Programmable repression and activation of bacterial gene expression using an RNA-guided DNA binding protein

Supplementary Materials

Supplementary figures.

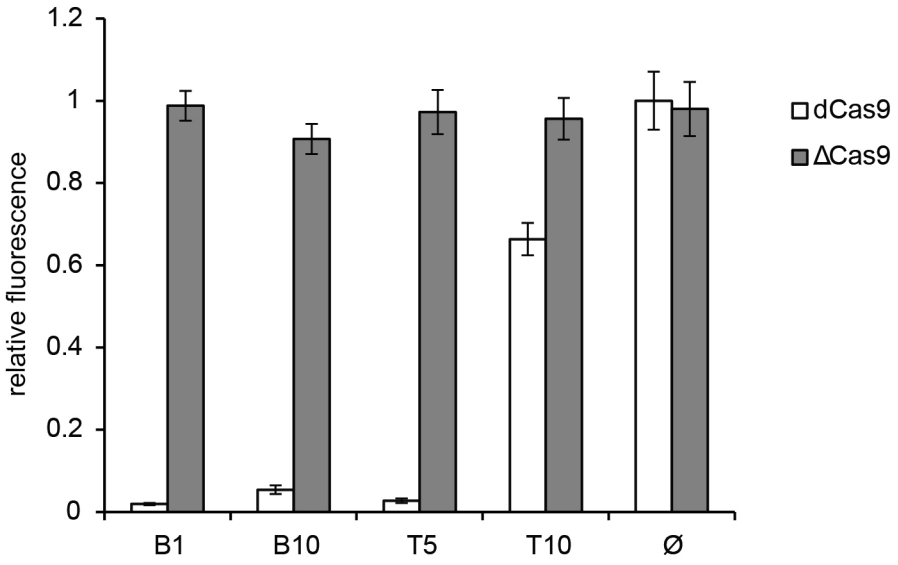

Figure S1. Repression in the absence of dCas9. To determine whether the expression of antisense crRNAs can exert *gfp-mut2* repression in the absence of dCas9 we measured the fluorescence of strains containing a plasmid expressing the crRNA but lacking the d*cas9* gene. Fluorescence of the different strains is reported relative to the fluorescence of a control strain lacking a targeting crRNA (Ø). The average of three independent experiments is indicated; error bars indicate one standard deviation.

