

1 \*Supporting Information:

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4 **Engineering *Escherichia coli* strains with symbiotic plasmid for production of phenylpyruvic acid**

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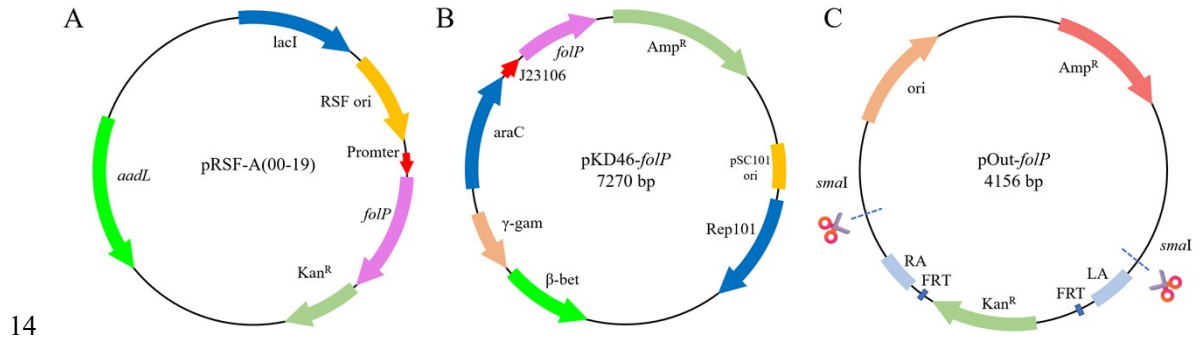
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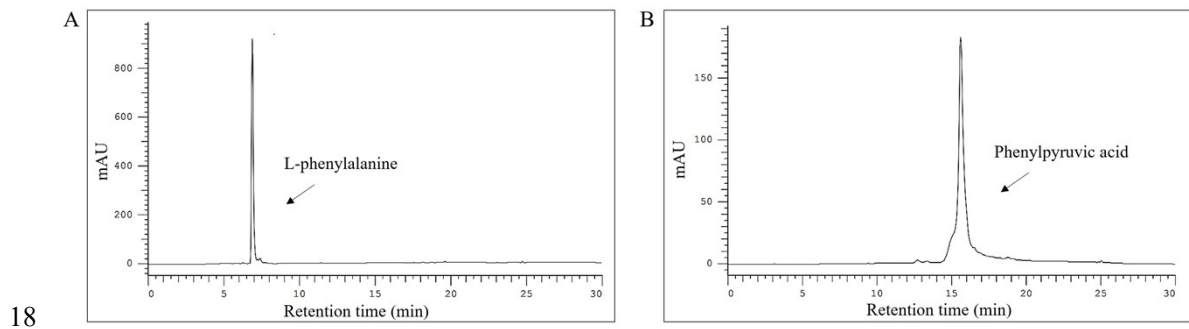
11 Address: Xinyang Normal University, 237 Nanhu Road, Xinyang, Henan 464000, China

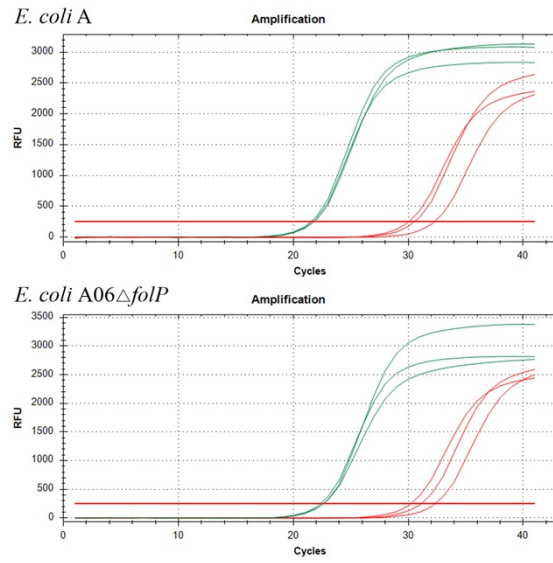
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13 **Supporting Figures**



