

## Supporting Information

### **A chromatogram-simplified *Streptomyces albus* host for heterologous production of natural products**

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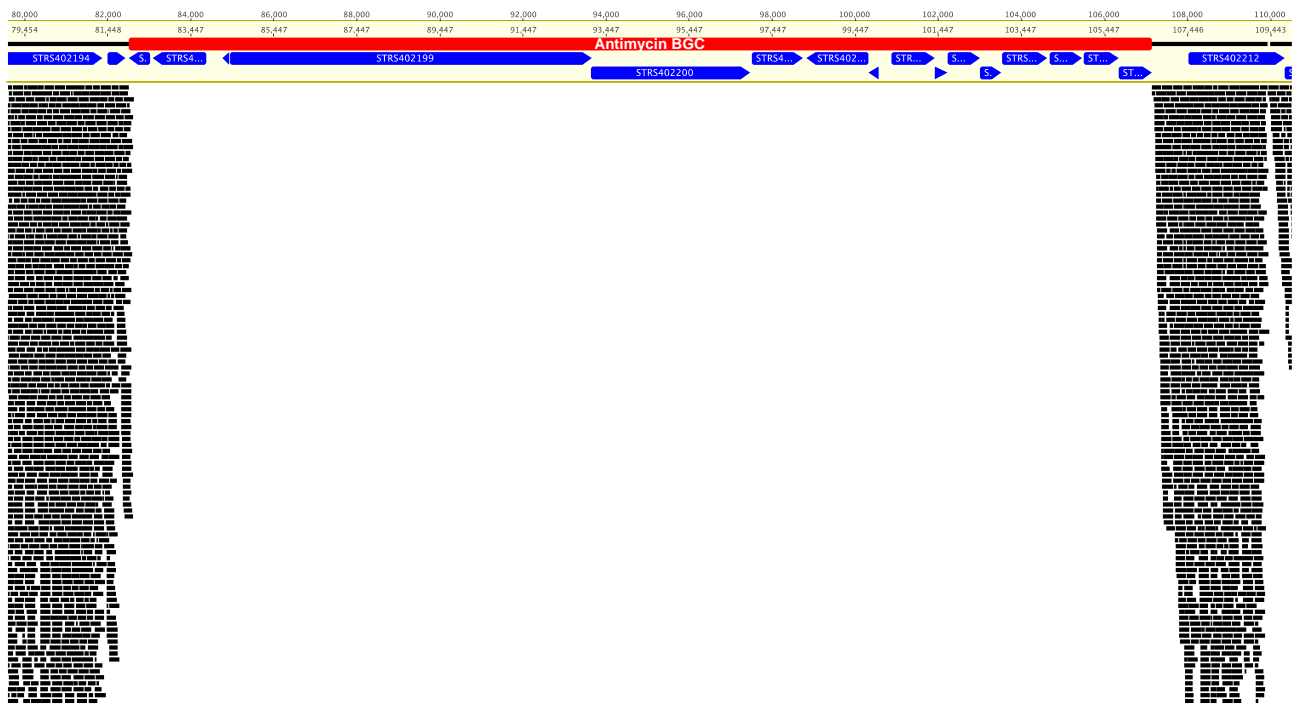
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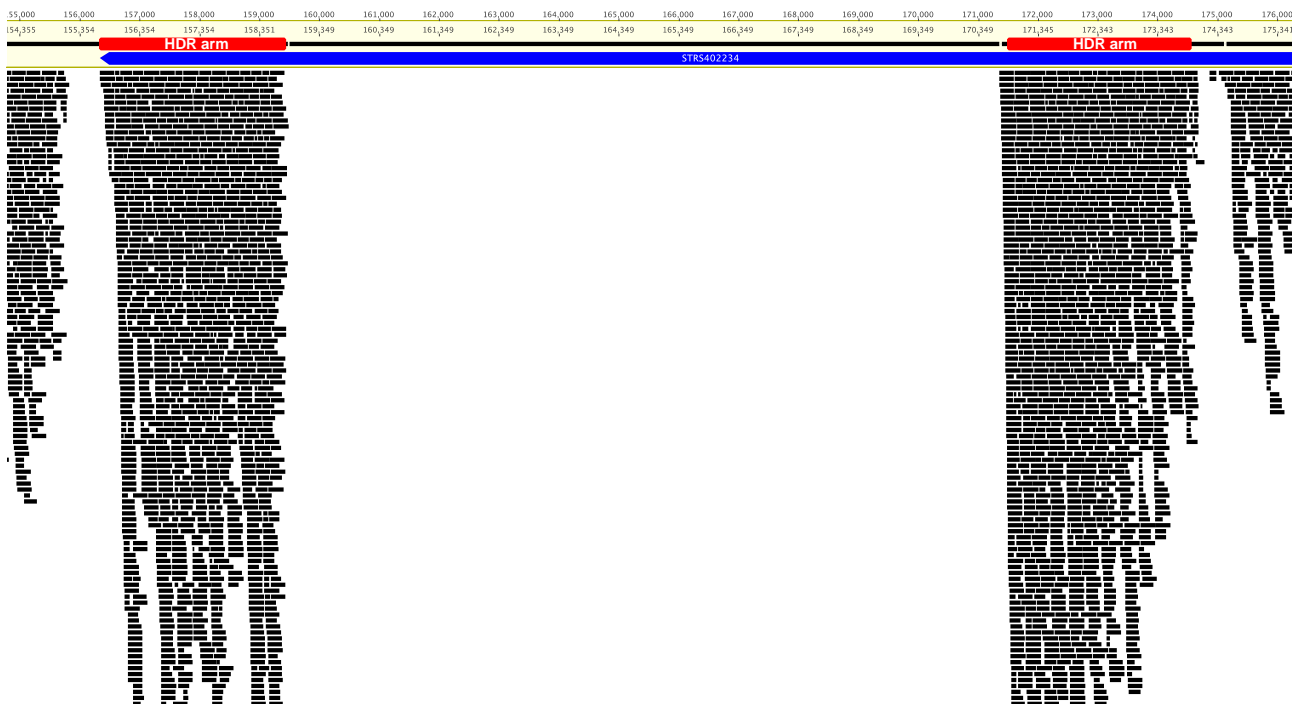
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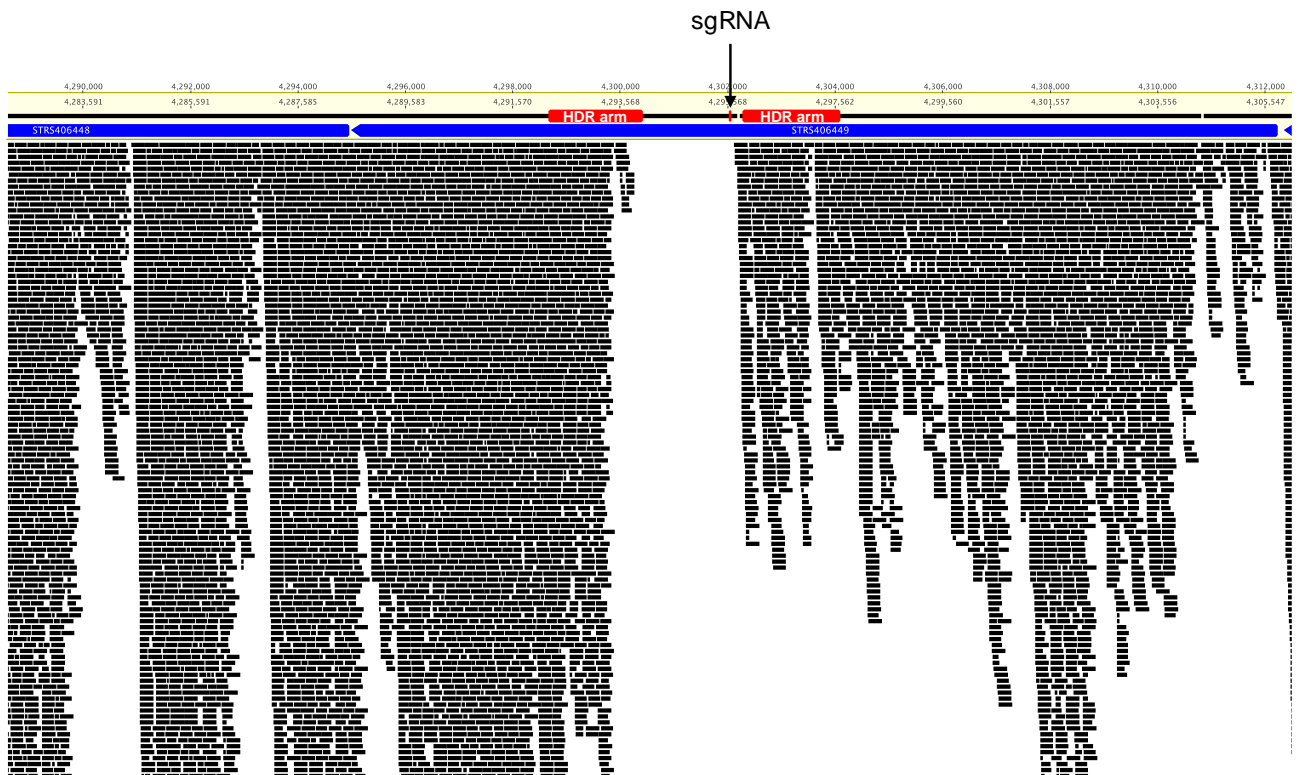
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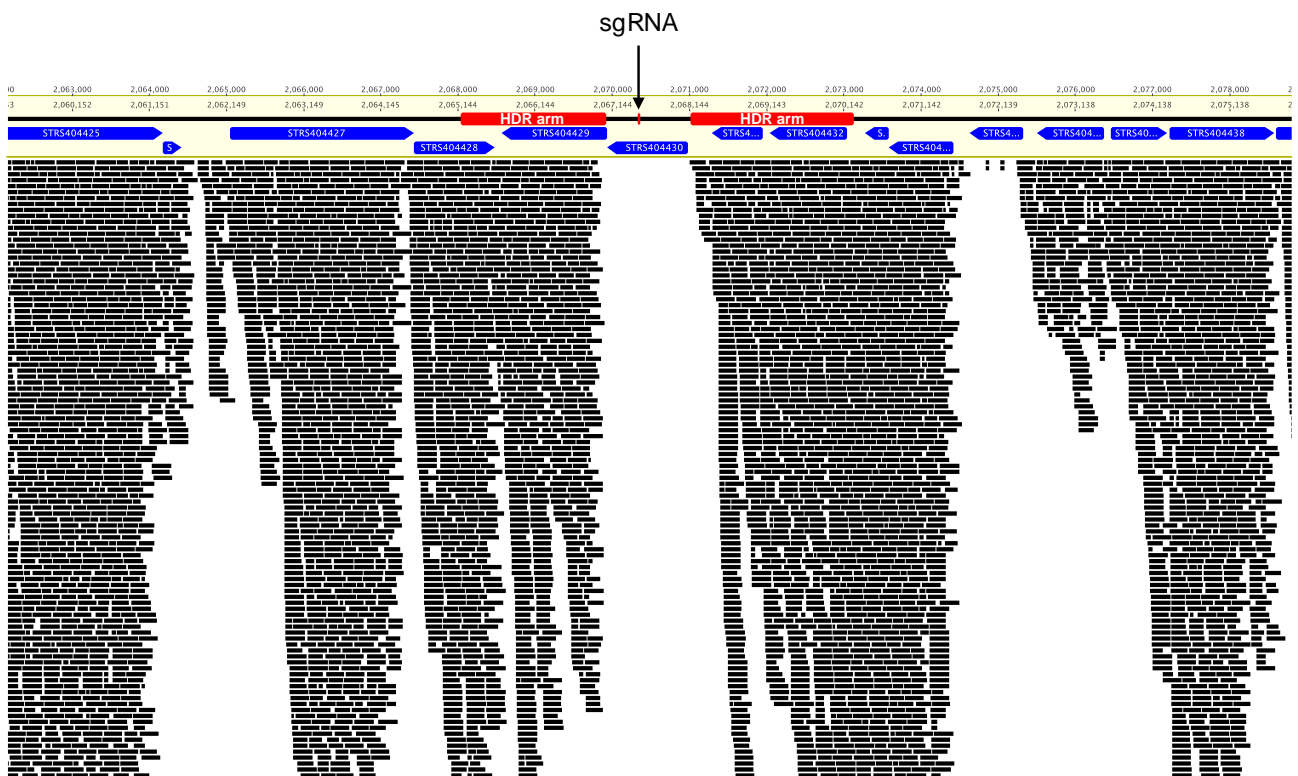
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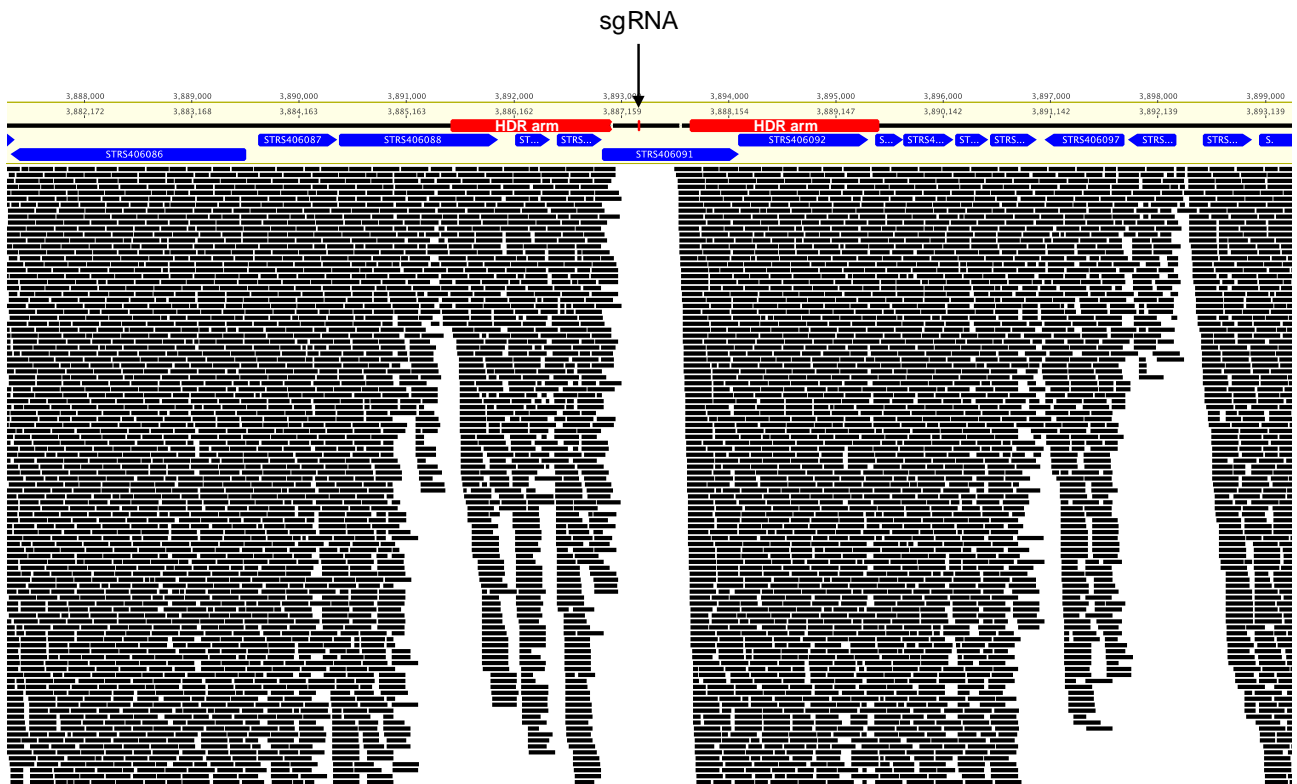
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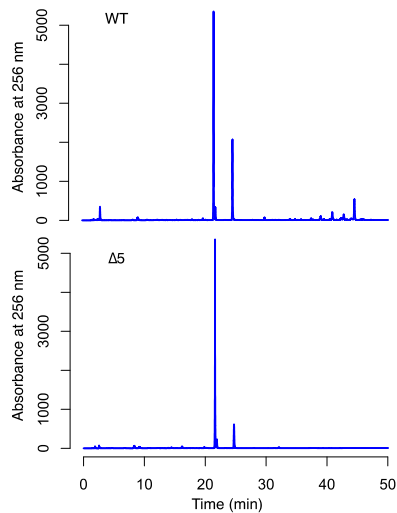
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**Figure S4.** Mutagenesis of the albaflavenone BGC in *Streptomyces albus* S4  $\Delta$ 5. Black rectangles represent Illumina MiSeq reads mapped to the *S. albus* S4  $\Delta$ 5 genome. Red rectangles indicate the homology-directed repair arms used to repair the double strand break generated by the Cas9 targeting the *STRS404430* gene. HDR, homology-directed repair arm; sgRNA, single guide RNA.



**Figure S5.** Mutagenesis of the fredericamycin BGC in *Streptomyces albus* S4  $\Delta 5$ . Black rectangles represent Illumina MiSeq reads mapped to the *S. albus* S4  $\Delta 5$  genome. Red rectangles indicate the homology-directed repair arms used to repair the double strand break generated by the Cas9 targeting the *STRS406091* gene. HDR, homology-directed repair arm; sgRNA, single guide RNA.



**Figure S6.** Full scale version of the HPLC chromatogram depicted in Figure 3 in the main text.

**Table S1.** Bacterial strains, cosmids and plasmids used in this study

| Strain/cosmid/plasmid             | Description <sup>a</sup>   | Reference           |
