# SUPPLEMENTARY DATA

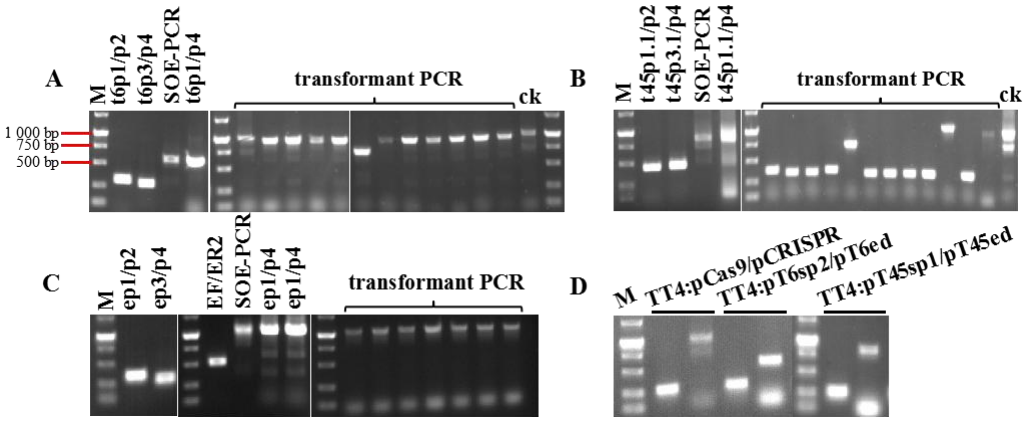


**FIG S1** Construction of strains with recombination template plasmid, or recombination template and targeted plasmids. M is DL5000 DNA Marker. The vertical text in the figure illustrats the primers used in PCR, except for SOE-PCR, which was the result of the second round of SOE-PCR (gene splicing by overlap extension- PCR). (A and B) The construction of TT4::pT6ed and TT4::pT45ed, respectively. The primers used in the PCR of transformants were pCRISPR-F/R. Ck is the result of TT4::pCRISPR amplified with primer pCRISPR-F/R. (C) The construction of TT4::pT6ed-e. Gene *e* was amplified with primers EF/ER2. The primers used for PCR of transformants were ep1/p4. (D) The verification of the strains containing double plasmids. Plasmid pCas9 was verified with primers DR-F/R. pCRISPR, pT6ed and pT45ed were verified with primers pCRISPR-F/R, t6p1/p4 and t45p1.1/p4, respectively.

