# Fine tuning the regulation of Cas9 expression levels for efficient CRISPR-Cas9 mediated recombination in *Streptomyces*

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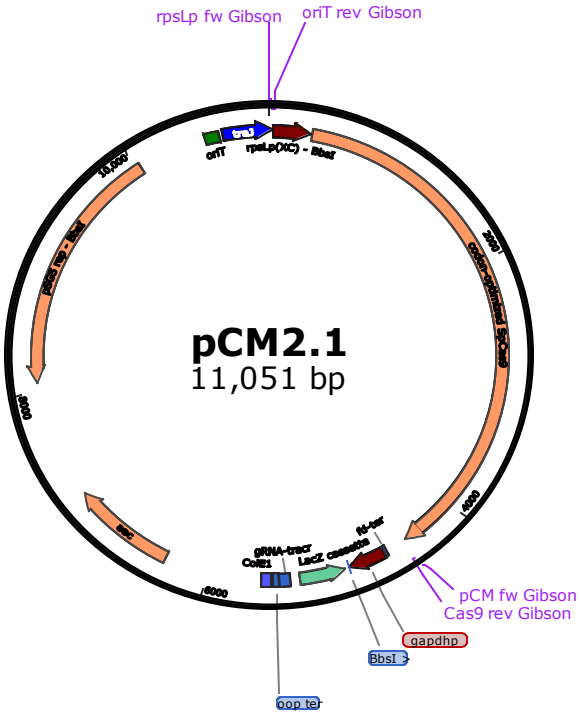
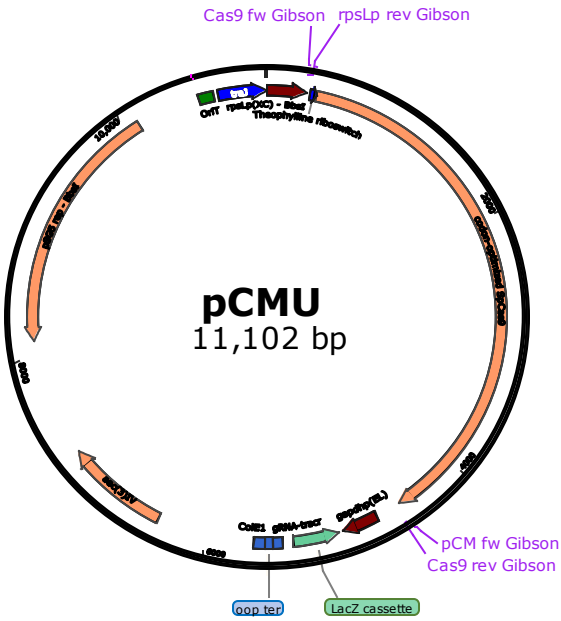