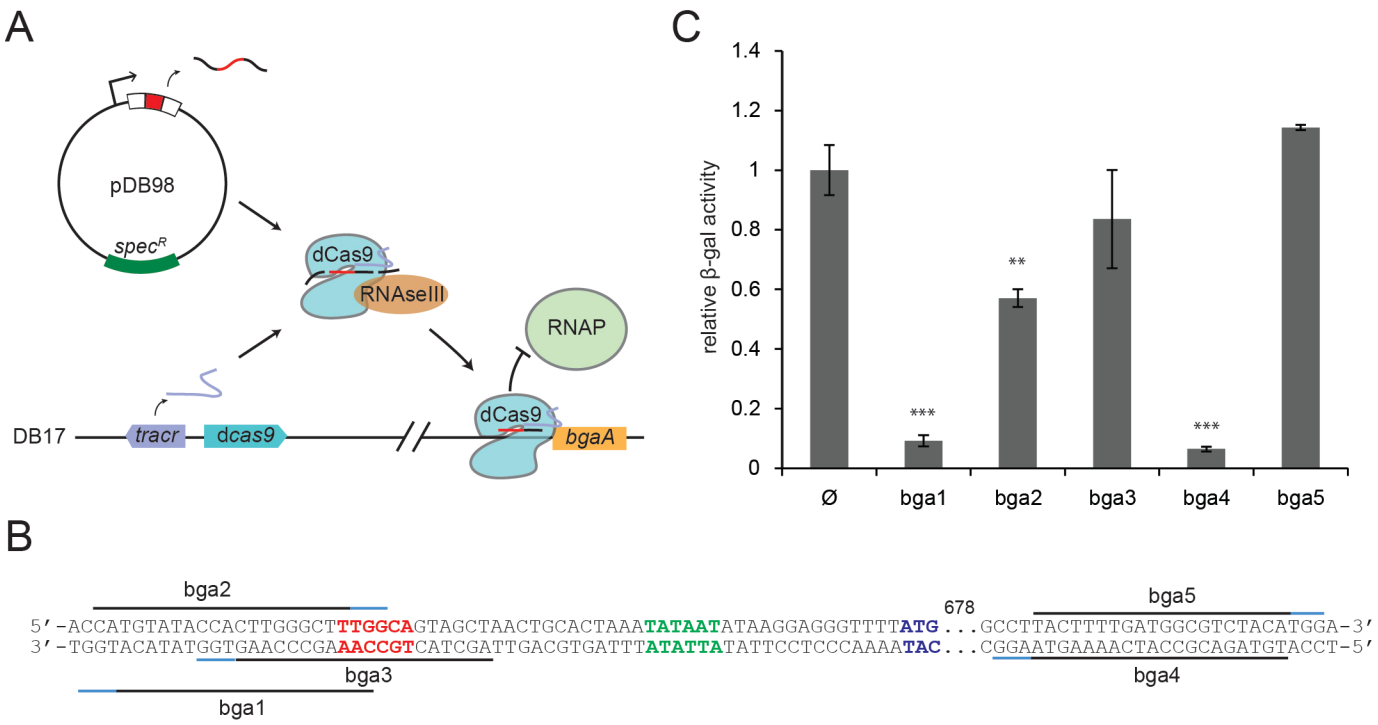

Figure S2. CrRNA-guided dCas9 repression in *S. pneumoniae*. (**A**) Strain DB17 contains chromosomal copies of the d*cas9* and tracrRNA genes. To direct dCas9 to the β-galactosidase gene (*bgaA*) promoter, a plasmid providing the different crRNA guides (pDB98) was introduced in this strain. (**B**) dCas9 targets in the *bgaA* promoter (bga1-5); underlined in light blue are the different NGG PAMs. Putative -35, -10 as well as the *bgaA* start codon are in red, green and blue letterhead, respectively. (**C**) β-galactosidase activity measured in Miller units for cells expressing the different crRNA guides and normalized against the value for cells expressing a non-targeting crRNA (Ø). The average of three independent experiments is indicated; error bars indicate one standard deviation. Asterisks indicate the *p*-values associated with each measurement, compared to the no crRNA guide control (Ø). $^{**}p \leq 0.005$; $^{***}p \leq 0.001$.

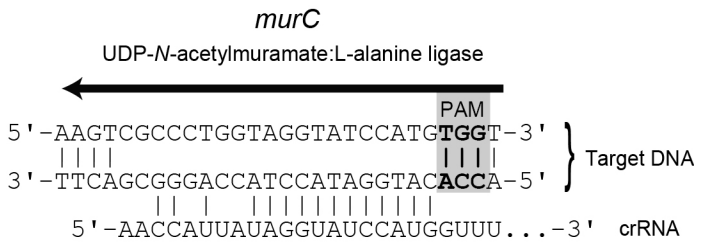

Figure S3. In the course of this study we were unable to clone a spacer with a perfect match to the *gfp-mut2* promoter region into pdCas9. BLAST search revealed a possible secondary target in the *murC* gene, encoding UDP-*N*-acetylmuramate:L-alanine ligase, an essential gene involved in peptidoglycan systhesis. We speculate that the transcriptional block generated produces the death of the transformants.

Supplementary sequences.

-35, -10 and start codon sequences are highlighted in red, green and blue, respectively.

> Promoter region of *gfp-mut2* on pDB127

AAGCAGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAA
CAGCTACGGAACTCTTGTGCGTAAGGAAAAGTAAGGAAAACGATTCCTTCTAACAGAAATGTCCTGAGCAATCACCT
ATGAACTGTCGACTCGAGGCC**TTGACA**GGTACCTCATGGATACC**TATAAT**GGTTCCGGAATTCCTTTAAAGAGGAGA
AATCTAG**ATG**

> Promoter region of *gfp-mut2* on pWJ89

AAGCAGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAA
CAGCTACGGAACTCTTGTGCGTAAGGAAAAGTAAGGAAAACGATTCCTTCTAACAGAAATGTCCTGAGCAATCACCT
ATGAACTGTCGACTCGAGCCTCTATGGATTATCACCTTGGCTGCAGGCCGGATCTTCCACAACACGCACGGTGTTAC
ATTAGGCATACCGGTC**TTGACA**GCTAGCTCAGTCCTAGG**GATTGT**GCTAGCGAATTCCTTTAAAGAGGAGAAATCTA
G**ATG**

