

Supporting Information for the manuscript:

An engineered aminoacyl-tRNA synthetase for cell-selective analysis of mammalian protein synthesis

Alborz Mahdavi^{‡*}, Graham D. Hamblin[†], Granton A. Jindal^{‡^}, John D. Bager^{‡*^}, Cathy Dong^{*}, Michael J. Sweredoski[¶], Sonja Hess[¶], Erin M. Schuman[†] and David A. Tirrell[‡]

[‡]Division of Chemistry and Chemical Engineering, ^{*}Division of Biology and Biological Engineering, [¶]Proteome Exploration Laboratory, Beckman Institute, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA, 91125. [†]Max Planck Institute for Brain Research, Frankfurt am Main, 60438, Germany

Materials and Methods:

Development of Mammalian Vectors for Expression of *E. coli* NLL-MetRS/tRNA^{Met}. For PCR and cloning purposes, unless otherwise stated, we used chemically competent *E. coli* MegaX DH10B (Zymo Research). Plasmid DNA was purified using a Miniprep kit (Qiagen); colony selection was performed on LB-agar plates with 100 µg/mL ampicillin. All plasmids were verified by sequencing (Laragen). The *E. coli* NLL-MetRS was obtained from pAM1 (Addgene plasmid 51401) through PCR amplification using a Nhe1 forward primer and an Xho1 reverse primer and inserted into the multiple cloning site of the mammalian expression vector pCDNA3.1+ (Invitrogen). This vector contains a CMV promoter, a bovine growth hormone (BGH) transcriptional stop sequence, and a neomycin resistance gene for G418 selection. The resulting plasmid was designated pMetRSNLL_G. For simultaneous expression of *E. coli* tRNA^{Met} and NLL-MetRS, the *E. coli* elongator tRNA^{Met} sequence was synthesized (Integrated DNA Technologies) with the 5' and 3' flanking sequences of human tRNA^{fMet} as well as flanking BglII restriction sites. This construct was inserted into the BglII site in pMetRSNLL_G to produce pMetRSNLLtRNA_G (sequence included in Figure S8). pMetRSNLLtRNA_G is a pCDNA3.1+-based vector expressing the NLL-MetRS under CMV promoter control as well as the *E. coli* tRNA^{Met}. A second tRNA sequence lacking the C-terminal CCA tail was synthesized (Integrated DNA Technologies) and inserted into the BglII restriction site of MetRSNLL_G to make pMetRSNLLtRNAdcca_G (sequence included in Figure S9).

Introduction of Mutations into MaRS and Development of Associated Mammalian Expression Vectors.

MaRS was obtained from a cDNA clone from American Type Culture Collection clone ID 6414029 (ATCC). Site-directed mutagenesis (Agilent) was used to introduce the NLL, CLL, PLL and SLL mutations at residues L274, Y527 and H562 respectively. The L274G mutation was also introduced. These sequences were PCR amplified with a Nhe1 forward primer and an Xho1 reverse primer and inserted into pCDNA3.1+ (Invitrogen), resulting in plasmids pMaRSWT_G, pMaRSL274G_G/pMaRS_G, pMaRSSLL_G, pMaRSPLL_G, pMaRSCLL_G, pMaRSNLL_G (sequences in Figures S1-S6, respectively).

Development of Expression Vectors for Cell-Selective Proteomic Labeling and Cre-Lox Mediated Expression of L274GMmMetRS. To prepare pMaRSC, the L274G MaRS coding sequence was connected to a mCherry sequence through a T2A linker by sewing PCR. Briefly, the MaRS sequence (obtained from pMaRSL274G_G/pMaRS) was PCR amplified using a Nhe1 forward primer containing a Flag-Tag sequence and a reverse primer containing a T2A linker. The mCherry coding sequence was amplified using a matching T2A sequence in the forward primer and a reverse primer encoding a C-terminal Myc-

tag and stop codon. Sewing PCR was used to amplify the final product with the following sequence components: Nhe1-FlagTag-MaRS-T2A-mCherry-MycTag-Xho1. This sequence was inserted between the Nhe1 and Xho1 sites of a pCDNA3.1+ vector containing a hygromycin resistance cassette (Invitrogen) to yield pMaRSC (sequence in Figure S12). For Cre-Lox mediated recombination, a LoxP-flanked transcriptional stop sequence was inserted after the CMV promoter in the pMaRSC plasmid. The LoxP-flanked transcriptional stop sequence consisting of forward LoxP (ATAACTTCGTATAGCATACATTATACGAAGTTAT) sequences flanking the transcriptional stop sequence was synthesized with Nhe1 restriction sites on both ends (Integrated DNA Technologies). The resulting fragment was ligated into the Nhe1 site of pMaRSC to yield the Cre-Lox plasmid pMaRSC_lox_H (sequence in Figure S13). The correct orientation of the insert into the Nhe1 cut site was verified by sequencing.

Cell Culture. Cells were passaged every three days on tissue-culture plates and incubated at 37°C and 5% CO₂. CHO-K1 cells were maintained in RMPI (Invitrogen) medium with 10% fetal bovine serum, and supplemented with Pen/Strep, L-glutamine and non-essential amino acids (Invitrogen). HeLa and COS7 cells were cultured in DMEM (Invitrogen) medium with 10% fetal bovine serum, and supplemented with Pen/Strep, L-glutamine and non-essential amino acids (Invitrogen).

Cell Transfection, Selection and Conditional Transgene Activation. Mammalian expression plasmids were amplified in *E. coli* strain MegaX DH10B and purified by using endotoxin-free plasmid Maxi-kits (Qiagen). Lipofectamine 2000 (Invitrogen) was used for all transfections according to the manufacturer's recommended procedures. For identification of MetRS variants that charge Anl and to study Anl incorporation, all cells were transiently transfected 30 hours prior to Anl labeling. For the Cre-Lox transgene activation study, pMaRSC_lox_H was linearized with BglII and transfected into CHO cells. After selection on hygromycin at 100 µg/ml for 10 days, surviving colonies were picked and expanded to yield a stable cell line. Cre-mediated recombination in these cells was achieved through transient transfection with a plasmid expressing eGFP-Cre under control of an EF1α promoter (Addgene plasmid 11923).

Synthesis of Azidonorleucine. Azidonorleucine synthesis was based on a previous protocol for azidohomoalanine synthesis, using Boc-lysine as the starting material.¹

Copper-Catalyzed Reaction of Alkyne-TAMRA with Anl-labeled Proteins in Cell Lysates and Detection by In-gel Fluorescence. Cells were lysed with 4% SDS in phosphate-buffered saline (PBS).

Ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor (Roche) was added to the lysates to reduce protease activity. PBS was added to dilute the SDS concentration to 1%, and cell lysates were centrifuged at 14,000 rcf for 10 minutes to remove cellular debris. Protein concentrations were measured by using a bicinchoninic protein quantification kit (BCA assay; Pierce). The same amount of protein was used for each condition; concentrations ranged from 0.1 to 0.4 mg/mL. Copper-catalyzed reactions were performed using the Click-IT TAMRA protein analysis kit (Invitrogen). Proteins were precipitated with chloroform/methanol, washed with methanol to remove unreacted dye and resuspended in protein loading buffer containing 2% SDS and 10 mM 2-mercaptoethanol. Proteins were separated by electrophoresis on 12% Bis-Tris polyacrylamide gels (Invitrogen). TAMRA ($\lambda_{\text{excitation}} = 555$ nm and $\lambda_{\text{emission}} = 580$ nm) was excited at 532 nm and detected with a 580 band-pass 30 nm filter. In-gel fluorescence images were acquired on a Typhoon 9400 molecular imager (GE Healthcare).

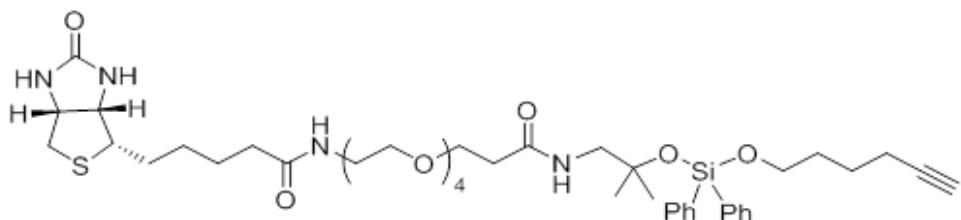
Detection of Proteins in Gels and Western Blots. Bicinchoninic acid protein quantification (Pierce) was used to equalize the amounts of proteins analyzed under different conditions. After dye labeling via the copper-catalyzed click reaction described above, proteins were washed with methanol to remove unreacted dye and then separated on Novex 12% Bis-Tris polyacrylamide gels (Invitrogen). Colloidal blue dye (Invitrogen) was used for nonspecific protein detection. For Western blots the proteins were transferred to nitrocellulose membranes (GE Healthcare), and probed with a Myc-tag-Alexa Fluor 488 conjugate monoclonal antibody (Cell Signal Technologies) at 1:1000 dilution in PBS with 0.2% v/w Tween20 (Sigma). Imaging of Western blots and gels was performed with a Typhoon 9400 molecular imager (GE Healthcare).

Copper-Catalyzed Reaction of Alkyne-TAMRA in Adherent Cells and Fluorescence Confocal Microscopy. Copper-catalyzed azide-alkyne cycloaddition reactions and synthesis of requisite THPTA ligand were performed as described previously^{1,2}. Adherent CHO cells seeded onto glass bottom tissue culture plates (MatTek) were incubated in fresh CHO medium, as described above, supplemented with Anl at 1.5 mM, for 6 hours. Cells were washed twice with PBS, fixed with 3.7% formaldehyde in PBS for 15 minutes at room temperature, permeabilized with 4°C methanol for 10 minutes, and washed three times with PBS at room temperature. Labeling with alkyne-TAMRA (Invitrogen) was performed at room temperature in pH 7.4 PBS for 2 hours, using a final concentration of 0.1 mM copper sulfate, 0.5 mM THPTA, 5 mM sodium ascorbate, 5 mM aminoguanidine and 10 μ M alkyne-TAMRA. To remove unreacted dye and other reaction components, cells were washed five times at 30-min intervals with PBS. Cell nuclei were stained with 300 nM DAPI in PBS for 30 min at room temperature and washed

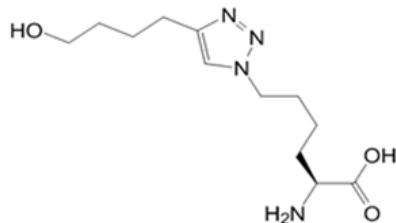
three times with PBS before imaging. Fluorescence confocal images were obtained on a Zeiss LSM 510 microscope.

Identification of Anl Incorporation Sites in Cellular Proteins.

HeLa cells expressing L274GMmMetRS were labeled for a total of 10 hours with 2mM Anl before proteomic analysis. Cells were lysed with 1% SDS in PBS supplemented with EDTA-free protease inhibitor (Roche). Lysates was sonicated using a tip sonicator to reduce viscosity and centrifuged at 14,000 rcf for 15 min to remove cellular debris and reacted with an acid-cleavable biotin-alkyne enrichment tag for 2 hours, using a final concentration of 0.1 mM copper sulfate, 0.5 mM THPTA ligand, 5 mM sodium ascorbate, 5 mM aminoguanidine, and 100 µM alkyne probe. To identify sites of Anl incorporation in proteins, we used a cleavable enrichment tag that would allow detection of Anl and tagged-Anl residues at Met positions. This acid-cleavable biotin-alkyne enrichment tag was previously reported by us³ and has the following structure:



After click reaction with this tag, proteins were precipitated with acetone, dissolved in 250 µl of 4% SDS in PBS, and diluted to 0.1% SDS by addition of PBS supplemented with EDTA-free protease inhibitor (Roche). Proteins were incubated with 400 µl Streptavidin Plus Ultralink resin (Pierce) for 1.5 hours at room temperature. Affinity purification was performed according to a previously published protocol⁴ and the tag was cleaved in mild acidic solution, the resulting cleaved tag structure after reaction with Anl residue is as follows:



This tag structure introduces a mass shift of 121.12 amu at each methionine position, this variable mass modification includes the mass shift from methionine to Anl and the triazole conjugate containing the

cleaved tag moiety. We used this mass shift in our mass spectrometric analysis as a variable mass modification to search for Anl incorporation at Met positions in proteins.

Tryptic Digest for Identification of Anl Incorporation Sites. After enrichment as described above, elution fractions were combined with Amicon Ultra 0.5 centrifuge filters (3 kDa MWCO) (Millipore), and resuspended in 200 μ L Tris-HCl pH 8.5. Lysyl endopeptidase (Wako; 10 μ L of 0.1 μ g/ μ L solution in 100 mM Tris-HCl pH 8.5) was added, and the sample was incubated for 4 hours at room temperature in the dark. Thereafter 20 μ L of 0.5 μ g/ μ L trypsin (Wako) in water was added and the sample was incubated in the dark overnight at room temperature. The eluent was centrifuged at 14000 rcf for 20 min using a 10 kDa molecular weight cutoff spin filter (Pierce) to remove undigested proteins as well as trypsin which remains in the filter. The flow-through, which contained tryptic peptides, was retained and acidified to 0.2% CF₃COOH. The peptide solution was desalted as described by Mann and coworkers⁵ using a 3 mL MILI-SPE C18-SD extraction disk cartridge (3M) as follows. The cartridge was washed with 1 mL CH₃OH and centrifuged at 1500 rcf for 1 min, washed with 0.5 mL 0.1% CF₃COOH, 70% CH₃CN in water, and centrifuged at 1500 rcf for 1 min. The cartridge was washed with 0.1% CF₃COOH in water and centrifuged at 1500 rcf for 1 min. The peptide sample was loaded into the cartridge and passed through three times; each time the cartridge was centrifuged at 150 rcf for 3 min. The cartridge was washed twice with 0.5 mL 0.1% CF₃COOH in water and centrifuged at 150 rcf for 3 min. To elute the desalted peptides, the cartridge was washed with 0.5 mL of CH₃CN in water and centrifuged at 150 rcf for 3 min. The desalted peptides were lyophilized and stored at 4°C before analysis by mass spectrometry.

Affinity Enrichment of Anl-labeled Proteins for Shotgun Proteomics. Cells were labeled for a total of 24 hours with 2 mM Anl, then washed with PBS and lysed with 1% SDS in PBS supplemented with EDTA-free protease inhibitor (Roche) and 100 mM chloroacetamide. Lysates were boiled for 10 minutes at 95°C, and centrifuged at 14,000 rcf for 30 minutes to remove cellular debris. The supernatant protein content was quantified using the bicinchoninic acid assay. For each enrichment, 3 mg of lysate was combined with 50 μ L of azadibenzocyclooctyne resin (50% slurry by volume; Click Chemistry Tools) that had been washed three times with 0.8% SDS in PBS. This copper-free on-resin cycloaddition reaction was incubated at room temperature on a rotating table for 3 hours, and then unreacted DBCO groups were quenched by addition of 20 μ L 100 mM Anl for 30 minutes. The supernatant was then removed, and the beads washed with 1 mL H₂O, reduced with 0.5 mL DTT (1 mM, 15 minutes at 70°C), and alkylated with 0.5 mL iodoacetamide (40 mM, 30 minutes at room temperature, protected from light). The resin was

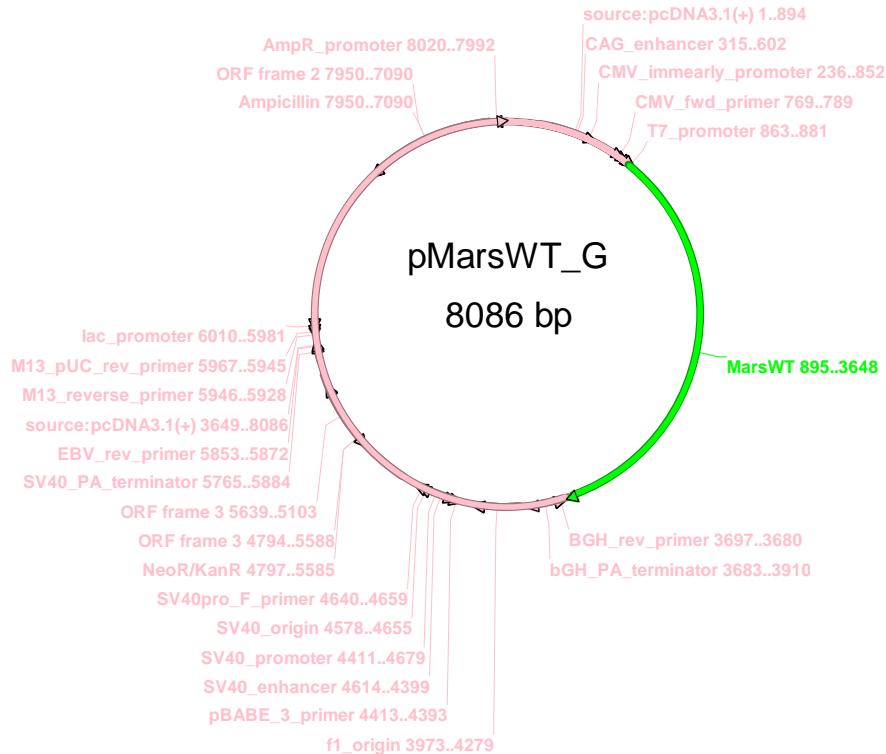
then washed extensively to remove non-specifically bound proteins, using 40 mL each of 0.8% SDS in PBS, 8 M urea in 100 mM tris (pH 8.0), and 20% acetonitrile (in 5 mL aliquots). After washing, the resin was transferred to an eppendorf with 100 μ L trypsin digestion buffer (10% acetonitrile in 50 mM ammonium bicarbonate). This was supplemented with 0.1 ug of sequencing grade trypsin, and incubated with shaking at 37°C overnight to digest the resin-bound proteins. The supernatant was then collected, combined with two washes of the resin (150 μ L 10% acetonitrile each), and lyophilized. To investigate the non-specific background of this enrichment, this same protocol was also carried out on cells that had been pulsed with 2 mM methionine instead of Anl.

Sample Preparation for Shotgun Proteomics. After affinity enrichment, digested peptides were resuspended in 100 μ L of 50 mM ammonium bicarbonate, treated with a HiPPR detergent removal spin column (Pierce) to remove trace SDS, and desalted with a C18 ZipTip (EMD Millipore). The eluate was lyophilized, and resuspended in 0.2% formic acid for LC-MS/MS analysis. An aliquot of each sample was diluted in water and quantified using the LavaPep Fluorescent Protein and Peptide Quantification Kit (Gel Company), to estimate the appropriate volume to analyze by mass spectrometric analysis.

Mass Spectrometry. Analyses were performed on a hybrid LTQ-Orbitrap Elite (Thermo Fisher Scientific) equipped with a nanoelectrospray ion source connected to an EASY-nLC II instrument (Thermo Fisher Scientific) as described previously⁶. Separation of peptides was performed using a 15-cm reversed phase analytical column (75 μ m ID) with 3 μ m C18 beads (ReproSil-Pur C18-AQ) with a gradient of 2% solvent B for 5 minutes followed by an increase from 2% to 30% in 115 min and lastly a sharp rise to 100% B in 1 min. Solvent A was 0.2% formic acid, 2% acetonitrile, 97.8% LC-MS water and solvent B was 0.2% formic acid, 80% acetonitrile, 19.8% LC-MS water. The mass spectrometer was operated in data-dependent mode. Survey full scan mass spectra were acquired with a resolution of 120,000 at 400 m/z. The top 20 most intense ions from the survey scan were isolated and, after the accumulation of 5000 ions, fragmented in the linear ion trap by collision induced dissociation. Precursor ion charge state screening was enabled and singly charged and unassigned charge states were rejected. The dynamic exclusion list was enabled with a relative mass window of 10 ppm. An additional exclusion list included common trypsin peptide masses. Data analysis was performed using MaxQuant software (v. 1.5.3.8).⁷ Spectra were extracted from the raw files using MaxQuant with match between runs. Spectra were searched against UniProt Chinese Hamster database (23888 entries) and a contaminant database (246 entries). Digestion enzyme was specified as trypsin with up to two missed cleavages. Variable modifications

included methionine oxidation (+15.9949), N-terminal protein acetylation (+42.0106), Met to Anl (+23.0450), and Met to Anl to Lys modification (-2.9455) and a fixed modification of cysteine carbamidomethylation (+57.0215). The Met to Anl to Lys modification is for residues in which Anl is incorporated at Met codons, wherein the azide side chain of this Anl does not form a triazole linkage during cycloaddition reaction, and it is reduced to a primary amine during proteomics workup and sample preparation. Precursor mass tolerance was less than 4.5 ppm after mass recalibration by MaxQuant. Fragment ion tolerance was 0.5 Da. Protein and peptide false discovery rates were fixed at 1% and estimated using a decoy database search performed by MaxQuant. Annotation of proteins into different cellular components was performed using STRAP software.⁸

Figure S1. The pMaRSWT_G vector for expression of wild-type *MmMetRS* under CMV promoter control. Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



GACGGATCGGAGATCTCCGATCCCTATGGTCACTCTCAGTACAATCTGCTGTGCCGCATAGTTAACGCCAG
 TATCTGCTCCCTGCTTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAAGGCAAGGCTT
 GACCGACAATTGCATGAAGAATCTGCTTAGGGTAGGCCTTGCCTGCGATGTACGGGCCAGATATACGC
 GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATAATGGAGTTC
 CGCGTTACATAACTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGAC
 GTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACT
 TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
 TATGCCAGTACATGACCTTATGGACTTCCATTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
 GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGTTGACTCACGGGATTCCAAGTCTCCACCCATTG
 ACGTCAATGGAGTTGTTGGCACCAAAATCAACGGACTTCCAAATGTCGAACAACCTGCCCTATTGACG
 CAAATGGCGGTAGGCGTGTACGGTGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCACTGCTTA
 CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTG**GCTAGC****GCCACC**ATGGCCACCATCACC
 CACCAT**ATGAGACTGT****CGTGAGCGAGGGT****CCCCGGGGAGCCTG****CCCGT****GCTGGCTGCCGCCGCGAGGGCCGGGG**
TCGGGCGGAGCT**GCTCATCAGCACCGTAGGCCCGAAGAGTGTGGTACCATT****CCCTACCCGGCTTAAGGTCCCTG**
TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGC**AACTCTGCCGATATT****TTTCTGTTATGTGGCTGG**
GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGAGGCAACAGAACTG**CAGCCAGTTCTGCTGCCCTACA**
CTGTCTAGTGGTCAAGGCAAGAAAGGGGAAGATATACTTGGCCCACTCG**GAGAGTCTGACTCACATTGATCACA**
GCTTGAGTCGTCAAAACTGTC**TTCTGGCTGGGACACAGAACTCTAGTGC****ACATTGTTGGGGAGCACTG**
TATCCTTACTGCAAGACCCAGCTTACCTCCCTGAGGAGTTGGGTGCCCTG**CAAAGTTGGTCCAGACACTGAGTAC**
CCAGGAACCGTGT**CAGCGAGCTGCAGAGACGGTGCTAAAACAGCAGGGTGT****CCACTTCGTC****TGTACCTCCAGA**
AACAGGCCACAGCCTCAGCCCCGCCCTCCTGAGGGAGAACTGTCAGCAACGAGCTGGAGGAAGAGGA**ACTGGCTAC**

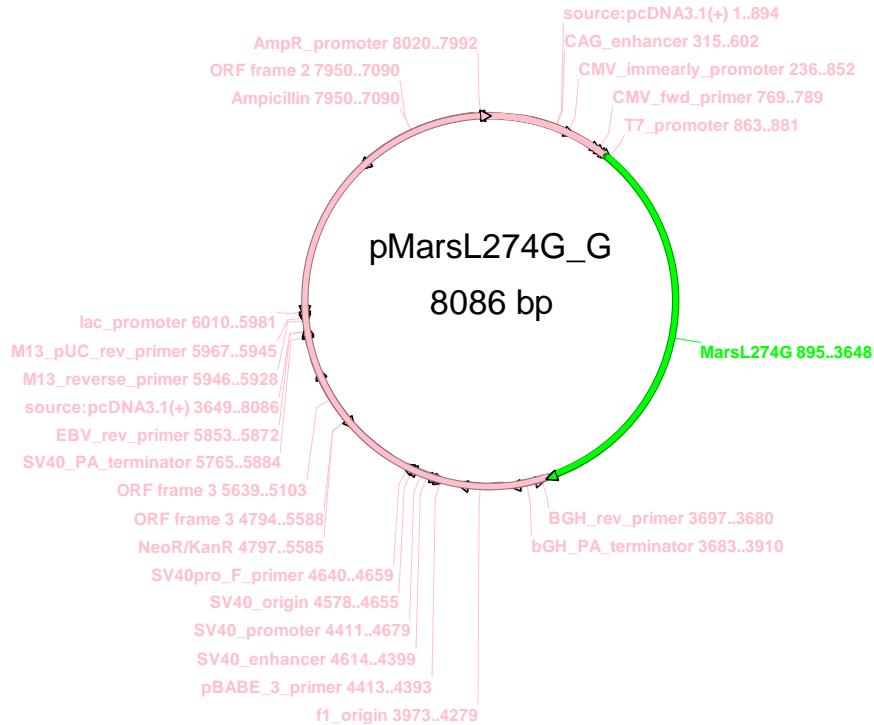
TTGTCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGAGAAGGGTCTGGAAAGCCTGCCTCCGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCCTGGAGAGAGGAATGTTCTCATCACCACTGCCCTCCCTATGTCAACAATGTCC
CCCACCTTGAAACATCATTGGCTGTGCTCAGTGTGATGTCCTTGCAAGGTATTGTCGCCCTGCCAGTGGAAAT
ACCCCTATCTGTGTGGTACAGATGAGTATGGTACTGCGACAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTCGGCATATGTTGACTTTCCGG
GCACTACCACCTCAGCAGACCAAAATACCCAGGACATCTTCAGAGGTGCTGACCCGGGGTTGTGCTGCCA
GATACTGTGGAGCAGCTTCGGTGTGAGCGGTGTGACGTTCTGGCTGACCGCTTGAGGGGTGTGTCCCTT
CTGTGGCTATGAAGAGGCCGAGGTGACAGTGTGACAGGTGTGCAAGCTCATCAATGCCATTGAGCTCAAGAAC
CACAGTGAAAATCTGCCGCTCTGCCCTGTGGTGAGGTGCTCACAGCACCTGTTCTAGACTTGCTTAAGTGGAA
AAGCGTCTGGAGGACTGGTTGGGAAGACAGTGCCTGCAGTGACTGGACACCCAAATGCCAGGTTATTACGTT
CTGGCTTCGAGATGGCCTCAAGCCACGATGCATCACCAAGAGACCTCAAATGGGAACGCCGTGCCCCGG
TTGAGGACAAGGTATTTCAGTCTGGTTGATGCTACTATTGGCTACGTGTCATCACAGCAAACATACAGACCA
TGGGAGAAATGGTGGAGAACCCAGAACAAAGTGGACCTTACAGTTCATGCCAAAGACAATGTTCCCTCCATGG
CTTGGCTTCCCGTGTTCAGTCCTAGGAGCTGAGGACAACATACCCCTGGTCAAGCACATATTGCTACAGAGTAC
TGAACATGAGGATGGAAATTCTCTAACAGAGCCGGGATAGGAGTGTGAGACATGCCAAGGATAACAGGAATC
CCTGCTGACATCTGGCATTCTATCTGCTATACATTGGCCTGAGGGCCAGGACAGTGCCTCTGGACAGACTT
GTGATTAAAAACAATTCTGAGCTGCTAACAAACCTGGCAACTTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTGGCGTTGTGTCCTGAGATGGCCTAACCCCTGATGACAGACGCCCTGGTGGCCCATGTCCTGGAAACTC
CAGCACTATCACCAGCTGGAAAAGGTTGGATCCGGATCCGGATGCCCTGCGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGGAAACGGATTAAAGGTGGTGGAGATGGCACAGGCAGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGGCTGCCTGCTGTCATGTCAGGCCATACATGCCACAGTCAGCTTAC
ATCCAGACCCAGCTGCAGCTCCCACCTGCAGCCTGCCATCCTGCCACAAGCTTCATTGTCACCTTGCCAGCAGG
CCACCGAATTGGCACAGTCAGTCTTGTCCAAAAACTGGAAAATGACCAGATTGAAAATTGAGGCAGCGCTTGG
GAGGGGGTCAGGCTAAAGGCTCCCCAAGCCAGCAGCTGTGGAGGCAGTTACAGCAGCAGGCTCGCAGCACATACAA
ACGCTGACGGATGAGGTGACCAAGCAGGGCACGTCCTGGGAACTGAAAGCACAGAAGGCAGACAAGAACAGG
TGCTGCAGAGGTGGCTAAACTCTGGATCTAAAGAAACAGTGGCTTGCTGAGGGAAACCCATTGAAACTCTA
AAGGCAAGAAGAAAAGTATAACTCGAGTCTAGAGGCCGTTAAACCCGCTGATCAGCCTGACTGTGCCCT
AGTGCAGGCCATCTGGTGTGCTGCCCTCCCCCGTGCCTCCCTGACCCCTGGAAGGTGCCACTCCACTGTCC
CTAATAAAATGAGGAAATTGCATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGGGGGGGGGGGGGG
GCAAGGGGGAGGATTGGAGAACAAATAGCAGGCATGCTGGGATGCCGCTATGGCTTGAGGCGAAAGA
ACCAGCTGGGCTCTAGGGGTATCCCCACCGCCCTGTAGCGGCCATTAAGCGCGGGGTGGTGGTTACGCG
CAGCGTACCGCTACATTGCCAGGCCCTAGGCCGCTCCTTCGCTTCTCCCTTCTGCCACGTTCG
CCGGCTTCCCGTCAAGCTCTAAATGGGGCTCCCTTAGGGTCCGATTAGTGCCTTACGGCACCTCGACCCCC
AAAAAAACTTGATTAGGGTGTGGTACGTAGTGGCATGCCCTGATAGCGTTTTCGCCCTTGACGTTGGA
GTCCACGTTCTTAATAGTGGACTCTTGTGCTTAAACTGGAAACAACACTCAACCCATTCTCGGTCTATTCTTGATT
TATAAGGGATTTGCCATTGCGCTATTGGTAAAAAAATGAGCTGATTAAACAAATTAAACGCGAATTAA
TGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCATGC
AATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCATGC
GTCAGCAACCATTGCGCCCTAATCCGCCCTAATCCGCCCTAATCCGCCCTGAGCTATTCCAGAAGTGTGAGGA
ATGGCTGACTAATTCTTATTGAGGCCGAGGCCGCTCTGCCCTGAGCTATTCCAGAAGTGTGAGGA
GGCTTTGGAGGCCCTAGGCTTTGCAAAAGCTCCGGGAGCTGTATATCCATTTCGGATCTGATCAAGAGAC
AGGATGAGGATCGTTGCATGATTGAACAAAGATGGATTGCACGAGGTTCTCCGGCCGTTGGTGGAGAGGCTAT
TCGGCTATGACTGGCACAACAGACAATCGGCTGCTCTGATGCCCGTGTCCGGCTGTCAGCGCAGGGGCCCG
GTCTTTGTCAAGACCGACCTGCGCTGCCCTGAATGAACTGCAGGAGGCCAGCGCGGCTATGTGGCTGG
CACGACGGCGTCTGCCCTGCGCAGCTGTGCTGACGTTGTCAGTGAAGCGGGAAAGGGACTGGCTGCTATTGGCG
TGCCTGGGGCAGGATCTCTGTCATCTCACCTGCTCTGCCGAGAAAGTATCCATTGGCTGATGCAATGCC
CTGCATACGCTTGATCCGGTACCTGCCATTGACCAAGGAAACATCGCATGAGCGAGCACGTACTCGGAT
GGAAGCCGGTCTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGCGCAGCCGAACACTGTTGCC
TCAAGGCGCGATGCCGACGGGAGGATCTCGTGCCTGACCCATTGGCGATGCCATTGGCTGCTTGCGAATAT
CATGGTGGAA

AATGGCCGCTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTAC
CCGTATATTGCTGAAGAGCTTGGCGCGAATGGCTGACCGCTTCTCGTCTTACGGTATGCCGCTCCGATT
CGCAGCGCATGCCTCTATGCCTCTTGACGAGTTCTGACGGGGACTCTGGGTTGAAATGACCGACCAAG
CGACGCCAACCTGCCATCACGAGATTCGATTCCACCGCCGCTCTATGAAAGGGTGGCTCGGAATCGTTTC
CGGGACGCCGGCTGGATGATCCTCAGCGCGGGATCTCATGCTGGAGTCTCGCCACCCAACTTGTATTG
AGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTACAATAAGCATTTTCACTGCATTCTAGTT
GTGGTTGTCAAACACTCATCAATGTATCTTATCATGCTGTATACCGTCACCTCTAGCTAGAGCTGGCGTAATCA
TGGTCATAGCTGTTCTGTGAAATTGTATCCGCTCACAACTTACACAAACATACCGAGCCGGAAAGCATAAGTG
TAAAGCCTGGGTGCCTAATGAGTGAGCTAACACATTAATTGCGTGCCTACTGCCGCTTCCAGTCGGAA
ACCTGTCGTGCCAGCTGCTTAATGAATGCCAACCGCGGGAGAGCGGTTGCGTATTGGCGCTTCCGCT
TCCTCGCTCACTGACTCGCTGCCGCTGGCTGCGCGAGCGGTATCAGCTCACTCAAAGGCCGTAATACG
GTTATCCACAGAACGAGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCCTAAAA
AGGCCGCGTTGCTGGCTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAATGACGCTCAAGTCAGAGG
TGGCAGAACCGACAGGACTATAAGATACCAGGCGTTCCCCCTGGAAGCTCCCTGCGCTCCGTGCGAC
CCTGCCGCTACCGGATACCTGTCGCCCTTCTCCCTCGGAAAGCGTGGCGTTCTCATAGCTACGCTGTAGGT
ATCTCAGTCGGTAGGTCGTTCTCCAAGCTGGCTGTGACGAACCCCCCGTTGAGCTCCCTGCGCTCCGTGCG
TTATCCGTAACTATCGTCTTGAGTCAACCGGTAAGACACGACTATGCCACTGGCAGCAGCACTGGTAACAG
GATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTTCTGAAGTGGTGGCTAACACTACGGCTACACTAGAAGAA
CAGTATTTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGCTTGTGATCCGGAAACAA
ACCACCGCTGGTAGCGTTTTTGCAAGCAGCAGATTACCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
GATCTTCTACGGGCTGACGCTCAGTGGAACGAAAACACGTTAACGGGATTTGGTATGAGATTATCAAAAA
GGATCTTCACCTAGATCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCT
GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTGTTGCTCATCCATAGTGCCTGACT
CCCCGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGCCCGAGTGCTGCAATGATACCGCAGACCCAC
GCTCACCGGCTCCAGATTATCAGCAATAAACAGCCAGCGAAGGGCCAGCGCAGAAAGTGGCCTGCAACTTA
TCCGCCTCCATCCAGTCTATTGTTGCGGGAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGAACGT
TGGTGCCTTGCTACAGGCATGTTGTCAGCTCGTGTGTTGATGGCTTCACTCAGCTCCGGTCCAAACGAT
CAAGGCAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCCGATCGTTGTCAGAAGT
AAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCGTAAGATG
CTTTCTGTGACTGGTAGTACTCAACCAAGTCATTGAGAATAGTGTATGCGCGACCGAGTTGCTCTGCCGG
CGTCAATACGGATAATACCGGCCACATAGCAGAACTTAAAAGTGTATCATTGAAAACGTTCTCGGGCGA
AAACTCTCAAGGATCTACCGCTGGAGATCCAGTTGATGTAACCCACTCGTCACCCAACTGATCTCAGCATC
TTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAAGGGAATAAGGGCAGAC
GGAAATGTTGAATACTCATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGA
TACATATTGAATGTATTTAGAAAATAACAAATAGGGTCCGCGACATTCCCGAAAAGTGCCACCTGACGT
C

Protein Sequence

MAHHHHHHMRLFVSEGPSGLPVAAAARARGRAELLIStVGPEECVPFLTRPKVPVLQLDSGNYLFSAICRYF
FLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVVQGKKGEDILGPLRRVLTHIDHSLSRQNCPFLAGTESLADI
VLWGALYPLLQDPAYLPEELGALQSWFQTLSTQEPCQRAAETVLKQQGVLAIRLYLQKQPQPPPEGRTVSNELE
EEEELATLSEEDIVTAVAWEKGLESPLPLKLQQHPVLPVPGERNVLITSALPYVNNVPHLGNIIGCVLSADVAFARYC
RLRQWNTLYLCGTDEYGTATETKAMEEGLTPREICDKYHAIHADIRWFGISFDTFGRRTTPQQTKITQDIFQRLLT
RGFVLRDTVEQLRCERCARFLADRVEGVCPFCGYEEARGDQCDCRGKLINAIELKKPQCKICRSCPVRSSQHLFL
DLPKLEKRLEDWLGKTVPGSDWTPNARFIIRSWLRLGKPRCITRDLKWGTPVPLEGFEDKVYVWFDATIGYVSIT
ANYTDQWEKKWNPEQVDLYQFMAKDNVPFHGLVFPCSVLGAEDNYTLVKHIIMATEYLNEYDGKFSKSRIGVFGDM
AKDTGIPADIWRFYLLYIRPEGQDSAFSWTLLIKNNSELLNNLGNFINRAGMFVSKFFGGCVPEMALTPDDRLVA
HVSWELOHYHQLEKVRIRDALRSILTISRHNQYIQVNEPWKRIKGGEMLDRQRAGTVTGMAVNMAALLSVMILQPYM
PTVSSTIQTQLQLPPAACRILATSFIGTLPAGHRIGTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTAA
GSQHIQTLTDEVTKQGNVVRELKAQKADKNQVAEVAKLLDKLQLALAEGKPIETPKGKKK-

Figure S2. The pMarsL274G_G vector for expression of L274GMmMetRS under CMV promoter control. Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence. This construct is available through Addgene.



```

GACGGATGGGAGATCTCCGATCCCTATGGTCACTCTCAGTACAATCTGCTGTGCCGCATAGTTAACCGAG
TATCTGCTCCCTGCTTGTGTGGAGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAAGGCAAGGCTT
GACCGACAATTGCATGAAGAATCTGCTTAGGGTAGGCCTTGCCTGCGATGTACGGGCCAGATATACGC
GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATAATGGAGTC
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
TATGCCAGTACATGACCTTATGGACTTCCATTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTGACG
ACGTCAATGGGAGTTGTTGGCACCAAAATCAACGGACTTCCAAATGCGTAACAACCTGCCCTATTGACG
CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCACTGCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGCGCCACCATGGCCACCATCACCATT
CACCATGAGACTGTCGTGAGCGAGGGTCCCCGGGGAGCCTGCCGTGCTGGCTGCGCCGCGAGGGCCGGGG
TGGGGCGGAGCTGCTCATCAGCACCGTAGGCCCGAAGAGTGTGGTACCATTCCCTACCCGGCTAAGGTCCCTG
TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGCAATCTGCCGATATTTTTCTGTTATGTGGCTGG
GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGGAGGCAACAGAACGAACTGCAGCCAGTTCTGCTGCCCTACA
CTGTCTAGTGGTCAAGGCAAGAAAGGGGAAGATATACTTGGCCACTTCGGAGAGTCCTGACTCACATTGATCACA
GCTTGAGTCGTCAAAACGTCCTTCTGGCTGGGACACAGAACGAACTCTGACTGACATTGTTGGGGAGCACTG
TATCCTTACTGCAAGACCCAGCTTACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGGAGTAC
CCAGGAACCGTGTCAGCGAGCTGCAGAGACGGTGCTAAACAGCAGGGTGTCCCTGGCACTTCGTGTACCTCCAGA
AACAGGCCACAGCCTCAGCCCCGCCCTCCTGAGGGAGAACTGTCAGCAACGAGCTGGAGGAAGAGGAACTGGCTAC

```

TTGTCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGAGAAGGGTCTGGAAAGCCTGCCCTCCGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCCCTGGAGAGAGGAATGTTCTCATCACCACTGCCGGACCCCTATGTCAACAATGTCC
CCCACCTTGAAACATCATTGGCTGTGCTCAGTGTGATGTCCTTGCAAGGTATTGTCGCCCTGCCAGTGGAAAT
ACCCCTATCTGTGTGGTACAGATGAGTATGGTACTGCGACAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GGAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTCGGCATATGTTGACTTTCCGG
GCACTACCACCTCAGCAGACCAAAATACCCAGGACATCTTCAGAGGTGCTGACCCGGGGTTGTGCTGCC
GATACTGTGGAGCAGCTTCGGTGTGAGCGGTGTGACGTTCTGGCTGACCGCTTGAGGGGTGTGTCCCTT
CTGTGGCTATGAAGAGGCCGAGGTGACAGTGTGACAGGTGTGCAAGCTCATCAATGCCATTGAGCTCAAGAAC
CACAGTGAAAATCTGCCGCTCTGCCCTGTGGTGAGGTGCTCACAGCACCTGTTCTAGACTTGCTTAAGTGGAA
AAGCGTCTGGAGGACTGGTTGGGAAGACAGTGCCTGCAGTGACTGGACACCCATGCCAGGTTATTACGTT
CTGGCTTCGAGATGGCCTCAAGCCACGATGCATCACCAAGAGACCTCAAATGGGAACGCCGTGCCCCGG
TTGAGGACAAGGTATTTCAGTCTGGTTGATGCTACTATTGGCTACGTGTCATCACAGCCAACATACAGACCA
TGGGAGAAATGGTGGAGAACCCAGAACAAAGTGGACCTTACAGTTCATGCCAAAGACAATGTTCCCTCCATGG
CTTGGCTTCCCGTGTTCAGTCCTAGGAGCTGAGGACAACATACCCCTGGTCAAGCACATATTGCTACAGAGTACC
TGAACATGAGGATGGAAATTCTCTAACAGAGCCGGGATAGGAGTGTGAGACATGCCAAGGATAACAGGAATC
CCTGCTGACATCTGGCATTCTATCTGCTATACATTGGCCTGAGGGCCAGGACAGTGCCTCTCTGGACAGACTT
GTGATTAAAAACAATTCTGAGCTGCTAACAAACCTGGCAACTTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTGGCGTTGTGTCCTGAGATGGCCTAACCCCTGATGACAGACGCCCTGGTGGCCCATGTCCTGGAAACTC
CAGCACTATCACCAGCTGGAAAAGGTTGGATCCGGATCCGGATGCCCTGCGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGGAAACGGATTAAAGGTGGTGGAGATGGCACAGGCAGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGGCTGCCTGCTGTCATGTCAGGCCATACATGCCACAGTCAGCTTAC
ATCCAGACCCAGCTGCAGCTCCCACCTGCAGCCTGCCATCCTGCCACAAGCTTCATTGTCACCTTGCCAGCAGG
CCACCGAATTGGCACAGTCAGTCCTTGTCCAAAAACTGGAAAATGACCAGATTGAAAATTGAGGCAGCGCTTG
GAGGGGGTCAGGCTAAAGGCTCCCCAAGCAGCAGCTGTGGAGGCAGTTACAGCAGCAGGCTCGCAGCACATACAA
ACGCTGACGGATGAGGTGACCAAGCAGGGCACGTCGCGGAACTGAAAGCACAGAAGGCAGACAAGAACAGG
TGCTGCAGAGGTGGCTAAACTCTGGATCTAAAGAAACAGTGGCTTGCTGAGGGAAACCCATTGAAACTCTA
AAGGCAAGAAGAAAAAGTATAACTCGAGTCTAGAGGCCGTTAAACCCCTGATCAGCCTGACTGTGCCCT
AGTGCAGGCCATCTGGTGTGCTGCCCTCCCCCGTGCCTCCCTTGACCCCTGGAAGGTGCCACTCCACTGTCC
CTAATAAAATGAGGAAATTGCATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGGGGGGGGGGGGGG
GCAAGGGGGAGGATTGGAAGACAATAGCAGGCATGCTGGGATGCCGTGCTATGGCTTGAGGCGAAAGA
ACCAGCTGGGCTCTAGGGGTATCCCCACCGCCCTGTAGCGGCGATTAAGCGCGGGGTGGTGGTTACGCG
CAGCGTACCGCTACATTGCCAGGCCCTAGGCCCGCTCCTTCGCTTCTCCCTTCTGCCACGTTCG
CCGGCTTCCCGTCAAGCTCTAAATCGGGGCTCCCTTAGGGTCCGATTAGTGCCTTACGGCACCTCGACCCCC
AAAAAAACTTGATTAGGGTGTGGTACGTAGTGGCATGCCCTGATAGCGTTTTCGCCCTTGACGTTGGA
GTCCACGTTCTTAATAGTGGACTCTTGTGCTTAAACTGGAACAAACACTCAACCCATTCTCGGTCTATTCTTGATT
TATAAGGGATTGCGCTTGCCTATTGGTAAAAAAATGAGCTGATTAAACAAATTAAACGCGAATTAA
TGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCATGC
AATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCATGC
GTCAGCAACCATTGCGCCCTAATCCGCCCTAATCCGCCCTAATCCGCCCTGAGCTATTCCAGAAGTGTGAGGA
ATGGCTGACTAATTCTTATTGAGGCCGAGGCCGCTCTGCCCTGAGCTATTCCAGAAGTGTGAGGA
GGCTTTGGAGGCCCTAGGCTTGTGAAAAAGCTCCGGAGCTGTATATCCATTTCGGATCTGATCAAGAGAC
AGGATGAGGATCGTTCGCTGATTGAACAAAGATGGATTGACGCCAGGTTCTCCGGCCCTGGGGAGAGGCTAT
TCGGCTATGACTGGCACAACAGACAATCGGCTGCTCTGATGCCCGTGTCCGGCTGTCAGCGCAGGGGCCCG
GTCTTTGTCAAGACCGACCTGCGCTGCCCTGAATGAACAGTCAGGAGGCCAGCGCGGCTATGTGGCTGG
CACGACGGCGTCTGCCCTGCGCAGCTGTGCTGACGTTGACTGAAGCGGGAAAGGGACTGGCTGCTATTGGCG
TGCCTGGGGCAGGATCTCTGTCATCTCACCTGCTCTGCCAGAGAAAGTATCCATTGCGTGTGCAATGCC
CTGCATACGCTTGATCCGGTACCTGCCATTGACCAAGCAGAACATCGCAGGCCAGGAGCACGTA
GGAAGCCGGTCTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGCGCAGCCGA
TCAAGGCGCGATGCCGACGGGAGGATCTCGTGAACCATGGCGATGCCCTGCTTGCGAATATCATGGTGGAA

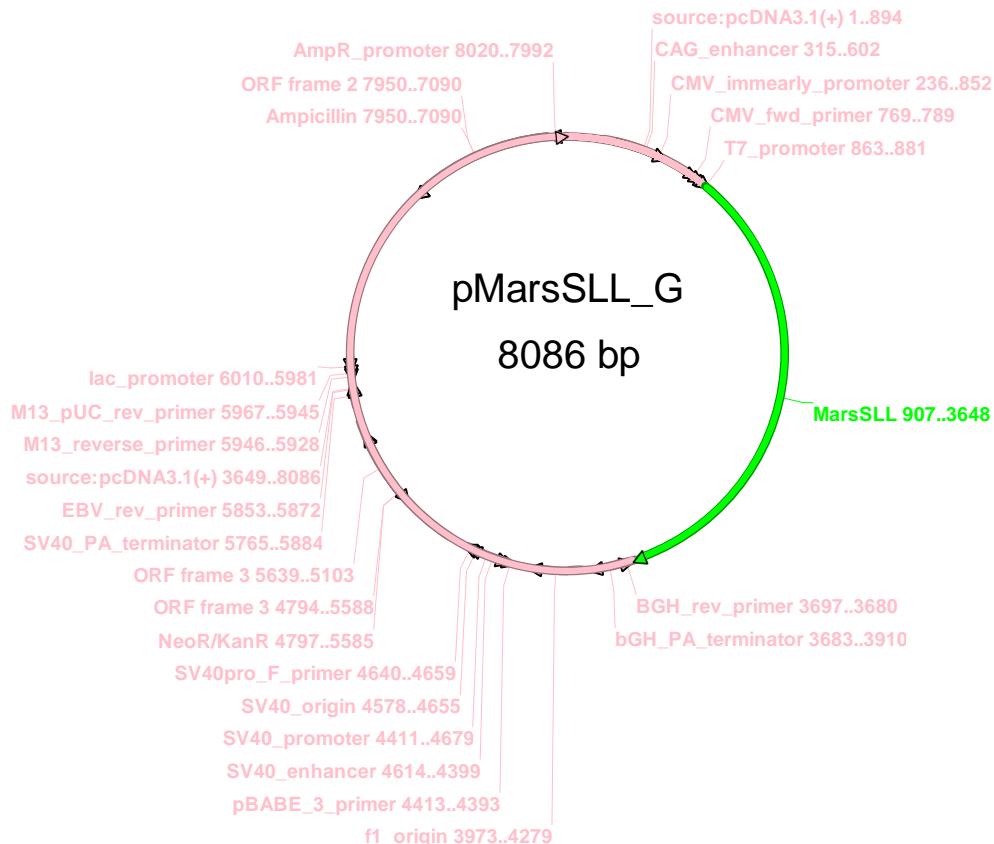
AATGGCCGCTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTAC
CCGTATATTGCTGAAGAGCTTGGCGCGAATGGCTGACCGCTTCTCGTCTTACGGTATGCCGCTCCGATT
CGCAGCGCATGCCTCTATGCCTCTTGACGAGTTCTGAGCGGGACTCTGGGTTGAAATGACCGACCAAG
CGACGCCAACCTGCCATCACGAGATTCGATTCCACCGCCGCTCTATGAAAGGGTGGCTCGGAATCGTTTC
CGGGACGCCGCTGGATGATCCTCAGCGCGGGATCTCATGCTGGAGTCTCGCCACCCAACTTGTATTGC
AGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTACAATAAGCATTTTCACTGCATTCTAGTT
GTGGTTGTCAAACACTCATCAATGTATCTTATCATGCTGTATACCGTCACCTCTAGCTAGAGCTGGCGTAATCA
TGGTCATAGCTGTTCTGTGAAATTGTATCCGCTCACAACTTACACAAACATACCGAGCCGAAAGCATAAGTG
TAAAGCCTGGGTGCCTAATGAGTGAGCTAACACATTAATTGCGTGCCTACTGCCGCTTCCAGTCGGAA
ACCTGTCGTGCCAGCTGCTTAATGAATGCCAACCGCGGGAGAGCGGTTGCGTATTGGCGCTTCCGCT
TCCTCGCTCACTGACTCGCTGCCGCTGGCTGCGCGAGCGGTATCAGCTCACTCAAAGGCCGTAATACG
GTTATCCACAGAACGAGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCCTAAAA
AGGCCGCGTTGCTGGCTTTCCATAGGCTCCGCCCTGACGAGCATCACAAATGACGCTCAAGTCAGAGG
TGGCAGAACCGACAGGACTATAAGATAACCAGGCGTTCCCCCTGGAAGCTCCCTGCGCTCCGTGAC
CCTGCCGCTACCGGATACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCCTTCTCATAGCTACGCTGTAGGT
ATCTCAGTCGGTAGGTCGTTCTCCAAGCTGGCTGTGACGAACCCCCGTTGAGCTCCCTGCGCTCCGTGAC
TTATCCGTAACTATCGTCTTGAGTCAACCGGTAAGACACGACTATGCCACTGGCAGCAGCACTGGTAACAG
GATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTTCTGAAGTGGTGGCTAACACTACGGCTACACTAGAAGAA
CAGTATTTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGAAAAGAGTTGGTAGCTTGTGATCCGGAAACAA
ACCACCGCTGGTAGCGTTTTTGCAAGCAGCAGATTACCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
GATCTTCTACGGGCTGACGCTCAGTGAACGAAAACACGTTAACGGATTGGTATGAGATTATCAAAAA
GGATCTTCACCTAGATCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCT
GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTGTTGCTCATCCATAGTGCCTGACT
CCCCGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGCCCGAGTGCTGCAATGATACCGCAGACCCAC
GCTCACCGGCTCCAGATTATCAGCAATAAACAGCCAGCGAAGGGCGAGCGCAGAAAGTGGCCTGCAACTTA
TCCGCCTCCATCCAGTCTATTAAATTGTTGCCGGAAAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGAACGT
TGGTGCCTTGCTACAGGCATGTTGTCAGCTCGTGTGTTGATGGCTTCACTCAGCTCCGGTCCAAACGAT
CAAGGCAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCCGATCGTTGTCAGAAGT
AAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCGTAAGATG
CTTTCTGTGACTGGTAGTACTCAACCAAGTCATTGAGAATAGTGTATGCGCGACCGAGTTGCTCTGCCGG
CGTCAATACGGATAATACCGGCCACATAGCAGAACTTAAAAGTGTATCATTGAAAACGTTCTCGGGCGA
AAACTCTCAAGGATCTACCGCTGGAGATCCAGTTGATGTAACCCACTCGTCACCCACTGATCTCAGCATC
TTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAAAGGAAATAAGGGCAGAC
GGAAATGTTGAATACTCATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGA
TACATATTGAATGTATTTAGAAAATAACAAATAGGGTCCGCGACATTCCCGAAAAGTGCCACCTGACGT
C

Protein Sequence

MAHHHHHHMRLFVSEGPSGLPVAAAARARGRAELLIStVGPEECVPFLTRPKVPVLQLDSGNYLFSAICRYF
FLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVVQGKKGEDILGPLRRVLTHIDHSLSRQNCPFLAGTESLADI
VLWGALYPLLQDPAYLPEELGALQSWFQTLSTQEPCQRAAETVLKQQGVLAIRLYLQKQPQPPPEGRTVSNELE
EEEELATLSEEDIVTAVAWEKGLESPLPLKLQQHPVLPVPGERNVLITSAGPYVNNVPHLGNIIGCVLSADVAFARYC
RLRQWNTLYLCGTDEYGTATETKAMEEGLTPREICDKYHAIHADIRWFGISFDTFGRRTTPQQTKITQDIFQRLLT
RGFVLRDTVEQLRCERCARFLADRVEGVCPFCGYEEARGDQCDCRGKLINAIELKKPQCKICRSCPVRSSQHLFL
DLPKLEKRLEDWLGKTVPGSDWTPNARFIIRSWLRLGKPRCITRDLKWGTPVPLEGFEDKVYVWFDATIGYVSIT
ANYTDQWEKKWNPEQVDLYQFMAKDNVPFHGLVFPCSVLGAEDNYTLVKHIIMATEYLNEYDGKF SKSRIGVFGDM
AKDTGIPADIWRFYLLYIRPEGQDSAFSWTLLIKNNSELLNNLGNFINRAGMFVSKFFGGCVPEMALTPDDRLVA
HVSWELOHYHQLLEKVRIRDALRSILTISRHNQYIQVNEPWKRIKGGE DRQRA GTVTGMAVNMAALLSVMILQPYM
PTVSSTIQTQLQLPPAACRILATSFIGTLPAGHRIGTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTAA
GSQHIQTLTDEVTKQGNVVRELKAQKADKNQVAEVAKLLDKLQLALAEGKPIETPKGKKK-

Figure S3. The pMaRSSLL_G vector for expression of SLL-MmMetRS under CMV promoter control.

Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



```

GACGGATCGGGAGATCTCCGATCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGATAGTTAACCCAG
TATCTGCTCCCTGCTTGTGTTGGAGGTGCGCTGAGTAGTGCGCGAGCAAAATTAAAGCTACAACAAGGCAAGGCTT
GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCCTTGGCTGCTCGCGATGTACGGGCCAGATAACGC
GTTGACATTGATTATTGACTAGTTATTAAAGTAATCAATTACGGGTCATTAGTCATAGCCCATAATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTTTACGGTAAACTGCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCGCTGGCAT
TATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTG
ACGTCAATGGGAGTTGTTGGCACCATAACCGGACTTTCAAATGTCGTAACAACCTCCGCCCCATTGACG
CAAATGGGCGGTAGGCGTGTACGGTGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCCACTGCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGC[GCCACCATGGCCCAACATCACC]AT
[CACCAT]ATGAGACTGTCGTGAGCGAGGGTCCCCGGGAGCCTGCCGTGCTGGCTGGCCGGAGGGCCGGGG
[TCGGGCGGAGCTGCTCATCAGCACCGTAGGCCCGAAGAGTGTGGTACCATCTTACCGGCCCTAAGGTCCCTG
TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGCATCTGCCATATTCTGTGCTGGCTGG
GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGGAGGCAACAGAACTGCAGCCAGTTCTGTCTGCTGCCCTACA
CTGTCTAGTGGTTCAAGGCAAGAAAGGGAGATATACTTGGCCCACTTCGAGAGTCTGACTCACATTGATCACA
GCCTTGAGTCGTAAAATGTCCTTCCGGCTGGGACACAGAACTCTAGCTGACATTGTTGTGGGAGCACTG
TATCCTTACTGCAAGACCCAGCTTACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAGTAC

```

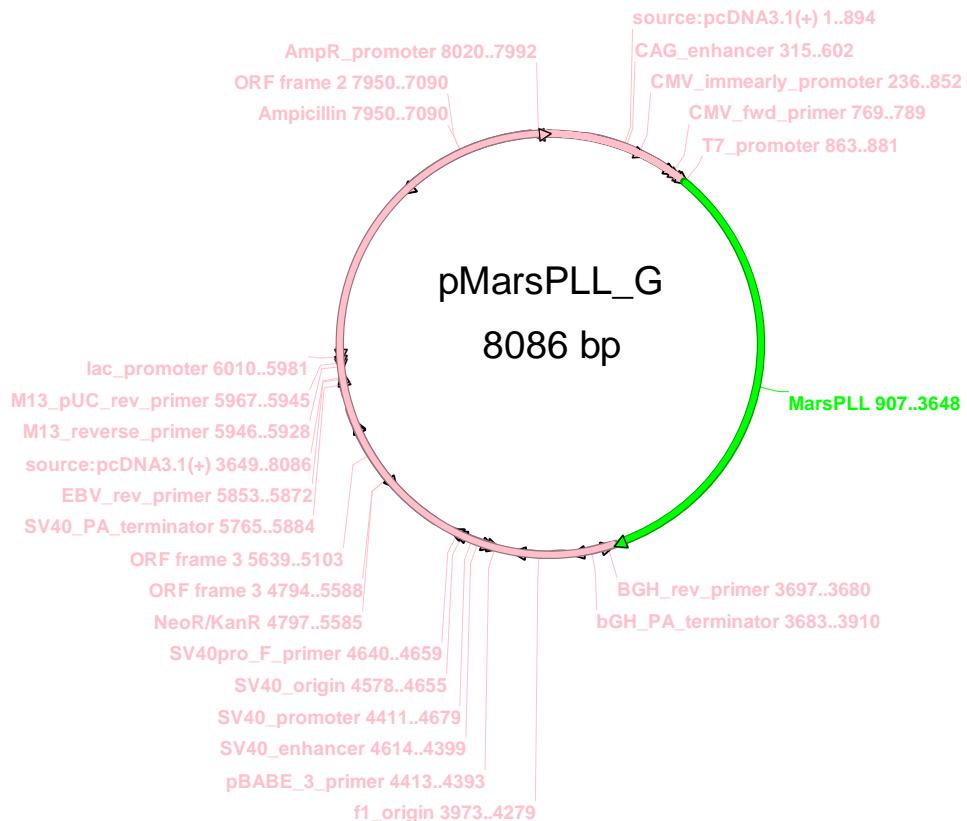
CCAGGAACCGTGTAGCGAGCTGCAGAGACGGTGTAAACAGCAGGGTGTCTGGCACTTCGTCTGTACCTCCAGAACAGCCACAGCCTGAGGGAGAAGACTGTCAACGAGCTGGAGGAAGAGGAACGGCTACCTTGCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGAGAAGGGTCTGGAAAGCCTGCCCTCGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCTGGAGAGAGGAATGTTCTCATCACCACTGCCCTCCCCCTATGTCAACAATGTCC
CCCACCTGGAAACATCATTGGCTGTGCTCAGTGCTGATGTTCTGCAGGATTGTCGCTTCGGCTGGAGAAGGGTCTGGAAAGCCTGCCCTCGCTAAAGCTCCA
ACCCCTATCTGTGTGGTACAGATGAGTATGGTACTGCAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GGAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTCCGGATATCGTGTGACATCTTCGGGC
GCACTACCCTCAGCAGACCAAAATCACCCAGGACATCTCCAGAGGTTGCTGACCCGGGGTTGTGCTGCCCTGGCTGG
GATACTGTGGAGCAGCTCGGTGTGAGCGGTGTGACGTTCTGGCTGACCGTTGTGGAGGGTGTGTCCT
CTGTGGCTATGAAGAGGCCAGGTGACAGTGTGACAGGTGTGGCAAGCTCATCAATGCCATTGAGCTCAAGAAC
CACAGTCAAATCTGCCCTGCCCTGTGGTGAGGTCCACAGCACCTGTTCTAGACTTGCTTAAGTTGGAA
AAGCGTCTGGAGGACTGGTTGGGAAGACAGTGCTGGCAGTGACTGGACACCCATGCCAGGTTATTACGTT
CTGGCTTCGAGATGGCTCAAGCCACGATGCATCACAGAGACCTCAAATGGGAACGCCCTGTGCCCTTGGAAAGGT
TTGAGGACAAGGTATTTACGTCTGGTTGATGCTACTATTGGCCTGGTGTCCATCACAGCCAACCTACACAGACCAA
TGGGAGAAATGGTGAAGAACCCAGAACACAAGTGGACCTTACCAAGTTCATGGCAAAGACAATGTTCCCTCCTGG
CTTGGTCTTCCGTGTTCAAGTCTAGGAGCTGAGGACAACCTACACCCCTGGTCAAGCACATCATTGCTACAGAGTACC
TGAACATAGGATGGAAATTCTCTAAGAGCCGGGATAGGAGTGTGGAGACATGGCCAAGGATACAGGAATC
CCTGCTGACATCTGGCATTCTATCTGCTATACATTGGCCTGAGGGCCAGGACAGTGCTTCTCCTGGACAGACTT
GTTGATAAAAACAATTCTGAGCTGCTCAACAAACCTGGCAACTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTTGGCGGTTGTCGCTGAGATGGCGCTAACCCCTGATGACAGACGCCCTGGTGGCCCATGTCCTTGGGAACTC
CAGCACTATCACCAAGCTGGAAAAGGTTGGATCCGGATCCGGATGCCCTGGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGGAAACGGATTAAAGGTGGTGGAGATGGACAGGAGCGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGGCTGCCCTGCTGTCATGCTGCCAGCCATACATGCCAACAGTCAGCTTAC
ATCCAGACCCAGCTGCAGCTCCACCTGCAGCCTGCCCATCCTTGCCACAAGCTCATTGTCACCTGCCAGCAGG
CCACCGAATTGGCACAGTCAGTCTTGTCCAAAAACTGGAAAATGACCAGATTGAAAATTGAGGCAGCGCTTGG
GAGGGGGTCAAGCTAAAGCTCCCCAAGCCAGCAGCTGTGGAGGCAGTTACAGCAGCAGGCTCGCAGCACATACAA
ACGCTGACGGATGAGGTGACCAAGCAGGGCAACGTCGCTCCGGAACTGAAAGCACAGAAGGCAGACAAGAAC
TGCTGAGGGTGGCTAAACTCTGGATCTAAAGAAACAGTGTGGCTTGCTGAGGGAAACCCATTGAAACTCCTA
AAGGCAAGAAGAAAAAGTGTATACTCGAGCTAGAGGGCCCGTTAAACCCGCTGATCAGCTCGACTGTGCTTCT
AGTGCAGCCATCTGGTTGCCCTCCCCCGTGCCTCCTTGACCTGGAAAGGTGCCACTCCACTGTCTTCT
CTAATAAAATGAGGAAATTGCATCGCATTGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGTGGGCAGGACA
GCAAGGGGGAGGATTGGAAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTCATGGCTCTGAGGGTGGTTACCG
ACCAGCTGGGCTCTAGGGGTATCCCCACCGCCCTGTAGCGCGCATTAGCGCCGGGTGTGGTGGTTACCG
CAGCGTACCGCTACATTGCCAGCGCCCTAGCGCCGCTCCTTCGCTTCTCCCTTCTCGCCACGTTG
CCGGCTTCCCCGTCAAGCTAAATGGGGCTCCCTTAGGGTCCGATTAGTGTGCTTACGGCACCTCGACCCCC
AAAAAAACTGATTAGGTGATGGTCACGTAGTGGCCATGCCCTGATAGCGTTTCGCCCCTTGACGTTGGA
GTCCACGTTCTTAATAGTGGACTCTGGTCAAAGTGGAAACAACACTCAACCCATTCTCGGTCTATTCTTGATT
TATAAGGGATTTGCCGATTGCGCTATTGGTAAAAAATGAGCTGATTAAACAAAATTAAACGCAATTAA
TGTGGATGTGTCAAGTGGAGGCTCCAGGCTCCCCAGCAGGAGAGTATGCAAAGCATGCATCT
AATTAGTCAGCAACCAGGTGGAAAGTCCCCAGCAGCTCCAGCAGGAGAGTATGCAAAGCATGCATCTCAATT
GTCAGCAACCCTAGTCCGCCCTAACCGCCCATCCGCCCTAACCTCCGCCAGTCCGCCATTCTCGGCC
ATGGCTGACTAATTCTTGATGAGGGCGAGGCGCTCTGGCTCTGAGCTATTCCAGAAGTAGTGGAGG
GGCTTTGGAGGCCTAGGCTTGTGAAAAAGCTCCGGAGCTGTATATCCATTGAGCTGATCAAGAGAC
AGGATGAGGATGTTCGCATGATTGAACAAGATGGATTGCACGAGGTTCTCCGGCGCTTGGGTGGAGGAGGCTAT
TCGGCTATGACTGGCACACAGACAATCGGCTGCTGTGATGCCCGTGTGTCAGCGCAGGGCGCC
GTTCTTTGTCAAGACCGACCTGTCGGTGCCTGAATGAACTGCAAGTAGCAGGAGGAGCGCAGGGCTATGTGGCTGG
CACGACGGCGTCTGCGAGCTGTGCTGACGTTGTCAGTGAAGCGGAAGGGACTGGCTGCTATTGGCGAAG
TGCCGGGAGGATCTCTGTCACTCACCTGCTCTGCCAGAGAAAGTATCCATCATGGCTGATGCAATGCGCG
CTGCATACGCTTGTGATCCGGTACCTGCCATTGACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGAT

GGAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCCACTGTCGCCAGGC
TCAAGGCGCGCATGCCGACGGCGAGGATCTCGTGTGACCATGGCATGCCTGCTTGCAGAATATCATGGTGGAA
AATGGCGCTTTCTGGATTATCGACTGTGGCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTAC
CCGTGATATTGCTGAAGAGCTTGGCGGGAATGGCTGACCGCTTCCCTCGTCTTACGGTATCGCGCTCCCGATT
CGCAGCGCATGCCCTCATCGCCTTCTTGACGAGTTCTCTGAGCGGGACTCTGGGGTCGAAATGACCGACCAAG
CGACGCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCTTCTATGAAAGGTTGGCTCGGAATGTTTC
CGGGACGCCGGCTGGATGATCCTCCAGCCGGGATCTCATGCTGGAGTTCTCCGCCACCCAACCTGTTTATTGC
AGCTTATAATGGTACAAATAAGCAATAGCATCACAAATTCAACAAATAAGCATTTTTCACTGCATTCTAGTT
GTGGTTGTCAAACTCATCAATGTATCTTATCATGTCGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCA
TGGTCATAGCTGTTCTGTGTGAAATTGTTATCCGCTCACAAATTCCACACAACATACGAGCCGGAAGCATAAAGTG
TAAAGCCTGGGGTGCCTAATGAGTGAGCTAACATCACATTAAATTGCGTTGCCTCACTGCCGCTTCCAGTCGGAA
ACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACCGCGGGAGAGGGCGTTGCGTATTGGCGCTTCCGCT
TCCTCGCTCACTGACTCGCTCGCTCGTGTGCGAGCGGTATCAGCTCACTCAAAGGCCGTAATACG
GTTATCCACAGAACATCAGGGATAACCGCAGGAAAGAACATGTGAGCAGAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
AGGCCCGTGTGGCTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGG
TGGCGAAACCGACAGGACTATAAGATACCAGGCCTTCCCTGGAAGCTCCCTCGTGCCTCCGTGAC
CCTGCCCTACGGATACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGT
ATCTCAGTTGGTGTAGGCTTCGCTCAAGCTGGCTGTGACGAAACCCCCGTTCAAGCCGACCCGCTGCGCC
TTATCCGTAACTATCGTCTGAGTCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAG
GATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAAGTGGTGGCTAACTACGGCTACACTAGAAGAA
CACTGGTATCTCGCTCTGTAAGCCAGTTACCTCGAAAAGAGTTGGTAGCTCTGATCCGAAACAA
ACCACCGCTGGTAGCGGTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTAAGAAGATCCTT
GATCTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTACGTTAAGGGATTGGTCAAGGATTATCAA
GGATCTCACCTAGATCCTTAAATTAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCT
GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTGCTTCATCCAGTTGCTGACT
CCCCCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCAGACCCAC
GCTCACCGGCTCCAGATTTACAGCAATAAACCGCAGCCAGCGAAGGGCGAGCGCAGAAGTGGTCTGCAACTTAA
TCCGCCCTCATCCAGTCTATTAAATTGTTGCCGGAAAGCTAGAGTAAGTAGTTGCGCAGTTAATAGTTGCGAACGTT
TGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGGTATGGCTTCATTCACTCCGGTCCCAACGAT
CAAGGCGAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCCGATCGTGTGAGAAGT
AAGTTGCCCGCAGTGTATCACTCATGGTTATGGCAGCAGTCATAATTCTTACTGTCATGCCATCCGTAAGGAT
CTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCAGCGAGTTGCTTGGCCGG
CGTCAATACGGGATAATACCGGCCACATAGCAGAACCTTAAAGTGTGATCATGGAAAAGCTTCCGGGCGA
AAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGGTTGCGATGTAACCCACTCGTGCACCCACTGATCTCAGCATC
TTTACTTCAACCGCGTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAAAAAAGGAAATAAGGGCGACAC
GGAAATGTTGAATACTCATACTCTCCCTTTCAATATTGAGCATTATCAGGGTTATGTCATGAGCGGA
TACATATTGAAATGTATTAGAAAATAACAAATAGGGGTTCCGCGCACATTCCCCGAAAAGTGCCACCTGACGT
C

MAHHHHHHMRLFVSEGPGSLPVAAAARARGRAELLISTVGPEECVVPFLTRPKVPVLQLDSGNYLFASASAICRYF
FLLCGWEQDDLTNQWLEWEATELQPVLSSAALHCLVVQGKKGEDILGPLRRVLTHIDHSLSRQNCPFLAGDTESLADI
VLWGALYPLLQDPAYLPPEELGALQSWFQTLSTQEPCQRRAETVLKQQGVLAIRLYLQKQPQFQPPPPEGRTVSNELE
EEELATLSEEDIVTAVAWEKGLESPLKLQQHPVLPVPGERNVLITSASPYVNVPHLGNIIGCVLSADVFARYC
RLRQWNLTLYLCGTDEYGATETKAMEEGLTPREICDKYHAIHADIYRWFGISFDTFGRTTTPQQTKITQDIFQRLLT
RGFVLRDTVEQLRCERCARFLADRFVEGVCFCGYEEARGDQCDCRGKLINAIELKKPQCKICRSCPVRSSQHFL
DLPKLEKRLEDWLGKTVPGSDWTPNARFIIRSWL RDGLKPRCITRDLKWGTVPLEGFEDKVFYVWF DATIGLVSI
ANYTDQWEKWWKNEQVDLYQFMKDNPFLGLVFPCSVLGAE NYTLVKHIIATEYLNYE DGKFSKSRSIGVFGDM
AKDTGIPADIWRFYLLYIRPEGQDSAFTWDLLIKNNSELLNLGNFINRAGMFVSKFFGGCVPEMALTPDDRRLVA
HVSWEQHYZHQLLEKVRIRDALRSILTISRHGNQYIQVNEPWKRIGGEMDRQRAGTVTGMAVNMAALLSVMLQPYM
PTVSSTIQTQLQLPPAACRILATSFIGTLPAGHRIGTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTAA
GSOHIQTLTDEVTKOGNVVRELKAOKADKNovaAEVAKL LDKKOLALAEGKPIETPKGKKK--

Figure S4. The pMaRSPLL_G vector for expression of PLL-MmMetRS under CMV promoter control.

Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



GACGGATCGGGAGATCTCCGATCCCTATGGTGCACCTCACTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAG
TATCTGCTCCCTGCTTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAGGCAGGCTT
GACCGACAATTGCATGAAGAACATCTGCTTAGGGTTAGCGCTGCTCGCGATGTACGGGCCAGATATACGC
GTTGACATTGATTATTGACTAGTTATTAAAGTAATCAATTACGGGTCATTAGTCATAGCCCATAATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACCCAATAGGGACTTCCATTGACGTCAATGGGTTGGAGTATTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCGCTGGCAT
TATGCCCAAGTACATGACCTTATGGACTTCCATTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
GATGCGGTTTGGCAGTACATCAATGGCGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTG
ACGTCAATGGGAGTTGGTACGGTGGAGGTCTATATAAGCAGAGCTCTGGCTAAGTACAGAACCACGTCTTA
CAAATGGCGGTAGGCGTGTACGGTGGAGGTCTATATAAGCAGAGCTCTGGCTAAGTACAGAACCACGTCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGC **GCCACCA**TGGCCACCATCACC
CACC**ATGAGACTGTCGTGAGCGAGGGT**CCCCGGGAGCCTGCCGTGCTGGCTGCCGCCGAGGGCCGGGG
**TCGGCGGAGCTGCTCATCAGCACCGTAGGGCCCGAAGAGTGTGTTACCATTCCTACCCGGCTAACGGTCCCTG
TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGCATCTGCCGATATT**TTCTGTTATGTGGCTGG
GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGAGGCAACAGAACTGCAGCCAGTTCTGCTGCCCTACA
CTGTCTAGTGGTCAAGGCAAGAAAGGGGAAGATATACTTGGCCACTTCGAGAGTCTGACTCACATTGATCACA
GCTTGAGTCGTCAAAACTGTCCTTCCGGTGGGACACAGAATCTCTAGCTGACATTGTTGTGGGAGCACTG

TATCCTTACTGCAAGACCCAGCTTACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAGTAC
CCAGGAACCGTGTCAAGCAGAGACGGTGCTAAAACAGCAGGGTGCCTGGCACCTCGTCTGTACCTCCAGA
AACAGCCACAGCCTCAGCCCCCGCCTCCTGAGGGAGAACTGTCAAGCAACGAGCTGGAGGAAGAGGAACGGTACCC
TTGTCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGAGAAGGGCTGAAAGCCTGCCTCCGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCTGGAGAGAGGAATGTTCTCATCACCACTGCCACCCCTATGTCAACAATGTCC
CCCACCTTGAAACATCATTGGCTGTGCTCAGTGTGATGTCTTGCAAGGTATTGTGCGCTTCGCCAGTGGAAAT
ACCCCTATCTGTGTGTAACAGATGAGTATGGTACTGCGACAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GGAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTCCGCATATCGTTGATACTTCCGGC
GCACTACCACCTCAGCAGACCAAAATCACCCAGGACATCTTCCAGAGGTGCTGACCCGGGGTTGTGCTGCC
GATACTGTGGAGCAGCTTCGGTGTGAGCGGTGTCACGTTCTGGCTGACCGCTTGAGGGTGTGTGTCCTT
CTGTGGCTATGAAGAGGCCGAGGTGACCACTGTGACAGGTGCAAGCTCATCAATGCCATTGAGCTCAAGAAC
CACAGTGCACAAATCTGCCGCTCTGCCCTGTGGTGAGGTCTCACAGCACCTGTTCTAGACTTGCTAACGGAA
AAGCGTCTGGAGGACTGGTGGGAAGACAGTCCTGGCAGTGACTGGACACCCAAATGCCAGGTTCATTACGTTC
CTGGCTTCGAGATGGCCTCAAGCCACGATGCATCACCAAGAGACCTCAAATGGGAACGCCGTGCCCCGG
TTGAGGACAAGGTATTTACGTCTGGTTGATGCTACTATTGGCTGGTCCATCACAGCAACTACACAGACCAA
TGGGAGAAATGGTGGAAAGAACCCAGAACACAAGTGGACCTTACCAAGTTCATGCCAAAGACAATGTTCCCTCTGG
CTTGGTCTTCCGTGTTCACTGCTTAGGAGCTGAGGACAACTAACCCCTGGTCAAGCACATCATTGCTACAGAGTAC
TGAACATGAGGATGGAAATTCTCTAACAGGCCGGGATAGGAGTGGTGGAGACATGCCAGGATACAGGAATC
CCTGCTGACATCTGGCATTCTATCTGCTATACATTCCGGCTGAGGGCCAGGACAGTGCTTCTCTGGACAGACTT
GTTGATTAACAAATTCTGAGCTGCTCAACAAACCTGGCAACTTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTTGGCGTTGTGCGCTGAGATGGCGCTAACCCCTGATGACAGACGCCGGTGGCCCATGTCCTTGGAAACTC
CAGCACTATCACCAGCTGTTGGAAAAGGTTGGATCCGGATGCCCTGGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGAAACGGATTAAAGGTGGTGGAGATGGACAGGCAGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGGCTGCCCTGCTGTCATGCTGCAGCCATACATGCCACAGTCAGCTTAC
ATCCAGACCCAGCTGCAGCTCCCACCTGCAGCCTGCCATCCTGCCACAAGCTTCAATTGACCTTGCCAGCAGG
CCACCGAATTGGCACAGTCAGTCCTTGTCCAAAAACTGAAAGACAGGATTGAAATTTGAGGCAGCGCTT
GAGGGGGTCAGGCTAAAGGCTCCCCAAGCCAGCAGCTGAGGAGGAGTTACAGCAGCAGGCTCGCAGCACATA
ACGCTGACGGATGAGGTGACCAAGCAGGGCAACGTCGCTGGGAACGTGAAAGCACAGAAGGAGACAAGAAC
TGCTGCAGAGGTGGCTAAACTCTGGATCTAAAGAAACAGTTGGCTTGGCTGAGGGAAACCCATTGAAACTC
AAGGCAAGAAGAAAAGTGTATAACTCGAGTCTAGAGGCCGTTAAACCCGCTGATCACCTGACTGTGCC
AGTTGCCAGCCATCTGTTGTTGCCCTCCCCCGTGCCTCCCTGACCCTGGAAGGTGCCACTCCACTGT
CTAATAAAATGAGGAAATTGCATCGCATTGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGCAGGACA
GCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTCTATGGCTCTGAGGCGGAAAGA
ACCAAGCTGGGCTCTAGGGGATCCCCACGCCCTGAGCGCCCTCCTTCGCTTCTCCCTTCTGCCACGTT
CAGCGTACCGCTACACTGCCAGGCCCTAGGCCGCTCCTTCGCTTCTCCCTTCTGCCACGTT
CCGGCTTCCCGTCAAGCTCTAAATGGGGCTCCCTTAGGGTCCGATTAGTGCCTTACGGCACCTCGAC
AAAAAACTTGATTAGGGTGTGGTACGTAGTGGCCATGCCCTGATAGACGGTTTCGCCCTTGACGTT
GTCACGTTCTTAATAGTGGACTCTGTTCAAAGTGGAAACAACACTCAACCCATTCTGGCTATTCT
TATAAGGGATTTGCCATTGCGCTATTGGTAAAAAAATGAGCTGATTTAACAAAATTAA
TACCGCAATTTC
TGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCAGCAGCAGAAGTATGCAAAGCAT
GCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGCAGGAGCTTGTATATCCATTTCGGATCTGAT
ATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGCAGGAGCTTGTATATCCATTTCGG
GTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGCAGGAGCTTGTATATCCATTTCGG
ATGGCTGACTAATTTCATTGAGGAGGCCGAGGCCGCTCTGCCCTGAGCTATTCCAGAAGTAGTGAG
GGCTTTGGAGGCCAGGCTAGGCTTTGCAAAAGCTCCGGAGCAGCTTGTATATCCATTTCGG
AGGATGAGGATCGTTGCATGATTGAAACAAGATGGATTGCAAGCAGGTTCTCCGGCCGCTTGG
TCGGCTATGACTGGCACAACAGACAATCGCCTGCTGATGCCCGTGTCCGGCTGTCAGCG
GTCAGGGCTTGTCAAGACCGACCTGTCGGTCCGCTGAATGAACTGCAGGAGGCCGAGGCC
CACGACGGCGTCTTGCAGCTGTCAGCTGTCAGCTGACGGTCACTGAAGC
GGAGGACTGGCTATTGGCTGCTATTGGCGAAG
TGCCGGGGCAGGATCTCCTGTCATCTCACCTGCTGCCGAGAAAGTATCC
CATGGCTGATGCAATGCCGG

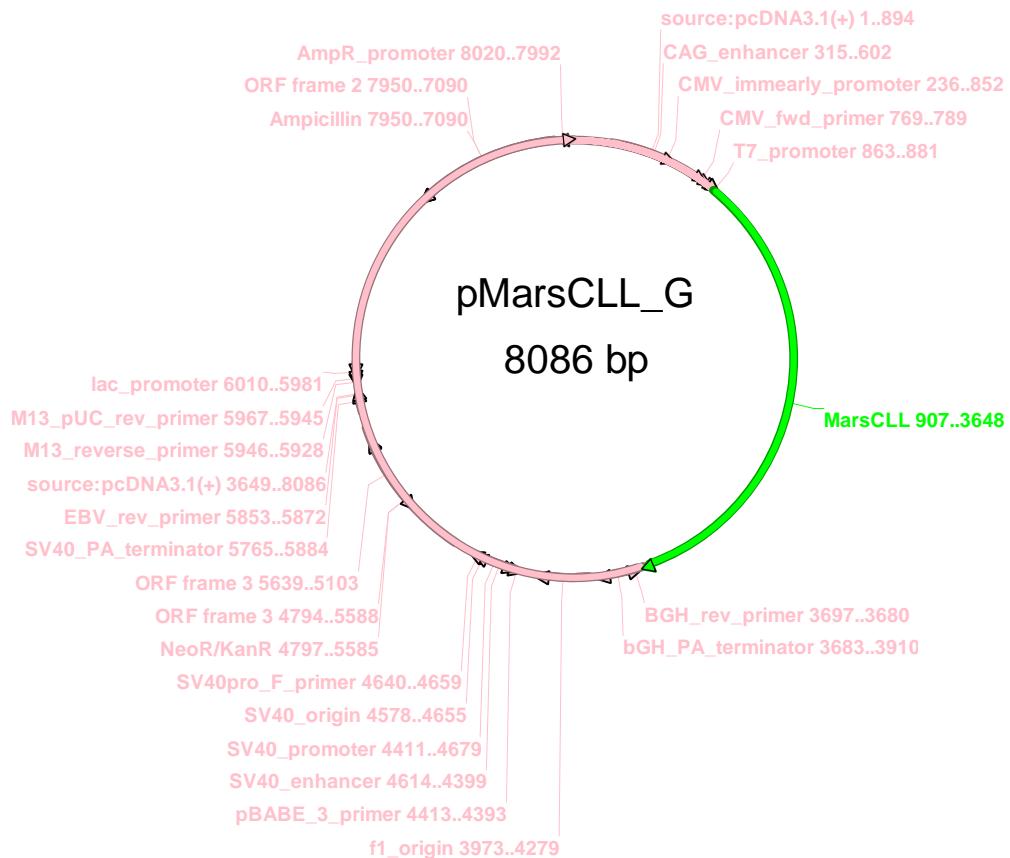
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAACATCGCATCGAGCAGCACGTACTCGGAT
GGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCGAACCTGTTGCCAGGC
TCAAGGCGCGATGCCGACGGCAGGATCTCGTCGTGACCCATGGCGATGCCGCTTACGGTATGCCGCTCCGATT
AATGGCCGCTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTAC
CCGTGATATTGCTGAAGAGCTTGGCGGAATGGCTGACCGCTTCTCGTCTTACGGTATGCCGCTCCGATT
CGCAGCGCATCGCCTCTATCGCCTCTTGACGAGTTCTGAGCGGGACTCTGGGGTCGAAATGACCGACCAAG
CGACGCCAACCTGCCATCACGAGATTTGATTCCACCGCCGCTTCTATGAAAGGGTGGCTCGGAATCGTTTC
CGGGACGCCGGCTGGATGATCCTCCAGCGGGGATCTCATGCTGGAGTTCTGCCAACCCAACTGTTATTGC
AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTCACTGCATTCTAGTT
GTGGTTGTCAAAATCTCATCAATGTATCTTATCATGCTGTATACCGTCACCTCTAGCTAGAGCTGGCGTAATCA
TGGTCATAGCTGTTCTGTGAAATTGTTATCCGCTCACAATCCACACAAACATACCGAGGCCAACGATAAGTG
TAAAGCCTGGGTGCTTAATGAGTGAGCTAACACATTAATTGCGTTCGCTCACTGCCGCTTCCAGTCGGAA
ACCTGTCGTGCCAGCTGCATTAATGAATCGCCAACCGCGGGAGAGGCAGTTGCGTATTGGCGCTTCCGCT
TCCTCGCTCACTGACTCGCTCGCTCGGCTGCGCGAGCGGTATCAGCTCACTCAAAGCGGTAAATACG
GTTATCCACAGAACGAGGGATAACGAGGAAAGAACATGTGAGCAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
AGGCCGCGTTGCTGGCTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAATGACGCTCAAGTCAGAGG
TGGCGAAACCCGACAGGACTATAAAGATACCGCGTTCCGAGCTCCCTCGTGCCTCTGTTCCGAC
CCTGCCGCTACCGATAACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGT
ATCTCAGTTCGGTGTAGGTCGCTCCAAGCTGGCTGTGACGAACCCCCCGTTCAAGCCACTGGCAGCAGCACTGGTAAACAG
TTATCCGTAACTATCGTCTTGAGTCCAACCGGTAAGACACGACTTATGCCACTGGCAGCAGCACTGGTAAACAG
GATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAACACTACGGTACACTAGAAGAA
CAGTATTTGGTATCTCGCCTGCTGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGCTTGTATCCGGCAAACAA
ACCACCGCTGGTAGCGTTTTTGCAAGCAGCAGATTACCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
GATCTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAACGGATTGGTATGAGATTATCAAAAA
GGATCTTCACCTAGATCTTAAATTAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCT
GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCAGTGTCTATTGTTGTTGCTCATCCATAGTGCCTGACT
CCCCGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGCCCACTGCTGCAATGATACCGCAGACCCAC
GCTCACGGCTCCAGATTATCAGCAATAAACAGCCAGCCGAAGGGCCAGCGCAGAACGGTCTGCAACTTTA
TCGCCCTCCATCCAGTCTATTAAATTGTCGGGAAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGCAACGT
TGGGCCATTGCTACAGGCATCGGGTGTACGCTCGTCTGGTATGGCTTCATTAGCTCCGGTCCAAACGAT
CAAGGCAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCTCGATCGTTGCTCAGAAGT
AAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCCGTAAGATG
CTTTCTGTGACTGGTGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGCGACCGAGTTGCTCTGGCCGG
CGTCAATACGGATAATACCGGCCACATAGCAGAACCTAAAGTGTCTCATTTGAAAAGTCTTGGGGCGA
AAACTCTCAAGGATCTACCGCTTGAGATCCAGTTGATGTAACCCACTCGTCACCAACTGATCTCAGCATC
TTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCAGAC
GGAAATGTTGAATACTCATACTCTCCCTTTCAATATTATGAAGCATTTATCAGGGTATTGTCATGAGCGGA
TACATATTGAATGTATTAGAAAATAACAAATAGGGTCCCGCACATTCCCCGAAAAGTGCACCTGACGT
C

MAHHHHHHMRLFVSESPGSLPVAAAARARGRAELLIISTVGPEECVVPFLTRPKVPVLQLDSGNYLF
SASAICRYF
FLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVVQGKKGEDIILGPLRRVLTHIDHSLSRQNCPFLAGDTE
SLADI
VILWGALYPLLQDPAYLPEELGALQSWFQTLSQEPCQRAAETV
LKQQGVLA
RLYLQKQPQPPPEGR
TVSNELE
EEELATLSEEDIVTA
VA
AAWEKGLES
LPPLKLQQHPVLPVPGERNVLITSAPPVNNVPH
LGN
IIGCVLSADVFARYC
RLRQWNTLYLCGTDEYGTATE
TKAMEEGLT
PRE
ICDKYHAI
HADIYRWFGIS
FDT
FGRT
TT
PQ
QTKIT
QD
IF
QR
LLT
RGFVLRDTVEQLRCER
CARFLADR
FVE
GCP
FCGY
EE
ARGDQC
DRCG
KLIN
IA
IEL
KKP
QCK
ICR
SCP
V
RSS
QH
FL
DLP
KLE
KR
LED
WL
GK
TV
PG
SD
WT
PN
AR
FI
IIR
S
WL
RD
GL
K
P
RC
I
TR
DL
K
W
G
T
P
V
LEG
FED
KV
FY
V
WF
D
AT
I
GL
VS
IT
ANY
TD
Q
WE
K
WW
KN
P
E
Q
V
D
IY
Q
F
MA
K
DN
V
P
F
L
G
L
V
F
P
C
S
V
L
G
A
E
D
N
Y
T
L
V
K
H
I
I
A
T
E
Y
L
N
Y
E
D
G
K
F
S
K
R
G
I
G
V
F
G
D
M
AK
DT
G
I
P
A
D
I
W
R
F
Y
L
L
Y
I
R
P
E
G
Q
D
S
A
F
S
W
T
D
L
L
I
K
N
N
S
E
L
N
N
L
G
N
F
I
N
R
A
G
M
F
V
S
K
F
F
G
C
V
P
E
M
A
L
T
P
D
D
R
R
L
V
A
H
V
S
W
E
L
Q
H
Y
H
Q
L
L
E
K
V
R
I
R
D
A
L
R
S
I
L
T
I
S
R
H
G
N
Q
Y
I
Q
V
N
E
P
W
K
R
I
K
G
G
E
M
D
R
Q
R
A
G
T
V
T
G
M
A
V
N
M
A
L
L
S
V
M
L
Q
P
Y
M

PTVSSTIQTQLQLPPAACRILATSFIGTLPAHGHRIGTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTAA
GSQHIQTLTDEVTKQGNVRELKAQKADKNQVAEVAKLDDLKKQLALAEGKPIETPKGKKK--

Figure S5. The pMaRSCLL_G vector for expression of CLL-MmMetRS under CMV promoter control.

Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



GACGGATCGGGAGATCTCCGATCCCTATGGTCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAG
TATCTGCTCCCTGCTTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAGGCAAGGCTT
GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGCGCTGCTCGCGATGTACGGGCAGATATACGC
GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATAATGGAGTTC
CGCGTTACATAACTACGGTAAATGGCCCGCTGGCTGACGCCAACGACCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGTGGAGTATTACGGTAAACTGCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
TATGCCAGTACATGACCTTATGGACTTCCATTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTG
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTG
ACGTCAATGGAGTTGGCACCATAACCGGACTTCCAAATGTCGTAACAACCGCCATTGACG
CAAATGGCGGTAGGCGTGTACGGTGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCACTGCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGC **GCCACC** ATGGCCACCATCACCATTG
CACCATT**ATGAGACTGTCGTGAGCGAGGGTCCCCGGGAGCCTGCCCCTGCTGGCTGCCGCGAGGGCCGGGG**
TCGGGCGGAGCTGCTCATCAGCACCGTAGGCCCCGAAGAGTGTGGTACCATTCCTACCCGGCTAAGGTCCCTG
TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGCAATCTGCCGATATTTTCTGTTATGTGGCTGG

GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGGAGGCAACAGAACTGCAGCCAGTTCTGCTGCCCTACA
CTGTCTAGTGGTTCAAGGCAAGAAAGGGGAAGATATACTTGGCCACTTCGGAGAGTCTGACTCACATTGATCACA
GCTTGAGTCGTCAAAACTGTCCTTCTGGCTGGGACACAGAACTCTAGCTGACATTGTTGTGGGAGCACTG
TATCCTTACTGCAAGACCCAGCTAACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAGTAC
CCAGGAACCGTGTCAAGCGAGCTGCAGAGACGGTCTAAAACAGCAGGGTGTCTGGCACTTCGTCTGTACCTCCAGA
AACAGCCACAGCCTAGCCCCCCTCCTGAGGGGAGAACTGTCAGCAACGAGCTGGAGGAAGAGGAACGGTAC
TTGTCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGGAGAAGGGTCTGAAAGCCTGCCCTCCGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCTGGAGAGAGGAATGTTCTCATCACCAGTGCCTGCCCTATGTCACAAATGTC
CCCACCTTGAAACATCATTGGCTGTGCTCAGTGTGATGTTGCAAGGTATTGTCGCCCTGCCAGTGGAAAT
ACCCCTATCTGTGTGTAAGATGAGTATGGTACTGCGACAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GGAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTTCGGCATATGTTGATACTTCCGGC
GCACTACCACCTCAGCAGACCAAAATCACCCAGGACATCTTCAGAGGTGCTGACCCGGGTTTGTGCTGCGA
GATACTGTGGAGCAGCTTCGGTGTGAGCGGTGTGACGTTCTGGCTGACCGCTTGAGGGTGTGTCCTT
CTGTGGCTATGAAGAGGCCGAGGTGACAGTGTGACAGGTGTGCAAGCTCATCAATGCCATTGAGCTAACAAAC
CACAGTGCACAAATCTGCCGCTCTGCCCTGCGGTGAGGTCTCACAGCACCTGTTCTAGACTTGCTAACGGTAA
AAGCGTCTGGAGGACTGGTGGGAAGACAGTGCCTGGCAGTGACTGGACACCCAAATGCCAGGTTATTACGTT
CTGGCTTCGAGATGCCCTCAAGCCACGATGCATCACCAGAGACCTCAAATGGGAACGCCGTGCCCCGGAA
TTGAGGACAAGGTATTTCAGTCTGGTTGATGCTACTATTGGCTGGTGTCCATCACAGCAACTACACAGACAA
TGGGAGAAATGGTGGAAAGAACCCAGAACAAAGTGGACCTTACCAAGTTCATGCCAAAGACAATGTTCCCTCCTGG
CTTGGTCTTCGTTGAGCTTAGGAGCTGAGGACAACACTACACCCGGTCAAGCACATCATTGCTACAGAGTAC
TGAACATGAGGATGGAAATTCTCTAACAGAGCCGGGCATAGGAGTGGTGGAGACATGCCAACGGATA
CCTGCTGACATCTGGCATTCTATCTGCTATACATTGGCCTGAGGGCCAGGACAGTGCCCTCTCTGGACAGACTT
GTGATTAAAAAACATTCTGAGCTGCTCAACAACTGGCAACTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTTGGCGTTGTGTCCTGAGATGGCGCTAACCCCTGATGACAGACGCCGGTGGCCCATGTCCTTGGGAAACTC
CAGCACTATCACAGCTGTTGGAAAAGGTTGGATCCGGATGCCCTGCGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGAAACGGATTAAAGGTGGTGGAGATGGACAGGCAGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGGCTGCCCTGCTGTGTCATGCTGCAGCCATACATGCCAACAGTCAGCTTAC
ATCCAGACCCAGCTGCAGCTCCACCTGCAGCCTGCCATCCTGCCACAAGCTTCTATTGTACCTTGCCAGCAGG
CCACCGAATTGGCACAGTCAGTCTTGTGTCCTTAAAGGACTGGAAAATGACCAAGATTGAAAATTGAGGCAGCG
GAGGGGGTCAGGCTAACGGCTCCCCAAGCCAGCAGCTGGAGGGACTGAAAGCACAGAAGGCAGACAAGAAC
ACGCTGACGGATGAGGTGACCAAGCAGGGCAACGTCGCGGGACTGAAAGCACAGAAGGCAGACAAGAAC
TGCTGCAGAGGTGGCTAAACTCTGGATCTAACAGAAACAGTGGCTTGCGTGGAGGGAAACCCATTGAAACTCTA
AAGGCAAGAAGAAAAGTGTAAACTCGAGTCTAGAGGGCCGTTAACCCCGCTGATCAGCCTCGACTGTGCC
AGTTGCCAGCCATCTGTTGTTGCCCTCCCCCGTCCTCTGACCCCTGGAAGGTGCCACTCCACTGTC
CTAATAAAATGAGGAAATTGCACTGCATTGCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGCAGGACA
GCAAGGGGGAGGATTGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGCTCTATGGCTCTGAGGCGGAAAGA
ACCAGCTGGGCTCTAGGGGATCCCCACCGGCCCTGAGCGCGCATTAGCGCGGGGTGGTGGTTAC
CAGCGTGACCGCTACACTGCCAGGCCCTAGGCCCTCCTCGCTTCTCCCTTCGCCACGTT
CCGGCTTCCCCGTCAAGCTCTAACATGGGGCTCCCTTGGGCTTACGGCACCTCGAC
AAAAAACTTGATTAGGGTGTGGTACGTAGTGGCCATGCCCTGATAGCGGTTTCGCCCTTGACGGTGA
GTCCACGTTCTTAATAGTGGACTCTTGTGTCCTTAAACTGGAACAAACACTCAACCCCTATCTCGGTCTATT
TATAAGGGATTTGCCATTGCGCTATTGGTAAAAAAATGAGCTGATTTAACAAAATTAAACGCAATTAA
TGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCAT
AATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCAT
GTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCAT
ATGGCTGACTAATTTCGCTATTGCAAGAGGCCAGGCGAGGGCTGCTGAGCTATTCCAGAAGT
GGCTTTGGAGGCCAGGCTTGCACGGCTGGGAGCTGTATATCCATTTCGGATCTGATCAAGAGAC
AGGATGAGGATCGTTGCATGATTGAACAAGATGGATTGCACGCCAGGTTCTCCGGCCGCTGGG
TCGGCTATGACTGGCACAACAGACAATCGGCTGCTGATGCCCGTGTCCGGCTGTCAGCG
CAGGGCGCCCG

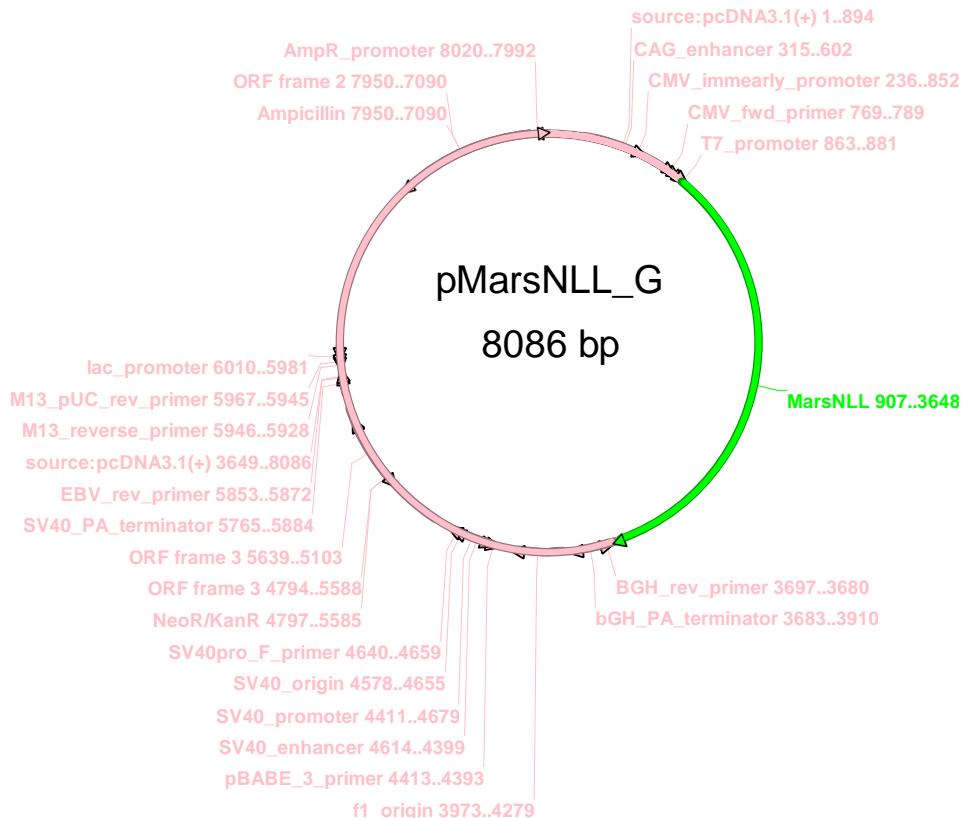
GTTCTTTTGTCAAGACCGACCTGTCGGTGCCTGAATGAACACTGCAGGACGAGGCAGCGCGCTATCGTGGCTGGC
 CACGACGGGCAGTCCTGCGCAGCTGTGCTGACGTTGCACTGAAGCGGGAAAGGGACTGGCTGCTATTGGCGAAG
 TGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCTGCCAGAAAAGTATCCATCATGGCTGATGCAATGCGCG
 CTGCATACGCTTGATCCGGTACCTGCCATTGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGAT
 GGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCGAACGTTGCCAGGC
 TCAAGGCAGCAGTCCCCAGGGAGGATCTCGTGTGACCCATGGCGATGCCGCTTGCCTGCCGAAATATCATGGGAA
 AATGGCCGCTTTCTGGATTATCGACTGTTGCCGGCTGGGTGTTGGCGGACCGTATCAGGACATAGCGTTGCCG
 CGGTGATATTGCTGAAGAGCTTGGCGGAATGGCTGACCGCTCCTCGTCTTACGGTATGCCGCTCCGATT
 CGCAGCGCATGCCCTCTATGCCCTCTTGACGAGTTCTGATTCCACCGCCGCTTATGAAAGGGCTCGGAATCGTT
 CGACGCCAACCTGCCATCACGAGATTCGATTCCACCGCCGCTTATGAAAGGGCTCGGAATCGTT
 CGGGACGCCGGCTGGATGATCCTCCAGCGGGGATCTCATGCTGGAGTTCTGCCAACCCAACTGTTATTGC
 AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTCAAAATAAAGCATTTTCACTGCATTCTAGTT
 GTGGTTGCCAAACTCATCAATGTATCTTATCATGTCGTGACCTCTAGCTAGAGCTGGCGTAATCA
 TGGTCATAGCTGTTCTGTGAAATTGTTATCCGCTCACAATTCCACACAAACATACCGAGCCGAAAGCATAAGTG
 TAAAGCCTGGGGGCCATGAGCTAACATCACATTAATTGCGTGCCTACTGCCGCTTCCAGTCGGAA
 ACCTGTCGCCCCAGCTGATTAATGAATGCCAACCGCGGGAGAGGCGGTTGCGTATTGGCGCTTCCGCT
 TCCTCGCTACTGACTCGCTGCCCGTGGCTGCGCAGCGGTATCAGCTACTCAAAGCGGTAATACG
 GTATCCACAGAATCAGGGATAACGCAAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAA
 AGGCCGCGTGTGGCTTCCATAGGCTCCGCCCCCTGAGGAGCATCACAAATCAGCCTAACGAG
 TGGCGAAACCGACAGGACTATAAGATACCAGGCGTTCCCCCTGGAAGCTCCCTGCGCTCCGCT
 CCTGCCGCTTACCGGATACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCTTCTCATAGCTACGCTGTAGGT
 ATCTCAGTTCGGTAGGTCGCTCCAAGCTGGCTGTGACGAGCTTCTGAGTGGTGGCTAACACTACGGTACACTAGAAGAA
 CAGTATTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAGGAGCTTGTAGCTTGTGATCCGGAAACAA
 ACCACCGCTGGTAGCGGTTTTGCAAGCAGCAGATTACCGCAGAAAAAGGATCTCAAGAAGATCCTT
 GATCTTCTACGGGCTGACGCTAGTGAACGAAACTCACGTTAAGGATTGTCATGAGATTATCAAAAA
 GGATCTCACCTAGATCTTAAATTAAAGTAACTAAAGTATATGAGTAAACTGGTCT
 GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTAGCGATCTGCTATTGCTCATCCATAGTGCCTGACT
 CCCCGTGTAGATAACTACGATAACGGGAGGGCTTACCATCTGCCCGAGCTGCAATGATAACCGCAGACCCAC
 GCTCACCGGCTCCAGATTACGCAATAAACAGCCAGCCGAAGGGCCGAGCGCAGAGTGGCCTGCAACTTA
 TCCGCCATCCAGTCTATTAAATTGCGGGAGCTAGAGTAAAGTAGTCTGCCAGTTAATAGTTGCGAACGT
 TGGTGCCTGCTACAGGCATCGGTGTCAGCTCGTGGTATGGCTTCTAGCTCCGATCGTGTAGAAGT
 CAAGCGAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCCGATCGTGTAGAAGT
 AAGTTGGCCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCGTAAGATG
 CTTTCTGTGACTGGTAGTACTCAACCAAGTCATTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTGCCGG
 CGTCAATACGGATAATACCGGCCACATAGCAGAACCTTAAAGTGTCTCATCTGGAAAACGTTCTCGGGCGA
 AAACTCTCAAGGATCTACCGCTGGTAGCAAGGAAAACAGGAAGGCAAAATGCCGAAAAAGGAAATAAGGCGACAC
 GAAAATGTTGAATACTCATACTCTCCCTTTCAATATTGAGCATTACGGTTATTGTCATGAGCGGA
 TACATATTGAATGTATTAGAAAATAACAAATAGGGTCCCGCACATTCCCCGAAAAGTGCACCTGACGT
 C

MAHHHHHHMRLFVSESPGSLPVAAAARARGRAELLIISTVGPEECVVFTRPKVPVLQLDGNYLFSASAICRYF
 FLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVQGKKGEDILGPLRRLVTHIDHSLSRQNCPFLAGDTESLADI
 VLWGALYPLLQDPAYLPEELGALQSWFQLSTQEPCQRAETVLKQQGVLAIRLYLQKQPQPPPEGRTVSNELE
 EEELATLSEEDIVTAVAAWEKGLESPPPLKLQQHPVLPVPGERNVLITSACPYVNNVPHLGNIIIGCVLSADVARYC
 RLRQWNTLYLCGTDEYGTATETKAMEEGLTPREICDKYHAIHADYRWFGISFDTGRTTPQQTKITQDIFQRLLT
 RGFVLRDTVEQLRCERCARFLADRFVEGVCPFCGYEARGDQCDRCGKLINAIELKKPQCKICRSCPVRSSQHLFL
 DLPKLEKRLEDWLGTKVPGSDWTPNARFIIRSWLDRGKPRCITRDLKWGTPVPLEGFEDKVFYVWFATIGLVSIT
 ANYTDQWEKWWKNPEQVDLYQFMAKDNVPFLGLVFPCSVLAEDNYTLVKHIIATEYLNEYDGKF SKSRGIGVFGDM

AKDTGIPADIWRFYLLYIRPEGQDSAFSWTDLLIKNNSELLNNLGNFINRAGMFVSKFFGGCVPEMALTPDDRLVA
 HVSWEIQHYHQLEKVRIRDALRSILTISRHGNQYIQVNEPWKRICKGGEMDRQRAGTVTGMAVNMAALLSVMQLQPYM
 PTVSSTIQTQLQLPPAACRILATSFICLPLPAGHRIGTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTAA
 GSQHIFTLTDEVTKQGNVRELKAQKADKNQVAEEVAKLLDLKKQALALAEGKPIETPKGKKKK--

Figure S6. The pMaRSNLL_G vector for expression of NLL-MmMetRS under CMV promoter control.

Restriction enzymes are highlighted in yellow and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



GACGGATCGGGAGATCTCCGATCCCCTATGGTCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAACGCCAG
 TATCTGCTCCTGCTTGTGTTGGAGGTGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAGGCAAGGGCTT
 GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCCTTGCCTGCTGCGATGTACGGGCCAGATAACGC
 GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATAATGGAGTC
 CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGAC
 GTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACT
 TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
 TATGCCAGTACATGACCTTATGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
 GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCCCATTG
 ACGTCAATGGGAGTTGTTGGCACAAATCAACGGGACTTCCAAATGTGTAACAACCTCCCCCCATTGACG
 CAAATGGCCGGTAGGCGTGTACGGTGGAGGTCTATAAGCAGAGCTCTGGCTAAGTACAGAGAACCCACTGCTTA
 CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGC GCCACCATGGCCACCACCAT
 CACCATATGAGACTGTCGTGAGCGAGGGTCCCCGGGAGCCTGCCGTGCTGGCTGGCCGCGAGGGCCGGGG
 TCGGGCGGAGCTGCTCATCAGCACCGTAGGCCCGAAGAGTGTGGTACCATCCTTACCCGGCTAAGGTCCCTG

TCTTGCAGCTGGATAGTGGCAACTACCTCTCTGCTAGTGCAATCTGCCATATTTTCTGTTATGTGGCTGG
GAACAAGATGATCTCACCAACCAGTGGCTGGAATGGGAGGCAACAGAACTGCAGCCAGTCTGCTGCTGCCCTACA
CTGTCTAGTGGTTCAAGGCAAGAAAGGGGAGATATACTTGGCCACTTCGGAGAGTCTGACTCACATTGATCACA
GCTTGAGTCGTCAAAACGTGCTTCTGGCTGGGACACAGAACTCTAGTGACATTGTTGGGGAGCACTG
TATCCTTAAGTGCAGAACCCAGCTTACCTCCCTGAGGAGTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAGTAC
CCAGGAACCGTGTAGCGAGCTGCAGAGACGGTGTCTAAAACAGCAGGGTGTCTGGCACTTCGCTGTACCTCCAGA
AACAGCCACAGCCTCAGCCCCCCTGAGGGAGAACTGTCAAGCAACAGAGCTGGAGGAAGAGGAACGGTACCG
TTGCTGAGGAGGACATCGTTACAGCTGTTGCCCGTGGAGAAGGGTCTGAAAGCCTGCCCTGGCTAAAGCTCCA
GCAGCATCCAGTGTGCTGTGCTGGAGAGAGGAATGTTCTCATCACCAGTGCAACCCCTATGTCAACAATGTCC
CCCACCTGGAAACATCATTGGCTGTGCTCAGTGCTGATGTTGCAAGGTATTGTCGCCCTGCCAGTGGAAAT
ACCCCTATCTGTGTGGTACAGATGAGTATGGTACTGCCAGAGACCAAGGCCATGGAGGAGGGCTAACCCACG
GGAAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTCCGGATATCGTTCGATACTTCCGGC
GCACTACCACTCCTCAGCAGACAAAATCACCAGGACATCTTCCAGAGGTTGCTGACCCGGGGTTGTGCTGCGA
GATACTGTGGAGCAGCTCGGTGTGAGCGGTGTGCACGTTCTGGCTGACCGCTTGAGGGTGTGTCCCTT
CTGTGGCTATGAAGAGGCCAGGGTGACAGTGTGACAGGTGTGCAAGCTCATCAATGCCATTGAGCTCAAGAAC
CACAGTCAAAATCTGCCGCTCTGCCCTGTTGAGGTCCTCACAGCACCTGTTCTAGACTGCCCTAAGTGGAA
AAGCGTCTGGAGGACTGGTGGGAAGACAGTGCTGGCAGTGACTGGACACCCAAATGCCAGGTTCATTACGTT
CTGGCTTCGAGATGGCTCAAGCACGATGCATCACCAGAGACCTCAAATGGGAACGCCGTGCCCCGGAGGTT
TTGAGGACAAGGTATTTCAGTCTGGTTGATGCTACTATTGGCTGGTGTCCATCACAGCAAACACAGACCAA
TGGGAGAAATGGTGGAAAGAACCCAGAACACAAGTGGACCTTACCAAGTTCATGCCAAAGACAATGTTCCCTGG
CTTGGCTTTCCGTGTCAGTCAGGAGCTGAGGACAACACTACACCCCTGGTCAAGCACATCATTGCTACAGAGTAC
TGAACATGAGGATGGAAATTCTCTAACAGGCCGGGCTAGGAGTGGTGGAGACATGCCAAGGATACAGGAATC
CCTGCTGACATCTGGCATTCTATGCTATACATTCCGCTGAGGGCCAGACAGTGCTTCTCTGGACAGACTT
GTTGATTAACAAATCTGAGCTGCTCAACAAACCTGGCAACTCATCAACAGAGCTGGCATGTTGTTCTAAGT
TTTTGGCGTTGTGCTGAGATGGCTAACCCCTGATGACAGACGCCGGTGGCCATGTCCTGGAAACTC
CAGCACTATCACCAGCTGGAAAGGTTGGATCCGGATGCCCTGCGCAGTATCCTCACCATATCTGCCATGG
CAACCAATACATTCAAGTGAATGAGCCCTGAAACGGATTAAAGGTGGTGGAGATGGCACAGGCAGGGCAGGCACAG
TGACAGGCATGGCAGTGAACATGCCCTGCTGTCATGCTGCAGCCATACATGCCACAGTCAGCTCTAC
ATCCAGACCCAGCTGCAGCTCCACCTGCAGCCTGCCATCCTGCCACAAGCTTCTTGTACCTGCCAGCAG
CCACCGAATTGGCACAGTCAGTCCTTGTCCAAAAACTGGAAATGACCAAGATTGAAAATTGAGGCAGCCTGG
GAGGGGGTCAGGCTAAAGCTCCCCAAGCCAGCAGCTGTGGAGGCAGTTACAGCAGCAGGCTCGCAGCACACAA
ACGCTGACGGATGAGGTGACCAAGCAGGGCAACGTCGCGGGAACTGAAAGCACAGAAGGAGACAAGAAC
TGCTGCAGAGGTGGCTAAACTCTGGATCTAAAGAAACAGTTGGCTTGGCTGAGGGAAACCCATTGAAA
AAGGCAAGAAGAAAAAGTATAACTCGAGTCTAGAGGCCGTTAACCCGCTGATCAGCCTGACTGTGCC
AGTTGCCAGCCATCTGTTGCTTGGCCCTCCCCGTGCCCTCCTGACCCCTGGAAGGTGCCACTCCACTGCT
CTAATAAAATGAGGAAATTGATCGCATCGATTGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGCAGGACA
GCAAGGGGAGGATTGGAAGACAATAGCAGGCATGCTGGGGATGCCCTGAGCAGGCTCTATGGCTCTGAGGCG
ACCAAGCTGGGCTCTAGGGGTATCCCCACGCCCTGAGCAGGCTTAAGCGCGGGTGTGGTGGTTACCG
CAGCGTGACCGCTACACTGCCAGGCCCTAGCGCCCTCCTTGCTTCTCCCTTCGCCACGTT
CCGGCTTCCCGTCAAGCTCAAATGGGGCTCCCTTAGGGTCCGATTAGTGCTTACGGCACCTGACCCCC
AAAAAACTTGATTAGGGTGTGGAAAGTCCCAGGCTCCCAGCAGCAGGAGTATGCAAAGCATGCATCT
GTCCACGTTCTTAATAGTGGACTCTGTTCCAAACTGGAAACAACACTCAACCCATTCTCGGTCTATT
TATAAGGGATTTGCCGATTGCGCTATTGGTAAAAAAATGAGCTGATTAAACAAAATTAAACGCAATT
TGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCAGGCTCCCAGCAGCAGGAGTATGCAAAGCATGC
AATTAGTCAGCAACCAGGTGTGGAAAGTCCCAGGCTCCCAGCAGGAGTATGCAAAGCATGCATCT
GTCAGCAACCATTCTCGCCCTAAGTCCGCCATTCCGCCCCACTCCGCCCCAGTCCGCCATTCT
ATGGCTGACTAATTCTGGTGGAGGCCCTAGGCTTTGCAAAAGCTCCGGGAGCTTGATATCCATT
GGCTTTGGAGGCCCTAGGCTTTGCAAAAGCTCCGGGAGCTTGATATCCATTTCGGATCTGATCAAGAGAC
AGGATGAGGATCGTTGTCATGATTGAACAAGATGGATTGACCGCAGGTTCTCCGGCCGCTGGGTGGAGAGGCTAT

TCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCCGTGTCCGGCTGTCAGCGCAGGGGCCCG
 GTCTTTTGTCAGACCGACCTGTCGGTGCCTGAATGAAGTGCAGGAGGCAGCGCGCTATCGTGGCTGGC
 CACGACGGCGTCCTGCGCAGCTGTGCTGACGTTGCACTGAAGCAGGGAAAGGGACTGGCTGCTATTGGCGAAG
 TGCCGGGCAGGATCTCCTGTCATCTCACCTGCTCCTGCCAGAAAAGTATCCATCATGGCTGATGCAATGCGCG
 CTGCATACGCTGATCCGGTACCTGCCATTGACCACCAAGCGAACATCGCATCGAGCGAGCACGTACTCGGAT
 GGAAGCCGGCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCGAACACTGTCGCCAGGC
 TCAAGGCGCGATGCCGACGGCAGGATCTGTCGTGACCCATGGCGATGCCGCTTGCGAATATCATGGTGGAA
 AATGGCCGCTTCTGATTGACTCGACTGTCGGCCGGCTGGGTGTCGGGACCGCTATCAGGACATAGCGTTGGCTAC
 CCGTGAATTGCTGAAGAGCTTGGCGGAATGGGTGACCGCTTCTCGTCTTACGGTATGCCGCTCCGATT
 CGCAGCGCATGCCCTATGCCCTTGTGACGAGTTCTGAGCAGGGACTCTGGGGTCGAAATGACCGACCAAG
 CGACGCCAACCTGCCATCACGAGATTCGATTCCACCGCCGCTTCTATGAAAGGGGGCTTCGGAATCGTTTC
 CGGGACGCCGGCTGGATGATCCTCAGCGGGGATCTCATGTCGGAGTTCTCGCCCACCCAACCTGTTATTG
 AGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTTCACAAATAAGCATTTCAGTGCATTCTAGTT
 GTGGTTGTCACACTCATCAATGTATTTATCATGTCGTATACCGTCACCTCTAGCTAGAGCTGGCGTAATCA
 TGGTCATAGCTGTTCTGTCAGGAAATTGTTATCCGCTCACAAATTCCACACACATACGAGCGGAAGCATAAGTG
 TAAAGCCTGGGTGCTTAATGAGTGAGCTAACACATTAATTGCGTGCCTCACTGCCGCTTCCAGTGGAA
 ACCTGTCGTGCCAGCTGCATTAATGAATCGCCAACGCGCGGGAGAGGCAGGTTGCGTATTGGCGCTTCCGCT
 TCCTCGCTCACTGACTCGCTCGCTCGGCTGCGGAGCGGTATCAGCTCACTCAAAGGCGTAATACG
 GTTATCCACAGAACGAGGATAACGAGGAAAGAACATGTGAGCAGAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
 AGGCCGCGTGGCTGGGTTTCCATAGGCTCCGCCCCCTGACGAGCATTACAAAATCGACGCTCAAGTCAGAGG
 TGGCGAAACCCGACAGGACTATAAGATAACCGCGTTCCCCCTGGAAGCTCCCTCGCGCTTCTCATAGCTCACGCTGAGG
 CCTGCCGCTTACCGGATACCTGTCGCCCTTCTCCCTCGGGAAAGCGTGGCGCTTCTCATAGCTCACGCTGAGG
 ATCTCAGTCGGTAGGCTCGCTCCAGCTGGGCTGTGACGAGCATTACGCCACTGGCAGCAGCACTGGTAACAG
 GATTAGCAGAGCGAGGTATGAGGCTGCTACAGAGTTCTGAAGTGGTGCCTAACTACGGCTACACTAGAAAGAA
 CAGTATTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGCTTGTATCCGGAAACAA
 ACCACCGCTGGTAGCGGTTTTGCAAGCAGATTACCGCAGAAAAAGATCTCAAGAAGATCCTT
 GATCTTCTACGGGCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATTGGTATGAGATTATCAAAAA
 GGATCTTCACCTAGATCTTAAATTAAAAAGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCT
 GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTAGCGATCTGTCTATTGTTGTCATCCATAGTGCCTGACT
 CCCGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGCCCAAGTGCTGCAATGATACCGCAGACCCAC
 GCTCACCGGCTCCAGATTATCAGCAATAAACAGCCAGCCGGAAAGGGCCGAGCGCAGAAGTGGCCTGCAACTTA
 TCCGCTCCATCCAGTCTATTAAATTGTCGGGAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGAACGT
 TGGTGCCTGCTACAGGCATGTCGGTGTACGCTCGTGTGTTGGTATGGCTTCAATTGCTCCGTTCCAAACGAT
 CAAGGCAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCTCCGATCGTTGTCAGAAGT
 AAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCGTAAGATG
 CTTTCTGTGACTGGTAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGCGACCGAGTTGCTCTGGCCGG
 CGTCAATACGGATAATACCGGCCACATAGCAGAACCTAAAGTGCATCATTGAAAAGCTCTCGGGCGA
 AAACCTCAAGGATCTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCGTCACCCAACGTATCTCAGCATC
 TTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAAGGGATAAGGGCAGAC
 GGAATGTTGAATACTCATACTCTCCCTTTCAATATTATTGAAGCATTATCAGGGTATTGTCATGAGCGGA
 TACATATTGAATGTATTAGAAAATAACAAATAGGGTCCCGCACATTCCCCGAAAAGTGCACCTGACGT
 C

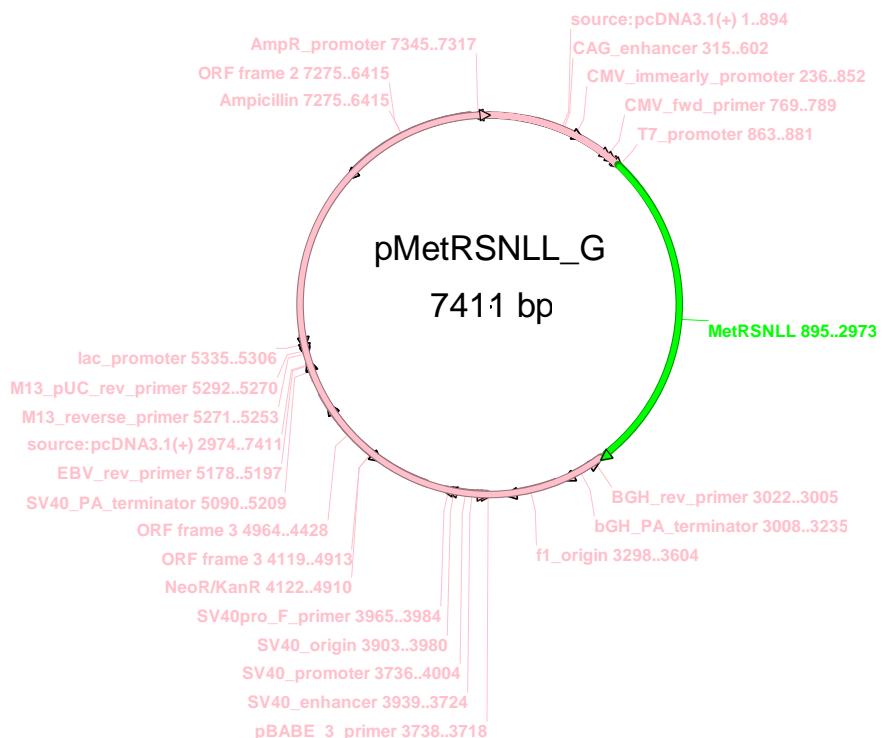
MAHHHHHHMRLFVSEGSPGSLPVAAAARARGAE~~L~~I~~S~~T~~V~~GPEECVVPFLTRPKVPVLQLD~~S~~GNYLFSASAICRYF
 FLLCGWEQDDLTNQWLEWEATELQPVL~~S~~AALHCLVVQGKKG~~E~~DILGPLRRVLTHIDHSLSRQNCPFLAGDTE~~S~~LADI
 VLWGALYPLLQDPAYLPEELGALQS~~W~~F~~Q~~T~~L~~STQEP~~C~~QRAAET~~V~~LKQQGV~~L~~R~~L~~Y~~L~~Q~~K~~Q~~P~~Q~~P~~PP~~P~~PEGRTV~~S~~NELE
 EEE~~L~~ATLSEEDIVTAVAAWEKGLES~~P~~PLKLQQHPVL~~P~~VG~~E~~RN~~V~~LITSANPYVNNVPHLGNIIGCVLSADVFARYC
 RLRQWNTLYLCGTDEYGTATE~~T~~KAMEEG~~L~~TPRE~~I~~CDKYHAI~~H~~ADIYRWFGIS~~F~~DT~~F~~GRT~~T~~TPQQT~~K~~ITQDIFQRLLT
 RG~~F~~VL~~R~~D~~T~~VEQLRCER~~C~~ARFLADRF~~V~~EG~~V~~CPFCGYEE~~A~~RGDQC~~D~~RCGKL~~I~~N~~A~~I~~E~~LKKPQCKICRSCP~~V~~RSSQHLFL

```

DLPKLEKRLEDWLGKTVPGSDWTPNARFIIRSWLRDGLKPRCITRDLKWGTPVPLEGFEDKVFYVWFATIGLVSIT
ANYTDQWEKWWKNPEQVDLYQFMAKDNVPFLGLVFPCSVLGAEDNYTLVKHI IATEYLNEDGKF SKSRGIGVFGDM
AKDTGIPADIWRFYLLYIRPEGQDSAFTDILLIKNNSELLNNLGNFINRAGMFVSKFFGGCVPEMALTPDDDRLLVA
HVSWELOQHYHQLLEKVRIRDALRSILTISRHNQYIQVNEPWKR KIKGGEMDRQRAGTVTGM MAVNMAALLSVM LQPYM
PTVSSTIQTOLQPLPAAACRILATSFIGTLPAGHRIGTVSPLFQKLENDQIENLRQRFGGQAKGSPKPAAVEAVTAA
GSQHIQTLTDEVTQGNVVRELKAQKADKNQVAEEVAKL LDKKQLALAEGKPIETPKGKKKK--

```

Figure S7. The pMetRSNLL_G vector for expression of the *E. coli* NLL-MetRS under CMV promoter control. Restriction enzymes are highlighted in yellow, start codon in red, and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence.



```

GACGGATCGGGAGATCTCCCGATCCCTATGGTCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAACGCCAG
TATCTGCTCCCTGCTTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAGGCAAGGCTT
GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGCGCTTGCCTGCGATGTACGGGCAGATATACGC
GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATAATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
TATGCCCAGTACATGACCTTATGGGACTTCCATTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTGACG
ACGTCAATGGGAGTTGGCACC AAAATCAACGGACTTCCAAAATGTCGTAACAACCGCCATTGACG
CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCACTGCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGT GCTAGC GCC ACC ATGGCCACCATCACC AT
CACC AT ACT CAAGT CGCG AAG AAA ATTCTGGT GAC GTG CGC AA ACC CGT AC GCT AAC CG CT AAC CC AC CT CGG CCA
TATGCTGGAGCACATCCAGGCTGATGTCTGGTCCGT TAC CAG CGA AT GC CGG CC AC GAG GT CA ACT TT CAT CT GCG
CCGACGATGCCACGGTACACCGATCATGCTGAAAGCTCAGCAGCTGGTATCACCCGGAGCAGATGATTGGCGAA

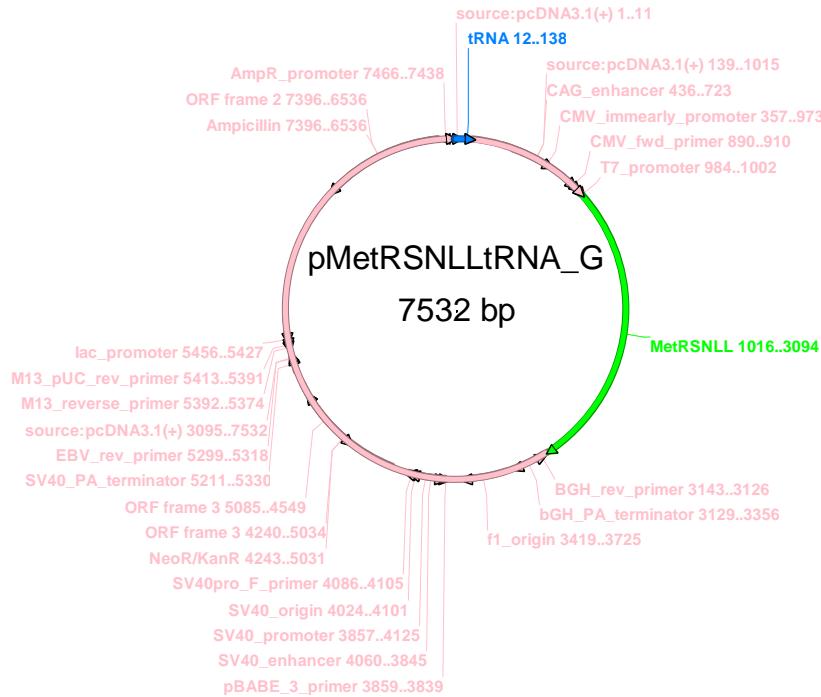
```

ATGAGTCAGGAGCATCAGACTGATT CGCAGGTTAACATCAGCTATGACAAC TACTCGACGCACAGCGAAGA
GAACCGCCAGTTGTCAGAACTTATCTACTCTCGCCTGAAAGAAAACGGTTTATTAAAAACCGCACCATCTCTCAGC
TGTACGATCCGAAAAAGGCATGTTCTGCGCGACC GTTGTGAAAGGCACCTGCCCGAAATGTAATCCCCGGAT
CAATACGGCGATAACTCGAAGTCTCGCGCGACCTACAGCCC ACTGAAC TGATCGAGCCGAAATCGGTGGTTTC
TGGCGCTACGCCGTAATCGTGATTCTGAACACTTCTTGTGATCTGCCCTTTCA CGGAAATGTTGCAGGCAT
GGACCCGCAGCGGTGCGTTGCGAGGAGCAGGTGGCAAATAAAATG CAGGAGTGGTTGAATCTGCCGCAACAGTGG
GATATCTCCCGCAGCCCCCTACTCGGTTTGAAATT CGAACCGCGCCGGCAAATATTCTACGTCTGGCTGGA
CCGACCGATGGCCTGATGGTTCTTCAAGAATCTGCGACAAGCCGCGACAGCGTAAGCTCGATGAATACT
GGAAGAAAAGACTCCACCGCGAGCTGTACCACTTCATCGTAAAGATATTGTTACTTCTGAGCCTGTTCTGGCCT
GCCATGCTGGAAGGCAGCAACTTCCGCAAGCGTCCAACCTGTTATGGCTATGTGACGGTAACGGCGCAA
GATGTCCAAGTCTCGCGCACCTTATTAAAGCCAGCACCTGGCTGAATCATT TGACCGAGACAGCCTGC GTTA
ACTACACTGCGAAACTCTTCGCGCATTGATGATATCGATCTCAACCTGGAAGATT CGTTCAGCGTGTGAATGCC
GATATCGTTAACAAAGGTTAACCTGCCCTCCCGTAATGCGGGTTTATCAACAAGCGTTTGACGGCGTGTGGC
AAGCGAACTGGCTGACCCGCACTGTGATACAAACCTTCACTGATGCCGCTGAAGTGATTGGTGAAGCGTGGGAAAGCC
GTGAATTGTTAACAGCGTGC GCGAAATCATGGCGTGGCTGATCTGGCTAACCGCTATGTCGATGAACAGCGTCCG
TGGGTGGTGGCGAAACAGGAAGGCCGCGATGCCGACCTGCGAGGCAATTGCTCAATGGCATCAACCTGTTCCCGT
GCTGATGACTTACCTGAAGCGGTACTGCCGAAACTGACCGAGCGTGCAGAACGATT CCTCAATACGGAACTGACCT
GGGATGGTATCCAGCAACCGCTGCTGGCCACAAAGTGAATCCGTTCAAGGCCTGTATAACCGCATCGATATGAGG
CAGGTTGAAGCACTGGTGGAAAGCCTCTAAAGAAGAAGTAAAAGCCGCTGCCGCCGTAACTGCCGCTGGCAGA
TGATCCGATTAGGAAACCATCACCTTGACGACTTCGCTAAAGTTGACCTGCGTGGCGCTGATTGAAAACGCA
AGTTTGTGAAGGTTCTGACAAACTGCTGCCCTGACGCTGGATCTGGCGGTGAAAAAACGCAATGCTTCTCCGGT
ATTGCTTCTGCTTACCGGATCCGCAAGGCACTGATTGGTGTGTCACACCATTATGGTGGCTAACCTGGCACCACGTAA
AATGCGCTTCGGTATCTCTGAAGCATGGTATGGCTGCCGGTCTGGCGGGAAAGATATTCTGCTAACGCCGG
ATGCCGGTCTAAACCGGGTCACTAGGTGAAATAAGGCTCCTGAGGTTCTAGAGGGCCGTTAACCCGCTGATCA
GCCTCGACTGTGCCCTTAGTTGCCAGCCATCTGTTGCCCCCTCCCCGTGCCCTTGACCCGATTAAGGGTGC
CACTCCCACGTCTTCTCTAAATAAAATGAGGAAATTGCATCGCATTGCTGAGTAGGTGTCAATTCTATTCTGGGG
GTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCACTGGGGATGCCGCTCTATG
GCTTCTGAGGCGGAAAGAACCGCTGGGCTCTAGGGGTATCCCCACGCCCTGTAGCGCGCATTAAGGCCG
GGGTGTGGTGGTTACCGCGAGCGTACACTTGCCAGGCCCTAGGCCGCTCTTGTGTTCTCCCT
CTTTCTGCCACGTTGCCGGTTCCCGTCAAGCTCTAAATGGGGCTCCCTTAGGGTCCGATTAGTGT
TTACGGCACCTGACCCAAAAACTTGATTAGGGTGTGTTCACTGAGTGGCCATGCCCTGATAGCGTTT
TCGCCCTTGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTGTTCCAAACTGGAACAAACACTCAACCCATCT
CGGTCTATTCTTTGATTATAAGGGATT TGCCGATTGCGCTATTGGTAAAAATGAGCTGATTAAACAAAAA
TTAACGCGAATTAAATTCTGTGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAAGT
ATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAAGTATGCA
AAGCATGCATCTCAATTAGTCAGCAACCAGTCAGGCCCTAACCTCCGCCCCATCCGCCCTAACCTCCGCCCCAGTT
CCGCCCATCTCCGCCCATGGCTGACTAATT TATGCAAGAGGCCAGGCCCTGCTCTGAGCT
TTCCAGAAGTAGTGAGGAGGCTTTGGAGGCC TAGGCTTTGCAAAAGCTCCGGAGCTGTATATCCATT
CGGATCTGATCAAGAGACAGGATGAGGATCGTTCGCA TGATTGAAAGATGGATTGACCGAGTTCTCCGGCG
CTTGGGTGGAGAGGCTATTGCGCTATGACTGGCACAACAGACAATCGGCTGCTGTGATGCCGCCGTTCCGGCT
TCAGCGCAGGGCGCCCGTTTTGTCAAGACCGACCTGTCGGTGCCTGAATGAACTGCAGGACGAGGCAGC
GCGGCTATCGTGGCTGCCACGACGGCGTCCCTGCCAGCTGTGCTGACGTTGTCACTGAAGCGGAAGGGACT
GGCTGCTATTGGCGAAGTGCCGGGCAGGATCTCTGTCATCTCACCTGCTCTGCCAGAAAGTATCCATCATG
GCTGATGCAATCGGCGCTGCATACGCTTGATCCGGTACCTGCCATTGACCCACCAAGCGAAACATCGCATCGA
GCGAGCACGTA CGGATGGAAGCCGGTCTGCGATCAGGATGATCTGGACGAAGAGCATCAGGGCTGCC
CCGAACGTGCGCAGGCTCAAGCGCGCATGCCGACGGCGAGGATCTGCGTGAACCATGGCGATGCCCTGCTT
CCGAATATCATGGTGGAAAATGCCGTTTCTGGATT CATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCA
GGACATAGCGTTGGCTACCGTGATATTGCTGAAGAGCTGGCGGAATGGGCTGACCGCTTCTCGTGTGTTACG
GTATGCCGCTCCGATTGCGACCGCATGCCCTCTGACGAGTTCTGAGCAGGACTCTGGGT

TCGAAATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGGTG
GGCTTCGGAATCGTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGGGGATCTCATGCTGGAGTTCTCGCCCA
CCCCAACTGTTATTGCAGCTATAATGGTTACAAATAAGCAATAGCATCACAAATTCAAAATAAGCATT
TTTCACTGCATTCTAGTTGTGGTTGTCCTCAAACCTCATCAATGTATCTTATCATGTCGTATACCGTCGACCTCTAGC
TAGAGCTTGGCGTAATCATGGTCAGCTGTTCTGTGAAATTGTTATCGCTCACAAATTCCACACAACATACG
AGCGGAAAGCATAAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTACTGC
CCGCTTCCAGTCGGAAACCTGTCGCCAGCTGCATTAATGAATCGCCAACGCCGGGGAGAGGCCGGTTGCGT
ATTGGCGCTTCCGCTTCGCTCACTGACTCGCTGCGCTCGGTGTTGCGCTGCGCGAGCGGTATCAGCTCA
CTCAAAGGCGGTAACTGGTTATCCACAGAACAGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAA
AGGCCAGGAACCGTAAAAGGCCGCGTGTGCTGGCGTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAT
CGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGACTATAAGATAACCAGGCGTTCCCCCTGGAAGCTCCCTCGT
GCGCTCCGTGTCGACCCCTGCCGTTACCGGATACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCGTTCTC
ATAGCTACGCTGTAGGTATCTCAGTCGGGTAGGTCGTCAGCTCCAGCTGGGTGTGACGAACCCCCCGTT
CAGCCCGACCGCTGCCCTTATCCGTAACACTATCGTCTTGAGTCCAACCCGTAAGACACGACTATGCCACTGGC
AGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAACT
ACGGCTACACTAGAAGAACAGTATTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGTTGGTAGC
TCTTGATCCGCAACAAACCAACCGCTGGTAGCGGTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGG
ATCTCAAGAAGATCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAACGAAAACACTACGTTAAGGGATTTGG
TCATGAGATTATCAAAAGGATCTCACCTAGATCTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATA
TATGAGTAAACTGGTCTGACAGTTACCAATGCTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTCGTT
ATCCATAGTGCCTGACTCCCCGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCAGTGTGCAA
TGATACCGCGAGACCCACGCTCACCGGCTCCAGATTATCAGCAATAAACCGAGCCAGCCGAAGGGCCAGCGCAGA
AGTGGTCTGCAACTTATCCGCTCCATCCAGTCTATTAAATTGTCGGGAAGCTAGAGTAAGTAGTCTGGCCAGT
TAATAGTTGCGCAACGTTGTCAGGCTACAGGCATCGGTGTCAGCTCGTGTGTTGGTATGGCTTCAATTCA
GCTCCGGTCCCAACGATCAAGGCAGGTTACATGATCCCCATGTTGCAAAAAAGCGGTTAGCTCCTCGGTCT
CCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCCTCATGGTTATGGCAGCAGTGCATAATTCTTACTGT
CATGCCATCCGTAAGATGCTTCTGTGACTGGTAGTACTCAACCAAGTCATTCTGAGAATAGTGTATCGGGCAG
CGAGTTGCTCTGCCGGCGTAATACGGGATAATACCGGCCACATAGCAGAACCTAAAGTGTACATCATTGG
AAACGTTCTCGGGCGAAAACCTCAAGGATCTACCGCTGGTAGATCCAGTGCATGTAACCCACTCGTCGACC
CAACTGATCTCAGCATCTTACTTACCTTACCCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAA
AGGGAAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCAATATTATTGAAGCATTATCAGGGT
TATTGTCTCATGAGCGGATAACATATTGAATGTATTAGAAAAATAACAAATAGGGTCCGCGCACATTCCCG
AAAAAGTGCCACCTGACGTC

MAHHHHHTQVAKKILVTCANPYANGSIHLGHMLEHIQADVVWVRYQRMRGHEVNFIGACDDAHGTPIMLKAQQQLGITPEQMIGEMSQEHQTDAGFNISYDNYHSTHSEENRQLSELIYSRLKENGFIKNRTISQLYDPEKGMLPDRFVKGTCPKCKSPDQYGDNCEVCAGATSYPTELIEPKSVVSGATPVMDSEHFFFDLPSFSEMLQAWTRSGALQEJVANKMQEWFESGLQQWDISRDAPYFGFEIPNAPGKYFYVWLDAPIGLMGSFKNLCDKRGSVSFDEYWKKDSTAELYHFIGKDIVYFLSLFWPAMLEGNSFRKPSNLFVHGVTVNGAKMSKSRTFIKASTWLNFADDSLRYYYTAKLSSRRIDDIDLNLEDFVQRVNADIVNKVVNLASRNAGFINKRFDGVLASELADPQLYKTFTDAAEVIGEAWESREFGKAVREIMALADLANRYVDEQAPWVVAKQEGRDA_DLQAICSMGINLFRVLMTYLKPVLPKLTERAEAFLNTELWDGIQQPLLGHKVNPFKALYNRIDMRQVEALVEASKEEVKAAAAPVTGPLADDPIQETITFDDFAKVDLVALIENAEGVEGSDKLLRLTLDBGGEKRNFGSGIRSAVPDQALIGRHTIMVANLAPRKMRFGISEGMVMAAGPGKDIFLLSPDAGAKPGHQVK-

Figure S8. The pMetRSNLLtRNA_G vector for expression of the *E. coli* NLL-MetRS under CMV promoter control. Restriction enzymes are highlighted in yellow, start codon in red, and the enzyme coding sequence is highlighted in green. Kozak sequence is highlighted in blue. The expressed protein sequence is included after the plasmid sequence. This plasmid also contains the tRNA expression cassette as outlined in Figure 1b of the main text. The tRNA cassette was inserted into the BgIII restriction site in the plasmid and is lighted in pink.



```

GACGGATCGGGAGATCTGATCCGACCGTGTGCTTGGCAGAACGGCTACGTAGCTCAGTTGGTTAGAGCACATCACTC
ATAATGATGGGTACACAGGTTGAATCCCCCGTAGGCCACCAGGCTTTTTTGAGATCTCCGATCCCCTATGGT
GCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGGAGGTCGCT
GAGTAGTGCAGGAAATTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAAATCTGCTTAGGGT
TAGGCCTTTCGCGCTGCTCGCGATGTACGGGCCAGATAACGCGTTGACATTGATTATTGACTAGTTATTAAAGT
AAATCAATTACGGGTCAATTAGTCATAGCCCATATATGGAGTTCCCGTTACATAACTACGGTAAATGGCCGCCT
GGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT
CCATTGACGTCAATGGTGGAGTATTACGTAAACTGCCACTGGCAGTACATCAAGTGTATCATATGCCAAGTA
CGCCCCCTATTGACGTCAATGACGGTAAATGGCCGCCATTGACGTCAATGACCTTATGGACTTTCCT
ACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGCGGTTTGGCAGTACATCAATGGCGTGG
ATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTGACGTCAATGGAGTTGGCTTGGCACAAAATC
AACGGGACTTCCAAAATGTCGAACAACCTCCGCCATTGACGAAATGGCGGTAGGCAGTACGGTGGGAGGTC
TATATAAGCAGAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACGACTCACTATAG
GGAGACCCAAGCTGGCTAGCGCCACATGGCCACCATCACCACCATACTCAAGTCGCGAAGAAAATTCTGGT
ACGTGCGCAAACCCGTACGCTAACGGCTCATCCACCTCGGCCATATGCTGGAGCACATCCAGGCTGATGTCGGT
CCGTTACCGCGAATGCGCGGCCACGAGGTCAACTTCATCTGCGCCGACGATGCCACGGTACACCGATCATGCTGA
AAGCTCAGCAGCTGGTATACCCCGGAGCAGATGATTGGCAAATGAGTCAGGAGCATCAGACTGATTTCGAGGC
TTAACATCAGCTATGACAACATCACTCGACGCACAGCGAAGAGAACGCCAGTTGTCAGAACTTATCTACTCTCG
CCTGAAAGAAAACGGTTTATTAAAAACCGCACCATCTCAGCTGTACGATCCGGAAAAGGCATGTTCTGCCGG
ACCGTTTGAAAGGCACCTGCCGAAATGTAATCCCCGGATCAATACGGCATAACTGCGAAGTCTGCCGGCG
ACCTACAGCCCGACTGAACTGATCGAGCCGAAATCGTGGTTCTGGCGCTACGCCGGTAATGCGTGATTCTGAACA

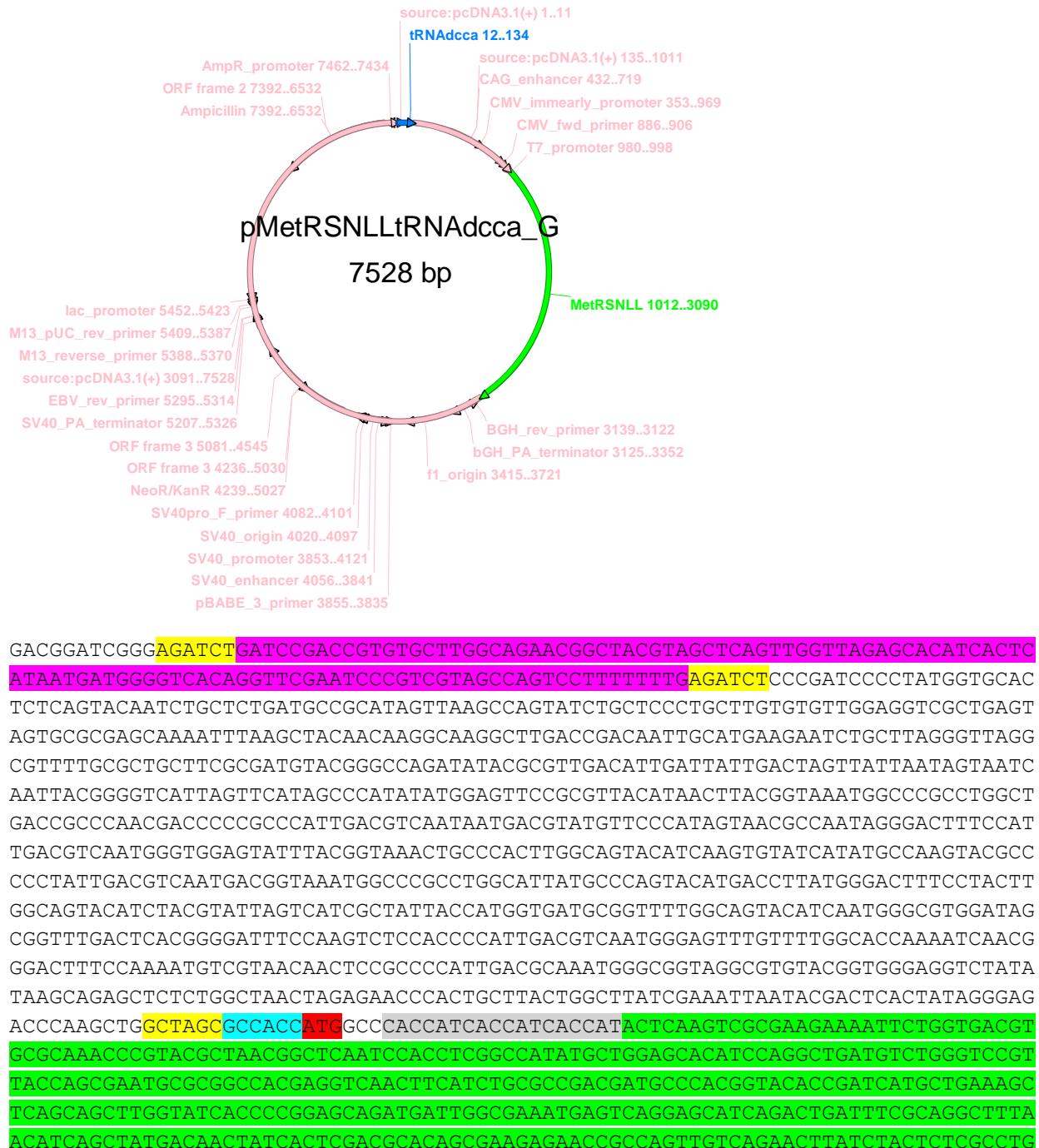
```

