CRISPR-Cpf1 assisted multiplex genome editing and transcriptional repression in *Streptomyces*

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Supplementary materials

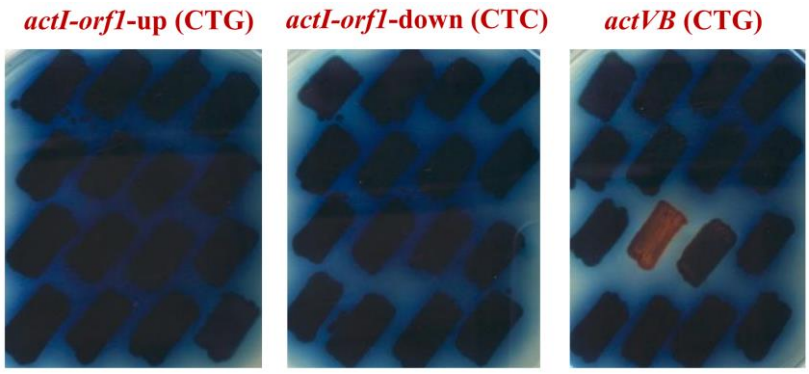

Fig. S1. Phenotypic analysis of exoconjugants in which 5′-CTV-3′ PAM-targeted crRNAs were used to guide the cleavage of *actI-orf1* or *actVB*. The strains with *actI-orf1 or actVB* inactivation only produced red-pigmented RED on R2YE plates (imaged at 72 h). 15 random exoconjugants were randomly picked for visual inspections and the strain in the top right corner represented the wild type in each group.

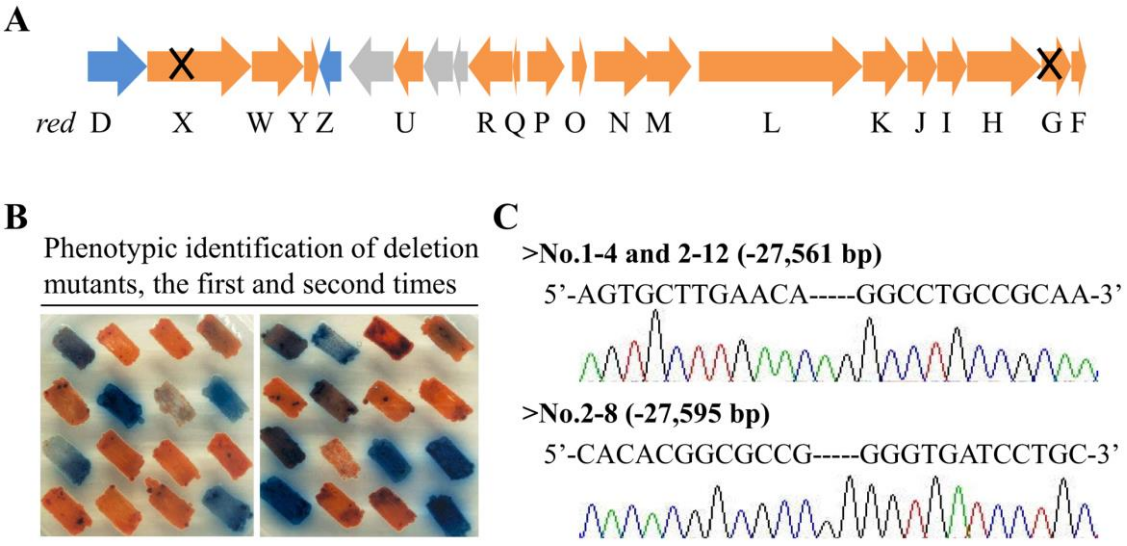

Fig. S2. One-step deletion of the prodiginines (RED) biosynthetic gene cluster (BGC) by *Fn*Cpf1-based reconstituted NHEJ editing system. (A) The map of the RED biosynthetic gene cluster. Two "X" marks represent the cleavage sites of CRISPR-Cpf1 system. (B) Phenotypic identification of the mutants with deletion of the RED BGC. For each trial, 15 colonies were selected. The images were photographed after growth on R2YE plates for 48 h at 30 ℃. The strain on the top right corner represents the wild-type *S. coelicolor* M145. These strains in which the RED BGC has been broken or completely deleted only produce blue-pigmented actinorhodin (ACT). (C) Sequence analysis of the mutants with deletion of the RED BGC. The numbers in brackets represent the deleted lengths.