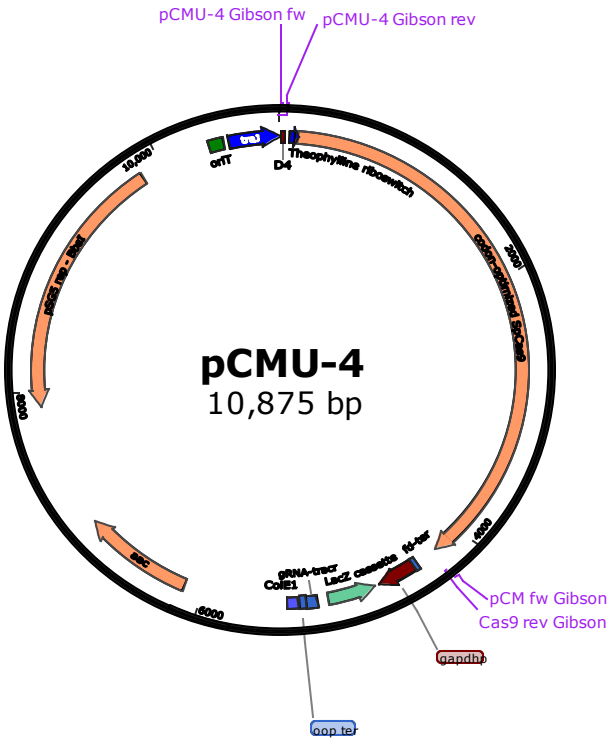
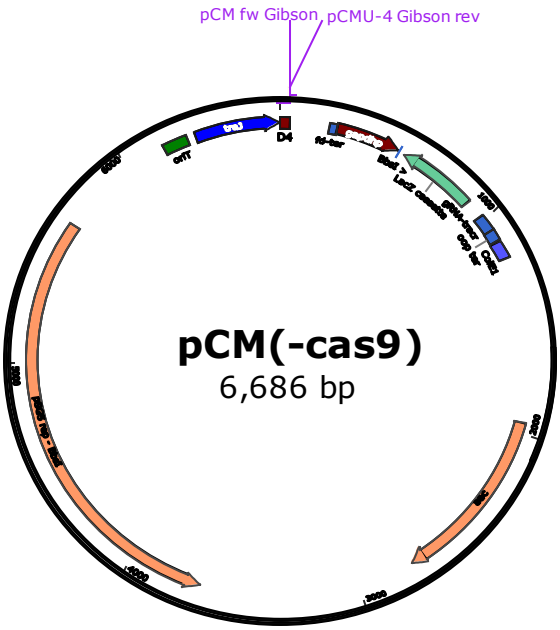
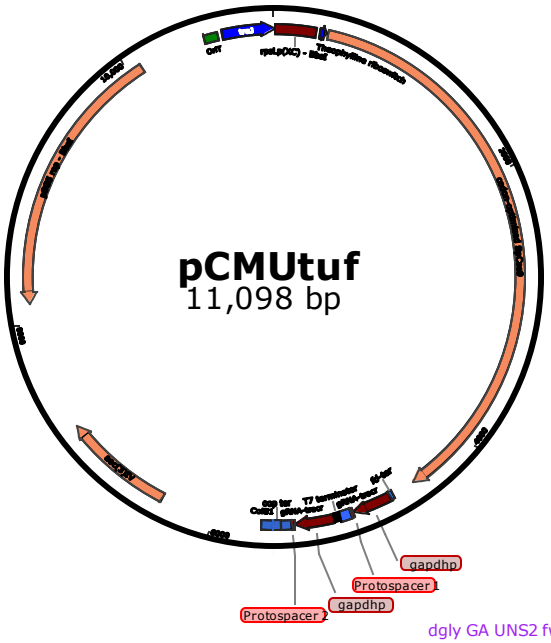
**Figure S1**. Maps of plasmids used in this study. Annealing sites for primers used for their assembly are indicated.

