

## Supplementary Information

### **Sesterterpene ophiobolin biosynthesis involving multiple gene clusters in *Aspergillus ustus***

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**Table S1.** *Aspergillus ustus* 094102 genome assembly and main feature

<b>Statistic</b>	<b>Value</b>
Coverage	>68×
Genome assembly size (Mb)	40.09
Number of scaffolds	174
Scaffold N50(Kb)	1,814
Number of contigs	301
Contig N50(Kb)	675
CDS total length (Mb)	20.44 (50.98% of genome)
CDS average size (bp)	1461.62
Predicted protein-coding genes (Number)	13,982
Predicted protein-coding genes, total length (Mb)	23.19
Predicted protein-coding genes, mean length (kb)	1658.33
Exon total length (Mb)	20.44
Exon total number	43,400
Exon average length (bp)	470.88
Intron total length (Mb)	2.75
Intron total number	29,418
Intron average length (bp)	93.50
GC content (%)	51.22

**Note:** The gene density is 349 genes/ Mb scaffold. On average, the predicted genes contain 3.10 introns that are 471 bp long. This is in the range seen for other fungi, where the average intron densities range from just over 1.0 intron/ kb coding sequence (cds) in *Schizosaccharomyces pombe* to approximately 5.0 introns/ kb cds in *Cryptococcus neoformans*. For all of the predicted proteins, 8,882 had hits in the SwissProt database, 7,929 genes were mapped in the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database, and 5,061 were classified in the Clusters of Orthologous Groups (COG) database, 8,336 were classified in the Gene Ontology (GO) database, 12,785 in the non-redundant database of NCBI (NR), and 10,928 in the Tremble database, 13,982 were hit in all the above databases.

**Table S2 Blastnp of the presumed 27 terpene synthases in *A. ustus* 094102 genome (done in Oct, 2012)**

Presumed AUPTS type	AUPTS ID (Au)	AA No.	Intron No.	RTS	Max score	Query coverage	Max identity
<b>Sesqui AUPTS</b>	226	382	8	[EEA21257.1] pentalenene synthase, putative [ <i>Penicillium marneffe</i> ATCC 18224]	125	95%	24%
	12847	408	8		116	83%	24%
	13884	363	8		141	93%	29%
	6852	390	8	[XP001390370.2] pentalenene synthase [ <i>Aspergillus niger</i> CBS 513.88]	457	91%	62%
	11092	340	0	[ACZ56398.1] trichodiene synthase [ <i>Fusarium incarnatum</i> ]	126	69%	31%
<b>Di AUPTS</b>	6061	343	0	[BAF45925.1] fusicoccadiene synthase [ <i>Phomopsis amygdali</i> ]	207	97%	37%
	6241	744	7		288	89%	29%
	7240	762	8		258	94%	28%
	8003	725	3		517	100%	39%
	11189	710	5		292	94%	30%
	13192	708	8		425	98%	36%
	13606	755	7		307	85%	31%
	13676	717	9		294	85%	30%
<b>Tri AUPTS</b>	1332	430	2	[EIT75610.1] squalene synthetase [ <i>Aspergillus oryzae</i> 3.042]	501	91%	59%
	7332	470	3		816	97%	83%
	1334	642	2	[XP001388569.2] squalene-hopene-cyclase [ <i>Aspergillus niger</i> CBS 513.88]	655	95%	51%
	1561	382	1	[GAA92416.1] squalene/phytoene synthase [ <i>Aspergillus kawachii</i> IFO 4308]	560	100%	71%
	5911	735	3	[EAW24229.1] oxidosqualene:lanosterol cyclase [ <i>Neosartorya fischeri</i> NRRL 181]	1407	100%	89%
	11571	731	2	[EAL89589.1] lanosterol synthase, putative [ <i>Aspergillus fumigatus</i> Af293]	1153	96%	76%
<b>Unknown AUPTS</b>	606	387	1	[EAA65430.1] hypothetical protein AN0654.2 [ <i>Aspergillus nidulans</i> FGSC A4]	696	98%	86%
	7669	362	2	gb[EAA59165.1] hypothetical protein AN8143.2 [ <i>Aspergillus nidulans</i> FGSC A4]	593	100%	83%
	11565	692	7	[EAA64518.1] hypothetical protein AN2407.2 [ <i>Aspergillus nidulans</i> FGSC A4]	759	99%	53%
	13624	689	7	[EAU32979.1] conserved hypothetical protein [ <i>Aspergillus terreus</i> NIH2624]	1036	100%	72%
	329	336	2	[CAK48316.1] unnamed protein product [ <i>Aspergillus niger</i> ]	529	99%	74%
	3446	456	1	[EAA63201.1] hypothetical protein AN2767.2 [ <i>Aspergillus nidulans</i> FGSC A4]	832	98%	90%
	6064	404	3	[CCD54927.1] BcSTC5, similar to sesquiterpene cyclase [ <i>Botryotinia fuckeliana</i> ]	38.1	18%	31%
	6298	345	1	[EAA59634.1] hypothetical protein AN8012.2 [ <i>Aspergillus nidulans</i> FGSC A4]	643	99%	88%

**Note: AUPTS: *Aspergillus ustus* presumed terpene synthases; Prefix “Au” for all the ID of the AUPTS**

**Table S3 Primers used for gene deletion and complementation of *Au3446***

Type	Full name	Sequence: 5'—3' (restriction endonuclease)	Product size (bp)
pUC18	M13-47	CGCCAGGGTTTTCCAGTCACGAC	156
	RV-M	GAGCGGATAACAATTCACACAGG	
Selective marker	<i>Sh ble-s</i>	CCCACACACCATAGCTTCAAAAT	1172
	<i>Sh ble-a</i>	AGCTTGCAAATTAAGCCTTCG	
	<i>Hpt II-s</i>	AAATTGACGCTTAGACAACCTAA	2146
	<i>Hpt II -a</i>	GCAGCTTGCCAACATGGTG	
<i>Au3446</i> deletion	<i>Au3446L'-s</i>	CGGAATTCATCTTGACGGATTC ( <i>EcoRI</i> )	1224
	<i>Au3446L'-a</i>	GGGGTACCTTGCGTGTGG ( <i>KpnI</i> )	1657
	<i>Au3446R'-s</i>	AACTGCAGCTAATCGAGATGTGC ( <i>PstI</i> )	
	<i>Au3446R'-a</i>	CCCAAGCTTCCTTCAAAATGG ( <i>HindIII</i> )	
	<i>Au3446-s</i>	AAAGATGTTTCTCCCTTTGGCG	1167
	<i>Au3446-a</i>	GAGGTCGCGGGCCTGATA	
<i>Au3446</i> complementation	<i>Au3446L'- Au3446-s</i>	GGGGTACCATACACTCTCATCCTAC ( <i>KpnI</i> )	2502
	<i>Au3446L'- Au_3446-a</i>	CTGCAGAACCAATGCATTGGCCGGTTCATGGCC ( <i>BstXI</i> )	
	<i>Au_3446R'-s</i>	CTGCAGAAAAAAGCTCGCTTTTGCAC ( <i>PstI</i> )	1012
	<i>Au_3446R'-a</i>	AAGCTTTGAACTTTTCTAGCCCCGCC ( <i>HindIII</i> )	

**Table S4 Plasmids used for gene deletion and complementation of *Au3446***

Type	Serial number / Full name	Characteristics
Expression vectors	pGAPZ $\alpha$ A	$P_{GAP}$ , $\alpha$ -factor signal, myc epitope tag, C-terminal polyhistidine tag, $T_{Aox1}$ , $P_{Tef1}$ , $P_{Em7}$ , <i>Sh ble</i> (Zeocin <sup>r</sup> ), $T_{Cyc1}$ , pUC ori (ColE1 ori)
Cloning vectors	pMD18-T	Derived from pUC18, pUC ori (ColE1 ori), $O_{LacZ}$ , <i>bla</i> (Amp <sup>r</sup> )
	pMD18-T-Simple	Derived from pMD18-T, without multiple cloning site
	pCAMBIA1301	$P_{CAMV35S}$ , <i>Gus</i> , $T_{Nos\ polyA}$ , $P_{CAMV35S}$ , <i>HptII</i> (HmB <sup>r</sup> ), $T_{CAMV35S\ polyA}$ , pVS1 rep, pVS1 sta, pBR322 ori, pBR322 born, Kan <sup>r</sup>
	pTFCM	Derived from pCAMBIA1301, $P_{CAMV35S}$ and <i>HptII</i> (HmB <sup>r</sup> ) were substituted with $P_{TrpC}$ , <i>HptII</i> (HmB <sup>r</sup> ), and $T_{TrpC}$
Random insertion vectors	JS	$P_{TrpC}$ , <i>eGFP</i> , $T_{TrpC}$ , <i>HptII</i> (HmB <sup>r</sup> ), pVS1 rep, pVS1 sta, Kan <sup>r</sup>
Deletion and complementation vectors	pWHU2201 / pUC18 / $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$	Derived from pMD18-T and pGAPZ $\alpha$ A, pUC ori (ColE1 ori), $O_{LacZ}$ , <i>bla</i> (Amp <sup>r</sup> ), $P_{Tef1}$ , $P_{Em7}$ , <i>Sh ble</i> (Zeocin <sup>r</sup> ), $T_{Cyc1}$
	pWHU2202 / pUC18 / $P_{CAMV35S}$ - <i>HptII</i> - $T_{CAMV35S\ polyA}$	Derived from pMD18-T and pCAMBIA1301, pUC ori (ColE1 ori), $O_{LacZ}$ , <i>bla</i> (Amp <sup>r</sup> ), $P_{CAMV35S}$ , <i>HptII</i> (HmB <sup>r</sup> ), $T_{CAMV35S\ polyA}$
Genetic deletion and complementation system (gene <i>Au_3446</i> )	pUC18 / <i>Au_3446L</i>	Derived from pMD18-T-Simple
	pUC18 / <i>Au_3446R</i>	Derived from pMD18-T-Simple
	pUC18 / <i>Au_3446L'</i>	Derived from pMD18-T-Simple
	pUC18 / <i>Au_3446R'</i>	Derived from pMD18-T-Simple
	pUC18 / <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$	Derived from pUC18 / $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$
	pWHU2203 / pUC18 / <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$ - <i>Au_3446R'</i>	Derived from pUC18 / <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$
	pWHU2204 / JS / <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$ - <i>Au_3446R'</i>	Derived from JS and pUC18 / <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$ - <i>Au_3446R'</i> , $P_{TrpC}$ , <i>eGFP</i> , and $T_{TrpC}$ were substituted with <i>Au_3446L'</i> - $P_{Tef1}$ - $P_{Em7}$ - <i>Sh ble</i> - $T_{Cyc1}$ - <i>Au_3446R'</i>
	pUC18 / <i>Au_3446</i> - <i>Au_3446L'</i> - $P_{CAMV35S}$ - <i>HptII</i> - $T_{CAMV35S\ polyA}$	Derive from pUC18 / $P_{CAMV35S}$ - <i>HptII</i> - $T_{CAMV35S\ polyA}$
	pWHU2205 / pUC18 / <i>Au_3446</i> - <i>Au_3446L'</i> - $P_{CAMV35S}$ - <i>HptII</i> - $T_{CAMV35S\ polyA}$ - <i>Au_3446R'</i>	Derive from pUC18 / <i>Au_3446</i> - <i>Au_3446L'</i> - $P_{CAMV35S}$ - <i>HptII</i> - $T_{CAMV35S\ polyA}$

