Supplemental Material for:

Reconstitution and Minimization of a Micrococcin Biosynthetic Pathway in *Bacillus subtilis*

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LIST OF SUPPLEMENTAL TABLES AND FIGURES

Table S1: Primers used for multi-locus sequence analysis

Primer name	**Primers Sequence (5' - 3')**[a]	**Product Size (bp)**
groEL for	GAAATTGCTGGTGACGGTACGACAAC	601
groEL rev	GGTCACCAAATCCTGGTGCTT	
ef4 For	CAACGTTTAAAGCGTCAGGAACG	1267
ef4 Rev	CAGCACCCACATAATCATTCGG	
rplK for	GCACGTATATGAGTGGGAGGG	578
rplK rev	GGTTTTAGCGGAATTTCCTCCC	
ctaD for	GCCGTTACTGTTTGCATATATG	1664
ctaD rev	GCCATTATTTAACACCTCTTTCG	
dnaK for	GTCAATGGTTAAAGTAAACGAATG	1975
dnaK rev	TACTATATCAGTGTATCGCATGTT	
gyrR for	ATTCAATGTATCAGAAGGACAGA	2027
gyrR rev	CAATTTTGAATCATGGTCTAAAGC	

[a] Primers were designed using the *M. caseolyticus* JCSC5402 genome (Genbank ref: NC_011999.1)

TABLE S2. BLASTp output from multi-locus sequence typing of strain 115

Target	**Top Hits**[a]	**Coverage (%)**	**Identity (%)**
groEL (heat shock protein 60)	1. *Macrococcus caseolyticus* JCSC5402	100	90
	2. *Staphylococcus caseolyticus* ATCC 13548	96	89
	3. *Macrococcus* sp. 1123N2A	95	89
ef4 (elongation factor 4)	1. *Macrococcus caseolyticus* JCSC5402	100	88
	2. *Bacillus thuringiensis* str. Bt185	90	77
	3. Bacillus cereus str. NJ-W	97	76
rplK (50S ribosomal protein L11)	1. *Macrococcus caseolyticus* JCSC5402	100	99
	2. *Staphylococcus haemolyticus* str. S167	96	88
	3. *Staphylococcus haemolyticus* str. Sh29/312/L2	96	88
ctaD (cytochrome C oxidase)	1. *Macrococcus caseolyticus* JCSC5402	99	89
	2. Bacillus sp. FJAT-22090	68	81
	3. Lysinibacillus sphaericus str. 2362	88	78
dnaK (mol. chaperone DnaK)	1. *Macrococcus caseolyticus* JCSC5402	100	95
	2. *Staphylococcus agnetis* str. 908	100	84
	3. *Staphylococcus hyicus* ATCC 11249	100	84
gyrB (DNA gyrase subunit B)	1. *Macrococcus caseolyticus* JCSC5402	100	93
	2. *Staphylococcus capitis* str. AYP1020	90	78
	3. *Staphylococcus hominis* str. DAR3383	94	77

[a] For simplicity, only top three BLASTp hits are shown

FIG S1 Plasmid map and sequence of pPxyl_U used to generate *B. subtilis thrC*::*xylR*-P_{xylA}-*tclU*-*spc* mutants strains. Key features are annotated, as well as relevant unique restriction sites (bold). The complete nucleotide sequence of the plasmid is provided below. The sequence of *tclU* is distinguished by lowercase font and the sequence of P_{xylA} is underlined.

