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Gokarn et al.

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(54) **3-HYDROXYPROPIONIC ACID AND OTHER ORGANIC COMPOUNDS**

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Related U.S. Application Data

(60) Continuation of application No. 13/926,054, filed on Jun. 25, 2013, now Pat. No. 8,999,700, which is a division of application No. 13/467,165, filed on May 9, 2012, now Pat. No. 8,501,455, which is a division of application No. 13/294,820, filed on Nov. 11, 2011, now Pat. No. 8,198,066, which is a division of application No. 12/127,700, filed on May 27, 2008, now Pat. No. 8,076,120, which is a division of application No. 11/539,856, filed on Oct. 9, 2006, now Pat. No. 7,393,676, which is a division of application No. 10/432,443, filed as application No. PCT/US01/43607 on Nov. 20, 2001, now Pat. No. 7,186,541.

(60) Provisional application No. 60/252,123, filed on Nov. 20, 2000, provisional application No. 60/285,478, filed on Apr. 20, 2001, provisional application No. 60/306,727, filed on Jul. 20, 2001, provisional application No. 60/317,845, filed on Sep. 7, 2001.

(51) **Int. Cl.**

C12N 1/20	(2006.01)
C12N 9/00	(2006.01)
C12P 7/42	(2006.01)
C12N 9/02	(2006.01)
C12N 15/52	(2006.01)
C12P 7/52	(2006.01)
C12P 7/62	(2006.01)
C12P 7/18	(2006.01)
C12P 7/40	(2006.01)

(52) **U.S. Cl.**

CPC ***C12P 7/42*** (2013.01); ***C12N 9/0004*** (2013.01); ***C12N 15/52*** (2013.01); ***C12P 7/18*** (2013.01); ***C12P 7/40*** (2013.01); ***C12P 7/52*** (2013.01); ***C12P 7/625*** (2013.01); *Y02P 20/52* (2015.11)

(58) **Field of Classification Search**

CPC C12N 9/0004; C12N 15/52

USPC 435/252.3, 183

See application file for complete search history.

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(57) **ABSTRACT**

Methods and materials related to producing 3-HP as well as other organic compounds are disclosed. Specifically, isolated nucleic acids, polypeptides, host cells, and methods and materials for producing 3-HP and other organic compounds are disclosed.

37 Claims, 105 Drawing Sheets

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Figure 1

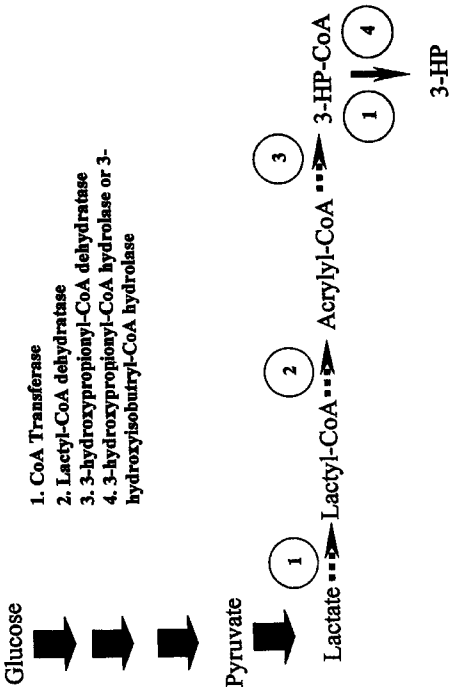
1. CoA Transferase
2. Lactyl-CoA dehydratase
3. 3-hydroxypropionyl-CoA dehydratase
4. 3-hydroxypropionyl-CoA hydrolase or 3-hydroxyisobutiryl-CoA hydrolase
Glucose
Pyruvate
Lactate
Lactyl-CoA
Acrylyl-CoA
3-HP-CoA
3-HP
1
2
3
4
1

Figure 2

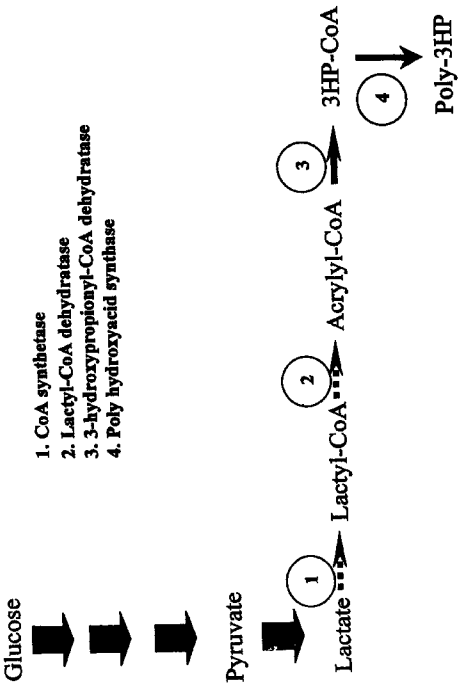
1. CoA synthetase
2. Lactyl-CoA dehydratase
3. 3-hydroxypropionyl-CoA dehydratase
4. Poly hydroxyacid synthase
Glucose
Pyruvate
Lactate
Lactyl-CoA
Acrylyl-CoA
3HP-CoA
Poly-3HP
1
2
3
4

