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Method for Obtaining Antifungal and Herbicidal Compounds that Target the First Committed Step in Shingolipid Long-Chain Base Biosynthesis

Robert C. Dickson
University of Kentucky, bobd@uky.edu

Robert L. Lester
University of Kentucky, rlester@email.uky.edu

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United States Patent [19]

Dickson et al.

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[45] **Date of Patent: Dec. 10, 1996**

[54] **METHOD FOR OBTAINING ANTIFUNGAL AND HERBICIDAL COMPOUNDS THAT TARGET THE FIRST COMMITTED STEP IN SHINGOLIPID LONG-CHAIN BASE BIOSYNTHESIS**

[75] Inventors: **Robert C. Dickson**; **Robert L. Lester**, both of Lexington, Ky.

[73] Assignee: **The University of Kentucky Research Foundation**, Lexington, Ky.

[21] Appl. No.: **365,981**

[22] Filed: **Dec. 28, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 906,899, Jun. 30, 1992, abandoned.

[51] **Int. Cl.**[6] **C12N 1/15**; C12N 1/19; C12N 1/21; C12N 5/10; C12N 15/54; C12N 15/63

[52] **U.S. Cl.** **435/240.1**; 435/252.3; 435/254.11; 435/320.1; 435/172.3; 435/193; 536/23.2

[58] **Field of Search** 536/23.2, 23.74; 435/240.1, 252.3, 254.11, 256, 320.1, 193

[56] **References Cited**

PUBLICATIONS

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Pinto et al., (1986) Federation Proceedings 45: 1826.

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Primary Examiner—Robert A. Wax

Assistant Examiner—Gabriele E. Bugaisky

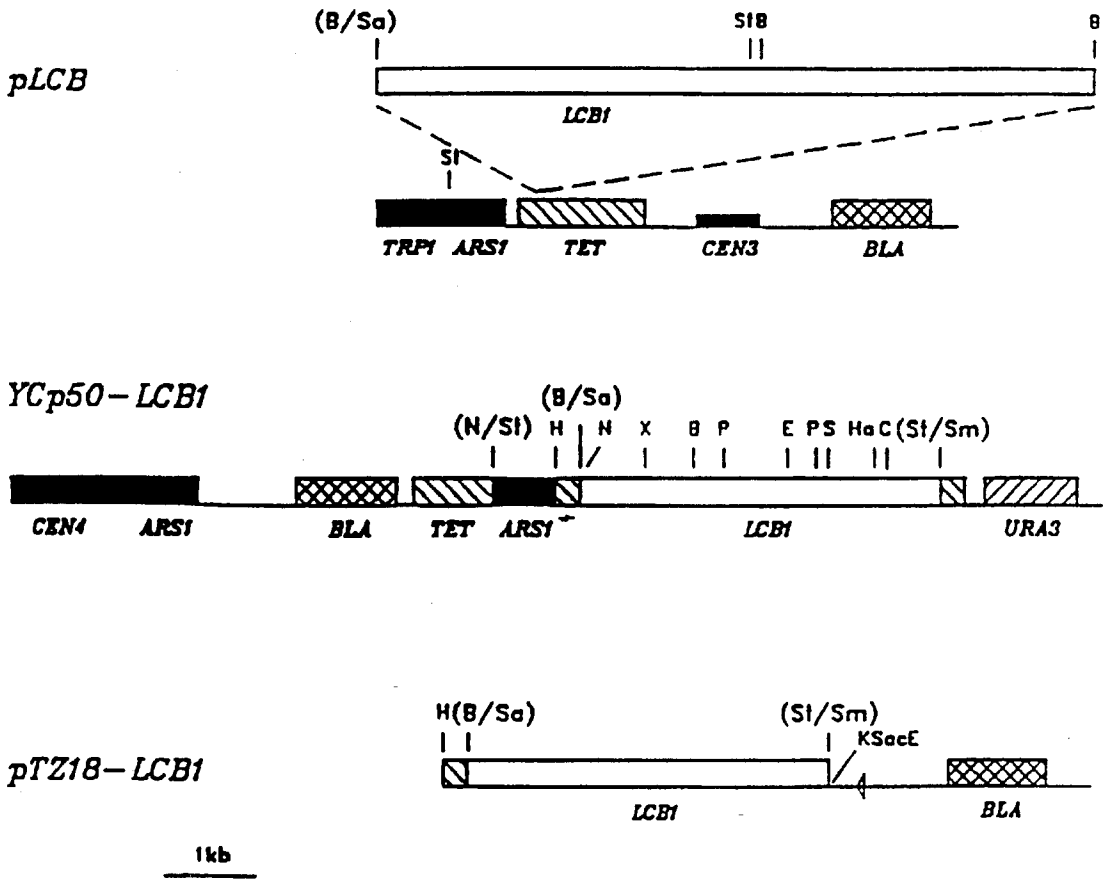
Attorney, Agent, or Firm—Lowe, Price, LeBlanc & Becker

[57] **ABSTRACT**

The invention provides the LCB1 and LCB2 genes of the yeast *Saccharomyces cerevisiae* that encode subunits of the enzyme serine palmitoyltransferase (SPT), the first enzyme leading to synthesis of the long-base component of the sphingolipids. The present specification describes the isolation of the LCB1 and LCB2 genes. The invention further relates to methods of using these genes to either inhibit SPT activity or to inhibit synthesis of the enzyme. Furthermore, the invention relates to methods for constructing strains of *S. cerevisiae* or other organisms that can be used to select and to test for compounds that either inhibit SPT activity or to inhibit synthesis of the enzyme.

12 Claims, 11 Drawing Sheets

FIGURE 1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SIB
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TRP1
ARS1
TET
CEN3
BLA
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CEN4
ARS1
BLA
TET
ARS1
LCB1
URA3
pTZ18-LCB1
H(B/Sa)
(SI/Sm)
KSacE
LCB1
BLA
1kb

FIGURE 2A

```
CGC GTA TTT TTT TTT TTT TGA GGC GCC ATG ATT TCT TAC ACG GTT TCT TTT TTT TTT
                -319                -304                -289
CCT TCT TTC CTT CTT GCT TCT CTG CTA ACA AAT TTT TCA CTC ATT CTT TTT TAT AGG
-274                -259                -244                -229
GGC ATA TTG CTG CGG TTA ACT GTA GTG AAC GAA AGT AAG ATT GAG AAA ATA TAG TAC
   -214                -199                -184                -169
TTA AGA AAA AGA AAA GGA AAA ATA AAA AAA ATT CTT TTC AAC ATC ATC GAG TAG CAC
       -154                -139                -124                -109
AGT ATA AGA GCG CTC TAA CCT TCT GCC TGG CCT CCA ATA TAC ACA TTT TGC TCG TGT
            -94                 -79                 -64                 -49
                                * *                                 M   A   H
AGG GTT ATT TAT CCT TTT TTC TTC CTT CCC ACC CAA AAA AAA AAA GCA ATG GCA CAC
                -34                 -19                 -4  +1
 I   P   E   V   L   P   K   S   I   P   I   P   A   F   I   V   T   T   S
ATC CCA GAG GTT TTA CCC AAA TCA ATA CCG ATT CCG GCA TTT ATT GTT ACC ACC TCA
12                  27                  42                  57
 S   Y   L   W   Y   Y   F   N   L   V   L   T   Q   I   P   G   G   Q   F
TCG TAC CTA TGG TAC TAC TTC AAT CTG GTG TTG ACT CAA ATC CCG GGA GGC CAA TTC
    72                  87                  102                 117
 I   V   S   Y   I   K   K   S   H   H   D   D   P   Y   R   T   T   V   E
ATC GTT TCG TAC ATC AAG AAA TCG CAT CAT GAC GAT CCA TAC AGG ACC ACG GTT GAG
        132                 147                 162                 177
 I   G   L   I   L   Y   G   I   I   Y   Y   L   S   K   P   Q   Q   K   K
ATA GGG CTT ATT TTA TAC GGG ATC ATC TAT TAC TTG TCC AAG CCA CAA CAG AAA AAG
            192                 207                 222                 237
 S   L   Q   A   Q   K   P   N   L   S   P   Q   E   I   D   A   L   I   E
AGT CTT CAA GCA CAG AAG CCC AAC CTA TCG CCC CAG GAG ATT GAC GCG CTA ATT GAG
                252                 267                 282
 D   W   E   P   E   P   L   V   D   P   S   A   T   D   E   Q   S   W   R
GAC TGG GAG CCC GAG CCT CTA GTC GAC CCT TCT GCC ACC GAT GAG CAA TCG TGG AGG
297                 312                 327                 342
 V   A   K   T   P   V   T   M   E   M   P   I   Q   N   H   I   T   I   T
GTG GCC AAA ACA CCC GTC ACC ATG GAA ATG CCC ATT CAG AAC CAT ATT ACT ATC ACC
    357                 372                 387                 402
 R   N   N   L   Q   E   K   Y   T   N   V   F   N   L   A   S   N   N   F
AGA AAC AAC CTG CAG GAG AAG TAT ACC AAT GTT TTC AAT TTG GCC TCG AAC AAC TTT
        417                 432                 447                 462
 L   Q   L   S   A   T   E   P   V   K   E   V   V   K   T   T   I   K   N
TTG CAA TTG TCC GCT ACG GAG CCC GTG AAA GAA GTG GTC AAG ACC ACT ATC AAG AAT
            477                 492                 507                 522
 Y   G   V   G   A   C   G   P   A   G   F   Y   G   N   Q   D   V   H   Y
TAC GGT GTG GGC GCC TGT GGT CCC GCC GGG TTC TAC GGT AAC CAG GAC GTT CAT TAC
                537                 552                 567
 T   L   E   Y   D   L   A   Q   F   F   G   T   Q   G   S   V   L   Y   G
ACG TTG GAA TAT GAT TTA GCA CAG TTC TTT GGC ACC CAA GGT TCC GTT CTG TAC GGG
582                 597                 612                 627
 Q   D   F   C   A   A   P   S   V   L   P   A   F   T   K   R   G   D   V
CAA GAC TTT TGT GCC GCA CCC TCT GTT CTG CCT GCT TTC ACA AAG CGT GGT GAT GTT
    642                 657                 672                 687
 I   V   A   D   D   Q   V   S   L   P   V   Q   N   A   L   Q   L   S   R
ATC GTG GCA GAC GAC CAG GTG TCA TTA CCA GTG CAA AAT GCT CTG CAA CTA AGC AGA
        702                 717                 732                 747
 S   T   V   Y   Y   F   N   H   N   D   M   N   S   L   E   C   L   L   N
TCC ACA GTC TAC TAC TTC AAC CAC AAC GAT ATG AAT TCG CTA GAA TGT TTA TTA AAC
            762                 777                 792                 807
 E   L   T   E   Q   E   K   L   E   K   L   P   A   I   P   R   K   F   I
GAG TTG ACC GAA CAG GAG AAA CTT GAG AAA CTG CCC GCC ATT CCA AGA AAA TTT ATC
                822                 837                 852
 V   T   E   G   I   F   H   N   S   G   D   L   A   P   L   P   E   L   T
GTC ACT GAG GGT ATT TTC CAC AAC TCG GGC GAT TTA GCT CCG TTG CCT GAG TTG ACT
867                 882                 897                 912
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FIGURE 2B

