

Supplementary Information

Identification of a Biosynthetic Gene Cluster Responsible for the Production of a New Pyrrolopyrimidine Natural Product—Huimycin

Hui Shuai ¹, Maksym Myronovskiy ¹, Suvd Nadmid ^{1,2} and Andriy Luzhetskyy ^{1,3,*}

¹ Pharmazeutische Biotechnologie, Universität des Saarlandes, 66123 Saarbrücken, Germany; hui.shuai@uni-saarland.de (H.S.); maksym.myronovskiy@uni-saarland.de (M.M.)

² School of Pharmacy, Mongolian National University of Medical Sciences, S. Zorig street, 14210 Ulaanbaatar, Mongolia; suvdn@yahoo.com (S.N.)

³ Helmholtz-Institut für Pharmazeutische Forschung Saarland, 66123 Saarbrücken, Germany

* Correspondence: a.luzhetskyy@mx.uni-saarland.de; Tel.: +49-0681-70223

Received: 21 June 2020; Accepted: 16 July 2020; Published: 12 August 2020

Figures

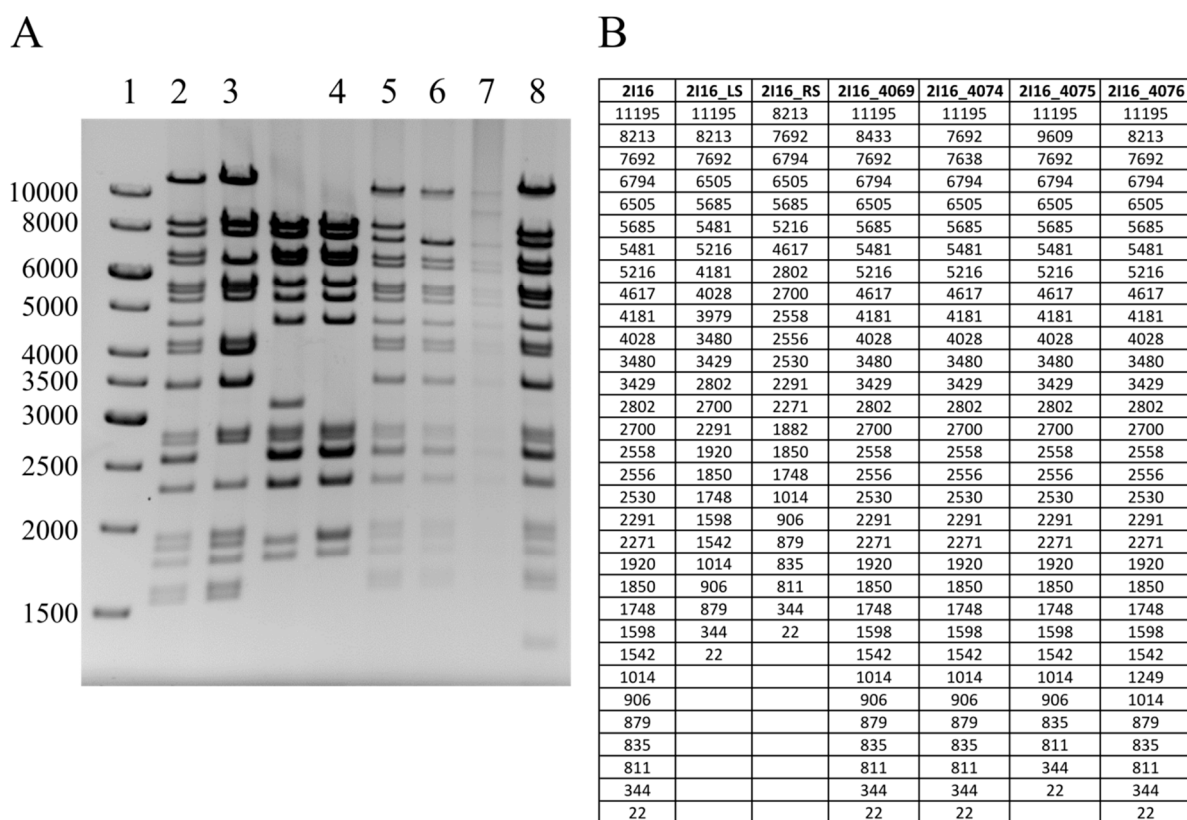
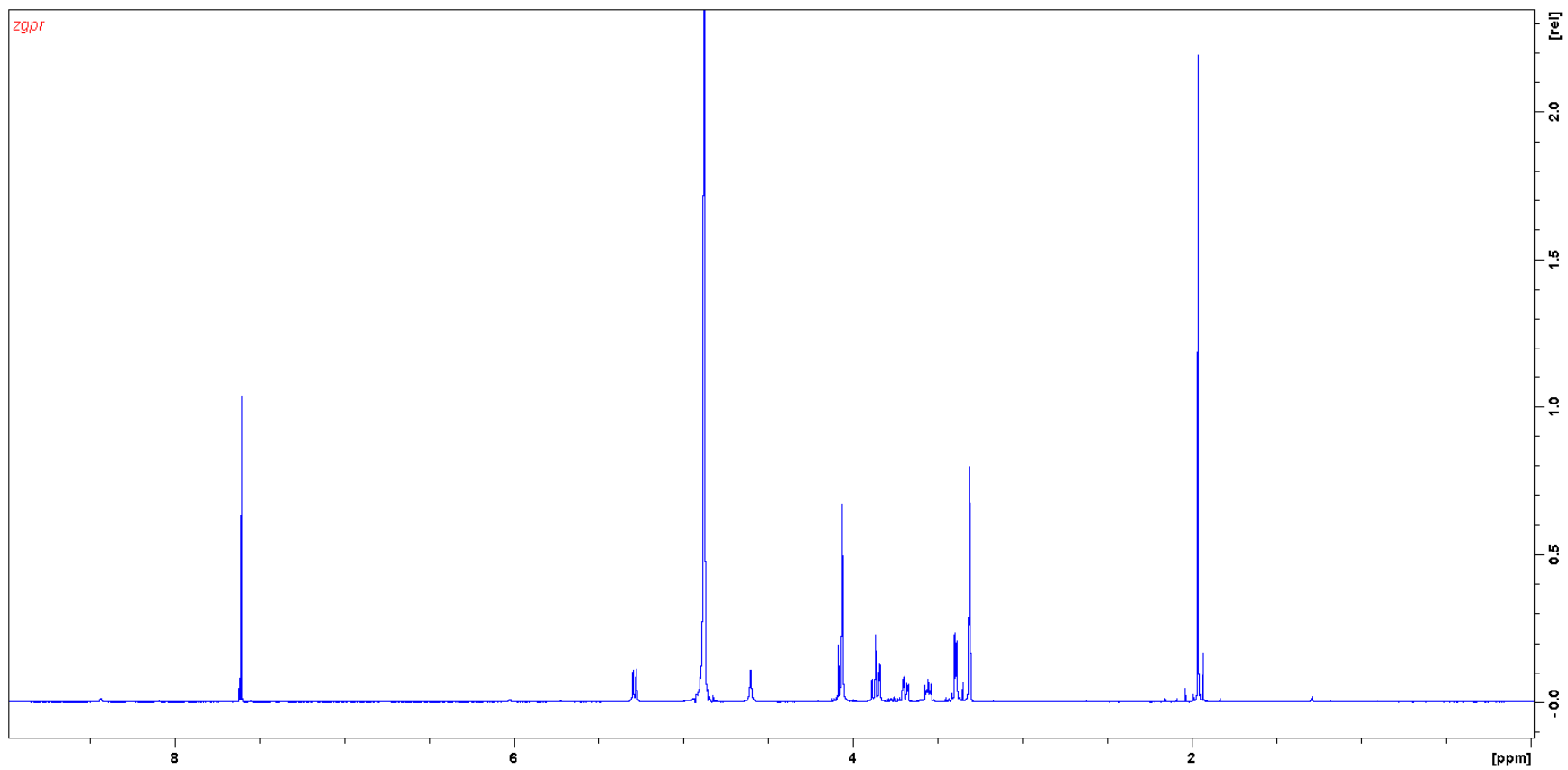


