**Combining directed evolution of pathway enzymes and dynamic pathway regulation using a quorum-sensing circuit to improve the production of 4-hydroxyphenylacetic acid in *Escherichia coli***

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Figure S1. (A) Esa quorum-sensing (QS) plasmid. (B) Activation and repression of genes using quorum-sensing circuit. The QS signaling molecule (3-oxohexanoyl-homoserine lactone, AHL) is produced by the AHL synthase encoded by *esaI*. In the absence of AHL or at low AHL concentration, the transcriptional regulator EsaRI70V binds the PesaS/or PesaR promoter and activates the transcription of GFP, and/or represses the transcription of mCherry. As cell density increases, AHLs accumulate, resulting in disruption of EsaRI70V binding and repressing of the transcription of GFP, and/or activating of the transcription of mCherry. (C) Gene expression from PesaS/or PesaR promoter in the Esa quorum-sensing circuit. As cell growth, the transcription level of GFP driven by the PesaS promoter decreased, and the transcription level of mCherry driven by the PesaR promoter increased.

Table S1. Mutations on the evolved gene

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| --- | --- | --- | --- |
| Plasmid | gene | Base mutation | Amino acid mutaion |
| 2E1 | *ARO10* | C1869T | No |
|  | *feaB* | T72G, T1195C, A1491T | I24M |
| 4F3 | *ARO10* | A1682G, A1842G | No |
|  | *feaB* | C165G, A609T, T1296 | N55K |
| 6D5 | *ARO10* | T412C, A653G, T948C, A1815T | F138L, D218G |
| 9F5 | *ARO10* | T695C, A1599G, A1776G | No |
| 10A3 | *ARO10* | T989C, G1351A, A1638G | V451I |
| 10G5 | *ARO10* | C1722T | No |

Table S2. The sequence of the TIGR

GCCTAGCAAGATCTCCTGATCCCGGTGCGCGACCACCCGGACATCTGCATAGTCTGGGCCAGTCTGAGGACTGGCGGATCAGGGCCTTGAATTTACAGTATTTTAGTGGCCTTACGCTATACTATTCGGTCACCTTATCCGCTCAAGACATGCACTCGGAACGCATCTAGGGTACCGCAGATACTGTATCC