Supplementary information

Engineering of methionine-auxotroph *Escherichia coli* via parallel evolution of two enzymes from *Corynebacterium glutamicum's* direct-sulfurylation pathway enables its recovery in minimal medium

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Figure S1. Sequence alignment of MetX of the following bacterial strains: *Geobacter lovleyi* (B3E278), *Leptospira interrogans* (Q8F4I0), *Thermus thermophilus* (Q9RA51), *Anabaena variabilis* (Q3M5Q6), *Deferribacter desulfuricans* (D3P9D1), *Chloroflexus aurantiacus* (A9WKM8), *Salinibacter ruber* (Q2S5A6), *Mycobacterium* hassiacum (K5B926), *Mycobacterium*smegmatis (A0QSZ0), *Anabaena variabilis* (Q3M5Q6) and *Chroococcidiopsis thermalis* (K9TW70).

Table S1. Protein sequences.

Protein name	Sequence
MetX CG	MPTLAPSGQLEIQAIGDVSTEAGAIIKNAEIAYHRWGEYRVDKEGRSNVVLIEHALTGDSN AADWWADLLGPGKAINTDIYCVICTNVIGGCNGSTGPGSMHPDGNFWGNRFPATSIRDQ VNAEKQFLDALGITTVAAVLGGSMGGARTLEWAAMYPEIVGAAAVLAVSARASAWQIGIQ SAQIKAIENDHHWHEGNYYESGCNPATGLGAARRIAHLTYRGELEIDERFGTKAQKNEN PLGPYRKPDQRFAVESYLDYQADKLVQRFDAGSYVLLTDALNRHDIGRDRGGLNKALESI KVPVLVAGVDTDILYLYHQQEHLSRNLGNLLAMAKIVSPVGHDAFLTESRQMDRIVRNFF SLISPDENNPSTYIEFYI
MetX CG R46H, L315P	MPTLAPSGQLEIQAIGDVSTEAGAIIKNAEIAYHRWGEYRVDKEGHSNVVLIEHALTGDSN AADWWADLLGPGKAINTDIYCVICTNVIGGCNGSTGPGSMHPDGNFWGNRFPATSIRDQ VNAEKQFLDALGITTVAAVLGGSMGGARTLEWAAMYPEIVGAAAVLAVSARASAWQIGIQ SAQIKAIENDHHWHEGNYYESGCNPATGLGAARRIAHLTYRGELEIDERFGTKAQKNEN PLGPYRKPDQRFAVESYLDYQADKLVQRFDAGSYVLLTDALNRHDIGRDRGGLNKALESI KVPVLVAGVDTDILYPYHQQEHLSRNLGNLLAMAKIVSPVGHDAFLTESRQMDRIVRNFF SLISPDENNPSTYIEFYI
MetY CG	MGPKYDNSNADQWGFETRSIHAGQSVDAQTSARNLPIYQSTAFVFDSAEHAKQRFALE DLGPVYSRLTNPTVEALENRIASLEGGVHAVAFSSGQAATTNAILNLAGAGDHIVTSPRLY GGTETLFLITLNRLGIDVSFVENPDDPESWQAAVQPNTKAFFGETFANPQADVLDIPAVAE VAHRNSVPLIIDNTIATAALVRPLELGADVVVASLTKFYTGNGSGLGGVLIDGGKFDWTVE KDGKSVFPYFVTPDAAYHGLKYADLGAPAFGLKVRVGLLRDTGSTLSAFNAWAAVQGID TLSLRLERHNENAIKVAEFLNNHEKVEKVNFAGLKDSPWYATKEKLGLKYTGSVLTFEIKG GKDEAWAFIDALKLHSNLANIGDVRSLVVHPATTTHSQSDEAGLARAGVTQSTVRLSVGI ETIDDIIADLEGGFAAI
MetY-del	MGPKYDNSNADQWGFETRSIHAGQSVDAQTSARNLPIYQSTAFVFDSAEHAKQRFALE DLGPVYSRLTNPTVEALENRIASLEGGVHAVAFSSGQAATTNAILNLA
CAB0498630	MPTKYDNSNANKWGFETRSIHAGQSVDSDTGARNLPIYLTSSYVFNDAEHAANRFNLSD AGPVYSRLTNPTVAAVEERLANLEGGVHAVLFASGMAAETAAILNIARAGSHIVSSPRIYG GTETLFAVTLHAWASKPLS
Link to sequencing results of Naive and enriched library	https://drive.google.com/drive/folders/1t8xtPBNrBfUSPrZRAVRsKONzSCJ5YzMx?usp=sharing

Table S2. Vitamin mix

Vitamin mix	500 mL
p-aminobenzoic acid	0.5 gr
Niacin	0.5 gr
Pyridoxine	0.5 gr
Riboflavin	0.5 gr
Thiamin HCl	0.5 gr
Choline HCl	0.5 gr
d-Biotin	1 mg
Store at 4 °C in the dark after autoclaving	

Table S3. Trace element solution

Trace elements solution	250 mL (H2O
H_3BO_3	100 mg
$CuSO_4\ 5H_2O$	100 mg
Iron chloride $4H_2O$	200 mg
$MnCl_2$	200 mg
$NaMoO_4\ 2H_2O$	200 mg
$ZnSO_4\ 7H_2O$	2 gr
Add HCl until the brown murky solution turns clear and yellow, filter sterile through 0.2 µM, keep in the dark.	