|-----------------------------------|--|---------------------|
| <i>Candida albicans</i>           | <i>Candida albicans</i> strain CA-6  | [1]                 |
| <i>Micrococcus luteus</i>         | <i>Micrococcus luteus</i> NCTC 9379  | NCTC                |
| <i>Streptomyces</i>               |  |                     |
| S4                                | Wild type <i>Streptomyces albus</i> S4   | [2]                 |
| Δ1                                | S4 harbouring a complete deletion of the antimycin BGC   | This study          |
| Δ2                                | Δ1 harbouring a deletion in the <i>STRS402234</i> ( <i>fscC</i> ) of the candicidin BGC  | This study          |
| Δ3                                | Δ2 harbouring a deletion in the <i>STRS406449</i> gene of the surugamide BGC   | This study          |
| Δ4                                | Δ3 harbouring a deletion in the <i>STRS404430</i> gene of the albaflavenone BGC  | This study          |
| Δ5                                | Δ4 harbouring a deletion in the <i>STRS406091</i> gene of the fredericamycin BGC   | This study          |
| Δ5/Act                            | <i>S. albus</i> S4 Δ5 harbouring the actinorhodin BGC ( <i>attB</i> ΦC31::pAH77); Apr <sup>R</sup>   | This study          |
| Δ5/Cin                            | <i>S. albus</i> S4 Δ5 harbouring the cinnamycin BGC ( <i>attB</i> ΦC31::pIJ10109) ; Apr <sup>R</sup>   | This study          |
