

# **Metabolic Engineering for Efficient Production of Z,Z-Farnesol in *E. coli***

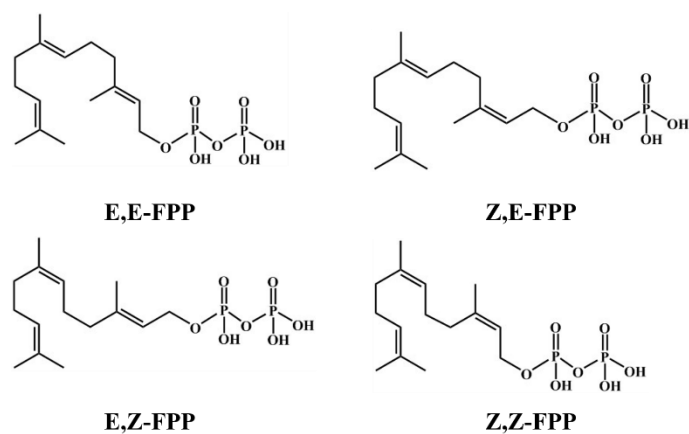
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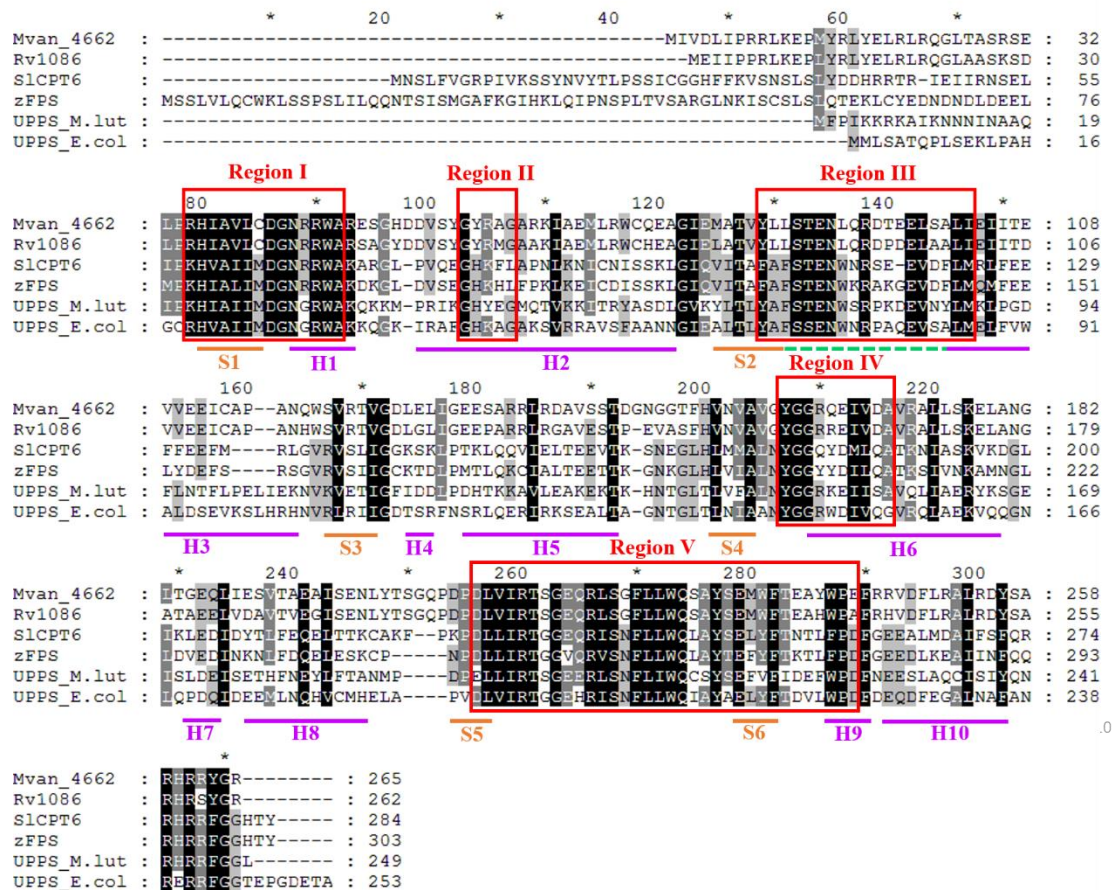
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**Figure S1.** Chemical Structure of Four Isomers of Farnesyl Diphosphate. (E,Z-FPP synthase has not been isolated so far)



