Post-PKS tailoring steps of the spiramycin macrolactone ring in *Streptomyces ambofaciens*

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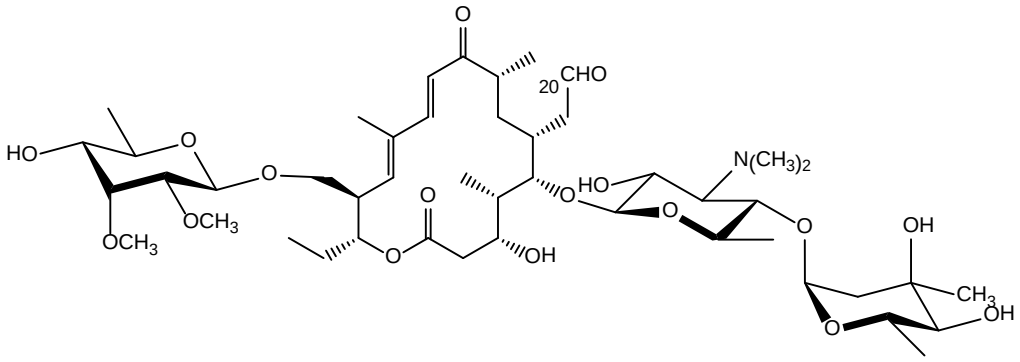
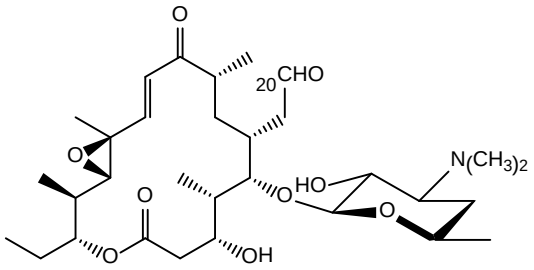
SUPPLEMENTARY DATA

<u>Figure S1 :</u> Structures of A) tylosin and B) rosamicin. The C_{20} carbons modified by TylI (tylosin) or RosC (rosamicin) are indicated.

