

Highly efficient synthesis of lysergic acid by engineered budding yeast

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Contains:

Table S1:	Plasmids used in this study.
Table S2:	Strains constructed in this study.
Table S3:	Primers used in this study.
Table S4:	gRNA target sequences used in this study.
Table S5:	¹ H NMR data of compounds lysergic acid (LA).
Figure S1:	¹ H NMR spectrum of LA in D ₂ O (500 MHz).
Figure S2:	MS spectra of sample LA and standard LA.
Figure S3:	Comparative analysis results of different sequences alignment of cloA from <i>Claviceps purpurea</i> source.
Figure S4:	The results of fermentation mass spectrometry of suspected LA were consistent with those of reported LA mass spectrometry.
Figure S5:	The results of growth and LA production in control strain (Sc251) and Δ sch9 strain (Sc560).
Figure S6:	The effect of Δ sch9 on LA production.
Figure S7:	Results of CpCPR transmembrane domain analysis.

Table S1 Plasmids used in this study.

Plasmids	Description	Source
pWN416	The cassette T_{FBAI} - $P_{GALL.10}$ - T_{TDH2} was cloned and inserted into pRS416.	This study
pWN041	The fragment <i>HSP12</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN042	The fragment <i>OLE1</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN043	The fragment <i>SNF1</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN044	The fragment <i>TIP1</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN045	The fragment <i>MPP10</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN046	The fragment <i>CpCloA(1)</i> (BsaI), <i>CpCPR</i> (BsmBI) were inserted into pWN416 with standardized restriction sites.	This study
pWN047	The fragment <i>CpCloA(1)_HDEL</i> (BsaI), <i>CpCPR_HDEL</i> (BsmBI) were inserted into pWN416 with standardized restriction sites.	This study
pWN048	The fragment <i>CpCloA(1)_KKYL</i> (BsaI), <i>CpCPR_KKYL</i> (BsmBI) were inserted into pWN416 with standardized restriction sites.	This study
pWN049	The fragment <i>easC</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN050	The fragment <i>easC(SKL)</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN051	The fragment <i>fzo1</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN052	The fragment <i>pex34</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN053	The fragment <i>easC(SKL)</i> (BsaI), <i>pex34</i> (BsmBI) were inserted into pWN416 with standardized restriction sites.	This study
pWN054	The fragment <i>CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN055	The fragment <i>t26CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN056	The fragment <i>t31CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN057	The fragment <i>t36CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN058	The fragment <i>CpCloA(1)</i> (BsaI), <i>t36CpCPR</i> (BsmBI) were inserted into pWN416 with standardized restriction sites.	This study
pWN059	The fragment <i>CpCloA(1)-L1-t36CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN060	The fragment <i>CpCloA(1)-L2-t36CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN061	The fragment <i>CpCloA(1)-L3-t36CpCPR</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study
pWN062	The fragment <i>CpCloA(1)-L2-t36CpCPR_KKYL</i> (BsaI) was inserted into pWN416 with standardized restriction sites.	This study

Table S2 Strains constructed in this study.

Strains	Description	Source
SyBE_Sc06130096	$\Delta GAL80::P_{GAL1,10}-IDII-His, ERG20p_{ERG20}::HXT1p_{ERG20}, \Delta 22::LUE2-T_{CYC1-dmaW}-P_{GAL1,10}-easF-T_{ADH1}, \Delta 15::KanR-T_{CYC1}-easE-P_{GAL1,10}-easC-T_{ADH2}, \Delta 14::hphMX6-T_{ADH2}-fadI-P_{GAL1,10}-eroI-T_{CYC1}, \Delta GAL1\backslash 7\backslash 10::P_{GAL7}-easD_{Cp}-T_{GPM1}, ho::T_{TDH2}-easG_{Cp}-P_{gal110}-easA_{Cf}-T_{FBA1}$	1
SyBE_Sc06130244	SyBE_Sc06130096, <i>YMRW</i> $\Delta 15:: Trp-T_{CYC1}-Cp_{cloA(1)}-P_{Gal110}-Cp_{CPR}-T_{ADH1}$	This study
SyBE_Sc06130245	SyBE_Sc06130096, <i>YMRW</i> $\Delta 15:: Trp-T_{CYC1}-Cp_{cloA(2)}-P_{Gal110}-Cp_{CPR}-T_{ADH1}$	This study
SyBE_Sc06130246	SyBE_Sc06130096, <i>YMRW</i> $\Delta 15:: Trp-T_{CYC1}-Cp_{cloA(3)}-P_{Gal110}-Cp_{CPR}-T_{ADH1}$	This study
SyBE_Sc06130251	SyBE_Sc06130244 with pWN416	This study
SyBE_Sc06130295	SyBE_Sc06130244 with pWN041	This study
SyBE_Sc06130296	SyBE_Sc06130244 with pWN042	This study
SyBE_Sc06130297	SyBE_Sc06130244 with pWN043	This study
SyBE_Sc06130298	SyBE_Sc06130244 with pWN044	This study
SyBE_Sc06130302	SyBE_Sc06130244 with pWN045	This study
SyBE_Sc06130560	SyBE_Sc06130244, $\Delta sch9$	This study
SyBE_Sc06130561	SyBE_Sc06130244 with pWN046	This study
SyBE_Sc06130562	SyBE_Sc06130244 with pWN047	This study
SyBE_Sc06130563	SyBE_Sc06130244 with pWN048	This study
SyBE_Sc06130564	SyBE_Sc06130560 with pWN046	This study
SyBE_Sc06130565	SyBE_Sc06130560 with pWN047	This study
SyBE_Sc06130566	SyBE_Sc06130560 with pWN048	This study
SyBE_Sc06130567	SyBE_Sc06130560 with pWN416	This study
SyBE_Sc06130568	SyBE_Sc06130244 with pWN049	This study
SyBE_Sc06130569	SyBE_Sc06130244 with pWN050	This study
SyBE_Sc06130570	SyBE_Sc06130560 with pWN049	This study
SyBE_Sc06130571	SyBE_Sc06130560 with pWN050	This study
SyBE_Sc06130580	SyBE_Sc06130244 with pWN051	This study
SyBE_Sc06130581	SyBE_Sc06130244 with pWN052	This study
SyBE_Sc06130582	SyBE_Sc06130560 with pWN051	This study
SyBE_Sc06130583	SyBE_Sc06130560 with pWN052	This study
SyBE_Sc06130584	SyBE_Sc06130244 with pWN053	This study
SyBE_Sc06130585	SyBE_Sc06130560 with pWN053	This study
SyBE_Sc06130287	SyBE_Sc06130244 with pWN054	This study
SyBE_Sc06130288	SyBE_Sc06130244 with pWN055	This study
SyBE_Sc06130289	SyBE_Sc06130244 with pWN056	This study
SyBE_Sc06130290	SyBE_Sc06130244 with pWN057	This study
SyBE_Sc06130400	SyBE_Sc06130244 with pWN058	This study
SyBE_Sc06130401	SyBE_Sc06130244 with pWN059	This study
SyBE_Sc06130402	SyBE_Sc06130244 with pWN060	This study
SyBE_Sc06130403	SyBE_Sc06130244 with pWN061	This study
SyBE_Sc06130404	SyBE_Sc06130560 with pWN058	This study