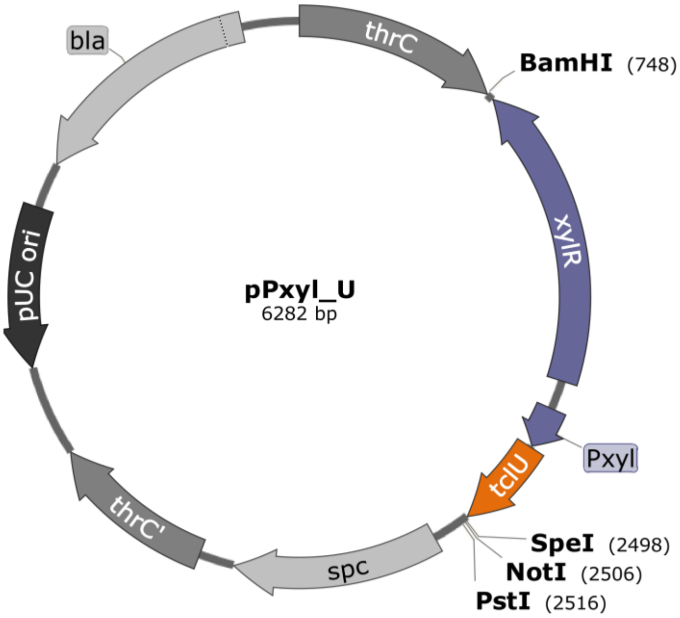

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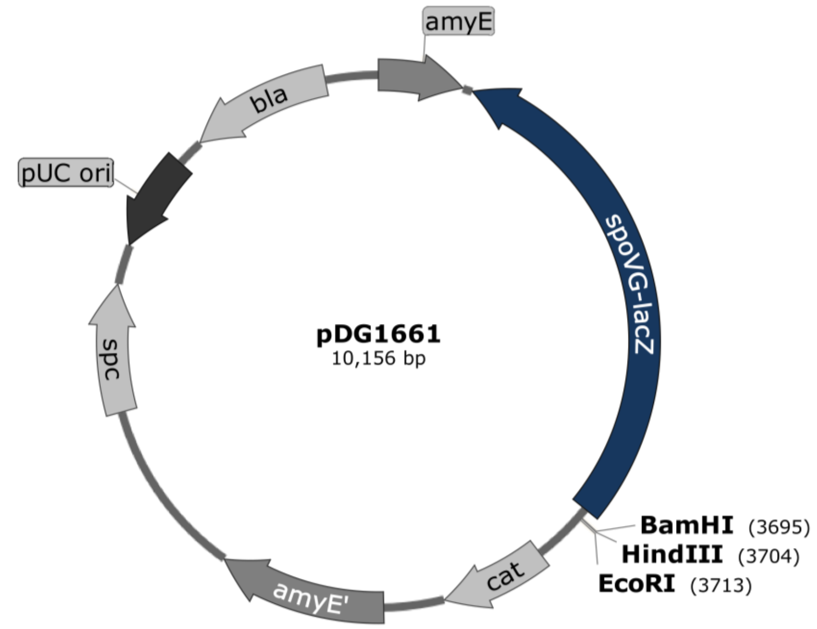
FIG S2 Plasmid map and sequences of the constructs used for reporter assays. (A) The plasmid pDG1661, which was modified to produce the plasmids pLacZ_E, pLacZ_Q and pLacZ_I. These derivatives were used to generate the *B. subtilis* 168 *lacZ* reporter strains shown in Fig. 2A. Shown on the plasmid map are key features including the *spoVG-lacZ* reporter gene and polylinker (bold). The complete nucleotide sequence of pDG1661 is provided below with *spoVG-lacZ* (3'–5') shown in lowercase font and the EcoRI, HindIII and BamHI sites in the polylinker are highlighted in yellow. (B) The *tcl* promoter sequences introduced into pDG1661. Restriction sites utilized are highlighted in yellow. Promoter sequences are in 5'-3' orientation.

A



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B pLacZ_E Insert (5’-3’):

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pLacZ_Q Insert (5’-3’):

