Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

A terpene cyclase from *Aspergillus ustus* is involved in the biosynthesis of geosmin precursor germacradienol

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Supplementary Tables

Table S1: Strains used in this study

Strain	Genotype	Created with plasmid	Reference
E. coli DH5α	F- *endA1 glnV44 thi-1 recA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^-m_K^+$), λ^-	-	[2]
E. coli BL21 (DE3)	F^-*ompT hsd*S_B (r_B^-, m_B^-) *galdcmrne*131	-	Merck, KGaA, Darmstadt, Germany
S. cerevisiae HOD114-2B	*MATα ura3-52 his3Δ1 leu2-3112*	-	[4]
A. ustus 3.3904	wildtype	-	CGMCCC
A. nidulans:			
LO8030	*pyroA4, riboB2, AfpyrG89, nkuA::argB*, deletion of secondary metabolite clusters: (AN7804-AN7825)Δ, (AN2545-AN2549)Δ, (AN1039-AN1029)Δ, (AN10023-AN10021)Δ, (AN8512- AN8520)Δ, (AN8379-AN8384)Δ, (AN9246-AN9259)Δ, (AN7906-AN7915)Δ, (AN6000-AN6002)Δ.	-	[1]
MP02	*wA-PKS::gpdA(p)-gdlS* 3'UTR-*Afribo* in LO8030	pMP008	This study
BK06	*wA-PKS::gpdA(p)* + 500bp 3'UTR-*Afribo* in LO8030	-	[5]

CGMCCC: China General Microbiological Culture Collection Center (Beijing, China)

Table S2: Plasmids used in this study

Plasmid	Genotype	Description	Reference
pJN017	*URA3, wA flanking, AfRiboB, Amp, gpdA(p)*	standard-vector for heterologous expression in *A. nidulans* LO8030	[3]
pMP008	*URA3, wA flanking, AfRiboB, Amp, gpdA(p), gdlS*	Heterlogous expression of *gdlS* in *A. nidulans* LO8030	This study
pET-28a(+)	*Kan , T7(p), 6xHis, MCS,* T7 Terminator*, lacI*	Expression vector for *E. coli* with both N- and C-terminal His6-tag	Merck, Novagen, Darmstadt, Germany
pMP014	*Kan , T7(p), 6xHis, gdlS,* T7 Terminator*, lacI*	Overexpression of GdlS protein in *E. coli* BL21 (DE3)	This study

Table S3: Oligonucleotide primers used in this study

Primers	Sequence (5‘-3‘)	Description
MP3.1_TSfor	TACCCCGCTTGAGCAGACATCACCGGCATGTTCTCTAATAAAACGAAACAGGACG	Cloning *gdlS* in pJN017 = pMP008
MP3.1_TSrev	CCATATTTTAATCCCATGTGGGCCTATCTAGTAAGAGATAAAACCCGAGATACAG	
Sst81	GCGAGCCTTCCATAGTTACG	Verification of MP02
MP3.1gene_midrev	CGAGAGCCAGAAATCGTCC	
MP3.1gene_midfor	GGACGTGCTGTATCTCGGG	
JN015ctrl_01	GCACTCTGGAAACGAACTCC	
MPTSforBamHI	GGATCCCTATCTAGTAAGAGATAAAACCCGAGATACAG	Cloning of CDS *gdlS* in pET-28a(+) =pMP014
MPTSrevNdeI	CATATGATGTTCTCTAATAAAACGAAACAGGACG	

Supplementary Figures

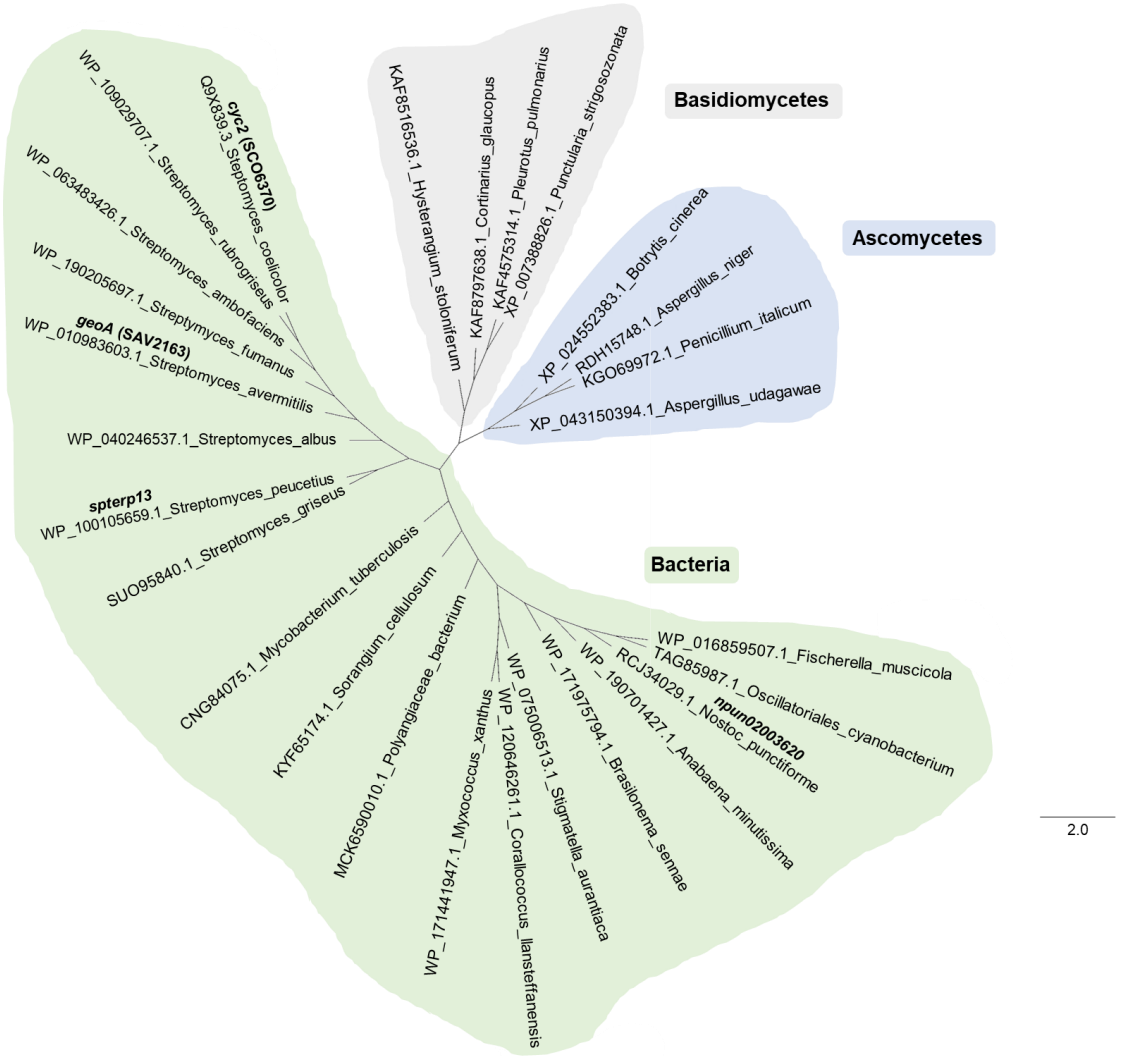

Fig. S1 Phylogenetic analysis of the C-terminus (amino acid residues 374−726) of the germacradienol/geosmin synthase SCO6073 from *Streptomyces coelicolor* and selected homologues from bacteria and fungi. The protein sequences were downloaded from NCBI database.

