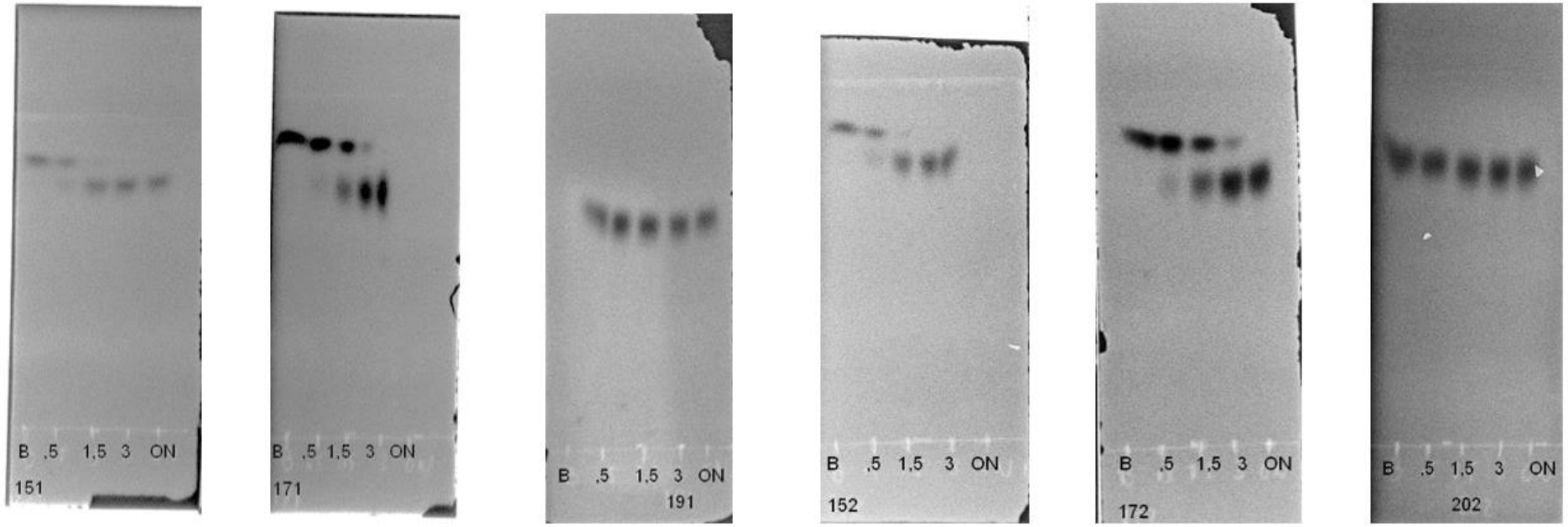


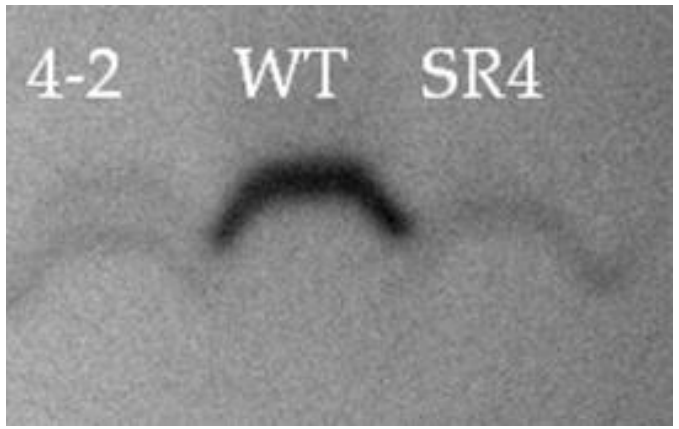
Sample code	Strain	BDP-acceptor
151	*C. coli* 76339	Lac
171	*C. coli* 76339	LacNAc
191	*C. coli* 76339	SiaLac
152	*C. coli* 76339 Δ*cstV*	Lac
172	*C. coli* 76339 Δ*cstV*	LacNAc
192	*C. coli* 76339 Δ*cstV*	SiaLac

Supplemental Figure 1. Example of enzymatic test. Sialyltransferase activity of protein extracts was tested on BODIPY labelled Lac, LacNac, and 3'Siayllactose or FCHASE labelled α-GalNAc, β-GalNAc, GM3, α-Gal, β-GlcNAc, α-Glc, β-Glc, Hep, as donors. Reactions were performed at 37 °C in 10 µl volumes containing 50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 1 mM CMP-NeuAc, 0.5 mM labelled acceptor, and 6 µl of extract. Enzymatic activity was assessed by thin-layer chromatography on silica using a solvent system of ethyl acetate/methanol/water/acetic acid 4:2:1:0.1.