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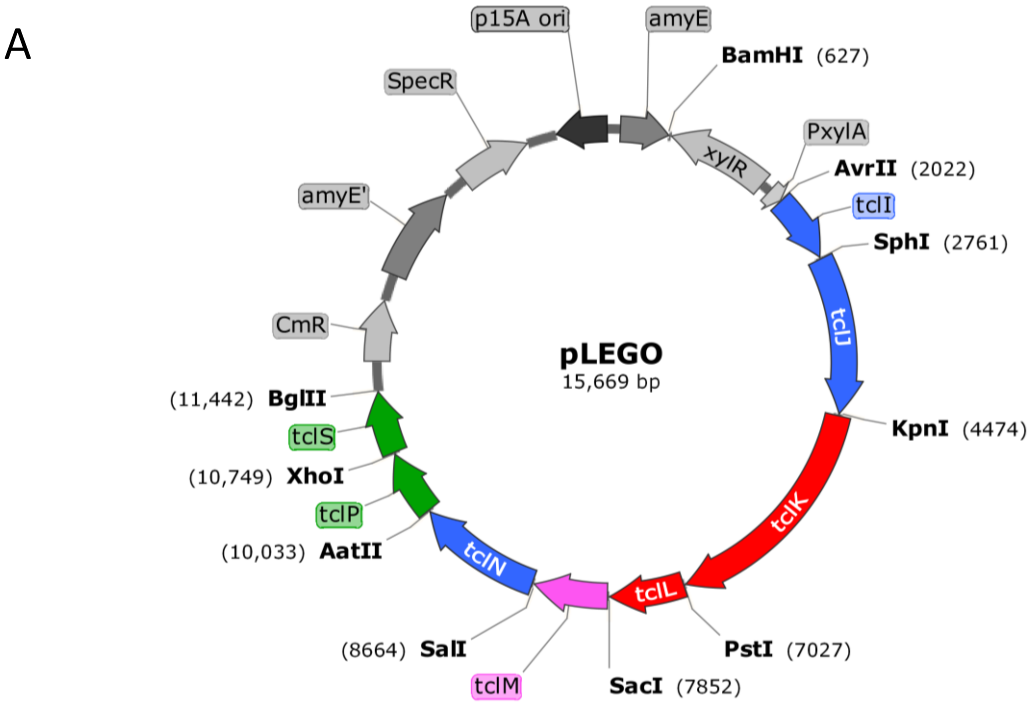
FIG S3 Plasmid map and sequences of pLEGO and its tagged derivatives. (A) pLEGO was engineered for xylose-inducible expression of the Tcl processing machinery. The genes encoding processing enzymes are colored by predicted function (see Fig. 1A) and are each flanked by unique restriction sites (bold). The nucleotide sequence of pLEGO is shown below. All *tcl* genes are distinguished by lowercase font and adjacent restriction sites are highlighted in yellow. (B) Sequence context (5'-3') of all successfully installed N- and C-terminal *Strep*-tags. In each case, the nucleotide sequence of the *Strep*-tag is underlined, *tcl* coding sequences are distinguished by lowercase font and relevant restriction sites are highlighted in yellow. For N-terminal tags, only the 5' end of the gene is shown. For C-terminal tags, the 3' end is shown.