Figure 3

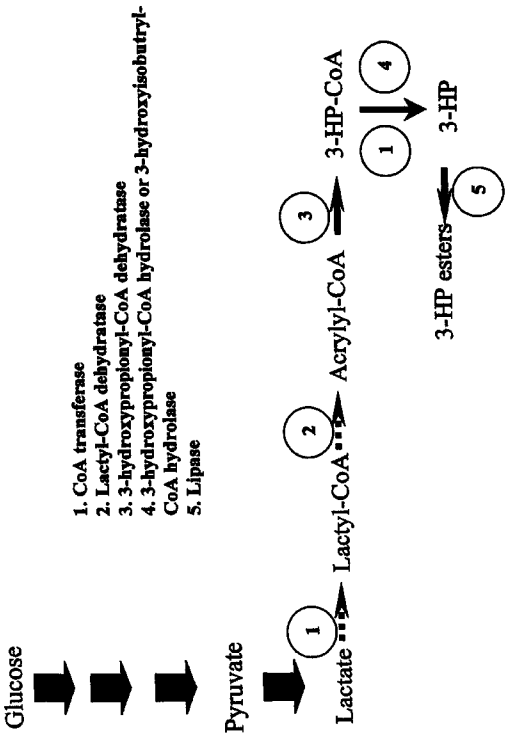
1. CoA transferase
2. Lactyl-CoA dehydratase
3. 3-hydroxypropionyl-CoA dehydratase
4. 3-hydroxypropionyl-CoA hydrolase or 3-hydroxyisobutryl-CoA hydrolase
5. Lipase
Glucose
Pyruvate
Lactate
Lactyl-CoA
Acrylyl-CoA
3-HP-CoA
3-HP
3-HP esters
1
2
3
4
5

Figure 4

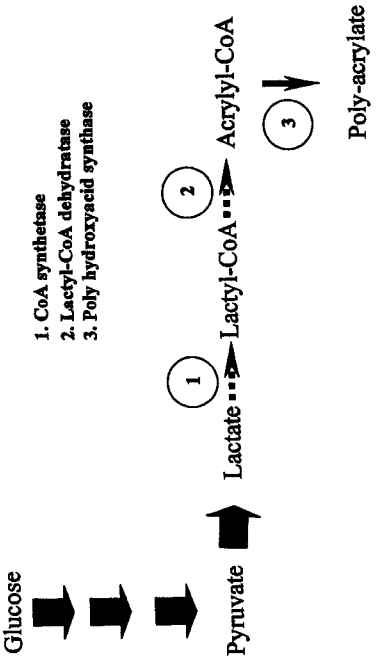
1. CoA synthetase
2. Lactyl-CoA dehydratase
3. Poly hydroxyacid synthase
Glucose
Pyruvate
Lactate
1
Lactyl-CoA
2
Acrylyl-CoA
3
Poly-acrylate

Figure 5

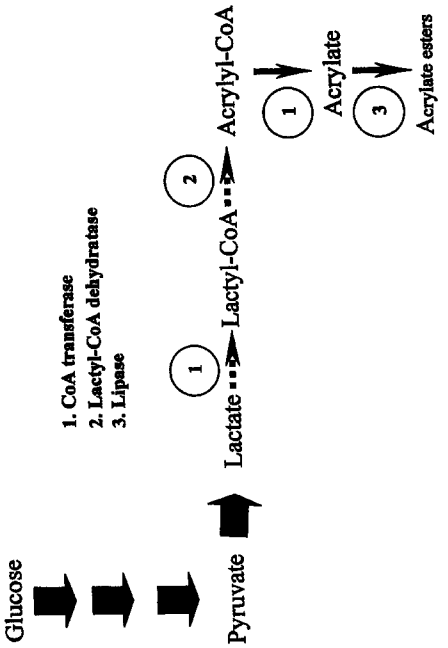
1. CoA transferase
2. Lactyl-CoA dehydratase
3. Lipase
Glucose
Pyruvate
Lactate
Lactyl-CoA
Acrylyl-CoA
Acrylate
Acrylate esters
1
2
1
3