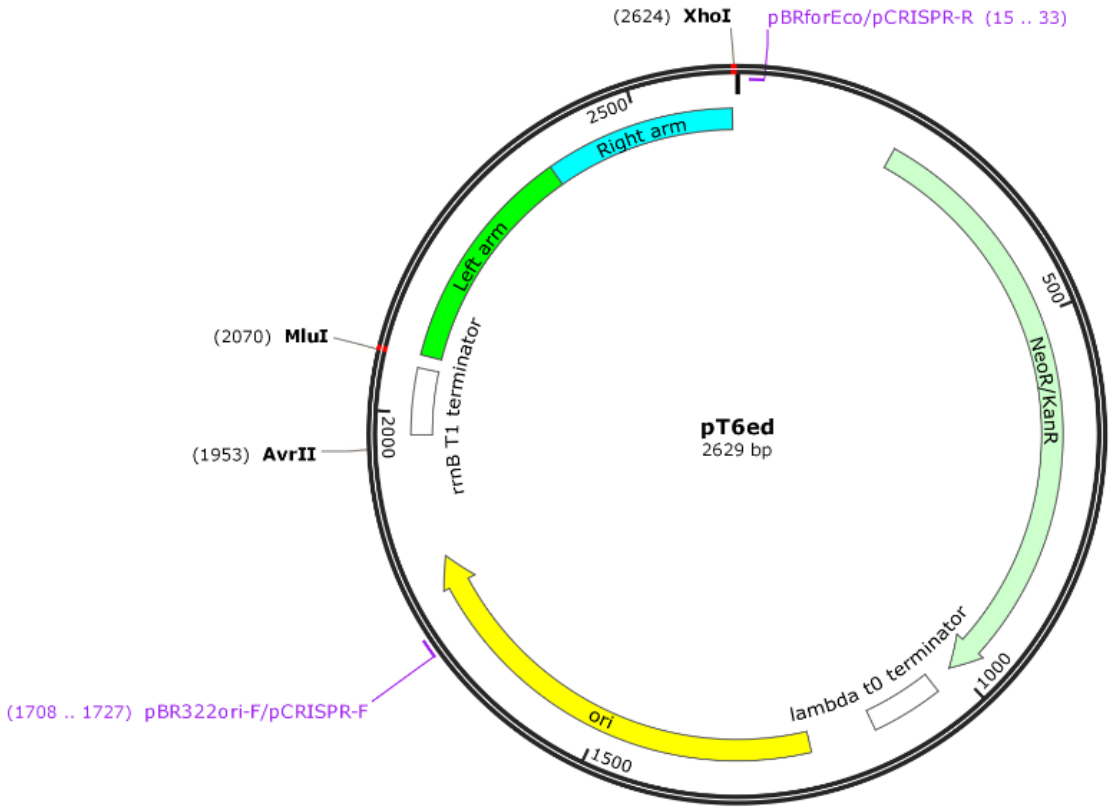


**FIG S2** Map of plasmid pT6ed. The left and the right arm were the gene sequence of phage TT4P2, which can be linked to plasmid pCRISPR after enzyme digestion (*Mlu*I and *Xho*I marked with red) to construct homologous recombination plasmid pT6ed. The plasmid pT6ed can promote the homologous recombination of phage TT4P2, when performed the deletion of phage TT4P2-*orf6*.