```
 K   L   K   N   K   Y   K   F   R   L   F   V   D   E   T   F   S   I   G
AAG CTG AAG AAC AAG TAC AAG TTC AGA CTA TTT GTT GAC GAA ACC TTC TCC ATT GGT
    927                 942                 957                 972
 V   L   G   A   T   G   R   G   L   S   E   H   F   N   M   D   R   A   T
GTT CTT GGC GCT ACG GGC CGT GGG TTG TCA GAG CAC TTC AAC ATG GAT CGC GCA ACT
        987                1002                1017                1032
 A   I   D   I   T   V   G   S   M   A   T   A   L   G   S   T   G   G   F
GCC ATT GAC ATT ACC GTT GGG TCC ATG GCC ACC GCG TTG GGG TCC ACC GGT GGT TTT
           1047                1062                1077                1092
 V   L   G   D   S   V   M   C   L   H   Q   R   I   G   S   N   A   Y   C
GTC CTG GGT GAC AGT GTT ATG TGT TTG CAC CAG CGT ATT GGT TCC AAT GCA TAT TGT
               1107                1122                1137
 F   S   A   C   L   P   A   Y   T   V   T   S   V   S   K   V   L   K   L
TTT TCT GCC TGT TTG CCG GCT TAC ACC GTC ACA TCC GTC TCC AAA GTC TTG AAA TTG
1152                1167                1182                1197
 M   D   S   N   N   D   A   V   Q   T   L   Q   K   L   S   K   S   L   H
ATG GAC TCC AAC AAC GAC GCC GTC CAG ACG CTG CAA AAA CTA TCC AAA TCT TTG CAT
    1212                1227                1242                1257
 D   S   F   A   S   D   D   S   L   R   S   Y   V   I   V   T   S   S   P
GAT TCC TTT GCA TCT GAC GAC TCC TTG CGT TCA TAC GTA ATC GTC ACG TCC TCT CCA
        1272                1287                1302                1317
 V   S   P   V   L   H   L   Q   L   T   P   A   Y   R   S   R   K   F   G
GTG TCT CCT GTC CTA CAT CTG CAA CTG ACT CCC GCA TAT AGG TCT CGC AAG TTC GGA
           1332                1347                1362                1377
 Y   T   C   E   Q   L   F   E   T   M   S   A   L   Q   K   K   S   Q   T
TAC ACC TGC GAA CAG CTA TTC GAA ACC ATG TCA GCT TTG CAA AAG AAG TCC CAG ACA
               1392                1407                1422
 N   K   F   I   E   P   Y   E   E   E   E   K   F   L   Q   S   I   V   D
AAC AAA TTC ATT GAG CCA TAC GAA GAG GAG GAA AAA TTT CTG CAG TCC ATA GTA GAT
1437                1452                1467                1482
 H   A   L   I   N   Y   N   V   L   I   T   R   N   T   I   V   L   K   Q
CAT GCT CTT ATT AAC TAC AAC GTT CTC ATC ACA AGA AAC ACT ATT GTT TTA AAA CAG
    1497                1512                1527                1542
 E   T   L   P   I   V   P   S   L   K   I   C   C   N   A   A   M   S   P
GAG ACG CTA CCA ATT GTC CCT AGC TTG AAA ATC TGC TGT AAC GCC GCC ATG TCC CCA
        1557                1572                1587                1602
 E   E   L   K   N   A   C   E   S   V   K   Q   S   I   L   A   C   C   Q
GAG GAA CTC AAA AAT GCT TGC GAA AGT GTC AAG CAG TCC ATC CTT GCC TGT TGC CAA
           1617                1632                1647                1662
 E   S   N   K
GAA TCT AAT AAA TAA AAA TAG AAA GCC AGT ATA TGC ACA CGC ACA TAT ATA TAT AAA
               1677                1692                1707
 TAT TTA TAC AAT AAT ACA AAT AAT CGT AAC ATC ATC TCT GTC AAA TTG ACG TGG TGC
1722                1737                1752                1767
 ACG GCG CCC AGA GAA TGC GCT AAA AAT TTT CGG ATC CGA AAT TTT CTT TCC TTT TAC
    1782                1797                1812                1827
 CAT CGA GGC AAA GCA ACC TGT ATT ATT TAT TTG TTT ATT TAT TAA TAG AAA AGA AAG
        1862                1857                1872                1887
 GAG TAC TTT CGT GGT ACG CTT TCT TGA GCA TTT TCG GTT TCA CTA GGC AGA GAA CTA
           1922                1917                1932                1967
 ACA CAA GAG ACA CAG CAA ACA TCA AAC AAG GTT AAA ACA GCA CAC CAA GGC AAT ATG
               1982                1977                1992
 ATG CAT TTT AGA AAG AAA TCC AGT ATC AGT AAC ACG AGT GAT CAT GAC GGA GCG AAC
2007                2022                2037                2052
 CGT GCC TCA GAT GTC AAG ATT TCT GAA GAT GAC AAG GCA AGA TTG AAG ATG CGT ACT
    2067                2082                2097                2112
 GCT TCC GTT GCT GAT CCT A
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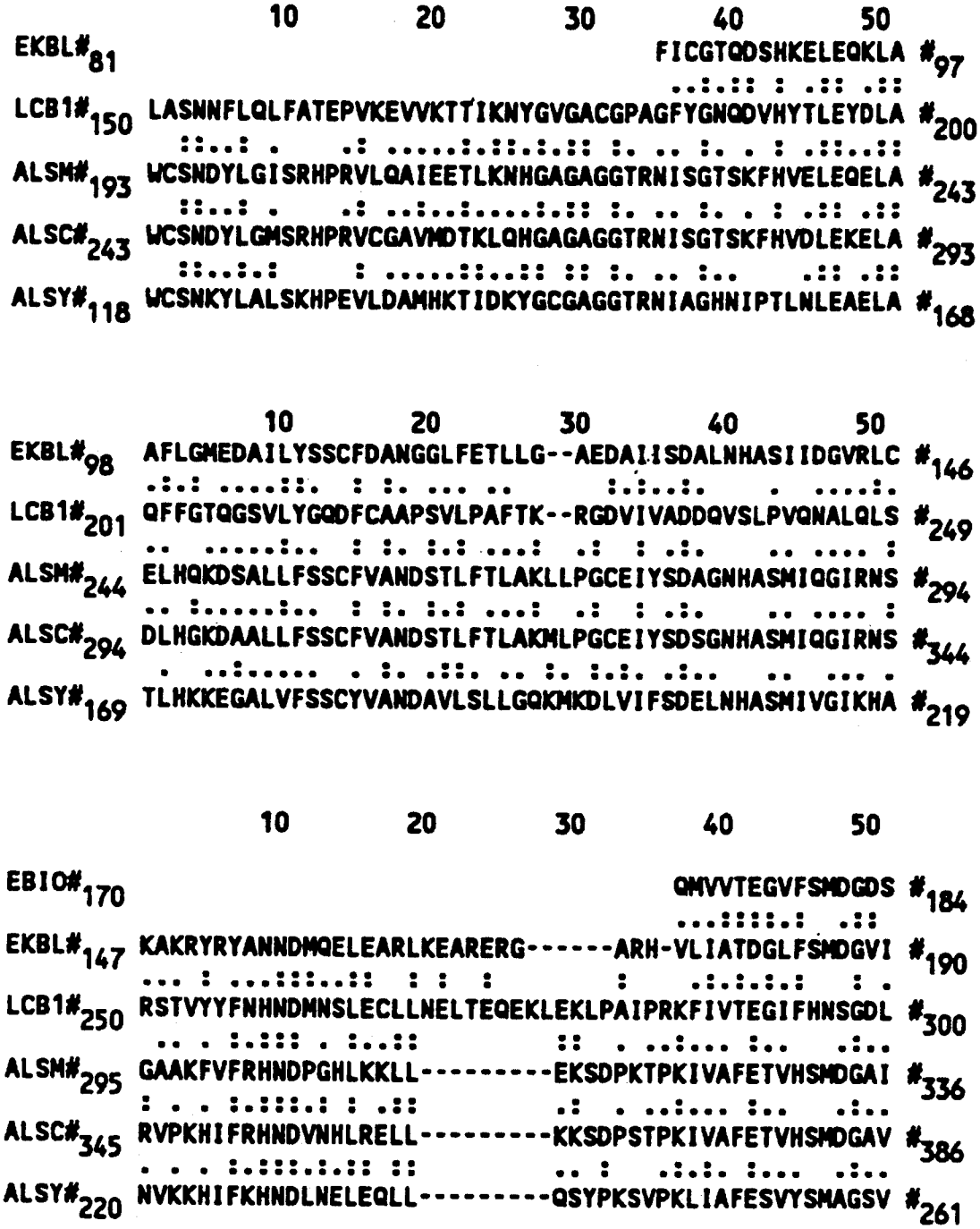
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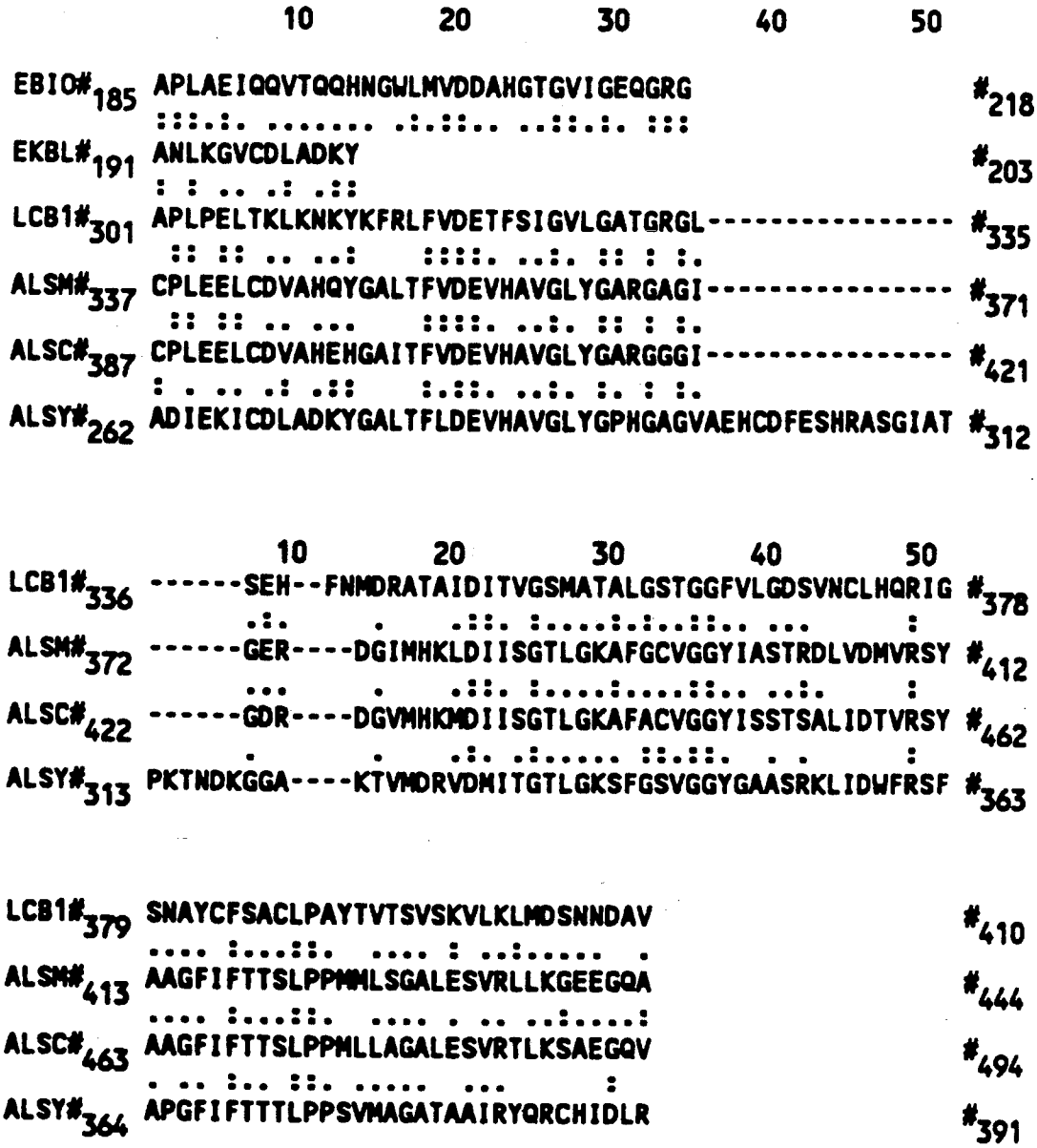
LCB1#150 LASNNFLQLFATEPVKEVVKTTIKNYGVGACGPAGFYGNQDVHYTLEYDLA #200
ALSM#193 WCSNDYLGISRHPRVLQAIEETLKNHGAGAGGTRNISGTSKFHVELEQELA #243
ALSC#243 WCSNDYLGMSRHPRVCGAVMDTKLQHGAGAGGTRNISGTSKFHVDLEKELA #293
ALSY#118 WCSNKYLALSKHPEVLDAMHKTIDKYGCGAGGTRNIAGHNIPTLNLEAELA #168
10 20 30 40 50
EKBL#98 AFLGMEDAILYSSCFDANGGLFETLLG--AEDAIISDALNHASIIDGVRLC #146
LCB1#201 QFFGTQGSVLYGQDFCAAPSVLPAFTK--RGDVIVADDQVSLPVQNALQLS #249
ALSM#244 ELHQKDSALLFSSCFVANDSTLFTLAKLLPGCEIYSDAGNHASMIQGIRNS #294
ALSC#294 DLHGKDAALLFSSCFVANDSTLFTLAKMLPGCEIYSDSGNHASMIQGIRNS #344
ALSY#169 TLHKKEGALVFSSCYVANDAVLSLLGQKMKDLVIFSDELNHASMIVGIKHA #219
10 20 30 40 50
EBIO#170 QMVVTEGVFSMDGDS #184
EKBL#147 KAKRYRYANNDMQELEARLKEARERG------ARH-VLIATDGLFSMDGVI #190
LCB1#250 RSTVYYFNHNDMNSLECLLNELTEQEKLEKLPAIPRKFIVTEGIFHNSGDL #300
ALSM#295 GAAKFVFRHNDPGHLKKLL---------EKSDPKTPKIVAFETVHSMDGAI #336
ALSC#345 RVPKHIFRHNDVNHLRELL---------KKSDPSTPKIVAFETVHSMDGAV #386
ALSY#220 NVKKHIFKHNDLNELEQLL---------QSYPKSVPKLIAFESVYSMAGSV #261

FIGURE 3B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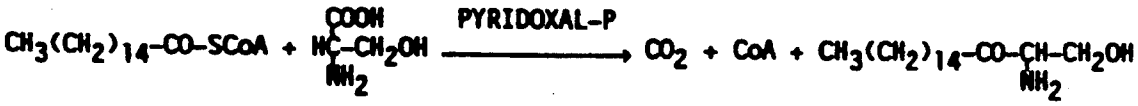
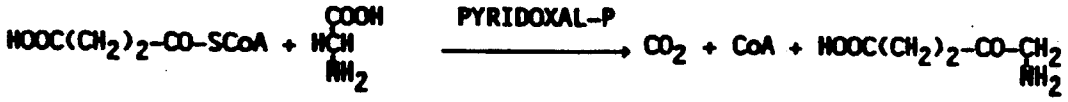
EBIO#185 APLAEIQQVTQQHNGWLMVDDAHGTGVIGEQGRG #218
EKBL#191 ANLKGVCDLADKY #203
LCB1#301 APLPELTKLKNKYKFRLFVDETFSIGVLGATGRGL #335
ALSM#337 CPLEELCDVAHQYGALTFVDEVHAVGLYGARGAGI #371
ALSC#387 CPLEELCDVAHEHGAITFVDEVHAVGLYGARGGGI #421
ALSY#262 ADIEKICDLADKYGALTFLDEVHAVGLYGPHGAGVAEHCDFESHRASGIAT #312
10 20 30 40 50
LCB1#336 ------SEH--FNMDRATAIDITVGSMATALGSTGGFVLGDSVNCLHQRIG #378
ALSM#372 ------GER----DGIMHKLDIISGTLGKAFGCVGGYIASTRDLVDMVRSY #412
ALSC#422 ------GDR----DGVMHKMDIISGTLGKAFACVGGYISSTSALIDTVRSY #462
ALSY#313 PKTNDKGGA----KTVMDRVDMITGTLGKSFGSVGGYGAASRKLIDWFRSF #363
LCB1#379 SNAYCFSACLPAYTVTSVSKVLKLMDSNNDAV #410
ALSM#413 AAGFIFTTSLPPMMLSGALESVRLLKGEEGQA #444
ALSC#463 AAGFIFTTSLPPMLLAGALESVRTLKSAEGQV #494
ALSY#364 APGFIFTTTLPPSVMAGATAAIRYQRCHIDLR #391

FIGURE 4

SERINE PALMITOYLTRANSFERASE:



5-AMINOLEVULINIC ACID SYNTHASE:



2-AMINO-3-KETOBUTYRATE LIGASE:

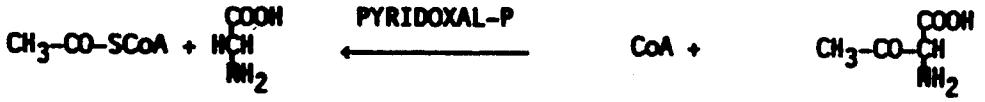
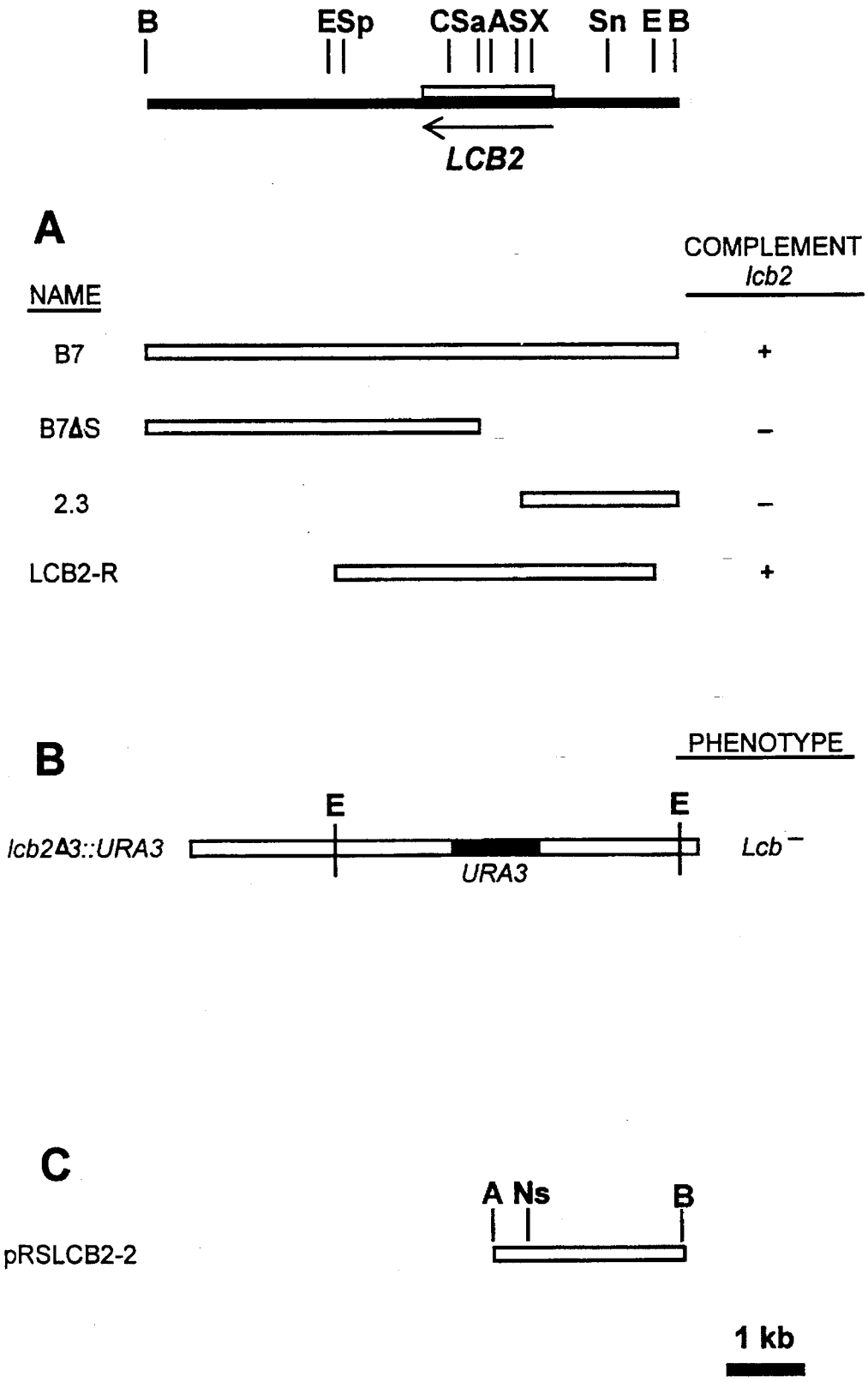


FIGURE 5


B
ESp
CSaASX
Sn
E
B
LCB2
A
NAME
COMPLEMENT
lcb2
B7
+
B7ΔS
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2.3
–
LCB2-R
+
B
PHENOTYPE
E
E
lcb2Δ3::URA3
URA3
Lcb⁻
C
A
Ns
B
pRSLCB2-2
1 kb

FIGURE 6A

```
-883 GCAAATATTGATTCTCGATGAGGCATTTTCTGGAATGGAGGTAGAACCTATGATGCGTTG
-823 TCATGAATTTTTAGAGGAGTGGCCTGGAACAGTCCTTGTAGTGGCACACGTTGCCGAAGA
-763 GACACCAAAATGTGCCCATTACTTAAGGCTCATATCTCCTGGAGAGTATGAAATAGGCGA
-703 TATGGAAAATTAAAGTTTTCTGTTGTGTGGCAGCAAGAGACAGAACCTCGATAATTTGAC
-643 ATACGTATATAATAGTACATGTACATAAAAACGTACGCAAATATCGTATATCTGTTATAC
-583 TACAAAACAATTACTTCTATATCATAGCCAGTTAGCGGGAACGACTTCAGCTAAATGGAC
-523 TATCCATGCTTTAGGCAGAGGCGAAGCGCGGTGATTGGGTGTAACATCATCTCCTTTTCT
-463 CTACGACAAATTCCCAAAAAAAAAATTTATGCTATGTTAATACCTGCACAATTCAACCGT
-403 GCTGAAACGTAAAATTAAGGTGATTATACGGATAGTATACGATATTATCAATCTCATAAG
-343 AAAAATCTCTTTTGAATTTAACGGAGGGATTATTCATTAGAAAGCGTTCTTACCATTCAC
-283 TAGGAGCGAATCCGTGGAAGGTGTTTTAACGTTGCCACGAAAAACAGCTCTACATCGAAA
-223 TAAAAGACAACAATCAGTGCCCGTAAGTTTCATTACTATTTTCTATTATTATCTGCAACT
-163 TTTTATTAGTTAGGTTTTTTTTGTTTGTTTGTTTGTTTTCAATTGATTAATTTACAAGAC
-103 AAAGAACCTTATATTTCGTGTTTTTCATTCTAAAGGAAAAAAAGCATAAAGAAGATTCCA
 -43 CACACTTTATTGTGATAGTTTTCAAAGTAAAAAGTAATAGATTATGAGTACTCCTGCAAA
                                                    M  S  T  P  A  N    6

  18 CTATACCCGTGTGCCCCTGTGCGAACCAGAGGAGCTGCCAGACGACATACAAAAAGAAAA
       Y  T  R  V  P  L  C  E  P  E  E  L  P  D  D  I  Q  K  E  N   26

  78 TGAATATGGTACACTAGATTCTCCGGGGCATTTGTATCAAGTCAAGTCACGTCATGGGAA
       E  Y  G  T  L  D  S  P  G  H  L  Y  Q  V  K  S  R  H  G  K   46

 138 GCCACTACCTGAGCCCGTTGTCGACACCCCTCCTTATTACATTTCTTTGTTAACATATCT
       P  L  P  E  P  V  V  D  T  P  P  Y  Y  I  S  L  L  T  Y  L   66

 198 AAATTATTTGATTCTGATTATATTAGGTCATGTTCACGACTTCTTAGGTATGACCTTCCA
       N  Y  L  I  L  I  I  L  G  H  V  H  D  F  L  G  M  T  F  Q   86

 258 AAAAAACAAACATCTGGATCTTTTAGAGCATGATGGGTTAGCACCTTGGTTTTCAAATTT
       K  N  K  H  L  D  L  L  E  H  D  G  L  A  P  W  F  S  N  F  106

 318 CGAGAGTTTTTATGTCAGGAGAATTAAAATGAGAATTGATGATTGCTTTTCTAGACCAAC
       E  S  F  Y  V  R  R  I  K  M  R  I  D  D  C  F  S  R  P  T  126

 378 TACTGGTGTTCCTGGTAGATTTATTCGTTGTATTGATAGAATTTCTCATAATATAAATGA
       T  G  V  P  G  R  F  I  R  C  I  D  R  I  S  H  N  I  N  E  146

 438 GTATTTTACCTACTCAGGCGCAGTGTATCCATGCATGAACTTATCATCATATAACTATTT
       Y  F  T  Y  S  G  A  V  Y  P  C  M  N  L  S  S  Y  N  Y  L  166

 498 AGGCTTCGCACAAAGTAAGGGTCAATGTACCGATGCCGCCTTGGAATCTGTCGATAAATA
       G  F  A  Q  S  K  G  Q  C  T  D  A  A  L  E  S  V  D  K  Y  186

 558 TTCTATTCAATCTGGTGGTCCAAGAGCTCAAATCGGTACCACAGATTTGCACATTAAAGC
       S  I  Q  S  G  G  P  R  A  Q  I  G  T  T  D  L  H  I  K  A  206

 618 AGAGAAATTAGTTGCTAGATTTATCGGTAAGGAGGATGCCCTCGTTTTTTCGATGGGTTA
       E  K  L  V  A  R  F  I  G  K  E  D  A  L  V  F  S  M  G  Y  226

 678 TGGTACAAATGCAAACTTGTTCAACGCTTTCCTCGATAAAAAGTGTTTAGTTATCTCTGA
       G  T  N  A  N  L  F  N  A  F  L  D  K  K  C  L  V  I  S  D  246

 738 CGAATTGAACCACACCTCTATTAGAACAGGTGTTAGGCTTTCTGGTGCTGCTGTGCGAAC
       E  L  N  H  T  S  I  R  T  G  V  R  L  S  G  A  A  V  R  T  266

 798 TTTCAAGCATGGTGATATGGTGGGTTTAGAAAAGCTTATCAGAGAACAGATAGTACTTGG
       F  K  H  G  D  M  V  G  L  E  K  L  I  R  E  Q  I  V  L  G  286

 858 TCAACCAAAAACAAATCGTCCATGGAAGAAAATTTTAATTTGCGCAGAAGGGTTGTTTTC
       Q  P  K  T  N  R  P  W  K  K  I  L  I  C  A  E  G  L  F  S  306
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FIGURE 6B