Figure 6

ATGAGAAAAGTAGAAATCATTACAGCTGAACAAGCAGCTCAGCTCGTAAAAGACAACGAC
ACGATTACGTCTATCGGCTTTGTCAGCAGCGCCCATCCGGAAGCACTGACCAAAGCTTTG
GAAAAACGGTTCCTGGACACGAACACCCCGCAGAACTTGACCTACATCTATGCAGGCTCT
CAGGGCAAACGCGATGGCCGTGCCGCTGAACATCTGGCACACACAGGCCTTTTGAAACGC
GCCATCATCGGTCACTGGCAGACTGTACCGGCTATCGGTAAACTGGCTGTCGAAAACAAG
ATTGAAGCTTACAACTTCTCGCAGGGCACGTTGGTCCACTGGTTCCGCGCCTTGGCAGGT
CATAAGCTCGGCGTCTTCACCGACATCGGTCTGGAAACTTTCCTCGATCCCCGTCAGCTC
GGCGGCAAGCTCAATGACGTAACCAAAGAAGACCTCGTCAAACTGATCGAAGTCGATGGT
CATGAACAGCTTTTCTACCCGACCTTCCCGGTCAACGTAGCTTTCCTCCGCGGTACGTAT
GCTGATGAATCCGGCAATATCACCATGGACGAAGAAATCGGGCCTTTCGAAAGCACTTCC
GTAGCCCAGGCCGTTCACAACTGTGGCGGTAAAGTCGTCGTCCAGGTCAAAGACGTCGTC
GCTCACGGCAGCCTCGACCCGCGCATGGTCAAGATCCCTGGCATCTATGTCGACTACGTC
GTCGTAGCAGCTCCGGAAGACCATCAGCAGACGTATGACTGCGAATACGATCCGTCCCTC
AGCGGTGAACATCGTGCTCCTGAAGGCGCTACCGATGCAGCTCTCCCCATGAGCGCTAAG
AAAATCATCGGCCGCCGCGGCGCTTTGGAATTGACTGAAAACGCTGTCGTCAACCTCGGC
GTCGGTGCTCCGGAATACGTTGCTTCTGTTGCCGGTGAAGAAGGTATCGCCGATACCATT
ACCCTGACCGTCGAAGGTGGCGCCATCGGTGGCGTACCGCAGGGCGGTGCCCGCTTCGGT
TCGTCCCGCAATGCCGATGCCATCATCGACCACACCTATCAGTTCGACTTCTACGATGGC
GGCGGTCTGGACATCGCTTACCTCGGCCTGGCCCAGTGCGATGGCTCGGGCAACATCAAC
GTCAGCAAGTTCGGTACTAACGTTGCCGGCTGCGGCGGTTTCCCCAACATTTCCCAGCAG
ACACCGAATGTTTACTTCTGCGGCACCTTCACGGCTGGCGGCTTGAAAATCGCTGTCGAA
GACGGCAAAGTCAAGATCCTCCAGGAAGGCAAAGCCAAGAAGTTCATCAAAGCTGTCGAC
CAGATCACTTTCAACGGTTCCTATGCAGCCCGCAACGGCAAACACGTTCTCTACATCACA
GAACGCTGCGTATTTGAACTGACCAAAGAAGGCTTGAAACTCATCGAAGTCGCACCGGGC
ATCGATATTGAAAAAGATATCCTCGCTCACATGGACTTCAAGCCGATCATTGATAATCCG
AAACTCATGGATGCCCGCCTCTTCCAGGACGGTCCCATGGGACTGAAAAAATAA (SEQ
ID NO:1)

Figure 7

MRKVEIITAEQAAQLVKDNDTITSIGFVSSAHPEALTKALEKRFLDTNTPQNLTYIYAGS
QGKRDGRAAEHLAHTGLLKRAIIGHWQTVPAIGKLAVENKIEAYNFSQGTLVHWFRALAG
HKLGVFTDIGLETFLDPRQLGGKLNDVTKEDLVKLIEVDGHEQLFYPTFPVNVAFLRGTY
ADESGNITMDEEIGPFESTSVAQAVHNCGGKVVVQVKDVVAHGSLDPRMVKIPGIYVDYV
VVAAPEDHQQTYDCEYDPSLSGEHRAPEGATDAALPMSAKKIIGRRGALELTENAVVNLG
VGAPEYVASVAGEEGIADTITLTVEGGAIGGVPQGGARFGSSRNADAIIDHTYQFDFYDG
GGLDIAYLGLAQCDGSGNINVSKFGTNVAGCGGFPNISQQTPNVYFCGTFTAGGLKIAVE
DGKVKILQEGKAKKFIKAVDQITFNGSYAARNGKHVLYITERCVFELTKEGLKLIEVAPG
IDIEKDILAHMDFKPIIDNPKLMDARLFQDGPMGLKK (SEQ ID NO:2)

```
SEQ ID NO:1      1 atgagaaaagtagaaatcattacagctgaacaagcagctc--agctcgta
SEQ ID NO:3      1 ---------gtgccggtcctgtcggcacaggaagcggtga--attatatt
SEQ ID NO:4      1 atgccgattctctcaaaaatatgggcggctccagcagctggaatcttgag
SEQ ID NO:5      1 ------------------------atgaa----------------tgca

SEQ ID NO:1     49 aaagacaacgacacgattacgtctatcggctttgtcagcagcgcccatcc
SEQ ID NO:3     40 cccgacgaagcaacactttgtgtgttaggcgctg---gcggcggtattct
SEQ ID NO:4     51 aaaaactccgagaaatgctcatcaaatgaggctaatctcaatga-catcc
SEQ ID NO:5     10 aaaga----------atta-----atcg----------------------

SEQ ID NO:1     99 ggaagcactgaccaaagctttggaaaaacggttcctg-------------
SEQ ID NO:3     87 ggaag------ccaccacgtt--aattactgctcttgctgataaatataa
SEQ ID NO:4    100 tcgatgaaagcaaaagtcttt-----aactctgc----------------
SEQ ID NO:5     23 ----------------------------------------------------

SEQ ID NO:1    136 ---gacacgaacaccccgcagaacttgacctacatctatgcag-gctctc
SEQ ID NO:3    129 acagactcaaacaccacgt--aatttatcgattattagtccaa-cagggc
SEQ ID NO:4    129 -------cgaagaagccgtgaaggatattccagat-aatgcaaagctttt
SEQ ID NO:5     23 --------------ctcgccgaatt------------------------

SEQ ID NO:1    182 agggcaaacgcgatggccgtgccgctgaacatctggcacacacaggcctt
SEQ ID NO:3    176 ttggcgatcgcgccgaccgtggtattagtcctctggcgcaagaaggtctg
SEQ ID NO:4    171 a----------gttggc--ggcttcggactatgcgg-aatcccagaaaat
SEQ ID NO:5     34 ---------gcgatgg-----------------------------------

SEQ ID NO:1    232 ttgaaacgcgccatcatcggtcactggcagactgtaccggc-tatcggta
SEQ ID NO:3    226 gtgaaatgggcattatgtggtcactgg-ggacaatcgccgcgtatttctg
SEQ ID NO:4    208 ctcatccaagctatca-caaaaactggtcaa-----------aaaggtc
SEQ ID NO:5     41 --------------------aattacatgatgga---ga-tattgtta

SEQ ID NO:1    281 aactggctgtcgaaaacaagattgaagcttacaacttctcgcagggcacg
SEQ ID NO:3    275 aactcgcagaacaaaataaaattattgcttataactacccacaaggtgta
SEQ ID NO:4    245 ttacatgtgtatcaaacaatgcgggagttgataatt-------ggggac-
SEQ ID NO:5     65 atctcggt-----------attg--gtttac---------caacacagg

SEQ ID NO:1    331 ttggtccactggttccgcgccttggcaggtcataagctcggcgtcttcac
SEQ ID NO:3    325 cttacacaaacctacgcgccgccgcagcccaccagcctggtattattag
SEQ ID NO:4    287 ttggcttgctccttc--aaactcgacaaatc--aagaaaatgatctcatc
SEQ ID NO:5     92 ttgt-----taattatttacctgataatgtcaata----------ttac

SEQ ID NO:1    381 cgacatcggtct----ggaaa---ctttcctcgatccccgtcagctcggc
SEQ ID NO:3    375 tgatattggcat----cggga---catttgtcgatccacgccagcaaggc
SEQ ID NO:4    333 gtacgtcggtgaaaacggaga---atttgctcga----caatatcttagc
SEQ ID NO:5    126 --acttcaatca----gaaaatggctttcttggtttaactgca-------

SEQ ID NO:1    424 ggcaagctcaatgacgtaacca------aagaagacctcgtcaaactgat
SEQ ID NO:3    418 ggcaaactgaatgaagtcacta------aagaagacctgattaaactggt
SEQ ID NO:4    376 ggagagctcgagttggaattcacaccacaaggaacactcgccgaacgaat
SEQ ID NO:5    163 ---------tttgac----cca------gaaaatgctaattcaaact---

SEQ ID NO:1    468 cgaagtcgatggtca---tgaacagcttttctacccgacc----------
SEQ ID NO:3    462 cgagtttgataacaa---agaatatctctattacaaagcg----------
SEQ ID NO:4    426 tcgtgcagctggtgccggtgttcccgcattctacac-accaacaggatac
SEQ ID NO:5    191 --tagtaaatgctgg---tggtcagcctt---------------------
```

