

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

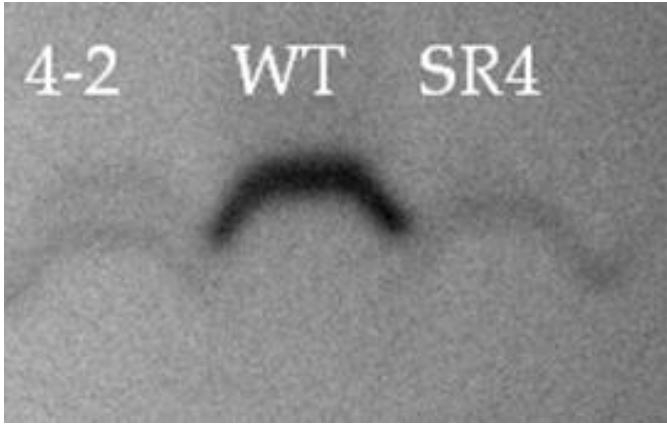
Supplemental Figure 1. Example of enzymatic test. Sialyltransferase activity of protein extracts was tested on BODIPY labelled Lac, LacNAc, and 3'Siayllactose or FCHASE labelled α -GalNAc, β -GalNAc, GM3, α -Gal, β -GlcNAc, α -Glc, β -Glc, Hep, as donors. Reactions were performed at 37 °C in 10 μ l volumes containing 50 mM HEPES pH 7.5, 10 mM MgCl₂, 1 mM CMP-NeuAc, 0.5 mM labelled acceptor, and 6 μ l 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)

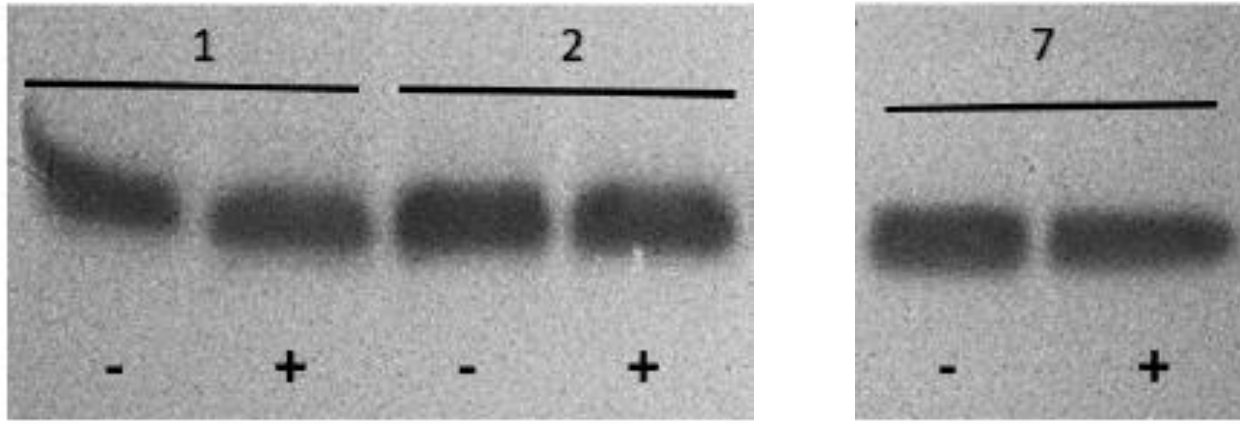


Primer name	Sequence
ProEx-cstIV-F-NdeI	GGGGGGGGCCATATGAATAAAAATTTAAATTTTACA
ProEx-cstIV-RW-SalI	ACCGTCGACCTATCTTGTATATCTTGTTTTTGA
ProEx-cstV-R-NdeI	CCATATGATAGAAAACAATGCAGTTGTTGTTGCA
ProEx-cstV-FW-SalI	ACCGTCGACTTATTTTGCCTTTTTTATATTTTAAAA