> Promoter region of *gfp-mut2* on pWJ96

AAGCAGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAA
CAGCTACGGAACTCTTGTGCGTAAGGAAAAGTAAGGAAAACGATTCCTTCTAACAGAAATGTCCTGAGCAATCACCT
ATGAACTGTCGACTCGAGCCTCTATGGATTATCACCTTGGCTGCAGGCCGGATCTTCCACAACACGCACGGTGTTAC
ATTAGGCATACCGGTC**TTGACA**GCTAGCTCAGTCCTAGG**GACTAT**GCTAGCGAATTCCTTTAAAGAGGAGAAATCTA
G**ATG**

> Promoter region of *gfp-mut2* on pWJ97

AAGCAGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAA
CAGCTACGGAACTCTTGTGCGTAAGGAAAAGTAAGGAAAACGATTCCTTCTAACAGAAATGTCCTGAGCAATCACCT
ATGAACTGTCGACTCGAGCCTCTATGGATTATCACCTTGGCTGCAGGCCGGATCTTCCACAACACGCACGGTGTTAC
ATTAGGCATACCGGTC**TTTACG**GCTAGCTCAGTCCTAGG**TACAAT**GCTAGCGAATTCCTTTAAAGAGGAGAAATCTA
G**ATG**

> *gfp-mut2*

ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAA
ATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAA
AACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGCGTATGGTCTTCAATGCTTTGCGAGATACCCAGATCAT
ATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGA
CGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTG
ATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCA
GACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGA
CCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTG
CCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGATCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACAT
GGCATGGATGAACTATACAAATAA

Supplementary Tables.

Table S1. Oligonucleotides

Number	Sequence (5'-3')	Purpose
B337	GACGCTATTTGTGCCGATAGCTAAGCCTATTGAGTATTTC	Cas9D10A-R
B338	GAAATACTCAATAGGCTTAGCTATCGGCACAAATAGCGTC	Cas9D10A-F
B339	GGAAACTTTGTGGAACAATGGCATCGACATCATAATCACT	Cas9H840A-R
B340	AGTGATTATGATGTCGATGCCATTGTTCCACAAAGTTTCC	Cas9H840A-F
B368	TTTGGATCCTTATTTGTATAGTTCATCCATGCC	BamHI-GFPmut2-R
B369	tcgaggccTTGACAggtacctcatggataccTATAATggttccgg	PAM-rich promoter-F
B370	aattccggaaccATTATAggtatccatgaggtaccTGTCAAggcc	PAM-rich promoter-R
B371	ACAGAATTCCTTTAAAGAGGAGAAATCTAGATGAGTAAAGGAGAAGAACTTTTC	EcoRI-RBS-XbaIGFPmut2-F
B441	TCCTGAACAGTTACGCGTGCAGCTGCGTCACCTCCTAGCTGACTCAAATC	pdCas9 (C-terminal)
B442	TGAGTCAGCTAGGAGGTGACGCAGCTGCACGCGTAACTGTTCAGGACGCTG	rpoZ (C-terminal)
B446	CTATTGCTGAAGGTCGTCGTGCTGCAGATAAGAAATACTCAATAGGCTTAG	pdCas9 (N-terminal)
B448	CCTATTGAGTATTTCTTATCTGCAGCACGACGACCTTCAGCAATAGCGG	rpoZ (N-terminal)
B507	TCGGCGCTACGGCGTTTCACTTCTGAGTTCGG	5s rRNA probe
L402	TTTCCCTTGAACTAGTCGAAGG	pCEP-BM105-F
L403	AGTCATCCCAGCAACAAATGG	pCEP-BM112-R
P510	AAACTTGACTTCAGCACGTGTCTTGTAGTTCC	3'probe GFP
P511	CAACAAGAATTGGGACAACTCCAGTGAAAAGTTC	5'probe GFP
H001	GGGCACTTTTTCACTCATTTTAGCTTCCTTAGCTCCTGAAAATC	(spec)-pCas9-F
H002	GGTGCCAGCCAATGATTTTTTTAAGGCAGTTATTGG	(spec)-pCas9-R
H003	GCTAAGGAAGCTAAAATGAGTGAAAAAGTGCCCGCC	(pCas9)-spec -F
H004	ACTGCCTTAAAAAAATCATTGGCTGGCACCAAGCAG	(pCas9)-spec-R
W573	TCATGCCCAGTCATTTCTTCACCTGTGGAGCTTTTTAAGTcctgttgataccgggaagcc	rpoZ-spec-F
W574	TCAGGCTTTCAAACAGATACAAGGGCGACCCGCTTTGTGAtctaggcaccaataactgcc	rpoZ-spec-R
W551	TTGCTGAAGGTCGTCGTTAATACTCTTAATAAATGCAGTAATACAGGG	pdCas9 (C-terminal)
W552	CAGGTTCAGTCTGCCAACAATTTGTTCAATAATAGTTTTAATGACCTCCG	pdCas9 (N-terminal)
W550	CTGTATTACTGCATTTATTAAGAGTATTAACGACGACCTTCAGCAATAG	rpoZ (C-terminal)
W553	CGGAGGTCATTAAAACTATTATTGAACAAATTGTTGGCAGACTGAACCTG	rpoZ (N-terminal)

