molecules

MDPI

Supplementary Materials

Characterization of a *Cis*-Prenyltransferase from *Lilium Longiflorum* Anther

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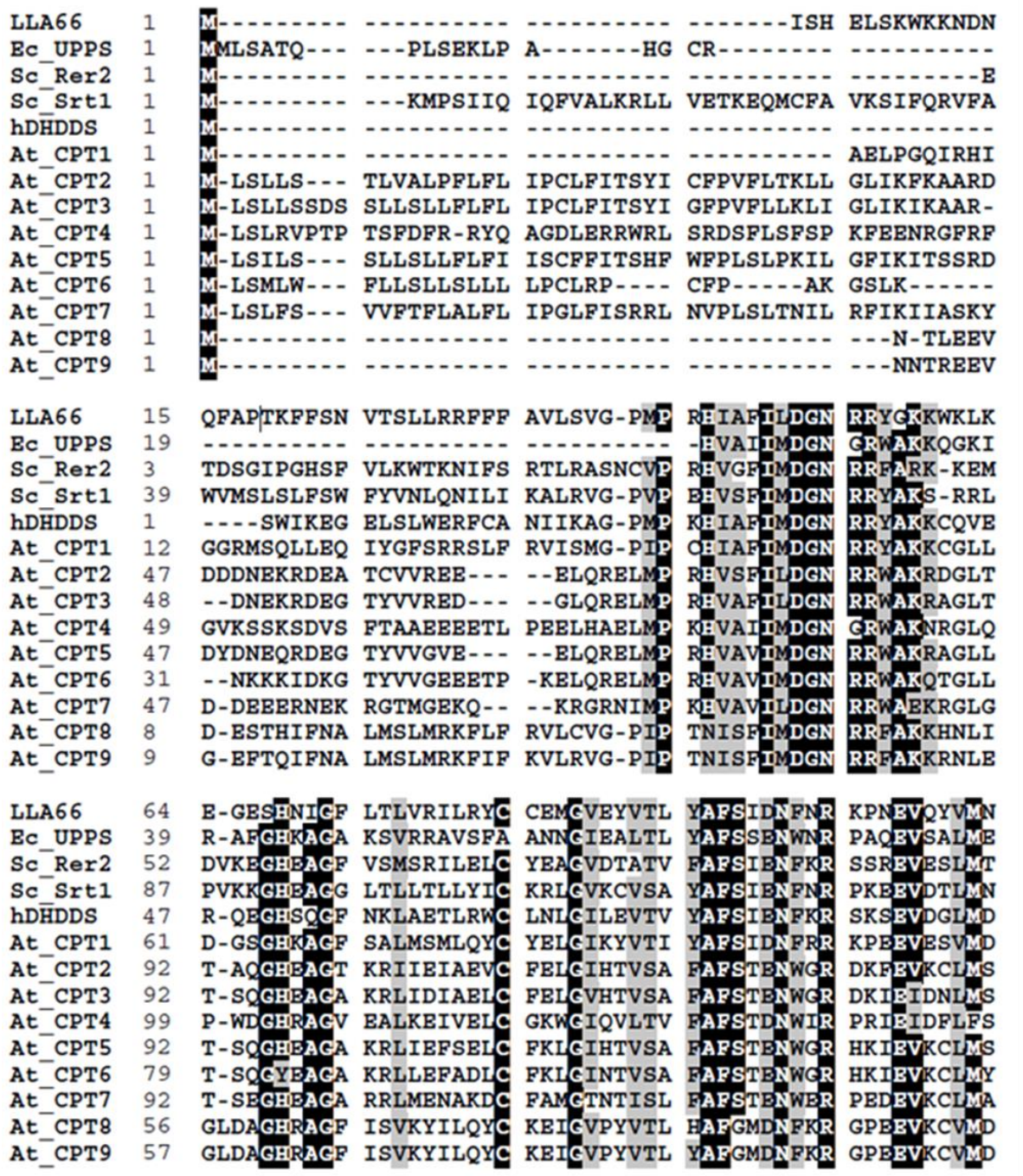