SyBE_Sc06130405	SyBE_Sc06130560 with pWN059	This study
SyBE_Sc06130406	SyBE_Sc06130560 with pWN060	This study
SyBE_Sc06130407	SyBE_Sc06130560 with pWN061	This study
SyBE_Sc06130593	SyBE_Sc06130560 with pWN416	This study
SyBE_Sc06130576	SyBE_Sc06130560, <i>YPRCΔτ3::P_{gal1}-CpcoA(1)-L2-t36CpCPR</i> - <i>T_{FBA1}</i>	This study
SyBE_Sc06130577	SyBE_Sc06130576 with pWN416	This study
SyBE_Sc06130578	SyBE_Sc06130576, <i>Δint11::T_{TDH2}-easC(SKL)-P_{gal110}-pex34-T_{FBA1}</i>	This study
SyBE_Sc06130590	SyBE_Sc06130578 with pWN416	This study
SyBE_Sc06130591	SyBE_Sc06130578 with pWN060	This study
SyBE_Sc06130592	SyBE_Sc06130578 with pWN062	This study

Table S3 Primers used in this study.

Primers	Sequence (5'-3')
For cloning <i>cloA(1)</i>	
W-1F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAAACTAG
W-2R	agtaacttaaggagttaaatTTAAGTCCATCTATCAGCCAAGATTTGTAATTC
For cloning <i>cloA(2)</i>	
W-3F	gtcaaggagaaaaactataATGGCTGTTTCATGTTGTTAAATCTTACTC
W-4R	agtaacttaaggagttaaatTTAGACATTACCAGATTTATACATTGATCTAGAACC
For cloning <i>cloA(3)</i>	
W-5F	gtcaaggagaaaaactataATGAAGTTGCATGTTGTTCAATCTTACC
W-6R	agtaacttaaggagttaaatTTATTCTTCGATTAACATCTTTTACCTTTTGGAG
For cloning <i>CpCPR</i>	
W-7F	aaaaagtaagaatTTTgaaaattcaataaaATGGCAGAATTGGATACCTTGGATATG
W-8R	aaaaactatatcaattaattgaattaacTTAGGACCAAACATCTTCTTGGTATTGGTTAG
For cloning <i>Hsp12</i>	
W-9F	gtcaaggagaaaaactataATGTCTGACGCAGGTAGAAAAGGATTC
W-10R	agtaacttaaggagttaaatTTACTTCTTGGTTGGGTCTTCTTCACC
For cloning <i>W-11Fning OLE1</i>	
W-11F	gtcaaggagaaaaactataATGCCAATTCTGGAACACTATTGAATTG
W-12R	agtaacttaaggagttaaatTTAAAAGAACTTACCAGTTTCGTAGATTTACCTC
For cloning <i>SNF1</i>	
W-13F	gtcaaggagaaaaactataATGAGCAGTAACAACAACACAAACACAG
W-14R	agtaacttaaggagttaaatTCAATTGCTTTGACTGTTAACGGCTAATTCC
For cloning <i>TIP1</i>	
W-15F	gtcaaggagaaaaactataATGTCCGTTTCCAAGATTGCTTTTCG
W-16R	agtaacttaaggagttaaatTTATAACAATAAAGCAGCTGCACCTGC
For cloning <i>MPP10</i>	
W-17F	gtcaaggagaaaaactataATGTCAGAACTCTTTGGAGTATTGAAATCTAATG
W-18R	agtaacttaaggagttaaatTCAAAGTTTATATTTGTGCTATCTGGCCC
For cloning <i>cloA(1)_HDEL</i>	
W-19F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAAACTAG
W-20R	agtaacttaaggagttaaatTTACAATTCGTCTGTGAACCACCACCAGTCCATCTATCAGCC AAGATTTGTAATTC
For cloning <i>CpCPR_HDEL</i>	
W-21F	aaaaagtaagaatTTTgaaaattcaataaaATGGCAGAATTGGATACCTTGGATATG
W-22R	atcaattaattgaattaacTTACAATTCGTCTGTGAACCACCACCGGACCAAACATCTTCT TGGTATTGGTTAG
For cloning <i>cloA(1)_KKYL</i>	
W-23F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAAACTAG
W-24R	agtaacttaaggagttaaatTTACAAGTACTTCTTTGAACCACCACCAGTCCATCTATCAGCC AAGATTTGTAATTC
For cloning <i>CpCPR_KKYL</i>	
W-25F	aaaaagtaagaatTTTgaaaattcaataaaATGGCAGAATTGGATACCTTGGATATG
W-26R	atcaattaattgaattaacTTACAAGTACTTCTTTGAACCACCACCGGACCAAACATCTTCTT GGTATTGGTTAG
For cloning <i>easC</i>	
W-27F	AGTTGAATATTCCTCAAAAATGGCACCAGACTCCAAACCAAC
W-28R	aatcattcattctcagacTTACAATCTAGCTGGAATAAAAGATTCTTCAGC
For cloning <i>easC(SKL)</i>	
W-29F	gtcaaggagaaaaactataATGGCTCCAGATTCTAAGCCAACCTAC
W-30R	agtaacttaaggagttaaatTTATAGTTTAGATGGAATAAAAGATTCTTCTGCTGGAGCTTC

