Supplementary Materials for

Probing the Limits of Genetic Recoding in Essential Genes

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Materials and Methods:

All DNA oligonucleotides (table S8) were purchased with standard purification and desalting from Integrated DNA Technologies. The Oligo Library Synthesis (OLS) array used for synthesizing radically recoded genes was generated on a DNA microchip, processed, and delivered as a ~1-10 pmol lyophilized pool of oligos by Agilent Technologies (Carlsbad, CA).

Cultures were grown at 34 °C with aeration in LB-Lennox (LB^L; 10 g/L Bacto tryptone, 5 g/L sodium chloride, 5 g/L yeast extract) and colonies were grown on LB^L-agar plates (LB^L with 15 g/L Bacto agar). LB^L media was supplemented with one or more of the following selective agents: carbenicillin (50 μg/mL), sodium dodecyl sulfate (SDS; 0.005% w/v), kanamycin (30 μg/mL). Colicin E1 was obtained via expression in strain JC411 (*31*), and purified as previously described.

NAT_*kan*^{R} cassette preparation

Kanamycin resistance (*kan*^{R}) cassettes were inserted *via* λ Red recombination (*26*, *32*) downstream of essential ribosomal genes, in order to test whether polar effects from inserting *kan*^{R} impair fitness. These "NAT_*kan*^{R}" cassettes were PCR amplified using primers that introduced 50 bp of genomic homology on either side of the intended *kan*^{R} insertion site (Kapa HiFi Ready Mix; manufacturer's protocols). PCR products were SPRI purified as previously described (*33*), eluted in deionized water (dH_2O), and checked on a 1% agarose gel for correct size and purity before being recombined as described below.

Recoded gene cassette preparation

Recoded essential genes (table S9) were synthesized from an Agilent OLS array as previously described (*24*). Due to their size, the *prfB* and *rpsA* genes were difficult to synthesize in one piece, so they were each synthesized in two pieces, which were then assembled *via* isothermal assembly (*34*). All synthesized recoded cassettes were fused to a downstream kanamycin resistance gene (*kan*^{R}) *via* isothermal assembly (*34*). The crude isothermal assemblies were PCR amplified using primers (table S8) that introduced 50 bp of genomic homology on either side of the recoded gene and *kan*^{R} (Kapa HiFi Ready Mix; manufacturer's protocols). Full-length cassettes were SPRI purified as previously described (*33*), eluted in dH_2O, and checked on a 1% agarose gel for correct size and purity before being recombined as described below.

Partially recoded cassette preparation

Partially recoded gene cassettes were prepared using the full-length recoded gene cassettes (described above) as template (Kapa HiFi Ready Mix; manufacturer's protocols). While the same reverse primers were used, new forward primers were designed to hybridize inside the recoded cassette and to introduce 50 bp homology regions matching the natural sequence, so that only the C-terminal portion of the gene would be recoded (Figure 1B).

We prepared two types of partially recoded cassettes. The less stringent version recoded exactly half of the gene. The more stringent version recoded all except for the first 30 codons of the gene. Partially recoded cassettes were SPRI purified as previously described (*33*), eluted in

dH_2O, and checked on a 1% agarose gel for correct size and purity before being recombined as described below.

CoS-MAGE selectable marker preparation

To maximize the number of alleles that could simultaneously be replaced per recombinant, we used Co-Selection Multiplex Automated Genome Engineering (CoS-MAGE) with *tolC* or *bla* as co-selectable markers (*18, 27*). In most cases, 90 nt MAGE oligos were designed to replace several forbidden codons. We performed CoS-MAGE in an EcNR2.*xseA*$^-$ background, which has ExoVII inactivated in order to minimize allele loss near the 3' end of the MAGE oligos (*35*). Since the ribosomal genes are clustered in different regions of the genome, selectable markers needed to be placed in multiple different genomic locations in order to provide co-selection in adequate proximity (~500 kb) to the target ribosomal genes. Therefore, we prepared two *tolC* cassettes (*tolC*.3502900 for *rpsL, rplQ, rplO, rpsG, rplD, rpsD, rpsC,* and *rplB*; *tolC*.4427600 for *rpsR*, *rplL*, and *rplJ*) using Kapa HiFi Ready Mix (manufacturer's protocols) and PCR primers that introduced 50 bp of flanking genomic homology (table S8). The *tolC* cassettes were purified using Qiagen's PCR purification kit (manufacturer's protocols, eluted in dH_2O) before being recombined as described in the "gene and allele replacement" methods section. For *rpsA* co-selection, *bla* was already present in the λ prophage of EcNR2.

Gene and allele replacement

All CoS-MAGE oligonucleotides and Nat_*Kan*^{R}, fully recoded, and partially recoded cassettes (described above) were recombined into EcNR2 (*E. coli* MG1655 Δ*mutS*::*cat* Δ(*ybhB-bioAB*)::[λcI857 N(*cro-ea59*)::*tetR-bla*]) as previously described (*18*). Briefly, EcNR2 was grown to mid-log phase (OD_{600} between 0.4 and 0.6), induced to express λ Red for 15 minutes in a 42 °C shaking water bath, and chilled on ice. For each recombination, 1 mL of induced culture was washed twice in 1 mL cold dH_2O, and then the cell pellet was resuspended in 50 µL of dH_2O containing the DNA to be recombined. For PCR products, 1-2 ng/µL was used; to inactivate selectable markers for CoS-MAGE, a 90mer oligonucleotide was used at a final concentration of 1 µM; for CoS-MAGE, 90mer oligonucleotides were pooled at a final concentration of ≤ 5 µM. A BioRad GenePulser™ was used for electroporation (0.1 cm cuvette, 1.78 kV, 200 Ω, 25 µF), and electroporated cells were allowed to recover in 3 ml LB^L in a rotator drum at 34°C for at least 3 hours before plating on appropriate selective media.

Recombinant clones were selected on LB^L-agar supplemented with kanamycin, and then re-streaked on fresh LB^L-agar supplemented with kanamycin to ensure monoclonality. Monoclonal colonies were then grown in a 96-well format (150 µL LB^L supplemented with kanamycin) in preparation for genetic analysis.

To prepare the EcNR2.*xseA*$^-$ strains for CoS-MAGE, we deleted the endogenous *tolC* from the genome using the tolC.90.del oligo and selected for recombinants *via* Colicin E1 selection (*18*). We then separately introduced the *tolC* co-selection cassettes (one per CoS-MAGE strain) and selected on LB^L supplemented with SDS. Finally, we inactivated *tolC* by introducing a nonsense mutation and a frameshift using the tolC-r_null_mut* oligo. For *bla* co-selection, we used the

bla_mut* oligo to inactivate *bla* (present in the λ prophage) and screened for carbenicillin-sensitive recombinants by replica plating on LB^L supplemented with carbenicillin.

CoS-MAGE: CoS-MAGE was performed as previously described (*27*), using 0.5 μM of each MAGE oligo and 0.5 μM of the appropriate co-selection oligo to revert *tolC*.3502900 (*rpsL*, *rplQ*, *rplO*, *rpsG*, *rplD*, *rpsD*, *rpsC*, *rplB*), *tolC*.4427600 (*rpsR*, *rplL*, *rplJ*), or *bla* (*rpsA*). MAGE (without co-selection) (*19*) was performed on *rpsP* and *rpsB* because they were distant from the available co-selectable markers and only had 4 codons to be removed. CoS-MAGE recombinants were selected on LB^L-agar supplemented with SDS (for *tolC*) or LB^L-agar supplemented with carbenicillin (for *bla*), and MAGE recombinants were grown on LB^L-agar without selection. Monoclonal colonies were picked into a 96-well plate and grown under the appropriate selection at 34 °C with shaking.

Recombinant clone genotyping

Recombinant clones were first screened by PCR, then validated by Sanger sequencing.

PCR screens: For the fully recoded genes, we performed 3 PCR reactions for each clone. As diagramed in Fig. 1B, the three sets of primers hybridized to the natural gene sequence (NAT), the recoded gene sequence (SYN), and the flanking genomic region (BND). PCR reactions (10 μL each) were performed with Kapa 2G Fast Ready Mix according to the manufacturer's protocols. Adequate primer specificity was observed with a 58 °C annealing temperature. Desired recombinants had no NAT amplicon, a gene-sized SYN amplicon, and a BND amplicon 847 bp larger than that of the wild type negative control. Partially (C-terminally) recoded recombinants were screened using the NAT forward and SYN reverse primers (desired recombinants had a gene-sized amplicon) and BND primers (desired recombinants showed an 847 bp increase in amplicon size). All putative recombinants that passed the PCR assay were Sanger sequenced (Genewiz or Eton Bioscience Inc.) using the forward BND primers and/or kanR.seqOUT-Nr2.

CoS-MAGE recombinants were typically sequenced without initial Multiplex Allele Specific Colony PCR (MASC-PCR (*18*)) screening because the targeted alleles were too close together to allow for the amplification of discrete bands. However, well-separated alleles were screened via MASC-PCR with standard protocols (*18*) prior to Sanger sequencing validation.

Doubling time analysis

Doubling times (Figure 2, Tables S4 - S5) were determined for all recoded clones using LB^L and Teknova HiDef Azure media. Kinetic growth curves were performed in triplicate on a Biotek H4 plate reader with OD_{600} measurements at 5 minute intervals. Cultures were grown in a flat-bottom 96-well plate (in 150 μL of LB^L supplemented with carbenicillin) with shaking at 34 °C. Doubling times were determined by $t_{double} = c*\ln(2)/m$, where c = 5 minutes per time point and m is the maximum slope of $\ln(OD_{600})$ smoothed across 5 contiguous time points (20 minutes). We typically calculate doubling time in this manner so as to accommodate strains that achieve lower maximum optical densities. Each data point in Figure 2 represents the average doubling time of an individual strain with one ribosomal gene partially or fully recoded (n = 3). Each

replicate was prepared by passaging from the previous one. All strains are based on EcNR2 or EcNR2.xseA$^-$ (doubling times under assay conditions for these strains are 49 +/- 4 minutes in LB^L and 84 +/- 5 minutes in Teknova HiDef Azure Media (12 replicates per condition)).

Supplementary information:

Design parameters for radically recoded genes

- We removed all instances of 13 rare codons (UAG, AGA, AGG, CUU, CUC, CCC, ACC, AUA, GUC, GCC, UCC, CGG, UGA) by replacing them with synonymous codons. Since our goal is to radically change the genetic code, codon removal is merely the first step toward removing and/or reassigning anticodon function, and all codons uniquely recognized by a tRNA or release factor must be changed prior to deletion. Therefore, rather than choosing the 13 rarest codons, we instead targeted codons that are recognized by the least frequently used anticodons. Removing all instances of these codons from the genome would permit the deletion of 10 anticodons (three less than the 13 codons removed, as both CUC and CUU correspond to the same Leu anticodon, both AGG and AGA correspond to the same Arg anticodon, and RF2 is still necessary to terminate UAA in the absence of RF1) and the introduction of 4 nonstandard amino acids into the genetic code (AUA, UAG, CGG, and AGA/AGG codons can be unambiguously reassigned to encode a new amino acid; the introduction of tRNAs for the other codons would cause ambiguous amino acid incorporation due redundant anticodon specificities – see Figure 1A).
- All start codons were changed to AUG (*rpsM* GUG→AUG).
- All non-forbidden codons in radically recoded genes (blue segments in Fig. 3) were swapped with a synonymous codon to reduce nucleotide identity to the natural sequence (see *rpmH* example below, page 4 of SOM). We randomly chose the synonymous replacement codon from a weighted distribution of the remaining possible codons based on their frequency in the *E. coli* MG1655 genome.
- Genes that encoded overlapping coding DNA sequences were modified to remove these overlaps. If another gene overlapped at the start of the coding sequence, the end of this gene was duplicated, and the start codon was removed. If the start codon could not be removed, an in-frame stop-codon was added to prevent translation initiation from the undesired start codon. If a gene overlapped at the end of the coding sequence, we removed the start codon from the recoded sequence. We ensured that the subsequent gene was still translated by duplicating the natural sequence downstream of the recoded sequence. table S10 provides a list of these refactored overlaps.
- Genetically encoded frameshifts were removed (*prfB* CUUU73CUU).
- The following restriction sites were removed: BtsI, BsaI, BsmBI, BspQI, XbaI, and AatII.
- The mRNA secondary structure near the ribosomal binding site was minimized. To accomplish this, we used UNAFold (*36*) to calculate the ΔG for the secondary structure of a 42 bp window centered at the translation start site. The initial design was optimized in order to reduce secondary structure if one of the following two conditions were met: (1) the recoded secondary structure was stronger than the original secondary structure and less than ΔG -7.0 kcal/mol, or (2) the recoded secondary structure was less than ΔG = -10 kcal/mol. To optimize the recoded sequence, all available synonymous codons were varied individually and a new sequence with reduced secondary structure was selected.

For example, below is the comparison between the natural and recoded sequences for *rpmH*. Nucleotide abbreviations are according to IUPAC notation. Yellow highlighting indicates nucleotides that differ in the recoded gene.