| Δ5/Prun                           | <i>S. albus</i> S4 Δ5 harbouring the neoantimycin/prunustatin BGC split over two cosmids ( <i>attB</i> ΦC31::cosmid813 <i>attB</i> ΦBT1::cosmid69); Apr <sup>R</sup> , Hyg <sup>R</sup> , Kan <sup>R</sup>           | [3]                 |
| <i>Escherichia coli</i>           |  |                     |
| BW25113                           | Host for REDIRECT PCR targeting system   | [4]                 |
| NEBα                              | General cloning host   | New England Biolabs |
| ET12567                           | Non-methylating host for transfer of DNA into <i>Streptomyces</i> spp. ( <i>dam</i> , <i>dcm</i> , <i>hsdM</i> ); Cam <sup>R</sup>   | [5]                 |
| GB05-red                          | Host for RecET recombination   | [6]                 |
| <b>Cosmids and BACs</b>           |  |                     |
| Supercos1                         | Cosmid backbone for <i>S. albus</i> S4 Cosmid 213; Carb <sup>R</sup> , Kan <sup>R</sup>  | Stratagene          |
| Cosmid 213                        | Supercos1 derivative containing the entire antimycin biosynthetic gene cluster; Carb <sup>R</sup> , Kan <sup>R</sup>   | [7]                 |
| Cosmid 213Δ <i>antF</i>           | Cosmid 213 derivative harbouring an apramycin-marked deletion of <i>antF</i> ; Carb <sup>R</sup> , Kan <sup>R</sup> , Apr <sup>R</sup>   | This study          |
