Supplementary Note

Oligonucleotides for genome editing

- *Lactococcus Lactis*

Rifampicin resistance 4bp mismatch:	C*G*AgagataccaccaggtcctaaggcagagaaacgacgtttgtTGCTaagctcagacaaaggattatgttggtccataaattgtgacaac
Rifampicin resistance 1bp mismatch:	C*G*AgagataccaccaggtcctaaggcagagaaacgacgtttgtTtgaaagctcagacaaaggattatgttggtccataaattgtgacaac
Streptomycin resistance (K56R):	G*A*AAGACGTACACGCGCGAATTTACGAAGCGCTGAGTTAGGTTTTCTAGGAGTCATTGTACCAACACGAGTTGCTACTCCACGTTTTTGT
NNKx5 oligo Spectinomycin resistance	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGMNNMNNMNNMNNMNNAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance (AA 32)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGMNNTCCACCTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance (AA 31)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGMNNACCTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance (AA 30)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCMNNTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance (AA 29)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCMNNTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance (AA 28)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCTTTMNNAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance mutation matching *E. coli* (G27D matches G30D)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCATCTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
NNKx5 oligo Spectinomycin resistance Validation 1 (RTNAR)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACG CCGCGCATTCGTACG AACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
NNKx5 oligo Spectinomycin resistance Validation 2 (NGTRF)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACG AAACCTAGTACCATT AACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance Validation 1 (V28P)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCTTTAGGAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
5x1NNK oligo Spectinomycin resistance Validation 2 (K29I)	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCAATTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT

Spectinomycin resistance validation R at 28	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCTTTCCTAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation R at 28, NN at 30-31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGATTATTTTTCCTAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation R at 28, N at 31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGATTACCTTTCCTAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation R at 28, N at 30	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCATTTTTCCTAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation NN at 30-31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGATTATTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation N at 31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGATTACCTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation N at 30	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCATTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation R at 31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGCCTACCTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation N at 30, R at 31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGCCTATTTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation MNR at 29-31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGCCTATTCATTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation M at 29	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCCATTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation M at 29, R at 31	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGCCTACCCATTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation M at 29, N at 30	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCATTCATTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation SNNRK	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGTTTACGATTATTTGAAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation FNGGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCGTTAAAAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT

Spectinomycin resistance validation FKGGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCTTTAAAAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation VNGGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCGTTTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation PNGGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCGTTCGGAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation V-GGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCTACAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT
Spectinomycin resistance validation FIGGR	C*G*GTCACCAACAACAACAAGTGCTGCAAAGCGAAGACGACGTCCACCAATAAAAACTTTAGTAACGCGGTTAATTGAAACTACGCGTTCT

- *Escherichia coli*

4bp mutation conferring SDS resistance (tolC targeting)	A*G*CAAGCACGCCTTAGTAACCCGGAATTGCGTAAGTCTGCCGCTAAATCGTGATGCTGCCTTTGAAAAATTAATGAAGCGCGCAGTCCA

- *Mycobacterium smegmatis*

1bp Streptomycin resistance (targets rpsL):	G*T*CAGCTTCACGCGCGCGACCTTCCGGAGCGCCGAGTTCGGCTTCCTCGGAGTGGTGGTGTAAACGCGCGTGCACACGCCGCGACGCTGC

- *Lactobacillus rhamnosus*

3bp rifampacin resistance (targets RpoB):	A*C*GAGTCAAGCCACCAGGTCCAAGGGCAGACAGACGCCGTTTGTCCGTTAATTCGCCCAACGGATTGGTCTGATCATGAACTGTGACAG

- *Caulobacter crescentus*

Rifampicin resistance 4bp mismatch:	C*G*CGGGTCAGACCGCCCGGGCCGAGGGCCGAGAGACGACGCTTCCTCGTGATTTCCGACAGCGGGTTCGTCTGGTCCATGAACTGCGACA
Rifampicin resistance 1bp mismatch:	C*G*CGGGTCAGACCGCCCGGGCCGAGGGCCGAGAGACGACGCTTGCGGGTGATTTCCGACAGCGGGTTCGTCTGGTCCATGAACTGCGACA

Oligos used for fluorescence quenching:

Quenching oligo: tolC-f.null.mut-5'IBFQ	/5'IBFQ/TGGACTGCGCGCTTCATTAATTTTTTCAAAGGCAGCATCACGATTTAGCGGCAGACTTACGCAATTCCGGGTTACTAAGGCGTGCTTGCT

Fluorescent oligo: tolC-r.null.mut-3'6FAM	AGCAAGCACGCCTTAGTAACCCGGAATTGCGTAAGTCTGCCGCTAAATCGTGATGCTGCCTTTGAAAAAATTAATGAAGCGCGCAGTCCA/3'FAM/

Primers

- MASC PCR (*L. lactis*)

Rifampacin 1bp PCR F WT	AGAGAAACGACGTTTGTG
Rifampacin 1bp PCR F Mut	CAGAGAAACGACGTTTGTT
Rifampacin 1bp PCR R Common	CGTTCAGTTGGTGAATTACT

- dsDNA validation of genome integration (*L. lactis*)

Colony PCR primer for integration into genome F	GTAGATAAATTATTAGGTATACTACTGACAGC
Colony PCR primer for integration into genome R	CGTACCAAGCTCAGTTAATTG

- Sanger sequencing for spectinomycin resistance mutants (*L. lactis*)

L. lactis RpsE sequencing primer R	ACACTTGCATCAGCTTCATC
L. lactis RpsE sequencing primer F	ATTTTGATGTTACGTCAGCAACAC

- Next-generation sequencing of spectinomycin resistance mutants (*L. lactis*)

Next gen. seq. primer Spectinomycin resistance F 1	ctttccctacacgacgctcttccgatctATGGCAGAAAACAGAAGAAATG
Next gen. seq. primer Spectinomycin resistance F 2	ctttccctacacgacgctcttccgatctNATGGCAGAAAACAGAAGAAATG
Next gen. seq. primer Spectinomycin resistance F 3	ctttccctacacgacgctcttccgatctNNATGGCAGAAAACAGAAGAAATG
Next gen. seq. primer Spectinomycin resistance F 4	ctttccctacacgacgctcttccgatctNNNATGGCAGAAAACAGAAGAAATG
Next gen. seq. primer Spectinomycin resistance R	ggagttcagacgtgtgctcttccgatctTTTTACCACCACCGAAGAC

Nucleotide sequences of codon optimized Genes: SSAPs

- *L. lactis* / *L. rhamnosus*

λ-Red β	ATGAGTACTGCACTTGCAACATTAGCTGGCAAGTTAGCAGAGCGTGTTGGTATGGATTCAGTCGACCCTCAGGAGCTTATAACTACCTTACGTCAAACAGCGTTCAAGTGTGACGCCTCTGATGCACAATTTATCGCTTTGCTTATCGTAGCTAACCAGTATGGGTTGAATCCTTGGACGAAGGAGATATACGCTTTCCCGGATAAGCAGAACGGTATTGTTCCTGTAGTAGGTG

	TCGATGGATGGAGTAGAATTATCAATGAAAATCAACAGTTCGATGG CATGGACTTCGAGCAGGATAATGAATCATGTACCTGCCGTATATATA GAAAAGACCGAAATCACCCAATTTGTGTGACTGAATGGATGGATGA GTGCAGACGTGAGCCGTTCAAGACCCGAGAAGGCCGTGAAATCACT GGTCCGTGGCAATCACATCCAAAGAGAATGTTGCGTCACAAGGCGA TGATTCAGTGCGCCCGTTTAGCTTTTGGGTTTGCTGGCATTTACGACA AGGACGAAGCTGAAAGAATCGTTGAAAACACTGCATATACCGCTGA ACGACAACCGGAGCGTGACATTACGCCAGTGAATGACGAGACAATG CAGGAAATTAACACGTTGTTGATTGCTTTGGACAAAACGTGGGACGA CGACTTGTTACCACTTTGTAGCCAAATTTTTCGTCGAGACATTAGAG CTTCATCTGAGCTTACACAAGCTGAAGCCGTCAAGGCATTGGGGTTT TTGAAACAAAAAGCTACCGAACAGAAGGTAGCGGCATAA
PapRecT	ATGGGAACCGCCCTTACACCTCTTTTGACAAAGTTCGCAACCAGATA TGAGATGGGAACGACCCCTGAAGAGGTTGCTAATACATTGAAACAA ACATGCTTCAAGGGACAGGTCAACGACAGTCAAATGGTAGCCCTTTT GATAGTCGCTGACCAGTACAAGTTAAACCCGTTCACCAAGGAGTTGT ATGCATTCCCTGACAAGAATAATGGAATCGTGCCAGTTGTTGGTGTC GATGGATGGGCGAGAATAATAAACGAGAACCCTCAGTTTGATGGTA TGGAATTTTCTATGGACCAGCAGGGCACTGAGTGCACGTGCAAAATC TATCGTAAGGATCGTTCTCACGCAATAAGCGCTACGGAATATATGGC CGAATGTAAGAGAAATACGCAACCTTGGCAAAGTCACCCACGACGT ATGTTAAGACATAAAGCCATGATCCAGTGTGCGCGATTAGCATTCGG CTTCGCTGGTATCTACGATCAAGACGAAGCGGAACGAATAGTCGAA AGAGACGTTACTCCGGCGGAGCAGTACGAAGATGTCAGCGAAGCTA TATGCTTGATTAAGGACAGCCCGACGATGGAGGATTTACAGGCAGC GTTCAGCAATGCCTGGAAGGCGTATAAAACCAAAGGTGCAAGAGAC CAATTGACAGCCGCCAAGGATCAGCGTAAAAAGGAATTACTTGATG CCCCAATAGATGTCGAGTTCGAAGAAACTGGCGATGATAGAGCAGC ATAA
MspRecT	ATGGCAGAAAACGCCGTGACGAAACAAGACTCACCTAAAGCCCCAG AAACGATATCACAGGTCCTTCAAGTGTTAGTACCTCAATTAGCTCGA GCCGTACCTAAGGGAATGGATCCTGATAGAATAGCTCGAATCGTCCA GACCGAGATCAGAAAGAGTAGAAATGCGAAAGCGGCGGGAATCGC CAAACAGTCATTGGATGACTGCACGCAAGAAAGCTTCGCCGGGGCG TTACTTACAAGTGCGGCATTGGGCCTTGAGCCAGGCGTTAACGGTGA GTGTTATCTTGTTCCATACAGAGACACAAGAAGAGGTGTCGTCGAGT GCCAGTTAATTATCGGGTATCAAGGAATCGTTAAATTGTTTTGGCAA CATCCGCGAGCCTCTCGAATAGACGCCCAGTGGGTGGGGGCAAACG ATGAATTCCATTATACAATGGGTCTTAACCCAACCTTAAAACATGTA AAGGCTAAGGGAGACCGAGGAAACCCAGTATATTTTTATGCTATCGT AGAGGTCACGGGTGCCGAGCCTTTGTGGGATGTCTTCACAGCTGATG AGATTAGAGAGTTGAGAAGAGGTAAAGTCGGTTCAAGCGGGGATAT TAAGGATCCGCAGAGATGGATGGAGCGAAAGACAGCGTTAAAACAA GTGCTTAAGCTTGCTCCAAAAACTACTCGTTTGGACGCAGCAATACG AGCGGACGATAGACCGGGAACAGATTTGTCTCAAAGCCAGGCGTTG GCATTACCTAGTACAGTTAAGCCAACAGCAGACTACATAGACGGTG