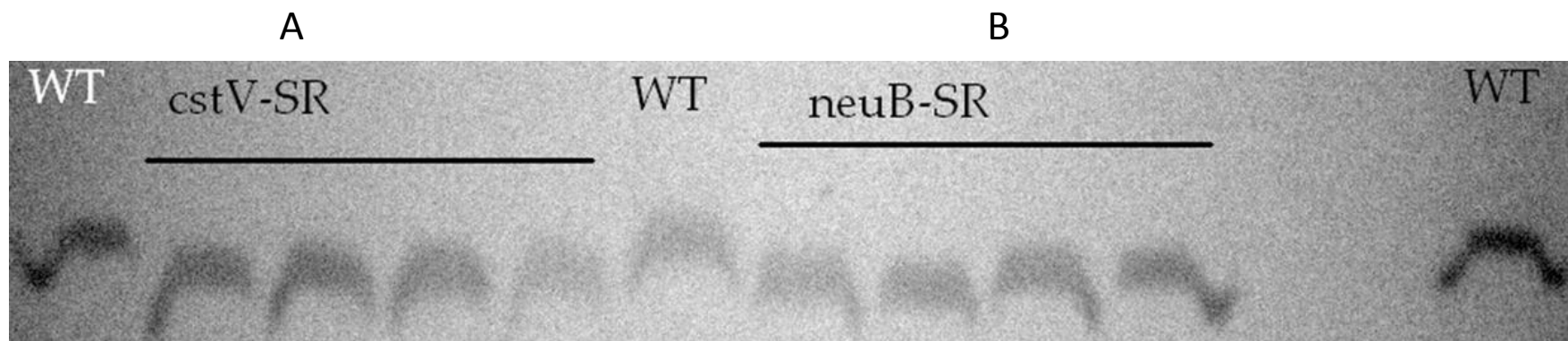
Supplemental Figure 2. Graphic representation of CstV protein expression construct. Gene *cstIV* from *C. coli* 73 and *cstV* from *C. coli* 76339 inserted into pCw and pCw-MalET plasmids. *E. coli* AD202 or *E. coli* BL21 were used as expression hosts



Supplemental Figure. 3 Electrophoresis mobility comparison of *C. coli* 76339 Δ *cstV* Δ *ggt:cstV* (4-2), *C. coli* 76339 WT, and *C. coli* 76339 Δ *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, $\Delta cstV$, and $\Delta neuB$. **A;** *C. coli* 76339 WT, $\Delta cstV$ -SR (erythromycin resistance cassette cloned in same direction as *cstV*). **B;** $\Delta neuB$ -SR (erythromycin resistance cassette cloned in same direction as *neuB*).

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Bi-Cj-cstII -----MKKVIILAGNGPSLKEIDY SRLPND FDFVFRNCQFYFEDKYYLGKKCKAVFNPILFEQYITLKKHL 65
Cj-cstI MTRTRMENELIVSKNMQNI I ILAGNGPSLKNIN YKRLPRE YDVFRCNQFYFEDKYYLGKKIKAVFFNPGVFLCQYHTAKQL 80
cstIV -----MKNKLNFTKRSVLLIAGNGPSLKEIDY SLLPKEDYDFRCN E FYFEDKYYLGKKIKAAFFW P YFE E YIMQKM 73
cstV -----MIENNAVAVAGNG ILSLKEIDY SRLPKE FDFVFRNCQFYFEDKYYLGRKVKAAFSFPGVFEFEQYITLNTL 68
HAC1267 -----MKNKPLI ILAGNGPSLKDLDYALFPKDFDVFRCNQFYFEDKYYLGR EIKGVFFNAHVFDLQMKITKAI 67
HAC1268 -----MGTIKKPLI ILAGNGPSLKDLDYALFPKDFDVFRCNQFYFEDKYYLGR EIKGVFFNPNCLSSQMCITVQYL 69
HBS-02 -----MPLKPLI ILAGNGPSLKDLDYSLF EFD FDFVFRNCQFYFEDKYYLGR EIVKGVFFNAQVFDLQMKITAREL 67
Pm70 -----MDKFAEHE I KAVI I VAGNGESLSQIDYRLLPKNYDVFRCNQFYFEEFYFLGNKIKAVFFTPGVLEQYITLYHL 74

