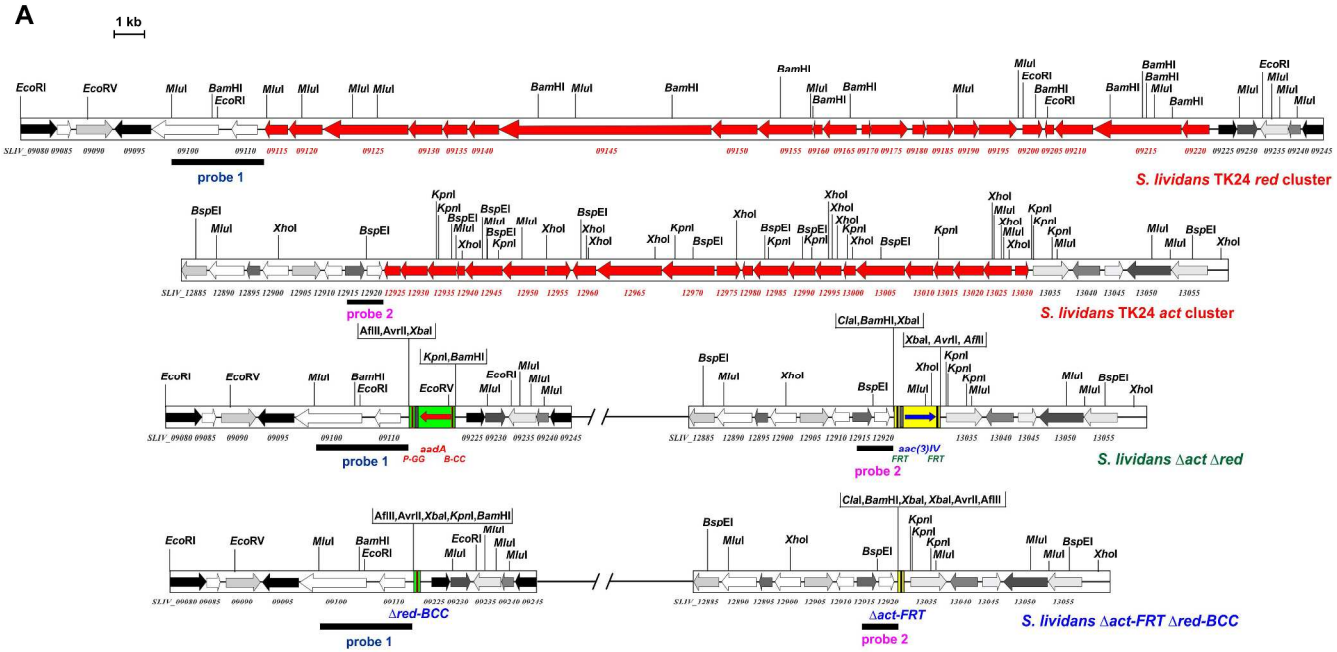
Supporting Information

Sun et al. **Development of a Biosensor Concept to Detect Production of Cluster-Specific Secondary Metabolites**

Figure S1. (A) Physical maps of chromosomal DNA containing the wild-type *S. lividans* TK24 *act* cluster for actinorhodin and *red* cluster for undecylprodigiosin, and the deleted alleles of the *S. lividans*, Δ*act* Δ*red* and *S. lividans*, Δ*act-FRT* Δ*red-BCC* strains. Thick arrows denote the direction and size of genes. Red arrows represent the *act* and *red* genes. Gene labelling is based on the genomic sequence of *S. lividans* TK24 (GenBank Acc. No. CP009124)[1]. The black bars below the maps represent the probes used for Southern hybridization analysis. Relevant restriction sites are indicated.

(**B)** Southern hybridization analysis of chromosomal DNA from the indicated strains verifying the correct integration and removing of the antibiotic cassette. 1 μg of gDNA from the corresponding strain was digested with the restriction enzymes indicated, separated by electrophoresis in 0.8% (w/v) agarose gel and transferred on Hybond N (Amersham) as described in Ausubel et al.[2] Hybridization followed the standard DIG protocol (Roche, Mannheim, Germany) using the DIG-labelled probes 1 and 2 for verification of correct *red* and *act* deletion, respectively. Lambda DNA-*Bst*EII digest was used as the size standard.


