**Supplementary File 1a. Strains**

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| **Bacterial Strains** | | |
| **Name** | **Genotype** | **Source** |
| ML6 | MG1655 |  |
| DH5α | Cloning strain | Invitrogen |
| ML3326 | MG1655 pBAD33-*toxN* pEXT20-*toxI* | Guegler and Laub, 2021 |
| ML3328 | MG1655 pBR322-*toxIN* | Guegler and Laub, 2021 |
| ML3330 | MG1655 pBR322 | Guegler and Laub, 2021 |
| ML3772 | MG1655 pBR322-*toxIN* pKVS45 | This study |
| ML3773 | MG1655 pBR322-*toxIN* pKVS45-*dmdT4* | This study |
| ML3774 | MG1655 pBR322-*toxIN* pKVS45-*tifAT4* | This study |
| ML3775 | MG1655 pBR322-*toxIN* pKVS45-*tifAT2* | This study |
| ML3776 | MG1655 pBR322-*toxIN* pKVS45-*tifAT6* | This study |
| ML3777 | MG1655 pBR322-*toxIN* pKVS45-*tifARB69* | This study |
| ML3778 | MG1655 pBAD33-*toxN* pEXT20-*tifAT2* | This study |
| ML3779 | MG1655 pBAD33-*toxN* pEXT20-*tifAT4* | This study |
| ML3780 | MG1655 pBAD33-*toxN* pEXT20-*tifAT6* | This study |
| ML3781 | MG1655 pBAD33-*toxN* pEXT20-*tifARB69* | This study |
| ML3782 | MG1655 pBAD33-*toxN* pEXT20 | This study |
| ML3783 | MG1655 pBR322-*toxIN* pKVS45-*tifA T4* ΔATG | This study |
| ML3784 | MG1655 pBR322-*toxIN* pKVS45-*tifA T4* recoded | This study |
| ML3785 | MG1655 pBR322-*toxIN* pKVS45-*tifAT4* ablated toxN-site | This study |
| ML3786 | MG1655 pBR322-*toxI-toxN*-His6 pKVS45-*tifAT4*-FLAG | This study |
| ML3787 | MG1655 pBR322- *toxI-toxN*-His6 pKVS45-*tifA T4* | This study |
| ML3788 | MG1655 pBR322-*toxI-toxN* pKVS45-*tifA T4*-FLAG | This study |
| ML3789 | MG1655 *attBλ::toxIN* | This study |
| ML3790 | MG1655 *attBλ::toxI-toxN(K55A)* | This study |
| ML3791 | MG1655 λ-lysogen | This study |
| ML3792 | MG1655 λ-lysogen pBR322-*rIIAT4* pKVS45-*rIIBT4* | This study |
| ML3793 | *E. coli str C* | Félix d’Hérelle Reference Center for Bacterial Viruses, Université Laval |
| ML3794 | ECOR17 | Thomas S. Whittam STEC Center at Michigan State University |
| ML3795 | ECOR17 pEXT20-*ipIIIT4* | This study |
| ML3796 | ECOR17 pEXT20-*ipIIIT4ΔCTS* | This study |
| ML3797 | ECOR17 *ΔRM-typeI* | This study |
| ML3798 | ECOR17 *ΔRM-typeIII* | This study |
| ML3799 | ECOR17 *Δabi2* | This study |
| ML3800 | ECOR17 *Δdsr1* | This study |
| ML3801 | ECOR17 *Δhhe* | This study |
| ML3802 | ECOR17 *Δcas3* | This study |
| ML3803 | ECOR71 | Thomas S. Whittam STEC Center at Michigan State University |
| ML3804 | ECOR13 | Thomas S. Whittam STEC Center at Michigan State University |
| ML3805 | ECOR16 | Thomas S. Whittam STEC Center at Michigan State University |
| ML3806 | MG1655 pBR322-*Dsr1ECOR17 pKVS45* | This study |
| ML3807 | MG1655 pBR322-*Dsr1ECOR17 pKVS45-nrdC.5T4* | This study |
| ML3808 | MG1655 pCas9-*tifAT4*-cr4 | This study |
| ML3343 | DH5α pBAD33-*toxN* | Guegler and Laub, 2021 |
| ML3345 | DH5α pEXT20-*toxI* | Guegler and Laub, 2021 |
| ML1978 | DH5α pEXT20 | E. coli Genetic Stock Center, #12325 |
| ML3346 | DH5α pBR322-*toxIN* | Guegler and Laub, 2021 |
| ML3348 | DH5α pBR322 | Guegler and Laub, 2021 |
| ML3349 | DH5α pBR322-*toxI-toxN-*His6 | Guegler and Laub, 2021 |
| ML3809 | DH5α pKVS45-*dmdT4* | This study |
| ML3810 | DH5α pKVS45-*tifAT4* | This study |
| ML3811 | DH5α pKVS45-*tifAT2* | This study |
| ML3812 | DH5α pKVS45-*tifAT6* | This study |
| ML3813 | DH5α pKVS45-*tifARB69* | This study |
| ML3814 | TOP10 pEXT20-*tifAT2* | This study |
| ML3815 | TOP10 pEXT20-*tifAT4* | This study |
| ML3816 | TOP10 pEXT20-*tifAT6* | This study |
| ML3817 | TOP10 pEXT20-*tifARB69* | This study |
| ML3818 | DH5α pKVS45-*tifAT4* ΔATG | This study |
| ML3819 | DH5α pKVS45-*tifA T4* recoded | This study |
| ML3820 | DH5α pKVS45-*tifA T4* ablated toxN-site | This study |
| ML3821 | TOP10 pKVS45-*tifAT4-*FLAG | This study |
| ML3822 | DH5α pBR322-*rIIAT4* | This study |
| ML3823 | DH5α pKVS45-*rIIBT4* | This study |
| ML3824 | DH5α pEXT20-*ipIIIT4* | This study |
| ML3825 | DH5α pEXT20-*ipIIIT4ΔCTS* | This study |
| ML3826 | DH5α pBR322-*Dsr1ECOR17* | This study |
| ML3827 | DH5α pKVS45-*nrdC.5T4* | This study |
| ML3828 | DH5α pCas9-*tifAT4*-cr4 | This study |
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| **Phage Strains** | | |
| **Name** | **Genotype** | **Source** |
| phML31 | T4 ancestor | Guegler and Laub, 2021 |
| phML32 | T4 control evo round 25 clone 1 | This study |
| phML33 | T4 evo 1 round 25 clone 1 | This study |
| phML34 | T4 evo 2 round 25 clone 1 | This study |
| phML35 | T4 evo 3 round 25 clone 1 | This study |
| phML36 | T4 evo 4 round 25 clone 1 | This study |
| phML37 | T4 evo 5 round 25 clone 3 | This study |
| phML38 | T2 | ATCC Cat #: 11303-B2 |
| phML39 | T6 | ATCC Cat #: 11303-B6 |
| phML40 | RB69 | Laval Collection, HER #  158 |
| phML41 | T4 *tifA-1* | This study |
| phML42 | T4 *tifA-2* | This study |

