediated UAG reversion
hda-TAG-f	cgttgttgaaaaagcaccagatttag	mascPCR primer to screen for conjugation-mediated UAG reversion
srlE-TAG-f	ggtttgtctccggttttatctatcaatag	mascPCR primer to screen for conjugation-mediated UAG reversion
nlpI-TAG-f	gcagaatcggaccagcaatag	mascPCR primer to screen for conjugation-mediated UAG reversion
mreC-TAG-f	cgccgggagggcaatag	mascPCR primer to screen for conjugation-mediated UAG reversion
xylR-TAG-f	gatgtaaatagcgaggtcatgttgtag	mascPCR primer to screen for conjugation-mediated UAG reversion
coaD-TAG-f	ctgatggcgaagttagcgtag	mascPCR primer to screen for conjugation-mediated UAG reversion
yidS-TAG-f	gcacatattccacagttgaaggattag	mascPCR primer to screen for conjugation-mediated UAG reversion
rbsB-TAG-f	atctgaaactggttgttaagcagtag	mascPCR primer to screen for conjugation-mediated UAG reversion
b4283-TAG-f	tggagaatgaaatattaaaaaggctactgtag	mascPCR primer to screen for conjugation-mediated UAG reversion
murF-TAA-f	ctttacaggagaatgggacatgttaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
yaiS-TAA-f	cttgctgcattaaaaaaacataaaagctaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
lon-TAA-f	tatgcaggttgtgactgcaaaataa	mascPCR primer to screen for conservation of UAA alleles during conjugation
adk.TAG.mut-f_correct1	ccatcagatgacngcaccgta	mascPCR primer to screen for conservation of adk dependence during conjugation
ybhS-TAA-f	ccaaacgtcggctggattaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
lolA-TAA-f	tcacggtagatgatcaacgtaagtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
ycaI-TAA-f	tgccagtggataacgggtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
fabH-TAA-f	cgcgctggttcgtttctaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
hemA-TAA-f	gcctcgggctggagtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
pspF-TAA-f	cgttgttgaaaaagcaccagatttaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
tyrS.TAG.mut-f	cgcgcgcccagtatgtata	mascPCR primer to screen for conservation of tyrS dependence during conjugation
ydiE-TAA-f	cgcgctggttcgtttctaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
rcsC-TAA-f	ggaaatcgcgggattcgtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
hda-TAA-f	cgttgttgaaaaagcaccagatttaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
srlE-TAA-f	ggtttgtctccggttttatctatcaataa	mascPCR primer to screen for conservation of UAA alleles during conjugation
nlpI-TAA-f	gcagaatcggaccagcaataa	mascPCR primer to screen for conservation of UAA alleles during conjugation
mreC-TAA-f	cgccgggagggcaataa	mascPCR primer to screen for conservation of UAA alleles during conjugation
xylR-TAA-f	gatgtaaatagcgaggtcatgttgtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
coaD-TAA-f	ctgatggcgaagttagcgtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
yidS-TAA-f	gcacatattccacagttgaaggattaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
rbsB-TAA-f	atctgaaactggttgttaagcagtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
b4283-TAA-f	tggagaatgaaatattaaaaaggctactgtaa	mascPCR primer to screen for conservation of UAA alleles during conjugation
murF-r	gacgggtaagcccacagc	reverse mascPCR primer for conjugation assay
yaiS-r	agtagagggaatgccgcc	reverse mascPCR primer for conjugation assay
lon-r	ccttacaacaccccttaataaggg	reverse mascPCR primer for conjugation assay
adk.TAG-r	ggaatagcgcagattgagg	reverse mascPCR primer for conjugation assay
ybhS-r	ctgttgttaggtttcggttttcct	reverse mascPCR primer for conjugation assay
lolA-r	ctgaccaccatagccacgg	reverse mascPCR primer for conjugation assay
ycaI-r	acgcacaacagtaatcagtgc	reverse mascPCR primer for conjugation assay
fabH-r	gttaaaggtgattttcgctaattctactg	reverse mascPCR primer for conjugation assay
hemA-r	atgttgtgaacgttcgtcctg	reverse mascPCR primer for conjugation assay
global.prfA-r	aggcagttctggcattacc	reverse mascPCR primer for conjugation assay
pspF-r	aaagaaagcgttccggcc	reverse mascPCR primer for conjugation assay
tyrS.TAG-r	tcaaagaaccgctgaacagg	reverse mascPCR primer for conjugation assay
ydiE-r	catttgatgactggtttgccc	reverse mascPCR primer for conjugation assay
rcsC-r	agtgaccttaagtccggcag	reverse mascPCR primer for conjugation assay
hda-r	cgaatactatggattaacttcgcgtag	reverse mascPCR primer for conjugation assay
srlE-r	ggatggagtgcacctttcaac	reverse mascPCR primer for conjugation assay
nlpI-r	tgaaattgatcagcaattttcattgaaaag	reverse mascPCR primer for conjugation assay
mreC-r	gacgttaatcactaaaaactctgccc	reverse mascPCR primer for conjugation assay
xylR-r	ggataaggcgttcacgcc	reverse mascPCR primer for conjugation assay
coaD-r	gatggtaaaccgggctatttcg	reverse mascPCR primer for conjugation assay
yidS-r	ttacctggtcgctggtttct	reverse mascPCR primer for conjugation assay
rbsB-r	cacgctagcccatacacca	reverse mascPCR primer for conjugation assay
b4283-r	cgaatatcacctgagacaatttataaaacg	reverse mascPCR primer for conjugation assay
mutS_null_mut-2*	a*c*cccatgagtgcaatagaaaatttcgacgcccatacgcccatgatgcagcagtgatagtcgctgaaagcccagcatcccgagatcctgc	MAGE oligo to inactivate mutS
mutS_null_revert-2*	a*c*cccatgagtgcaatagaaaatttcgacgcccatacgcccatgatgcagcagtatctcaggctgaaagcccagcatcccgagatcctgc	MAGE oligo to reactivate mutS
mutS-2_ascPCR_wt-f	ccatgatgcagcagtatctcag	Wild-type forward ascPCR primer for mutS reversion
mutS-2_ascPCR_mut-f	ccatgatgcagcagtgatagtc	Mutant forward ascPCR primer for mutS inactivation
mutS-2_ascPCR-r	aggttgtcctgacgctcctg	reverse ascPCR primer for mutS toggling
pEVOL-UCUA1-f	agggcagaacggcggacttctaaatccgcatggcaggggt	primers for isothermal construction of quaruplet codon variant of pEVOL-bipA
PEVOL-UCUA1-r	gagtatgaaaagtatggctgaagcgcaaaatgatcccctg	primers for isothermal construction of quaruplet codon variant of pEVOL-bipA

pEVOL-UCUA2-f	caggggatcattttgcgcttcagccatacttttcatactc	primers for isothermal construction of quaruplet codon variant of pEVOL-bipA
pEVOL-UCUA2-r	acccctgccatgcggatttagaagtccgccgttctgccct	primers for isothermal construction of quaruplet codon variant of pEVOL-bipA
adk(tdk)-Nf	atcgctttttcaaaaaattcgacacattttaaggg	primers for adk UAGA cassette construction
adk_uaga_variant-Nr	tttggaaacgtagccgattctacggtgctgtcatctgatgg	primers for adk UAGA cassette construction
adk_uaga_variant-Cf	ccatcagatgacagcaccgtagaatcggctacgtttcca	primers for adk UAGA cassette construction
adk.DEP.seq-r	ggaatagcgcagattgagg	primers for adk UAGA cassette construction
tyrS.d8-A70V-Nr	gacccgtcgcgccgcctaccaggacaaccggcttgtggcc	primers for isothermal construction of tyrS.d8-A70V variant to confirm escape mechanism
tyrS.d8-A70V-Cf	ggccacaagccggttgtcctggtaggcggcgcgacgggtc	primers for isothermal construction of tyrS.d8-A70V variant to confirm escape mechanism
Dlon:kanR-f	cagtcgtgtcatctgattacctggcggaaattaaactaagagagagctctcctgtgacggaagatcactt	primers for deletion of Lon protease (lambda red recombination)
Dlon:kanR-r	cccgaattagcctgccagccctgtttttattagtgcattttgcgcgaggtcaaaccagcaatagacataagcg	primers for deletion of Lon protease (lambda red recombination)
lon.seq-f	ctctattctcggcgttgaatg	primers to validate Dlon::kanR recombinants
lon.seq-r	cacttgaatccttcaaggtacg	primers to validate Dlon::kanR recombinants
adk.d6-TAG178TGG	c*t*tctttggaAACgtagccgatCCAcggtgcTGTcatctgatggtattcaaccagacgtttacgtacggtctcttcctgatcatctttac	MAGE oligo to convert adk.d6 bipA->Trp
adk.d6-TAG178CTG	c*t*tctttggaAACgtagccgatCAGcggtgcTGTcatctgatggtattcaaccagacgtttacgtacggtctcttcctgatcatctttac	MAGE oligo to convert adk.d6 bipA->Leu
tyrS.d7-TAG303TGG	t*g*taaaccttcttcaccgtgaaccagacgagtTACctgctccgcCCAtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d7 bipA->Trp
tyrS.d7-TAG303CTG	t*g*taaaccttcttcaccgtgaaccagacgagtTACctgctccgcCAGtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d7 bipA->Leu
tyrS.d8-TAG303TGG	t*g*taaaccttcttcaccgtgaaccagacgagtTGCctgctccgcCCAtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d8 bipA->Trp
tyrS.d8-TAG303CTG	t*g*taaaccttcttcaccgtgaaccagacgagtTGCctgctccgcCAGtacatactgggcgcgcggtgctttaccgctgtttttatcttct	MAGE oligo to convert tyrS.d8 bipA->Leu
Dtdk:ParaBAD-aaRS-f	aggcttcgtaagggagaacgcatatacccacttctgtgcatactgcaggggatcattttgcg	primers for Dtdk:bipARS genomic integration (lambda red recombination)
Dtdk:bipARS-r	gagaagggtatatagcccggaagaagtgcgtaaaacgaactgacattacagacgtttacggatcgg	primers for Dtdk:bipARS genomic integration (lambda red recombination)
tdk_tRNA+cat-f	ttgcggaagaactgatcaaaatcctggaaccgatccgtaaacgtctgtaagccagtttgctcaggctc	primers for Dtdk:tRNA$_{bipA}$+cat genomic integration (lambda red recombination)
tdk_tRNA+cat-r	gagaagggtatatagcccggaagaagtgcgtaaaacgaactgacactgaacaggagggacagct	primers for Dtdk:tRNA$_{bipA}$+cat genomic integration (lambda red recombination)
C-term2-vector-f	aaaaacgcgtaggcggaagaactgatcaaaatcctggaaccgatccgtaaacgtctgtaactgcagtttcaaacgcta	primers for amplifying pEVOL backbone to prepare pEVOL-bipARS.d7
C-term2-vector-r	cgggttaccttcaacaacac	primers for amplifying pEVOL backbone to prepare pEVOL-bipARS.d7
C-term2-VAG-f	gaaaatcaaaaaagcgtactgcccggcgggtgttgttgaaggtaacccggtngcngaaatcggnaaatacttcctggaataccc	primers used to produce library of bipA-dependent bipARS genes from which we isolated bipARS.d7
C-term2-TAG-f	gaaaatcaaaaaagcgtactgcccggcgggtgttgttgaaggtaacccgacngcngaaatcggnaaatacttcctggaataccc	primers used to produce library of bipA-dependent bipARS genes from which we isolated bipARS.d7
C-term2-AAG-f	gaaaatcaaaaaagcgtactgcccggcgggtgttgttgaaggtaacccggcngcngaaatcggnaaatacttcctggaataccc	primers used to produce library of bipA-dependent bipARS genes from which we isolated bipARS.d7
C-term2-GAB-r	attttgatcagttcttccgcctacgcgtttttngcacgcatcgggtgcagttctttgtttttgaacagagattcnccttcttcgtaagag	primers used to produce library of bipA-dependent bipARS genes from which we isolated bipARS.d7
C-term2-GGB-r	attttgatcagttcttccgcctacgcgtttttnccacgcatcgggtgcagttctttgtttttgaacagagattcnccttcttcgtaagag	primers used to produce library of bipA-dependent bipARS genes from which we isolated bipARS.d7
aaRS1.seq-f	ttttcatactcccgccattc	Sanger sequencing primers to validate endineered bipARS genotypes
aaRS1.seq-r	ggagaccgtttaaactcaatgatg	Sanger sequencing primers to validate endineered bipARS genotypes
aaRS2.seq-f	ggtcatcaatcatccccataatcc	Sanger sequencing primers to validate endineered bipARS genotypes
aaRS2.seq-r	ggtgctacgcctgaataagtg	Sanger sequencing primers to validate endineered bipARS genotypes