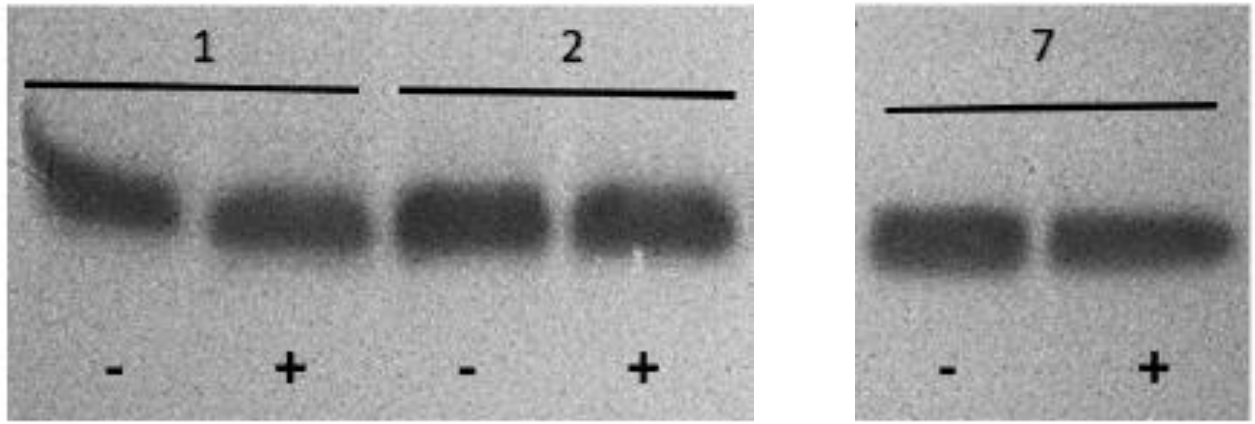
Samples were incubated for 0.5, 1, 1.5, and 3 hours, and overnight (ON)

Primer name	Sequence
ProEx-cstIV-F-NdeI	GGGGGGGGCCATATGAATAAAAATTTAAATTTTACA
ProEx-cstIV-RW-SalI	ACCGTCGACCTATCTTGTATATCTTGTTTTTGA
ProEx-cstV-R-NdeI	CCATATGATAGAAAACAATGCAGTTGTTGTTGCA
ProEx-cstV-FW-SalI	ACCGTCGACTTATTTTGCCTTTTTTATATTTTTAAAA

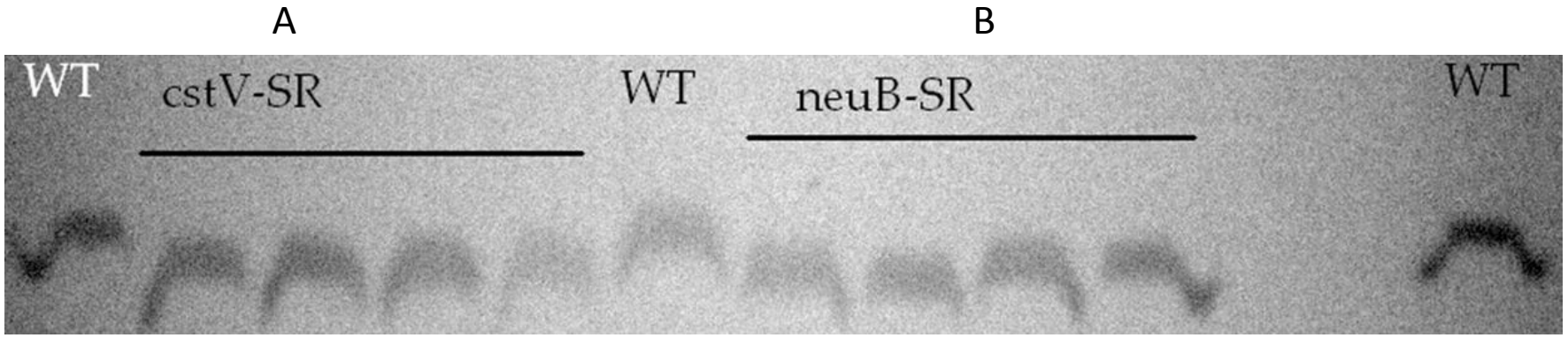
Supplemental Figure 2. Graphic representation of CstV protein expression construct. Gene *cstIV* from *C. coli* 73 and *cstV* from *C. coli* 76339 inserted into pCw and pCw-MalET plamids. *E. coli* AD202 or *E. coli* BL21 were used as expression hosts