Bi-Cj-cstII I C N Q E Y E T L I M C S N Y N Q A H L E N E N - - - - F V K T I R Y D Y F P D A H L G Y - D F F K C I K D F N A Y F K F H E I Y F N Q - - R I T S G V Y M C 137
Cj-cstI I L K N E Y E I K N I F C S T F N L P F I E S N D - - - - F L H Q R Y N F F P D A K L G Y - E V I E N L K E F Y A Y I K Y N E I Y F N K - - R I T S G V Y M C 152
cstIV L Q N G D Y E C E N I V C K M Y N F Q D R K E - - - - K I F R E N R K Y F F P A A I N G Y D A F F Y K I K E L S N M I D F D C Y E Y N T T E I T T Y V I 148
cstV M Q N K E Y Y C E N I V C K L F P L Q H E I N Q K S L - R N F K K I R P L F F P Y A L D G N E H Y F N K L K E L N S F I N F N L Y D E G - L Q I T T G M Y A I 146
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HBS-02 S L R Q E Y Y F E D I F C S T I A P F M N F G N H Y T - - H A Q D Y L D K H Y P G A R N T Y A L L Q S L E P F Y K L Y T T R R N F Y Q Q - - H F T T G V M M I 142
Pm70 K R N E Y F V D N V I L S S F N H P T V D L E K - - - - S Q K I Q A L F I D V I N G Y E K Y L S K I T A F D V Y L R Y K E L Y E N Q - - R I T S G V Y M C 146

Bi-Cj-cstII A V A I A L G Y K E I Y L S G I D F Y Q N - G S S Y A F D T K Q K N L L K L A P N F K N D N S H Y I G H S K N T D I K A L E F F E K T Y K I K L Y C L C P N S L 216
Cj-cstI A A I A I A L G Y K T I Y L C G I D F Y E G - D V I Y P F E A M S T N I K T I F P G I K - D F K P S N C H S K E Y D I E A L K L K S I Y K V N I Y A L C D D S I 230
cstIV C C A V A C G Y K E I Y L A G M D F G D E - K Y N Y F S E K I E K - - - - I K E S K K R - - T K M H H K S I D E K I I D F T Q Q Q Y N V K I F S I C P N S S 220
cstV A C A V A C G Y K E I Y I T G I D F Y S T - Q E Y A F D I K D K I G L Y A L N P S F K I Q - - Y L K S H S K E T D E I L S F I K Q T Y N A N F S I S P K S P 223
HAC1267 L V A I Q L G Y K E I Y L C G I D F Y E N G F G H F Y E - - - - - N Q G G F F E E D S D P M H D K N I D I C A L E L A K K Y - - A K I Y A L V P N S A 210
HAC1268 L S A V V L G Y K E I Y L V G I D F G A S S W G H F Y D E - - - - - S Q S Q H F S N H M A D C E N I Y Y D M L T I C L C Q K Y - - A K I Y A L V P N S P 216
HBS-02 I V A I V L G Y K E I Y C A G I D F Y L E G L G H F Y H - - - - - V K S P H F T L A P D C Q H T K D L D I K G I E V K Q Y - - A C I Y A L V P N S A 210
Pm70 A V A L A M G Y T D I Y L T G I D F Y Q A S E E N Y A F D N K K P N I I R L L P D F R K E K T L F S Y H S K D I D E A L S F I Q Q H Y H V N E Y S I S P M S P 226

Bi-Cj-cstII L A N F I E L A P N - - - - - 226
Cj-cstI L A N H F P L S I N - - - - - 240
cstIV I N A F I P L H P I - - - - - 230
cstV M T K Y I P L A P K - - - - - 233
HAC1267 L V K M I P L S S Q K G V L E K V K D R I G L G E F K R E K F G Q K E L E R Q K E L E R Q K E L E R Q K E L E R Q K E L E R Q K E L E R Q K E L E R 290
HAC1268 L S H L L T I N P Q A K Y P F E L L D K P - I G Y T S D L I I S S P L E E K L L E F K N I E E K L L E F K N I E E K L L E F K N I E E K L L E F K N I E E K L L 295
HBS-02 L S A I L P L S P H K N A L S Q E K - - - - - 228
Pm70 L S K H F P I P T V - - - - - 236

Bi-Cj-cstII -----LNSN--FIIQEKNN-YTKDIIIPSS EAYGKFSKNIN F----- 260
Cj-cstI -----INNN--FTLENKHNN SINDIILTDNTPGVS FYKNQLKAD----NKIMLN FYNILH 289
cstIV -----QNNENI FKP IERPKDAIKTQLTPPIKAVR RYKR-LYLES----NI I IKFFH ELVQ 280
cstV -----QNY S--FDIEEKSS ESIKDELIPSKKAYR NYSRALYLQN----NMFY NFIHDCLK 282
HAC1267 QKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELERQKELER 370
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HBS-02 -----M C A L K L G D P K P N G Y I D D V C V D F V E F S M R A T L I Q A T Q S V G I T P D N L I Y K G L N M V W R 283
Pm70 -----E D D C E T T F V A P L K E N Y I N D I L P P H F V Y E K L G T I V S K K S R F H S N L I V R L I R I L L K 291