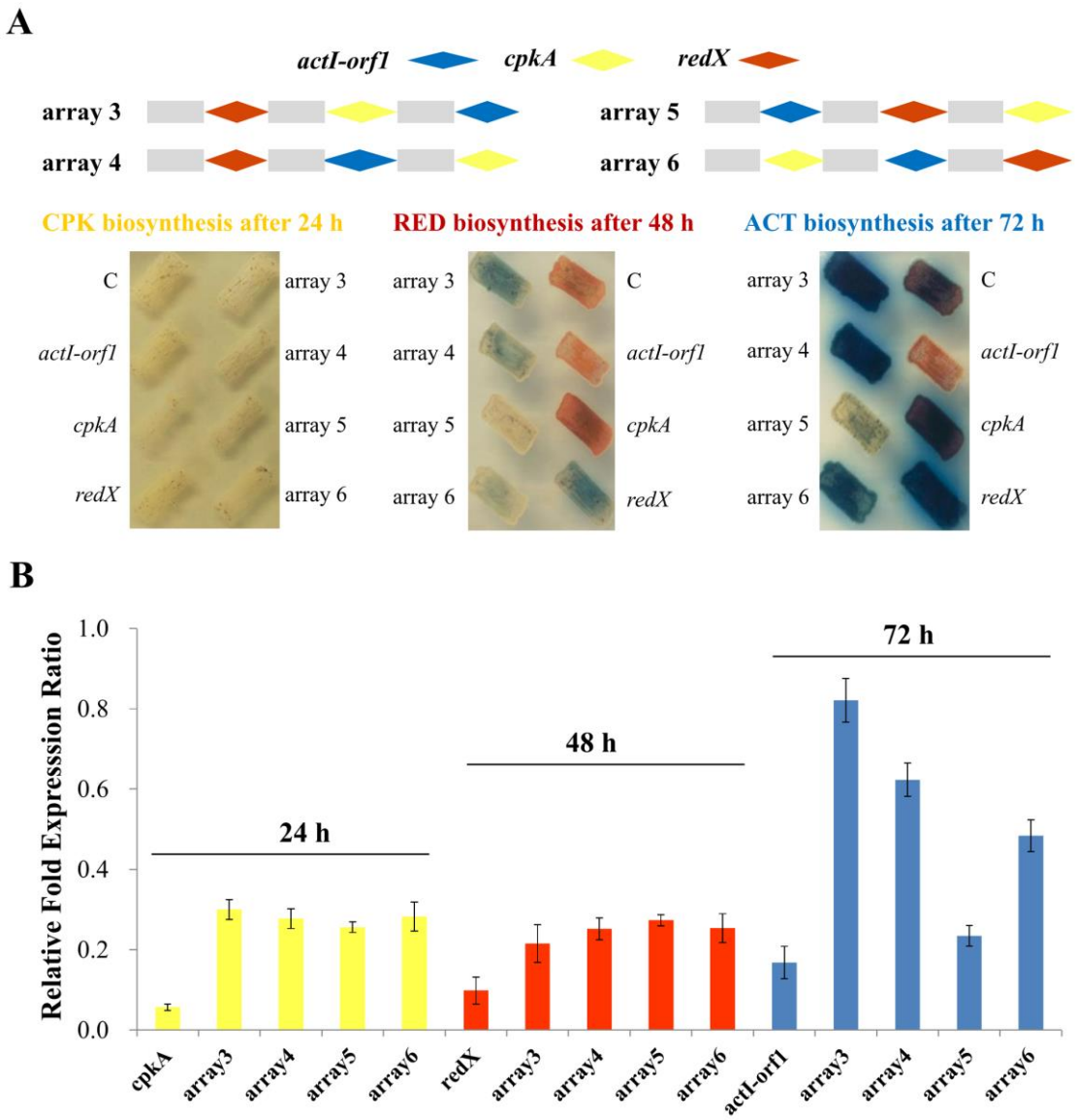

Fig. S3. ddCpf1-mediated simultaneous repression of three genes using a single customized crRNA array in *S. coelicolor*. (A) Phenotypic analysis of the exoconjugants with the individual editing plasmid containing single or multiple crRNAs . Three target genes (*cpkA*, *actI-orf1* and *redX*) were selected for simultaneous repression. The order of crRNAs targeting three genes is designed as array 3, array 4, array 5 or array 6. The strain only expressing ddCpf1 was used as the control (indicated as C). Images for antibiotics production (CPK, RED and ACT) was photographed at the time indicated. (B) Transcriptional analysis of *cpkA*, *redX* and

actI-orf1 in the strains with the individual editing plasmid containing single or multiple crRNAs. RNA samples for the analysis of *cpkA*, *redX* and *actI-orf1* transcription were isolated from the cultures after growth for 24, 48 and 72 h, respectively. The transcriptional levels of each gene were analyzed in the engineered strains expressing ddCpf1 with individual crRNA or crRNA arrays, and the strain only expressing ddCpf1 was used as the control (indicated as C).

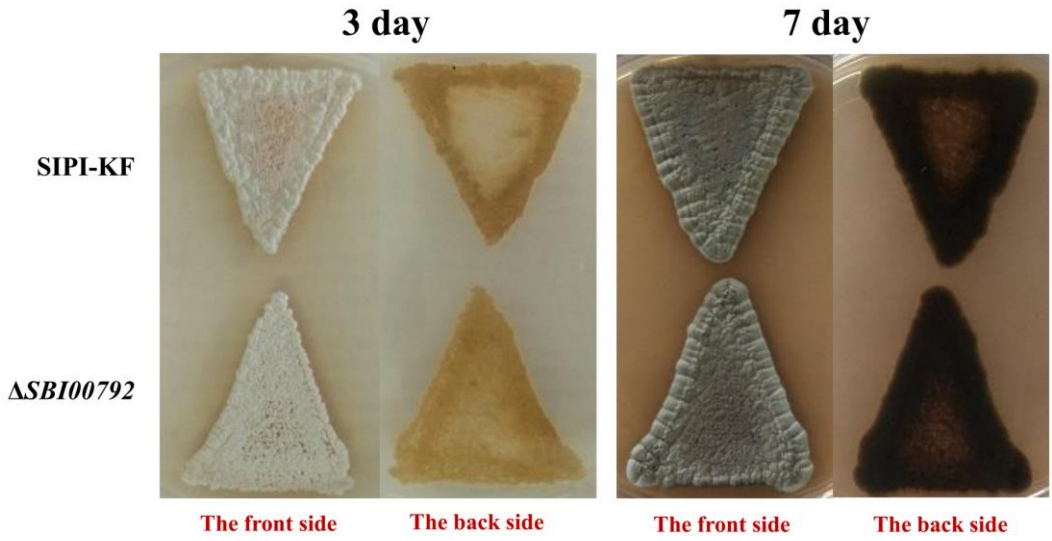

Fig. S4. Effect of deletion of *SBI00792* on bacterial growth. The partenal strain *S. hygroscopicus* SIPI-KF and the Δ*SBI00292* mutant were grown on MB plates and images were photographed from the front and back sides at 3 and 7 days.

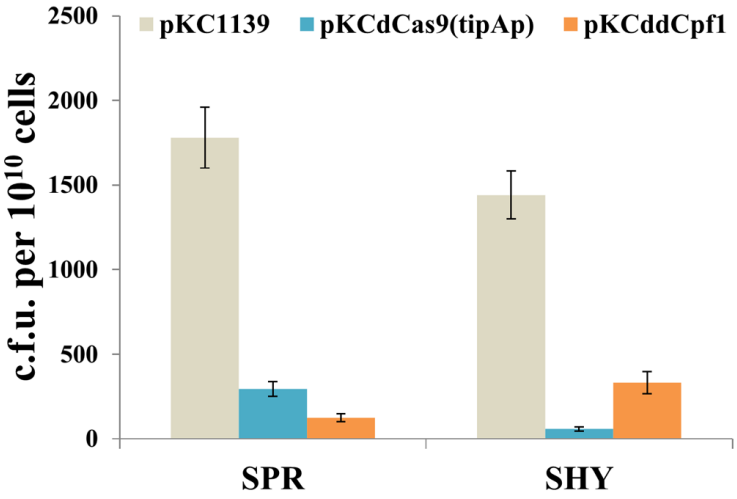

Fig. S5. Growth of the two important industrial *Streptomyces* species only expressing dCas9 or ddCpf1. pKC1139 was used as the control. c. f. u. represents colony-forming unit. SPR: *S. pristinaespiralis* HCCB10218, SHY: *S. hygroscopicus* SIPI-KF.