A

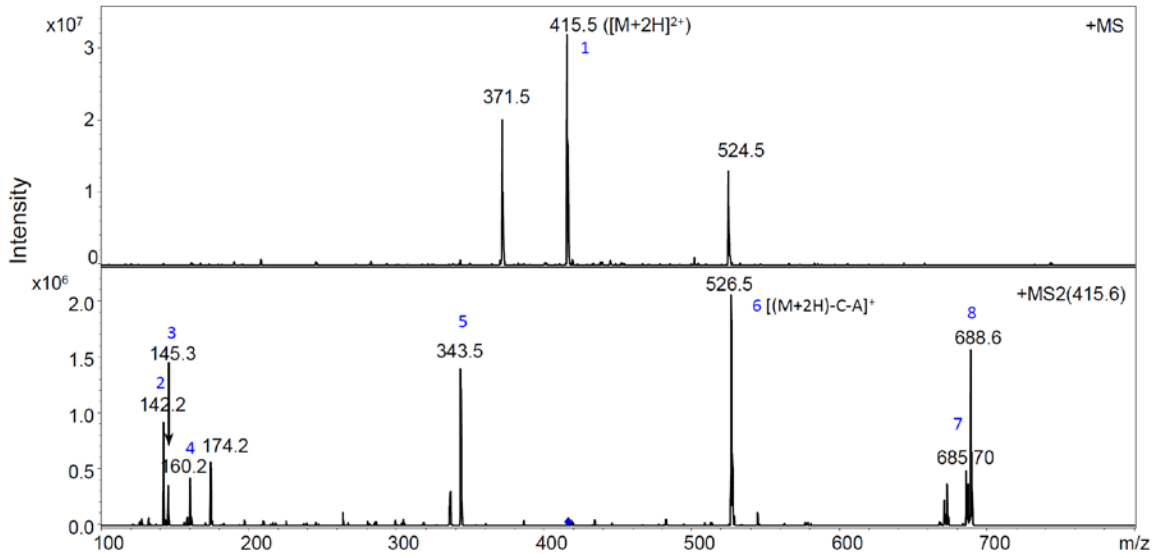


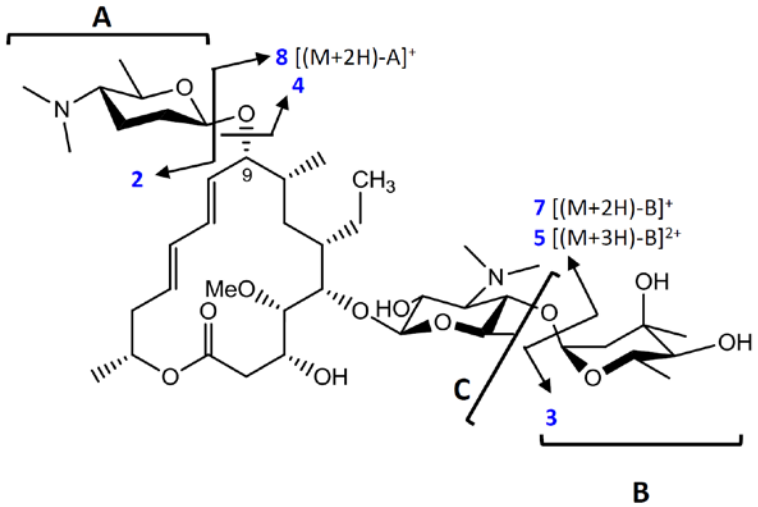
B



<u>Figure S2:</u> Identification of the metabolites 10, 11 and 12 (Figures 2C and 2D) by MS and MS-MS. A) MS and MS-MS fragmentation of peak 10 (C_{19} methyl-spiramycin I, labeled 1 (m/z = 415.5) in MS spectrum) B) MS and MS-MS fragmentation of peak 11 (C_{19} methyl-spiramycin II labeled 1 (m/z = 436.5) in MS spectrum) and C) MS and MS-MS fragmentation of peak 12 (C_{19} methyl-spiramycin III, labeled 1 (m/z = 443.5) in MS spectrum).