>radical recoding *rpmH*

```
ATG AAR CGY ACK TTY CAR CCK WST GTW YTG AAR CGY AAY CGY TCW CAY
GGY TTY CGY GCK CGY ATG GCW ACK AAR AAY GGY CGY CAR GTK YTG GCR
CGY CGY CGY GCW AAR GGY CGY GCK CGY YTR ACS GTK TCW AAR TAA
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Partial replacement with full-length recoded cassettes

Fully recoded *rpmH* and *rplT* cassettes repeatedly produced partially recoded recombinants (wild type until C81 and T147, respectively). In both cases, the position of the crossover was shifted upstream by using partially recoded constructs that preserved the wild type N-termini (Figure S2). This indicates that the full-length cassette 1) had a lethal design element in its N-terminus and/or 2) had poorly recominogenic homology sequence.

Double mutants with full-length recoded cassettes

To understand the effect of combining multiple recoded genes in a single strain, we transcriptionally fused the recoded rplM_syn1 variant (third slowest doubling time) or rpsI_syn1 variant (fourth slowest doubling time) to a spectinomycin resistance gene, generated double mutants in rplP_syn1 (slowest doubling time and contains ATA forbidden codon), rpmC_syn1 (second slowest doubling time), and rplE_syn1 (normal doubling time), and selected the highest fitness recombinant exhibiting the desired genotype. When grown in LB^L without antibiotic supplementation, all double mutants grew faster than expected assuming additive fitness defects for independent mutations (fig. S1, table S11). It is possible that compensatory off-target mutations facilitated by inactive mismatch repair may alleviate growth impairment, and double mutants may exhibit varying fitness effects due to ribosomal protein autoregulation (*23*).

Remaking rplP_syn2, rpsS_syn2, and rpmD_syn2

We re-sequenced all gene replacement strains (table S4) to confirm that no mutations had occurred during extended growth. We observed a G36A mutation in rplP_syn1, which introduced a forbidden AUA codon; a C5T mutation in rpsS_syn1, which introduced a forbidden CUU codon; and a putative duplication in rpmD_syn1, resulting in the presence of both a natural and recoded copy of *rpmD* in the same genome.

For *rplP*, the wild type AUG codon resulted in an extreme fitness disadvantage, which provided a strong selection for the spontaneous G36A mutation (AUG→AUA change). Therefore, we attempted to change the forbidden AUA codon to all other Ile (AUC and AUU) and Met (AUG) codons using MAGE. While AUG was not observed, presumably due to an extreme fitness disadvantage, AUU and AUC were well-tolerated, leading to rplP_syn2. Since this mutation was intended, it was not counted as a synthesis error or represented by a yellow line in Fig. 3.

For *rpsS*, resequencing revealed a forbidden CUU codon. We re-amplified the *rpsS* gene with primers that changed this forbidden CUU to all permitted Pro (CCA, CCG, and CCU) and Leu (UUA, UUG, CUA, and CUG) codons. Codons CCA and CCG (but not CCU) were observed for Pro. Codons UUA, UUG, CUA, and CUG were observed for Leu. Additionally, we allowed the

subsequent CGC Codon be shuffled to CGA, CGU, and CGC. We selected a clone, rpsS_syn2, with intended mutations T6A and C9A. Since these mutations were intended, they were not counted as synthesis errors or represented by yellow lines in Fig. 3.

For *rpmD*, we repeated the insertion of the original synthetic *rpmD* gene, yielding a clone, rpmD_syn2, with the correct genotype.

Supplemental Figures:

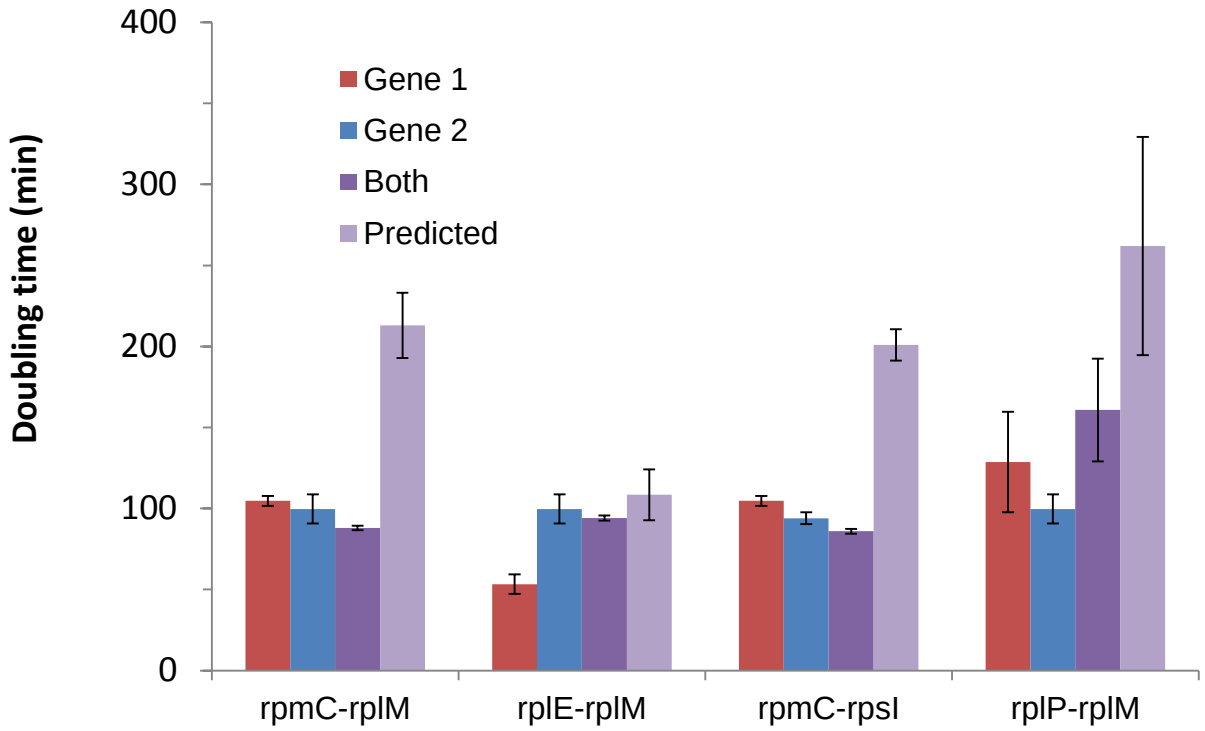

fig. S1. Doubling times of double mutants compared to single mutants. Synthetic gene 2 (*rplM* or *rpsI*) was transcriptionally fused to a spectinomycin resistance gene and recombined into strains rpmC_syn1, rplE_syn1, and rplP_syn1. Double mutants that were resistant to both kanamycin and spectinomycin were isolated, Sanger sequence verified, and assayed for doubling time in LB^L without antibiotic supplementation. The double mutant exhibiting the fastest doubling time for each gene pair was chosen. Data bars represent the doubling times of the gene 1 syn strain (red), gene 2 syn strain (blue), double mutant (dark purple), and predicted doubling time of the double mutant assuming that fitness defects are independent (light purple). Error bars are the standard deviation of 3 technical replicates; variances for the predictions are the square root of the summed squared variances of the two measured strains.

Supplemental Tables:

table S1. Recoded essential gene design attributes

Gene	Length	Forbidden codons	Number of changes	% nt identity	Total codons	Identical codons	Changed codons	% codon identity
rpmH	141	1	46	67.4%	47	3	44	6.38%
rpmD	180	4	59	67.2%	60	5	55	8.33%
rpmC	192	2	71	63.0%	64	3	61	4.69%
rpsR	228	6	84	63.2%	76	2	74	2.63%
rpmB	237	7	84	64.6%	79	4	75	5.06%
rpsP	249	1	86	65.5%	83	3	80	3.61%
rpsQ	255	5	88	65.5%	85	4	81	4.71%
rpmA	258	3	92	64.3%	86	2	84	2.33%
rpsS	279	6	96	65.6%	93	5	88	5.38%
rplW	303	10	101	66.7%	101	6	95	5.94%
rpsN	306	6	106	65.4%	102	7	95	6.86%
rplU	312	3	105	66.3%	104	5	99	4.81%
rpsJ	312	10	104	66.7%	104	3	101	2.88%
rplX	315	10	111	64.8%	105	4	101	3.81%
rplV	333	8	119	64.3%	111	3	108	2.70%
rplS	348	8	127	63.5%	116	4	112	3.45%
rplR	354	10	124	65.0%	118	2	116	1.69%
rplT	357	3	126	64.7%	119	4	115	3.36%
rpsM	357	13	119	66.7%	119	4	115	3.36%
rplL	366	1	122	66.7%	122	7	115	5.74%
rplN	372	10	132	64.5%	124	6	118	4.84%
rpsL	375	9	131	65.1%	125	3	122	2.40%
rplQ	384	4	138	64.1%	128	5	123	3.91%
rpsK	390	8	130	66.7%	130	5	125	3.85%
rpsH	393	13	135	65.6%	131	8	123	6.11%
rpsI	393	8	140	64.4%	131	6	125	4.58%
rplP	411	7	137	66.7%	137	9	128	6.57%
rplM	429	7	134	68.8%	143	10	133	6.99%
rplO	435	5	152	65.1%	145	7	138	4.83%
rplJ	498	11	176	64.7%	166	7	159	4.22%
rpsE	504	15	169	66.5%	168	9	159	5.36%
rplF	534	12	182	65.9%	178	5	173	2.81%
rplE	540	14	186	65.6%	180	8	172	4.44%
rpsG	540	14	192	64.4%	180	8	172	4.44%
rplD	606	11	210	65.3%	202	10	192	4.95%
rpsD	621	12	224	63.9%	207	6	201	2.90%
rplC	630	14	216	65.7%	210	8	202	3.81%
rpsC	702	9	237	66.2%	234	11	223	4.70%
rpsB	726	12	250	65.6%	242	14	228	5.79%
rplB	822	13	284	65.5%	274	10	264	3.65%
prfB	1098	46	388	64.7%	366	17	349	4.64%
rpsA	1674	34	583	65.2%	558	17	541	3.05%
Total	**18759**	**405**	**6496**	**65.4%**	**6253**	**269**	**5984**	**4.44%**

table S2. Genes with forbidden codons successfully removed after each phase of recoding

Gene	Operon location[a]	*Kan*^{R} only[b]	Full cassettes[c]	Partial cassettes[d]	CoS-MAGE
rpmH	Start				
rpmD	Middle				
rpmC	Middle				
rpsR	Middle			5/6	
rpmB	Complex				
rpsP	Start			0/1	
rpsQ	End				
rpmA	End				
rpsS	Middle				
rpsN	Middle				
rplU	Start				
rpsJ	Start				
rplX	Middle				
rplW	Middle				
rplV	Middle				
rplS	End				
rplR	Middle				
rplT	Complex				
rpsM	Start				
rplL	Complex			0/1	
rplN	Start				
rpsL	Start			6/9	
rplQ	End				
rpsK	Middle				
rpsI	End				
rpsH	Middle				
rplM	Start				
rplP	Middle				
rplO	Middle	Not observed			
rplJ	Complex				
rpsE	Middle				
rplF	Middle				
rplE	Middle				
rpsG	Middle				
rplD	Middle				
rpsD	Middle			7/12	
rplC	Middle				
rpsC	Middle			3/9	
rpsB	Middle			9/12	
rplB	Middle				
prfB	Complex				
rpsA	Complex				

[a] Start = first ORF in operon, Middle = flanked on both sides by other ORFs in same operon, End = last ORF in operon, Complex = multiple overlapping transcriptional units

[b] Purple indicates successful insertion of *kan*^{R} into the operon without recoding

[c] Dark green indicates genes with all forbidden codons removed during that phase

[d] Lime green indicates genes that had all forbidden codons removed in a previous phase; light green indicates genes with a subset of their forbidden codons removed (instances removed/total instances) by partially recoded cassettes

table S3. Forbidden codons remaining after each phase of recoding

Codon removed	Natural assignment	Instances in genome	Instances in targeted essential genes	Fully recoded cassettes[a]	Partially recoded cassettes[b]	CoS-MAGE[c]
UAG	STOP (RF1)	321	0	0	0	0
AGA/AGG	Arg	4,228	5	1	1	0
CUU/CUC	Leu	30,030	50	19	14	0*
CCC	Pro	7,401	3	1	1	0
ACC	Thr	31,766	133	53	37	0
AUA	Ile	5,797	1	0	0	0
GUC	Val	20,757	59	12	10	0
GCC	Ala	34,747	65	22	18	0
UCC	Ser	11,672	82	35	27	0
CGG	Arg	7,273	3	1	1	0
UGA	STOP (RF2)	1,232	4	2	2	0
	Total remaining	**155,224**	**405**	**146**	**111**	**0**

[a] Instances of each forbidden codon remaining after recombination with fully recoded cassettes
[b] Instances of each forbidden codon remaining after recombination with partially recoded cassettes
[c] Instances of each forbidden codon remaining after CoS-MAGE
*Original desired *rplQ* U162G (CUU→CUG) change was not observed, but this was overcome using diversity (see table S7)

table S4. Gene replacement strain summary and doubling times

							NAT_*Kan*^{R} LB^L doubling time (min)[c]			NAT_*Kan*^{R} Azure doubling time (min)[c]			SYN_*Kan*^{R} LB^L doubling time (min)[d,e]			SYN_*Kan*^{R} Azure doubling time (min)[d,e]		
Strain name[a]	**Gene**	**Switch-over index**[b]	**Forbidden codons removed**	**Unintended mismatches**	**Unintended deletions**	**Total mutations**	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**
rpmH_chim1	rpmH	69	1	0	0	17	56	60	59	93	102	104	52	52	48	80	77	77
rpmD_syn1	rpmD	0	4	0	0	59	75	77	76	111	122	130	55	57	46	113	117	116
rpmD_syn2	rpmD	0	4	0	0	59	75	77	76	111	122	130	89	94	91	169	175	171
rpmC_syn1	rpmC	0	2	0	0	71	55	56	55	105	102	83	108	104	102	128	110	117
rpsR_chim1	rpsR	90	5	0	0	54	49	50	52	91	90	79	78	61	61	95	78	92
rpmB_syn1	rpmB	0	7	2	0	84	51	49	51	106	87	85	58	59	63	137	131	137
rpsP_chim1	rpsP	92	0	0	0	54	49	49	50	105	79	83	54	53	61	104	115	104
rpsQ_syn1	rpsQ	0	5	0	0	88	61	60	55	103	98	69	70	68	65	102	97	93
rpmA_syn1	rpmA	0	3	1	0	91	47	48	47	80	78	79	66	66	74	85	93	90
rpsS_syn1	rpsS	0	6	1	0	96	51	51	53	100	105	97	64	57	63	123	97	99
rpsS_syn2	rpsS	0	6	0	0	96	51	51	53	100	105	97	49	50	50	80	79	76
rplW_syn1	rplW	0	10	2	0	102	42	54	52	114	110	109	67	69	61	113	111	124
rpsN_syn1	rpsN	0	6	3	0	107	52	52	53	86	92	84	68	69	69	69	91	89
rplU_syn1	rplU	0	3	0	0	105	49	49	49	85	89	65	74	74	74	140	129	138
rpsJ_syn1	rpsJ	0	10	1	0	105	54	55	55	77	81	73	59	61	63	82	113	107
rplX_syn1	rplX	0	10	0	0	111	91	93	88	121	117	113	57	55	53	105	87	83
rplV_syn1	rplV	0	8	0	0	119	52	53	52	97	101	90	90	69	61	119	107	119
rplS_syn1	rplS	0	8	2	0	127	48	50	51	97	87	84	57	58	56	105	120	90
rplR_syn1	rplR	0	10	3	0	125	51	50	50	57	80	75	54	53	47	87	96	79
rplT_chim1	rplT	92	3	1	0	96	61	56	56	104	96	96	69	67	66	98	94	88
rpsM_syn1	rpsM	0	13	1	1	119	56	57	59	92	88	81	69	62	62	95	85	104
rplL_chim1	rplL	90	1	0	0	94	63	63	61	92	110	106	54	56	51	90	88	68
rplN_syn1	rplN	0	10	2	0	132	61	63	53	60	77	61	49	51	48	92	70	90
rpsL_chim1	rpsL	186	6	1	1	65	56	54	57	71	83	80	75	78	77	103	101	95
rpsK_syn1	rpsK	0	8	0	0	130	54	52	54	79	87	93	95	79	83	107	111	95
rpsI_syn1	rpsI	0	8	1	0	141	51	51	42	92	90	85	95	97	90	104	107	124
rpsH_syn1	rpsH	0	13	0	0	135	53	55	50	101	77	78	64	63	58	97	86	99
rplP_syn1	rplP	0	7	1	0	137	58	59	54	93	92	110	164	116	106	158	112	158
rplP_syn2	rplP	0	7	0	0	137	58	59	54	93	92	110	67	65	61	134	136	138
rplM_syn1	rplM	0	7	1	0	135	58	59	52	95	98	83	110	94	95	141	149	134
rpsE_syn1	rpsE	0	15	0	0	169	58	62	50	111	134	95	51	54	51	108	105	102
rplF_syn1	rplF	0	12	1	0	183	71	75	57	139	134	153	60	84	72	105	110	89
rplE_syn1	rplE	0	14	0	0	186	60	65	46	123	142	125	47	59	54	101	81	89
rpsD_chim1	rpsD	90	7	0	0	190	70	72	67	98	77	93	74	72	66	119	120	130
rplC_syn1	rplC	0	14	1	0	216	58	61	58	104	88	83	73	77	97	151	127	127
rpsC_chim1	rpsC	351	3	0	1	118	57	58	54	93	82	105	60	58	57	129	109	164

rpsB_chim1	rpsB	90	9	2	0	225	50	45	45	93	87	92	46	53	48	90	79	69
prfB_syn1	prfB	0	46	1	0	389	55	57	55	86	91	75	56	60	49	110	97	82
	Total:	**294**		**26**	**3**	**4375**												

[a] Strains are named for their recoded genes; "syn" indicates fully recoded; "chim" indicates partially recoded; the original "syn" strains rpmD_syn1, rpsS_syn1, and rplP_syn1 gained forbidden codons, so an additional clone was generated and characterized for each gene (rpmD_syn2, rpsS_syn2, and rplP_syn2). Although the original rpmD_syn1, rpsS_syn1, and rplP_syn1 strains are still reported in gray letters, their forbidden codons removed, unintended mismatches, and unintended deletions were not included in the totals at the bottom of the table.

[b] Beginning of radically recoded portion in partially recoded genes

[c] NAT_*Kan*^{R} indicates that a kanR gene was inserted without recoding the target gene

[d] SYN_*Kan*^{R} indicates that the target gene was radically recoded