CTTCTTCTTGATCTGCCCTTTCAGCGAAATGTTGAGGCATGGACCCGAGCGGTGCGTTGCAGGAGCAGGTGG
 CAAATAAAATGCAGGAGTGGTTGAATCTGCCCTGCAACAGTGGATATCTCCCGCACGCCCTACTTCGTTTT
 GAAATTCCGAACCGCGCGGGCAAATATTCACGTCTGGCTGGACGCACCGATTGGCCTGATGGTTCTTCAGAAG
 TCTGTGCGACAAGCGCGCGACAGCGTAAGCTCGATGAATACTGGAAGAAAGACTCCACCGCGAGCTGTACCACT
 TCATCGTAAAGATATTGTTACTTCCTGAGCCTGTTCTGGCCTGCCATGCTGGAAGGAGCAGCAACTCCGCAAGCG
 TCCAACCTGTTGTTCATGGCTATGTGACGGTAACGGCGAAAGATGTCAGTCTCGCGGACCTTATTAAAGC
 CAGCACCTGGCTGAATCATTTGACGAGACAGCCTGCGTTACTACTACACTGCGAAACTCTTCGCGCATTGATG
 ATATCGATCTCAACCTGGAAGATTGTTCAACCGCTGTAATGCCGATATCGTTAACAAAGTGGTTAACCTGGCCTCC
 CGTAATGCGGGCTTTATCAACAAGCGTTTGACGGCGTGTGGCAAGCGAAACTGGCTGACCCGAGTTGACAAAAC
 CTCACGTGCGCTGAAGTATTGGTGAAGCGTGGAAAGCCGTAATTGGTAAAGCCGTGCGCAGGAAATCATGG
 CGCTGGCTGATCTGGCTAACCGCTATGTCGATGAACAGGCTCCGTGGGTGGCGAAACAGGAAGGCCGCGATGCC
 GACCTGCAGGCAATTGCTCAATGGCATTCAACCTGTCGCTGATGACTTACCTGAAGCCGTTACTGCCGAA
 ACTGACCGAGCGTGCAGAAGCATTCTCAATACGGAACCTGACCTGGGATGGTATCCAGCAACCGCTGCTGGCCACA
 AAGTGAATCCGTTCAAGGCCTGTATAACCGCATCGATATGAGGCAGGTTGAAGCACTGGTGGAAAGCCTCTAAAGAA
 GAAGTAAAAGCCGCTGCCGCCGTAACTGGCCGCTGGCAGATGATCCGATTCAAGGAAACCATCACCTTGACGA
 CTTCGCTAAAGTTGACCTGCGCTGGCGTATTGAAAACGCAATGTTCTCCGGTATTGTTCTGACAAACTGCTGCGCC
 TGACGCTGGATCTGGCGGTGAAAAACGCAATGTTCTCCGGTATTGTTCTGCTTACCCGGATCCGAGGCACTG
 ATTGGTCGTACACCATTATGGTGGCTAACCTGGCACACGTAAAATGCGCTCGGTATCTGAAGGCATGGTGT
 GGCTGCCGGCTGGCGGGAAAGATATTTCCTGCTAACGCCGGATGCCGGCTAACCGGGTCATCAGGTGAAAT
 AAAGAGCTCCTCGAGTCTAGAGGGCCGTTAACCCGCTGATCACGCCGACTGTGCCCTTAGTTGCCAGCCATCT
 GTTGTGTTGCCCTCCCCGTGCCCTTGACCCCTGAAAGGTGCCACTCCCACGTGCTTTCTTAATAAAATGAGGA
 AATTGCATCGCATTGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGTGGGCAGGACAGCAAGGGGAGGATT
 GGGAAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTATGGCTCTGAGGCGGAAAGAACAGCTGGGCTCT
 AGGGGGTATCCCCACCGGCCCTGTAGCGGCCGATTAAGCGCGGGGTGTGGTTACGCGCAGCGTGACCGCTAC
 ACTTGCAGGCCCTAGCGCCGCTCTTCGCTTCTCCCTTCTGCCACGTTGCCGCTTCCCCGTC
 AAGCTCTAAATCGGGGCTCCCTTAGGGTCCGATTAGTGTCTTACGGCACCTGACCCAAAAAAACTTGATTAG
 GGTGATGGTCACGTAGTGGCCATGCCCTGATAGACGGTTTCGCCCCCTTGACGTTGGAGTCCACGTTCTTAA
 TAGTGGACTCTGTTCCAAACTGGAACAAACTCAACCTATCTGGCTATTCTTTGATTATAAGGGATTG
 CGATTTCGGCCTATTGGTAAAAAAATGAGCTGATTAAACAAAATTAAACGCAATTAAATTCTGTTGAAATGTTG
 AGTTAGGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGGAGTATGCAAAGCATGCAATTAGTCAGCAACC
 AGGTGTGGAAAGTCCCCAGGCTCCAGCAGGCAGAAGTATGCAAAGCATGCAATTAGTCAGCAACCAGT
 CCCGCCCTAACTCCGCCATCCGCCCTAACTCCGCCAGTCCGCCATTCTCCGCCCATGGCTGACTAATT
 TTTTTATTTATGCAGAGGCCGAGGCCCTGAGCTATTCCAGAAGTAGTGTGAGGAGGCTTTGGAGGC
 CTAGGCTTTGCAAAAGCTCCCCGGAGCTGTATATCCATTTCGGATCTGATCAAGAGACAGGATGAGGATCGTT
 TCGCATGATTGAAACAAGATGGATTGACGCAGGTTCTCCGGCGCTGGGTGGAGAGGCTATTGCGCTATGACTGG
 CACAACAGACAATCGGTGCTGATGCCCGTGTCCGGCTGTCAGCGCAGGGGCCCGGTTCTTGTCAAG
 ACCGACCTGTCCGGTGCCTGAATGAACTGCAAGGACAGGAGCAGCGCGCTATCGTGGCTGGCCACGACGGCGTCC
 TTGCGCAGCTGTGCTGACGTTGCACTGAAGCGGAAGGGACTGGCTGCTATTGGCGAAGTGCCGGGCAGGATC
 TCCTGTCATCTCACCTGCTCTGCCGAGAAAGTATCCATGCGCTGATGCAATGCCGGCTGCATACGCTTGT
 CCGGCTACCTGCCATTGACCAAGCGAAACATGCCATGAGCAGCACGTAACGGATGGAAGCCGGTCTGT
 CGATCAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCGAACTGTTGCCAGGCTCAAGCGCGCATGC
 CCGACGGCGAGGATCTGCGTGCACCATGGCGATGCCCTGCGAATATCATGGTGGAAATGGCGCTTCTT
 GGATTGATCGACTGTGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCGTGATATTGCTGA
 AGAGCTTGGCGCGAATGGGCTGACCGCTTCTCGTGTCTTACGGTATGCCGCTCCGATTGCGAGCGCATCGCCT
 TCTATGCCCTTCTGACGAGTTCTCTGAGCGGACTCTGGGTTGCAATGACCGACCAAGCGACGCCAACCTGC
 CATCACGAGATTGATCCACCGCCGCTCTATGAAAGGTTGGGCTCGAATCGTTCCGGACGCCGGCTGG
 ATGATCCTCAGCGCGGGATCTCATGCTGGAGTTCTCGCCACCCAACTTGTATTGCAAGCTTATAATGGTTA
 CAAATAAAGCAATAGCATCACAAATTTCACAAATAAGCATTTCGCACTGCAATTCTAGTTGTGGTTGTCAAAC
 TCATCAATGTATCTCATGTCGACCTCTAGCTAGAGCTGGCGTAATCATGGTCAGCTGTT

CCTGTGTGAAATTGTTATCCGCTACAATTCCACACAACATA CGAGCCGAAGCATAAAGTGTAAAGCCTGGGTGC
CTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGAAACCTGTCGTGCCAGC
TGCATTAATGAATCGGCCAACCGCGGGGAGAGGCGGTTGCGTATTGGGCCTCTTCCGCTTCGCTACTGAC
TCGCTCGCTCGTCGGCTCGGCGAGCGGTATCAGCTACTCAAAGCGGTAAACGGTTATCCACAGAATC
AGGGATAACCGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGTTGCTGG
CGTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
GGACTATAAAGATAACCAGCGTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCGCTTACCGG
ATACCTGTCCGCCCTTCTCCCTCGGAAGCGTGGCCTTCTCATAGCTCACGCTGTAGGTATCTCAGTCGGTGT
AGGTCGTTCGCTCCAAGCTGGGCTGTGACGAACCCCCCGTTAGCCCAGCCGCTGCCCTATCCGTAACACTAT
CGTCTGAGTCCAACCCGTAAGACACGACTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCAG
GTATGTAGGGGGTGTACAGAGTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCT
GCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTGGTAGCTCTGATCCGCAAACAAACACCAGCTGGTAGC
GGTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTGATTTCTACGGG
GTCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATTTGGCATGAGATTATAAAAAGGATCTTCACCTAGA
TCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTTGGTGTACAGTTACCAATGC
TTAATCAGTGAGGCACCTATCTCAGCAGTGTCTATTGTTGATCCATAGTTGCCCTGACTCCCCGCTGTAGAT
AACTACGATAACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAACCGCAGACCCACGCTACCGGCTCCAG
ATTATCAGCAATAAACAGCCAGCCGGAAGGGCCAGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAG
TCTATTAAATTGTTGCCGGAAAGCTAGAGTAAGTAGTTGCCAGTTAATAGTTGCCACGTTGTCAGAAGTAAGTTGGCGAGTG
TTATCACTCATGGTTATGGCAGCAGTGCATAATTCTTACTGTCATGCCATCCGTAAGATGCTTTCTGTGACTGG
TGAGTACTCAACCAAGTCATTGAGAATAGTGTATGCCGAGCGAGTTGCTTGCCTGGCGTCAATACGGATA
ATACCGGCCACATAGCAGAACTTAAAGGCTCATATTGAAAACGTTCTCGGGCGAAAACCTCTCAAGGATC
TTACCGCTGTTGAGATCCAGTCAGTGAACCCACTCGTCACCCACTGATCTTCAGCATCTTACCCAG
CGTTCTGGGTGAGAAAAACAGGAAGGAAAATGCCGAAAAAGGAATAAGGGCAGACGGAAATGTTGAATAC
TCATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTATTGTCATGAGCGGATACATATTGAATGT
ATTAGAAAAATAACAAATAGGGTTCCGCGCACATTCCCCGAAAAGTGCCACCTGACGTC

MAHHHHHHTQVAKKILVTCANPYANGSIHLGHMLEHIQADWVWRYQMRGHEVNFI CADDAGTPIMLKAQQLGITP
EQMIGEMSQEHQTDAGFNISYDNYHSTHSEENRQLSELIYSRLKENGFIKNRTISQLYDPEKGMLPDRFVKGTCP
KCKSPDQYGDNCEVCAGATSPTELIEPKSVSGATPVMDSEHFFDLPSFSEMLQAWTRSGALQEJVANKMQEWFE
SGLQQWDISRDAPYFGFEIPNAPGKYFYVWLDAPIGLMGSFKNLCDKRGDSVSFDEYWKKDSTAELYHFIGKDIVYF
LSLFWPAMLEGSNFRKPSNLVHGYTVNGAKMSKSRTFIKASTWLNFADSLRYYTAKLSSRIDDIDLNLED
VQRVNADIVNKVVNLASRNAGFINKRFDGVLASELADPQLYKTFDAAEVIGEAWESREFGKAVREIMALADLANRY
VDEQAPWVVAKQEGRDADLQAICSMGINLFRVLMTYLKPVLPLTERAEAFNTELTWDGIQQPLLGHKVNPFKALY
NRIDMRQVEALVEASKEEVKAAPVTGPLADDPIQETITFDDFAKVDLVALIENAEGVEGSDKLLRLTLDBGKE
RNVFSGIRSAVPDPQALIGRHTIMVANLAPRKMRFGISEGMVMAAGPGGKDIFLLSPDAGAKPGHQVK-

Figure S9. The pMetRSNLLtRNA_{dccca}_G vector for expression of the *E. coli* NLL-MetRS under CMV promoter control. Restriction enzymes are highlighted in yellow, start codon in red, and the enzyme coding sequence is highlighted in green. The expressed protein sequence is included after the plasmid sequence. This plasmid also contains the tRNA expression cassette as outlined in Figure 1b of the main text, wherein the CCA tail of the tRNA was removed in this sequence. The tRNA cassette was inserted into the BgIII restriction site in the plasmid and is lighted in pink.



AAAGAAAACGGTTTATTAAAAACCGCACCATCTCTCAGCTGTACGATCCGAAAAGGCATGTTCTGCCGGACCG
TTTGTGAAAGGCACCTGCCGAAATGTAAATCCCCGATCAATACGGCATAACTGCCAAGTCTGCCGCCGACCT
ACAGCCCACACTGAACGTGAGGCCAAATCGTGGTTCTGCCCTACGCCGTAATCGTGATTCTGAACACTTC
TTCTTGATCTGCCCTTTCAGCGAAATGTTGCAGGCATGGACCGCAGCGTGCCTGCAGGAGCAGGTGGCAA
TAAAATGCAGGAGTGGTTGAATCTGCCCTGCAACAGTGGATATCTCCCGCAGCCCTTACTCGTTTGAAA
TTCGAAACGCCGGGCAAATATTCTACGCTGGCTGGACGCCGATTGCCCTATGGTTCTTCAGAATCTG
TGCACAGCGCGCACAGCGTAAGCTCGATGAATACTGGAAGAAAGACTCCACCGCAGCTGTACCACTTC
CGTAAAGATAATTGTTACTTCCTGAGCCTGTTCTGCCATGCTGGAAGGCAGAACCTCCGAAGCCGCTCA
ACCTGTTGTCATGGCTATGTGACGGTGAACGCCGAAAGATGTCAGCTGCCGACCTTATTAAAGCCAGC
ACCTGGCTGAATCATTGACGCAGACAGCCTGCCACTACTACACTGCAAACCTCTTCGCGATTGATGATAT
CGATCTCAACCTGGAAGATTGTTGCTGCGTGTGAATGCCGATATCGTTAACAAAGTGGTTAACCTGCCCTCCGTA
ATGCGGGCTTATCAACAAGCGTTTGACGGCGTGTGGCAAGCGAACTGGCTGACCCGAGTTGACAAAACCTTC
ACTGATGCCGCTGAAGTGATTGGTGAAGCGTGGAAAGCCGTGAATTGGTAAAGCCGTGCGCAAATCATGGCGCT
GGCTGATCTGGCTAACCGCTATGTCGATGAAACAGGCTCCGTGGTGGTGGCAGAACAGGAAGGCCGATGCCGACC
TGCAGGCAATTGCTCAATGGCATCAACCTGTTCCCGTGTGATGACTTACCTGAAGCCGGTACTGCCGAAACTG
ACCGAGCGTGCAGAACGATTCTCAATACGAACTGACCTGGATGGTATCCAGCAACCGCTGCTGGCCACAAAGT
GAATCCGTTCAAGGCCGTGTATAACCGCATCGATATGAGGCAGGTTGAAGCACTGGTGAAGCCTAAAGAAGAAG
TAAAAGCCGCTGCCGCCGGTAACTGGCCGCTGGCAGATGATCCGATTCAAGGAAACCATCACCTTGACGACTTC
GCTAAAGTTGACCTGCCGTGGCGCTGATTGAAAACGAGAGTTGTTGAAGGTTCTGACAAACTGCTGCCGCTGAC
GCTGGATCTGGCGGTAAAAACGAATGTTCTCCGGTATTGCTGCTTACCCGATCCGAGGCAGTGGT
GTCGTACACCAATTGGTGGCTAACCTGCCACCGTAAACCGCTTCGGTATCTGAAGGCATGGTGAAGGCT
GCCGGTCTGGCGGGAAAGATATTCTGCTAACGCCGGATGCCGGTGTAAACCGGGTACAGGTGAAATAAGA
GCTCTCGAGTCTAGAGGGCCCCTTAAACCGCTGATCAGCCTGACTGTGCCCTCTAGTTGCCAGCCATCTGTTG
TTGCCCTCCCCCGTGCCTCCCTGACCCCTGGAAAGGTGCCACTCCACTGCTCCTTCCAATAAAATGAGGAAATT
GCATCGCATGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGAGACAGCAAGGGGAGGATTGGGA
AGACAATAGCAGGCATGCTGGGATGCCGTGGCTCATGGCTCTGAGGCCGAAAGAACAGCTGGGCTCTAGGG
GGTATCCCCACGCCCTGTAGCCGCATTAAGGCCGGGTGTGGTGGTACGCCAGCGTACCGCTACACTT
GCCAGGCCCTAGGCCGCTCTTCTGCTTCTCCCTTCGCCACGTTGCCGGCTTCCCCGTCAAGC
TCTAAATGGGGCTCCCTTAGGGTCCGATTTAGTGTCTTACGGCACCTCGACCCAAAAAAACTTGATTAGGGT
ATGGTTACGTAGTGGGCATGCCCTGATAGACGTTTCTGCCCTTGACGTTGGAGTCCACGTTCTTAATAGT
GGACTCTTGTCCAAACTGGAACACACTCAACCCATCTCGGTCTATTCTTGATTATAAGGATTGCGAT
TTCGGCCTATTGGTAAAAATGAGCTGATTTAACAAAATTAAACGCAATTATTCTGGAATGTGTGTCAGTT
AGGGTGTGGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGT
GTGGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGTCCC
CCCTAACTCCGCCATCCGCCCTAACCTCCGCCAGTCCGCCATTCTCCGCCATGGCTGACTAATT
TATTATGCAAGAGGCCGAGGCCGCTCTGCCCTGAGCTATTCCAGAAGTAGTGGAGGAGGCTTTGGAGGCTAG
GCTTTGCAAAAGCTCCGGAGCTGTATATCCATTTCGGATCTGATCAAGAGACAGGATGAGGATGTTG
ATGATTGAACAAGATGGATTGACGCAGGTTCTCCGCCGTTCCGGCTGTCAGCGCAGGGGCCGGTTCTTGTCAAGACCG
ACAGACAATCGGCTGCTCTGATGCCGCGTGTCCGGCTGTCAGCGCAGGGGCCGGTTCTTGTCAAGACCG
ACCTGTCCGGTGCCCTGAATGAACACTGCAAGGACGAGGCAGCGCGCTATCGTGGCTGCCACGACGGCGTCTTGC
GCAGCTGTGCTGACGTTGTCAGTGAAGCGGAAGGGACTGGCTGCTATTGGCGAAGTGCCTGGCAGGATCTCCT
GTCATCTCACCTTGCTCTGCCGAGAAAGTATCCATGCTGATGCAATGCCGGCTGCATACGCTGATCCGG
CTACCTGCCATTGACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTG
CAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGCGAAGTGCCTGCCAGGCTCAAGGCCG
CGCGAGGATCTCGTGTGACCCATGGCGATGCCCTGCCGAATATCATGGTGGAAAGTGGCGCTTCTGG
TCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTGGCTACCGT
GATATTGCTGAGAGCTGGGACTCTGGGTTGAAATGACCGACCAAGCGACGCCAACCTGCCATC
ACGAGATTGATCCACCGCCCTTATGAAAGGTTGGCTCGGAATCGTTCCGGACGCCGGCTGGATGA

TCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTGCCACCCAACTTGTATTGCAGCTATAATGGTTACAAA
TAAAGCAATAGCATCACAAATTCAACAAATAAAGCATTTCACTGCATTCTAGTTGGTTGTCAAACACTCAT
CAATGTATCTTATCATGCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTCTG
TGTGAAATTGTTATCCGCTACAATTCCACACAAACATACGAGCGGAAGCATAAAGTGTAAAGCCTGGGTGCCTAA
TGAGTGAGCTAACACATTAATTGCGTGCCTCACTGCCGCTTCCAGTCGGAAACCTGCGCAGCTGCA
TTAATGAATCGGCCAACCGCGGGGAGAGGCGGTTGCGTATTGGCGCTCTCCGCTCGCTCACTGACTCGC
TGCCTCGGTGCGTCCGCTGCGGAGCGGTATCAGCTCACTCAAAGGCGGTAAACGTTATCCACAGAACAGGG
GATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCGTTGCTGGCGTT
TTCCATAGGCTCCGCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGTGGCGAAACCCGACAGGAC
TATAAGATACCAGGCCTTCCCCCTGGAAGCTCCCTGCGCTCTCTCATAGCTCACGCTGTAGGTATCTCAGTTGGTAGGT
CTGTCGCCCTTCTCCCTCGGGAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTGGTAGGT
CGTCGCTCCAAGCTGGCTGTGACGAACCCCCGTTAGCCCACCGCTGCGCTTATCCGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTAT
GTAGGCGGTGCTACAGAGTTCTGAAGTGGCTTAACACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGC
TCTGCTGAAGCCAGTTACCTCGAAAAAGAGTTGGTAGCTCTGATCCGCAAACAAACCACCGCTGGTAGCGGTT
TTTTGTTGCAAGCAGCAGATTACGCGAGAAAAAAAGGATCTAAGAAGATCCTTGATCTTCTACGGGTCT
GACGCTCAGTGGAACGAAAACACGTTAAGGGATTTGGTCATGAGATTATCAAAAGGATCTCACCTAGATCCT
TTAAATTAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACCTGGTCTGACAGTTACCAATGTTAA
TCAGTGAGGCACCTATCTAGCGATCTGTCTATTGTTCATCCATAGTGGCTGACTCCCGCTGTAGATAACT
ACGATACGGGAGGGCTTACCATCTGGCCCGAGCGCAGAAGTGGCCTGCAACTTATCCGCTCCATCAGTCTA
TTAATTGTTGCCGGAGCTAGAGTAAGTAGTTGCGCAGTTAATAGTTGCGCAACGTTGCTTGCCTACAGGC
ATCGTGGTGTCACTCGCTGTTGGTATGGCTTCACTCAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATC
CCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGCTCCGATCGTTGTCAGAAGTAAGTGGCGCAGTGTAT
CACTCATGGTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCGTAAGATGTTCTGTGACTGGTAG
TACTCAACCAAGTCATTGAGAATAGTGTATGCGGCCAGGAGTTGCTCTGCCGGGTCAATACGGGATAATAC
CGGCCACATAGCAGAACTTTAAAGTGTCTCATTTGGAAAACGTTCTCGGGCGAAAACCTCAAGGATCTTAC
CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGCACCCAACTGATCTCAGCATCTTACCTTACCGCGTT
TCTGGGTGAGCAAAACAGGAAGGCAAAATGCGCAAAAAGGAAATAAGGGCGACACGGAAATGTTGAATACTCAT
ACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGATACATATTGAATGTATT
AGAAAATAACAAATAGGGTCCGCGCACATTCCGAAAAGTGCACCTGACGTC

MAHHHHHHTQVAKKILVTCANPYANGSIHLGHMLEHIQADVWVRYQMRGHEVNFIACADDAGTPIMLKAQQLGITP
EQMIGEMSQEHQDFAGFNISYDNYHSTHSEENRQLSELIYSRLKENGFIKNRTISQLYDPEKGMFLPDRFKGTCP
KCKSPDQYGDNEVCAGATSYPTELIEPKSVVSGATPVMRDSEHFFDLPSFSEMLQAWTRSGALQEJVANKMQEWFE
SGLQQWDISRDAPYFGFEIPNAPGKYFYVLDAPIGLMGSFKNLCDKRGDSVSFDEYWKDSTAELYHFIGKDIVYE
LSLFWPAMLEGNSFRKPSNLVHGVTVNGAKMSRSRTFIKASTWLNFADSLRYYTAKLSSRIDDIDLNLEDF
VQRVNADIVNKVVNLASRNAGFINKRFDGVLASELADPQLYKTFDAAEVIGEAWESREFGKAVREIMALADLANRY
VDEQAPWVVAKQEGRDADLQAICSMGINLFRVLMTYLKPVLPKLTERAEAFNTELTWDGIQQPLLGHKVNPFKALY
NRIDMRQVEALVEASKEEVKAAAAPVTGPLADDPIQETITFDDFAKVDLVALIENAEGSDKLLRLTLIDLGGEK
RNVFSGIRSAYPDPQALIGRHTIMVANLAPRKMRFGISEGMVMAAGPGGKDIFLLSPDAGAKPGHQVK-

Figure S10. Structure of the alkyne-TAMRA dye.

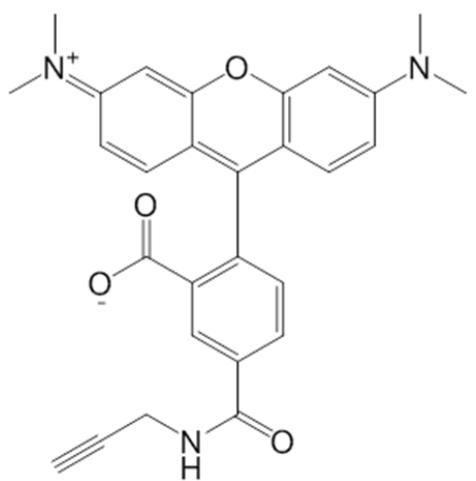


Figure S11.

Protein loading controls corresponding to Figure 1(d) of the main text. Cell lysate proteins which were labeled with TAMRA-alkyne dye and electrophoresed on a polyacrylamide SDS gel were subsequently stained with colloidal blue dye to determine the relative abundances of proteins in lysates derived from different labeling conditions (indicated above the gel) for each cell line.