Table S1 PAM occurrence frequencies of four different class 2 CRISPR-Cas systems in *Streptomyces coelicolor* M145

	***Sp*Cas9**	***Fn*Cpf1**	***As*Cpf1/*Lb*Cpf1**
PAM sequence	NGG	TTV	TTTV
Occurrence frequency	0.26	0.0334	0.0047
PAM numbers in 100-bp DNA region	26	3.34	0.47
PAM numbers in single gene	257.7	33.1	4.7

Table S2 Bacterial plasmids and strains used in this study

Plasmids or Strains	Relevant features	Source/Reference
Plasmids		
pCB003	pMB1*ori*, *aadA*, the promoter j23119 was used to express the synthetic guide RNA(sgRNA)	Huang et al., 2015
pKC1139	A replicative vector in actinomycetes harboring a temperature sensitive replicon pSG5, *oriT*, and *aac(3)IV*	Kieser et al., 2000
pAH91*kasOp*-cmlR*	pAH91 with *cmlR* under the control of the strong promoter *kasOp**	Li et al., 2017
pIB139	An integrative plasmid containing *oriT*, *attP*, *int*, *aac(3)IV* and *ermEp**	Kieser et al., 2000
pIB-00792	pIB139 with *SBI00792* under the control of the strong promoter *ermEp**	This study
pKCCas9(*tipAp*)	pKC1139 with the *scocas9* gene under the control of the inducible promoter *tipAp*	Huang et al., 2015
pKCCas9(*tipAp*)-*actI-orf1*	pKCCas9(*tipAp*) with the sgRNA transcription cassette for editing *actI-orf1*	This study
pKCdCas9(*tipAp*)	pKC1139 with the *scocas9* (D10A and H840A) gene under the control of the inducible promoter *tipAp*	This study
pKCCpf1(*tipAp*)	pKC1139 with the *scocpf1* gene under the control of the inducible promoter *tipAp* and the crRNA repeat unit under the control of *kasOp**	This study
pKCCpf1(*tipAp*)-*actI-orf1*	pKCCpf1(*tipAp*) with the crRNA transcription cassette for editing *actI-orf1*	This study
pKCCpf1	pKC1139 with the *scocpf1* gene under the control of *ermEp** and the crRNA repeat unit under the control of the strong promoter *kasOp**	This study
pKCddCpf1	pKC1139 with the *scocpf1* (E1006A) gene under the control of the promoter *ermEp**	This study
pKCCpf1-*actI-orf1*	pKCCpf1 with the crRNA transcription cassette for editing *actI-orf1*	This study
pKCCpf1-*actI-orf1*-up (TTC)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and PAM is TTC	This study
pKCCpf1-*actI-orf1*-up (CTG)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and PAM is CTG	This study
pKCCpf1-*actI-orf1*-down (TTG)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and PAM is TTG	This study
pKCCpf1-*actI-orf1*-down (CTC)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and PAM is CTC	This study
pKCCpf1-*actVB* (TTC)	pKCCpf1 with the crRNA transcription cassette for editing *actVB* and PAM is TTC	This study
pKCCpf1-*actVB* (CTG)	pKCCpf1 with the crRNA transcription cassette for editing *actVB* and PAM is CTG	This study

pKCCpf1-*actI-orf1*-up (23 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 23 nt	This study
pKCCpf1-*actI-orf1*-up (22 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 22 nt	This study
pKCCpf1-*actI-orf1*-up (21 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 21 nt	This study
pKCCpf1-*actI-orf1*-up (20 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 20 nt	This study
pKCCpf1-*actI-orf1*-up (19 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 19 nt	This study
pKCCpf1-*actI-orf1*-up (18 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 18 nt	This study
pKCCpf1-*actI-orf1*-up (17 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the length of spacer is 17 nt	This study
pKCCpf1-*actI-orf1*-up (16 nt)	pKCCpf1 with the crRNA transcription cassette for editing the upstream of *actI-orf1* and the spacer length of crRNA is 16 nt	This study
pKCCpf1-*actI-orf1*-down (23 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 23 nt	This study
pKCCpf1-*actI-orf1*-down (22 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 22 nt	This study
pKCCpf1-*actI-orf1*-down (21 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 21 nt	This study
pKCCpf1-*actI-orf1*-down (20 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 20 nt	This study
pKCCpf1-*actI-orf1*-down (19 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 19 nt	This study
pKCCpf1-*actI-orf1*-down (18 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 18 nt	This study
pKCCpf1-*actI-orf1*-down (17 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer	This study

	length of crRNA is 17 nt	
pKCCpf1-*actI-orf1*-down (16 nt)	pKCCpf1 with the crRNA transcription cassette for editing the downstream of *actI-orf1* and the spacer length of crRNA is 16 nt	This study
pKCCpf1-*actI-orf1*-HR	pKCCpf1 with the crRNA transcription cassette for deleting *actI-orf1* and two homologous arms	This study
pKCCpf1-*redX*-HR	pKCCpf1 with the crRNA transcription cassette for deleting *redX* and two homologous arms	This study
pKCCpf1-*actI-orf1-redX*-HR	pKCCpf1 with two-pair homologous arms and the crRNA transcription cassettes for simultaneously deleting *actI-orf1* and *redX*	This study
pZX09	NHEJ expression vector harboring the *ligD* and *ku* genes from *Mycobacterium smegmatis*	Zheng et al., 2017
pGH-*gadphp*-Sda-LK	NHEJ cloning vector harboring the *ligD* and *ku* genes from *Streptomyces daghestanicus* under the control of the ultrastrong promoter *gadphp*	This study
pKCCpf1-MsmP	pKCCpf1 with the *ligD* and *Ku* genes from *Mycobacterium smegmatis* under the control of the ultrastrong promoter *gadphp*	This study
pKCCpf1-MsmE	pKCCpf1 with the *ligD* and *Ku* genes from *Mycobacterium smegmatis* under the control of the strong promoter *ermEp**	This study
pKCCpf1-SdaP	pKCCpf1 with the *ligD* and *Ku* genes from *Streptomyces daghestanicus* under the control of the ultrastrong promoter *gadphp*	This study
pKCCpf1-SdaE	pKCCpf1 with the *ligD* and *Ku* genes from *Streptomyces daghestanicus* under the control of the strong promoter *ermEp**	This study
pKCCpf1-PpuP	pKCCpf1 with the *ligD* and *Ku* genes from *Pseudomonas putida* KT2440 under the control of the ultrastrong promoter *gadphp*	This study
pKCCpf1-PpuE	pKCCpf1 with the *ligD* and *Ku* genes from *Pseudomonas putida* KT2440 under the control of the strong promoter *ermEp**	This study
pKCCpf1-MsmE-*redX*	pKCCpf1-MsmE with the crRNA transcription cassette for editing *redX*	This study
pKCCpf1-SdaE-*redX*	pKCCpf1-SdaE with the crRNA transcription cassette for editing *redX*	This study
pKCCpf1-PpuE-*redX*	pKCCpf1-PpuE with the crRNA transcription cassette for editing *redX*	This study
pKCCpf1-MsmE-*actI-orf1*	pKCCpf1-MsmE with the crRNA transcription cassette for editing *actI-orf1*	This study
pKCCpf1-SdaE-*actI-orf1*	pKCCpf1-SdaE with the crRNA transcription cassette for editing *actI-orf1*	This study