	AGATTGCAGAGCCACACGAGGTTGACACTCCGCCTAAAAGCAGTCG AGCACAACGAGCTCAGAGAGCGACTGCCCCAGCCCCAGACGTTCAG ATGGCCAATCCGGATCAATTAAAGCGTTTGGGAGAGATTCAAAAAG CCGAAAAGTACAATGATGCCGACTGGTTTAAGTTCTTAGCTGATAGT GCAGGGGTGAAAGCGACAAGAGCAGCTGATCTTACATTTGATGAAG CAAAAGCTGTAATAGATATGTTCGACGGCCCAAATGCTTGA
LrpRecT	ATGGCTAATCAAGTAGCACAACAGCAGAAACCGACTAAGCTAACCG ATCTTGTATTAGATCGTGTTAAACAAATGCAAGACACGCAGGACTTG TCACTACCCAAGAATTACAACGCTTCTAATGCGTTGAATGCAGCCTT TCTCGAATTACAAAAAGTACAAGACCGTAATCATCGGCCAGCCTTAG AAGTATGTTCTCATGACTCGATTGTTAAGTCCTTGTTAGATATGACAC TGCAAGGGCTATCCCCAGCAAAAGATCAATGCTACTTCATCGTATAC GGCAATGAGCTTCAAATGCAACGGAGCTATTTCGGTACTGTTGCAGC AGTTAAGCGACTGGATGGTGTTAAGAAAGTTAGGGCAGAAGTTGTT CACGAAAAAGATGACTTTGAAATTGGTGCTAATGAAGACATGGAGC TAGTCGTTAAGAGGTTCGTTCCTAAGTTTGAAAATCAAGATAATCAA ATTATTGGAGCTTTTGCCATGATTAAGACTGATGAAGGTACTGACTT TACTGTTATGACTAAGAAAGAGATTGATCAGTCATGGGCACAAACA CGTCAAAAAAATAACAAAGTACAGCAGAATTTTAGCCAAGAAATGG CAAAGCGTACTGTGCTTAATCGTGCCGCTAAGATGTTTATTAACACG TCTGATGATAGTGACCTTTTAACTGGTGCTATCAACGATACAACAAG CAACGAATACGATGATGAGCGTCGAGATGTAACGCCCGTTGAGGAT GAAAAACAAAGTACTGATAAATTGCTAGAAGGATTTCAAAAGTCAC AAGAAGCGAAGGCTAAGTGGGTAAGTAATGATGGCAACAGCAACGA AGGCAAAGAAACCAGTGAAGAAGTCGCAGACGGACAAACAGAACT CTTCAGCGAAGGGACAATCAAACCAGCCGATGAAGCTGACAGCTAA

- *E. coli*

λ-Red β	ATGAGTACTGCACTCGCAACGCTGGCTGGGAAGCTGGCTGAACGTGT CGGCATGGATTCTGTCGACCCACAGGAACTGATCACCACTCTTCGCC AGACGGCATTTAAAGGTGATGCCAGCGATGCGCAGTTCATCGCATTA CTGATCGTTGCCAACCAGTACGGCCTTAATCCGTGGACGAAAGAAAT TTACGCCTTTCCTGATAAGCAGAATGGCATCGTTCCGGTGGTGGGCG TTGATGGCTGGTCCCGCATCATCAATGAAAACCAGCAGTTTGATGGC ATGGACTTTGAGCAGGACAATGAATCCTGTACATGCCGGATTTACCG CAAGGACCGTAATCATCCGATCTGCGTTACCGAATGGATGGATGAAT GCCGCCGCGAACCATTCAAAACTCGCGAAGGCAGAGAAATCACGGG GCCGTGGCAGTCGCATCCCAAACGGATGTTACGTCATAAAGCCATGA TTCAGTGTGCCCGTCTGGCCTTCGGATTTGCTGGTATCTATGACAAGG ATGAAGCCGAGCGCATTGTCGAAAATACTGCATACACTGCAGAACG TCAGCCGGAACGCGACATCACTCCGGTTAACGATGAAACCATGCAG GAGATTAACACTCTGCTGATCGCCCTGGATAAAACATGGGATGACGA CTTATTGCCGCTCTGTTCCCAGATATTTCGCCGCGACATTCGTGCATC GTCAGAACTGACACAGGCCGAAGCAGTAAAAGCTCTTGGATTCCTG AAACAGAAAGCCGCAGAGCAGAAGGTGGCAGCATGA

PapRecT	ATGGGTACTGCTCTAACGCCGTTATTAACCAAGTTTGCCACCCGCTA TGAGATGGGAACTACCCCCGAAGAGGTCGCTAATACGCTGAAACAG ACTTGTTTCAAGGGCCAAGTGAACGATAGTCAGATGGTAGCCCTTTT GATCGTTGCGGATCAATATAAGCTCAATCCATTCACAAAAGAGCTCT ACGCGTTCCCTGACAAAAATAATGGTATTGTTCCAGTTGTGGGAGTC GATGGTTGGGCTAGAATTATTAACGAGAATCCCCAGTTTGATGGGAT GGAATTCAGTATGGATCAACAGGGAACTGAATGCACTTGTAAAATTT ACCGCAAAGACCGCTCGCACGCCATCAGCGCCACCGAGTACATGGC TGAGTGCAAAAGGAACACTCAACCTTGGCAGTCTCACCCGCGACGTA TGCTGCGTCATAAGGCTATGATTCAATGCGCCAGACTAGCCTTTGGT TTCGCGGGGATCTACGATCAGGATGAGGCCGAACGCATTGTTGAACG AGATGTAACTCCCGCCGAGCAATACGAGGATGTATCCGAAGCGATTT GTCTGATCAAAGACTCACCAACTATGGAGGACTTGCAGGCCGCGTTC TCAAACGCGTGGAAAGCTTACAAGACTAAAGGTGCCCGTGATCAAC TGACTGCTGCTAAAGACCAGAGAAAAAAGGAGCTGTTGGATGCGCC CATTGATGTCGAATTCGAAGAAACTGGAGATGATCGTGCTGCGTAA
MspRecT	ATGGCCGAGAATGCCGTCACGAAACAGGATTCCCCTAAGGCACCGG AAACCATTAGTCAAGTGCTTCAGGTGCTGGTCCCACAATTGGCTCGT GCAGTACCTAAAGGCATGGATCCTGATCGTATTGCACGTATCGTACA GACGGAGATTCGCAAATCCCGCAACGCAAAAGCCGCTGGAATCGCA AAGCAAAGTTTAGACGATTGCACACAGGAGTCCTTTGCGGGAGCCTT ACTGACCTCAGCGGCTTTAGGGTTAGAGCCAGGCGTCAATGGGGAGT GTTATCTGGTACCCTATCGTGATACACGCCGTGGTGTGGTCGAGTGC CAACTGATTATTGGATATCAAGGGATTGTCAAACTTTTTTGGCAACA TCCGCGCGCGAGCCGCATCGATGCGCAATGGGTTGGCGCGAACGAC GAGTTCCATTATACGATGGGACTTAATCCTACCTTGAAACATGTAAA GGCAAAAGGTGATCGTGGAAACCCGGTCTACTTTTACGCCATCGTCG AGGTGACCGGTGCTGAGCCCTTATGGGATGTTTTTACTGCTGATGAA ATTCGTGAACTTCGTCGTGGCAAGGTTGGATCGTCTGGAGATATTAA GGACCCCCAGCGTTGGATGGAACGCAAGACAGCATTGAAACAGGTA CTGAAATTGGCTCCCAAAACCACACGCCTGGATGCGGCGATCCGCGC TGATGATCGTCCAGGGACTGACCTTTCACAGTCGCAGGCTCTGGCCT TACCGTCTACCGTTAAGCCTACCGCAGACTATATTGATGGGGAGATC GCCGAACCGCATGAAGTCGATACACCACCAAAGAGTTCACGTGCTC AACGCGCACAACGTGCCACGGCACCGGCTCCTGATGTGCAAATGGC CAACCCCGACCAATTGAAGCGTCTGGGGGAGATCCAAAAGGCGGAG AAGTACAATGATGCCGACTGGTTCAAGTTTTGGCGGACTCCGCCGG TGTGAAAGCGACGCGTGCTGCTGATCTTACGTTTGATGAAGCAAAGG CTGTAATCGACATGTTTGATGGCCAAACGCGTGA
LrpRecT	ATGGCGAATCAAGTTGCACAGCAACAAAAACCGACAAAATTAACCG ATCTGGTTTTGGATAGAGTCAAGCAGATGCAAGACACCCAGGACCTT AGCCTTCCGAAAAACTATAACGCATCCAATGCACTGAATGCCGCGTT TTTAGAATTGCAGAAGGTACAAGACCGGAACCACAGACCAGCACTG GAAGTCTGCTCGCACGATTCTATTGTAAAATCGCTGTTGGACATGAC TTTGCAGGGCTTATCCCCTGCGAAGGATCAGTGTTACTTCATAGTAT ATGGCAATGAGTTACAGATGCAGAGATCTTATTTCGGGACTGTCGCG

	GCAGTTAAAAGATTAGATGGGGTGAAGAAGGTCCGGGCGGAAGTCG TGCATGAAAAGGACGACTTCGAAATTGGCGCCAATGAAGACATGGA GCTGGTAGTGAAACGGTTTGTACCAAAGTTCGAAAATCAAGACAAC CAAATAATAGGGGCGTTCGCAATGATTAAAACGGATGAAGGTACGG ACTTCACAGTTATGACGAAAAAGGAAATAGATCAAAGTTGGGCGCA AACACGCCAGAAGAACAATAAGGTACAGCAGAACTTTAGTCAAGAA ATGGCGAAACGTACAGTCCTTAATCGTGCCGCTAAGATGTTTATAAA CACTTCAGACGATTCGGACTTATTAACCGGGGCCATAAATGACACGA CCTCAAACGAGTATGACGATGAAAGAAGAGATGTGACACCAGTCGA GGACGAAAAACAGAGCACGGATAAATTACTGGAGGGGTTTCAGAAG TCGCAGGAGGCGAAAGCAAAAGGGGTTAGTAACGACGGAAACAGTA ATGAGGGAAAAGAGACAAGCGAGGAGGTGGCCGATGGACAGACGG AACTGTTCTCTGAAGGTACTATTAAACCAGCAGATGAAGCGGATAGC TAA

- *M. smegmatis*

λ-Red β	ATGAGCACGGCGCTCGCGACCTTGGCGGGGAAACTGGCGGAGCGTG TCGGCATGGATTCCGTCGACCCGCAAGAACTCATCACCACGCTGCGC CAGACGGCGTTCAAATGCGACGCCTCGGACGCCCAGTTTATCGCGCT CCTGATCGTCGCGAACCAGTATGGGCTGAACCCATGGACCAAGGAA ATTTATGCCTTTCCCGACAAGCAGAATGGGATTGTGCCCGTCGTGGG GGTGGATGGGTGGTCGCGTATCATTAACGAAAACCAACAGTTCGAC GGCATGGATTTTGAGCAGGATAACGAATCGTGTACCTGTCGGATTTA CCGCAAAGACCGGAACCACCCGATTTGTGTGACGGAGTGGATGGAC GAGTGCCGGCGGGAGCCATTCAAGACGCGGGAAGGGCGTGAGATTA CCGGCCCCTGGCAGTCCCATCCAAAGCGTATGCTCCGTCATAAGGCC ATGATCCAATGTGCGCGGCTGGCCTTCGGGTTTGCGGGGATTTATGA CAAGGACGAGGCGGAGCGGATTGTGGAGAACACCGCGTATACCGCC GAGCGGCAGCCGGAACGCGATATTACGCCAGTCAATGACGAAACCA TGCAGGAGATCAATACGCTCCTCATCGCGTTGGACAAAACGTGGGAT GATGACTTGTTGCCGCTCTGCTCCCAGATTTTCCGCCGTGACATTCGG GCCTCCTCGGAGCTGACCCAGGCGGAGGCGGTCAAAGCCTTGGGCTT CCTCAAGCAGAAGGCGACCGAACAGAAGGTGGCCGCCTAA
LrpRecT	ATGGCGAACCAAGTCGCGCAACAACAGAAGCCGACGAAGCTGACCG ACTTGGTCTTGGACCGTGTCAAACAAATGCAGGACACGCAGGACCTC AGCTTGCCGAAGAATTATAATGCCTCCAATGCCTTGAACGCCGCGTT CCTGGAATTGCAAAAGGTCCAAGATCGCAATCACCGTCCAGCCCTGG AAGTGTGTAGCCACGACTCCATCGTCAAAAGCCTCCTGGATATGACG CTGCAGGGTTTGAGCCCCGCCAAAGATCAATGCTATTTTATCGTGTA CGGTAATGAGTTGCAAATGCAGCGCTCGTACTTTGGCACCGTGGCCG CCGTGAAGCGCCTGGACGGCGTGAAGAAAGTCCGTGCGGAGGTCGT CCATGAAAAGGACGATTTCGAGATTGGCGCCAACGAGGACATGGAG TTGGTGGTCAAGCGTTTCGTCCCAAAATTCGAGAATCAAGATAACCA AATCATCGGGGCGTTTGCGATGATTAAAACGGACGAGGGCACGGAT TTCACGGTCATGACCAAGAAAGAGATCGACCAAAGCTGGGCCCAAA CGCGCCAGAAGAACAATAAGGTCCAGCAGAACTTTAGCCAAGAGAT