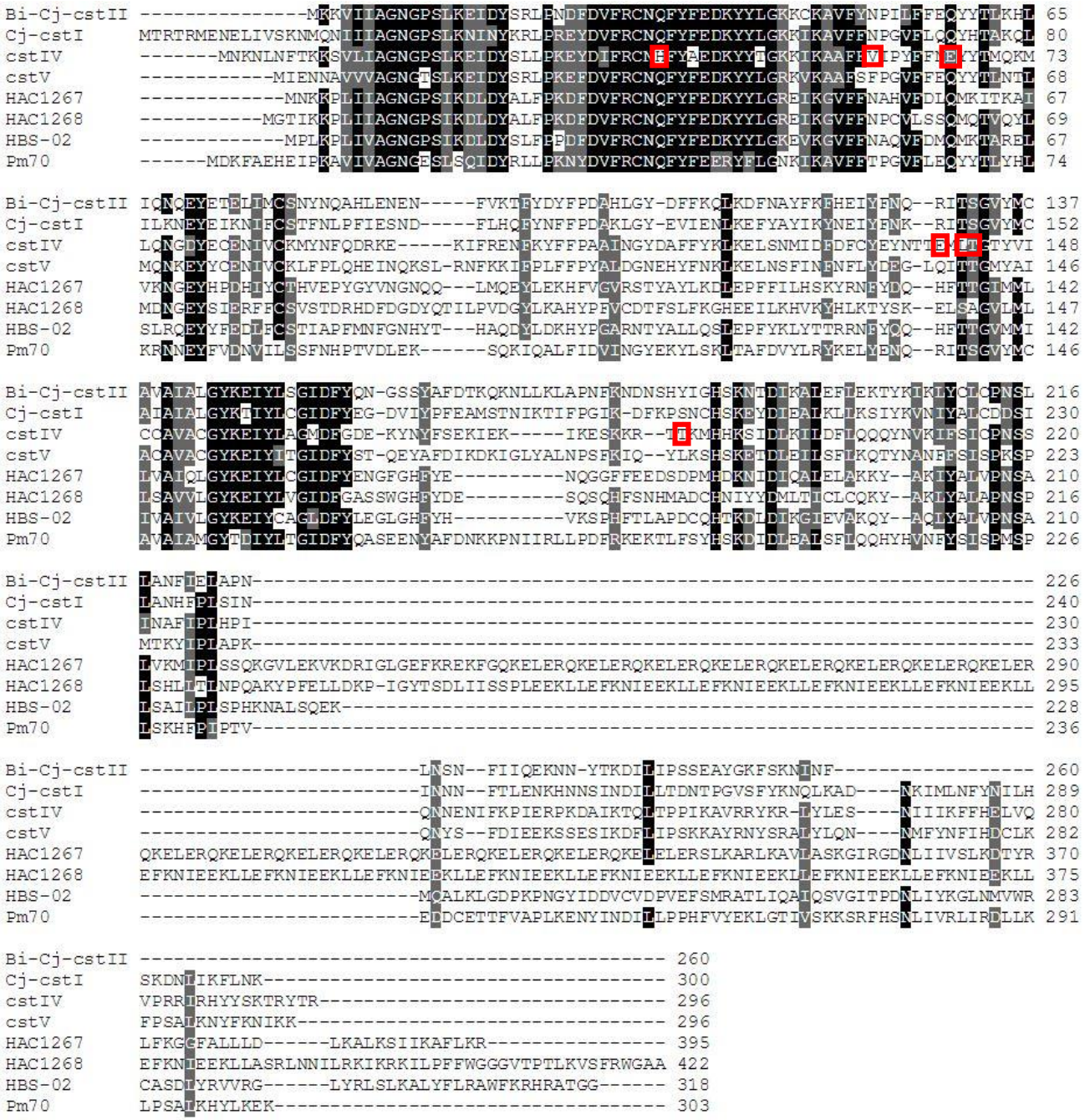
Supplemental Figure. 3 Electrophoresis mobility comparison of *C. coli* 76339Δ*cstV*Δ*ggt*:*cstV* (4-2), *C. coli* 76339 WT, and *C. coli* 76339Δ*cstV* (SR4).



Supplemental Figure 4. Silver stain analysis of neuraminidase treated crude LOS. 1. *C. coli* 76339 (*cstV*); 2. *C. coli* 65 (*cstIV*); 7. *C. coli* 73 (*cstIV*); (+) neuraminidase treated, (-) control.



Supplemental Figure 5. Electrophoresis mobility comparison of *C. coli* 76339 WT, Δ*cstV,* and Δ*neuB*. **A;** *C. coli* 76339 WT, Δ*cstV*-SR (erythromycin resistance cassette cloned in same direction as *cstV*). **B;** Δ*neuB*-SR (erythromycin resistance cassette cloned in same direction as *neuB*).



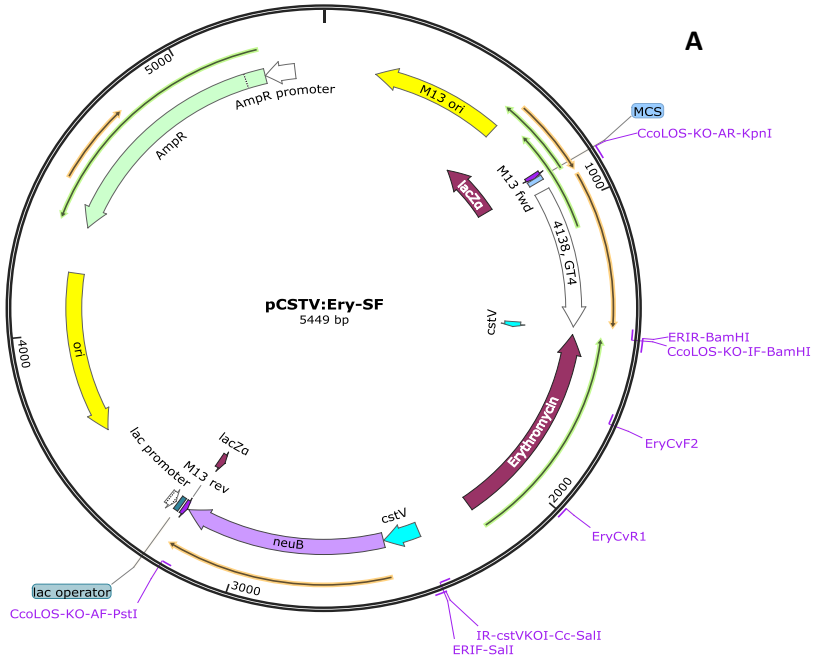
Important residues for CMP	
CstII	**CstIV**
Thr131	Leu142
Ser132	Thr143
Important residues for Neu5Ac	
CstII	**CstIV**
Gln32	His40
Asn51	Val59
Gln58	Glu66
Arg129	Glu140
Ser132	Thr143
Tyr185	Thr189