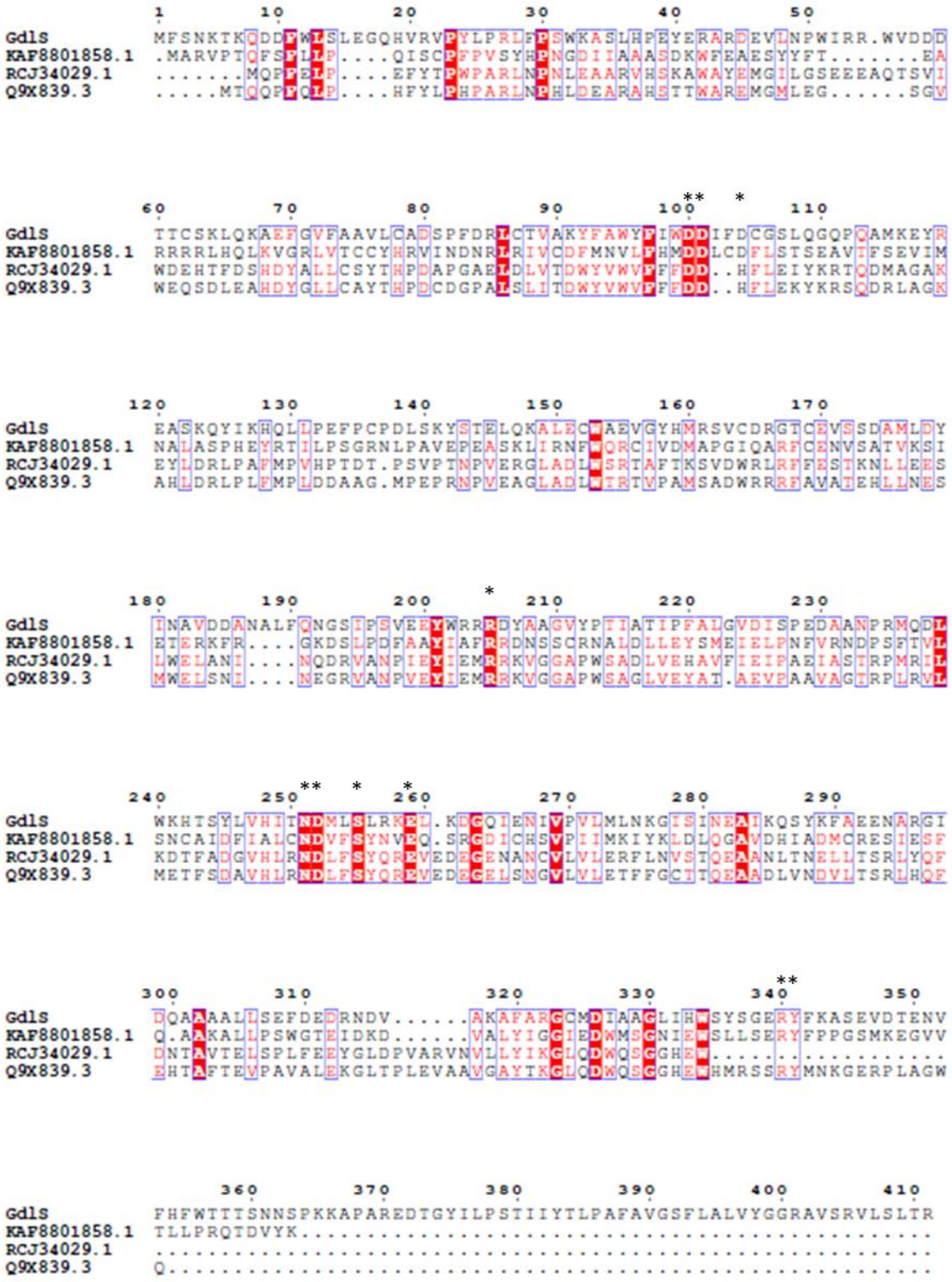

Fig. S2 Multiple sequence alignments of GdlS. The fungal terpene synthase KAF8801858.1 is from *Cortinarius glaucopus*. The N-terminal sequences of the bacterial germacradienol/geosmin synthases are from RCJ34029.1 (*Nostoc punctiforme*) and Q9X839.3 (*Streptomyces coelicolor*). Amino acid residues marked with * are responsible for catalytic activity of terpene synthases.