**Supplementary File 1b. Plasmids**

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| **Plasmid** | **Description** | **Source** |
| pBR322 empty vector | Derivative of pBR322 with pTet removed | Guegler and Laub, 2021 |
| pBR322-*toxIN* | Full *toxIN* locus | Guegler and Laub, 2021 |
| pBR322-*toxI-toxN-*His6 | *toxIN* locus with C-terminal His6-tagged ToxN | Guegler and Laub, 2021 |
| pBR322-*rIIAT4* | *rIIA* under native T4 middle promoter | This study |
| pBR322-*Dsr1ECOR17* | *Dsr1* from ECOR17 under native promoter region | This study |
| pKVS45 | aTc inducible vector |  |
| pKVS45-*tifA T4* | aTc inducible TifA expression | This study |
| pKVS45-*dmd T4* | aTc inducible Dmd expression | This study |
| pKVS45-*tifA T2* | aTc inducible TifA expression | This study |
| pKVS45-*tifA T6* | aTc inducible TifA expression | This study |
| pKVS45-*tifA RB69* | aTc inducible TifA expression | This study |
| pKVS45-*tifAT4* ΔATG | aTc inducible TifAΔATG expression | This study |
| pKVS45-*tifA T4* recoded | aTc inducible TifArecoded expression | This study |
| pKVS45-*tifAT4* ablated toxN-site | aTc inducible TifAablated toxN-site expression | This study |
| pKVS45-*tifAT4*-FLAG | aTc inducible TifA with C-terminal FLAG tag | This study |
| pKVS45-*rIIBT4* | aTc inducible *rIIA* expression | This study |
| pKVS45-*nrdC.5T4* | aTc inducible *nrdC.5* expression | This study |
| pBAD33-*toxN* | Arabinose inducible ToxN expression | Guegler and Laub, 2021 |
| pEXT20 | IPTG inducible vector | E. coli Genetic Stock Center, #12325 |
| pEXT20-*toxI* | IPTG inducible *toxI* expression | Guegler and Laub, 2021 |
| pEXT20-*tifAT2* | IPTG inducible *tifAT2* expression | This study |
| pEXT20-*tifAT4* | IPTG inducible *tifAT4* expression | This study |
| pEXT20-*tifAT6* | IPTG inducible *tifAT6* expression | This study |
| pEXT20-*tifARB69* | IPTG inducible *tifARB69* expression | This study |
| pEXT20-*ipIIIT4* | IPTG inducible *ipIIIT4* expression | This study |
| pEXT20-*ipIIIT4ΔCTS* | IPTG inducible *ipIIIT4ΔCTS* expression | This study |
| pCas9 | Cas9 with restriction site to clone guideDNA | Addgene #42876 |
| pCas9-*tifAT4*-cr4 | Cas9 with guide targeting *tifAT4* | This study |
| pKD46 | L-ara inducible λ-red recombinase | Datsenko and Wanner, 2000 *E. coli* stock center CGSC# 7669 |
| pKD4 | Kanamycin resistance cassette | Datsenko and Wanner, 2000 *E. coli* stock center CGSC# 7632 |