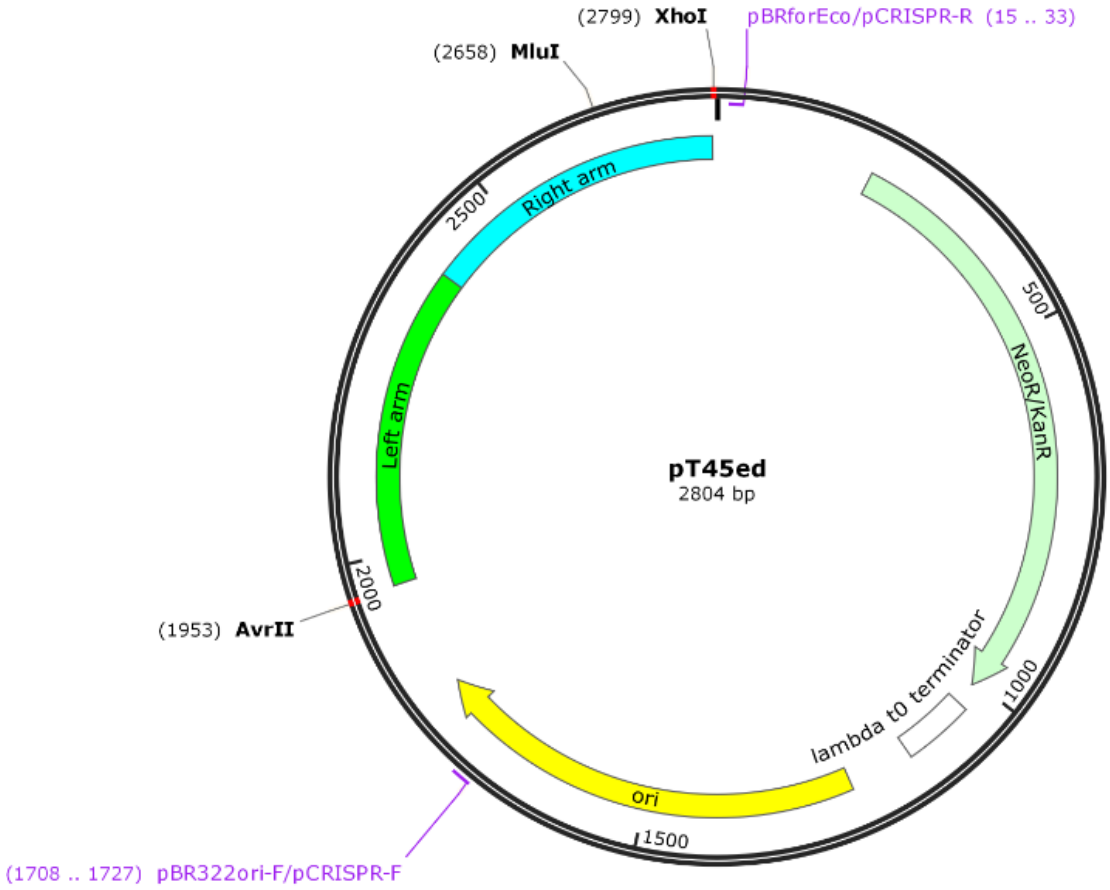


**FIG S3** Map of plasmid pT45ed. The left and the right arm were the gene sequence of phage TT4P2, which can be linked to plasmid pCRISPR after enzyme digestion (*Avr*II and *Xho*I marked with red) to construct homologous recombination plasmid pT45ed. The plasmid pT45ed can promote the homologous recombination of phage TT4P2, when performed the deletion of phage TT4P2-*orf45*.