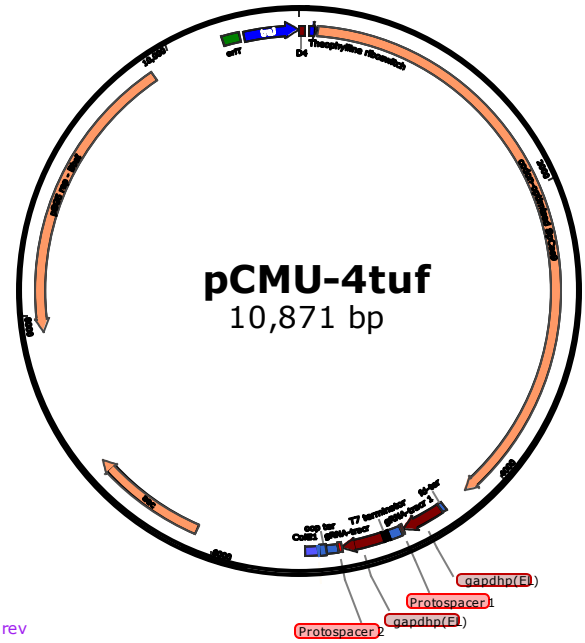


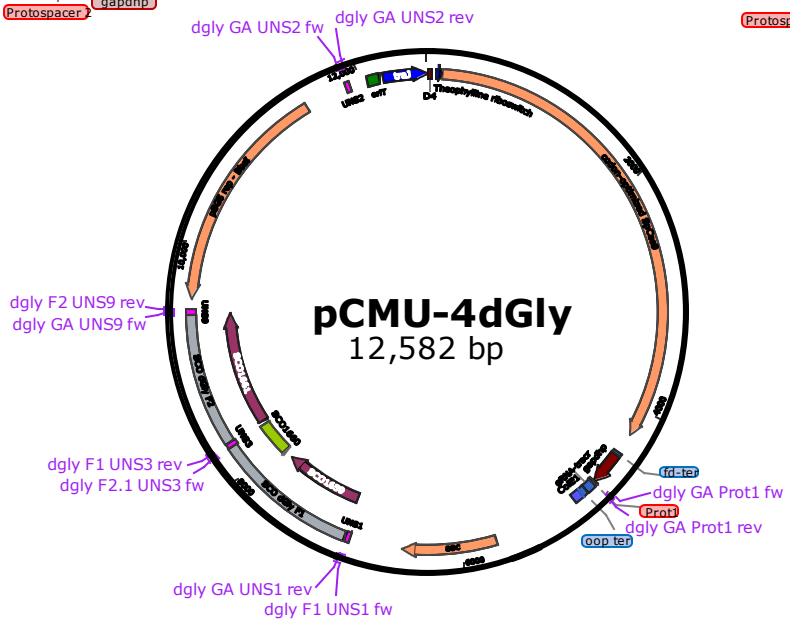



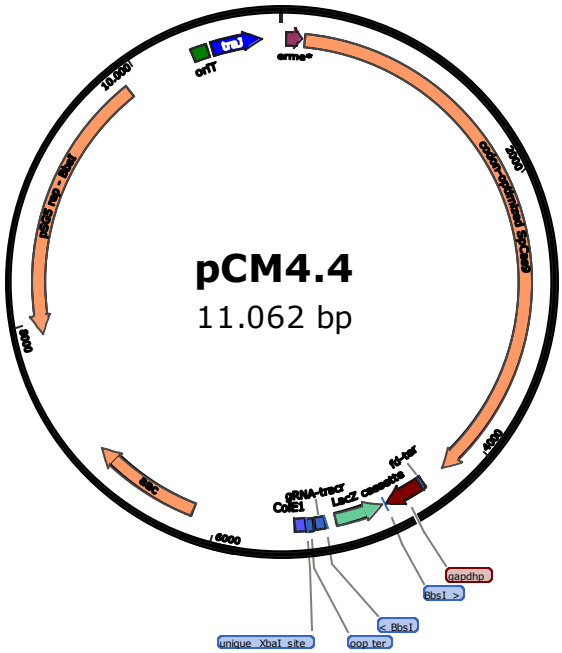




pCMUtuf
11,098 bp
gapdhp
Protospacer 1
gapdhp
Protospacer 2


pCMU-4tuf
10,871 bp
gapdhp(E1)
Protospacer 1
gapdhp(E1)
Protospacer 2


pCMU-4dGly
12,582 bp
dgly GA UNS2 fw
dgly GA UNS2 rev
dgly F2 UNS9 rev
dgly GA UNS9 fw
dgly F1 UNS3 rev
dgly F2.1 UNS3 fw
dgly GA UNS1 rev
dgly F1 UNS1 fw
fd-ter
dgly GA Prot1 fw
Prot1
dgly GA Prot1 rev
oop ter


pCM4.4
11.062 bp
gRNA-tracr
LacZ cassette
fd-ter
gapdhp
BbsI >
< BbsI
unique_XbaI site
oop ter