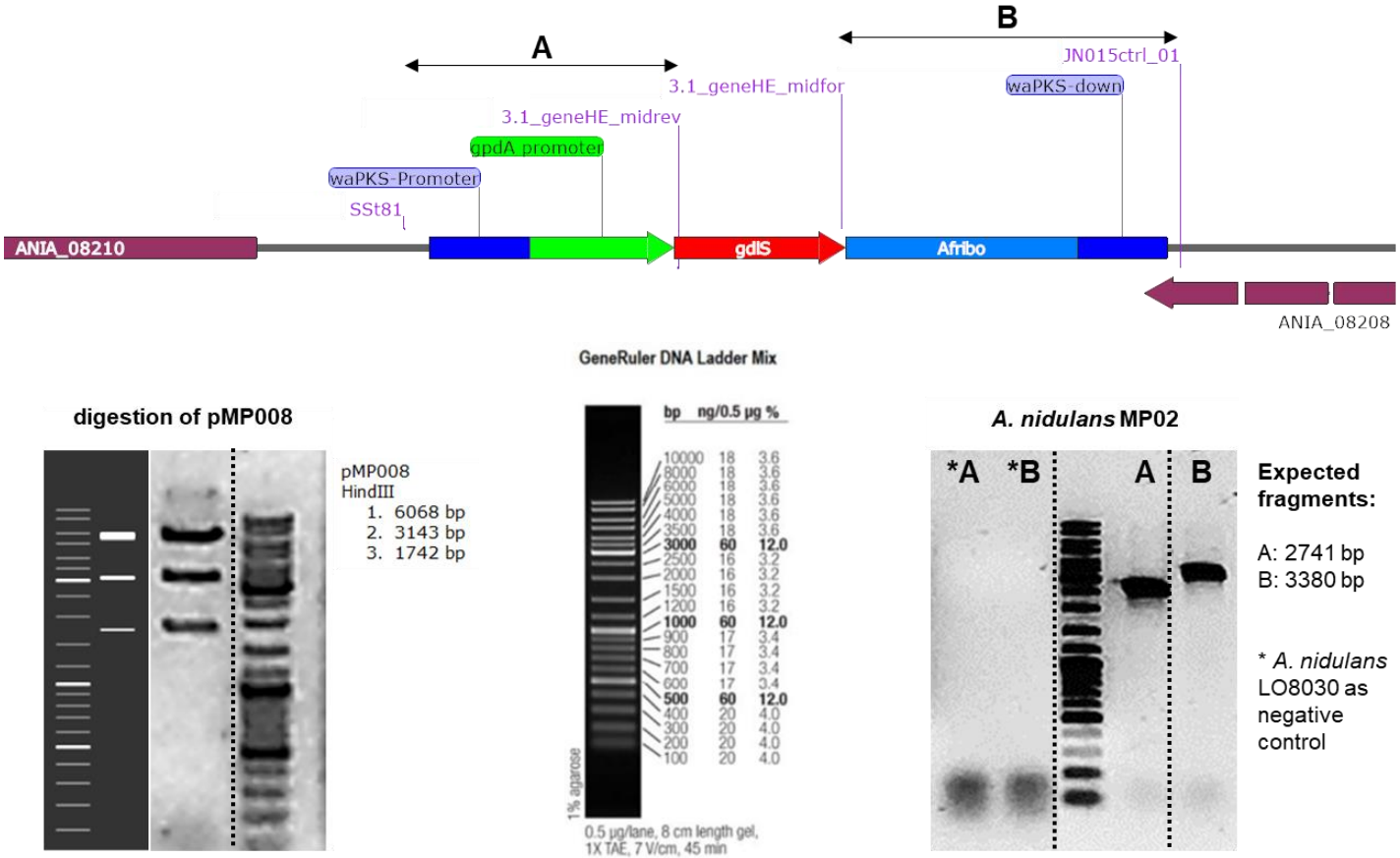

Fig. S3 Verification of plasmid pMP008 for heterologous expression of *gdlS* in *A. nidulans* LO8030 and correct integration in *wA* locus. The GeneRuler DNA Ladder mix by Thermo Fisher was used as size standard for DNA fragments. Verification of transformants *via* PCR was performed with primers binding outside of the integration construct in genomic DNA and inside of integrated gene. Verification of pMP008 was carried out by digestion with restriction enzyme HindIII. Gel image of pMP008 digestion was mirrored and cut. The electrophoretic gel of the verification of putative transformants was assembled out of two gels and rearranged at the dashed lines.

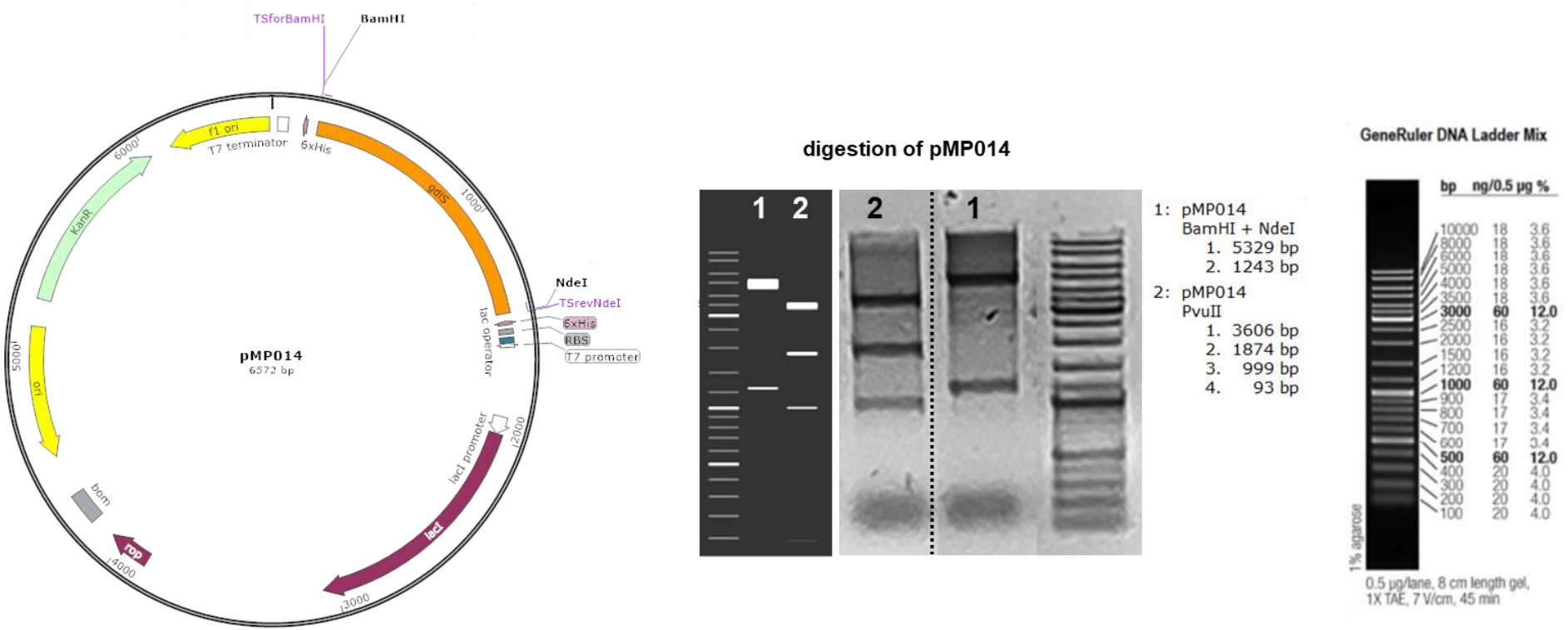

Fig. S4 Verification of plasmid pMP014 for GdlS overproduction in *E. coli* BL21 (DE3). GeneRuler DNA Ladder mix by Thermo Fisher served as size standard for DNA fragments. The verification of the plasmid was done *via* restriction with BamHI, NdeI and PvuII and by sequencing. The gel image for the digestion of pMP014 was cut and rearranged at the dashed lines.