**Table S5 Primers used for gene cluster inactivation**

Type	Full name	Sequence: 5'—3' (restriction endonuclease)	Product size (bp)
pUC18	M13-47	CGCCAGGGTTTTCCAGTCACGAC	156
	RV-M	GAGCGGATAACAATTCACACAGG	
Selective marker	<i>Sh ble-s</i>	CCCACACACCATAGCTTCAAAAAT	1172
	<i>Sh ble-a</i>	AGCTTGCAAATTAAGCCTTCG	
	<i>HptII-s</i>	AAATTGACGCTTAGACAACCTAA	2146
	<i>HptII-a</i>	GCAGCTTGCCAACATGGTG	
POC3446 deletion	POC3446L-s	GAATTCACAGATAGTATGCGAGTC ( <i>EcoRI</i> )	1419
	POC3446L-a	TCTAGACAAACGCCAGAATCAGC ( <i>XbaI</i> )	
	POC3446R-s	GTCGACTAGGGTGGTATTTGCGT ( <i>SalI</i> )	1128
	POC3446R-a	AAGCTTGGGCATCCGTATGATGTCG ( <i>HindIII</i> )	
POC8003 deletion	POC8003L-s	GAATTCGTCGTACAGTCAGGTAGGGA ( <i>EcoRI</i> )	1085
	POC8003L-a	GGTACCAAGGAGGAGTGCAAGGAA ( <i>KpnI</i> )	
	POC8003R-s	CCTGCAGGTTGCCGAGGACAAGG ( <i>SbfI</i> )	1061
	POC8003R-a	AAGCTTCATCTGTGGACGGAGTAAAG ( <i>HindIII</i> )	
	<i>Au_8003-s</i>	CGCCCACCACTCATTCTTTTAG	1063
	<i>Au_8003-a</i>	CGACCTGATCCGATGCTGTC	
POC13192 deletion	POC13192L-s	GAATTCTAACTTCAAATCAGCGAGGA ( <i>EcoRI</i> )	1224
	POC13192L-a	TCTAGAATACCCACGGACCCAAC ( <i>XbaI</i> )	
	POC13192R-s	CCTGCAGGAGCAGCATCTTCGATTGG ( <i>SbfI</i> )	1077
	POC13192R-a	AAGCTTATTCTGGGACAGGAACTAG ( <i>HindIII</i> )	
	<i>Au_13192-s</i>	AAGAACAAGCACGGACTGCC	1096
	<i>Au_13192-a</i>	CCTGCCTCGTCCAATACAGAT	
POC11565 deletion	POC11565L-s	GAATTCTTGTTGAGGCGTTTTGTG ( <i>EcoRI</i> )	1058
	POC11565L-a	TCTAGAGTCATCGTGCGAGTAGG ( <i>XbaI</i> )	
	POC11565R-s	CCTGCAGGCATCCGAGTCATAGTAGT ( <i>SbfI</i> )	1018
	POC11565R-a	AAGCTTCGCCTCCACGTTG ( <i>HindIII</i> )	
	<i>Au_11565-s</i>	CTCGTATTCGCTTTGGGTTGA	1106
	<i>Au_11565-a</i>	CCAGCATTGTCGGGTTTCG	
POC6298 deletion	POC6298L-s	GAATTCGCAGCCAGTTCCTCCCTA ( <i>EcoRI</i> )	1508
	POC6298L-a	GGATCCTTTTTACCCAAGCCCATC ( <i>BamHI</i> )	
	POC6298R-s	CCTGCAGGAGGGAAATACCGCCTCAAAAG ( <i>SbfI</i> )	1208
	POC6298R-a	AAGCTTAGCCTCCTCCTCGTTC ( <i>HindIII</i> )	
	<i>Au_6298-s</i>	TTTGAGGCGGTATTTCCC	1038
	<i>Au_6298-a</i>	ACCTCCTTCTTCAGTCCCT	

**Table S6 Plasmids used for gene cluster inactivation**

Type	Serial number / Full name	Characteristics
Expression vectors	pGAPZαA	<i>P<sub>GAP</sub></i> , α-factor signal, myc epitope tag, C-terminal polyhistidine tag, <i>T<sub>Aox1</sub></i> , <i>P<sub>Tef1</sub></i> , <i>P<sub>Em7</sub></i> , <i>Sh ble</i> (Zeocin <sup>r</sup> ), <i>T<sub>Cyc1</sub></i> , pUC ori (ColE1 ori)
Cloning vectors	pMD18-T	Derived from pUC18, pUC ori (ColE1 ori), <i>O<sub>LacZ</sub></i> , <i>bla</i> (Amp <sup>r</sup> )
	pMD18-T-Simple	Derived from pMD18-T, without multiple cloning site
	pCAMBIA1301 (CAMBIA, Canberra, Australia)	<i>P<sub>CAMV35S</sub></i> , <i>Gus</i> , <i>T<sub>Nos polyA</sub></i> , <i>P<sub>CAMV35S</sub></i> , <i>HptIII</i> (HmB <sup>r</sup> ), <i>T<sub>CAMV35S polyA</sub></i> , pVS1 rep, pVS1 sta, pBR322 ori, pBR322 born, Kan <sup>r</sup>
	pTFCM	Derived from pCAMBIA1301, <i>P<sub>CAMV35S</sub></i> and <i>HptIII</i> (HmB <sup>r</sup> ) were substituted with <i>P<sub>TTPC</sub></i> , <i>HptIII</i> (HmB <sup>r</sup> ), and <i>T<sub>TTPC</sub></i>
POC3446 deletion	pUC18 / POC3446L	Derived from pMD18-T-Simple
	pUC18 / POC3446R	Derived from pMD18-T-Simple
	pUC18 / POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>	Derived from pUC18 / <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2206 / pUC18 / POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC3446R	Derived from pUC18 / POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2207 / JS / POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC3446R	Derived from JS and pUC18 / POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC3446R, <i>P<sub>TTPC</sub></i> , <i>eGFP</i> , and <i>T<sub>TTPC</sub></i> were substituted with POC3446L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC3446R
POC8003 deletion	pUC18 / POC8003L	Derived from pMD18-T-Simple
	pUC18 / POC8003R	Derived from pMD18-T-Simple
	pUC18 / POC8003L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>	Derived from pUC18 / <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2212 / pUC18 / POC8003L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC8003R	Derived from pUC18 / POC8003L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
POC13192 deletion	pUC18 / POC13192L	Derived from pMD18-T-Simple
	pUC18 / POC13192R	Derived from pMD18-T-Simple
	pUC18 / POC13192L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>	Derived from pUC18 / <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2213 / pUC18 / POC13192L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC13192R	Derived from pUC18 / POC13192L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
POC11565 deletion	pUC18 / POC11565L	Derived from pMD18-T-Simple
	pUC18 / POC11565R	Derived from pMD18-T-Simple
	pUC18 / POC11565L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>	Derived from pUC18 / <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2214 / pUC18 / POC11565L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC11565R	Derived from pUC18 / POC11565L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
POC6298 deletion	pUC18 / POC6298L	Derive from pMD18-T-Simple
	pUC18 / POC6298R	Derive from pMD18-T-Simple
	pUC18 / POC6298L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>	Derived from pUC18 / <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>
	pWHU2223 / pUC18 / POC6298L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i> -POC6298R	Derived from pUC18 / POC6298L- <i>P<sub>Tef1</sub></i> - <i>P<sub>Em7</sub></i> - <i>Sh ble</i> - <i>T<sub>Cyc1</sub></i>

