Supporting information

Evolutionary engineering of cyanobacteria to enhance the production of α-farnesene from CO_2

Napisa Pattharaprachayakul[1,2], Hyun Jeong Lee[1], Aran Incharoensakdi[2], Han Min Woo[1]*

[1]Department of Food Science and Biotechnology, Sungkyunkwan University (SKKU), 2066 Seobu-ro, Jangan-gu, Suwon 16419, Republic of Korea

[2]Laboratory of Cyanobacterial Biotechnology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330 Thailand

*Corresponding author:

Han Min Woo

Department of Food Science and Biotechnology, Sungkyunkwan University (SKKU),

2066 Seobu-ro, Jangan-gu, Suwon 16419, Republic of Korea

Tel: +82 31 290 7808; Fax: +82 31 290 7882;

E-mail address: hmwoo@skku.edu (H. M. Woo)

ORCID: 0000-0002-8797-0477

Table S1. RBS sequences and oligonucleotides used for gene cloning in this study.

Name	Relevant characteristics (5' → 3')	Source
RBS-1	CAAAagatctAATAAAGGAGGTTTAAAGCT**ATGgaatttcgcgtgcacctgcag**	This study
RBS-2	CAAAagatctTAGCATCGAACATAGAGAGGTCAGAC**ATGgaatttcgcgtgcac**	This study
RBS-3	CAAAagatctTGTATTCGTAGGGTACAGTTT**ATGgaatttcgcgtgcacct**	This study
RBS-R	TTGGATGCTCTTGAATTGCC	This study

Note: The restriction enzyme sites were shown as lower cases. RBS and coding regions of AFS were underlined and bold letters, respectively.

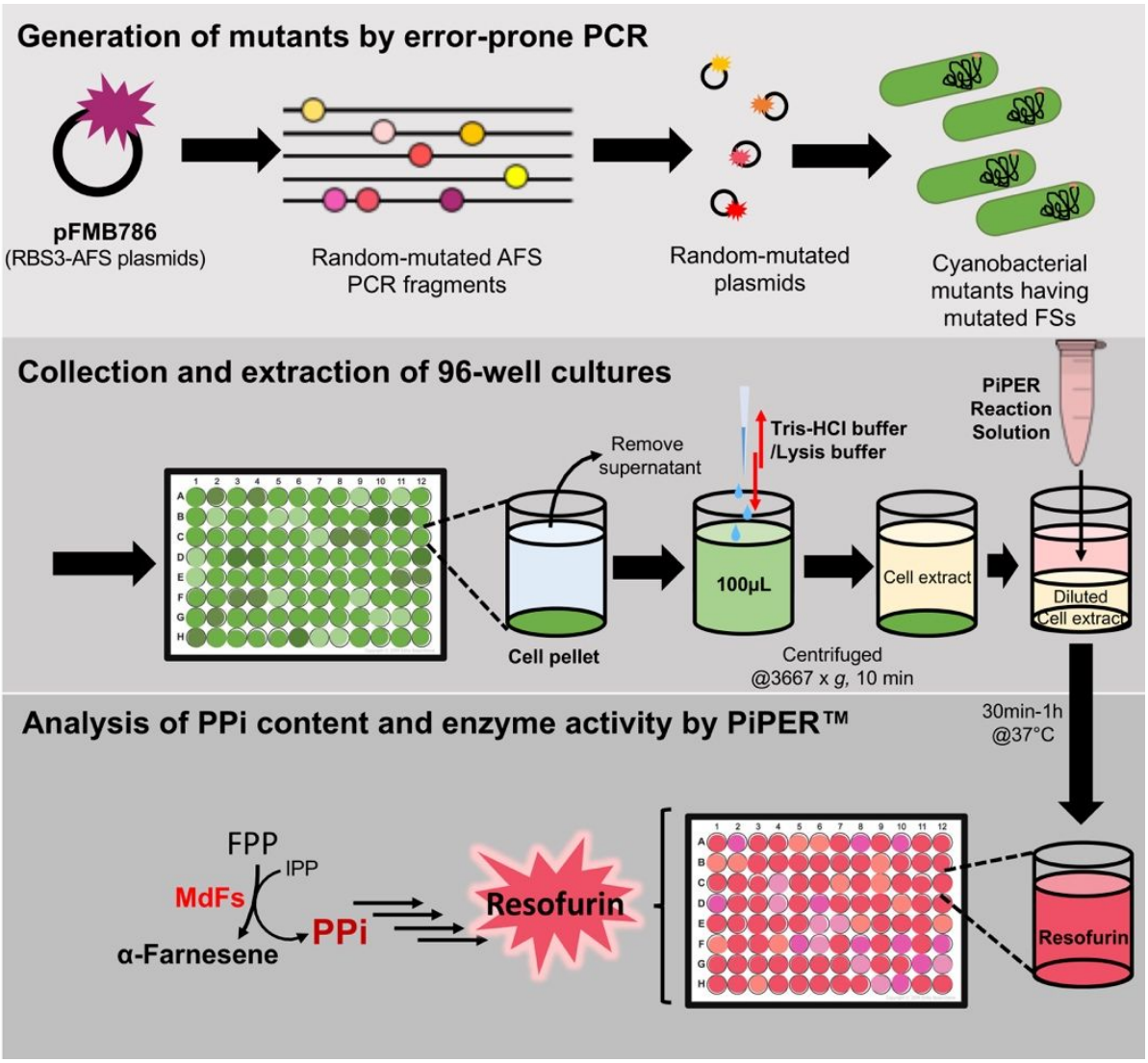
Table S2. Oligonucleotides used for DNA sequencing.