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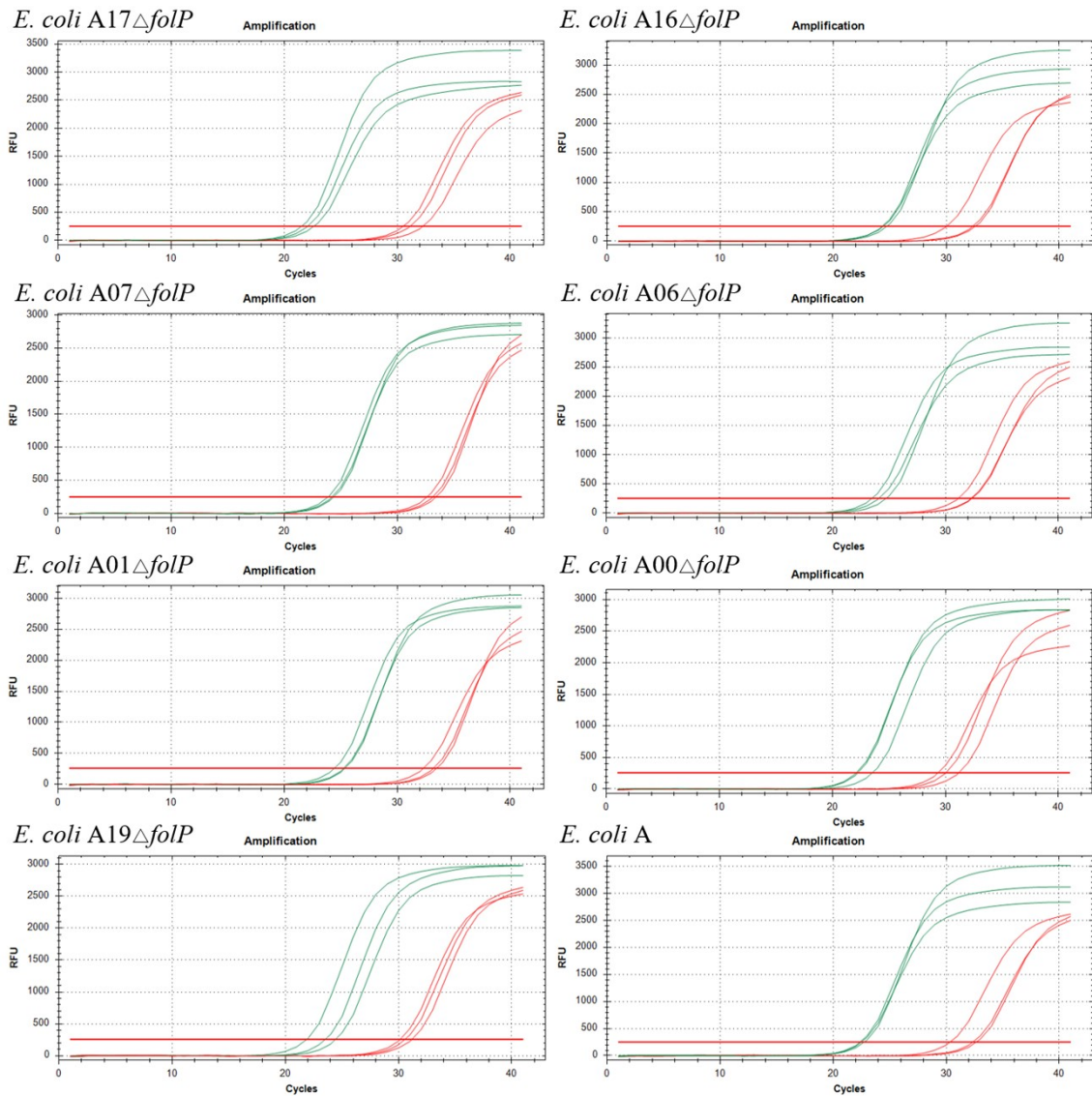




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23 **Figure S3.** The amplification curve of quantitative PCR from *E. coli A* and *E. coli A06 $\Delta$ folP*. The red  
24 line is threshold line, the amplification curve of 16S is marked in red and the amplification curve of *aadL*  
25 is marked in green.