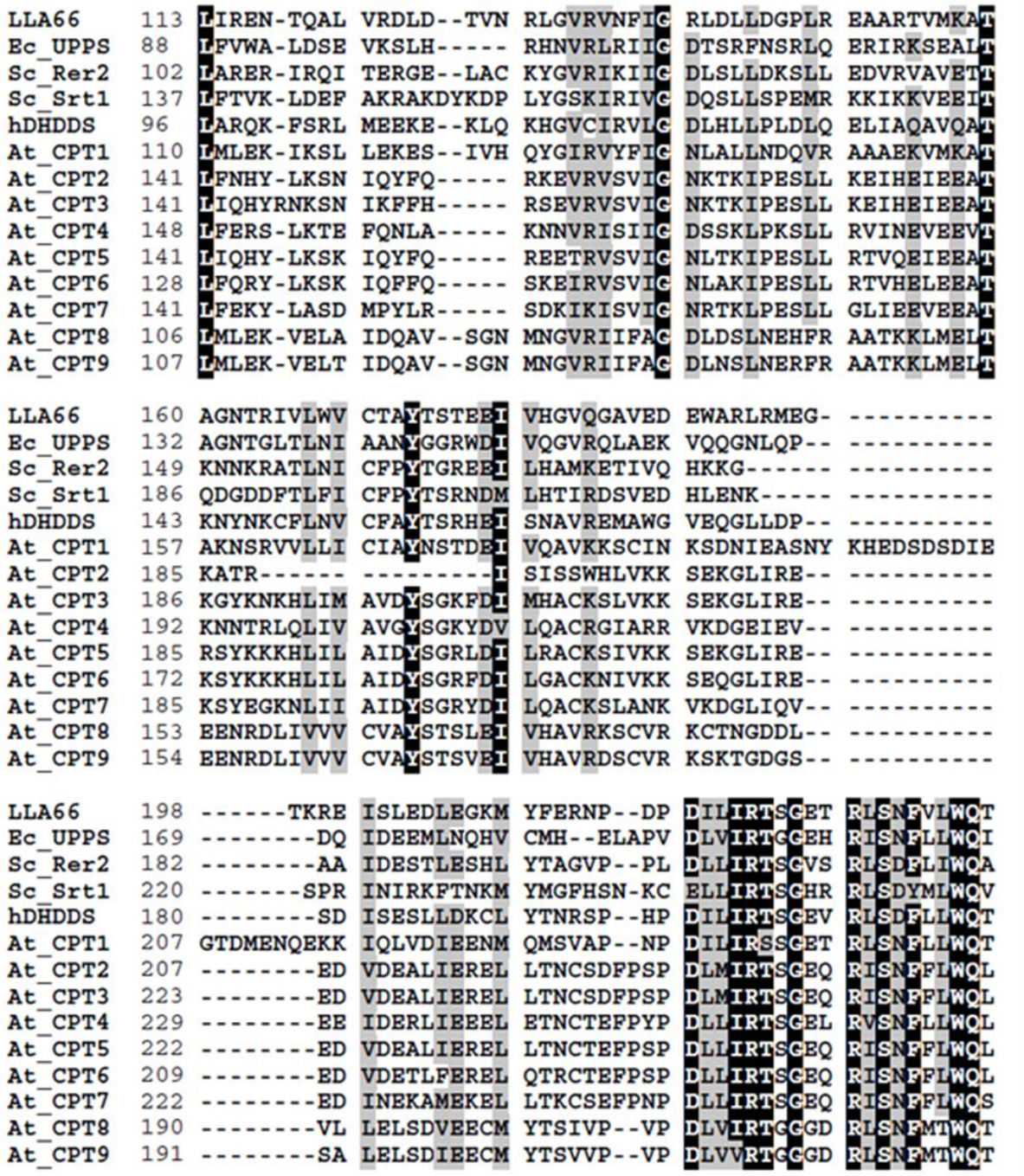
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Ec_UPPS 88 LFVWA-LDSE VKSLH----- RHNVRLRIIG DTSRFNSRLQ ERIRKSEALT
Sc_Rer2 102 LARER-IRQI TERGE--LAC KYGVRIKIIG DLSLLDKSLL EDVRVAVETT
Sc_Srt1 137 LFTVK-LDEF AKRAKDYKDP LYGSKIRIVG DQSLLSPEMR KKIKKVEEIT
hDHDDS 96 LARQK-FSRL MEEKE--KLQ KHGVCIRVLG DLHLLPLDLQ ELIAQAVQAT
At_CPT1 110 LMLEK-IKSL LEKES--IVH QYGIRVYFIG NLALLNDQVR AAAEKVMKAT
At_CPT2 141 LFNHY-LKSN IQYFQ----- RKEVRVSVIG NKTKIPESLL KEIHEIEEAT
At_CPT3 141 LIQHYRNKSN IKFFH----- RSEVRVSVIG NKTKIPESLL KEIHEIEEAT
At_CPT4 148 LFERS-LKTE FQNLA----- KNNVRISIIG DSSKLPKSLL RVINEVEEVT
At_CPT5 141 LIQHY-LKSK IQYFQ----- REETRVSVIG NLTKIPESLL RTVQEIEEAT
At_CPT6 128 LFQRY-LKSK IQFFQ----- SKEIRVSVIG NLAKIPESLL RTVHELEEAT
At_CPT7 141 LFEKY-LASD MPYLR----- SDKIKISVIG NRTKLPESLL GLIEEVEEAT
At_CPT8 106 LMLEK-VELA IDQAV--SGN MNGVRIIFAG DLDSLNEHFR AATKKLMELT
At_CPT9 107 LMLEK-VELT IDQAV--SGN MNGVRIIFAG DLNSLNERFR AATKKLMELT
LLA66 160 AGNTRIVLWV CTAYTSTEEI VHGVQGAVED EWARLRMEG- ----------
Ec_UPPS 132 AGNTGLTLNI AANYGGRWDI VQGVRQLAEK VQQGNLQP-- ----------
Sc_Rer2 149 KNNKRATLNI CFPYTGREEI LHAMKETIVQ HKKG------ ----------
Sc_Srt1 186 QDGDDFTLFI CFPYTSRNDM LHTIRDSVED HLENK----- ----------
hDHDDS 143 KNYNKCFLNV CFAYTSRHEI SNAVREMAWG VEQGLLDP-- ----------
At_CPT1 157 AKNSRVVLLI CIAYNSTDEI VQAVKKSCIN KSDNIEASNY KHEDSDSDIE
At_CPT2 185 KATR------ ---------I SISSWHLVKK SEKGLIRE-- ----------