[e] Some doubling times appear to decrease across subsequent replicates, possibly indicating that spontaneous mutagenesis improves fitness. We note that each strain was passaged at least twice prior to sequence verification.

table S5. CoS-MAGE strain summary and doubling times

				NAT_*Kan*^{R} LB^L doubling time (min)[c]			NAT_*Kan*^{R} Azure doubling time (min)[c]			CoS-MAGE LB^L doubling time (min)			CoS-MAGE Azure doubling time (min)		
Strain name[a]	**Gene**	**Alleles targeted**	**Alleles converted**[b]	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**	**Rep1**	**Rep2**	**Rep3**
rpsR_CoS1	rpsR	1	1	49	50	52	91	90	79	50	50	51	58	72	56
	rpsP	1	1	49	49	50	105	79	83						
	rpsB	3	3	50	45	45	93	87	92						
rpsR_CoS2	rpsR	1	1	49	50	52	91	90	79	50	50	53	60	72	61
	rplL	1	1	63	63	61	92	110	106						
	rpsB	3	3	50	45	45	93	87	92						
rpsL_CoS1	rpsL	3	3	56	54	57	71	83	80	49	49	49	44	64	62
rplQ_CoS1	rplQ	4	1	64	66	66	113	109	100	54	50	50	110	90	79
rplQ_CoS2	rplQ	4	1	64	66	66	113	109	100	53	49	50	62	92	74
rplQ_CoS3	rplQ	4	1	64	66	66	113	109	100	49	49	48	80	72	78
rplQ_CoS4	rplQ	4	1	64	66	66	113	109	100	50	50	47	65	80	68
rplQ_CoS5	rplQ	4	1	64	66	66	113	109	100	48	48	48	85	85	71
rplQ_CoS6	rplQ	4	1	64	66	66	113	109	100	49	48	48	75	72	86
rplQ_CoS7	rplQ	4	1	64	66	66	113	109	100	51	50	48	87	74	65
rplQ_CoS8	rplQ	4	1	64	66	66	113	109	100	48	49	48	76	92	92
rplQ_CoS9	rplQ	4	1	64	66	66	113	109	100	50	49	48	69	76	92
rplQ_CoS10	rplQ	4	1	64	66	66	113	109	100	53	52	52	106	95	96
rplQ_CoS11	rplQ	4	1	64	66	66	113	109	100	53	49	50	78	74	40
rplQ_CoS12	rplQ	4	1	64	66	66	113	109	100	49	54	53	80	116	114
	rpsC	6	2	57	58	54	93	82	105						
rplO_CoS1	rplO	5	2	nr	nr	nr	nr	nr	nr	46	51	49	101	91	100
rplO_CoS2	rplO	5	2	nr	nr	nr	nr	nr	nr	48	51	50	72	66	72
rplO_CoS3	rplO	5	1	nr	nr	nr	nr	nr	nr	48	54	51	70	65	72
rplJ_CoS1	rplJ	11	3	51	51	49	114	87	90	48	49	49	67	58	49
rplJ_CoS2	rplJ	11	3	51	51	49	114	87	90	60	57	56	84	59	56
rplJ_CoS3	rplJ	11	3	51	51	49	114	87	90	53	51	52	89	102	87
rplJ_CoS4	rplJ	11	3	51	51	49	114	87	90	52	51	52	83	68	62
rplJ_CoS5	rplJ	11	2	51	51	49	114	87	90	55	51	53	88	78	68
rplJ_CoS6	rplJ	11	2	51	51	49	114	87	90	51	48	49	70	78	68
rplJ_CoS7	rplJ	11	1	51	51	49	114	87	90	49	51	54	93	88	99
	rpsA	34	1	48	50	44	93	95	100						
rpsG_CoS1	rpsG	14	10	49	50	44	84	83	84	51	53	52	61	79	97
rpsG_CoS2	rpsG	14	7	49	50	44	84	83	84	51	53	53	76	82	63
rpsG_CoS3	rpsG	14	7	49	50	44	84	83	84	48	53	52	86	106	72
rpsD_CoS1	rpsD	5	3	70	72	67	98	77	93	52	55	56	98	100	99
rpsD_CoS2	rpsD	5	3	70	72	67	98	77	93	56	51	51	84	94	85
rplD_CoS1	rplD	11	2	52	53	54	104	108	105	51	50	52	97	77	60

rplD_CoS2	rplD	11	4	52	53	54	104	108	105	49	53	49	86	92	90
rplD_CoS3	rplD	11	7	52	53	54	104	108	105	48	52	51	51	78	73
rplD_CoS4	rplD	11	1	52	53	54	104	108	105	49	48	49	55	83	86
	rpsA	34	1	48	50	44	93	95	100						
rpsC_CoS1	rpsC	6	4	57	58	54	93	82	105	52	52	49	102	85	70
rpsC_CoS2	rpsC	6	4	57	58	54	93	82	105	50	54	54	62	56	57
rplB_CoS1	rplB	13	3	50	53	45	88	75	87	50	54	54	73	58	59
rplB_CoS2	rplB	13	1	50	53	45	88	75	87	61	63	63	84	95	93
rplB_CoS3	rplB	13	4	50	53	45	88	75	87	50	56	50	71	77	65
rplB_CoS4	rplB	13	7	50	53	45	88	75	87	50	54	51	76	50	68
rpsA_CoS1	rpsA	34	19	48	50	44	93	95	100	45	50	43	76	53	61
rpsA_CoS2	rpsA	34	7	48	50	44	93	95	100	47	50	44	90	79	91
rpsA_CoS3	rpsA	34	12	48	50	44	93	95	100	47	51	44	103	84	77
rpsA_CoS4	rpsA	34	16	48	50	44	93	95	100	46	51	43	89	85	89
rpsA_CoS5	rpsA	34	15	48	50	44	93	95	100	45	49	45	88	90	89

[a] 45 total CoS-MAGE strains

[b] 187 total forbidden codons (111 unique positions) removed across 45 CoS-MAGE strains

[c] nr indicates that no recombinants were observed

table S6. Oligos used to replace rplQ CUU 160-162

Oligo	Sequence
rplQ_CTT162YTR*	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggc**YAR**agtaatcagcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162ATY*	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggc**RAT**agtaatcagcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162GTD*	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggc**HAC**agtaatcagcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162GCD*	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggc**HGC**agtaatcagcggctcaactacgcggcgcagctctttcgct

table S7. Successful rplQ CUU 160-162 replacements

Amino acid	Codon	Codon count
Leu (unchanged)	CUU	57
Leu (synonymous)	CUA	4
	CUG	0
	UUA	4
	UUG	0
Ile	AUC	5
	AUU	6
Val	GUU	8
	GUA	5
	GUG	0
Ala	GCU	2
	GCA	2
	GCG	3
	Total	**96**

Codons are color-coded for each amino acid. CUG, UUG, and GUG codons (red) were never observed to replace CUU at positions 160-162.

table S8. Primers and oligonucleotides used in this study.

An asterisk (*) indicates a phosphorothioate bond used to protect against exonuclease activity.

Oligo name	Purpose	Oligo sequence
gfp-rpmH	forward gene primer for generating rpmH recoded cassette	ggatatccaataaagccattga
gfp-rpmD	forward gene primer for generating rpmD recoded cassette	tcgctgccaagcgtggtaa
gfp-rpmC	forward gene primer for generating rpmC recoded cassette	gcagcgaaactgccga
gfp-rpsR	forward gene primer for generating rpsR recoded cassette	ggttttgcatgccgagcag
gfp-rpmB	forward gene primer for generating rpmB recoded cassette	gccaataccccatacgaag
gfp-rpsP	forward gene primer for generating rpsP recoded cassette	actccgttcctcgatgg
gfp-rpsQ	forward gene primer for generating rpsQ recoded cassette	cgcgatgtcgcacgcg
gfp-rpmA	forward gene primer for generating rpmA recoded cassette	gatgtgaaaattactggcatca
gfp-rpsS	forward gene primer for generating rpsS recoded cassette	tgataaattcatcgtacgtcgcc
gfp-rpsN	forward gene primer for generating rpsN recoded cassette	ctgctggctgcctttg
gfp-rplU	forward gene primer for generating rplU recoded cassette	atagcgcactctgaatcattgaaaa
gfp-rpsJ	forward gene primer for generating rpsJ recoded cassette	gtctgaggagtaatcattttcgtt
gfp-rplX	forward gene primer for generating rplX recoded cassette	gttcatgaaaattatctctctggc
gfp-rplW	forward gene primer for generating rplW recoded cassette	acaaagtcgtaatgactgctg
gfp-rplV	forward gene primer for generating rplV recoded cassette	ggccacgctgctgataaa
gfp-rplS	forward gene primer for generating rplS recoded cassette	atggcgtaagcccccg
gfp-rplR	forward gene primer for generating rplR recoded cassette	ccgacgaagtcgtgcgta
gfp-rplT	forward gene primer for generating rplT recoded cassette	cgttaacgtttttaacttttaattagaatataga
gfp-rpsM	forward gene primer for generating rpsM recoded cassette	aaacgggcttttcagca
gfp-rplL	forward gene primer for generating rplL recoded cassette	aacgcattcgcttacgtataaa
gfp-rplN	forward gene primer for generating rplN recoded cassette	cgacctgattttcgggtctc
gfp-rpsL	forward gene primer for generating rpsL recoded cassette	acgttttattacgtgtttacgaag
gfp-rplQ	forward gene primer for generating rplQ recoded cassette	tgacgagtaaccggatcac
gfp-rpsK	forward gene primer for generating rpsK	ccgtaagggtccgcgc

	recoded cassette	
gfp-rpsI	forward gene primer for generating rpsI recoded cassette	acgcggcacagcaacc
gfp-rpsH	forward gene primer for generating rpsH recoded cassette	aaaaggctagctggtaattgt
gfp-rplM	forward gene primer for generating rplM recoded cassette	agacgtttgggtgttca
gfp-rplP	forward gene primer for generating rplP recoded cassette	ctcagcctaaaaagcagca
gfp-rplO	forward gene primer for generating rplO recoded cassette	gcggtatgatcaacgcgg
gfp-rplJ	forward gene primer for generating rplJ recoded cassette	tgaagtgagttccagagatttt
gfp-rpsE	forward gene primer for generating rpsE recoded cassette	gcagatgctgcccgtg
gfp-rplF	forward gene primer for generating rplF recoded cassette	ctggtcttggtggcgaa
gfp-rplE	forward gene primer for generating rplE recoded cassette	cgtttcttcaagtctaacagcg
gfp-rpsG	forward gene primer for generating rpsG recoded cassette	ctaaactcgtagagttttggacaa
gfp-rpsD	forward gene primer for generating rpsD recoded cassette	aaaaacgtcgcgtataacgcc
gfp-rplD	forward gene primer for generating rplD recoded cassette	cggtagcgacctgatcgt
gfp-rplC	forward gene primer for generating rplC recoded cassette	cagatcagcctgggtt
gfp-rpsC	forward gene primer for generating rpsC recoded cassette	agccacatcactgtggttg
gfp-rpsB	forward gene primer for generating rpsB recoded cassette	tatgggatacgtggaggca
gfp-rplB	forward gene primer for generating rplB recoded cassette	cagaatctggacttcgttgg
gfp-prfB	forward gene primer for generating prfB recoded cassette	tcccgctcttatcaccg
gfp-rpsA	forward gene primer for generating rpsA recoded cassette	catccggcatggagcc
grp-rpmH	reverse gene primer for generating rpmH recoded cassette	gacgtctttctagattattttgacacc
grp-rpmD	reverse gene primer for generating rpmD recoded cassette	gacgtctttctagattattcttccac
grp-rpmC	reverse gene primer for generating rpmC recoded cassette	gacgtctttctagattaagcg
grp-rpsR	reverse gene primer for generating rpsR recoded cassette	gacgtctttctagattattggtga
grp-rpmB	reverse gene primer for generating rpmB recoded cassette	gacgtctttctagattaatatttctcacc
grp-rpsP	reverse gene primer for generating rpsP recoded cassette	gacgtctttctagattatgcag
grp-rpsQ	reverse gene primer for generating rpsQ recoded cassette	gacgtctttctagattacaacaca
grp-rpmA	reverse gene primer for generating rpmA recoded cassette	gacgtctttctagattactccg

grp-rpsS	reverse gene primer for generating rpsS recoded cassette	gacgtctttctagattacttttttttg
grp-rpsN	reverse gene primer for generating rpsN recoded cassette	gacgtctttctagattaccacga
grp-rplU	reverse gene primer for generating rplU recoded cassette	gacgtctttctagattacgcc
grp-rpsJ	reverse gene primer for generating rpsJ recoded cassette	gacgtctttctagattagccca
grp-rplX	reverse gene primer for generating rplX recoded cassette	gacgtctttctagattatttaatcgtttc
grp-rplW	reverse gene primer for generating rplW recoded cassette	gacgtctttctagattattctgcac
grp-rplV	reverse gene primer for generating rplV recoded cassette	gacgtctttctagattatcggtc
grp-rplS	reverse gene primer for generating rplS recoded cassette	gacgtctttctagattaattcaggc
grp-rplR	reverse gene primer for generating rplR recoded cassette	gacgtctttctagattaaaattgcag
grp-rplT	reverse gene primer for generating rplT recoded cassette	gacgtctttctagattacgctaac
grp-rpsM	reverse gene primer for generating rpsM recoded cassette	gacgtctttctagattacttttttaataggc
grp-rplL	reverse gene primer for generating rplL recoded cassette	gacgtctttctagattacttcacc
grp-rplN	reverse gene primer for generating rplN recoded cassette	gacgtctttctagattataacacct
grp-rpsL	reverse gene primer for generating rpsL recoded cassette	gacgtctttctagattacgcttt
grp-rplQ	reverse gene primer for generating rplQ recoded cassette	gacgtctttctagattattcagcc
grp-rpsK	reverse gene primer for generating rpsK recoded cassette	gacgtctttctagattacactcgg
grp-rpsI	reverse gene primer for generating rpsI recoded cassette	gacgtctttctagattagcg
grp-rpsH	reverse gene primer for generating rpsH recoded cassette	gacgtctttctagattacgcaac
grp-rplM	reverse gene primer for generating rplM recoded cassette	gacgtctttctagattaaatatccag
grp-rplP	reverse gene primer for generating rplP recoded cassette	gacgtctttctagattacataacag
grp-rplO	reverse gene primer for generating rplO recoded cassette	gacgtctttctagattactcctca
grp-rplJ	reverse gene primer for generating rplJ recoded cassette	gacgtctttctagattacgctg
grp-rpsE	reverse gene primer for generating rpsE recoded cassette	gacgtctttctagattacttacct
grp-rplF	reverse gene primer for generating rplF recoded cassette	gacgtctttctagattatttttttttcg
grp-rplE	reverse gene primer for generating rplE recoded cassette	gacgtctttctagattatttacgaaatgg
grp-rpsG	reverse gene primer for generating rpsG recoded cassette	gacgtctttctagattagttcagataa
grp-rpsD	reverse gene primer for generating rpsD recoded cassette	gacgtctttctagattattttgaatac

grp-rplD	reverse gene primer for generating rplD recoded cassette	gacgtctttctagattacgcc
grp-rplC	reverse gene primer for generating rplC recoded cassette	gacgtctttctagattatgctttaac
grp-rpsC	reverse gene primer for generating rpsC recoded cassette	gacgtctttctagattactttcgt
grp-rpsB	reverse gene primer for generating rpsB recoded cassette	gacgtctttctagattattccgc
grp-rplB	reverse gene primer for generating rplB recoded cassette	gacgtctttctagattacttcgat
grp-prfB	reverse gene primer for generating prfB recoded cassette	gacgtctttctagattataggc
grp-rpsA	reverse gene primer for generating rpsA recoded cassette	tgacgtctttctagattattcacc