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agaaaatacaagtaatagagatagtagattctataatatctttcctaaataatagtattacattttgtgagataaaattacttcct
taaAGATCTCAGGAATTCTCATGTTTGACAGCTTATCATCGGCAATAGTTACCCTTATTATCAAGATAAGAAAGAAAAGGATTTTT
CGCTACGCTCAAATCCTTTAAAAAAACACAAAAGACCACATTTTTTAATGTGGTCTTTTATTCTTCAACTAAAGCACCCATTAGTT
CAACAAACGAAAATTGGATAAAGTGGGATATTTTTAAAATATATATTTATGTTACAGTAATATTGACTTTTAAAAAAGGATTGATT
CTAATGAAGAAAGCAGACAAGTAAGCCTCCTAAATTCACTTTAGATAAAAATTTAGGAGGCATATCAAATGAACTTTAATAAAATT
GATTTAGACAATTGGAAGAGAAAAGAGATATTTAATCATTATTTGAACCAACAAACGACTTTTAGTATAACCACAGAAATTGATAT
TAGTGTTTTATACCGAAACATAAAACAAGAAGGATATAAATTTTACCCTGCATTTATTTTCTTAGTGACAAGGGTGATAAACTCAA
ATACAGCTTTTAGAACTGGTTACAATAGCGACGGAGAGTTAGGTTATTGGGATAAGTTAGAGCCACTTTATACAATTTTTGATGGT
GTATCTAAAACATTCTCTGGTATTTGGACTCCTGTAAAGAATGACTTCAAAGAGTTTTATGATTTATACCTTTCTGATGTAGAGAA
ATATAATGGTTCGGGGAAATTGTTTCCCAAAACACCTATACCTGAAAATGCTTTTTCTCTTTCTATTATTCCATGGACTTCATTTA
CTGGGTTTAACTTAAATATCAATAATAATAGTAATTACCTTCTACCCATTATTACAGCAGGAAAATTCATTAATAAAGGTAATTCA
ATATATTTACCGCTATCTTTACAGGTACATCATTCTGTTTGTGATGGTTATCATGCAGGATTGTTTATGAACTCTATTCAGGAATT
GTCAGATAGGCCTAATGACTGGCTTTTATAATATGAGATAATGCCGACTGTACTTTTTACAGTCGGTTTTCTAATGTCACTAACCT
GCCCCGTTAGTTGAAGAAGGTTTTTATATTACAGCTCCAGATCCTCTACGCCGGACGCATCGTGGCCGGCATCACCGGCGCCACAG
GTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGC
GTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCATGGCGGCCGCTCGACATGGATGAGCGATGA
TGATATCCGTTTAGGCTGGGCGGTGATAGCTTCTCGTTCAGGCAGTACGCCTCTTTTCTTTTCCAGACCTGAGGGAGGCGGAAATG
GTGTGAGGTTCCCGGGGAAAAGCCAAATAGGCGATCGCGGGAGTGCTTTATTTGAAGATCAGGCTATCACTGCGGTCAATAGATTT
CACAATGTGATGGCTGGACAGCCTGAGGAACTCTCGAACCCGAATGGAAACAACCAGATATTTATGAATCAGCGCGGCTCACATGG
CGTTGTGCTGGCAAATGCAGGTTCATCCTCTGTCTCTATCAATACGGCAACAAAATTGCCTGATGGCAGGTATGACAATAAAGCTG
GAGCGGGTTCATTTCAAGTGAACGATGGTAAACTGACAGGCACGATCAATGCCAGGTCTGTAGCTGTGCTTTATCCTGATGATATT
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GTTAAAAGAGATCCAGCGTCGGCCAAAACCATCGGCTATCAAAATCCGAATCATTGGAGCCAGGTAAATGCTTATATCTATAAACA
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AAGCTCGATCGAGCCACCTACTGAAGTGGATTTCTTTAAGCTAGAGGATCGATCTGTATAATAAAGAATAATTATTAATCTGTAGA
CAAATTGTGAAAGGATGTACTTAAACGCTAACGGTCAGCTTTATTGAACAGTAATTTAAGTATATGTCCAATCTAGGGTAAGTAAA
TTGAGTATCAATATAAACTTTATATGAACATAATCAACGAGGTGAAATCATGAGCAATTTGATTAACGGAAAAATACCAAATCAAG
CGATTCAAACATTAAAAATCGTAAAAGATTTATTTGGAAGTTCAATAGTTGGAGTATATCTATTTGGTTCAGCAGTAAATGGTGGT
TTACGCATTAACAGCGATGTAGATGTTCTAGTCGTCGTGAATCATAGTTTACCTCAATTAACTCGAAAAAAACTAACAGAAAGACT
AATGACTATATCAGGAAAGATTGGAAATACGGATTCTGTTAGACCACTTGAAGTTACGGTTATAAATAGGAGTGAAGTTGTCCCTT
GGCAATATCCTCCAAAAAGAGAATTTATATACGGTGAGTGGCTCAGGGGTGAATTTGAGAATGGACAAATTCAGGAACCAAGCTAT
GATCCTGATTTGGCTATTGTTTTAGCACAAGCAAGAAAGAATAGTATTTCTCTATTTGGTCCTGATTCTTCAAGTATACTTGTCTC
CGTACCTTTGACAGATATTCGAAGAGCAATTAAGGATTCTTTGCCAGAACTAATTGAGGGGATAAAAGGTGATGAGCGTAATGTAA
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TTACCTAAAGAGCATGTAACTTTACTGGATATAGCTAGAAAAGGCTATCGGGGAGAGTGTGATGATAAGTGGGAAGGACTATATTC
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TGCTTCATGTGGCAGGAGAAAAAAGGCTGCACCGGTGCGTCAGCAGAATATGTGATACAGGATATATTCCGCTTCCTCGCTCACTG
ACTCGCTACGCTCGGTCGTTCGACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATA
CTTAACAGGGAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATCACGAAATCTGACGCTCA
AATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGC
CTTTCGGTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGGTAGGCAGTTCGC
TCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGA
AAGACATGCAAAAGCACCACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAA
ACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAA
ACCGCCCTGCAAGGCGGTTTTTTCGTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATC
AGATAAAATATTTCTAG

B Sequence context (5’-3’) of all successfully installed N-terminal *Strep*-tags.