Figure 8A

```
SEQ ID NO:1     505 --ttcccgg--tcaacgtagctttcctccgcggtacgtatgctga---tg
SEQ ID NO:3     499 --attgcgc--cagatattgccttcattcgcgctaccacctgcga---ca
SEQ ID NO:4     475 ggtacccagattcaagaaggaggtgctccga-ttaagtacagtaaaactg
SEQ ID NO:5     215 ----------------------------gtggaa------ttaa---aa

SEQ ID NO:1     548 aatccggcaatatc-accatggacg--------aagaaatcgggccctttc
SEQ ID NO:3     542 gtgaaggctacgcc-acttttgaag--------atgaggtgatgtatctc
SEQ ID NO:4     524 aaaaaggaaagattgaagttgcaagtaaagcgaaagaaacacgacaattc
SEQ ID NO:5     227 aaggcggctcta-----------------------------------ctttt

SEQ ID NO:1     589 ga---aagcacttccgta---gcccaggccgttcac--aactgtggcggt
SEQ ID NO:3     583 ga------cgcattggttattgcccaggcggtgcac--aataacggcggt
SEQ ID NO:4     574 aatggaattaattatgtaatggaagaggctatttggggagattttgcatt
SEQ ID NO:5     244 ga---tagtgctt----------------------t--ttctttcgcttt

SEQ ID NO:1     631 aaagtcgtcgtccaggtcaaagacgtcgtcgc-----tcacggcagcctc
SEQ ID NO:3     625 attgtgatgatgcaggtgcagaaaatggttaa-----gaaagccacgctg
SEQ ID NO:4     624 gatcaaggcgtggagagcagatac-tcttggaaatattcaattcagacat
SEQ ID NO:5     267 aa-------------------------------------------------ttc

SEQ ID NO:1     676 gacccgcgcatggtcaagatccctg--------gcatctatgtcgactac
SEQ ID NO:3     670 catcctaaatctgtccgtattccgg--------g----ttatctggtggat
SEQ ID NO:4     673 gctgctggaaatttcaataatccaatgtgcaaagcctctaaatgcac--c
SEQ ID NO:5     272 gtggcggtcatgtt---gatgcctg--------tgtgctaggtggact--

SEQ ID NO:1     718 gtcgtcgtagcagctccggaagaccatcagcag--acgtatgactgcgaa
SEQ ID NO:3     709 attgtggtggtcgatccg---gatcaaacccaa--ctgtatggcggtgca
SEQ ID NO:4     721 atcgtcgaagtag---aggaaatcgtcgaaccgggagtaattgctccaaa
SEQ ID NO:5     309 ------------------------------------------------------

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SEQ ID NO:3     754 c-------cggttaaccgctttatttctggtgacttcacccttg-atgac
SEQ ID NO:4     768 cgatgtgcacattccatcaatctattgtcatcgtctagttttgggaaaga
SEQ ID NO:5     309 ------------------------------------------tg-aagtt

SEQ ID NO:1     808 gctac--------cgatgcagc---------tctccccatgagcgctaaga
SEQ ID NO:3     796 agtac--------caaacttag---------cctgcccctaaac-caacgt
SEQ ID NO:4     818 actacaaaaaaccaatcgaacggccaatgttcgcacacgaaggaccaata
SEQ ID NO:5     316 gatca--------agaagcaaa---------tctcgc--------------

SEQ ID NO:1     842 aaatcatcggc-cgccgcggcgctttggaattgactgaaaacgctgtcgt
SEQ ID NO:3     829 aaattagttgcgcggcgcgcattattcgaaatgcgtaaaggcgcggtggg
SEQ ID NO:4     868 aaaccatctac-atcggc--tgctggaaaatcgagagaaatcattg-cag
SEQ ID NO:5     336 ---------------------------------taactgga----------

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SEQ ID NO:3     879 gaatgtcggcgtcggtattgc-----tgacg--gcattggcctggtcgcc
SEQ ID NO:4     914 cacgtgcagctttggagttcacagatggaatgtacgccaatttgggtatc
SEQ ID NO:5     344 ----------------------------------------tggtgcc

SEQ ID NO:1     934 gg--tgaagaaggtatcgccga------tacca-------ttaccctgac
SEQ ID NO:3     922 cg--agaagaaggttgtgctga------tgact-------ttattctgac
SEQ ID NO:4     964 gggattccgactttggcgccaaattatataccaaatggatttactgttca
SEQ ID NO:5     351 tg--gcaaaatggta-------------------------------------

SEQ ID NO:1     969 cgtcgaaggtg------gcgccatcggtggcgt-accgcagggcggtgcc
SEQ ID NO:3     957 ggtagaaacag------gtccgattggcggaattacttcacaggggatcg
SEQ ID NO:4    1014 tttgcaaagtgagaatggtattattggagtggg-accata------tcca
SEQ ID NO:5     364 ------------------------------------------------------
```