Figure S1. Restriction mapping of the BAC 2I16 and its derivatives with gene deletions. **(A)** Gel electrophoresis of KpnI digests of the following BACs: 2—2I16, 3—2I16_LS, 4—2I16_RS, 5—2I16_4069, 6—2I16_4074, 7—2I16_4075, 8—2I16_4076, 1—DNA ladder. Size of individual fragments in base pairs is shown. **(B)** The expected length of DNA fragments in base pairs obtained after KpnI digestion of the following BACs: 2I16, 2I16_LS, 2I16_RS, 2I16_4069, 2I16_4074, 2I16_4075, and 2I16_4076.



1

2

3

Figure S2. ¹H NMR (500 MHz, MeOD₄) spectrum of huimycin 1.

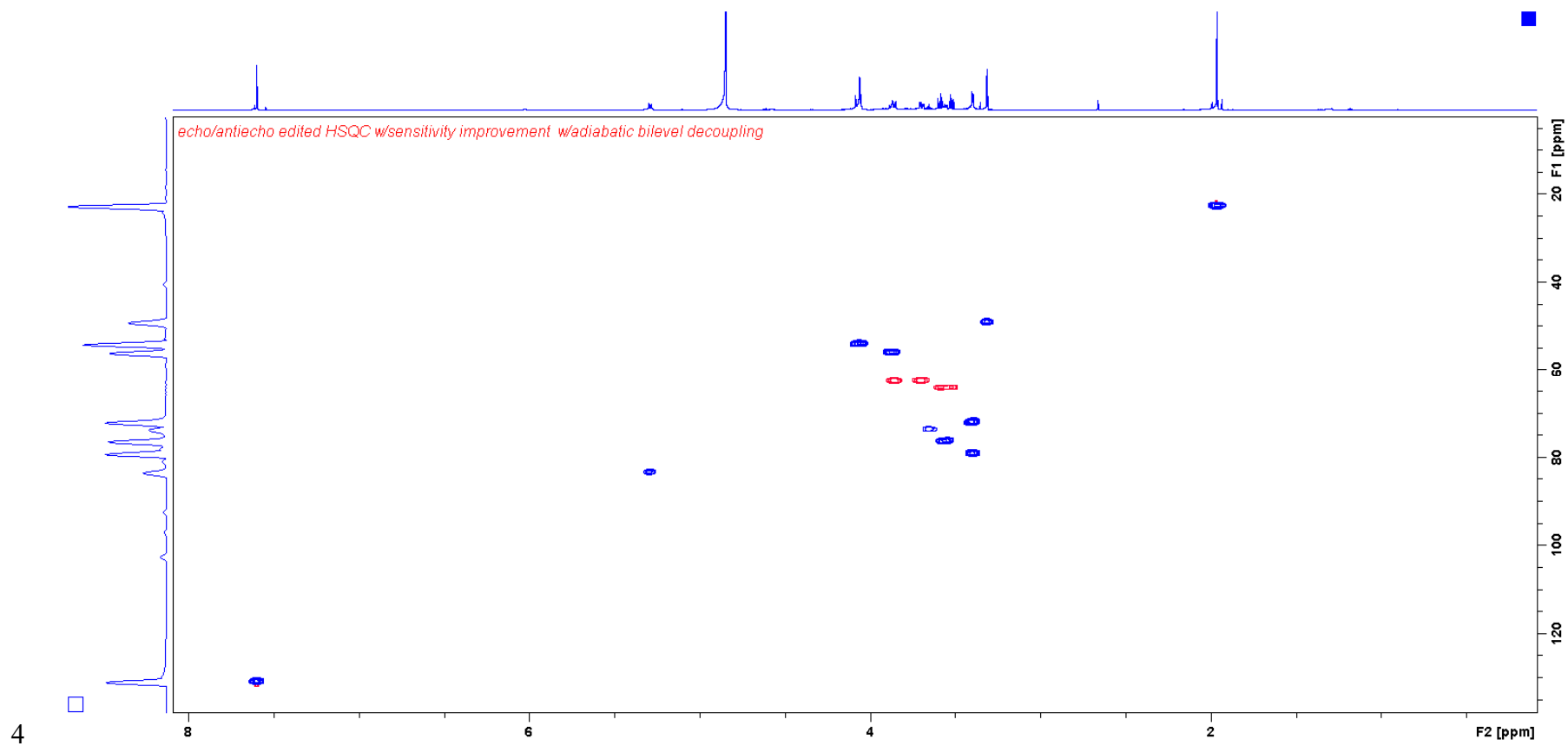
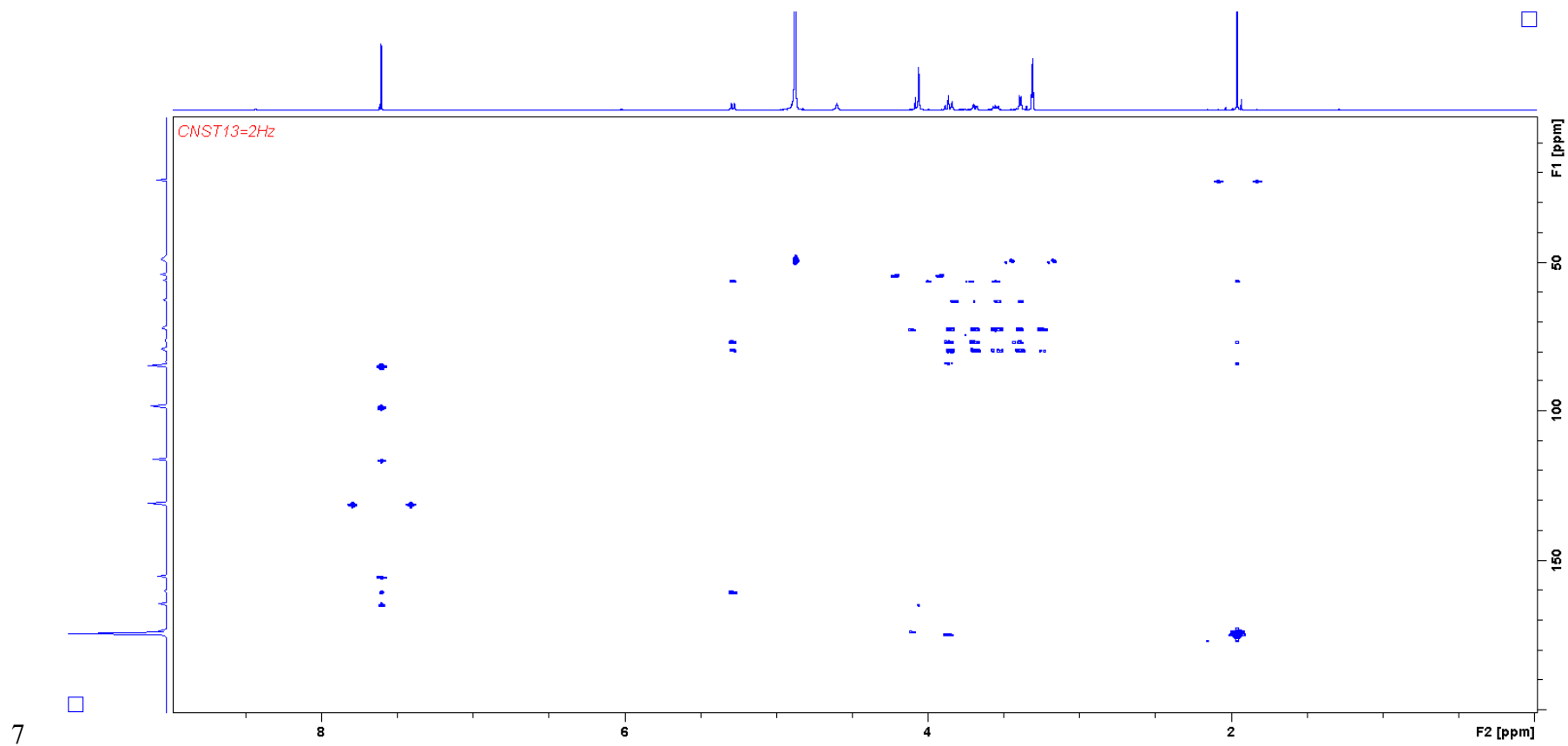


Figure S3. HSQC (500 MHz, MeOD₄) spectrum of huimycin 1.