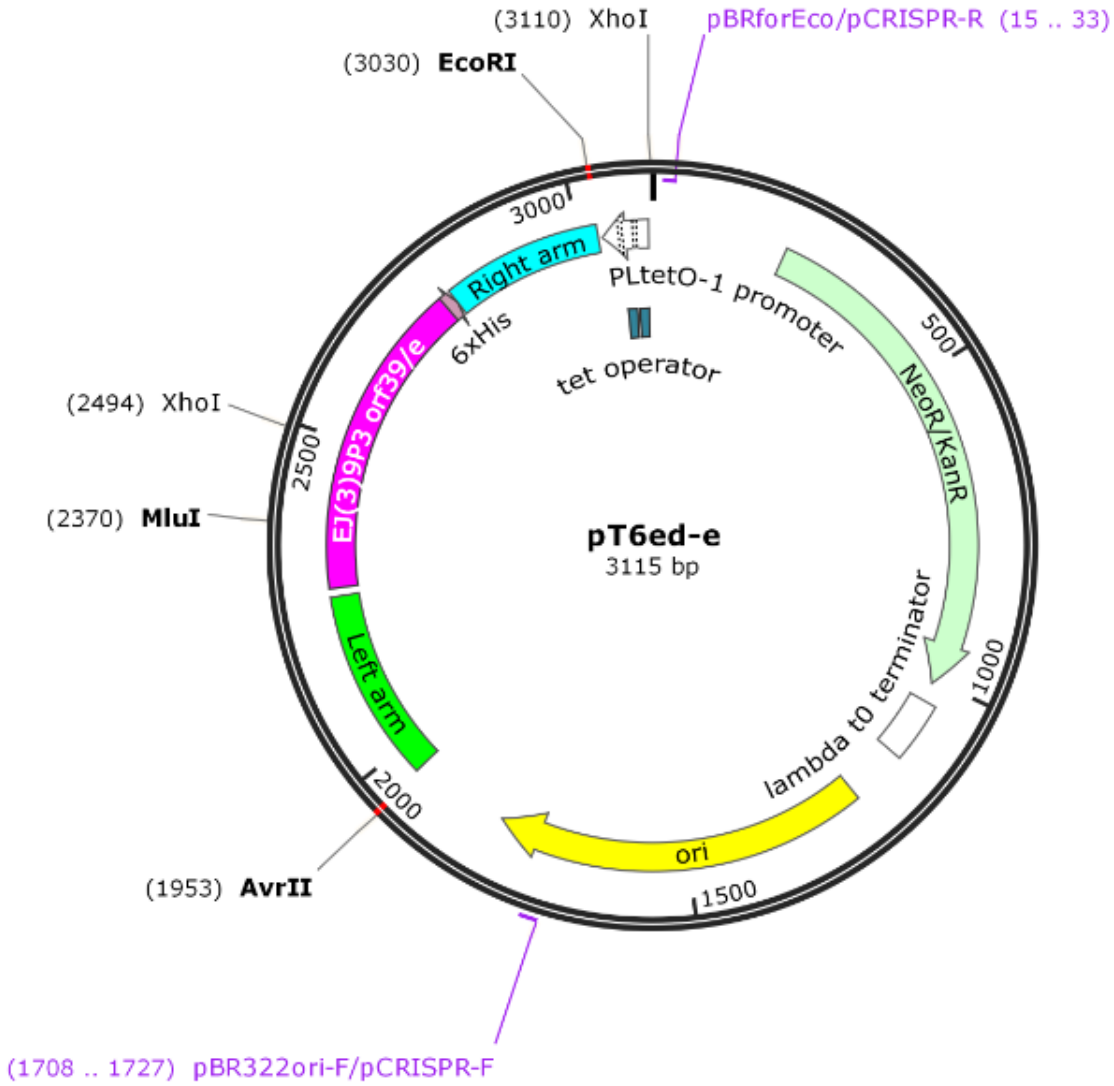


**FIG S4** Map of plasmid pT6ed-e. The left and the right arm were the gene sequence of phage TT4P2, which can be linked to plasmid pCRISPR after enzyme digestion (*Avr*II and *Eco*RI marked with red) to construct plasmid pT6ed-e. EJ(3)9P3-*orf39* is lysozyme *e*. The plasmid pT6ed-e was used for the gene replacement between TT4P2-*orf6* and EJ(3)9P3-*orf39*.

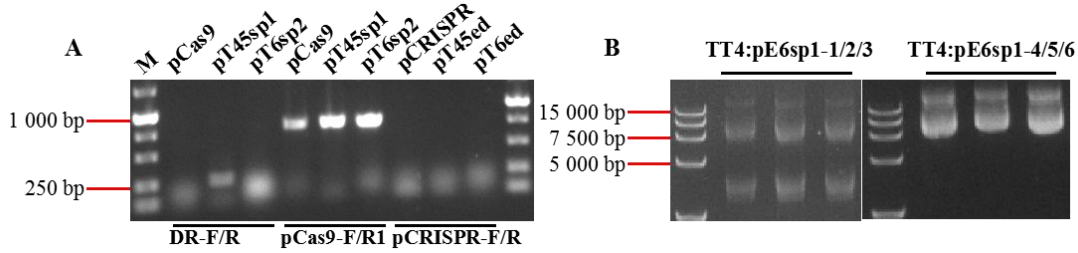


**FIG S5** PCR of bacterial suspensions and extraction of pE6sp1 for plasmid stability Assay. M is DL5000 DNA Marker. (A) PCR results of plasmids were used to verify their stability. The labels below the picture were primers. (B) The extraction of pE6sp1. A sequence in pCas9 was replaced with a spacer1 sequence targeting EH(3)23P1-*orf6* to form plasmid pE6sp1. Plasmids were extracted from TT4::pE6sp1. 1/2/3 were extracted from TT4::pE6sp1, which was taken out at -20 ℃ and directly subcultured. 4/5/6 were extracted from TT4::pE6sp1, which was taken out at -20 ℃ and streaking on the plate to picked single colony.

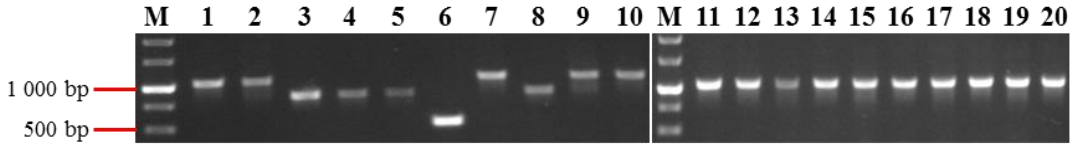


**FIG S6** Replacement of phage TT4P2-*orf6* with lysozyme gene *e* of phage EJ(3)9P3 (*orf39*). M is DL5000 DNA Marker. 1-20 were plaques. The first 10 plaques were picked from TT4::pT6sp2, and the last 10 plaques were picked from TT4::pT6sp1. The plaques of 3, 4, 5 and 8 were wild-type. The plaque of 6 was a nontarget recombinant.

**TABLE 1** Strains used in this study

| Strain | Source or Reference |
|---|---|
| *Vibrio natriegens* TT4 | Preserved in this laboratory |
| *Enterobacter* spp. EJ(3)9 | Preserved in this laboratory |
| *Escherichia coli* DH5α::pCas9 | Addgene |
| *E. coli* DH5α::pCRISPR | Addgene |
| *V. natriegens* TT4::pCas9 | TT4 contains plasmid pCas9 |
| *V. natriegens* TT4::pCRISPR | TT4 contains plasmid pCRISPR |
| *V. natriegens* TT4::pCas9/pCRISPR | TT4 contains plasmid pCas9 and pCRISPR |
| *V. natriegens* TT4::pT6sp1 | TT4 contains plasmid pT6sp1 |
| *V. natriegens* TT4::pT6sp2 | TT4 contains plasmid pT6sp2 |
| *V. natriegens* TT4::pT6ed | TT4 contains plasmid pT6ed |
| *V. natriegens* TT4::pT6ed-*e* | TT4 contains plasmid pT6ed-*e* |
| *V. natriegens* TT4::pT45sp1 | TT4 contains plasmid pT45sp1 |
| *V. natriegens* TT4::pT45sp2 | TT4 contains plasmid pT45sp2 |
| *V. natriegens* TT4::pT45ed | TT4 contains plasmid pT45ed |
| *V. natriegens* TT4::pT6sp2/pT6ed | TT4 contains plasmid pT6sp2 and pT6ed |
| *V. natriegens* TT4::pT45sp1/pT45ed | TT4 contains plasmid pT45sp1 and pT45ed |
| *V. natriegens* TT4::pE6sp1 | TT4 contains plasmid pE6sp1 |

