Supplemental Material

Engineering CRISPR Interference System to Repress Class 1 Integron in *E. coli*

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Legends

Fig. S1 Partial sequencing results of seven CRISPRi recombinant plasmids. The base-pairing regions of all CRISPRi recombinant plasmids plv-dCas9-R(0-6) were the same as designed, whereby "R(0-6)" represent different sgRNAs.

Fig. S2 Partial sequencing results of ARG cassettes integrated into R388 class 1 integron. (**A-B**) Partial sequence of integrated fragment *intI1-attI-aadA1* (A) or *intI1-attI-aadB* (B) through conjugation from the control donor *E. coli* C600(R388+plv-dCas9-R0+pINT-cassette) to the recipient *E. coli* J53 without CRISPRi induction.

Fig. S3 Plasmid loss of the CRISPRi recombinant plasmid plv-dCas9-R3 in serial passage. (**A**) Colony counts were enumerated on LB+CM plates to assess plasmid loss of every ten generations continuously passaged in liquid medium containing CM. (**B**) Colony counts were enumerated on LB+CM plates to determine plasmid loss of every ten generations in serial passage without exposure to CM. CM, chloramphenicol; G, generation.

Fig. S4 Schematic outline of construction of pINT-cassette. (**A**) Sequence of BmgBI-P_{tac} for recombinant plasmid pINT-cassette. (**B**) Schematic diagram of pINT-cassette. (**C-D**) The resulting recombinant plasmids pINT-*aadA1* (C) and pINT-*aadB* (D).

Table S1 Strains and vectors used in this study.

Table S2 Oligonucleotides and primers used in this study.

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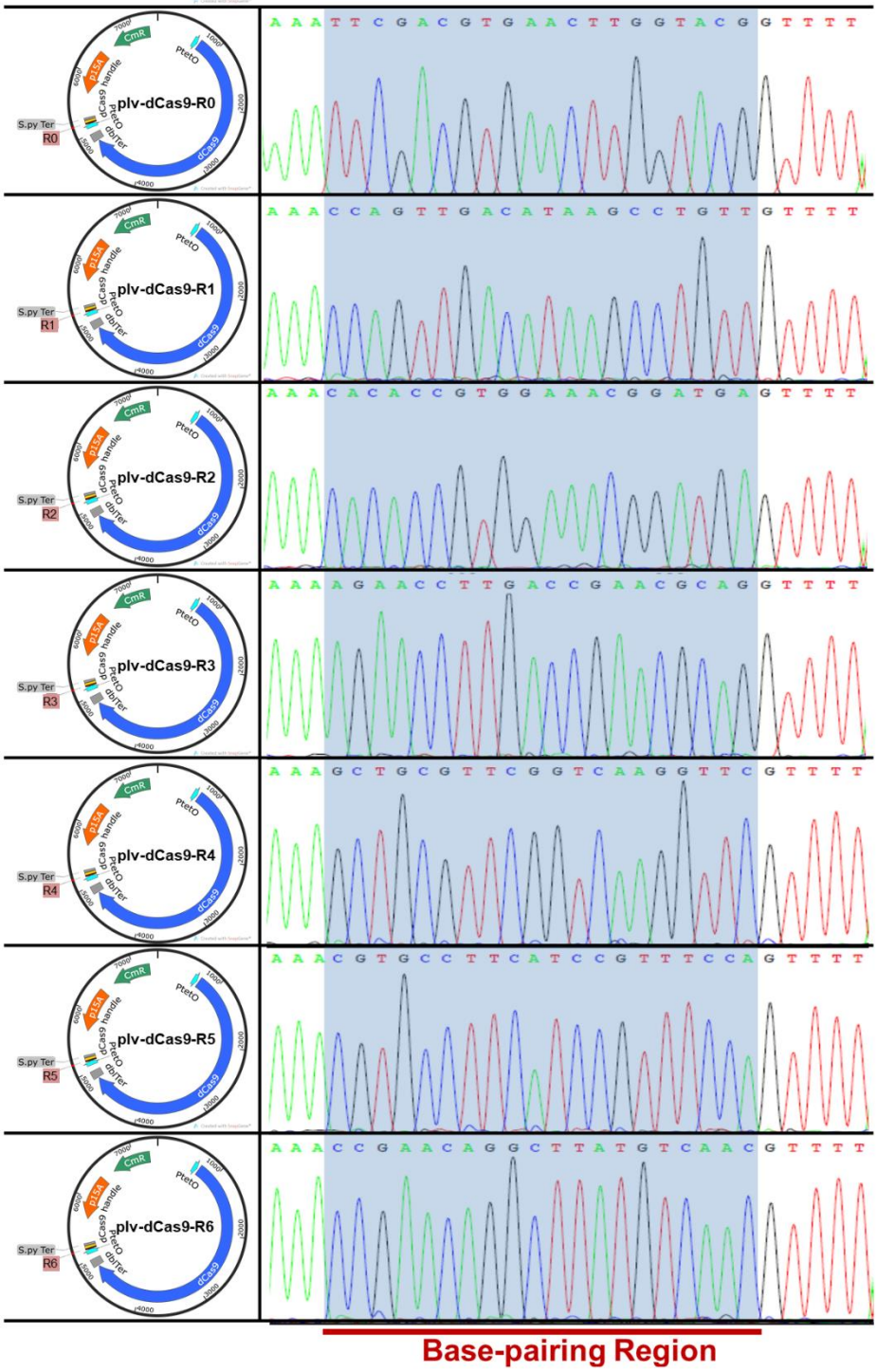