A
1 kb
probe 1
S. lividans TK24 red cluster
probe 2
S. lividans TK24 act cluster
probe 1
probe 2
S. lividans Δact Δred
probe 1
probe 2
S. lividans Δact-FRT Δred-BCC

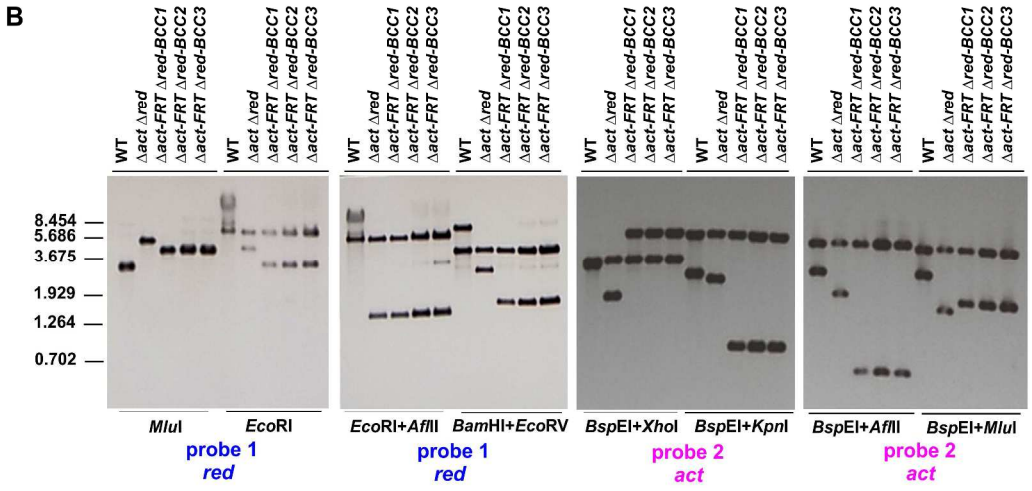

B
WT
Δact Δred
Δact-FRT Δred-BCC1
Δact-FRT Δred-BCC2
Δact-FRT Δred-BCC3
8.454
5.686
3.675
1.929
1.264
0.702
MluI
EcoRI
probe 1
red
EcoRI+AflII
BamHI+EcoRV
probe 1
red
BspEI+XhoI
BspEI+KpnI
probe 2
act
BspEI+AflII
BspEI+MluI
probe 2
act

Figure S2. Absorptions, accurate mass and MS/MS profile of metabolite detected in the extract of *S. lividans* RedStrep1 [pYQS040/pYQS060] strain. Detected compound has m/z of 349.1213 $[M+H]^+$ (calculated for coelimycin P1 (C17H20N2)4S) 349.1215 $[M+H]^+$) and three UV maximums at 225, 275 and 360 nm with peak at 360 nm being characteristic for coelimycin P1[3]. The MS/MS fragmentation pattern is the same as described for coelimycin P1[4].