| Cosmid 213Δ <i>antA-antO</i>      | Cosmid 213 derivative harbouring an apramycin-marked deletion in the entire antimycin biosynthetic gene cluster ( <i>antABCDEFGHIJKLMNO</i> ); <i>antF</i> ; Carb <sup>R</sup> , Kan <sup>R</sup> , Apr <sup>R</sup> | This study          |
| Cosmid 213Δ <i>antA-antO</i> -FLP | Cosmid 213Δ <i>antA-antO</i> derivative in which the apramycin resistance cassette was removed by the FLP recombinase; Carb <sup>R</sup> , Kan <sup>R</sup>  | This study          |

|   |   |            |
|---|---|------------|
| Cosmid 213 $\Delta$ <i>antA-antO</i> -FLPHyg <sup>R</sup> <i>oriT</i> | Cosmid 213 $\Delta$ <i>antA-antO</i> -FLP derivative in which the <i>bla</i> resistance gene on the cosmid backbone was disrupted with a <i>hyg<sup>R</sup>-oriT</i> cassette                           | This study |
| pIJ10109  | Derivative of pOJ436 harbouring the cinnamycin biosynthetic gene cluster from <i>Streptomyces cinnamoneus</i> ; Carb <sup>R</sup> , Apr <sup>R</sup>  | [8]        |
| Cosmid 813  | Supercos1 derivative harbouring <i>natABCDEF</i> from the neoantimycin biosynthetic gene cluster; Carb <sup>R</sup> , Kan <sup>R</sup>  | [3]        |
| Cosmid 69   | Supercos1 derivative harbouring a partial <i>natB</i> gene and <i>natCDEFGQF'G'HIJKLNO</i> P genes from the neoantimycin biosynthetic gene cluster; Carb <sup>R</sup> , Kan <sup>R</sup>                | [3]        |
| Cosmid 69- $\Phi$ BT1   | Cosmid 69 derivative engineered to integrate into the $\Phi$ BT1 <i>attB</i> site; Carb <sup>R</sup> , Hyg <sup>R</sup>   | [3]        |
| <b>Plasmids</b>   |   |            |
| pCRISPomyces-2  | pGM1190 derivative harbouring the CRISPR/Cas9 machinery; Apr <sup>R</sup>   | [9]        |