kfp-rpmH	forward kanR primer for generating rpmH recoded cassette	gtgcgcgcttaacggtgtcaaaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpmD	forward kanR primer for generating rpmD recoded cassette	cgtttatggtgaaggtggaagaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpmC	forward kanR primer for generating rpmC recoded cassette	tgttgaatgaaaaagctggcgcttaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsR	forward kanR primer for generating rpsR recoded cassette	taccttatacggaccgtcaccaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpmB	forward kanR primer for generating rpmB recoded cassette	tacgcgcgcgcggtgagaaatattaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsP	forward kanR primer for generating rpsP recoded cassette	ttaaggaggtgaataaggctgcataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsQ	forward kanR primer for generating rpsQ recoded cassette	gtgtggtggaaaaggctgtgttgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpmA	forward kanR primer for generating rpmA recoded cassette	agttcatttcgattgaggcggagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsS	forward kanR primer for generating rpsS recoded cassette	cagacaagaaggcaaaaaaaaagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsN	forward kanR primer for generating rpsN recoded cassette	caggcctaaagaaagcatcgtggtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplU	forward kanR primer for generating rplU recoded cassette	taaagatcacaggtatttcggcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsJ	forward kanR primer for generating rpsJ recoded cassette	tggatgttcaaattagtttgggctaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplX	forward kanR primer for generating rplX recoded cassette	aaagcaattcggaaacgattaaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplW	forward kanR primer for generating rplW recoded cassette	tggattttgtaggaggtgcagaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplV	forward kanR primer for generating rplV recoded cassette	ttacggttgtggtaagcgaccgataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplS	forward kanR primer for generating rplS recoded cassette	cgcgaattaaggaacgcctgaattaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplR	forward kanR primer for generating rplR recoded cassette	cacgcgaggcgggactgcaattttaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplT	forward kanR primer for generating rplT recoded cassette	agaaggctaaggctgcgttagcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsM	forward kanR primer for generating rpsM recoded cassette	gcccacgtaagcctattaaaaagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplL	forward kanR primer for generating rplL recoded cassette	caggtgcagaggtagaggtgaagtaatctagaaagacgttgagttgtcgagattttcagg

kfp-rplN	forward kanR primer for generating rplN recoded cassette	ttagcttggcgccggaggtgttataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsL	forward kanR primer for generating rpsL recoded cassette	acggtgttaaacgaccgaaagcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplQ	forward kanR primer for generating rplQ recoded cassette	aaaaggctgaggcagcggctgaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsK	forward kanR primer for generating rpsK recoded cassette	gccctccaaagaagcgccgagtgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsI	forward kanR primer for generating rpsI recoded cassette	gccgacctcaatttagtaagcgctaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsH	forward kanR primer for generating rpsH recoded cassette	gtgagatcatttgttatgttgcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplM	forward kanR primer for generating rplM recoded cassette	aacagcctcaggtgctggatatttaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplP	forward kanR primer for generating rplP recoded cassette	cattcgtgacgaaaactgttatgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplO	forward kanR primer for generating rplO recoded cassette	cggcgggtggcaagattgaggagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplJ	forward kanR primer for generating rplJ recoded cassette	tgcgtgacgctaaggaggcagcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsE	forward kanR primer for generating rpsE recoded cassette	gcgtggaggagatcctaggtaagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplF	forward kanR primer for generating rplF recoded cassette	gcactaaggaagcgaaaaaaaaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplE	forward kanR primer for generating rplE recoded cassette	cgttcgattttccatttcgtaaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsG	forward kanR primer for generating rpsG recoded cassette	aaccggcgttaggttatctgaactaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsD	forward kanR primer for generating rpsD recoded cassette	taattgtggaattgtattcaaaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplD	forward kanR primer for generating rplD recoded cassette	aacaggtagaagaaatgttggcgtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rplC	forward kanR primer for generating rplC recoded cassette	ttgtgaagccggcggttaaagcataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsC	forward kanR primer for generating rpsC recoded cassette	aacaacaacgcaagggacgaaagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsB	forward kanR primer for generating rpsB recoded cassette	aggagtcttttgttgaggcggaataatctagaaagacgttgagttgtcgagattttcagg
kfp-rplB	forward kanR primer for generating rplB recoded cassette	ttattgtgcgccgtcgatcgaagtaatctagaaagacgttgagttgtcgagattttcagg
kfp-prfB	forward kanR primer for generating prfB recoded cassette	aggcgtctttaaaggcgggcctataatctagaaagacgttgagttgtcgagattttcagg
kfp-rpsA	forward kanR primer for generating rpsA recoded cassette	atttaaggcggcgaagggtgaataatctagaaagacgtctgagttgtcgagattttcagg
krp-rpmH	reverse kanR primer for generating rpmH recoded cassettes and NAT_*kan*^{R}	agcgtaactccctgggaaatgcgagcttaaccactcaggggttagctttattagaaaaactcatcgagcatc
krp-rpmD	reverse kanR primer for generating rpmD recoded cassettes and NAT_*kan*^{R}	cccgccttttggagccttcggccggagacagagtatttaaacgcatctcttagaaaaactcatcgagcatc
krp-rpmC	reverse kanR primer for generating rpmC recoded cassettes and NAT_*kan*^{R}	cattttgtcgctaacaacgcgaccttgcagagtacggattttatcggtcattacgcacccgccttcttagaaaaactcatcgagcatc
krp-rpsR	reverse kanR primer for generating rpsR recoded cassettes and NAT_*kan*^{R}	aacttgcattaccttatcctctcaaagtcgtattaatggaccgtgaccgattagaaaaactcatcgagcatc
krp-rpmB	reverse kanR primer for generating rpmB recoded cassettes and NAT_*kan*^{R}	cttgattttctcacgaataccttagccatgatttatttcctctaagtacttagaaaaactcatcgagcatc

krp-rpsP	reverse kanR primer for generating rpsP recoded cassettes and NAT_*kan*^{R}	acaggtgcttgcgcggtgagttgtttgctcatcatgaccaccgtgacagattagaaaaactcatcgagcatc
krp-rpsQ	reverse kanR primer for generating rpsQ recoded cassettes and NAT_*kan*^{R}	taaacggctcatttctgagccgtttattcgtattgagagagtgtactgtattagaaaaactcatcgagcatc
krp-rpmA	reverse kanR primer for generating rpmA recoded cassettes and NAT_*kan*^{R}	gccccgcaacgtgttgcggggctttcatccgttaccgggacgcgaaaaacttagaaaaactcatcgagcatc
krp-rpsS	reverse kanR primer for generating rpsS recoded cassettes and NAT_*kan*^{R}	agaacgagcatggcgatgtttagcgatagtttccatctcttcctcctaccttagaaaaactcatcgagcatc
krp-rpsN	reverse kanR primer for generating rpsN recoded cassettes and NAT_*kan*^{R}	ggatcttgcatgctcatctgtctttactcccgtgattcaattggtgacaattagaaaaactcatcgagcatc
krp-rplU	reverse kanR primer for generating rplU recoded cassettes and NAT_*kan*^{R}	tgtggagccgccagccttttatgtgccatttgaaatctctcctcaggtcttagaaaaactcatcgagcatc
krp-rpsJ	reverse kanR primer for generating rpsJ recoded cassettes and NAT_*kan*^{R}	accgactaaaccaatcattgtttcaacctctcaatcgctcaatgacctgattagaaaaactcatcgagcatc
krp-rplX	reverse kanR primer for generating rplX recoded cassettes and NAT_*kan*^{R}	tacttcgtctttgtagtaatcatgcagtttcgccatcgtactactccaaattagaaaaactcatcgagcatc
krp-rplW	reverse kanR primer for generating rplW recoded cassettes and NAT_*kan*^{R}	cggagatgtcggtttacatttaacaactgccattgtattactcctccgacttagaaaaactcatcgagcatc
krp-rplV	reverse kanR primer for generating rplV recoded cassettes and NAT_*kan*^{R}	gcgaataccattaggatgtactttctgacccattgctagtctccagagtcttagaaaaactcatcgagcatc
krp-rplS	reverse kanR primer for generating rplS recoded cassettes and NAT_*kan*^{R}	gccagccaattggccagcccttcttaacaggatgtcgcttaagcgaaatcttagaaaaactcatcgagcatc
krp-rplR	reverse kanR primer for generating rplR recoded cassettes and NAT_*kan*^{R}	ctgcagttcgccagcttgtttttcgatgtgagccatcttacacctctaccttagaaaaactcatcgagcatc
krp-rplT	reverse kanR primer for generating rplT recoded cassettes and NAT_*kan*^{R}	tgatggcgttgaaacgaaaagagggagactagctccctctttcaactggcttagaaaaactcatcgagcatc
krp-rpsM	reverse kanR primer for generating rpsM recoded cassettes and NAT_*kan*^{R}	cacgtttacgtgcacgaattggtgcctttgccattattcaatcaccccgattagaaaaactcatcgagcatc
krp-rplL	reverse kanR primer for generating rplL recoded cassettes and NAT_*kan*^{R}	agtcaccagccatcagcctgatttctcaggctgcaaccggaagggttggcttagaaaaactcatcgagcatc
krp-rplN	reverse kanR primer for generating rplN recoded cassettes and NAT_*kan*^{R}	acacgataacttcgtcatcacgacggattttcgctgccatgattcgctccttagaaaaactcatcgagcatc
krp-rpsL	reverse kanR primer for generating rpsL recoded cassettes and NAT_*kan*^{R}	ttagtttgacatttaagttaaaacgtttggccttacttaacggagaaccattagaaaaactcatcgagcatc
krp-rplQ	reverse kanR primer for generating rplQ recoded cassettes and NAT_*kan*^{R}	tacgggtataaaaaaacccgccggggcgggttttttacgttgcttcagattagaaaaactcatcgagcatc
krp-rpsK	reverse kanR primer for generating rpsK recoded cassettes and NAT_*kan*^{R}	cccaaatatcttgccattttctttctccaacaaacctggaaaacgaggcgttagaaaaactcatcgagcatc
krp-rpsI	reverse kanR primer for generating rpsI recoded cassettes and NAT_*kan*^{R}	cgccgaagcgggtttttcgaaaattgttttctgccggagcagaagccaattagaaaaactcatcgagcatc
krp-rpsH	reverse kanR primer for generating rpsH recoded cassettes and NAT_*kan*^{R}	gcaggaacaacgaccggtgctttagcaacacgagacatttttttcctccgattagaaaaactcatcgagcatc
krp-rplM	reverse kanR primer for generating rplM recoded cassettes and NAT_*kan*^{R}	cggcgaccagtgccgtagtattgattttcagccattgcctataatcccgattagaaaaactcatcgagcatc
krp-rplP	reverse kanR primer for generating rplP recoded cassettes and NAT_*kan*^{R}	ctcggtgttcagctcttcaacgctcttctcacgcagctcttttgctttcattacatcaccgtcttattagaaaaactcatcgagcatc
krp-rplO	reverse kanR primer for generating rplO recoded cassettes and NAT_*kan*^{R}	caccttttggcactttgaaaatctaatcccggttgtttagccatctgctacttagaaaaactcatcgagcatc
krp-rplJ	reverse kanR primer for generating rplJ recoded cassettes and NAT_*kan*^{R}	tatcagaataagtttatacgtaagcgaatgcgttaaaaagataactgcgattagaaaaactcatcgagcatc
krp-rpsE	reverse kanR primer for generating rpsE recoded cassettes and NAT_*kan*^{R}	cgaccgattgcactgcgggtttgagtaattttaatagtctttgccatggtttagaaaaactcatcgagcatc
krp-rplF	reverse kanR primer for generating rplF recoded cassettes and NAT_*kan*^{R}	gcgcgggtcgcacgacggatacgagcagatttcttatccatagtgttaccttagaaaaactcatcgagcatc

krp-rplE	reverse kanR primer for generating rplE recoded cassettes and NAT_*kan*^{R}	ttttacttcgcgtgctttcattgattgcttagccatttagtaaccctaccttagaaaaactcatcgagcatc
krp-rpsG	reverse kanR primer for generating rpsG recoded cassettes and NAT_*kan*^{R}	gcgatgggtgttgtacgagccatttgtttcctcgtttatcttttaggcgtttagaaaaactcatcgagcatc
krp-rpsD	reverse kanR primer for generating rpsD recoded cassettes and NAT_*kan*^{R}	gaaactctgtcacagaaccctgcattgtgtcctctctttggtactaagctttagaaaaactcatcgagcatc
krp-rplD	reverse kanR primer for generating rplD recoded cassettes and NAT_*kan*^{R}	tcagaaacgtgcggtgcacgcagcaccttcagcagacgttcttcacgaatcatgccagcatcttcctcattagaaaaactcatcgagcatc
krp-rplC	reverse kanR primer for generating rplC recoded cassettes and NAT_*kan*^{R}	cagtcagcgcgctctgcgcgtctttcaatactaattccattgctatctccttagaaaaactcatcgagcatc
krp-rpsC	reverse kanR primer for generating rpsC recoded cassettes and NAT_*kan*^{R}	tgcattttacggaattttgtacgctttggttgtaacatcagcgacgctccttagaaaaactcatcgagcatc
krp-rpsB	reverse kanR primer for generating rpsB recoded cassettes and NAT_*kan*^{R}	ttgccgcctttctgcaactcgaactattttgggggagttatcaagccttattagaaaaactcatcgagcatc
krp-rplB	reverse kanR primer for generating rplB recoded cassettes and NAT_*kan*^{R}	caataaaaggacctttcttgagagaacgtggcatggcttatcctctaaaattagaaaaactcatcgagcatc
krp-prfB	reverse kanR primer for generating prfB recoded cassettes and NAT_*kan*^{R}	tcgactaccgcgtcagcgccctgtgcgtgttgttcagacatgttggttccttagaaaaactcatcgagcatc
krp-rpsA	reverse kanR primer for generating rpsA recoded cassettes and NAT_*kan*^{R}	tcaagtaaactcaacaaacttcggaataaaaatcccgaagagtcagagaattagaaaaactcatcgagcatc
prfB-1r	prfB split synthesis N-terminus reverse primer	cacccgaaatcttaatagtaacgct
prfB-2f	prfB split synthesis C-terminus forward primer	gacggagattattgaggaatctgag
rpsA-1r	rpsA split synthesis N-terminus reverse primer	gtaacacgccctgttaacttcg
rpsA-2f	rpsA split synthesis C-terminus forward primer	gcaattgcgaagcgctac
rpmH-early	C-terminal cassette forward primer for rpmH; All but 90 bp is recoded	gttctcacggcttccgtgctcgtatggctactaaaaatggtcgtcaggttttggcgcgccgccgc