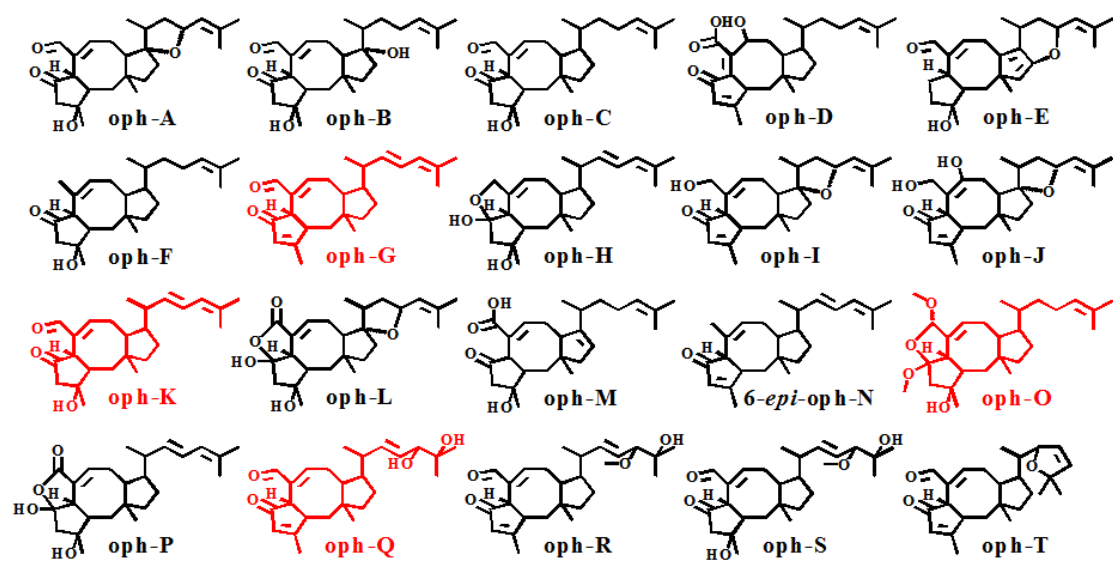
**Table S7 BlastnP result in GenBank database of 5 TS/TC proteins with reference sequences.** Underlined sequences were added in 2015; shaded were unidentified sequences

	sequence	sources	description		
			Max score	Query cover	Ident
Au8003	AcOS	<i>Aspergillus clavatus</i>	979	100%	65%
	<u>EvVS</u>	<u><i>Emericella varicolor</i></u>	<u>273</u>	<u>85%</u>	<u>45%</u>
	<u>EvSS</u>	<u><i>Emericella varicolor</i></u>	<u>552</u>	<u>100%</u>	<u>41%</u>
	PaPS	<i>Phomopsis amygdali</i>	516	100%	40%
	PaFS	<i>Phomopsis amygdali</i>	517	100%	39%
	AbFS	<i>Alternaria brassicicola</i>	466	98%	36%
	<u>NfSS</u>	<u><i>Neosartorya fischeri</i></u>	<u>290</u>	<u>96%</u>	<u>30%</u>
Au13192	<u>EvVS</u>	<u><i>Emericella varicolor</i></u>	<u>1115</u>	<u>99%</u>	<u>78%</u>
	<u>NfSS</u>	<u><i>Neosartorya fischeri</i></u>	<u>218</u>	<u>83%</u>	<u>46%</u>
	PaFS	<i>Phomopsis amygdali</i>	425	98%	36%
	PaPS	<i>Phomopsis amygdali</i>	394	97%	35%
	<u>EvSS</u>	<u><i>Emericella varicolor</i></u>	<u>412</u>	<u>98%</u>	<u>34%</u>
	AbFS	<i>Alternaria brassicicola</i>	359	96%	34%
	AcOS	<i>Aspergillus clavatus</i>	402	98%	33%
Au11565	<u>NfSS</u>	<u><i>Neosartorya fischeri</i></u>	<u>191</u>	<u>87%</u>	<u>40%</u>
	AcOS	<i>Aspergillus clavatus</i>	263	96%	32%
	<u>EvSS</u>	<u><i>Emericella varicolor</i></u>	<u>274</u>	<u>96%</u>	<u>30%</u>
	<u>EvVS</u>	<u><i>Emericella varicolor</i></u>	<u>237</u>	<u>75%</u>	<u>29%</u>
	PaFS	<i>Phomopsis amygdali</i>	209	73%	29%
	AbFS	<i>Alternaria brassicicola</i>	206	71%	28%
	PaPS	<i>Phomopsis amygdali</i>	259	98%	28%
Au6298	<u>WcFPPS</u>	<u><i>Wolfiporia cocos</i></u>	<u>397</u>	<u>98%</u>	<u>58%</u>
	<u>CpFPPS</u>	<u><i>Chimonanthus praecox</i></u>	<u>326</u>	<u>100%</u>	<u>50%</u>
	EpFPPS	<i>Euphorbia pekinensis</i>	318	100%	49%
	OsaFPPS	<i>Ornithogalum longibracteatum</i>	315	98%	48%
	RpFPPS1	<i>Ornithogalum longibracteatum</i>	310	97%	48%
	RpFPPS2	<i>Ornithogalum longibracteatum</i>	315	97%	47%
	RsFPPS	<i>Rhizosolenia setigera</i>	319	99%	46%
Au3446	<u>Nf</u>	<u><i>Neosartorya fischeri</i></u>	<u>786</u>	<u>100%</u>	<u>86%</u>
	<u>Ao</u>	<u><i>Aspergillus oryzae</i></u>	<u>776</u>	<u>100%</u>	<u>85%</u>
	<u>An</u>	<u><i>Aspergillus niger</i></u>	<u>745</u>	<u>100%</u>	<u>79%</u>
	<u>Ak</u>	<u><i>Aspergillus kawachii</i></u>	<u>742</u>	<u>100%</u>	<u>79%</u>
	Ml	<i>Micrococcus luteus</i>	125	60%	32%
	Ss	<i>Sulfolobus solfataricus</i>	49.3%	34%	26%



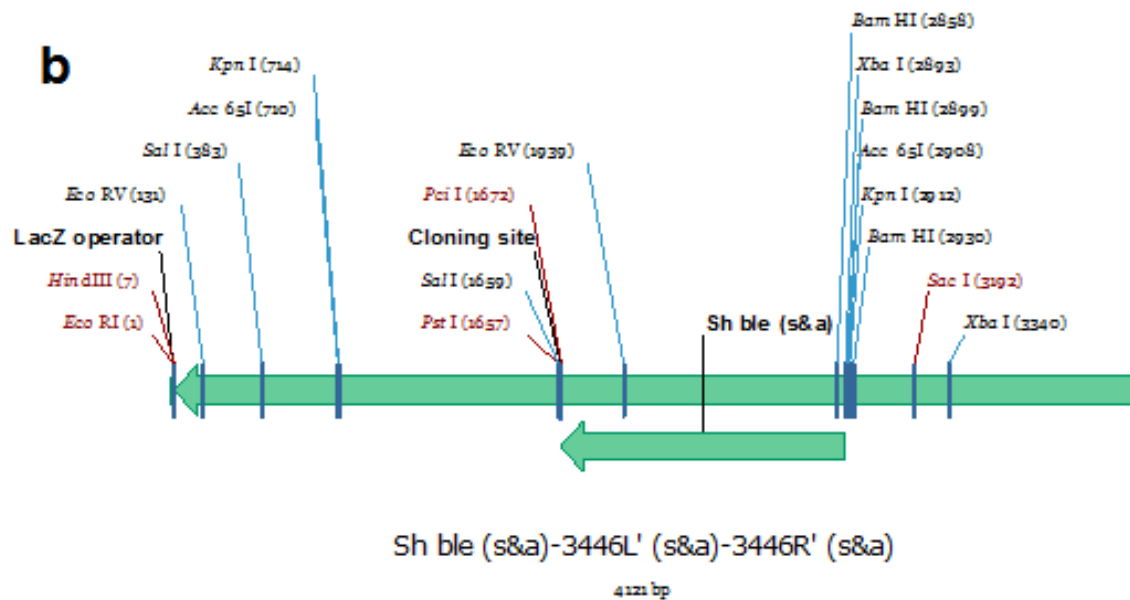
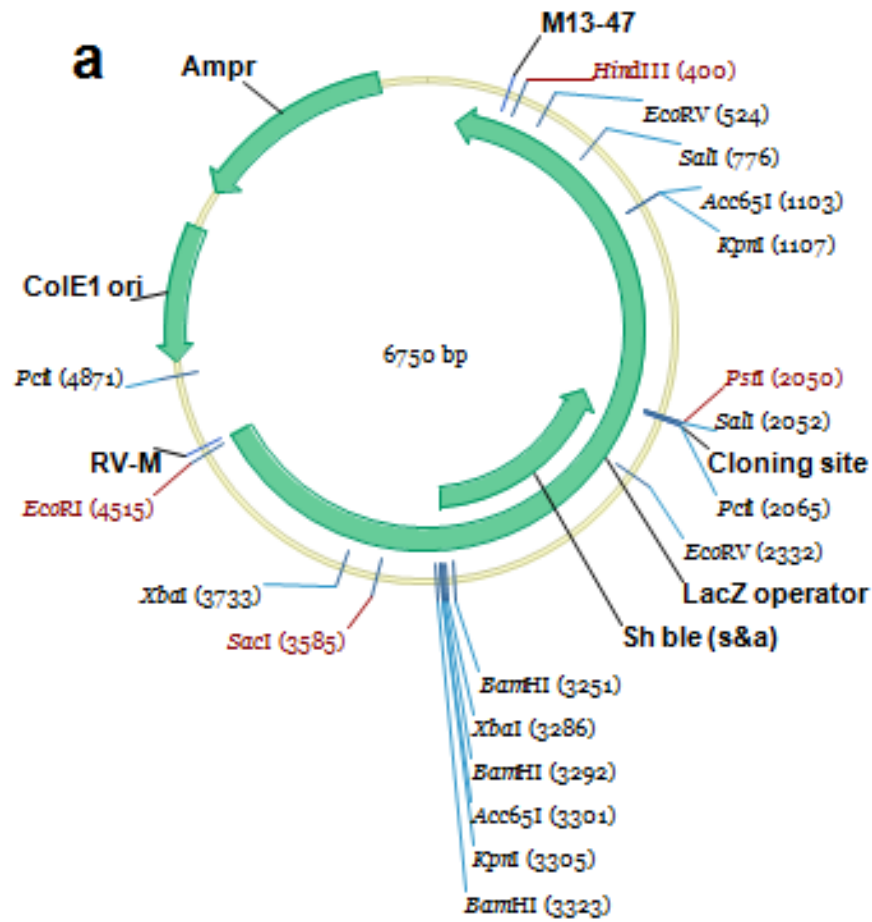
**Table S8 Primers used for gene expression**