Figure 8B

```
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SEQ ID NO:3    1001 c-ctttggcgcgaacgtga-atacccgtgccattc----tggatatgacg
SEQ ID NO:4    1057 agaaaag----gaacagaagacgccgatctcattaatgctggaaaagagc
SEQ ID NO:5     364 ------------ccagga-atg----------------------------

SEQ ID NO:1    1057 tatcagttcgacttctacgatggcggc----------ggtctggacatcg
SEQ ID NO:3    1045 tcccagtttgatttttatcacggtggc----------ggtctggatgttt
SEQ ID NO:4    1103 ---caattactcttct-caaaggagcttcaattgttggttctgatgaatc
SEQ ID NO:5     373 ----------------------ggcgga----------gcaatggacttag

SEQ ID NO:1    1097 cttacctcggcctgg-----cccagtgcgatg-------gctcgggcaac
SEQ ID NO:3    1085 gttatttgagttttg-----ctgaagtcgacc-------agcacggtaac
SEQ ID NO:4    1149 attcgcaatgattcgtggttctcatatggatattactgtgctcggtgcac
SEQ ID NO:5     392 ------------------------------tg-------actggtgcaa-

SEQ ID NO:1    1135 atcaacgtcagca-agttcggtactaacgttgccggctgcggcggtttcc
SEQ ID NO:3    1123 gtcggcgtgcata-aattcaatggtaaaatcatgggcaccggtggattta
SEQ ID NO:4    1199 ttca--gtgctcacagtttgg----agatttagcgaattggatgattccg
SEQ ID NO:5     404 --------------------------------------------------

SEQ ID NO:1    1184 ccaacatt--tcccagcagacaccgaatgtttacttctgcggcacct-tc
SEQ ID NO:3    1172 ttgatatcagtgccacttcgaagaaaatcatt--ttctgcggcacat-ta
SEQ ID NO:4    1243 ggaaaatt-------ggtga-aaggaatgggcggtgcaatggatcttgtc
SEQ ID NO:5     404 ----------------------aaaaagtgattatt-----ggca------

SEQ ID NO:1    1231 acggctggcggcttgaaaatcgctgtcgaagacggcaaagtcaagatcct
SEQ ID NO:3    1219 actgcgggcagtttaaaaacagaaattaccgacggcaaattaaatatcgt
SEQ ID NO:4    1285 tctgctcccgg------agcccgtgt-gatcgttgtaatggagcatgtat
SEQ ID NO:5     422 ------------tggaacattg-----------tgccaagtcaggttcct

SEQ ID NO:1    1281 ccaggaaggcaaagccaagaagttcatcaaagctgtcgaccagatcactt
SEQ ID NO:3    1269 ccaggaaggacgggtgaagaaatttattcgggaactaccggaaattactt
SEQ ID NO:4    1328 cgaagaacggagagccaaaaatt----------------ctagagcactg
SEQ ID NO:5     449 caaaaattctaaag---aaatgtacattaccgct------cacagcaagt

SEQ ID NO:1    1331 tcaacgg-----ttcctatgcagc---ccgcaacggcaaacacgttctct
SEQ ID NO:3    1319 tcagcggaaaaatcgctctcgagc---gagggctgg-----atgttcgtt
SEQ ID NO:4    1362 cgaac-------ttcctctga--c---cggcaaagg--agtaatttcccg
SEQ ID NO:5     490 aaaaaag-----ttgccatggtggttaccgaattggca-----gtattta

SEQ ID NO:1    1373 a--catcacagaacgctgcgtatttgaactgacca--aagaa-ggcttga
SEQ ID NO:3    1361 a--tatcactgagcgcgcagtattcacgctgaaag--aagac-ggcctgc
SEQ ID NO:4    1398 aatcattactgatatggcagttttcgacgtggacacaaagaacggattga
SEQ ID NO:5     530 a--cttcattgaaggcagattagttcta------a--aagaa---catgc

SEQ ID NO:1    1418 aactcatcgaagtcgcaccgggcatcgatattgaaaaagatatcctcgct
SEQ ID NO:3    1406 atttaatcgaaatcgcccctggcgtcgatttacaaaaagatattctcgac
SEQ ID NO:4    1448 cattgatcgaagt--caggaaggatc-ttactgtagatgatat-------
SEQ ID NO:5     567 tcctcat----------------gtggatttagaaaca---attaaagcc

SEQ ID NO:1    1468 cacatggacttcaagccgat--cattgata---atccga--aactcatgg
SEQ ID NO:3    1456 aaaatggatttcaccccagt--gatttcgccagaactca--aactgatgg
SEQ ID NO:4    1488 --caagaaactca--ccg-----cttgcaa---attcga--aatttccga
SEQ ID NO:5     598 aaaacag-----aagccgatttcattgtt-----gccgatgatttcaaag

SEQ ID NO:1    1511 atgcccgcctcttccaggacggtcccatggga------ctgaaaaaa---
SEQ ID NO:3    1502 acgaaagattatttatcgatgcggcgatgggttttgtcctgcctgaagcg
SEQ ID NO:4    1524 aaatctgaagccaatgggacaggctcctctta------atcaaggataa-
SEQ ID NO:5     638 aaatgcaaatcagccag---------aaagga------cttgaattatga
```