pKCCpf1-PpuE-*actI-orf1*	pKCCpf1-PpuE with the crRNA transcription cassette for editing *actI-orf1*	This study
pKCCpf1-MsmE-RED-BGC	pKCCpf1-MsmE with the artificial CRISPR array for transcribing two crRNAs for deleting RED biosynthetic gene cluster	This study
pSET152	pUC19 *ori, ΦC31 int*/*attP, aac(3)IV, lacZα,* and *oriT* RK2	Bierman et al., 1992
pSETddCpf1	pSET152 with *Scocpf1*(E1006A) gene under the control of the strong promoter *ermEp** and the crRNA repeat unit under the control of the strong promoter *kasOp**	This study
pSETddCpf1-*redX*-T1 (also as pSETddCpf1-*redX*)	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *redX*, No.1	This study
pSETddCpf1-*redX*-T2	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *redX*, No.2	This study
pSETddCpf1-*redX*-T3	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *redX*, No.3	This study
pSETddCpf1-*redX*-T4	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *redX*, No.4	This study
pSETddCpf1-*redX*-NT1	pSETddCpf1 with the crRNA transcription cassette targeting the non-template strand of *redX*, No.1	This study
pSETddCpf1-*redX*-NT2	pSETddCpf1 with the crRNA transcription cassette targeting the non-template strand of *redX*, No.2	This study
pSETddCpf1-*redX*-NT3	pSETddCpf1 with the crRNA transcription cassette targeting the non-template strand of *redX*, No.3	This study
pSETddCpf1-*redX*-NT4	pSETddCpf1 with the crRNA transcription cassette targeting the non-template strand of *redX*, No.4	This study
pSETddCpf1-*actI-orf1*	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *actI-orf1*	This study
pSETddCpf1-*cpkA*	pSETddCpf1 with the crRNA transcription cassette targeting the template strand of *cpkA*	This study
pSETddCpf1-*array* 1	pSETddCpf1 with the artificial CRISPR array for transcribing three crRNAs targeting the template strands of *redX*, *cpkA* and *actI-orf1*	This study
pSETddCpf1-*array* 2	pSETddCpf1 with the artificial CRISPR array for transcribing three crRNAs targeting the template strands of *actI-orf1*, *redX* and *cpkA*	This study
pSETddCpf1-*array* 3	pSETddCpf1 with the artificial CRISPR array for transcribing three crRNAs targeting the template strands of *actI-orf1*, *cpkA* and *redX*	This study
pSETddCpf1-*array* 4	pSETddCpf1 with the artificial CRISPR array for transcribing three crRNAs targeting the template strands of *cpkA* , *actI-orf1* and *redX*	This study
pSETddCpf1-*array* 5	pSETddCpf1 with the artificial CRISPR array for	This study

	transcribing three crRNAs targeting the template strands of *cpkA* , *redX* and *actI-orf1*	
pSETddCpf1-*array* 6	pSETddCpf1 with the artificial CRISPR array for transcribing three crRNAs targeting the template strands of *redX* , *actI-orf1* and *cpkA*	This study
pKCCpf1-*SBI00792*	pKCCpf1 with the crRNA transcription cassette for editing *SBI00792*	This study
pKCCpf1-*SBI00792*-HR1.0	pKCCpf1 withthe crRNA transcription cassette for deleting *SBI00792* and two 1-kb homologous arms	This study
pKCCpf1-*SBI00792*-HR1.5	pKCCpf1 with the crRNA transcription cassette for deleting *SBI00792* and two 1.5-kb homologous arms	This study
pKCCpf1-*SBI00792*-HR2.0	pKCCpf1 with the crRNA transcription cassette for deleting *SBI00792* and two 2-kb homologous arms	This study
pKCCpf1-*SBI00792*-HR2.5	pKCCpf1 with the crRNA transcription cassette for deleting *SBI00792* and two 2.5-kb homologous arms	This study
Escherichia coli		
DH5α	F¯80Φ*dlacZD*M15Δ(*lacZYA−argF*)*U169deoR recA1 endA1 hsdR17*($rk^- mk^+$) *supE44* λ^- *thi*$^-$*1gyrA96 relA1*	GIBCO-BRL
ET12567/pUZ8002	ET12567 containing the non-transmissible RP4 derivative plasmid pUZ8002	GIBCO-BRL
S17-1	*supE44*, Δ*lacU169* (Φ*lacZ*ΔM15), *recA1*, *endA1*, *hsdR17*, *thi-1*, *gyrA96*, *relA1*, *par* phage lysogenic	GIBCO-BRL
Streptomyces coelicolor		
M145	SCP1- SCP2-, aderivative from *S. coelicolor* A3(2) [wild type]	Kieser et al., 2000
M145/pKC1139	M145 carrying the plasmid pKC1139	This study
M145/pKCCas9(*tipAp*)	M145 carrying the plasmid pKCCas9(*tipAp*)	This study
M145/pKCCpf1(*tipAp*)	M145 carrying the plasmid pKCCpf1(*tipAp*)	This study
M145/pKCCpf1	M145 carrying the plasmid pKCCpf1	This study
Δ*actI-orf1*	Mutant with in-frame deletion of the *actI-orf1* gene	This study
Δ*redX*	Mutant with in-frame deletion of the *redX* gene	This study
Δ*actI-orf1-redX*	Mutant with in-frame deletion of both *actI-orf1* and *redX*	This study
M145/pKCCpf1-MsmP	M145 carrying the plasmid pKCCpf1-MsmP	This study
M145/pKCCpf1-MsmE	M145 carrying the plasmid pKCCpf1-MsmE	This study
M145/pKCCpf1-SdaP	M145 carrying the plasmid pKCCpf1-SdaP	This study
M145/pKCCpf1-SdaE	M145 carrying the plasmid pKCCpf1-SdaE	This study
M145/pKCCpf1-PpuP	M145 carrying the plasmid pKCCpf1-PpuP	This study
M145/pKCCpf1-PpuE	M145 carrying the plasmid pKCCpf1-PpuE	This study
M145/pSETddCpf1	M145 carrying the plasmid pSETddCpf1	This study
M145/pSETddCpf1-*redX*-T1	M145 carrying the plasmid pSETddCpf1-*redX*-T1	This study
M145/pSETddCpf1-*redX*-T2	M145 carrying the plasmid pSETddCpf1-*redX*-T2	This study
M145/pSETddCpf1-*redX*-T3	M145 carrying the plasmid pSETddCpf1-*redX*-T3	This study
M145/pSETddCpf1-*redX*-T4	M145 carrying the plasmid pSETddCpf1-*redX*-T4	This study

