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Supplementary Information for

CRISPR-BEST: highly efficient DSB-free base editors for streptomycetes

Yaojun Tong, Christopher M. Whitford, Helene L. Robertsen, Kai Blin, Tue S. Jørgensen, Andreas K. Klitgaard, Tetiana Gren, Xinglin Jiang, Tilmann Weber, Sang Yup Lee

Correspondence should be addressed to: Sang Yup Lee or Tilmann Weber
Email: leesy@kaist.ac.kr or tiwe@biosustain.dtu.dk

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Supplementary Materials and Methods

Strains, plasmids, and culture conditions

The strains and plasmids used in this study are listed in *SI Appendix*, Table S2. Plasmids were maintained in *E. coli* DH5alpha and Mach1 $T1^R$. All *E. coli* strains and *Bacillus subtilis* 168 were grown in LB medium (agar and liquid) at 37 °C. *Streptomyces* strains were grown at 30 °C in either ISP2 (Yeast extract 4 g/l, Malt extract 10 g/l, Dextrose 4 g/l, 20 g/l Agar for solidification) for seed culture and DNA preparation, or in MS-$MgCl_2$ (20 g/l each D-mannitol, soya flour, agar, and 10 mM $MgCl_2$) for sporulation and conjugation. Kirromycin production medium (10 g/l low-fat soy flour, 10 g/l D-mannitol, and 5 g/l $CaCO_3$, dissolved in tap water and pH adjusted to 7.4 prior to autoclaving) was used for kirromycin production assay. Appropriate antibiotics were supplemented as necessary (50 μg/ml apramycin; 50 μg/ml nalidixic acid; 0.5 μg/ml thiostrepton; 25 μg/ml kanamycin; and 25 μg/ml chloramphenicol). *E. coli* ET12567/pUZ8002 was used for conjugating plasmids into streptomycetes as described previously (1).

DNA manipulation

All primers and spacers used in this work are listed in *SI Appendix* Table S3 and *SI Appendix* Table S4, respectively. All kits and enzymes were used according to the manufacturers' recommendations. Standard protocols were used for DNA purification, PCR, and cloning, unless the modifications were indicated. PCR was performed using Phusion High-Fidelity PCR Kit (Thermo Fisher Scientific, USA), and Q5 High-Fidelity DNA Polymerase (New England Biolabs, USA). Digestion was carried out using FastDigest restriction enzymes (Thermo Fisher Scientific, USA). Cloning was carried out using the Gibson Assembly® Master Mix kit and NEBuilder® HiFi DNA Assembly kit (New England Biolabs, USA). Genomic DNA was prepared by the Blood & Cell Culture DNA Kit (QIAGEN, Germany). Mix2Seq kit (Eurofins Genomics, Germany) was used for Sanger sequencing.

Construction of CRISPR-BEST plasmids

All primers used in this study are listed in *SI Appendix* Table S3.

A self-replicating pSG5-based thermosensitive *E.coli-Streptomyces* shuttle vector pGM1190 (2) was used as the backbone plasmid to construct the CRISPR-BEST plasmid (Fig. 1*A*). The sgRNA cassette design is similar to our previous pCRISPR-Cas9 system (3). In order to simplify the 20nt-spacer cloning step and increase the cloning efficiency, we modified the original sgRNA cassette to be compatible with single strand DNA (ssDNA) oligo

bridging method (Fig. 1*B*) by removal of a G from the pGM1190-sgRNA (3) plasmid with primers removalG_F and removalG_R, resulting in plasmid pGM1190-sgRNAnoG. The transcription of the sgRNA is controlled by a constitutive promoter PermE*, and terminated by a *to* terminator. Due to the huge differences of codon usage between streptomycetes and other organisms, the cytidine deaminase rAPOBEC1 (apolipoprotein B mRNA editing enzyme catalytic subunit 1 from *Rattus norvegicus*, genbank accession number: NM_012907.2), the Cas9n (D10A), and the UGI from *Bacillus* phage AR9 (genbank accession number: YP_009283008) and the adenosine deaminase ecTadA (genbank accession number: NP_417054.2) were codon optimized to *S. coelicolor* A3(2) using Genscript's OptimumGene^{TM} algorithm (*SI Appendix* Fig. S4) and then synthesized by Genscript. The stop codon removed rAPOBEC1 was fused to the N-terminus of the start and stop codons removed Cas9n (D10A) using a 16-amino acid flexible linker (SGSETPGTSESATPES, the encoding DNA sequence was also Streptomyces codon optimized). The start codon removed UGI was then fused to the C-terminus of Cas9n (D10A) by a SGGS linker. Gibson assembly was used to assemble the DNA fragment encoding the N-rAPOBEC1-16aa linker-Cas9n-4aa linker-UGI-C fusion protein into *Nde*I and *Xba*I digested pGM1190-sgRNAnoG plasmid, the fusion protein is under control of the thiostrepton inducible *tipA* promoter, resulting in the final pCRISPR-cBEST plasmid. The construction of pCRISPR-aBEST is very similar to the construction of pCRISPR-cBEST. The only difference is that pCRISPR-aBEST does contain a UGI. Both plasmids have been deposited to Addgene (Plasmid #125689 and #131464).

Construction of the multiplexing CRISPR-cBEST plasmid

To facilitate the multiplexing applications of base editing, we modified the CRISPR-cBEST plasmid with following steps. As the codon usage between streptomycetes and *Pseudomonas* is very similar, we decided to use the original *P. aeruginosa csy4* gene (*SI Appendix*, Table S12). First, we amplified *csy4* from the genomic DNA of *P. aeruginosa* PA14. Then we use this fragment to replace the original sgRNA cassette. Second, the above-generated plasmid was further adapted by replacing the *fd* terminator region with the landing pad for the Golden Gate Assembly compatible sgRNA array with SP_{19} promoter (4), followed by a NcoI restriction site, a strong RBS (Registry of Standard Biological Parts: BBa_B0034), a mCherry from pGM1192 (2), an NheI restriction site, and finally a t0 terminator, ending up with the final plasmid pCRISPR-McBEST. Golden Gate assembly was used to clone the sgRNA array.

Single-strand DNA based PCR-free spacer cloning protocol

To use the pCRISPR-BEST for base editing applications, only one cloning step is required, which is the insertion of a 20nt-spacer into the sgRNA scaffold. A ssDNA oligo based, PCR-free method was adopted for spacer cloning in this study. The oligo was designed as CGGTTGGTAGGATCGACGGC**N20**GTTTTAGAGCTAGAAATAGA. As designed, the pCRISPR-BEST plasmid can be linearized by *Nco*I. By mixing the linearized pCRISPR-BEST plasmid and chemically synthesized spacer containing oligo with the NEBuilder (New England Biolabs, USA). The linearized pCRISPR-BEST plasmid then will be bridged by the spacer containing oligo, ending up with the desired pCRISPR-BEST. Mach1™-T1^{R} *E. coli* (Life Technologies, UK) was used for cloning. Because of the high bridging efficiency, 4-8 clones were directly Sanger sequenced using primer "stre_spacer_seq" to screen for the correct constructs. All plasmids generated and used are listed in *SI Appendix*, Table S2.

***In vivo* spacer-matrix design using PatScan**

As two key components of the protospacer-matrix are the positions and the variants of T**C**G**C**A**CC** or T**A**GA**C**A**A** in the 23nt protospacer plus PAM sequence. The pattern of the CRISPR-cBEST matrix is $N_{2\text{-}3}(TC_nGC_nAC_nC_n)N_{12\text{-}11}GG$, where n = 1 to 7, while the pattern of the CRISPR-aBEST matrix is $N_{2\text{-}3}(TA_nGA_nCA_nA_n)N_{12\text{-}11}GG$, where n = 1 to 7. Therefore, in theory, each matrix should contain in total seven pieces of protospacer. PatScanUI (5) (https://patscan.secondarymetabolites.org) was used to locate all possible protospacers in the genome of *S. coelicolor* A3(2). However, in the case of CRISPR-aBEST, we could not find all seven protospacers in the *S. coelciolor* genome sequence data. Each found protospacer was cross-compared with all spacers of *S. coelicolor* found by CRISPy-web (6), then the ones with less off-target effects were manually checked if they are inside of essential genes or not, based on the genome annotation of *S. coelicolor* A3(2). Base on the following rules for matrix protospacer selection: not in essential gene; all from the same DNA strand; not located too close to the chromosome end; less off-target effects; and if possible, select the ones sharing the same PAM sequence, seven and six pieces of protospacers for CRISPR-cBEST matrix and CRISPR-aBEST matrix were finally selected, respectively (Fig. 2*A,* Fig. 2*C* and *SI Appendix*, Table S4).

CRISPR-BEST support in CRISPy-web

For the updated CRISPy-web, sgRNAs are identified using the regular CRISPy-web algorithm published previously (6). All sgRNAs in the region of interest where the potential edit window overlaps with an annotated CDS region are then selected for CRISPR-BEST analysis. The CDSs with overlap to the sgRNA edit windows

are split into individual codons. The codons are filtered for overlaps with the edit window again. For sgRNAs on the same strand as the CDS, all possible C to T mutations for CRISPR-cBEST and A to G mutations for CRISPR-aBEST are recorded, for sgRNAs on the opposite strand, accordingly, all possible G to A mutations and T to C mutations are recorded. Non-conservative mutations changing the encoded amino acid are finally reported in the CRISPy-web interface.