For cloning <i>Fzo1</i>	
W-31F	gtcaaggagaaaaactataATGTCTGAAGGAAAACAACAATTCAAAGACAG
W-32R	agtaacttaaggagtaaataCTAATCGATGTCTAAATTTATTCTCCACCATCAATTTTG
For cloning <i>Pex34</i>	
W-33F	gtcaaggagaaaaactataATGGTTTCGAAGAAAATACGGCTGAAATC
W-34R	agtaacttaaggagtaaataTTATACAATTATTCTACAAAGTGTTATTATGTTAGATAATCTTT
For cloning <i>t26CpCPR</i>	
W-35F	gtcaaggagaaaaactataATGACTATTTGGGGTGTGTTAAGGACCC
W-36R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAGC
For cloning <i>t31CpCPR</i>	
W-37F	gtcaaggagaaaaactataATGGTTAAGGACCCTTACGCTAATACCTTCG
W-38R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAGC
For cloning <i>t36CpCPR</i>	
W-39F	gtcaaggagaaaaactataATGGCTAATACCTTCGCTAACGTTAACGG
W-40R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAGC
For cloning <i>cloA(1)-L1-t36CpCPR</i>	
W-41F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAACTAGAC
W-42R	CTTAGCAGCAGCTTCCTTAGCAGCAGCTTCAGTCCATCTATCAGCCAAGATTTGTA ATTC
W-43F	CTAAGGAAGCTGCTGCTAAGATGGCTAATACCTTCGCTAACGTTAAC
W-44R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAG
For cloning <i>cloA(1)-L2-t36CpCPR</i>	
W-45F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAACTAGAC
W-46R	TGGAGTTGGAGTTGGAGTTGGAGTTGGAGTCCATCTATCAGCCAAGATTTGTAATT C
W-47F	CAACTCCAACTCCAACTCCAATGGCTAATACCTTCGCTAACGTTAAC
W-48R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAG
For cloning <i>cloA(1)-L3-t36CpCPR</i>	
W-49F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAACTAGAC
W-50R	AGAACCACCACCAGTCCATCTATCAGCCAAGATTTGTAATTC
W-51F	GGACTGGTGGTGGTGGTTCTATGGCTAATACCTTCGCTAACGTTAAC
W-52R	agtaacttaaggagtaaataTTAGGACCAAACATCTTCTTGGTATTGGTTAG
For cloning <i>cloA(1)-L2-t36CpCPR_KKYL</i>	
W-53F	gtcaaggagaaaaactataATGTCTTTGCAATGGTTACAACAACTAGAC
W-54R	agtaacttaaggagtaaataTTACAAGTACTTCTTTGAACCACCACCGACCAAACATCTTC TTGGTATTGG

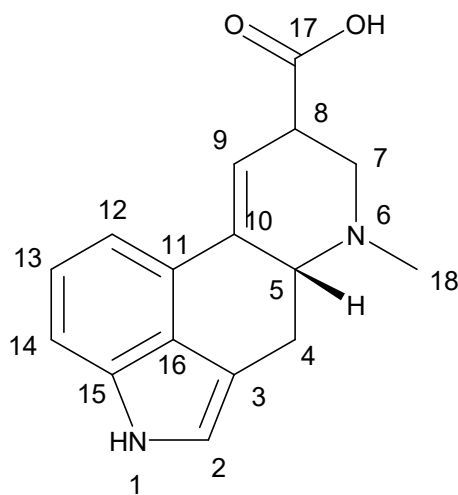
Sequences underlined and highlighted in bold locate signal peptides for ER and Peroxisomes

Table S4 gRNA target sequences used in this study.