At_CPT3 186 KGYKNKHLIM AVDYSGKFDI MHACKSLVKK SEKGLIRE-- ----------
At_CPT4 192 KNNTRLQLIV AVGYSGKYDV LQACRGIARR VKDGEIEV-- ----------
At_CPT5 185 RSYKKKHLIL AIDYSGRLDI LRACKSIVKK SEKGLIRE-- ----------
At_CPT6 172 KSYKKKHLIL AIDYSGRFDI LGACKNIVKK SEQGLIRE-- ----------
At_CPT7 185 KSYEGKNLII AIDYSGRYDI LQACKSLANK VKDGLIQV-- ----------
At_CPT8 153 EENRDLIVVV CVAYSTSLEI VHAVRKSCVR KCTNGDDL-- ----------
At_CPT9 154 EENRDLIVVV CVAYSTSVEI VHAVRDSCVR KSKTGDGS-- ----------
LLA66 198 ------TKRE ISLEDLEGKM YFERNP--DP DILIRTSGET RLSNFVLWQT
Ec_UPPS 169 --------DQ IDEEMLNQHV CMH--ELAPV DLVIRTGGEH RISNFLLWQI
Sc_Rer2 182 --------AA IDESTLESHL YTAGVP--PL DLLIRTSGVS RLSDFLIWQA
Sc_Srt1 220 -------SPR INIRKFTNKM YMGFHSN-KC ELLIRTSGHR RLSDYMLWQV
hDHDDS 180 --------SD ISESLLDKCL YTNRSP--HP DILIRTSGEV RLSDFLLWQT
At_CPT1 207 GTDMENQEKK IQLVDIEENM QMSVAP--NP DILIRSSGET RLSNFLLWQT
At_CPT2 207 --------ED VDEALIEREL LTNCSDFPSP DLMIRTSGEQ RISNFFLWQL
At_CPT3 223 --------ED VDEALIEREL LTNCSDFPSP DLMIRTSGEQ RISNFFLWQL
At_CPT4 229 --------EE IDERLIEEEL ETNCTEFPYP DLLIRTSGEL RVSNFLLWQL
At_CPT5 222 --------ED VDEALIEREL LTNCTEFPSP DLLIRTSGEQ RISNFFLWQL
At_CPT6 209 --------ED VDETLFEREL QTRCTEFPSP DLLIRTSGEQ RISNFFLWQL
At_CPT7 222 --------ED INEKAMEKEL LTKCSEFPNP DLLIRTSGEQ RISNFFLWQS
At_CPT8 190 --------VL LELSDVEECM YTSIVP--VP DLVIRTGGGD RLSNFMTWQT
At_CPT9 191 --------SA LELSDIEECM YTSVVP--VP DLVVRTGGGD RLSNFMTWQT