	GGCGAAGCGGACCGTGTTGAACCGTGCGGCCAAGATGTTTATCAATA CCAGCGACGACTCGGATCTCCTCACCGGGGCCATCAACGACACCACC TCGAACGAGTATGACGACGAGCGGCGCGATGTGACGCCAGTCGAAG ATGAGAAACAGAGCACCGATAAGCTCCTGGAGGGCTTCCAGAAGTC GCAAGAAGCGAAAGCGAAAGGCGTGAGCAACGATGGGAACAGCAA CGAGGGTAAGGAGACGTCGGAAGAAGTGGCGGATGGTCAAACGGA GCTGTTCTCGGAGGGGACCATTAAACCCGCCGACGAAGCGGACAGC
MspRecT	ATGGCAGAAAACGCTGTAACCAAGCAAGACAGTCCCAAAGCGCCCG AGACCATATCGCAGGTATTGCAAGTGTTAGTGCCTCAATTAGCAAGA GCAGTCCCCAAAGGGATGGATCCTGACAGAATAGCACGCATAGTGC AGACCGAAATACGTAAGTCCCGTAATGCCAAAGCTGCCGGCATCGC AAAACAATCGTTAGATGATTGTACCCAGGAGAGTTTTGCCGGGGCGC TGCTTACCTCAGCAGCCTTAGGTCTGGAACCAGGAGTTAACGGAGAG TGTTATTTGGTCCCATACCGGGATACTCGTCGCGGAGTTGTTGAGTG CCAACTTATTATCGGTTACCAGGGAATAGTGAAGTTGTTCTGGCAAC ACCCTCGTGCGTCCCGGATTGACGCGCAGTGGGTAGGTGCAAACGAC GAATTCCACTACACTATGGGCCTTAATCCGACACTTAAACACGTCAA AGCGAAAGGGGATAGAGGAAACCCGGTGTACTTTTATGCAATTGTTG AGGTTACTGGAGCAGAGCCGTTATGGGATGTCTTTACTGCCGATGAG ATACGCGAGCTGCGTCGTGGCAAGGTCGGGAGTTCAGGGGACATTA AAGATCCCCAACGGTGGATGGAGCGGAAGACTGCGCTGAAACAGGT GTTGAAGTTGGCCCCCAAAACGACCCGCCTTGACGCTGCAATCCGGG CGGATGATCGTCCTGGGACTGACCTGTCCCAAAGCCAAGCCTTAGCC CTTCCAAGTACTGTCAAGCCAACCGCAGATTACATTGACGGGGAAAT CGCAGAACCGCACGAAGTTGACACTCCTCCGAAGAGTAGCCGCGCA CAACGTGCCCAGCGCGCGACGGCACCAGCGCCGGATGTGCAGATGG CAAATCCTGACCAACTTAAAAGACTGGGAGAGATACAGAAAGCAGA GAAGTACAACGACGCAGATTGGTTTAAGTTTTTGGCGGACAGCGCTG GCGTCAAAGCAACTCGTGCGGCCGACTTGACCTTTGACGAAGCGAA GGCGGTCATAGATATGTTTGATGGTCCAAACGCCTGA
PapRecT	ATGGGCACCGCCCTGACCCCACTCTTGACCAAGTTTGCCACGCGGTA TGAGATGGGCACCACCCCAGAGGAAGTGGCGAACACCCTCAAGCAG ACCTGCTTTAAGGGTCAGGTCAATGATAGCCAGATGGTGGCCTTGCT GATCGTCGCGGACCAATATAAACTGAATCCATTTACCAAGGAACTCT ATGCGTTTCCGGATAAGAACAATGGTATTGTCCCCGTCGTCGGCGTG GACGGTTGGGCGCGGATCATTAACGAGAACCCCCAATTCGATGGCAT GGAATTTTCGATGGACCAGCAAGGGACCGAATGCACCTGCAAAATC TACCGGAAAGACCGTAGCCATGCCATTAGCGCCACGGAGTATATGG CCGAATGTAAACGTAATACGCAGCCATGGCAATCCCATCCACGCCGG ATGTTGCGCCACAAGGCGATGATCCAGTGTGCGCGGTTGGCCTTTGG TTTCGCGGGCATCTATGACCAGGACGAAGCGGAACGCATCGTCGAG CGGGATGTGACCCCGGCCGAACAGTATGAGGACGTGTCGGAGGCGA TTTGTCTCATCAAAGATAGCCCAACGATGGAGGATTTGCAGGCCGCC TTCAGCAACGCCTGGAAGGCGTACAAGACCAAAGGTGCCCGTGACC AACTGACGGCCGCGAAGGACCAGCGTAAGAAAGAACTGTTGGATGC

	CCCAATTGATGTCGAATTTGAGGAAACCGGGGACGATCGGGCGGCG TAA

- *C. crescentus*

λ-Red β	ATGAGCACGGCGCTCGCGACGCTCGCGGGGAAGCTGGCCGAGCGTG TGGGCATGGATTCGGTCGATCCGCAGGAGCTCATCACCACGCTCCGG CAGACGGCCTTTAAGTGTGACGCGAGCGATGCCCAGTTTATCGCCCT CCTGATCGTGGCCAATCAGTACGGCCTGAACCCGTGGACGAAGGAA ATCTACGCCTTTCCCGACAAGCAAAACGGGATCGTGCCGGTGGTCGG CGTCGATGGGTGGTCCCGTATCATCAATGAAAATCAGCAATTTGATG GCATGGATTTCGAGCAAGACAATGAATCCTGCACGTGCCGCATCTAT CGGAAGGACCGCAACCATCCGATCTGCGTGACGGAATGGATGGATG AGTGCCGCCGGGAGCCCTTTAAGACGCGGGAGGGCCGGGAAATCAC CGGGCCCTGGCAGTCGCACCCCAAGCGGATGCTCCGTCATAAGGCG ATGATCCAATGTGCCCGCCTCGCCTTCGGGTTCGCGGGCATCTACGA CAAGGATGAAGCCGAGCGCATCGTGGAAAATACGGCCTACACGGCG GAGCGTCAGCCGGAACGGGATATCACGCCGGTCAATGACGAAACGA TGCAGGAAATCAATACCCTGCTCATCGCGCTCGACAAGACCTGGGAC GATGATCTGCTGCCCCTGTGTAGCCAAATCTTCCGTCGTGATATCCG CGCCTCGTCCGAACTGACCCAAGCGGAGGCGGTGAAGGCCCTGGGG TTCCTGAAGCAGAAGGCCACCGAGCAAAAGGTCGCGGCCTAA
PapRecT	ATGGGCACGGCGCTCACGCCGCTGCTCACCAAGTTTGCCACCCGTTA CGAGATGGGGACCACCCCCGAAGAAGTGGCGAACACCCTGAAGCAA ACGTGCTTCAAGGGCCAGGTCAACGACTCGCAGATGGTGGCCCTGCT CATCGTGGCCGATCAGTATAAGCTCAATCCGTTCACCAAGGAACTCT ACGCGTTTCCCGATAAGAACAATGGGATCGTGCCGGTCGTCGGCGTC GACGGCTGGGCGCGTATCATCAATGAAAATCCGCAGTTCGACGGCA TGGAATTCTCGATGGACCAACAAGGGACCGAATGTACGTGCAAGAT CTATCGTAAGGATCGTTCGCACGCGATCAGCGCCACGGAATACATGG CGGAGTGTAAGCGGAATACGCAGCCGTGGCAATCCCACCCCCGCCG TATGCTGCGCCATAAGGCGATGATCCAATGTGCCCGCCTGGCGTTTG GGTTCGCCGGCATCTACGATCAAGATGAAGCGGAGCGGATCGTCGA ACGCGATGTGACGCCCGCCGAACAATATGAAGACGTGTCGGAAGCG ATCTGCCTGATCAAGGACAGCCCCACGATGGAAGATCTCCAAGCGG CCTTTAGCAATGCCTGGAAGGCCTACAAGACGAAGGGGGCGCGTGA CCAACTGACGGCGGCCAAGGATCAACGGAAGAAGGAGCTGCTGGAT GCGCCGATCGATGTCGAATTCGAGGAAACGGGGGACGATCGTGCCG CGTAA

<u>Nucleotide sequences of codon optimized Genes: SSB</u>

- *L. lactis / L. rhamnosus*

EcSSB	ATGGCAAGCCGTGGGGTTAACAAAGTTATTCTTGTTGGAAACTTAGG ACAAGACCCGGAAGTGCGTTATATGCCTAATGGAGGCGCGGTAGCC AATATCACCTTGGCCACAAGCGAGTCTTGGCGAGACAAAGCAACAG GTGAAATGAAAGAACAAACTGAATGGCACAGAGTAGTTTTGTTTGG AAAATTGGCAGAGGTAGCCTCAGAATACTTGCGAAAGGGCAGTCAG

	GTCTATATAGAGGGCCAATTGCGTACCCGTAAGTGGACAGACCAGA GCGGACAAGATCGTTATACGACCGAGGTCGTTGTTAATGTAGGAGG CACAATGCAGATGTTGGGGGGGAGACAGGGCGGAGGCGCTCCGGCT GGAGGCAATATCGGGGGTGGCCAACCTCAAGGTGGGTGGGGGCAGC CACAGCAACCGCAAGGAGGTAATCAATTTAGTGGAGGAGCCCAATC ACGTCCGCAGCAGTCTGCGCCTGCCGCCCCTTCTAATGAACCGCCGA TGGATTTTGACGATGATATACCTTTCTGA
PaSSB	ATGGCCCGTGGAGTGAACAAAGTAATTCTTGTCGGTAATGTGGGTG GGGATCCAGAGACGCGATACATGCCAAACGGAACGCCGTGACAAA TATCACCTTAGCCACGAGCGAATCTTGGAAGGACAAACAAACAGGT CAGCAACAAGAACGAACCGAATGGCATAGAGTTGTATTTTTGGCC GACTTGCTGAGATCGCGGGTGAGTACCTTAGAAAGGGTTCTCAGGTT TATGTCGAGGGCTCATTAAGAACACGTAAGTGGCAGGGGCAGGACG GGCAAGACCGATATACAACTGAAATAGTAGTGGACATAAACGGCAA CATGCAACTTCTTGGTGGCAGACCGAGTGGGGACGATTCACAGAGA GCTCCAAGAGAACCTATGCAGCGACCACAGCAGGCTCCTCAACAGC AGTCTCGTCCGGCCCCTCAGCAGCAACCGGCTCCGCAACCTGCACAA GATTACGATAGTTTTGATGATGATATTCCATTCTAA
MsSSB	ATGGCGGGAGACACAACAATTACGGTTGTGGGAAACTTGACAGCCG ATCCTGAATTGCGATTCACCCCATCAGGCGCTGCGGTGGCGAATTTC ACAGTCGCGAGCACCCCACGAATGTTTGATAGACAATCAGGCGAAT GGAAGGATGGCGAAGCGTTGTTTTACGATGCAACATCTGGAGAGA GGCGGCCGAGAACGTCGCCGAAAGCCTTACCCGTGGCAGTCGAGTG ATTGTAACCGGACGATTAAAGCAAAGAAGTTTTGAGACGAGAGAAG GAGAGAAACGAACTGTGGTAGAGGTAGAGGTGGATGAAATAGGTCC TAGTTTGCGTTATGCCACAGCGAAAGTAAACAAAGCCTCTCGTAGTG GTGGCGGGGGGGGCGGCTTTGGTTCAGGGGGTGGAGGTTCACGACA GAGCGAGCCAAAGGATGATCCTTGGGGCAGTGCCCCTGCATCAGGC AGTTTTAGCGGAGCAGATGACGAGCCGCCTTTTTGA
LrSSB	ATGCTTAATCGTGCAGTCTTAACTGGGCGTTTAACAAGAGATCCCGA GTTGCGGTACACAACCAGCGGGACAGCAGTTGTTTCATTTACGTTAG CTGTTGATCGGCAATTCCGAAACCAAAATGGTGATCGTGATGCTGAT TTTATCAATTGCGTTATTTGGCGTAAATCCGCTGAAAACTTTAGTAA CTTTACGCATAAGGGTTCACTTGTTGGAATTGAAGGGCGTATTCAAA CCCGGAATTATGAAAACCAACAGGGTAACCGTGTGTATGTTACCGA AGTTGTTGTAGATAATTTTGCATTGTTAGAACCTCGTCAAAATGGTG GCATGAACCAATCAGGGGTTCAACAACCATTTAACAGCAACCAACA ATCATTTGGTGCTCAGGCTCCACAATATGGTAGTCAACCACAACCTG GAAATAATGCTCCTCAAAGTAATCCGTCACCAAGTATGGATAATGGT TTCGATCCCAATCAAAATGCTGGCAACCAGTTCCCTGGAAGCAGTGA TGATGGTGGTCAATCCATTGATTTAGCTGATGACGAATTACCATTCT AA
LlSSB	ATGATTAACAATGTTGTATTAGTGGGACGCATTACTCGCGATCCTGA ACTTCGTTACACCCCTCAAAATCAAGCTGTTGCAACTTTTTCATTGGC TGTAAATCGTCAATTTAAAAATGCTAACGGTGAACGTGAGGCTGATT TCATTAACTGCGTTATTTGGCGCCAACAAGCTGAAAATTTGGCAAAT