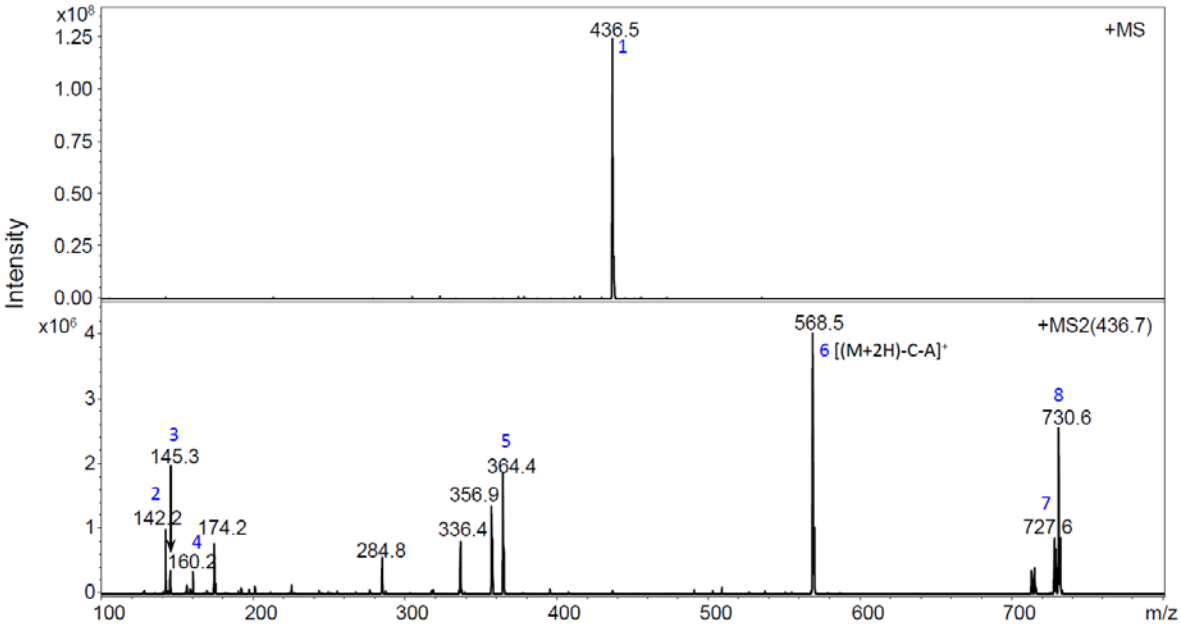
A

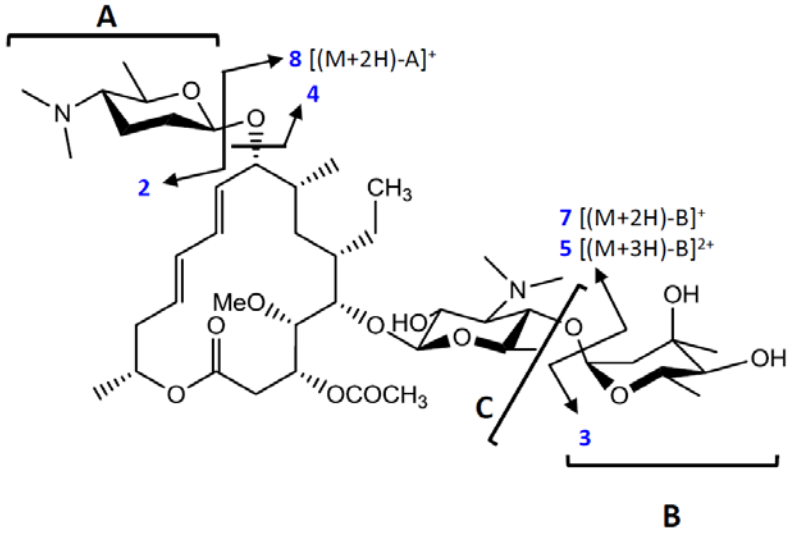




1 $[M+2H]^{2+}$: m/z = 415.25

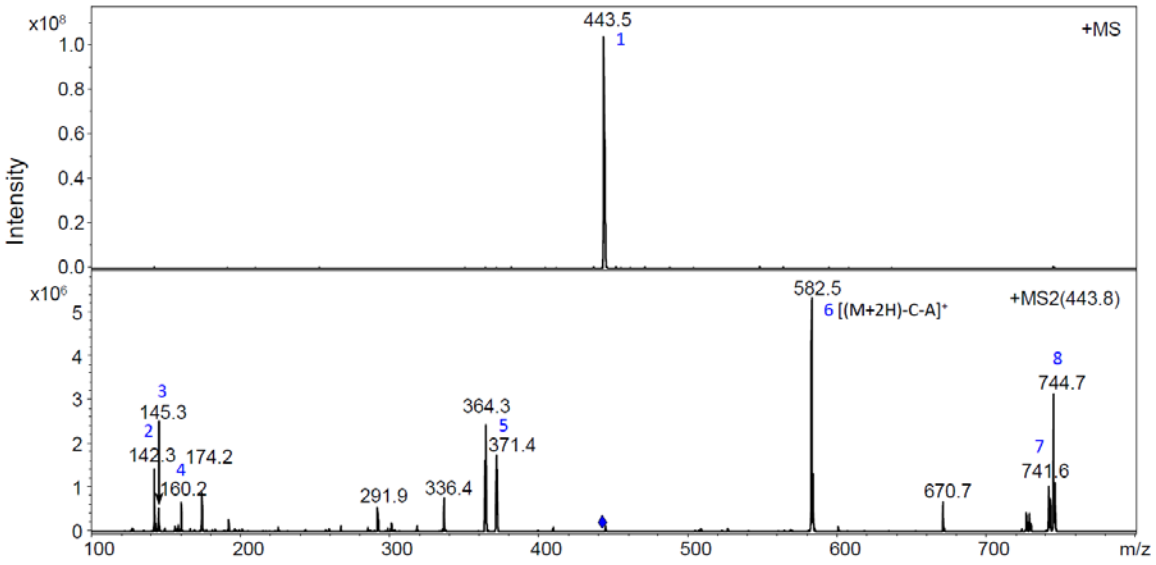
B

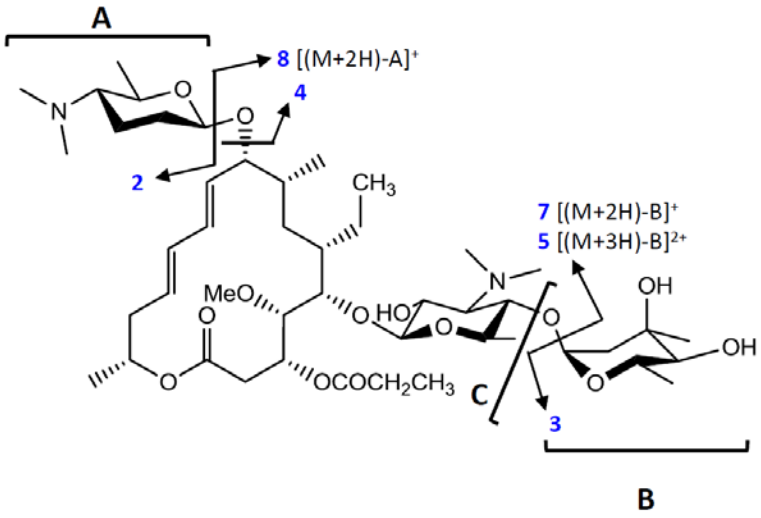




1 $[M+2H]^{2+}$: m/z = 436.28

C





1 $[M+2H]^{2+}$: m/z = 443.28

Figure S3: LC and LC-MS analyses of the *srm43* (SPM543) and the *srm42* deletion mutant (SPM225) culture supernatants. a) UV and b) EIC traces of m/z = 422.2 ($[M+2H]^{2+}$, [**6**]), m/z = 443.2 ($[M+2H]^{2+}$, [**7**]) and m/z = 450.2 ($[M+2H]^{2+}$, [**8**]) for SPM543, and c) UV and d) EIC traces of m/z = 422.2 ($[M+2H]^{2+}$, [**6**]), m/z = 443.2 ($[M+2H]^{2+}$, [**7**]) and m/z = 450.2 ($[M+2H]^{2+}$, [**8**]) for SPM225. Peak numbers correspond to molecule numbers in Figure 1