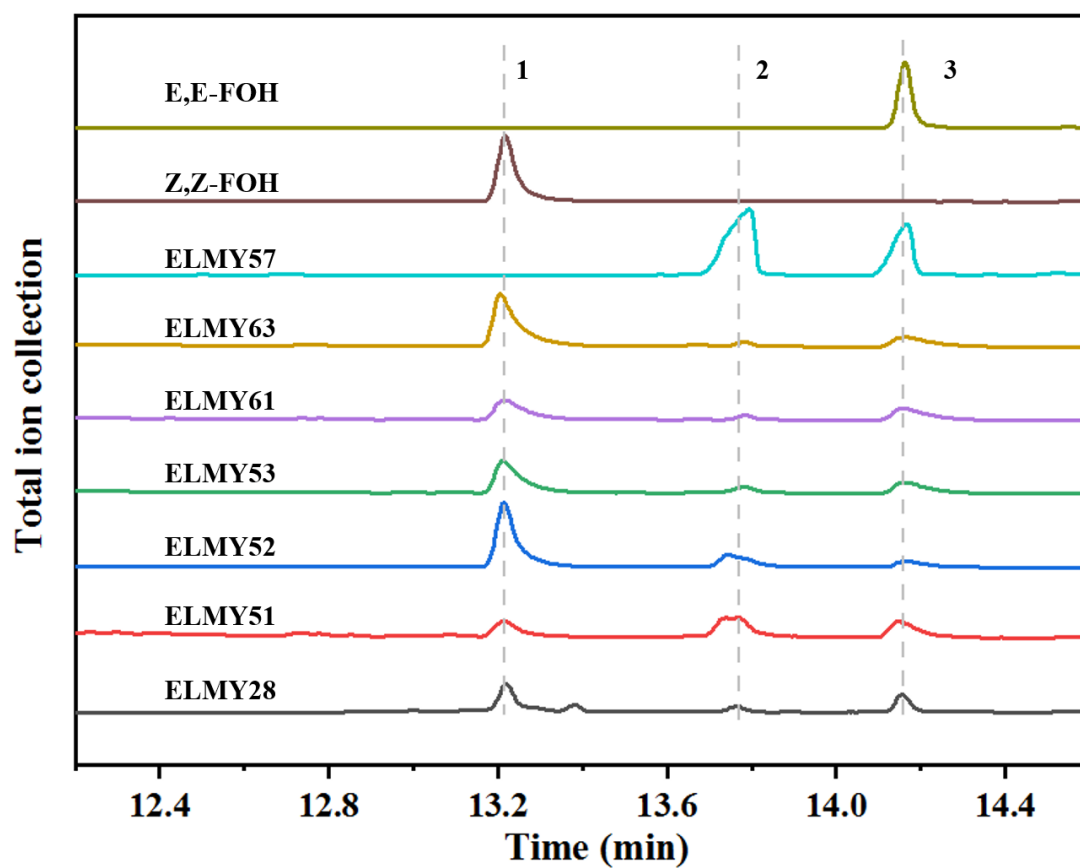


Figure S3. GC chromatogram of cultures by engineered strains and FOH isomers standard. Peak 1 represents Z,Z-FOH, peak 2 represents Z,E-FOH, peak 3 represents E,E-FOH.