Supplementary Table 2 | Enzyme variants from computational design for MAGE oligos and gene synthesis

Enzyme	Template (chain_ID)	Variants				
holB	1A5T_A	A190bipA	F205A,V	W210G,A	E214E,L	
alaS	3HY0_A	F89F,A	F292F,A	L337bipA	M341M,A	L348L,V,A
metG	3H99_A	F481A	M485A	F502A,G,V	L503bipA	
pgk	1ZMR_A	V185V,A	I187V,A	I211A,G	L293A	L297bipA
		I303V,A	V309V,A	V324A	I328V,A	
adk	1E4V_A	I4A	L6V,A	M96M,L	V103V,A	V106V,A
		T175T,F	L178bipA	Y182V,A	T191T,M	
tyrS	2YXN_A	L49L,A,M	L52L,V	F236F,A,M	W260A,F,G	T263T,A,V
		F271F,L,W	F275G	L303bipA	V307A,G,L,M,V	
bipARS	2PXH_A	I240V,T,A	M241A	A244G	L272G	L286A,G
		V290bipA				

Supplementary Table 3 | DNA sequences for construction of synthetic genes and *asd* and *thyA* natural metabolic auxotrophs

>tyrS-1 (fragment 1 of tyrS dependence variant pool with codons targeted for diversification highlighted)
AAAAGTCGTGTACCGGCAAAGGTGCAGTCGTTATATACATGGAGATTTTGatgGCAAGCAGTAACTTG
ATTAAACAATTGCAAGAGCGGGGGCTGGTAGCCCAGGTGACGGACGAGGAAGCGTTAGCAGAGCG
ACTGGCGCAAGGCCCGATCGCGCTCTATTGCGGCTTCGATCCTACCGCTGACAGCTTGCATTTGGG
GCATCTTGTTCCATTGTTATGCCTGAAACGCTTCCAGCAGGCGGGCC

>tyrS-2 (fragment 1 of tyrS dependence variant pool with codons targeted for diversification highlighted)
GTTCCATTGTTATGCCTGAAACGCTTCCAGCAGGCGGGCCACAAGCCGGTTGCGCTGGTAGGCGGC
GCGACGGGTCTGATTGGCGACCCGAGCTTCAAAGCTGCCGAGCGTAAGCTGAACACCGAAGAAACT
GTTCAGGAGTGGGTGGACAAAATCCGTAAGCAGGTTGCCCCGTTCCTCGATTTCGACTGTGGAGAA
AACTCTGCTATCGCGGCGAACAACTATGACTGGTTCGGCAATATGAATGTGCTGACCTTCCTGCGCG
ATATTGGCAAACACTTCTCCGTTAACCAGATGATCAACAAAGAAGCGGTTAAGCAGCGTCTCAACCG
TGAAGATCAGGGGATTTCGTTCACTGAGTTTTCCTACAACCTGTTGCAGGGTTATGACTTCGCCTGTC
TGAACAAACAGTACGGTGTGGTGCTGCAAATTGGTGGTTCTGACCAGTGGGGTAACATCACTTCTGG
TATCGACCTGACCCGTCGTCTGCATCAGAATCAGGTGTTTGGCCTGACCGTTCCGCTGATCACTAAA
GCAGATGGCACCAAATTTGGTAAAACTGAAGGCGGCGCAGTCTGGTTGGATCCGAAGAAAACCAGC
CCGTACAAA

>tyrS-3 (fragment 1 of tyrS dependence variant pool with codons targeted for diversification highlighted)
CGCAGTCTGGTTGGATCCGAAGAAAACCAGCCCGTACAAATTCTACCAGTTCTGGATCAACACTGCG
GATGCCGACGTTTACCGCTTCCTGAAGTTCTTCACCTTTATGAGCATTGAAGAGATCAACGCCCTGG
AAGAAGAAGATAAAAACAGCGGTAAAGCACCGCGCGCCCAGT

>tyrS-4 (fragment 1 of tyrS dependence variant pool with codons targeted for diversification highlighted)
GAAGAAGATAAAAACAGCGGTAAAGCACCGCGCGCCCAGTATGTACTGGCGGAGCAGGTGACTCGT
CTGGTTCACGGTGAAGAAGGTTTACAGGCGGCAAAACGTATTACCGAATGCCTGTTCAGCGGTTCTT
TGAGTGCGCTGAGTGAAGCGGACTTCGAACAGCTGGCGCAGGACGGCGTACCGATGGTTGAGATG
GAAAAGGGCGCAGACCTGATGCAGGCACTGGTCGATTCTGAACTGCAACCTTCCCGTGGTCAGGCA
CGTAAAACTATCGCCTCCAATGCCATCACCATTAACGGTGAAAAACAGTCCGATCCTGAATACTTCTT
TAAAGAAGAAGATCGTCTGTTTGGTCGTTTTACCTTACTGCGTCGCGGTAAAAAGAATTACTGTCTGA
TTTGCTGGAAAtaaTGCA

>tyrS_block1 (gBlock 1 for constructing recoded tyrS gene for tyrS+tdk cassette)
gcccgatcgcgctctattgcggcttcgatcctaccgctgacagcttgcatCTGGGACACCTGGTCCCGCTTTTGTGTCTCAAG
AGGTTTCAACAAGCTGGTCATAAACCAGTCGCTCTAGTCGGTGGAGCTACCGGGCTAATCGGGGAT
CCCTCCTTTAAGGCAGCTGAACGCAAACTTAATACAGAGGAGACGGTGCAAGAATGGGTCGATAAGA
TACGAAAACAAGTAGCGCCATTTTTGGACTTTGATTGCGGCGAGAATTCGGCAATAGCCGCTAATAA
TTACGATTGGTTTGGAAACATGAACGTACTTACTTTTTTGCGGGACATAGGGAAGCATTTTAGCGTGA
ATCAAATGATAAATAAGGAGGCCGTCAAACAAAGACTGAATCGCGAGGACCAAGGTATCAGCTTTAC
AGAATTCTCGTATAATCTTTTACAAGGGTACGATTTTGCATGCCTTAATAAGCAATATGGAGTCGTCCT
ACAGATAGGAGGAAGTGATCAATGGGGAAATATTACGTCGGGAATAGATTTGACACGCCGCCTCCAC
CAAAACCAAGTCTTCGGTCTCACGGTGCCTCTTATTACGAAGGCTGACGGGACGAAGTTCGGCAAG
ACCGAGGGTGGTGCCGTGTGGCTAGACCCAAAAAAGACTTCGCCTTATAAGTTTTATCAATTTTGGA
TTAATACGGCCGACGCTGATGTCTATCGTTTTCTCAAATTTTTTACTTTCATGTCCATCGAGG

>tyrS_block2 (gBlock 2 for constructing recoded tyrS gene for tyrS+tdk cassette)
TATCGTTTTCTCAAATTTTTTACTTTCATGTCCATCGAGGAAATTAATGCGCTCGAGGAGGAGGACAA
GAATTCCGGAAAGGCGCCCCGTGCACAATACGTCCTTGCAGAACAAGTCACAAGGCTAGTGCATGG
CGAGGAGGGCTTGCAAGCAGCGAAGCGGATAACTGAGTGTTTGTTTAGTGGAAGTCTTTCCGCCCT
CTCGGAGGCAGATTTTGAGCAACTTGCACAAGATGGGGTTCCCATGGTGGAAATGGAGAAAGGAGC
CGATCTTATGCAAGCGCTTGTTGACTCGGAGTTGCAGCCATCACGCGGACAAGCGCGAAAGACGAT
TGCTTCGAACGCTATTACAATCAATGGGGAGAAGCAATCGGACCCGGAGTATTTTTTCAAGGAGGAG

GACAGACTCTTCGGCAGGTTCACACTTTTAAGGCGTGGCAAGAAAAACTATTGCCTAATATGTTGGA
AGTAACTGTTGAGCTGAAAAACTGACGAATTATG

>adk_block1 (gBlock for constructing recoded adk gene for adk+tdk cassette)
atcgctttttcaaaaaattcgacacattttaaggggattttcgcaATGCGAATTATATTATTAGGAGCCCCCGGTGCTGGTAA
GGGCACGCAAGCACAATTTATTATGGAAAAGTACGGCATACCACAGATATCAACGGGTGACATGCTT
AGGGCCGCTGTTAAGTCGGGATCTGAATTAGGGAAGCAGGCTAAGGATATAATGGACGCGGGGAAG
TTAGTTACTGATGAGCTAGTTATAGCCTTAGTAAAGGAAAGGATCGCACAAGAGGATTGTCGGAACG
GATTTCTCTTAGATGGATTTCCCCGGACTATACCCCAAGCCGATGCAATGAAGGAGGCCGGTATTAA
CGTGGACTATGTACTTGAGTTTGATGTTCCAGATGAGTTAATAGTAGATAGGATAGTAGGAAGACGT
GTACACGCACCCAGTGGCAGGGTCTACCATGTCAAGTTTAACCCACCCAAGGTTGAGGGTAAGGAT
GATGTCACTGGGGAGGAGCTTACCACACGCAAGGACGACCAAGAGGAAACGGTGCGAAAGAGGCT
TGTAGAGTATCACCAAATGACTGCCCCACTAATAGGGTATTATTCAAAGGAGGCTGAGGCAGGGAAC
ACAAAGTATGCCAAGGTCGATGGGACAAAACCCGTAGCCGAGGTCAGAGCCGACTTGGAGAAGATT
TTGGGTTAACTGTTGAGCTGAAAAACTGACGAATTATGATAAACTCCAG