CRISPR-cBEST compatible protospacers identification using CRISPy-web

The procedure is based on our previous report (6). Briefly, a custom genome or an antiSMASH generated job id needs to be uploaded to CRISPy-web (https://crispy.secondarymetabolites.org). Taking *kirN* of *S. collinus* Tü365 as an example (*SI Appendix*, Fig. 5*A*), all possible protospacers from both DNA strands will be displayed for the *kirN* gene (*SI Appendix*, Fig. 5*A*). By choosing the "CRISPR-BEST mode" box, it goes to a new page with three more options, "C to T", "A to G", and "Show only STOP mutations" (*SI Appendix*, Fig. 5*B*). If one wants to have all CRISPR-aBEST compatible spacers displayed, click "A to G" button, then all possible amino acid substitutions will be displayed as well (*SI Appendix*, Fig. 5*E*). If one needs to design CRISPR-cBEST compatible sgRNAs, click "C to T" button, then an additional option is available, "Show only STOP mutations" (*SI Appendix*, Fig. 5*C*). By subsequentially choosing this box, all possible STOP codon introductions will be displayed (*SI Appendix*, Fig. 5*D*), and the selected protospacers can be downloaded as CSV file by clicking the shopping basket located in the up-right corner.

In-frame deletion of *kirN* using CRISPR-Cas9 based homologous recombination strategy

The in-frame deletion of *kirN* in *S. collinus* Tü365 using CRISPR-Cas9 based homologous recombination approach was carried out as we described in (7). USER cloning approach was used for the sgRNA cloning (7). The 20nt spacer region GATCGCATTTCGCCAACTAC that specifically targeted on *kirN* was predicted by CRISPy-web (6). The 462 bp sgRNA-*kirN* cassette was then designed and ordered as a gBlocks® Gene Fragment from IDT (Integrated DNA Technologies, US), the full sequence of this gBlock can be found in (*SI Appendix*, Table S5). The directional assembly of the sgRNA and the two 1kb editing templates, interspaced by the promoter PermE*, in the linearized pCRISPR-USER-Cas9 was ensured by the uracil-containing overhangs generated by PCR amplification with primer pair pHR1/pHR2 for the sgRNA gBlocks® gene fragment, pHR3/pHR4 for the promoter PermE*, and primer pairs pHR5/pHR6 and pHR7/pHR8 for the 1kb editing templates up- and down-stream of *kirN*, respectively. The 1 kb editing templates were amplified from genomic DNA of *S. collinus* Tü365.

Upon the USER assembly, correct clones of pCRISPR-Δ*kirN* were identified with control PCR with pHR9/pHR10 and confirmed by Sanger sequencing with primer pairs pHR9 and pHR13. The resulting pCRISPR-Δ*kirN* was introduced into *S. collinus* Tü365 by intergeneric conjugation as reported previously (1).

Validating base pair changes by Sanger sequencing

First, primers that can amplify a several-hundred base pairs region containing the base editing window were designed (*SI Appendix*, Table S3). Secondly, colony PCR approach was used to amplify the designed regions directly from streptomycete colonies. The protocol was modified from our previous publication (3): about four-square-millimeter actively growing mycelia (for example, 3-day old *S. coelicolor*, before sporulation) of the selected colonies were scraped from the agar plate using a sterile toothpick into 20 µl pure DMSO in PCR tubes. The tubes were top-speed shaken and boiled for 20 min at 100 °C in a heating block. After cooling down to room temperature, the solution was centrifuged at top speed for 30 seconds, 1 µl of the supernatant was used as the PCR template in a 20 µl-reaction with Q5 High-Fidelity DNA Polymerase (New England Biolabs, US). Lastly, the PCR products were cleaned up by GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) and then Sanger sequenced by Mix2Seq kit (Eurofins Genomics, Germany).

Genome-wide off-targets identification of CRISPR-BEST in streptomycetes

Illumina sequencing libraries were constructed from each strain using the HyperPlus library preparation kit from KAPA (Roche, Basel, Switzerland) according to protocol, but in half volume. Input DNA from each strain was adjusted to 2 ng/ul and 11 PCR cycles were run with multiplex indexing primers from Pentabase (PentaBase Aps, Denmark). Seramag Select magnetic beads (GE healthcare, USA) were used to size select ca 600 nt fragments prior to PCR amplification. Libraries were quantified with a Fragment Analyzer (Agilent Technologies, USA) and qubit (Thermo Fisher Scientific, USA). Libraries were sequenced on a single Illumina MiSeq 2x150nt flowcell. Raw sequencing reads were adapter and quality trimmed using AdapterRemoval (v2.1.17) (8) with the switches --trimns –trimqualities. The WT coelicolor genome was polished using the polishing module of Unicycler (v0.4.8-beta) and the programs ALE (9), pilon (v1.22) (10), minimap2 (v2.16-r922) (11), bowtie2(v2.3.5) (12), and samtools (v1.9) (13). Prokka (v1.13) (14) was used for annotation of the polished genome. Breseq (v0.33.2) (15) was used for SNP calling with the parameters –p and --polymorphism-frequency-cutoff 0.2 to allow variants existing in 20% to 100% of the reads. Bcftools from the Samtools package was used

to summarise the SNP data. All raw data and the polished genome sequence of *S. coelicolor* WT_{NBC} have been deposited at NCBI under project accession PRJNA557658.

Illumina whole genome sequencing and analysis of *S. collinus* strains

Illumina sequencing was carried out as we described before (3). Briefly, a 10 ml five days old *S. collinus* cluture was used for genomic DNA isolation. The genomic library was generated using the TruSeq ®Nano DNA LT Sample Preparation Kit (Illumina Inc., USA). The whole genome sequencing protocol was carried out as we described in (3). The reads obtained from the Illumina sequencing were mapped to the WT *S. collinus* Tü365 reference genome (Genome Accession: CP006259) (16) using the software BWA (17) with the BWA-mem algorithm. The data was inspected and visualized using readXplorer (18) and Artemis (19).

Kirromycin fermentation and chemical analysis

The protocol was modified from (20). Four-day old seed cultures (grown in ISP2), normalized according to wet weight, were inoculated into kirromycin production medium ending up with in total 50 ml in 250 ml-shake flasks. The fermentations were carried out for six days at 30°C in a rotary shaker at 180 rpm. 30 ml of each culture was extracted with 1:1 ethyl acetate for 2 h using a magnetic stirrer at room temperature. The extracts were then dried, re-dissolved in 200 µl methanol, and stored in -20°C for further applications.

LC-MS analysis was performed using an ultra-high-performance liquid chromatography (UHPLC) UV/Vis diode array detector (DAD) high-resolution mass spectrometer (HRMS) Orbitrap Fusion mass spectrometer connected to a Dionex Ultimate 3000 UHPLC pumping system (Thermo Fisher Scientific, USA). UV-Vis detection was done using a DAD-3000 set to the range 190 nm - 700 nm. Injections of 3 µL of each sample was separated using an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm) (Waters, USA) at a flow rate of 0.4 ml/min, and a temperature of 30.0 °C. Mobile phases A and B were 0.1 % formic acid in water and acetonitrile, respectively. Elution was performed with a 30 min multistep system. After 5 % B for 1 min, a linear gradient started from 5 % B to 100 % B in 21 min, which was held for another 5 min and followed by re-equilibration to 5 % B until 30 min. HRMS was performed in ESI- from a range (*m/z*) 200 to 2,000 at a resolution of 120,000, RF Lens 60 %, and AGC target 5.0e4.

Data analyses were performed with the software Xcalibur 3.1.2412.17 (Thermo Fisher Scientific, USA).

Bioactivity assay of kirromycin

Wild type *Bacillus subtilis* 168 was used as indicator strain. An overnight *B. subtilis* colony of approximately four-square-millimeter was transferred from LB agar plate into 1 ml LB liquid medium in a 1.5 ml Eppendorf tube. The suspension was mixed by vortexing. 200 µl of the above suspension was plated onto a LB agar plate, air drying for 5 min under sterile conditions. Sterilized paper disks were placed onto the *B. subtilis* plated LB agar plate, then 20 µl of each exact was added onto the paper disks. The resulting LB plate was incubated at 37°C incubator for 24 h, the image was taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany).

Assay for actinorhodin extraction

Exconjugants were picked and streaked onto apramycin containing ISP2 agar plate and incubated for five days at 30 °C. The photos of related plates were taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany). Actinorhodin extraction assay was carried as follows: 10 ml of seven days old *S. coelicolor* ISP2 culture was mixed 1:1 with 1 N NaOH. Extracting for 4 h using a magnetic stirrer at room temperature. The suspensions were centrifuged at 10,000 g for 5 min, the supernatants were transferred into PCR tubes. All tubes were placed under the same filed for photo taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany), so that their color could be directly compared.