```
 918 CATGGAAGGTACTTTGTGTAACTTGCCAAAATTGGTTGAATTGAAGAAGAAATATAAATG
       M  E  G  T  L  C  N  L  P  K  L  V  E  L  K  K  K  Y  K  C  326

 978 TTACTTGTTTATCGATGAAGCCCATTCTATAGGCGCTATGGGCCCAACTGGTCGCGGTGT
       Y  L  F  I  D  E  A  H  S  I  G  A  M  G  P  T  G  R  G  V  346

1038 TTGTGAAATATTTGGCGTTGATCCCAAGGACGTCGACATTCTAATGGGTACTTTCACTAA
       C  E  I  F  G  V  D  P  K  D  V  D  I  L  M  G  T  F  T  K  366

1098 GTCGTTTGGTGCTGCTGGTGGTTACATTGCTGCTGATCAATGGATTATCGATAGACTGAG
       S  F  G  A  A  G  G  Y  I  A  A  D  Q  W  I  I  D  R  L  R  386

1158 GTTGGATTTAACCACTGTGAGTTATAGTGAGTCAATGCCGGCTCCTGTTTTAGCTCAAAC
       L  D  L  T  T  V  S  Y  S  E  S  M  P  A  P  V  L  A  Q  T  406

1218 TATTTCCTCATTACAAACCATTAGTGGTGAAATATGTCCCGGACAAGGTACTGAAAGATT
       I  S  S  L  Q  T  I  S  G  E  I  C  P  G  Q  G  T  E  R  L  426

1278 GCAACGTATAGCCTTTAATTCCCGTTATCTACGTTTAGCTTTGCAAAGGTTAGGATTTAT
       Q  R  I  A  F  N  S  R  Y  L  R  L  A  L  Q  R  L  G  F  I  446

1338 TGTCTACGGTGTGGCTGACTCACCAGTTATTCCCTTACTACTGTATTGTCCCTCAAAGAT
       V  Y  G  V  A  D  S  P  V  I  P  L  L  L  Y  C  P  S  K  M  466

1398 GCCCGCATTTTCGAGAATGATGTTACAAAGACGGATTGCTGTTGTTGTTGTTGCTTATCC
       P  A  F  S  R  M  M  L  Q  R  R  I  A  V  V  V  V  A  Y  P  486

1458 TGCTACTCCGCTGATCGAATCAAGAGTAAGATTCTGTATGTCTGCATCTTTAACAAAGGA
       A  T  P  L  I  E  S  R  V  R  F  C  M  S  A  S  L  T  K  E  506

1518 AGATATCGATTATTTACTGCGTCATGTTAGTGAAGTTGGTGACAAATTGAATTTGAAATC
       D  I  D  Y  L  L  R  H  V  S  E  V  G  D  K  L  N  L  K  S  526

1578 AAATTCCGGCAAATCCAGTTACGACGGTAAACGTCAAAGATGGGACATCGAGGAAGTTAT
       N  S  G  K  S  S  Y  D  G  K  R  Q  R  W  D  I  E  E  V  I  546

1638 CAGGAGAACACCTGAAGATTGTAAGGACGACAAGTATTTTGTTAATTGAATTTTACCTAA
       R  R  T  P  E  D  C  K  D  D  K  Y  F  V  N                561

1698 TTGCTAGTTAGGTGAAAAATTACAAAATTTCTGGAAGACGTTGGAAACACGCAACGTCTT
1758 TTTGACATAAACTTAAAACTGCCAAAAGTCAAACAAAAATTGCAAAAAAAGTAAAAAAAG
1818 TTACGAAAAAAAAAACATTTAAAAGAAAGAAGAAGTTAAAAGTGCACGCAATATGTTCCA
1878 GGATATGAAATGAAATACCTTTTGTTTCACCTTTTAAATAATTTAATGTTATATATACAA
1938 CTTTATCGTATCATATTCGCAATTACATTATACAAGAATGAGTTTTTTTTCGCGACAAAG
```

FIGURE 7A

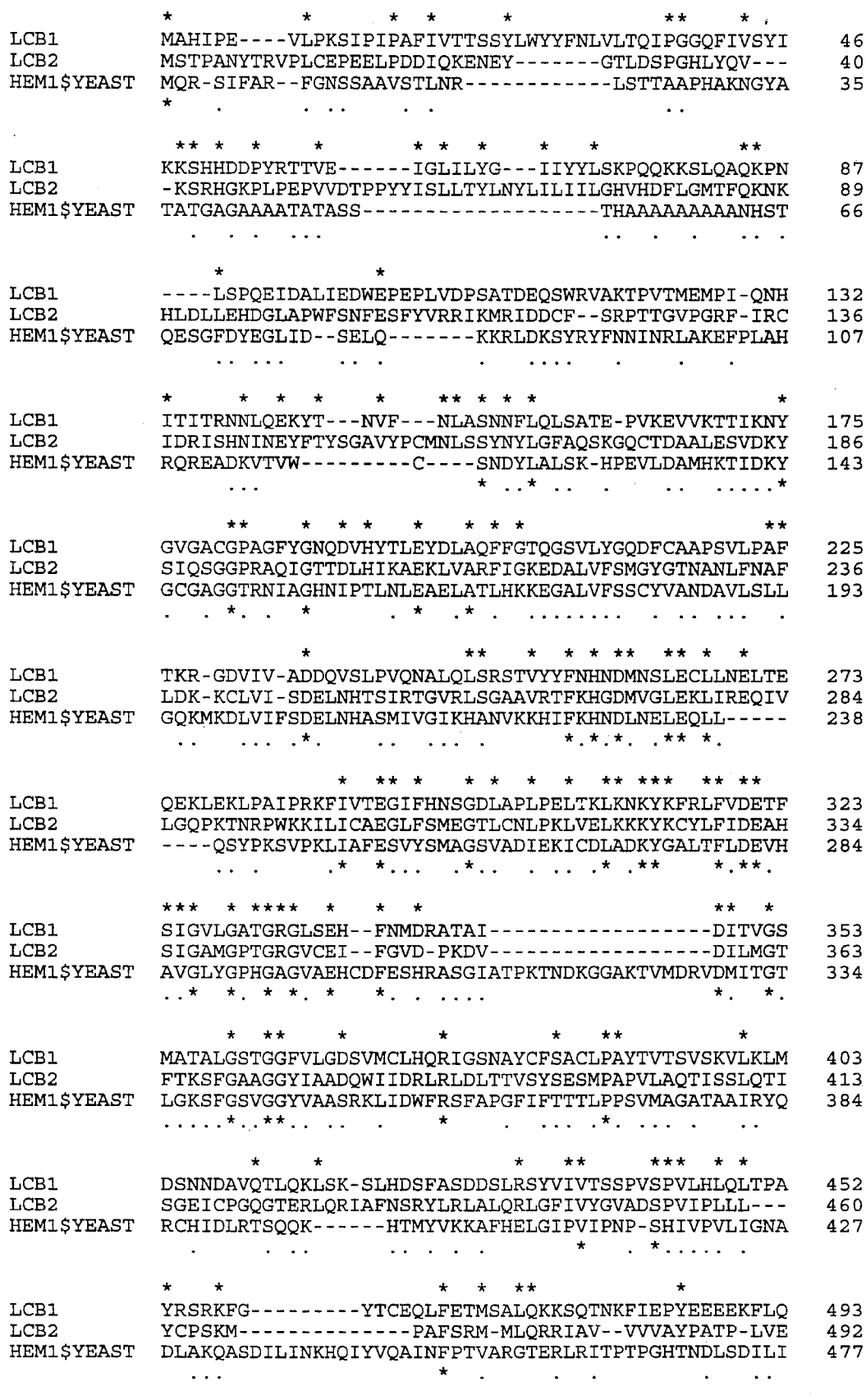

FIGURE 7B

```
            * *          *            *           *    *   *    *
LCB1        SIVDHALINYNVLITRN----TIVLKQETLPIVPSLKICCNAAMSPEELK   539
LCB2        SRVRFCMSA--SLTKED----IDYLLRHVSEVGDKLNLKSNSGKSSYDGK   536
HEM1$YEAST  NAVDDVFNELQLPRVRDWESQGGLLGVGESGFVEESNLWTSSQLSLTNDD   527
            . *   . .        .        *          . ..  ..  *  . .

                  * *        *
LCB1        NA---CESVKQSILACCQESN---K   558
LCB2        RQRWDIEEVIRRTPEDCKDDKYFVN   561
HEM1$YEAST  LNP----NVRDPIVKQLEVSSGIKQ   548
              .     .*  ..    . ..   .
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METHOD FOR OBTAINING ANTIFUNGAL AND HERBICIDAL COMPOUNDS THAT TARGET THE FIRST COMMITTED STEP IN SHINGOLIPID LONG-CHAIN BASE BIOSYNTHESIS

This application is a continuation of application Ser. No. 07/906,899 filed Jun. 30, 1992, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

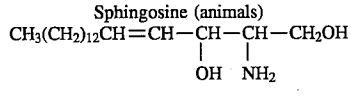
This invention relates to the isolation of the LCB1 and LCB2 genes of the year *Saccharomyces cervisiae* that encode subunits of the enzyme serine palmitoyltransferase (SPT), the first enzyme leading to synthesis of the long-base component of sphingolipids. The invention further relates to method of using these genes to either inhibit SPT activity or to inhibit synthesis of the enzyme. Furthermore, the invention relates to methods for construction strains of *S. cervisiae* or other organisms that can be used to select and test for compounds that either inhibit SPT activity or to inhibit synthesis of the enzyme.

2. Description of the Background

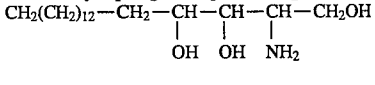
Sphingolipids are abundant in the membranes of fungi (Brennah, P. J., & Losel, D. M. 1978. Fungal lipids, in *Microbial Physiology,* Rose, A. H. & Morris, P. G., Eds. 17, 47–179, Acad. Press., N.Y.), animals (Seeley, C. C. and Siddiqui, B. 1977; the Glycojungates, Horowitz, M. I. and Pigman, W., eds., Acad. Press, N.Y. 1:495), and higher plants (Laine, R. a., Hsieh, T. C.-Y., & Lester, R. L. Glycophosphoceramides from plants, in *Cell Surface Glycolipids,* p.65, Am. Chem. Soc. Symp. Ser. 128, Am. Chem. Soc. Wash, D.C.) 1980. In spite of much effort, it has been difficult to understand the exact biological role(s) of sphingolipids and their mode of action at the molecular level. In animals, sphingolipids are thought to play a role in such general cellular events as cell-to-cell recognition, regulation of cell growth, and differentiation. The prevalence of sphingolipids suggests that they play vital roles in cells and direct proof that sphingolipids are essential cellular components has been obtained with the discovery of mutants of *S. cervisiae* that absolutely require a sphingolipid long-chain base (see below) for growth (Wells, G. B. and Lester, R. L.; J.Biol. Chem. 258: pages 10200–10203 (1983)) and viability (Pinto, W. J., Wells, G. B., Williams, A. C., Anderson, K. A., Teater, E. C., and Lester, R. L., *Fed. Proc.* 45: 1826 (1986)).

sphingolipids are derivatives of ceramides containing sugars and sometimes phosphates. Ceramides usually contain a fatty acid of 20–26 carbons connected via an amide linkage to a long-chain base. The major long-chain bases and their predominant distribution are:

Sphingosine (animals)

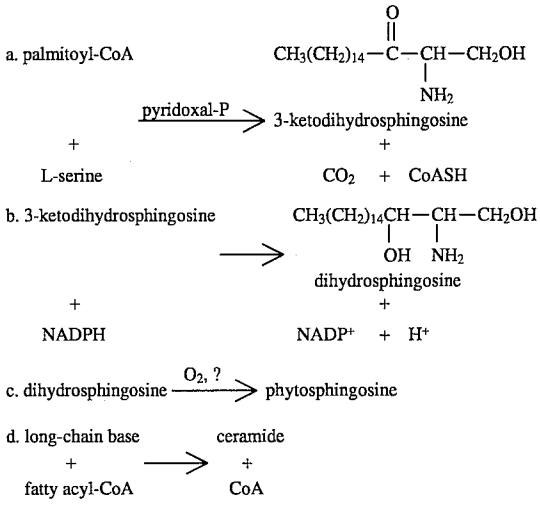


Phytosphingosine (plants, fungi)



-continued

The route of sphingolipid biosynthesis is proposed to be:



Reaction (a), the first committed step in sphingolipid biosynthesis (reviewed in Merrill, A. H. and Jones, D. D. 1990. *Biochemica et Biophysica Acta.* 1044:1–12) is catalyzed by serine palmitoyltransferase (SPT, also called 3-ketodihydrosphingosine synthetase). This enzyme has been shown to occur in the fungus *Hansenula ciferri* (Snell, E. E., Di Mari, S. J., and Brady, R. N. 1970. *Chem. Phys. Lipids,* 5:116–138), in beef liver (Stoffel, W. 1970. *Chem. Phys. Lipids.* 5:139–158), and in the bacterium *Bacteroides melaninogenicus* (Lev, M., and Milford, A. F. 1973. *Arch. Biochem. Biophys.* 157:500–508). Other evidence for this reaction comes from our own work in *S. cervisiae* (Pinto et al., 1986; Pinto W. J., Wells, G. W. and Lester, R. L. 1992. *J. Bacteriol.* 174:2575–2581). The enzyme has never been purified to homogenity and characterized in any detail (reviewed in Merrill, A. H. and Jones, D. D. 1990. *Biochemica et Biophysica Acta.* 1044:1–12).

In reaction (d) the long-chain base is attached to a fatty acid to form a ceramide. In all organisms ceramides are converted to complex derivatives, the sphingolipids, by the addition of polar groups to the 1-hydroxyl. The sphingolipids in animals contain various oligosaccharides inked glycosidically to the ceramide to yield glycosphingolipids and also contain choline linked by a phosphodiester bond to ceramide to yield the abundant compound sphingomyelin. Certain sphingolipids in fungi and plants differ from the sphingolipids in animals because the 1-hydroxyl is linked through a phosphoryl group to inositol (myo-inositol) rather than directly to a sugar. This core structure, inositol-phosphorylceramide, or inositol-P-ceramide ("IPC", Smith, S. W., and Lester, R. L. 1974. *J. Biol. Chem.* 249:3395–3405), along with mannose-inositol-P-ceramide, (MIPC, ibid) and mannose-(inositol-P)$_2$-ceramide (M(IP)$_2$C, (Steiner, S., Smith, S. Waechter, C. J., and Lester, R. L. 1969. *Proc. Natl. Acad. Sci. U.S.A.* 64:1042–1048) collectively constitute the sphingolipids in *S. cervisiae* (Smith, S. W., and Lester, R. L. 1974. *J. Biol. Chem.* 249:3395–3405). Phosphoinositol sphingolipids are also a major class of lipids in plants (for references see Kaul, K. and Lester, R. L. 1975. *Plant Physiol.,* 55:120–129) and parasites (Singh, B. N., Costello, C. E., and Beach, D. H. 1991. *Arch. Biochem. Biophys.* 286:409–418).

Because sphingolipids are vital for *S. cerevisiae,* the long-chain base biosynthesis pathway would appear to be a good target for antifungal compounds. In fact, sphingolipids

may be vital for all organisms that contain them, and therefore, any compound that would inhibit long-chain base biosynthesis might inhibit growth of an organism that contained sphingolipids.

