Industrializing a Bacterial Strain for L-Serine Production through Translation Initiation Optimization

Rennig, Maja; Mundhada, Hemanshu; Wordofa, Gossa Garedew; Gerngross, Daniel; Wulff, Tune; Worberg, Andreas; Nielsen, Alex Toftgaard; Nørholm, Morten H.H.
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Supporting information for:

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

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³Mycropt IVS, Copenhagen, Denmark

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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. 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 RedLibs 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 Calculator.
Supplementary Figure S3. Proteomics data of SerA<sup>mut</sup>, SerB and SerC. (a) LFQ intensities for the original assembled operon and all optimized variants as well as the high copy plasmid system. (b) LFQ intensities of the same samples normalized to GAPDH-A expression.
Supplementary Figure S4. Protein ratios of different *ser*A*mut*CB operons in comparison.

Ratios of SerA to SerC and SerA to SerB were calculated for the original synthetic operon pSEVA27-sl-*ser*A*mut*CB (black), the pSEVA27-sl-*ser*A*mut*CB TIR 1 operon (orange), the pSEVA27-sl-*ser*A*mut*CB RedLibs 4 operon (blue) and the high copy two plasmid system (grey).
Supplementary Figure S5. Growth of all selected operons during batch fermentations.

The optical density (OD$_{600}$) of all strains was measured during a 24-hour serine production experiment.
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**

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<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tr>
<td><em>E. coli</em> NEB5a</td>
<td>fluA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15</td>
<td>a</td>
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<tr>
<td></td>
<td>gyrA96 recA1 relA1 endA1 thi-1 hsdR17</td>
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<tr>
<td><em>E. coli</em> MC1061</td>
<td>araD139, Δ(ara, leu)7697, ΔlacX74, galU+, galK-, hsr-, hsm+, strA</td>
<td>In house</td>
</tr>
<tr>
<td><em>E. coli</em> BL21(DE3)</td>
<td>F− ompT hsdS6(r− m−) gal dcm (DE3)</td>
<td>b</td>
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<tr>
<td><em>E. coli</em> ALE-5(DE3)</td>
<td>MG1655 ΔsdaA ΔsdaB ΔtdcG ΔglyA thrA&lt;sup&gt;mut&lt;/sup&gt; λ(DE3)</td>
<td>3</td>
</tr>
<tr>
<td><em>E. coli</em> ALE-5(DE3)</td>
<td>MG1655 ΔsdaA ΔsdaB ΔtdcG ΔglyA thrA&lt;sup&gt;mut&lt;/sup&gt; glsmS&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pstS:&lt;sup&gt;−&lt;/sup&gt;serA&lt;sup&gt;mut&lt;/sup&gt;CB λ(DE3)</td>
<td>This study</td>
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</table>

*NEB, Ipswich, MA, USA; b Novagen, Merck KGaA, Darmstadt, Germany*
**Supplementary Table S2.**

**Supplementary Table S2. Plasmids used in this study**

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<td>pCDFDuet-1-serAmut-serC</td>
<td>vector encoding <em>serA</em> and <em>serC</em>, Sp&lt;sup&gt;R&lt;/sup&gt;</td>
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<tr>
<td>pACYC-1-serB</td>
<td>vector encoding <em>serB</em>, Cm&lt;sup&gt;R&lt;/sup&gt;</td>
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<tr>
<td>pSEVA27-sl3-nark-gfp</td>
<td>vector encoding <em>nark-gfp</em>, Km&lt;sup&gt;R&lt;/sup&gt;, used to amplify pSEVA27-sl backbone</td>
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<tr>
<td>pACYC-dashergfp-hp-bla</td>
<td>vector encoding <em>dashergfp</em> translationally coupled to <em>bla</em>, Cm&lt;sup&gt;R&lt;/sup&gt;, used to amplify hp-bla</td>
<td>5</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-hp-bla</td>
<td>vector encoding <em>serA</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmutCB-hp-bla</td>
<td>vector encoding a synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-opt-serCB-hp-bla</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-opt-serCBopt-hp-bla</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-serCB-hp-bla RedLibs1</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-serCB-hp-bla RedLibs2</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-serCB-hp-bla RedLibs3</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pSEVA27-sl3-serAmut-serCB-hp-bla RedLibs4</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em> translationally coupled to <em>bla</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
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<tr>
<td>pSEVA27-sl3-serAmutCB</td>
<td>vector encoding a synthetic operon <em>serACB</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
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<td>pSEVA27-sl3-serAmut-opt-serCB</td>
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<td>pSEVA27-sl3-serAmut-serCB RedLibs3</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
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<tr>
<td>pSEVA27-sl3-serAmut-serCB RedLibs4</td>
<td>vector encoding an optimized synthetic operon <em>serACB</em>, Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
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</table>
pGEM-FRT-KanR vector encoding Km\textsuperscript{R} flanked by FRT sites for integration into the genome, Amp\textsuperscript{R} 
In house

pGEM-FRT-KanR-FRT-\textit{serAmut-serCB} TIR 1 vector encoding the optimized synthetic operon \textit{serACB} TIR 1 and Km\textsuperscript{R} flanked by FRT sites for integration into the genome, Amp\textsuperscript{R} 
This study

Supplementary Table S3.