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LLA66     241 SFC--LLYAP RCLWPDLSLR HLVWAVLLYQ RSYAYLEKAK KYKLEVNGQG
Ec_UPPS   210 AYA--ELYFT DVLWPDFDEQ DFEGALNAFA NRERRFGGTE PGDETA----
Sc_Rer2   223 SSKGVRIELL DCLWPEFGPI RMAWILLKFS FHKSFLNKEY RLEEGDYDEE
Sc_Srt1   263 HEN-ATIEFS DTLWPNFSFF AMYLMILKWS FFS-TIQKYN EKNHSLFEKI
hDHDDS    221 SHS--CLVFQ PVLWPEYTFW NLFEAILQFQ MNHSVLQKAR DMYAEERKRQ
At_CPT1   255 GNT--QLCSP AALWPEIGLR HLLWAILNFQ RNHSYLEKRK KQL-------
At_CPT2   250 AYT--ELFYS PVLWPDFDKD KLLEALASYQ GRERRFGCRV ----------
At_CPT3   266 AYS--ELFFS PVEWPDFDKD KLLEALASYQ RRERRFGCRV ----------
At_CPT4   272 AYT--ELFFA QELWPDFGRS GFIEALMSFQ QRQRRFGGRK S---------
At_CPT5   265 AYT--ELFFS PVLWPDFDKD KLLEALVSYQ RRERRFGCRV ----------
At_CPT6   252 AYT--EFFFS PVLWPDFDKQ KFIEALVSYQ RRDRRFGSRL ----------
At_CPT7   265 AYT--ELYFP TVLWPDFGEA EYLEALTWYQ QRQRRFGRRV ----------
At_CPT8   231 SRS--LLHRT EALWPELGLW HLVWAILKFQ RMQDYLTKKK KLD-------
At_CPT9   232 SRA--LLHRT EALWPELGLW HLVWAILKFQ RMQDYLQKKK KLH-------