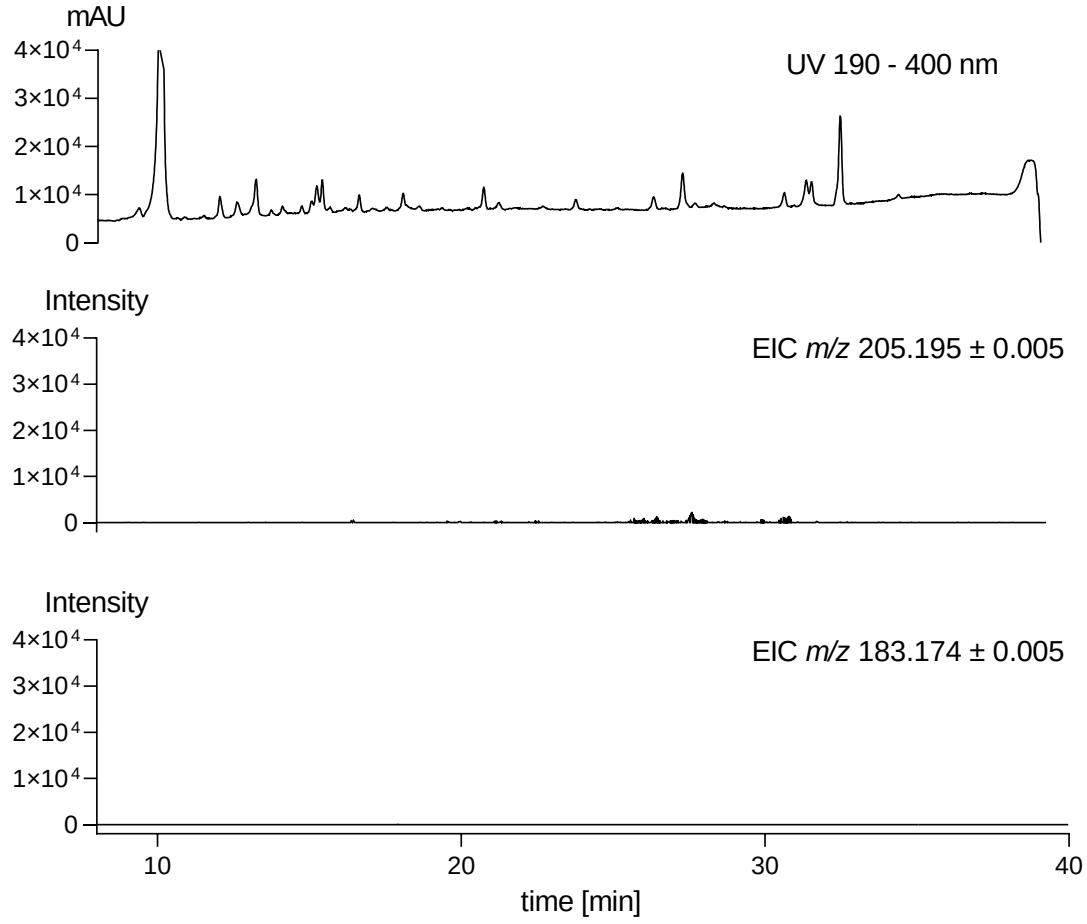

Fig. S5 LC-MS analysis of the wildtype *A. ustus* 3.3904. Illustrated are UV absorptions at 190 − 400 nm, EICs with the main fragment ion of germacradienol at *m/z* 205.195 and the $[M+H]^+$ ion of geosmin at *m/z* 183.174.

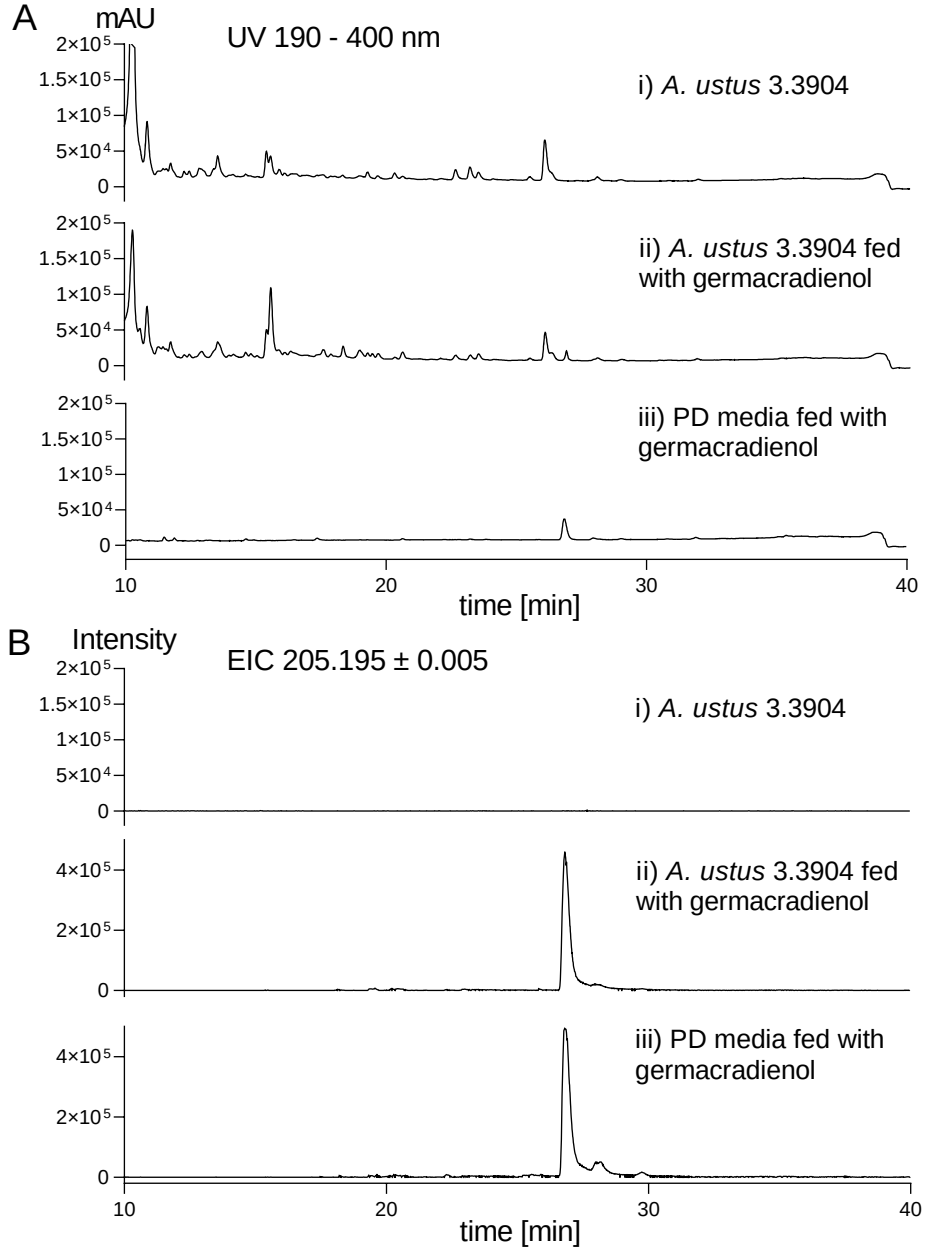

Fig. S6 LC-MS analysis of feeding *A. ustus* 3.3904 with germacradienol. UV absorption at 190 − 400 nm is illustrated in A. EIC of the main fragment of germacradienol is shown in B.