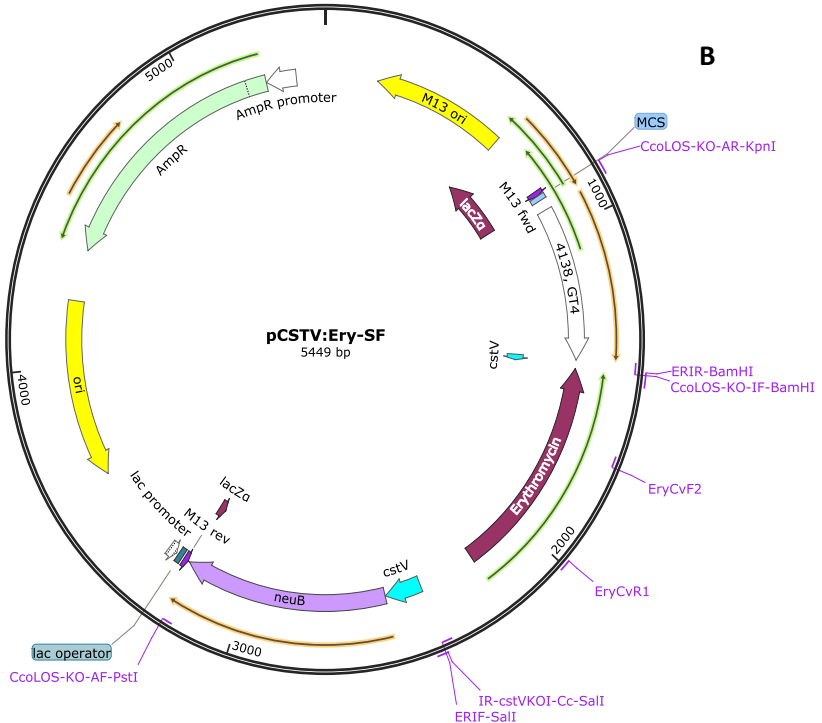
Supplemental Figure 6. Multiple sequence alignment of characterized GT-42 sialyltransferases and *C. coli* 76339 *cstV* and *C.coli* 73 *cstIV.*

Supplemental Material

Supplemental Table 1. Bacterial strains and protein expression constructs

Strain or plasmid	**Genotype and/or phenotype**	**Reference or source**
C. coli 76339	*cstI*, *cstV*, *neuB*	[1]
C. coli 76339 Δ*cstV*-SF1	*cstI*, Δ*cstV*: Ery, *neuB*	This study
C. coli 76339 Δ*cstV*-SR4	*cstI*, Δ*cstV*: Ery, *neuB*	This study
C. coli 76339 Δ*neuB*-SR1	*cstI*, Δ*neuB*: Ery, *cstV*	This study
C. coli 76339 Δ*cstI*-XR3	Δ*cstI*: CAT, *cstV*, *neuB*	This study
C. coli 76339 Δ*cstV*-SR4 Δ*cstI*-XR1	Δ*cstI*: CAT, Δ*cstV*: Ery, *neuB*	This study
C. coli 76339 Δ*neuB*-SR2	*cstI*, Δ*neuB*: Ery, *cstV*	This study
C. coli 76339 Δ*cstV*-SR4 Δ*ggt:cstV*-2	*cstI*, Δ*cstV*: Ery, *neuB*, Δggt:*cstV:*CAT	This study
C. coli 65	*cstIV*	[2]
C. coli 65 Δ*cstIV*-SF5	Δ*cstIV*:Ery	This study
C. coli 73	*cstIV*	[2]
C. coli 73 Δ*cstIV*-SF3	Δ*cstIV*:Ery	This study
C. coli 73 Δ*neuB2*	Δ*neuB2*:Ery	This study
C. jejuni 81-176		
E. coli AD202		[3]
E. coli BL21		
Protein expression constructs		
pCwMal-ET		[3]
pCw		
pCwMal-ET_51cstV		This study
pCwMal-ET_73cstIV		This study
pCw_51cstV		This study
pCw_73cstIV		This study