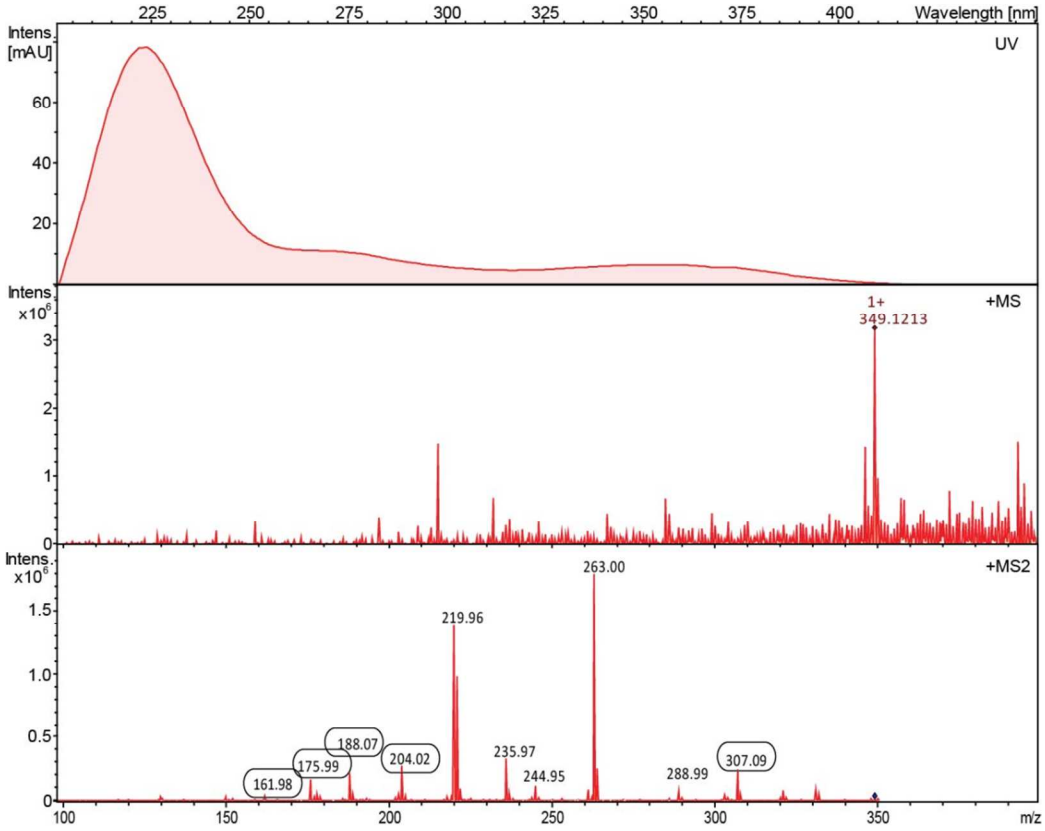


Table S1. Plasmids used in this study.

Plasmid name	Description	Construction details	Source
pSET152	Integrative *E.coli-Streptomyces* vector, *ϕC31* attachment site, Am^R		5
pUWL-oriT	*E.coli- Streptomyces* shuttle vector, Amp^R, Tsr^R		6
pBpsA	Integrative *E.coli-Streptomyces* vector, *ϕBT1* attachment site, Am^R		7
pKC1218 hyg_mut	*E.coli- Streptomyces* shuttle vector, Hyg^R	Am^R marker replaced with HygR, one *Eco*RI site eliminated	This study
pTSA101	Intermediate/construction vector	pKC1218hyg_mut backbone with *bpsA* gene under control of P21 promoter and CymR operator	This study
pYQS031	Intermediate/construction vector	Ligation of PCR PermE-*dnaQ2* with pKC1218 hyg_mut (HindIII/EcoRI)	This study
pYQS040	SLIV_06710 PO - BpsA in pSET152 backbone	Ligation: pSET152 (BamHI/EcoRI) + PCR SLIV_06710 PO (BamHI/SpeI) + pTSA101 (SpeI/EcoRI, *bpsA*)	This study
pYQS041	SLIV_06815 PO - BpsA in pSET152 backbone	Ligation: pSET152 (BamHI/EcoRI) + PCR SLIV_06815 PO (BamHI/SpeI) + pTSA101 (SpeI/EcoRI, *bpsA*)	This study
pYQS042	SLIV_09080 PO - BpsA in pSET152 backbone	Ligation: pSET152 (BamHI/EcoRI) + PCR SLIV_09080 PO (BamHI/SpeI) + pTSA101 (SpeI/EcoRI, *bpsA*)	This study
pYQS046	Backbone was used for double crossover to knockout target gene	Ligation: PCR dnaQ1 flanking A (AgeI/BamHI) + PCR *dnaQ1* flanking B (BamHI/HindIII) + pTSA101 (HindIII/AgeI)	This study
pYQS056	SLIV_31825 PO - *bpsA* in pSET152 backbone	Ligation: pSET152 (BamHI/EcoRI) + PCR SLIV_31825 PO (BamHI/SpeI) + pTSA101 (SpeI/EcoRI, *bpsA*)	This study
pYQS060	Cluster specific positive regulator: PermE-SLIV_06705 in pKC1218, Hyg^R	Ligation of pYQS031 with PCR SLIV_06705 (EcoRI/XbaI)	This study