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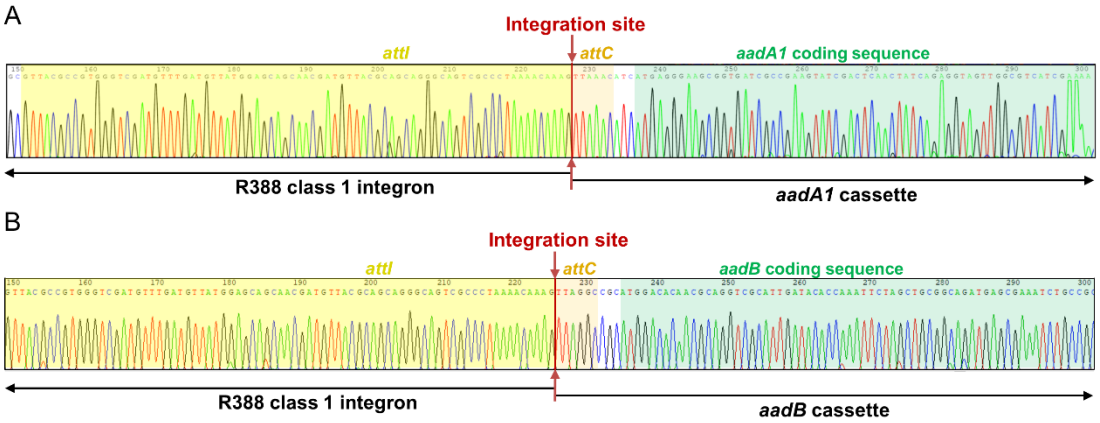

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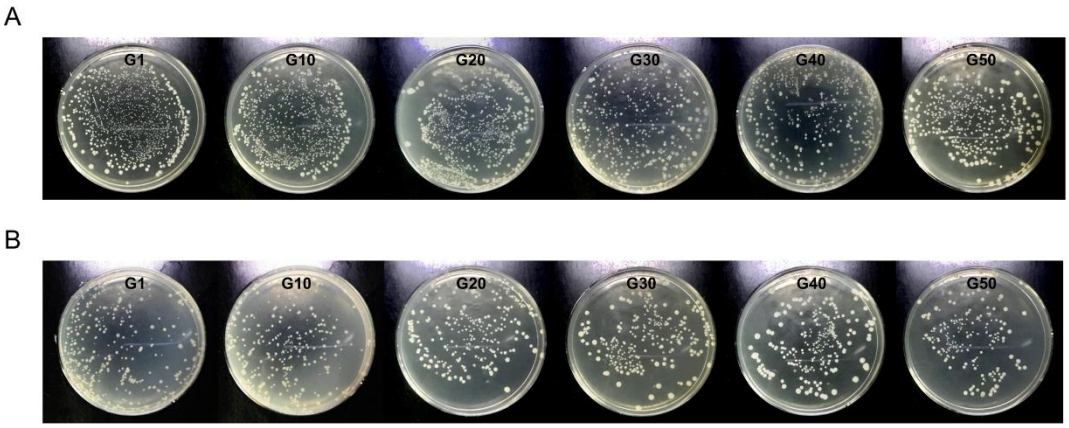