>adk.d4
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AATATGGTATTCCGCAAATCTCCACTGGCGATATGCTGCGTGCTGCGGTCAAATCTGGCTCCGAGCT
GGGTAAACAAGCAAAAGACATTATGGATGCTGGCAAACTGGTCACCGACGAACTGGTGATCGCGCT
GGTTAAAGAGCGCATTGCTCAGGAAGACTGCCGTAATGGTTTCCTGTTGGACGGCTTCCCGCGTAC
CATTCCGCAGGCAGACGCGATGAAAGAAGCGGGCATCAATGCCGATTACGCTCTGGAATTCGACGT
ACCGGACGAACTGATCGTTGACCGTATCGTCGGTCGCCGCGTTCATGCGCCGTCTGGTCGTGTTTA
TCACGTTAAATTCAATCCGCCGAAAGTAGAAGGCAAAGACGACGTTACCGGTGAAGAACTGACTACC
CGTAAAGATGATCAGGAAGAGACCGTACGTAAACGTCTGGTTGAATACCATCAGATGACTGCACCGT
AGATCGGCTACGTTTCCAAAGAAGCAGAAGCGGGTAATACCAAATACGCGAAAGTTGACGGCACCA
AGCCGGTTGCTGAAGTTCGCGCTGATCTGGAAAAAATCCTCGGCtaa

>adk.d6
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TGGGTAAACAAGCAAAAGACATTATGGATGCTGGCAAACTGGTCACCGACGAACTGGTGATCGCGC
TGGTTAAAGAGCGCATTGCTCAGGAAGACTGCCGTAATGGTTTCCTGTTGGACGGCTTCCCGCGTAC
CATTCCGCAGGCAGACGCGATGAAAGAAGCGGGCATCAATGCTGATTACGTTCTGGAATTCGACGT
ACCGGACGAACTGATCGTTGACCGTATCGTCGGTCGCCGCGTTCATGCGCCGTCTGGTCGTGTTTA
TCACGTTAAATTCAATCCGCCGAAAGTAGAAGGCAAAGACGACGTTACCGGTGAAGAACTGACTACC
CGTAAAGATGATCAGGAAGAGACCGTACGTAAACGTCTGGTTGAATACCATCAGATGACAGCACCGT
AGATCGGCTACGTTTCCAAAGAAGCAGAAGCGGGTAATATTAAATACGCGAAAGTTGACGGCACCAA
GCCGGTTGCTGAAGTTCGCGCTGATCTGGAAAAAATCCTCGGCtaa

>tyrS.d6
atgGCAAGCAGTAACTTGATTAAACAATTGCAAGAGCGGGGGCTGGTAGCCCAGGTGACGGACGAGG
AAGCGTTAGCAGAGCGACTGGCGCAAGGCCCGATCGCGCTCTATTGCGGCTTCGATCCTACCGCTG
ACAGCTTGCATCTGGGGCATTTAGTTCCATTGTTATGCCTGAAACGCTTCCAGCAGGCGGGCCACAA
GCCGGTTGCGCTGGTAGGCGGCGCGACGGGTCTGATTGGCGACCCGAGCTTCAAAGCTGCCGAGC
GTAAGCTGAACACCGAAGAAACTGTTCAGGAGTGGGTGGACAAAATCCGTAAGCAGGTTGCCCCGT
TCCTCGATTTCGACTGTGGAGAAAACTCTGCTATCGCGGCGAACAACTATGACTGGTTCGGCAATAT
GAATGTGCTGACCTTCCTGCGCGATATTGGCAAACACTTCTCCGTTAACCAGATGATCAACAAAGAA
GCGGTTAAGCAGCGTCTCAACCGTGAAGATCAGGGGATTTCGTTCACTGAGTTTTCCTACAACCTGT
TGCAGGGTTATGACTTCGCCTGTCTGAACAAACAGTACGGTGTGGTGCTGCAAATTGGTGGTTCTGA
CCAGTGGGGTAACATCACTTCTGGTATCGACCTGACCCGTCGTCTGCATCAGAATCAGGTGTTTGGC
CTGACCGTTCCGCTGATCACTAAAGCAGATGGCACCAAAGCCGGTAAAACTGAAGGCGGCGCAGTC
TGGTTGGATCCGAAGAAAACCAGCCCGTACAAATTCTACCAGTTCTTCATCAACGTAGCGGATGCCG
ACGTTTACCGCTTCCTGAAGTTCGGTACCTTTATGAGCATTGAAGAGATCAACGCCCTGGAAGAAGA
AGATAAAAACAGCGGTAAAGCACCGCGCGCCCAGTATGTATAGGCGGAGCAGGCCACTCGTCTGGT
TCACGGTGAAGAAGGTTTACAGGCGGCAAAACGTATTACCGAATGCCTGTTCAGCGGTTCTTTGAGT

GCGCTGAGTGAAGCGGACTTCGAACAGCTGGCGCAGGACGGCGTACCGATGGTTGAGATGGAAAA
GGGCGCAGACCTGATGCAGGCACTGGTCGATTCTGAACTGCAACCTTCCCGTGGTCAGGCACGTAA
AACTATCGCCTCCAATGCCATCACCATTAACGGTGAAAAACAGTCCGATCCTGAATACTTCTTTAAAG
AAGAAGATCGTCTGTTTGGTCGTTTTACCTTACTGCGTCGCGGTAAAAAGAATTACTGTCTGATTTGC
TGGAAAtaa

>tyrS.d7
atgGCAAGCAGTAACTTGATTAAACAATTGCAAGAGCGGGGGCTGGTAGCCCAGGTGACGGACGAGG
AAGCGTTAGCAGAGCGACTGGCGCAAGGCCCGATCGCGCTCTATTGCGGCTTCGATCCTACCGCTG
ACAGCTTGCATGCAGGGCATTTGGTTCCATTGTTATGCCTGAAACGCTTCCAGCAGGCGGGCCACAA
GCCGGTTGCGCTGGTAGGCGGCGCGACGGGTCTGATTGGCGACCCGAGCTTCAAAGCTGCCGAGC
GTAAGCTGAACACCGAAGAAACTGTTCAGGAGTGGGTGGACAAAATCCGTAAGCAGGTTGCCCCGT
TCCTCGATTTCGACTGTGGAGAAAACTCTGCTATCGCGGCGAACAACTATGACTGGTTCGGCAATAT
GAATGTGCTGACCTTCCTGCGCGATATTGGCAAACACTTCTCCGTTAACCAGATGATCAACAAAGAA
GCGGTTAAGCAGCGTCTCAACCGTGAAGATCAGGGGATTTCGTTCACTGAGTTTTCCTACAACCTGT
TGCAGGGTTATGACTTCGCCTGTCTGAACAAACAGTACGGTGTGGTGCTGCAAATTGGTGGTTCTGA
CCAGTGGGGTAACATCACTTCTGGTATCGACCTGACCCGTCGTCTGCATCAGAATCAGGTGTTTGGC
CTGACCGTTCCGCTGATCACTAAAGCAGATGGCACCAAAGCCGGTAAAACTGAAGGCGGCGCAGTC
TGGTTGGATCCGAAGAAAACCAGCCCGTACAAATTCTACCAGTTCGCTATCAACGCTGCGGATGCCG
ACGTTTACCGCTGGCTGAAGTTCGGCACCTTTATGAGCATTGAAGAGATCAACGCCCTGGAAGAAGA
AGATAAAAACAGCGGTAAAGCACCGCGCGCCCAGTATGTATAGGCGGAGCAGGTAACTCGTCTGGT
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Supplementary Table 4 | Templates, mutations, escape frequencies in SC, doubling times, and escapee doubling times of synthetic auxotrophs