**Table S1.** Primers used in this study

Primer	sequence
SltNPPS-F	ATAAGGAGATATACCATGTCTGCTAGAGGTTTGAACA
SltNPPS-R	GCGGCCGCAAGCTTTTAGTAAGTGTGACCACCGAATC
SltNPPS-Vec-F	ACACTTACTAAAAGCTTGCGGCCG
SltNPPS-Vec-R	TAGCAGACATGGTATATCTCCTTATTAAAGTTAAACAAAATTATTT
PstIDS3-F	AGATATACATATGACCCTGGCGAGC
PstIDS3-R	AGACTCGAGGTTAGCAAACGCTCTTGCTG
PstIDS3-Vec-F	AGCAAGAGCGTTTGCTAACCTCGAGTCTGGTAAAGAAAC
PstIDS3-Vec-R	AAAGCTCGCCAGGGTCATATGTATATCTCCTTCTTATACTTAACTAA TATACTAAG
SltCPT6-F	AGATATACATATGAGCAGCTACAACG
SltCPT6-R	AGACTCGAGGTTAATAGGTGTGGCCACC
SltCPT6-Vec-F	GGTGGCCACACCTATTAACCTCGAGTCTGGTAAAGAAAC
SltCPT6-Vec-R	CACGTTGTAGCTGCTCATATGTATATCTCCTTCTTATACTTAACTAAT ATACTAAG
tZFPS-F	AGATATACATATGGCGCGTGGTCTGAAC
tZFPS-R	AGACTCGAGGTTAATAGGTGTGGCCACCAAAACGAC
tZFPS-Vec-F	GGTGGCCACACCTATTAACCTCGAGTCTGGTAAAGAAAC
tZFPS-Vec-R	G TTCAGACCACGCGCCATATGTATATCTCCTTCTTATACTTAACTAA TATACTAAG
Mvan_4662-F	GAAGGAGATATACATATGATCGTTGACCTGATTCC
Mvan_4662-R	TTACCAGACTCGAGGTTAACGTCCATAACGACGATGAC
Mvan_4662-Vec-F	TGGACGTTAACCTCGAGTCTGGTAAAGAAAC
Mvan_4662-Vec-R	CAACGATCATATGTATATCTCCTTCTTATACTTAACTAATATACTAA G
Rv1086-F	GAAGGAGATATACATATGGAGATCATTCGCCG
Rv1086-R	AGACTCGAGGTTAACGACCATAGCTACGGTG
Rv1086-Vec-F	AGCTATGGTCGTTAACCTCGAGTCTGGTAAAGAAAC
Rv1086-Vec-R	TGATCTCCATATGTATATCTCCTTCTTATACTTAACTAATATACT
rdgB-F	GAGATATACCATGCAAAAAGTTGTCCTCGC

rdgB-R	GACTTAAGCATTAAACCATTACGTAAAGCGTCCA
rdgB-Vec-F	GCTTTACGTAATGGTTAATGCTTAAGTCGAACAGAAAGTAATC
rdgB-Vec-R	GAGGACAACCTTTTTGCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
phoA-F	GAGATATACCATGCCTGTTCTGGAAAACC
phoA-R	GACTTAAGCATTATTTTCAGCCCCAGAGCG
phoA-Vec-F	GCTCTGGGGCTGAAATAATGCTTAAGTCGAACAGAAAGTAATC
phoA-Vec-R	GTTTTCCAGAACAGGCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
ppa-F	GAGATATACCATGAGCTTACTCAACGTCCC
ppa-R	GACTTAAGCATTATTTATCTTTGCGCGCTCG
ppa-Vec-F	CGCGCAAAGAATAAATAATGCTTAAGTCGAACAGAAAGTAATC
ppa-Vec-R	GACGTTGAGTAAGCTCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
cdh-F	GAGATATACCATGAAAAAAGCGGGTCTTCTT
cdh-R	GACTTAAGCATTAAACGCAAAATCTCACACTGAT
cdh-Vec-F	TGTGAGATTTTGCGTTAATGCTTAAGTCGAACAGAAAGTAATC
cdh-Vec-R	AAGACCCGCTTTTTTCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
lpxH-F	GAGATATACCATGGCGACACTCTTTATTGC
lpxH-R	GACTTAAGCATTAAAACGGAAAATGAATCAGCTCA
lpxH-Vec-R	AATAAAGAGTGTCGCCATGGTATATCTCCTTATTAAAGTTAAACAA AATTATTTC
lpxH-Vec-F	ATTCATTTTCCGTTTTAATGCTTAAGTCGAACAGAAAGTAATC
mutT-F	GAGATATACCATGAAAAAGCTGCAAATTGCG
mutT-R	GACTTAAGCATTACAGACGTTTAAGCTTCGC
mutT-Vec-F	AAGCTTAAACGTCTGTAATGCTTAAGTCGAACAGAAAGTAATC
mutT-Vec-R	AATTTGCAGCTTTTTTCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
nudc-F	GAGATATACCATGGATCGTATAATTGAAAAATTAGATCAC
nudC-R	GACTTAAGCATTACTCATACTCTGCCCCGACA
nudC-Vec-F	CGGGCAGAGTATGAGTAATGCTTAAGTCGAACAGAAAGTAATC
nudC-Vec-R	TTCAATTATACGATCCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTC
nudF-F	GAGATATACCATGCTTAAGCCAGACAACC

