Supplementary Table S1. LB components and contents

Components	Contents
yeast extract powder	0.5%
peptone	1%
NaCl	1%

Supplementary Table S2. LBG components and contents

Components	Contents
yeast extract powder	0.5%
peptone	1%
NaCl	1%
glucose	0.5%

Supplementary Table S3. CgXII components and contents

Components	Contents
$(NH_4)_2SO_4$	2%
urea	0.5%
KH_2PO_4	0.1%
$MgSO_4 \cdot 7H_2O$	0.025%
$K_2HPO_4 \cdot 3H_2O$	0.1%
3-(n-morphine) -propanesulfonic acid	4.2%
$CaCl_2$	0.001%
$FeSO_4 \cdot 7H_2O$	0.001%
$MnSO_4 \cdot H_2O$	0.001%
$ZnSO_4 \cdot 7H_2O$	0.001%
$CuSO_4 \cdot 5H_2O$	0.00002%
$NiCl_2 \cdot 6H_2O$	0.00002%
biotin	0.00002%
Thick catechuic acid	0.000003%

Supplementary Table S4. EPO components and contents

Components	Contents
yeast extract powder	0.5%
peptone	1%
NaCl	1%
Tween 80	0.1%
glycine	3%

Supplementary Table S5. LBHISG components and contents

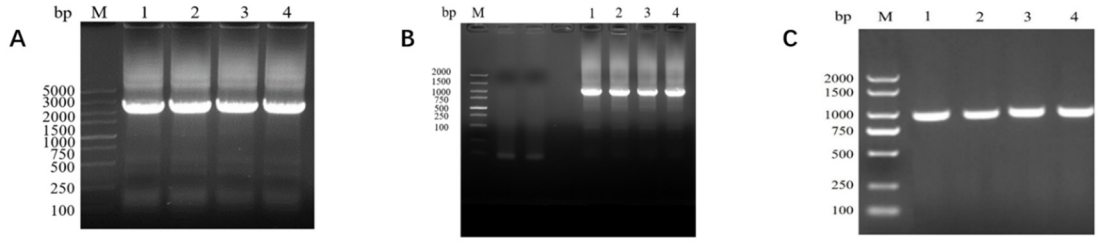
Components	Contents
yeast extract powder	0.25%
peptone	0.5%
NaCl	0.5%
Brain and heart extract	1.85%
glucose	0.25%
sorbitol	9.1%

Supplementary Table S6. Composition and content of fermentation medium

Components	Contents
glucose	2.0%
corn steep liquor	0.05%
molasses	1.6%
phosphoric acid	0.06%
$(NH_4)_2SO_4$	1.20%
$MgSO_4$	0.20%
KCl	0.05%
betaine	0.05%
$FeSO_4$	0.03%
$MnSO_4$	0.03%
$ZnSO_4$	0.005%
$CuSO_4$	0.005%
vitamin B1	0.0005%
L-threonine	0.025%