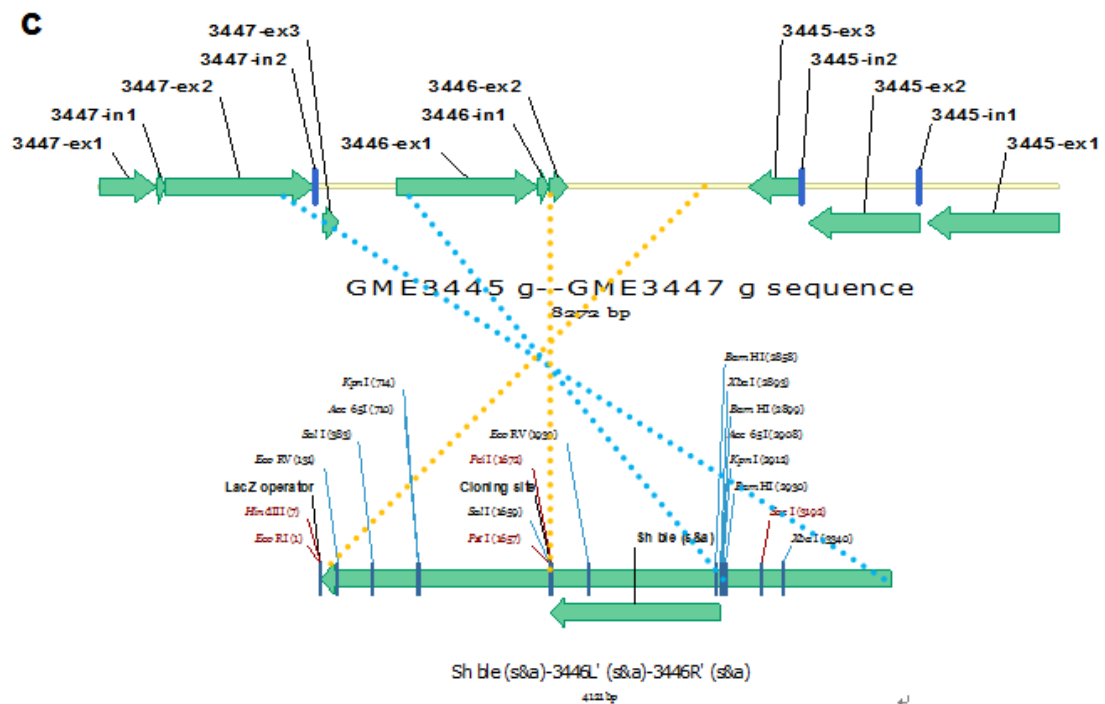
Type	Full name	Sequence: 5'—3' (restriction endonuclease)	Product size (bp)
Au8003 expression	Au8003-F	CATATGATGGAGTATAAGTACTCGACC( <i>Nde</i> I)	2178
	Au8003-R	AAGCTT TCAAACCTTCAGCAGCTCCA( <i>Hind</i> III)	
Au6298 expression	Au6298-F	CCCATATGATGTCTTCACCGCGTGCC ( <i>Nde</i> I)	1038
	Au6298-R	CCAAGCTTTTATTTGGTGCCTTGTA ( <i>Hind</i> III)	
Au11565 expression	Au11565	GGGGTACCGCGATGTACACCCTCGA ( <i>Kpn</i> I)	2405
	Au11565	GCTCTAGATACACTTTTCAGCAAAAAGCA ( <i>Xba</i> I)	
Au13192 exon fusion and expression	Au13192E1-s	AAGCTTATGCCACAAA CACAATCC( <i>Hind</i> III)	326
	Au13192E1-a	GCCGACTCGACAACATTATCATAACAGAAAC GCATACTCAAAGATATAACAAATAA	301
	Au13192E3-s	TGATAATGTTGTCGAGTCGGCGCCAATTC TACGTTGAACATGGACACGGACAA	
	Au13192E3-a	CATGAGCATGTGCGACGAAGGGGGCGCCGGTATCGAT	766
	Au13192E4-s	CTTCGTCGACATGCTCATG	
	Au13192E4-a	GCCGGGGCGAGGAGGATCTCGTCTCCTAGCTGCAC	175
	Au13192E5-s	ATCCTCCTCGCCCCGGC	
	Au13192E5-a	AGTCTTCGATGTCGTCGAGCATGAGCGAGGCATTGTGTA	309
	Au13192E6-s	CTCGACGACATCGAAGACT	
	Au13192E6-a	CTGCACCATCAACCTCGTAAGCAACCGGAAC AACCCACCGTCTTCTGCCTAACCATCTCCAAAT	126
	Au13192E8-s	TTACGAGGTTGATGGTGCAGATTGCGCCGGT GCGGCGGAAAGATCTCGACGGCATCTTATCAT	
	Au13192E8-a	CCCTTTTGGCCGGTGTACTCTTCAGTTAGGTTCTTGTAGT	333
	Au13192E9-s	AGTACACCGGCCAAAAGGG	
	Au13192E9-a	GCGGCCGCTACACCTTCAACCTCTGCAC( <i>Not</i> I)	



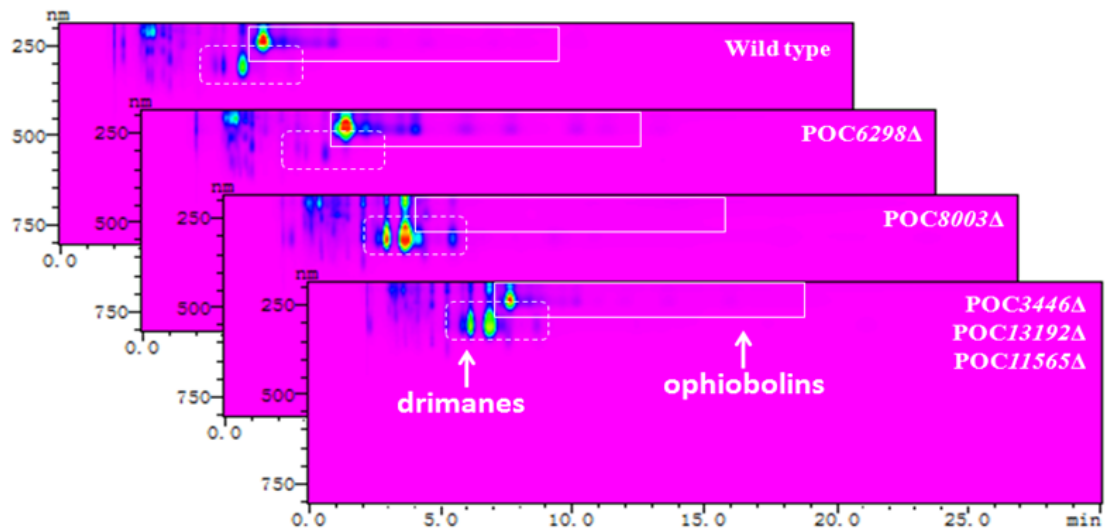
**Figure S1** Twenty reported subgroups (1957–2013) of ophiobolins with 5-8-5 ring scaffolds. These structures were from references 12,14,15 and 16; red color shows ophiobolins identified from *Aspergillus ustus* 094102







**Figure S3 Gene deletion of Au3446.** (a) Plasmid pMD18-T-3446L'-Sh ble- 3446R' constructed with the selective marker and the left and right homologous sequences for recombination; (b) The detailed targeting fragment; (c) Homologous recombinant of the cassette with the genomic DNA to delete the target gene.



**Figure S4 Full wavelength range from 210–400 nm analysis collected for ophiobolin and drimane on the fermentation products of wild type strain and the five gene cluster inactivated mutants.** Ophiobolin did not appear for  $\Delta$ POC8003, increased for  $\Delta$ POC6298, partially decreased for  $\Delta$ POC13192,  $\Delta$ POC3446 and  $\Delta$ POC11565; drimane was increased for  $\Delta$ POC8003,  $\Delta$ POC13192,  $\Delta$ POC3446 and  $\Delta$ POC11565, but decreased for  $\Delta$ POC6298.

Au8003 1 .....MEYKYSTIVDSSKWDPEGLIEGIPLRKHEAGDLEEVGSFRVQEDWRRLVGPVEN-PFRGSL  
AcOS 1 .....MACKYSTLIDSSLYDREGLCPGIDRRHRVAGELEEVGAFAREQEDWRRLVGPPLPK-PYAGLL  
EvSS 1 .....MEYKFSYVVDPGTYETHGLCEGYEYRHKNAELEDIGCLRCQEHWRQSVGPLG-AFKGTL  
PaPS 1 .....MEYRYSYVIDPSSYDNQGLCNGIPVYVHRNADIEEYATISLRNDWRKHVGLPLTISYGGNL  
PaFS 1 .....MEFKYSSEVVEPSTYYTEGLCEGIDVRKSKFTTLEDRGAIRAHEDWNKHIGPCG-EYRGTL  
AbFS 1 .....MKYQFSIIVDPATYDNEGLSNGIDRRKNNFTLEDRGAIRAQQDWAHIAPIK-QFKGTL  
Au13192 1 MPQQTQSESYLNPSLTPVPRSTPDIAFGCAGYELRRHHHEDKANEGSLACRADWEKYGPIER-WGSCN  
EvS 1 MSQS-SDFILNSTLSVYVERSTPDIAFGCSYELRRHHHELANEGSLACRDRDWEQFIPGIER-WGSCN  
Au11565 1 ---MYTLDDFPFKYSGLPNPEAERPAAFFNSLPVVRVTIEDDCGSTIDRVVAEWKAITGKGV-DAVG  
NfSS 1 .....MEVWEHSRPIADDTIKKTPSFTTLPRIINQKNDVADAATRRLRDWYLYLHDGLAERALISI  
Au6298 1 .....  
RpFPPS1 1 .....  
RpFPPS2 1 .....  
EpFPPS 1 .....  
OsaFPPS 1 .....  
Au3446 1 .....  
SsHexPPS 1 .....  
MHexPPS 1 .....  
  