Target	Sequence (5'-3')
<i>G418</i>	AGGCAGTTCATAGGATGGC
<i>YPRCτ3</i>	tattccatggcctcttagtt
<i>Int11</i>	gaacaagaacaacaaactcc
<i>Sch9</i>	TTGGTCACTGATTATATGAG
<i>Ras2</i>	AGGAAGCAGCAAGCTGCACC
<i>PEX5</i>	GCTCAACAACCGCATCAAGC
<i>NCP1</i>	cgtccatgtcacctccattg

Table S5 ^1H NMR data of compounds lysergic acid (LA).

Proton serial number	^1H NMR (δ , ppm)
14	7.87 (1H, br s)
12	7.68 (1H, d, $j=8.24$)
13	7.49 (1H, t, $j=7.69$)
2	7.36 (1H, s)
9	7.22 (1H, br s)
8	4.39 (1H, ov)
5	4.39 (1H, ov)
7	3.42-3.89 (2H, ov)
18	3.32 (3H, ov)
4	3.17-3.25 (2H, ov)



Numbered LA chemical structure

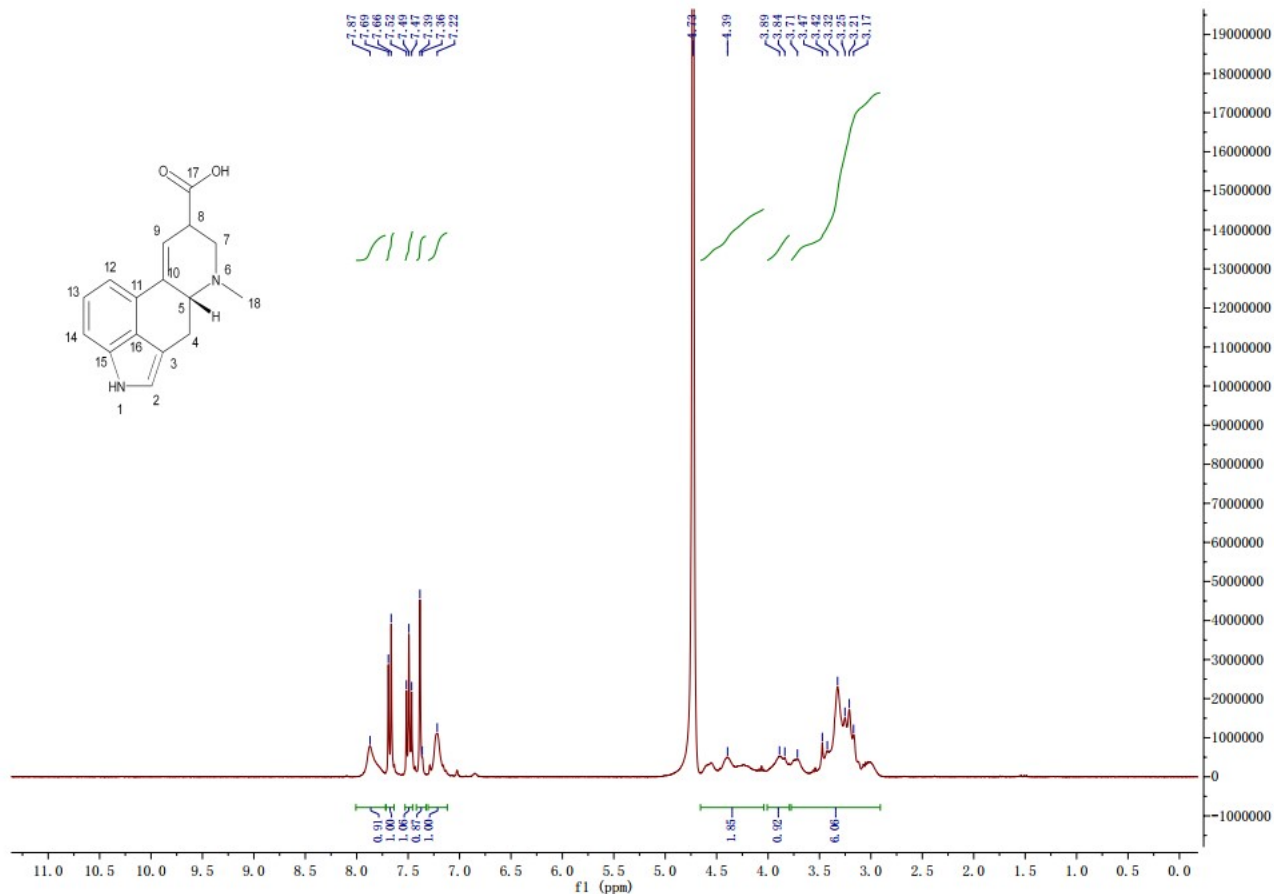


Figure S1 ¹H NMR spectrum of LA in D₂O (500 MHz).