nudF-R	GACTTAAGCATTATGCCCACTCATTTTTTAACGC
nudF-Vec-F	AAAAATGAGTGGGCATAATGCTTAAGTCGAACAGAAAGTAATC
nudF-Vec-R	GTTGTCTGGCTTAAGCATGGTATATCTCCTTATTAAAGTTAAACAAA ATTATTTT
nudj-F	GAGATATACCATGTTTAAACCGCACGTTACC
nudj-R	GACTTAAGCATTAGATGACACCCTTTGTAAAAGGC
nudj-Vec-F	ACAAAGGGTGTCTAATGCTTAAGTCGAACAGAAAGTAATCG
nudj-Vec-R	AACGTGCGGTTTAAACATGGTATATCTCCTTATTAAAGTTAAACAAA ATTAT
C41A-F	CGTTCTGGCAGATGGAAATCGTCGTTGGGCAC
C41A-R	GATTTCCATCTGCCAGAACGGCAATGTGGCGC
C41F-F	CGTTCTGTTTGATGGAAATCGTCGTTGGGCAC
C41F-R	GATTTCCATCAAACAGAACGGCAATGTGGCGC
C41L-F	CGTTCTGCTGGATGGAAATCGTCGTTGGGCAC
C41L-R	GATTTCCATCCAGCAGAACGGCAATGTGGCGC
C41M-F	CGTTCTGATGGATGGAAATCGTCGTTGGGCAC
C41M-R	GATTTCCATCCATCAGAACGGCAATGTGGCGC
C41S-F	CGTTCTGAGCGATGGAAATCGTCGTTGGGCAC
C41S-R	GATTTCCATCGCTCAGAACGGCAATGTGGCGC
C41W-F	CGTTCTGTGGGATGGAAATCGTCGTTGGGCAC
C41W-R	GATTTCCATCCCACAGAACGGCAATGTGGCGC
C41Y-F	CGTTCTGTATGATGGAAATCGTCGTTGGGCAC
C41Y-R	GATTTCCATCATACAGAACGGCAATGTGGCGC
Y85A-F	GCAACCGTTGCACTGCTGAGCACAGAAAATCTGCAG
Y85A-R	GCTCAGCAGTGCAACGGTTGCCATTTCAATACCTGC
Y85F-F	GCAACCGTTTTTCTGCTGAGCACAGAAAATCTGCAG
Y85F-R	GCTCAGCAGAAAAACGGTTGCCATTTCAATACCTGC
Y85L-F	GCAACCGTTCTGCTGCTGAGCACAGAAAATCTGCAG
Y85L-R	GCTCAGCAGCAGAACGGTTGCCATTTCAATACCTGC
Y85S-F	GCAACCGTTAGCCTGCTGAGCACAGAAAATCTGCAG
Y85S-R	GCTCAGCAGGCTAACGGTTGCCATTTCAATACCTGC