Bi-Cj-cstII ----- 260
Cj-cstI SKDN I I K F L N K - - - - - 300
cstIV V P R R I R H Y Y S K T R Y T R - - - - - 296
cstV F P S A I K N Y F K N I K K - - - - - 296
HAC1267 L F K G C F A L L D - - - - L K A L K S I I K A F L K R - - - - - 395
HAC1268 E F K N I E E K L L A S R L N N I L R K I K R K I L P F W G G V T P T L K V S F R W G A A 422
HBS-02 C A S D I Y R V V R G - - - - - L Y R L S L K A L Y F L R A W F K R H R A T G G - - - - - 318
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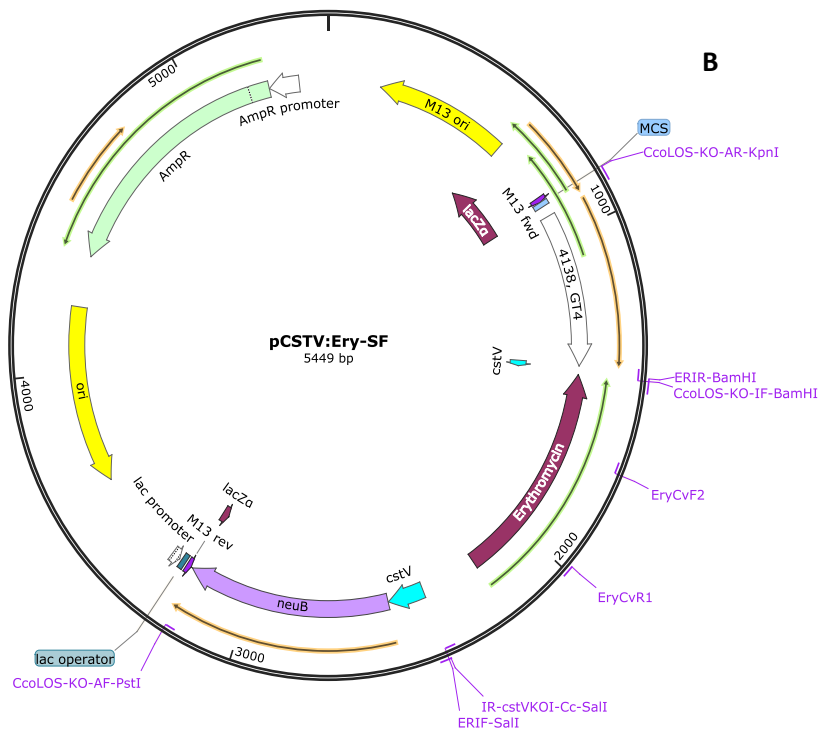
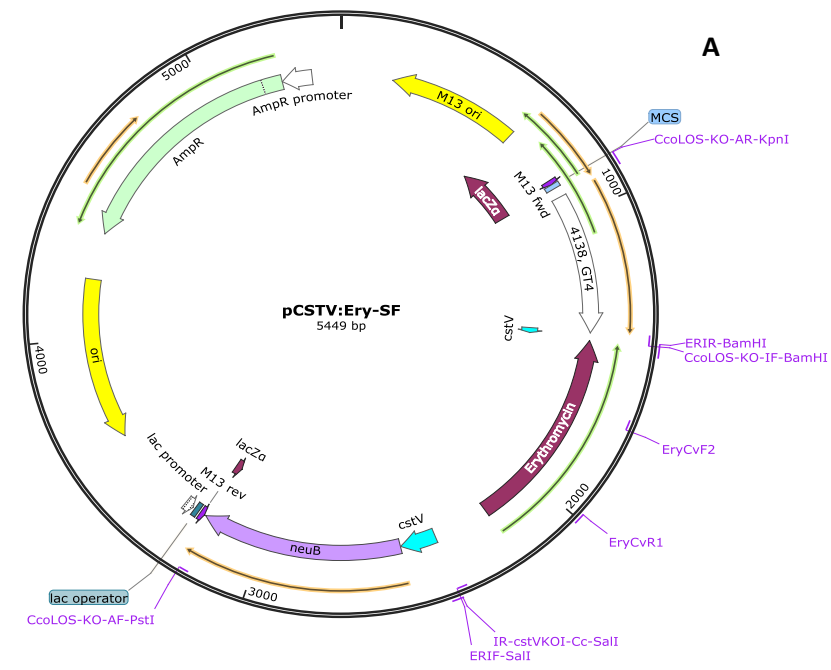
Important residues for CMP	
CstII	CstIV
Thr131	Leu142
Ser132	Thr143
Important residues for Neu5Ac	
CstII	CstIV
Gln32	His40
Asn51	Val59
Gln58	Glu66
Arg129	Glu140
Ser132	Thr143
Tyr185	Thr189