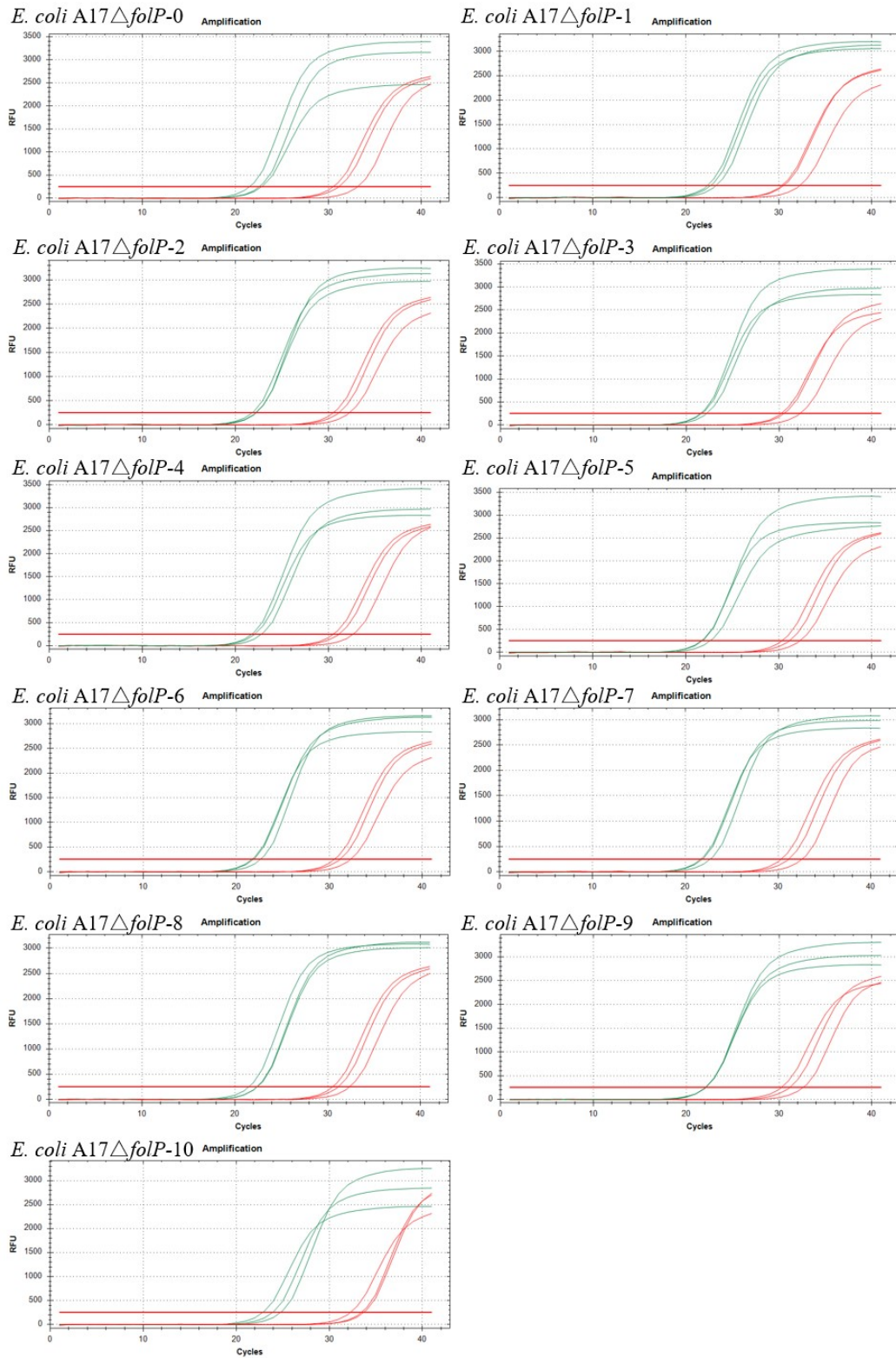
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28 **Figure S4.** The amplification curve of quantitative PCR from engineered *Escherichia coli* with promoters  
 29 of differing strength. The red line is threshold line, the amplification curve of 16S is marked in red and  
 30 the amplification curve of *aadL* is marked in green.