Table S4. Primers used in this study

Primer name	Sequence	Use
cTrc_Ins_Fw	ATAAGGAGGAATAAACCATGGGTCCG	Error-prone PCR and sequencing
cTrc_Ins_Rev	GGTACCAGCTGCAGATCTCGAGTTA	Error-prone PCR and sequencing
metY_F_SEQ	AAAGTTCGTGTGGGCCTGCT	Sequencing
metY_R_SEQ	AGCAGGCCCACACGAACTTT	Sequencing
metX_F_SEQ	TGGTTCTGATCGAACACGCGC	Sequencing

Table S5. Sequence of MetYX CG construct

Gene	Sequence
Wld-type MetXY gene of CG that was cloned and transformed to the E. coli	> metYX_CG_ OQ291222 gaattcTTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTT AACTTTAAGAGAGGTATATATTACCATGGGTCCGAAGTACGACAACAGC AACGCGGATCAGTGGGGCTTCGAGACCCGTAGCATCCACGCGGGTCA GAGCGTTGACGCGCAAACCAGCGCGCGTAACCTGCCGATTTACCAGA GCACCGCGTTCGTGTTTGACAGCGCGGAGCACGCGAAACAACGTTTC GCGCTGGAAGATCTGGGCCCGGTTTATAGCCGTCTGACCAACCCGAC CGTGGAGGCGCTGGAAAACCGTATTGCGAGCCTGGAGGGTGGCGTT CATGCGGTGGCGTTTAGCAGCGGTCAGGCGGCGACCACCAACGCGAT CCTGAACCTGGCGGGTGCGGGTGACCACATTGTTACCAGCCCGCGTC TGTATGGTGGCACCGAAACCCTGTTCCTGATCACCCTGAACCGTCTGG GCATTGATGTTAGCTTTGTGGAGAACCCGGATGATCCGGAAAGCTGGC AGGCGGCGGTTCAACCGAACACCAAGGCGTTCTTTGGCGAGACCTTT GCGAACCCGCAAGCGGACGTGCTGGATATCCCGGCGGTTGCGGAAG TGGCGCACCGTAACAGCGTTCCGCTGATCATTGACAACACCATTGCGA CCGCGGCGCTGGTGCGTCCGCTGGAGCTGGGTGCGGATGTGGTTGT GGCGAGCCTGACCAAGTTCTACACCGGTAACGGCAGCGGTCTGGGTG GCGTTCTGATCGACGGTGGCAAATTTGATTGGACCGTGGAAAAGGAC GGCAAAAGCGTTTTCCCGTATTTTGTTACCCCGGATGCGGCGTACCAC GGTCTGAAGTATGCGGATCTGGGTGCGCCGGCGTTTGGTCTGAAAGT TCGTGTGGGCCTGCTGCGTGACACCGGTAGCACCCTGAGCGCGTTTA ACGCGTGGGCGGCGGTTCAAGGCATCGATACCCTGAGCCTGCGTCTG GAGCGTCACAACGAAAACGCGATTAAGGTGGCGGAGTTCCTGAACAA CCACGAGAAGGTTGAAAAAGTGAACTTTGCGGGTCTGAAGGATAGCC CGTGGTACGCGACCAAGGAAAAACTGGGCCTGAAATATACCGGTAGC GTGCTGACCTTCGAGATCAAGGGTGGCAAAGACGAAGCGTGGGCGTT TATTGATGCGCTGAAACTGCACAGCAACCTGGCGAACATCGGCGACGT TCGTAGCCTGGTTGTGCATCCGGCGACCACCACCCATAGCCAAAGCG ATGAGGCGGGCCTGGCGCGTGCGGGTGTGACCCAAAGCACCGTTCG TCTGAGCGTGGGTATCGAGACCATTGACGATATCATTGCGGACCTGGA AGGTGGCTTCGCGGCGATTTAAGGATCCAGAGGTATATATTAatgCCGAC CCTGGCGCCGAGCGGTCAGCTGGAGATCCAAGCGATTGGTGACGTTA GCACCGAGGCGGGCGCGATCATTACCAACGCGGAAATTGCGTACCAC CGTTGGGGTGAGTATCGTGTGGACAAAGAAGGCCGTAGCAACGTGGT TCTGATCGAACACGCGCTGACCGGTGATAGCAACGCGGCGGACTGGT GGGCGGATCTGCTGGGTCCGGGCAAGGCGATCAACACCGACATTTAC TGCGTTATCTGCACCAACGTGATCGGTGGCTGCAACGGCAGCACCGG TCCGGGCAGCATGCACCCGGATGGTAACTTCTGGGGCAACCGTTTTC CGGCGACCAGCATTCGTGACCAGGTTAACGCGGAGAAACAATTCCTG GATGCGCTGGGTATTACCACCGTTGCGGCGGTGCTGGGTGGCAGCAT GGGTGGCGCGCGTACCCTGGAGTGGGCGGCGATGTATCCGGAAACC GTTGGTGCGGCGGCGGTGCTGGCGGTTAGCGCGCGTGCGAGCGCGT GGCAGATCGGCATTCAGAGCGCGCAAATCAAGGCGATTGAAAACGATC ACCACTGGCACGAGGGTAACTACTATGAAAGCGGCTGCAACCCGGCG ACCGGTCTGGGTGCGGCGCGTCGTATTGCGCACCTGACCTACCGTGG TGAGCTGGAAATCGACGAGCGTTTTGGCACCAAGGCGCAGAAAAACG AAAAACCCGCTGGGTCCGTATCGTAAGCCGGATCAACGTTTCGCGGTTG

AGAGCTACCTGGACTATCAGGCGGATAAACTGGTTCAACGTTTTGACG
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CCAGCAAGAACACCTGAGCCGTAACCTGGGTAACCTGCTGGCGATGG
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GTCAAATGGATCGTATTGTGCGTAACTTCTTTAGCCTGATCAGCCCGGA
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CCTGGAGGCGGGCGCAGGCCCGCCTTTTaagctt