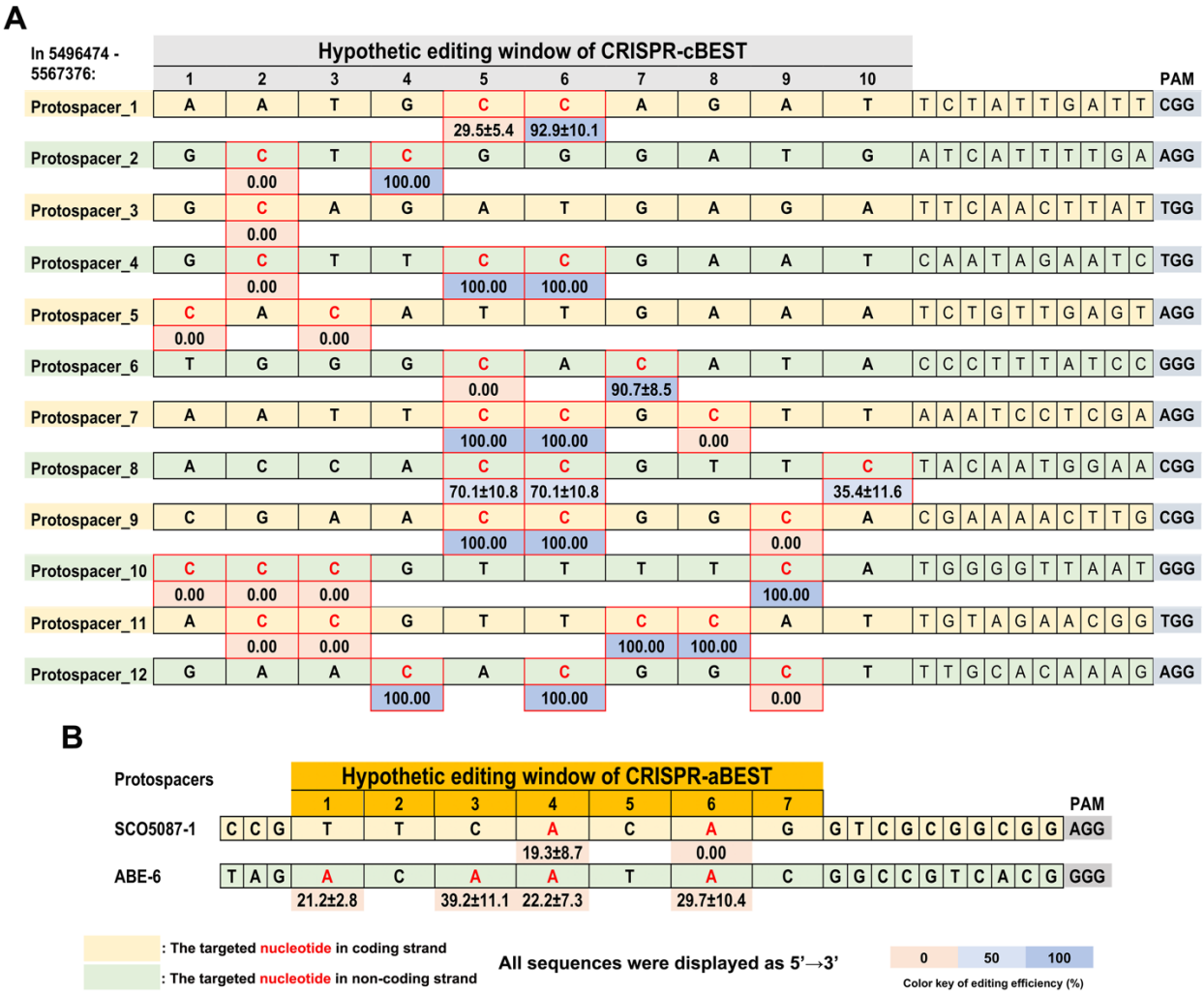

Fig. S1. CRISPR-BEST evaluation by targeting the selected protospacers from *S. coelicolor* A3(2) genome.

A. Results for CRISPR-cBEST. **B.** Results for CRISPR-aBEST.

The entire 20nt protospacer plus 3nt PAM is displayed in 5' to 3' direction. The cytidines in the 10-nt hypothetic editing window are highlighted in red. The targeted C in coding and non-coding strand showed as light yellow and light green, respectively. The editing efficiencies are shown as the mean and standard deviation. PCR fragments of five to ten exconjugants from each of two to three independent conjugations were subjected to Sanger sequencing.

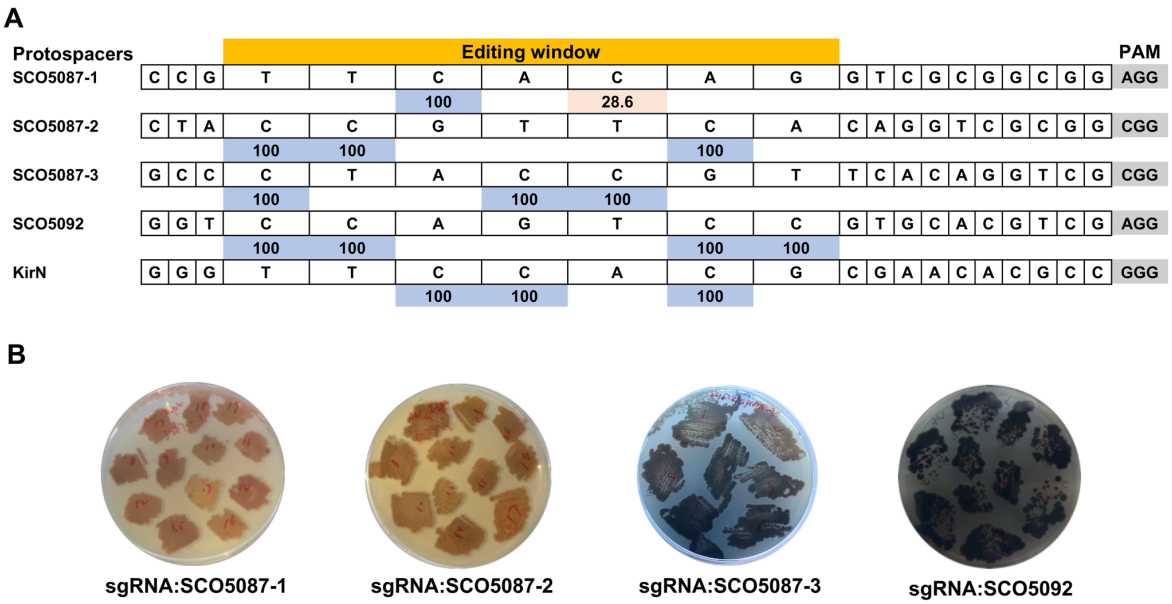

Fig. S2. Editing efficiency of different protospacers.

(*A*) The entire 20nt protospacer plus 3nt PAM is displayed. The 7nt editing window is shown in yellow. The numbers indicate the editing efficiency of the target cytidine. (*B*) Seven to twelve of the exconjugants with different sgRNAs were randomly picked and streaked onto the apramycin and nalidixic acid containing ISP2 plates, and incubated for five days at 30 °C. The photos were taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany) at day 6.

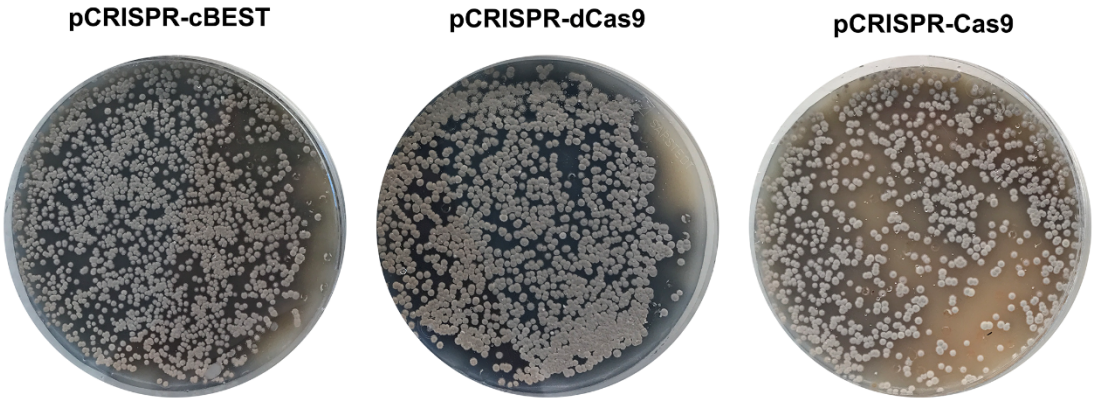

Fig. S3. The conjugation results of three plasmids carrying sgRNA from the same region.

The photos were taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany) at day 5 after antibiotics overlaying.