4

5

6

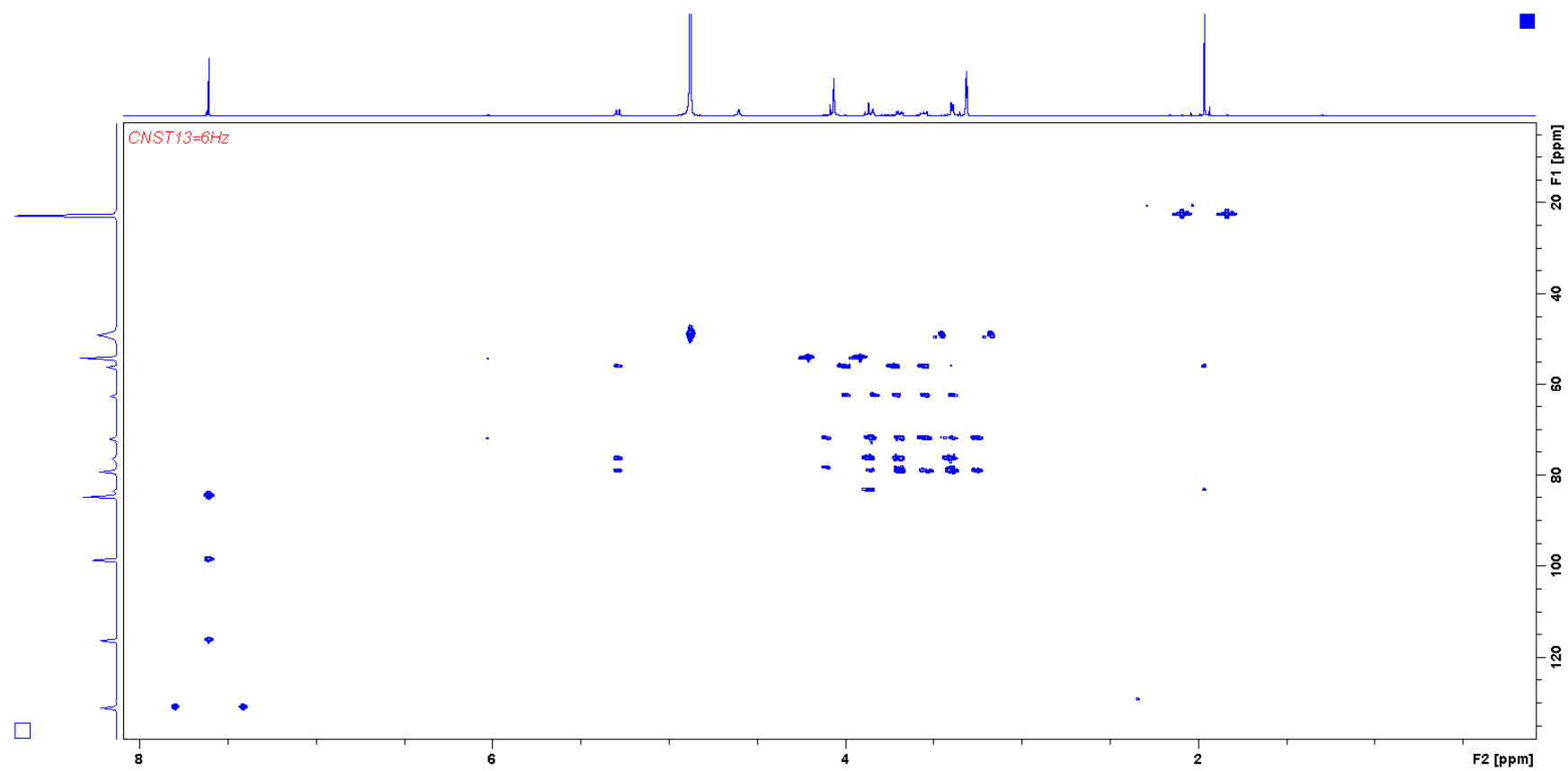


7

8

Figure S4. HMBC (500 MHz, MeOD₄) spectrum (CNST 13=2 Hz) of huimycin 1.