**Supplementary File 1c. Primers**

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| Name | Purpose | Sequence |
| SS-1 | Primer to amplify *tifA* locus (forward) | ATAAAGCATTATTCACCTACCACTTCAGCG |
| SS-2 | Primer to amplify *tifA* locus (reverse) | GAGCTAGCAGCTGCTGAACAACAACT |
| SS-3 | Divergent primer to amplify *tifA* segmental amplification (forward) | TTCCTGACGTTATACGGAGTAGG |
| SS-4 | Divergent primer to amplify *tifA* segmental amplification (reverse) | TTCCTGACGTTATACGGAGTAGG |
| SS-5 | Construct pKVS45-*tifA* (forward) | cgaattcgagctcggtacccATGCATATTGTTTTATTTAAACCTAC |
| SS-6 | Construct pKVS45-*tifA* (reverse) | ggtcgactctagaggatccccTTATTTTAAAATTTCTGCGTAATCAC |
| SS-7 | Construct pKVS45-*dmd* (forward) | cgaattcgagctcggtacccATGGAATTGGTAAAGGTAGT |
| SS-8 | Construct pKVS45-*dmd* (reverse) | ggtcgactctagaggatccccTTATCCTCGGCAATCCACTT |
| SS-9 | Construct pKVS45-*tifA*-FLAG (forward) | ggggatcctctagagtcgacc |
| SS-10 | Construct pKVS45-*tifA*-FLAG (reverse) | TTActtgtcatcgtcgtccttgtagtcGCTTCCGCTTCCTTTTAAAATTTCTGCGTAATCACATGTTACAAACTGTTTC |
| SS-11 | Construct pEXT20-*tifAT2*(forward) | AGGTTTCTCCATACAGGAGGTACCCATGCATATTGTTTTATTTAAACCTACTC |
| SS-12 | Construct pEXT20-*tifAT2* (reverse) | TGCAGGTCGACTCTAGAGGATCCCCTTATTTTAAAACTTTTGCGTAATCAC |
| SS-13 | Construct pEXT20-*tifAT6*(forward) | AGGTTTCTCCATACAGGAGGTACCCATGCATATTGTTTTATTTAAACCTACTC |
| SS-14 | Construct pEXT20-*tifAT6* (reverse) | TGCAGGTCGACTCTAGAGGATCCCCCTATTTTAAAACTTTTGCATAATCAC |
| SS-15 | Construct pEXT20-*tifARB69* (forward) | AGGTTTCTCCATACAGGAGGTACCCATGTATTCAACTGTGTTTAAACCATC |
| SS-16 | Construct pEXT20-*tifARB69* (reverse) | TGCAGGTCGACTCTAGAGGATCCCCCTATTTTAAACTTTTGCGAAATTTG |
| SS-17 | Construct pEXT20-*tifAT2*(forward) | CACACAGGAAACAGAATTCGAGCTCATGCATATTGTTTTATTTAAACCTACTCC |
| SS-18 | Construct pEXT20-*tifAT2* (reverse) | GAAGCTTGCATGCCTGCAGGTCGACTTATTTTAAAACTTTTGCGTAATCACATG |
| SS-19 | Construct pEXT20-*tifAT4*(forward) | CACACAGGAAACAGAATTCGAGCTCATGCATATTGTTTTATTTAAACCTACTC |
| SS-20 | Construct pEXT20-*tifAT4* (reverse) | GAAGCTTGCATGCCTGCAGGTCGACTTATTTTAAAATTTCTGCGTAATCACATG |
| SS-21 | Construct pEXT20-*tifAT6*(forward) | CACACAGGAAACAGAATTCGAGCTCATGCATATTGTTTTATTTAAACCTACTCC |
| SS-22 | Construct pEXT20-*tifAT6* (reverse) | GAAGCTTGCATGCCTGCAGGTCGACCTATTTTAAAACTTTTGCATAATCACATG |
| SS-23 | Construct pEXT20-*tifARB69* (forward) | CACACAGGAAACAGAATTCGAGCTCATGTATTCAACTGTGTTTAAACCATC |
| SS-24 | Construct pEXT20-*tifARB69* (reverse) | GAAGCTTGCATGCCTGCAGGTCGACCTATTTTAAACTTTTGCGAAATTTGCG |
| SS-25 | Construct pAH150-*toxIN* (*toxIN* forward) | GCGAGAGTAGGGAACTGCCAGGCATTTATGGCCGCGTTTATCTCATTCCACG |