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

Supplemental Material

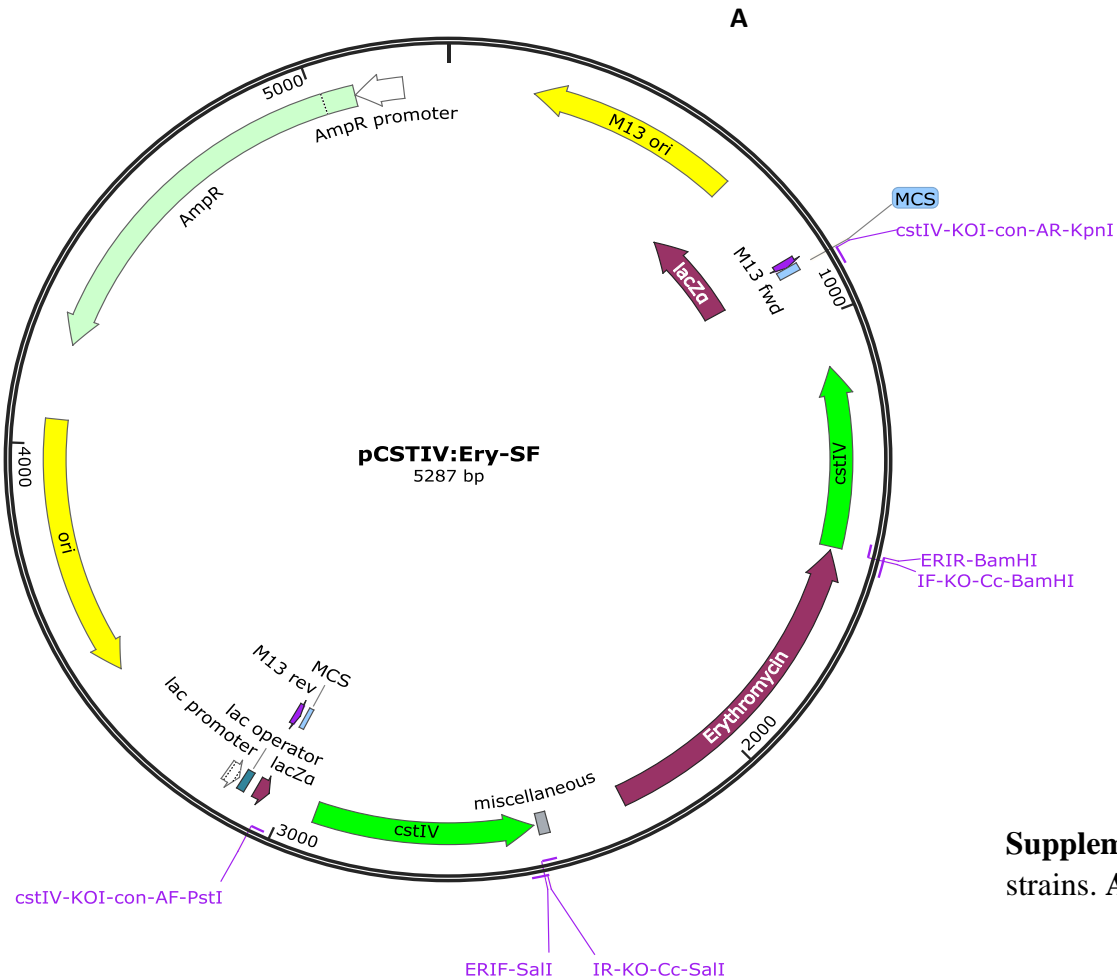
Supplemental Table 1. Bacterial strains and protein expression constructs