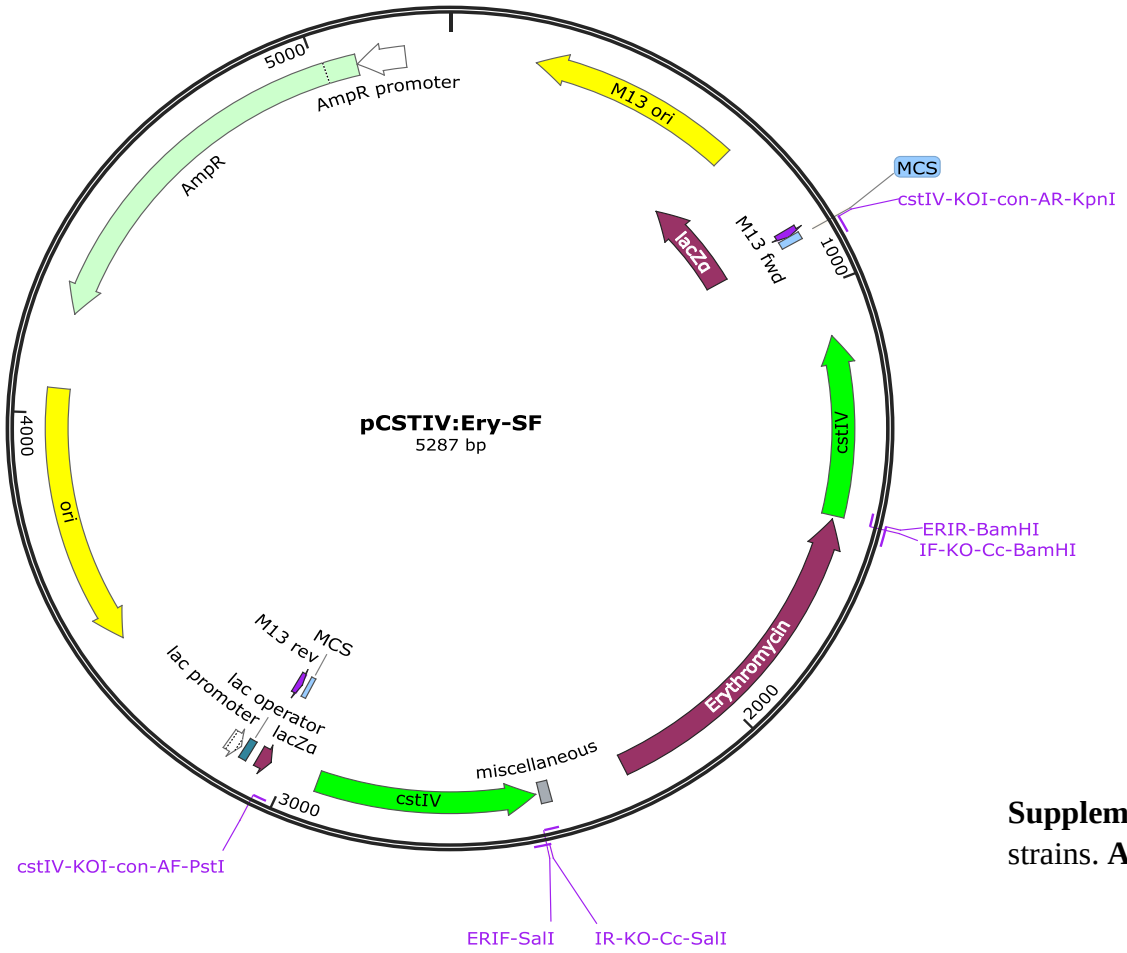




Primer name	Sequence
cstV amplification	
CcoLOS-KO-AF-PstI	ATCTGCAGCCTAATGCAACCGCACCCAAGC
CcoLOS-KO-AR-KpnI	AGGTACCATTTATCCCAAAAAGTTGTCTTAAGCGTGG
Inverse PCR	
IR-cstVKOI-Cc-SalI	ACCGTCGACCATATCGAAATTATTCTAGAGCTC
CcoLOS-KO-IF-BamHI	AGGATCCGTTTTCTATCATATCTGTCCTCATAG
Erythromycin cassette	
ERIF-SalI	ACCGTCGACAGTATAAAACCTTTAAGAACTTTC
ERIR-BamHI	ACCGGATCCACTTACTTATTAAATAATTTATAGCTATTG
ERIR-SalI	ACCGTCGACACTTACTTATTAAATAATTTATAGCTATTG
ERIF-BamHI	ACCGGATCCAGTATAAAACCTTTAAGAACTTTC
Mutant verification	
EryCvF1	CCTCTTATTATAGCCATTTGTTTGC
EryCvF2	ATATTTTCATCCTAAACCTAAAGTGAATAGC
EryCvR1	TTATTTTCTGTAGTTTTGCATAATTTATGG
EryCvR2	GGAGAAAGAGTTTGTGCTAATCTC
Gene expression verification	
09870cFv	TAA GTT TTT GGT AGT TTT TGC CTC G
09870cRv	ATTAGCACTAGATGATACAACCAGTG
09880cFv	CAACTAAGATCCCATTTATGCCAAG
09880cRv	TAGAAGGTGGAGAGCTTTCAGG
09890cFv	ACATAGCCTGTAATCTTACTAAATACG
09890cRv	ATAGGTTCAGGAGAATGTAATAACTATCC
09910cFv	GGC TAG TAA GTG CAT AAA TAC TTG C
09910cRv	CGATCAAAGAAAGTGATTTATCCCAA
09930cFv	TGCGTGGTAGAGCTAGGATG
09930cRv	ACAATGAAAGCACTGATGACACTC

Supplemental Figure 7. Plasmids and primers for the generation of *C. coli* 76339 Δ*cstV* mutant strains. **A;** pCSTV:Ery-SF plasmid, **B;** pCSTV:Ery-RF plasmid, C; primer list