9

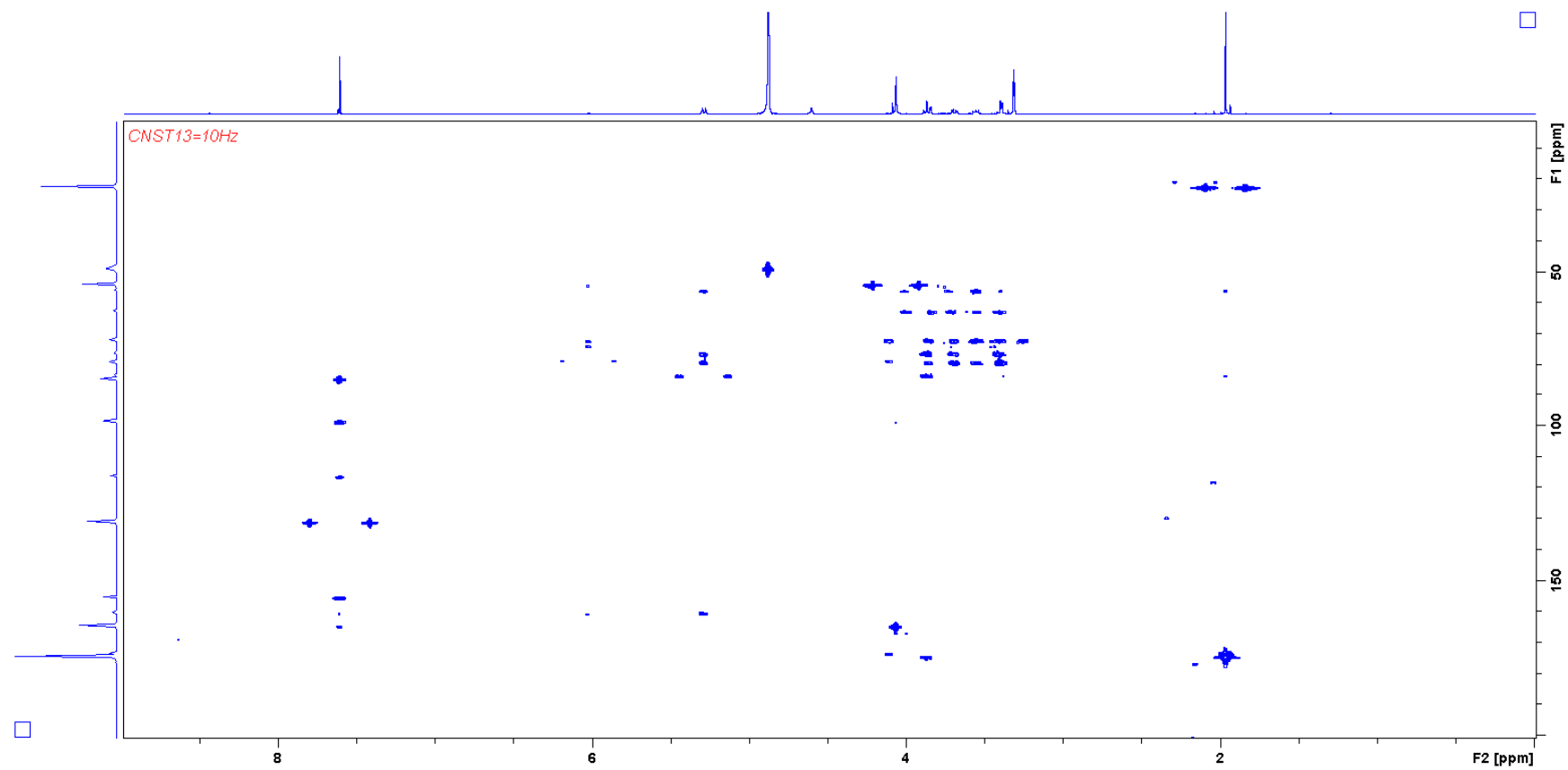


10

11

Figure S5. HMBC (500 MHz, MeOD₄) spectrum (CNST 13=6 Hz) of huimycin 1.