LLA66     289 RSLTPECMAA FTAASSYIQF ---------- ---------- ----------
Ec_UPPS   253 ---------- ---------- ---------- ---------- ----------
Sc_Rer2   273 TNGDPIDLKE KKLN------ ---------- ---------- ----------
Sc_Srt1   311 HESVPSIFKK KKTAMSLYNF PNPPISVSVT GDE------- ----------
hDHDDS    269 QLERDQATVT EQLLREGLQA SGDAQLRRTR LHKLSARREE RVQGFLQALE
At_CPT1   295 ---------- ---------- ---------- ---------- ----------
At_CPT2   287 ---------- ---------- ---------- ---------- ----------
At_CPT3   303 ---------- ---------- ---------- ---------- ----------
At_CPT4   310 ---------- ---------- ---------- ---------- ----------
At_CPT5   302 ---------- ---------- ---------- ---------- ----------
At_CPT6   289 ---------- ---------- ---------- ---------- ----------
At_CPT7   302 ---------- ---------- ---------- ---------- ----------
At_CPT8   271 ---------- ---------- ---------- ---------- ----------
At_CPT9   272 ---------- ---------- ---------- ---------- ----------

LLA66     308 ---------- -----
Ec_UPPS   253 ---------- -----
Sc_Rer2   286 ---------- -----
Sc_Srt1   343 ---------- -----
hDHDDS    319 LKRADWLARL GTASA
At_CPT1   295 ---------- -----
At_CPT2   287 ---------- -----
At_CPT3   303 ---------- -----
At_CPT4   310 ---------- -----
At_CPT5   302 ---------- -----
At_CPT6   289 ---------- -----
At_CPT7   302 ---------- -----
At_CPT8   271 ---------- -----
At_CPT9   272 ---------- -----
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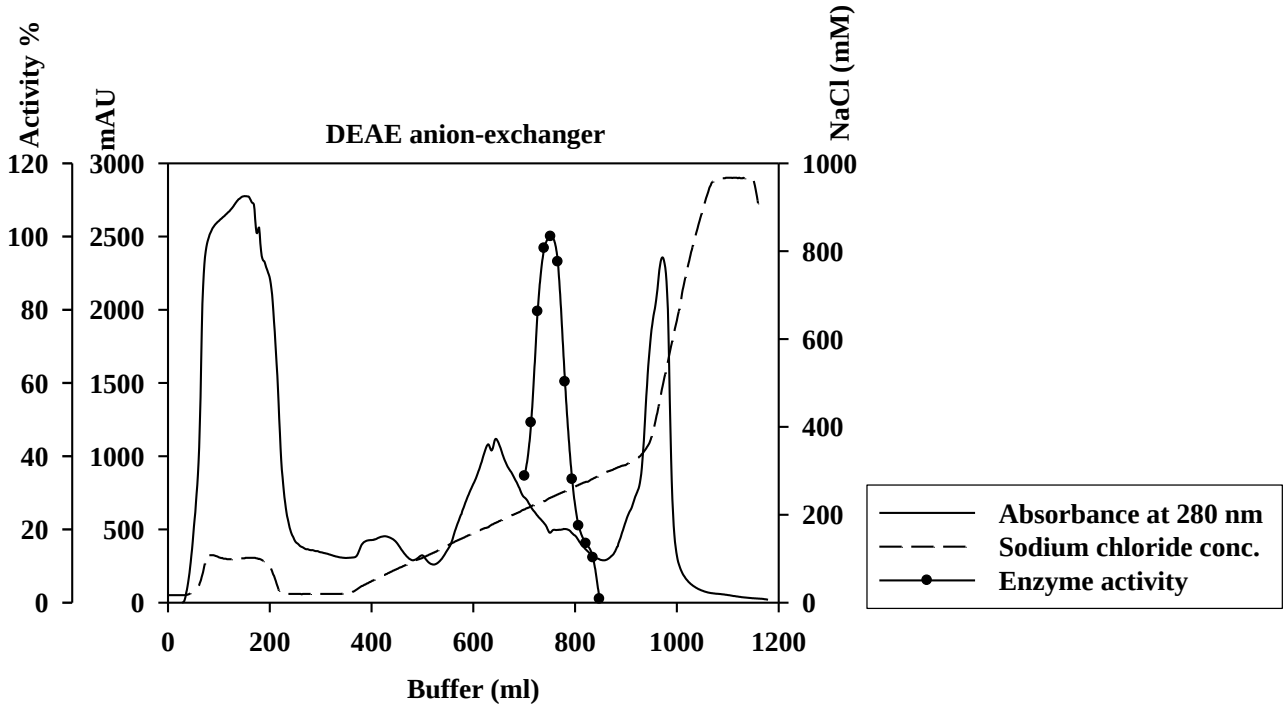
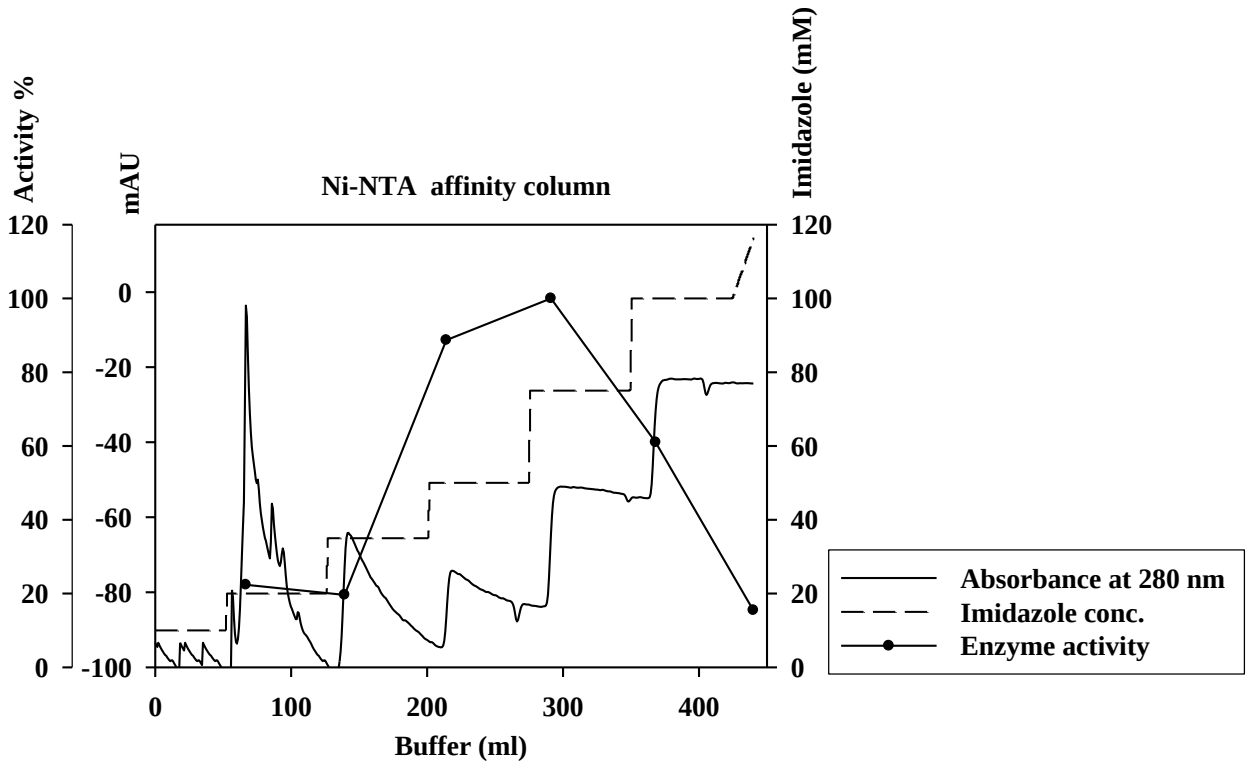
Figure S1. Sequence homology of LLA66 with other *cis*-prenyltransferases, including UPPS from *E. coli*, Rer2 and Srt1 from yeast, DHDDS from human, and 9 *cis*-prenyltransferases from *A. thaliana*.