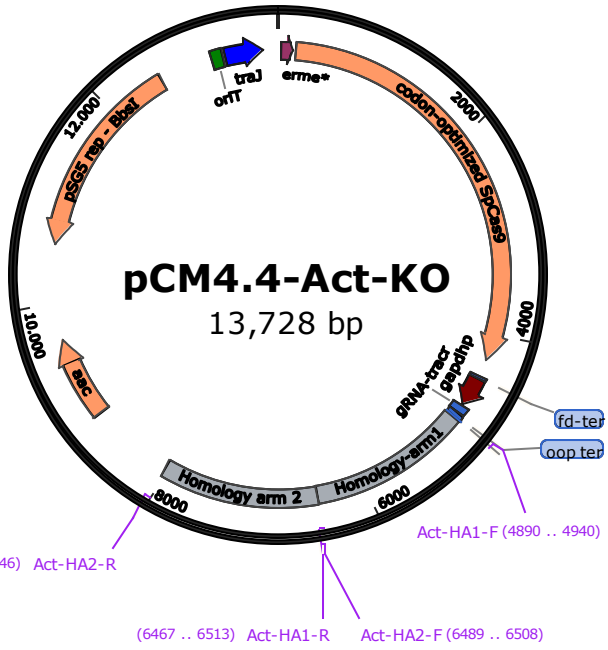

pCM4.4-Act-KO
13,728 bp
gRNA-tracr
gapdhp
fd-ter
oop ter
Homology arm 2
Homology-arm1
Act-HA1-F (4890 .. 4940)
(7998 .. 8046) Act-HA2-R
(6467 .. 6513) Act-HA1-R
Act-HA2-F (6489 .. 6508)

**Figure S2**. Representation of glycerol uptake operon in wild type (WT) and mutant strains, showing annealing sites for primers used to amplify homologous arms for pCMU-4dGly assembly as well as the cutting site for the designed protospacer. Primers used for mutants DNA verification (green and blue) and expected size for the corresponding PCR fragments are shown.

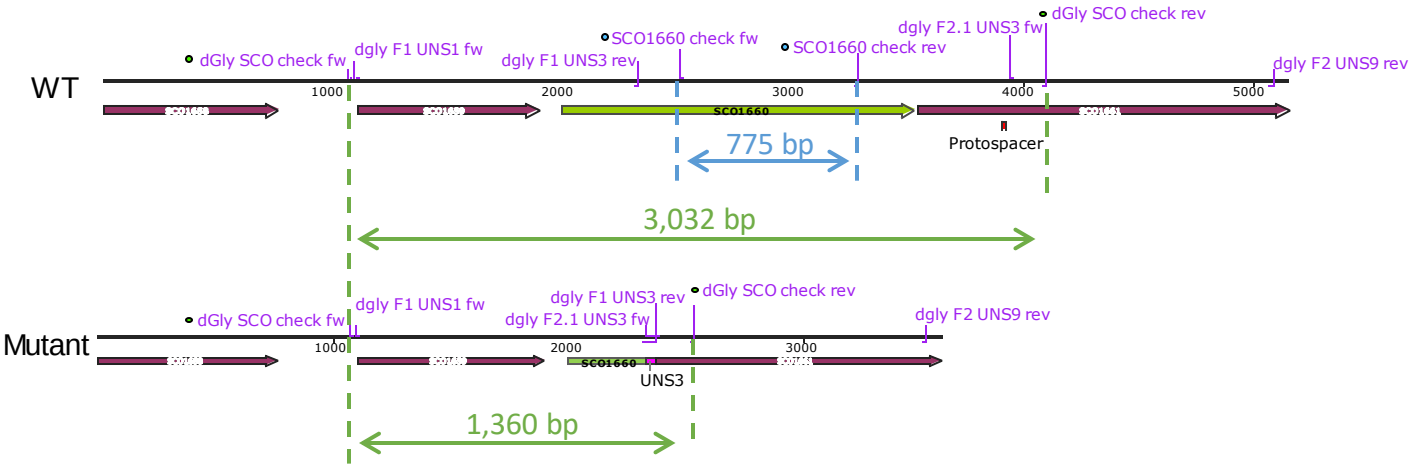


**Figure S3**. Plates incubated for 6 days against 0, 0.5, 1 and 2 mM theophylline from conjugations with pCM(-cas9) (control of conjugation, lacks *cas9*), pCMU (theophylline riboswitch between *rpsL*p and *cas9*) or pCMUtuf (control of Cas9 activity, pCMU containing 2 protospacers targeting *tuf1* gene but no homology repair template), of A) *S. coelicolor* and B) *S. lividans.* Main features of each plasmid are depicted next to each set of plates for better understanding.