| pCRISPomyces-2-sur  | Derivative of pCRISPomyces-2 derivative containing the <i>STRS406449</i> -targeting protospacer cloned into the BbsI site and homology-directed repair arms cloned into the XbaI site; Apr <sup>R</sup> | This study |
| pCRISPomyces-2-alb  | Derivative of pCRISPomyces-2 derivative containing the <i>STRS404430</i> -targeting protospacer cloned into the BbsI site and homology-directed repair arms cloned into the XbaI site; Apr <sup>R</sup> | This study |
| pCRISPomyces-2-fdm  | Derivative of pCRISPomyces-2 derivative containing the <i>STRS406091</i> -targeting protospacer cloned into the BbsI site and homology-directed repair arms cloned into the XbaI site; Apr <sup>R</sup> | This study |
| pIJ773  | ReDirect PCR template plasmid harbouring an apramycin resistance cassette and <i>oriT</i> ; Carb <sup>R</sup> , Apr <sup>R</sup>  | [4]        |
| pIJ10701  | ReDirect PCR template plasmid harbouring a hygromycin resistance cassette and <i>oriT</i> ; Hyg <sup>R</sup>  | [4]        |
| pKC1132-UpDn  | Derivative of suicide plasmid pKC1132 [10] containing ~3kb of homologous DNA upstream and downstream of the region of <i>fscC</i> targeted for deletion; Apr <sup>R</sup>                               | [11]       |
| pUZ8002   | Encodes conjugation machinery for mobilisation of plasmids from <i>E. coli</i> to <i>Streptomyces</i> ; Kan <sup>R</sup>  | [5]        |

<sup>a</sup> Carb, carbenicillin; Apr, apramycin; Hyg, hygromycin, Kan, kanamycin; Cam, chloramphenicol; *oriT*, origin of conjugal transfer