M145/pSETddCpf1-*redX*-NT1	M145 carrying the plasmid pSETddCpf1-*redX*-NT1	This study
M145/pSETddCpf1-*redX*-NT2	M145 carrying the plasmid pSETddCpf1-*redX*-NT2	This study
M145/pSETddCpf1-*redX*-NT3	M145 carrying the plasmid pSETddCpf1-*redX*-NT3	This study
M145/pSETddCpf1-*redX*-NT4	M145 carrying the plasmid pSETddCpf1-*redX*-NT4	This study
M145/pSETddCpf1-*array*1	M145 carrying the plasmid pSETddCpf1-*array* 1	This study
M145/pSETddCpf1-*array*2	M145 carrying the plasmid pSETddCpf1-*array* 2	This study
M145/pSETddCpf1-*array*3	M145 carrying the plasmid pSETddCpf1-*array* 3	This study
M145/pSETddCpf1-*array*4	M145 carrying the plasmid pSETddCpf1-*array* 4	This study
M145/pSETddCpf1-*array*5	M145 carrying the plasmid pSETddCpf1-*array* 5	This study
M145/pSETddCpf1-*array*6	M145 carrying the plasmid pSETddCpf1-*array* 6	This study
Other *Streptomyces* species		
S. albus J1074	Model *Strptomyces*, *S. albus* G mutant	Chater and Wilde, 1976
S. venezuelae ATCC10712	Model *Streptomyces* [Wild type]	Bush et al., 2013
S. avermitilis NRRL8165	Avermectin-producing industrial strain	Ōmura et al., 2001
S. pristinaespiralis HCCB10218	Pristinamycin-producing industrial strain	Li et al., 2015
S. roseosporus SIPI-DT51	Daptomycin-producing industrial strain	SIPI
S. hygroscopicus SIPI-KF	5-oxomilbemycin A3/A4-producing industrial strain, derived from milbemycin-producing *S. hygroscopicus* strain (with deletion of the *milF* gene)	SIPI
S. verticillus SIPI-BL	Bleomycin-producing industrial strain	SIPI
J1074/pKC1139	*S. albus* J1074 carrying the plasmid pKC1139	This study
J1074/pKCCas9(*tipAp*)	*S. albus* J1074 carrying the plasmid pKCCas9(*tipAp*)	This study
J1074/pKCCpf1	*S. albus* J1074 carrying the plasmid pKCCpf1	This study
10712/pKC1139	*S. venezuelae* ATCC10712 carrying the plasmid pKC1139	This study
10712/pKCCas9(*tipAp*)	*S. venezuelae* ATCC10712 carrying the plasmid pKCCas9(*tipAp*)	This study
10712/pKCCpf1	*S. venezuelae* ATCC10712 carrying the plasmid pKCCpf1	This study
8165/pKC1139	*S. avermitilis* NRRL8165 carrying the plasmid pKC1139	This study
8165/pKCCas9(*tipAp*)	*S. avermitilis* NRRL8165 carrying the plasmid pKCCas9(*tipAp*)	This study
8165/pKCCpf1	*S. avermitilis* NRRL8165 carrying the plasmid pKCCpf1	This study
10218/pKC1139	*S. pristinaespiralis* 10218 carrying the plasmid pKC1139	This study
10218/pKCCas9(*tipAp*)	*S. pristinaespiralis* 10218 carrying the plasmid	This study

	pKCCas9(*tipAp*)	
10218/pKCCpf1	*S. pristinaespiralis* 10218 carrying the plasmid pKCCpf1	This study
10218/pKCdCas9(*tipAp*)	*S. pristinaespiralis* 10218 carrying the plasmid pKCdCas9(*tipAp*)	This study
10218/pKCddCpf1	*S. pristinaespiralis* 10218 carrying the plasmid pKCddCpf1	This study
SIPI-DT51/pKC1139	*S. roseosporus* SIPI-DT51 carrying the plasmid pKC1139	This study
SIPI-DT51/pKCCas9(*tipAp*)	*S. roseosporus* SIPI-DT51 carrying the plasmid pKCCas9(*tipAp*)	This study
SIPI-DT51/pKCCpf1	*S. roseosporus* SIPI-DT51 carrying the plasmid pKCCpf1	This study
SIPI-KF/pKC1139	*S. hygroscopicus* SIPI-KF carrying the plasmid pKC1139	This study
SIPI-KF/pKCCas9(*tipAp*)	*S. hygroscopicus* SIPI-KF carrying the plasmid pKCCas9(*tipAp*)	This study
SIPI-KF/pKCCpf1	*S. hygroscopicus* SIPI-KF carrying the plasmid pKCCpf1	This study
SIPI-KF/pKCdCas9(*tipAp*)	*S. hygroscopicus* SIPI-KF carrying the plasmid pKCdCas9(*tipAp*)	This study
SIPI-KF/pKCddCpf1	*S. hygroscopicus* SIPI-KF carrying the plasmid pKCddCpf1	This study
SIPI-BL/pKC1139	*S. verticillus* SIPI-BL carrying the plasmid pKC1139	This study
SIPI-BL/pKCCas9(*tipAp*)	*S. verticillus* SIPI-BL carrying the plasmid pKCCas9(*tipAp*)	This study
SIPI-BL/pKCCpf1	*S. verticillus* SIPI-BL carrying the plasmid pKCCpf1	This study
Δ*SBI00792*	Mutant with in-frame deletion of the *SBI00792* gene in *S. hygroscopicus* SIPI-KF	This study
SIPI-KF/pIB139	*S. hygroscopicus* SIPI-KF carrying the empty vector pIB139	This study
Δ*SBI00792*/pIB139	*SBI00792* deletion mutant carrying the empty vector pIB139	This study
Δ*SBI00792*/pIB-00792	*SBI00792* deletion mutant carrying the complemented plasmid pIB-00792	This study

Note: SIPI represents Shanghai Institute of Pharmaceutical Industry.