A

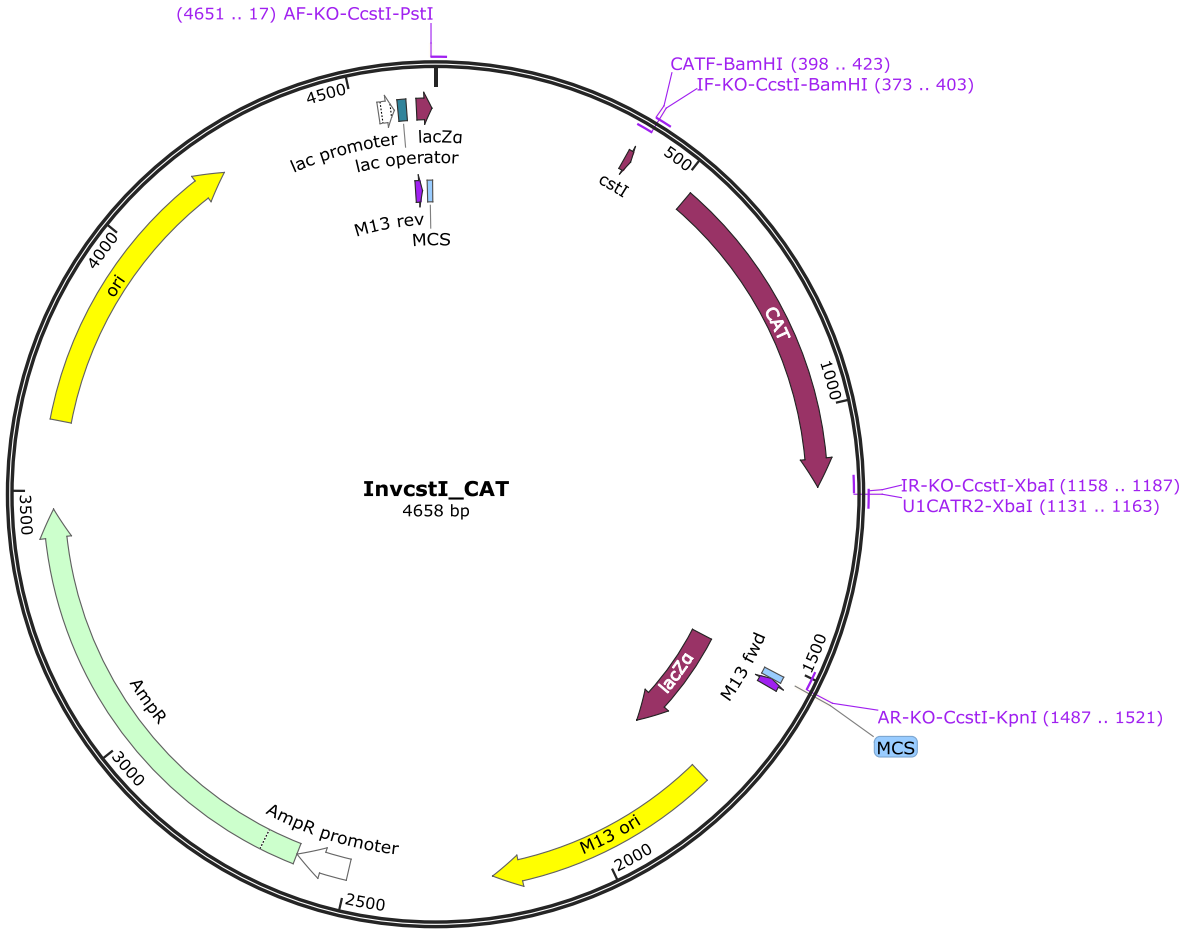


B

Primer name	Sequence
***cstIV* amplification**	
cstIV-KOI-con-AF-PstI	ATCTGCAGGCCAAAACCACTCACTTAAAAG
cstIV-KOI-con-AR-KpnI	AGGTACCGCAATCGATACTGATAATTTTAACGCT
Inverse PCR	
IF-KO-Cc-BamHI	AGGATCCGCTGGCATGGATTTTGGAGATG
IR-KO-Cc-SalI	ATAGTCGACTCTTTATAGCCACATGCAACAGC
Erythromycin cassette	
ERIF-SalI	ACCGTCGACAGTATAAAACCTTTAAGAACTTTC
ERIR-BamHI	ACCGGATCCACTTACTTATTAAATAATTTATAGCTATTG
Mutant verification	
CcCstIVvF	AACAGAAATGCTTACTGGCAC
EryCvF2	ATATTTTCATCCTAAACCTAAAGTGAATAGC
EryCvR1	TTATTTTCTGTAGTTTTGCATAATTTATGG

Supplemental Figure 8. Plasmids and primers for the generation of *C. coli* 65 and 73 Δ*cstIV* mutant strains. **A;** pCSTIV:Ery-SF plasmid, **B;** primer list