Au8003 61 GPEISFITYTVPECLPERLEAISYGLDYGFLHDDDEITTKIEE---AELDDVGAAALAQGGSTG---KIQE  
AcOS 61 GPDFSFIGAVPECHPDRMEIVAYALEFGFMHDDVIDTQVNH---ASLDEVGHTLDQS-RTG---KIED  
EvSS 60 GNPFNLLSLVIEPECLPDRLSIVGFANELAFIHDDBVTDIQVYG-DAHNNDKFAFNMSATTTG---SM  
PaPS 62 GKPYNFTAVTLPECPDRLEIVSYIMEFAFLMDDDLVDTAQVDEALANDTWRDGIETGLDTT---SA  
PaFS 60 GPRFSYISVAVPEICIPERLEIVSYANEFALMDDDBVTDHVGHDTGEVENDEMMTVFLEAHTGAI DTSNKKV  
AbFS 60 GHDSYFMTVCVPEICIPERLEIVSYANEFALMDDDBVTDLDTENNTSAENDKLMGTFLAGTQG---WSPQ  
Au13192 69 PWEHFGAVVLPFCFKPERLAVICYIFEYAFLYDDVVEESAANSTLNMMDTNDIALDETEYRTVR---  
EvS 68 PWEHFGAVVLPFCFKPERLAVICYIFEYAFLYDDVVEESAANSTLNLNMDTNDIALDETEYRTVR---  
Au11565 65 GHASDLIRYVLPFCPVEFVAPVTRLIYILLLWDDATDILDPVSHENLMLDFRVGVVSMKLG---YCG  
NfSS 63 SELGNLGAFAPEVPPERLAIVTYLTDLGIHDDGYEAMDMQARTEHREFGALFDPHEQLP---SRR  
Au6298 1 .....  
RpFPPS1 1 .....  
RpFPPS2 1 .....  
EpFPPS 1 .....  
OsaFPPS 1 .....  
Au3446 1 .....  
SsHexPPS 1 .....  
MHexPPS 1 .....  
  
Au8003 124 GTKSSGKRKMAAQLLREMMALDPERAMTLAKSWAQ-GVQHSARRVEEKDWKSLDEYIPFRCMDLGYMHWH  
AcOS 122 -KGS DGKRQMTVQIIREMMAIDPERAMTVAKSWAS-GVRHSSRRKEDTNFKALEQYIPEYRALDVGMYLWH  
EvSS 122 ENAASGKRALQAYAREMVRIDKERAIPTIKAWAK-FVDYGG-RQETTRFTSEKEYTEYRIQDILGWFWY  
PaPS 126 KGKKS GEGELLRNLKEVTAIDPVRAELMFKWKR-DLQVSRDRKH--FRDFDDYMEYRIVDCASYFLI  
PaFS 130 DIRRAGKRRIQSFLLEMLAIDPECAKTTMKSWAR-FVEVGSRRQHETRFVFLAKYIPYRIMDVGEMFWF  
AbFS 126 DQSSSGKTRILKQLFSEMMEIDKECAIATMKAWAE-FLRVGSSRRQHGTVFTLRKDYLPYRIMDVGEMFWF  
Au13192 120 -SILGTKQKSKMLLELLAIDARCAEVVINSWKE-MISTTAKQDKTQAFDLEEYVDRYRIDTGAPFFD  
EvS 129 -SILGTKQKSKMLLELLAIDAPRAEVVINSWKE-MISTTAKQDKTQAFDLEEYVDRYRIDTGAPFFD  
Au11565 130 CELEMNRIYQSVLALVVQAGRNLDGGDFKRGLEVFDDSTARGQAPPQTITWEEYKRHRITTSIGRLIT  
NfSS 128 GTRAAKLKLKLSVQLLELEAIRIDRDMGMYFDMYNGWLSVAGGEGKVPQFKSVEEYQAYRRDDFGIRAFW  
Au6298 1 .....  
RpFPPS1 1 .....  
RpFPPS2 1 .....  
EpFPPS 1 .....  
OsaFPPS 1 .....  
Au3446 1 -MKARTVPARRLVLASPIKQPTTLICWQCLRNDLFLERIQCQTRKYHPTRRKDVSPFGAAVSAQAQTIK  
SsHexPPS 1 .....  
MHexPPS 1 .....  
  
Au8003 193 GLVTFGCAITVPEEEEEERTLEPAVIACLMTNDLFSYEKEKNDNPNQ-----NAVAVIMKIKHCSE  
AcOS 191 GLVTFGCAITVPEEEEEAKRILIPALVQASLNDLFSFEKFNKNDANVQ-----NAVLIIVMNEHGCSSE  
EvSS 190 GLLSFAMALDVEPEHEREMCHECRTAYQOIMLVLDLASEWEKFKLNAALG-KDVTINIFVLMEEHGISE  
PaPS 192 ALSFTFAMALTPAEDKDEVFHTTRPVMAAALNDVQSWKEKDKLFQKDN-ATDMTNGWMLMKQYSIGV  
PaFS 199 GLVTFGLGHPDHELELCREIMANAWI AVGLOND IWSWPKERDAATLHG-KDHVVNAIWWLMQEHQTDV  
AbFS 195 GVVTFGMAHHPDHEMDACHKIMPAWI AVGLANDVFSWPKERDASQRLG-RTHVVNAIWWLMQEHGTSQV  
Au13192 198 MLMRFGMGLLTPPEEQARTAPVWPCYAAAGL ANDVFSWPKERDASQREGAKTTMTNAWVLMQEWGLSV  
EvS 197 MLMRFGMGLLTPPEEQARTAPVWPCYAAAGL ANDVFSWPKERDASQREGAKTTMTNAWVLMQEWGLSV  
Au11565 200 ALIPSMRKLRLDQDALDSVSHLAIECYLIAGLNDVFSWPKERDASQREGAKTTMTNAWVLMQEWGLSV  
NfSS 198 PMVVEFGMARLSDDEKKLIEPVMPEIDKAIWITNDVFSWPKERDASQREGAKTTMTNAWVLMQEWGLSV  
Au6298 1 .....  
RpFPPS1 1 ---MNKMLTFTRLSRRSAYFLS-DAAAVREMLFR-PMSTVTRAPPVP-----PA  
RpFPPS2 1 ---MNKMLSTFTRTLNRPATFLFRATANRDNHFRSMTMSVHVHSPVPS-----PV  
EpFPPS 1 .....  
OsaFPPS 1 .....  
Au3446 69 GLPKAPPGSMDPLRIVGKELKFLSKNIRQLGSGHPHLDKVKAKYYTRSE-----GKHMRPLVLLMSQ  
SsHexPPS 1 .....  
MHexPPS 1 .....  
  
Au8003 256 EEARDICQRIRLECRKYARIVKETLART-DISLDLKRYYEIMQYTVSGNWAWSSTQCPRYHADAKFNELQ  
AcOS 254 EEARDILKRRIRLECANYLRNKKETNARA-DVSDLEKRYINVMQYTLSGNAAWSTNCPRYNGPTKFNELQ  
EvSS 259 EEAKEKRCRETAKTLAADYLVKIVVEYKARD-DLSLDSRKYESWLTYLISGNTVWSFICPRYNSGGSFSDHQ  
PaPS 261 EEAKRRI LGKAREHVAEFVKTLISQIHNRLLDLSLDSRLFVEAMQYMLISGNTVWSFICPRYNSGGSFSDHQ  
PaFS 268 DGAMQICRKLIVVEYVAKYLEVIEATKNDE-SISLDLRLKYLDAMLYSISGNTVWSFICPRYNSGGSFSDHQ  
AbFS 264 EQARQYCRELAAQFVAQYLDNIRNINKEE-SISPLDRTYVEAMQYSISGNTVWSFICPRYNSGGSFSDHQ  
Au13192 268 EDKRRVREVTNMYERQYVKNVEEFCASEGKDEPKLOEYVLAQQHGVPGNVAVSLRCPRYHPWLCKEAAE  
EvS 267 EQKRRVREVTNMYERQYVKNVEEFCASEGKDEPKLOEYVLAQQHGVPGNVAVSLRCPRYHPWLCKEAAE  
Au11565 270 SEAEIIMKLEILSAERTLMEKYE EWKSSSAPKSDLELHFLVLSILAVGGASVWSFISPRYQANLSTTVE  
NfSS 266 DEAKAAVRQLLVNLEQQYLERKRAIYAONPSIPSHLRKWLIEVVGITVAGTHFWASCSPREHAWRNNSRNG  
Au6298 1 -MSS---PRAAEVAFPSLAQDILDHAKKYNLPQ-----NALEWLEKNLNTN-----  
RpFPPS1 45 ITGTAVSKDETRDFMAVFPDVVRDLTDGTRNLDIP-----DVTKWLKVLGYN-----  
RpFPPS2 49 LIGTSISKDELGDFMAVFPDVKDLTDGTRNLDIP-----DVTKWLKVLGYN-----  
EpFPPS 1 -MAD---LKSTFLDVSQSLKTELLNDT-AFEWSP-----DSRQWVERMLDYN-----  
OsaFPPS 1 -MADSNGLDTKSRFLQIYGRKSELLEDP-AFDFTD-----DARQWIDRMLDYN-----  
Au3446 133 ATALTTPRNRPVSEADSTATVNDPITSP-----  
SsHexPPS 4 IEFWLEAKATIDRLIEQLNSNRDWDLVD-----  
MHexPPS 1 -MIALSYKAFLNPIIIEVEKRYECIQSD-----  
  