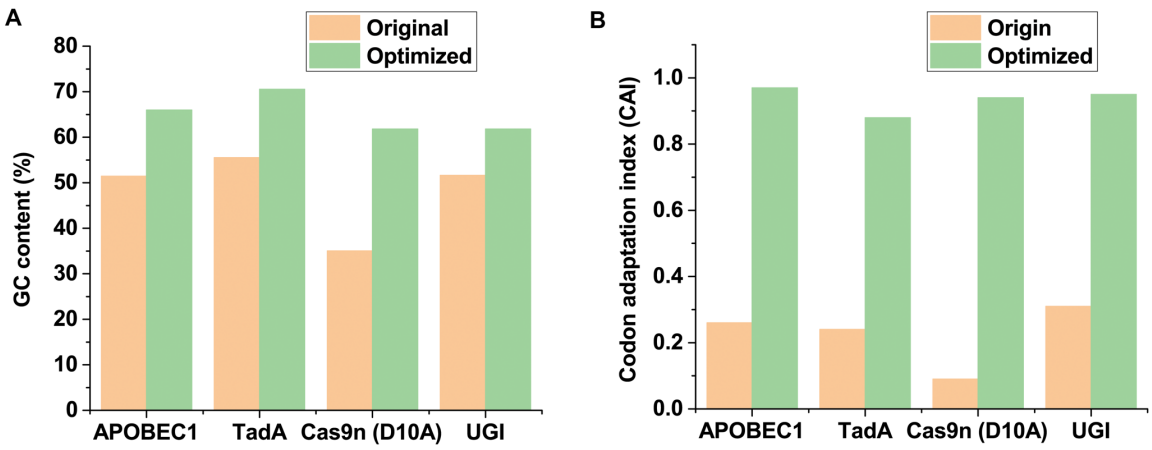

Fig. S4. **Codon optimization results of the four proteins**.

(*A*) The GC content changes of APOBEC1, TadA, Cas9n, and UGI before and after codon optimization using *S. coelicolor* A3(2) codon usage frequency. (*B*) The codon adaptation index (CAI) changes of APOBEC1, TadA, Cas9n, and UGI before and after codon optimization using *S. coelicolor* A3(2) codon usage frequency.

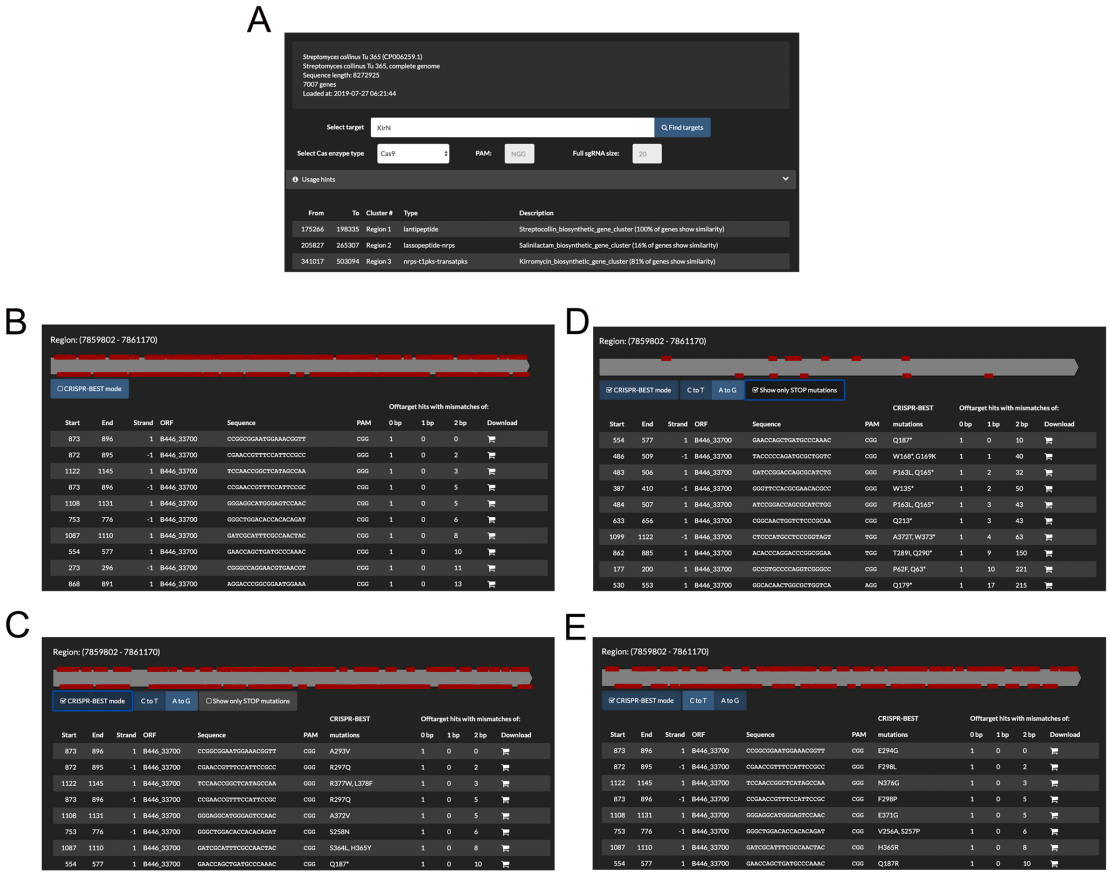

Fig. S5. An example output of CRISPy-web run to identify CRISPR-cBEST compatible protospacers in the *kirN* gene of *S. collinus* Tü365.

(*A*) After genome sequence uploaded, the overview of protospacers identified in the BGCs of *S. collinus* Tü365 was displayed. One can search the gene-of-interest, for example, this case was *kirN*. (*B*) By clicking "Find Targets" button, a zoom view of protospacers for *kirN* was shown. A new box named "CRISPR-BEST mode" was available as well. (*C*) After clicking the "CRISPR-BEST mode" box, the default setting showed users the selected view of CRISPR-cBEST compatible protospacers. (*D*) By further selecting the "Show only STOP mutations" box, a view of the protospacers that could introduce STOP codons into *kirN* gene were displayed. (*E*) Either from step (*C*) or step (*D*), users could click "A to G" button, to display the CRISPR-aBEST compatible protospacers.

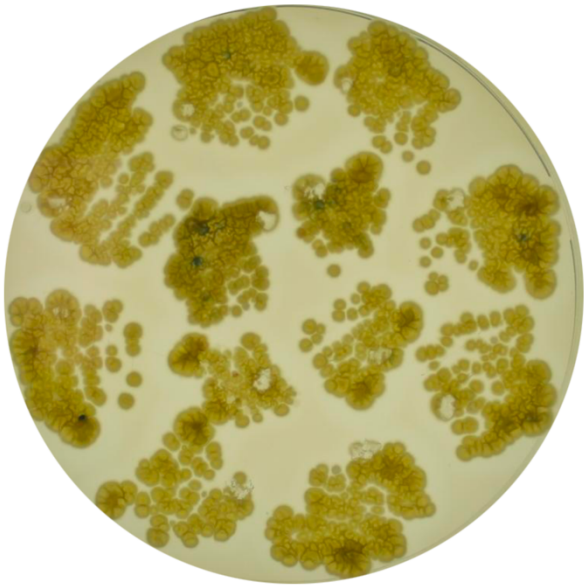

Fig. S6. Randomly picked exconjugants bearing pCRISPR-McBEST-ScoTri.

Twelve pCRISPR-McBEST-ScoTri conjugated exconjugants were randomly picked and streaked onto the apramycin and nalidixic acid containing ISP2 plates, and incubated for five days at 30 °C. The photos were taken by a ColonyDoc-It™ Imaging Station (Analytik Jena AG, Germany) at day 6.

Table S1[a]. A direct comparison between CRISPR-BEST and CRISPR-(d)Cas9.

	CRISPR-Cas9	CRISPR-dCas9 (CRISPRi)	CRISPR-BEST (Base editor)
Conjugation efficiency[b]	Medium	Medium	High
Editing efficiency[c]	High	High	High
Spacer cloning (day)	3-5	3-5	1-2
Editing templates cloning (day)	8-15	Not required	Not required
Null mutant generation	Yes	No	Yes
Stress on chromosome	High	Low	Low
SNP generation	Hard	No	Easy
Editing repeats	No[d]	Yes	Yes

a: Information of this table compiled from (3) and this study; b: Please refer to *SI Appendix*, Fig. S3; c: Editing efficiency of CRISPR-(d)Cas9 is sgRNA dependent (3), editing efficiency of CRISPR-BEST is also sgRNA dependent, but additional constraints apply (see main text and Fig. 2); d: Only one case (*kirN* in *S. collinus* Tü365) was tested in this study. It might be strain dependent.

Table S2. Strains and plasmids used/generated in this study.