A

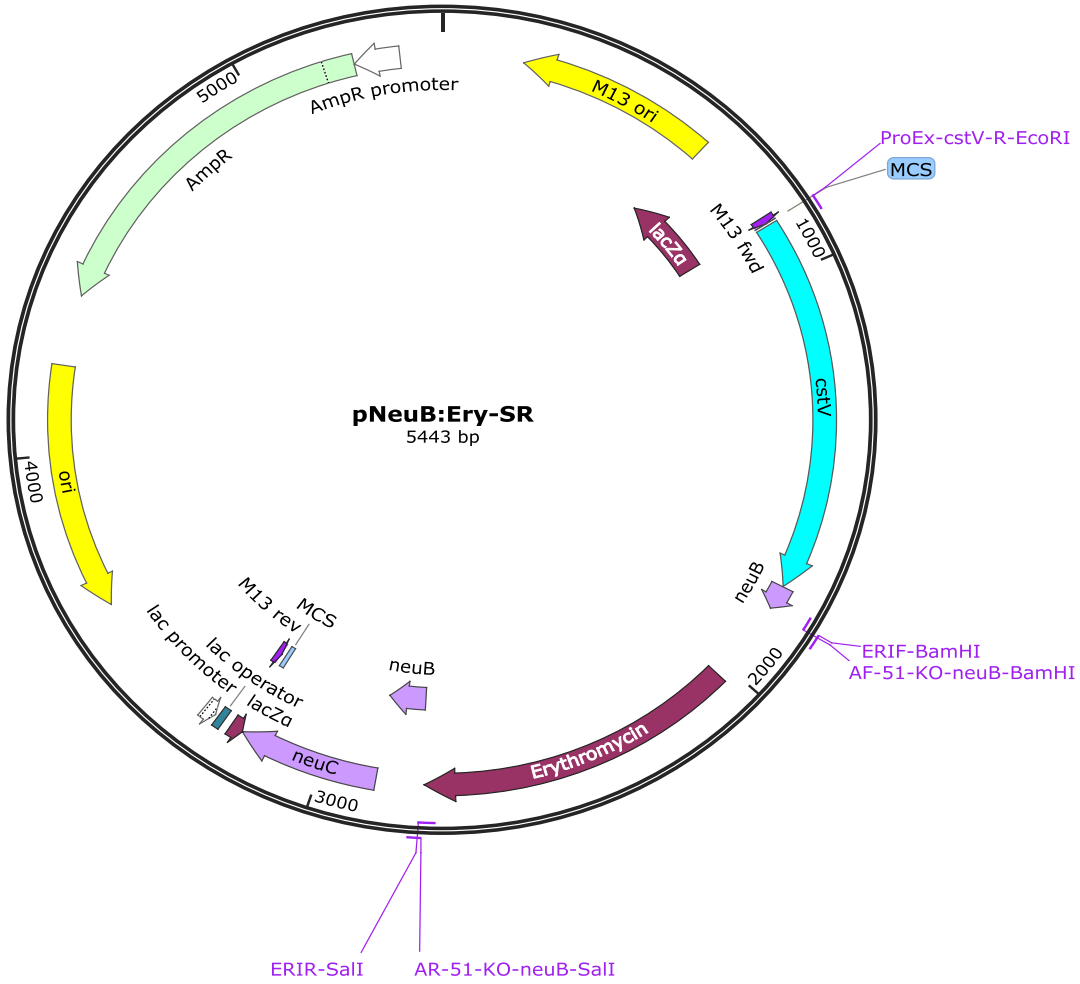


B

Primer name	Sequence
cstI amplification	
AF-KO-CcstI-PstI	ATCTGCAGAGGAAATGGGCCTAGTCTT
AR-KO-CcstI-KpnI	AGGTACCCACTATGGATTTCATAAGAATTTCTACCT
Inverse PCR	
IR-KO-CcstI-XbaI	ATTCTAGATGAATTTGGCTAAGACTGTAGGTG
IF-KO-CcstI-BamHI	ACCGGATCCGCTATGGCGCACATATAAATACCAG
CAT cassette	
U1CATR2-XbaI	ATTCTAGAGGGATTTTATTTATTCAGCAAGTCTTG
CATF-BamHI	AGGATCCCGGCGGTGTTCCTTTCCAAG
Mutant verification	
cstI-CvR	GCATTCTTCTATACAATTTAACTCCC
cstI-CvF	ACTAAAAAACGGGAGGGAAGC
CAT-CvR	CTCAGTCCAAATACTCGAAAAGG
CAT-CvF	TCTATGATACCGTGGACAAGC

Supplemental Figure 9. Plasmids and primers for the generation of *C. coli* 76339 Δ*cstI* mutant strains. A; InvcstI_CAT plasmid, B; primer list