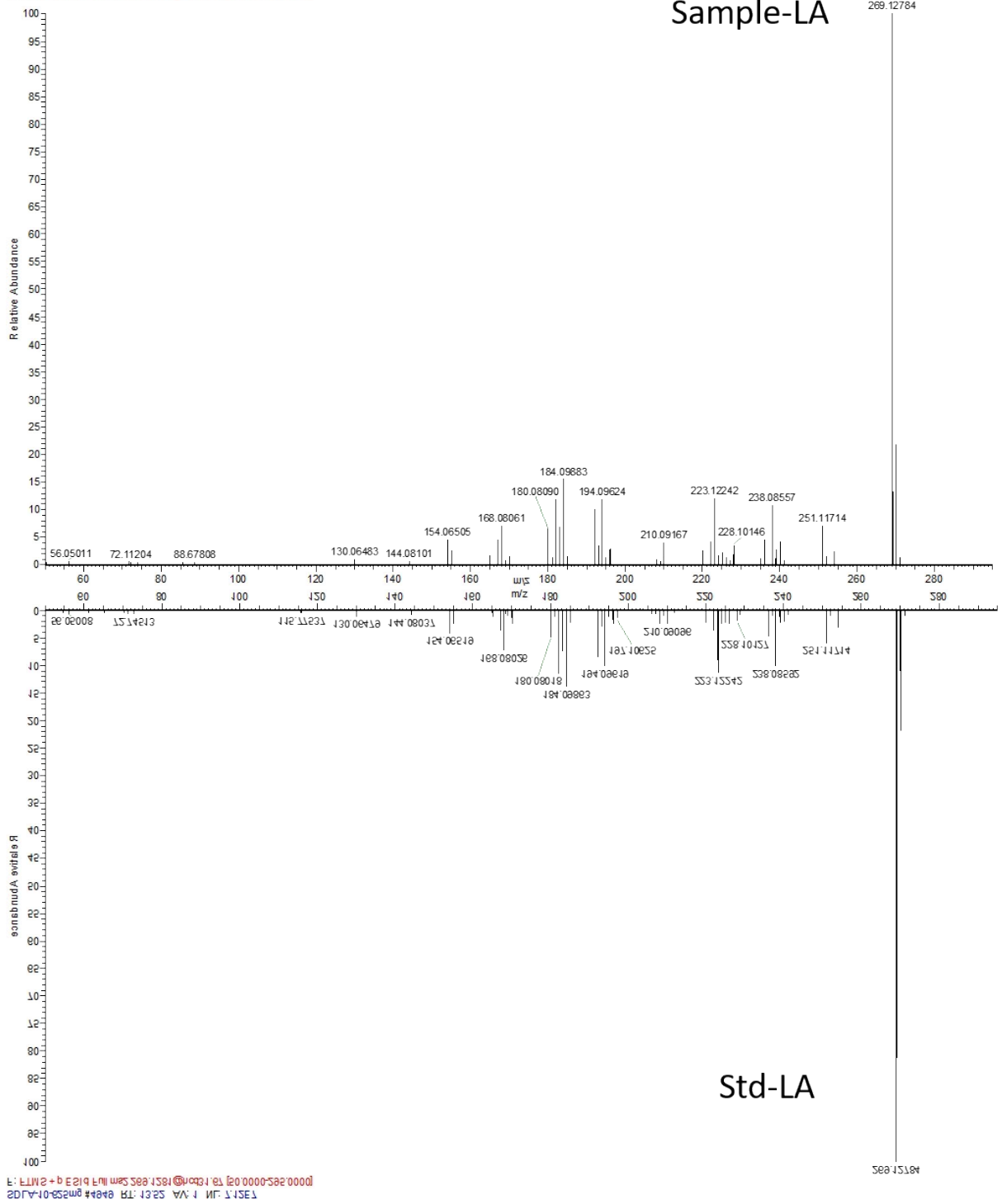


Figure S2 MS spectra of sample LA and standard LA.