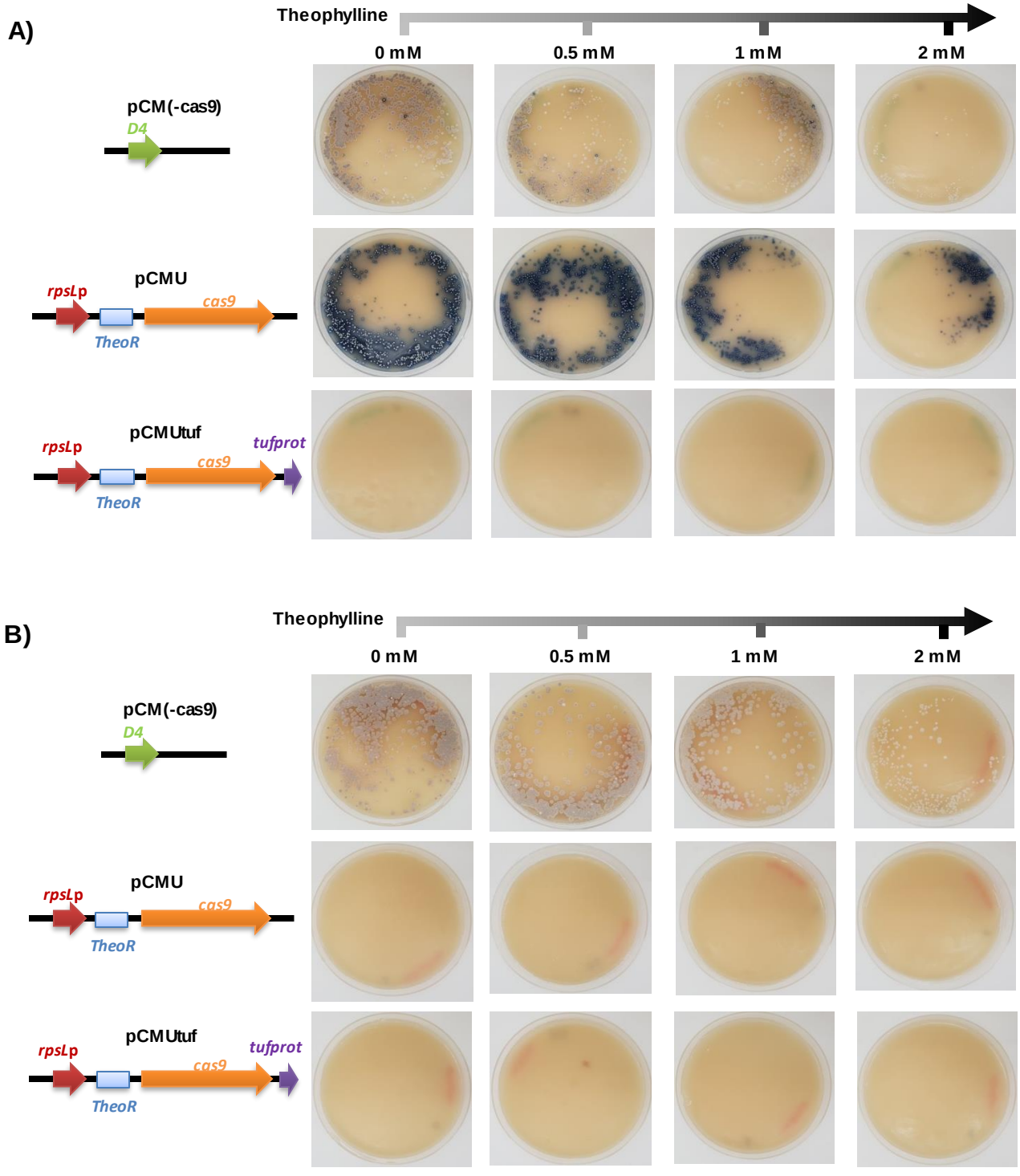


**Figure S4**. Plates incubated for 6 days against 0, 0.5, 1 and 2 mM theophylline from conjugations with pCM(-cas9) (control of conjugation, lacks *cas9*), pCMU-4 (theophylline riboswitch between *D4* promoter and *cas9*) or pCMUtuf (control of Cas9 activity, pCMU-4 containing 2 protospacers targeting *tuf1* gene but no homology repair template), of A) *S. coelicolor* and B) *S. lividans.* Main features of each plasmid are depicted next to each set of plates for better understanding.