Strains	Description	Source
***E. coli* DH5alpha**	For routine plasmids maintenance and cloning	New England Biolabs
***E. coli* Mach1™-T1R**	For routine plasmids maintenance and cloning	Thermo Fisher Scientific
***E. coli* ET12567/pUZ8002**	For conjugating plasmids into streptomycetes	Maintained in lab
***Bacillus subtilis* 168**	For kirromycin bioactivity testing	ATCC
***S. coelicolor* WT_{NBC}, CFB_NBC_0001**	Wild type strain of *S. coelicolor* A3(2), some mutations have been accumulated during time, therefore, a new name *S. coelicolor* WT_{NBC} was given and used in this study	Maintained in lab
ΔSCO5087 (Q91*)	Base edited *S. coelicolor* A3(2), a stop codon was introduced in SCO5087	This study
SCO5087 (R89C, S90L)	Base edited *S. coelicolor* A3(2), two amino acids were exchanged in SCO5087	This study
SCO5087 (R89C)	Base edited *S. coelicolor* A3(2), an amino acid was exchanged in SCO5087	This study
ΔSCO5092 (Q136*)	Base edited *S. coelicolor* A3(2), a stop codon was introduced in SCO5092	This study
Tri-edited *S. coelicolor*	Multiplexed base edited *S. coelicolor* A3(2) strain, stop codons were introduced in SCO3230 (W3361*) and SCO5892 (Q495*), and a rare leucin codon in SCO5087 (S90L)	This study
***S. collinus* Tü365**	Wild type strain of *S. collinus*	Maintained in lab
Δ*kirN*-Cas9	*S. collinus* Tü365 with pCRISPR-ΔkirN	This study
ΔkirN (W135*)-1	Base edited *S. collinus* Tü365, a stop codon was introduced in KirN, clone no. 1	This study
ΔkirN (W135*)-2	Base edited *S. collinus* Tü365, a stop codon was introduced in KirN, clone no. 2	This study
Tü365_Empty vector	*S. collinus* Tü365 with pCRISPR-cBEST without any spacer coloned	This study
***Streptomyces griseofuscus* DSM40191**	For cluster inactivation in a non-standard *Streptomyces*	DSMZ
Plasmids	**Description**	**Source**
pJET1.2	For cloning PCR fragments of Sanger sequencing	Thermo Fisher Scientific
pCRISPR-USER-Cas9	Empty vector, USER cloning compatible pCRISPR-Cas9	(7)
pCRISPR-McBEST	Based on pCRISPR-cBEST, sgRNA scaffold replaced with *csy4* from *P. aeruginosa*; *fd* terminator replaced with P_{SP19}, RBS, mCherry, and t0 terminator	This study
pCRISPR-Δ*kirN*	pCRISPR-Cas9 with KirN spacer and editing templates for KirN deletion	This study
pCRISPR-cBEST	Empty vector, cytidine deaminase based base editor	This study
pCRISPR-cBEST-KirN	pCRISPR-cBEST with sgRNA:KirN	This study
pCRISPR-cBEST-5087-1	pCRISPR-cBEST with sgRNA:SCO5087-1	This study
pCRISPR-cBEST-5087-2	pCRISPR-cBEST with sgRNA:SCO5087-2	This study
pCRISPR-cBEST-5087-3	pCRISPR-cBEST with sgRNA:SCO5087-3	This study
pCRISPR-cBEST-5092	pCRISPR-cBEST with sgRNA:SCO5092	This study
pCRISPR-cBEST-Matrix_1	pCRISPR-cBEST with sgRNA:Matrix1	This study

pCRISPR-cBEST-Matrix_2	pCRISPR-cBEST with sgRNA:Matrix2	This study
pCRISPR-cBEST-Matrix_3	pCRISPR-cBEST with sgRNA:Matrix3	This study
pCRISPR-cBEST-Matrix_4	pCRISPR-cBEST with sgRNA:Matrix4	This study
pCRISPR-cBEST-Matrix_5	pCRISPR-cBEST with sgRNA:Matrix5	This study
pCRISPR-cBEST-Matrix_6	pCRISPR-cBEST with sgRNA:Matrix6	This study
pCRISPR-cBEST-Matrix_7	pCRISPR-cBEST with sgRNA:Matrix7	This study
pCRISPR-cBEST-spacer_1	pCRISPR-cBEST with sgRNA:spacer1	This study
pCRISPR-cBEST-spacer_2	pCRISPR-cBEST with sgRNA:spacer2	This study
pCRISPR-cBEST-spacer_3	pCRISPR-cBEST with sgRNA:spacer3	This study
pCRISPR-cBEST-spacer_4	pCRISPR-cBEST with sgRNA:spacer4	This study
pCRISPR-cBEST-spacer_5	pCRISPR-cBEST with sgRNA:spacer5	This study
pCRISPR-cBEST-spacer_6	pCRISPR-cBEST with sgRNA:spacer6	This study
pCRISPR-cBEST-spacer_7	pCRISPR-cBEST with sgRNA:spacer7	This study
pCRISPR-cBEST-spacer_8	pCRISPR-cBEST with sgRNA:spacer8	This study
pCRISPR-cBEST-spacer_9	pCRISPR-cBEST with sgRNA:spacer9	This study
pCRISPR-cBEST-spacer_10	pCRISPR-cBEST with sgRNA:spacer10	This study
pCRISPR-cBEST-spacer_11	pCRISPR-cBEST with sgRNA:spacer11	This study
pCRISPR-cBEST-spacer_12	pCRISPR-cBEST with sgRNA:spacer12	This study
pCRISPR-aBEST-Matrix_1	pCRISPR-aBEST with sgRNA:Matrix1	This study
pCRISPR-aBEST-Matrix_2	pCRISPR-aBEST with sgRNA:Matrix2	This study
pCRISPR-aBEST-Matrix_3	pCRISPR-aBEST with sgRNA:Matrix3	This study
pCRISPR-aBEST-Matrix_4	pCRISPR-aBEST with sgRNA:Matrix4	This study
pCRISPR-aBEST-Matrix_5	pCRISPR-aBEST with sgRNA:Matrix5	This study
pCRISPR-aBEST-Matrix_6	pCRISPR-aBEST with sgRNA:Matrix6	This study
pCRISPR-aBEST-Matrix_7	pCRISPR-aBEST with sgRNA:Matrix7	This study
pCRISPR-McBEST-ScoTri	pCRISPR-MCBE targeting SCO5087, SCO3230, and SCO5892 in *S. coelicolor* A3(2)	This study

Table S3. Primers used in this study.
Forward primers were used as Sanger sequencing primers unless specifically indicated.

Names	sequence (5' to 3')	Purpose
ssDNA_spacer1	CGGTTGGTAGGATCGACGGCAATGCCAGATTCTATTGATTGTTTTAGAGCTAGAAATAGA	For spacer cloning using ssDNA oligo bridging For CRISPR-BEST validation
ssDNA_spacer2	CGGTTGGTAGGATCGACGGCGCTCGGGATGATCATTTTGAGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer3	CGGTTGGTAGGATCGACGGCGCAGATGAGATTCAACTTATGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer4	CGGTTGGTAGGATCGACGGCGCTTCCGAATCAATAGAATCGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer5	CGGTTGGTAGGATCGACGGCCACATTGAAATCTGTTGAGTGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer6	CGGTTGGTAGGATCGACGGCTGGGCACATACCCTTTATCCGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer7	CGGTTGGTAGGATCGACGGCAATTCCGCTTAAATCCTCGAGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer8	CGGTTGGTAGGATCGACGGCCGAACCGGCACGAAAACTTGGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer9	CGGTTGGTAGGATCGACGGCACCACCGTTCTACAATGGAAGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer10	CGGTTGGTAGGATCGACGGCCCCGTTTTCATGGGGTTAATGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer11	CGGTTGGTAGGATCGACGGCGCTCGGGATGATCATTTTGAGTTTTAGAGCTAGAAATAGA	
ssDNA_spacer12	CGGTTGGTAGGATCGACGGCGAACACGGCTTTGCACAAAGGTTTTAGAGCTAGAAATAGA	
ssDNA_ABE6	CGGTTGGTAGGATCGACGGCTAGACAATACGGCCGTCACGGTTTTAGAGCTAGAAATAGA	
Check_F1	CTCGACACCATGATCGTGCG	For amplifying sequencing fragment containing spacers 1, 3, 4, 5, 7
Check_R1	CTCGTCGATCAGGGCGAAGT	
Check_F2	TGGCTCGACCAGGACGTA	For amplifying sequencing fragment containing spacers 6, 10
Check_R2	AGACCTCCACCAGCAGGA	
Check_F3	ATCGTATGGCATGAACGGGC	For amplifying sequencing fragment containing spacer 8, 11
Check_R3	ACGGTGATCCACTGGATG	
Check_F4	TCCTTCAGTCGTTTCGGC	For amplifying sequencing fragment containing spacers 2, 9
Check_R4	GCAGCGACGTGTCGAACT	
Check_F5	TACCACCTGCCCGTCAGGTA	For amplifying sequencing fragment containing spacers 12
Check_R5	GACCGTGCTGTCGTTCATCG	
Matrix_oligo_1	CGGTTGGTAGGATCGACGGCGCTC1GC3AC5C6CAAGGACGCAGGTTTTAGAGCTAGAAATAGA	