Strain	Template PDB (id_chain)	Mutations (template numbering)	Day 1 escape rate (escapees/c.f.u.)	Day 2 escape rate (escapees/c.f.u.)	Day 3 escape rate (escapees/c.f.u.)	Day 4 escape rate (escapees/c.f.u.)	Day 5 escape rate (escapees/c.f.u.)	Day 6 escape rate (escapees/c.f.u.)	Day 7 escape rate (escapees/c.f.u.)	Doubling time in 10 uM bipA (min)	Doubling time in 100 uM bipA (min)	Doubling time of fittest escapee in 10 uM bipA (min)	Doubling time of fittest escapee in SC (min)
holB.d1	1A5T_A	A190bipA	not determined	not determined	not determined	not determined	not determined	not determined	not determined	not determined	not determined	not determined	not determined
alaS.d5	3HY0_A	F90A, F293A, L338bipA, M342A, L349P	4.93E-02 ± 3.57E-02	7.75E-02 ± 4.35E-02	1.20E-01 ± 6.17E-02	1.20E-01 ± 6.17E-02	1.20E-01 ± 6.17E-02	1.20E-01 ± 6.17E-02	1.20E-01 ± 6.17E-02	not determined	not determined	not determined	not determined
alaS.d5. startUAG	3HY0_A	startUAG, F90A, F293A, L338bipA, M342A, L349P	1.34E-06 ± 3.46E-07	3.86E-02 ± 1.14E-03	above detection limit	above detection limit	above detection limit	above detection limit	3.63E-01 ± 7.95E-02*	66 ± 0	100 ± 1	66 ± 0	109 ± 0
metG.d3	3H99_A	M485A, F502G, L503bipA	7.29E-07 ± 7.32E-07	2.87E-05 ± 1.23E-05	above detection limit	above detection limit	above detection limit	above detection limit	8.00E-02 ± 1.67E-02*	104 ± 1	no growth	136 ± 16	142 ± 2
pgk.d4	1ZMR_A	V185A, I187A, I211G, L297bipA	1.69E-06 ± 6.12E-07†	2.24E-06 ± 8.06E-07†	8.33E-07 ± 8.34E-07	2.08E-06 ± 4.24E-07	2.92E-06 ± 4.32E-07	2.92E-06 ± 4.32E-07	2.92E-06 ± 4.32E-07	94 ± 1	111 ± 1	135 ± 9	125 ± 6
adk.d4	1E4V_A	V103A, V106A, L178bipA, Y182V	2.38E-07 ± 2.39E-07	1.24E-05 ± 1.06E-05	1.35E-05 ± 1.16E-05	1.49E-05 ± 1.29E-05	1.51E-05 ± 1.30E-05	1.54E-05 ± 1.31E-05	1.57E-05 ± 1.33E-05	75 ± 1	222 ± 11	113 ± 0	153 ± 3
adk.d6	1E4V_A	I4A, L6V, V103A, L178bipA, Y182V, T191I	5.98E-07 ± 1.41E-07	4.35E-06 ± 1.47E-06	6.79E-06 ± 2.61E-06	9.99E-06 ± 3.89E-06	1.07E-05 ± 4.08E-06	1.11E-05 ± 4.20E-06	1.14E-05 ± 4.36E-06	79 ± 1	99 ± 2	77 ± 0	225 ± 3
tyrS.d6	2YXN_A	F236A, W260F, T263V, F275G, L303bipA, V307A	< 2.36E-09	1.27E-07 ± 9.05E-08	2.48E-07 ± 1.46E-07	9.98E-07 ± 3.99E-07	2.54E-06 ± 6.50E-07	4.67E-06 ± 6.39E-07	8.84E-06 ± 1.63E-06	73 ± 0	103 ± 3	not determined	not determined
tyrS.d7	2YXN_A	L49A, F236A, W260A, T263A, F271W, F275G, L303bipA	1.94E-08 ± 1.98E-08	2.31E-06 ± 5.62E-07	> 7.08E-05	> 7.08E-05	> 7.08E-05	> 7.08E-05	2.38E-01 ± 1.28E-01*	72 ± 0	98 ± 2	69 ± 0	300 ± 11
tyrS.d8	2YXN_A	L49A, F236A, W260A, T263A, F271W, F275G, L303bipA, V307A	< 3.47E-09	1.04E-08 ± 7.85E-08	5.90E-08 ± 1.70E-07	1.84E-06 ± 5.22E-07	2.75E-06 ± 6.46E-07	5.47E-06 ± 1.30E-06	7.09E-06 ± 1.52E-06	72 ± 1	91 ± 1	74 ± 0	177 ± 5
adk.d6_tyrS.d6	n/a	adk.d6 + tyrS.d6	< 7.39E-11†	2.04E-07 ± 9.03E-08	5.69E-07 ± 1.99E-07	1.44E-06 ± 4.28E-07	3.36E-06 ± 5.23E-07	7.60E-06 ± 1.15E-06	1.02E-05 ± 1.29E-06	82 ± 0	103 ± 2	83 ± 0	353 ± 2
adk.d6_tyrS.d7	n/a	adk.d6 + tyrS.d7	< 8.30E-11†	3.48E-07 ± 3.58E-07	5.57E-07 ± 4.90E-07	5.01E-06 ± 2.74E-06	5.97E-06 ± 2.89E-06	8.10E-06 ± 3.60E-06	9.14E-06 ± 3.94E-06	83 ± 2	113 ± 3	81 ± 0	229 ± 1
adk.d6_tyrS.d8	n/a	adk.d6 + tyrS.d8	< 7.44E-11†	< 7.44E-11†	< 7.44E-11†	< 7.44E-11†	2.90E-06 ± 2.10E-06	5.00E-06 ± 2.37E-06	6.86E-06 ± 2.30E-06	86 ± 1	120 ± 5	80 ± 0	348 ± 7
adk.d6_tyrS.d6_ asd	n/a	adk.d6 + tyrS.d6 + Δasd	< 6.41E-11	< 6.41E-11	< 6.41E-11	< 6.41E-11	< 6.41E-11	< 6.41E-11	< 6.41E-11	81 ± 0	107 ± 4	none observed	none observed
adk.d6_tyrS.d7_ asd	n/a	adk.d6 + tyrS.d7 + Δasd	< 5.51E-11	< 5.51E-11	< 5.51E-11	< 5.51E-11	< 5.51E-11	< 5.51E-11	< 5.51E-11	81 ± 0	109 ± 3	none observed	none observed
adk.d6_tyrS.d8_ asd	n/a	adk.d6 + tyrS.d8 + Δasd	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	89 ± 0	137 ± 4	none observed	none observed
adk.d6_quad	n/a	adk.d6-UAGA + pEVOL-bipA-UAGA	3.01E-06 ± 7.53E-07	1.88E-05 ± 4.20E-06	3.53E-05 ± 1.40E-05	4.88E-05 ± 2.08E-05	5.22E-05 ± 2.30E-05	5.45E-05 ± 2.38E-05	5.45E-05 ± 2.38E-05	not determined	not determined	not determined	not determined
adk.d6_ bipARS.d7	1E4V_A	adk.d6 + pEVOL bipARS: I241T, M242A, A245G, L273G, D286R, L287A, V291bipA	4.07E-09 ± 4.07E-09	4.07E-09 ± 4.07E-09	4.07E-09 ± 4.07E-09	8.13E-09 ± 4.08E-09	8.13E-09 ± 4.08E-09	3.66E-08 ± 2.54E-08	5.69E-08 ± 2.86E-08	not determined	not determined	not determined	not determined
adk.d6_int	1E4V_A	adk.d6 + Δtdk: pEVOL-bipA	< 3.47E-08	< 3.47E-08	< 3.47E-08	3.47E-08 ± 3.47E-08	3.47E-08 ± 3.47E-08	3.47E-08 ± 3.47E-08	3.47E-08 ± 3.47E-08	not determined	not determined	not determined	not determined
adk.d6_tyrS.d8_ bipARS.d7	n/a	adk.d6_ bipARS.d7 + tyrS.d8	< 2.25E-12	< 2.25E-12	< 2.25E-12	< 2.25E-12	< 2.25E-12	< 2.25E-12	< 2.25E-12 (monitored for 14 days)	102 ± 0	88 ± 1 (55, R^2=0.998)	none observed	none observed
adk.d6_tyrS.d8_ int	n/a	adk.d6_int + tyrS.d8	< 2.18E-12	< 2.18E-12	< 2.18E-12	< 2.18E-12	< 2.18E-12	< 2.18E-12	< 2.18E-12 (monitored for 14 days)	427 ± 38	81 ± 1 (57, R^2=0.999)	none observed	none observed

Errors reported are s.e.m. (Methods)

* Determined from additional plating at low density

† Determined from additional plating at high density

< Indicates escape frequency below given detection limit

Doubling times in parentheses are from growth in culture tubes rather than in a microplate reader (Methods)

Supplementary Table 5 | Escape frequencies of synthetic auxotrophs in SCA

Strain	Day 1 escape rate (escapees/c.f.u.)	Day 2 escape rate (escapees/c.f.u.)	Day 3 escape rate (escapees/c.f.u.)	Day 4 escape rate (escapees/c.f.u.)	Day 5 escape rate (escapees/c.f.u.)	Day 6 escape rate (escapees/c.f.u.)	Day 7 escape rate (escapees/c.f.u.)
holB.d1	not determined	not determined	not determined	not determined	not determined	not determined	not determined
alaS.d5	not collected	1.88E-01 ± 5.73E-02	1.97E-01 ± 5.57E-02	1.97E-01 ± 5.57E-02	1.97E-01 ± 5.57E-02	1.97E-01 ± 5.57E-02	1.97E-01 ± 5.57E-02
alaS.d5. startUAG	1.04E-05 ± 8.42E-06	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit
metG.d3	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit
pgk.d4	4.17E-07 ± 4.17E-07	2.08E-06 ± 1.11E-06	1.38E-05 ± 9.45E-06	1.26E-04 ± 1.09E-04	1.66E-04 ± 1.16E-04	1.96E-04 ± 1.16E-04	2.06E-04 ± 1.18E-04
adk.d4	1.07E-06 ± 1.01E-06	1.04E-04 ± 5.62E-05	1.07E-04 ± 5.66E-05	1.09E-04 ± 5.74E-05	1.09E-04 ± 5.74E-05	1.09E-04 ± 5.73E-05	1.09E-04 ± 5.74E-05
adk.d6	1.16E-05 ± 2.27E-06	2.28E-05 ± 2.11E-06	2.89E-05 ± 2.96E-06	3.35E-05 ± 3.47E-06	4.03E-05 ± 4.67E-06	4.43E-05 ± 4.91E-06	4.43E-05 ± 4.91E-06
tyrS.d6	< 2.36E-09	3.58E-07 ± 2.32E-07	8.25E-07 ± 2.92E-07	2.04E-06 ± 3.38E-07	2.57E-06 ± 2.58E-07	2.83E-06 ± 2.14E-07	3.26E-06 ± 1.73E-07
tyrS.d7	< 2.16E-09	1.79E-06 ± 4.14E-07	above detection limit	above detection limit	above detection limit	above detection limit	above detection limit
tyrS.d8	3.47E-09 ± 3.55E-09	1.51E-06 ± 5.86E-07	2.14E-06 ± 7.61E-07	4.94E-06 ± 1.33E-06	5.83E-06 ± 1.54E-06	7.65E-06 ± 1.98E-06	8.49E-06 ± 2.09E-06
adk.d6_tyrS.d6	< 6.41E-11	1.38E-07 ± 5.58E-08	2.96E-07 ± 7.68E-08	2.39E-06 ± 1.12E-06	3.87E-06 ± 9.64E-07	6.02E-06 ± 1.27E-06	6.92E-06 ± 1.54E-06
adk.d6_tyrS.d7	< 3.16E-09	2.06E-07 ± 1.86E-07	6.90E-07 ± 3.90E-07	7.53E-06 ± 2.61E-06	7.85E-06 ± 2.68E-06	8.03E-06 ± 2.73E-06	8.19E-06 ± 2.78E-06
adk.d6_tyrS.d8	< 4.24E-09	2.97E-08 ± 2.49E-08	8.47E-08 ± 2.95E-08	1.06E-07 ± 4.38E-08	2.33E-06 ± 1.54E-06	7.23E-06 ± 4.00E-06	8.40E-06 ± 4.25E-06
adk.d6_tyrS.d6_ asd	< 6.41E-11	1.09E-09 ± 8.19E-10	1.54E-09 ± 8.89E-10	1.67E-09 ± 1.00E-09	1.79E-09 ± 1.00E-09	1.86E-09 ± 1.01E-09	1.92E-09 ± 1.02E-09
adk.d6_tyrS.d7_ asd	6.62E-10 ± 4.41E-10	2.59E-09 ± 2.29E-09	3.75E-09 ± 3.29E-09	3.86E-09 ± 3.40E-09	3.92E-09 ± 3.37E-09	3.92E-09 ± 3.37E-09	3.97E-09 ± 3.42E-09
adk.d6_tyrS.d8_ asd	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11	< 4.46E-11
adk.d6_quad	6.24E-05 ± 1.25E-05	2.84E-04 ± 5.83E-05	3.97E-04 ± 4.44E-05	5.36E-04 ± 7.02E-05	5.80E-04 ± 7.08E-05	6.03E-04 ± 7.26E-05	6.12E-04 ± 7.09E-05
adk.d6_ bipARS.d7	0.00E+00	4.07E-09 ± 4.07E-09	3.25E-08 ± 4.31E-09	3.66E-08 ± 7.22E-09	3.66E-08 ± 7.22E-09	3.66E-08 ± 7.22E-09	3.66E-08 ± 7.22E-09
adk.d6_int	3.82E-07 ± 2.84E-07	3.82E-07 ± 2.84E-07	4.17E-07 ± 2.62E-07	4.86E-07 ± 2.78E-07	5.21E-07 ± 2.62E-07	5.90E-07 ± 2.28E-07	5.90E-07 ± 2.28E-07
adk.d6_tyrS.d8_ bipARS.d7	< 4.63E-11	< 4.63E-11	< 4.63E-11	< 4.63E-11	< 4.63E-11	< 4.63E-11	< 4.63E-11
adk.d6_tyrS.d8_ int	< 4.54E-11	< 4.54E-11	< 4.54E-11	< 4.54E-11	< 4.54E-11	< 4.54E-11	< 4.54E-11