Accordingly, there is a need to begin to identify or design such inhibitory antifungal compounds to target the long-chain base biosynthesis pathway, which would appear to be a good target for antifungal compounds. Therefore we isolated two *S. cerevisiae* genes, LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6), that most likely encode subunits of SPT. These are the first genes involved in long-chain base biosynthesis to be isolated from any organism. The genes provide a unique opportunity to identify compounds that block SPT activity or synthesis in specific organisms.

SUMMARY OF THE INVENTION

One objective of the present invention is to provide the LCB1 (SEQ ID NOS.: 1–3), and the LCB2 ((SEQ ID NOS.: 4–6) genes of *S. cerevisiae* and to demonstrate that they provide SPT enzyme activity to a strain that lacks such enzyme activity.

Another objective of the present invention is to provide the LCB1 ((SEQ ID NOS.: 1–3), and the LCB2 ((SEQ ID NOS.: 4–6), genes of *S. cerevisia* for use in constructing a genetically engineered strain of *S. cerevisiae* that has increased SPT protein and therefore enzyme activity.

Another objective of the present invention is to provide the DNA sequence of the LCB1 ((SEQ ID NOS.: 1–3) and LCB2 ((SEQ ID NOS.: 4–6) genes for use as targets for antisense or triple-helix-forming oligonucleotides which will inhibit the production of SPT protein.

Another objective of the present invention is to provide the DNA sequence of the LCB1 (SEQ ID NOS.: 1–3) and LCB2(SEQ ID NOS.: 4–6) genes for use in overexpression of the genes and subsequent overproduction of the SPT enzyme.

Another objective of the present invention is to provide the DNA sequence of the LCB1 ((SEQ ID NOS.: 1–3) and LCB2 ((SEQ ID NOS.: 4–6) genes for use in isolating the homolog of these genes from other organisms.

Other objectives and advantages of the invention will become apparent as the description thereof proceeds.

In satisfaction of the foregoing objects and advantages, the present invention provides the LCB1 ((SEQ ID NOS.: 1–3) and LCB2 ((SEQ ID NOS.: 4–6) genes and their DNA sequence. The genes are shown to restore SPT activity to a lcb1((SEQ ID NOS.: 1–3)-defective and lcb2((SEQ ID NOS.: 4–6)-defective strain, respectively.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** represents a schematic diagram of plasmids carrying the LCB1 (SEQ ID NOS.: 1–3) gene of *S. cerevisiae.*

FIG. **2** sets forth the DNA sequence of the LCB1 (SEQ ID NOS.: 4–6) gene and the predicted protein product.

FIG. **3** sets forth a comparison of the LCB1 (SEQ ID NOS.: 1–3) protein sequence with other proteins that catalyze a chemical reaction that is similar to the one catalyzed by SPT.

FIG. **4** sets forth a comparison of the reaction catalyzed by SPT and other enzymes.

FIG. **5**(A–C) represents a schematic diagram of plasmids carrying the LCB2 (SEQ ID NOS.: 4–6) gene of *S. cerevisiae* or portions of the gene.

FIG. **6** sets forth the DNA sequence of the LCB2 (SEQ ID NOS.: 4–6) gene and the predicted protein sequence.

FIG. **7** sets forth a comparison of LCB1 (SEQ ID NOS.: 1–3), LCB2 (SEQ ID NOS.: 4–6), and the *S. cerevisiae* HEM1 protein sequences.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to the isolation of the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes of *S. cerevisiae.*

The present invention provides a DNA sequence LCB1 having a nucleotide sequence as set forth in FIG. **2**. It also provides a plasmid comprising the LCB1 sequence according to the invention. Particularly preferred is a plasmid according to the invention which is the plasmid pTZ18-LCB1 (SEQ ID NOS.: 1–3) containing the LCB1 (SEQ ID NOS.: 1–3) sequence. Also, particularly preferred is a plasmid according to the invention which is plasmid YIpLCB1-1 containing the LCB1 sequence.

In another embodiment the present invention provides a host cell line transformed by a plasmid containing the LCB1 (SEQ ID NOS.: 1–3) sequence according to the present invention.

In another aspect the present invention provides a DNA sequence LCB2 (SEQ ID NOS.: 4–6) having a nucleotide sequence as set forth in FIG. **6**. It also provides a plasmid comprising the LCB2 (SEQ ID NOS.: 4–6) sequence according to the invention. Particularly preferred is a plasmid according to the invention which is the plasmid pRSLCB2-2 containing the LCB2 (SEQ ID NOS.: 4–6) sequence.

In another embodiment the present invention provides a host cell line transformed by a plasmid containing the LCB2 (SEQ ID NOS.: 4–6) sequence according to the present invention.

The present invention further provides a genetically engineered strain of *S. cerevisiae* which has increased production of Serine Palmitoyltransferase protein and therefore increased enzyme activity as compared to the wild type *S. cerevisiae.*

In another aspect the present invention provides an antisense or triple helix forming oligonucleotide specific for the LCB1 (SEQ ID NOS.: 1–3) sequence, which will inhibit the production of Serine Palmitoyltransferase protein.

In still another aspect the present invention provides an antisense or triple-helic-forming oligonucleotide specific for the LCB2 (SEQ ID NOS.: 4–6) sequence, which will inhibit the production of Serine Palmitoyltransferase protein.

The present invention also provides a genetically engineered microbial strain transformed by a plasmid comprising either the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequence, or both the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) sequences, which overexpresses the gene(s) with which it is transformed and subsequently overproduces the Serine Palmitoyltransferase enzyme.

Also the present invention provides a method for testing an oligonucleotide or organic compound for the ability to block Serine Palmitoyltransferase activity or synthesis, which method comprises:

exposing the oligonucleotide or the organic compound being tested to a host cell or host cell extract, which host cell has been transformed to include either a LCB1 (SEQ ID

NOS.: 1–3) gene or LCB2 (SEQ ID NOS.: 4–6) gene (or both genes), and

testing for an absence of Serine Palmitoyltransferase enzyme or its activity, which diminished activity is indicated by the absence or lower concentration of sphingolipids.

The present invention further provides an oligonucleotide DNA sequence, which is a complement to either the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequences, or to portions thereof.

In yet another aspect the present invention provides a method of testing for and/or isolating closely related sequences (similar to LCB1 (SEQ ID NOS.: 1–3)) which comprises

producing or obtaining an oligonucleotide which is a complement to a portion of the LCB1 (SEQ ID NOS.: 1–3) gene, and

using the complement as an oligonucleotide probe by

exposing a target nucleotide sequence to the said nucleotide probe and testing for binding to said probe, and optionally

isolating and separating the nucleotide probe from the DNA sequence to which it has bound.

In still another aspect, the present invention provides a method of testing for and/or isolating closely related sequences (similar to LCB2 (SEQ ID NOS.: 4–6)) which comprises

producing or obtaining an oligonucleotide which is a complement to a portion of the LCB2 (SEQ ID NOS.: 4–6) gene, and

using the complement as an oligonucleotide probe by

exposing a target nucleotide sequence to the said nucleotide probe and testing for binding to said probe, and optionally

isolating and separating the nucleotide probe from the DNA sequence to which it has bound.

The LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) sequences according to the present invention, plasmids comprising either of the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequences, transformed host cells having a sequence according to the present invention, and sequences which are complements are all useful in screening potential antifungal agents, or for producing reagents useful in screen potential antifungal agents, (both oligonucleotides and organic chemical agents, which are potential antifungal agents may be screened).

The sequences according to the present invention are also useful to provide oligonucleotides which have complementary DNA sequences, which complementary sequences can be used as probes to screen for sequences which are homologs of the claimed sequences and/or used in a process to isolate and ultimately sequence such homologs of LCB1 (SEQ ID NOS.: 1-3) or LCB2 (SEQ ID NOS.: 4–6).

In accordance with present invention, as a preliminary step, a mutant strain of *S. cerevisiae* blocked in sphingolipid biosynthesis was obtained. For example, strains of *S. cerevisiae* carrying the mutant allele, lcb1-1, are absolute auxotrophs and grow only when a long-chain base (lcb, phytosphingosine but not sphingosine) is added to the culture medium.

The genes were isolated from a *S. cerevisiae* genomic DNA library by complementation for growth on medium lacking a long-chain base (such as phytosphingosine) of an lcb1 or an lcb2-defective strain.

The original lcb mutant MCGA (MATα lcb1-1 inol (J. Biol. Chem. 258, 10200–10203 (1983) was crossed with strain W303-1B (MATα ade2-1 can1-100 ura3-1 his3-11,15 trp1-1 leu2-3,112; obtained from R. J. Rothstein, Columbia University). Progeny from this cross were backcrossed to W303-1B, and several offspring were selected for further study, including strains X2A1B (MATa lcb1-1 ura3-1 trp1-1 his3-11,15). Strain SL1 was derived from strain SJ21R (MATa ura3-52 leu2-3,112 ade1 MEL1) by replacement of the LCB1 allele with a mutant allele that was disrupted by inserting a 1.1-kb URA3 DNA fragment at the SalI site of LCB1. The LCB1:: URA3-disrupted allele was prepared by transferring 4.3-kb HindIII-StuI fragment, carrying LCB1, from pLCB to pTZ18 (Pharmacia) cleaved with HindIII and SmaI. The resulting plasmid, pTZ18-LCB1 was cleaved with SalI and ligated with a 1.1-kb URA3 DNA fragment having SalI cohesive ends (obtained from pUC-URA3 cut with SalI) to yield pTZ18-LCB1::URA3.

To replace the LCB1 chromosomal allele with the URA3-disrupted allele, 10 μg of pTZ18-LCB1::URA3 DNA was cleaved with XbaI and ClaI, extracted with phenol, phenol-chloroform, and chloroform and precipitated with ethanol. The DNA was transformed into strain SJ21R with selection for Ura+ transformants. Replacement of the LCB1 chromosomal allele with the URA3-disrupted allele was verified by Southern blot analysis. YIpLCB1-1 was constructed by inserting TRP1 of *S. cerevisiae,* as a 1.4-kb HindIII fragmentm into the HindIII site of pTZ18-LCB1. YIpLCB1-1 was cleaved at its unique BAMHI site located on the 3'side of LCB1, and the linear DNA was used to transform strain 24D5 with selection for Ura+transformants. Integration at the expected chromosomal location was verified by Southern blotting. Transformants were crossed to strain YPH1 (MATa ura3-52 lys2-801 ade2-101 (See, for example, Genetics, 122, 19–27 (1989)).

The plasmid pLCB was isolated from a *S. cerevisiae* genomic DNA library carried in a CEN vector. The 6.44-kb vector was pBR322 with a 0.63-kb Sau3A CEN3 DNA fragment inserted into the PvuII site of the vector and a 1.4-kb TRTRP1 ARS1 fragment inserted into the EcoRI site of the vector. The ligations were done with molecules whose ends were made blunt ended so that the original restriction sites were destroyed. Sau3A genomic DNA fragments of 8-kb average size from strain X2180 (a/α gal2/gal2) were cloned into the BamHI site of the vector (the library was obtained from ZymoGenetics, Seattle, Wash.). DNA fragments from pLCB were subcloned into YCp50 (see, Methods Enzymol., 152, 481–504 (1987)).

Plasmids were propagated in *Escherichia coli* DH5α. The lcb-defective strains were propagated in several media as described later in the detailed section which follows.

For example, to isolate LCB1, strain X2A1B (relevant genotype lcb1-1, trp1) was transformed with a genomic DNA library which was carried in a vector containing CEN3 and ARS1, for single-copy propagation in yeast cells, and TRP1, for selection of Trp^+ yeast that had been transformed with the vector. Ten thousand Trp^+ transformants were selected on minimal medium plates containing phytosphingosine but lacking tryptophan. Transformants were pooled and reselected on minimal medium plates lacking both tryptophan and phytosphingosine. About one per thirty-five hundred Trp^+ colonies was able to grow without added phytosphingosine and thus had an Lcb^+ phenotype.

Plasmid DNA was isolated from several Lcb^+ yeast transformants and transformed into *E. coli* with selection for ampicillin resistant cells. Plasmid DNA from *E. coli* transformants was isolated and digested with restriction endonucleases. The pattern of restriction fragments indicated that

the original Lcb$^+$ yeast transformants all contained the same plasmid which carried an insert of about 8 kb.

To localize the LCB1 gene on the 8 kb DNA insert we subcloned parts of the insert into the CEN4 vector YCp50 and tested the resulting plasmids for their ability to confer a Lcb$^+$ phenotype on strain X2A1B. The experiments localized LCB1 to a subclone of 4.0 kb (FIG. 1).

Further localization of LCB1 was achieved by chromosomal disruption. For these experiments the 4 kb insert was disrupted at the unique SalI site (FIG. 1) by insertion of the URA3 gene of *S. cerevisiae* to create the lcb1::URA3-disruption allele. The lcb1::URA3-disruption allele was used to replace the wild type LCB1 allele in strain SJ21R (relevant phenotype Lcb$^+$ Ura$^-$) by homologous recombination as described in EXAMPLE 2. These procedures produced a strain, SL1, having the chromosome disrupted at the expected SalI site. If this procedure had disrupted the LCB1 gene then the strain SL1should require long-chain base (phytosphingosine) for growth and, therefore, having an Lcb$^-$ phenotype. This expectation was verified because strain SL1 had an Lcb$^-$ phenotype. We conclude that the SalI site shown in plasmid YCp50-LCB1 between the PstI and HpaI sites is located within the LCB1 gene.

Genetic complementation analysis was used to verify that the lcb1::URA3 disruption mutation in strain SL1 was allelic to the original lcb1-1 mutation carried in strain X2A1B. Strain SL1 was crossed to strain 24D5. The resulting diploids had an Lcb$^-$ phenotype, suggesting allelism of the cloned gene and lcb1. Strong support for allelism would be obtained by sporulating these diploids and showing that all tetrads give four Lcb$^-$ spores. However, such diploids failed to sporulate under a variety of conditions suggesting that sphingolipids are needed for sporulation. An alternative genetic approach was used to demonstrate allelism. The putative LCB1 allele, carried on the integrating vector YIpLCB1-1, was directed to integrate into its homologous chromosomal locus as described in EXAMPLE 3. The host strain for integration of YIpLCB1 was strain 24D5 which carried the lcb1-1 mutation. If YIpLCB1-1 did indeed carry the wild type LCB1 gene then the host strain should have this plasmid integrated next to the lcb1-1 allele. When this strain is crossed to an LCB1 strain (YPH1) all progeny should be Lcb$^+$ since YIpLCB1-1 should be tightly linked to lcb1-1 and there should be little if any recombination events that would separate the two alleles. In fourteen four-spored tetrads from such a cross, showing 2$^+$:2$^-$ segregation for the Ade, Ura and Leu phenotypes, all spores were Lcb$^+$ indicating that YIpLCB1 had been directed to integrate in close proximity to the lcb1-1 allele. We conclude that the LCB1 gene has been cloned and is carried on pTZ18-LCB1 gene as claimed.

To determine if SPT activity was missing in lcb1-defective strains and to determine if a plasmid carrying LCB1 restored such activity we assayed membranes for the enzyme. The parental strain MC6A contained 54.4 units of enzyme activity per mg of protein while the lcb1-defective strain X2A1B contained 2.5 units per mg of protein or about 20 times less enzyme activity that the parental strain: this level of activity is at the limit of detection and the actual enzyme activity may be lower. The cloned LCB1 allele carried in pLCB was able to restore enzyme activity to about 50% of the wild-type level since three independent transformants of strain X2A1B gave 22.7, 25.6, and 22.8 units of enzyme activity per mg of protein. These data support the claim that LCB1 encodes the SPT enzyme or a subunit of the enzyme.

Based upon the results of the lcb1::URA3 disruption experiments a region surrounding the SalI site shown in FIG. 1 was subjected to DNA sequence analysis and the sequence was analyzed by computer to locate large open reading frames which could encode the LCB1 protein. The sequence (FIG. 2) contained a single, large open reading frame, encoding 558 amino acids which was oriented in the same direction of transcription as the LCB1 mRNA (data not shown). This region must code for the LCB1 protein product because it is in the correct 5' to 3' orientation, because a URA3 disruption of the open reading frame at the unique SalI site created a Lcb$^-$ phenotype, and because it is genetically tightly linked to the lcb1-1 allele.

The nucleotide sequence of the open reading frame was used to product the amino acid sequence of the LCB1 peptide. The results of the prediction are illustrated above each codon of the nucleotide sequence (FIG. 2) beginning with the first ATG codon at position +1 and ending with the stop codon TAA at position +1675. Assuming that this ATG codon is the true translation initiation site, the product of the open reading frame is a protein of 558 amino acids. Since the amino terminus of the LCB1 protein has not been determined directly it is possible that the amino terminus of the actual protein is different than indicated in FIG. 2. The difference could occur either because of post-translational processing or because an ATG codon down stream of the one shown in FIG. 3 is used as the initiation codon.

Because SPT activity is present in the membrane fraction of lysed cells, we expected the LCB1 protein to be membrane-associated. The hydrophobicity of the deduced protein sequence was therefore examined for potential membrane spanning regions. According to the 5theory of Kyte, J., and Doolittle, R. F. 1982. *J. Mol. Biol.* 157:105–132, the Grand Average Hydropathy Score (GRAVY) for the predicted LCB1 protein is –1.39, a value that places the protein in the same class as globular proteins. A globular, rather than integral membrane, protein is also predicted by the procedure of Eisenberg, D., Schwartz, E., Komaromy, M., and Wall, R. 1984. *J. Mol. Biol.* 179:125–142. In addition, this analysis predicts two very hydrophobic, membrane-associated helices. Helix I spans amino acid residues 12–32 and has the sequence IPIPAFIVTTSSYLWYYFNLV, while Helix II spans residues 344–373 and has the sequence ATAIDITVGSMATALGSTGGFVLG.