Figure 8C

```
SEQ ID NO:1    1552 ------taa
SEQ ID NO:3    1552 gctcattaa
SEQ ID NO:4    1567 ---------
SEQ ID NO:5     673 ---------
```

Figure 8D

```
SEQ ID NO:2      1 -------------------------mrkveiit---------aeqaaqlv
SEQ ID NO:6      1 ----------------------------mpvls---------aqeavnyi
SEQ ID NO:7      1 mpilskiwaapaagilrktprnahqmrlismtssmkakvfnsaeeavkdi
SEQ ID NO:8      1 ------------------------mnakeli----------arriamel

SEQ ID NO:2     17 kdndtitsigfvssahpealt--kalekrfldtntpqnltyiyagsqgkr
SEQ ID NO:6     14 pdeatlcvlg-agggileattlitaladkykqtqtprnlsiisptglgdr
SEQ ID NO:7     51 pdnakllvggfglcgipenli--qai------tktgqkgltcvsnnagv-
SEQ ID NO:8     16 hdgd-ivnlg-----------------------------------------

SEQ ID NO:2     65 dgraaehlahtgllkraiighwqtvpaigklavenkieaynfsqgtlvhw
SEQ ID NO:6     63 adrgisplaqeglvkwalcghwgqspriselaeqnkiiaynypqgvltqt
SEQ ID NO:7     92 dnwglgllltqrqikkmissyvgengefarqylsgeleleftpqgtlaer
SEQ ID NO:8     25 -------------------------------------------------

SEQ ID NO:2    115 fralaghklgvftdigletfldprqlggklndvtkedlvkliev------
SEQ ID NO:6    113 lraaaahqpgiisdigigtfvdprqqggklnevtkedliklvef------
SEQ ID NO:7    142 iraagagvpafytptgygtqi---qeggapikysktekgk-ievaskake
SEQ ID NO:8     25 -------------igl---------------------------------

SEQ ID NO:2    159 ----dgheqlfyptfpvnvaflrgtyadesgnitmdeeigpfestsvaqa
SEQ ID NO:6    157 ----dnkeylyykaiapdiafirattcdsegyatfedevmyldalviaqa
SEQ ID NO:7    188 trqfnginyvmeeaiwgdfalikawradtlgniqfrhaagnfnnpmckas
SEQ ID NO:8     28 ------------ptqvvn-------ylpdnvnitlqsengflglta----

SEQ ID NO:2    205 vhncggkvvvqvkdvvahgsldprmvkipgiyvdyvvvaapedhqqtydc
SEQ ID NO:6    203 vhnnggivmmqvqkmvkkatlhpksvripgylvd-ivvvdpdqtqlygga
SEQ ID NO:7    238 --kc---tiveveeivepgviapndvhipsiychrlvlg------knykk
SEQ ID NO:8     55 -------------------------------------------------

SEQ ID NO:2    255 eydpslsgehrapegatdaalpmsakkiigrrgaleltenavvnlgvg--
SEQ ID NO:6    252 pvnrfisgdftl-ddstklslplnqrklvarralfemrkgavgnvgvg--
SEQ ID NO:7    277 pierpmfahegpikpstsaa--gksreiiaaraaleftdgmyanlgigip
SEQ ID NO:8     55 -fdp---------------------------------enansnl-vn--

SEQ ID NO:2    303 --apeyvasvageegiadtitltveggaig--gvpqggarfgssrnad--
SEQ ID NO:6    299 --iadgiglvareegcaddfiltvetgpig--gitsqgiafganvntr--
SEQ ID NO:7    325 tlapnyipn-------gftvhlqsengiigvgpyprkgtedadlinagke
SEQ ID NO:8     67 --a----------------------ggqpc--gikkggstf---------

SEQ ID NO:2    347 --------aiidhtyqfdfydgggldiaylglaqcdgsgni-nvskfgtn
SEQ ID NO:6    343 --------aildmtsqfdfyhgggldvcylsfaevdghgnv-gvhkfngk
SEQ ID NO:7    368 pitllkgasivgsdesfamirgshmditvlgalqcsqfgdlanwmipgkl
SEQ ID NO:8     82 -----------dsafsfalirgghvdacvlgglevdqeanlanwmvpgkm

SEQ ID NO:2    388 vagcggfpnisqqtpnvyfcgtftagglkiav------edgkvkilqegk
SEQ ID NO:6    384 imgtggfidisatskkiifcgtltagslktei------tdgklnivqegr
SEQ ID NO:7    418 vkgmggamdl------------vsapgarvivvmehvskngepkilehce
SEQ ID NO:8    121 vpgmggamdlvtgakkvii-------gmehca------ksgsskilk---

SEQ ID NO:2    432 akkfikavdqitfngsyaarngkhvl--yitercvfel-tkeglklieva
SEQ ID NO:6    428 vkkfirelpeitfsgkialergldvr--yiteravftl-kedglhlieia
SEQ ID NO:7    456 -----------------lpltgkgvisriitdmavfdvdtkngltlievr
SEQ ID NO:8    155 -----kctlplt-------askkvam--vvtelavfnf-iegrlvlkeha
```