Errors reported are s.e.m. (Methods)

< Indicates escape frequency below given detection limit

Supplementary Table 6 | Codon and amino acid frequencies for UAG saturation mutagenesis

alaS.d5 UAG->NNN codon selection

Codon	**1 hr**	**4 hr**	**Confluent 1**	**Confluent 2**	**Confluent 3**
AAA	434	280	234	35	26
AAC	470	567	397	2932	8470
AAG	827	630	679	385	166
AAT	569	677	572	4595	13072
ACA	512	523	334	2804	7940
ACC	513	518	347	2576	7108
ACG	414	464	305	2497	6853
ACT	575	614	456	3396	9492
AGA	401	281	266	89	44
AGC	441	444	358	2628	7247
AGG	366	257	180	83	84
AGT	468	556	487	3346	9450
ATA	628	657	539	4399	12010
ATC	727	776	694	3684	10547
ATG	758	731	581	3542	9641
ATT	802	774	665	5140	15402
CAA	471	508	370	2602	6656
CAC	469	522	361	2230	6508
CAG	744	688	663	2201	4723
CAT	616	694	534	3408	9372
CCA	421	279	217	47	37
CCC	407	260	165	81	31
CCG	582	444	399	277	277
CCT	426	234	177	40	19
CGA	433	264	247	108	146
CGC	429	252	191	42	41
CGG	347	216	173	63	63
CGT	694	362	323	121	94
CTA	565	681	512	3336	9816
CTC	468	549	421	2811	8046
CTG	453	470	429	2782	7622
CTT	626	629	570	3970	12187
GAA	382	452	376	2003	4059
GAC	659	650	491	916	713
GAG	571	537	537	1879	3469
GAT	627	627	563	1154	971
GCA	495	502	326	2677	6810
GCC	560	656	516	2296	6184
GCG	420	363	298	1867	4910
GCT	561	534	412	2925	7505
GGA	424	445	359	2148	5040
GGC	641	734	561	2332	5308
GGG	377	301	282	1425	3504
GGT	5057	4893	4962	5991	10615
GTA	704	771	660	3625	10506
GTC	5887	5894	5951	7840	14533
GTG	557	458	339	2523	7181

GTT	823	891	855	4446	12142
TAA	768	512	388	114	81
TAC	705	877	761	4385	12945
TAG	581822	562326	551652	305073	114316
TAT	902	1018	1033	5845	17549
TCA	646	624	560	3572	9647
TCC	2051	2106	2116	4899	11583
TCG	629	593	460	3235	9025
TCT	395	201	312	2398	6784
TGA	619	385	259	68	87
TGC	657	706	525	3867	10883
TGG	11110	10570	10425	11089	18011
TGT	1017	1205	1154	5443	14346
TTA	738	869	785	6500	18871
TTC	811	976	903	5252	14948
TTG	1109	1236	1129	5143	14591
TTT	749	864	914	6632	19313
Total	640529	619577	603710	485812	579620

adk.d4 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	153	455	60	17	11
AAC	237	502	105	45	50
AAG	403	727	123	52	81
AAT	312	927	143	36	43
ACA	340	965	4061	3568	2996
ACC	204	535	1862	1288	341
ACG	179	460	3974	2731	596
ACT	7086	8160	9936	7584	5538
AGA	239	538	87	34	56
AGC	223	604	622	529	492
AGG	214	600	48	8	10
AGT	375	1013	93	6	23
ATA	370	955	11111	9564	8685
ATC	347	1073	8974	7333	6806
ATG	428	1072	15608	16600	15749
ATT	749	2096	31875	27557	25765
CAA	154	441	49	9	16
CAC	250	557	184	111	144
CAG	904	1416	3794	3458	3292
CAT	315	885	112	27	28
CCA	163	433	54	11	20
CCC	185	467	55	19	15
CCG	234	503	139	46	44
CCT	298	893	128	30	33
CGA	172	497	55	15	11
CGC	172	520	62	27	23
CGG	762	1201	1770	1519	3048
CGT	415	1172	131	24	10
CTA	337	933	19021	19535	17501
CTC	386	929	20211	20813	20438

CTG	7804	8732	30312	29906	26691
CTT	745	2097	50471	51862	52212
GAA	774	1056	1860	1695	3394
GAC	6115	6693	8498	7209	6732
GAG	303	681	163	84	141
GAT	431	1091	172	66	105
GCA	370	709	353	279	269
GCC	199	548	95	22	32
GCG	303	655	262	106	148
GCT	436	1154	188	65	80
GGA	669	1178	1606	1339	2957
GGC	263	668	160	66	98
GGG	219	669	65	19	18
GGT	461	1234	119	22	22
GTA	361	1003	13513	14382	14658
GTC	374	1075	10041	10307	10877
GTG	348	1110	14207	15168	14869
GTT	816	2196	29537	31407	31643
TAA	405	992	116	23	38
TAC	375	940	3230	1345	747
TAG	299876	282570	36240	558	121
TAT	689	1723	10556	6426	4443
TCA	250	757	103	14	19
TCC	312	871	73	6	19
TCG	464	1008	133	57	56
TCT	577	1649	103	11	12
TGA	563	1379	448	325	322
TGC	1487	2275	4170	2372	1039
TGG	483	1200	403	68	11
TGT	693	2145	8670	4111	483
TTA	637	1835	48622	51252	47156
TTC	638	1667	4674	1925	845
TTG	850	2212	62408	65456	63594
TTT	1288	3644	6725	2865	1323
Total	347184	370945	482743	423414	397039

adk.d6 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	435	186	68	18	17
AAC	468	249	98	55	38
AAG	776	556	292	94	128
AAT	833	419	147	44	44
ACA	8859	7837	6954	3858	2803
ACC	478	209	102	74	56
ACG	531	160	69	12	18
ACT	1518	665	3303	5895	4436
AGA	446	259	135	60	75
AGC	1887	1488	1247	687	498
AGG	491	211	46	25	25
AGT	902	383	58	18	19
ATA	778	409	1805	3994	3012

ATC	864	416	1989	4238	3513
ATG	981	484	13653	17237	9526
ATT	1693	886	6950	16125	12856
CAA	385	188	60	17	10
CAC	538	277	223	152	113
CAG	10608	9240	7619	4205	3045
CAT	797	358	127	34	21
CCA	384	182	59	19	15
CCC	406	200	62	13	12
CCG	447	218	120	63	55
CCT	695	293	85	50	41
CGA	488	229	40	8	5
CGC	472	196	54	24	30
CGG	1124	713	1430	3238	4069
CGT	1055	432	108	34	19
CTA	810	404	13504	24116	17664
CTC	795	402	10116	14076	7790
CTG	1752	949	21830	41540	32554
CTT	1722	873	49594	106967	85277
GAA	864	674	1512	3439	4382
GAC	8515	7108	8589	7995	6091
GAG	739	374	199	174	217
GAT	1062	467	184	76	51
GCA	663	395	350	281	202
GCC	471	189	64	34	18
GCG	772	355	227	251	265
GCT	1032	413	171	106	63
GGA	883	517	1245	3071	3870
GGC	737	299	141	78	63
GGG	635	270	69	35	50
GGT	1063	436	113	21	11
GTA	884	418	9612	12564	7065
GTC	837	404	9706	14608	9821
GTG	997	479	14193	32387	40571
GTT	2020	956	20328	23316	11081
TAA	908	477	205	33	25
TAC	890	430	234	132	105
TAG	384963	350206	205074	4079	128
TAT	1664	747	242	35	13
TCA	714	260	63	26	9
TCC	795	310	74	11	20
TCG	1034	489	203	58	73
TCT	1482	601	162	14	4
TGA	1207	657	465	407	297
TGC	1095	450	668	1002	740
TGG	1193	534	158	7	9
TGT	1930	814	148	9	2
TTA	1582	843	32333	59986	37764
TTC	1593	635	196	89	62
TTG	2133	1154	33147	61493	43687
TTT	3285	1444	430	183	100

Total	473060	403776	482452	472990	354643

pgk.d4 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	12940	11993	9016	10859	9349
AAC	411	152	89	33	45
AAG	502	282	319	184	319
AAT	1960	1400	1068	1057	958
ACA	477	194	341	221	493
ACC	640	220	135	13	16
ACG	560	188	109	19	19
ACT	832	296	196	12	12
AGA	492	269	426	239	377
AGC	1045	922	6450	5621	9972
AGG	415	177	310	204	471
AGT	685	240	147	8	21
ATA	792	316	231	2227	674
ATC	1180	568	429	2810	915
ATG	988	404	286	29802	29054
ATT	1431	491	373	4839	1259
CAA	443	174	132	48	66
CAC	997	663	4964	4369	7873
CAG	631	404	502	227	326
CAT	761	323	202	33	35
CCA	528	203	185	72	103
CCC	784	241	201	59	133
CCG	712	247	159	36	39
CCT	1091	362	242	33	43
CGA	564	283	378	246	457
CGC	657	230	148	32	49
CGG	632	327	247	105	98
CGT	1301	819	2024	1513	2258
CTA	928	416	1689	14719	10765
CTC	1005	379	330	11669	6971
CTG	1205	662	478	19570	11733
CTT	1648	620	576	27985	17350
GAA	5929	5404	4665	5525	5070
GAC	411	182	174	90	136
GAG	591	357	818	611	1141
GAT	696	295	236	50	91
GCA	1148	1004	6668	5817	10780
GCC	567	213	141	48	115
GCG	834	656	2012	1695	2413
GCT	907	362	198	9	13
GGA	525	314	217	132	113
GGC	536	191	180	72	180
GGG	433	170	96	5	8
GGT	821	266	166	7	11
GTA	678	266	162	163	29
GTC	859	360	648	724	752
GTG	945	367	267	263	49