A

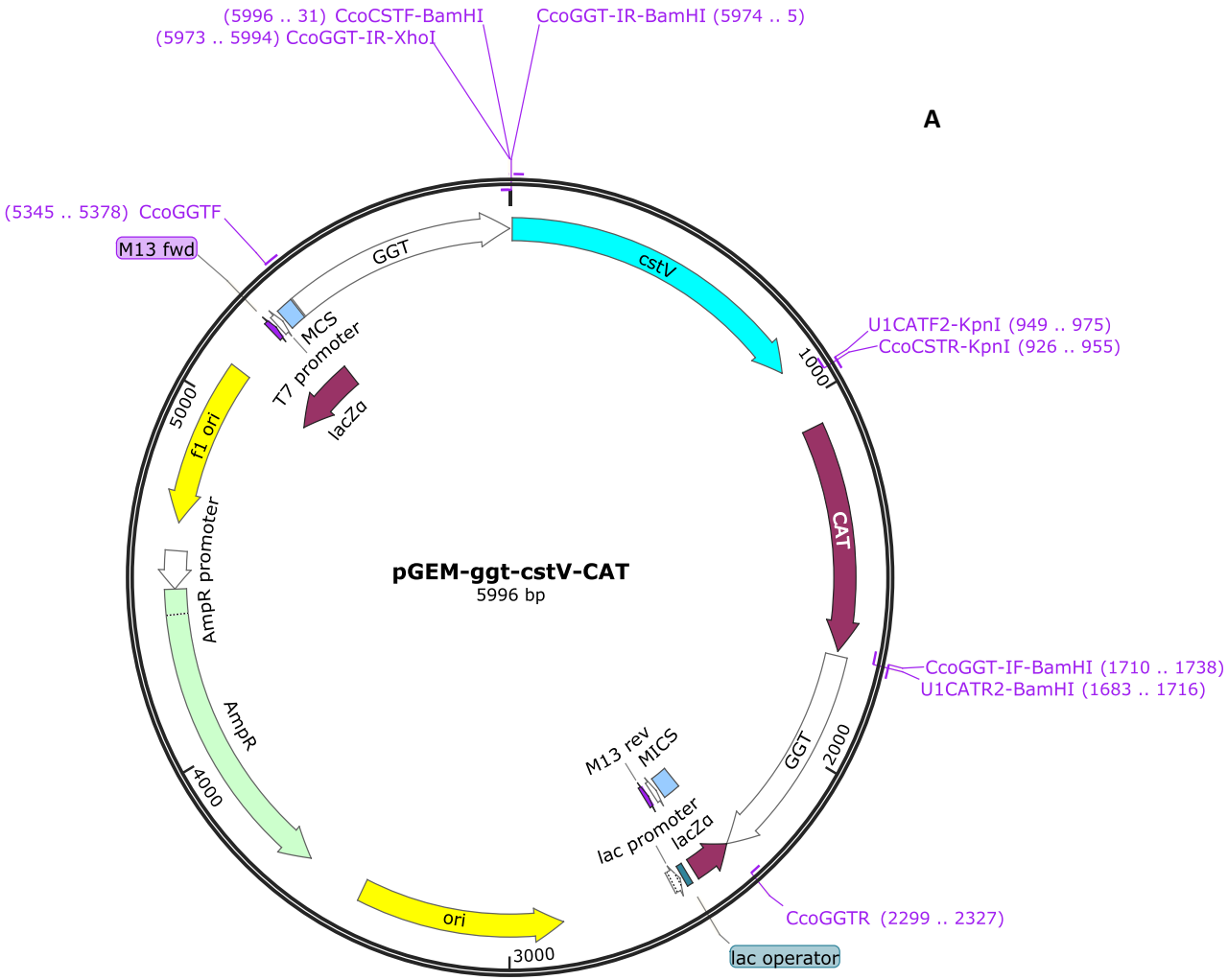


B

Primer name	Sequence
neuB amplification	
AF-51-KO-neuB-PstI	ATCTGCAGCTCTCCACCTTCTATATGTGCTAC
ProEx-cstV-R-EcoRI	ACTGAATTCATAGAAAACAATGCAGTTGTTGTT
Inverse PCR	
AR-51-KO-neuB-SalI	ACCGTCGACGGGACTCGGTGGAATCAGC
AF-51-KO-neuB-BamHI	AGGATCCCTAAAGGTGGGTTTTCTTGGGATATG
Erythromycin cassette	
ERIR-SalI	ACCGTCGACACTTACTTATTAAATAATTTATAGCTATTG
ERIF-BamHI	ACCGGATCCAGTATAAAACCTTTAAGAACTTTC
Mutant verification	
EryCvF2	ATATTTTCATCCTAAACCTAAAGTGAATAGC
EryCvR1	TTATTTTCTGTAGTTTTGCATAATTTATGG

Supplemental Figure 10. Plasmids and primers for the generation of *C. coli* 76339 Δ*neuB1* mutant strains. A; pNeuB:Ery-SR plasmid, B; primer list

A



B

Primer name	Sequence
ggt amplification	
CcoGGTF	ATTTAGTTATATTTGTGATTTCAATCACGCTAGG
CcoGGTR	AAAACTGTTGTGATGATTCTAGAGCCACC
Inverse PCR	
CcoGGT-IF-BamHI	AGGATCCTTCTATGTCGCCACCTAGTAGCG
CcoGGT-IR-BamHI	AGGATCCCAGGGCCTTCTTTGGCGATGAG
cstV amplification	
CcoCSTR-KpnI	ACCGGTACCTTCTTGGGATATGGTTAATTTATC
CcoCSTF-BamHI	AGGATCCAATGATAGAAAACAATGCAGTTGTTG
CAT cassette	
U1CATR2-KpnI	AGGTACCGGGATTTTATTTATTCAGCAAGTCTTG
U1CATR2-BamHI	AGGATCCGGGATTTTATTTATTCAGCAAGTCTTG

Supplemental Figure 11. Plasmids and primers for the generation of *cstV* complemented strain. A; pGEM-ggt-cstV-CAT plasmid, B; primer list