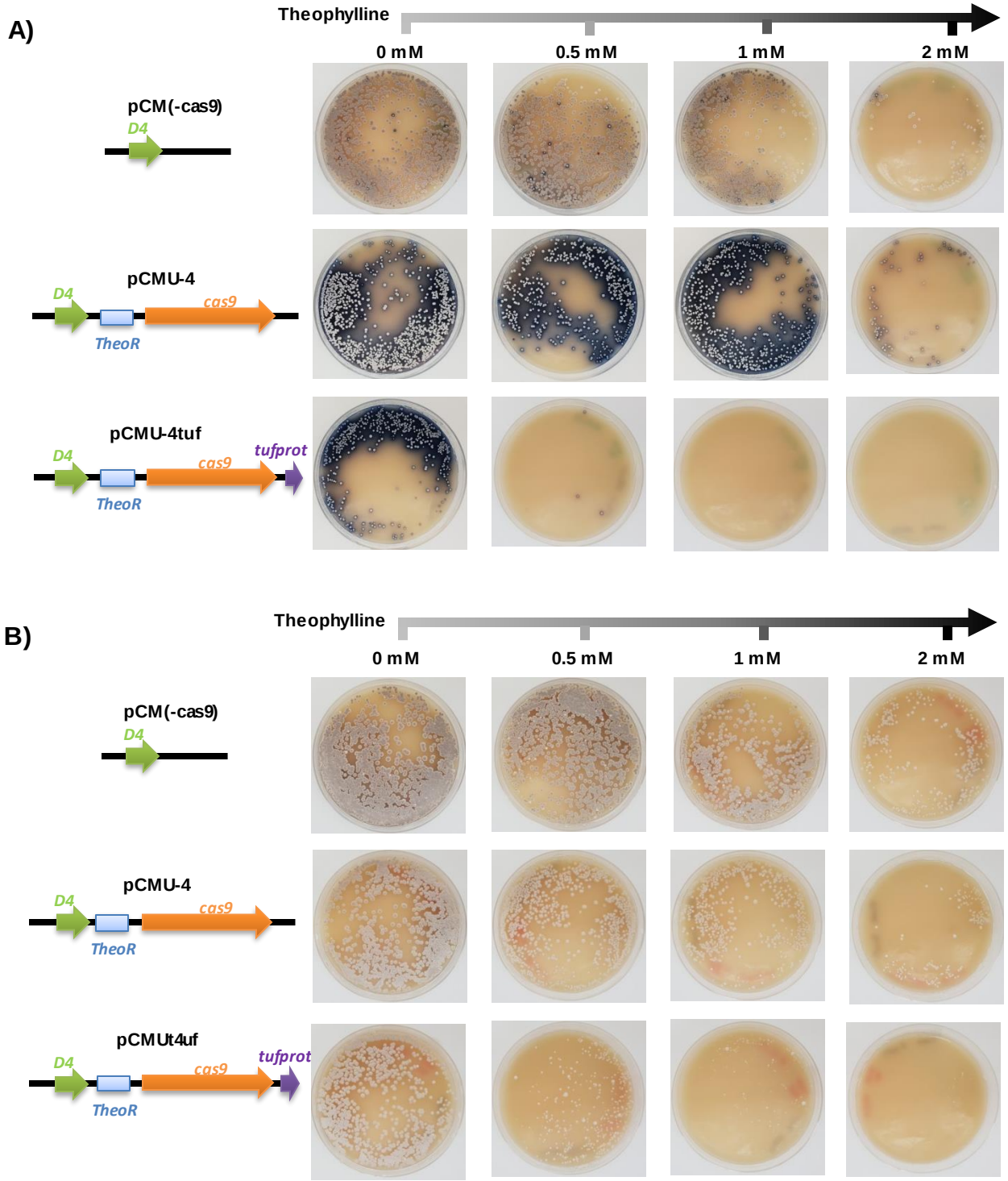


**Figure S5**. Plates incubated for 10 days against 0, 0.5, 1 and 2 mM theophylline from conjugations with pCM(-cas9) (control of conjugation, lacks *cas9*), pCMU-4 (theophylline riboswitch between *D4* promoter and *cas9*) or pCMUtuf (control of Cas9 activity, pCMU-4 containing 2 protospacers targeting *tuf1* gene but no homology repair template), of *S. lividans.* Main features of each plasmid are depicted next to each set of plates for better understanding.