GTT	1698	900	1801	1560	1731
TAA	721	383	240	77	55
TAC	843	412	347	20622	20549
TAG	595312	588683	413664	60275	5963
TAT	1264	613	509	37015	37711
TCA	708	287	403	256	451
TCC	947	342	382	178	332
TCG	986	501	466	191	349
TCT	1342	643	1686	1333	2381
TGA	5099	4321	3639	4330	3790
TGC	1050	410	300	3913	1250
TGG	995	455	307	22	15
TGT	1422	569	459	8027	2448
TTA	1219	456	445	22558	13746
TTC	1629	868	1161	65049	75582
TTG	1819	794	634	31758	19803
TTT	2356	974	1160	137316	164670
Total	674527	636483	476633	548555	483980

metG.d3 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	141	140	105	33	32
AAC	131	116	111	92	125
AAG	562	549	576	419	493
AAT	123	119	96	90	87
ACA	109	124	215	2626	1759
ACC	64	62	129	1360	864
ACG	61	56	104	1267	945
ACT	44	41	128	1376	981
AGA	191	214	310	250	492
AGC	2493	2489	2425	2475	3429
AGG	1039	1194	1058	1037	1356
AGT	104	109	150	142	175
ATA	122	181	485	43156	64191
ATC	460	618	1183	24708	37555
ATG	176	308	411	38425	68619
ATT	63	87	294	26417	40023
CAA	93	93	69	28	48
CAC	84	43	89	86	203
CAG	4952	5128	6009	5446	7333
CAT	105	126	132	120	118
CCA	93	55	43	26	49
CCC	37	21	28	12	38
CCG	26	22	29	20	27
CCT	44	25	19	10	11
CGA	51	41	42	8	15
CGC	66	48	38	26	49
CGG	63	49	175	262	467
CGT	37	23	34	7	16
CTA	60	92	266	24982	34855
CTC	57	60	178	14504	21542

CTG	66	77	154	12951	18632
CTT	48	51	166	15826	23083
GAA	336	424	841	1343	2644
GAC	41	38	30	22	43
GAG	142	176	151	99	142
GAT	144	153	244	265	453
GCA	9952	9829	9453	9630	12309
GCC	52	41	49	126	141
GCG	44	49	42	111	174
GCT	49	31	45	103	87
GGA	238	409	336	372	760
GGC	185	117	859	91	168
GGG	56	58	34	17	41
GGT	57	55	68	29	61
GTA	114	122	259	11797	18735
GTC	81	87	168	7012	10760
GTG	68	66	192	9983	15497
GTT	56	66	162	6361	9734
TAA	201	208	175	85	64
TAC	89	100	170	8220	10119
TAG	440300	435794	353213	70412	8273
TAT	206	338	346	12272	14071
TCA	414	551	928	967	1226
TCC	25	26	45	37	41
TCG	107	110	206	233	438
TCT	47	34	37	33	26
TGA	4884	1885	11177	1267	2375
TGC	80	60	143	6663	7634
TGG	150	150	130	84	131
TGT	148	244	238	7212	8804
TTA	65	108	323	27847	41861
TTC	31	44	133	7053	9445
TTG	174	180	839	19597	30468
TTT	51	52	147	10039	12515
Total	470352	463966	396434	437569	546852

tyrS.d6 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	213	153	97	19	22
AAC	392	250	109	12	7
AAG	505	422	469	47	120
AAT	782	607	309	77	49
ACA	649	562	361	226	171
ACC	8019	8493	8291	6230	5016
ACG	650	411	203	14	17
ACT	1600	1109	464	106	121
AGA	329	221	131	6	14
AGC	534	327	163	19	31
AGG	474	292	137	5	11
AGT	1367	866	3219	882	2575
ATA	828	637	385	114	76

ATC	1347	925	603	46	27
ATG	1336	889	2107	1140	294
ATT	2906	2120	1163	105	30
CAA	578	508	344	201	145
CAC	917	722	536	265	208
CAG	1341	1325	1439	859	707
CAT	1534	1093	386	138	90
CCA	6012	4962	7932	10955	9367
CCC	6574	5432	8992	11176	9989
CCG	1449	1063	1201	422	626
CCT	3389	2576	1456	473	552
CGA	693	461	189	15	15
CGC	555	279	248	145	132
CGG	1060	616	256	25	31
CGT	2539	1723	652	72	95
CTA	1628	1304	6930	29792	19840
CTC	2756	2084	10971	48854	30926
CTG	2585	1906	14710	56499	48050
CTT	5919	4542	26800	115601	72987
GAA	398	322	166	88	66
GAC	751	607	337	133	108
GAG	612	453	3078	780	2521
GAT	1335	887	344	19	24
GCA	888	838	1510	3257	1339
GCC	5621	4610	9721	14723	10505
GCG	849	597	1686	4573	1875
GCT	2508	1958	7435	13635	14450
GGA	543	360	136	11	15
GGC	690	456	266	41	33
GGG	761	457	213	5	2
GGT	1901	1308	511	31	71
GTA	1189	872	455	65	46
GTC	2320	1691	793	241	398
GTG	2042	1333	918	168	232
GTT	4835	3587	1517	105	38
TAA	982	726	377	78	19
TAC	1546	1078	504	27	8
TAG	480221	557142	520026	29487	748
TAT	3431	2451	1159	96	54
TCA	1446	1012	458	114	108
TCC	3193	2310	987	280	218
TCG	2341	1637	743	66	27
TCT	5409	3942	1318	62	46
TGA	1491	1102	6488	2360	4861
TGC	2200	1380	3384	6112	3109
TGG	2355	1503	981	150	120
TGT	5298	3668	9502	17836	10468
TTA	2946	2092	12455	56753	34964
TTC	5331	3782	1591	192	95
TTG	5244	3839	20447	108780	67635
TTT	11424	8421	3202	398	214

Total	623561	665301	713961	545206	356758

tyrS.d7 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	27	13	7	0	0
AAC	56	30	11	0	0
AAG	260	126	332	6	1
AAT	116	42	27	0	0
ACA	60	15	15	0	0
ACC	111	49	22	0	0
ACG	116	42	30	1	0
ACT	197	66	65	25	9
AGA	30	18	11	0	0
AGC	19	11	8	0	1
AGG	82	31	18	20	11
AGT	123	54	22	1	0
ATA	82	45	27	0	0
ATC	137	50	32	14	6
ATG	278	90	69	15	14
ATT	341	117	108	28	5
CAA	43	20	14	0	0
CAC	65	19	21	1	0
CAG	402	198	403	15	14
CAT	217	76	74	0	0
CCA	120	39	24	1	1
CCC	143	60	38	2	1
CCG	244	82	43	4	6
CCT	313	127	99	3	0
CGA	57	25	20	1	0
CGC	14	7	8	2	0
CGG	129	90	20	54	30
CGT	223	108	42	0	0
CTA	151	54	46	10	10
CTC	262	120	62	47	14
CTG	418	172	158	19597	32691
CTT	674	280	202	58	43
GAA	49	16	15	0	0
GAC	47	24	20	1	1
GAG	186	88	161	0	10
GAT	205	76	60	1	0
GCA	48	25	9	1	1
GCC	101	47	32	0	1
GCG	131	50	28	1	1
GCT	215	95	71	3	5
GGA	62	15	11	1	0
GGC	24	18	9	1	0
GGG	145	56	23	7	6
GGT	185	77	56	1	0
GTA	134	55	40	1	0
GTC	144	88	39	11	3
GTG	326	98	61	2	1

GTT	554	185	128	13	3
TAA	283	175	193	8	0
TAC	303	147	129	73	34
TAG	411640	403939	447328	26255	1126
TAT	963	499	602	137	47
TCA	168	66	56	2	0
TCC	329	200	119	17	8
TCG	786	342	247	16	6
TCT	656	260	231	44	15
TGA	211	68	112	58	61
TGC	115	72	34	35	26
TGG	1123	398	677	155810	81173
TGT	846	321	168	150	78
TTA	440	185	114	65	33
TTC	684	267	399	78152	36911
TTG	1991	777	683	896	409
TTT	1611	640	873	148211	70444
Total	430215	411645	454806	429878	223260

tyrS.d8 UAG->NNN codon selection

Codon	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3
AAA	276	125	94	0	0
AAC	494	228	159	3	0
AAG	434	178	170	2	2
AAT	1018	490	294	7	0
ACA	541	249	142	2	0
ACC	1088	501	284	7	0
ACG	747	312	233	6	0
ACT	1956	912	510	4	1
AGA	471	189	126	2	0
AGC	721	288	171	3	0
AGG	651	250	210	31	69
AGT	1666	680	433	7	0
ATA	1114	467	373	7	1
ATC	1909	779	564	12	1
ATG	1654	612	564	5	3
ATT	4117	1858	1062	27	4
CAA	512	230	160	6	0
CAC	989	361	232	4	0
CAG	782	352	349	11	23
CAT	1992	917	504	4	0
CCA	1066	576	226	4	0
CCC	2253	950	483	9	7
CCG	1649	685	428	11	5
CCT	4266	1980	955	10	0
CGA	832	359	219	3	1
CGC	748	187	188	2	0
CGG	1265	532	376	60	153
CGT	3237	1387	780	11	9
CTA	1995	878	564	18	4
CTC	3719	1495	815	25	16

CTG	3190	1277	989	528	1052
CTT	8112	3416	2133	70	33
GAA	433	196	119	3	0
GAC	742	356	258	2	0
GAG	627	269	287	2	38
GAT	1674	792	485	3	2
GCA	828	355	194	3	7
GCC	1554	774	373	8	2
GCG	1173	470	310	4	1
GCT	3022	1399	664	17	12
GGA	674	317	194	3	0
GGC	983	373	273	3	0
GGG	997	391	298	20	25
GGT	2539	1184	729	9	0
GTA	1618	750	432	5	1
GTC	3022	1347	800	12	3
GTG	2086	854	621	12	4
GTT	6224	2963	1547	25	3
TAA	1040	436	325	8	1
TAC	2097	821	587	31	8
TAG	12084	10879	8894	1224	423
TAT	3950	1735	1067	52	24
TCA	1867	785	515	9	0
TCC	4061	1812	1061	21	2
TCG	2668	1094	761	29	31
TCT	7008	3123	1784	31	13
TGA	1854	738	783	103	341
TGC	2988	1127	659	49	81
TGG	2665	1065	1205	159892	394007
TGT	6867	3011	1840	212	431
TTA	3955	1685	1233	50	24
TTC	7511	3231	2173	1256	739
TTG	6160	2577	1846	927	1218
TTT	15458	6994	4496	15124	12233
Total	165893	77603	51603	180050	411058

alaS.d5 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	**1 hr**	**4 hr**	**Confluent 1**	**Confluent 2**	**Confluent 3**	**# codons**
*	194403	187741	184100	101752	38161	3
A	509	514	388	2441	6352	4
C	837	956	840	4655	12615	2
D	643	639	527	1035	842	2
E	477	495	457	1941	3764	2
F	780	920	909	5942	17131	2
G	1625	1593	1541	2974	6117	4
H	543	608	448	2819	7940	2
I	719	736	633	4408	12653	3
K	631	455	457	210	96	2
L	660	739	641	4090	11856	6
M	758	731	581	3542	9641	1
N	520	622	485	3764	10771	2