Y85W-F	GCAACCGTTTGGCTGCTGAGCACAGAAAATCTGCAG
Y85W-R	GCTCAGCAGCCAAACGGTTGCCATTTCAATACCTGC
D95A-F	TGCAGCGTGCAACAGAAGAAGTCTGAGCGCACTGA
D95A-R	GTTCTTCTGTTGCACGCTGCAGATTTTCTGTGCT
D95P-F	TGCAGCGTCCAACAGAAGAAGTCTGAGCGCACTGA
D95P-R	GTTCTTCTGTTGGACGCTGCAGATTTTCTGTGCT
D95S-F	TGCAGCGTAGCACAGAAGAAGTCTGAGCGCACTGA
D95S-R	GTTCTTCTGTGCTACGCTGCAGATTTTCTGTGCT
E97D-F	GTGATACAGATGAACTGAGCGCACTGATTGAAATCA
E97D-R	CGCTCAGTTCATCTGTATCACGCTGCAGATTTTCTGTGC
E97G-F	GTGATACAGGTGAACTGAGCGCACTGATTGAAATCA
E97G-R	CGCTCAGTTCACCTGTATCACGCTGCAGATTTTCTGTGC
E97Q-F	GTGATACACAGGAAGTCTGAGCGCACTGATTGAAATCA
E97Q-R	CGCTCAGTTCCTGTGTATCACGCTGCAGATTTTCTGTGC
L92A-F	CAGAAAATGCACAGCGTGATACAGAAGAAGTCTGAGC
L92A-R	TATCACGCTGTGCATTTTCTGTGCTCAGCAGATAAACGG
L92F-F	CAGAAAATTTTCAGCGTGATACAGAAGAAGTCTGAGC
L92F-R	TATCACGCTGAAAATTTTCTGTGCTCAGCAGATAAACGG
L92S-F	CAGAAAATAGCCAGCGTGATACAGAAGAAGTCTGAGC
L92S-R	TATCACGCTGGCTATTTTCTGTGCTCAGCAGATAAACGG
L92W-F	CAGAAAATTGGCAGCGTGATACAGAAGAAGTCTGAGC
L92W-R	TATCACGCTGCCAATTTTCTGTGCTCAGCAGATAAACGG
L99V-F	CAGAAGAAGTTAGCGCACTGATTGAAATCATTACCG
L99V-R	TCAGTGCGCTAACTTCTTCTGTATCACGCTGCAGATTTTCT
Q93A-F	AAATCTGGCACGTGATACAGAAGAAGTCTGAGCGC
Q93A-R	TCTGTATCACGTGCCAGATTTTCTGTGCTCAGCAGATAAACGG
Q93G-F	AAATCTGGGTCGTGATACAGAAGAAGTCTGAGCGC
Q93G-R	TCTGTATCACGACCCAGATTTTCTGTGCTCAGCAGATAAACGG
Q93K-F	AAAATCTGAAACGTGATACAGAAGAAGTCTGAGCGC
Q93K-R	CTGTATCACGTTTCAGATTTTCTGTGCTCAGCAGATAAACGG



Q93S-F	AAAATCTGAGCCGTGATACAGAAGAACTGAGCGC
Q93S-R	CTGTATCACGGCTCAGATTTTCTGTGCTCAGCAGATAAACG
Q93N-F	AAAATCTGAATCGTGATACAGAAGAACTGAGCGC
Q93N-R	CTGTATCACGATTCAGATTTTCTGTGCTCAGCAGATAAACG

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**Table S2.** Codon-optimized sequences of *mvan4662*, *rv1086* and truncated *PsIDS3*, *SLCPT6*, *ShzFPS* and *TcNudix1* in this study.