	TGGGCTAAAAAAGGAGCTTTGATCGGTGTAACTGGTCGAATTCAAA CACGTAATTATGAAAATCAACAAGGTCAACGCGTTTATGTGACTGA GGTTGTGGCTGATAGTTTCCAAATGTTGGAAAGTAGATCTGCTCGCG ATGGTATGGGAGGCGGAGCTTCTGCCGGTTCATATTCTGCACCAAGC CAATCTACAAATAATACTCCACGTCCACAAACGAATAATAATAGTG CAACACCGAATTTCGGTCGTGATGCTGACCCATTTGGTAGCTCACCT ATGGAAATCTCGGATGATGATCTTCCATTCTAA
PapSSB	ATGCGTGGGGTTAATAAGGTAATCTTAGTTGGTAACGTGGGTGGGG ACCCGGAGACCCGATATATGCCAAATGGAAACGCGGTAACAAACAT CACCCTTGCAACTAGTGAGAGTTGGAAAGATAAACAAACTGGCCAA CAGCAAGAACGTACTGAATGGCACAGAGTGGTGTTTTTGGCAAATT AGCCGAAATTGTCGGCCAACACGTTAAGAAAGGCCAGCAGCTTTAC GTCGAAGGGTCATTGCGAACCCGTAAGTGGCAAGCGCAGGATGGTC AGGACAGATATACGACAGAAATCATAGTAGATATGCACGGACAAAT GCAAATGTTCGGGGGAAAACCTGGGAATGAGCAGGCCGCACAGTCA AGATCATCTACCCAACAACAAAGCGCCCCGCAACAACGATCAGCAC AGGATGAATTTGATGATGATATACCTTTATAA

- *E. coli*

EcSSB	ATGGCCAGCAGAGGCGTAAACAAGGTTATTCTCGTTGGTAATCTGG GTCAGGACCCGGAAGTACGCTACATGCCAAATGGTGGCGCAGTTGC CAACATTACGCTGGCTACTTCCGAATCCTGGCGTGATAAAGCGACCG GCGAGATGAAAGAACAGACTGAATGGCACCGCGTTGTGCTGTTCGG CAAACTGGCAGAAGTGGCGAGCGAATATCTGCGTAAAGGTTCTCAG GTTTATATCGAAGGTCAGCTGCGTACCCGTAAATGGACCGATCAATC CGGTCAGGATCGCTACACCACAGAAGTCGTGGTGAACGTTGGCGGC ACCATGCAGATGCTGGGTGGTCGTCAGGGTGGTGGCGCTCCGGCAG GTGGCAATATCGGTGGTGGTCAGCCGCAGGGCGGTTGGGGTCAGCC TCAGCAGCCGCAGGGTGGCAATCAGTTCAGCGGCGGCGCGCAGTCT CGCCCGCAGCAGTCCGCTCCGGCAGCGCCGTCTAACGAGCCGCCGA TGGACTTTGATGATGACATTCCGTTCTGA
PaSSB	ATGGCTCGCGGGGTAAATAAGGTCATTTTGGTTGGCAATGTTGGTGG TGATCCCGAGACACGCTATATGCCTAACGGGAACGCCGTCACTAAT ATCACACTGGCAACGTCCGAGTCATGGAAGGATAAACAGACAGGTC AACAGCAAGAACGCACGGAGTGGCACCGCGTGGTATTTTTCGGGCG TCTTGCTGAGATCGCCGGAGAGTATTTACGCAAAGGATCGCAGGTAT ACGTTGAGGGTTCTTTACGCACGCGCAAGTGGCAGGGTCAGGATGG TCAGGACCGTTATACTACCGAAATTGTAGTCGACATTAACGGGAAC ATGCAATTATTAGGTGGTCGTCCGAGCGGAGATGACTCCCAGCGCG CCCCCCGCGAGCCCATGCAGCGTCCGCAACAGGCTCCACAGCAGCA GAGCCGCCCTGCCCCTCAACAACAACCCGCTCCTCAACCCGCGCAA GATTACGATTCGTTTGACGACGATATTCCTTTTTAA
MsSSB	ATGGCAGGGGATACCACGATAACCGTTGTCGGTAACTTAACCGCGG ACCCTGAACTTCGTTTCACACCATCCGGTGCAGCGGTTGCAAACTTC ACGGTCGCTTCTACGCCTCGTATGTTCGACAGACAGTCTGGTGAGTG GAAAGATGGGGAAGCACTGTTTTTAAGATGCAATATATGGCGCGAA

	GCAGCAGAGAATGTAGCCGAGAGTTTAACCAGAGGTTCACGTGTGA TCGTAACTGGCCGTTTGAAACAACGCTCCTTTGAAACACGCGAAGGC GAGAAACGCACGGTAGTTGAGGTCGAAGTCGACGAGATAGGCCCGT CCTTACGCTATGCCACAGCGAAAGTCAACAAAGCGTCTCGCAGCGG AGGCGGTGGGGGCGGGTTTGGTAGTGGTGGGGGGGGTAGTCGTCAA TCGGAACCCAAGGATGACCCGTGGGGGTCGGCACCAGCTTCAGGAA GTTTTTCTGGGGCCGATGACGAGCCGCCATTTTGA
LrSSB	ATGCTGAACCGTGCCGTGCTTACTGGTCGCCTTACTCGTGACCCTGA ATTGCGCTATACGACATCAGGGACTGCAGTAGTGTCCTTTACATTGG CGGTCGATCGTCAATTTCGTAACCAAAACGGCGACCGCGACGCCGA TTTTATCAACTGTGTGATTTGGAGAAAGAGCGCCGAGAACTTTAGCA ATTTCACTCATAAAGGGAGTTTAGTTGGAATCGAGGGGCGTATCCAA ACGAGAAACTACGAAAACCAGCAAGGCAATCGCGTCTACGTAACCG AAGTCGTAGTAGATAACTTCGCCCTGTTGGAACCACGGCAAAACGG TGGGATGAACCAATCTGGAGTTCAACAACCCTTCAACAGTAACCAG CAGTCTTTCGGGGCTCAGGCACCTCAATATGGCAGTCAGCCACAACC TGGAAACAATGCCCCACAGTCTAACCCAAGTCCCTCTATGGACAATG GGTTTGACCCCAACCAGAATGCGGGGAACCAATTCCCTGGGAGCTC GGATGACGGCGGCCAATCAATTGATCTGGCTGACGATGAATTACCCT TTTAA

- *M. smegmatis*

PaSSB	ATGGCGCGTGGGGTGAACAAAGTCATCCTCGTGGGGAATGTCGGTG GCGATCCCGAAACGCGTTATATGCCGAATGGGAATGCGGTCACCAA TATCACGCTCGCCACCAGCGAGTCCTGGAAAGATAAACAAACGGGT CAACAGCAGGAGCGTACGGAGTGGCATCGGGTGGTCTTCTTCGGGC GCCTCGCCGAGATCGCCGGGGAATACCTCCGTAAAGGTTCGCAGGT CTATGTGGAGGGCTCGCTGCGGACCCGTAAATGGCAAGGTCAGGAT GGCCAGGATCGGTACACGACGGAAATCGTCGTGGACATTAACGGTA ATATGCAATTGCTCGGTGGCCGCCCCTCCGGCGATGATAGCCAGCGT GCCCCGCGTGAACCGATGCAACGCCCGCAACAAGCGCCCCAACAGC AATCGCGGCCCGCGCCGCAGCAGCAGCCGGCCCCGCAACCAGCCCA GGACTACGATTCGTTTGATGATGACATTCCATTTTAA

- *C. crescentus*

PaSSB	ATGGCGCGTGGGGTCAATAAGGTGATCCTGGTCGGCAACGTGGGGG GCGATCCCGAAACCCGGTACATGCCGAACGGCAACGCGGTCACCAA CATCACCCTGGCGACCAGCGAGAGCTGGAAGGATAAGCAAACGGGC CAGCAGCAAGAACGTACGGAATGGCATCGTGTGGTCTTTTTCGGCC GGCTGGCGGAGATCGCGGGGGAATACCTCCGTAAGGGGTCCCAAGT CTACGTGGAGGGCTCGCTGCGGACCCGGAAGTGGCAAGGGCAAGAT GGGCAAGATCGCTACACCACGGAGATCGTCGTCGACATCAACGGGA ACATGCAGCTCCTCGGGGGGCGTCCCTCGGGGGACGATTCCCAACG CGCCCCCCGTGAGCCCATGCAACGCCCGCAGCAAGCGCCCCAGCAA CAATCGCGTCCCGCCCCCCAGCAACAGCCGGCGCCCCAACCGGCGC AGGACTACGACTCGTTCGACGATGATATCCCCTTTTAA

Nucleotide sequences of codon optimized Genes: phage exonuclease

- *L. lactis*

PapExo	ATGATAGAACAGCGTAGTGATGAATGGTTCGCGCAGCGACTTGGCC GAGTCACCGCGAGTAAAGTAAAGGATGTCATGGCGAAGGGGCGATC AGGTGCGCCATCAGCCACCAGACAGAATTACATGATGCAATTGTTAT GTGAGAGACTTACCGGGAAACGAGAAGAGGGGTTCACGAGTGCGGC GATGCAGCGTGGGACGGACCTTGAACCAATAGCGCGATCAGCTTAT GAGTTTAACGCAGGAGTAATGACTATAGAAACAGGCCTTATTATCC ATCCACGTATCGACGGTTTCGGAGCTAGTCCGGATGGGCTTGCGGGA GAGCATGGATTAGTGGAAATTAAGTGCCCGTCAACAGCAACGCACA TTTATACCATGCAAAGTGGTAAGCACGACCCTCAGTACGAATGGCA AATGCTTGCTCAAATGAGTTGCTCAGGCAGAGAGTGGGTGGATTTCG TGTCATTCGACGATAGATTGCCAGACGAATTGCAATATGTTTGTTTC CGTTATCACCGTGATGAAGAGAGAATAAGAGAAATGGAAAGCGAA GTTAAGGCATTCTTGGAGGAATTAGCTGAATTGGAACACCAAATGC GTGAACGTATGAGAAAGGCGGCCTAA
LrpExo	ATGAAACTTACGGCCAACAATTACTATAGCCATGAGACTGACTGGC AATATATGTCAGTTTCATTGTTCAAAGACTTCGAAAAGTGCGAAGCG CGTGCATTAGCAAAGTTGAAGGAAGATTGGCAACCTGTTTCTAGTCC AGTTCCGCTTTTGGTTGGGAACTATGTACACAGTTATTTCGAAAGTG CTAAGAGCCACCAAGATTTTATAGAGGCGAATAAGAAAGAGCTTAT GACCAGACCTACTAAGACAAACCCGAACGGCCATCTTAGAGCGGAA TTTAAGGGGGCAAACTCAATGATTCAGACCTTGCAAGCCGACGATA TGTTTAACTACTTTTATGCACCAGGGGACAAAGAAGTTATCGTTACC GGAGAGATAGACGGCTATTTGTGGAAGGGAAAAATAGACTCTTTAG TTCTTGACAAAGGCTATTTTTGCGATCTTAAGACGGTAGACGACATT CATAAGGGACATTGGAATACGTATGAACACAGATACGTCCCGTTCA TTCAAGACCGAGAATATGATTTACAAATGGCTGTTTATAGAGAGTTA ATCAAGCAGACGTTCGGGAAAAAGTGCCAACCTTTAATTTTTGCCAT CTCTAAGCAAACTCCGCCTGACAAGATGGCCATCGACTTTAATGGCG TTGATGACGACTATCAGATGCAGGCCGATCTTGATAAGGTCAAAGA GCTTCAACCACACTTTTGGAAAGTAATGACGGGAGAGGAAGAGCCT GTCCACTGTGGTAAGTGCGACTATTGTAGAGAAACGAAAATGTTGA GCGGCTTCATCCACGCATCAGAAATAGAGGTTTAA