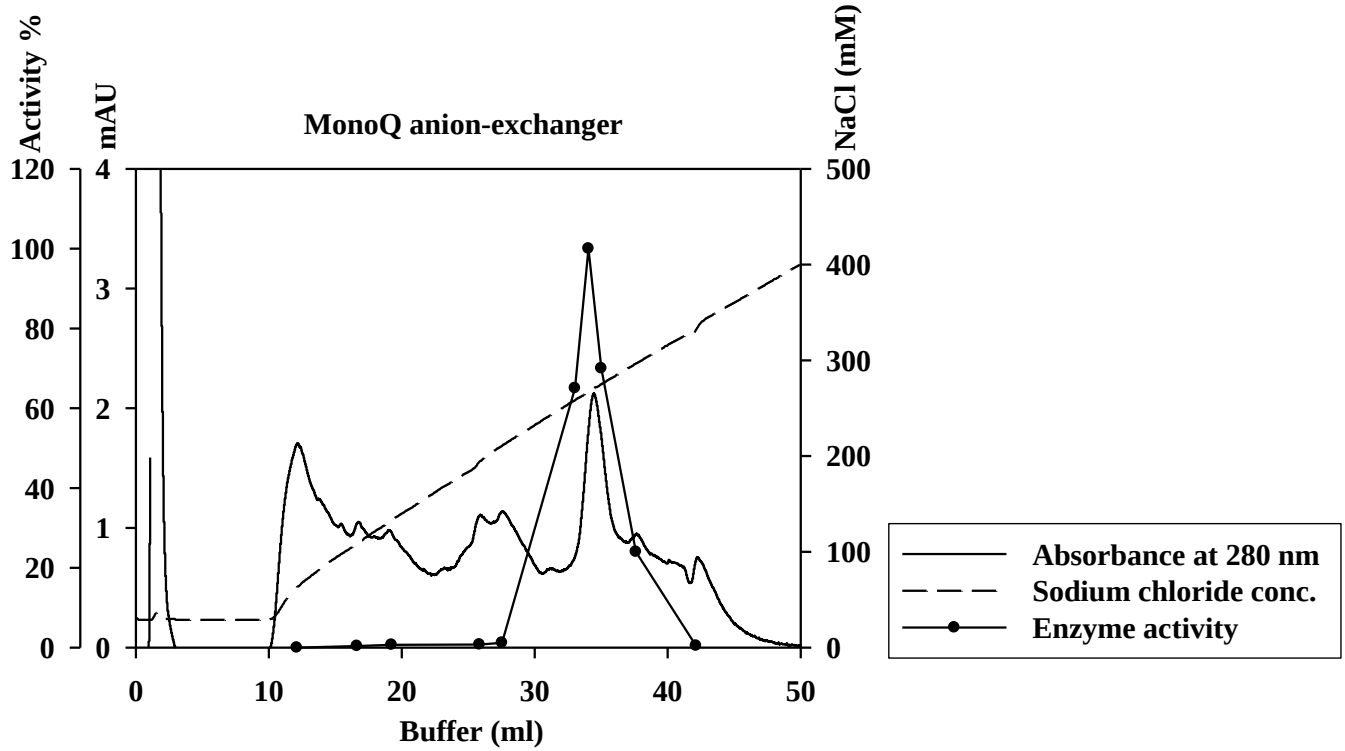
(a)


DEAE anion-exchanger
Activity %
mAU
NaCl (mM)
120
100
80
60
40
20
0
3000
2500
2000
1500
1000
500
0
1000
800
600
400
200
0
0
200
400
600
800
1000
1200
Buffer (ml)
Absorbance at 280 nm
Sodium chloride conc.
Enzyme activity

(b)


Ni-NTA affinity column
Activity %
mAU
Imidazole (mM)
120
100
80
60
40
20
0
0
-20
-40
-60
-80
-100
120
100
80
60
40
20
0
0
100
200
300
400
Buffer (ml)
Absorbance at 280 nm
Imidazole conc.
Enzyme activity

(c)


MonoQ anion-exchanger
Activity %
mAU
NaCl (mM)
120
100
80
60
40
20
0
4
3
2
1
0
500
400
300
200
100
0
0
10
20
30
40
50
Buffer (ml)
Absorbance at 280 nm
Sodium chloride conc.
Enzyme activity

(d)