Figure 9A

```
SEQ ID NO:2     479 pgidiekdi--lahmdfkpiidnp-klmdarlfqdgpmglkk-----
SEQ ID NO:6     475 pgvdlqkdi--ldkmdftpvispelklmderlfidaamgfvlpeaah
SEQ ID NO:7     489 kdltvd-dikkltackfe-isenl-kpmgqaplnqg----------
SEQ ID NO:8     190 phvdle-ti--kakteadfivad-----dfkemqisqkglel-----
```

Figure 9B

Figure 10

GTGAAAACTGTGTATACTCTCGGAATCGACGTTGGTTCTTCTTCTTCCAAGGCAGTCATC
CTGGAAGATGGCAAGAAGATCGTCGCCCATGCCGTCGTTGAAATCGGCACCGGTTCGACC
GGTCCGGAACGCGTCCTGGACGAAGTCTTCAAAGATACCAACTTAAAAATTGAAGACATG
GCGAACATCATCGCCACAGGCTATGGCCGTTTCAATGTCGACTGCGCCAAAGGCGAAGTC
AGCGAAATCACGTGCCATGCCAAAGGGGCCCTCTTTGAATGCCCCGGTACGACGACCATC
CTCGATATCGGCGGTCAGGACGTCAAGTCCATCAAATTGAATGGCCAGGGCCTGGTCATG
CAGTTTGCCATGAACGACAAATGCGCCGCTGGTACGGGCCGTTTCCTCGACGTCATGTCG
AAGGTACTGGAAATCCCCATGTCTGAAATGGGGGACTGGTACTTCAAATCGAAGCATCCC
GCTGCCGTCAGCAGTACCTGCACGGTTTTTGCTGAATCGGAAGTCATTTCCCTTCTTTCC
AAGAATGTCCCGAAAGAAGATATCGTAGCCGGTGTCCATCAGTCCATCGCCGCCAAAGCC
TGCGCTCTCGTGCGCCGCGTCGGTGTCGGTGAAGACCTGACCATGACCGGCGGTGGCTCC
CGCGATCCCGGCGTCGTCGATGCCGTATCGAAAGAATTAGGTATTCCTGTCAGAGTCGCT
CTGCATCCCCAAGCGGTGGGTGCTCTCGGAGCTGCTTTGATTGCTTATGATAAAATCAAG
AAATAA (SEQ ID NO:9)