pYQS061	Cluster specific positive regulator: PermE-SLIV_06745 in pKC1218, Hyg^R	Ligation of pYQS031 with PCR SLIV_06745 (EcoRI/XbaI)	This study
pYQS062	Cluster specific positive regulator: PermE-SLIV_09200 in pKC1218, Hyg^R	Ligation of pYQS031 (EcoRI/XbaI) with PCR SLIV_09200 (MfeI/XbaI)	This study
pYQS063	Cluster specific positive regulator: PermE-SLIV_09220 *redD* in pKC1218, Hyg^R	Ligation of pYQS031 with PCR SLIV_09220 RedD (EcoRI/XbaI)	This study
pYQS064	Cluster specific positive regulator: PermE-SLIV_31695 in pKC1218, Hyg^R	Ligation of pYQS031 with PCR SLIV_31695 (EcoRI/XbaI)	This study
pYQS065	Used to construct hybrid repressor HR1: REC SLIV_09200 - linker SLIV_09200 - HTH SLIV_09075	Gibson assembly: Reverse PCR pYQS046 + PCR SLIV_09075 flanking A1 + PCR HR1-frag4 + PCR HR1-frag1 + PCR SLIV_09075 flanking B1	This study
pYQS066	Used to construct hybrid repressor HR2: HTH SLIV_09075 - linker SLIV_09075 - REC SLIV_09200	Gibson assembly: Reverse PCR pYQS046 + PCR SLIV_09075 flanking A2 HR2-frag2 + PCR HR2-frag3 + PCR SLIV_09075 flanking B2	This study
pYQS070	single crossover plasmid for insertion knockout of SLIV_06770, Amp^R and Tsr^R	Ligation PCR SLIV_06770 middle with pUWL (NdeI/BamHI)	This study
pYQS079	PermE-HR1, pKC1218 backbone, Hyg^R	Ligation of PCR Hybrid Repressor 1 (XbaI/MfeI) with pYQS060 (XbaI/EcoRI)	This study
pYQS080	PermE-HR2, pKC1218 backbone, Hyg^R	Ligation PCR Hybrid Repressor 2 (XbaI/MfeI) with pYQS060 (XbaI/EcoRI)	This study

PO: promoter-operator. HR: hybrid repressor. PR: putative positive regulator

Table S2. *Escherichia coli* and *Streptomyces lividans* strains used in this study.