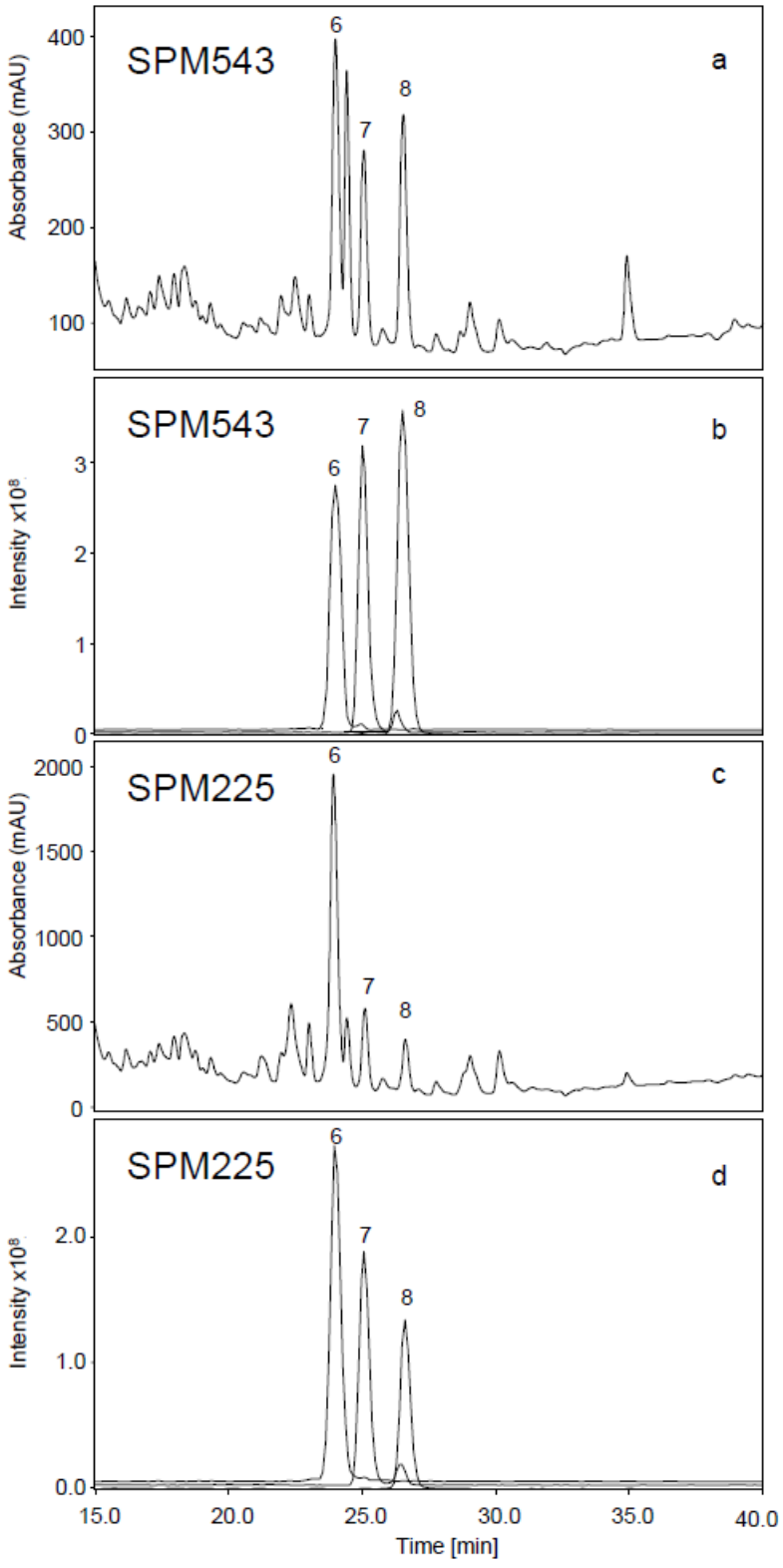


Figure S4 : **Structure of spiramycin IV**

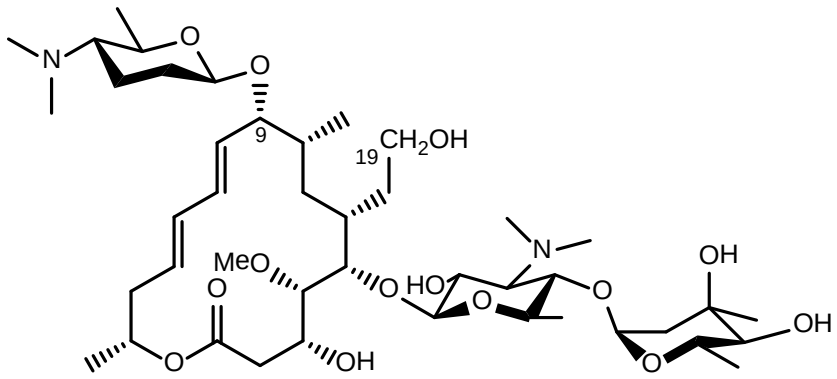


Table S1: Strains and plasmids used in this study

Strains	**Description**	**Source/Reference**
Escherichia coli		
DH5α	General cloning host strain	Promega
S17.1	Host strain for conjugation from *E. coli* to *S. ambofaciens*	(1)
ET12567/pUZ8002	Host strains for conjugation from *E. coli* to *Streptomyces*	(2)
KS272/pKOBEG	Strain used for PCR-targeting by λ RED-mediated recombination	(3)
M. luteus Cg^R	Mutant strain of *M. luteus* DSM1790, resistant to congocidine	(4)
Streptomyces		
OSC2	*S. ambofaciens* strain derived from ATCC23877 devoid of pSAM2	(5)
SPM108	Δ*srm29*::*aat3* in OSC2	(6)
SPM121	Δ*srm5*::*aat3* in OSC2	(6)
SPM212	Δ*srm28*::*aat2* in OSC2	(6)
SPM225	Δ*srm42*::*att2aac* in OSC2	This study
SPM234	Δ*srm26*::*att2aac* in OSC2	This study
SPM235	Δ*srm26*::*att2* in OSC2	This study
SPM512	Δ*srm13*::*att3aac* in OSC2	This study
SPM513	Δ*srm13*::*att3* in OSC2	This study
SPM514	Δ*srm13*::*att3aac, srm26*::*att2* in OSC2	This study
SPM515	Δ*srm13*::*att3, srm26*::*att2* in OSC2	This study
SPM543	Δ*srm43*::*att3aac* in OSC2	This study
Plasmids	**Description**	**Source/Reference**
pWED2	Amp^R, Pur^R, *oriT*, *E. coli* cosmid used for construction of the *S. ambofaciens* genomic library.	(7)
pOSV206	Amp^R, Apr^R, *oriT*, *E. coli-Streptomyces* shuttle plasmid for gene expression in *Streptomyces*, derived from pUWL201	This study
pOSV234	Amp^R, Apr^R, *E. coli-Streptomyces* shuttle plasmid, source of the *att3aac* cassette	(8)
pOSV236	Amp^R, Pur^R, *oriT*, *E. coli-Streptomyces* shuttle plasmid expressing the Xis and Int proteins for site-specific excision of excisable cassettes.	(6)
pSPM266	Coding region of *srm26* cloned into pOSV206	This study
pSPM36	DNA fragment containing the region of spiramycin cluster spanning from *srm24* to the end of the cluster cloned in pWED2	(7)
pSPM265	DNA fragment containing the *att2aac* cassette inserted in *srm42* cloned in pWED2	This study
pSPM267	DNA fragment containing the *att2aac* cassette inserted in *srm26* cloned in pWED2	This study
pSPM543	pSPM36 containing the *att3aac* cassette inserted in *srm43*	This study
pSPM573	DNA fragment containing the a*tt3aac* cassette inserted in *srm13* cloned in pWED2	This study