cloA-1	MSLQWLQQTRHEL SWTWLILTTTCIA ISPLVLKGIYNYVF	40
cloA-2MAVHVVKSYSQLAGLTVVA..LFYFIFLVLS	30
cloA-3MKLHVVCSPCLAGVAVV..HIFGSLEAYV	30
Consensus	1 1	
cloA-1	HPLRNIPGPKLAALTD FYAFYWNWIRDE GYSKQFSRLHEQ	80
cloA-2	RPTSKLPGFWYSKWTDL.VERYQWFYGK..QTFYTHDLHK	67
cloA-3	QGAWEMVFRMDRRYVPK.TCRAKVSKE..DVWLLIDVAH	67
Consensus		
cloA-1	YNSPIIRIGENN WHTTQVEFYDVI FKSGSKWIKDRSFYKY	120
cloA-2	KYGPVIRVAENQAVADLET VKSVYTIKET YRKT.FYEL	106
cloA-3	.IGP VIRVAEDKVI VADLD AVKSIYTIKET YRKT.D.FYKL	105
Consensus	pi r p v k fy	
cloA-1	FNG.....LDAMIEFYQYRTY RTHL PLYAQRAIDGLAEK	155
cloA-2	LVS RPIQTVFSTADVEL FRKLRRLMASQMSSESSLKSMLEPQ	146
cloA-3	ITS RPIQTI FSTGDV DLFRKLR RELLASQMSSESSLKTMMEQ	145
Consensus	r r a p	
cloA-1	LRSDLTNSASGMMRQT RNGQTVNMA RVLRITLSTSMILHNL	195
cloA-2	VTSHVELAIQRMK EEKDRGVIDVFR WCLFMTT DV IGELS	186
cloA-3	IKSHVDLAIQRMK EEKARGVIDVFR WLSL FMTT DIIGELS	185
Consensus	s m k k t i	
cloA-1	ESLDISLNDGDEYHFFL DAFQ LMTQ SWL FV TYEMVPMVL	235
cloA-2	EGESFQMLEKGGKKTQY EDL EN VSSV SADRIT FFLFFHLA	226
cloA-3	EGESFQMLEKGGKNQY EDL DISSIAAYRV IFFFFWLA	225
Consensus	f e e t p	
cloA-1	SLIPGTSFARFNSS YTTFSNYCTAWN DEDMRKCRESEEQS	275
cloA-2	DKYD..KILPLFRSKIA INRRIVEYSR QSLGRYQKCVESD	264
cloA-3	DRYS..NILPLFKGIS ITRRLKEYSR QSIGRYQK QV EN	263
Consensus	e	
cloA-1	TRDSHTKRYLSLKDD DARKKTAI PYEDD VFN FVAGGSDT	315
cloA-2	ATSAKYTFVEKLFQAE KDKITFED ITNAQTFIIAGSDT	304
cloA-3	PANAKQTFVAKMFQAE KDKIAFGD ITNAQTLIVAGSDT	303
Consensus	k l gsdt	
cloA-1	TAYITACAFYHILSS ETVREN VVELDEHSSIRDEEDYN	355
cloA-2	TATILTYLIWSICKR PELRDALL KELR TL P...GFTEP	340
cloA-3	TASILTYLIWSVCKR EDLRDALL KELR TL P...GFTEA	339
Consensus	st a t p r l e l f	
cloA-1	KIQNLHYLN AVIRETLRIS V PV EGSLPRIVEGGITIGSF	395
cloA-2	DLRLELELN CTV BETLRVYSS PEASL PRAVEKGGAHVCGY	380
cloA-3	DLRLELELN RTI BETLRVYSS VEALL PRAVEKGGAHIGGY	379
Consensus	lp n eclr p lpr vp gg g	
cloA-1	SLPAGTGV SITQQAISFNEK IFLPHSFL ER WIC E KSVG	435
cloA-2	YLSEGT TVAACAYTMHR DP AI ENED VE FCRWANE.TRA	419
cloA-3	YLSEGT TVAACAYTMHR DP AI ENED VE FCRWVNE.TKA	418
Consensus	l gt v if p f p rw p	
cloA-1	LDKWNIAFSRGR QCIGITL AYLELR CVIA YFFSRFD.MA	474
cloA-2	MKEAFM FCRGR SRSMYKSGNV.....	440
cloA-3	MKEASM FCRGR VCIGIHLAYIELRLAAAR FL LEFPNAR	458
Consensus	f rg r	
cloA-1	LTGNCGDQLRWV DRFVAVN LDDELQILADRW	506
cloA-2	440
cloA-3	VSAKEGMSDR DM STVYFLMSPKGRCLIEE.	489
Consensus		

Figure S3 Comparative analysis results of different sequences alignment of *cloA* from *Claviceps purpurea* source.