12

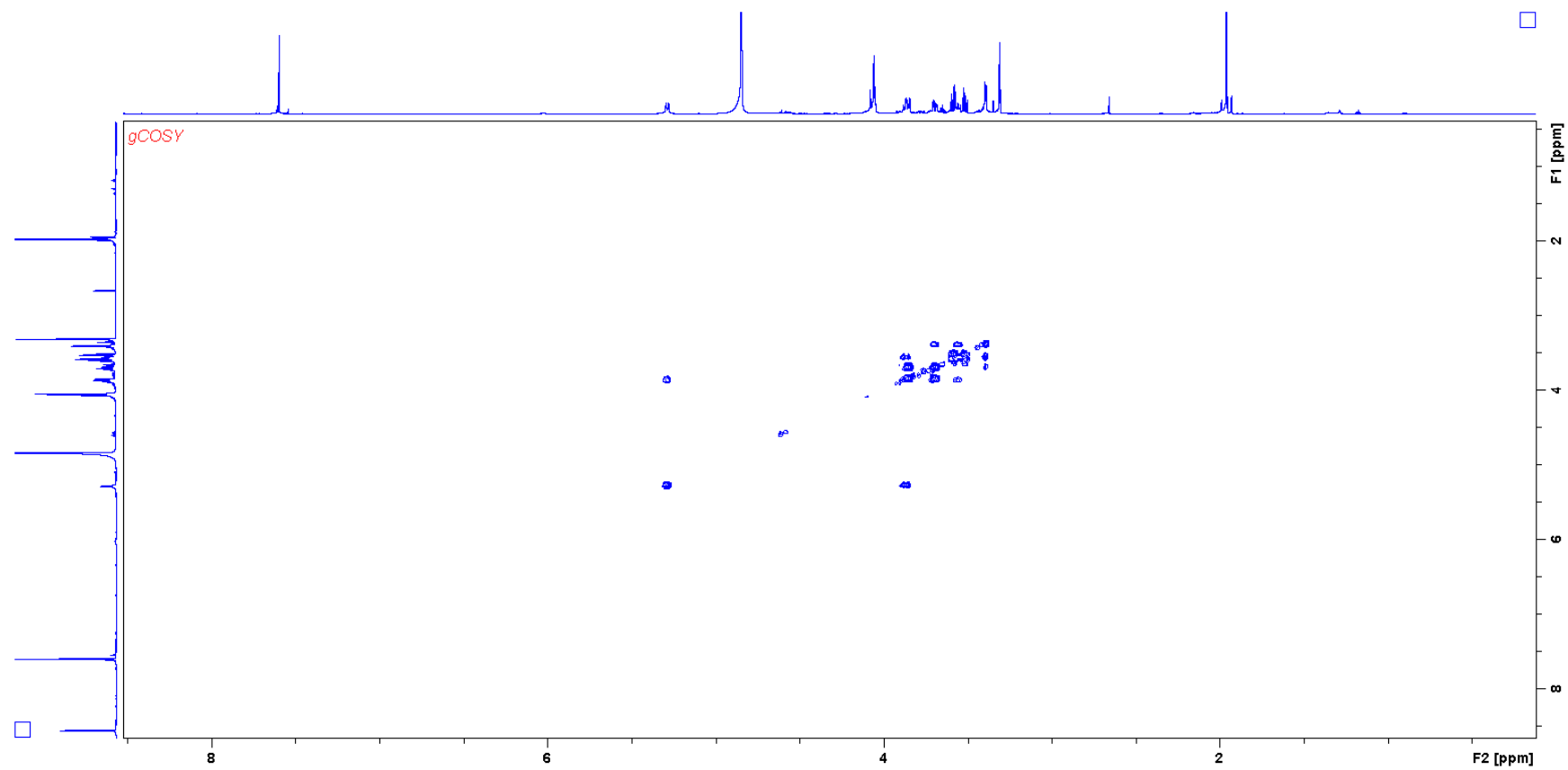


13

14

Figure S6. HMBC (500 MHz, MeOD₄) spectrum (CNST 13=10 Hz) of huimycin 1.

15

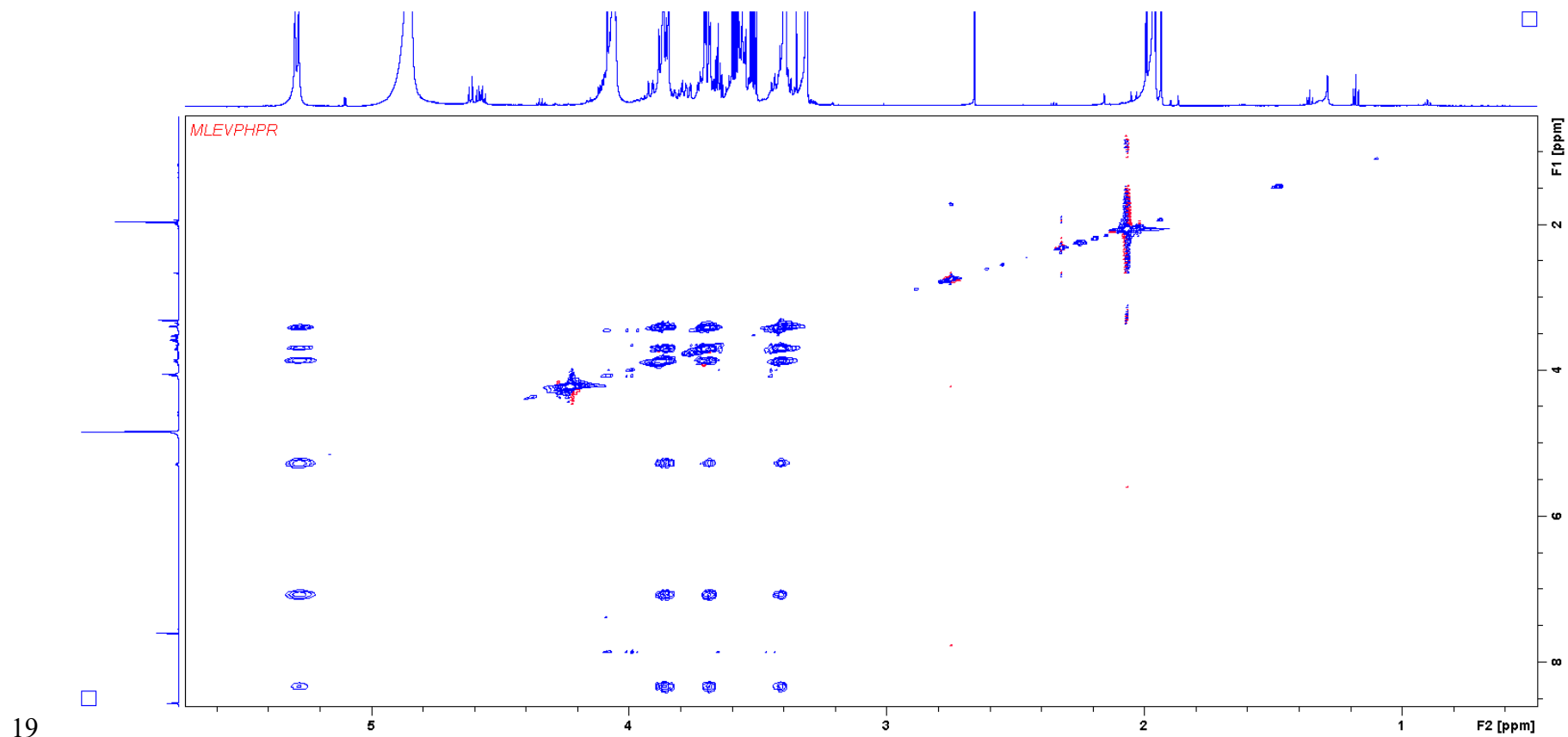


16

17

Figure S7. ^1H - ^1H COSY (500 MHz, MeOD_4) spectrum of huimycin 1.