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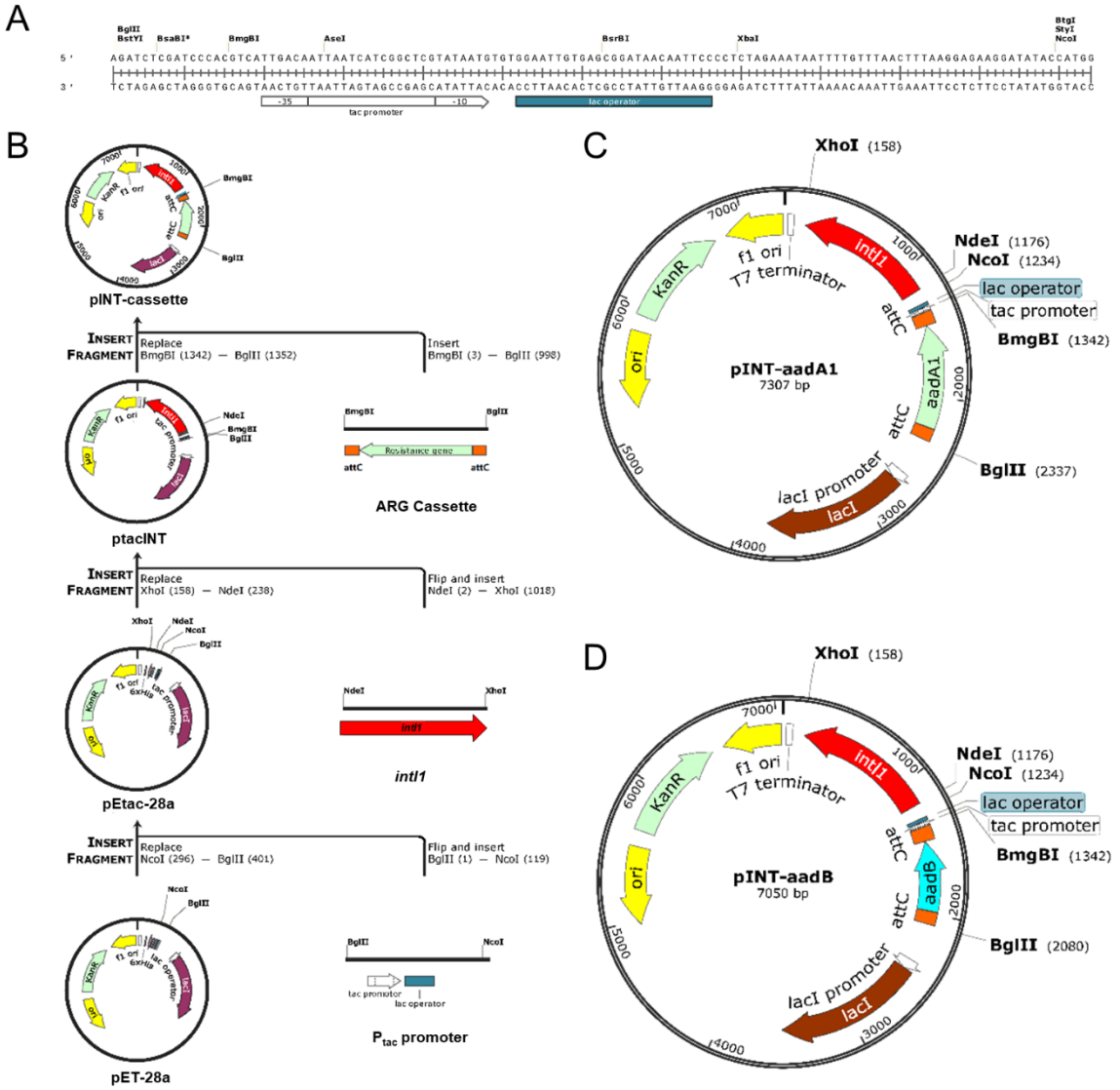