**Table S2.** Oligonucleotide primers used in this study

| <b>Primer alias</b> | <b>Sequence (5'-3')<sup>a</sup></b>                                  | <b>Description</b>   |
|---------------------|--|--|
| RFS196              | <b>ctcgtgctgttctcaggtggagaggtgctcgcgcctcatgtaggc</b><br>tggagctgcttc | PCR: <i>antF</i> REDIRECT knockout cassette                          |
| RFS236              | cgctacaacaccggtagt   | PCR: confirmation of $\Delta ant$ mutation                           |
| RFS237              | aggggacgatgttgacgacc   | PCR: confirmation of $\Delta ant$ mutation                           |
| RFS197              | <b>gacggccccggcggccgggacggccggcggtgctgatgattcc</b><br>gggatccgtcgacc | PCR: <i>antF</i> REDIRECT knockout cassette                          |
| RFS219              | <b>caacgccccgctgtctcaccgccatggtggccgcaattccg</b><br>gggatccgtcgacc   | PCR: <i>antABCDEFGHIJKLMNO</i> REDIRECT knockout cassette            |
| RFS203              | <b>ccgctcggcgggtcgggagacatctggcggcggtcatgtag</b><br>gctggagctgcttc   | PCR: <i>antABCDEFGHIJKLMNO</i> REDIRECT knockout cassette            |
| RFS242              | atcacgcggtgatcgacca  | PCR: confirmation of $\Delta antF$ mutant strain                     |
| RFS243              | tggaggaactcgggaccatc   | PCR: confirmation of $\Delta antF$ mutant strain                     |
| EH_S3               | acgctgccgggcccgcgcgagaaa   | CRISPR protospacer targeting <i>STRS4_04430</i> (albaflavenone BGC)  |
| EH_S4               | aaactttctcgcggcggcccggca   | CRISPR protospacer targeting <i>STRS4_04430</i> (albaflavenone BGC)  |
| EH_S7               | acgcgtacgctgctccatggaga  | CRISPR protospacer targeting <i>STRS4_06091</i> (fredericamycin BGC) |