rpsR-early	C-terminal cassette forward primer for rpsR; All but 90 bp is recoded	ccgcggaaggcgttcaagagatcgactataaagatatcgctacgctgaaaaattatattacggagtctggcaaaa
rpsP-early	C-terminal cassette forward primer for rpsP; All but 90 bp is recoded	gtccgttctaccaggttgttgtcgctgacagccgtaatgcacgcaacggtcgttttattgaacgtgtgggc
rplT-early	C-terminal cassette forward primer for rplT; All but 90 bp is recoded	acaagaaaattttgaaacaagctaaaggctactacggtgcgcgttctcgcgtgtatcgtgtagcttttcaagc
rplL-early	C-terminal cassette forward primer for rplL; All but 90 bp is recoded	ctatgtctgtaatggacgttgtagaactgatctctgcaatggaagaaaaatttggcgtatcagcagcg
rpsL-early	C-terminal cassette forward primer for rpsL; All but 90 bp is recoded	gcaaagttgcgaaaagcaacgtgcctgcgctggaagcatgcccgcaaaaacgcggggttgcacg
rplQ-early	C-terminal cassette forward primer for rplQ; All but 90 bp is recoded	gcagccatcgccaggctatgttccgcaatatggcaggttcactggttcgtcacgagattattaaaactacattaccga
rplO-early	C-terminal cassette forward primer for rplO; All but 90 bp is recoded	aggcgggtaaacgcctgggtcgtggtatcggttctggcctcggtaaaaccggcggacgcggccat
rplJ-early	C-terminal cassette forward primer for rplJ; All but 90 bp is recoded	aagtcagcgaagtagccaaaggcgcgctgtctgcagtagttgcggattcccgcggtgtgacagtgg
rpsG-early	C-terminal cassette forward primer for rpsG; All but 90 bp is recoded	cggatccgaagttcggatcagaactgctggctaaatttgtaaatatcctgatggtggacgggaaaaaga
rpsD-early	C-terminal cassette forward primer for rpsD; All but 90 bp is recoded	gtgagggcaccgacttattccttaagtctggcgttcgcgcgatcgataccaaatgcaagatcgagcaggc
rplD-early	C-terminal cassette forward primer for rplD; All but 90 bp is recoded	tttccgaaactaccttcggtcgtgatttcaacgaagcgctggttcaccaggtagtggtggcgtacgc
rpsC-early	C-terminal cassette forward primer for rpsC; All but 90 bp is recoded	ttgtaaaaccatggaactctacctggtttgcgaacaccaaagaattcgctgataatttagatagtgacttcaaggttcg

rpsB-early	C-terminal cassette forward primer for rpsB; All but 90 bp is recoded	ttcacttcggtcaccagacccgttactggaacccgaaaatgaagccgttcatttttggcgcacg caa
rplB-early	C-terminal cassette forward primer for rplB; All but 90 bp is recoded	gccacgtagttaaagtggttaaccctgagctgcacaagggcaaacctttgcgccattattaga gaagaattct
rpsA-early	C-terminal cassette forward primer for rpsA; All but 90 bp is recoded	aagaaatcgaaacccgcccgggttctatcgttcgtggcgttgttgttgctattgataaggatgtt gtgttggtg
rpmH-middle	C-terminal cassette forward primer for rpmH; Half of gene is recoded	cgtctgtactgaagcgcaaccgttctcacggcttccgtgctcgtatggctacgaagaacggcc gc
rpsR-middle	C-terminal cassette forward primer for rpsR; Half of gene is recoded	actataaagatatcgctacgctgaaaaactacatcaccgaaagcggtaagatcgttccttcac gcattaca
rpsP-middle	C-terminal cassette forward primer for rpsP; Half of gene is recoded	gtaatgcacgcaacggtcgcttcatcgagcgcgttggtttcttcaacccaattgcgtctgagaa ggagg
rplT-middle	C-terminal cassette forward primer for rplT; Half of gene is recoded	gtcagtatgcttaccgtgaccgtcgtcaacgtaagcgtcagttccgtcaattatggatcgcacg cattaatg
rplL-middle	C-terminal cassette forward primer for rplL; Half of gene is recoded	cggttgaagctgctgaagaaaaaactgaattcgacgtaattctgaaagctgcgggggcgaat aagg
rpsL-middle	C-terminal cassette forward primer for rpsL; Half of gene is recoded	actccgcgctgcgtaaagtatgccgtgttcgtctgactaacggtttcgaagttacgtcttatattg gcggag
rplQ-middle	C-terminal cassette forward primer for rplQ; Half of gene is recoded	ttgagccgctgattactcttgccaagactgatagcgttgctaatcgtcgtttggcgtttgctcgca
rplO-middle	C-terminal cassette forward primer for rplO; Half of gene is recoded	ctctgtaccgtcgtctgccgaaattcggcttcacttctcgtaaagcagcgattactgcggagat ccgc
rplJ-middle	C-terminal cassette forward primer for rplJ; Half of gene is recoded	ctccgttcgagtgcctgaaagacgcgtttgttggtccgaccctgattgcatatagcatggagca tcctgg
rpsG-middle	C-terminal cassette forward primer for rpsG; Half of gene is recoded	aagttaagtctcgccgcgttggtggttctacttatcaggtaccagttgaagtgcgacctgtacg cc
rpsD-middle	C-terminal cassette forward primer for rpsD; Half of gene is recoded	gtgaaaacctgttggctctgctggaaggtcgtctggacaacgttgtataccgcatggggtttgg cg
rplD-middle	C-terminal cassette forward primer for rplD; Half of gene is recoded	ttgctgctcgtccgcaggaccacagtcaaaaagttaacaagaagatgtaccgaggggcatta aagtctatttt
rpsC-middle	C-terminal cassette forward primer for rpsC; Half of gene is recoded	tcaacatcgccgaagttcgtaagcctgaactggacgcaaaactggttgctgatagtattacaa gccaattagagcg
rpsB-middle	C-terminal cassette forward primer for rpsB; Half of gene is recoded	aaaccgttcgtcagtccatcaaacgtctgaaagacctggaaactcagtctcaagatggcacgt ttgataaattaa
rplB-middle	C-terminal cassette forward primer for rplB; Half of gene is recoded	atgctgcaatcaaaccaggtaacaccctgccgatgcgcaacatcccggttggaagcacggt gcaca
rpsA-middle	C-terminal cassette forward primer for rpsA; Half of gene is recoded	gcgaagatccgtgggtagctatcgctaaacgttatccggaaggtaccaaattaacagggcgt gttactaatttg
NAT_krp-rpmH	forward kanR primer for generating rpmH NAT_*kan*^{R} cassette	tcgtgctaaaggccgcgctcgtctgaccgtttctaagtaatgagttgtcgagattttcagg
NAT_krp-rpmD	forward kanR primer for generating rpmD NAT_*kan*^{R} cassette	gatcaacgcggtttccttcatggttaaagttgaggagtaatgagttgtcgagattttcagg
NAT_krp-rpmC	forward kanR primer for generating rpmC NAT_*kan*^{R} cassette	acgcgttaagactttactgaacgagaaggcgggtgcgtaatgagttgtcgagattttcagg
NAT_krp-rpsR	forward kanR primer for generating rpsR NAT_*kan*^{R} cassette	ctacctgtccctgctgccgtacactgatcgccatcagtaatgagttgtcgagattttcagg
NAT_krp-rpmB	forward kanR primer for generating rpmB NAT_*kan*^{R} cassette	agttctggctgaactgcgtgcccgtggcgaaaagtactaatgagttgtcgagattttcagg
NAT_krp-rpsP	forward kanR primer for generating rpsP NAT_*kan*^{R} cassette	cgttgctgcgctgatcaaagaagtaaacaaagcagcttaatgagttgtcgagattttcagg
NAT_krp-rpsQ	forward kanR primer for generating rpsQ NAT_*kan*^{R} cassette	ctggacgctggttcgcgttgtagagaaagcggttctgtaatgagttgtcgagattttcagg
NAT_krp-rpmA	forward kanR primer for generating rpmA NAT_*kan*^{R} cassette	cccgaaaaaccgtaaatttatcagcatcgaagctgaataatgagttgtcgagattttcagg

NAT_krp-rpsS	forward kanR primer for generating rpsS NAT_*kan*^{R} cassette	tcgcggccacgctgctgataaaaaagcgaagaagaaataatgagttgtcgagattttcagg
NAT_krp-rpsN	forward kanR primer for generating rpsN NAT_*kan*^{R} cassette	gcgcggtgaaatcccgggtctgaaaaaggctagctggtaatgagttgtcgagattttcagg
NAT_krp-rplU	forward kanR primer for generating rplU NAT_*kan*^{R} cassette	gtggttcactgatgtgaaaattactggcatcagcgcctaatgagttgtcgagattttcagg
NAT_krp-rpsJ	forward kanR primer for generating rpsJ NAT_*kan*^{R} cassette	tctggctgccggtgtagacgtgcagatcagcctgggttaatgagttgtcgagattttcagg
NAT_krp-rplX	forward kanR primer for generating rplX NAT_*kan*^{R} cassette	agtccgtttcttcaagtctaacagcgaaactatcaagtaatgagttgtcgagattttcagg
NAT_krp-rplW	forward kanR primer for generating rplW NAT_*kan*^{R} cassette	agaaggccagaatctggacttcgttggcggcgctgagtaatgagttgtcgagattttcagg
NAT_krp-rplV	forward kanR primer for generating rplV NAT_*kan*^{R} cassette	gcgcaccagccacatcactgtggttgtgtccgatcgctgatgagttgtcgagattttcagg
NAT_krp-rplS	forward kanR primer for generating rplS NAT_*kan*^{R} cassette	tactggtaaggctgctcgtatcaaagagcgtcttaactaatgagttgtcgagattttcagg
NAT_krp-rplR	forward kanR primer for generating rplR NAT_*kan*^{R} cassette	actggcagatgctgcccgtgaagctggccttcagttctaatgagttgtcgagattttcagg
NAT_krp-rplT	forward kanR primer for generating rplT NAT_*kan*^{R} cassette	caccgctctggttgaaaaagcgaaagcagctctggcataatgagttgtcgagattttcagg
NAT_krp-rpsM	forward kanR primer for generating rpsM NAT_*kan*^{R} cassette	acgtacccgtaagggtccgcgcaaaccgatcaagaaataatgagttgtcgagattttcagg
NAT_krp-rplL	forward kanR primer for generating rplL NAT_*kan*^{R} cassette	agctctggaagaagctggcgctgaagttgaagttaaataatgagttgtcgagattttcagg
NAT_krp-rplN	forward kanR primer for generating rplN NAT_*kan*^{R} cassette	gttcatgaaaattatctctctggcaccagaagtactctaatgagttgtcgagattttcagg
NAT_krp-rpsL	forward kanR primer for generating rpsL NAT_*kan*^{R} cassette	ggctcgttccaagtatggcgtgaagcgtcctaaggcttaatgagttgtcgagattttcagg
NAT_krp-rplQ	forward kanR primer for generating rplQ NAT_*kan*^{R} cassette	ggttgatcgttcagagaaagcagaagctgctgcagagtaatgagttgtcgagattttcagg
NAT_krp-rpsK	forward kanR primer for generating rpsK NAT_*kan*^{R} cassette	tcataacggttgtcgtccgccgaaaaaacgtcgcgtataatgagttgtcgagattttcagg
NAT_krp-rpsI	forward kanR primer for generating rpsI NAT_*kan*^{R} cassette	gcgtaaagcacgtcgtcgtccgcagttctccaaacgttaatgagttgtcgagattttcagg
NAT_krp-rpsH	forward kanR primer for generating rpsH NAT_*kan*^{R} cassette	ggctggtcttggtggcgaaattatctgctacgtagcctaatgagttgtcgagattttcagg
NAT_krp-rplM	forward kanR primer for generating rplM NAT_*kan*^{R} cassette	caaccacgcggcacagcaaccgcaagttcttgacatctaatgagttgtcgagattttcagg
NAT_krp-rplP	forward kanR primer for generating rplP NAT_*kan*^{R} cassette	gccgattaaaaccacctttgtaactaagacggtgatgtaatgagttgtcgagattttcagg
NAT_krp-rplO	forward kanR primer for generating rplO NAT_*kan*^{R} cassette	tgctgctatcgaagctgctggcggtaaaatcgaggaataatgagttgtcgagattttcagg
NAT_krp-rplJ	forward kanR primer for generating rplJ NAT_*kan*^{R} cassette	tactctggctgctgtacgcgatgcgaaagaagctgcttaatgagttgtcgagattttcagg
NAT_krp-rpsE	forward kanR primer for generating rpsE NAT_*kan*^{R} cassette	caagcgtggtaaatccgttgaagaaattctggggaaataatgagttgtcgagattttcagg
NAT_krp-rplF	forward kanR primer for generating rplF NAT_*kan*^{R} cassette	cgacgaagtcgtgcgtaccaaagaggctaagaagaagtaatgagttgtcgagattttcagg
NAT_krp-rplE	forward kanR primer for generating rplE NAT_*kan*^{R} cassette	cgctctgctggctgcctttgacttcccgttccgcaagtaatgagttgtcgagattttcagg
NAT_krp-rpsG	forward kanR primer for generating rpsG NAT_*kan*^{R} cassette	cgcttccagtaagcagcccgctttgggctacttaaattgatgagttgtcgagattttcagg
NAT_krp-rpsD	forward kanR primer for generating rpsD NAT_*kan*^{R} cassette	cattaacgaacacctgatcgtcgagctttactccaagtaatgagttgtcgagattttcagg

NAT_krp-rplD	forward kanR primer for generating rplD NAT_*kan*^{R} cassette	tgctgatgctgttaagcaagttgaggagatgctggcatgatgagttgtcgagattttcagg
NAT_krp-rplC	forward kanR primer for generating rplC NAT_*kan*^{R} cassette	cggtagcgacctgatcgttaaaccagctgtgaaggcgtaatgagttgtcgagattttcagg
NAT_krp-rpsC	forward kanR primer for generating rpsC NAT_*kan*^{R} cassette	tgctcagcctaaaaagcagcagcgtaaaggccgtaaataatgagttgtcgagattttcagg
NAT_krp-rpsB	forward kanR primer for generating rpsB NAT_*kan*^{R} cassette	ggcttcccaggcggaagaaagcttcgtagaagctgagtaatgagttgtcgagattttcagg
NAT_krp-rplB	forward kanR primer for generating rplB NAT_*kan*^{R} cassette	gcgtactgataaattcatcgtacgtcgccgtagcaaataatgagttgtcgagattttcagg
NAT_krp-prfB	forward kanR primer for generating prfB NAT_*kan*^{R} cassette	ggatcaatttatcgaagcaagtttgaaagcagggttatgatgagttgtcgagattttcagg
NAT_krp-rpsA	forward kanR primer for generating rpsA NAT_*kan*^{R} cassette	cgcaatggctgaagctttcaaagcagctaaaggcgagtaatgagttgtcgagattttcagg
bndfp-rpmH	rpmH forward boundary primer	tcggtgtccatcgtttca
bndfp-rpmD	rpmD forward boundary primer	ttgatggcctggaaaatatgaat
bndfp-rpmC	rpmC forward boundary primer	tgaagcattcaagctggc
bndfp-rpsR	rpsR forward boundary primer	caaagaacggactgagcaaa
bndfp-rpmB	rpmB forward boundary primer	gctgtaaagcctgacgag
bndfp-rpsP	rpsP forward boundary primer	ttcgggcttttaatatgacacc
bndfp-rpsQ	rpsQ forward boundary primer	gtctcacctgttgaagcaag
bndfp-rpmA	rpmA forward boundary primer	gccatcgtcagtggttca
bndfp-rpsS	rpsS forward boundary primer	taagaagacccgcagcaa
bndfp-rpsN	rpsN forward boundary primer	tgcgaaatctgacgaagaag
bndfp-rplU	rplU forward boundary primer	tattcgcgccctattgtga
bndfp-rpsJ	rpsJ forward boundary primer	cactctcccatcaatcgtaatg
bndfp-rplX	rplX forward boundary primer	ctcgtgagcttcgtagtga
bndfp-rplW	rplW forward boundary primer	ggttagcctgatcgcctt
bndfp-rplV	rplV forward boundary primer	aattcgcaccgactcgta
bndfp-rplS	rplS forward boundary primer	cgcacaacagcaacataaac
bndfp-rplR	rplR forward boundary primer	ccttataaaggcaagggtgttc
bndfp-rplT	rplT forward boundary primer	ctggtaatcgcgtgcctg
bndfp-rpsM	rpsM forward boundary primer	tctgtgcgtttccatttgag
bndfp-rplL	rplL forward boundary primer	gaagctgcttaatcgcagt
bndfp-rplN	rplN forward boundary primer	gccctcgatatggggatt
bndfp-rpsL	rpsL forward boundary primer	aaattcggcgtcctcatattg
bndfp-rplQ	rplQ forward boundary primer	catgcgcctggaaaactg
bndfp-rpsK	rpsK forward boundary primer	gtaccaagaccaacgcac
bndfp-rpsI	rpsI forward boundary primer	gtttacgcgggtaacgag
bndfp-rpsH	rpsH forward boundary primer	ctatgcgcggtgaaatcc
bndfp-rplM	rplM forward boundary primer	tttgtcgtgtgaacctcaac
bndfp-rplP	rplP forward boundary primer	ctgttgaacaaccggaaaaac

bndfp-rplO	rplO forward boundary primer	gcgaggatactcctgctatt
bndfp-rplJ	rplJ forward boundary primer	attaagacgctctctccgtt
bndfp-rpsE	rpsE forward boundary primer	caatatcatggtcgtgtccag
bndfp-rplF	rplF forward boundary primer	acctctaaaggtgttatgactga