18

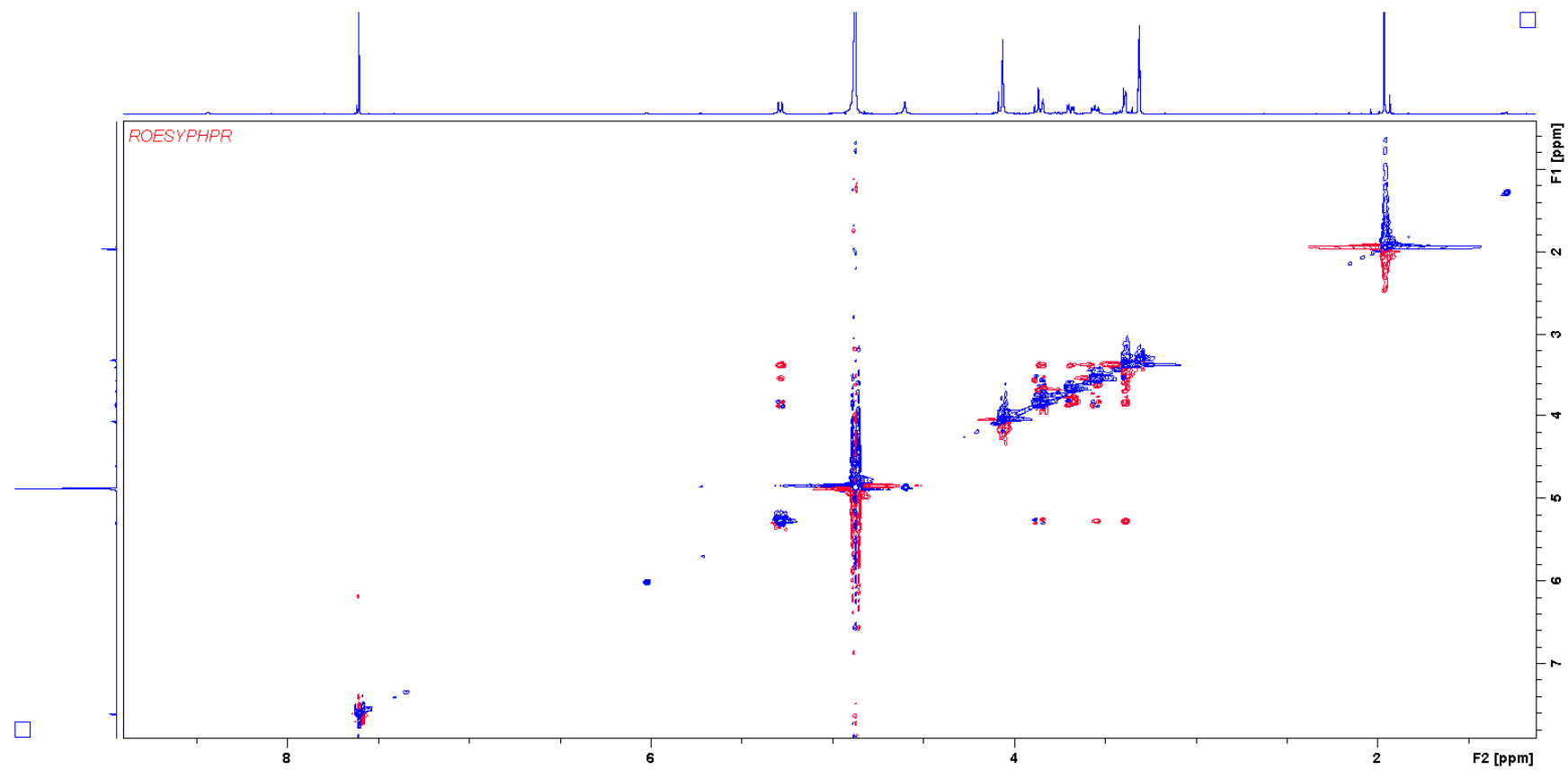


19

20

Figure S8. TOSCY (500 MHz, MeOD₄) spectrum of huimycin 1.

21



22

23

Figure S9. ROESY (500 MHz, MeOD₄) spectrum of huimycin 1.

Tables

Table S1. Bacterial strains used in this work.

Strain	Description	Reference or source
<i>Kutzneria albida</i> DSM 43870	The wild type strain; the source of the huimycin cluster	[1]
<i>Streptomyces albus</i> Del14	The heterologous host strain; cluster-free derivative of the <i>S. albus</i> J1074	[2]
<i>S. albus</i> 2I16	Derivative of <i>S. albus</i> Del14 harboring the 2I16 BAC	This work
<i>S. albus</i> 2I16_LS	Derivative of <i>S. albus</i> Del14 harboring the 2I16_LS BAC	This work
<i>S. albus</i> 2I16_RS	Derivative of <i>S. albus</i> Del14 harboring the 2I16_RS BAC	This work
<i>S. albus</i> 2I16_4069	Derivative of <i>S. albus</i> Del14 harboring the 2I16_4069 BAC	This work
<i>S. albus</i> 2I16_4074	Derivative of <i>S. albus</i> Del14 harboring the 2I16_4074 BAC	This work
<i>S. albus</i> 2I16_4075	Derivative of <i>S. albus</i> Del14 harboring the 2I16_4075 BAC	This work
<i>S. albus</i> 2I16_4076	Derivative of <i>S. albus</i> Del14 harboring the 2I16_4076 BAC	This work
<i>Escherichia coli</i> ET12567 pUB307	Donor strain for intergeneric conjugation	[3]
<i>Escherichia coli</i> DH10 β	General cloning strain	[4]

Table S2. Plasmids and BACs used in this work.