Negative control (no recombinase)

- All organisms

mCardinal **RBS** eGFP	ATGCACCATCATCACCACCACGGTTCCGGCATGGTTTCTAAAGGTGA AGAACTGATCAAGGAAAACATGCACATGAAGCTGTATATGGAAGGT ACCGTTAACAACCACCATTTCAAATGCACCACTGAAGGTGAAGGTAA ACCGTACGAGGGTACGCAGACCCAACGTATTAAAGTTGTTGAGGGT GGTCCGCTGCCGTTCGCGTTCGACATCCTGGCGACCTGTTTCATGTAC GGCTCTAAAACCTTCATCAACCACACCCAGGGTATCCCTGACTTCTT CAAACAGTCTTTCCCGGAGGGTTTCACCTGGGAACGTGTTACCACCT ACGAAGACGGTGGTGTACTGACCGTTACCCAGGACACTTCTCTGCAG

	GACGGTTGCCTGATCTACAACGTTAAACTCCGCGGTGTTAATTTCCC GTCTAACGGTCCGGTTATGCAAAAAAAGACGCTGGGTTGGGAAGCG ACTACGGAAACTCTCTACCCTGCCGATGGCGGCCTCGAAGGTCGTTG TGATATGGCGCTGAAACTGGTTGGTGGCGGTCACCTGCACTGCAATC TGAAAACTACCTACCGTTCTAAAAAACCAGCTAAAAACCTCAAAATG CCGGGTGTTTACTTTGTTGATCGTCGTCTGGAACGTATCAAAGAAGC AGACAACGAAACTTACGTTGAACAGCACGAAGTTGCGGTGGCGCGT TACTGCGACCTGCCATCTAAACTGGGTCACAAAGGTATGGACGAACT GTACAAATAA**AAAAAATAGGAGGAAAAACAT**ATGGGTTCTCACCA CCATCACCACCACAGCGGCTCTAAAGGTGAAGAATTATTCACTGGTG TTGTCCCAATTTTGGTTGAATTAGATGGTGATGTTAATGGTCACAAAT TTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACGTACGGTAAATTG ACCTTAAAATTTATTTGTACTACTGGTAAATTGCCAGTTCCATGGCCA ACCTTAGTCACTACTTTCACTTATGGTGTTCAATGTTTTTCTAGATAC CCAGATCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGA AGGTTATGTTCAAGAAAGAACTATTTTTTTCAAAGATGACGGTAACT ACAAGACCAGAGCTGAAGTCAAGTTTGAAGGTGATACCTTAGTTAAT AGAATCGAATTAAAAGGTATTGATTTTAAAGAAGATGGTAACATTTT AGGTCACAAATTGGAATACAACTATAACTCTCACAATGTTTACATCA TGGCTGACAAACAAAAGAATGGTATCAAAGTTAACTTCAAAATTAG ACACAACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAAC AAAATACTCCAATTGGTGATGGTCCAGTCTTGTTACCAGACAACCAT TACTTATCCACTCAATCTGCCTTATCCAAAGATCCAAACGAAAAGAG AGACCACATGGTCTTGTTAGAATTTGTTACTGCTGCTGGTATTACCCA TGGTATGGATGAATTGTACAAATAA

Nucleotide sequences of codon optimized Genes: dominant negative mutL proteins

- *L. lactis*

E. coli MutL E32K	ATGCCTATACAAGTGTTGCCTCCACAGTTGGCCAACCAAATCGCG GCAGGCGAGGTGGTCGAACGTCCGGCTTCAGTCGTTAAGGAATTG GTAAAAAATTCTTTGGATGCAGGGGCAACGAGAATTGATATTGAC ATCGAACGAGGCGGGGCCAAGTTAATCAGAATCCGAGACAATGG GTGTGGGATTAAAAAGGATGAACTTGCTTTGGCGTTGGCACGTCA CGCGACCAGCAAAATAGCGTCTCTTGACGACTTGGAAGCTATTAT CAGTCTTGGTTTCCGTGGGGAAGCCTTAGCATCTATTAGCTCTGTG TCACGTTTGACTTTGACTAGCAGAACGGCGGAACAGCAGGAAGC ATGGCAAGCGTATGCGGAAGGACGAGACATGAACGTCACGGTTA AGCCGGCAGCCCACCCGGTCGGCACGACCTTGGAGGTCTTGGACT TGTTCTATAATACCCCTGCACGTCGTAAATTCTTACGAACCGAAA AGACCGAATTTAACCATATAGATGAGATAATAAGAAGAATTGCG TTAGCACGTTTCGATGTTACTATAAATTTGAGTCATAACGGAAAA ATCGTTAGACAGTATCGAGCCGTGCCTGAGGGCGGGCAGAAGGA AAGAAGATTAGGGGCTATTTGTGGCACTGCTTTTCTTGAACAAGC ACTTGCGATCGAATGGCAACATGGGGACCTTACCTTGCGAGGTTG GGTAGCGGACCCGAATCATACAACACCAGCGTTGGCAGAGATAC AATATTGCTATGTAAACGGACGAATGATGAGAGATCGTTTGATCA

	ACCACGCAATACGACAGGCTTGCGAAGATAAGTTGGGGGCGGAT CAACAGCCAGCTTTCGTCCTTTATCTTGAAATTGACCCTCATCAGG TAGATGTGAATGTACATCCGGCCAAACACGAGGTTCGTTTTCATC AAAGTCGACTTGTGCATGATTTTATATACCAGGGTGTCTTAAGTG TCTTGCAGCAGCAGCTTGAGACACCTTTACCTTTAGATGATGAGC CGCAGCCAGCTCCGCGTAGTATCCCTGAGAATCGAGTTGCCGCCG GCAGAAATCATTTCGCAGAACCGGCAGCCCGTGAACCTGTAGCAC CGAGATACACCCCGGCTCCTGCCTCTGGATCACGTCCTGCTGCCC CGTGGCCTAACGCACAACCGGGCTATCAGAAGCAGCAGGGTGAA GTTTATCGTCAATTGTTACAAACTCCGGCACCAATGCAAAAACTT AAGGCCCCGGAGCCGCAGGAACCGGCGCTTGCTGCAAATTCACA ATCTTTCGGACGAGTTTTAACAATAGTGCATAGTGACTGCGCATT ACTTGAGCGTGACGGCAACATTAGTTTGCTTTCATTGCCTGTTGCC GAGCGTTGGTTGAGACAAGCACAATTAACCCCTGGTGAAGCACC AGTCTGTGCACAGCCATTATTGATCCCATTGCGTTTAAAGGTCTCA GCCGAGGAAAAGAGTGCTTTGGAAAAAGCCCAAAGTGCCCTTGC AGAGCTTGGAATTGATTTCCAAAGCGACGCACAACACGTTACGAT AAGAGCGGTTCCATTACCGTTAAGACAGCAAAACTTACAAATTCT TATACCAGAGCTTATCGGGTATTTGGCGAAACAGAGCGTATTCGA ACCAGGTAATATCGCCCAGTGGATAGCGCGTAACCTTATGTCAGA ACACGCGCAGTGGAGTATGGCGCAAGCTATCACATTGTTAGCCGA CGTTGAGCGTTTGTGCCCACAGTTGGTGAAAACGCCTCCGGGTGG ACTTCTTCAAAGTGTGGACTTACATCCAGCAATTAAGGCTCTTAA AGATGAATAA
L. lactis MutL E33K	GTGGGAAAAATTATTGAACTAAATGAAGCGCTCGCCAATCAAATT GCTGCTGGAGAGGTGGTTGAGCGGCCTGCTAGTGTTGTCAAAGAA TTAGTCAAAAACTCAATTGATGCTGGAAGCAGTAAAATTATTATC AATGTTGAAGAAGCAGGTTTGCGATTAATTGAAGTCATTGATAAT GGTTTGGGCTTAGAAAAAGAAGATGTGGCTTTGGCTTTGCGTCGT CATGCGACAAGTAAAATCAAAGATTCAGCTGATTTATTTCGAATT AGAACGCTCGGTTTTCGGGGTGAGGCTCTGCCGTCAATCGCTTCT GTCAGTCAGATGACGATTGAAACAAGTAATGCTCAGGAAGAAGC TGGGACAAAACTGATTGCTAAAGGTGGGACGATTGAAACTTTAG AACCTCTTGCAAAGCGGTTAGGGACAAAAATTTCTGTTGCGAATC TTTTTTATAATACACCAGCAAGGCTCAAGTATATCAAGTCTTTACA GGCTGAACTTTCTCATATTACAGATATTATCAATCGTTTGAGCCTC GCTCATCCAGAGATTTCTTTTACTTTAGTTAATGAGGGTAAAGAA TTTTTGAAAACGGCGGGAAATGGAGACTTGCGCCAAGTGATTGCT GCAATTTATGGCATTGGAACGGCGAAAAAAATGCGTGAGATTAA TGGCTCGGACTTAGATTTTGAACTGACAGGTTATGTCAGTTTACCC GAGCTGACAAGAGCGAATCGCAACTATATCACGATTTTGATTAAT GGTCGATTTATCAAGAATTTTTTGTTGAATCGAGCAATTTTAGAA GGTTACGGGAACCGATTGATGGTTGGACGTTTTCCTTTTGCTGTTT TATCAATTAAAATTGACCCTAAATTAGCAGATGTCAATGTCCATC CGACAAAACAAGAAGTACGTTTGTCTAAGGAACGTGAATTGATG ACTTTAATTTCTAAAGCGATTGATGAGACCTTATCAGAAGGGGTT

	TTGATTCCAGAAGCTTTGGAAAATTTGCAAGGTAGAGCCAAGGAA AAGGGGACTGTTTCTGTTCAAACGGAACTTCCTTTACAGAATAAT CCTTTATACTATGACAATGTTCGTCAAGATTTTTTGTCAGAGAAG AAGCGATTTTTGAAATCAATAAAAACGATAATTCAGATTCTCTGA CTGAACAAAATTCTACTGATTATACAGTTAATCAGCCAGAAACTG GTTCTGTCAGTGAAAAAATTACGGACAGAACTGTCGAAAGTTCAA ATGAATTTACTGACAGAACCCCAAAAAATTCTGTCAGTAACTTTG GAGTTGATTTTGATAATATTGAGAAGCTGAGTCAGCAATCAACTT TTCCCCAACTAGAATACTTGGCACAATTGCATGCGACTTATTTACT TTGTCAGTCAAAAGAGGGTCTTTATTTGGTTGACCAACATGCGGC TCAGGAGCGAATCAAGTATGAATATTGGAAAGATAAAATCGGCG AAGTGAGCATGGAGCAACAAATTTTACTTGCGCCATATTTATTTA CTTTACCCAAAAATGATTTTATTGTTTTAGCTGAGAAAAAGGATTT ATTACATGAAGCAGGGGTTTTCTTGGAAGAATACGGAGAAAATC AATTCATATTAAGAGAGCATCCGATTTGGTTAAAAGAAACTGAGA TAGAGAAATCAATTAATGAAATGATTGATATTATTCTCTCATCAA AAGAATTTTCACTCAAAAAATATCGGCATGATTTAGCCGCAATGG TTGCTTGTAAAAGCTCAATCAAAGCCAACCATCCCCTTGATGCCG AGTCTGCTAGAGCTTTGCTTAGAGAATTATCAACTTGTAAAAATC CTTATAGTTGTGCGCATGGACGGCCAACGATTGTCCATTTTTCAG GAGATGACATTCAAAAAATGTTCCGCAGAATTCAAGAAACGCAT CGTTCAAAAGCGGCCTCTTGGAAAGATTTTGAGTAA
L. lactis dsDNA template (Erythromycin resistance gene) Homology arm, promoter, gene, terminator, homology arm	ATGATTGAACTTAGTGGCAAAGATAGAAAGTATTTGTATAAACTA GTAAAATCCAAAAAACTAAATTATGAACAAGGTAATTTATCGCAT CAAGTTTTAATTGAAAACAAGTTAGCAAAAGTTTACTTTACAAGC GATAAATATGATCCTGACTTAGGGGAACACATAAATCCACAAAAT ATTATTGCTCCAACTAGTACAGGTTTAAGATATAAAAATATTTAT CGTGAACAATTATGGGAAAAATATTTTACTCCTATTTGGGTATCT ACGGCAACAACGACTCTAATATGGTTAGCAAAATATTTACTAGAG AACTTGCTGTAACGCTAAGTAAGATTACTATCCATAGCTCTTTTTT ATCTTTTCTCATCTTTCCACCTCCTAGCCCACTCGGGCTTTTTAATT TAAAAATTGTTTAATCTCATGAAACGCCATGCCTATTTCTAACAGT AAGATAATGCTGTCAGTATAGCGCCTAAGCGTTTCTTTTTGTTCTG ATTTTTTAATGTGGTCTTTATTCTTCAACTAAAGCACCCATTAGTT CAACAAACGAAAATTGGATAAAGTGGGATATTTTTAAAATATATA TTTATGTTACAGTAATATTGACTTTTAAAAAAGGATTGATTCTAAT GAAGAAAGCAGACAAGTAAGCCTCCTAAATTCACTTTAGATAAA AATTTAGGAGGCATATCAAATGAACAAAAATATAAAATATTCTCA AAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAAC AATTGAATTTAAAAGAAACCGATACCGTTTACGAAATTGGAACAG GTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAG GTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAG AAAAATTAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATA TTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTG GGAGTATTCCTTACCATTTAAGCACACAAATTATTAAAAAAGTGG TTTTTGAAAGCCATGCGTCTGACATCTATCTGATTGTTGAAGAAG

	GATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGC TCTTGCACACTCAAGTCTCGATTCAGCAATTGCTTAAGCTGCCAG CGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAA AACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGC TATATACGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTC AACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCA AAGTAAACAATTTAAGTACCGTTACTTATGAGCAAGTATTGTCTA TTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATAATATG AGATAATGCCGACTGTACTTTTTACAGTCGGTTTTCTAATGTCACT AACCTGCCCCGTTAGTTGAAGAAGGTTTTTATATTACAGCTCCAC GGTTAAATTTGTCGCCTGACTGTTTAAAGCTCGTTAGACTACGAT ATTTTCCGCTTGTCGTAAGTTGTACAAGTAAATCAAGAATGATTTT GTGATAGTACGGTTTAGACTGCCTGCTTTGCATGATTGCGGTGTCT AGTTTGTTCATGGTTAGTTATCCTTAACTTGCAAAAAAATCAAGTT AATAGTTAAAATTTTTCATCAAGTCATAAATAGAATTTTCTTCTAA ATTTGCTGCTCTTTCTAATTCTTTAACCTTATCAAGTGTTAATTTAT TCGGAGCTAATCTAATGCGATATAGAGCATTATATGTGATTCCCA TATTCTTCGCTATCGCCTCATATCTTACCCCTGATTGTTTTAAAAT CTCATCAAGTGGTTTATAAGTTTTACTCATTTTATCTCCTTTCTGAT TTTTATGTTTTTCATTCTAACATTAACTTGATTTTTATGCAAGTAAT AACTTTACTTTTTTGCAAGTTTTCTCTTGAAAGTAGTT

Plasmids:

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LOCUS       pARC8                   3497 bp    DNA     circular UNA 15-NOV-
2018
DEFINITION  whole plasmid sequencing.
ACCESSION   urn.local...3f-8p8b27t
VERSION     urn.local...3f-8p8b27t
KEYWORDS    .
SOURCE      E. coli
  ORGANISM  E. coli.
FEATURES             Location/Qualifiers
     promoter        4..133
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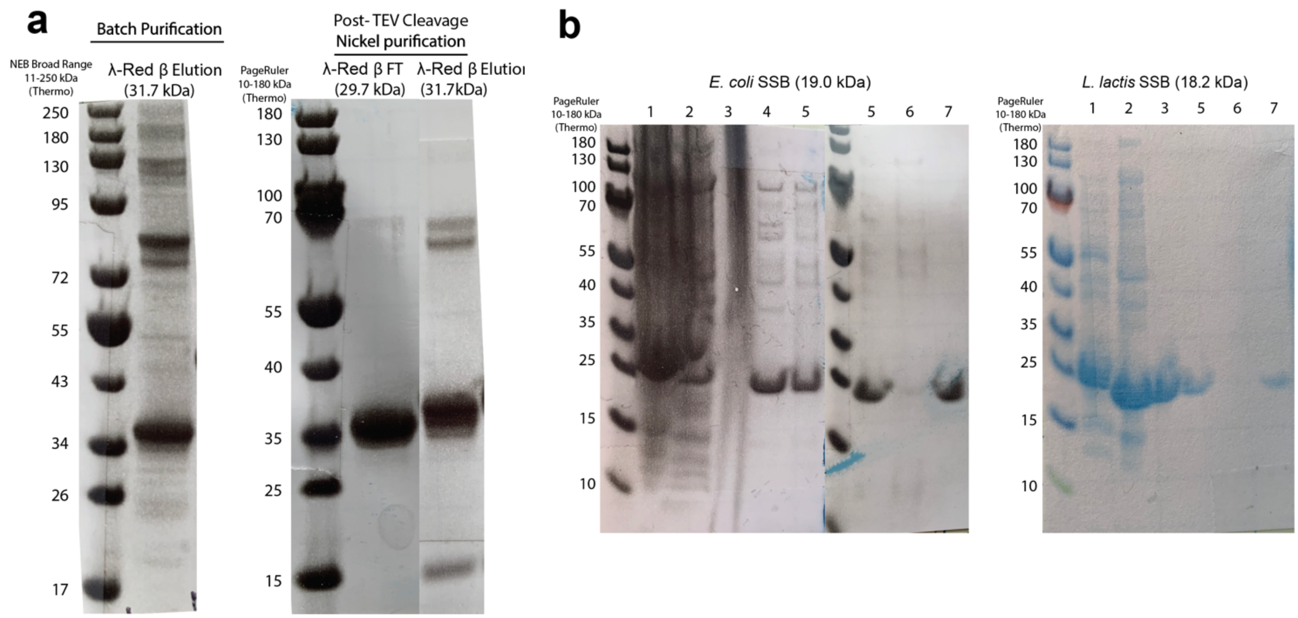
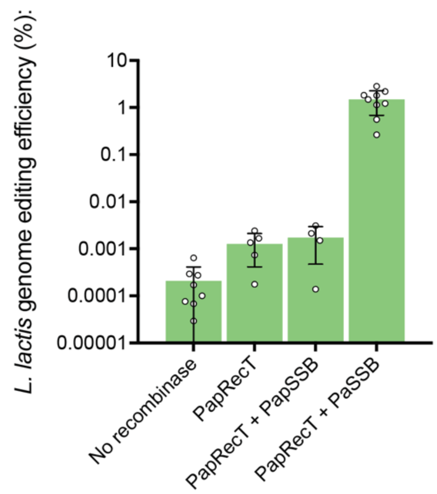
Additional Supplementary Figures



Supplementary Figure 1.

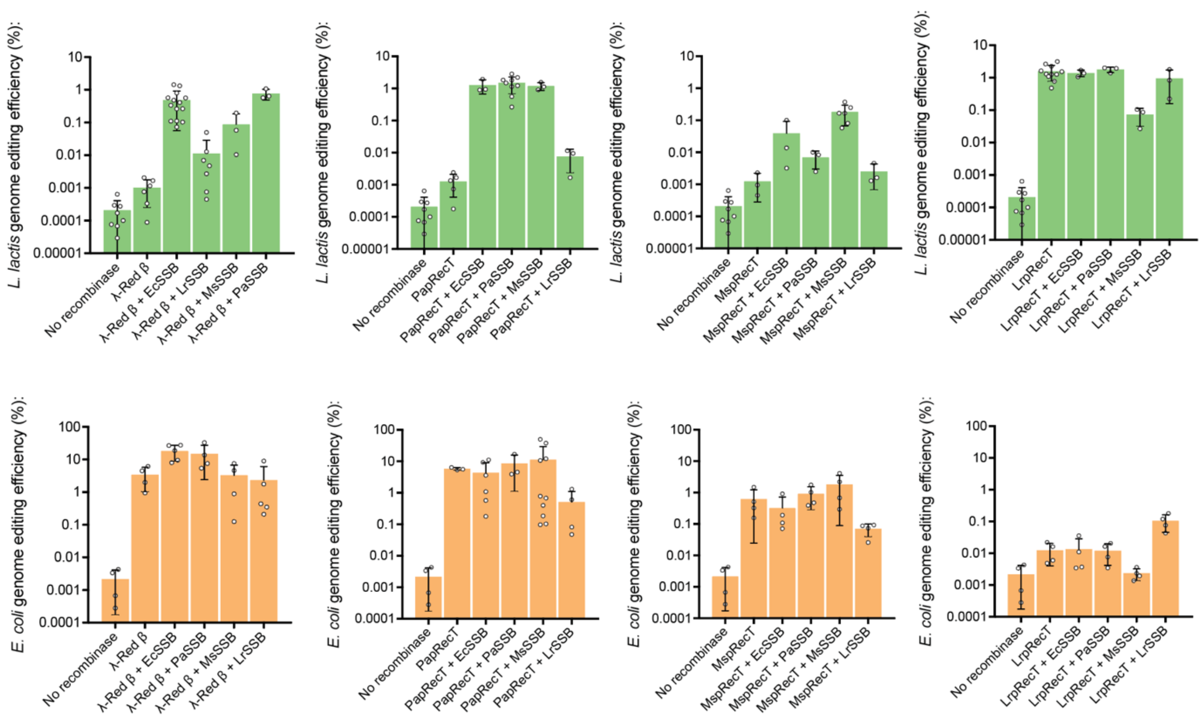
Bis-Tris SDS-PAGE denaturing gels during purification of λ-Red β, EcSSB, and LlSSB as described in "Methods". In (b), the lanes are as follows: 1. Lysed cell culture, 2. Fraction I (pellet after clarification), 3. Polymin P precipitation supernatant, 4. Resuspended Polymin P pellet, 5. Fraction II (Polymin P supernatant), 6. Supernatant after ammonium sulfate precipitation, 7. Fraction III (Final resuspension of ammonium sulfate pellet after clarification). This experiment was performed once to generate the presented gel images.



Supplementary Figure 2.

A number of phage operons containing a SSAP also include a phage SSB protein, as is the case for the *P. aeruginosa* phage. However, co-expression of this phage SSB protein (PapSSB) along with the phage SSAP (PapRecT) did not allow recovery of editing activity in *L. lactis*, unlike co-expression of a paired bacterial SSB (PaSSB). Error bars indicate SD from the mean of at least four biologically independent replicates.

One note is that the PapSSB C7 amino acids "FDDDIP**L**" do not match the C7 amino acids of the bacterial SSBs that PapRecT is compatible with ("FDDDIP**F**"). The terminal lysine (L) in the PapSSB is a single nucleotide mutation away from the phenylalanine-(F)-containing cognate C7 tail, so we speculate that this gene may be mutated since the phage SSB is carried in a likely non-essential prophage operon within *P. aeruginosa.*

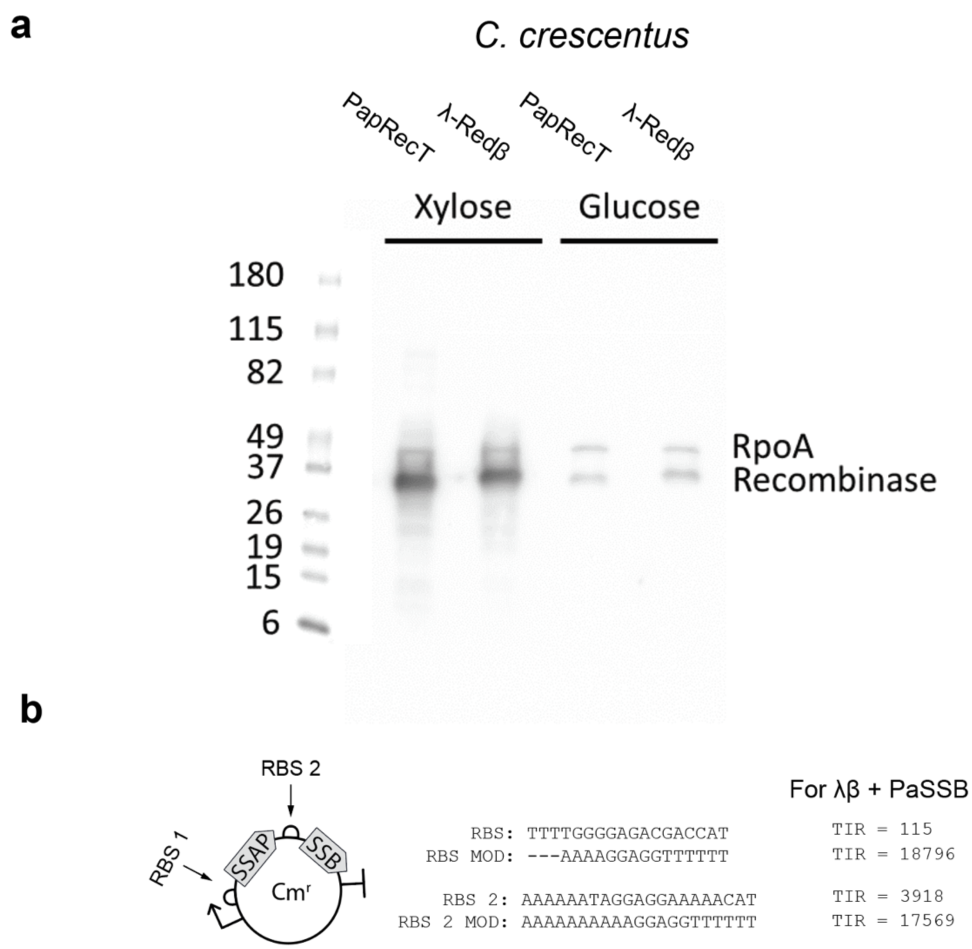


Supplementary Figure 3.