Strain or plasmid	Genotype and/or phenotype	Reference or source
<i>C. coli</i> 76339	<i>cstI</i> , <i>cstV</i> , <i>neuB</i>	¹
<i>C. coli</i> 76339 Δ <i>cstV</i> -SF1	<i>cstI</i> , Δ <i>cstV</i> : Ery, <i>neuB</i>	This study
<i>C. coli</i> 76339 Δ <i>cstV</i> -SR4	<i>cstI</i> , Δ <i>cstV</i> : Ery, <i>neuB</i>	This study
<i>C. coli</i> 76339 Δ <i>neuB</i> -SR1	<i>cstI</i> , Δ <i>neuB</i> : Ery, <i>cstV</i>	This study
<i>C. coli</i> 76339 Δ <i>cstI</i> -XR3	Δ <i>cstI</i> : CAT, <i>cstV</i> , <i>neuB</i>	This study
<i>C. coli</i> 76339 Δ <i>cstV</i> -SR4 Δ <i>cstI</i> -XR1	Δ <i>cstI</i> : CAT, Δ <i>cstV</i> : Ery, <i>neuB</i>	This study
<i>C. coli</i> 76339 Δ <i>neuB</i> -SR2	<i>cstI</i> , Δ <i>neuB</i> : Ery, <i>cstV</i>	This study
<i>C. coli</i> 76339 Δ <i>cstV</i> -SR4 Δ ggt: <i>cstV</i> -2	<i>cstI</i> , Δ <i>cstV</i> : Ery, <i>neuB</i> , Δ ggt: <i>cstV</i> :CAT	This study
<i>C. coli</i> 65	<i>cstIV</i>	²
<i>C. coli</i> 65 Δ <i>cstIV</i> -SF5	Δ <i>cstIV</i> :Ery	This study
<i>C. coli</i> 73	<i>cstIV</i>	²
<i>C. coli</i> 73 Δ <i>cstIV</i> -SF3	Δ <i>cstIV</i> :Ery	This study
<i>C. coli</i> 73 Δ <i>neuB2</i>	Δ <i>neuB2</i> :Ery	This study
<i>C. jejuni</i> 81-176		
<i>E. coli</i> AD202		³
<i>E. coli</i> BL21		
Protein expression constructs		
pCwMal-ET		³
pCw		
pCwMal-ET_51 <i>cstV</i>		This study
pCwMal-ET_73 <i>cstIV</i>		This study
pCw_51 <i>cstV</i>		This study
pCw_73 <i>cstIV</i>		This study