Note: In order to select plasmid pCas9 from *E. coli* DH5α::pCas9, chloramphenicol was added to medium at a final concentration of 25μg/ml. The selection of plasmid pCRISPR from *E.coli* DH5α::pCRISPR was added to medium at a kanamycin final concentration of 50μg/ml. *V. natriegens* phage TT4P2-*orf6* is indicated by T6; TT4P2-*orf6*-spacer is indicated by T6sp, which was connected to plasmid pCas9 to form plasmid pT6sp; TT4P2-*orf6*-editing is indicated by T6ed, which was connected to plasmid pCRISPR to form plasmid pT6ed. The same is as TT4P2-*orf45*.

**TABLE 2** Bacteriophages used in this study

| Bacteriophage | Source or Reference |
|---|---|
| *Vibrio natriegens* siphophage TT4P2 | Preserved in this laboratory |
| *Enterobacter* spp. myophage EJ(3)9P3 | Preserved in this laboratory |
| TT4P2Δ*orf6* | TT4P2 *orf6* deleted 292 bp, constructed in this study |
| TT4P2Δ*orf5*Δ*orf6*Δ*orf7* | TT4P2 deleted 820 bp, including the entire *orf6*, 121 bp at the back of *orf5*, and 182 bp at the front of *orf7*, constructed in this study |
| TT4P2Δ*orf6*Δ*orf7* | TT4P2 deleted 347 bp, occurring between two short repeats in the same direction. One of the two repetitive sequences is in *orf6*, and the latter is in *orf7*, constructed in this study |
| TT4P2Δ*orf6*::*e* | The 292 bp of phage TT4P2-*orf6* was replaced with *orf39*(*e*) of phage EJ(3)9P3, constructed in this study |
| TT4P2Δ*orf*45 | TT4P2 *orf45* deleted 162 bp, constructed in this study |

**TABLE 3** Plasmids used in this study

| Plasmid | Description | Source or Reference |
|---|---|---|
| pCas9 | Low copy, $Cam^R$, 9326 bp | Addgene |
| pCRISPR | High copy, $Kan^R$, 2707 bp | Addgene |
| pT6sp1 | The original sequence in pCas9 was replaced with a spacer (sp1) targeting TT4P2 *orf6* | This study |
| pT6sp2 | The original sequence in pCas9 was replaced with a spacer (sp2) targeting TT4P2 *orf6* | This study |
| pT6ed | Transformed by pCRISPR to delete 292 bp of TT4P2 *orf6* | This study |
| pT6ed-*e* | Transformed by pCRISPR to replace 292 bp of TT4P2 *orf6* with EJ(3)9P3-*orf39* | This study |
| pT45sp1 | The original sequence in pCas9 was replaced with a spacer (sp1) targeting TT4P2 *orf45* | This study |
| pT45sp2 | The original sequence in pCas9 was replaced with a spacer (sp2) targeting TT4P2 *orf45* | This study |
| pT45ed | Transformed by pCRISPR to delete 162 bp of TT4P2 *orf45* | This study |

Note: *V. natriegens* phage TT4P2-*orf45* is indicated by T45; TT4P2-*orf45*-spacer is indicated by T45sp, which was connected to plasmid pCas9 to form plasmid pT45sp; TT4P2-*orf45*-editing is indicated by T45ed, which was connected to plasmid pCRISPR to form plasmid pT45ed.