Table S2: Oligonucleotides used in this study.

Name	Sequence	Description
SRM5	ATAAAGCTTGCGAGGAACTCGATGCCG	Forward primer for amplification of the left-flanking sequence of *srm42*
SRM6	ATAAGATCTACTGCGCCCAGCTCACCG	Reverse primer for amplification of the left-flanking sequence of *srm42*
SRM7	ATAAGATCTTCATCGCCACGACGTCCA	Forward primer for amplification of the right-flanking sequence of *srm42*
SRM8	ATAGATATCGCACCTCGTCCAGCTGCT	Reverse primer for amplification of the right-flanking sequence of *srm42*
SRM9	ATAAAGCTTCCGGATCGAGGAGTTCAC C	Forward primer for amplification of the left-flanking sequence of *srm26*.
SRM10	ATAGATATCAGTGCCTCGGCGGCGAAG	Reverse primer for amplification of the left-flanking sequence of *srm26*
SRM11	ATAGATATCTGAGCCGGGAGAAGGAG CTG	Forward primer for amplification of the right-flanking sequence of *srm26*
SRM12	ATAGCTAGCGAAGTCCGCCGTCAGGTC GAG	Reverse primer for amplification of the right-flanking sequence of *srm26*
SRM13	ATAAAGCTTCCTGACCCGTGAACAACA CC	Forward primer for amplification of the left-flanking sequence of *srm13*
SRM14	ATAGATATCCGGGCGTCCGCGTCCTTG	Reverse primer for amplification of the left-flanking sequence of *srm13*
SRM15	ATAGATATCACCAGCTCACTCCGAAGT ACC	Forward primer for amplification of the right-flanking sequence of *srm13*
SRM16	ATAGCTAGCTCGGCGTAGGTGATGTCG AG	Reverse primer for amplification of the right-flanking sequence of *srm13*
EDR71	CGTCATCGACGTGCGGGGAAGACAGA GGTGATACCGATGCATCGCGCGCGCT TCGTTCGG	PCR-targeting of *srm43*. The sequence identical to the beginning of the *srm43* coding sequence is underlined
EDR72	GGGGGTGAGTTCCGTCGAGCAGCTGG ACGAGGTGCTGGCATCTGCCTCTTCGT CCCGAA	PCR-targeting of *srm43*. The sequence identical to the end of the *srm43* coding sequence is underlined
OE.srm26F	ATAAAGCTTTGGGGGGTTCGAGAGCGT TC	Forward primer for amplication of *srm26* coding sequence
OE.srm26R	ATAGAATTCCCGAGACCTTCACCGTGT AG	Reverse primer for amplication of *srm26* coding sequence

Construction of the pOSV206 vector

To construct pOSV206, the 1.7 kb *Bam*HI/*Hin*dIII/Klenow fragment from pPAOI6 (9) was ligated with pULW201 (10) digested with *Pvu*II. Orientation of the insert was determined by digesting the resulting plasmids with *Xba*I and *Sal*I.

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