Supplementary materials

High-level Bio-Based Production of Coproporphyrin in *Escherichia coli*

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Materials and methods

Bacterial strains and plasmids

Plasmid pK-hemABCD was used from our previous study to serve as the template for the remaining plasmids [1]. Briefly, the *hemA* gene was amplified by polymerase chain reaction (PCR) from the genomic DNA of *Rhodobacter sphaeroides* DSM 158 using primers P001/P002. Similarly, *hemB, hemC,* and *hemD* genes were amplified from the genomic DNA of *Escherichia coli* MG1655 using primer sets P003/P004, P005/P006, and P007/P008, respectively. The backbone, consisting of p15A ori and *trc* promoter with a kanamycin resistance marker, was amplified from a lab-made plasmid using primers P009/P010. Subsequently, these five fragments were Gibson-assembled to construct pK-hemABCD.

pK-hemABD was constructed by amplifying *hemA* and *hemB* using primers P001/P004 and amplifying *hemD* along with the backbone using primers P017/P018 and pK-hemABCD as template. These two fragments were Gibson-assembled to form pK-hemABD.

Table S1: Oligomers used in this study

Primers/oligo name	**Primer/oligo sequence (5' → 3')**
P001	AAGGAGGAATAGAAAATGGACTACAATCTGGCACTCG
P002	TTTCGTACCTCCTTTGCTAGCTCAGGCAACGACCTCGGC
P003	CTAGCAAAGGAGGTACGAAATATGACAGACTTAATCCAACGC
P004	ATGTAAATTTCCTCCTTTGGTACCTTAACGCAGAATCTTCTTCTCAG
P005	CCAAAGGAGGAAATTTACATGTTAGACAATGTTTTAAGAATTGCCAC
P006	TAATAATCCTCCTTTGCATGCTCATGCCGGGGCGTCTCC
P007	CATGCAAAGGAGGATTATTATATGAGTATCCTTGTCACCC
P008	CTGCGGATCCTTATTGTAATGCCCGTAAAAGC
P009	ATTACAATAAGGATCCGCAGCCCGCCTAATG
P010	TCCATTTTCTATTCCTCCTTTAATTGTTATCCGCTC
P011	GTATCACCGCTAAGGATCCGCAGCCCGCCTAATGAGC
P012	TTTCGGATGTTATTGTAATGCCCGTAAAAGCGCATCG
P013	GGAATCAATAATGACCGAACTTAAAAACGATCG
P014	CATCGACTGTCTGAACAGTATCACCGCTAAGGATCCG
P015	CGGGCATTACAATAACATCCGAAAAGAATGATGGATC
P016	TTAAGTTCGGTCATTATTGATTCCTCCTTTAATTGG
P017	TACCAAAGGAGGAAATTTACATGAGTATCCTTGTCACCCG
P018	GTCCATTTTCTATTCCTCCTTTAATTGTTATCCG
P019	TCTTTTCGGATGTTAACGCAGAATCTTCTTCTCAG
P020	TCTGCGTTAACATCCGAAAAGAATGATGG
P021	GGATACTCATTATTGATTCCTCCTTTAATTGGG
P022	GGAGGAATCAATAATGAGTATCCTTGTCACCCG
P023	AAATTTCCTCCTTTGGTACCTTATTGTAATGCCCGTAAAAGCGCATCG
P024	GGTACCAAAGGAGGAAATTTACATGACCGAACTTAAAAACGATCG
P025	TTGAATTTTCCTCCTTTTCATGATTAGCGGTGATACTGTTCAG
P026	TGAAAAGGAGGAAAATTCAATGAGTGACGGCAAAAAACATG
P027	CTGCGGATCCTTAGCTGAATAAATAGGTAAGCG
P028	TTATTCAGCTAAGGATCCGCAGCCCGCCTAATGAG
P029	GTCCATTTTCTATTCCTCCTTTAATTGTTATCCGC
P030	TGAAAAGGAGGAAAATTCAATGCTAAAAGTGTTATTGCTCTTTGTG
P031	CTGCGGATCCCTACTGTGGCGGGTTATTCTGC
P032	GCCACAGTAGGGATCCGCAGCCCGCCTAATGAGC
P033	CAATTAAAGGAGGAATAGAAAATGGAC
P034	TTTCTATTCCTCCTTTAATTGTTATCCG

References

1. Arab, B.; Westbrook, A.W.; Moo-Young, M.; Liu, Y.; Chou, C.P. Bio-Based Production of Uroporphyrin in Escherichia coli. *Synthetic Biology and Engineering* **2024**, *2*, 10002.