Matrix_oligo_2	CGGTTGGTAGGATCGACGGCCGTTC2GC4AC6C7GGGGGACGCGGTTTTAGAGCTAGAAATAGA	For spacer cloning using ssDNA oligo bridging For CRISPR-BEST characterization
Matrix_oligo_3	CGGTTGGTAGGATCGACGGCAGCC1TC3GC5AC7TGCTCCAGTTGTTTTAGAGCTAGAAATAGA	
Matrix_oligo_4	CGGTTGGTAGGATCGACGGCCAAC1C2TC4GC6TCCCTCCTCCAGTTTTAGAGCTAGAAATAGA	
Matrix_oligo_5	CGGTTGGTAGGATCGACGGCGATAC2C3TC5GC7CACATCGCATGTTTTAGAGCTAGAAATAGA	
Matrix_oligo_6	CGGTTGGTAGGATCGACGGCCCGC1AC3C4TC6ACGGTTGGGTGGTTTTAGAGCTAGAAATAGA	
Matrix_oligo_7	CGGTTGGTAGGATCGACGGCTCTGC2AC4C5TC7ACCCAACCGGGTTTTAGAGCTAGAAATAGA	
Matrix_test_1F	TTCGTGCGGAGCCGTTCATC	For amplifying sequencing fragment containing spacers of the Matrix
Matrix_test_1R	ACACGGGACTCGGTCACAGA	
Matrix_test_2F	ATCGACGACGCGGACTTCTC	
Matrix_test_2R	ATAGGCCGTTGAGGCGCTGA	
Matrix_test_3F	TCTACGAACTCACCTGGCCC	
Matrix_test_3R	GAACAGGCCCAGCAGGAGT	
Matrix_test_4F	GTACCTCGTCGGCACCATC	
Matrix_test_4R	CCGGTGAGCAGTTCCTCGT	
Matrix_test_5F	TATTCAGGGCGTACAGGTAG	
Matrix_test_5R	TGGGCGAACGTCGTCGAATT	
Matrix_test_6F	GATGGTCTCGACGGGACTC	
Matrix_test_6R	GGCATTCTGCTGACTCCGC	
Matrix_test_7F	AGTGTCAACGCGTGGCACG	
Matrix_test_7R	CTGTGCGCGTGCACCAGAT	
ssDNA_SCO5087-1	CGGTTGGTAGGATCGACGGCCCGTTCACAGGTCGCGGCGGGTTTTAGAGCTAGAAATAGA	For spacer cloning using ssDNA oligo bridging Spacers were from SCO5087 and SCO5092
ssDNA_SCO5087-2	CGGTTGGTAGGATCGACGGCCTACCGTTCACAGGTCGCGGGTTTTAGAGCTAGAAATAGA	
ssDNA_SCO5087-3	CGGTTGGTAGGATCGACGGCGCCCTACCGTTCACAGGTCGGTTTTAGAGCTAGAAATAGA	
ssDNA_SCO5092	CGGTTGGTAGGATCGACGGCGGTCCAGTCCGTGCACGTCGGTTTTAGAGCTAGAAATAGA	
5087-check_F	CCGACGATGACGACGACCAC	For amplifying sequencing fragment containing spacers of SCO5087 or SCO5092
5087-check_R	CGCTGGGCACCAGGTAGTC	
5092-check_F	GTTCGTTCGTGTCCGTCTCG	

5092-check_R	GGCTGCTCAACCACCTGACC	
ssDNA_kirN1	CGGTTGGTAGGATCGACGGCGGGTTCCACGCGAACACGCCGTTTTAGAGCTAGAAATAGA	For spacer cloning using ssDNA oligo bridging Spacer was from KirN
KirN_checkF	CGGCACGACCTCCCCTAC	For amplifying sequencing fragment containing KirN spacer
KirN_checkR	CGAACCGTTTCCATTCCGC	
stre_spacer_seq	TGTACGCGGTCGATCTTGA	For validation of spacer cloning
removalG_F	ACGGCCATGGTTTTAGAGC	For one G removal of sgRNA cassette from pCRISPR-Cas9
removalG_R	GGAACATCGTAGCTGACG	
pHR1	CGTGCGAUGCTAGCAAAGCGGTCGAT	For pCRISPR-Δ*kirN* construction and validation
pHR2	AGGTGACCUCAGAACTCCATCTGGATTTGTTC	
pHR3	ACAGGCGGUCGATCTTGACGGCTGGCG	
pHR4	AGGTCTTCGUCGGCCGTCGATCCTACCAAC	
pHR5	AGGTCACCUGAGGCGACCCCGACCAG	
pHR6	ACCGCCTGUGCTCCTCCGTGTACGCGA	
pHR7	ACGAAGACCUCGTCACGGTCCACTCCGA	
pHR8	CACGCGAUATCTCCGACGACGCGTGG	
pHR9	GGCGGCAACGCAGCG	
pHR10	CGTACCGCTTCGGGCC	
pHR11	GGACCGCCATGAGACCG	
pHR12	GCGTGGTGGCCGGAG	
pHR13	GTGCTCCTCCGTGTACGCG	
ABE_matrix_oligo_1	CGGTTGGTAGGATCGACGGCTCTAGACAAACCCAACATAGGTTTTAGAGCTAGAAATAGA	For spacer cloning using ssDNA oligo bridging for CRISPR-aBEST validation
ABE_matrix_oligo_2	CGGTTGGTAGGATCGACGGCAGCTAGACAACGTGTGAGCCGTTTTAGAGCTAGAAATAGA	
ABE_matrix_oligo_3	CGGTTGGTAGGATCGACGGCGGAATAGATCCCCGGTCGCGGTTTTAGAGCTAGAAATAGA	
ABE_matrix_oligo_4	CGGTTGGTAGGATCGACGGCCTCAATAGACTTGTTGCGATGTTTTAGAGCTAGAAATAGA	
ABE_matrix_oligo_5	CGGTTGGTAGGATCGACGGCCCTCAATAGACTTGTTGCGAGTTTTAGAGCTAGAAATAGA	
ABE_matrix_oligo_6	CGGTTGGTAGGATCGACGGCCATGACAATACGGCGCCGCCGTTTTAGAGCTAGAAATAGA	

ABE-mtrx1-coel-fwd	GGCCTCATCCTCATCAGGTC	For amplifying sequencing fragment containing spacers of the ABE Matrix
ABE-mtrx1-coel-rev	CGTCATGGCGTCATTCTGCG	
ABE-mtrx2-coel-fwd	GCCTGAAGGATGTCGTAGAAC	
ABE-mtrx2-coel-rev	GGAACCAGACAACTGACTGC	
ABE-mtrx3-coel-fwd	CTCACGGGTGCGTCCGTCAC	
ABE-mtrx3-coel-rev	CCGTCCTGACCACGGGGTTC	
ABE-mtrx4-coel-fwd	GTCGAGGAGAGCGGAGATCG	
ABE-mtrx4-coel-rev	CACTCCATTGCAACGACAAC	
ABE-mtrx6-coel-fwd	CATCGCCGTGTTTCGACCGG	
ABE-mtrx6-coel-rev	ATCTGTTCGCGCAGCGAGTG	
ABE-mtrx7-coel-fwd	CACCACGTGAGATGCCCCGT	
ABE-mtrx7-coel-rev	CGGCAAGGAGAGGCTTGTTC	
CW138_CBE-cl2	CGGTTGGTAGGATCGACGGCCGCACCGTCCAACCCAGCAGGTTTTAGAGCTAGAAATAGC	For spacer cloning using ssDNA oligo bridging, *S. griseofuscus* pCRISPR-cBEST plasmids
CW140_CBE-cl5	CGGTTGGTAGGATCGACGGCACCGCCCGAATAGTCCTTGAGTTTTAGAGCTAGAAATAGC	
CW141_CBE-cl6	CGGTTGGTAGGATCGACGGCGCGATCCAGTCCTCGGTCTTGTTTTAGAGCTAGAAATAGC	
CW143_CBE-cl31	CGGTTGGTAGGATCGACGGCTTCCAGCCGTGTTCCATCGCGTTTTAGAGCTAGAAATAGC	
CW151_CBE-cl2-check_fwd	GACGCTGTGTCCCAGGACG	For amplifying sequencing fragments of the edited clusters in *S. griseofuscus*
CW152_CBE-cl2-check-rev	CGAACTCCTCGGCAGGGAC	
CW155_CBE-cl5-check_fwd	GTAGATCACATAGGCAGGCG	
CW156_CBE-cl5-check_rev	TGCCGATCGACCCTGACTAC	
CW157_CBE-cl6-check_fwd	GTTCTGCTCGCTGTGCTACC	
CW158_CBE-cl6-check_rev	CGAGTGGCTCCATGGTGGTC	
CW161_CBE-cl31-check_fwd	GTGACCTGAACTCGTAGCGG	
CW162_CBE-cl31-check_rev	CGTAGACGGTGATGGCCTTG	
CW132_sgRNA-SCO5087-fwd	GATCAGGTCTCGCATGGTTCACTGCCGTATAGGCAGCTAAGAAAC	
	CGTTCACAGGTCGCGGCGGGTTTTAGAGCTAGAAATAGCAAGT	

CW133_sgRNA-SCO5087-rev	GATCGGGTCTCACCGCTTTCTTAGCTGCCTATACGG	
CW134_sgRNA-SCO3230-fwd	GATCGGGTCTCAGCGGCGAACCAGCCCATCATGTTTTAGAGCTAGAAATAGCAAGT	Primers for construction of the multiplexed CBE plasmid for *S. coelicolor* A3(2)
CW135_sgRNA-SCO3230-rev	GATCGGGTCTCAGGGGTTTCTTAGCTGCCTATACGG	
CW136_sgRNA-SCO5892-fwd	GATCAGGTCTCACCCCCAGGACGTGGAACAGAGTTTTAGAGCTAGAAATAGCAAGT	
CW137_sgRNA-SCO5892-rev	GATCAGGTCTCGCTAGTTTCTTAGCTGCCTATACGG	
	TGCGGGACGTCTTCGAACAC	
3230-check_F	TACGGCGTCGAAGCGGACCA	
3230-check_R	AGGTCTTCGGCACCCGGATC	For amplifying sequencing fragments of the multiplexed CBE edited *S. coelicolor* A3(2)
5892-check_F	AGTTCCAGGTCGAGCAGGCG	
5892-check_R		

Table S4. The spacers used in this study.