The predicted amino acid sequence of the LCB1 protein shows high similarity to the enzyme 5-aminolevulinic acid synthase (ALA synthase) whose structural gene has been sequenced from many organisms including *S. cerevisiae* (ALSY (SEQ ID NO.: 12), FIG. 3, Urban-Grimal, D., Wollard, C., Garnier, T., Dehoux, P., and Labbe-Boise, R. 1986. *Eur. J. Biochem.* 156:511–519), mouse (ALSM (SEQ ID NO.: 10), FIG. 3, Schoenhaut, D. S., and Curtis, P. J. 1986. *Gene* 48:55–63) and chicken (ALSC (SEQ ID NO.: 11), FIG. 3, Riddle, R. D., Yamamoto, M., and Engel, J. D. 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:792–796). The predicted LCB1 protein also shows high similarity to the *Escherichia coli* enzymes 2-amino-3-ketobutyrate CoA ligase (EKBL (SEQ ID NO.: 8), FIG. 3, Aronson, B. A., Ravnikar, P. D., and Somerville, R. L. 1988. *Nucleic Acids Res.* 16:3586) and biotin synthetase (EBIO, FIG. 3, Otsuka, A. J., Buoncristiani, M. R., Howard, P. K., Flamm, J., Johnson, C., Yamamoto, R., Uchida, K., Cook, C., Ruppet, J., and Matsuzaki, J. 1988. *J. Biol. Chem.* 263:19577–19585).

The similarity of the LCB1 protein to ALA synthase and to 2-amino-3-ketobutyrate CoA ligase seems particularly significant since these enzymes catalyze a reaction (FIG. 4) that is very similar to that catalyzed by SPT. In addition, the *E. coli* 2-amino-3-ketobutyrate CoA ligase uses pyridoxal

phosphate as a cofactor (Mukherjee, J. J., Dekker, E. E. 1987. *J. Biol. Chem.* 262:14441–14447) as do serine palmitoyltransferase (Brady, R. O. and Koval, G. J. 1957. *J. Am. Chem. Soc.* 79:2648–2649) and ALA synthase (Warnich, G. R., and Burnham, B. F. 1971. *J. Biol. Chem.* 246:6880–6885). The similarity of the amino acid sequences (FIG. **3**) and the reactions catalyzed by these enzymes (FIG. **4**) argue that the product of LCB1 is most likely SPT or a catalytic subunit of the enzyme, rather than a regulatory protein that regulates transcription of LCB1 or the enzymatic activity of SPT.

Besides lcb1-mutant strains, lcb2-mutant strains also lack SPT enzyme activity (Pinto, W. J., Wells, G. W., and Lester, R. L. 1992. *J. Bateriol.* 174:2575–2581). The LCB2 gene was isolated from a *S. cerevisiae* genomic DNA library of complementation for growth on medium lacking phytosphingosine of the lcb2 mutation carried in strain BS238. The strain was transformed with the same recombinant DNA library that was used for isolation of LCB1. Ura^+ transformants were selected, pooled, and replated on plates lacking phytosphingosine to select transformants that could grow in the absence of phytosphingosine (Lcb^+). Plasmid DNA was recovered from Lcb^+ cells by transformation into *E. coli.* Plasmid DNA isolated from *E. coli* was analyzed by restriction digestion. The pattern of restriction fragments indicated that all plasmids carried the same insert of about 7-kb which we designated B7 (FIG. **5**).

LCB2 was localized by subcloning and testing the subclones for their ability to complement the lcb2 mutation in strain BS238 and allow the strain to grow in the absence of phytosphingosine (EXAMPLE 4). These data localized the LCB2 gene to a region near the ApaI site shown in FIG. **1**. DNA around this site was sequenced and the sequence was scanned by computer in all reading frames. There was only one large open reading frame, indicated by the open box at the top of FIG. **5**. The determined DNA sequence and the translated open reading frame representing the putative LCB2 protein are indicated in FIG. **6**.

To prove that this open reading was the LCB2 gene we used the cloned gene to make a chromosomal deletion allele lcb2Δ3::URA3 (EXAMPLE 5), as shown in FIG. **5**. The deletion allele was originally introduced into the diploid strain YPH501 and Southern blotting was used to verify that the deletion strain carried one normal allele and the deletion allele (data not shown). The diploid was sporulated and spores were tested for their Lcb phenotype. All 17 four-spored tetrads showed 2:2 segregation for the Lcb^+:Lcb^- phenotype and all the Lcb^- spores were Ura^+ as expected for a URA3 gene disruption. Thus, the deleted region is needed for long-chain base synthesis as would be expected if the region was the LCB2 gene. To verify that the putative LCB2 gene indicated in FIG. **5** is allelic to the authentic LCB2 gene, we used the integrating vector pRSLCB2-2 (EXAMPLES 6 and FIG. **5**) which only carries the 5' half of the putative LCB2 gene. The plasmid was directed, by digestion with NaiI, to integrate into the genome of strain BS238 (relevant genotype lcb2), at the homologuos NsiI site located in the putative LCB2 gene. Integration at the correct chromosomal location was verified by Southern blotting (data not shown). The strain carrying the integrated plasmid was crossed to strain YPH-500, diploids were selected, and sporulated. Twenty-five four-spored tetrads gave 2 Lcb^+:2 Lcb^- segregation and all of the Lcb^+ spores were Leu^- while the Lcb^+ spores were Leu^+. These data demonstrate that the cloned DNA fragment directs integration at or near the lcb2 allele carried in strain BS238. Taken as a whole the data demonstrate that the LCB2 gene has been cloned.

The predicted sequence of the LCB2 protein is shown in FIG. **6**. The protein contains 561 amino acid residues. Since the amino terminus of the LCB2 protein has not been determined directly it is possible that the amino terminus of the actual protein is different than indicated in FIG. **6**. The difference could occur either because of post-translational processing or because an ATG codon down stream of the one shown in FIG. **6** is used as the initiation codon. A membrane-associated helix is predicted for residues 57 to 77 (PYYISLLTYLNYLILIILGHV) and 443–463 (LGFIVYGVADSPVIPLLLYCP) by the algorithm of Eisenberg et al., (1984).

Comparison of the LCB2 protein sequence against other sequences in GenBank using the FASTA search procedure of Pearson, W. R. and Lipman, D. J., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444–2448 revealed that the sequence was homologous to the LCB1 protein and to various ALA synthases including the one from *S. cerevisiae* (FIG. **7**). In addition, the sequence was homologuos to the BACBIOXWF (Genbank) and the ECOKBLTDH (Genbank, called EKBL (SEQ ID NO.: 8) in FIG. **3**) sequences (data not shown).

The similarity of the LCB2 protein to the ALA synthases and to 2-amino-3-ketobutyrate CoA ligase (EKBL FIG. **3**, ECOKBLTDH Gen Bank) seems particularly significant since these enzymes catalyze a reaction (FIG. **4**) that is very similar to that catalyzed by SPT. In addition, the *E. coli* 2-amino-3-ketobutyrate CoA ligase uses pyridoxal phosphate as a cofactor (Mukherjee and Dekker, 1987) as do serine palmitoyltransferase and ALA synthase. The similarity of the amino acid sequences (FIG. **6**) and the reactions catalyzed by these enzymes (FIG. **4**) argue that the product of LCB2 is most likely SPT or a catalytic subunit of the enzyme, rather than a regulatory protein that regulates transcription of LCB2 or the enzymatic activity of SPT.

Potential uses of the LCB1 and LCB2 genes.

One use of the genes is to construct strains of *S. cerevisiae* or other organisms or cell lines that can be used to screen for inhibitors of SPT enzyme activity or inhibitors of expression of the LCB1 or LCB2 gene at the transcriptional or translational level. To construct a strain for screening inhibitors of SPT activity, one can use the LCB1 and LCB2 genes to overproduce their protein product. Overproduction will yield a host organism relatively more resistant to SPT inhibitors compared to a host that does not overproduce the proteins. This principle was first demonstrated in *S. cerevisiae* by Rine, J., Hansen, W., Hardeman, E., and Davis, R. W. 1983. *Proc. Natl. Acad. Sci. U.S.A.* 80:6750–6754. In the case of an inhibitor of transcription or translation, for example a triple helix-forming oligonucleotide or an antisense koligonucleotide, one can construct a strain carrying multiple copies of the LCB1 and LCB2 genes. Multiple copies should make the strain more resistant to the inhibitor than a strain having only one copy of each gene. A variation of this approach could be used for inhibitors of translation (an antisense oligonucleotide) in which the LCB1 and LCB2 coding regions would be fused to a strong promoter-enhancer region so that a single copy of the fusion genes would give high levels of LCB1 and LCB2 mRNA.

Another use of the LCB1 and LCB2 genes is to overexpress them and overproduce their protein product. Such overproduction usually makes it possible to purify the proteins. Expression and overproduction could be achieved in any number of organisms including *E. coli, S. cerevisiae,* or insect cells or other hosts for baculovirus vectors. The purified protein could then be used to identify or design inhibitors of SPT enzyme activity.

Finally, the LCB1 and LCB2 genes can be used to isolate their homologs from other organisms. Homologs can be isolated by complementation of the lcb1 and lcb2 mutation in appropriate *S. cerevisiae* host strains such as those presented in this application. Alternatively, degenerate primers for the polymerase chain reaction (PCR) could be designed based upon the sequence of LCB1 and LCB2 and used to prime a PCR reaction using genomic or cDNA from the organism whose LCB genes are to be cloned. LCB1 and LCB2 homologs from particular organisms would enable the design of highly specific triple-helix forming or antisense oligonucleotides or for inhibitors of SPT activity unique to a particular organism.

In the examples the following materials were used:

S. cerevisiae: The original lcb mutant MC6A (MATa lcb1-1 inol; Wells and Lester, 1983), was crossed with strain W303-1B (MATa ade2-1 can1-100 ura3-1 his3-11,15 trp1-1 leu2-3,112; obtained from R. J. Rothstein, Columbia, Univ.). Progeny from this cross were backcrossed to W303-1B and several offspring were selected for further study including strains X2A1B (MATa lcb1-1 ura3-1 trp1-1 his3-11,15) and 24D5 (MATα lcb1-1 ura3-1 trp1-1 leu2-3,112 his3-11,15). Strains YPH1(MATa ura3-52 lys2-801 ade2-101,), YPH500 (MATa ura3-52 leu2-801^{amber} leu2-Δ101^{ochre} trp1-Δ63 his3-Δ20 leu2-Δ1), and YPH501 (MATa/a ura3-52 leu2-801^{amber} leu2-101^{ochre} trp1-Δ63 his3Δ20 leu2-d1, were obtained from Sikorski, R. S. and Hieter, P., 1989, *Genetics,* 122:19–27. Strain BS238 (MATa lcb2 ura3-52 leu2-3,112 ade1) was from Pinto, W. J., Srinivasan, B., Shepherd, S., Schmidt, A., Dickson, R. C., and Lester, R. L. 1992. *J. Bacteriol.* 174:2565–2574. Strain SJ21R (MATa ura3-52 leu2-3,112 ade1 MEL1) was described in Dickson, R. C., Wells, G. B., Schmidt, A., and Lester, R. L. 1990. *Mol. Cell. Biol.* 10:2176–2181. The YPH strains are sensitive to the long-chain base phytosphingosine and in order to transform them with DNA it is necessary to use 12.5 μM phytosphingosine and 0.025% tergitol (half of the normal concentrations) in selection plates. Likewise, for genetic crosses involving YPH strains it is necessary to make the same adjustments for dissection plates (minimal medium, Sherman, F., Fink, G. R., and Hicks, T. B. 1986. *Methods in Yeasts Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.) otherwise spores will not germinate.

Escherichia coli: strain DH5a was used for propagation of plasmids.

Media: PYED contained 1% peptone, 1% yeast extract, 2% agar (for plates), 50 mM sodium succinate (pH 5), inositol (50 mg/l), potassium phosphate monobasic (50 mg/ml), and 2% or 4% glucose. Minimal medium contained 1× Difco Yeast Nitrogen Base without amino acids, 50 μM sodium succinate (pH 5), 2% glucose, 1.5% agar (for plates), inositol (50 mg/ml), valine (150 mg/ml), isoleucine (30 mg/ml), threonine (200 mg/ml) and these supplements at 20 mg/l: adenine sulfate, arginine-HCl, histidine-HCl, leucine, lysine-HCl, methionine, tryptophan, and uracil. One or more supplements were omitted from minimal medium for selection of yeast transformants. For strains requiring long chain base the medium was supplemented with 25 μM phytosphingosine (Sigma, St. Louis, Mo.). A 10× stock solution of phytosphingosine was prepared by adding 0.25 ml of 100 μM phytosphingosine (dissolved in 95% ethanol) to 99.75 ml of a 0.5% solution of tergitol (Sigma, St. Louis, Mo.).

DNA sequencing: Synthetic oligonucleotide primers were used for dideoxynucleotide sequencing with Sequenase Version 2.0 DNA Polymerase (USB, Cleveland, Ohio) essentially as recommended by the supplier. The LCB1 sequence (FIG. **2**) has been deposited in the Gen Bank and given accession number M63674. The LCB2 sequence (FIG. **6**) has been deposited in the Gen Bank and given accession number M95669.

Serine palmitoyltransferase activity assays were done as described in Buede, R., Rinker-Schaffer, C., Pinto, W. J., Lester, R. L., and Dickson, R. C. 1991. *J. Bacteriol.* 173:4325–4332.

Miscellaneous Procedures—Yeast were transformed by the lithium acetate procedure described by Sherman, F., Fink, G. R., and Hicks, T. B. 1986. *Methods in Yeasts Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. Genetic crosses and tetrad analysis were done by standard procedures (ibid). Southern blots were done essentially as described by Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. For Southern blots [^{32}P]dATP-labeled probes were prepared by the method of Feinberg, A. P., and Vogelstein, B. 1983. *Anal. Biochem.* 132:6–13.

EXAMPLE 1

The plasmid pLCB (FIG. **1**) was isolated from a *S. cerevisiae* genomic DNA library carried in a vector containing the CEN3 region of *S. cerevisiae* DNA. The 6.44 kb vector was pBR322 with a 0.63 kb Sau3A CEN3 DNA fragment inserted into the PvuII site of the vector and a 1.4 kb TRP1ARS1 fragment inserted into the EcoRI site of the vector. These ligations were done with molecules whose ends were made blunt-ended so that the original restriction sites were destroyed. Sau3A genomic DNA fragments of 8 kb average size from strain X2180 (a/a gal2/gal2) were cloned into the BamHI site of the vector (the library was a gift from Zymogenetics, Seattle, Wash.). To construct YCp50-LCB1, a 4.7 kb StuI fragment from pLCB1 containing the LCB1 region, was subcloned into the NruI site of YCp50 (Rose, M. D. 1987. *Meth. Enzymology.* 152:481–504).

EXAMPLE 2

Strain SL1 as derived from strain SJ21R by replacement of the LCB1 allele with a mutant allele that was disrupted by inserting a 1.1 kb URA3 DNA fragment from *S. cerevisiae* into the SalI site of LCB1 (FIG. **1** shows the SalI site). The lcb1::URA3 -disrupted allele was prepared by ligating a 4.3 kb HindIII-StuI fragment, carrying LCB1, derived from pLCB (FIG. **1**) to pTZ18 (Pharmacia) which had been cleaved with the restriction endonucleases HindIII and SmaI. The resulting plasmid, pTZ18-LCB1 (FIG. 1), was cleaved with SalI and ligated with a 1.1 kb URA3 DNA fragment having SalI cohesive ends to yield pTZ18-LCB1::URA3. To replace the LCB1 chromosomal allele with the URA3-disrupted allele, ten micrograms of pTZ18-LCB1::URA3 DNA was cleaved with XbaI and ClaI, extracted with phenol, phenol:chloroform and chloroform, and precipitated with ethanol. The DNA was transformed into strain SJ21R with selection for Ura$^+$ transformants. Replacement of the LCB1 chromosomal allele with the URA3-disrupted allele was verified by Southern blot analysis. Total DNA isolated from SL1 and the non-disrupted parental strain SJ21R was cleaved with the restriction endonucleases NruI and StuI. Following Southern blot analysis, the parental strain showed a 4 kb band of hybridization, as expected, when the blot was probed with a ^{32}P-labeled NruI to StuI DNA probe containing the LCB1 region (FIG. **1**). If the lcb1::URA3-disrupted allele had replaced the wild type allele of LCB1 in strain SL1 then the Southern blot of strain

SL1 should show two bands that hybridize to the ^{32}P-probe because URA3 contains a StuI cleavage site. The fragments should be 2.1 kb and 3 kb in length. The Southern blot (data not shown) contained the two expected bands of hybridization indicating that strain SL1 carried the lcb1::URA3-disruption allele.

EXAMPLE 3

YIpLCB1-1was constructed by inserting TRP1 of *S. cerevisiae,* as a 1.4 kb Hind III fragment, into the Hind III site of pTZ18-LCB1. YIpLCB1-1 was cleaved at its unique BamHI site (FIG. 1), located on the 3' side of LCB1, and the linear DNA was used to transform strain 24D5 with selection for Ura$^+$ transformants. Integration at the expected chromosomal location was verified by southern blotting. Transformants were crossed to strain YPH1.

EXAMPLE 4

Plasmids carrying all of or portions of LCB2 (FIG. 2) were constructed using standard molecular cloning techniques as follows. Insert B7 is a 7 kb BamHI *S. cerevisiae* DNA fragment cloned into the BamHI site of pRS315 (Sikorski and Hieter, 1989). Insert B7ΔS is a 4.9 kb BamHI-SalI fragment cloned into pRS315 at the BamHI-SalI sites of the polylinker. Insert 2.3 is a 2.3 kb BamHI-SacI fragment cloned into pRS316 (Sikorski and Hieter, 1989) at the BamHI-SacI sites of the polylinker. Insert LCB2-R is a 4.3-kb EcoRI fragment made blunt-ended by filling in the ends with the Klenow fragment of DNA polymerase I and ligated into the SmaI site of pRS315.

EXAMPLE 5

S. cerevisiae strain LCB25, carrying the lcb2Δ3::URA3 allele (FIG. 5), was derived from strain YPH501 as follows: The LCB2-R insert, carried in pIC20R, Marsh, J. L., Erfle, M. and Wykes, E. J., 1984, *Gene* 32:481–485, at the EcoRI site of the polylinker, was cleaved with the restriction endonucleases ClaI and XbaI (FIG. 5), the ends of the molecules were made blunt by treatment with the Klenow fragment of DNA polymerase I, and the fragment was ligated to a 1.1 kb URA3 fragment having blunt ends to give the lcb2Δ3::URA3 allele (FIG. 5).