Strep-tclI: …CCTAGGatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcatatttacta…

Strep-tclJ: …GCATGCAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcagaaattttt…

Strep-tclK: …GGTACCAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcaataaaaaaa…

Strep-tclL: …CTGCAGAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcaaattggaca…

Strep-tclM: …GAGCTCAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcaaaaacaaaa…

Strep-tclP: …GACGTCAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcaaatatacta…

Strep-tclS: …CTCGAGAAGGAGGTGTGTCTCatgtcatggagccatccgcaatttgaaaaaggcgcaggcgcagataattgt…

Sequence context (5’-3’) of all successfully installed C-terminal *Strep*-tags:

tclK-Strep: …gaaggcgtaGGCGCAGGCGCATGGAGCCATCCGCAATTTGAAAAAtaaCTGCAG…

tclL-Strep: …agtaataaaGGCGCAGGCGCATGGAGCCATCCGCAATTTGAAAAAtaaGAGCTC…

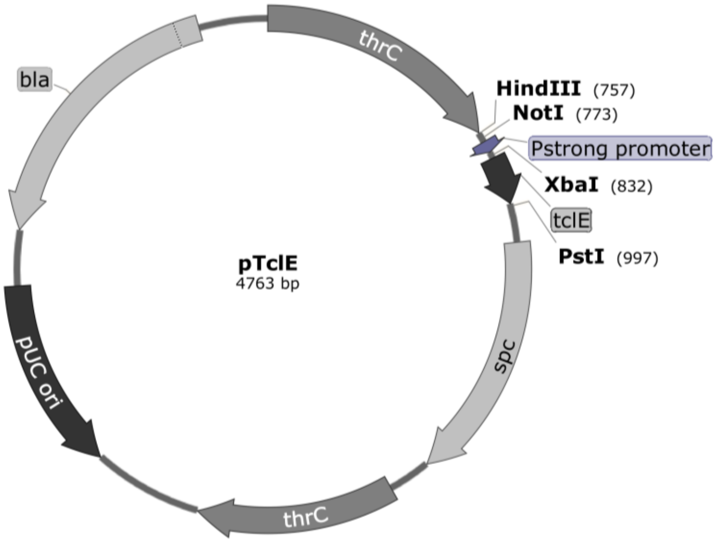
tclN-Strep: …agtgatttaGGCGCAGGCGCATGGAGCCATCCGCAATTTGAAAAAtaaGACGTC…

tclP-Strep: …acaatatggGGCGCAGGCGCATGGAGCCATCCGCAATTTGAAAAAtaaCTCGAG…

tclS-Strep: …ttacttcctGGCGCATGGAGCCATCCGCAATTTGAAAAAtaaAGATCT…

FIG S4 Plasmid map and sequence of pTclE and its derivatives. (A) pTclE was used to introduce *tclE* into the *thrC* locus of *B. subtilis* 168R. The plasmid map shows key features including the P_{strong} promoter and the micrococcin precursor gene, *tclE* (black arrow). The complete plasmid sequence is shown below with the P_{strong} promoter underlined, *tclE* distinguished in lowercase font, and useful restriction sites highlighted yellow. (B) Relevant portions of the *His₆-tclE* and *GST-tclE* inserts in the pTclE derivatives pTclE_His and pTclE_GST. In each case the upstream restriction site is highlighted in yellow, affinity tags are underlined, and the first three codons of *tclE* are shown. The GST tag includes a TEV cleavage site (italicized) for tag removal.