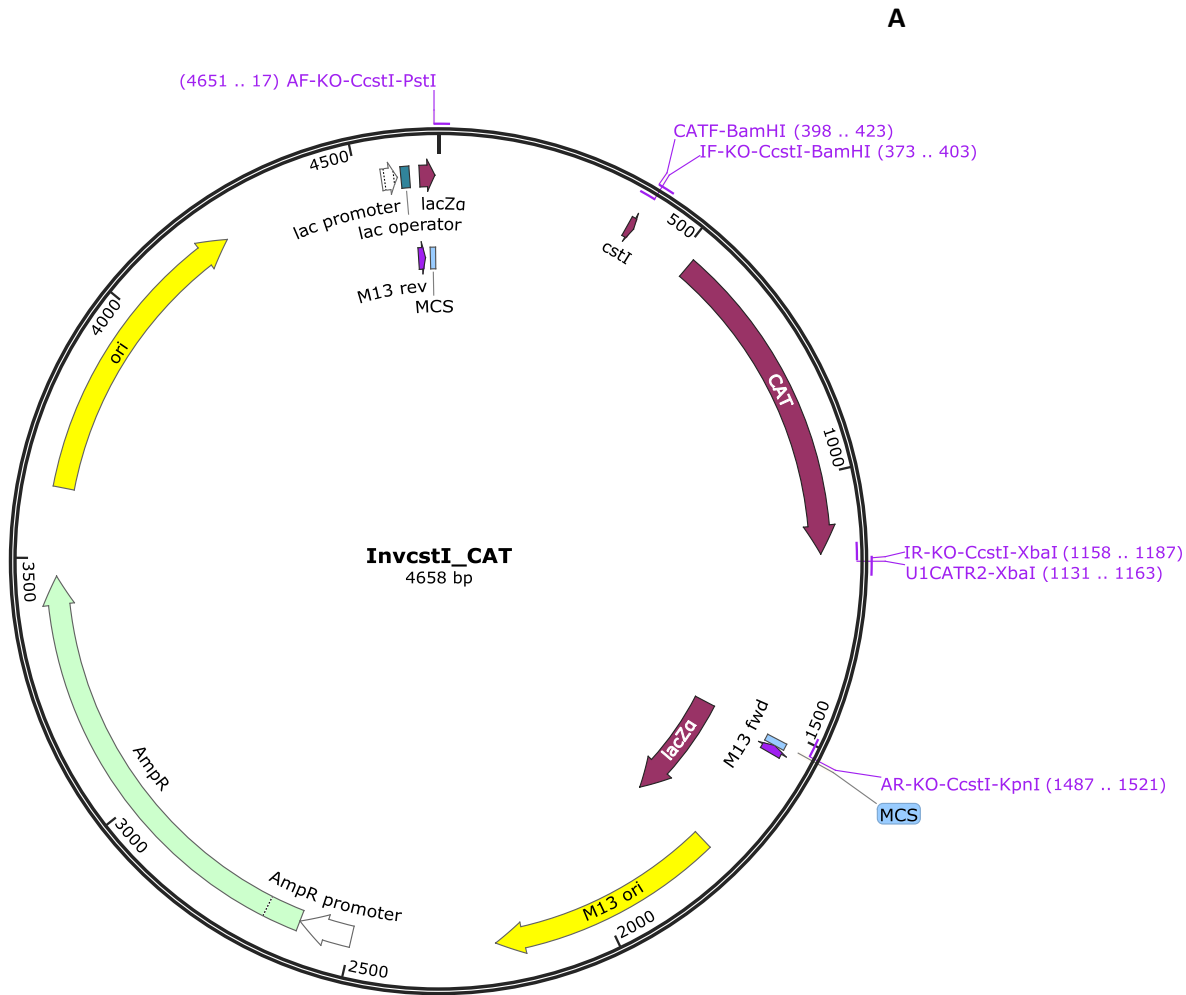
Primer name	Sequence
cstV amplification	
CcoLOS-KO-AF-PstI	ATCTGCAGCCTAATGCAACCGCACCCAAGC
CcoLOS-KO-AR-KpnI	AGGTACCATTTATCCCAAAAAGTTGTCTTAAGCGTGG
Inverse PCR	
IR-cstVKOI-Cc-SalI	ACCGTCGACCATATCGAAATTATTCTAGAGCTC
CcoLOS-KO-IF-BamHI	AGGATCCGTTTTCTATCATATCTGTCCTCATAG
Erythromycin cassette	
ERIF-SalI	ACCGTCGACAGTATAAAACCTTTAAGAACTTTC
ERIR-BamHI	ACCGGATCCACTTACTTATTAATAATTTATAGCTATTG
ERIR-SalI	ACCGTCGACACTTACTTATTAATAATTTATAGCTATTG
ERIF-BamHI	ACCGGATCCAGTATAAAACCTTTAAGAACTTTC
Mutant verification	
EryCvF1	CCTCTTATTATAGCCATTTGTTTGC
EryCvF2	ATATTTTCATCCTAAACCTAAAGTGAATAGC
EryCvR1	TTATTTTCTGTAGTTTGCATAATTTATGG
EryCvR2	GGAGAAAGAGTTTGTGCTAATCTC
Gene expression verification	
09870cFv	TAA GTT TTT GGT AGT TTT TGC CTC G
09870cRv	ATTAGCACTAGATGATACAACCAGTG
09880cFv	CAACTAAGATCCCATTATGCCAAG
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



Primer name	Sequence
<i>cstIV</i> 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	ACCGGATCCACTTACTTATTAATAATTTATAGCTAT TG
Mutant verification	
CcCstIVvF	AACAGAAATGCTTACTGGCAC
EryCvF2	ATATTTTCATCCTAAACCTAAAGTGAATAGC
EryCvR1	TTATTTTCTGTAGTTTTGCATAATTTATGG