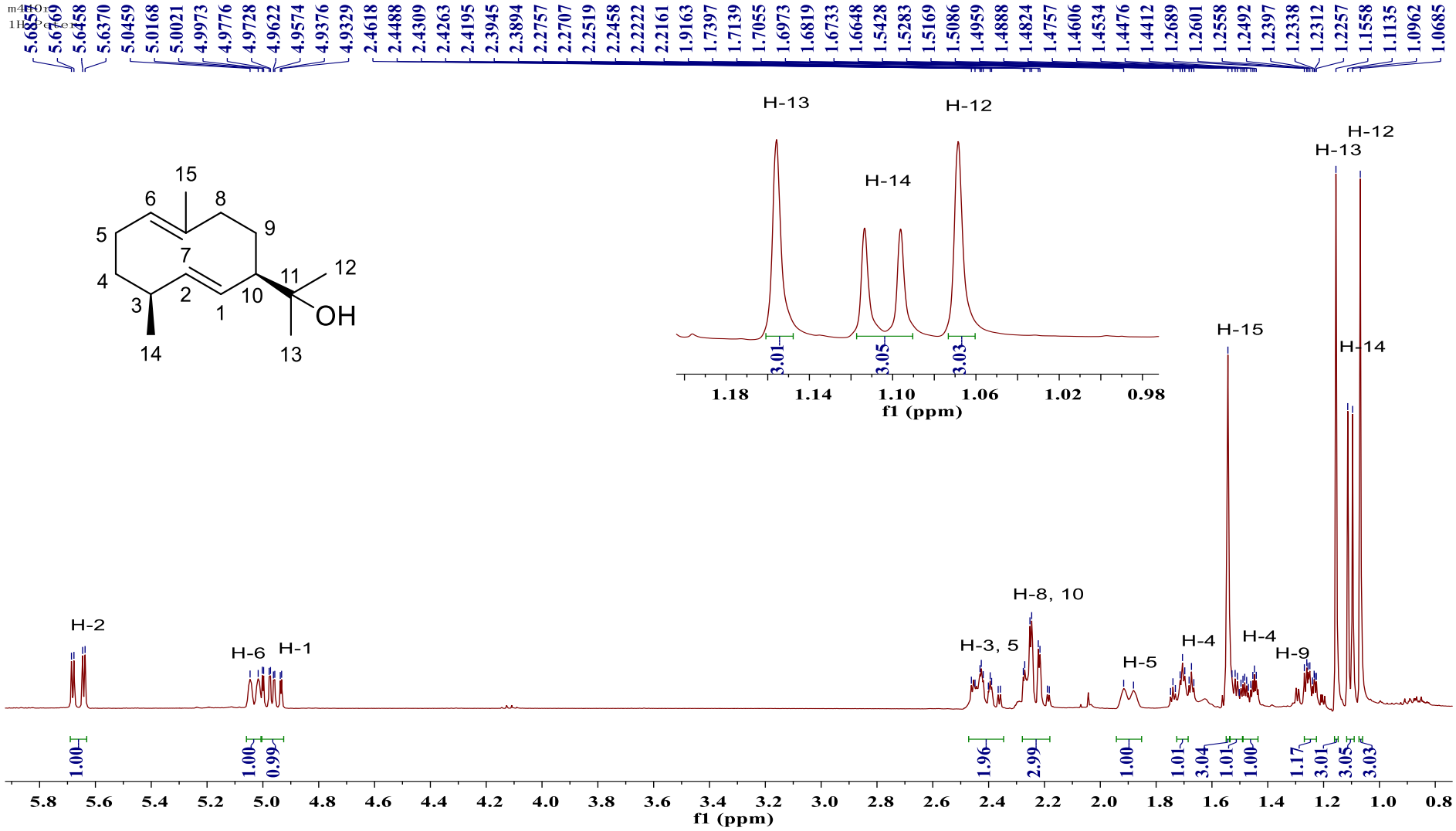

Fig. S7 ^{1}H NMR spectrum of germacradienol in $CDCl_3$ (400 MHz).