Bar graphs showing individual data points and SEM for mean values plotted in Fig 3a and Fig 3b. Error bars indicate SD from the mean of at least three biologically independent replicates.

We note there are four cases of SSB co-expression lowering SSAP-mediated genome editing efficiencies. Specifically, LrSSB reduces the activity of MspRecT and PapRecT in *E. coli*, while MsSSB reduces the activity of LrpRecT in *E. coli* and *L. lactis*. We speculate that this may occur if the supplied SSB is close to compatible with the SSAP but not fully compatible. In each of these cases, the 7 amino acid C-terminal tail of a compatible SSB is one amino acid different from that of the supplied SSB (Fig 3c).

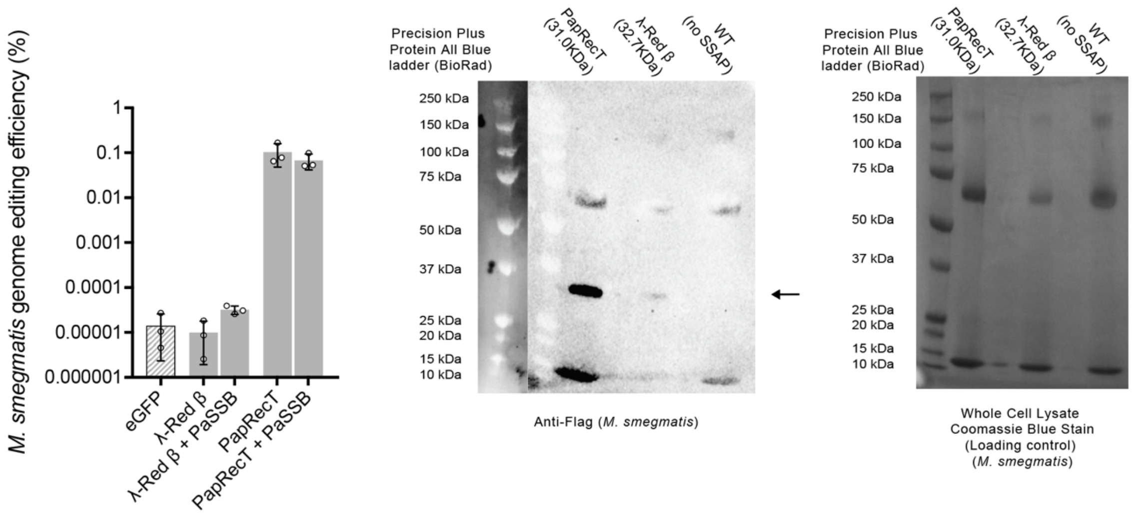
We also observe two instances of SSB co-expression improving an otherwise active SSAP (In *E. coli*: λ-Redβ + EcSSB, and λ-Redβ + PaSSB). This is unlikely to be caused simply by the reduction in toxicity (Fig S4), as some constructs have significantly reduced toxicities but similar genome editing activities (PapRecT with and without PaSSB).



Supplementary Figure 4.

(A) Western blot of a 3xFLAG tagged PapRecT and λ-Red β in *C. crescentus* under induced (Xylose) and non-induced (Glucose) conditions, along with a RpoA control. With the 3xFLAG tag, PapRecT should be 31 kDA, while λ-Redβ should be 32.7 kDA, showing that they are both expressed at similar levels under induction conditions. This experiment was performed once to generate the presented gel images.

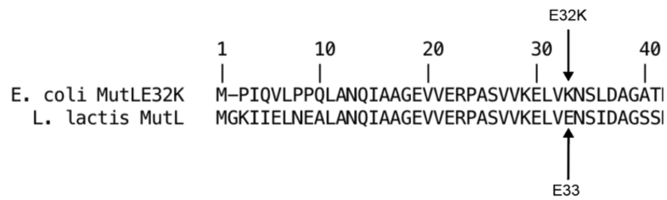
(B) Using the Salis RBS calculator we designed RBSs conferring a greater translation rate in order to increase SSAP and SSB protein expression for the Caulobacter constructs.[1,2]



Supplementary Figure 5.

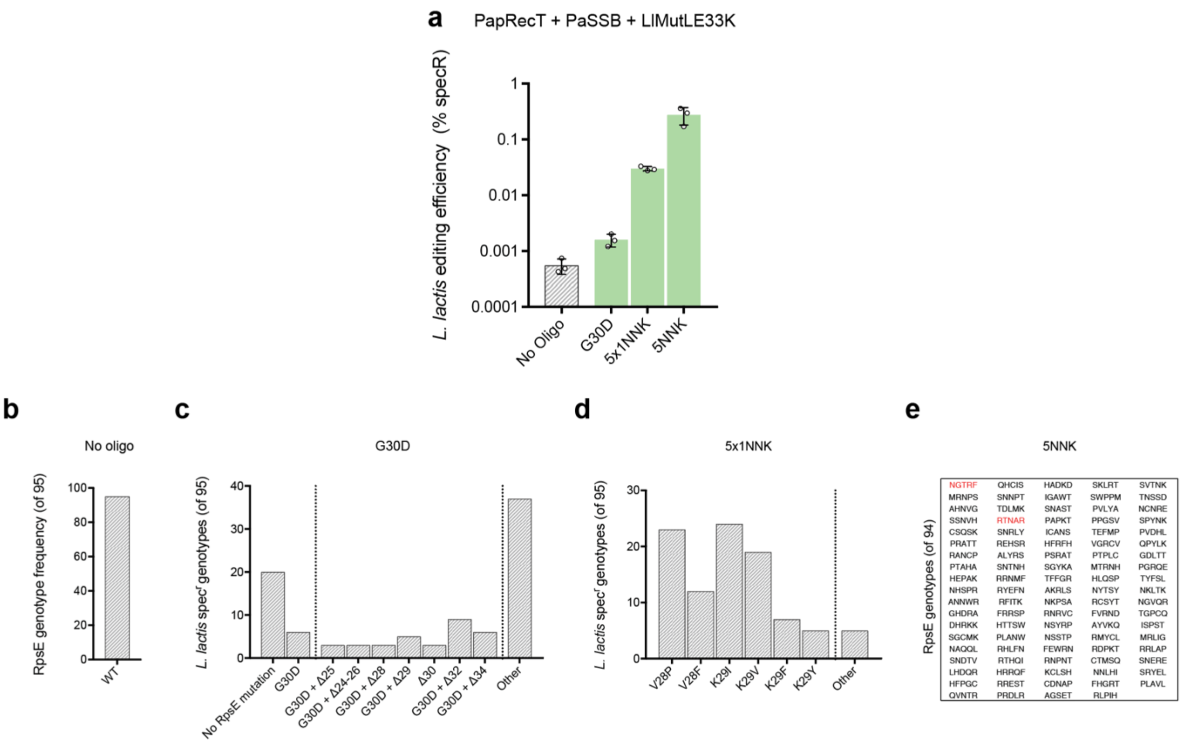
Recombineering using select SSAPs and SSAP-SSB pairs in *M. smegmatis*. Recombineering using λ-Redβ + PaSSB is not substantially improved over λ-Redβ in *M. smegmatis*. PapRecT has basal activity in *M. smegmatis*, and PapRecT + PaSSB performs at approximately the same level. Error bars indicate SD from the mean of at least three biologically independent replicates.

In this case, the lack of any effect of λ-Redβ, or λ-Redβ + PaSSB in *M. smegmatis* may be due to far lower expression of λ-Redβ in the strain, as compared to PapRecT. This protein expression experiment was performed once to generate the presented gel images.



Supplementary Figure 6.

To generate a dominant negative *L. lactis* MutL, we aligned the host MutL protein with the dominant negative E. coli gene (MutL.E32K) and made the corresponding change in *L. lactis* (E33K).



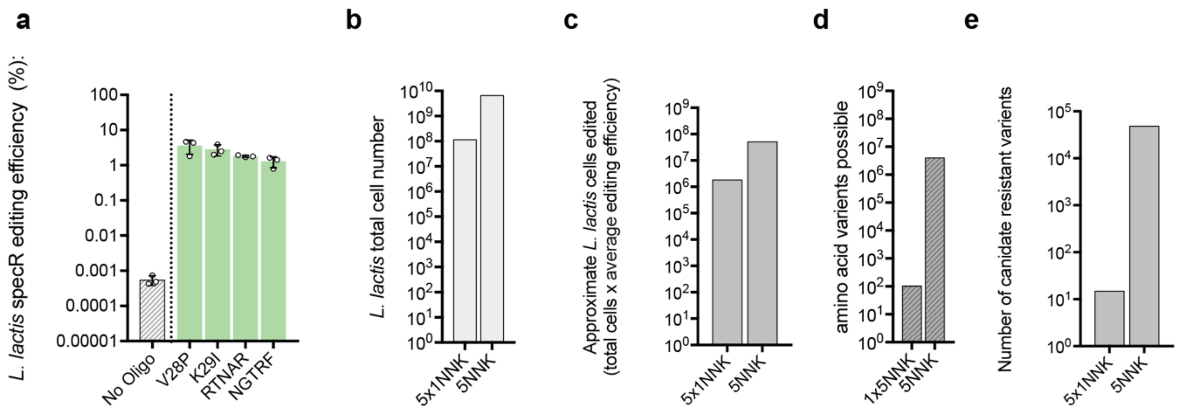
Supplementary Figure 7.
Preliminary experiment for spectinomycin resistance at *L. lactis* RpsE

(A), Efficiencies of spectinomycin resistance for different oligo designs measured in triplicate. Error bars indicate SD from the mean of at least three biologically independent replicates.

(B-E), Sanger sequencing of spectinomycin resistance mutants.

The amino acid conversion corresponding to a *E. coli* spectinomycin resistant mutant G27D (G30D) did not generate a resistant phenotype in *L. lactis*. The higher rate of resistant alleles using a G30D targeting oligo compared to background levels may be attributed to a rare occurrence of small deletions that occur during mutagenesis (b) which result in a resistant phenotype. Low levels of unintended deletions may be due to oligonucleotide synthesis errors, or an aberrant effect of remaining mismatch repair proteins.[3]

Sanger sequencing results from the 5x1NNK and 5NNK plates reflect allele mutations seen in the next-generation sequencing results



Supplementary Figure 8.