EXAMPLE 6

The integrating vector pRSLCB2-2 (FIG. 5) was constructed by cloning a 2.6-kb BamHI-ApaI fragment from the B7 insert into the BamHI-ApaI region of the polylinker in pRS305 (Sikorski and Hieter, 1989). pRS305 carries the LEU2 marker gene that was used for selection of transformants in *S. cerevisiae* strain BS238.

DETAILED DESCRIPTION OF THE FIGURES

FIG. 1

Structure of Plasmids. The plasmid pLCB carrying the LCB1 gene is shown. The approximate location of LCB1 is indicated. Not all restriction endonuclease sites are indicated in a given plasmid. The open arrowhead in pTZ18-LCB1 represents the T7 promoter. DNA sequences are: open box, *S. cerevisiae;* TRP1, a marker gene for selection in *S cerevisiae;* ARS1, a *S. cerevisiae* autonomous replication sequence; CEN3 a centromere for maintenance of a single-copy of the vector in yeast; BLA and TET confer ampicillin and tetracycline resistance in *E. coli,* respectively. Abbreviations for restriction endonucleases are: B, BamHl, C, ClaI: E, EcoRI; H, HindIII; Ha, HpaI; K, KpnI; P, PstI; S, SalI; Sa, Sau3A; Sac, SacI; Sm, SmaI; St, StuI; X, XbaI.

FIG. 2

DNA sequence of LCB1. The nucleotide sequence of the LCB1 gene of *S. cervisiae* is presented along with the deduced protein sequence of the 558 amino acids. The predicted translation start codon is indicated by +1.

FIG. 3

Comparison of the deduced amino acid sequence of LCB1 to other proteins. The protein sequences of LCB1 and the mouse (ALSM ((SEQ ID. NO.:10)), chicken (ALSC ((SEQ ID. NO.:11)), and yeast (ALSY ((SEQ ID. NO.:12)) 5-aminolevulinic acid synthases were compared using the procedure of Pearson and Lipman (1988) and aligned for maximum similarity. The 2-amino-3-ketobutyrate CoA ligase (EKBO ((SEQ ID. NO.:8)) and the biotin synthetase (EBIO ((SEQ ID. NO.:7)) sequences were identified and aligned by using the FASTA algorithm (ibid). Colons (:) represent identity between residues while dots (.) denote conservative replacements by similar residues. Insertions made during the alignment optimization process are indicated by dashes (—).

FIG. 4

Comparison of the reactions catalyzed by serine palmitoyltransferase, ALA synthase, and 2-amino-3ketobutyrate CoA ligase.

FIG. 5

Structure of Plasmids. A restriction map of the 7 kb BamHI fragment carrying the LCB2 gene is shown at the top of the figure and the approximate location of LCB2 and the direction of transcription are indicated. Not all of the cutting sites for a particular restriction endonuclease are indicated. A. Portions of the region carrying LCB2 were tested for their ability to complement the Lcb$^-$ phenotype of an lcb2-defective strain. B. Structure of a deletion allele. C. Structure of the chromosomal insert carried in pRSLCB2-2. Vector sequences are not shown. Abbreviations for restriction endonucleases are: A, ApaI; B, BamHl; C, ClaI; E, EcoRI; Ns, NsiI; Sa, SalI; S, SacI; Sn, SnaBl; Sp, SspI; X, XbaI.

FIG. 6

DNA sequence of LCB2. The nucleotide sequence of the LCB2 gene of *S. cervisiae* is presented along with the deduced protein sequence of the 561 amino acids. Numbers on the right side of the figure indicate amino acid residues while numbers on the left indicate nucleotides. The A of the predicted ATG initiation codon has been designated as +1.

FIG. 7

Comparison of the predicted LCB1 ((SEQ ID. NO.:13) and LCB2 ((SEQ ID. NO.:14) protein sequences with each other (identical residues indicated by an asterisk above the sequence) and with the ALA synthase of *S. cervisiae* (HEM1$Yeast ((SEQ ID. NO.:15)). Asterisks below the sequence indicate amino acids that are identical in all three sequences while dots (.) indicate amino acids that are similar in the three sequences. Dashes (—) indicate gaps in the sequence introduced to improve alignment.

The invention now being fully described it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth therein. The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept and therefore such adaptations are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description only and not of limitation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 15

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polynucleotide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CGCGTATTTT TTTTTTTTTG AGGCGCCATG ATTTCTTACA CGGTTTCTTT TTTTTTTCCT   60

TCTTTCCTTC TTGCTTCTCT GCTAACAAAT TTTTCACTCA TTCTTTTTTA TAGGGGCATA  120

TTGCTGCGGT TAACTGTAGT GAACGAAAGT AAGATTGAGA AAATATAGTA CTTAAGAAAA  180

AGAAAAGGAA AAATAAAAAA AATTCTTTTC AACATCATCG AGTAGCACAG TATAAGAGCG  240

CTCTAACCTT CTGCCTGGCC TCCAATATAC ACATTTTGCT CGTGTAGGGT TATTTATCCT  300

TTTTTCTTCC TTCCCACCCA AAAAAAAAAA GCA                               333
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1674
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG GCA CAC ATC CCA GAG GTT TTA CCC AAA TCA ATA CCG ATT CCG GCA    48
MET Ala His Ile Pro Glu Val Leu Pro Lys Ser Ile Pro Ile Pro Ala
                5                  10                  15

TTT ATT GTT ACC ACC TCA TCG TAC CTA TGG TAC TAC TTC AAT CTG GTG    96
Phe Ile Val Thr Thr Ser Ser Tyr Leu Trp Tyr Tyr Phe Asn Leu Val
            20                  25                  30

TTG ACT CAA ATC CCG GGA GGC CAA TTC ATC GTT TCG TAC ATC AAG AAA   144
Leu Thr Gln Ile Pro Gly Gly Gln Phe Ile Val Ser Tyr Ile Lys Lys
        35                  40                  45

TCG CAT CAT GAC GAT CCA TAC AGG ACC ACG GTT GAG ATA GGG CTT ATT   192
Ser His His Asp Asp Pro Tyr Arg Thr Thr Val Glu Ile Gly Leu Ile
    50                  55                  60

TTA TAC GGG ATC ATC TAT TAC TTG TCC AAG CCA CAA CAG AAA AAG AGT   240
Leu Tyr Gly Ile Ile Tyr Tyr Leu Ser Lys Pro Gln Gln Lys Lys Ser
65                  70                  75                  80

CTT CAA GCA CAG AAG CCC AAC CTA TCG CCC CAG GAG ATT GAC GCG CTA   288
Leu Gln Ala Gln Lys Pro Asn Leu Ser Pro Gln Glu Ile Asp Ala Leu
                85                  90                  95

ATT GAG GAC TGG GAG CCC GAG CCT CTA GTC GAC CCT TCT GCC ACC GAT   336
Ile Glu Asp Trp Glu Pro Glu Pro Leu Val Asp Pro Ser Ala Thr Asp
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-continued

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                100                 105                 110

GAG CAA TCG TGG AGG GTG GCC AAA ACA CCC GTC ACC ATG GAA ATG CCC   384
Glu Gln Ser Trp Arg Val Ala Lys Thr Pro Val Thr MET Glu MET Pro
        115                 120                 125

ATT CAG AAC CAT ATT ACT ATC ACC AGA AAC AAC CTG CAG GAG AAG TAT   432
Ile Gln Asn His Ile Thr Ile Thr Arg Asn Asn Leu Gln Glu Lys Tyr
    130                 135                 140

ACC AAT GTT TTC AAT TTG GCC TCG AAC AAC TTT TTG CAA TTG TCC GCT   480
Thr Asn Val Phe Asn Leu Ala Ser Asn Asn Phe Leu Gln Leu Ser Ala
145                 150                 155                 160

ACG GAG CCC GTG AAA GAA GTG GTC AAG ACC ACT ATC AAG AAT TAC GGT   528
Thr Glu Pro Val Lys Glu Val Val Lys Thr Thr Ile Lys Asn Tyr Gly
                165                 170                 175

GTG GGC GCC TGT GGT CCC GCC GGG TTC TAC GGT AAC CAG GAC GTT CAT   576
Val Gly Ala Cys Gly Pro Ala Gly Phe Tyr Gly Asn Gln Asp Val His
            180                 185                 190

TAC ACG TTG GAA TAT GAT TTA GCA CAG TTC TTT GGC ACC CAA GGT TCC   624
Tyr Thr Leu Glu Tyr Asp Leu Ala Gln Phe Phe Gly Thr Gln Gly Ser
        195                 200                 205

GTT CTG TAC GGG CAA GAC TTT TGT GCC GCA CCC TCT GTT CTG CCT GCT   672
Val Leu Tyr Gly Gln Asp Phe Cys Ala Ala Pro Ser Val Leu Pro Ala
    210                 215                 220

TTC ACA AAG CGT GGT GAT GTT ATC GTG GCA GAC GAC CAG GTG TCA TTA   720
Phe Thr Lys Arg Gly Asp Val Ile Val Ala Asp Asp Gln Val Ser Leu
225                 230                 235                 240

CCA GTG CAA AAT GCT CTG CAA CTA AGC AGA TCC ACA GTC TAC TAC TTC   768
Pro Val Gln Asn Ala Leu Gln Leu Ser Arg Ser Thr Val Tyr Tyr Phe
                245                 250                 255

AAC CAC AAC GAT ATG AAT TCG CTA GAA TGT TTA TTA AAC GAG TTG ACC   816
Asn His Asn Asp MET Asn Ser Leu Glu Cys Leu Leu Asn Glu Leu Thr
            260                 265                 270

GAA CAG GAG AAA CTT GAG AAA CTG CCC GCC ATT CCA AGA AAA TTT ATC   864
Glu Gln Glu Lys Leu Glu Lys Leu Pro Ala Ile Pro Arg Lys Phe Ile
        275                 280                 285

GTC ACT GAG GGT ATT TTC CAC AAC TCG GGC GAT TTA GCT CCG TTG CCT   912
Val Thr Glu Gly Ile Phe His Asn Ser Gly Asp Leu Ala Pro Leu Pro
    290                 295                 300

GAG TTG ACT AAG CTG AAG AAC AAG TAC AAG TTC AGA CTA TTT GTT GAC   960
Glu Leu Thr Lys Leu Lys Asn Lys Tyr Lys Phe Arg Leu Phe Val Asp
305                 310                 315                 320

GAA ACC TTC TCC ATT GGT GTT CTT GGC GCT ACG GGC CGT GGG TTG TCA  1008
Glu Thr Phe Ser Ile Gly Val Leu Gly Ala Thr Gly Arg Gly Leu Ser
                325                 330                 335

GAG CAC TTC AAC ATG GAT CGC GCA ACT GCC ATT GAC ATT ACC GTT GGG  1056
Glu His Phe Asn MET Asp Arg Ala Thr Ala Ile Asp Ile Thr Val Gly
            340                 345                 350

TCC ATG GCC ACC GCG TTG GGG TCC ACC GGT GGT TTT GTC CTG GGT GAC  1104
Ser MET Ala Thr Ala Leu Gly Ser Thr Gly Gly Phe Val Leu Gly Asp
        355                 360                 365

AGT GTT ATG TGT TTG CAC CAG CGT ATT GGT TCC AAT GCA TAT TGT TTT  1152
Ser Val MET Cys Leu His Gln Arg Ile Gly Ser Asn Ala Tyr Cys Phe
    370                 375                 380

TCT GCC TGT TTG CCG GCT TAC ACC GTC ACA TCC GTC TCC AAA GTC TTG  1200
Ser Ala Cys Leu Pro Ala Tyr Thr Val Thr Ser Val Ser Lys Val Leu
385                 390                 395                 400

AAA TTG ATG GAC TCC AAC AAC GAC GCC GTC CAG ACG CTG CAA AAA CTA  1248
Lys Leu MET Asp Ser Asn Asn Asp Ala Val Gln Thr Leu Gln Lys Leu
                405                 410                 415

TCC AAA TCT TTG CAT GAT TCC TTT GCA TCT GAC GAC TCC TTG CGT TCA  1296
Ser Lys Ser Leu His Asp Ser Phe Ala Ser Asp Asp Ser Leu Arg Ser
```

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                420                 425                 430

TAC GTA ATC GTC ACG TCC TCT CCA GTG TCT CCT GTC CTA CAT CTG CAA      1344
Tyr Val Ile Val Thr Ser Ser Pro Val Ser Pro Val Leu His Leu Gln
        435                 440                 445

CTG ACT CCC GCA TAT AGG TCT CGC AAG TTC GGA TAC ACC TGC GAA CAG      1392
Leu Thr Pro Ala Tyr Arg Ser Arg Lys Phe Gly Tyr Thr Cys Glu Gln
    450                 455                 460

CTA TTC GAA ACC ATG TCA GCT TTG CAA AAG AAG TCC CAG ACA AAC AAA      1440
Leu Phe Glu Thr MET Ser Ala Leu Gln Lys Lys Ser Gln Thr Asn Lys
465                 470                 475                 480

TTC ATT GAG CCA TAC GAA GAG GAG GAA AAA TTT CTG CAG TCC ATA GTA      1488
Phe Ile Glu Pro Tyr Glu Glu Glu Glu Lys Phe Leu Gln Ser Ile Val
                485                 490                 495

GAT CAT GCT CTT ATT AAC TAC AAC GTT CTC ATC ACA AGA AAC ACT ATT      1536
Asp His Ala Leu Ile Asn Tyr Asn Val Leu Ile Thr Arg Asn Thr Ile
            500                 505                 510

GTT TTA AAA CAG GAG ACG CTA CCA ATT GTC CCT AGC TTG AAA ATC TGC      1584
Val Leu Lys Gln Glu Thr Leu Pro Ile Val Pro Ser Leu Lys Ile Cys
        515                 520                 525

TGT AAC GCC GCC ATG TCC CCA GAG GAA CTC AAA AAT GCT TGC GAA AGT      1632
Cys Asn Ala Ala MET Ser Pro Glu Glu Leu Lys Asn Ala Cys Glu Ser
    530                 535                 540

GTC AAG CAG TCC ATC CTT GCC TGT TGC CAA GAA TCT AAT AAA              1674
Val Lys Gln Ser Ile Leu Ala Cys Cys Gln Glu Ser Asn Lys
545                 550                 555
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 463
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polynucleotide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
TAAAAATAGA AAGCCAGTAT ATGCACACGC ACATATATAT ATAAATATTT ATACAATAAT      60

ACAAATAATC GTAACATCAT CTCTGTCAAA TTGACGTGGT GCACGGCGCC CAGAGAATGC     120

GCTAAAAATT TTCGGATCCG AAATTTTCTT TCCTTTTACC ATCGAGGCAA AGCAACCTGT     180

ATTATTTATT TGTTTATTTA TTAATAGAAA AGAAAGGAGT ACTTTCGTGG TACGCTTTCT     240

TGAGCATTTT CGGTTTCACT AGGCAGAGAA CTAACACAAG AGACACAGCA AACATCAAAC     300

AAGGTTAAAA CAGCACACCA AGGCAATATG ATGCATTTTA GAAAGAAATC CAGTATCAGT     360

AACACGAGTG ATCATGACGG AGCGAACCGT GCCTCAGATG TCAAGATTTC TGAAGATGAC     420

AAGGCAAGAT TGAAGATGCG TACTGCTTCC GTTGCTGATC CTA                       463
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 884
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polynucleotide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GCAAATATTG ATTCTCGATG AGGCATTTTC TGGAATGGAG GTAGAACCTA TGATGCGTTG      60

TCATGAATTT TTAGAGGAGT GGCCTGGAAC AGTCCTTGTA GTGGCACACG TTGCCGAAGA     120
```

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```
GACACCAAAA TGTGCCCATT ACTTAAGGCT CATATCTCCT GGAGAGTATG AAATAGGCGA   180

TATGGAAAAT TAAAGTTTTC TGTTGTGTGG CAGCAAGAGA CAGAACCTCG ATAATTTGAC   240

ATACGTATAT AATAGTACAT GTACATAAAA ACGTACGCAA ATATCGTATA TCTGTTATAC   300

TACAAAACAA TTACTTCTAT ATCATAGCCA GTTAGCGGGA ACGACTTCAG CTAAATGGAC   360

TATCCATGCT TTAGGCAGAG GCGAAGCGCG GTGATTGGGT GTAACATCAT CTCCTTTTCT   420

CTACGACAAA TTCCCAAAAA AAAAATTTAT GCTATGTTAA TACCTGCACA ATTCAACCGT   480

GCTGAAACGT AAAATTAAGG TGATTATACG GATAGTATAC GATATTATCA ATCTCATAAG   540

AAAAATCTCT TTTGAATTTA ACGGAGGGAT TATTCATTAG AAAGCGTTCT TACCATTCAC   600

TAGGAGCGAA TCCGTGGAAG GTGTTTTAAC GTTGCCACGA AAAACAGCTC TACATCGAAA   660

TAAAAGACAA CAATCAGTGC CCGTAAGTTT CATTACTATT TTCTATTATT ATCTGCAACT   720

TTTTATTAGT TAGGTTTTTT TTGTTTGTTT GTTTGTTTTC AATTGATTAA TTTACAAGAC   780

AAAGAACCTT ATATTTCGTG TTTTTCATTC TAAAGGAAAA AAAGCATAAA GAAGATTCCA   840

CACACTTTAT TGTGATAGTT TTCAAAGTAA AAAGTAATAG ATTA                     884
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1683
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TGA GTA CTC CTG CAA ACT ATA CCC GTG TGC CCC TGT GCG AAC CAG AGG      48
Met Ser Thr Pro Ala Asn Tyr Thr Arg Val Pro Leu Cys Glu Pro Glu
                5                  10                  15