P	459	304	240	111	91	4
Q	608	598	517	2402	5690	2
R	445	272	230	84	79	6
S	772	754	716	3346	8956	6
T	504	530	361	2818	7848	4
V	1993	2004	1951	4609	11091	4
W	11110	10570	10425	11089	18011	1
Y	804	948	897	5115	15247	2

adk.d4 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	100281	94980	12268	302	160	3
A	327	767	225	118	132	4
C	1090	2210	6420	3242	761	2
D	3273	3892	4335	3638	3419	2
E	539	869	1012	890	1768	2
F	963	2656	5700	2395	1084	2
G	403	937	488	362	774	4
H	283	721	148	69	86	2
I	489	1375	17320	14818	13752	3
K	278	591	92	35	46	2
L	1793	2790	38508	39804	37932	6
M	428	1072	15608	16600	15749	1
N	275	715	124	41	47	2
P	220	574	94	27	28	4
Q	529	929	1922	1734	1654	2
R	329	755	359	271	526	6
S	367	984	188	104	104	6
T	1952	2530	4958	3793	2368	4
V	475	1346	16825	17816	18012	4
W	483	1200	403	68	11	1
Y	532	1332	6893	3886	2595	2

adk.d6 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	129026	117113	68581	1506	150	3
A	735	338	203	168	137	4
C	1513	632	408	506	371	2
D	4789	3788	4387	4036	3071	2
E	802	524	856	1807	2300	2
F	2439	1040	313	136	81	2
G	830	381	392	801	999	4
H	668	318	175	93	67	2
I	1112	570	3581	8119	6460	3
K	606	371	180	56	73	2
L	1466	771	26754	51363	37456	6
M	981	484	13653	17237	9526	1
N	651	334	123	50	41	2
P	483	223	82	36	31	4
Q	5497	4714	3840	2111	1528	2
R	679	340	302	565	704	6

S	1136	589	301	136	104	6
T	2847	2218	2607	2460	1828	4
V	1185	564	13460	20719	17135	4
W	1193	534	158	7	9	1
Y	1277	589	238	84	59	2

pgk.d4 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	200377	197796	139181	21561	3269	3
A	864	559	2255	1892	3330	4
C	1236	490	380	5970	1849	2
D	554	239	205	70	114	2
E	3260	2881	2742	3068	3106	2
F	1993	921	1161	101183	120126	2
G	579	235	165	54	78	4
H	879	493	2583	2201	3954	2
I	1134	458	344	3292	949	3
K	6721	6138	4668	5522	4834	2
L	1304	555	692	21377	13395	6
M	988	404	286	29802	29054	1
N	1186	776	579	545	502	2
P	779	263	197	50	80	4
Q	537	289	317	138	196	2
R	677	351	589	390	618	6
S	952	489	1589	1265	2251	6
T	627	225	195	66	135	4
V	1045	473	720	678	640	4
W	995	455	307	22	15	1
Y	1054	513	428	28819	29130	2

metG.d3 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	148462	145962	121522	23921	3571	3
A	2524	2488	2397	2493	3178	4
C	114	152	191	6938	8219	2
D	93	96	137	144	248	2
E	239	300	496	721	1393	2
F	41	48	140	8546	10980	2
G	134	160	324	127	258	4
H	95	85	111	103	161	2
I	215	295	654	31427	47256	3
K	352	345	341	226	263	2
L	78	95	321	19285	28407	6
M	176	308	411	38425	68619	1
N	127	118	104	91	106	2
P	50	31	30	17	31	4
Q	2523	2611	3039	2737	3691	2
R	241	262	276	265	399	6
S	532	553	632	648	889	6
T	70	71	144	1657	1137	4
V	80	85	195	8788	13682	4

W	150	150	130	84	131	1
Y	148	219	258	10246	12095	2

tyrS.d6 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	160898	186323	175630	10642	1876	3
A	2467	2001	5088	9047	7042	4
C	3749	2524	6443	11974	6789	2
D	1043	747	341	76	66	2
E	505	388	1622	434	1294	2
F	8378	6102	2397	295	155	2
G	974	645	282	22	30	4
H	1226	908	461	202	149	2
I	1694	1227	717	88	44	3
K	359	288	283	33	71	2
L	3513	2628	15386	69380	45734	6
M	1336	889	2107	1140	294	1
N	587	429	209	45	28	2
P	4356	3508	4895	5757	5134	4
Q	960	917	892	530	426	2
R	942	599	269	45	50	6
S	2382	1682	1148	237	501	6
T	2730	2644	2330	1644	1331	4
V	2597	1871	921	145	179	4
W	2355	1503	981	150	120	1
Y	2489	1765	832	62	31	2

tyrS.d7 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	137378	134727	149211	8774	396	3
A	124	54	35	1	2	4
C	481	197	101	93	52	2
D	126	50	40	1	1	2
E	118	52	88	0	5	2
F	1148	454	636	113182	53678	2
G	104	42	25	3	2	4
H	141	48	48	1	0	2
I	187	71	56	14	4	3
K	144	70	170	3	1	2
L	656	265	211	3446	5533	6
M	278	90	69	15	14	1
N	86	36	19	0	0	2
P	205	77	51	3	2	4
Q	223	109	209	8	7	2
R	89	47	20	13	7	6
S	347	156	114	13	5	6
T	121	43	33	7	2	4
V	290	107	67	7	2	4
W	1123	398	677	155810	81173	1
Y	633	323	366	105	41	2

tyrS.d8 UAG->NNN amino acid selection (normalized for number of codons decoding a given amino acid)

aa	1 hr	4 hr	Confluent 1	Confluent 2	Confluent 3	# codons
*	4993	4018	3334	445	255	3
A	1644	750	385	8	6	4
C	4928	2069	1250	131	256	2
D	1208	574	372	3	1	2
E	530	233	203	3	19	2
F	11485	5113	3335	8190	6486	2
G	1298	566	374	9	6	4
H	1491	639	368	4	0	2
I	2380	1035	666	15	2	3
K	355	152	132	1	1	2
L	4522	1888	1263	270	391	6
M	1654	612	564	5	3	1
N	756	359	227	5	0	2
P	2309	1048	523	9	3	4
Q	647	291	255	9	12	2
R	1201	484	317	18	39	6
S	2999	1297	788	17	8	6
T	1083	494	292	5	0	4
V	3238	1479	850	14	3	4
W	2665	1065	1205	159892	394007	1
Y	3024	1278	827	42	16	2

Supplementary Table 7 | Doubling times of leucine and tryptophan variants of adk.d6, tyrS.d7 and tyrS.d8

			Permissive media			Nonpermissive media		
Strain[a]	**Variant**	**Clone**	**Avg. Tdouble (min.)**	**Tdouble s.e.m. (min)**	**Max OD_{600}**	**Avg. Tdouble (min.)**	**Tdouble s.e.m. (min)**	**Max OD_{600}**
adk.d6	Leu	1	70	1	0.98	124	2	0.85
adk.d6	Leu	2	68	0	0.98	141	1	0.58
adk.d6	Leu	3	75	1	0.93	133	1	0.70
tyrS.d7	Trp	1	156	6	0.96	248	9	0.66
tyrS.d7	Trp	2	179	4	0.98	288	12	0.57
tyrS.d7	Trp	3	198	9	0.95	288	9	0.59
tyrS.d8	Trp	1	161	4	1.10	148	7	0.95
tyrS.d8	Trp	2	276	13	1.13	267	8	0.66
tyrS.d7[b]	Leu	1	nd	nd	nd	no growth	no growth	no growth
tyrS.d7[b]	Leu	2	nd	nd	nd	no growth	no growth	no growth
tyrS.d7[b]	Leu	3	nd	nd	nd	no growth	no growth	no growth

[a] We were unable to produce adk.d6 bipA->Trp or tyrS.d8 bipA->Leu variants

[b] tyrS.d7 Leu variants were confirmed by Sanger sequencing, but did not grow in nonpermissive liquid media

Supplementary Table 8 | Viability of *adk* synthetic auxotrophs in stationary phase

Condition	**Measured OD_{600}**	**Viable c.f.u./ml**
adk.d4-SCAB10-mid log	0.6	2.83×10^8
adk.d4-SCAB10-late log	0.96	5.43×10^8
adk.d4-SCAB10-stationary	1.2	1.60×10^7
adk.d4-SCAB50-mid log	0.65	2.47×10^8
adk.d4-SCAB50-late log	1.2	6.33×10^8
adk.d4-SCAB50-stationary	2	3.13×10^8
adk.d6-SCAB10-late log	1.1	6.20×10^8
adk.d6-SCAB10-early stationary	2.1	1.83×10^9
adk.d6-SCAB10-late stationary	2.6	3.30×10^9
adk.d6-SCAB50-mid log	0.5	2.30×10^8
adk.d6-SCAB50-stationary	2.4	1.27×10^9

Supplementary Table 9 | Escape mechanisms uncovered by whole-genome sequencing

Strain name	Media condition	Escape mechanism
adk.d6_esc-1	permissive	frame shift in lon at nt 459009
adk.d6_esc-2	non-permissive	structural event upstream of lon
adk.d6_esc-3	non-permissive	structural event upstream of lon
adk.d6_esc-4	non-permissive	structural event upstream of lon
tyrS.d8_esc-1	permissive	tyrS A70V mutation at nt 1721829
tyrS.d8_esc-2	non-permissive	tyrS A70V mutation at nt 1721829
tyrS.d8_esc-3	non-permissive	tyrS A70V mutation at nt 1721829
tyrS.d8_esc-4	non-permissive	tyrS A70V mutation at nt 1721829
adk.d6_tyrS.d8_esc-1	permissive	mutation in lon (nt:T459768C aa:L611P)
adk.d6_tyrS.d8_esc-2	permissive	structural event upstream of lon
adk.d6_tyrS.d8_esc-3	permissive	structural event upstream of lon

Supplementary Table 10 | tyrS.A70V growth rates

	bipA concentration (μM)					
Strain	**0**	**0.5**	**1**	**5**	**10**	**50**
tyrS.A70V_esc-1	295.9 ± 8.9	86.9 ± 0.7	72.4 ± 0.3	62.9 ± 1.8	66.0 ± 0.2	90.6 ± 0.6
tyrS.A70V_esc-2	318.7 ± 25.1	97.1 ± 0.8	73.2 ± 0.5	64.7 ± 0.2	66.0 ± 0.4	96.1 ± 0.4
tyrS.A70V_esc-3	484.1 ± 16.0	89.5 ± 0.6	70.5 ± 0.3	66.6 ± 0.2	69.3 ± 0.2	116.5 ± 1.6
tyrS.d8	no growth	465.0 ± 177.0	84.2 ± 0.8	68.2 ± 0.3	68.9 ± 0.3	130.4 ± 1.5
C321.ΔA	64.4 ± 0.1	62.6 ± 0.2	62.3 ± 0.2	60.6 ± 0.2	61.8 ± 0.4	89.2 ± 1.2

Supplementary Table 11 | Escape and growth rates of natural metabolic auxotrophs

Strain	Escape assay plate media	Day 1 esc. frequency	Day 2 esc. frequency	Day 7 esc. frequency	Doubling time in LB^L + DAP + thymidine
EcNR1ΔthyA	LB^L	< 3.51E-11	2.70E-09 ± 1.20E-10	7.40E-08 ± 5.14E-09	43.6 ± 0.2
EcNR1Δasd	LB^L	< 5.98E-10	5.98E-10 ± 5.38E-10	8.97E-09 ± 5.46E-09	47.7 ± 0.3
EcNR1ΔasdΔthyA	LB^L	< 1.83E-10	< 1.83E-10	8.79E-09 ± 4.81E-09	57.5 ± 0.2
EcNR1ΔasdΔthyA	LB^L + thymidine	< 5.49E-10	1.10E-09 ± 5.65E-10	2.86E-08 ± 4.40E-09	n.a.