Table S3 The conditions of conjugal transfer for different *Streptomyces* species

***Streptomyces* species**	**Abbreviation**	**Donor *E. coli***	**Medium**
***S. coelicolor* M145**	SCO	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}
***S. albus* J1074**	SAL	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}
***S. venezuelae* ATCC10712**	SVEN	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}
***S. avermitilis* NRRL8165**	SAV	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}
***S. roseosporus* SIPI-DT51**	SRO	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}
***S. pristinaespiralis* HCCB10218**	SPR	S17-1	M-Isp4+10 mM Mg^{2+}
***S. hygroscopicus* SIPI-KF**	SHY	S17-1	M-Isp4+60 mM Mg^{2+}
***S. verticillus* SIPI-BL**	SVER	ET12567/pUZ8002	M-Isp4+10 mM Mg^{2+}

Table S4 Oligonucleotide sequences used in this study

Primers	Sequence (5'-3')
Primers for the construction pKCCpf1(*tipAp*), pKCCpf1 and pKCddCpf1	
*kasOp**-crRNA-fw	gcTCTAGAtgttcacattcgaaccgtc
*kasOp**-crRNA-rev	ggACTAGTatctacaacagtagaaatttggccacgactttacaacac
*ermEp**-fw	aagcagagacggttcgaatgtgaacaGGATCCctctagtatgcatgcgagtg
*ermEp**-rev	ttgttgacgaactcctggtagatggaCATATGtggatcctaccaaccggcac
ddcpf1-fw	ggaattcCATATGtccatctaccaggagttcgtca
ddcpf1-rev	gGAATTCtcagttgttgcggttctgcacgaa
Primers for the construction of pKCCas9(*tipAp*)-*actI-orf1*, pKCCpf1(*tipAp*)-*actI-orf1* and pKCCpf1-*actI-orf1*	
cas9-actI-orf1-fw	cccAAGCTTgcagatctcaaaaaaagcaccgact
cas9-actI-orf1-rev	gACTAGTgaagcgcagagtcgtcatcagttttagagctagaaatagca
cpf1-actI-orf1-fw	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
cpf1-actI-orf1-rev	ggaattcCATATGtggatcctaccaaccggcacgatt
Primers for the construction of the plasmids for determining PAM compatibility in *S. coelicolor*	
crRNA-*actI-orf1*-up-fw (TTC)	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (CTG)	gACTAGTacgactctgcgcttcaatccgaaatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (TTG)	gACTAGTgacgcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (CTC)	gACTAGTagtggccgaccatcgacttgatcatctacaacagtagaaatttgg
crRNA-*actVB*-fw (TTC)	gACTAGTgctccatcgagacggacacgaacatctacaacagtagaaatttgg
crRNA- *actVB*-fw (CTG)	gACTAGTttggccgtacgagccaggcagacatctacaacagtagaaatttgg
crRNA-rev	ggaattcCATATGtggatcctaccaaccggcacgatt
Primers for the construction of the plasmids for determining the efficient spacer lengths of crRNA in *S. coelicolor*	
crRNA-*actI-orf1*-up-fw (23 nt)	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (22 nt)	gACTAGTtgacgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (21 nt)	gACTAGTgacgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (20 nt)	gACTAGTacgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (19 nt)	gACTAGTcgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (18 nt)	gACTAGTgactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (17 nt)	gACTAGTactctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-up-fw (16 nt)	gACTAGTctctgcgcttcaatccatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (23 nt)	gACTAGTgacgcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (22 nt)	gACTAGTacgcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (21 nt)	gACTAGTcgcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (20 nt)	gACTAGTgcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (19 nt)	gACTAGTcgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (18 nt)	gACTAGTgacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (17 nt)	gACTAGTacggacatcgactacatatctacaacagtagaaatttgg
crRNA-*actI-orf1*-down-fw (16 nt)	gACTAGTcggacatcgactacatatctacaacagtagaaatttgg
crRNA-rev	ggaattcCATATGtggatcctaccaaccggcacgatt
Primers for the construction and identification of Δ*actI-orf1*, Δ*redX* and Δ*actI-orf1-redX*	

Del-*actI-orf1*-up-fw	gACTAGTacggtgagaaggtgctcgtgtagca
Del-*actI-orf1*-up-rev	aacgtagtcgagatcgcactcggggtcgctgaaggccgatacgggacccctcgat
Del-*actI-orf1*-down-fw	agcgaccccgagtgcgatctcgacta
Del-*actI-orf1*-down-rev	tgtagatggattgaagcgcagagtcgtcattcggcgaacacgacgtcgacgtcct
actI-orf1-crRNA-fw	atgacgactctgcgcttcaatccatctacaacagtagaaatttgg
actI-orf1-crRNA-rev	ggaattcCATATGtggatcctaccaaccggcacgatt
ID-*actI-orf1*-fw	aggcgctggaatcgtatcggaatct
ID-*actI-orf1*-rev	acatcagggcggtgaccacgtcga
Del-*redX*-up-fw	gACTAGTacctgcctcggcctggaccggcagta
Del-*redX*-up-rev	cgtcgaagtcgaagttcatcgcgttgcgtcggcttcctccaggagcacgtggcata
Del-*redX*-down-fw	gacgcaacgcgatgaacttcgactt
Del-*redX*-down-rev	tgtagatccacctgttgatcgaggaaggcaaactggacggcgtccagtccgagtt
redX-crRNA-fw	tgccttcctcgatcaacaggtggatctacaacagtagaaatttgg
redX-crRNA-rev	cccAAGCTTatcctaccaaccggcacgattgtgc
ID-*redX*-fw	acatcgaggtcgacgtggcacggtt
ID-*redX*-rev	gatctcgttggtgccggagaagat
Primers for the construction of NHEJ expression vector and the identification of deletion mutants	
Msm-ligD-fw	cgGATATCatggagcgctatgagcgggttcgcctgacgaa
Msm-ligD-rev	gcTCTAGAgcctattcccacacaacctcatcgggtgt
Msm-ku-fw	gcTCTAGAaaggagtgtccatatgaaccgtgcggtacgccatactg
Msm-ku-rev	cccAAGCTTctacgacttcttcgcagctgccttcttg
Ppu-ligD-fw	gACTAGTagctttGTTTAAACatggccaagcccctgcaggaatac
Ppu-ligD-rev	catatggacactccttTCTAGAgctcattcgagccctagctgcttgcgcat
Ppu-ku-fw	gcTCTAGAaaggagtgtccatatggctcgggcaatctggaaaggcgccatcagt
Ppu-ku-rev	cccAAGCTTtcatgaagcctttcgcgtcttcttcac
gapdhp-fw	gACTAGTgctgctccttcggtcggacgtgcgtcta
gapdhp-rev	agctttGTTTAAACgcgtatccccttcagatactcgca
*ermEp**-fw (*Spe* I)	gACTAGTcatgcgagtgtccgttcgagt
*ermEp**-rev (*Eco*R V)	cgGATATCcatatgtggatcctaccaac
*ermEp**-rev (*Pme* I)	agctttGTTTAAACcatatgtggatcctaccaac
crRNA-RED-BGC-fw	gACTAGTcggtgcacgtaggtcacgtggtaatctacaacagtagaaatttgccttcctcgatcaacaggtggatctacaacagtagaaatttgg
crRNA-rev	ggaattcCATATGtggatcctaccaaccggcacgatt
ID-NHEJ-*redX*-fw	tcactgaccggcaccgtatgccacgt
ID-NHEJ-*redX*-rev	gatctcgttggtgccggagaagat
ID-NHEJ-RED-BGC-fw	tcactgaccggcaccgtatgccacgt
ID-NHEJ-RED-BGC-rev	gatctgtggaggggatctgtggat
Primers for the construction of a series of CRISPRi plasmids	
ddcpf1-up-fw	aagcttgggctgcaggtcgacTCTAGAtcagttgttgcggttctgcacgaa
ddcpf1-up-E1006A-rev	atcgagtacaacgccatcgtcgtcttcgccgacctgaacttcggcttcaag
ddcpf1-down-fw	ggcgaagacgacgatggcgttgt
ddcpf1-down-rev	ttgccgccgggcgtttttttattggtgaGTTTAAACtatgcttaattaatcaag
CRISPRi-*redX*-T1-fw	gACTAGTctgtggctgtgtcgttgtctgaatctacaacagtagaaatttgg
CRISPRi-*redX*-T2-fw	gACTAGTtgcgcggccgacagcgagtagcgatctacaacagtagaaatttgg