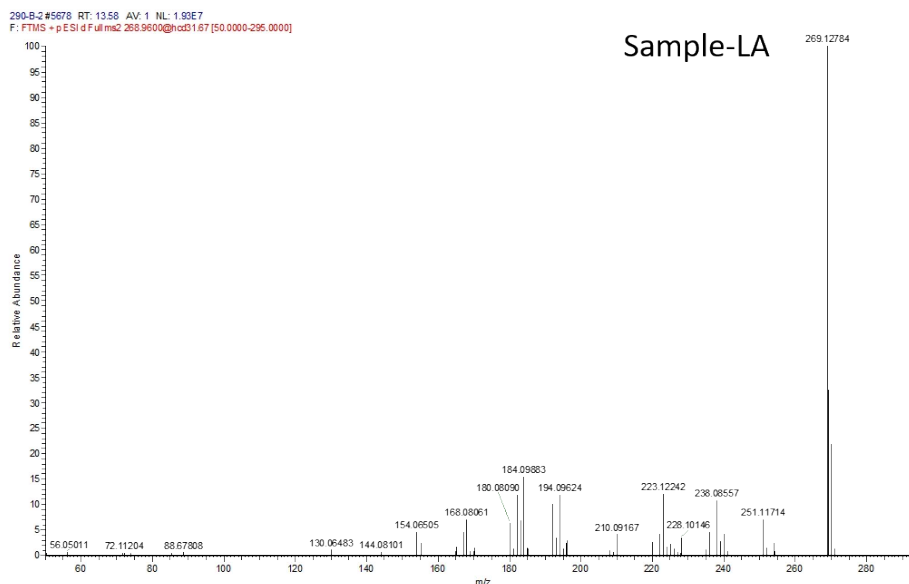
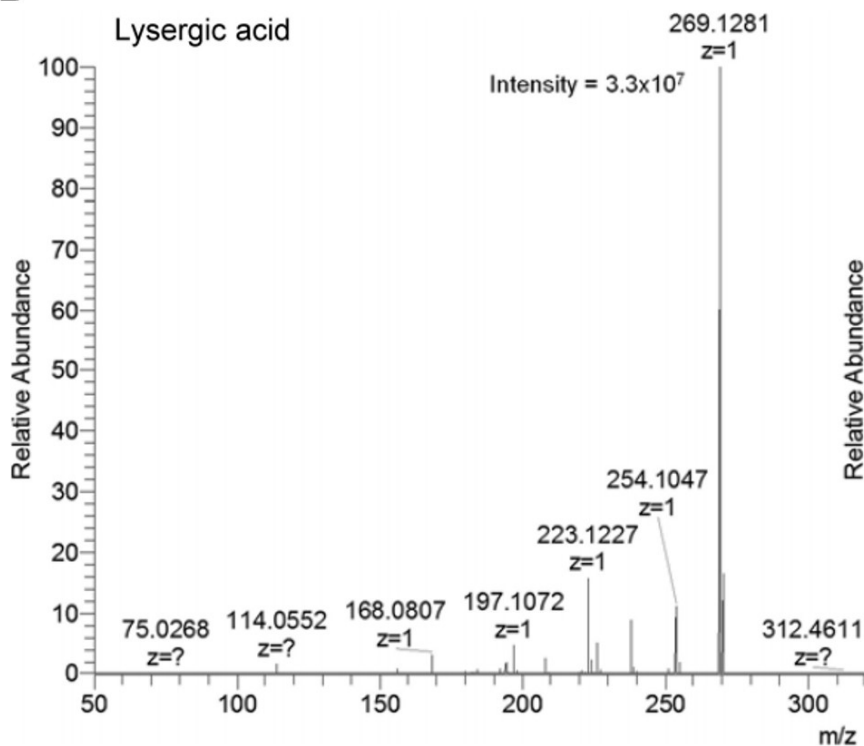
A**B**

Figure S4 The results of fermentation mass spectrometry of suspected LA were consistent with those of reported LA mass spectrometry. A. LA mass spectrometry results in this study. B. LA mass spectrometry results in the literature².

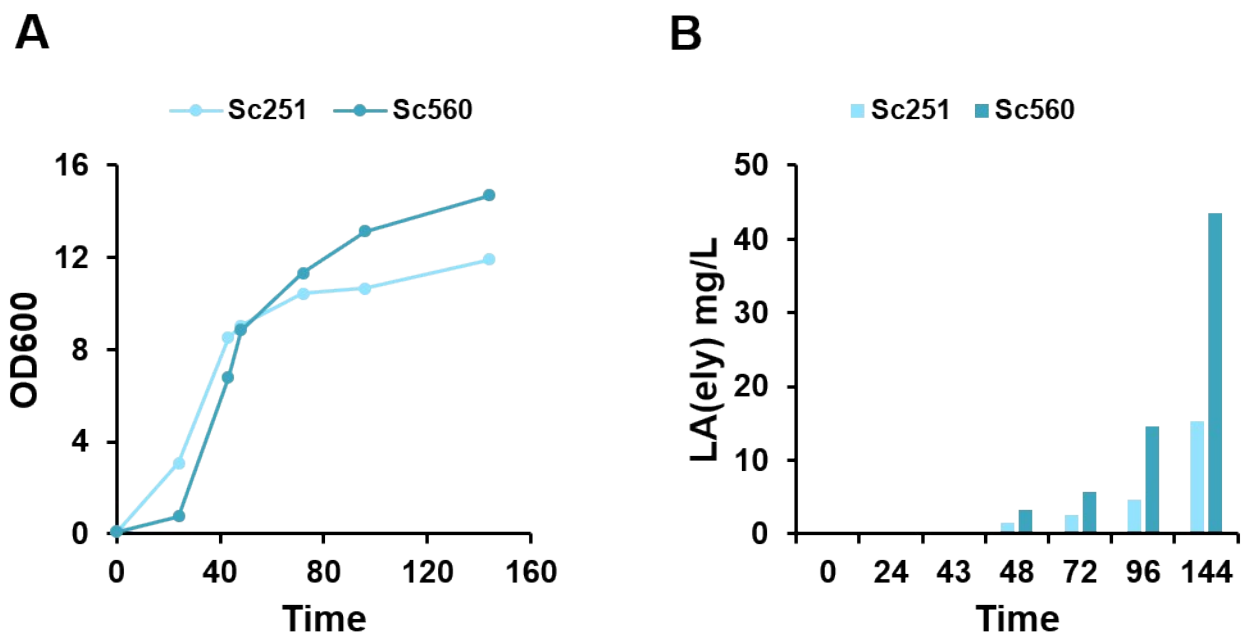


Figure S5 The results of growth and LA production in control strain(Sc251) and Δ sch9 strain(Sc560). A. The OD600 in strains Sc251 and Sc560. B. The titers of LA in strains Sc251 and Sc560.

