Supplementary Table 1. Strains and plasmids used in this study.

Strain and Plasmid	Relevant characteristics	Source or reference
Strains		
E. coli		
DH5α	*supE*44, Δ*lacU*169(φ80 *lacZ*ΔM15), *HsdR*17, *recA*1, *endA*1, *gyrA*96, *relA*1	
BL21(DE3) CodonPlus RIL	*E. coli* B F^- *ompT hsdS*($r_B^-m_B^-$) *dcm*$^+$ Tet^r *gal* λ (DE3) *endA* Hte (*argU ileY leuW* Cam^r)	
T. kodakaraensis		
KOD1		(8, 9)
KUW1	Δ*pyrF*, Δ*trpE*	(12)
ΔTK2141 (Δ*pok*)	KUW1 ΔTK2141	This study
ΔTK1686 (Δ*pps*)	KUW1 ΔTK1686	This study
ETK0939	KUW1 P_{TK0939}::P_{csg}::TK0939	This study
ETK2141	KUW1 Δ*chiA*::TK2141 overexpression cassette (P_{csg}::TK2141)	This study
Plasmids		
pUC118	Amp^r general cloning vector	Takara
pET-21a(+)	Amp^r general expression vector in *E. coli*	Novagen
pET-TK0939	pET-21a(+) derivative; TK0939	This study
pET-TK1473	pET-21a(+) derivative; TK1473	This study
pET-TK2141	pET-21a(+) derivative; TK2141	This study
pET-TK2242	pET-21a(+) derivative; TK2242	This study
pET-TK1686	pET-21a(+) derivative; TK1686	This study
pET-*EccoaA*	pET-21a(+) derivative; *EccoaA*	This study
pUD3	pUC118 derivative; *pyrF* marker cassette (P*pyrF*::*pyrF*)	This study
pUD3-TK0939	pUD3 derivative; P_{csg}::TK0939::*pyrF*	This study
pUD3-TK2141	pUD3 derivative; Δ*chiA*::TK2141 overexpression cassette (P_{csg}::TK2141)::*pyrF*	This study
pUD3-Δ*pok*	pUD3 derivative; Δ*pok* cassette::*pyrF*	This study
pUD3-Δ*pps*	pUD3 derivative; Δ*pps* cassette::*pyrF*	

Supplementary Table 2. Primers used in this study.

Primer names	Primer sequences
Construction of plasmids for gene expression in *E. coli*	
TK0939EF	5'- TGGTGAG**CATATG**ATAGTGCGCACGCCTAGGA -3'
TK0939ER	5'- GCTCCA**GGATCC**TCACTGACCACCGGC -3'
TK1473F	5'- AGGTGGA**CATATG**ATACTCATCAAGATCGGCGGT -3'
TK1473R	5'- CGAACA**GAATTC**TCACCTCAGCCTCGTTCCGA -3'
TK2141EF	5'- GGTGAA**CATATG**CTCATCAGGGCGTTTATTCC -3'
TK2141ER	5'- AAGGGT**GAATTC**CTACCCGACCCAGCGCCCGA -3'
TK2242F	5'- GGTGAGG**CATATG**GATTCGATCAGAATCAAAG -3'
TK2242R	5'- ATACCA**GGATCC**TTAACCATCCAGCACCACCA -3'
TK1686F	5'- AGGTGAT**CATATG**GTGAACATCCCGAAGAGT -3'
TK1686R	5'- AAAGGA**GAATTC**TCACTCAAGCTCCTTTCTCCT -3'
*EccoaA*F	5'- GATAGA**CATATG**CACCACCACCACCACCACATGAGTATAAAAGAGCAAACG-3'
*EccoaA*R	5'- CTCCCC**GGATCC**TTATTTGCGTAGTCTGACCT-3'
Construction of plasmids for gene expression in *T. kodakaraensis*	
TK0939TF1	5'- TG**GGATCC**CTCGTCACGAGTCTCGGCTACC -3'
TK0939TR1	5'- CA**GGATCC**TCACTGACCACCGGCGACGCT-3'
TK0939TF2	5'- *CACCACCACCACCACCAC*GTGATAGTGCGCACGCC-3'
TK0939TR2	5'- TTTCTCACCACCATAGAAGCAAAGAAG -3'
P_{csg}F1	5'- TATCGGCAAAAGGCGAATTATGTGTAGGCA -3'
P_{csg}R	5'- *GTGGTGGTGGTGGTGGTG***CATATG**ACAACACCTCCTTGGGTT -3'
TK2141TF	5'- AAAAA**CATATG**CACCACCACCACCACCACATGCTCATCAGGGCGT -3'
TK2141TR	5'- *CTACCCGACCCAGCGC*CCGACCTT -3'
*chiA*TF1	5'- *GCGCTGGGTCGGGTAG*CCCCTCTCTTCTCCTCTTT -3'
*chiA*TR1	5'- AAAA**GGATCC**TCTGAGCCCCTGGAGAAGGGGG -3'
*chiA*TF2	5'- AAAA**GAATTC**ACGAACCTTATTCCTTCTGCAT -3'
*chiA*TR2	5'- *GCCGATAACACAAC*ACCCCTTGAGCTTTGAAT-3'
P_{csg}F2	*5'-GTTGTGTTATCGGC*AAAAGGCGAATTATGTGT-3'
Construction of plasmids for gene disruption in *T. kodakaraensis*	
TK2141DF1	5'- AA**GGATCC**AACCGACCTTGGCGCTCCACA -3'
TK2141DR1	5'- AA**GGATCC**ACGTATTGGAAGGGCCTTGGCCG -3'
TK2141DF2	5'- CCCGCTACCCTTTTATTCTCCTTGC -3'
TK2141DR2	5'- GCTTTCACCCCAGAATTGTTCAGA -3'
TK1686DF1	5'- GG**GGATCC**TGCTCGGCAAAGCGGGAGTC -3'
TK1686DR1	5'- GC**GGATCC**CCAGATGAACGTCTTTAAC -3'
TK1686DF2	5'- AGCCTTTCCTTTTGTTACCTTTTTGT -3'
TK1686DR2	5'- ACTATCACCTGCTCACAGTTTGCTG -3'
Examination of gene disruption	
TK2141CF	5'- TTACTGTGGCGAGCATAGGTGCAGT -3'
TK2141CR	5'- CTTTCCGAGGATTAGGTATGGAA -3'
TK1686CF	5'- ACAGTGCACCACCTCGAACACGTGA -3'
TK1686CR	5'- TGCCGTAGTCAGTGCCCTTGAGAA -3'

Underlined sequences correspond to those found in the *T.kodakaraensis* genome sequence. Bold sequences represent restriction enzyme sites.

Italic sequences are regions used to connect DNA fragments via fusion PCR.

Legend to Supplementary Fig. 1.

Supplementary Fig. 1. The plasmid and recombination strategies to construct the over-expression strains of TK0939 (A) and TK2141 (B). *T. kodakaraensis* KUW1 (Δ*pyrF*, Δ*trpE*) was used as the host strain. A strong, constitutive promter (P_{csg}) and a His-Tag sequence was incorporated into the plasmids. Cells that had undergone the initial single crossover insertion were enriched by growing cells in medium without uracil. Cells that had undergone the second, pop-out recombination were selected with resistance towards 5-FOA.

SUPPLEMENTARY FIGURE 1