Supplementary Table S3. Oligonucleotides used in this study

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<td>1</td>
<td>pSEVA27_fwd</td>
<td>AGCTGAGGUGUCGCTCACGC</td>
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<td>2</td>
<td>pSEVA27_T7_rev</td>
<td>AAACCTGGTCCUCCCTCTAAAGTTAACAAAATTATTTTCTAGAG</td>
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<td>3</td>
<td>SerA_fwd</td>
<td>AGACCAGTTUATGGGAAGGTATCGC</td>
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<tr>
<td>4</td>
<td>SerA_rev</td>
<td>ACCTCCTAUGTCAGTACAGCAGC</td>
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<tr>
<td>5</td>
<td>hp_Bla_fwd</td>
<td>ATAGGAGGUCCTCCTATGTAATCAATACATTTCCGTGTC</td>
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<tr>
<td>6</td>
<td>hp_Bla_rev</td>
<td>ACCUCAGCUTACAAATGCTTAATCAATG</td>
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<td>7</td>
<td>SerA-C_rev</td>
<td>ATTCGCCGUGACCGCTGCTGCTGACTGATAAAA</td>
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<td>8</td>
<td>SerC-B_rev</td>
<td>AATCCGCGUGACCACATTCCGCCCCTGCTGACTGATCGGTTTTTTTTTATGTC</td>
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<td>10</td>
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<td>SerA_RedLibs_fwd</td>
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<td>14</td>
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<td>16</td>
<td>SerB_remove_fwd</td>
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<td>SerA_remove_rev</td>
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<td>SerC_remove_fwd</td>
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<td>pGEM_BB_fwd</td>
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<td>SerB_seq_rev</td>
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**Supplementary Table S4.**

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

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<th>Gene /Operon name</th>
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<td><strong>A. TIR variants of individual (\text{serA}^{\text{mut}}) gene</strong></td>
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<td>1. (\text{serA}^{\text{mut}})</td>
<td>AAGGAGACCAGTTT(\text{ATG})GCAAAG</td>
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<tr>
<td>(\text{serA}^{\text{mut opt}})</td>
<td>AAGGAGACTGACT(\text{ATG})GCCAAA</td>
<td>variant 7 and 8</td>
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<td><strong>B. TIR variants of (\text{serA}^{\text{mut opt}})-(\text{serCB}) operon</strong></td>
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<tr>
<td>1. Original operon</td>
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<td>(\text{serA}^{\text{mut}})</td>
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<tr>
<td>(\text{serC})</td>
<td>AAGGAGACCAGTTT(\text{ATG})GCTCAA</td>
<td>pSEVA27-sl-(\text{serA}^{\text{mut CB}})</td>
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<tr>
<td>(\text{serB})</td>
<td>AAGGAGACCAGTTT(\text{ATG})CCTAAC</td>
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<td>2. Operon 1</td>
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<tr>
<td>(\text{serA}^{\text{mut}})</td>
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<td>(\text{serC})</td>
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<td>(\text{serB})</td>
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<td>(\text{serB})</td>
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<td><strong>C. RBS variants of (\text{serA}^{\text{mut}})-(\text{serCB}) operon</strong></td>
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<tr>
<td>1. Operon 1</td>
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<tr>
<td>(\text{serA}^{\text{mut}})</td>
<td>GGGGTAGGAGCAGTTT(\text{ATG})GCAAAG</td>
<td>pSEVA27-sl-(\text{serA}^{\text{mut CB}}) RedLibs 1</td>
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<td>(\text{serC})</td>
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<td>(\text{serB})</td>
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<tr>
<td>2. Operon 2</td>
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<tr>
<td>(\text{serA}^{\text{mut}})</td>
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<td>pSEVA27-sl-(\text{serA}^{\text{mut CB}}) RedLibs 2</td>
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<td>(\text{serC})</td>
<td>GAGGTAACCAGTTT(\text{ATG})GCTCAA</td>
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<td>(\text{serB})</td>
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<td>3. Operon 3</td>
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<td>(\text{serA}^{\text{mut}})</td>
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<td>pSEVA27-sl-(\text{serA}^{\text{mut CB}}) RedLibs 3</td>
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<tr>
<td>(\text{serC})</td>
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<td>(\text{serC})</td>
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**Supplementary Table S5. LFQ data**

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<th>SD</th>
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<td></td>
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<td>SerA</td>
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<td>1,610,900,000</td>
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<tr>
<td>SerC</td>
<td>1,926,000,000</td>
<td>1,813,600,000</td>
<td>1,869,800,000</td>
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<tr>
<td>SerB</td>
<td>418,160,000</td>
<td>513,240,000</td>
<td>465,700,000</td>
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<tr>
<td>GAPDH</td>
<td>20,366,000,000</td>
<td>20,626,000,000</td>
<td>20,496,000,000</td>
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<tr>
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<td>SerC</td>
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