Strain name	Description	Plasmid(s) used for conjugation	Source/reference
Escherichia coli XL1-blue	General cloning host: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q} ZΔ*M15* Tn*10* (Tet^{R})]		Stratagene
Escherichia coli ET125671 (pUZ8002)	Mediates conjugative DNA transfer from RP4 *oriT* with helper plasmid pUZ8002 (Kan^{R}, Cm^{R}). Methylation deficient (*dam*$^{-}$, *dcm*$^{-}$, *hsdM*$^{-}$)		8
S. lividans TK24	*S. lividans* wild-type strain		2
RedStrep1	TK24 *Δact Δred*	Deleted clusters for actinorhodine and undecylprodigiosin	This study
TK24 + Reporter 1	TK24 *attB$_{C31}$ :: SLIV_06710$_{PO}$-bpsA*	pYQS040, *attB$_{C31}$* integrative	This study
TK24 + Reporter 2	TK24 *attB$_{C31}$ :: SLIV_06815$_{PO}$-bpsA*	pYQS041, *attB$_{C31}$* integrative	This study
TK24 + Reporter 3	TK24 *attB$_{C31}$ :: SLIV_09080$_{PO}$-bpsA*	pYQS042, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 1	RedStrep 1 *attB$_{C31}$ :: SLIV_06710$_{PO}$-bpsA*	pYQS040, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 2	RedStrep 1 *attB$_{C31}$ :: SLIV_06815$_{PO}$-bpsA*	pYQS041, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 3	RedStrep 1 *attB$_{C31}$ :: SLIV_09080$_{PO}$-bpsA*	pYQS042, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 4	RedStrep 1 *attB$_{C31}$ :: SLIV_31825$_{PO}$-bpsA*	pYQS056, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 1+ PR1	RedStrep 1 SLIV_06710$_{PO}$-BpsA + ermEp-SLIV_06705	pYQS060, replicative	This study
RedStrep 1 + Reporter 1+ PR2	RedStrep 1 SLIV_06710$_{PO}$-BpsA + ermEp-SLIV_06745	pYQS061, replicative	This study
RedStrep 1 + Reporter 4+ PR5	RedStrep 1 SLIV_31825$_{PO}$-BpsA + ermEp-SLIV_31695	pYQS064, replicative	This study
TK24 + Reporter 3 + PR3	RedStrep 1 SLIV_09080$_{PO}$-BpsA + ermEp-SLIV_09200	pYQS062, replicative	This study

TK24 + Reporter 3 + PR4	RedStrep 1 SLIV_09080$_{PO}$-BpsA + ermEp-SLIV_09220 RedD	pYQS063, replicative	This study
TK24 *ΔSLIV_09075 :: HR1*	TK24 *ΔSLIV_09075 :: Hybrid repressor_1*	pYQS065, for deletion knockout by double crossover	This study
TK24 *ΔSLIV_09075 :: HR2*	TK24 *ΔSLIV_09075 :: Hybrid repressor_2*	pYQS066, for deletion knockout by double crossover	This study
TK24 *ΔSLIV_09075 :: HR1* + Reporter 3	TK24 *ΔSLIV_09075 :: Hybrid repressor_1* + *SLIV_09080$_{PO}$-BpsA*	pYQS042, *attB$_{C31}$* integrative	This study
TK24 *ΔSLIV_09075 :: HR2* + Reporter 3	TK24 *ΔSLIV_09075 :: Hybrid repressor_1* + *SLIV_09080$_{PO}$-BpsA*	pYQS042, *attB$_{C31}$* integrative	This study
RedStrep 1 + Reporter 1 + PR1 *ΔSLIV_06770*	SLIV_06770 was knocked out in "RedStrep 1 + Reporter 1 + PR1"	pYQS070, for insertion knockout by single crossover	This study
RedStrep 1 + Reporter 3+ *HR1*	RedStrep 1 *SLIV_09080$_{PO}$-BpsA* + *ermEp-HR1*	pYQS079, replicative	This study
RedStrep 1 + Reporter 3 + *HR2*	RedStrep 1 *SLIV_09080$_{PO}$-BpsA* + *ermEp-HR2*	pYQS080, replicative	This study

$_{PO}$: promoter-operator. *HR*: hybrid repressor. PR: putative Positive Regulator

HR1: REC SLIV_09200 - linker SLIV_09200 - HTH SLIV_09075

HR2: HTH SLIV_09075 - linker SLIV_09075 - REC SLIV_09200

Table S3. Primers for plasmid construction used in this study.