Fig S4 Schematic diagram of construction of pINT-cassette. (**A**) Sequence of BmgBI-P_{tac} for recombinant plasmid pINT-cassette. (**B**) Schematic diagram of pINT-cassette. (**C-D**) The resulting recombinant plasmids pINT-*aadA1* (C) and pINT-*aadB* (D).

Table S1 Strains and vectors used in this study.

Vectors/Strains	Description	Source
Vectors		
R388	33 kb conjugative plasmid containing a class 1 integron with *dfrB2-orfA* cassette array, TMP^R, SUL^R	(1) Stokes& Hall,1989
plv-dCas9-sgRNA	CRISPRi encoding vector, CM^R	(2) Lv et al. 2015
plv-dCas9-R0	CRISPRi recombinant plasmid, sgRNA R0, CM^R	This study
plv-dCas9-R1	CRISPRi recombinant plasmid, sgRNA R1, CM^R	This study
plv-dCas9-R2	CRISPRi recombinant plasmid, sgRNA R2, CM^R	This study
plv-dCas9-R3	CRISPRi recombinant plasmid, sgRNA R3, CM^R	This study
plv-dCas9-R4	CRISPRi recombinant plasmid, sgRNA R4, CM^R	This study
plv-dCas9-R5	CRISPRi recombinant plasmid, sgRNA R5, CM^R	This study
plv-dCas9-R6	CRISPRi recombinant plasmid, sgRNA R6, CM^R	This study
pET-28a	Cloning vector, KAN^R	Novagen
pEtac-28a	pET-28a with original T7 promoter replaced by *tac* promoter, KAN^R	This study
ptacINT	pEtac-28a with *intI1* gene overexpressing under *tac* promoter, KAN^R	This study
pINT-*aadA1*	ptacINT with *aadA1* cassette added, KAN^R	This study
pINT-*aadB*	ptacINT with *aadB* cassette added, KAN^R	This study
R388-*aadA1*	R388 with *aadA1* cassette inserted, TMP^R, SUL^R, STR^R	This study
R388-*aadB*	R388 with *aadB* cassette inserted, TMP^R, SUL^R, GEN^R	This study
Strains		

***E. coli* C600**	Wild-type strain, F^- *ton*A21 *thi*$^-$1 *thr*$^-$1 *leu*B6 *lac*Y1 *gln*V44 *rfb*C1 *fhu*A1 λ^-	Lab stock
***E. coli* Top10**	Competent cells	Biomed
***E. coli* DH5α(R388)**	*E. coli* DH5α carrying plasmid R388	Lab stock
***E. coli* ATCC 25922**	Quality control strain for determination of antibiotic resistance	ATCC
***Salmonella sp.*S084**	Wild-type strain of *aadB* cassette	Lab stock
***Salmonella sp.*S010**	Wild-type strain of *aadA1* cassette	Lab stock
***E. coli* C600(R388+plv-dCas9-R0)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R0	This study
***E. coli* C600(R388+plv-dCas9-R1)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R1	This study
***E. coli* C600(R388+plv-dCas9-R2)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R2	This study
***E. coli* C600(R388+plv-dCas9-R3)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R3	This study
***E. coli* C600(R388+plv-dCas9-R4)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R4	This study
***E. coli* C600(R388+plv-dCas9-R5)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R5	This study
***E. coli* C600(R388+plv-dCas9-R6)**	Recombinant *E. coli* C600 carrying R388 and plv-dCas9-R6	This study
***E. coli* C600(R388+plv-dCas9-R0+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R0 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R1+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R1 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R2+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R2 and pINT-*aadA1*	This study