Name	Relevant characteristics (5' → 3')	Source
Promoter checking		
Native MEP		
dxs-proF	GGGTCGACAGACTGAGCCCA	This study
dxs-proR	TGTCGTTGAGCACGACCAAC	This study
dxr-proF	CGAGACCGGCCAAACCAG	This study
dxr-proR	CAGTTACTTGCGACAGCCGT	This study
ispD-proF	GCACTCAAATTCCAGCCTCC	This study
ispD-proR	GCTCAACGACGACATCAATC	This study
ispE-proF	CGCTTCCAGTTCTGGTTGTG	This study
ispE-proR	TTGGCCTTCGTAAAGTTGG	This study
ispF-proF	TGGGATCGAAACGTACGCTG	This study
ispF-proR	CGCCTCCTCCACGGACTGAT	This study
ispG-proF	CTCGCGCAGGCTGGTGACTA	This study
ispG-proR	CTCAGAAGCCGATAAAGAC	This study
ispH-proF	CTTTCCGCAGCTCCAAG	This study
ispH-proR	GAGCTGGTGGCGACCGTCTC	This study
idi-proF	CAACAAGCCCTTCGGCTCCA	This study
idi-proR	TGCCTGTGAACGTCGGTACC	This study
ispA-proF	TCTGACGACCGATTCGAC	This study
ispA-proR	CCTGGAATGGGTTGAGATG	This study
NSI		
UP-pTrcI	CGACAGGTTTCCCGACTGGA	This study
Pr_AFS-R1	CAGCCGTTGCGCAGGTATTC	This study