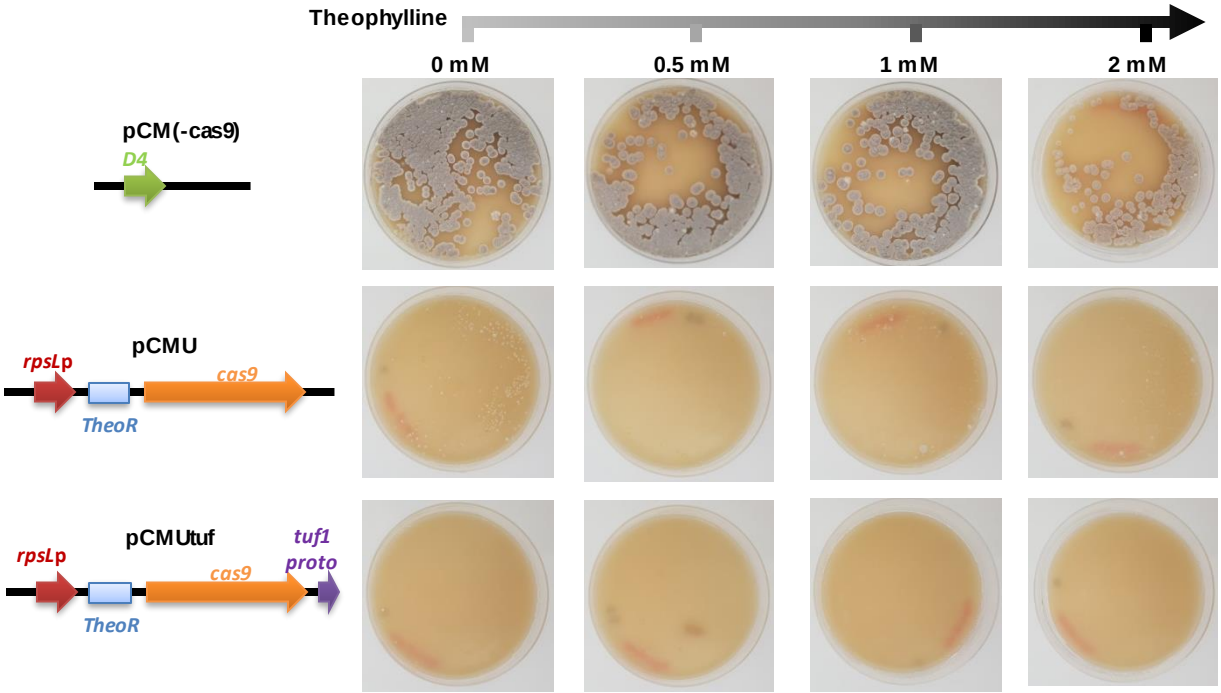


**Table S1**. Primers and synthetic DNA used in this study

| **Primers used for constructs assembly and sequencing** | |
|---|---|
| **Name** | **Sequence (5' – 3')** |
| oriT rev Gibson | ctgacttccgcctgcagggctcatggctctgccctcgg |
| pCM fw Gibson | tgagaattcagatctacgc |
| rpsLp fw Gibson | gcccgagggcagagccatgagccctgcaggcggaagtc |
| Cas9 rev Gibson | cagggagtcaaaggccgc |
| rpsLp rev Gibson | aacctatagtgagtcgtatttacgtctccgtcgtctactcg |
| Cas9 fw Gibson | gagtagacgacggagacgtaaatacgactcactataggttccggtgatacc |
| pCMU-4 Gibson fw | cccgtcggctggaaacacttgatccaacttaggatacttccggtaccaatacgactcac |
| pCMU-4 Gibson rev | ggaagtatcctaagttggatcaagtgtttccagccgacgggtcatggctctgccctcgg |
| OriT-F | ttgggcagtgcagaaagtgcagaaacctaggtgcaggtcgacggatcttttcc |
| OriT-R | ccaaaaggagcctttaattgctagctgcaggtccccgggga |
| dgly GA Prot1 fw | gtatctgaaaggggatacgcgtccgcccttgtacacgggggttttagagctagaaatagc |
| dgly GA UNS1 rev | gagacgagacgagacagcctgagaatggatgcgagtaatgtcttcttccaaagttgagaa |
| dgly F1 UNS1 fw | cattactcgcatccattctcaggctgtctcgtctcgtctcgagtcgcgggtgtccagct |
| dgly F1 UNS3 rev | cgaccttgatgtttccagtgcgattgaggaccttcagtgctgcgggtgtcctgccagac |
| dgly F2.1 UNS3 fw | gcactgaaggtcctcaatcgcactggaaacatcaaggtcgaagctcggggccggcgtctt |
| dgly F2 UNS9 rev | cagtgctcttgtgggtccgattcgccagatgataaggaacgtacggcggcgcagcacgtc |
| dgly GA UNS9 fw | gttccttatcatctggcgaatcggacccacaagagcactgggcagaccctcggctttc |
| dgly GA UNS2 rev | gcttggattctgcgtttgtttccgtctacgaactcccagcctaggtttctgcactttctgcac |
| dgly GA UNS2 fw | gctgggagttcgtagacggaaacaaacgcagaatccaagcgtgcaggtcgacggatctt |
| dgly GA Prot1 rev | ccccgtgtacaagggcggacgcgtatccccttcagatac |
| Fragment seq rev | gcgtcgatttttgtgatgct |
| Act-gRNA-F | acgccgaaagcaatatcgcgcacc |
| Act-gRNA-R | aaacggtgcgcgatattgctttcg |
