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Supporting information for:

Industrializing a bacterial strain for L-serine production through translation initiation optimization

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Supplementary Figure S1.

Supplementary Figure S1. Development and validation of a medium throughput HPLC method for the detection of L-serine in microbial culture supernatant. (a) Overlaid HPLC chromatogram of L-serine standards. (b) The recovery of L-serine from spiked samples (n=5). (c) Reproducibility of the method (n=5) for different L-serine concentrations. (d) Calibration curve for L-serine.

Supplementary Figure S2.

Supplementary Figure S2. Translation initiation rate prediction of the input library and library reduced by RedLibs. Histograms of the translation initiation rate predictions for fully degenerate input libraries at the Shine-Dalgarno sequence for *serA* (**a**) and *serC* (**b**) and the output libraries reduced by RedLibs¹ to 144-member libraries with a uniform target distribution for *serA* (**c**) and *serC* (**d**). In the bottom panels, dashed lines mark the translation initiation rates of the variants that were chosen for further analysis. The individual predictions for the fully degenerate input libraries were calculated using the RBS Calculator².

a

Supplementary Figure S4.

Supplementary Figure S4. Protein ratios of different *serA^{mut}CB* operons in comparison.

Ratios of SerA to SerC and SerA to SerB were calculated for the original synthetic operon pSEVA27-sl-*serA^{mut}CB* (black), the pSEVA27-sl-*serA^{mut}CB* TIR 1 operon (orange), the pSEVA27-sl-*serA^{mut}CB* RedLibs 4 operon (blue) and the high copy two plasmid system (grey).

Supplementary Figure S5.

Supplementary Figure S5. Growth of all selected operons during batch fermentations.

The optical density (OD₆₀₀) of all strains was measured during a 24-hour serine production experiment.

Supplementary Figure S6.

Supplementary Figure S6. Assessment of effects of expression optimization of *serC*-hp-*bla*. Expression of *serC* was translationally coupled to the ampicillin resistance gene *bla*. Expression levels of the original construct (orig) and selected expression library variants (var 1-8) were estimated by resistance to ampicillin (**a**). The optical density of cultures expressing the original construct or a selected library variant was analysed after 20 hours of expression in the absence of ampicillin (**b**).

Supplementary Table S1.

Supplementary Table S1. Strains used in this study

Strain	Genotype	Source/Reference
<i>E. coli</i> NEB5 α	fhuA2 Δ (argF-lacZ)U169 phoA glnV44 Φ 80 Δ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17	^a
<i>E. coli</i> MC1061	araD139, Δ (ara, leu)7697, Δ lacX74, galU-, galK-, hsr-, hsm+, strA	In house
<i>E. coli</i> BL21(DE3)	F ⁻ <i>ompT</i> <i>hsdS</i> _B (r _B ⁻ m _B ⁻) <i>gal dcm</i> (DE3)	^b
<i>E. coli</i> ALE-5(DE3)	MG1655 Δ <i>sdaA</i> Δ <i>sdaB</i> Δ <i>tdcG</i> Δ <i>glyA</i> <i>thrA</i> ^{mut} λ (DE3)	³
<i>E. coli</i> ALE-5(DE3) <i>serA</i> ^{mut} CB TIR1	MG1655 Δ <i>sdaA</i> Δ <i>sdaB</i> Δ <i>tdcG</i> Δ <i>glyA</i> <i>thrA</i> ^{mut} <i>glsmS</i> - <i>pstS</i> :: <i>serA</i> ^{mut} CB λ (DE3)	This study

^aNEB, Ipswich, MA, USA; ^bNovagen, Merck KGaA, Darmstadt, Germany

Supplementary Table S2.