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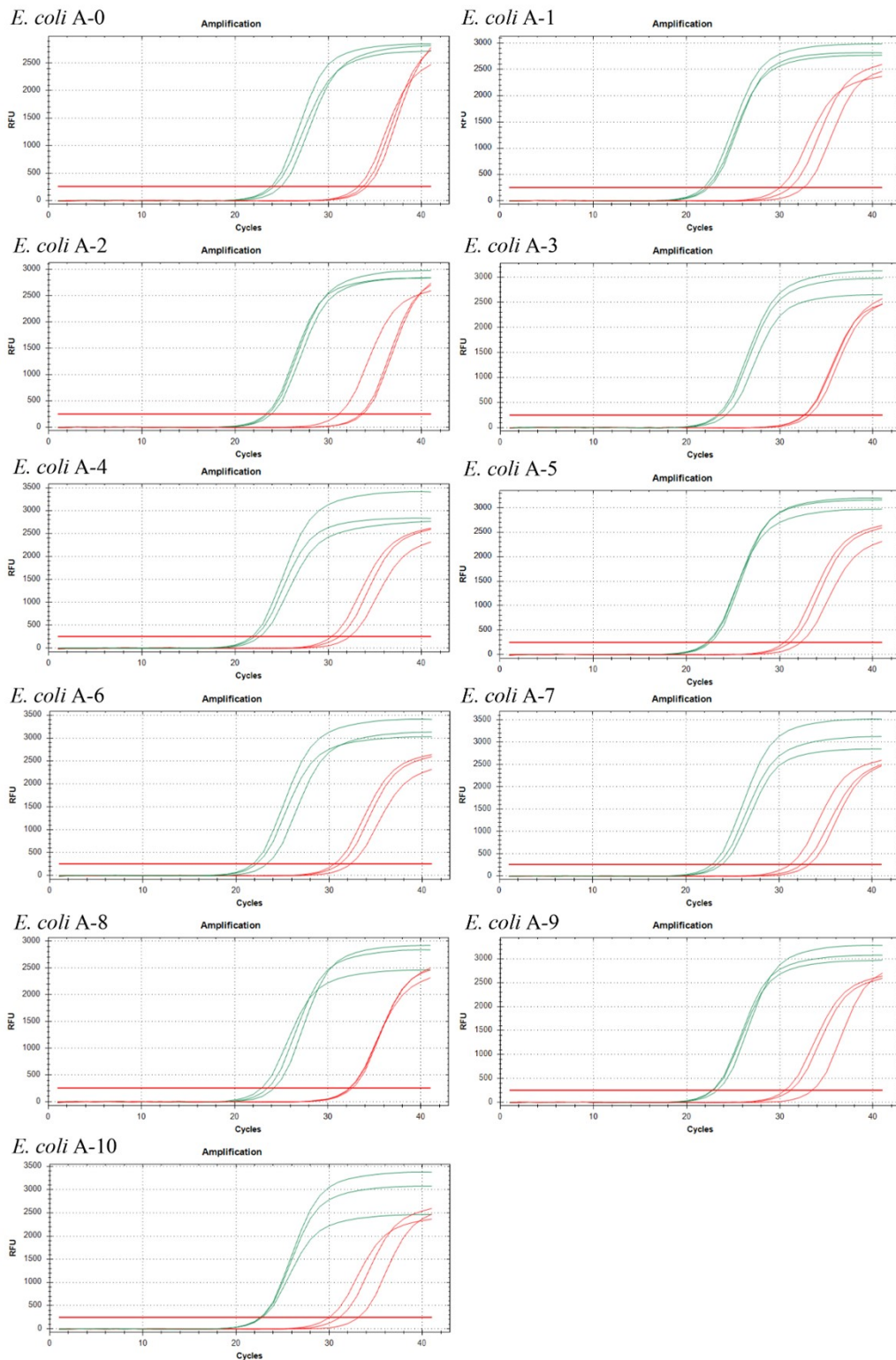