Names	Protospacer sequence (5' to 3')	PAM
Spacer1	AATGCCAGATTCTATTGATT	CGG
Spacer2	GCTCGGGATGATCATTTTGA	GGG
Spacer3	GCAGATGAGATTCAACTTAT	TGG
Spacer4	GCTTCCGAATCAATAGAATC	TGG
Spacer5	CACATTGAAATCTGTTGAGT	AGG
Spacer6	TGGGCACATACCCTTTATCC	GGG
Spacer7	AATTCCGCTTAAATCCTCGA	AGG
Spacer8	CGAACCGGCACGAAAACTTG	CGG
Spacer9	ACCACCGTTCTACAATGGAA	CGG
Spacer10	CCCGTTTTCATGGGGTTAAT	GGG
Spacer11	GCTCGGGATGATCATTTTGA	AGG
Spacer12	GAACACGGCTTTGCACAAAG	AGG
SCO5087-1	CCGTTCACAGGTCGCGGCGG	AGG
SCO5087-2	CTACCGTTCACAGGTCGCGG	CGG
SCO5087-3	GCCCTACCGTTCACAGGTCG	CGG
SCO5092	GGTCCAGTCCGTGCACGTCG	AGG
CBE_matrix_1	GCTCGCACCCAAGGACGCAG	CGG
CBE_matrix_2	CGTTCGCACCGGGGGACGCG	CGG
CBE_matrix_3	AGCCTCGCACTGCTCCAGTT	CGG
CBE_matrix_4	CAACCTCGCTCCCTCCTCCA	TGG
CBE_matrix_5	GATACCTCGCCACATCGCAT	CGG
CBE_matrix_6	CCGCACCTCACGGTTGGGTG	CGG
CBE_matrix_7	TCTGCACCTCACCCAACCGG	TGG
KirN	GGGTTCCACGCGAACACGCC	GGG
ABE_matrix_1	TCTAGACAAACCCAACATAG	TGG
ABE_matrix_2	AGCTAGACAACGTGTGAGCC	GGG
ABE_matrix_3	GGAATAGATCCCCGGTCGCG	CGG
ABE_matrix_4	CTCAATAGACTTGTTGCGAT	GGG
ABE_matrix_5	CCTCAATAGACTTGTTGCGA	TGG
ABE_matrix_6	CATGACAATACGGCGCCGCC	CGG

ABE_ NC_6	TAGACAATACGGCCGTCACG	GGG
Sg_CBE-cl2	CGCACCGTCCAACCCAGCAG	TGG
Sg_CBE-cl5	ACCGCCCGAATAGTCCTTGA	CGG
Sg_CBE-cl6	GCGATCCAGTCCTCGGTCTT	CGG
Sg_CBE-cl31	TTCCAGCCGTGTTCCATCGC	CGG
MCBE_SCO5087	CCGTTCACAGGTCGCGGCGG	AGG
MCBE_SCO5892	CCCCCAGGACGTGGAACAGA	CGG
MCBE_SCO3230	GCGGCGAACCAGCCCATCAT	CGG

Table S5. sgRNA-kirN gBlock sequence.

sgRNA-*kirN* gBlock sequence	GCTAGCAAAGCGGTCGATCTTGACGGCTGGCGAGAGGTGCGGGGAGGATCTGACC GACGCGGTCCACACGTGGCACCGCGATGCTGTTGTGGGCACAATCGTGCCGGTTGG TAGGATCGACGGACTAGTGATCGCATTTCGCCAACTACGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGT GCTTTTTTTACCCTAGGAAAAGCTACGATGTTCCGGGGACTGCTGATCCGGTCAGCA GGTGGAAGAGGGACTGGATTCCAAAGTTCTCAATGCTGCTTGCTGTTCTTGAATGG GGGGTCGTTGACGACGACATGGCTCGATTGGCGCGACAAGTTGCTGCGATTCTCAC CAATAAAAAACGCCCGGCGGCAACGCAGCGTTCTGAACAAATCCAGATGGAGTTC TGAGGTCACCTGAGG

Table S6. Detailed amino acid substitution list by CRISPR-cBEST.
Coding strand was masked with light blue, while non-coding strand was masked with light yellow.

AA	Codon		AA
	From	*To*	
Ala	GCA	GTA	Val
	GCC	GCT	Ala
	GCC	GTC	Val
	GCC	GTT	Val
	GCG	GTG	Val
	GCT	GTT	Val
	GCA	ACA	Thr
	GCC	ACC	Thr
	GCG	ACA	Thr
	GCG	ACG	Thr
	GCG	GCA	Ala
	GCT	ACT	Thr
Arg	CGA	TGA	STOP
	CGC	TGT	Cys
	CGC	CGT	Arg
	CGC	TGC	Cys
	CGG	TGG	Trp
	CGT	TGT	Cys
	AGA	AAA	Lys
	AGG	AGA	Arg
	AGG	AAG	Lys
	AGG	AAA	Lys
	CGA	CAA	Gln
	CGC	CAC	His
	CGG	CAG	Gln
	CGG	CGA	Arg
	CGG	CAA	Gln
	CGT	CAT	His
Asn	AAC	AAT	Asn
Asp	GAC	GAT	Asp

	GAC	AAC	Asn
	GAT	AAT	Asn
Cys	TGC	TGT	Cys
	TGT	TAT	Tyr
	TGC	TAC	Tyr
Gln	CAA	TAA	STOP
	CAG	TAG	STOP
	CAG	CAA	Gln
Glu	GAG	AAA	Lys
	GAG	GAA	Glu
	GAG	AAG	Lys
	GAA	AAA	Lys
Gly	GGC	GGT	Gly
	GGA	AAA	Lys
	GGA	GAA	Glu
	GGA	AGA	Arg
	GGC	AAC	Asn
	GGC	AGC	Ser
	GGC	GAC	Asp
	GGG	AAA	Lys
	GGG	GAA	Glu
	GGG	AGA	Arg
	GGG	AAG	Lys
	GGG	GGA	Gly
	GGG	GAG	Glu
	GGG	AGG	Arg
	GGT	AAT	Asn
	GGT	AGT	Ser
	GGT	GAT	Asp
His	CAC	TAC	Tyr
	CAC	TAT	Tyr
	CAC	CAT	His
	CAT	TAT	Try

Ile	ATC	ATT	Ile
Leu	CTA	TTA	Leu
	CTC	TTT	Phe
	CTC	CTT	Leu
	CTC	TTC	Phe
	CTG	TTG	Leu
	CTT	TTT	Phe
	CTG	CTA	Leu
	TTG	TTA	Leu
Lys	AAG	AAA	Lys
Met	ATG	ATA	Ile
Phe	TTC	TTT	Phe
Pro	CCA	TTA	Leu
	CCA	TCA	Ser
	CCA	CTA	Leu
	CCC	TTC	Phe
	CCC	TCC	Ser
	CCC	TCT	Ser
	CCC	TTT	Phe
	CCC	CTT	Leu
	CCC	CCT	Pro
	CCC	CTC	Leu
	CCG	TTG	Leu
	CCG	TCG	Ser
	CCG	CTG	Leu
	CCT	TTT	Phe
	CCT	CTT	Leu
	CCT	TCT	Ser
	CCG	CCA	Pro
Ser	AGC	AGT	Ser
	TCA	TTA	Leu
	TCC	TTT	Phe
	TCC	TCT	Ser

	TCC	TTC	Phe
	TCG	TTG	Leu
	TCT	TTT	Phe
	TCG	TCA	Ser
	AGC	AAC	Asn
	AGT	AAT	Asn
Thr	ACA	ATA	Ile
	ACC	ACT	Thr
	ACC	ATC	Ile
	ACC	ATT	Ile
	ACG	ATG	Met
	ACT	ATT	Ile
	ACG	ACA	Thr
Trp	TGG	TGA	STOP
	TGG	TAA	STOP
	TGG	TAG	STOP
Tyr	TAC	TAT	Tyr
Val	GTC	GTT	Val
	GTA	ATA	Ile
	GTC	ATC	Ile
	GTG	ATA	Ile
	GTG	GTA	Val
	GTG	ATG	Met
	GTT	ATT	Ile
STOP	TGA	TAA	STOP
	TAG	TAA	STOP

Table S7. Detailed amino acid substitution list by CRISPR-aBEST.
Coding strand was masked with light blue, while non-coding strand was masked with light yellow.