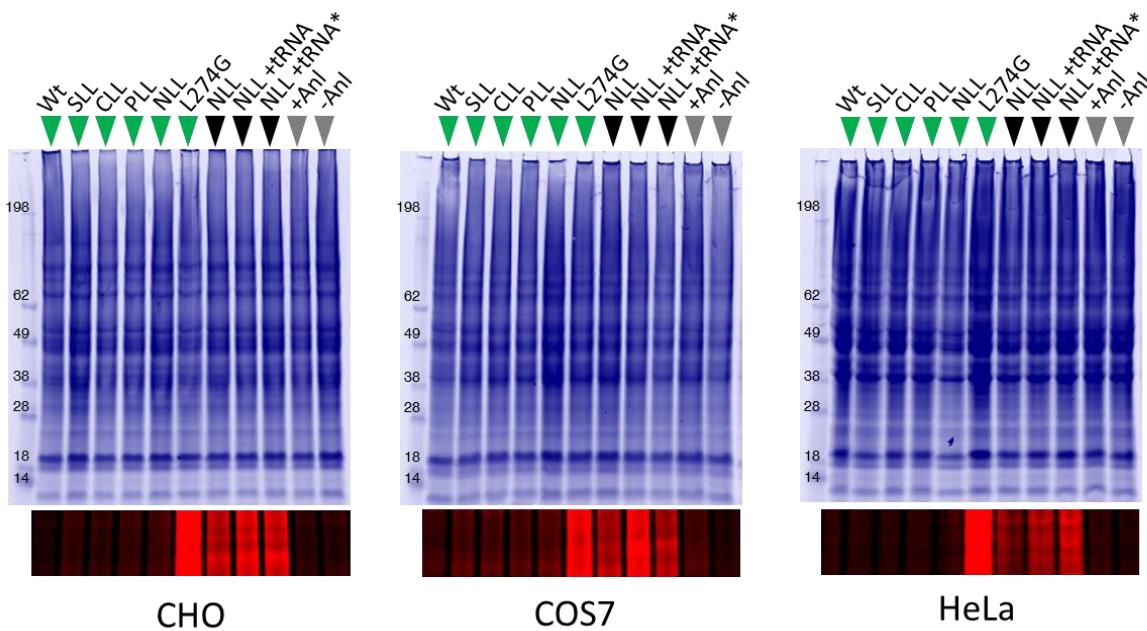
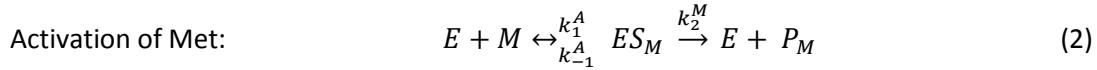
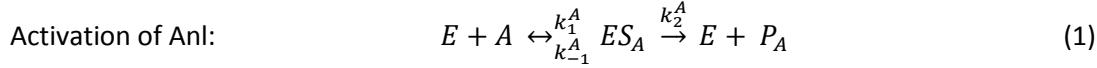


Figure S12. Selectivity of L274GMmMetRS. In-gel fluorescence was used as a measure of Anl incorporation into cellular proteins. At a fixed concentration of Met, changes in Anl concentration determine the extent of protein labeling, which depends on the relative rates of activation of Anl and Met by L274GMmMetRS.



where ES_A is the complex of L274GMmMetRS with Anl (A) and ES_M is the complex of L274GMmMetRS with Met (M). P_A is tRNA^{Anl} charged with Anl; P_M is tRNA^{Met} charged with Met.

Let $K^A = \left(\frac{k_{cat}}{K_M}\right)^{Anl}$ and $K^M = \left(\frac{k_{cat}}{K_M}\right)^{Met}$

Because A and M compete for L274GMmMetRS (see Fersht (Eq. 3.44)⁹):

$$\frac{V_A}{V_M} = \frac{A}{M} \frac{K^A}{K^M} \quad (3)$$

where V_A and V_M are the rates of activation of Anl and Met, respectively.

For a fixed Anl concentration the total rate of substrate activation by L274GMmMetRS is a constant (C):

$$C = V_A + V_M$$

Rearranging (3) gives: $K^A = \frac{V_A}{V_M} \frac{M}{A} K^M$

Eliminating V_M : $K^A = \frac{V_A}{C - V_A} \frac{M}{A} K^M$ or $V_A = \frac{K^A C}{\frac{M}{A} K^M + K^A} \quad (4)$

Dividing numerator and denominator by K^M yields:

$$V_A = \frac{A \left(\frac{K^A}{K^M} \right) C}{M + A \left(\frac{K^A}{K^M} \right)} \quad (5)$$

where $\left(\frac{K^A}{K^M} \right)$ is the selectivity, C is a constant and the Met and Anl concentrations are known. Measured values of in-gel fluorescence were fit to Equation 5 by least-squares methods at different concentrations of Anl and Met to determine the selectivity of L274GMmMetRS.

The following Matlab functions were used to fit the data from Figure 2A in the main text. The resulting parameter fits yielded a selectivity of 0.2508 and C = 1.089.

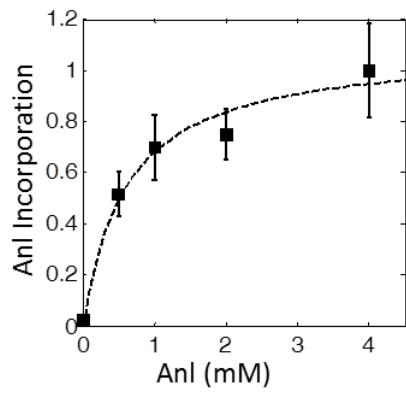


Figure S12A. Selectivity of L274GMmMetRS. In-gel fluorescence was used as a measure of Anl incorporation into cellular proteins. At a fixed concentration of Met, changes in Anl concentration determine the extent of protein labeling, which depends on the relative rates of activation of Anl and Met by L274GMmMetRS.

```

function fitfluorescenceR
function m = FF(x , xdata)
for i=1:size(xdata)
m(i)=((xdata(i)*x(1)*x(2)) / ((0.15+xdata(i)*x(1))) );
end
m=m';
end

A=[0; 0.5; 1; 2; 4];
f1=[0.022871044; 0.516731636; 0.699062794; 0.752107911; 1]
err=[0.002135075; 0.086032748; 0.127708537; 0.098922244; 0.183627695];
errorbar(A,f1,err,'ro');

% initial guess for parameters
x0=[0.5 1] % This is the selectivity parameters
% second 1 this is constant C
[x,resnorm, residual,~,exitflag,output]=lsqcurvefit(@FF,x0,A,f1);
hold on
counter=0; for i=0:0.1:4.5, counter=counter+1; II(counter)=i;
F(counter)=(i*0.2508*1.089)/(0.15+i*0.2508);end
plot(II,F)

SStotal = (length(f1)-1) * var(f1);
SSresid=sum(residual.^2);
rsq = 1 - SSresid/SStotal
end
-----
```

We tested the selectivity parameters that we obtained from Figure 2A to compare the predicted and observed Anl incorporation levels at the different Met concentrations used in Figure 2B. The following Matlab functions were used for Figure 2B in the main text. The data in Figure 2B were first normalized so that at a Met concentration of 0.15 mM, the level of Anl incorporation corresponds to that of Figure 2A; the concentration of Met used in Figure 3A is a constant 0.15 mM.

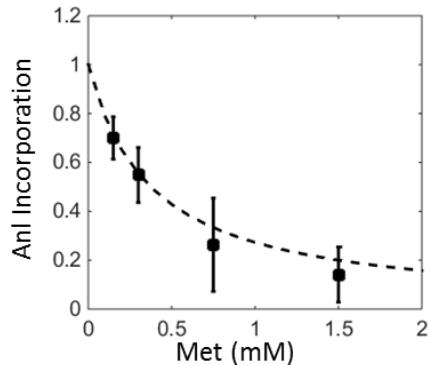


Figure S12B. Selectivity of L274GMMmMetRS. In-gel fluorescence was used as a measure of Anl incorporation into cellular proteins at a fixed Anl concentration with varying concentrations of Met. Selectivity parameters that were obtained from Figure 2A were used to obtain the predicted Anl incorporation levels shown in dotted line.

```

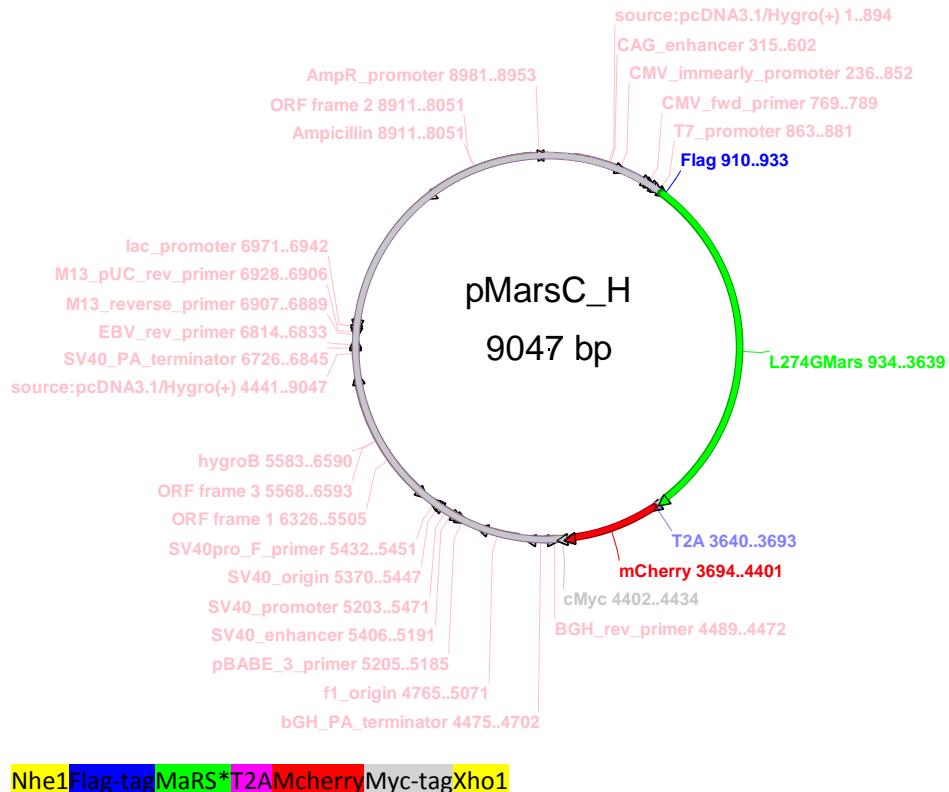
-----
function fitfluorescencetst
function m = FF(x , xdata)
for i=1:size(xdata)
m(i)=((1.5*x(1))/(xdata(i)+1.5*x(1)));
end
m=m';
end

M=[0.15;      0.3;      0.75;      1.5];
f1=[0.7;      0.548851071;    0.263135683;    0.14119813];
err=[0.086722315;    0.112658654;    0.19100414;  0.11272512];
errorbar(M,f1,err,'ro');

counter=0; for i=0:0.1:2, counter=counter+1; II(counter)=i;
F(counter)=(1.5*0.25)/(i+1.5*0.25);end
plot(II,F)

SStotal = (length(f1)-1) * var(f1);
SSresid=sum(residual.^2);
rsq = 1 - SSresid/SStotal
end
-----
```

Figure S13. The pMaRSC vector for expression of L274GMmMetRS and mCherry proteins. The cassette inserted into the Nhe1/Xho1 restriction sites of the pcDNA3.1 plasmids is color coded corresponding to highlighted sequences. Kozak sequence is highlighted in light blue. The pMaRS plasmid is the same construct as pMaRSL274G, and below is the sequence for pMaRSC which contains a T2A-Mcherry sequence appended to the C-terminal of L274GMmMetRS. This construct is available through Addgene.



Nhe1Flag-tagMaRS*T2AMcherryMyc-tagXho1

```

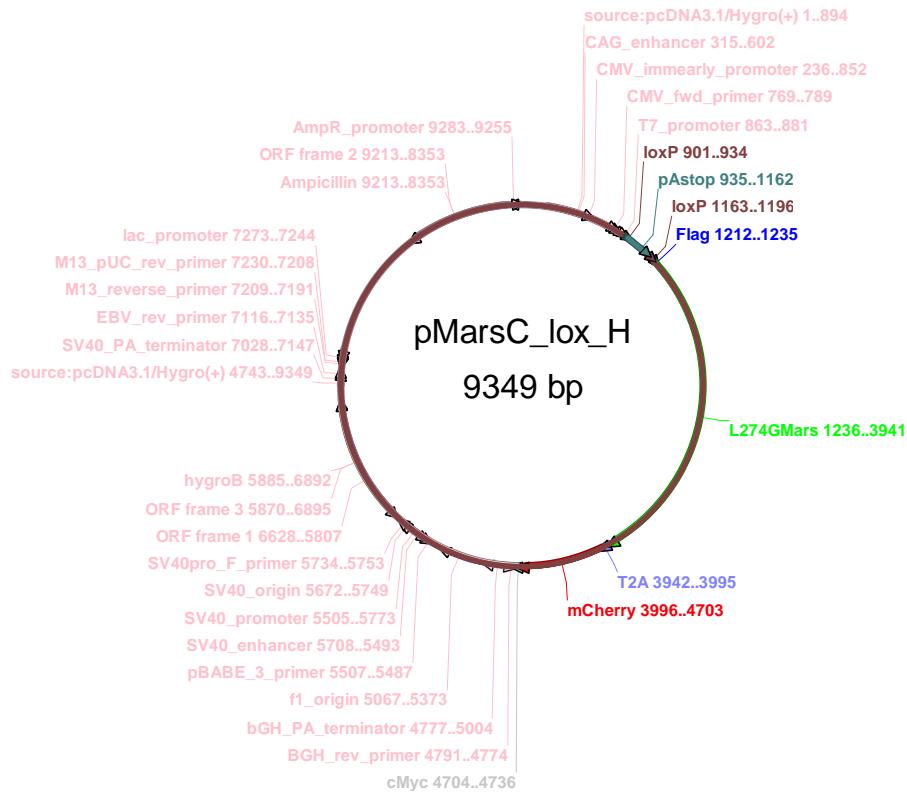
GACGGATCGGGAGATCTCCGATCCCTATGGTCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAACGCCAG
TATCTGCTCCCTGCTTGTGTTGGAGGTGCGCTGAGTAGTGCGCGAGCAAAATTAAAGCTACAAACAAGGCAGAGCTT
GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCCTTGGCGCTGCTCGCGATGTACGGGCCAGATATACGC
GTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
TATGCCAGTACATGACCTTATGGGACTTCCATTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTGACTCACGGGATTCCAAGTCTCCACCCATTG
ACGTCAATGGGAGTTGTTGGCACAAATCAACGGGACTTCCAAAATGTCGTAACAACCTCGCCCCATTGACG
CAAATGGGCGGTAGGCCTGTACGGTGGGAGGTCTATAAAGCAGAGCTCTGGCTAAGAGAACCAACTGCTTA
CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGCGCCACCATGGATTACAAGGATGAT
GATGATAAGATGAGACTGTTCGTGAGCGAGGGTTCCCGGGGAGCCTGCCCGTGTGGCTGCCCGCGAGGGCCG
GGGTGGGGAGCTGCTCATCAGCACCGTAGGCCCGAAGAGTGTGTTGACCATTCCATTCCCTAACCGGCCTAAGGTCC
CTGCTTGCAAGCTGGATACTGGCAACTACCTCTCTGCTAGTGCATCTGGCATAATTGTTCTGTTATGTGGC
TGGGAACAAGATGATCTCACCAACCAGTGGCTGGAATGGGAGGCAACAGAACTGCAGCCAGTTCTGTCTGCTGCCCT
ACACTGTCTAGTGGTCAAGGCAAGAAAGGGGAAGATATACTTGGCCCACAGAGAATCTCTAGTGCACATTGTTGTGGC
ACAGCTTGAGTCGTAAAATGTCCTTCTGGCTGGGACACAGAAATCTCTAGTGCACATTGTTGTGGGAGGCA
CTGTATCCTTACTGCAAGACCCAGTTACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAG
TACCCAGGAACCGTGTAGCGAGCTGCAGAGACGGTGTCTAAACAGCAGGGTGTCTGGCACTTCGTCTGTACCTCC
AGAAACAGCCACAGCTCAGCCCCGCCCTCTGAGGGGAGAACTGTCAGCAACGAGCTGGAGGAAGAGGAACACTGGCT
ACCTGTCTGAGGAGGACATCGTTACAGCTGTCAGCTGCCCGTGGGAGAAGGTCTGAAAGCCTGCCCTAAAGCT

```


CCAGCCGAACCTGTCGCCAGGCTAAGGCAGCGCATGCCGACGGGAGGATCTCGTCGTACCCATGGCGATGCCTG
 CTTGCCGAATATCATGGTGGAAAATGGCCGTTTCTGGATTATCGACTGTGGCCGGCTGGGTGCGGACCGCT
 ATCAGGACATAGCGTGGCTACCGTGATATTGCTGAAGAGCTGGCGGCAATGGGCTGACCCTCCTCGTCT
 TACGGTATGCCGCTCCGATTCCGAGCGCATGCCCTCTATGCCCTCTGACGAGTTCTGAGCAGGGACTCTG
 GGGTCGAATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATTGATCCACCGCCCTCTATGAAAG
 GTTGGGCTTCGGAATCGTTCCGGACGCCGGCTGGATGATCCTCCAGCGGGGATCTCATGCTGGAGTTCTCG
 CCCACCCAACTTGTTATTGAGCTTATAATGGTACAATAAGCAATAGCATCACAAATTCAAAATAAGCA
 TTTTTTCACTGCATTCTAGTTGTTGTCCAACACTCATCAATGTATCTTATCATGCTGTATACCGTCACCTC
 TAGCTAGAGCTGGCTAATCATGGTCAAGCTGTTCTGTGAAATTGTTATCCGCTCACAAATTCCACACA
 TACGAGCCGAAGCATAAAAGTAAAGCCTGGGTGCTTAATGAGTGAAGCTAACATCACATTAAATTGCGTTGCGCTCA
 CTGCCCCTTCAGTCGGAAACCTGTCGTGCCAGCTGCTTAATGAATCGGCCAACCGCGGGGAGAGGCGGTT
 GCGTATTGGCGCTCTCCGCTTCGCTACTGACTCGCTGCCGCTCGGTGCGCTGCGGAGCGGTATCAG
 CTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGATAACCGAGAAAGAACATGTGAGCAAAGGCCAG
 CAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCTGGCTTTCCATAGGCTCCGCCCCCTGACGAGCATACAA
 AAATCGACGCTCAAGTCAGAGGTTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCTTCCCCCTGGAAGCTCC
 TCGTGCCTCCTGTCCGACCCCTGCCGCTTACCGGATACTGTCCGCTTTCTCCCTGGGAAGCGTGGCGCTT
 TCTCATAGCTCACGCTGTAGGTATCTCAGTCGGTGTAGGTCGTCGCTCCAAGCTGGCTGTGACGAACCCCC
 CGTTCAGCCGACCGCTGCGCCTATCCGTAACTATCGTCTGAGTCCAACCCGTAAGACACGACTTATGCCAC
 TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCAGGGTATGAGGCGGTGCTACAGAGTTCTGAAAGTGGTGGCCT
 AACTACGGTACACTAGAAGAACAGTATTTGTATCTGCGCTGCTGAAGCCAGTTACCTTCGGAAAAGAGTTGG
 TAGCTCTTGATCCGCAAACAAACCACCGCTGGTAGCGGTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAA
 AAGGATCTCAAGAAGATCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAACGAAAACACGTTAACGTTAAGGGATT
 TTGGTCATGAGATTATCAAAAAGATCTCACCTAGATCCTTAAATTAAAATGAAGTTAAATCAATCTAAAG
 TATATATGAGTAAACTGGTCTGACAGTACCAATGCTTAATCAGTGAGGACACTATCTCAGCGATCTGTCTATTTC
 GTTCATCCATAGTTGCTGACTCCCCGTCGTGAGATAACTACGATAACGGGAGGGCTTACCATCTGGCCCAGTGCT
 GCAATGATAACCGCGAGACCCACGCTACCGGCTCCAGATTACAGCAATAAACAGCCAGCCGGAAAGGGCCGAGCG
 CAGAAGTGGCCTGCAACTTTATCCGCTCCATCCAGTCTATTAAATTGTTGCCGGGAAGCTAGAGTAAGTAGTC
 CAGTTAATAGTTGCGAACGTTGTGCCATTGCTACAGGCATCGTGTGTCAGCTCGTCGTTGGTATGGCTCA
 TTCAGCTCCGGTCCCAAACGATCAAGGCAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTCGG
 TCCTCCGATCGTGTGAGAAGTAAGTGGCGCAGTGTATCACTCATGTTATGGCAGCACTGCTACATTCTGAGA
 ATAGTGTATGCG
 CGACCGAGTTGCTCTGCCCGGTCAATACGGATAATACCGGCCACATAGCAGAACTTTAAAGTGTCTCATCAT
 TGAAAACGTTCTCGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTCAGTGAACCCACTCGT
 CACCCAACTGATCTTCAGCATCTTACCTTACCAAGCGTTCTGGGTGAGCAAAACAGGAAGGAAAATGCCGA
 AAAAAGGAAATAAGGGCAGACCGAAATGTGAATACTCATACTCTCCTTTCAATATTATTGAAGCATTATCA
 GGTTATTGCTCATGAGCGGATACATATTGAATGTATTAGAAAATAACAAATAGGGTTCCGCGCACATTTC
 CCCGAAAAGTGCCACCTGACGTC

MBYKDDDDKMRLFVSEGPGLPVAAAARARGAELLISTVGPEECVVPFLTRPKVPVLQLDGNYLFSASAI
 CRYFFLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVVQGKKGEDILGPLRRVLTHIDHLSRQNCPFLAGDTE
 SADIVLWGALYPLLQDPAYLPEELGALQSWFQTLSQEPQCRAAETVLIKQQGVLA
 RLRLQKQPQPPPEGRVSNEL
 EEEELATLSEEDIVTAVA
 AWEKGLESLPPLQLQQHPVLPVPGERNVLITSAGPYVNNVPHLGN
 IIGCVLSADV
 FARYCRLQWNTLYLCGTDEYGTATEKAMEEGLTPREICDKYHAIHADIYRWF
 GISMFDTFGR
 TTPQQT
 KITQDIFQRL
 LTRGFVLRDTVEQLRCERCARFLADRFVEGVC
 PFCGYEARGDQCDRCGKL
 INAI
 ELLKKPQCKI
 CRSCP
 VVRSSQHLF
 LDLPKLEKRLEDWL
 GKTVPGSDWTPNARFI
 IIRSLRDGLKPRC
 ITRDL
 KWGT
 PVP
 LEGFEDKV
 FYVWF
 DATIGY
 VSITANYTDQWE
 KWKNP
 E
 QVDLYQFM
 MAKDN
 VPFHGLV
 FPC
 SVLGA
 EDNY
 TLV
 KHI
 I
 ATE
 Y
 N
 Y
 EDG
 K
 F
 SK
 R
 G
 I
 G
 V
 F
 G
 D
 MAKDTGIP
 A
 DI
 W
 RF
 Y
 L
 LY
 I
 R
 PEG
 Q
 D
 S
 A
 F
 S
 W
 T
 D
 L
 L
 I
 K
 N
 N
 S
 E
 L
 L
 N
 L
 G
 N
 F
 I
 N
 R
 A
 G
 M
 F
 V
 S
 K
 F
 F
 G
 G
 C
 V
 P
 E
 M
 A
 L
 T
 P
 D
 D
 R
 L
 V
 AHV
 SW
 EL
 Q
 H
 Y
 H
 Q
 L
 L
 E
 K
 V
 R
 I
 R
 D
 A
 L
 R
 S
 I
 L
 T
 I
 S
 R
 H
 G
 N
 Q
 Y
 I
 Q
 V
 N
 E
 P
 W
 K
 R
 I
 K
 G
 G
 E
 M
 D
 R
 Q
 R
 A
 G
 T
 V
 T
 G
 M
 A
 V
 N
 M
 A
 A
 L
 L
 S
 V
 M
 L
 Q
 P
 Y
 M
 P
 T
 V
 S
 S
 T
 I
 Q
 T
 Q
 L
 L
 P
 P
 A
 C
 R
 I
 L
 A
 T
 S
 F
 I
 C
 T
 L
 P
 A
 G
 H
 R
 I
 G
 T
 V
 S
 P
 L
 F
 Q
 K
 L
 E
 N
 D
 Q
 I
 E
 N
 L
 R
 Q
 R
 F
 G
 G
 Q
 A
 K
 G
 S
 P
 K
 P
 A
 A
 V
 E
 A
 V
 T
 A
 AGSQH
 I
 Q
 T
 L
 D
 E
 V
 T
 K
 Q
 G
 N
 V
 V
 R
 E
 L
 K
 A
 Q
 K
 A
 D
 K
 N
 Q
 V
 A
 A
 E
 V
 A
 K
 I
 L
 D
 L
 K
 Q
 L
 A
 L
 A
 E
 G
 K
 P
 I
 E
 T
 P
 K
 G
 K
 K
 K
 E
 G
 R
 G
 S
 L
 L
 T
 C
 G
 D
 V
 E
 ENP
 GPM
 VSK
 GE
 EDN
 M
 A
 I
 I
 K
 E
 F
 M
 R
 F
 K
 V
 H
 M
 E
 G
 S
 V
 N
 G
 H
 E
 F
 E
 I
 E
 G
 E
 G
 E
 R
 P
 Y
 E
 G
 T
 Q
 T
 A
 K
 L
 K
 V
 T
 K
 G
 G
 P
 L
 P
 F
 A
 W
 D
 I
 L
 S
 P
 Q
 F
 M
 Y
 G
 S
 K
 A
 Y
 V
 K
 H
 P
 A
 D
 I
 P
 D
 Y
 L
 K
 L
 S
 F
 P
 E
 G
 F
 K
 W
 E
 R
 V
 M
 N
 F
 E
 D
 G
 G
 V
 V
 T
 V
 T
 Q
 D
 S
 S
 L
 Q
 D
 G
 E
 F
 I
 Y
 K
 V
 K
 L
 R
 G
 T
 N
 F
 P
 S
 D
 G
 P
 V
 M
 Q
 K
 K
 T
 M
 G
 W
 E
 A
 S
 S
 E
 R
 M
 Y
 P
 E
 D
 G
 A
 L
 K
 G
 E
 I
 K
 Q
 R
 L
 K
 L
 D
 G
 G
 H
 Y
 D
 A
 E
 V
 K
 T
 T
 Y
 K
 A
 K
 K
 P
 V
 Q
 L
 P
 G
 A
 Y
 N
 V
 N
 I
 K
 L
 D
 I
 T
 S
 H
 N
 E
 D
 Y
 T
 I
 V
 E
 Q
 Y
 E
 R
 A
 E
 G
 R
 H
 STGGM
 D
 E
 L
 Y
 K
 E
 Q
 Q
 K
 L
 I
 S
 E
 E
 D
 L
 -

Figure S14. The pMarlox vector for expression of L274GMmMetRS and mCherry proteins. The cassette inserted into the Nhe1/Xho1 restriction sites of the pcDNA3.1 plasmids is color coded corresponding to highlighted sequences. Kozak sequence is highlighted in blue. The loxP sequences and transcriptional stop sequence are inserted into the Nhe1 site of this plasmid, thereby introducing a second Nhe1 site.