Supplementary Table S2. Plasmids used in this study

Plasmid	Property	Source/Reference
pCDFDuet-1- <i>serAmut-serC</i>	vector encoding <i>serA</i> and <i>serC</i> , Sp ^R	3
pACYCDuet-1- <i>serB</i>	vector encoding <i>serB</i> , Cm ^R	3
pSEVA27-sl3- <i>narK-gfp</i>	vector encoding <i>narK-gfp</i> , Km ^R , used to amplify pSEVA27-sl backbone	4
pACYC- <i>dasher</i> <i>gfp</i> -hp- <i>bla</i>	vector encoding <i>dasher</i> <i>gfp</i> translationally coupled to <i>bla</i> , Cm ^R , used to amplify hp- <i>bla</i>	5
pSEVA27-sl3- <i>serAmut</i> -hp- <i>bla</i>	vector encoding <i>serA</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> -hp- <i>bla</i>	vector encoding a synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-opt-serCB</i> -hp- <i>bla</i>	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-opt-serCB</i> opt-hp- <i>bla</i>	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> -hp- <i>bla</i> RedLibs1	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> -hp- <i>bla</i> RedLibs2	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> -hp- <i>bla</i> RedLibs3	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> -hp- <i>bla</i> RedLibs4	vector encoding an optimized synthetic operon <i>serACB</i> translationally coupled to <i>bla</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i>	vector encoding a synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-opt-serCB</i>	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-opt-serCB</i> opt	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> RedLibs1	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> RedLibs2	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> RedLibs3	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study
pSEVA27-sl3- <i>serAmut-serCB</i> RedLibs4	vector encoding an optimized synthetic operon <i>serACB</i> , Km ^R	This study

pGEM-FRT-KanR	vector encoding Km ^R flanked by FRT sites for integration into the genome, Amp ^R	In house
pGEM-FRT-KanR-FRT- <i>serAmut-serCB</i> TIR 1	vector encoding the optimized synthetic operon <i>serACB</i> TIR 1 and Km ^R flanked by FRT sites for integration into the genome, Amp ^R	This study

Supplementary Table S3.

Supplementary Table S3. Oligonucleotides used in this study

No.	Name	Sequence (5' --> 3')
Cloning oligonucleotides		
1	pSEVA27_fwd	AGCTGAGGUCGCCTCAGC
2	pSEVA27_T7_rev	AAACTGGTCUCCTTCTTAAAGTTAAACAAAATTATTTCTAGAG
3	SerA_fwd	AGACCAGTTUATGGCAAAGGTATCGC
4	SerA_rev	ACCTCCTAUGTCAGTACAGCAGACG
5	hp_Bla_fwd	ATAGGAGGUCCTCCTATGTCAATTCAACATTTCCTGTC
6	hp_Bla_rev	ACCUCAGCUTACCAATGCTTAATCAGTG
7	SerA-C_rev	ACCCGGAUAGCTTTCATTGCCTGC
8	SerA-C_fwd	ATTCCGGGUACCATTTCGCGCCCGTCTGCTGTACTGATAAAA GAAGGAGACCAGTTTATGGCTCAAATCTTCAATTTAGTTC
9	SerC-B_rev	ATCAACCGUGACGGCGTTTCGAACTCAACCATGAAGTCTGTC AGCG
10	SerC-B_fwd	ACGGTTGAUAAAAGAAGGAGACCAGTTTATGCCTAACATTA CCTG
11	SerB_rev	ACCTCCTAUGTCACTTCTGATTCAGGC
12	SerA_lib_rev	TCTCCTTCTTAAAGTTAAACAAAATTATTCTAGAGGGGAATT GTTATCCG
13	SerA_TIRlib_fwd	ACTTTAAGAAGGAGACNNNNNNATGGCNAARGTATCGCTGG AG
14	SerB_TIRlib_fwd	ACGGTTGAUAAAAGAAGGAGACNNNNNNATGCCNAAYATT ACCTG
15	SerC_TIRlib_fwd	ATTCCGGGUACCATTTCGCGCCCGTCTGCTGTACTGATAAAA GAAGGAGACNNNNNNATGGCNCARATCTTCAATTTAGTTC
16	SerA_RedLibs_fwd	AATTTTGTTUAACTTTAAGGDGGTNNDCAGTTTATGGCAAA GGTATCGCTGGA
17	SerC_RedLibs_fwd	ATCTTCAATUTTAGTTCTGGTCCGGCA
18	SerC_RedLibs_rev	AATTGAAGAUTTGAGCCATAAACTGNDNACCHCCTTTTATC AGTACAGCAGACGGGCGCGAA
19	SEVA27_BB_rev	AAACAAAATUATTCTAGAGGGGAATTGTTATCCG
20	Bla_remove_fwd	AGCTGAGGUCGCCTCAGCGCCCGGCC
21	Bla_remove_rev	ACCTCAGCUTTACTTCTGATTCAGGCTGCCTG
22	SerACB_in_pGEM_fwd	ACTTCCTCAUTTGTCCTACTCAGGAGAG
23	SerACB_in_pGEM_rev	ATTTTCTCCUATTCTCACCAATAAAAAACGCCCCG
24	pGEM_BB_fwd	AGGAGAAAAUTGCGTTGCGCTCACTGC