(A), Editing efficiency of select 1 amino acid and 5 amino acid variants. Error bars indicate SD from the mean of at least three biologically independent replicates.
(B), Total cell number after editing with the 5x1NNK oligo library (a single transformation), and the 5NNK oligo library (30 pooled transformations) targeting L. lactis RpsE.
(C). Calculation of expected number of edited cells using the average editing efficiency for 1AA changes and 5AA changes given in (A)
(D), Number of possible amino acid variants and stop codons in the two libraries (5 x 21 for 1x5NNK), (21^5 for 5NNK)

Pre-selection 1NNK (WT reads removed)

	NNK1	NNK2	NNK3	NNK4	NNK5
D	0.007	0.007	0.009	0.008	0.007
E	0.006	0.005	0.004	0.005	0.005
K	0.009	0.786	0.008	0.009	0.009
R	0.013	0.015	0.015	0.013	0.802
H	0.009	0.011	0.011	0.009	0.011
N	0.013	0.017	0.014	0.015	0.014
Q	0.005	0.008	0.006	0.006	0.006
Y	0.009	0.010	0.011	0.010	0.010
C	0.006	0.006	0.007	0.006	0.006
G	0.007	0.006	0.797	0.798	0.006
S	0.019	0.020	0.019	0.018	0.020
T	0.016	0.020	0.017	0.016	0.017
A	0.012	0.010	0.009	0.011	0.009
M	0.007	0.008	0.007	0.007	0.006
I	0.011	0.011	0.010	0.010	0.010
L	0.018	0.019	0.017	0.017	0.019
V	0.803	0.008	0.009	0.010	0.010
F	0.007	0.007	0.008	0.007	0.008
W	0.004	0.004	0.004	0.004	0.003
P	0.013	0.016	0.014	0.013	0.014
*	0.007	0.007	0.006	0.008	0.006

Pre-selection 5NNK (WT removed)

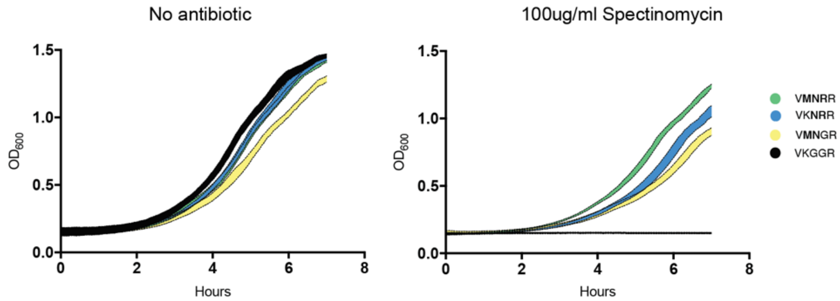
	NNK1	NNK2	NNK3	NNK4	NNK5
D	0.035	0.034	0.037	0.036	0.036
E	0.024	0.023	0.024	0.023	0.023
K	0.042	0.044	0.041	0.041	0.040
R	0.064	0.073	0.064	0.067	0.074
H	0.045	0.045	0.048	0.048	0.047
N	0.064	0.060	0.065	0.068	0.066
Q	0.030	0.032	0.032	0.030	0.030
Y	0.046	0.044	0.047	0.049	0.050
C	0.025	0.028	0.025	0.027	0.029
G	0.030	0.033	0.033	0.034	0.032
S	0.090	0.090	0.087	0.089	0.090
T	0.088	0.083	0.083	0.082	0.079
A	0.053	0.051	0.051	0.047	0.046
M	0.033	0.033	0.032	0.031	0.032
I	0.043	0.043	0.045	0.048	0.047
L	0.083	0.086	0.087	0.084	0.085
V	0.048	0.043	0.045	0.045	0.043
F	0.033	0.033	0.034	0.035	0.037
W	0.017	0.019	0.016	0.017	0.018
P	0.074	0.074	0.073	0.067	0.065
*	0.030	0.030	0.031	0.030	0.032

Supplementary Figure 9.

Normalized frequencies of next-generation sequencing reads in the 1x5NNK library, as well as the 5NNK library after editing and 1hr of recovery, before selection with spectinomycin.

a

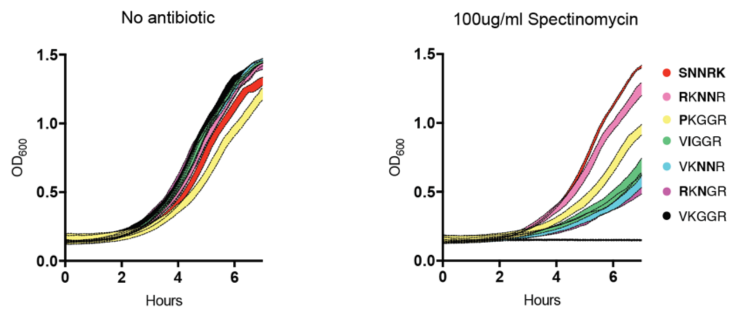
5NNK	Genotype	Resistant 100ug/ml
3 AA mutant	VMNRR	Yes
2 of 3	VMNGR	Yes
2 of 3	VMGRR	No
2 of 3	VKNRR	Yes
1 of 3	VMGGR	No
1 of 3	VKNGR	No
1 of 3	VKGRR	No



b

5NNK	Genotype	Resistant 100ug/ml
Most enriched	SNNRK	Yes (red)
3 AA mutant	RKNNR	Yes (pink)
2 of 3	RKNGR	Yes (purple)
2 of 3	RKGNR	No
2 of 3	VKNNR	Yes (cyan)
1 of 3	RKGGR	No
1 of 3	VKNGR	No
1 of 3	VKGNR	No

1NNK	Genotype	Resistant 100ug/ml
Most enriched	PKGGR	Yes (yellow)
2nd most enriched	VIGGR	Yes (green)

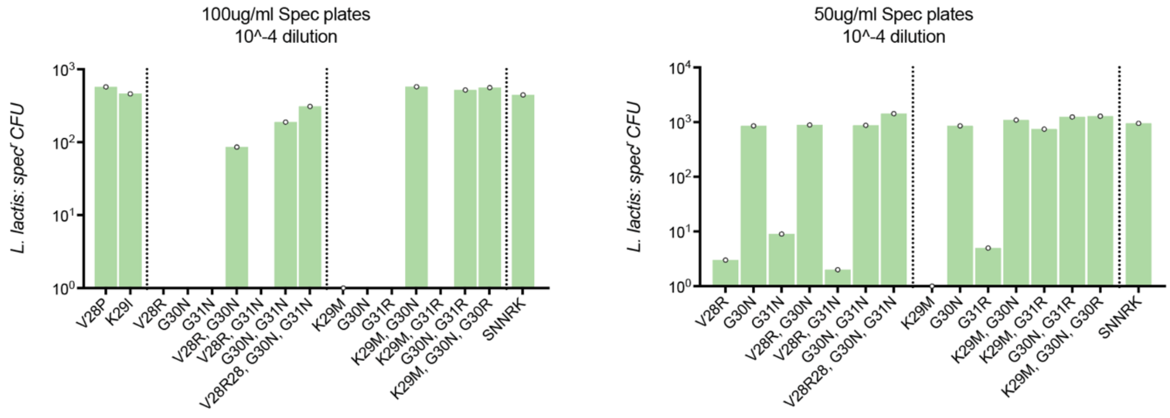


Supplementary Figure 10.

Doubling times of additional combination mutants and their resistant constituent variants (a, VMNRR) (b, RKNNR, as well at the most enriched variant in the entire pool SNNRK).

The triple mutant VMNRR is more resistant than its two constituent two double mutants (VKNRR, and VMNGR). Neither the remaining double mutant VMGRR nor any of the single mutants grew on 100 µg/mL Spec plates.

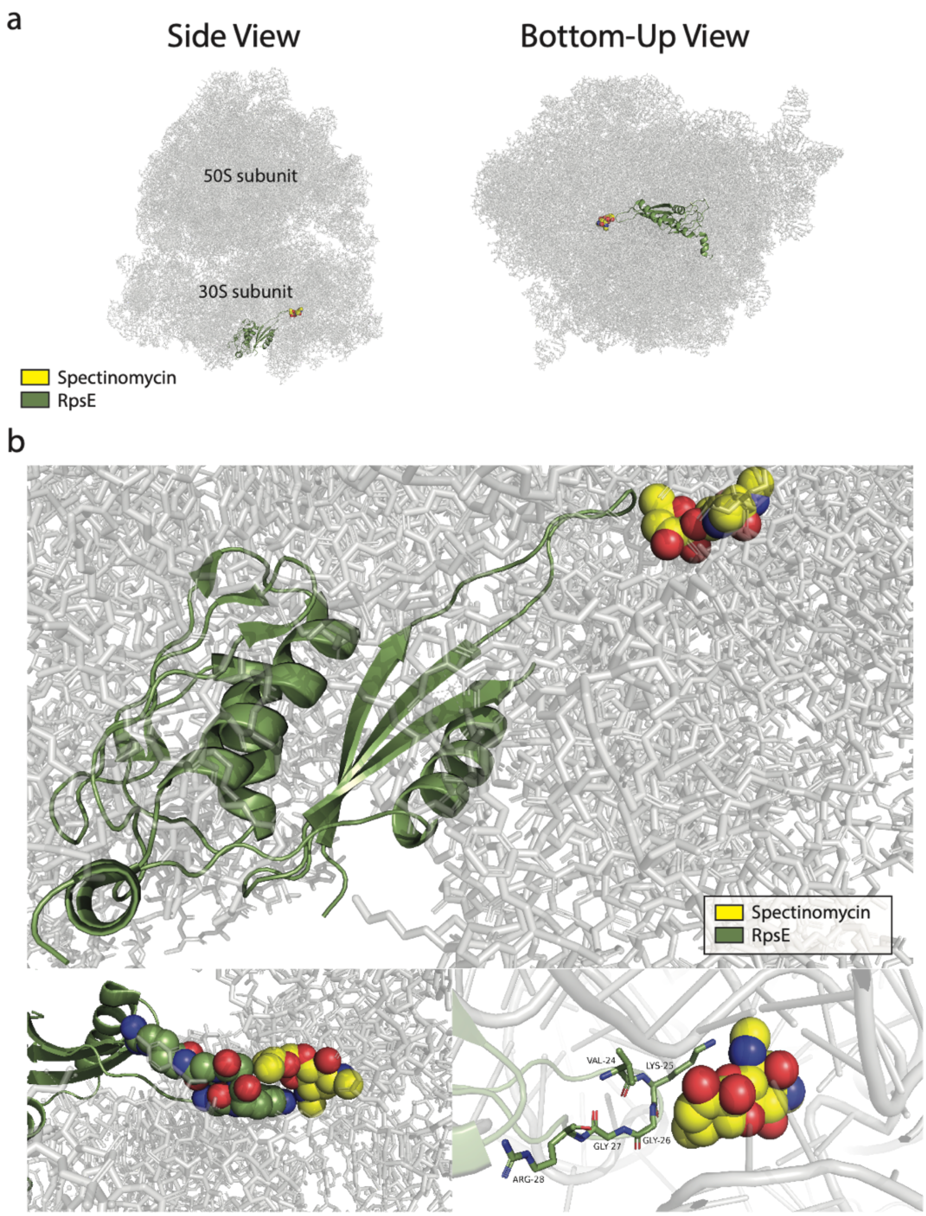
SNNRK grew faster in the presence of antibiotic than any of the other variants, and had less of a fitness defect than PKGGR even though all 5 positions are mutated from wildtype. Again, the triple mutant RKNNR was more resistant than its constituent double mutants and none of the signal mutants grew on 100 µg/mL Spec plates.



Supplementary Figure 11.

Validation of resistance for two genotypes (VMNRR, RKNNR) after two days of growth on 100 µg/mL spectinomycin plates or 50 µg/mL spectinomycin plates.

One of most enriched alleles that appeared in the 5NNK combination mutant library is G30N. This mutation alone did not allow colony formation after 2 days on 100 µg/mL Spec plates, but did on 50 µg/mL Spec plates, indicating that weaker selection pressure may provide additional paths to high resistance phenotypes.



Supplementary Figure 12. Structure Supplement. (a) Side (left) and bottom-up (right) views of the *E. coli* ribosome bound to spectinomycin (PDB ID: 4V56). Spectinomycin is shown in yellow spheres, RpsE (the protein product of ribosomal protein S5) in green ribbon, and the rest of the RNA and protein components of the ribosome in gray, partially transparent sticks. (b) The bottom-up view is magnified to show the interaction between RpsE and Spectinomycin. Three different views are shown: (top) the full RpsE protein is visible in green ribbon, (bottom left) the loop that contacts spectinomycin is focused on with the five varied residues shown in green spheres, and (bottom right) this same loop is focused on with residues labeled and shown in green sticks.

Supplemental References:

1. Espah Borujeni, A., Channarasappa, A. S. & Salis, H. M. Translation rate is controlled by coupled trade-offs between site accessibility, selective RNA unfolding and sliding at upstream standby sites. *Nucleic Acids Res* **42**, 2646–2659 (2014).
2. Salis, H. M., Mirsky, E. A. & Voigt, C. A. Automated design of synthetic ribosome binding sites to control protein expression. *Nature Biotechnology* **27**, 946–950 (2009).
3. Oppenheim, A. B., Rattray, A. J., Bubunenko, M., Thomason, L. C. & Court, D. L. In vivo recombineering of bacteriophage λ by PCR fragments and single-strand oligonucleotides. *Virology* **319**, 185–189 (2004).