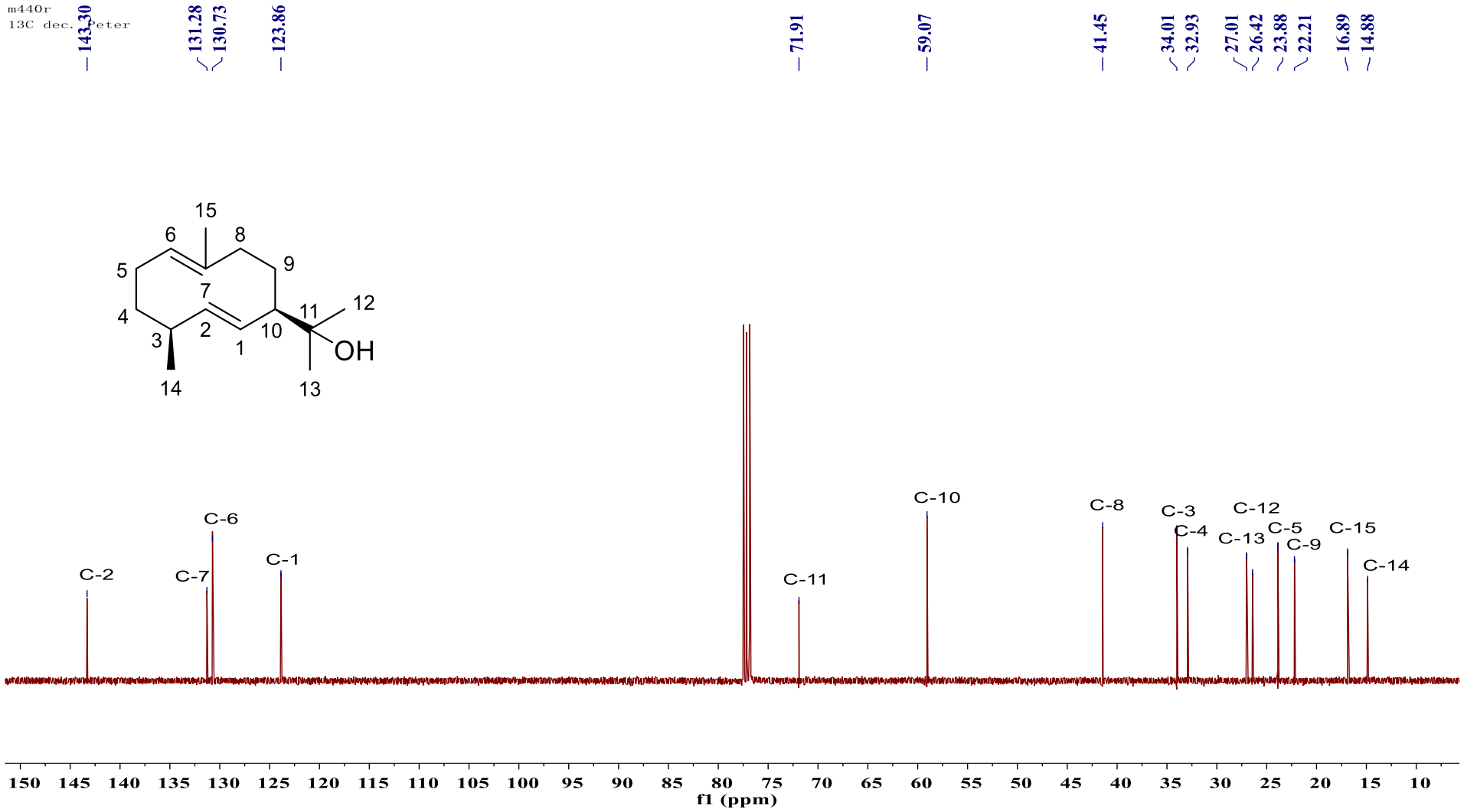

Fig. S8 ^{13}C NMR spectrum of germacradienol in $CDCl_3$ (100 MHz).

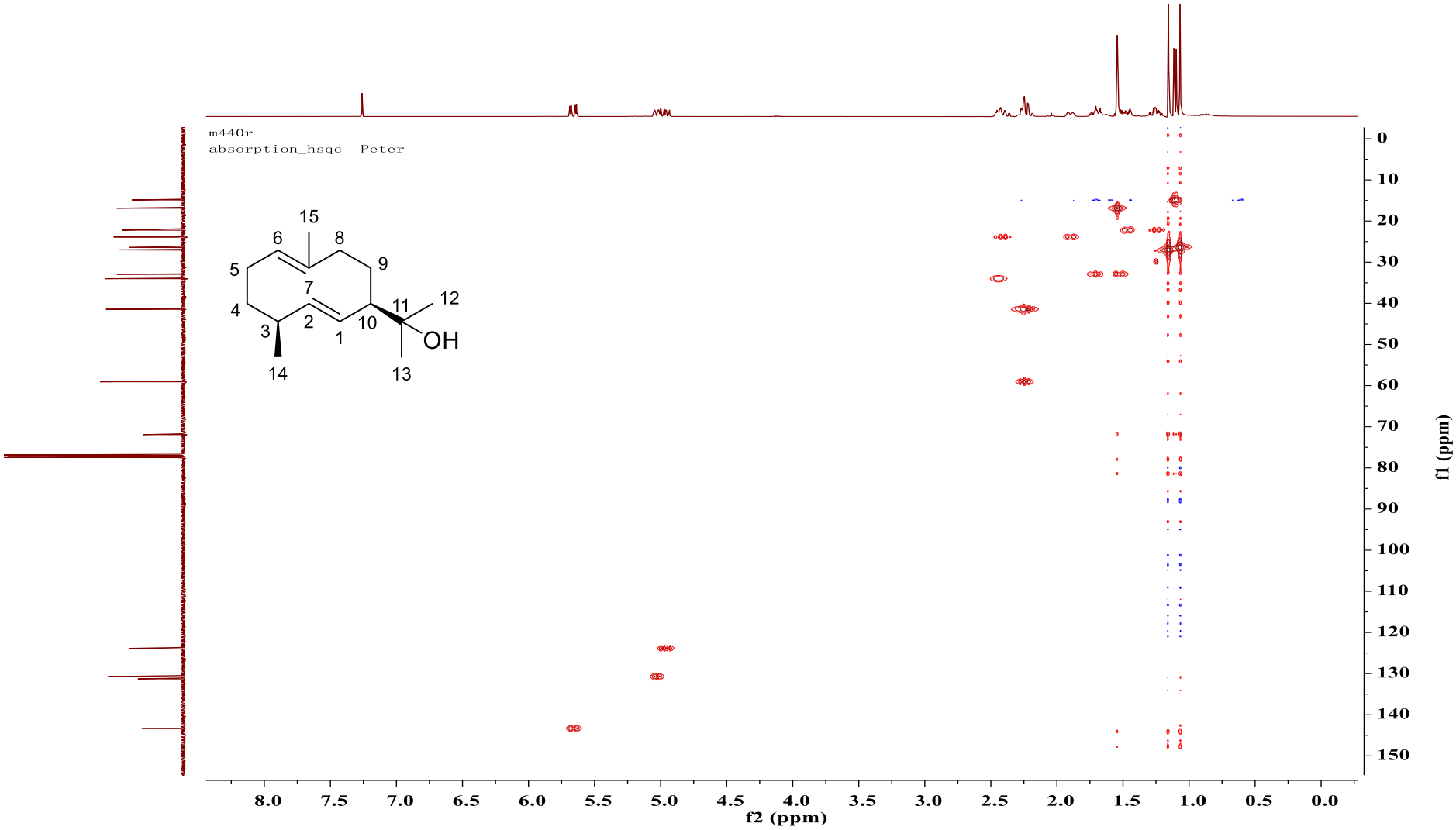

Fig. S9 HSQC spectrum of germacradienol in $CDCl_3$.