AGC TGC CAG ACG ACA TAC AAA AAG AAA ATG AAT ATG GTA CAC TAG ATT      96
Glu Leu Pro Asp Asp Ile Gln Lys Glu Asn Glu Tyr Gly Thr Leu Asp
            20                  25                  30

CTC CGG GGC ATT TGT ATC AAG TCA AGT CAC GTC ATG GGA AGC CAC TAC     144
Ser Pro Gly His Leu Tyr Gln Val Lys Ser Arg His Gly Lys Pro Leu
        35                  40                  45

CTG AGC CCG TTG TCG ACA CCC CTC CTT ATT ACA TTT CTT TGT TAA CAT     192
Pro Glu Pro Val Val Asp Thr Pro Pro Tyr Tyr Ile Ser Leu Leu Thr
    50                  55                  60

ATC TAA ATT ATT TGA TTC TGA TTA TAT TAG GTC ATG TTC ACG ACT TCT     240
Tyr Leu Asn Try Leu Ile Leu Ile Ile Leu Gly His Val His Asp Phe
65                  70                  75                  80

TAG GTA TGA CCT TCC AAA AAA ACA AAC ATC TGG ATC TTT TAG AGC ATG     288
Leu Gly Met Thr Phe Gln Lys Asn Lys His Leu Asp Leu Leu Glu His
                85                  90                  95

ATG GGT TAG CAC CTT GGT TTT CAA ATT TCG AGA GTT TTT ATG TCA GGA     336
Asp Gly Leu Ala Pro Trp Phe Ser Asn Phe Glu Ser Phe Tyr Val Arg
            100                 105                 110

GAA TTA AAA TGA GAA TTG ATG ATT GCT TTT CTA GAC CAA CTA CTG GTG     384
Arg Ile Lys Met Arg Ile Asp Asp Cys Phe Ser Arg Pro Thr Thr Gly
        115                 120                 125

TTC CTG GTA GAT TTA TTC GTT GTA TTG ATA GAA TTT CTC ATA ATA TAA     432
Val Pro Gly Arg Phe Ile Arg Cys Ile Asp Arg Ile Ser His Asn Ile
    130                 135                 140

ATG AGT ATT TTA CCT ACT CAG GCG CAG TGT ATC CAT GCA TGA ACT TAT     480
Asn Glu Tyr Phe Thr Tyr Ser Gly Ala Val Tyr Pro Cys Met Asn Leu
145                 150                 155                 160

CAT CAT ATA ACT ATT TAG GCT TCG CAC AAA GTA AGG GTC AAT GTA CCG      528
```

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```
Ser Ser Tyr Asn Tyr Leu Gly Phe Ala Gln Ser Lys Gly Gln Cys Thr
                165                 170                 175

ATG CCG CCT TGG AAT CTG TCG ATA AAT ATT CTA TTC AAT CTG GTG GTC   576
Asp Ala Ala Leu Glu Ser Val Asp Lys Tyr Ser Ile Gln Ser Gly Gly
            180                 185                 190

CAA GAG CTC AAA TCG GTA CCA CAG ATT TGC ACA TTA AAG CAG AGA AAT   624
Pro Arg Ala Gln Ile Gly Thr Thr Asp Leu His Ile Lys Ala Glu Lys
        195                 200                 205

TAG TTG CTA GAT TTA TCG GTA AGG AGG ATG CCC TCG TTT TTT CGA TGG   672
Leu Val Ala Arg Phe Ile Gly Lys Glu Asp Ala Ley Val Phe Ser Met
    210                 215                 220

GTT ATG GTA CAA ATG CAA ACT TGT TCA ACG CTT TCC TCG ATA AAA AGT   720
Gly Tyr Gly Thr Asn Ala Asn Leu Phe Asn Ala Phe Leu Asp Lys Lys
225                 230                 235                 240

GTT TAG TTA TCT CTG ACG AAT TGA ACC ACA CCT CTA TTA GAA CAG GTG   768
Cys Leu Val Ile Ser Asp Glu Leu Asn His Thr Ser Ile Arg Thr Gly
                245                 250                 255

TTA GGC TTT CTG GTG CTG CTG TGC GAA CTT TCA AGC ATG GTG ATA TGG   816
Val Arg Leu Ser Gly Ala Ala Val Arg Thr Phe Lys His Gly Asp Met
            260                 265                 270

TGG GTT TAG AAA AGC TTA TCA GAG AAC AGA TAG TAC TTG GTC AAC CAA   864
Val Gly Leu Glu Lys Leu Ile Arg Glu Gln Ile Val Leu Gly Gln Pro
        275                 280                 285

AAA CAA ATC GTC CAT GGA AGA AAA TTT TAA TTT GCG CAG AAG GGT TGT   912
Lys Thr Asn Arg Pro Trp Lys Lys Ile Leu Ile Cys Ala Glu Gly Leu
    290                 295                 300

TTT CCA TGG AAG GTA CTT TGT GTA ACT TGC CAA AAT TGG TTG AAT TGA   960
Phe Ser Met Glu Gly Thr Leu Cys Asn Leu Pro Lys Leu Val Glu Leu
305                 310                 315                 320

AGA AGA AAT ATA AAT GTT ACT TGT TTA TCG ATG AAG CCC ATT CTA TAG  1008
Lys Lys Lys Tyr Lys Cys Tyr Leu Phe Ile Asp Glu Ala His Ser Ile
                325                 330                 335

GCG CTA TGG GCC CAA CTG GTC GCG GTG TTT GTG AAA TAT TTG GCG TTG  1056
Gly Ala Met Gly Pro Thr Gly Arg Gly Val Cys Glu Ile Phe Gly Val
            340                 345                 350

ATC CCA AGG ACG TCG ACA TTC TAA TGG GTA CTT TCA CTA AGT CGT TTG  1104
Asp Pro Lys Asp Val Asp Ile Leu Met Gly Thr Phe Thr Lys Ser Phe
        355                 360                 365

GTG CTG CTG GTG GTT ACA TTG CTG CTG ATC AAT GGA TTA TCG ATA GAC  1152
Gly Ala Ala Gly Gly Tyr Ile Ala Ala Asp Gln Trp Ile Ile Asp Arg
    370                 375                 380

TGA GGT TGG ATT TAA CCA CTG TGA GTT ATA GTG AGT CAA TGC CGG CTC  1200
Leu Arg Leu Asp Leu Thr Thr Val Ser Tyr Ser Glu Ser Met Pro Ala
385                 390                 395                 400

CTG TTT TAG CTC AAA CTA TTT CCT CAT TAC AAA CCA TTA GTG GTG AAA  1248
Pro Val Leu Ala Gln Thr Ile Ser Ser Leu Gln Thr Ile Ser Gly Glu
                405                 410                 415

TAT GTC CCG GAC AAG GTA CTG AAA GAT TGC AAC GTA TAG CCT TTA ATT  1296
Ile Cys Pro Gly Gln Gly Thr Glu Arg Leu Gln Arg Ile Ala Phe Asn
            420                 425                 430

CCC GTT ATC TAC GTT TAG CTT TGC AAA GGT TAG GAT TTA TTG TCT ACG  1344
Ser Arg Tyr Leu Arg Leu Ala Leu Gln Arg Leu Gly Phe Ile Val Tyr
        435                 440                 445

GTG TGG CTG ACT CAC CAG TTA TTC CCT TAC TAC TGT ATT GTC CCT CAA  1392
Glu Val Ala Asp Ser Pro Val Ile Pro Leu Leu Leu Tyr Cys Pro Ser
    450                 455                 460

AGA TGC CCG CAT TTT CGA GAA TGA TGT TAC AAA GAC GGA TTG CTG TTG  1440
Lys Met Pro Ala Phe Ser Arg Met Met Leu Gln Arg Arg Ile Ala Val
465                 470                 475                 480

TTG TTG TTG CTT ATC CTG CTA CTC CGC TGA TCG AAT CAA GAG TAA GAT  1488
```

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```
Val Val Val Ala Tyr Pro Ala Thr Pro Leu Ile Glu Ser Arg Val Arg
                485                 490                 495

TCT GTA TGT CTG CAT CTT TAA CAA AGG AAG ATA TCG ATT ATT TAC TGC      1536
Phe Cys Met Ser Ala Ser Leu Thr Lys Glu Asp Ile Asp Tyr Leu Leu
            500                 505                 510

GTC ATG TTA GTG AAG TTG GTG ACA AAT TGA ATT TGA AAT CAA ATT CCG      1584
Arg His Val Ser Glu Val Gly Asp Lys Leu Asn Leu Lys Ser Asn Ser
        515                 520                 525

GCA AAT CCA GTT ACG ACG GTA AAC GTC AAA GAT GGG ACA TCG AGG AAG      1632
Gly Lys Ser Ser Tyr Asp Gly Lys Arg Gln Arg Trp Asp Ile Glu Glu
    530                 535                 540

TTA TCA GGA GAA CAC CTG AAG ATT GTA AGG ACG ACA AGT ATT TTG TTA      1680
Val Ile Arg Arg Thr Pro Glu Asp Cys Lys Asp Asp Lys Tyr Phe Val
545                 550                 555                 560

ATT                                                                  1683
Asn
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 313
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polynucleotide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GAATTTTACC TAATTGCTAG TTAGGTGAAA AATTACAAAA TTTCTGGAAG ACGTTGGAAA      60

CACGCAACGT CTTTTTGACA TAAACTTAAA ACTGCCAAAA GTCAAACAAA AATTGCAAAA     120

AAAGTAAAAA AAGTTACGAA AAAAAAAACA TTTAAAAGAA AGAAGAAGTT AAAAGTGCAC     180

GCAATATGTT CCAGGATATG AAATGAAATA CCTTTTGTTT CACCTTTTAA ATAATTTAAT     240

GTTATATATA CAACTTTATC GTATCATATT CGCAATTACA TTATACAAGA ATGAGTTTTT     300

TTTCGCGACA AAG                                                       313
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Gln Met Val Val Thr Glu Gly Val Phe Ser Met Asp Gly Asp Ser Ala
                5                  10                  15

Pro Leu Ala Glu Ile Gln Gln Val Thr Gln Gln His Asn Gly Trp Leu
            20                  25                  30

Met Val Asp Asp Ala His Gly Thr Gly Val Ile Gly Glu Gln Gly Arg
        35                  40                  45

Gly
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Ile Cys Gly Thr Gln Asp Ser His Lys Glu Leu Glu Gln Lys Leu
5 10 15

Ala Ala Phe Leu Gly Met Glu Asp Ala Ile Leu Tyr Ser Ser Cys Phe
20 25 30

Asp Ala Asn Gly Gly Leu Phe Glu Thr Leu Leu Gly Xaa Xaa Ala Glu
35 40 45

Asp Ala Ile Ile Ser Asp Ala Leu Asn His Ala Ser Ile Ile Asp Gly
50 55 60

Val Arg Leu Cys Lys Ala Lys Arg Tyr Arg Tyr Ala Asn Asn Asp Met
65 70 75 80

Gln Glu Leu Glu Ala Arg Leu Lys Glu Ala Arg Glu Arg Glu Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Ala Arg His Xaa Val Leu Ile Ala Thr Asp Gly Leu
100 105 110

Phe Ser Met Asp Gly Val Ile Ala Asn Leu Lys Gly Val Cys Asp Leu
115 120 125

Ala Asp Lys Tyr
130

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Ala Ser Asn Asn Phe Leu Gln Leu Ser Ala Thr Glu Pro Val Lys
5 10 15

Glu Val Val Lys Thr Thr Ile Lys Asn Tyr Gly Val Gly Ala Cys Gly
20 25 30

Pro Ala Gly Phe Tyr Gly Asn Gln Asp Val His Tyr Thr Leu Glu Tyr
35 40 45

Asp Leu Ala Gln Phe Phe Gly Thr Gln Gly Ser Val Leu Tyr Gly Gln
50 55 60

Asp Phe Cys Ala Ala Pro Ser Val Leu Pro Ala Phe Thr Lys Xaa Xaa
65 70 75 80

Arg Gly Asp Val Ile Val Ala Asp Asp Gln Val Ser Leu Pro Val Gln
85 90 95

Asn Ala Leu Gln Leu Ser Arg Ser Thr Val Tyr Tyr Phe Asn His Asn
100 105 110

Asp Met Asn Ser Leu Glu Cys Leu Leu Asn Glu Leu Thr Glu Gln Glu
115 120 125

Lys Leu Glu Lys Leu Pro Ala Ile Pro Arg Lys Phe Ile Val Thr Glu
130 135 140

Gly Ile Phe His Asn Ser Gly Asp Leu Ala Pro Leu Pro Glu Leu Thr
145 150 155 160

Lys Leu Lys Asn Lys Tyr Lys Phe Arg Leu Phe Val Asp Glu Thr Phe
165 170 175

Ser Ile Gly Val Leu Gly Ala Thr Gly Arg Gly Leu Xaa Xaa Xaa Xaa
180 185 190

-continued

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
195 200 205

Xaa Xaa Ser Glu His Xaa Xaa Phe Asn Met Asp Arg Ala Thr Ala Ile
210 215 220

Asp Ile Thr Val Gly Ser Met Ala Thr Ala Leu Gly Ser Thr Gly Gly
225 230 235 240

Phe Val Leu Gly Asp Ser Val Met Cys Leu His Gln Arg Ile Gly Ser
245 250 255

Asn Ala Tyr Cys Phe Ser Ala Cys Leu Pro Ala Tyr Thr Val Thr Ser
260 265 270

Val Ser Lys Val Leu Lys Leu Met Asp Ser Asn Asn Asp Ala Val
275 280 285

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Trp Cys Ser Asn Asp Tyr Leu Gly Ile Ser Arg His Pro Arg Val Leu
5 10 15

Gln Ala Ile Glu Glu Thr Leu Lys Asn His Gly Ala Gly Ala Gly Gly
20 25 30

Thr Arg Asn Ile Ser Gly Thr Ser Lys Phe His Val Glu Leu Glu Gln
35 40 45

Glu Leu Ala Glu Leu His Gln Lys Asp Ser Ala Leu Leu Phe Ser Ser
50 55 60

Cys Phe Val Ala Asn Asp Ser Thr Leu Phe Thr Leu Ala Lys Leu Leu
65 70 75 80

Pro Gly Cys Glu Ile Tyr Ser Asp Ala Gly Asn His Ala Ser Met Ile
85 90 95

Gln Gly Ile Arg Asn Ser Gly Ala Ala Lys Phe Val Phe Arg His Asn
100 105 110

Asp Pro Gly His Leu Lys Lys Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
115 120 125

Xaa Xaa Glu Lys Ser Asp Pro Lys Thr Pro Lys Ile Val Ala Phe Glu
130 135 140

Thr Val His Ser Met Asp Gly Ala Ile Cys Pro Leu Glu Glu Leu Cys
145 150 155 160

Asp Val Ala His Gln Tyr Gly Ala Leu Thr Phe Val Asp Glu Val His
165 170 175

Ala Val Gly Leu Tyr Gly Ala Arg Gly Ala Gly Ile Xaa Xaa Xaa Xaa
180 185 190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
195 200 205

Xaa Xaa Gly Glu Arg Xaa Xaa Xaa Xaa Asp Gly Ile Met His Lys Leu
210 215 220

Asp Ile Ile Ser Gly Thr Leu Gly Lys Ala Phe Gly Cys Val Gly Gly
225 230 235 240

Tyr Ile Ala Ser Thr Arg Asp Leu Val Asp Met Val Arg Ser Tyr Ala
245 250 255

Ala Gly Phe Ile Phe Thr Thr Ser Leu Pro Pro Met Met Leu Ser Gly

-continued

260 265 270

Ala Leu Glu Ser Val Arg Leu Leu Lys Gly Glu Glu Gly Gln Ala
275 280 285

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Trp Cys Ser Asn Asp Tyr Leu Gly Met Ser Arg His Pro Arg Val Cys
5 10 15

Gly Ala Val Met Asp Thr Lys Leu Gln His Gly Ala Gly Ala Gly Gly
20 25 30

Thr Arg Asn Ile Ser Gly Thr Ser Lys Phe His Val Asp Leu Glu Lys
35 40 45

Glu Leu Ala Asp Leu His Gly Lys Asp Ala Ala Leu Leu Phe Ser Ser
50 55 60

Cys Phe Val Ala Asn Asp Ser Thr Leu Phe Thr Leu Ala Lys Met Leu
65 70 75 80

Pro Gly Cys Gln Ile Tyr Ser Asp Ser Gly Asn His Ala Ser Met Ile
85 90 95

Gln Gly Ile Arg Asn Ser Arg Val Pro Lys His Ile Phe Arg His Asn
100 105 110

Asp Val Asn His Leu Arg Glu Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
115 120 125

Xaa Xaa Lys Lys Ser Asp Pro Ser Thr Pro Lys Ile Val Ala Phe Glu
130 135 140

Thr Val His Ser Met Asp Gly Ala Val Cys Pro Leu Glu Glu Leu Cys
145 150 155 160

Asp Val Ala His Glu His Gly Ala Ile Thr Phe Val Asp Glu Val His
165 170 175

Ala Val Gly Leu Tyr Gly Ala Arg Gly Gly Gly Ile Xaa Xaa Xaa Xaa
180 185 190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
195 200 205

Xaa Xaa Gly Asp Arg Xaa Xaa Xaa Xaa Asp Gly Val Met His Lys Met
210 215 220

Asp Ile Ile Ser Gly Thr Leu Gly Lys Ala Phe Ala Cys Val Gly Gly
225 230 235 240

Tyr Ile Ser Ser Thr Ser Ala Leu Ile Asp Thr Val Arg Ser Tyr Ala
245 250 255

Ala Gly Phe Ile Phe Thr Thr Ser Leu Pro Pro Met Leu Leu Ala Gly
260 265 270

Ala Leu Glu Ser Val Arg Thr Leu Lys Ser Ala Glu Gly Gln Val
275 280 285

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Trp Cys Ser Asn Lys Tyr Leu Ala Leu Ser Lys His Pro Glu Val Leu
5                                   10                  15

Asp Ala Met His Lys Thr Ile Asp Lys Tyr Gly Cys Gly Ala Gly Gly
            20                  25                  30