CRISPRi-*redX*-T3-fw	gACTAGTcgtggtagaggtcccggtcgaaatctacaacagtagaaatttgg
CRISPRi-*redX*-T4-fw	gACTAGTtgccttcctcgatcaacaggtggatctacaacagtagaaatttgg
CRISPRi-*redX*-NT1-fw	gACTAGTcacggacacacccaccgtcacgatctacaacagtagaaatttgg
CRISPRi-*redX*-NT2-fw	gACTAGTctggctctcgcgcacgccgtggaatctacaacagtagaaatttgg
CRISPRi-*redX*-NT3-fw	gACTAGTtcggaaccgtcgtcgtcggcagatctacaacagtagaaatttgg
CRISPRi-*redX*-NT4-fw	gACTAGTtcaattaccacctgttgatcgagatctacaacagtagaaatttgg
CRISPRi-*actI-orf1*-fw	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
CRISPRi-*cpkA*-fw	gACTAGTgaaccgctgtgcagcagctcccaatctacaacagtagaaatttgg
CRISPRi-*array* 1-fw	gACTAGTctgtggctgtgtcgttgtctgaatctacaacagtagaaattgaaccgctgtgcagcagct ccccaatctacaacagtagaaattatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
CRISPRi-*array* 2-fw	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaattctgtggctgtgtcgttgtc tgaatctacaacagtagaaattgaaccgctgtgcagcagctcccaatctacaacagtagaaatttgg
CRISPRi-*array* 3-fw	gACTAGTatgacgactctgcgcttcaatccatctacaacagtagaaattgaaccgctgtgcagca gctcccaatctacaacagtagaaattctgtggctgtgtcgttgtctgaatctacaacagtagaaatttgg
CRISPRi-*array* 4-fw	gACTAGTgaaccgctgtgcagcagctcccaatctacaacagtagaaattatgacgactctgcgct tcaatccatctacaacagtagaaattctgtggctgtgtcgttgtctgaatctacaacagtagaaatttgg
CRISPRi-*array* 5-fw	gACTAGTgaaccgctgtgcagcagctcccaatctacaacagtagaaattctgtggctgtgtcgttg tctgaatctacaacagtagaaattatgacgactctgcgcttcaatccatctacaacagtagaaatttgg
CRISPRi-*array* 6-fw	gACTAGTctgtggctgtgtcgttgtctgaatctacaacagtagaaattatgacgactctgcgcttca atccatctacaacagtagaaattgaaccgctgtgcagcagctcccaatctacaacagtagaaatttgg
Primers used in qRT-PCR analysis in *S. coelicolor*	
RT-*hrdB*-fw	agcctcaaccagatcctcga
RT-*hrdB*-rev	agcggtcgccttcctgctggtca
RT-*actI-orf1*-fw	agttctgggaactgctcacct
RT-*actI-orf1*-rev	acaggccacggcgaactgcga
RT-*redX* -fw	acccatcgccatcgtcgggatgt
RT-*redX*-rev	accgtgccacgtcgacctcgat
RT-*cpkA*-fw	agcagcggctcgtgctcgaact
RT-*cpkA*-rev	accgcctcgtccccgtactggta
Primers for the construction and identification of Δ*SBI00792*	
SBI00792-crRNA-fw	gtggccaaatttctactgttgtagatcgcaacaaggacgagctccttgccACTAGTgcgtcgatat ctcg
SBI00792-crRNA-rev	cgttgtaaaacgacggccagtgccaagcttCCATGGtgcgagtttaaactatgctt
Del-*SBI00792*-up-1kb-fw	caacaaggacgagctccttgccACTAGTctctgcgggtcgtagaagtcg
Del-*SBI00792*-down-1kb-rev	gttgtaaaacgacggccagtgccAAGCTTatcgtgttccgcaatcagc
Del-*SBI00792*-up-1.5 kb-fw	caacaaggacgagctccttgccACTAGTggcagcatggcgttcttgg
Del-*SBI00792*-down-1.5 kb-rev	gttgtaaaacgacggccagtgccAAGCTTtcgaggtacttgccgaacgag
Del-*SBI00792*-up-2 kb-fw	caacaaggacgagctccttgccACTAGTaagccctcggggacggtggt
Del-*SBI00792*-down-2 kb-rev	gttgtaaaacgacggccagtgccAAGCTTcgccacctcgcattcgtc
Del-*SBI00792*-up-2.5 kb-fw	caacaaggacgagctccttgccACTAGTgccgacggtgtagtagtgcg
Del-*SBI00792*-down-2.5 kb-rev	gttgtaaaacgacggccagtgccAAGCTTcctccacgatacggctcacct
Del-*SBI00792*-up-rev	ggctttggtgtagccgttctcg
Del-*SBI00792*-down-fw	agtttcgccgagaacggctacaccaaagccgtgctcaacgggctccaa
ID-*SBI00792*-fw	tgaggtcggcggagaagtcg

ID-*SBI00792*-rev	gcatcacgctggtttccct
Com-*SBI00792*-fw	ggaattcCATATGgtgagccagggaaaggcgcgt
Com-*SBI00792*-rev	gGAATTCtggagcggctggagaaccc

Note: The underlined letters stand for the restriction enzyme sites. The red, blue or yellow lowercase letters represent the guide sequences of crRNAs.