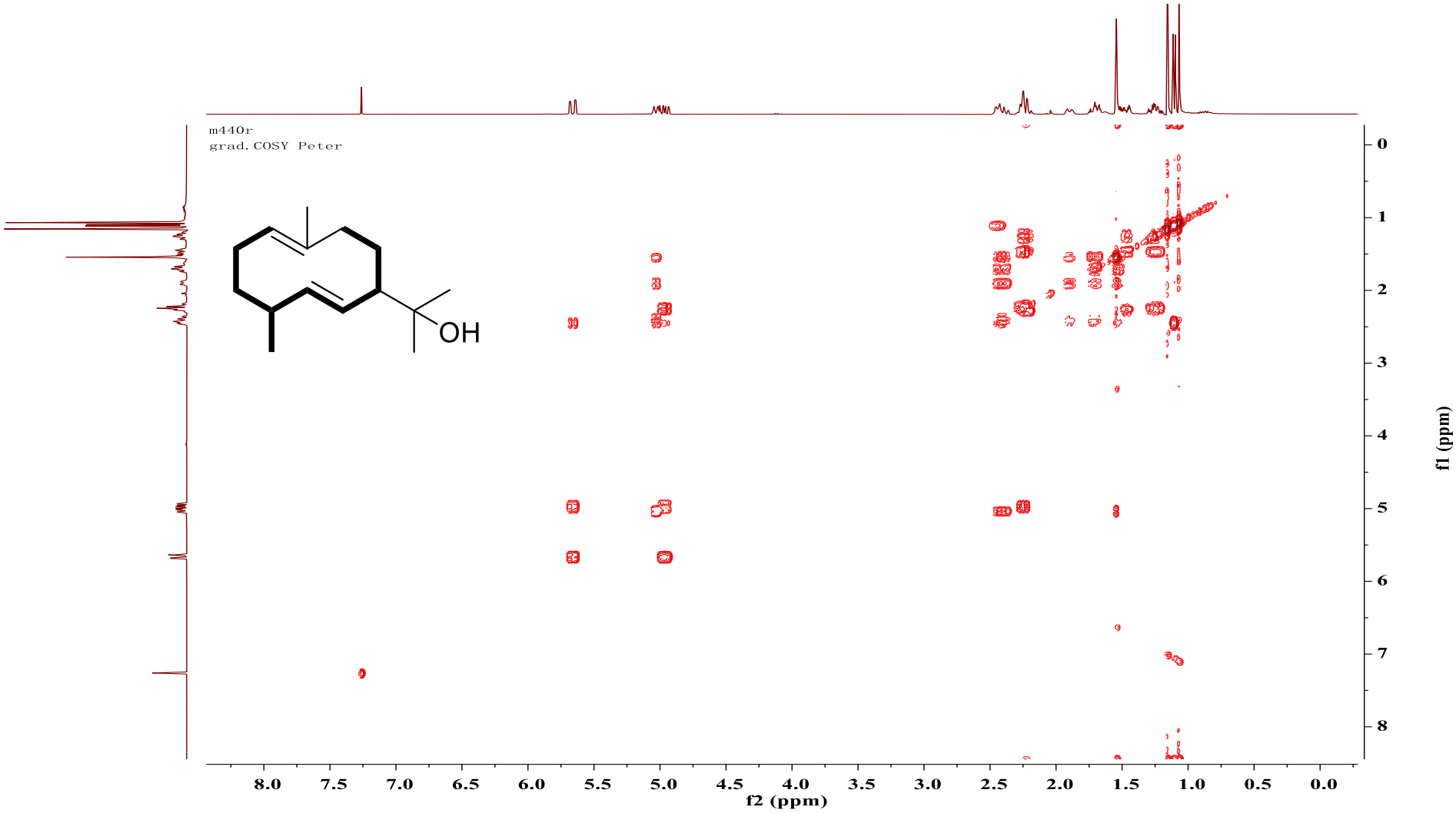

Fig. S10 ^{1}H-^{1}H COSY of germacradienol in $CDCl_3$.

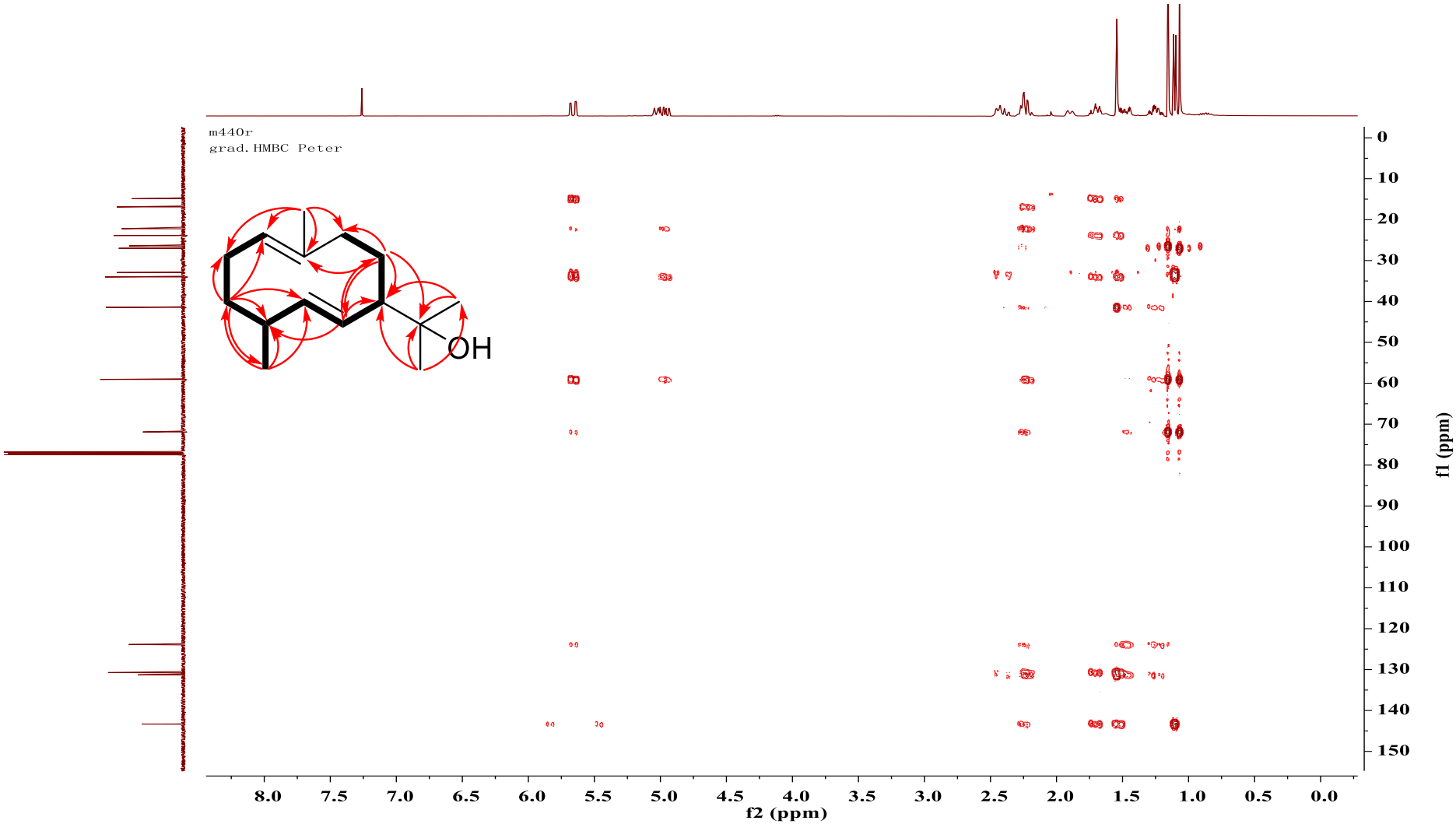

Fig. S11 HMBC spectrum of germacradienol in $CDCl_3$.