Au8003 325 MLRAEHGVAKYPARYSLENRKNANGVNGVNGVNGVNGVNGKRRSGEETA-----  
AcOS 323 LLRSEHGLAKYPSRWQENRSTGL-----VEGDCHESKPN-ELKRRKNGVSV-----  
EvSS 328 LELMKNGVPKDPASGSTNGTSNGT-----SNGTSHVAVNG-NGHVTNDDLSAN-----  
PaPS 330 VARMKYGWNHREVTKLTSLENRGTKRTHQDTEGVQSVKRFNGASTKNGINGTNGI-----  
PaFS 337 LEWMRQGLPSELESCPVLARSPED-----SDASVSPAD-----ESDSTEDSLGS-----  
AbFS 333 LDWMQNLGSPS-----TVE-----LDGASNTSSSF-----LSTSTHGSPAS-----  
Au13192 338 LLRDAIAESREEKPIYETETTRWGSSPSESSESDASEGSPTFWSSGSSRSVRSVSV-----  
EvS 337 LLHQDTIQELEAG-RKPALEEYRSRSHSESDLSASPTFWSSGSSRSARSSVSV-----  
Au11565 340 DRAQLVGRSYTAG-----LRLPNYPVPVAMTKSTNGYSESLTNR-----  
NfSS 336 LKPAHVAAPTLITPSNNLNSSKGSSEQMQDSNNGTRTQMCNPANDHEVMQLNAKLSLQKQDGGHAMRAAL  
Au6298 43 .....  
RpFPPS1 92 .....  
RpFPPS2 96 .....  
EpFPPS 42 .....  
OsaFPPS 47 .....  
Au3446 161 .....  
SsHexPPS 32 .....  
MHexPPS 28 .....

Au8003 378 ----- DDARTNG-NGIKKPAHVLEYR-DSLVLIEDIVALSLDWNLPDLSGVVYQPKYITSLPS  
 AcOS 369 ----- DEMRTNGTNGAKKPAHVSQPSTDSIVLEDMVQLARTCDLPDLSDTV LQPYRYITSLPS  
 EvSS 374 ----- GIKTDGELLSAITMEHLKNRNSFKLGDHQEVKSLHGHGQALDPRV LQAPYDYITSLPS  
 PaPS 386 ----- NGLNGINGNGVKIKRHKNKESYSGALTKDSDLVLVNDMLNGLS-SALCPYDYITSLPS  
 PaFS 382 ----- GSRQDSSLSTGLSLSPVHS-----NEGKD LQRVDTDHIFFEKAV EAPYDYITSLPS  
 AbFS 367 ----- GSQTTIESKDGWTD-----SSGIVSLLNCSLPLPSHKV SAPLTYITSLPS  
 Au13192 392 ----- -----SAASLHEKQT-EHVQLGDEILAPADYITSLPS  
 EvS 389 ----- SAFGPPDKISITPAILGDEHLAPAEYITSLPS  
 Au11565 378 ----- QASLITKDLLAPFEKAPAEVVLAPWKYITSLPS  
 NfSS 406 ALLSRAAEQCESLFDGMEHERARLLQSGEEKARLSWEGRSKGSQLEHSHWYKPAKTAQAPIHYITSLPS  
 Au6298 43 ----- -----VGGKLNRLSV  
 RpFPPS1 92 ----- -----VGGKLNRLSV  
 RpFPPS2 96 ----- -----VGGKLNRLSV  
 EpFPPS 42 ----- -----VGGKLNRLSV  
 OsaFPPS 47 ----- -----VGGKLNRLSV  
 Au3446 161 ----- SVLADANPDIDRFITSS  
 SsHexPPS 32 ----- ISSYILKDG  
 MfHexPPS 28 ----- SETLNKAHITLSSGG

DDXXD motif

Au8003 436 KGFRLDQADSLNTWLRVPTKTKMIDKVIKMLHSASLMLDDLEDSPLRRGKPSSTHVIYGNAQTNSATY  
 AcOS 429 KGFRLDQADSLNKWLKVPKSKMIDKVIKMLHSASLMLDDLEDSPLRRGKPSSTHVIYGMAQTNSATY  
 EvSS 434 KGLRQEAIDALNVWFRVPTAKLEIKSITTLHNASLMLDDVEDSSELRRAKPATHNFGLGQITNSANY  
 PaPS 445 KGLRDNVADALSILWDPKALEINQIKRATNLHNASLMLDDVQDSSELRRAKPATHNFGVGLTNSANY  
 PaFS 435 KGVRFDFDALNDWLRVDPVKGKIDAVRVLHNASLMLDDDFQDSSELRRAKPATHNFGVGLTNSANY  
 AbFS 416 KGVTRDMFLDALNHVLRVDEQRASQVKMAIRMLHNASLMLDDVQDSSELRRAKPATHNFGVGLTNSANY  
 Au13192 425 KGVREAFDGLNVWLVLPDHRVRLKNIQAQTLHNASLMLDDLEDSPLRRGKPSATHNFGVGLTNSANY  
 EvS 424 KGVREAFDGLNVWLVLPDHRVRLKNIQAQTLHNASLMLDDLEDSPLRRGKPSATHNFGVGLTNSANY  
 Au11565 413 KRTVGRMECLQAWFSLPVESKISEITTLHNASLMLDDIQDSSELRRAKPATHNFGVGLTNSANY  
 NfSS 476 KGVRSRMLEAFNYWLEVDSTLTKRRLVLDLHNASLMLDDLEDSPLRRGKPSATHNFGVGLTNSANY  
 Au6298 56 PDTGVALLKPK-LSEEQYKDLATLGMWTELLQAFFLVSDDIMDSSITRRGKPSATHNFGVGLTNSANY  
 RpFPPS1 105 VLS-FKMLSSPSDQDENLRLSYILGWCVELQAYQLVLDIMDAITRRGKPSATHNFGVGLTNSANY  
 RpFPPS2 109 VLS-FKMLSSPSDQDENLRLSYILGWCVELQAYQLVLDIMDAITRRGKPSATHNFGVGLTNSANY  
 EpFPPS 55 IDS-YKLLKQGEELTEEEIFLASALGWCEWLQAYFLVLDIMDSSHTRRGKPSATHNFGVGLTNSANY  
 OsaFPPS 60 VDS-YKLLREGIQLSDDEFFLASSLGWCEWLQAYFLVLDIMDSSHTRRGKPSATHNFGVGLTNSANY  
 Au3446 177 SSAADGQYDFAGDENILPSQ--RRLAEITELHNASLMLDDVIDAITRRGKPSATHNFGVGLTNSANY  
 SsHexPPS 42 KRFVRFGLTLMFVTLVGLGDKIDSYGGALAEILHSASLMLDDVIDAIDATRRGKPSATHNFGVGLTNSANY  
 MfHexPPS 45 KRVRPMFLVLSGLFNDTKQDDIRTAVALSELVHNASLMLDDVIDAIDSMRRGKPSATHNFGVGLTNSANY

Au8003 506 QYTEATGLAARLPNP-----TSRLRYLLEVQQLVYGGQSYDLYWTHNALC--PSPEY---LKMVDQKT  
 AcOS 499 QYITATDITAQLQNS-----ETFHIVFEEELQQHVVGGQSYDLYWTHNTLC--PTIAEY---LKMVDQKT  
 EvSS 504 QLVRALQEQKLGDA-----RSLLVFVEELHNLVYGGQSYDLYWTHNTLVC--PSMHEY---FQMLDKT  
 PaPS 515 QILQAMNEKRLGSD-----DCLDFSEELKLYVGGQSYDLYWVYNDSC--PTIEDY---FKMVDQKT  
 PaFS 505 SIIKAGQIMFESAG-----ESVQEVMSIMIFQGGAMDLFWYNGHV--PSEEEY---LQMLDKT  
 AbFS 486 LNVESIKLRLEAGD-----GGVAALVEKLTSEFVGGQADLHSSRNLSP--PSLTEY---QTLQDKT  
 Au13192 495 LLEVMDQIRKLLDDP-----GCLDIYFEMRNLFVGGQSEDLYWRQGECC--PSEEEY---LQMLDKT  
 EvS 494 LLDVMEKVRKLLDDP-----RCMDIYFEMRNLFVGGQSEDLYWRQGECC--PSEEEY---LQMLDKT  
 Au11565 483 LYVKGSRRLRQVVKHAD-----ECADALLELELAFGGQALELQWKYKQIC--PSTKQY---LVMIDQKT  
 NfSS 546 MFVQAVYQVARFRNP-----NAVDLLELEENLYGGQSYDLYWTHNALC--PSPEY---LKMVDQKT  
 Au6298 124 LLESGYIILKQKFRSHPAYIDIVELFETTWQTELGOLCPLITAPEDK--VDLNSMEKYMFVITYK  
 RpFPPS1 174 LLEQTYQLKQKFKKPYIHLLELVEYVTMKTSMGOCPLMTANSFKT--KKLEKVTMNYTAIVKYK  
 RpFPPS2 178 LLEQAYQLKQKFKKPYIHLLELVEYVTMKTSMGOCPLMTANSFKT--KKLEKVTMNYTAIVKYK  
 EpFPPS 124 LLRNHIPRILKQKFRGKPYVDLLELVEYVFTASGOMIDLITLEGE--QDLNKYTLSLHRRIVQYK  
 OsaFPPS 129 LLRNHIPRILKQKFRGKPYVDLLELVEYVFTASGOMIDLITLEGE--QDLNKYTLSLHRRIVQYK  
 Au3446 245 LLGRAVAALRSDP-----EVTELLATVIANVEGFEMLKNTAEDEKNPVFTDTEISYLLQKTYLKT  
 SsHexPPS 112 LLPTAIRIQTYSQD-----DALNTSILWKTDSVGLRDMYDN-----SDY---IRTLEKT  
 MfHexPPS 115 LLARALQNLATINNS-----KFHQITSEITLWCFGEFQADRFNYP-----VSFTAY---LRRINRKT