Data1:

Sequence of synthetic codon-optimized *Fncpf1*

>*scocpf1*, 3903 bp

atgtccatctaccaggagttcgtcaacaagtacagcctgagcaagaccctccgcttcgagctgatcccccagggcaagaccctcgagaac
atcaaggcccgcggcctgatcctggacgacgagaagcgggccaaggactacaagaaggccaagcagatcatcgacaagtaccaccagt
tcttcatcgaggagatcctgtcctccgtctgcatctccgaggacctgctgcagaactactccgacgtctacttcaagctgaagaagtccgacg
acgacaacctgcagaaggacttcaagtccgccaaggacaccatcaagaagcagatctccgagtacatcaaggactccgagaagttcaag
aacctgttcaaccagaacctgatcgacgccaagaagggccaggagtccgacctgatcctgtggctgaagcagtccaaggacaacggcat
cgagctgttcaaggccaactccgacatcaccgacatcgacgaggccctggagatcatcaagtccttcaagggctggaccacctacttcaag
ggcttccacgagaaccgcaagaacgtctactcctccaacgacatcccgacctcgatcatctaccggatcgtcgacgacaacctgcccaagt
tcctggagaacaaggccaagtacgagtccctgaaggacaaggccccggaggccatcaactacgagcagatcaagaaggacctggccg
aggagctgaccttcgacatcgactacaagaccagcgaggtcaaccagcgcgtcttctccctggacgaggtcttcgagatcgccaacttcaa
caactacctcaaccagtccggcatcaccaagttcaacaccatcatcggcggcaagttcgtcaacggcgagaacaccaagcggaagggca
tcaacgagtacatcaacctgtactcccagcagatcaacgacaagaccctgaagaagtacaagatgtccgtcctgttcaagcagatcctgtcc
gacaccgagtccaagtccttcgtcatcgacaagctggaggacgactccgacgtcgtcaccaccatgcagtccttctacgagcagatcgccg
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tctacttcaagaacgacaagtccctgaccgacctgtcccagcaggtcttcgacgactactccgtcatcggcaccgccgtcctggagtacatc
acccagcagatcgccccgaagaacctggacaacccctccaagaaggagcaggagctgatcgccaagaagaccgagaaggccaagtac
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ctgaagatcttccacatctcccagtccgaggacaaggccaacatcctggacaaggacgagcacttctacctggtcttcgaggagtgctactt
cgagctggccaacatcgtcccgctgtacaacaagatccgcaactacatcacccagaagccctactccgacgagaagttcaagctgaacttc
gagaactccaccctggccaacggctgggacaagaacaaggagccggacaacaccgccatcctgttcatcaaggacgacaagtactacct
gggcgtcatgaacaagaagaacaacaagatcttcgacgacaaggccatcaaggagaacaagggcgagggctacaagaagatcgtgtac
aagctgctgccgggcgccaacaagatgctgcccaaggtcttcttctccgccaagtccatcaagttctacaacccgtccgaggacatcctgc
gcatccggaaccactccacccacaccaagaacggctccccccagaagggctacgagaagttcgagttcaacatcgaggactgccgcaa
gttcatcgacttctacaagcagtccatctccaagcacccggagtggaaggacttcggcttccgcttctccgacacccagcggtacaactcca
tcgacgagttctaccgcgaggtcgagaaccagggctacaagctgaccttcgagaacatctccgagtcctacatcgactccgtcgtcaacca
gggcaagctgtacctgttccagatctacaacaaggacttctccgcctactccaagggccgccccaacctgcacaccctgtactggaaggcc

ctgttcgacgagcggaacctgcaggacgtcgtctacaagctgaacggcgaggccgagctgttctaccgcaagcagtccatcccgaagaa

gatcacccaccccgccaaggaagccatcgccaacaagaacaaggacaacccgaagaaggagtccgtcttcgagtacgacctgatcaag

gacaagcggttcaccgaggacaagttcttcttccactgccccatcaccatcaacttcaagtcctccggcgccaacaagttcaacgacgagat

caacctgctgctgaaggagaaggccaacgacgtccacatcctgtccatcgaccgcggcgagcggcacctggcctactacaccctggtcg

acggcaagggcaacatcatcaagcaggacaccttcaacatcatcggcaacgaccgcatgaagaccaactaccacgacaagctggccgc

catcgagaaggaccgcgactccgcccggaaggactggaagaagatcaacaacatcaaggagatgaaggaaggctacctgtcccaggtc

gtccacgagatcgccaagctggtcatcgagtacaacgccatcgtcgtcttcgaggacctgaacttcggcttcaagcgcggccggttcaagg

tcgagaagcaggtctaccagaagctggagaagatgctgatcgagaagctgaactacctggtcttcaaggacaacgagttcgacaagaccg

gcggcgtcctgcgggcctaccagctgaccgccccgttcgagaccttcaagaagatgggcaagcagaccggcatcatctactacgtcccg

gccggcttcacctcgaagatctgccccgtcaccggcttcgtcaaccagctgtaccccaagtacgagtccgtctccaagtcccaggagttctt

ctccaagttcgacaagatctgctacaacctggacaaggggtacttcgagttctccttcgactacaagaacttcggcgacaaggccgccaag

ggcaagtggaccatcgcctccttcggctcccgcctgatcaacttccggaactccgacaagaaccacaactgggacacccgcgaggtctac

ccgaccaaggagctggagaagctgctgaaggactactccatcgagtacggccacggcgagtgcatcaaggccgccatctgcggcgagt

ccgacaagaagttcttcgccaagctgacctcggtcctgaacaccatcctgcagatgcgcaactccaagaccggcaccgagctggactacc

tgatctcccccgtcgccgacgtcaacggcaacttcttcgactcccggcaggccccgaagaacatgccccaggacgccgacgccaacggc

gcctaccacatcggcctgaagggcctgatgctgctgggccgcatcaagaacaaccaggaaggcaagaagctcaacctggtcatcaagaa

cgaggagtacttcgagttcgtgcagaaccgcaacaactga

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