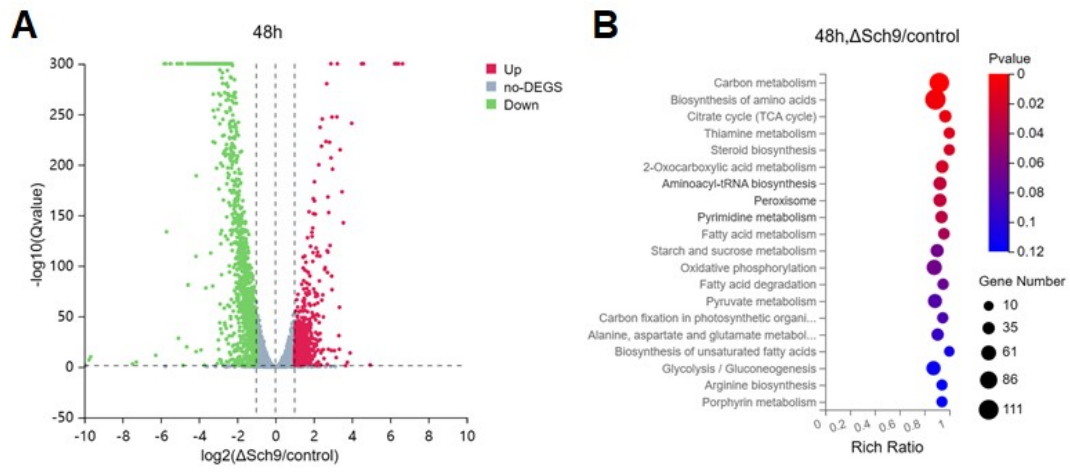


Figure S6 The effect of $\Delta sch9$ on LA production. A. Volcano map of differential genes at 48h between $\Delta sch9$ and control strains. B. KEGG pathway enrichment analysis of DEGs at 48h between $\Delta sch9$ and control strains.

Confidently predicted domains, repeats, motifs and features:

Name	Start ▲	End	E-value
transmembrane region	9	31	N/A

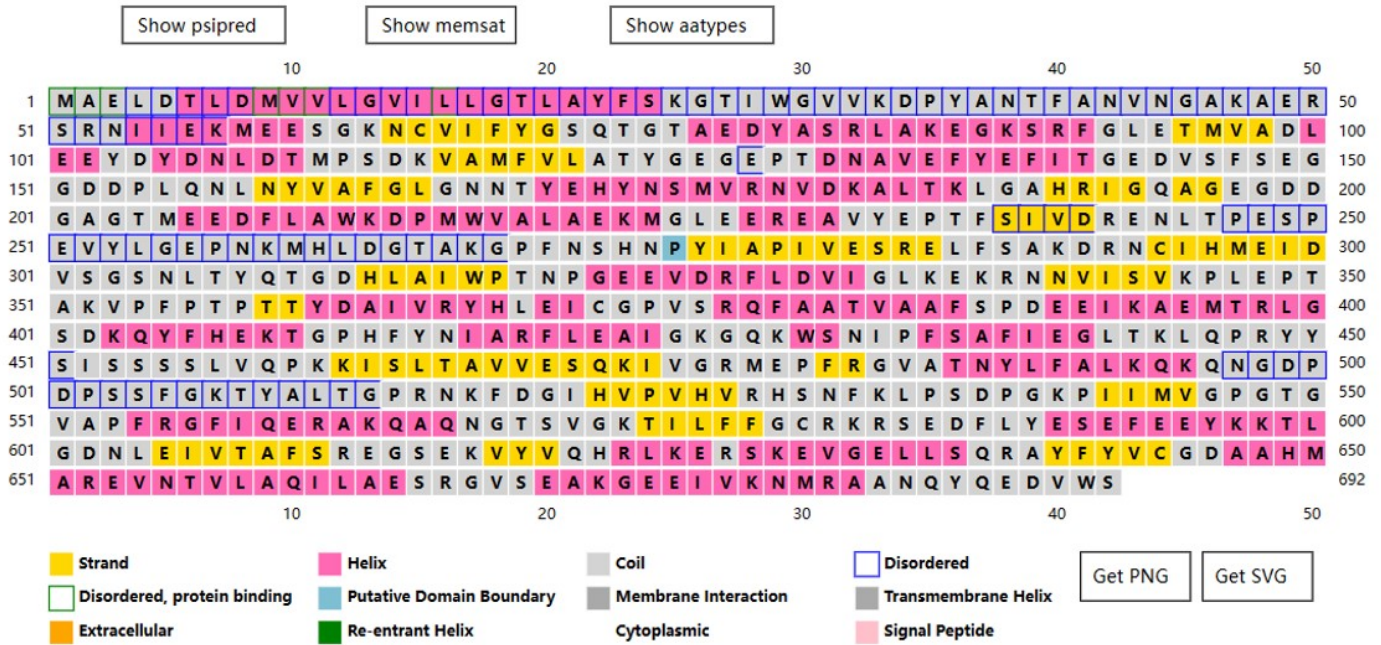


Figure S7 Results of CpCPR transmembrane domain analysis.

References

1. N. Wu, M. Yao, W. Xiao, T. Dong, H. Ma, X. Du, Y. Wang and Y. Yuan, *ACS Synth Biol*, 2023, **12**, 1133-1145.
2. D. Smith, L. Smith, W. Shafer, J. Klotz and J. Strickland, *J Agric Food Chem*, 2009, **57**, 7213-7220.