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33 **Figure S5.** The amplification curve of quantitative PCR from *E. coli* A17 $\Delta$ folP for different generation.

34 The red line is threshold line, the amplification curve of 16S is marked in red and the amplification curve

35 of *aadL* is marked in green.



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37 **Figure S6.** The amplification curve of quantitative PCR from *E. coli* A for different generation. The red  
 38 line is threshold line, the amplification curve of 16S is marked in red and the amplification curve of *aadL*  
 39 is marked in green.

40 **Supporting Tables**41 **Table S1.** Primers used in the work

Primers	Sequences, 5'-3'
<i>aadL</i> -BamHI (F)	G <b><u>CGGATCC</u></b> GATGAATATTTCTCGCCGTAAAC
<i>aadL</i> -EcoRI (R)	G <b><u>CGAATTC</u></b> TTACTTCTTGAAACGGTCAAGTG
<i>folP</i> -J23100 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTGACGGCTAGCTCAGTCCTAGGTACAGTGCT AGCTTAATTTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAAT GAAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23101 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTTACAGCTAGCTCAGTCCTAGGTATTATGCTA GCCAGTTTTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAATG AAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23106 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTTACGGCTAGCTCAGTCCTAGGTATAGTGCTA GCGATAGTTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAATG AAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23107 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTTACGGCTAGCTCAGCCCTAGGTATTATGCTA GCATGGATTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAATG AAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23116 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGGACTATGCT AGCAGGATTTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAAT GAAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23117 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGGATTGTGCT AGCCAATCTTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAAT GAAACTCTTTGCCCAGGGTAC
<i>folP</i> -J23119 (F)	G <b><u>CGCATGC</u></b> GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGTATAATGCT AGCACGAATTGTTTAACTTTAATTCACACAGGAAAGTACTAGATATAAT GAAACTCTTTGCCCAGGGTAC
<i>folP</i> (R)	CG <b><u>CCCGGG</u></b> TTACTCATAGCGTTTGTTCCTTTGC
pKD46- <i>folP</i> (F)	CCGTCAAGTTGTCATAATAAATCGGCGCGCCTTTACGGCTAGCTCAGTC CTAGGTATAGTGCTAGCGATAGTTGTTTAACTTTAATTCACACAGGAAA GTACTAGATATAATGAAACTCTTTGCCCAGGGTAC
pKD46- <i>folP</i> (R)	GCCACCTGCATCGATTTTTACTCATAGCGTTTGTTCCTTTG
pOut- <i>folP</i> (F)	<b><u>CCCGGG</u></b> ATGAAACTCTTTGCCCAGGGTACTTCACTGGACCTTAGCCATC CTCACGTAATGGATCAGTGATAAGCTGTCAAACATG

pOut- <i>folP</i> (R)	<u>CCCGGG</u> TTACTCATAGCGTTTGTTCCTTTGCAGACAGAGTGGCTTCC ACCACCCGTTGAGCGATTGTGTAGGCTGGAGCTG
qPCR-16S (F)	CTCTTGCCATCGGATGTGCCCA
qPCR-16S (R)	CCAGTGTGGCTGGTCATCCTCTCA
qPCR- <i>aadL</i> (F)	AATCATTAGTTACCAGACATC
qPCR- <i>aadL</i> (R)	GAGTGCGATAGGAAGTAT

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43 **Table S2. Plasmids and strains used in this study.**