***E. coli* C600(R388+plv-dCas9-R3+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R3 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R4+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R4 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R5+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R5 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R6+pINT-*aadA1*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R6 and pINT-*aadA1*	This study
***E. coli* C600(R388+plv-dCas9-R0+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R0 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R1+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R1 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R2+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R2 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R3+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R3 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R4+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R4 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R5+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R5 and pINT-*aadB*	This study
***E. coli* C600(R388+plv-dCas9-R6+pINT-*aadB*)**	Donor *E. coli* C600 carrying R388, plv-dCas9-R6 and pINT-*aadB*	This study
***E.coli* J53**	Wild-type recipient	Lab stock
***E. coli* J53 (R388)**	Transconjugant *E. coli* J53 carrying R388	This study
***E. coli* J53 (R388-*aadA1*)**	Transconjugant *E. coli* J53 carrying R388-*aadA1*	This study
***E. coli* J53 (R388-*aadB*)**	Transconjugant *E. coli* J53 carrying R388-*aadB*	This study

TMP, trimethoprim; SUL, sulfamethoxazole; CM, chloramphenicol; KAN, kanamycin; STR, streptomycin; GEN, gentamicin; ATCC, American type culture collection.

53 **Table S2 Oligonucleotides and primers used in this study.**

Primers	Sequences (5'-3')	Vectors
Oligonucleotides used for sgRNA synthesis		
BspQI-R0-F	aaaTTCGACGTGAACTTGGTACG	plv-dCas9-R0
R0-BspQI-R	aacCGTACCAAGTTCACGTCGAA	
BspQI-R1-F	aaaCCAGTTGACATAAGCCTGTT	plv-dCas9-R1
R1-BspQI-R	aacAACAGGCTTATGTCAACTGG	
BspQI-R2-F	aaaCACACCGTGGAAACGGATGA	plv-dCas9-R2
R2-BspQI-R	aacTCATCCGTTTCCACGGTGTG	
BspQI-R3-F	aaaAGAACCTTGACCGAACGCAG	plv-dCas9-R3
R3-BspQI-R	aacCTGCGTTCGGTCAAGGTTCT	
BspQI-R4-F	aaaGCTGCGTTCGGTCAAGGTTC	plv-dCas9-R4
R4-BspQI-R	aacGAACCTTGACCGAACGCAGC	
BspQI-R5-F	aaaCGTGCCTTCATCCGTTTCCA	plv-dCas9-R5
R5-BspQI-R	aacTGGAAACGGATGAAGGCACG	
BspQI-R6-F	aaaCCGAACAGGCTTATGTCAAC	plv-dCas9-R6
R6-BspQI-R	aacGTTGACATAAGCCTGTTCGG	
Primers used for construction of pINT-cassette		
NdeI-intI1-F	attactaacaacatatgAAAACCGCCACTGCGCC GTTACC	ptacINT
intI1-XhoI-R	tatactcgagCTACCTCTCACTAGTGAGGGG CGGC	
BglII-aadA1-F	aatagatctGGTTAACAAGTGGCAGC	pINT-*aadA1*
aadA1-BmgBI-R	acaagatctGACCTGATAGTTTGGCTG	
BglII-aadB-F	cgtagatctAAGCAGCAAGCGCGTTA	pINT-*aadB*
aadB-BmgBI-R	ataagatctGGCCGAGCGCTGGAATA	
Primers used for colony PCR		
BspQI-R0-F	aaaTTCGACGTGAACTTGGTACG	plv-dCas9-R0

sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R1-F	aaaCCAGTTGACATAAGCCTGTT	plv-dCas9-R1
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R2-F	aaaCACACCGTGGAAACGGATGA	plv-dCas9-R2
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R3-F	aaaAGAACCTTGACCGAACGCAG	plv-dCas9-R3
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R4-F	aaaGCTGCGTTCGGTCAAGGTTC	plv-dCas9-R4
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R5-F	aaaCGTGCCTTCATCCGTTTCCA	plv-dCas9-R5
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
BspQI-R6-F	aaaCCGAACAGGCTTATGTCAAC	plv-dCas9-R6
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
intI1-F	TGATGGCGACGCACGACA	R388
intI1-R	TCCTGGCTGGCGAACGAG	
BglII-aadA1-F	aatagatctGGTTAACAAGTGGCAGC	pINT-*aadA1*
intI1-CX-R	ATCCGGATATAGTTCCTCCTTTCAGCA AAAAACC	
BglII-aadB-F	cgtagatctAAGCAGCAAGCGCGTTA	pINT-*aadB*
intI1-CX-R	ATCCGGATATAGTTCCTCCTTTCAGCA AAAAACC	
Primers used for DNA sequencing		
sgRNA-CX-F	TGTTTGTCGGTGAACGCTCTCTACTAG	plv-dCas9-R(0-6)
sgRNA-CX-R	GCGGAATATATCCCTAGGCCTGCAG	
intI1-CX-F	GAAGGATATACCATGGGCAGCAGCC	ptacINT
intI1-CX-R	ATCCGGATATAGTTCCTCCTTTCAGCA AAAAACC	
Cassette-CX-F	GGCTGCTGCCCATGGTATATCCTTC	pINT-cassette

Cassette-CX-R	CACCATACCCACGCCGAAACAAGCG	
Integration-CX-F	ACGAACCCAGTTGACATAAG	
aadA1-CX-R	TCGATGACGCCAACTAC	R388-*aadA1*
Integration-CX-F	ACGAACCCAGTTGACATAAG	
aadB-CX-R	GGCAGATTTCGCTCATCTG	R388-*aadB*
Primers used for RT-qPCR		
16S rRNA-RT-F	CCTACGGGAGGCAGCAG	-
16S rRNA-RT-R	ATTACCGCGGCTGCTGG	
dCas9-RT-F	TGGTGGAAGAAGACAAGAA	plv-dCas9-R(0-6)
dCas9-RT-R	CGCAGATGATAGATAGTTGGA	
intI1-RT-F	ATCGTTTGTTCGCCCAGCTT	pINT-cassette and R388
intI1-RT-R	GTGCCGTGATCGAAATCCAG	
drfB2-RT-F	AGAAATCTGGTGCCGCTTG	R388
drfB2-RT-R	ACTGAGCCTGGGTGGGATT	
sul1-RT-F	ACGGTGTTCGGCATTCTGA	R388
sul1-RT-R	TCCGACTCGCAGCATTTCA	

Primers were designed by Primer Premier 5.0 software using sequences downloaded from NCBI (http://www.ncbi.nlm.nih.gov/) as templates. The nucleotides labeled with gray background are the restriction endonuclease sites.

Table S3 IC_{50} of TMP and SUL in recombinant *E. coli* with/without aTc induction.

Strains	aTc	IC_{50} of TMP (μg/ml)	IC_{50} of SUL (μg/ml)
E. coli ATCC25922	-	2	32
E. coli C600(R388)	-	512	4096
E. coli C600(R388+plv-dCas9-R0)	-	512	4096
	+	512	4096
E. coli C600(R388+plv-dCas9-R1)	-	512	4096
	+	64	1024
E. coli C600(R388+plv-dCas9-R2)	-	512	4096
	+	64	256
E. coli C600(R388+plv-dCas9-R3)	-	512	4096
	+	64	128
E. coli C600(R388+plv-dCas9-R4)	-	512	4096
	+	64	2048
E. coli C600(R388+plv-dCas9-R5)	-	512	4096
	+	64	2048
E. coli C600(R388+plv-dCas9-R6)	-	512	4096
	+	64	128

IC_{50}, half-maximal inhibitory concentration; TMP, trimethoprim; SUL, sulfamethoxazole; aTc, anhydrotetracycline.

1. Stokes HW, Hall RM. 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1699-1683.
2. Lv L, Ren YL, Chen JC, Wu Q, Chen GQ. 2015. Application of CRISPRi for prokaryotic metabolic engineering involving multiple genes, a case study: controllable P(3HB-co-4HB) biosynthesis. Metab Eng 29:160-8.