Primer name	5'-3' sequence (*Italic*: endonuclease restriction enzyme site)	PCR product (size bps, template)	Enzyme/paired primer
YQ30_SE	TAAT*GGATCC*GTCGTGCTCCGTGGTCGC	PCR SLIV_06710 PO (258, TK24)	*BamHI*
YQ30_AS	ACACC*ACTAGT*CGCCTTTTCCCCTTACCGTTC		*SpeI*
YQ31_SE	TATA*GGATCC*GCCTGCCTCCTTGTTCAT	PCR SLIV_06815 PO (137, TK24)	*BamHI*
YQ31_AS	CAGT*ACTAGT*GGGTCCCCCCCAGGAATC		*SpeI*
YQ32_SE	AGAT*GGATCC*TTTCTCCGCTTCACCTCG	PCR SLIV_09080 PO (164, TK24)	*BamHI*
YQ32_AS	AATT*ACTAGT*CGGGCAGCCTTCGGCAGGA		*SpeI*
YQ42_SE	CAAT*GGATCC*GACGCCTCCTTCCAGTG	PCR SLIV_31825 PO (120, TK24)	*BamHI*
YQ42_AS	ACACC*ACTAGT*GAACCCCCACCTCCTTAAG		*SpeI*
YQ47_SE	ATCA*TCTAGA*CATCCGCGGCACGGCAAAC	PCR SLIV_06705 (927, TK24)	*XbaI*
YQ47_AS	GTACT*GAATTC*CGAGATCGGGCGCGTCAC		*EcoRI*
YQ48_SE	CGCA*TCTAGA*AGTCGTGGCCAGGAGAATAC	PCR SLIV_06745 (1743, TK24)	*XbaI*
YQ48_AS	ATACT*GAATTC*GACGGCGGAGACGGTGGG		*EcoRI*
YQ49_SE	ATCA*TCTAGA*GCCCGCACGCCACGTACGGT	PCR SLIV_09200 (739, TK24)	*XbaI*
YQ49_AS	ATACT*CAATTG*GCCGGACCGGGGCATTCAGC		*MfeI*
YQ50_SE	ATCA*TCTAGA*CCGATCGTTCGGTGGATGAC	PCR SLIV_09220 *redD* (1097, TK24)	*XbaI*
YQ50_AS	ATACT*GAATTC*CCGGGTCGTCAGGCGCTGAG		*EcoRI*
YQ51_SE	GGCA*TCTAGA*ATGCGGGCACTGTATCCAGG	PCR SLIV_31695 (863, TK24)	*XbaI*
YQ51_AS	ATACT*GAATTC*CTCACGGCCTCCGCATGAGC		*EcoRI*
YQ54_SE	GATAAGCTTGGGCTGCAGGTC	Reverse PCR pYQS046 (7884, pYQS046)	
YQ54_AS	ACCGAGTCCCACCGGTCGAAAC		

YQ55_SE	TTCGACCGGTGGGACTCGGTGCTGGAGCGCAGGTTGTC	PCR SLIV_09075 flanking A1 (1530, TK24)	
YQ55_AS	GGGTCGTCATTTTCTCCGCTTCACCTCGG		
YQ56_SE	AGCGGAGAAAATGACGACCCGTGTCCTG	PCR HR1-frag4 (455, TK24)	
YQ56_AS	CAATCTTGGGTTCGGGGCGGAGGTTCTTC		
YQ57_SE	CCGCCCCGAACCCAAGATTGAAGCCGGC	PCR HR1-frag1 (218, TK24)	
YQ57_AS	GGGTGCGTCAGACGACGAGTTCGAGCAGG		
YQ58_SE	ACTCGTCGTCTGACGCACCCCGTCCAGG	PCR SLIV_09075 flanking B1 (1530, TK24)	
YQ58_AS	ACCTGCAGCCCAAGCTTATCCGGCGGTCCTCTCGTCGG		
YQ59_AS	CACGGGTCGTGCGAGCTGCCTGTTCGAG	PCR SLIV_09075 flanking A2 HR2-frag2 (1845, TK24)	YQ55_SE
YQ60_SE	GGCAGCTCGCACGACCCGTGTCCTGGTG	PCR HR2-frag3 (359, TK24)	
YQ60_AS	GGGTGCGTCAGATGGTTCTGATGACGTGCAC		
YQ61_SE	CAGAACCATCTGACGCACCCCGTCCAGG	PCR SLIV_09075 flanking B2 (1530, TK24)	YQ58_AS
YQ68_SE	ATCA*CATATG*AACTTCCCGCCAGCCTGAAC	PCR SLIV_06770 middle (2142, TK24)	*NdeI*
YQ68_AS	ATAA*GGATCC*AAGAAGTCGGCGTCGAACTC		*BamHI*
YQ75_SE	ACGGTGTCTCGTACACCTTC	Amplify SLIV_06770 to identify insertion knockout (9894)	
YQ75_AS	TTCCGTAGGTGGCCATGAGG		
YQ80_SE	GCCA*TCTAGA*GGTGAAGCGGAGAAAATGACGA	PCR Hybrid Repressor 1 (678, pYQS065)	*XbaI*
YQ80_AS	CTACT*CAATTG*GGTGCGTCAGACGACGAGTT		*MfeI*
YQ81_SE	GCCA*TCTAGA*AGGTGAAGCGGAGAAAATGC	PCR Hybrid Repressor 2 (696, pYQS066)	*XbaI*
YQ81_AS	CCGCT*CAATTG*CGTCAGATGGTTCTGATGAC		*MfeI*