bndfp-rplE	rplE forward boundary primer	ggctttagattcgaagacgg
bndfp-rpsG	rpsG forward boundary primer	ccgttaagtaaggccaaacg
bndfp-rpsD	rpsD forward boundary primer	ctcataacggttgtcgtcc
bndfp-rplD	rplD forward boundary primer	ctggttaaaggtgctgtcc
bndfp-rplC	rplC forward boundary primer	ctctgatgcgtctggatct
bndfp-rpsC	rpsC forward boundary primer	tgcagatcgcatcctgaa
bndfp-rpsB	rpsB forward boundary primer	cacatattccggggtgcc
bndfp-rplB	rplB forward boundary primer	agcttacgtcaccctgaaa
bndfp-prfB	prfB forward boundary primer	aaaaagagcgtggattggg
bndfp-rpsA	rpsA forward boundary primer	gaatgacagcgggtatgtt
bndrp-rpmH	rpmH reverse boundary primer	gaatgtgaattgactgggagtt
bndrp-rpmD	rpmD reverse boundary primer	gaaccgataccacgaccc
bndrp-rpmC	rpmC reverse boundary primer	gttcgatagcaacaacaatgga
bndrp-rpsR	rpsR reverse boundary primer	ctacccaggtttgctactttatc
bndrp-rpmB	rpmB reverse boundary primer	agtaccagcagaagaaacca
bndrp-rpsP	rpsP reverse boundary primer	catttttcccaaaacgatggg
bndrp-rpsQ	rpsQ reverse boundary primer	gcttcaaggatatgggtagaaaa
bndrp-rpmA	rpmA reverse boundary primer	gcatttttaccggttatcgaatg
bndrp-rpsS	rpsS reverse boundary primer	caacaaggcgaaccttctg
bndrp-rpsN	rpsN reverse boundary primer	cgttacggatacgggtca
bndrp-rplU	rplU reverse boundary primer	tctgaatcgcgaccgtta
bndrp-rpsJ	rpsJ reverse boundary primer	acgggtcatacccactttt
bndrp-rplX	rplX reverse boundary primer	aactcagtcatgagttttttaac
bndrp-rplW	rplW reverse boundary primer	ttaaccactttaactacgtggc
bndrp-rplV	rplV reverse boundary primer	caggtagagttccatggttttac
bndrp-rplS	rplS reverse boundary primer	caccagcaaacagataaaaaagg
bndrp-rplR	rplR reverse boundary primer	gtttaccgcgatcagcttt
bndrp-rplT	rplT reverse boundary primer	ctacggcgataaaagtcaatgt
bndrp-rpsM	rpsM reverse boundary primer	ccgtcagagacttgttttctt
bndrp-rplL	rplL reverse boundary primer	tacagcgcaaaaaggctg
bndrp-rplN	rplN reverse boundary primer	tttaccgcgtttacctttatctt
bndrp-rpsL	rpsL reverse boundary primer	ttcaggattgtccaaaactctac
bndrp-rplQ	rplQ reverse boundary primer	cagctattgtagataagtgggga
bndrp-rpsK	rpsK reverse boundary primer	gctcagcttgagcttagga

bndrp-rpsI	rpsI reverse boundary primer	tttacgctgattcagattttagc
bndrp-rpsH	rpsH reverse boundary primer	gttgatttttacgtcaacgcc
bndrp-rplM	rplM reverse boundary primer	cgagctgcggaacttttg
bndrp-rplP	rplP reverse boundary primer	caggttgaactgctcacg
bndrp-rplO	rplO reverse boundary primer	cagcagtctgcgtttcag
bndrp-rplJ	rplJ reverse boundary primer	gtgatagacatttaaattgttcc
bndrp-rpsE	rpsE reverse boundary primer	agcgttgccttgtgtttc
bndrp-rplF	rplF reverse boundary primer	ccagctcctggagcttgc
bndrp-rplE	rplE reverse boundary primer	gtttcgcgaagtatttatcagc
bndrp-rpsG	rpsG reverse boundary primer	cactgataccgatgttacgg
bndrp-rpsD	rpsD reverse boundary primer	tcgaactcacttgctcgata
bndrp-rplD	rplD reverse boundary primer	tggatttttccatcgcagtag
bndrp-rplC	rplC reverse boundary primer	ttcgttgaaatcacgaccg
bndrp-rpsC	rpsC reverse boundary primer	taacatccgtaccctgcg
bndrp-rpsB	rpsB reverse boundary primer	cggtcacttactgatgtaagc
bndrp-rplB	rplB reverse boundary primer	ctttctctaccttcttcagcaa
bndrp-prfB	prfB reverse boundary primer	cgacgcgttttcagttca
bndrp-rpsA	rpsA reverse boundary primer	tgcttgattacaggacgaaac
natfp-rpmH	rpmH forward natural sequence primer	ctgtactgaagcgcaacc
natfp-rpmD	rpmD forward natural sequence primer	cagtgcaatcggtcgtct
natfp-rpmC	rpmC forward natural sequence primer	gagcgttgaagagctgaac
natfp-rpsR	rpsR forward natural sequence primer	aagttctgccgtttcacc
natfp-rpmB	rpmB forward natural sequence primer	ccaagttactggcaagcg
natfp-rpsP	rpsP forward natural sequence primer	cgctaaaaagcgtccgttc
natfp-rpsQ	rpsQ forward natural sequence primer	gcaaggtcgcgttgttag
natfp-rpmA	rpmA forward natural sequence primer	gtaacggtcgcgattcag
natfp-rpsS	rpsS forward natural sequence primer	gtccttttattgacctgcact
natfp-rpsN	rpsN forward natural sequence primer	tgaaagcacgcgaagtaaaa
natfp-rplU	rplU forward natural sequence primer	acaacaccgagtaagcga
natfp-rpsJ	rpsJ forward natural sequence primer	tgaaagcgtttgatcatcgt
natfp-rplX	rplX forward natural sequence primer	gtgatgacgaagttatcgtgtta
natfp-rplW	rplW forward natural sequence primer	aacgtctgctgaaggtgc
natfp-rplV	rplV forward natural sequence primer	catgctcgttcttctgctc
natfp-rplS	rplS forward natural sequence primer	acttgaacaagagcagatgaag
natfp-rplR	rplR forward natural sequence primer	ctgctcgtatccgtcgtg
natfp-rplT	rplT forward natural sequence primer	cgtgcacgtcacaagaaa
natfp-rpsM	rpsM forward natural sequence primer	ctgatcataagcatgccgtaa
natfp-rplL	rplL forward natural sequence primer	ttgaagcagttgcagctatg

natfp-rplN	rplN forward natural sequence primer	gactatgctgaacgtcgc
natfp-rpsL	rpsL forward natural sequence primer	accagctggtacgcaaac
natfp-rplQ	rplQ forward natural sequence primer	tcaactgaaccgcaacag
natfp-rpsK	rpsK forward natural sequence primer	cacgtaaacgtgtaagaaaacaa
natfp-rpsI	rpsI forward natural sequence primer	atactacggcactggtcg
natfp-rpsH	rpsH forward natural sequence primer	gctgacccgtatccgtaa
natfp-rplM	rplM forward natural sequence primer	agaaaccgtaaaacgcgac
natfp-rplP	rplP forward natural sequence primer	aaattccgtaaaatgcacaaagg
natfp-rplO	rplO forward natural sequence primer	ccgaaggctccaaaaagg
natfp-rplJ	rplJ forward natural sequence primer	acaagcgattgttgctgaa
natfp-rpsE	rpsE forward natural sequence primer	ggcgaactgcaggaaaag
natfp-rplF	rplF forward natural sequence primer	taaagcaccggtcgttgt
natfp-rplE	rplE forward natural sequence primer	ctgcatgattactacaaagacga
natfp-rpsG	rpsG forward natural sequence primer	tcagcgtaaaattctgccg
natfp-rpsD	rpsD forward natural sequence primer	taagctcaagctgagccg
natfp-rplD	rplD forward natural sequence primer	gagcgcgctgactgtttc
natfp-rplC	rplC forward natural sequence primer	gtgggtatgacccgtatctt
natfp-rpsC	rpsC forward natural sequence primer	atggtattcgcctgggtatt
natfp-rpsB	rpsB forward natural sequence primer	caaggctggtgttcacttc
natfp-rplB	rplB forward natural sequence primer	gttaaatgtaaaccgacatctcc
natfp-prfB	prfB forward natural sequence primer	ttcaggacctcacggaac
natfp-rpsA	rpsA forward natural sequence primer	ctcaactctttgaagagtcctt
natrp-rpmH	rpmH reverse natural sequence primer	ggcctttagcacgacgac
natrp-rpmD	rpmD reverse natural sequence primer	ggaaaccgcgttgatcatac
natrp-rpmC	rpmC reverse natural sequence primer	agtcttaacgcgtgcgac
natrp-rpsR	rpsR reverse natural sequence primer	tcagtgtacggcagcagg
natrp-rpmB	rpmB reverse natural sequence primer	gttcagccagaactgtatcg
natrp-rpsP	rpsP reverse natural sequence primer	cagcaacgcgatcagaaa
natrp-rpsQ	rpsQ reverse natural sequence primer	aaccagcgtccaggattt
natrp-rpmA	rpmA reverse natural sequence primer	ggtttttcgggcctttaact
natrp-rpsS	rpsS reverse natural sequence primer	ggccgcgataagtacgag
natrp-rpsN	rpsN reverse natural sequence primer	ggatttcaccgcgcatag
natrp-rplU	rplU reverse natural sequence primer	atgccagtaattttcacatcagt
natrp-rpsJ	rpsJ reverse natural sequence primer	atctgcacgtctacaccg
natrp-rplX	rplX reverse natural sequence primer	gttagacttgaagaaacggactt
natrp-rplW	rplW reverse natural sequence primer	tctggccttctttcaggg
natrp-rplV	rplV reverse natural sequence primer	acagtgatgtggctggtg
natrp-rplS	rplS reverse natural sequence primer	cagccttaccagtacgct

natrp-rplR	rplR reverse natural sequence primer	atctgccagtgcctggac
natrp-rplT	rplT reverse natural sequence primer	ttcaaccagagcggtgaa
natrp-rpsM	rpsM reverse natural sequence primer	ttacgggtacgtgcgttg
natrp-rplL	rplL reverse natural sequence primer	cagcttcttccagagcttttt
natrp-rplN	rplN reverse natural sequence primer	ggtgccagagagataattttcat
natrp-rpsL	rpsL reverse natural sequence primer	catacttggaacgagcctg
natrp-rplQ	rplQ reverse natural sequence primer	tgctttctctgaacgatcaac
natrp-rpsK	rpsK reverse natural sequence primer	aaccgttatgagggatcgg
natrp-rpsI	rpsI reverse natural sequence primer	gacgtgctttacgcagac
natrp-rpsH	rpsH reverse natural sequence primer	cagataatttcgccaccaaga
natrp-rplM	rplM reverse natural sequence primer	gcgtggttgtgctcgtta
natrp-rplP	rplP reverse natural sequence primer	ttaatcggcagtttcgctg
natrp-rplO	rplO reverse natural sequence primer	gcttcgatagcagcacga
natrp-rplJ	rplJ reverse natural sequence primer	cagccagagtacgaacca
natrp-rpsE	rpsE reverse natural sequence primer	ttcttcaacggatttaccacg
natrp-rplF	rplF reverse natural sequence primer	cttcgtcggcgtaacgaa
natrp-rplE	rplE reverse natural sequence primer	gaagtcaaaggcagccag
natrp-rpsG	rpsG reverse natural sequence primer	aaagcgggctgcttactg
natrp-rpsD	rpsD reverse natural sequence primer	cgatcaggtgttcgttaatgtc
natrp-rplD	rplD reverse natural sequence primer	cttaacagcatcagcagtcatt
natrp-rplC	rplC reverse natural sequence primer	cagctggtttaacgatcagg
natrp-rpsC	rpsC reverse natural sequence primer	ttacgctgctgctttttagg
natrp-rpsB	rpsB reverse natural sequence primer	gaagctttcttccgcctg
natrp-rplB	rplB reverse natural sequence primer	ttatcagtacgcttgttgctg
natrp-prfB	prfB reverse natural sequence primer	gctttcaaacttgcttcgataaa
natrp-rpsA	rpsA reverse natural sequence primer	cttcagccattgcgttgt
synfp-rpmH	rpmH forward recoded sequence primer	gtgttttgaaacgtaatcgctc
synfp-rpmD	rpmD forward recoded sequence primer	acacagactcgttctgctatt
synfp-rpmC	rpmC forward recoded sequence primer	cgaaaaatctgtggaggaactaa
synfp-rpsR	rpsR forward recoded sequence primer	aattttgtcgctttacggct
synfp-rpmB	rpmB forward recoded sequence primer	gggaaacgcccagttaca
synfp-rpsP	rpsP forward recoded sequence primer	gcgaagaaacgcccatttt
synfp-rpsQ	rpsQ forward recoded sequence primer	acgtgtggtgtcggataa
synfp-rpmA	rpmA forward recoded sequence primer	gtggatcaactcgcaatgg
synfp-rpsS	rpsS forward recoded sequence primer	cgttcatcgatttgcatctgt
synfp-rpsN	rpsN forward recoded sequence primer	gtgaggtgaagcgagttg
synfp-rplU	rplU forward recoded sequence primer	cagcatcgtgtttcagagg
synfp-rpsJ	rpsJ forward recoded sequence primer	gactaaaggctttcgaccac

synfp-rplX	rplX forward recoded sequence primer	gatgaggtaattgttctgacgg
synfp-rplW	rplW forward recoded sequence primer	gcgcttgttgaaagtattgc
synfp-rplV	rplV forward recoded sequence primer	gcgatcaagtgcacaaaaag
synfp-rplS	rplS forward recoded sequence primer	agcaggaacaaatgaaacaaga
synfp-rplR	rplR forward recoded sequence primer	tacacgtgctcgtcgtaa
synfp-rplT	rplT forward recoded sequence primer	gcgctcgccataaaaaga
synfp-rpsM	rpsM forward recoded sequence primer	atatcccggaccacaaaca
synfp-rplL	rplL forward recoded sequence primer	ccagattatcgaggcggt
synfp-rplN	rplN forward recoded sequence primer	aaatgtagctgataatagtgggg
synfp-rpsL	rpsL forward recoded sequence primer	aatcaattagttcgtaagcctcg
synfp-rplQ	rplQ forward recoded sequence primer	cgccagttaaatcgtaattcatc
synfp-rpsK	rpsK forward recoded sequence primer	aagcgcgttcgtaagcag
synfp-rpsI	rpsI forward recoded sequence primer	cgtcgtaagtcaagtgctg
synfp-rpsH	rpsH forward recoded sequence primer	ctgacatgttaacgcgca
synfp-rplM	rplM forward recoded sequence primer	aacggtgaagcgtgattg
synfp-rplP	rplP forward recoded sequence primer	tttcgcaagatgcataaggg
synfp-rplO	rplO forward recoded sequence primer	tgcagagggaagcaagaa
synfp-rplJ	rplJ forward recoded sequence primer	gcaggataagcaggcaatc
synfp-rpsE	rpsE forward recoded sequence primer	gggtgagttacaagagaaattga
synfp-rplF	rplF forward recoded sequence primer	aaggctcctgtagtggtg
synfp-rplE	rplE forward recoded sequence primer	ataaggatgaggtggtgaagaa
synfp-rpsG	rpsG forward recoded sequence primer	gcaagatcttaccagaccct
synfp-rpsD	rpsD forward recoded sequence primer	attatctcgccgcgaagg
synfp-rplD	rplD forward recoded sequence primer	agtgcactaacggtatctgaa
synfp-rplC	rplC forward recoded sequence primer	atgacgcgcatttttactga
synfp-rpsC	rpsC forward recoded sequence primer	atccgtttgggcatcgtg
synfp-rpsB	rpsB forward recoded sequence primer	aagcaggcgtacattttgg
synfp-rplB	rplB forward recoded sequence primer	gcaagcctacgtcacctg
synfp-prfB	prfB forward recoded sequence primer	acaaccgtatccaagatttaaca
synfp-rpsA	rpsA forward recoded sequence primer	aggaaagcctgaaggagatt
synrp-rpmH	rpmH reverse recoded sequence primer	gttaagcgcgcacgacc
synrp-rpmD	rpmD reverse recoded sequence primer	tactgcattaatcattccacgg
synrp-rpmC	rpmC reverse recoded sequence primer	tgtttttacacgcgccac
synrp-rpsR	rpsR reverse recoded sequence primer	acggtccgtataaggtagtaaa
synrp-rpmB	rpmB reverse recoded sequence primer	gctagcaccgtgtcaatc
synrp-rpsP	rpsP reverse recoded sequence primer	cttaatcaatgctgccacac
synrp-rpsQ	rpsQ reverse recoded sequence primer	ccaatgtccaagacttcgttt
synrp-rpmA	rpmA reverse recoded sequence primer	attctttggtcccttcacct

synrp-rpsS	rpsS reverse recoded sequence primer	atgcccacggtacgttcg
synrp-rpsN	rpsN reverse recoded sequence primer	ggaatctcgcctcgcatc