| EH_S8               | aaactcctcatggagcaggcgtac   | CRISPR protospacer targeting <i>STRS4_06091</i> (fredericamycin BGC) |
| EH_S9               | acgccacctcacggcaccggga   | CRISPR protospacer targeting <i>STRS4_06449</i> (surugamide BGC)     |
| EH_S10              | aaactcccgtgccgctgaggtg   | CRISPR protospacer targeting <i>STRS4_06449</i> (surugamide BGC)     |
| EH_P7               | <b>tgccgccccggcgtttttat</b> gtgtactggttccgctc                        | PCR: <i>STRS4_04430</i> homology-directed repair arm                 |
| EH_P8               | <b>tttgttcgtgctgctttcc</b> gaatcaccaccgaac                           | PCR: <i>STRS4_04430</i> homology-directed repair arm                 |
| EH_P9               | <b>ggcgttcggtggtggattc</b> gggaaagcaggcacgaac                        | PCR: <i>STRS4_04430</i> homology-directed repair arm                 |
| EH_P10              | <b>cggccttttacggttctc</b> ggcctggttgatgcaagagg                       | PCR: <i>STRS4_04430</i> homology-directed repair arm                 |
| EH_P15              | <b>tgccgccccggcgtttttat</b> cgtcttctgctgttgg                         | PCR: <i>STRS4_06091</i> homology-directed repair arm                 |
| EH_P16              | <b>ggtcatgtgtagccgtt</b> ggccttcacgatcatctcc                         | PCR: <i>STRS4_06091</i> homology-directed repair arm                 |
| EH_P17              | <b>agatgatcgtgaaggcca</b> accaacggctaccatgacc                        | PCR: <i>STRS4_06091</i> homology-directed repair arm                 |