Nhe1|loxstop|loxpFlagMaRS*T2AMcherryMycXho1

GACGGATCGGGAGATCTCCGATCCCCTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAACGCCAG
 TATCTGCTCCCTGCTTGTGTTGGAGGTGCGTGAGTAGTGCGCGAGCAAAATTAAAGCTACAACAAGGCAGGCTT
 GACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCCTTGCCTGCTTCGCGATGTACGGCCAGATAACGC
 GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCTATTAGTTCATAGCCCATAATGGAGTC
 CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGAC
 GTATGTTCCCATAGTAACGCCATAGGGACTTCCATTGACGTCAATGGGGAGTATTACGGTAAACTGCCACT
 TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGCCCGCTGGCAT
 TATGCCAGTACATGACCTTATGGACTTTCTACTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGT
 GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGGGTTGACTCACGGGGATTCCAAGTCTCCACCCATTG
 ACGTCAATGGGAGTTTGGCACCATAACGGGACTTCCAAATGTCGTAACAACCTCCCCCCATTGACG
 CAAATGGCCGGTAGGCCGTACGGTGGAGGTCTATAAAGCAGAGCTCTGGCTAACTAGAGAACCCACTGCTTA
 CTGGCTTATCGAAATTAAACGACTCACTATAGGGAGACCAAGCTGGCTAGCATAACCTCGTATAGCATACATTAT
 ACGAAGTTATCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGCTTGCCTCCCTCCCGTGCCTTGACCTGGAA
 AGGTGCCACTCCCACTGTCCTTCTAATAAAATGAGGAAATTGCATCGCATTGCTGAGTAGGTGTATTCTATT
 TGGGGGGTGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGCGGTGGG
 TCTATGGATAACTTCGTATAGCATACATTATAAGAAGTTATGCTAGCAGGCCACCATGATTACAAGGATGATGATGA
 TAAGATGAGACTGTTCGTGAGCGAGGGTCCCCGGGAGCCTGCCGTGCTGGCTGCCGCGAGGGCCCGGGTC

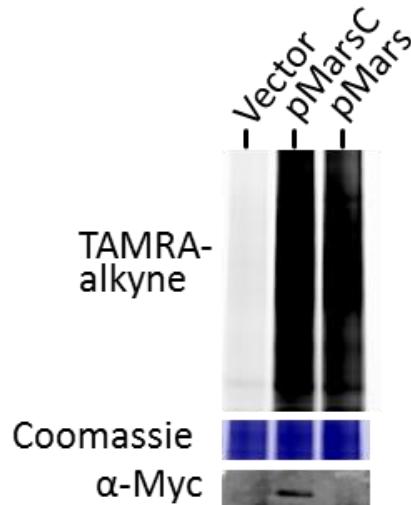
GGGCGGAGCTGCTCATCAGCACCGTAGGCCGAAGAGTGTGGTACCTCCTAACCGGCCTAAGGTCCCTGTC
 TTGCAGCTGGATAGTGGCAACTACCTCTGCTAGTGAATCTGCCATTTCTGTTATGTGGCTGGGA
 ACAAGATGATCTACCAACCAGTGGCTGGAATGGGAGGCAACAGAACTGCAGCCAGTCTGCTGCCCTACACT
 GTCTAGTGGTCAAGGCAAGAAAGGGAAAGATACTTGCCCCTCGAGAGTCCTGACTCACATTGATCACAGC
 TTGAGTCGTCAAAACTGTCCTTCTGGCTGGACACAGAACATCTAGTCAGTACATTGTTGTGGGAGCACTGTA
 TCCTTACTGCAAGACCAGCTAACCTCCCTGAGGAGTTGGGTGCCCTGCAAAGTTGGTCCAGACACTGAGTACCC
 AGGAACCGTGTAGCGAGCTGCAGAGACGGTCTAAAACAGCAGGGTGTCCGGACTCGTGTACCTCCAGAAA
 CAGCCACAGCCTCAGCCCCGCCCTGAGGGAGAACAGTCAGCAACGAGCTGGAGGAAGAGGAACGGTACCTT
 GTCTGAGGAGGACATCGTTACAGCTGTGCGCGTGGAGAAGGGTCTGGAAAGCCTGCCCTCGCTAAAGCTCCAGC
 AGCATCCAGTGTGCTGTGAGAGAGGAATGTTCTCATCACCACTGCCGACCCATGTCAACAATGTCCCC
 CACCTTGGAAACATCATTGGCTGTGCTCAGTGCAGTGTCTTGCAAGGTTATTGTCGCTTCGCCAGTGGAAATAC
 CCTCTATCTGTGTTACAGATGAGTATGGTACTGCAGAGACCAAGGCCATTGGAGGAGGGCTAACCCACGGG
 AAATCTGTGACAAGTACCATGCCATCCATGCTGACATCTACCGCTGGTTCGGCATATCGTTGATACTTCGGCGC
 ACTACCAACTCCTCAGCAGACCAAAATCACCAAGGACATCTTCCAGAGGTTGCTGACCCGGGGTTGTGCTGCGAGA
 TACTGTGGAGCAGCTCGGTGTGAGCGGTGTGCACGTTCTGGTGCACGGTTGTGGAGGGTGTGTCCTTCT
 GTGGCTATGAAGAGGCCAGGGTACAGTGTGACAGGTGTGGCAAGCTCATCAATGCCATTGAGCTCAAGAACCA
 CAGTGC AAAATCTGCCCTCCTGCCCTGTGGTGGAGGTTCTCACAGCACCTGTTCTAGACTTGCTAAGTTGAAAAA
 GCCTGAGGACTGGTGGGAAGACAGTGCCTGGCAGTGACTGGACACCCAATGCCAGGTTCAATTACGTTCT
 GGCTCGAGATGCCCTCAAGCCACGATGCATCACAGAGACCTCAAATGGGAACGCCGTGCCCCGGTGAAGGTTT
 GAGGACAAGGTATTTACGTCTGGTTGATGCTACTATTGGCTACGTGTCATCACAGCCAACATACAGACCAATG
 GGAGAAATGGTGGAAAGAACCCAGAACACAAGTGGACCTTACCAAGTICATGCCAAAGACAATGTTCCCTCCATGGCT
 TGGTCTTCCGTGTTCAAGCTCTAGGAGCTGAGGACAACACTACACCTGGTCAAGCACATCATTGCTACAGAGTACCTG
 AACTATGAGGATGGAAATTCTCTAACAGGCCGGCATAGGAGTTGGAGACATGCCAAGGATAACAGGAATCCC
 TGCTGACATCTGGCATTCTATGCTATACATTGCCCTGAGGGCCAGGACAGTGCCTTCTCTGGACAGACTGT
 TGATTAAAACAATTCTGAGCTGCTAACAAACCTGGCAACTTCATCAACAGAGCTGGATGTTGTTCTAAGTT
 TTGGCGGTTGTGCTGAGATGGCGCTAACCCCTGATGACAGACGCCGTGGCCCATGTCCTTGGAAACTCCA
 GCACTATCACCAGCTGTTGGAAAGGTTGCGATCCGGATGCCCTGCGCAGTATCCTCACCATATCTGCCATGGCA
 ACCAATACATTCAAGTGAATGAGCCCTGGAAACGGATAAGGTTGAGATGGACAGGCCAGGGCACAGTG
 ACAGGCATGGCAGTGAACATGGCTGCCCTGCTGTCATGCTGCAGCCATACGCCACAGTCAGCTTACCAT
 CCAGACCCAGCTGCAGCTCCACCTGCAGCCTGCCCATCCTGCCACAAGCTCATTGTAACCTGCCAGCAGGCC
 ACCGAATTGGCACAGTCAGTCCTTGTCCAAAATGGAAAATGACCAGATTGAAAATTGAGGCAGCGCTTGG
 GGGGGTCAGGCTAAAGCTCCCCAAGCCAGCAGCTGGAGGCAGTTACAGCAGCAGGCTCGCAGCACATAAAC
 GCTGACGGATGAGGTGACCAAGCAGGGCAACGTCGTCGGGAACGTGAAAGCACAGAAGGCAGACAAGAACCA
 CTGCAGAGGTGGCTAAACTCTGGATCTAAAGAAACAGTGGCTTGGCTGAGGGAAACCCATTGAAACTCTAAA
 GGCAAGAAGAAAAG **GAAGGGAGAGGAAGCCTCTAACATGCCGTGACGTGGAGGAGAACCCAGGACCA** ATGGTGAG
 CAAGGGCGAGGAGGATAACATGCCCATCATCAAGGAGTTGCTGCCCTCAAGGTGACATGGAGGGCTCCGTGAACG
 GCCACGAGTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCCTACGGAGGGCACCCAGACGCCAACGCTGAAGGTGACC
 AAGGGTGGCCCCCTGCCCTGCCCTGGACATCCTGCTCCCTCAGTTGATGTCAGGCTCAAGGCCCTACGTGAAGCA
 CCCCGCCGACATCCCCGACTACTTGAGCTGCTTCCCCGAGGGCTTCAAGTGGAGGCGCTGATGAACTTCGAGG
 ACGGCGCGTGGTGACCGTACCGACTCCTCCCTGCAGGACGGCGAGTTGATCTACAAGGTGAAGCTGCCGG
 ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAACAGACCATGGCTGGAGGCCTCCCGAGCGGATGTACCC
 CGAGGACGGCCCTGAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGCCACTACGACGCTGAGGTCA
 AGACCACCTACAAGGCCAAGAACGGCGTGCAGCTGCCGGCCTACAACGTCAACATCAAGTTGGACATCACCTCC
 CACAACGAGGACTACACCATCGTGAACAGTACGAACGCCGAGGGCCGCACTCCACCGCGGATGGACGAGCT
 GTACAAGGAACAAAAACTTATCTGAGAAGATCTGAA**CTCGAG**TCTAGAGGGCCGTTAACCCGCTGATCAG
 CCTCGACTGTGCCCTCTAGTTGCCAGCCATCTGTTGCCCTCCCCCGTGCCTTCCCTGACCTGGAGGTGCC
 ACTCCCCTGCTCTTCTAATAAAATGAGGAAATTGATCGCATTGCTGAGTAGGTGTCATTCTATTCTGGGGGG
 TGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGAAAGACAATAGCAGGACATGCTGGGATGCCGGTGGCTATGG
 CTCTGAGGCGGAAAGAACCCAGCTGGGCTAGGGGTATCCCCACGCCGTAGCGCGCATTAGCGCG

GGTGTGGTGGTTACGCGCAGCGTACCGCTACACTGCCAGGCCCTAGCGCCGCTCCTTCGCTTCTCCCTTC
CTTCTCGCACGTTGCCGGCTTCCCCGTCAAGCTAAATCGGGGGCTCCCTTAGGGTCCGATTTAGTGCTT
TACGGCACCTCGACCCCCAAAAAATTGATTAGGTGATGGTCACGTAGTGGGCATGCCCTGATAGACGGTTTT
CGCCCTTGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTGTTCAAACGGAAACAACACTCAACCCTATCTC
GGTCTATTCTTGATTTATAAGGGATTTGCCGATTGCCCTATTGGTAAAAAATGAGCTGATTAAACAAAAT
TTAACCGAATTAATTCTGTGGAATGTGTGCAAGTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAA
ACGATGCATCTCAATTAGTCAGCAACCAGTCAGGCCCTAACCTCCGCCATCCGCCCTAACCTCCGCCAGTTC
CGCCCATTCGCCCATGGCTGACTAAATTTTTATTGCAAGAGGCCCTAGGTTGCAAAAGCTCCCAGGCTGTATCCATTTC
TCCAGAAGTAGTGAGGAGGCTTTTGAGGCCCTAGGTTGCAAAAGCTCCCAGGCTGTATATCCATTTC
GGATCTGATCAAGAGACAGGATGAGGATCGTTGCACTGATTGAAACAAGATGGATTGCAACGAGGTTCTCCGCCGC
TTGGTGGAGAGGCTATTGGCTATGACTGGGACAACAGACAATCGGCTGCTGATGCCCGTGTCCGGCTGT
CAGCGCAGGGCGCCGGTCTTTGTCAAGACCGACCTGTCGGTGCCTGAATGAACACTGCAGGACGAGGCAGCG
CGGCTATCGGCTGGCCACGACGGCGTCTGCACTGACGCTGTGACGTTGCACTGAAGCGGGAAAGGACTG
GCTGCTATTGGCGAAGTGCCGGCAGGATCTCCTGTCATCTCACCTGCTCTGCCAGAAAGTATCCATCATGG
CTGATGCAATGCGCGCGCTGCATACGCTTGTGATCCGGTACCTGCCATTGACCAAGCGAAACATCGCATCGAG
CGAGCACGTACTCGGATGGAAGCCGGTCTGCACTGAGGATGATCTGGACGAAGAGCATCAGGGCTCGGCCAGC
CGAACTGTCGCCAGGCTCAAGGCGCGATGCCGACGGCGAGGATCTCGTGTGACCCATGGCGATGCCCTGCTTC
CGAATATCATGGTGGAAAATGGCGCTTTCTGGATTGATCGACTGTCGGCTGGGGATCTCATGCTGGAGTTCTCGCCAC
GACATAGCGTTGGCTACCGTGTATATTGCTGAAGAGCTTGGCGCGAATGGCTGACCGCTTCCTCGTGTACGG
TATCGCCGCTCCGATTCGAGCCATGCCCTCTGCCCTTGACGAGTTCTGAGCGGGACTCTGGGTT
CGAAATGACGACCAAGCGACGCCAACCTGCCATACGAGATTGATCCACCGCCCTCTATGAAAGGTTGG
GCTTCGGAATCGTTCCGGGACGCCGGCTGGATGATCTCCAGCGCGGGATCTCATGCTGGAGTTCTCGCCAC
CCCAACTGTTATTGAGCTTAAATGGTACAAATAAGCAATAGCATCACAAATTCAAAATAAGCATTTT
TTCACTGCATTCTAGTTGTTGCAACTCATCAATGTATTTATCATGCTGTATACCGTCACCTCTAGCT
AGAGCTTGGCGTAATCATGGTCACTGACTGTTCTGTCGAAATTGTTATCCGTCACAATTCCACACAATACGA
GCCCGAAGCATAAGTGTAAAGCTGGGTCATAATGAGTGGACTAACATTAATTGCGTTGCGCTACTGCC
CGCTTCCAGTCGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGAGAGGCAGGTTGCGTA
TTGGCGCTTCCGCTTCCGCTACTGACTCGCTCGCTCGTGGCTGCCGAGCGGTATCAGCTCAC
TCAAAGCGGTAAACGGTTATCCACAGAACGAGGATAACGAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA
GGCCAGGAACCGTAAAAGGCCGTTGCTGGCTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATC
GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCGAGGTTCCCCCTGGAAGCTCCCTCGTG
CGCTCTCCTGTTCCGACCCCTGCCCTACGGGATAACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCTTCTCA
TAGCTCACGCTGTAGGTATCTCAGTTGGCTAGGTGCTCGCTCAAGCTGGCTGTCACGAACCCCCCGTTC
AGCCCGACCGCTGCCCTATCCGTAACTATGCTTGAGTCCAACCCGGTAAGACACGACTATGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGAGGCGGTACAGAGTTCTGAAAGTGGGCTAACTA
CGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGTCGAAGCCAGTACCTTGGAAAAGAGTTGGTAGCT
CTGATCCGCAAACAAACCACCGCTGGTAGCGGTTTTGTTGCAAGCAGATTACGCGCAGAAAAAGGA
TCTCAAGAAGATCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAAACACTCACGTTAAGGGATTTGGT
CATGAGATTATCAAAAGGATCTCACCTAGATCCTTAAATTAAAATGAAGTTAAATCAATCTAAAGTATAT
ATGAGTAAACTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGGATCTGTCTATTGCTCA
TCCATAGTTGCCCTGACTCCCCGCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGCCCTAGTGTGCAAT
GATACCGCGAGACCCACGCTCACCGCTCCAGATTATCAGCAATAAACCGCCAGCCAGGGAAAGGCCGAGCGCAGAA
GTGGTCTGCAACTTATCCGCCATCCAGTCTATTAAATTGTCGGGAAGCTAGAGTAAGTAGTTGCCAGTT
AATAGTTGCGCAACGTTGTCATTGCTACAGGCATCGTGGTGTACGCTCGTGTGGTATGGCTTCTCATTGAG
CTCCGGTTCCAACGATCAAGGCAGGTTACATGATCCCCCATGTTGCAAAAGCGGTTAGCTCCTCGGTCTC
CGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGTTATGGCAGCAGTCAGCATAATTCTTACTGTC
ATGCCATCCGTAAGATGCTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTGAGAATAGTGTATGCCGAC
GAGTTGCTCTGCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTAAAGTGTCTCATCATTGAA

AACGTTCTCGGGCGAAAACTCTAAGGATCTTACCGCTGTTGAGATCCAGTCGATGTAACCCACTCGTCACCC
AACTGATCTCAGCATTTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCACCGCAAAAAA
GGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTT
ATTGTCTCATGAGCGGATAACATATTGAATGTATTTAGAAAATAACAAATAGGGTCCGCGCACATTCCCCGA
AAAGTGCCACCTGACGTC

MDYKDDDDKMRLFVSEGSPGSLPVAAAARARGRAELLISTVGPEECVVPFLTRPKVPVLQLDSGNYLFSASAICRY
FLLLCGWEQDDLTNQWLEWEATELQPVLSAALHCLVVQGKKGEDILGPLRRVLTHIDHSLSRQNCPFLAGDTESSLAD
IVLWGALYPLLQDPAYLPEELGALQSWFQTLSTQEPCQRAAEVLIKQQGVIALRLYQKQPQPPPEGRTVSNE
EEEELATLSEEDIVTAVAWEKGLESLPPKLQQHPVLPVPGERNVLITSAGPYVNNVPHLGNIIGCVLSADVARY
CRLRQWNTLYLCGTDEYGTATEKAMEEGLTPREICDKYHAIHADIYRWFGISFTFGRTTPQQTKITQDIFQRLL
TRGFVLRDTVEQLRCERCARFLADRVEGVCFCGYEARGDQCDRCGKLINAIELKKPQCKICRSCPVRSSQHLF
LDLPLKEKRLEDWLKGKTVPGSDWTPNARFIIRSWLRDGLKPRCITRDLKWGTPVPLEGEDKVFYVWFDATIGYVS
TANYTDQWEKKWKNPEQVDLYQFMAKDNVPFHGLVFPCSVLGAEDNYTLVKHIATEYLNEDGKFSKSRGIGVFGD
MAKDTGIPADIWRFYLLYIRPEGQDSAFSWTDLLIKNNSELLNNLGNFINRAGMFVSKFFGGCVPEMALTPDERRLV
AHVSWELQHYHQLLEKVRIRDALRSILTISRHGNQYIQVNEPWKRICKGEMDRQRAGTVTGMVNMAALLSVMQPY
MPTVSSTIQTQLQLPPAACRILATSFIGTLPAHRIGHTVSPLFQKLENDQIENLRQRFGGGQAKGSPKPAAVEAVTA
AGSQHQIQTLDDEVTKQGNVVRELKAQKADKNQVAEVAKLIDLKQLALAEGKPIETPKGKKKEGRGSLLTCGDVE
ENPGPMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGERPYEGTQAKLKVTKGGLPFAWDILSPQFMY
GSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGIFIYKVKLRTTNFPSDGPVMQKKTMGWE
ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRH
STGGMDELYKEQKLISEEDL-

Figure S15. Western blot for detection of L274GMmMetRS and Anl labeled proteins. Metabolic incorporation of Anl by pMaRS- and pMaRSC-transfected CHO cells. The in-gel fluorescence image on top shows TAMRA labeling, which indicates Anl incorporation. Western blot at the bottom using anti-Myc antibody shows the detection of mCherry at approximately 25 kDa.



Western blot using a Myc-tag-Alexa Fluor 488 conjugate monoclonal antibody was used to probe for mCherry in lysates of CHO cells transfected with pMaRSC and pMaRS vectors. The pMaRSC lane shows a protein band at approximately 25 kDa corresponding to mCherry and no other bands at higher molecular weights indicating that mCherry is not fused to L274GMmMetRS. The pMaRS vector lacks the mCherry sequence and as anticipated we do not observe a protein band corresponding to mCherry in the cell lysates.

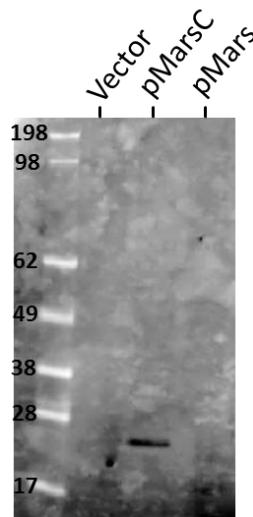
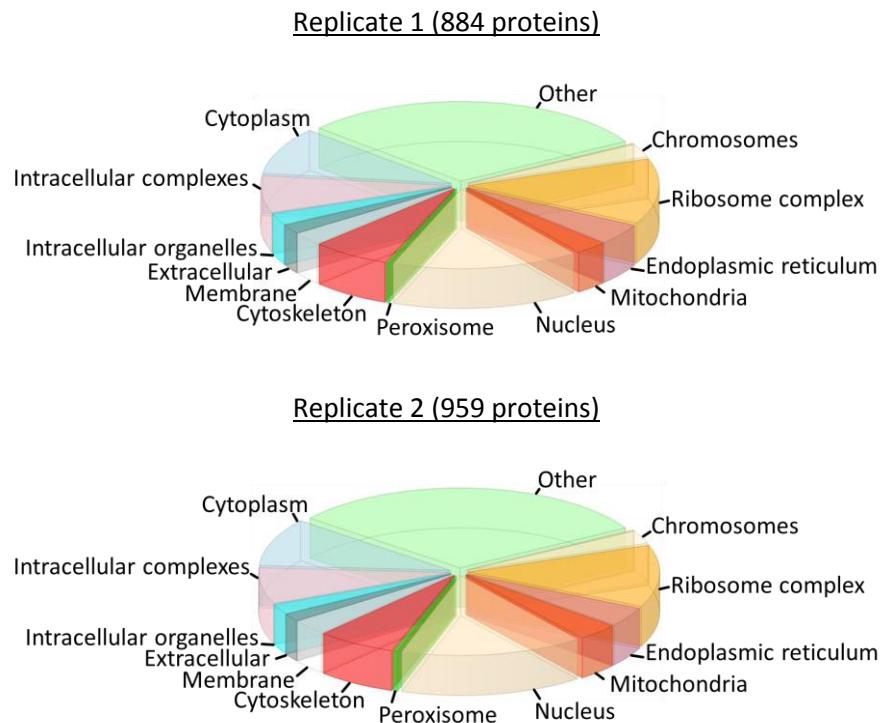


Figure S16. Identification of Anl-labeled proteins by tandem mass spectrometry and annotation in terms of cellular components using STRAP software. BONCAT was used to identify proteins made in CHO cells that constitutively express the L274G/MmMetRS under control of the CMV promoter. Cells were labeled in media containing either 2 mM Anl or with 2 mM Met. The Met samples were used to account for proteins that bind non-specifically to the resin during enrichment. Two biological replicates were performed for each amino acid with independent mass spectrometric runs for each. The charts below show the distributions of proteins identified in the Anl-labeled samples.



The proteins identified in either of the Met samples were removed from the list of proteins identified in either of the Anl samples. The result of this analysis is shown in the chart below and included in the main text in Figure 3d.

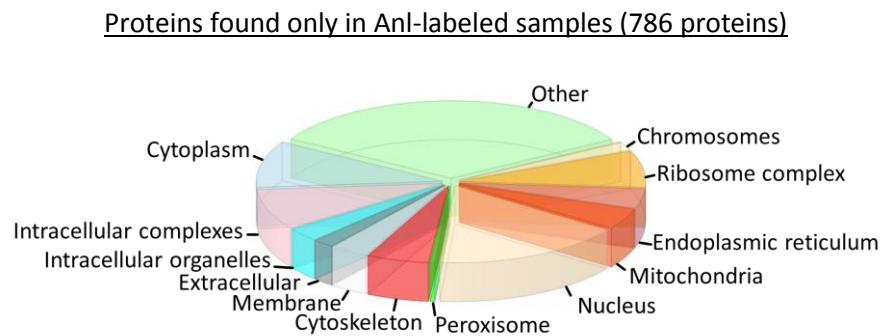


Figure S17. Assessment of enrichment and reproducibility in BONCAT analysis. The extent to which BONCAT allows enrichment of newly synthesized proteins was assessed by comparing the numbers of proteins found in Anl- and Met-treated samples, and by comparing spectral counts. We identified a total of 847 proteins that were found in both Anl replicates, of which 724 were not present in the Met controls. There were 129 proteins that were in both Met samples (a). Combining the total proteins identified in either of the Anl samples resulted in 996 proteins, 786 of which were not present in either of the Met samples (b). Comparison of the proteins identified in the two Anl replicates (c) and the two Met replicates (d) shows low sample-to-sample variability for the Anl replicates. Reproducibility of quantified protein levels across biological replicates was determined using MaxQuant's label-free quantification (LFQ) value. LFQ serves as a normalized measure of relative protein abundance. These results are shown as dot plots of LFQ levels between Anl replicates (e) and Met replicates (f). On average, we observed 19-fold more spectral counts for Anl samples than for Met samples (g). The numbers in panel g represent the spectral counts acquired in individual MS runs.

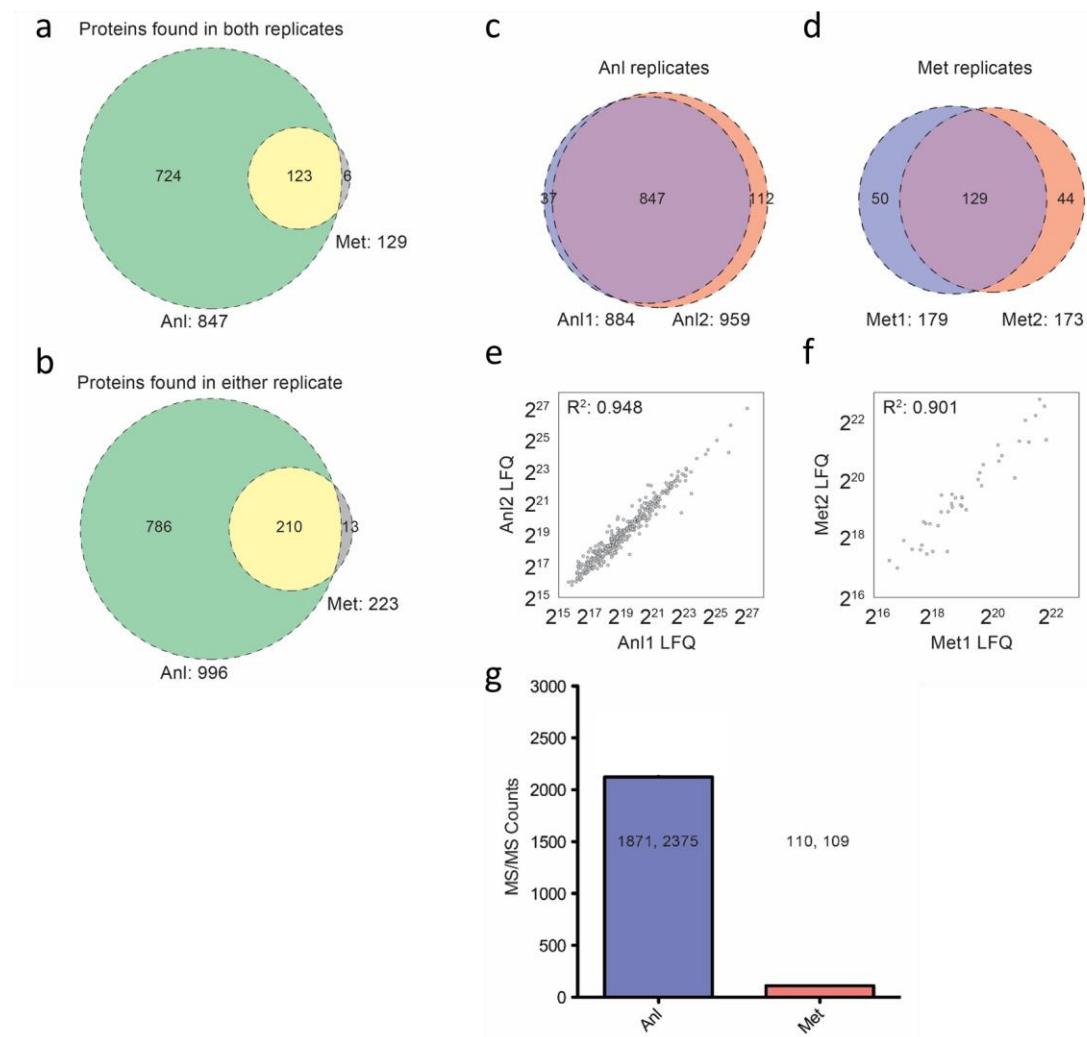
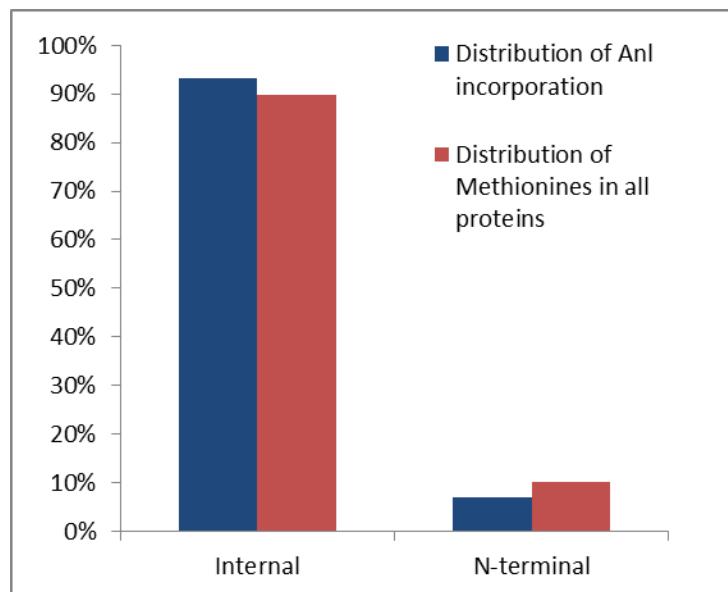


Figure S18. Incorporation of Anl at terminal and internal Met sites in HeLa cells. According to UniProt (<http://www.uniprot.org/>), there are 711,731 internal and 79,802 N-terminal Met residues in the human proteome; internal Met sites constitute 90% of the total. Mass spectrometric analysis of proteins made in HeLa cells expressing the L274GMmMetRS (see table S2 for protein list) identified 161 sites of Anl incorporation; 150 (93%) at internal Met positions.



SUPPLEMENTARY REFERENCES

- (1) Link, A. J.; Vink, M. K.; Tirrell, D. A. *Nat. Protoc.* **2007**, 2, 1884.
- (2) Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem. Int. Ed.* **2009**, 48, 9879.
- (3) Mahdavi, A.; Szychowski, J.; Ngo, J. T.; Sweredoski, M. J.; Graham, R. L.; Hess, S.; Schneewind, O.; Mazmanian, S. K.; Tirrell, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, 111, 433.
- (4) Szychowski, J.; Mahdavi, A.; Hodas, J. J.; Bagert, J. D.; Ngo, J. T.; Landgraf, P.; Dieterich, D. C.; Schuman, E. M.; Tirrell, D. A. *J. Am. Chem. Soc.* **2010**, 132, 18351.
- (5) Wisniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M. *Nat. Methods* **2009**, 6, 359.
- (6) Kalli, A.; Smith, G. T.; Sweredoski, M. J.; Hess, S. *J. Proteome Res.* **2013**, 12, 3071.
- (7) Cox, J.; Mann, M. *Nat. Biotechnol.* **2008**, 26, 1367.
- (8) Bhatia, V. N.; Perlman, D. H.; Costello, C. E.; McComb, M. E. *Anal. Chem.* **2009**, 81, 9819.
- (9) Fersht, A. *Structure and Mechanism in Protein Science : A Guide to Enzyme Catalysis and Protein Folding*; W.H. Freeman: New York, 1999.