Table S2. Plasmids

Name	Genotype	Reference
pLZ12spec	pLZ12, specR	(33)
pDB98	pLZ21spec, repeat-BsaI spacer-repeat	(23)
pCas9	pACYC184(CmR) with tracrRNA, *cas9*, repeat-BsaI spacer-repeat	(23)
pdCas9	pACYC184(CmR) with tracrRNA, *cas9*(D10A, H840A), repeat-BsaI spacer-repeat	this study
pWJ66	pACYC184(CmR) with tracrRNA, *cas9*(D10A, H840A)-ω, repeat-BsaI spacer-repeat	this study
pWJ68	pACYC184(CmR) with tracrRNA, *ω-cas9*(D10A, H840A), repeat-BsaI spacer-repeat	this study
pDB191	pACYC184(SpecR) with tracrRNA, *cas9*(D10A, H840A)-ω, repeat-BsaI spacer-repeat	this study
pDB192	pACYC184(SpecR) with tracrRNA, *ω-cas9*(D10A, H840A), repeat-BsaI spacer-repeat	this study
pZS*24-MCS1	pSC101*, KanR	(36)
pDB127	pZS*24-MCS1, PAM-rich promoter, *gfp-mut2*	this study
pWJ89	pZS*24-MCS1, PAM-rich 5' UTR region, J23117, *gfp-mut2*	this study
pWJ96	pZS*24-MCS1, PAM-rich 5' UTR region, J23116, *gfp-mut2*	this study
pWJ97	pZS*24-MCS1, PAM-rich 5' UTR region, J23110, *gfp-mut2*	this study

Table S3. Spacer sequences

Name	sequence (5'-3')	Target	Figure
T0	CTACGGAACTCTTGTGCGTAAGGAAAAGTA	pDB127	1
T1	CTGAGCAATCACCTATGAACTGTCGACTCG	pDB127	1
T3	TGTCGACTCGAGGCCTTGACAGGTACCTCA	pDB127	1
T4	CCTTGACAGGTACCTCATGGATACCTATAA	pDB127	1
T5	CAGGTACCTCATGGATACCTATAATGGTTC	pDB127	1
T6	ACCTATAATGGTTCCGGAATTCCTTTAAAG	pDB127	1
T7	CCTTTAAAGAGGAGAAATCTAGATGAGTAA	pDB127	1
T8	CTAGATGAGTAAAGGAGAAGAACTTTTCAC	pDB127	1
T9	TGGAGTTGTCCCAATTCTTGTTGAATTAGA	pDB127	1
T10	TCAAGAGTGCCATGCCCGAAGGTTATGTAC	pDB127	1
T11	GATACCCTTGTTAATAGAATCGAGTTAAAA	pDB127	1
B0	TCTTCAGAAATGAGCTTTTGCTCCTCTGCT	pDB127	1
B1	CCTTTACTCATCTAGATTTCTCCTCTTTAA	pDB127	1
B2	TAGGTGATTGCTCAGGACATTTCTGTTAGA	pDB127	1
B3	CTCGAGTCGACAGTTCATAGGTGATTGCTC	pDB127	1
B4	ACCTGTCAAGGCCTCGAGTCGACAGTTCAT	pDB127	1
B5	CCATTATAGGTATCCATGAGGTACCTGTCA	pDB127	1
B7	TCCTCTTTAAAGGAATTCCGGAACCATTAT	pDB127	1
B8	CATCTAGATTTCTCCTCTTTAAAGGAATTC	pDB127	1
B9	ATTAACATCACCATCTAATTCAACAAGAAT	pDB127	1
B10	CTTGAAAAAGTCATGCTGTTTCATATGATC	pDB127	1
B11	CAATACCTTTTAACTCGATTCTATTAACAA	pDB127	1
B1m2	CCTTTACTCAAGTAGATTTCTCCTCTTTAA	pDB127	2
B1m4	CCTTTACTCAAGATGATTTCTCCTCTTTAA	pDB127	2
B1m6	CCTTTACTCAAGATCTTTTCTCCTCTTTAA	pDB127	2
B1m8	CCTTTACTCAAGATCTAATCTCCTCTTTAA	pDB127	2
B1m10	CCTTTACTCAAGATCTAAAGTCCTCTTTAA	pDB127	2
B1m12	CCTTTACTCAAGATCTAAAGAGCTCTTTAA	pDB127	2
B1m14	CCTTTACTCAAGATCTAAAGAGGACTTTAA	pDB127	2
T5m2	CAGGTACCTCTAGGATACCTATAATGGTTC	pDB127	2
T5m4	CAGGTACCTCTACCATACCTATAATGGTTC	pDB127	2
T5m6	CAGGTACCTCTACCTAACCTATAATGGTTC	pDB127	2
T5m8	CAGGTACCTCTACCTATGCTATAATGGTTC	pDB127	2
T5m10	CAGGTACCTCTACCTATGGAATAATGGTTC	pDB127	2
T5m12	CAGGTACCTCTACCTATGGATAAATGGTTC	pDB127	2
T5m14	CAGGTACCTCTACCTATGGATATTTGGTTC	pDB127	2
B10m2	CTTGAAAAAGAGATGCTGTTTCATATGATC	pDB127	2
B10m4	CTTGAAAAAGAGTAGCTGTTTCATATGATC	pDB127	2
B10m6	CTTGAAAAAGAGTACGTGTTTCATATGATC	pDB127	2
B10m8	CTTGAAAAAGAGTACGACTTTCATATGATC	pDB127	2