| EH_P18              | <b>cggccttttacggttctc</b> ggcctatggtcttgaggtcgtgaagc                 | PCR: <i>STRS4_06091</i> homology-directed repair arm                 |
| EH_P19              | <b>tgccgccccggcgtttttat</b> gtacgtcatgtccacctcc                      | PCR: <i>STRS4_06449</i> homology-directed repair arm                 |
| EH_P31              | <b>cgtaagcctgggtgtt</b> gctctacagctcgctcagttcg                       | PCR: <i>STRS4_06449</i> homology-directed repair arm                 |
| EH_P27              | <b>cggcaactgagcgagct</b> gtaggacaacaccaggcttacg                      | PCR: <i>STRS4_06449</i> homology-directed repair arm                 |
| EH_P28              | <b>cggccttttacggttctc</b> ggcctctcctcaccgactcagc                     | PCR: <i>STRS4_06449</i> homology-directed repair arm                 |



|        |                               |  |
|--------|-------------------------------|--|
| EH_P35 | <u>gtcgtgaatctcctgatcg</u>    | PCR: confirmation of <i>STRS4_04430</i> mutation |
| EH_P36 | <u>tacggctacctctacatcgacc</u> | PCR: confirmation of <i>STRS4_04430</i> mutation |
| EH_P37 | <u>tgccgaacccttctactcc</u>    | PCR: confirmation of <i>STRS4_06091</i> mutation |
| EH_P38 | <u>ccatgtccagggtcgttcagc</u>  | PCR: confirmation of <i>STRS4_06091</i> mutation |
| EH_P39 | <u>caccaggacttctcacg</u>      | PCR: confirmation of <i>STRS4_06449</i> mutation |
| EH_P40 | <u>gagggagaagaagttgctgtgg</u> | PCR: confirmation of <i>STRS4_06449</i> mutation |

<sup>a</sup> non-homologous sequences are underlined and engineered restriction endonuclease sites are bolded

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