**TABLE 4** Oligos used in this

| Spacer oligo | Sequence (5'-3') | Description |
|---|---|---|
| t6oligo-sp1 I | AAACTAAACGAAAAGCCCGCTATTGAATCCGTTAG | The spacer (sp1) of TT4P2 *orf6*; Targeting TT4P2 *orf6* |
| t6oligo-sp1 II | AAAACTAACGGATTCAATAGCGGGCTTTTCGTTTA | |
| t6oligo-sp2 I | AAACCACGTTTTCGCAGGTTGTAAGATCAACTTAG | The spacer (sp2) of TT4P2 *orf6*; Targeting TT4P2 *orf6* |
| t6oligo-sp2 II | AAAACTAAGTTGATCTTACAACCTGCGAAAACGTG | |
| t45oligo-sp1 I | AAACCGACAAGCTACTAGACCTCGCGAGCCGTATG | The spacer (sp1) of TT4P2 *orf45*; Targeting TT4P2 *orf45* |
| t45oligo-sp1 II | AAAACATACGGCTCGCGAGGTCTAGTAGCTTGTCG | |
| t45oligo-sp2 I | AAACCCCATTCGTCCATCGTAGAATACCCCTAAAG | The spacer (sp2) of TT4P2 *orf45*; Targeting TT4P2 *orf45* |
| t45oligo-sp2 II | AAAACTTTAGGGGTATTCTACGATGGACGAATGGG | |

Note: The underscore at oligos was the sequence of spacers.

**TABLE 5** Primers used in this

| Primers title | Sequence (5'-3') | Description |
|---|---|---|
| e1/e_pET_ex_his_1 | ATAGGAGGTCCCCATGGACATT | Verified EJ(3)9P3 |
| e2/e_pET_ex_his_2 | AAATGTCGACTAGGTTTTCATATGC | |
| pBR322ori-F/pCas9-R1 | GGTGATGTCGGCGATATAGG | Verified pCas9 |
| CAT-R/pCas9-F | GCAACTGACTGAAATGCCTC | |
| DR-F | GCTGAGACAAATAGTGCG | Verified spacer |
| DR-R | GTATCCGACTGCTGGTATT | |
| pBR322ori-F/pCRISPR-F | GGGAAACGCCTGGTATCTTT | Verified pCRISPR |
| pBRforEco/pCRISPR-R | AATAGGCGTATCACGAGGC | |
| t6p1 | TACGACGCGTCATGAGACGCTAGGAGAAC | Delete TT4P2 *orf6* |
| t6p2 | ATGAGTGATCGAAGTGGG | |
| t6p3 | CGCCCACTTCGATCACTCATGCTTATCGCGTTTACTGG | |
| t6p4 | TCTACTCGAGTCCTTTAGGTTGGCTTCG | |
| t6Y-F | TCCCTGAACCTGACACCAG | Verified TT4P2Δ*orf6* |
| t6Y-R | TTAGGCTCGCACTGTCCCA | |
| ep1 | TACGCCTAGGCATGAGACGCTAGGAGAAC | Replaced TT4P2*orf6* |
| ep2 | ATGCCAAAAATGTCCATGGGGACCTCCTATATGAGTGATCGAAGTGGG | |
| ep3 | TATGAAAACCTACATCATCATCATCATCATGCTTATCGCGTTTACTGG | |
| ep4 | TCTAGAATTCTCCTTTAGGTTGGCTTCG | |
| EF/e1 | ATAGGAGGTCCCCATGGACATT | |
| ER2 | ATGATGATGATGATGATGTAGGTTTTCATATGCTTTCC | |
| t45p1.1 | TACGCCTAGGTGAACGCGATTAACTACGG | Delete TT4P2 *orf45* |
| t45p2 | ATCCAGCCTTGATCGTTG | |
| t45p3.1 | CCTGACAGCAACCAACGATCAAGGCTGGATCGATTACTACACGGGCACT | |
| t45p4 | TCTACTCGAGCAACGTATGACGCTCCAA | |
| t45Y-F | CGCCAGAAATTAACACGC | Verified TT4P2Δ*orf45* |
| t45Y-R | AAGCCCAAACACCCTCAA | |
| TT45-F | GATGAACTACGCAGGGAT | |
| TT45-R1 | GGTGTGATTGCCATATAAAG | |

Note: The underline at 6 bases was restriction endonuclease cleavage site. The underline at p2/p3 was the complementary sequence in overlap extension PCR. The wavy line at ER2 was tag of 6 ×His.