GXXOX motif, DDXNX motif

Au8003 564 GGLFRMLTRMLVSEPARSSIL--DQTYPFLSHLGRFQFRDDYQNLASAEYARQKGAEDLDEGKYSF  
 AcOS 557 GGLFRMLTRMLVSEPARSSIL--DQTYPFLSHLGRFQFRDDYQNLASAEYARQKGAEDLDEGKYSF  
 EvSS 562 GGLFRMLTRMLVSEPARSSIL--DQTYPFLSHLGRFQFRDDYQNLASAEYARQKGAEDLDEGKYSF  
 PaPS 573 GGLFRMLTRMLVSEPARSSIL--DQTYPFLSHLGRFQFRDDYQNLASAEYARQKGAEDLDEGKYSF  
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 SsHexPPS 162 GSKLSTVLSAYASKHYNTKQ--QMLDVGKYLGIIMQVDDVQYKTKKVEIEGSAKQLFK--  
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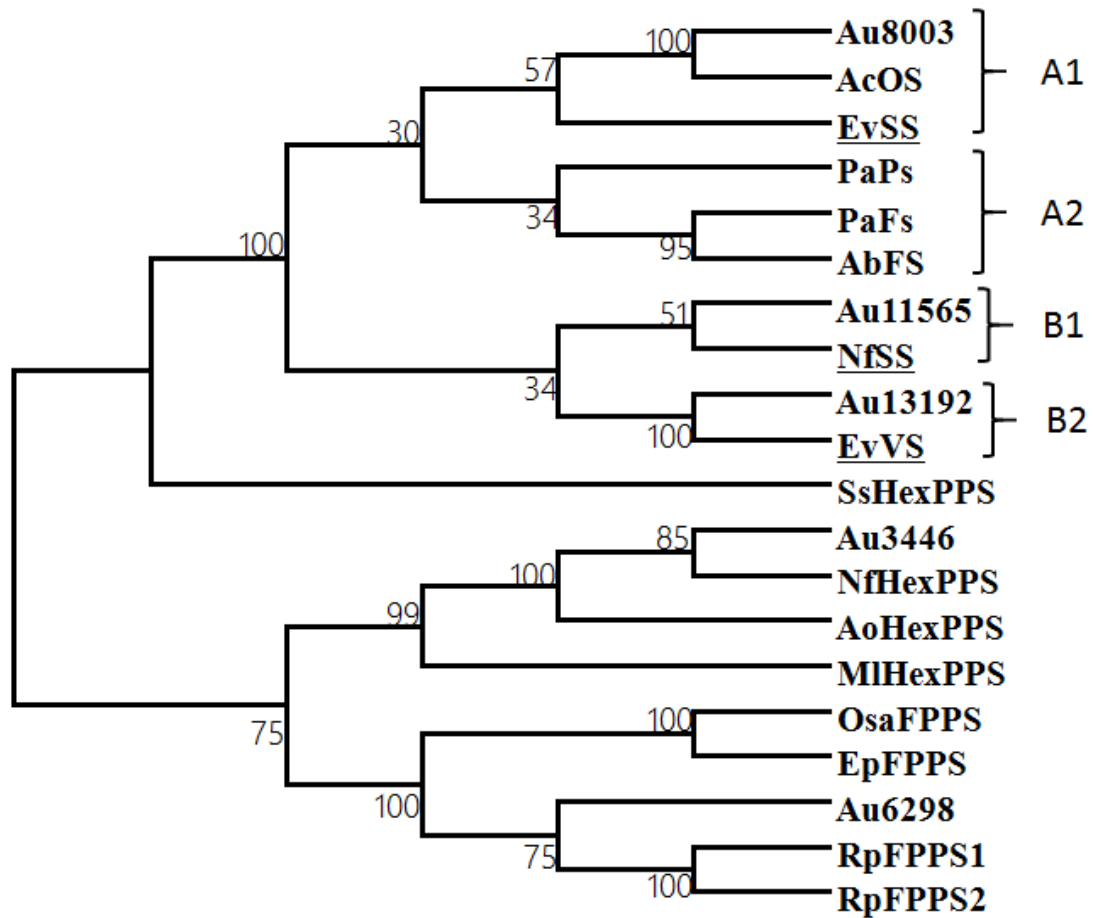
DDXXD motif

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 PaPS 638 TLHIAISAAP-----EPEALLRNLSGRNRNDGK--LSVQKNLALSIIEGARSLEYTAAVLQKLYKATV  
 PaFS 633 ALHIMHKQ-----RSHMALLNVLSTGRKHGG--MTLEKQKFLDIEIEKSLDYTRSVMDLHVQELR  
 AbFS 611 PLVYASISQ-----SENFLQNLNLSLRLAEGT--LDDDKRLALDQMQLVKTNELRKLIMDSLYDELR  
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 NfSS 669 PTVVCVANHADFR--DLIDGVRQRPATISGMQPLAPEIKRYVVEYLNTSGTFQHCREFLMLLESLTE  
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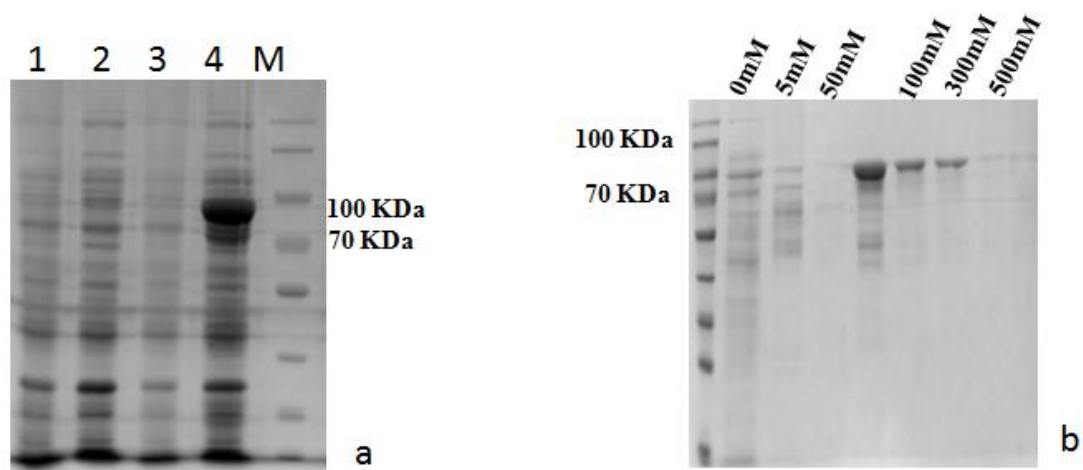
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 PaPS 701 RELESTERQFG-ENKPFRLSLKLV--  
 PaFS 694 AEIGREILLDSPNPAWRLLLELRLV--  
 AbFS 672 AELQCISSSFASENPQWELMLMMLKL--  
 Au13192 683 DSVVALEGETGTANVWVYRLLVQRLKV--  
 EvS 680 DSVVLEGETGSPNWWVYRLLHRLKV--  
 Au11565 668 GALDNVEAKLG-LNKKRIFLRLKLV--  
 NfSS 736 SEIDREKVTNEANPMLRLLLEKLSVKEN  
 Au6298 321 AVDESQGLKKEVFAFLGKIYKRTK--  
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 RpFPPS2 376 HL--SQGLSQDMFLKFLKIKYKRTL--  
 EpFPPS 320 AH--PSKAVQSVLKSFLGKIYKRRK--  
 OsaFPPS 325 AQ--PSKAVQEVLLKSFLLHKIYKRRK--  
 Au3446 433 SIFPGSEAKSGLIEMCVKAMNRRK--  
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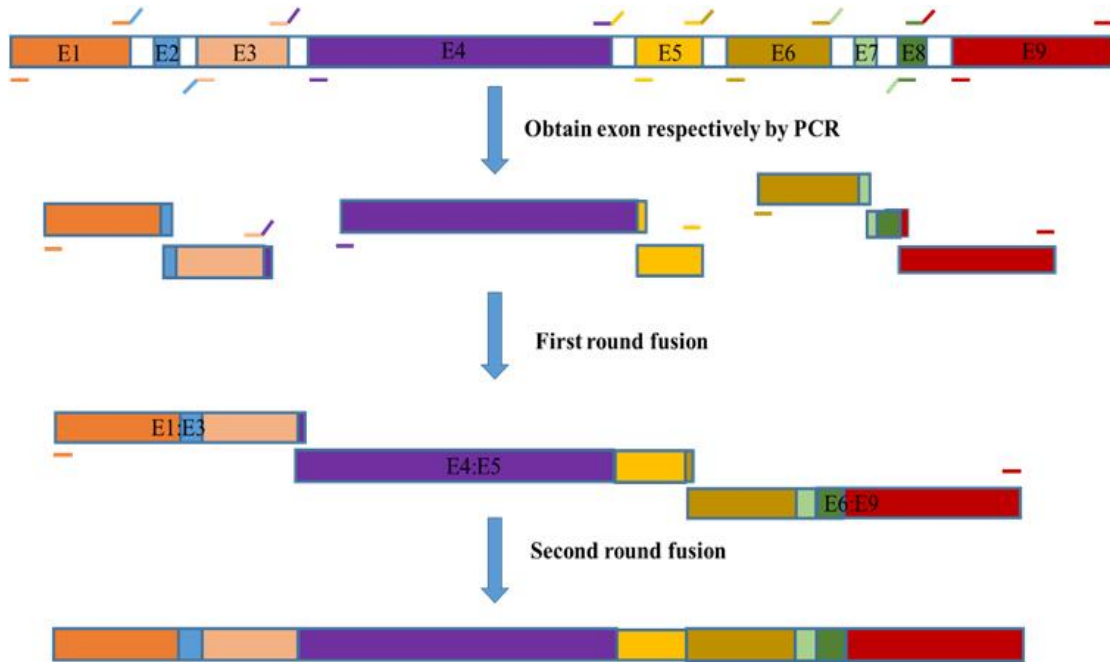
**Figure S5. Alignment of amino acid sequences of the five terpene synthesis related proteins in ophiobolin biosynthesis with identified functions.** Shown here are DDXXD/E (Terpene cyclase (TC) domain), DDXXD (Prenyltransferases (PT) domain), GQ(PT), DDXXN (bifunction PT only) DDXXD (single function PT only), *Aspergillus clavatus* ophiobolin F synthase (AcOS A1C8C3), *Emericella varicolor stellata*-2,6,19-triene Synthase (EvSS LC073704), *Phomopsis amygdali* phomopsene synthase (PaPS AB254159), *Phomopsis amygdali* fusicoccadiene synthase (PaFS AB267396), *Alternaria brassicicola* fusicoccadiene synthase (AbFS C9K2Q3), *Emericella varicolor* Variediene Synthase (EvVS LC063849), *Neosartorya fischeri* sesterfisherol synthase (NfSS EAW16201), *Rhopalosiphum padi* isoprenyl diphosphate synthase (RpFPPS1 and RpFPPS2 HQ850372 and HQ850373), *Ornithogalum saundersiae* farnesyl pyrophosphate synthase (OsaFPPS KF509889), *Euphorbia pekinensis* farnesyl diphosphate synthase (EpFPPS FJ755465), *Sulfolobus solfataricus* Hexaprenyl Pyrophosphate Synthase (SsHexPPS 2AZK\_B), and *Micrococcus luteus* heterodimeric hexaprenyl diphosphate synthase (MIHexPPS 3AQB\_B).