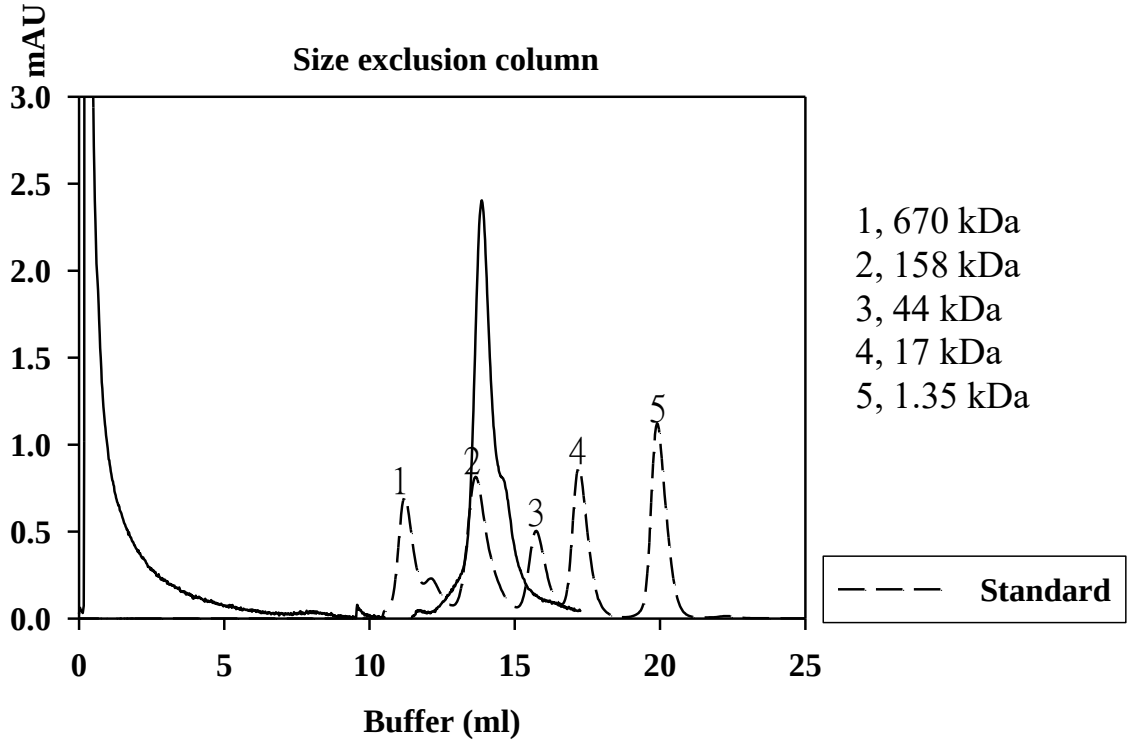

Size exclusion column
mAU
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Buffer (ml)
1
2
3
4
5
1, 670 kDa
2, 158 kDa
3, 44 kDa
4, 17 kDa
5, 1.35 kDa
Standard

Figure S2. Purification of the *S. cerevisiae* enzyme complex that produced C15 farnesol from [^{14}C]IPP alone. *S. cerevisiae* cell lysate was loaded onto a DEAE anion exchanger and the IPP-utilizing activity appeared between 200 to 300 mM NaCl eluting fractions (a). These fractions were collected and loaded onto Ni-NTA column and the activity was found from the 100 mM imidazole elution (b). The active protein complex was further purified with anion exchanger Mono-Q (c), and size exclusion chromatography (d).