synrp-rplU	rplU reverse recoded sequence primer	gatctttacgtccgtaaaccatt
synrp-rpsJ	rpsJ reverse recoded sequence primer	taatttgaacatccacgccc
synrp-rplX	rplX reverse recoded sequence primer	aaaaaagcgcaccttcttg
synrp-rplW	rplW reverse recoded sequence primer	atccaagttttgaccctcct
synrp-rplV	rplV reverse recoded sequence primer	acaaccgtaatatgagatgtacg
synrp-rplS	rplS reverse recoded sequence primer	cgcttttcctgtgcgttc
synrp-rplR	rplR reverse recoded sequence primer	cgtcagccaacgcttgta
synrp-rplT	rplT reverse recoded sequence primer	ctactaacgccgtaaatgcc
synrp-rpsM	rpsM reverse recoded sequence primer	gagtgcgagcattcgttt
synrp-rplL	rplL reverse recoded sequence primer	ctccaacgccttcttcaac
synrp-rplN	rplN reverse recoded sequence primer	ccaagctaatgatcttcataaat
synrp-rpsL	rpsL reverse recoded sequence primer	gtatttgctgcgcgcttg
synrp-rplQ	rplQ reverse recoded sequence primer	cgatcggtccaccaattca
synrp-rpsK	rpsK reverse recoded sequence primer	ccattgtgcggaattggc
synrp-rpsI	rpsI reverse recoded sequence primer	gcttactaaattgaggtcggc
synrp-rpsH	rpsH reverse recoded sequence primer	aaatgatctcaccgccca
synrp-rplM	rplM reverse recoded sequence primer	ctgttgcgctgcatgatta
synrp-rplP	rplP reverse recoded sequence primer	gtcgtcttgattggcaactta
synrp-rplO	rplO reverse recoded sequence primer	acccgccgcctcaatt
synrp-rplJ	rplJ reverse recoded sequence primer	gctaatgtgcgcactaact
synrp-rpsE	rpsE reverse recoded sequence primer	atctcctccacgctctttc
synrp-rplF	rplF reverse recoded sequence primer	acctcatcagcatatcgca
synrp-rplE	rplE reverse recoded sequence primer	caataatgcacgtccctcc
synrp-rpsG	rpsG reverse recoded sequence primer	tgttttgatgacgcaccag
synrp-rpsD	rpsD reverse recoded sequence primer	gctcattgatatctgcgctt
synrp-rplD	rplD reverse recoded sequence primer	tctacctgtttcacagcgt
synrp-rplC	rplC reverse recoded sequence primer	aatcactacccgtcgctc
synrp-rpsC	rpsC reverse recoded sequence primer	gtttctttggttgcgctg
synrp-rpsB	rpsB reverse recoded sequence primer	ttggcttgctaagtcttgtg
synrp-rplB	rplB reverse recoded sequence primer	aaacttgtctgtgcgtttatttg
synrp-prfB	prfB reverse recoded sequence primer	tcaatgaactggtccaaactc
synrp-rpsA	rpsA reverse recoded sequence primer	cttaaatgcctccgccatc
kanR.seqOUT-Nr2	Primer for sequencing the C-terminus of recoded essential genes (hybridizes near the N-terminus of kanR and faces toward the recoded essential gene)	gaatttaatcgcggcctc
3502900.tolC-f	forward primer for generating tolC insertion cassette at nt 3502900	gcgcgttgaattttacatcccgtacgttcccctcaccctaaccctctccttgaggcacattaacgcc

3502901.tolC-r	reverse primer for generating tolC insertion cassette at nt 3502900	atagcaccgtcaagctaaattccgtactgaacggtcccctcgccccttttgtctagggcggcgg att
4427600.tolC-f	forward primer for generating tolC insertion cassette at nt 4427600	tgaagtcgaactgctggaaatcctctaagcagcgcattctgttcccctcgttgaggcacattaa cgcc
4427601.tolC-r	reverse primer for generating tolC insertion cassette at nt 4427600	cgtttggcaaactgaagggtttattgctgaatgcctgctcccctctcgtttctagggcggcggat t
3502822.seq-f	forward primer for screening tolC insertion at nt 3502900	cattaaccgtaggccggataaga
3503081.seq-r	reverse primer for screening tolC insertion at nt 3502900	tcccgccgctcttttatcg
4427507.seq-f	forward primer for screening tolC insertion at nt 4427600	ctggcatatggcgagc
4427776.seq-r	reverse primer for screening tolC insertion at nt 4427600	tcgatattaggtaacaatacgcgg
tolC.90.del	deletes endogenous tolC	gaatttcagcgacgtttgactgccgtttgagcagtcatgtgttaaagcttcggccccgtctgaac gtaaggcaacgtaaagatacgggttat
tolC-r_null_mut*	inactivates tolC for CoS-MAGE	a*g*caagcacgccttagtaacccggaattgcgtaagtctgccgctaaatcgtgatgctgcct ttgaaaaaattaatgaagcgcgcagtcca
tolC-r_null_revert*	tolC co-selection oligo	c*a*gcaagcacgccttagtaacccggaattgcgtaagtctgccgccgatcgtgatgctgcc tttgaaaaaattaatgaagcgcgcagtcca
bla_mut*	inactivates bla in lambda prophage	g*c*c*a*catagcagaactttaaaagtgctcatcattggaaaacgttattaggggcgaaaa ctctcaaggatcttaccgctgttgagatccag
bla_restore*	bla co-selection oligo	g*c*c*a*catagcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaa ctctcaaggatcttaccgctgttgagatccag
rplJ_12-54	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*tactgcagacagcgcgcctttcgctacttcgcttacttcagcaacaatcgcttgtttgtctt gcagatttaaagccattagctttgct
rplJ_42-87	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*g*tcattttatctacagttacgccacgcgaatccgcaactactgcagacagcgcgcctttc gctacttcgcttacttcagcaacaatcgc
rplJ_321-333	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*g*tcgatctgagacgccgggatcagctcaccttcaaacgcagccgcttttacctcaaatttt gcattcgctttcgcgaactctttgaaca
rplJ_390-423	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*c*agtttgccagccgaagcttctttcatcgttgccatcaggcgtgcaattgcttcttcgtacg tcggcagagttgccaggcggtcgatct
rpsB_12-57	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*t*tttcgggttccagtaacgcgtctggtgaccgaagtgaacaccagccttcagcatgtcgc gcatagaaacagttgccatgattaaaacc
rplB_147-117	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*g*cttgtggccaccaccgatatgacgagtcgtgatacggccattgttgttacgaccaccac ttttgctgtttttttccagcaacggagca
rplB_240-261	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*cgtctttgtacagaaccagcgcgatgttcgccgaacggttcggatcgtactccagacgt tcaacaactgccgggataccgtctttgtt
rplB_468-516	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*c*accagaacgcagacgcagcgtcacataagcaccatcacgagcaacgatctgaacgt aagtaccagcactacgtgccagctgaccgcct
rplB_654-666	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*g*accttcaccaccaccatgtgggtggtctaccgggttcatcgccgtaccgcgaacagtc ggacgaacaccacgccagcgtgcagcacct
rplB_753-768	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*gtacgatgaatttatcagtacgcttgttgctgcgagtcttcttacctttcgtctgaacgccc cacggagttaccgggtgcttaccaaa
rplD_42-51	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*a*cctggtgaaccagcgcttcgttgaaatcacgaccgaatgtagtttcagaaacagtcag cgcgctctgcgcgtctttcaatactaattc
rplD_162-192	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*g*gctcttgatagaaccagaacgcgcacggccagtgcctttctggcgccacggtttttac ctgaaccagttacttcagcacgagtcttc
rplD_327-360	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*t*tcggcgcttctacagagaacttctctacaacgatcagacgatcctgacgtaccagttccg acaggatgcttttcagcgcgccgcggta

rplD_543-603	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*g*ttcttcacgaattatgccagcatctcctcaacttgcttaacagcatcagcagtcattacaactttgtcgaacgcgatcaggctaaccg
rplO_24-33	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*g*aaccgataccacgacccaggcgtttacccgccttttgctgccttctgccggagacagagtatttaaacgcatctcttactcctcaac
rplO_78-87	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*c*cagaacgagacttctgacctttgtgaccacgaccacccgttttacccaggccagaaccgataccacgacccaggcgtttacccgcctt
rplQ_159-201	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*gatctcgttatcacgagtacgagcgaatgccagacgacgattagcaacgctatcagtcttagccagagtaatcagcggctcaactacg
rpsC_60-75	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*t*ttaaaatcgctgtccaggttgtcagcgaattcttttgtgttcgcaaaccaagtagagttccatggttttacaatacccaggcgaatac
rpsC_150-198	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*c*gggcgagcagtgtgaatagttacacggatgctcttagccggacgctcgataacgatacgagatactgacgctttagccagttccttag
rpsC_267-309	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*c*cagttcaggcttacgaacttcagcgatgttgatctgtgcaggaacgccagcgatgtccgctactaccttacgcagtttttctacgtct
rpsD_6-60	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*ttcaggaataagtccgtgccctcacgacggctcagcttcagcttaggacccaaatagcgtgccatttttctttctccaacaaacctgga
rpsD_48-87	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*g*ctggccaggagcttgttcaattttacacttagtatcgatcgcgcgaacgccagacttcaggaataagtccgtgccctcacgacggctc
rpsG_144-195	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*g*tcgggcgcacgttttccagagctacttcgaatgcttccagttcagatttaccagagcgctgagccagtgtctccagcgcgctgtatac
rpsG_342-369	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*a*ctgcagtacctttgttttctgcagcatcagacagttcgttcgccaggcgcagagccatgcttttatcaccgcgtttacgagcagcttc
rpsG_432-438	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*g*cgcagagataaccaacggtagtgtgcgaacgccttgtttgcttcagccatacggtgaacgtcttcacgtttcttaactgcagtacctt
rpsG_471-516	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*t*ttaagtagcccaaagccggctgcttacttgaagcgcccgcctggtgactaaaactgcgcagagataaccaacggtagtgtgcgaacgc
rpsG_504-537	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*c*gatgggtgttgtacgagccatttgtttcctcgtttatcttttaggcgtttaatttaagtagccccaaagccggctgcttacttgaagcg
rpsA_21-51	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*aacgccacgaacgatagaacccgggcgcgtttcgatttcttttaagctctcttcaaataattgagcaaaagattcagtcatgtttaat
rpsA_135-165	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*c*acctacctggatttccagctcgccctgagcgtttttgaactgctcagccgggatcgcgctctcagatttcagaccagcgtcaaccagt
rpsA_252-324	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*c*agtaacagtttcagcatcttcgtaagctttttccagcgtgatccaagcttcgtgacgtttagctttctcacggctcagcagagtttca
rpsA_453	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*aacgttgttgcgcttctgatccagcttgattactttaaattccagctctttgccttccaggtgcagagtgtcacgcaccggacgaacg
rpsA_513-525	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*g*gttttccagcagctgatcgcgctctgcgctgttttccgattcgataacagcacgacgagaaacaacaacgttgttgcgcttctgatcc
rpsA_603	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*a*cgccgcccagatcaacgaatgcaccgtagtcagtcaggttcttaacgataccttttaacttccatgccttcctg
rpsA_669-702	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*t*taacagtgatttcgtcgcccacgttaacgatttcgctcggatgcttaacgcgtttccaagccatgtcagtgatgtgcagcaggccgtc
rpsA_756-765	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*a*tagctacccacggatcttcgcccagctgtttcaggcccagtgatacacgagtacgttcgcggtcgaacttcagcactttaacagtgat
rpsA_831-861	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*c*ttcgatttcaacgaagcagccgtagtcagtcaggttagtcacgcgaccagtcagtttcgtaccttccggataacgtttagcgatagct
rpsA_918-954	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*tacatcgccaacgttaacaactttactcgggtggatgtttttgttagtccagtccatttcgctaacgtgtaccaggccttcaacgcct

rpsA_1026-1077	CoS-MAGE oligo that simultaneously changes multiple nearby codons	a*c*ggtcgcccttgttgtgcgtttccgcgaactgctgccacgggttagctttgcactgtttcagacccagtgagatacgacgacgttcttc
rpsA_1188	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*t*ttttgtattcacgaactgcttcttcgcctgcaacgttccaagagatgtcagacaggtgaaccaggccgtcgatgccgccgtccaagcc
rpsA_1287-1305	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*t*tgttcagagcaacccagttgttgaacggatcttctgctaactgtttaacgcccagagagatacgttcacgttctgcgtcaacctgcag
rpsA_1362-1398	CoS-MAGE oligo that simultaneously changes multiple nearby codons	c*t*tcaacgccgtcagccagttctaccgttgcgcctttagcgtcaactgcagttactttaccagttacgatagcgccttttcttgttcagag
rpsA_1449-1515	CoS-MAGE oligo that simultaneously changes multiple nearby codons	g*a*tcaacgcccgtgaatttagcttcaacttcgtcgccaacgctcagaaccagcgtagcgtcttcaacgcggtcacgcgatgcttcagaag
rpsA_1626	CoS-MAGE oligo that simultaneously changes multiple nearby codons	t*t*actcgcctttagctgctttgaaagcttcagccattgcgttgttactgaagtttgcatcttcctgtttgttaacagttgcgattgcatc
rplJ_ACC171ACG	CoS-MAGE oligo that changes one codon	g*a*ccaacaaacgcgtctttcaggcactcgaacggagtaccttcaacagcacggcgcagcagcgtgttacgaacaacacgcatgtatacgc
rplJ_ACC237ACG	CoS-MAGE oligo that changes one codon	a*g*acgagcagcagcgcccgggtgttccatagagtatgcaatcagcgtcggaccaacaaacgcgtctttcaggcactcgaacggagtacct
rpsR_ACC39ACG	CoS-MAGE oligo that changes one codon	a*g*cgtagcgatatctttatagtcgatctcttgaacgccttccgccgtgaaacggcagaacttgcgacgacggaaataacgtgccatatgg
rplL_TCC99TCA	CoS-MAGE oligo that changes one codon	g*c*agcttcaaccgggccagcagctacagctacagcagcagcagctgaaacaccgaatttttcttccattgcagagatcagttctacaacg
rpsP_GTC60GTT	CoS-MAGE oligo that changes one codon	t*t*gaagaaaccaacgcgctcgatgaagcgaccgttgcgtgcattacggctgtcagcaacaacaacctggtagaacggacgctttttagcg
rplB_ACC384ACA	CoS-MAGE oligo that changes one codon	a*c*gttatgaacagtagaaccaaccgggatgttgcgcatcggcagtgtgttacctggtttgattgcagcatcaacgccagactgaatctgg
rplB_GCC315GCG	CoS-MAGE oligo that changes one codon	c*a*tcaacgccagactgaatctggtcgccagctttcaggcctttaggcgccaggatgtaacggcgttcaccgtctttgtacagaaccagcg
rplD_ACC249ACG	CoS-MAGE oligo that changes one codon	t*t*cttgttaacttttgactgtggtcctgcggacgagcagcaaacgtcacgccaccagaaccgccagatcgggctcttgatagaaccagaa
rplD_ACC447ACG	CoS-MAGE oligo that changes one codon	t*g*caggttgcgcgcagccaggaacaggttttcgtccagctcacccgtgatgatcagcacatcttccagagccatgtctttcagtttctgt
rplO_GCC222GCG	CoS-MAGE oligo that changes one codon	a*c*tacaccgccttctactttagccaggtcagacagacgaatttccgctgtaatcgctgcttacgagaagtgaagccgaatttcggcaga
rplQ_GCC270GCG	CoS-MAGE oligo that changes one codon	t*c*gcctgcacggaagccacacttcagaatacgagtgtaaccacccgcacggctcgcgaaacgcgggcccagttcgttaaacagttttgcc
rpsG_GTC15GTT	CoS-MAGE oligo that changes one codon	t*c*tgatccgaacttcggatccggcagaattttacgctgaccaataacgcgacgacgtggcatggaaatactccgttgttaattcaggatt