AA	Codon		AA
	From	*To*	
Ala	GCA	GCG	Ala
	GCT	GCC	Ala
Arg	AGA	GGA	**Gly**
	AGA	GGG	**Gly**
	CGA	CGG	**Arg**
	AGA	AGG	**Arg**
	CGT	CGC	Arg
Asn	AAT	GGT	**Gly**
	AAC	GGC	**Gly**
	AAT	AGT	**Ser**
	AAC	AGC	**Ser**
	AAT	GAT	**Asp**
	AAC	GAC	**Asp**
	AAT	AAC	Asn
Asp	GAT	GGT	**Gly**
	GAC	GGC	**Gly**
	GAT	GAC	Asp
Cys	TGT	CGT	**Arg**
	TGC	CGC	**Arg**
	TGT	TGC	Cys
Gln	CAA	CAG	Gln
	CAA	CGA	**Arg**
	CAG	CGG	**Arg**
Glu	GAA	GGA	**Gly**
	GAG	GGG	**Gly**
	GAA	GAG	Glu
Gly	GGA	GGG	Gly
	GGT	GGC	Gly
His	CAT	CGT	**Arg**
	CAC	CGC	**Arg**
	CAA	CGG	**Arg**
	CAG	CGG	**Arg**
	CAT	CAC	His
Ile	ATT	GTT	**Val**
	ATC	GTC	**Val**
	ATA	GTG	**Val**
	ATA	ATG	**Met**
	ATT	ACT	**Thr**
	ATC	ACC	**Thr**
	ATA	ACA	**Thr**
	ATT	ATC	Ile
Leu	TTA	TCA	**Ser**
	TTG	TCG	**Ser**
	TTA	CCA	**Pro**
	TTG	CCG	**Pro**
	CTT	CCT	**Pro**
	CTC	CCC	**Pro**

	CTT	CTC	Leu
	TTA	TTG	Leu
	CTA	CTG	Leu
Lys	AAA	GGG	**Gly**
	AAA	GGA	**Gly**
	AAA	GAA	**Glu**
	AAA	AAG	Lys
	AAA	AGG	**Arg**
	AAG	GGG	**Gly**
	AAG	GAG	**Glu**
	AAG	AGG	**Arg**
	AAA	AAG	Lys
Met	ATG	GTG	**Val**
	ATG	ACG	**Thr**
Phe	TTT	CTT	**Leu**
	TTT	CCT	**Pro**
	TTT	CCC	**Pro**
	TTT	TCC	**Ser**
	TTC	CTC	**Leu**
	TTC	TCC	**Ser**
	TTC	CCC	**Pro**
	TTT	TTC	Phe
Pro	CCA	CCG	Pro
	CCT	CCC	Pro
Ser	AGT	GGT	**Gly**
	AGC	GGC	**Gly**
	TCA	TCG	Ser
	TCT	CCT	**Pro**
	TCC	CCC	**Pro**
	TGA	CGA	**Pro**
	TCG	CCG	**Pro**
	AGT	AGC	Ser
Thr	ACT	GCT	**Ala**
	ACC	GCC	**Ala**
	ACA	GCA	**Ala**
	ACG	GCG	**Ala**
	ACA	ACG	Thr
	ACT	ACC	Thr
Trp	TGG	CGG	**Arg**
Tyr	TAT	TGT	**Cys**
	TAC	TGC	**Cys**
	TAT	TAC	Tyr
	TAT	CAT	**His**
	TAG	CAG	**His**
Val	GTT	GCT	**Ala**
	GTC	GCC	**Ala**
	GTA	GCA	**Ala**
	GTG	GCG	**Ala**
	GTT	GTC	Val
	GTA	GTG	Val

STOP	TAA	TGG	**Trp**
	TAG	TGG	**Trp**
	TGA	TGG	**Trp**
	TAA	TAG	STOP
	TAA	CAA	**Gln**
	TAG	CAG	**Gln**
	TGA	CGA	**Arg**

Table S8. Frequency of STOP codon introduction in the nonessential secondary metabolites biosynthesis genes of *S. coelicolor* A3(2).

***S. coelicolor* A3(2)**				
Cluster no.	**Cluster size (bp)**	**STOP count**	**gene count**	**STOP/gene**
1	53018	498	40	12.45
2	24764	286	24	11.92
3	23530	302	22	13.73
4	49828	541	36	15.03
5	8242	78	6	13.00
6	38823	409	40	10.23
7	10399	86	9	9.56
8	10570	95	13	7.31
9	10972	112	9	12.44
10	79080	741	36	20.58
11	70903	740	63	11.75
12	20562	221	20	11.05
13	72543	640	70	9.14
14	10278	106	7	15.14
15	45282	408	35	11.66
16	11317	103	10	10.30
17	19321	204	16	12.75
18	13208	139	11	12.64
19	70203	862	33	26.12
20	46636	463	32	14.47
21	18700	200	18	11.11
22	25810	250	24	10.42
23	48144	540	37	14.59
24	26454	329	25	13.16
25	40888	419	36	11.64
26	21128	186	22	8.45
27	73251	713	65	10.97
		sum	sum	average
		9671	**759**	**12.74**

Table S9. Frequency of STOP codon introduction in the nonessential secondary metabolites biosynthesis genes of *S. collinus* Tü365.

S. collinus Tü365				
Cluster no.	**Cluster size (bp)**	**STOP count**	**gene count**	**STOP/gene**
1	23069	187	16	11.69
2	59480	525	45	11.67
3	162077	1381	72	19.18
4	25263	252	22	11.45
5	43867	431	32	13.47
6	20659	229	20	11.45
7	55714	655	31	21.13
8	11926	91	7	13.00
9	43264	482	34	14.18
10	41041	409	40	10.23
11	52568	483	38	12.71
12	10405	79	9	8.78
13	69134	648	59	10.98
14	10411	69	12	5.75
15	11770	113	9	12.56
16	42498	441	41	10.76
17	56291	594	34	17.47
18	55031	564	54	10.44
19	58662	564	45	12.53
20	21014	234	18	13.00
21	12169	122	7	17.43
22	12145	121	7	17.29
23	52987	555	47	11.81
24	11419	83	10	8.30
25	22163	195	18	10.83
26	13158	149	12	12.42
27	21011	170	19	8.95
28	26758	283	23	12.30
29	41374	354	37	9.57
30	73684	704	29	24.28
31	162077	1381	72	19.18
32	59480	525	45	11.67
33	23069	187	16	11.69

	sum	sum	average
	13260	**980**	**13.53**

Table S10. Frequency of STOP codon introduction in the nonessential secondary metabolites biosynthesis genes of *S. griseofuscus* DSM40191.

S. griseofuscus DSM40191				
Cluster no.	**Cluster size (bp)**	**STOP count**	**gene count**	**STOP/gene**
1	24425	219	27	8.11
2	45158	419	40	10.48
3	155722	1018	50	20.36
4	46595	486	35	13.89
5	114646	1028	63	16.32
6	41065	371	43	8.63
7	51804	621	43	14.44
8	10411	108	12	9.00
9	42938	420	35	12.00
10	10597	66	12	5.50
11	10997	121	9	13.44
12	72512	763	75	10.17
13	10933	87	7	12.43
14	54105	539	55	9.80
15	19893	228	21	10.86
16	47491	525	40	13.13
17	56541	622	49	12.69
18	10801	118	10	11.80
19	20640	225	22	10.23
20	51706	554	40	13.85
21	71151	808	54	14.96
22	43318	410	39	10.51
23	23776	295	25	11.80
24	88583	888	63	14.10
25	52196	503	44	11.43
26	46340	437	31	14.10
27	69518	718	49	14.65
28	41295	435	41	10.61
29	10216	98	8	12.25
30	103599	978	67	14.60
31	41374	379	33	11.48
32	13322	96	8	12.00
33	42325	427	33	12.94

34	65327	633	63	10.05
		sum	sum	average
		15643	**1246**	**12.55**

Table S11. **The four selected BGCs of *S. griseofuscus* DSM40191.**

Cluster NO.	Cluster Location	Target Locus-Tag / Gene	Predicted Product	BGC Type
C2	198,665 - 243,822	LGGNEMGE_00214 / ppsD_1	Carbapenem MM 4550	NRPS, T1PKS
C5	1,388,264 - 1,502,909	LGGNEMGE_01214 / tycC_2	Laspartomycin	NRPS
C6	1,506,788 - 1,547,852	LGGNEMGE_01271	Herboxidiene	T1PKS+T3PKS
C31	8,282,447 - 8,323,820	LGGNEMGE_07491 / spkC	A-503083	Lanthipeptide

Table S12. Csy4 coding sequence from *Pseudomonas aeruginosa* PA14.

Csy4 coding sequence	ATGGACCACTACCTCGACATTCGCTTGCGACCGGACCCGGAATTTCCCCCGGCGC AACTCATGAGCGTGCTCTTCGGCAAGCTCCACCAGGCCCTGGTGGCACAGGGCGG GGACAGGATCGGCGTGAGCTTCCCCGACCTCGACGAAAGCCGCTCCCGGCTGGGC GAGCGCCTGCGCATTCATGCCTCGGCGGACGACCTTCGTGCCCTGCTCGCCCGGCC CTGGCTGGAAGGGTTGCGGGACCATCTGCAATTCGGAGAACCGGCAGTCGTGCCT CACCCCACACCGTACCGTCAGGTCAGTCGGGTTCAGGCGAAAAGCAATCCGGAAC GCCTGCGGCGGCGGCTCATGCGCCGGCACGATCTGAGTGAGGAGGAGGCTCGGA AACGCATTCCCGATACGGTCGCGAGAACCTTGGACCTGCCCTTCGTCACGCTACGC AGCCAGAGCACCGGACAGCACTTCCGTCTCTTCATCCGCCACGGGCCGTTGCAGG CGACGGCAGAGGAAGGAGGATTCACCTGTTACGGGTTGAGCAAAGGAGGTTCCGT TCCCTGGTTCTGA

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