Table S4. Primers used in construction of *S. lividans* RedStrep1.

Oligonucleotide	Sequence (5′ − 3′)
12925Dir	CCCCCAATTGCGCTGAGCAAGCAGATCCGGGCCC
129215Rev	CCCCATCGATGGAAGGCCGTCAGCGGCCCGTGGC
13030Dir	CCCCAAGCTTCGAAGACCGCCAGGGACGTCAGCC
13013Rev	CCCCCTTAAGTGACGCGCACCCTCGCCTTCGCGC
9220Dir	CCCCCAATTGCCGGTACGCTTCAGATATGAGTGC
9220Rev	CCCCGGATCCACCGTCAATGTGTTGAGGCCG
9115Dir	CCCCCTTAAGCGTGTACTGACCCCGCCCGTCACG
9115Rev	CCCCAAGCTTCGATCTCCTCGGCGACCCGGTCG

References

(1) Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.O., Seidman, J.S., Smith, J.A., and Struhl, K. (1995) Current Protocols in Molecular Biology. Wiley, New York.

(2) Ruckert, C., Albersmeier, A., Busche, T., Jaenicke, S., Winkler, A., Friethjonsson, O. H., Hreggviethsson, G. O., Lambert, C., Badcock, D., Bernaerts, K., Anne, J., Economou, A., and Kalinowski, J. (2015) Complete genome sequence of Streptomyces lividans TK24, *J Biotechnol 199*, 21-22.

(3) Whittle, M., Willett, P., Klaffke, W. and van Noort, P. (2003) Evaluation of similarity measures for searching the Dictionary of Natural Products database. *J. Chem. Inform. Comp. Sci. 43*, 449-457.

(4) Gomez-Escribano, J.P., Song, L., Fox, D.J., Yeo, V., Bibb, M.J., and Challis, G.L. (2012) Structure and biosynthesis of the unusual polyketide alkaloid coelimycin P1, a metabolic product of the *cpk* gene cluster of *Streptomyces coelicolor* M145. *Chem. Sci. 3*, 2716–2720.
(5) Bierman, M., Logan, R., O'Brien, K., Seno, E. T., Rao, R. N., and Schoner, B. E. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to *Streptomyces* spp. *Gene 116*, 43-49.

(6) Luzhetskyy, A., Zhu, L., Gibson, M., Fedoryshyn, M., Durr, C., Hofmann, C., Hoffmeister, D., Ostash, B., Mattingly, C., Adams, V., Fedorenko, V., Rohr, J., and Bechthold, A. (2005) Generation of novel landomycins M and O through targeted gene disruption, *Chembiochem 6*, 675-678.
(7) Knirschova, R., Novakova, R., Mingyar, E., Bekeova, C., Homerova, D., and Kormanec, J. (2015) Utilization of a reporter system based on the blue pigment indigoidine biosynthetic gene *bpsA* for detection of promoter activity and deletion of genes in *Streptomyces. J. Microbiol. Methods 113*, 1-3.
(8) Flett F., Mersinias, V., and Smith, C.P. (1997) High efficiency intergeneric conjugal transfer of plasmid DNA from *Escherichia coli* to methyl DNA-restricting streptomycetes. *FEMS Microbiol. Lett. 155*, 223-229.