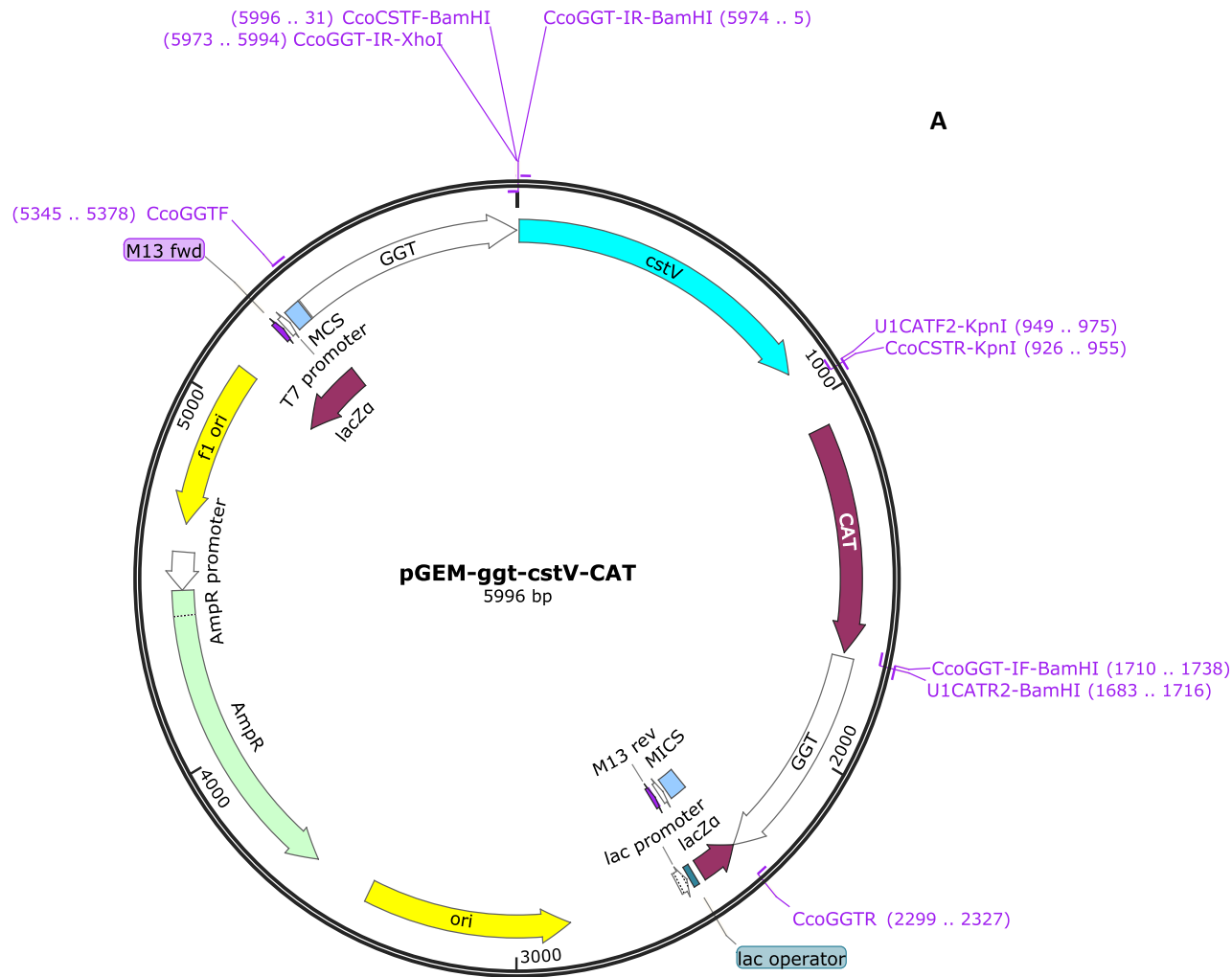
Supplemental Figure 8. Plasmids and primers for the generation of *C. coli* 65 and 73 Δ *cstIV* mutant strains. **A**; pCSTIV:Ery-SF plasmid, **B**; primer list



B

Primer name	Sequence
cstI amplification	
AF-KO-CcstI-PstI	ATCTGCAGAGGAAATGGGCCTAGTCTT
AR-KO-CcstI-KpnI	AGGTACCCACTATGGATTCATAAGAATTTCTACCT
Inverse PCR	
IR-KO-CcstI-XbaI	ATTCTAGATGAATTTGGCTAAGACTGTAGGTG
IF-KO-CcstI-BamHI	ACCGGATCCGCTATGGCGCACATATAAATACCAG
CAT cassette	
U1CATR2-XbaI	ATTCTAGAGGGATTTTATTTATTCAGCAAGTCTTG
CATF-BamHI	AGGATCCCGGCGGTGTTCCCTTTCCAAG
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; InvstI_CAT plasmid, B; primer list



Primer name	Sequence
ggt amplification	
CcoGGTF	ATTTAGTTATATTTGTGATTTCAATCACGCTAGG
CcoGGTR	AAAACCTGTTGTGATGATTCTAGAGCCACC
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