NSII		
Up-pTrcI	CGACAGGTTTCCCGACTGGA	This study
DXS-R1	TAAAGGCCAGGTGCAGGCGGG	This study
Gene checking		
Native MEP		
7942_dxs_F	ACGTTCACTGCAGCCAGCAG	This study
7942_dxs_R	CATGATCAGTCCAGGTCTTG	This study
7942_dxr_F	TCGCCTCCCTCGCTTCAGCA	This study
7942_dxr_R	TAGACCTCAGGCACATCGAT	This study
7942_ispD_F	ATCGGGGTAAGCTGAAGTC	This study
7942_ispD_R	GCACTCAAATTCCAGCCTCC	This study
7942_ispF_F	ATCTAGGCCACATGACGTCA	This study
7942_ispF_R	TGGGATCGAAACGTACGCTG	This study
7942_ispE_F	ATCAGATTGCGCCCGAAGC	This study
7942_ispE_R	GAGCTAGTGCTGGATGGGAT	This study
7942_ispG_F	TCGCCCAAGGGTTGGTCGAT	This study
7942_ispG_R	GCGAAAACCGCCTATCAGGA	This study
7942_ispH_F	GCAATTGGCGATCGCGGCTT	This study
7942_ispH_R	GTGGAGTCGAGAGTCACGAT	This study
7942_idi_F	CAGCACATGCCAGTTCAGAC	This study
7942_idi_R	GTTGGACCAATGACCGTGGC	This study
7942_ispA_F	GTCAAGCTGAAGGTGGGCAC	This study
7942_ispA_R	CGGTAGCGAAATAGCGATCG	This study
NSI		
DXS-F1	CGTGTCGCTCAAGGCGCACT	This study
DXS-R1	TAAAGGCCAGGTGCAGGCGGG	This study
idi-F1	AACCCGTGCCCGTGCTGA	This study
idi-R1	TACACCAGAAAGGGGCGCAG	This study

ispA-F1	ATCGATGCCACCCCCTGGG	This study
ispA-R1	AGCCGAACGCCCTAGGTATA	This study
NSII		
PrHW882F	TAATGTTTTTTGCGCCGACA	FMB lab collection
PrHW883R	TTGGATGCTCTTGAATTGCC	FMB lab collection
Pr_AFS-F1	CACCACTTTGCCCACCTGAA	This study
Pr_AFS-R1	CAGCCGTTGCGCAGGTATTC	This study
Pr_AFS-F2	CTACCTGATCAACCAGCGCC	This study
Pr_AFS-R2	CATCGCCATCTTTGTACAGG	This study

Figure S1. A Scheme of 96-well based screening and sample preparation for analyzing mutants generated from error-prone PCR.



Generation of mutants by error-prone PCR. The template used in error-prone PCR was pFMB786 (described in Table 1). Error-prone PCR was performed by using GeneMorph® II Random Mutagenesis Kit (Agilent) and following its manual instruction, which high- and low-rate of mutation were obtained from 25 and 500 ng of initial template, respectively. Randomly mutated

PCR libraries were cut and ligated with pSe2Bb1k plasmid backbone before transformed into *E. coli* DH10β**.** Randomly-mutated plasmids were continually collected and transformed into SeHL33. The colonies selection was done with cultivation on BG-11 supplemented with spectinomycin and kanamycin.

Collection and extraction of 96-well cultures. Each selective colony was inoculated in 96-well microplate along with controls until reaching OD_{730} >1 and then transferred 100 μL of culture to new 96-well plates (Clear 96-Well Plate, Greiner Bio-One) containing BG-11 with 1 mM IPTG addition. Preparation of samples before fluorescence screening was firstly collecting cell pellets and then removing supernatants. Cell pellets were then washed and lysed by mixing with pH 7.5 Tris-HCl and lysis buffer (B-PER™, Thermo Science) respectively. Diluted cell extracts from day-3 and -8 after induction were transferred to new 96-well plate (μClear® 96 Well Plate, Greiner Bio-One) for PPi signal detection and OD_{730} measurement.

Analysis of PPi content and enzyme activity by PiPER™. PiPER™ reaction solution must be prepared fresh before use (described in the instruction) and added into 96-well plate containing cell extracts (with 100 μL in total volume). The reaction was run for 30 – 60 min at 37°C in microplate reader. Resofurin fluorescence was measured and calculated at 560 nm with 590 nm excitation wavelength. Note that the enzyme activity assay is described in second part of instruction.