Plasmids and strains	Description	Source
<i>E. coli</i> DH5 $\alpha$	Wild type	Lab stock
<i>E. coli</i> BL21(DE3)	Wild type	Lab stock
pKD46	Recombinase, <i>AmpR</i>	Lab stock
PCP20	pSC101 ori, <i>AmpR</i>	Lab stock
pKD3	<i>CmR</i> flanked by FRT sites	Lab stock
pRSFDuet-1	Double T7 promoters, RSF ori, <i>KanR</i>	Novagen
T-Vector pMD19 (Simple)	T vector, <i>AmpR</i>	TaKaRa
pKD46- <i>folP</i>	Recombinase, <i>AmpR</i> and <i>folP</i>	This study
pOut- <i>folP</i>	T-Vector carrying <i>folP</i> , <i>CmR</i> homologous arms	This study
pRSF- <i>aadL</i>	pRSFDuet-1 carrying <i>aadL</i>	This study
pRSF-A00	pRSF- <i>aadL</i> carrying J23100, B0032 and <i>folP</i>	This study
pRSF-A01	pRSF- <i>aadL</i> carrying J23101, B0032 and <i>folP</i>	This study
pRSF-A06	pRSF- <i>aadL</i> carrying J23106, B0032 and <i>folP</i>	This study
pRSF-A07	pRSF- <i>aadL</i> carrying J23107, B0032 and <i>folP</i>	This study
pRSF-A16	pRSF- <i>aadL</i> carrying J23116, B0032 and <i>folP</i>	This study
pRSF-A17	pRSF- <i>aadL</i> carrying J23117, B0032 and <i>folP</i>	This study
pRSF-A19	pRSF- <i>aadL</i> carrying J23119, B0032 and <i>folP</i>	This study
<i>E. coli</i> pKD46- <i>folP</i>	<i>E. coli</i> BL21(DE3) harboring pKD46- <i>folP</i>	This study
<i>E. coli</i> pKD46 $\square$ <i>folP</i>	<i>E. coli</i> pKD with the deletion of <i>folP</i>	This study
<i>E. coli</i> BL21 $\square$ <i>folP</i>	<i>E. coli</i> BL21 deleting <i>folP</i> from chromosome	This study
<i>E. coli</i> A	<i>E. coli</i> BL21(DE3) harboring pRSF- <i>aadL</i>	This study
<i>E. coli</i> A00 $\square$ <i>folP</i>	<i>E. coli</i> BL21 $\square$ <i>folP</i> harboring pRSF-A00	This study
<i>E. coli</i> A01 $\square$ <i>folP</i>	<i>E. coli</i> BL21 $\square$ <i>folP</i> harboring pRSF-A01	This study
<i>E. coli</i> A06 $\square$ <i>folP</i>	<i>E. coli</i> BL21 $\square$ <i>folP</i> harboring pRSF-A06	This study



<i>E. coli</i> A07□ <i>folP</i>	<i>E. coli</i> BL21□ <i>folP</i> harboring pRSF-A07	This study
<i>E. coli</i> A16□ <i>folP</i>	<i>E. coli</i> BL21□ <i>folP</i> harboring pRSF-A16	This study
<i>E. coli</i> A17□ <i>folP</i>	<i>E. coli</i> BL21□ <i>folP</i> harboring pRSF-A17	This study
<i>E. coli</i> A19□ <i>folP</i>	<i>E. coli</i> BL21□ <i>folP</i> harboring pRSF-A19	This study

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45 **Table S3. Comparison of promoters used in this study**

Name	Sequence (5'-3')	number in library
J23117	GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGCCAATC	5
J23116	GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGGACTATGCTAGCAGGAT	14
J23107	GGCGCGCCTTTACGGCTAGCTCAGCCCTAGGTATTATGCTAGCATGGA	18
J23106	GGCGCGCCTTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGCGATAG	27
J23101	GGCGCGCCTTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGCCAGTT	44
J23100	GGCGCGCCTTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGCTTAAT	56
J23119	GGCGCGCCTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCACGAA	82
B0032	TCACACAGGAAAGTACTAG	29

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