Thr Arg Asn Ile Ala Gly His Asn Ile Pro Thr Leu Asn Leu Glu Ala
        35                  40                  45

Glu Leu Ala Thr Leu His Lys Lys Glu Gly Ala Leu Val Phe Ser Ser
    50                  55                  60

Cys Tyr Val Ala Asn Asp Ala Val Leu Ser Leu Leu Gly Gln Lys Met
65                  70                  75                  80

Lys Asp Leu Val Ile Phe Ser Asp Glu Leu Asn His Ala Ser Met Ile
                85                  90                  95

Val Gly Ile Lys His Ala Asn Val Lys Lys His Ile Phe Lys His Asn
            100                 105                 110

Asp Leu Asn Glu Leu Glu Gln Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        115                 120                 125

Xaa Xaa Gln Ser Tyr Pro Lys Ser Val Pro Lys Leu Ile Ala Phe Glu
    130                 135                 140

Ser Val Tyr Ser Met Ala Gly Ser Val Ala Asp Ile Glu Lys Ile Cys
145                 150                 155                 160

Asp Leu Ala Asp Lys Tyr Gly Ala Leu Thr Phe Leu Asp Glu Val His
                165                 170                 175

Ala Val Gly Leu Tyr Gly Pro His Gly Ala Gly Val Ala Glu His Cys
            180                 185                 190

Asp Phe Glu Ser His Arg Ala Ser Gly Ile Ala Thr Pro Lys Thr Asn
        195                 200                 205

Asp Lys Gly Gly Ala Xaa Xaa Xaa Xaa Lys Thr Val Met Asp Arg Val
    210                 215                 220

Asp Met Ile Thr Gly Thr Leu Gly Lys Ser Phe Gly Ser Val Gly Gly
225                 230                 235                 240

Tyr Gly Ala Ala Ser Arg Lys Leu Ile Asp Trp Phe Arg Ser Phe Ala
                245                 250                 255

Pro Gly Phe Ile Phe Thr Thr Thr Leu Pro Pro Ser Val Met Ala Gly
            260                 265                 270

Ala Thr Ala Ala Ile Arg Tyr Gln Arg Cys His Ile Asp Leu Arg
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Ala His Ile Pro Glu Xaa Xaa Xaa Xaa Val Leu Pro Lys Ser Ile
5                                   10                  15

Pro Ile Pro Ala Phe Ile Val Thr Thr Ser Ser Tyr Leu Trp Tyr Tyr
            20                  25                  30

Phe Asn Leu Val Leu Thr Gln Ile Pro Gly Gly Gln Phe Ile Val Ser
        35                  40                  45
```

-continued

Tyr Ile Lys Lys Ser His His Asp Asp Pro Tyr Arg Thr Thr Val Glu
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Ile Gly Leu Ile Leu Tyr Gly Xaa Xaa Xaa
65 70 75 80

Ile Ile Tyr Tyr Leu Ser Lys Pro Gln Gln Lys Lys Ser Leu Gln Ala
85 90 95

Gln Lys Pro Asn Xaa Xaa Xaa Xaa Leu Ser Pro Gln Glu Ile Asp Ala
100 105 110

Leu Ile Glu Asp Trp Glu Pro Glu Pro Leu Val Asp Pro Ser Ala Thr
115 120 125

Asp Glu Gln Ser Trp Arg Val Ala Lys Thr Pro Val Thr Met Glu Met
130 135 140

Pro Ile Xaa Gln Asn His Ile Thr Ile Thr Arg Asn Asn Leu Gln Glu
145 150 155 160

Lys Tyr Thr Xaa Xaa Xaa Xaa Asn Val Phe Xaa Xaa Xaa Asn Leu Ala Ser
165 170 175

Asn Asn Phe Leu Gln Leu Ser Ala Thr Glu Xaa Pro Val Lys Glu Val
180 185 190

Val Lys Thr Thr Ile Lys Asn Tyr Gly Val Gly Ala Cys Gly Pro Ala
195 200 205

Gly Phe Tyr Gly Asn Gln Asp Val His Tyr Thr Leu Glu Tyr Asp Leu
210 215 220

Ala Gln Phe Phe Gly Thr Gln Gly Ser Val Leu Tyr Gly Gln Asp Phe
225 230 235 240

Cys Ala Ala Pro Ser Val Leu Pro Ala Phe Thr Lys Arg Xaa Gly Asp
245 250 255

Val Ile Val Xaa Ala Asp Asp Gln Val Ser Leu Pro Val Gln Asn Ala
260 265 270

Leu Gln Leu Ser Arg Ser Thr Val Tyr Tyr Phe Asn His Asn Asp Met
275 280 285

Asn Ser Leu Glu Cys Leu Leu Asn Glu Leu Thr Glu Gln Glu Lys Leu
290 295 300

Glu Lys Leu Pro Ala Ile Pro Arg Lys Phe Ile Val Thr Glu Gly Ile
305 310 315 320

Phe His Asn Ser Gly Asp Leu Ala Pro Leu Pro Glu Leu Thr Lys Leu
325 330 335

Lys Asn Lys Tyr Lys Phe Arg Leu Phe Val Asp Glu Thr Phe Ser Ile
340 345 350

Gly Val Leu Gly Ala Thr Gly Arg Gly Leu Ser Glu His Xaa Xaa Phe
355 360 365

Asn Met Asp Arg Ala Thr Ala Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
370 375 380

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ile Thr Val Gly Ser
385 390 395 400

Met Ala Thr Ala Leu Gly Ser Thr Gly Gly Phe Val Leu Gly Asp Ser
405 410 415

Val Met Cys Leu His Gln Arg Ile Gly Ser Asn Ala Tyr Cys Phe Ser
420 425 430

Ala Cys Leu Pro Ala Tyr Thr Val Thr Ser Val Ser Lys Val Leu Lys
435 440 445

Leu Met Asp Ser Asn Asn Asp Ala Val Gln Thr Leu Gln Lys Leu Ser
450 455 460

Lys Xaa Ser Leu His Asp Ser Phe Ala Ser Asp Asp Ser Leu Arg Ser

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465 470 475 480

Tyr Val Ile Val Thr Ser Ser Pro Val Ser Pro Val Leu His Leu Gln
485 490 495

Leu Thr Pro Ala Tyr Arg Ser Arg Lys Phe Gly Xaa Xaa Xaa Xaa Xaa
500 505 510

Xaa Xaa Xaa Xaa Tyr Thr Cys Glu Gln Leu Phe Glu Thr Met Ser Ala
515 520 525

Leu Gln Lys Lys Ser Gln Thr Asn Lys Phe Ile Glu Pro Tyr Glu Glu
530 535 540

Glu Glu Lys Phe Leu Gln Ser Ile Val Asp His Ala Leu Ile Asn Tyr
545 550 555 560

Asn Val Leu Ile Thr Arg Asn Xaa Xaa Xaa Xaa Thr Ile Val Leu Lys
565 570 575

Gln Glu Thr Leu Pro Ile Val Pro Ser Leu Lys Ile Cys Cys Asn Ala
580 585 590

Ala Met Ser Pro Glu Glu Leu Lys Asn Ala Xaa Xaa Xaa Cys Glu Ser
595 600 605

Val Lys Gln Ser Ile Leu Ala Cys Cys Gln Glu Ser Asn Xaa Xaa Xaa
610 615 620

Lys
625

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ser Thr Pro Ala Asn Tyr Thr Arg Val Pro Leu Cys Glu Pro Glu
5 10 15

Glu Leu Pro Asp Asp Ile Gln Lys Glu Asn Glu Tyr Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Gly Thr Leu Asp Ser Pro Gly His Leu Tyr Gln Val Xaa
35 40 45

Xaa Xaa Xaa Lys Ser Arg His Gly Lys Pro Leu Pro Glu Pro Val Val
50 55 60

Asp Thr Pro Pro Tyr Tyr Ile Ser Leu Leu Thr Tyr Leu Asn Tyr Leu
65 70 75 80

Ile Leu Ile Ile Leu Gly His Val His Asp Phe Leu Gly Met Thr Phe
85 90 95

Gln Lys Asn Lys His Leu Asp Leu Leu Glu His Asp Gly Leu Ala Pro
100 105 110

Trp Phe Ser Asn Phe Glu Ser Phe Tyr Val Arg Arg Ile Lys Met Arg
115 120 125

Ile Asp Asp Cys Phe Xaa Xaa Ser Arg Pro Thr Thr Gly Val Pro Gly
130 135 140

Arg Phe Xaa Ile Arg Cys Ile Asp Arg Ile Ser His Asn Ile Asn Glu
145 150 155 160

Tyr Phe Thr Tyr Ser Gly Ala Val Tyr Pro Cys Met Asn Leu Ser Ser
165 170 175

Tyr Asn Tyr Leu Gly Phe Ala Gln Ser Lys Gly Gln Cys Thr Asp Ala
180 185 190

-continued

Ala Leu Glu Ser Val Asp Lys Tyr Ser Ile Gln Ser Gly Gly Pro Arg
195 200 205

Ala Gln Ile Gly Thr Thr Asp Leu His Ile Lys Ala Glu Lys Leu Val
210 215 220

Ala Arg Phe Ile Gly Lys Glu Asp Ala Leu Val Phe Ser Met Gly Tyr
225 230 235 240

Gly Thr Asn Ala Asn Leu Phe Asn Ala Phe Leu Asp Lys Xaa Lys Cys
245 250 255

Leu Val Ile Xaa Ser Asp Glu Leu Asn His Thr Ser Ile Arg Thr Gly
260 265 270

Val Arg Leu Ser Gly Ala Ala Val Arg Thr Phe Lys His Gly Asp Met
275 280 285

Val Gly Leu Glu Lys Leu Ile Arg Glu Gln Ile Val Leu Gly Gln Pro
290 295 300

Lys Thr Asn Arg Pro Trp Lys Lys Ile Leu Ile Cys Ala Glu Gly Leu
305 310 315 320

Phe Ser Met Glu Gly Thr Leu Cys Asn Leu Pro Lys Leu Val Glu Leu
325 330 335

Lys Lys Lys Tyr Lys Cys Tyr Leu Phe Ile Asp Glu Ala His Ser Ile
340 345 350

Gly Ala Met Gly Pro Thr Gly Arg Gly Val Cys Glu Ile Xaa Xaa Phe
355 360 365

Gly Val Asp Xaa Pro Lys Asp Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
370 375 380

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ile Leu Met Gly Thr
385 390 395 400

Phe Thr Lys Ser Phe Gly Ala Ala Gly Gly Tyr Ile Ala Ala Asp Gln
405 410 415

Trp Ile Ile Asp Arg Leu Arg Leu Asp Leu Thr Thr Val Ser Tyr Ser
420 425 430

Glu Ser Met Pro Ala Pro Val Leu Ala Gln Thr Ile Ser Ser Leu Gln
435 440 445

Thr Ile Ser Gly Glu Ile Cys Pro Gly Gln Gly Thr Glu Arg Leu Gln
450 455 460

Arg Ile Ala Phe Asn Ser Arg Tyr Leu Arg Leu Ala Leu Gln Arg Leu
465 470 475 480

Gly Phe Ile Val Tyr Glu Val Ala Asp Ser Pro Val Ile Pro Leu Leu
485 490 495

Leu Xaa Xaa Xaa Tyr Cys Pro Ser Lys Met Xaa Xaa Xaa Xaa Xaa Xaa
500 505 510

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Ala Phe Ser Arg Met Xaa Met
515 520 525

Leu Gln Arg Arg Ile Ala Val Xaa Xaa Val Val Val Ala Tyr Pro Ala
530 535 540

Thr Pro Xaa Leu Ile Glu Ser Arg Val Arg Phe Cys Met Ser Ala Xaa
545 550 555 560

Xaa Ser Leu Thr Lys Glu Asp Xaa Xaa Xaa Xaa Ile Asp Tyr Leu Leu
565 570 575

Arg His Val Ser Glu Val Gly Asp Lys Leu Asn Leu Lys Ser Asn Ser
580 585 590

Gly Lys Ser Ser Tyr Asp Gly Lys Arg Gln Arg Trp Asp Ile Glu Glu
595 600 605

Val Ile Arg Arg Thr Pro Glu Asp Cys Lys Asp Asp Lys Tyr Phe Val

-continued

610 615 620

Asn
625

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: polypeptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Gln Arg Xaa Ser Ile Phe Ala Arg Xaa Xaa Phe Gly Asn Ser Ser
5 10 15

Ala Ala Val Ser Thr Leu Asn Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Leu Ser Thr Thr Ala Ala Pro His Ala Lys Asn Gly
35 40 45

Tyr Ala Thr Ala Thr Gly Ala Gly Ala Ala Ala Ala Thr Ala Thr Ala
50 55 60

Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Thr His Ala Ala Ala Ala Ala Ala Ala Ala Ala
85 90 95

Asn His Ser Thr Gln Glu Ser Gly Phe Asp Tyr Glu Gly Leu Ile Asp
100 105 110

Xaa Xaa Ser Glu Leu Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Lys Arg
115 120 125

Leu Asp Lys Ser Tyr Arg Tyr Phe Asn Asn Ile Asn Arg Leu Ala Lys
130 135 140

Glu Phe Pro Leu Ala His Arg Gln Arg Glu Ala Asp Lys Val Thr Val
145 150 155 160

Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Ser
165 170 175

Asn Asp Tyr Leu Ala Leu Ser Lys Xaa His Pro Gln Val Leu Asp Ala
180 185 190

Met His Lys Thr Ile Asp Lys Tyr Gly Cys Gly Ala Gly Gly Thr Arg
195 200 205

Asn Ile Ala Gly His Asn Ile Pro Thr Leu Asn Leu Glu Ala Glu Leu
210 215 220

Ala Thr Leu His Lys Lys Glu Gly Ala Leu Val Phe Ser Ser Cys Tyr
225 230 235 240

Val Ala Asn Asp Ala Val Leu Ser Leu Leu Gly Gln Lys Met Lys Asp
245 250 255

Leu Val Ile Phe Ser Asp Glu Leu Asn His Ala Ser Met Ile Val Gly
260 265 270

Ile Lys His Ala Asn Val Lys Lys His Ile Phe Lys His Asn Asp Leu
275 280 285

Asn Glu Leu Glu Gln Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
290 295 300

Gln Ser Tyr Pro Lys Ser Val Pro Lys Leu Ile Ala Phe Glu Ser Val
305 310 315 320

Tyr Ser Met Ala Gly Ser Val Ala Asp Ile Glu Lys Ile Cys Asp Leu
325 330 335

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Ala Asp Lys Tyr Gly Ala Leu Thr Phe Leu Asp Glu Val His Ala Val
340 345 350

Gly Leu Tyr Gly Pro His Gly Ala Gly Val Ala Glu His Cys Asp Phe
355 360 365

Glu Ser His Arg Ala Ser Gly Ile Ala Thr Pro Lys Thr Asn Asp Lys
370 375 380

Gly Gly Ala Lys Thr Val Met Asp Arg Val Asp Met Ile Thr Gly Thr
385 390 395 400

Leu Gly Lys Ser Phe Gly Ser Val Gly Gly Tyr Val Ala Ala Ser Arg
405 410 415

Lys Leu Ile Asp Trp Phe Arg Ser Phe Ala Pro Gly Phe Ile Phe Thr
420 425 430

Thr Thr Leu Pro Pro Ser Val Met Ala Gly Ala Thr Ala Ala Ile Arg
435 440 445

Tyr Gln Arg Cys His Ile Asp Leu Arg Thr Ser Gln Gln Lys Xaa Xaa
450 455 460

Xaa Xaa Xaa Xaa His Thr Met Tyr Val Lys Lys Ala Phe His Glu Leu
465 470 475 480

Gly Ile Pro Val Ile Pro Asn Pro Xaa Ser His Ile Val Pro Val Leu
485 490 495

Ile Gly Asn Ala Asp Leu Ala Lys Gln Ala Ser Asp Ile Leu Ile Asn
500 505 510

Lys His Gln Ile Tyr Val Gln Ala Ile Asn Phe Pro Thr Val Ala Arg
515 520 525

Gly Thr Glu Arg Leu Arg Ile Thr Pro Thr Pro Gly His Thr Asn Asp
530 535 540

Leu Ser Asp Ile Leu Ile Asn Ala Val Asp Asp Val Phe Asn Glu Leu
545 550 555 560

Gln Leu Pro Arg Val Arg Asp Trp Glu Ser Gln Gly Gly Leu Leu Gly
565 570 575

Val Gly Glu Ser Gly Phe Val Glu Glu Ser Asn Leu Trp Thr Ser Ser
580 585 590

Gln Leu Ser Leu Thr Asn Asp Asp Leu Asn Pro Xaa Xaa Xaa Xaa Asn
595 600 605

Val Arg Asp Pro Ile Val Lys Gln Leu Glu Val Ser Ser Gly Ile Lys
610 615 620

Gln
625

What is claimed is:

1. A DNA sequence LCB1 having the nucleotide sequence of Sequence ID NOs: 1–3, wherein LCB stands for long chain base.

2. A plasmid comprising the LCB1 sequence according to claim **1**.

3. A plasmid according to claim **2**, which is the plasmid pTZ18-LCB1.

4. A plasmid according to claim **2**, which is YIpLCB1-1.

5. A host cell transformed by a plasmid to comprise and express an LCB1 sequence according to claim **1**.

6. A DNA sequence LCB2 having the nucleotide sequence of Sequence ID NOS: 4–6.

7. A plasmid comprising the DNA sequence according to claim **6**.

8. A plasmid according to claim **7**, which is pRSLCB2-2.

9. A host cell transformed by a plasmid to comprise and express an LCB2 sequence according to claim **6**.

10. A genetically engineered microbial strain transformed by a plasmid comprising both the LCB1 and LCB2 sequences of claims **1** and **6**, wherein said plasmid over expresses the genes with which it has transformed and overproduces the Serine Palmitoyltransferase enzyme.

11. A DNA sequence which is a complement to the sequence according to claim **1**.

12. A DNA sequence which is a complement to the sequence according to claim **5**.

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