A



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AATTCATGTAAAAGATGAGGTTGGTTCATTCTCGAAAATTACATCTGTGTTCTCAGAGCGGGGCGTGACGTTTGAAAAAATCCTTC
AGCTGCCAATTAAAGGCCATGATGAGTTAGCTGAAATCGTAATTGTCACACATCATACATCAGAAGCTGATTTCAGTGATATCCTG
CAAAACCTAAATGATTTGGAAGTCGTTCAAGAAGTCAAAAGCACATATCGTGTAGAAGGGAACGGTTGGAGCTAATGTGGAAAGGA
CTTATCCATCAATATAAAGAATTTTTACCTGTAACAGATCAAACACCGGCGCTAACTTTACATGAAGGAAACACACCTCTTATTCA
CCTGCCGAAGCTGTCTGAGCAGCTCGGAATTGAGCTTCATGTCAAAACGGAAGGCGTCAATCCTACGGGATCATTTAAAGATCGCG
GAATGGTTATGGCTGTGGCAAAGGCAAAAGAAGAAGGCAATGACACGATTATGTGCGCGTCAACAGGTAACACTTCCGCTGCTGCG
GCAGCATATGCAGCCCGTGCTAACATGAAATGCATTGTCATCATCCCGAACGGAAAAATTGCATTTGGAAAACTCGCTCAAGCTGT
CATGTACGGAGCCGAGATTATCGCAATTGACGGAAACTTTGACGATGCGCTTAAAATTGTCCGTTCCATCTGTGAGAAATCACCGA
TTGCCCTTGTCAACTCAGTCAACCCTTACCGCATTGAAGGCCAAAAAACTGCTGCCTTCGGATCCTAGAAGCTTATCGAATTCGCG
GCCGCTTCTAGGCTTGACAAATTGCAGTAGGCATGACAAAATGGACTCAAGGAGGTGTCTAGAATGGGAtccgaattccaaacaaa
caatatcgaaggtttagatgtcactgatttagaatttatcagtgaagaagttactgaaaaagacgagaaagaaatcatgggtgctt
cttgtactacatgtgtttgtacatgcagttgttgtacaacttaaGGCTGCAGGATAAAAAATTTAGAAGCCAATGAAATCTATAAA
TAAACTAAATTAAGTTTATTTAATTAACAACTATGGATATAAAATAGGTACTAATCAAAATAGTGAGGAGGATATATTTGAATACA
TACGAACAAATTAATAAAGTGAAAAAAATACTTCGGAAACATTTAAAAAATAACCTTATTGGTACTTACATGTTTGGATCAGGAGT
TGAGAGTGGACTAAAACCAAATAGTGATCTTGACTTTTTAGTCGTCGTATCTGAACCATTGACAGATCAAAGTAAAGAAATACTTA
TACAAAAAATTAGACCTATTTCAAAAAAAATAGGAGATAAAAGCAACTTACGATATATTGAATTAACAATTATTATTCAGCAAGAA
ATGGTACCGTGGAATCATCCTCCCAAACAAGAATTTATTTATGGAGAATGGTTACAAGAGCTTTATGAACAAGGATACATTCCTCA
GAAGGAATTAAATTCAGATTTAACCATAATGCTTTACCAAGCAAAACGAAAAAATAAAAGAATATACGGAAATTATGACTTAGAGG
AATTACTACCTGATATTCCATTTTCTGATGTGAGAAGAGCCATTATGGATTCGTCAGAGGAATTAATAGATAATTATCAGGATGAT
GAAACCAACTCTATATTAACTTTATGCCGTATGATTTTAACTATGGACACGGGTAAAATCATACCAAAAGATATTGCGGGAAATGC
AGTGGCTGAATCTTCTCCATTAGAACATAGGGAGAGAATTTTGTTAGCAGTTCGTAGTTATCTTGGAGAGAATATTGAATGGACTA
ATGAAAATGTAAATTTAACTATAAACTATTTAAATAACAGATTAAAAAAATTATAAAAAAATTGAAAAAATGGTGGAAACACTTTT
TTCAATTTTTTTGTTTTATTATTTAATATTTGGGAAATATTCATTCTAATTGGTAATCAGATTTTAGAAAACAATAAACCCTTGCA
TAGGGGGATCTCGAGGCCTTCCGAAAATGCGCGGCTTTGAAGCTGAGGGTGCGGCGGCAATCGTGCGCAATGAAGTGATTGAAAAT
CCGGAAACAATAGCGACAGCCATTCGTATCGGAAACCCGGCAAGCTGGGACAAAGCTGTAAAGGCAGCCGAGGAATCCAATGGGAA
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AATTGACGAAGTCACTGATGATGAAATCCTTCACGCATATCAGCTGATCGCCCGTGTAGAAGGCGTGTTTGCAGAACCAGGTTCTT
GCGCGTCTATCGCAGGAGTGCTGAAACAGGTGAAATCCGGAGAAATTCCGAAAGGCAGCAAGGTCGTAGCTGTGTTAACAGGAAAC
GGACTGAAAGATCCGAACACAGCGGTCGACATTTCAGAAATCAAGCCTGTCACATTGCCGACTGATGAAGACAGCATCCTTGAATA
TGTAAAAGGAGCGGCCCGTGTATGAACGAAGCCGACATGCTGTTCTCTGTCACTGTTCCCGGAAGCACAGCTAACCTAGGCCCCGG
CTTTGATTCAGTCGGAATGGCGCTCAGCAGATATTTGAAGCTGACCGTCTTTGAAAGCGACAAATGGTCTTTTGAGGCTGAAACAG
AAACAGTCGCCGGAATTAATTCGAGCTCCTTTAACTTCCTCACCAGTAGTTGTATCGGTACCATAAGTAGAAGCAGCAACCCAAGT
AGCTTTACCAGCATCCGGTTCAACCAGCATAGTAAGAATCTTACTGGACATCGGCAGTTCTTCGAACAGTGCGCCAACTACCAGCT
CTTTCTACAGTTCATTCAGGGCACCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTT
GATCCATGGATTACGCGCGACCTTCAGCATCGCCGGCATGTCCCCCTGGCGGACGGAAGTATCCAGCTCGAGGTCGGCCGCGTT
GCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC
TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC
TTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGG
CTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACT
TATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
AACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGATAGCTCTTG
ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAG
ATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGG
ATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCA
ATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA
CGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATA
AACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC
TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCCGGCATCGTGGTGTCACGCTCGTCGTTTGGTA
TGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGT
CCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCC
ATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCC
CGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTC
TCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAG
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCT
TCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAA
ATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAG
GCGTATCACGAGGCCCTTTCGTCTTCAAGAATT
```

B pTclE_His insert, His$_6$-**TclE:**