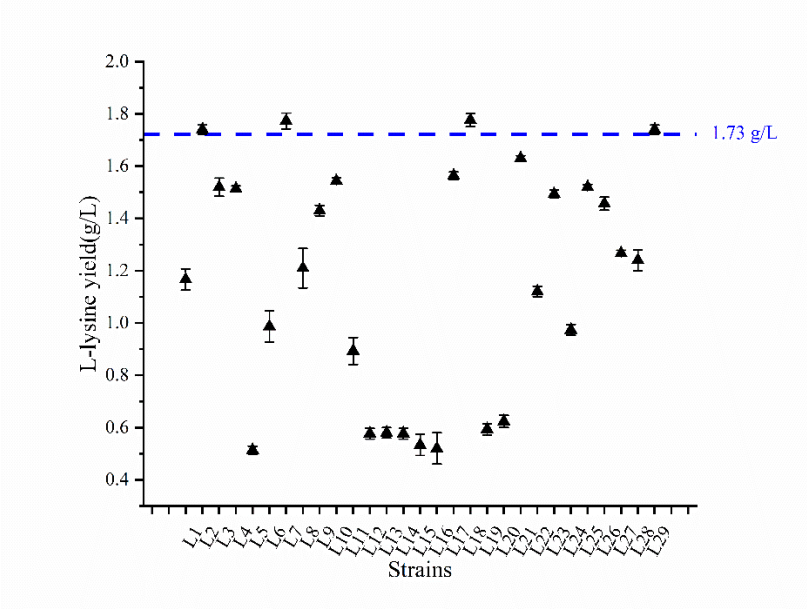
Supplementary Table S7 Primers list

Primer	Sequence(5'→3')	Size (bp)	Restriction site
t-Lys-F1	gtggtggtggtggtgctcgagGTGCAGGATAAATCCCGCC	40	*Xho*I
t-Lys-R1	ggtccacggagaattcACTTTTTCGTTGCTTTCGGTTT	38	
t-Lys-F2	cgagctcggtaccATGTAAAAAAGCGCCCTAAAGG	35	
t-Lys-R2	cagcaaatgggtcgcggatccATGCTTAACGGCGTCGGC	39	*Bam*HI
Kanr-F	aagtGAATTCTCCGTGGACCTGCA	24	
Kanr-R	tttacatGGTACCGAGCTCGGATCCG	27	
staygold^{r}-F	ATGGCTAGTACACCATTTAAATTTC	25	
rplx^{r}-R	TTATTTGATAGTTTCGCTGTTAGATTTG	28	
L21^{r}-R	TTAAGATTTTTTTGTGGTTTTTTTTGC	27	

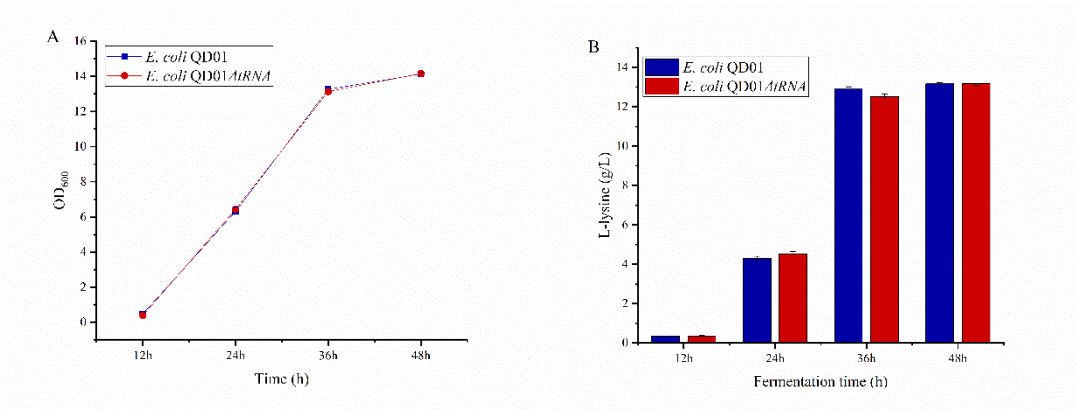


Supplementary Figure S1 Gel electrophoresis of PCR products.

(A) PCR gel showing the expected product insert size of 2324 bp for Knock out box gene fragments. M, DL5000 DNA marker. (B) PCR gel showing the expected product delete size of 1324 bp for Kanr fragments. M, DL2000 DNA marker. (C) PCR gel showing the expected product insert size of 1008 and 1026 bp for *staygold-rplx^{r}* and *staygold-L21^{r}* gene fragments separately. M, DL2000 DNA marker.



Supplementary Figure S2 The L-lysine produced by the original strain and the mutated strains. *29 mutant strains of E. coli ΔtRNA egfp^r were selected on 96-well plates, blue line indicates the highest L-lysine group three parallel fermentation yield of the starting E. coli QD01ΔtRNA, 1.73 g/L. Among the 29 mutant strains, 4 with improved L-lysine yields were screened.*



Supplementary Figure S3 OD_{600} values and L-lysine production of *E. coli* QD01 and *E. coli* QD01*ΔtRNA* (fermentation medium). *(A) These two strains had comparable OD_{600} values. (B) We detected no significant difference between the strains with respect to L-lysine pro-duction ($p > 0.05$).*