Gene	Encoding sequence
<i>mvan4662</i>	ATGATCGTTGACCTGATTCCGCGCCGTCTGAAAGAGCCGA TGTACCGTCTGTATGAACTGCGTCTGCGCCAGGGCCTGACC GCAAGTCGTTCCGAGCTGCCGCGCCACATTGCCGTTCTGTG TGATGGAAATCGTCGTTGGGCACGTGAAAGCGGTCACGAT GATGTTAGCTATGGTTATCGTGCAGGAGCACGAAAATTG CAGAAATGCTGCGTTGGTGTCAGGAAGCAGGTATTGAAAT GGCAACCGTTTATCTGCTGAGCACAGAAAATCTGCAGCGT GATACAGAAGAAGTCTGAGCGCACTGATTGAAATCATTACCG AGGTTGTTGAAGAAATTTGTGCACCAGCAAACCAAGTGGAG TGTTTCGTACCGTTGGTGATCTGGAAGTCTGTTGGAGAGGAA AGCGCACGTCGTCTGCGTGATGCAGTGAGCAGCACCGATG GGAATGGAGGGACCTTTCATGTGAATGTGGCAGTGGGTTA TGGTGGTCGTCAGGAAATTGTTGATGCGGTTCTGTCAGTGC TGAGTAAAGAACTGGCGAATGGTCTGACAGGCGAACAGCT GATTGAGTCAGTTACCGCAGAGGCAATTAGTGAAAATCTG TATACCAGCGGGCAGCCGGATCCTGATCTGGTTATTCGTAC AAGCGGAGAACAAACGCCTGAGCGGCTTTCTGCTGTGGCAG AGCGCATATAGCGAAATGTGGTTTACCGAAGCGTATTGGC CGGAGTTTCGTTCGTGTTGATTTTCTGCGTGCCCTGCGCGAT TATAGCGCACGTCATCGTCGTTATGGACGTTAA
<i>rv1086</i>	ATGGAGATCATTCCGCCGCGTCTGAAAGAGCCGCTGTACC GTCTGTATGAGCTGCGTCTGCGTCAAGGCCTGGCGGCGAG CAAGAGCGACCTGCCGCGTCACATTGCGGTTCTGTGCGAT GGTAACCGTCGTTGGGCGCGTAGCGCGGGTTACGACGATG TGAGCTACGGCTATCGTATGGGTGCGGCGAAGATCGCGGA GATGCTGCGTTGGTGCCACGAGGCGGGTATTGAACTGGCG ACCGTGTATCTGCTGAGCACCGAAAACCTGCAGCGTGACC CGGATGAGCTGGCGGCGCTGATCGAAATCATTACCGACGT GGTTGAGGAAATTTGCGCGCCGGCGAACCACTGGAGCGTT CGTACCGTGGGTGATCTGGGCCTGATTGGTGAAGAACCGG CGCGTCGTCTGCGTGGCGCGGTTGAGAGCACCCCGGAAGT

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AGGAACTGGCGAACGGTGCGACCGCGGAGGAACTGGTTG  
ATGCGGTTACCGTGAGGGGCATCAGCGAAAACCTGTACAC  
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GGCGAACAACGTCTGAGCGGTTTCCTGCTGTGGCAGAGCG  
CGTATAGCGAGATGTGGTTTACCGAAGCGCACTGGCCGGC  
GTTCCGTCACGTTGACTTTCTGCGTGCGCTGCGTGATTACA  
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*PstIDS3*

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CGAAGTACAAGACCGGCTTCTATACCTTTTACTATAGCGTG  
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*SlhCPT6*

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TTACCAACACCCTGTTCCCGGACTTTGGCGAGGAAGCGCT  
GATGGATGCGATCTTCAGCTTTCAACGTCGTCACCGTCGTT  
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*ShlzFPS*

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AAAGGTCTGCATCTGGTGATTGCGCTGAACTATGGTGGCT

ACTATGACATCCTGCAGGCGACCAAGAGCATTGTGAACAA  
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CGGACCTGCTGATTTCGTACCGGTGGCGATCAGCGTGTTAG  
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TTTGGTGGCCACACCTATTAA

*TctNudix1*

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CAATTTATCTGACCCAAACCAAACCTCCACAAACCATCGAA  
CCTCAAAGATGTGAAGGTTGGGACTGGTACGACTTGAAAA  
ACCTGCCAGAACCAATGTTCCAACCATTGAAAGAATTGTT  
GCAAGCTGGTTCTTTCAACATTTTCACCACTCACTCTTAA

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**Table S3.** Description of phosphatases used for Z,Z-FOH production in this study.

Names	Accessions	Descriptions
RdgB	NP_417429.1	dITP/XTP pyrophosphatase
PhoA	NP_414917.2	Periplasmic alkaline phosphatase
ScLPP1	NP_010791.3	lipid phosphate phosphatase
Ppa	NP_418647.1	Inorganic pyrophosphatase
Cdh	NP_418353.1	CDP-diacylglycerol pyrophosphatase
LpxH	NP_415057.1	UDP-2,3-diacylglucosamine hydrolase
MutT	NP_414641.1	8-Oxo-dGTP diphosphatase
NudC	YP_026280.1	NADH pyrophosphatase
NudF	NP_417506.1	ADP-ribose pyrophosphatase
Nudj	NP_415652.1	Nudix hydrolase
TctNudix1	MT12604.1	Truncated chloroplast nudix1
PgpB	NP_415794.1	Phosphatidylglycerophosphatase B
YbgG	NP_415362.1	Undecaprenyl (C55) pyrophosphate