B10m10	CTTGAAAAAGAGTACGACAATCATATGATC	pDB127	2
B10m12	CTTGAAAAAGAGTACGACAAAGATATGATC	pDB127	2
B10m14	CTTGAAAAAGAGTACGACAAAGTAATGATC	pDB127	2
B10m2	CTTGAAAAAGAGATGCTGTTTCATATGATC	pDB127	2
B10m4	CTTGAAAAAGAGTAGCTGTTTCATATGATC	pDB127	2
B10m6	CTTGAAAAAGAGTACGTGTTTCATATGATC	pDB127	2
B10m8	CTTGAAAAAGAGTACGACTTTCATATGATC	pDB127	2
B10m10	CTTGAAAAAGAGTACGACAATCATATGATC	pDB127	2
B10m12	CTTGAAAAAGAGTACGACAAAGATATGATC	pDB127	2
B10m14	CTTGAAAAAGAGTACGACAAAGTAATGATC	pDB127	2
Z1	ACAACACGCACGGTGTTACATTAGGCACCC	KS1ΔZ lacZ promoter	3
Z2	GATCTTCGACAACACGCACGGTGTTACATT	KS1ΔZ lacZ promoter	3
Z3	GGCTGCAGGTCGGATCTTCGACAACACGCA	KS1ΔZ lacZ promoter	3
Z4	ATTCTGTCGAAGATCAGCTTGGCTGCAGGT	KS1ΔZ lacZ promoter	3
bga1	TAGCTACTGCCAAAGCCCAAGTGGTATACA	S. pneumoniae bgaA	S2
bga2	GTTGTCATACCATGTATACCACTTGGGCTT	S. pneumoniae bgaA	S2
bga3	TAGTGCAGTTAGCTACTGCCAAAGCCCAAG	S. pneumoniae bgaA	S2
bga4	TGAGAATCCATGTAGACGCCATCAAAAGTA	S. pneumoniae bgaA	S2
bga5	ATGTTCGCCTTACTTTTGATGGCGTCTACA	S. pneumoniae bgaA	S2
W101	GATCTTCCACAACACGCACGGTGTTACATT	pWJ89	4
W102	GGCTGCAGGCCGGATCTTCCACAACACGCA	pWJ89	4
W103	CCTCTATGGATTATCACCTTGGCTGCAGGC	pWJ89	4
W104	GTCGACTCGAGCCTCTATGGATTATCACCT	pWJ89	4
W105	TCACCTATGAACTGTCGACTCGAGCCTCTA	pWJ89	4
W106	GGTATGCCTAATGTAACACCGTGCGTGTTG	pWJ89	4
W107	AATGTAACACCGTGCGTGTTGTGGAAGATC	pWJ89	4
W108	GCGTGTTGTGGAAGATCCGGCCTGCAGCCA	pWJ89	4
W109	CCGGCCTGCAGCCAAGGTGATAATCCATAG	pWJ89	4
W110	AATCCATAGAGGCTCGAGTCGACAGTTCAT	pWJ89	4