Raw data

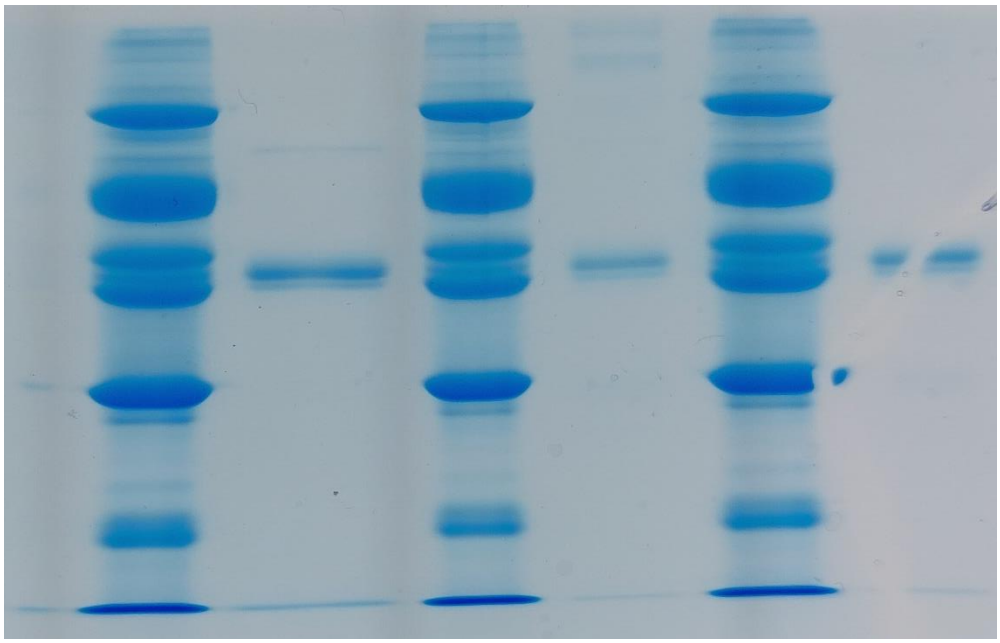

Fig. S12 SDS-PAGE of purified and recombinant GdlS in three different dilutions (1.1 µg, 0.6 µg, 0.3 µg).

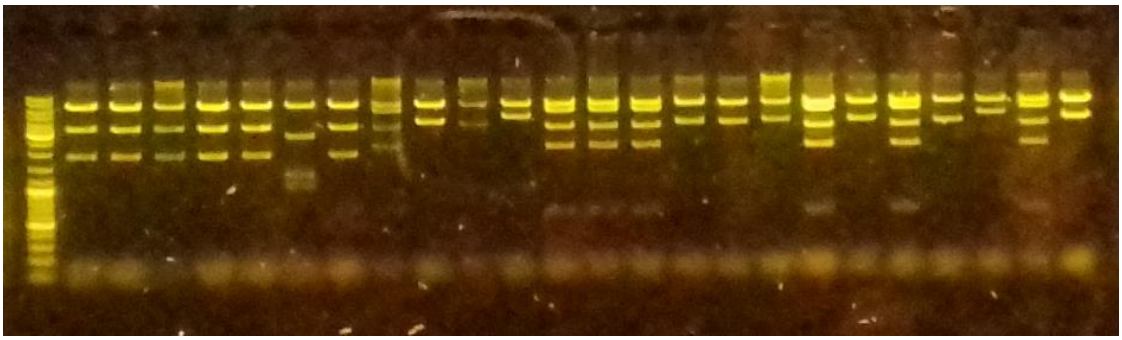

Fig. S13 Verification of plasmid pMP008 for heterologous expression of *gdlS* in *A. nidulans* LO8030. Plasmids isolated from 24 *E. coli* colonies were tested.

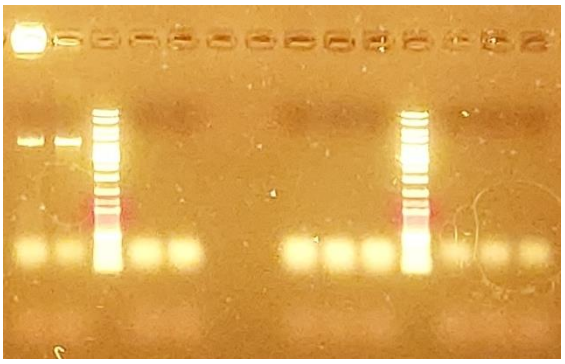

Fig. S14 Control of correct integration of *gpdA:gdlS* in *wA* locus in *A. nidulans* LO8030. Lane 4 and 5 represent the negative control.

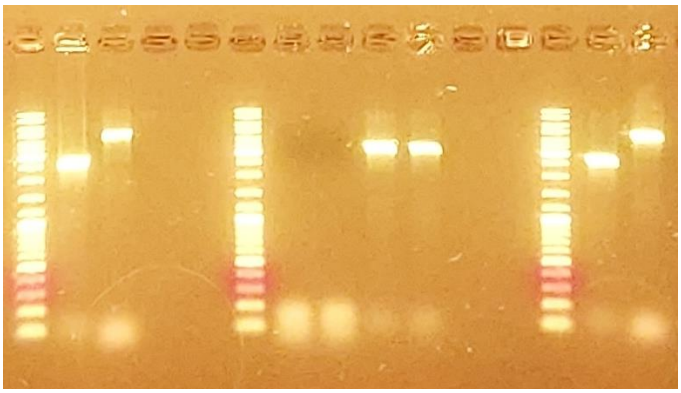

Fig. S15 Control of correct integration of *gpdA:gdlS* in *wA* locus in *A. nidulans* LO8030. Lane 13 represents the marker, lane 14 corresponds to the amplification of the upstream fragment A in the transformant strain MP02, lane 10 exhibits amplification of the downstream fragment B in the transformant strain MP02.

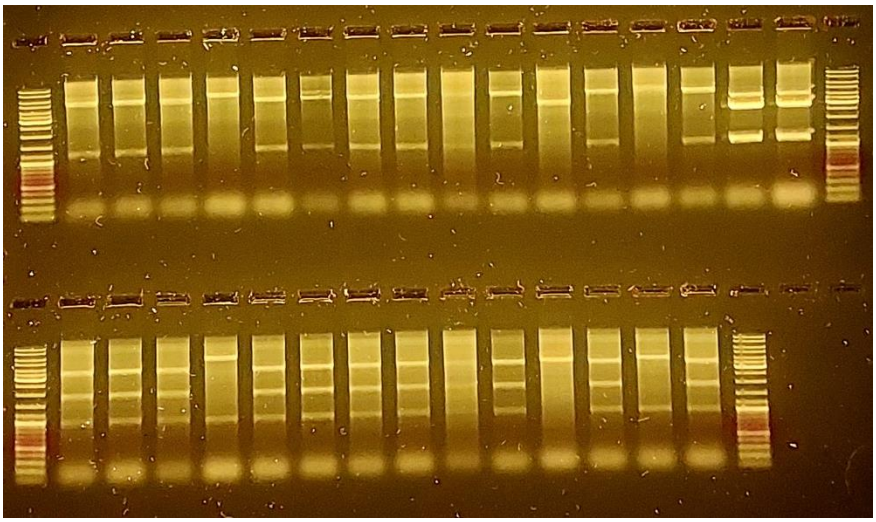

Fig. S16 Verification of plasmid pMP014. The upper image exhibit digestion with BamHI and NdeI with plasmids isolated from 16 different *E. coli* colonies, whereas digestion with PvuII in 14 distinct plasmids isolated from *E. coli* is shown in the lower image.

References

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