BAC	Description	Reference or source
2I16	The BAC containing 95 kb chromosomal fragment of <i>K. albidia</i> ; contains huimycin gene cluster	[1]
2I16_LS	The derivative of 2I16 with the deletion of 20 kb DNA fragment upstream the huiA gene	This work
2I16_RS	The derivative of 2I16 with the deletion of 36 kb DNA fragment downstream the KALB_4076 gene	This work
2I16_4069	The derivative of 2I16 with the deletion of KALB_4069 (<i>huiC</i>) gene	This work
2I16_4074	The derivative of 2I16 with the deletion of KALB_4074 gene	This work
2I16_4075	The derivative of 2I16 with the deletion of KALB_4075 gene	This work
2I16_4076	The derivative of 2I16 with the deletion of KALB_4076 gene	This work
pACS-hyg	The plasmid containing hygromycin resistance gene	[5]
pUC19	General cloning vector	Thermo Scientific

Table S3. Primers used in this study.

Primer	Sequence
LS_F	CGTTCCGTCTCTGTCACGAAGTGCGCCAGTTGGTAGAGGGCCGCTGCGCAATACTT GACATATCACTGT
LS_R	TACGAGCCCAGCTCACTGAGGCTGGTCTCGAGTGTGTTGGGCGCGTCAGTTCTAGAT CAGGCGCCGGGGCGGTGT
RS_F	ACGCAGAGGCAGCCCCGACGCGCCAGCCCACGGTCGTAGCAAGGAGCCAGGCGTCA GGTGGCACTTTTCG
RS_R	TGATGCAGCAGGGCACTGGGCAGGACGTA ACTGGTTGACCGCAGGATCGCGATATC TTACCAATGCTTAATCAGTG
4069_F	ATGAGCATGGGAACATCCCGGACAGCGGTGACTCCTCTCGACGCCGAGGGTTTAAA CCGTCAGGTGGCACTTTTCG
4069_R	CACAGCACCGAAGCCGTCGACGTGTGGGGGCGGGCGATCAGGACGGCGCCGTTTAA ACTTACCAATGCTTAATCAGTG
4074_F	ATGATCGTTCTCGGACTGATCGGACGGCCCGACGTCCCGCTCTGCCATGGTTTAAAC CGTCAGGTGGCACTTTTCG
4074_R	CAGGAGCGGCTCACCGCGTACGGCGGCATGACCAGCAGGTGCGACGTCCGGGTTTAA ACTTACCAATGCTTAATCAGTG
4075_F	ATGGGGAAGAGCTACGAGCGGATAGACGGCAGACTGCGTGCCTTCATCGGTTTAAA CCGTCAGGTGGCACTTTTCG
4075_R	TATTCAGCGCGGGAAGCGGTGGCAGCGGCAACGGCAACCCCGGCAGGCCGTTTAA ACTTACCAATGCTTAATCAGTG
4076_F	ATGGTCGACAACATGCGGAGGTGTGATGAGATACCCGATTCCACGATTGGTTTAAAC CGTCAGGTGGCACTTTTCG
4076_R	CAGGTCGGTGAGGTCGCTGTGGGGATTTCCGGCGATACGCCAAACGCCGATGTTTAAA CTTACCAATGCTTAATCAGTG
LS_chF	GTTCTCCCTTCCACCAGCC
LS_chR	TGTTCTTCAGGACGCGGAC
RS_chF	TGATTTTCGTCGCGGTGGAA
RS_chR	GCCGCATACGACAGGGAAT
4069_chF	AACCTGCGGAACCTGCTAC
4069_chR	AACTTGCTGAGTCCCGCTC
4074_chF	CGAACAGTTGTGGTGTGCG
4074_chR	GGTCTTGACAATTGCTCCGG
4075_chF	CGATCCCCTTGGCATGTGA
4075_chR	CGCTGCTGGATGAGATCGT
4076_chF	AACCAACAAGGGGCTGTCC
4076_chR	AGTTCACCGCGACGAAAT

References

1. Rebets, Y.; Tokovenko, B.; Lushchik, I.; Rückert, C.; Zaburannyi, N.; Bechthold, A.; Kalinowski, J.; Luzhetskyy, A. Complete genome sequence of producer of the glycopeptide antibiotic Aculeximycin *Kutzneria albida* DSM 43870T, a representative of minor genus of *Pseudonocardiaceae*. *BMC Genomics* **2014**, *15*, 885, doi:10.1186/1471-2164-15-885.
2. Myronovskyi, M.; Rosenkränzer, B.; Nadmid, S.; Pujic, P.; Normand, P.; Luzhetskyy, A. Generation of a cluster-free *Streptomyces albus* chassis strains for improved heterologous expression of secondary metabolite clusters. *Metab. Eng.* **2018**, *49*, 316–324, doi:10.1016/j.ymben.2018.09.004.
3. Flett, F.; Mersinias, V.; Smith, C.P. High efficiency intergeneric conjugal transfer of plasmid DNA from *Escherichia coli* to methyl DNA-restricting streptomycetes. *FEMS Microbiol. Lett.* **1997**, *155*, 223–229, doi:10.1111/j.1574-6968.1997.tb13882.x.
4. Grant, S.G.; Jessee, J.; Bloom, F.R.; Hanahan, D. Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4645–4649, doi:10.1073/pnas.87.12.4645.
5. Myronovskyi, M.; Brötz, E.; Rosenkränzer, B.; Manderscheid, N.; Tokovenko, B.; Rebets, Y.; Luzhetskyy, A. Generation of new compounds through unbalanced transcription of landomycin A cluster. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 9175–9186, doi:10.1007/s00253-016-7721-3.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).