Errors reported are s.e.m. of the rate (Methods)

< Indicates below given detection limit

n.a. = not applicable

Supplementary Table 12 | Allele reversion frequencies in conjugal escapees

		adk.d4		adk.d6		tyrS.d6		tyrS.d7		tyrS.d8		adk.d6_tyrS.d6		adk.d6_tyrS.d7		adk.d6_tyrS.d8		adk.d6_tyrS.d8_asd	
	N =	15	n.a.	51	6	16	n.a.	15	n.a.	44	7	8	n.a.	6	n.a.	8	59	5	38
Gene	**UAG Position**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**	**50 minute conj.**	**12 hour conj.**
murF	96008	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.542	1.000	0.895
yaiS	383283	0.867	n.d.	0.667	0.500	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.814	1.000	0.895
lon	460466	1.000	n.d.	0.745	0.833	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.966	1.000	0.947
adk	497043	1.000	n.d.	1.000	1.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	1.000	1.000	1.000
ybhS	825342	0.267	n.d.	0.078	0.000	0.000	n.d.	0.000	n.d.	0.039	0.143	1.000	n.d.	1.000	n.d.	1.000	0.949	1.000	0.974
lolA	937206	0.267	n.d.	0.039	0.000	0.000	n.d.	0.000	n.d.	0.039	0.143	1.000	n.d.	1.000	n.d.	1.000	0.949	1.000	0.947
ycaI	965807	0.267	n.d.	0.039	0.000	0.000	n.d.	0.000	n.d.	0.039	0.143	1.000	n.d.	1.000	n.d.	1.000	0.949	1.000	0.921
fabH	1148935	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.039	0.143	1.000	n.d.	1.000	n.d.	1.000	0.949	1.000	0.947
hemA	1264193	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.098	0.429	1.000	n.d.	1.000	n.d.	1.000	0.966	1.000	0.974
prfA	1265317	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.098	0.429	1.000	n.d.	1.000	n.d.	1.000	0.966	1.000	0.974
pspF	1364959	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.137	0.571	1.000	n.d.	1.000	n.d.	1.000	0.915	1.000	0.974
tyrS	1713972	0.000	n.d.	0.000	0.000	1.000	n.d.	1.000	n.d.	1.000	1.000	1.000	n.d.	1.000	n.d.	1.000	1.000	1.000	1.000
ydiE	1787828	0.267	n.d.	0.020	0.000	0.500	n.d.	0.200	n.d.	0.608	0.714	1.000	n.d.	1.000	n.d.	1.000	0.831	1.000	0.974
rcsC	2315049	0.267	n.d.	0.020	0.000	0.063	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.492	1.000	0.842
hda	2616097	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.373	1.000	0.789
srlE	2825373	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.271	1.000	0.816
nlpI	3306062	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.220	1.000	0.842
mreC	3396897	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.220	1.000	0.842
xylR	3734180	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.220	1.000	0.947
coaD	3808327	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.237	1.000	0.947
yidS	3868464	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.288	1.000	0.947
rbsB	3935191	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.305	1.000	0.921
insN-2	4505486	0.267	n.d.	0.020	0.000	0.000	n.d.	0.000	n.d.	0.020	0.000	1.000	n.d.	1.000	n.d.	1.000	0.339	1.000	0.842

N = number of escapees assayed by mascPCR

n.a. = not applicable

n.d. = not determined (12 hr conjugation escape assays were only performed for adk.d6_tyrS.d8_asd and its component single- and double-enzyme synthetic auxotrophs adk.d6, tyrS.d8 and adk.d6_tyrS.d8)

SUPPLEMENTARY DISCUSSION

Structure determination of a NSAA-dependent enzyme. We used X-ray crystallography to test the accuracy of our computational methods, and to explore the structural implications of introducing a NSAA into an enzyme core. We determined the crystal structure of tyrS.d7, one of the eight tyrS variants predicted from all-atom computational models, which showed strong dependence on bipA in both solid and liquid media assays, to 2.65 Å resolution (Extended Data Table 1). Two full-length protein constructs are present in the asymmetric unit (chain A and chain B), and their overall structures are comparable. Here we describe the refined model for chain A, which is considerably more ordered than the other molecule. The engineered enzyme is composed of two domains connected by a short α-helix (Extended Data Fig. 3a) and several flanking loops. The C-terminal tRNA recognition domain displays a topologically similar structure to the corresponding region of *T. thermophilus* TyrS[1] (PDB code 1H3E) (Extended Data Fig. 3b), the only full-length bacterial tyrosyl-tRNA synthetase with a determined crystal structure prior to this study. The N-terminal domain that catalyzes tyrosine activation is closely superimposable on the crystal structure of the wild-type enzyme[2] (Extended Data Fig. 3c; PDB code 1X8X, Cα root mean square deviation [r.m.s.d.] = 1.0 Å) except for a long loop consisting of residues 226-245. The loop is largely disordered, probably due to the introduction of compensatory substitution F236A that was predicted to resolve steric clashes for F236 with L303bipA and F271W. Although this region, known as the KMSKS loop, is important for the catalytic mechanism of the enzyme[3], the increased disorder of this loop observed in tyrS.d7 does not substantially impair viability (Fig. 2, Supplementary Table 4). Strong density consistent with a bound tyrosine molecule is observed in the substrate binding pocket as previously observed in the wild type TyrS-tyrosine co-crystal structure (Extended Data Fig. 3d)[2]. The presence of substrate indicates that the redesigned enzyme retains the ability to tightly bind *L*-tyrosine, since no additional tyrosine was added during protein purification or crystallization. Out of seven amino acid substitutions that were introduced in the engineered protein, six (including bipA at residue 303) are clearly visible and lead to very minor conformational changes in the backbone helices (Fig. 1b-d). The side chain rotameric conformations for all six of these redesigned positions agree with the computationally predicted model (Fig. 1d). Redesigned position F236A is on the disordered KMSKS loop and thus is not observed.

Competition between synthetic auxotroph escapees and prototrophs. Any biocontainment mechanism – however robust – is vulnerable to evolutionary pathways through the fitness landscape that lead to survival in nonpermissive conditions. Although escape pathways may be exceedingly rare, it is critical that genotypes along these pathways sufficiently decrease fitness so that escapees are outcompeted in natural ecosystems. In this respect toxin/antitoxin systems are disadvantaged because the primary escape mechanism – ejecting the toxic gene – typically improves fitness. In contrast, escapees of our synthetic auxotrophs are highly impaired under nonpermissive growth conditions (Supplementary Table 4). We quantified the ability of escaped synthetic auxotrophs to compete in an ecosystem using a flow cytometry-based competition assay (Fig. 4).

We clonally isolated escapee strains that emerged both from single-enzyme (pgk.d4) and double-enzyme (adk.d6_tyrS.d8) synthetic auxotrophs and competed them against C321.ΔA, used here as a proxy for prototrophic environmental competitors. From an initial seeding density 100-fold higher than the prototrophs, the single-enzyme pgk.d4 escapees maintain a significant presence (>50% of the total population) as resources become limiting after 8 hours of growth (Fig. 4a).

The continued presence of pgk.d4 in the 8 hour population arises from escapees growing in nonpermissive conditions, even though fitness is impaired in comparison to the prototroph. In contrast, under identical seeding conditions, the prototrophic strain completely overtakes the adk.d6_tyrS.d8 escapees, inverting their relative abundance within 8 hours (Fig. 4b). This extreme effect is largely due to the severe fitness impairment of the adk.d6_tyrS.d8 escapees in nonpermissive conditions. Further, these results emphasize that cross-feeding from prototrophic co-cultures cannot circumvent synthetic auxotrophies. Thus, while GMOs biocontained by multiple synthetic auxotrophies may explore mutations conferring nominal viability under nonpermissive conditions, the associated fitness impairment renders them readily outcompeted by prototrophic microbial competitors.

Quadruplet codon decoding. One possible class of escape mechanisms includes host modifications that suppress recoded UAG positions, such as mutations in endogenous tRNAs or ribosomal subunits. We attempted to safeguard against general suppression of the UAG triplet by mutating UAG in the designed enzymes to UAGA and introducing orthogonal quadruplet codon decoding machinery[4] in adk.d6. This strategy yielded a strain with reduced fitness (1.31-fold increase in doubling time) and a ~4-fold increase in 7 day escape frequency (adk.d6_quad, Supplementary Tables 4 and 5). The increase in escape frequency could arise from reduced fitness of the parental auxotroph, and suggests that host modifications to recognize UAG codons are not primary escape mechanisms for adk.d6.

adk.d4 exhibits poor survivability in stationary phase. Cultures of adk.d4 and adk.d6 were grown in permissive media containing 10 µM bipA (SCAB10) or 50 µM bipA (SCAB50). Cell density at different growth phases was monitored by comparing viable c.f.u. versus OD_{600} (Supplemental Table 8). Strain adk.d6 exhibited the best survival in permissive media containing SCAB10, while SCAB50 was slightly toxic. Strain adk.d4 grew to a lower maximum OD_{600} than adk.d6 under both growth conditions, and its c.f.u./ml did not track with OD_{600}. This suggests that a considerable fraction of the biomass (observed by OD_{600}) is no longer viable (observed by c.f.u.).

SUPPLEMENTARY REFERENCES

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