rpsG_GTC270GTG	CoS-MAGE oligo that changes one codon	t*c*aacgatccaacgcattgccagagcattacgacgaaccggacgcacttcaactggtacctgataagtagaaccaccaacgcggcgagac
rplJ_GCC333GCG	redesigned CoS-MAGE oligos to convert remaining forbidden codons	c*a*gagttgccaggcggtcgatctgagacgccgggatcagctcaccttcaaacgcagcggctttgacctcaaattttgcattcgctttcgc
rplD_TGA603TAA_refactor	redesigned CoS-MAGE oligos to convert remaining forbidden codons	g*t*gcggtgcacgcagcaccttcagcagacgttcttcacgaatcattatgccagcatctcctcaacttgcttaacagcatcagcagtcat
rplQ_CTT162CTG	redesigned CoS-MAGE oligos to convert remaining forbidden codons	c*g*ggcgaatgccagacgacgattagcaacgctatcagtcttggccagagtaatcagcggctcaactacgcggcgcagctctttcgcttta
rpsA_ACC324ACT	redesigned CoS-MAGE oligos to convert remaining forbidden codons	c*a*gctcaacagtgaagccgcccttaactttgccgttgataacaccagtaacagtttcagcatcttcgtaagctttttccagcgtgatcca
rpsL-1	redesigned CoS-MAGE oligos to convert remaining forbidden codons	a*g*tcagacgaacacggcatactttacgcagcgcgctgttcggttttttaggagtcgtagtatatacacgagtacatacgccacgtttttg

rpsL-2	redesigned CoS-MAGE oligos to convert remaining forbidden codons	a*c*cgccacggatcaggatcacactgtgctcctgcaggttgtgaccttcaccaccgatgtaagaagtcacttcgaaaccgttagtcagacg
rpsL-3	redesigned CoS-MAGE oligos to convert remaining forbidden codons	g*c*ttacggtctttaacgccagagcagtctaacgcaccacgtactgtgtggtaacgaacaccccggcaggtctttaacacgaccgccacg
rpsL-4	redesigned CoS-MAGE oligos to convert remaining forbidden codons	a*c*gcttcacgccatacttgctacgagcctgcttacggtctttaacgccagagcagtctaacgcaccacgtactgtgtggtaacgaacacc
rplQ_CTT162YTR*	CoS-MAGE oligo to change rplQ CUU 160-162 to all Leu codons	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggcyaragtaatcagcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162ATY*	CoS-MAGE oligo to change rplQ CUU 160-162 to all Ile codons	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggcratagtaatcagcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162GTD*	CoS-MAGE oligo to change rplQ CUU 160-162 to all Val codons	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggchacagtaatcaggcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162GCD*	CoS-MAGE oligo to change rplQ CUU 160-162 to all Ala codons	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggchgcagtaatcaggcggctcaactacgcggcgcagctctttcgct
rplQ_CTT162ATG*	CoS-MAGE oligo to change rplQ CUU 160-162 to the Met ATG codon	g*t*acgggcgaatgccagacgacgattagcaacgctatcagtcttggccauagtaatcaggcggctcaactacgcggcgcagctctttcgct
rplP_syn_fix_G	MAGE oligo to convert rplP_syn1 AUA to AUG, AUC, or AUU	c*g*acacgtcagttccttgagctaggccacgattgcgtcccttatgCatcttgcgaaacttcgttcgtttcggctgcagcatcagcgacgc
rplO_24-33_wt-f	wt forward mascPCR primer	cccgccttttggagccttcg
rplO_78-87_wt-f	wt forward mascPCR primer	ggttctggcctcggtaaaacc
rplO_GCC222GCG_wt-f	wt forward mascPCR primer	cgtaaagcagcgattacagcc
rplQ_159-201_wt-f	wt forward mascPCR primer	gcaacgctatcagtcttggcaag
rplQ_GCC270GCG_wt-f	wt forward mascPCR primer	cgtttcgcgagccgtgcc
rpsC_60-75_wt-f	wt forward mascPCR primer	cgaattctttggtgttcgcaaaccag
rpsC_150-198_wt-f	wt forward mascPCR primer	taaggaactggctaaagcgtcc
rpsC_267-309_wt-f	wt forward mascPCR primer	cctgcacagatcaacatcgcc
rpsD_6-60_wt-f	wt forward mascPCR primer	gcttgagcttaggacccaaatatct
rpsD_48-87_wt-f	wt forward mascPCR primer	gcgttcgcgcgatcgatacc
rpsR_ACC39ACG_wt-f	wt forward mascPCR primer	gtcgcaagttctgccgtttcacc
rplL_TCC99TCA_wt-f	wt forward mascPCR primer	gcaatggaagaaaaattcggtgtttcc
rpsP_GTC60GTT_wt-f	wt forward mascPCR primer	gtccgttctaccaggttgttgtc
rpsB_12-57_wt-f	wt forward mascPCR primer	actgtttccatgcgcgacatgctc
rplJ_GCC333GCG-wt-f	wt forward mascPCR primer	ttgaggtcaaagccgctgcc
rplD_TGA603TAA	wt forward mascPCR primer	aagttgaggagatgctggcatg

_refactor-wt-f		
rplQ_CTT162CTG-wt-f	wt forward mascPCR primer	gtagttgagccgctgattactctt
rpsA_ACC324ACT-wt-f	wt forward mascPCR primer	cttacgaagatgctgaaactgttacc
rplO_24-33_mut-f	mutant forward mascPCR primer	cccgccttttgctgccttct
rplO_78-87_mut-f	mutant forward mascPCR primer	ggttctggcctgggtaaaacg
rplO_GCC222GCG_mut-f	mutant forward mascPCR primer	cgtaaagcagcgattacagcg
rplQ_162-201_mut-f	mutant forward mascPCR primer	gcaacgctatcagtcttagccag
rplQ_GCC270GCG_mut-f	mutant forward mascPCR primer	cgtttcgcgagccgtgcg
rpsC_60-75_mut-f	mutant forward mascPCR primer	cgaattcttttgtgttcgcaaaccaa
rpsC_150-198_mut-f	mutant forward mascPCR primer	taaggaactggctaaagcgtca
rpsC_267-309_mut-f	mutant forward mascPCR primer	cctgcacagatcaacatcgct
rpsD_6-60_mut-f	mutant forward mascPCR primer	cttcagcttaggacccaaatagcg
rpsD_48-87_mut-f	mutant forward mascPCR primer	gcgttcgcgcgatcgatact
rpsR_ACC39ACG_mut-f	mutant forward mascPCR primer	gtcgcaagttctgccgtttcacg
rplL_TCC99TCA_mut-f	mutant forward mascPCR primer	gcaatggaagaaaaattcggtgtttca
rpsP_GTC60GTT_mut-f	mutant forward mascPCR primer	gtccgttctaccaggttgttgtt
rpsB_12-57_mut-f	mutant forward mascPCR primer	actgtttctatgcgcgacatgctg
rplJ_GCC333GCG-mut-f	mutant forward mascPCR primer	ttgaggtcaaagccgctgcg
rplD_TGA603TAA_refactor-mut-f	mutant forward mascPCR primer	aagttgaggagatgctggcata
rplQ_CTT162CTG-mut-f	mutant forward mascPCR primer	gtagttgagccgctgattactctg
rpsA_ACC324ACT-mut-f	mutant forward mascPCR primer	cttacgaagatgctgaaactgttact
rplO_24-33_rev	reverse mascPCR primer	tgaacgcgctctggaaaaagg
rplO_78-87_rev	reverse mascPCR primer	gcatctgaccaccctcgaaacc
rplO_GCC222GCG_rev	reverse mascPCR primer	cagcggtgaagtagaatgcaaagc
rplQ_159-201_rev	reverse mascPCR primer	ctgagaaggataaggtcatgcgc
rplQ_GCC270GCG_rev	reverse mascPCR primer	ggtaagcaaccggcattcttcag

rpsC_60-75_rev	reverse mascPCR primer	caagaaagttctggaatctgccattg
rpsC_150-198_rev	reverse mascPCR primer	cgccagcgatgtccgctac
rpsC_267-309_rev	reverse mascPCR primer	aggtgttgtagtcgatgtcagcac
rpsD_6-60_rev	reverse mascPCR primer	ggtccgcgcaaaccgatc
rpsD_48-87_rev	reverse mascPCR primer	aaactctgtcacagaaccctgc
rpsR_ACC39ACG_rev	reverse mascPCR primer	gtttcgagcgagtgccgcag
rplL_TCC99TCA_rev	reverse mascPCR primer	cgaatacgtttttctcggtataggagtaaacc
rpsP_GTC60GTT_rev	reverse mascPCR primer	tgataacctgcccatcgaggaac
rpsB_12-57_rev	reverse mascPCR primer	ccaggctgttttccagtttctcc
rplJ_GCC333GCG-r	reverse mascPCR primer	cctgaatatcagaataagtttatacgtaagcgaatg
rplD_TGA603TAA_refactor-r	reverse mascPCR primer	ccttgtgcagctcagggttaac
rplQ_CTT162CTG-r	reverse mascPCR primer	cgctggagatcgctttcggtatatag
rpsA_ACC324ACT-r	reverse mascPCR primer	caacgaatgcaccgtagtcagt
vsr_mut*	MAGE oligo that inactivates vsr by adding two in-frame stop codons	g*g*c*c*ctgcccggttaacagactggcgaggcgcttctcttatcacgtatcacgcgtggcaatcgcgcgcatatttttgctg
vsr_wt-f	wt forward mascPCR primer	ccacgcgtgatacggc
vsr_mut-f	mutant forward mascPCR primer	gccacgcgtgatacgtg
vsr-r	reverse mascPCR primer	cgcactcccagacaatcaatac
rplP_syn_fix_wt-f	wt forward mascPCR primer	acgaacgaagtttcgcaagatA
rplP_syn_fix_mut-f	mutant forward mascPCR primer	acgaacgaagtttcgcaagatB
rplP_syn_fix_305-r	reverse mascPCR primer	catccatctcgtataataccttgcc
rpsS-Leu-fix-f	Primer converts the rpsS_syn1 forbidden CUU codon to CUA, CUG, UUA, or UUG	GATAAATTCATCGTACGTCGCCGTAGCAAATAATTTTAGAGGATAAGCCATGytrCGhAGCTTAAAAAAGGGACC
rpsS-Pro-fix-f	Primer converts the rpsS_syn1 forbidden CUU codon to CCA, CCG, or CCU	GATAAATTCATCGTACGTCGCCGTAGCAAATAATTTTAGAGGATAAGCCATGccdCGhAGCTTAAAAAAGGGACC
rpsS-Syn-r	Reverse primer for rpsS_syn cassette	AGAACGAGCATGGCGATG

table S9. Recoded gene designs

>rpmH
ATGAAGCGTACGTTCCAGCCTAGTGTTTTGAAACGTAATCGCTCACATGGTTTTCGCGCGCGCATGGCAACGAAGAA
CGGCCGCCAAGTGTTGGCGCGCCGCCGCGCAAAGGGTCGTGCGCGCTTAACGGTGTCAAAATAATCTAGAAAGACGT
C

>rpmD
ATGGCGAAAACGATCAAGATCACACAGACTCGTTCTGCTATTGGGCGCTTGCCTAAGCATAAAGCTACATTACTGGG
GTTAGGCTTACGACGCATCGGACATACGGTTGAACGTGAGGACACGCCGGCAATCCGTGGAATGATTAATGCAGTAT
CGTTTATGGTGAAGGTGGAAGAATAATCTAGAAAGACGTC

>rpmC
ATGAAGGCGAAGGAATTGCGCGAAAAATCTGTGGAGGAACTAAATACGGAATTGTTAAATTTATTGCGAGAACAATT
TAATTTACGCATGCAAGCAGCGTCGGGACAATTGCAGCAAAGCCATCTACTGAAACAGGTACGCCGTGACGTGGCGC
GTGTAAAAACACTGTTGAATGAAAAAGCTGGCGCTTAATCTAGAAAGACGTC

>rpsR
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TGCGACTTTAAAGAATTATATTACGGAGTCTGGCAAAATCGTTCCTTCACGCATTACAGGCACTCGAGCTAAGTATC
AACGCCAATTAGCGCGTGCAATTAAGCGTGCGCGTTATTTAAGTTTACTACCTTATACGGACCGTCACCAATAATCT
AGAAAGACGTC

>rpmB
ATGTCTCGTGTATGTCAGGTGACGGGGAAACGCCCAGTTACAGGCAATAATCGCAGCCATGCTCTAAATGCTACGAA
GCGTCGCTTTTTGCCAAATTTACATAGCCATCGCTTTTGGGTGGAATCAGAAAAACGATTCGTGACTTTACGTGTGA
GCGCAAAGGGCATGCGCGTTATTGACAAGAAGGGGATTGACACGGTGCTAGCAGAGCTACGCGCGCGCGGTGAGAAA
TATTAATCTAGAAAGACGTC

>rpsP
ATGGTGACGATCCGACTGGCTCGCCATGGAGCGAAGAAACGCCCATTTTATCAAGTGGTGGTTGCAGATTCACGAAA
CGCTCGTAATGGCCGTTTTATTGAACGTGTGGGCTTTTTTAATCCGATTGCGTCTGAGAAGGAGGAGGGAACGCGTT
TAGACTTGGACCGTATTGCGCATTGGGTGGGTCAAGGTGCTACGATCTCGGACCGTGTGGCAGCATTGATTAAGGAG
GTGAATAAGGCTGCATAATCTAGAAAGACGTC

>rpsQ
ATGACGGACAAGATTCGCACATTACAGGGACGTGTGGTGTCGGATAAGATGGAAAAGAGCATCGTGGTGGCAATTGA
GCGCTTCGTAAAGCATCCTATTTATGGGAAGTTTATTAAACGCACTACGAAGTTACATGTTCACGATGAAAATAATG
AGTGTGGAATTGGCGATGTAGTGGAGATTCGAGAGTGTCGCCCATTAAGCAAAACGAAGTCTTGGACATTGGTGCGT
GTGGTGGAAAAGGCTGTGTTGTAATCTAGAAAGACGTC

>rpmA
ATGGCGCACAAGAAAGCGGGTGGATCAACTCGCAATGGCCGTGACTCTGAGGCGAAGCGATTGGGGGTAAAACGCTT
TGGCGGTGAGAGTGTGTTAGCTGGGTCAATTATTGTGCGACAGCGCGGAACGAAGTTTCATGCGGGGGCAAATGTGG
GATGTGGCCGCGATCATACGTTATTCGCGAAGGCTGATGGGAAGGTTAAGTTTGAGGTGAAGGGACCAAAGAATCGC
AAGTTCATTTCGATTGAGGCGGAGTAATCTAGAAAGACGTC

>rpsS
ATGCCTCGCAGCTTAAAAAAGGGACCGTTCATCGATTTGCATCTGTTAAAAAAAGTGGAAAAGGCTGTTGAGTCAGG
CGATAAAAAACCGTTGCGTACGTGGTCGCGACGCAGCACAATTTTCCCAAATATGATTGGCCTGACAATTGCGGTGC
ACAACGGACGCCAACATGTACCAGTGTTCGTTACGGATGAGATGGTGGGCCATAAGTTGGGCGAGTTTGCTCCAACG
CGAACGTACCGTGGGCATGCGGCAGACAAGAAGGCAAAAAAAAAAGTAATCTAGAAAGACGTC

>rplW
ATGATTCGCGAGGAGCGCTTGTTGAAAGTATTGCGCGCGCCACATGTGTCAGAGAAGGCTAGTACAGCAATGGAAAA
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TCTGAGATTGTTAATGTTGGTGATGAGATTACGGTGAAGGTTTTGAAATTTGATCGTGAGCGCACTCGCGTTTCATT
AGGGCTAAAGCAATTAGGTGAGGACCCATGGGTGGCAATTGCGAAGCGCTACCCTGAGGGAACGAAGTTAACAGGGC
GTGTTACTAATTTGACTGATTATGGTTGTTTTGTGGAGATTGAGGAGGGAGTGGAGGGATTAGTTCATGTGAGCGAG
ATGGATTGGACTAATAAGAATATTCATCCTAGTAAGGTGGTGAATGTGGGTGACGTGGTTGAGGTGATGGTGTTAGA
CATTGATGAGGAGCGCCGCCGCATTTCATTAGGATTAAAGCAATGTAAGGCGAATCCATGGCAACAATTTGCTGAAA
CGCATAATAAAGGGGATCGCGTGGAGGGCAAGATTAAATCGATTACGGATTTTGGCATTTTTATTGGACTGGATGGT
GGAATTGATGGTTTAGTGCATTTATCGGATATTTCTTGGAATGTAGCGGGTGAGGAGGCTGTGCGCGAGTATAAGAA
GGGTGATGAGATTGCAGCGGTGGTGTTGCAAGTAGATGCTGAGCGCGAGCGCATTTCTTTAGGTGTGAAGCAATTAG
CGGAGGACCCTTTTAATAATTGGGTGGCATTGAATAAAAAGGGTGCAATTGTGACTGGGAAGGTGACAGCGGTGGAT
GCGAAGGGTGCGACGGTGGAGTTAGCGGATGGGGTAGAGGGATATTTACGCGCATCGGAGGCGTCGCGCGATCGTGT
GGAGGATGCGACGTTGGTGTTGTCAGTGGGTGATGAGGTGGAGGCGAAGTTTACGGGTGTGGACCGCAAGAATCGTG
CGATTAGTTTGAGTGTGCGCGCTAAGGATGAGGCAGATGAAAAGGACGCGATTGCGACAGTAAATAAGCAAGAGGAC
GCTAATTTTAGTAATAATGCGATGGCGGAGGCATTTAAGGCGGCGAAGGGTGAATAATCTAGAAAGACGTCA

>kanR (with upstream ribosome binding site) used for transcriptional fusion with recoded genes (start codon is in lower case)
TGAGTTGTCGAGATTTTCAGGAGCTAAGGAAGCTAAAatgAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAA
ATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTAT
CGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGA
TGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATG
ATGCATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAA
AATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGA
TCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGC
GTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACT
CATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGG
AATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGC
TTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA

table S10. Refactored overlapping genes

Gene	Gene terminus overlapped	Length of overlap
rplD	C-terminus	4 bp
rplP	C-terminus	1 bp
rplW	N-terminus	4 bp
rpmC	N-terminus	1 bp
rpmC	C-terminus	1 bp
rpsQ	N-terminus	1 bp

table S11. Doubling times of double mutants compared to single mutants

Gene(s)	Actual fitness[a]	Predicted fitness[b]	Actual doubling time	Predicted doubling time[c]
rplP	0.381	-	128.7	-
rpmC	0.468	-	104.7	-
rplM	0.491	-	99.7	-
rplE	0.919	-	53.3	-
rpsI	0.521	-	94.0	-
rpmC-rplM-1	-	0.230	88.0	213.0
rplE-rplM-6	-	0.452	94.2	108.4
rpmC-rpsI-4	-	0.244	86	200.9
rplP-rplM-1	-	0.187	160.8	261.9

[a]Actual fitness is the measured doubling time divided by wild type doubling time (49 minutes under the conditions used in this study)

[b]Predicted fitness is the product of the actual fitness measured for each synthetic gene corresponding to a double mutant.

[c]Predicted doubling time is the wild type doubling time (49 minutes under the conditions used in this study) divided by the predicted fitness