| SS-26 | Construct pAH150-*toxIN* (*toxIN* reverse) | cgactctagaggatccccggGTACCCTTATATTGGATGAGAGCAAAAAAAATAGGTCC |
| SS-27 | Construct pAH150-*toxIN* (backbone forward) | GGTACccggggatcctctagagtcg |
| SS-28 | Construct pAH150-*toxIN* (backbone reverse) | ATGCCTGGCAGTTCCCTACTC |
| SS-29 | Construct pAH150-*toxIN(K55A)* (forward) | GCGGCATGGCATGCTAATGTAAAAGAGTCATC |
| SS-30 | Construct pAH150-*toxIN(K55A)* (reverse) | TGGCGATGTTAAAGGTGCTAAATATTTATGTCC |
| SS-31 | Construct pKVS45-*tifA* ΔATG (forward) | CATATTGTTTTATTTAAACCTACTCCG |
| SS-32 | Construct pKVS45-*tifA* ΔATG (reverse) | GGGTACCTCCTGTATGGAGAAACCTAGG |
| SS-33 | Construct pKVS45-*tifA* ΔtoxN-site (forward) | ATTACGCAGAGATTTTAAAATAAGGGGATCCTCTAGAG |
| SS-34 | Construct pKVS45-*tifA* ΔtoxN-site (reverse) | ATTTTAAAATCTCTGCGTAATCACATGTTACAAACTG |
| SS-35 | Construct pEXT20-*ipIIIT4* (forward) | CACACAGGAAACAGAATTCGAGCTCATGAAAACATATCAAGAATTTATTGCCG |
| SS-36 | Construct pEXT20-*ipIIIT4* (reverse) | GAAGCTTGCATGCCTGCAGGTCGACTTAAGAATTACCACGGGCTGCATTAG |
| SS-37 | Construct pBR322-*rIIAT4* (forward) | CTTTCGTCTTCAAGAATTCTCATGTTCGATATGGGAGAAGCCGAAG |
| SS-38 | Construct pBR322-*rIIAT4* (reverse) | CAAGAATTGATTGGCTCCAATTCTTTTATTTAAATTGTTCAGTAACGTCTTCAAC |
| SS-39 | Amplify pBR322 vector (forward) | AAGAATTGGAGCCAATCAATTCTTG |
| SS-40 | Amplify pBR322 vector (reverse) | ACATGAGAATTCTTGAAGACGAAAG |
| SS-41 | Construct pKVS45-*rIIBT4* (forward) | CGAATTCGAGCTCGGTACCCATGTACAATATTAAATGCCTGAC |
| SS-42 | Construct pKVS45-*rIIBT4* (reverse) | GGTCGACTCTAGAGGATCCCCTTATTTAAATTGTTCAGTAACGTCT |
| SS-43 | Construct pBR322-*Dsr1ECOR17* (forward) | CTTTCGTCTTCAAGAATTCTCATGTAGGTGTATGGCAAGTTTATGACAAGAGT |
| SS-44 | Construct pBR322- *Dsr1ECOR17* (reverse) | CAAGAATTGATTGGCTCCAATTCTTTCACACGCTGCGCCTT |
| SS-45 | Construct pKVS45-*nrdC.5T4* (forward) | CGAATTCGAGCTCGGTACCCATGAAAACTCGTTCTCAAATTG |
| SS-46 | Construct pKVS45-*nrdC.5T4* (reverse) | GGTCGACTCTAGAGGATCCCCCTAGTTCAGTGCATTTAGTGC |
| SS-47 | Construct pCas9-61.4-cr4 (forward) | AAACAATTCCACTCGACCAAATGGG |
| SS-48 | Construct pCas9-61.4-cr4 (reverse) | AAAACCCATTTGGTCGAGTGGAATT |

**Supplementary File 1d. Antibodies**

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| Reagent | Source | Catalogue Number | RRID |
| Anti-FLAG M2 magnetic beads | Sigma | M8823 | AB\_2637089 |
| Recombinant anti-6X His tag rabbit antibody | Abcam | AB200537 |  |
| DYKDDDDK tag rabbit mAb | Cell Signaling Technology | 14793 | AB\_2572291 |
| Goat anti-rabbit IgG (H+L) secondary antibody, HRP | ThermoFisher Scientific | 32460 | AB\_1185567 |