25	pGEM_BB_rev	ATGAGGAAGUTCCTATACTTTCTAGAGAATAGGAACTTCCG CGCCGCACACA
26	Genome_int_fwd	GCGTAACCTGGCAAAATCGGTTACGGTTGAGTAATAAATGG ATACTAGAGAATTAACCCTCACTAAAGGGCG
27	Genome_int_rev	TTACAAACATTAATAACGAAGAGATGACAGAAAAATTTTCA TTCTGTGACATTCTCACCAATAAAAAACG

Sequencing oligonucleotides

28	SerA_seq_fwd	CTCAGGTACAGCATCTTTCTG
29	SerA_seq_rev	GTACATGCAGACTCACCACATCG
30	SerC_seq_fwd	GGTCGCCGCTGACTTCTCTTC
31	SerC_seq_rev	CACCATAACGGCTGACGTCAA
32	SerB_seq_fwd	GGAAGTAACCGAACGGGCGATG
33	SerB_seq_rev	CAGCGTCGCCACACGGCTGCG
34	glmS-pstS_fwd	GGCTTACCATGTCGCGCTGATCA
35	glmS-pstS_rev	CGCGGCGACAACAGTTGCGAC

Supplementary Table S4.

Supplementary Table S4. Sequences of original clones and optimized TIR and RBS library variants

Gene /Operon name	Sequence	Comments
A. TIR variants of individual <i>serA</i> ^{mut} gene		
1. <i>serA</i> ^{mut}	AAGGAGACC <u>CAGTTT</u> ATG GCAAAG	
<i>serA</i> ^{mut} opt	AAGGAGACT <u>GATCT</u> ATG GCGAAA	variant 7 and 8
B. TIR variants of <i>serA</i> ^{mut} opt- <i>serCB</i> operon		
1. Original operon		
<i>serA</i> ^{mut}	AAGGAGACC <u>CAGTTT</u> ATG GCAAAG	pSEVA27-sl- <i>serA</i> ^{mut} CB
<i>serC</i>	AAGGAGACC <u>CAGTTT</u> ATG GCTCAA	
<i>serB</i>	AAGGAGACC <u>CAGTTT</u> ATG CCTAAC	
2. Operon 1		
<i>serA</i> ^{mut}	AAGGAGACT <u>GATCT</u> ATG GCGAAA	pSEVA27-sl- <i>serA</i> ^{mut} CB TIR 1
<i>serC</i>	AAGGAGACC <u>CAGTTT</u> ATG GCTCAA	
<i>serB</i>	AAGGAGACC <u>CAGTTT</u> ATG CCTAAC	
3. Operon 2		
<i>serA</i> ^{mut}	AAGGAGACT <u>GATCT</u> ATG GCGAAA	pSEVA27-sl- <i>serA</i> ^{mut} CB TIR 2
<i>serC</i>	AAGGAGACC <u>CAGTTT</u> ATG GCTCAA	
<i>serB</i>	AAGGAGACT <u>GACTA</u> ATG CCGAAT	
C. RBS variants of <i>serA</i> ^{mut} <i>serCB</i> operon		
1. Operon 1		
<i>serA</i> ^{mut}	<u>GGGGTAGGC</u> AGTTT ATG GCAAAG	pSEVA27-sl- <i>serA</i> ^{mut} CB RedLibs 1
<i>serC</i>	<u>GAGGTAACC</u> AGTTT ATG GCTCAA	
<i>serB</i>	<u>AAGGAGACC</u> AGTTT ATG CCTAAC	
2. Operon 2		
<i>serA</i> ^{mut}	<u>GGGGTGGGC</u> AGTTT ATG GCAAAG	pSEVA27-sl- <i>serA</i> ^{mut} CB RedLibs 2
<i>serC</i>	<u>GAGGTAAAC</u> AGTTT ATG GCTCAA	
<i>serB</i>	<u>AAGGAGACC</u> AGTTT ATG CCTAAC	
3. Operon 3		
<i>serA</i> ^{mut}	<u>GGGGTGTGC</u> AGTTT ATG GCAAAG	pSEVA27-sl- <i>serA</i> ^{mut} CB RedLibs 3
<i>serC</i>	<u>GGGGTGTGC</u> AGTTT ATG GCTCAA	
<i>serB</i>	<u>AAGGAGACC</u> AGTTT ATG CCTAAC	
4. Operon 4		
<i>serA</i> ^{mut}	<u>GAGGTGTGC</u> AGTTT ATG GCAAAG	pSEVA27-sl- <i>serA</i> ^{mut} CB RedLibs 4
<i>serC</i>	<u>GAGGTCACC</u> AGTTT ATG GCTCAA	