| Act-HA1-F | tcggttgccgccgggcgttttttatctagaatgatcctcacggtcctggtg |
| Act-HA1-R | ctgcggcgggctggggaactactgatcgagattctccgtctcctggg |
| Act-HA2-F | tcagtagttccccagcccgc |
| Act-HA2-R | gcggccttttacggttcctggcctctagattgccgacggagtccaggc |
| **Primers used for DNA verification of mutant strains** | |
| **Name** | **Sequence (5' – 3')** |
| dGly SCO check fw | ttcggctttccctggacg |
| dGly SCO check rev | cgtcgttcatctggtcgtcg |
| SCO1660 check fw | cctgggtcatctggaacct |
| SCO1660 check rev | atcagcaggttgttggagg |
| Act-edit-F | aggaatgccagattctattgattcgg |
| Act-edit-R | acatctgggaggtgtcgacc |
| Act-WT-F | ttggaatgcagctccgtatcgac |
| Act-WT-R | tcgtcgatcagggcgaagtag |
| **Synthetic DNA** | |
| **Name** | **Sequence (5' – 3')** |
| tuf1prot | gagacatctttgaagacaaacgcgtcgtgcagcaccttggtaagttttagagctagaaatagcaagttaaaataaggctagtccg<br>ttatcaacttgaaaaagtggcaccgagtcggtgctttttttagcataaccccttggggcctctaaacgggtcttgaggggttttttggc<br>tgctccttcggtcggacgtgcgtctacgggcaccttaccgcagccgtcggctgtgcgacacggacggatcgggcgaactggccga<br>tgctgggagaagcgcgctgctgtacggcgcgcaccgggtgcggagcccctcggcgagcggtgtgaaacttctgtgaatggcctgt<br>tcggttgctttttttatacggctgccagataaggcttgcagcatctgggcggctaccgctatgatcggggcgttcctgcaattcttag<br>tgcgagtatctgaaaggggatacgcggtccggccaccctcgcggagtttaagtcttctttcacgtggc |
| ter-ermE*p-RBS | atgagcacgtccgcgagctggcccgctagcaattaaaggctccttttggagccttttttttacgcggtcgatcttgacggctggcgag<br>aggtgcggggaggatctgaccgacgcggtccacacgtggcaccgcgatgctgttgtgggcacaatcgtgccggttggtaggatcc<br>agcggagcaacggaggtacggacatggacaagaagtacagcatcggcctggacatcggcaccaacagcgtgggctgggcggt<br>catcaccgacgagtacaaggtcccctccaagaagttcaaggtcctgggcaacaccgaccggcactcgatcaagaagaacctgat<br>cggcgccctgctcttcgacagcggcgaaaccgccgaggcgacccgcctgaagcggaccgcccgtcgccgctacacccggcgcaa<br>gaaccgcatctgctacctgcaggagatcttctccaacgagatggcc |