```
  XbaI
…TCTAGA ATG GGC TCA CAT CAT CAT CAT CAT CAT GGC GGC GGA tcc gaa ttc…
         M   G   S   H   H   H   H   H   H   G   G   G   S   G   F
```

pTclE_GST insert, GST-*TEV*-**TclE**:

```
  XbaI
…TCTAGAATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGAATATCTTGAAGAAA

AATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTT

CCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGG

TTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTA

AAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACA

TATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCT

GGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATA

TAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAA GGA GGA GAA AAC CTG TAT
                                                             G   G   E   N   L   Y
TTT CAA GGC GGA tca gaa ttc…
 F   Q   G   G   S   E   F
```

FIG S5 Alignment of tRNA^{glu} DNA sequences between relevant bacterial strains. Deviations from the *B. subtilis* trnSL_glu2 sequence are highlighted in yellow. All nucleotide sequences were obtained from GenBank. Sequences shown are representative of one of multiple identical copies of each tRNA^{glu} gene from each species. Abbreviations are: *Bs*, *B. subtilis* str. 168; *Mc*, *M. caseolyticus* JCSC5402; *Ec*, *E. coli* MG1655.

```
>Bs|trnSL_glu2     GGCCCGTTGG TCAAGCGGTT AAG-ACACCG CCCTTTCACG...
>Mc|MCCL_tRNA01    GGCCCCTTGG TCAAGCGGTT AAG-ACACCG CCCTTTCACG...
>Ec|gltT           GTCCCCTTCG TCTAGAGGCC CAGGACACCG CCCTTTCACG...

>Bs|trnSL_glu2    ...GCGGTAACAC GGGTTCGAAT CCCGTACGGG TCA(cca)
>Mc|MCCL_tRNA01   ...GCGGTAACAC GGGTTCGAAT CCCGTAGGGG TCA(cca)
>Ec|gltT          ...GCGGTAACAG GGGTTCGAAT CCCCTAGGGG ACG(cca)
```