<i>serB</i>	<u>AAGGAGACCAGTTT</u> ATGCCTAAC	
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Supplementary Table S5.

Supplementary Table S5. LFQ data

Operon/protein	LFQ intensity		Mean	SD
pSEVA27-sl-serA ^{mut} CB				
SerA	1,684,300,000	1,537,500,000	1,610,900,000	103,803,275.5
SerC	1,926,000,000	1,813,600,000	1,869,800,000	79,478,802.21
SerB	418,160,000	513,240,000	465,700,000	67,231,712.76
GAPDH	20,366,000,000	20,626,000,000	20,496,000,000	183,847,763.1
pSEVA27-sl-serA ^{mut} CB TIR 1				
SerA	3,828,200,000	3,568,800,000	3,698,500,000	183,423,499.0
SerC	13,410,000,000	14,857,000,000	14,133,500,000	1,023,183,512.4
SerB	4,805,600,000	5,978,700,000	5,392,150,000	829,506,965.0
GAPDH	20,214,000,000	33,060,000,000	26,637,000,000	9,083,493,711
pSEVA27-sl-serA ^{mut} CB TIR 2				
SerA	4,715,500,000	4,286,000,000	4,500,750,000	303,702,362.5
SerC	2,269,100,000	2,265,200,000	2,267,150,000	2,757,716.4
SerB	4,792,700,000	4,401,500,000	4,597,100,000	276,620,172.8
GAPDH	27,558,000,000	16,931,000,000	22,244,500,000	7,514,423,764
pSEVA27-sl-serA ^{mut} CB RedLibs 1				
SerA	4,271,600,000	4,496,700,000	4,384,150,000	159,169,736.4
SerC	6,349,100,000	5,783,200,000	6,066,150,000	400,151,727.5
SerB	2,003,300,000	1,824,600,000	1,913,950,000	126,359,981.8
GAPDH	23,726,000,000	22,918,000,000	23,322,000,000	571,342,279
pSEVA27-sl-serA ^{mut} CB RedLibs 2				
SerA	9,888,000,000	12,890,000,000	11,389,000,000	2,122,734,557.1
SerC	11,619,000,000	14,238,000,000	12,928,500,000	1,851,912,660
SerB	3,753,200,000	3,579,700,000	3,666,450,000	122,683,026.5
GAPDH	21,960,000,000	10,298,000,000	16,129,000,000	8,246,279,282
pSEVA27-sl-serA ^{mut} CB RedLibs 3				
SerA	7,770,200,000	8,497,200,000	8,133,700,000	514,066,629.9
SerC	4,161,900,000	4,419,600,000	4,290,750,000	182,221,417.5
SerB	1,792,600,000	1,883,100,000	1,837,850,000	63,993,163.7
GAPDH	24228000000	23696000000	23962000000	376,180,808
pSEVA27-sl-serA ^{mut} CB RedLibs 4				
SerA	6,972,600,000	6,631,300,000	6,801,950,000	241,335,544.4
SerC	6,513,200,000	6,378,900,000	6,446,050,000	94,964,440.7
SerB	2,052,700,000	2,052,300,000	2,052,500,000	282,842.7
GAPDH	24,392,000,000	24,046,000,000	24,219,000,000	244,658,946
pCDFDuet-1-serA ^{mut} -serC and pACYCDuet-1-serB				
SerA	13,649,000,000	13,976,000,000	17,337,000,000	14,987,333,333
SerC	176,040,000,000	180,410,000,000	184,470,000,000	180,306,666,666.7
				4,215,949,873

SerB	78,093,000,000	87,967,000,000	80,839,000,000	82,299,666,667	5,096,482,055
GAPDH	42,486,000,000	41,690,000,000	43,103,000,000	42,426,333,333	708,387,135

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