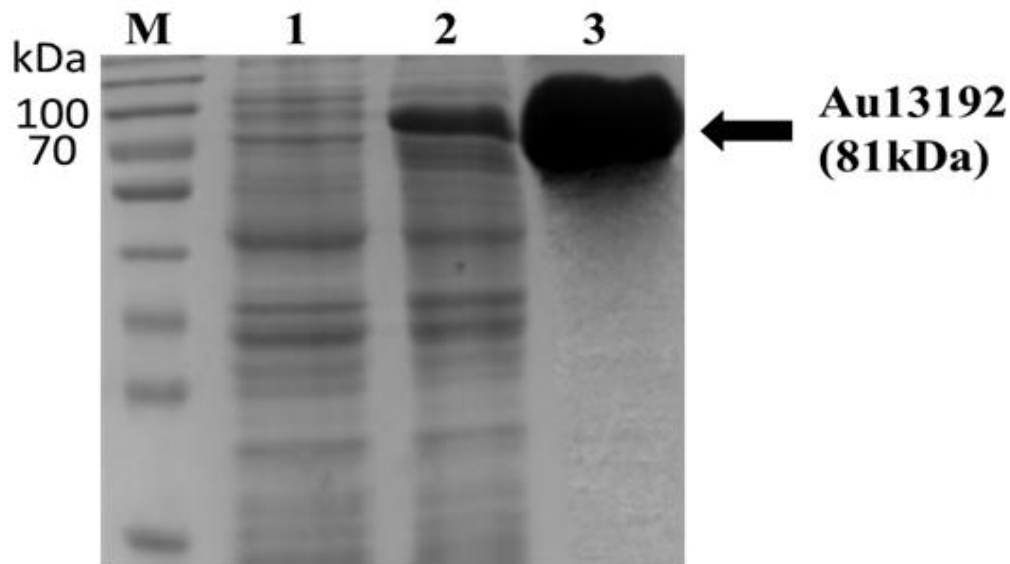
**Figure S6 Neighbour-joining phylogenetic tree based on amino acid sequences of bifunctional terpene synthases, farnesyl pyrophosphate synthases and hexprenyl pyrophosphate synthases.** The tree was generated based on Kimura 2-parameter matrix in MEGA software (version 6.0). Numbers at nodes are bootstrap values obtained using 1000 iterations. The scale bar indicates 0.2 substitutions per nucleotide position. Reference sequences were the same as in Figure S5.



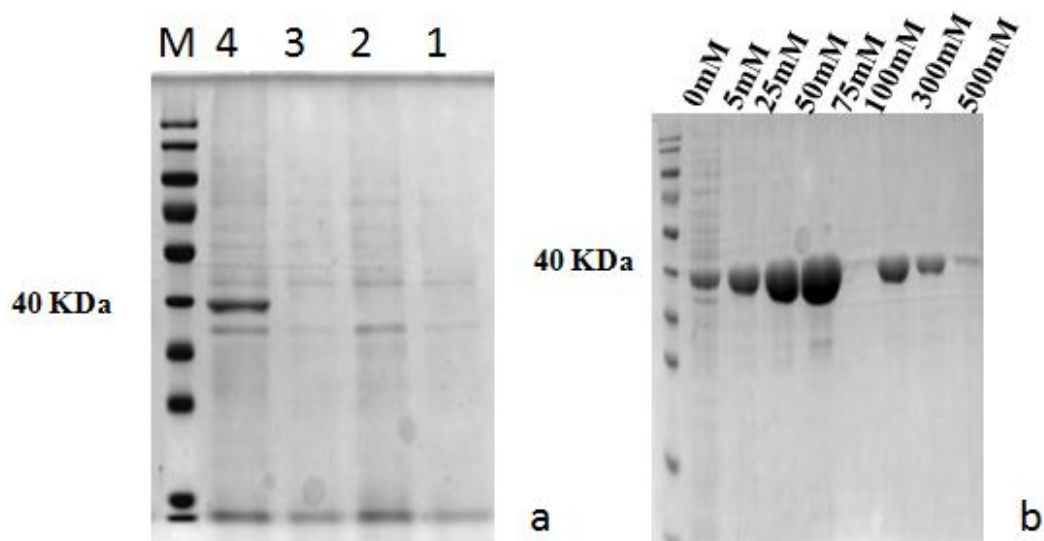
**Figure S7. SDS-PAGE analysis of expression and purification of Au8003.** (a) Overexpression of Au8003 gene in BL21(DE3). Lane M: Marker; Lane 1: uninduced (without isopropyl (3-D-thiogalactopyranoside) IPTG) bacterial protein carrying plasmid pET28a; Lane 2: induced (with IPTG) bacterial protein carrying plasmid pET28a, Lane 3: uninduced bacterial protein carrying the Au8003 cDNA; Lane 4: induced bacterial protein carrying the Au8003 cDNA; (b) purification with different concentrations of imidazole.



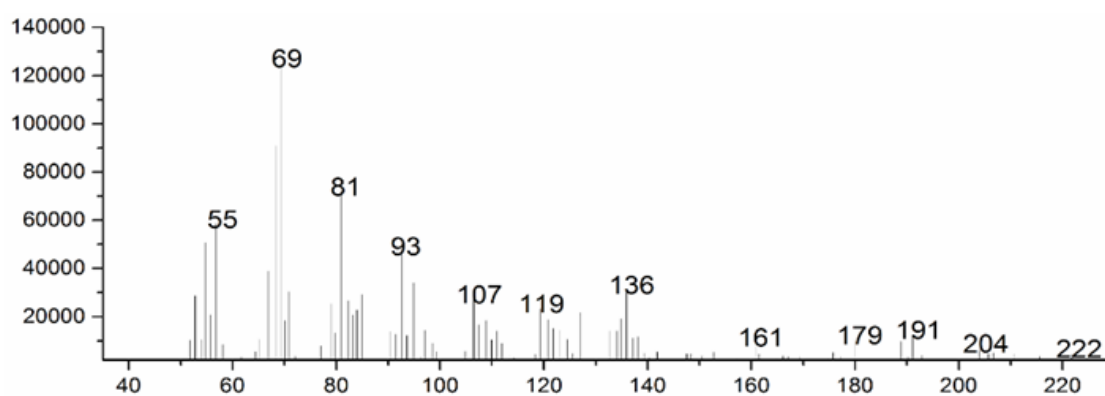
**Figure S8. Strategy of Au13192 multiple exon fusion by overlap extension PCR.** Stage 1: designing primers and amplifying exon 1 (E1) to exon 9 (E9) with overlap sequences through normal PCR (because the E2 and E7 segments are too short, the two gene sequences were designed according to the adjacent two exons of the reverse primer and forward primer, respectively); Stage 2: first round fusion obtained E1:E3/E4:E5/E6:E9, using adjacent exon as template with forward primers of front end exon and reverse primers of tail end exon; Stage3: second fusion obtained Au13192 cDNA following stage 2.



**Figure S9. SDS page gel detection of overexpression and purification of Au13192.** Lane M: Marker; Lane 1: uninduced (without isopropyl (3-D-thiogalactopyranoside) bacterial protein carrying the 2.1-kb Au13192 cDNA; Lane 2: induced (with isopropyl p-D-thiogalactopyranoside) bacterial protein carrying the 2.1-kb Au13192 cDNA; Lane3: purified Au13192



**Figure S10. SDS-PAGE analysis of expression and purification of Au 6298.** (a) Overexpression of Au6298 genes in BL21(DE3). Lane M: Marker; Lane 1: uninduced (without isopropyl (3-D-thiogalactopyranoside) IPTG) bacterial protein carrying plasmid pET28a; Lane 2: induced (with IPTG) bacterial protein carrying plasmid pET28a, Lane 3: uninduced bacterial protein carrying the Au6298 cDNA; Lane 4: induced bacterial protein carrying the Au6298 cDNA; (b) purification with different concentrations of imidazole.



**Figure S11 Mass spectrum of authentic farnesol.**