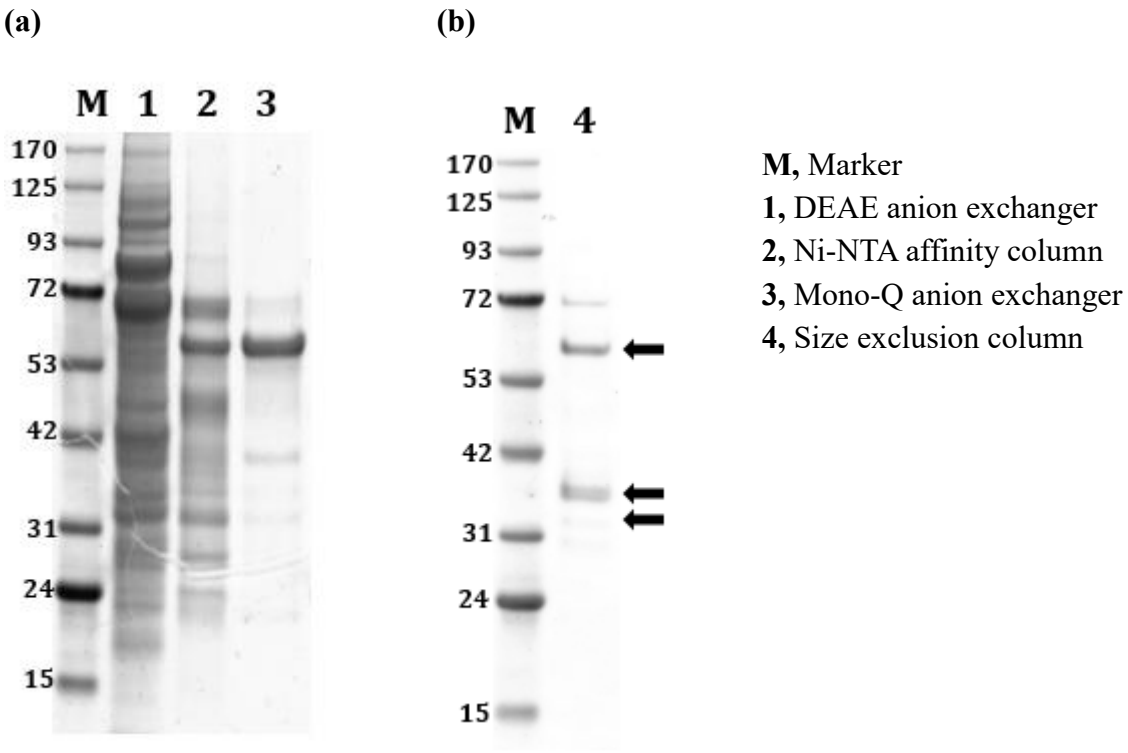


Figure S3. SDS-PAGE analysis of the *S. cerevisiae* enzyme complex from a series of purification steps. (a) and (b) show SDS-PAGE analysis of the proteins after the purification steps. Lane M, 1, 2, 3, and 4 indicate MW markers, after DEAE anion exchange column, Ni-NTA column, Mono-Q column, and size column, respectively. The arrows indicate the proteins taken for LC-MS/MS analysis.

(a)

1 MMTHTLPSEQ TRLVPGSDSS SRPKKRRISK RSKIIVSTVV CIGLLLVLVQ
51 LAFPSSFALR SASHKKK**NVI FFVTDGMGPA SLSMARSFNQ HVNDLPIDDI**
101 **LTLDEHFIGS SR**TR**SSDSLV TDSAAGATAF ACALKSYNGA IGVDPHHRPC**
151 **GTVLEAAKLA GYLTGLVVTT RITDATPASF SSHVDYRWQE DLIATHQLGE**
201 **YPLGRVVDLL MGGGRSHFYP QGEK**ASPYGH HGARK**DGRDL IDEAQSNGWQ**
251 **YVGDRKNFDS LLK**SHGENVT LPFLGLFADN DIPFEIDRDE K**EYPSLK**EQV
301 K**VALGALEK**A SNEDK**DSNGF FLMVEGSRID HAGHQNDPAS QVR**EVLAFDE
351 AFQYVLEFAE NSDTETVLVS TSDHETGGLV TSRQVTASYP QYVWYPQVLA
401 NATHSGEFLK R**KLVDFVHEH K**GASSK**IENF IKHEILEKDL GIYDYTDSDL**
451 **ETLIHLDDNA NAIQDKLNDM VSFRAQIGWT THGHSAVDVN IYAYANKK**AT
501 WSYVLNNLQG NHENTEVGQF LENFLELNLN EVTDLIRDTK HTSDFDATEI
551 ASEVQHYDEY YHELTN

(b)

1 MASEKEIRRE R**FLNVFPKLV EELNASLLAY GMPK**EACDWY AHSLNYNTPG
51 GK**LNRGLSVV DTYAILSNKT VEQLGQEEYE K**VAILGWCIE LLQAYFLVAD
101 DMMDKSITRR GQPCWYKVPE VGEIAINDAF MLEAAIYKLL KSHFRNEKYY
151 IDITELFHEV TFQTELGQLM DLITAPEDKV DLSKFSLKK**H SFIVTFK**TAY
201 YSFYLPVALA MYVAGITDEK DLKQARDVLI PLGEYFQIQD DYLDCFGTPE
251 QIGK**IGTDIQ DNK**CSWVINK **ALELASAEQR** K**TLDENYGK**K DSVAEAKCKK
301 IFNDLK**IEQL YHEYEESIAK** DLKAK**ISQVD ESRGFKADVL TAFLNK**VYKR
351 SK

(c)

1 **MTADNNSMPH GAVSSYAKLV QNQTPEDILE EFPEIIPLQQ R**PNTRSSETS
51 NDESGETCFS GHDEEQIKLM NENCIVLDWD DNAIGAGTKK **VCHLMENIEK**
101 GLLHRAFSVF IFNEQGELLL QQRATEKITF PDLWTNTCCS HPLCIDDELG
151 LKGKLDDKIK GAITAAVR**KL DHELGIPEDE TK**TRGK**FHFL NRIHYMAPSN**
201 **EPWGEHEIDY ILFYK**INAK**E NLTVNPNVNE VR**DFKWVSPN DLK**TMFADPS**
251 **YK**FTPWFKII CENYLFNWWE QLDDLSEVEN DRQIHRML

Figure S4. Identification of the proteins from LC-MS/MS analysis. After the protease digestions, the peptides detected (shown in red) represented alkaline phosphatase (a), FPPS (b), and IPP:DMAPP isomerase (c).

Table S1. Proteins identified by LC-MS/MS analysis from the protease digested SDS-PAGE bands

Bands on Gel	Protein hits	MW	Score/peptides/coverage
~60 kDa band	alkaline phosphatase	62965	3031/122/54%
~40 kDa band	Farnesyl pyrophosphate synthase	40458	1753/56/34%
~30 kDa band	IPP:DMAPP isomerase	33330	579/39/41%