Figure 11

VKTVYTLGIDVGSSSSKAVILEDGKKIVAHAVVEIGTGSTGPERVLDEVFKDTNLKIEDM
ANIIATGYGRFNVDCAKGEVSEITCHAKGALFECPGTTTILDIGGQDVKSIKLNGQGLVM
QFAMNDKCAAGTGRFLDVMSKVLEIPMSEMGDWYFKSKHPAAVSSTCTVFAESEVISLLS
KNVPKEDIVAGVHQSIAAKACALVRRVGVGEDLTMTGGGSRDPGVVDAVSKELGIPVRVA
LHPQAVGALGAALIAYDKIKK (SEQ ID NO:10)

```
SEQ ID NO:9       1 gtgaaaactgtgtatactctcggaatcgacgttggttcttcttcttccaa
SEQ ID NO:11      1 ---atgagtatctataccttgggaatcgatgttggatctactgcatccaa
SEQ ID NO:12      1 gtggcagtggcatattcgattggcattgattccggctcaaccgccaccaa
SEQ ID NO:13      1 ------------atgattttagggatagatgttggatctacaacaacgaa

SEQ ID NO:9      51 ggcagtcatcctggaagatggcaagaagatcgtcgc-ccatgccgtcgtt
SEQ ID NO:11     48 gtgcattatcctgaaagatggaaaagaaatcgtggc-gaaatccctggta
SEQ ID NO:12     51 agggatcttactggcagacggcgtgatta----cgcgccgtttcctcgtt
SEQ ID NO:13     39 gatggttctaatggaagatagc---aagataatttg-gtataagatagag

SEQ ID NO:9     100 gaaatcggcaccggttcgaccggtccggaacgcgtcctggacgaagtctt
SEQ ID NO:11     97 gccgtggggaccggaacttccggtcccgcacggtctatttcggaagtcct
SEQ ID NO:12     97 ccaa----ccccctttcgcccgg-caacagcaattact----gaagcctg
SEQ ID NO:13     85 gatattgg-agttgtta-----ttgaggaagatattttattaaaaatggt

SEQ ID NO:9     150 caaagatacc-aacttaaaaattgaagacatggcgaacatcatcgc-cac
SEQ ID NO:11    147 ggaaaatgcc-cacatgaaaaaagaagacatggcctttaccctggc-tac
SEQ ID NO:12    138 ggaa-actct-gcgcgaagggttagagacaacgccgtttctgacgctcac
SEQ ID NO:13    129 taaggagattgaacaaaaatatccaatagat----aaaatcgttgc-aac

SEQ ID NO:9     198 aggctatggccgtttcaatgtcg---------actgcgccaaaggcgaag
SEQ ID NO:11    195 cggctacggacg---caat-tcgctggaaggcattgccgacaagcaga--
SEQ ID NO:12    186 cggctacgggcggcaactggtgg---------attttgccgataaacagg
SEQ ID NO:13    174 tggatatggaaggcataaggtta---------gttttgcagataagatag

SEQ ID NO:9     239 tcagcgaaatcacgtgccatgccaaaggggcc---ctctttgaatgcccc
SEQ ID NO:11    239 tgagcgaactgagctgccatgccatgggcgcc---agctttatctggccc
SEQ ID NO:12    227 taacggaaatctcctgtcacgggctgggcgca---cggtttcttgcgcca
SEQ ID NO:13    215 ttccagaagtta-ttgcattgggaaaaggagctaactatttctttaacga

SEQ ID NO:9     286 ggtacgacga--ccatcctcgatatcggcggtcaggacgtcaa-gtccat
SEQ ID NO:11    286 --aacgtccataccgtcatcgatatcggcgggcaggatgtgaa-ggtcat
SEQ ID NO:12    274 gcaacgcgcg--cggtaatcgacatcggtggtcaggacagcaaagtgatt
SEQ ID NO:13    264 ggcagatgga----gttatagacattggagggcaagatacaaa-ggtctt

SEQ ID NO:9     333 caaattga--atggccagggcctggtcatgcagtttgcc-atgaacgaca
SEQ ID NO:11    333 ccatgtgg--aaaacgggaccatgacca---atttccag-atgaatgata
SEQ ID NO:12    322 cagcttgatgatgacggtaacctg----tgcgatttcctgatgaatgaca
SEQ ID NO:13    309 aaagattg--ataaaaacggaaaagttgttgattttatc-ctatcagata

SEQ ID NO:9     380 aatgcgccgctggtacgggccgtttcctcgacgtcatgtcgaaggtactg
SEQ ID NO:11    377 aatgcgctgccgggactggccgtttcctggatgttatggccaatatcctg
SEQ ID NO:12    368 aatgcgcggcgggcaccgggcgtttcctggaggtgatctcgcgcacgctt
SEQ ID NO:13    356 aatgtgccgctggaactggaaaattcttaga---------aaaggcatta

SEQ ID NO:9     430 gaaatccccatgtct-ga--aatgggggactggtactt-caaatcgaagc
SEQ ID NO:11    427 gaagtgaaggtttcc-ga--cctggctgagctgggagc-caaatccacca
SEQ ID NO:12    418 ggca--ccagcgtcgagc--aactcgacagcattaccg-aaaat---gtc
SEQ ID NO:13    397 gatattttaaaaatt-gataaaaatgagataaataaatacaaatcagata

SEQ ID NO:9     476 atcccgct-gccgtcagcagtacctgcacggtttttgctgaatcggaagt
SEQ ID NO:11    473 aacgggtg-gctatcagctccacctgtactgtgtttgcagaaagtgaagt
SEQ ID NO:12    460 acgccgcacgccatcacgagtatgtgcacagtgtttgctgaatcagaagc
SEQ ID NO:13    446 atatcgct-aaaatatcttcaatgtgtgctgtctttgctgaaagtgagat
```

Figure 12A

```
SEQ ID NO:9     525 catttcccttctttccaagaatgtcccgaaagaa--gatatcgtagccgg
SEQ ID NO:11    522 catcagccagctgtccaa--agggaccgacaagatcgacatcattgccgg
SEQ ID NO:12    510 gatcagcctgcgctcagcgggcgtcgcgccagaa--gcgattctcgcagg
SEQ ID NO:13    495 aataagcttactatcaaaaaaagttccaaaggaa--ggcattttaatggg

SEQ ID NO:9     573 tgtccatcagtccatcgccgccaaagcctgcgctctcgtgc-gccgcgtc
SEQ ID NO:11    570 gatccatcgttctgtagccagccgggtcattggtcttgcca-atcgggtg
SEQ ID NO:12    558 agtgattaacgcgat-ggcgcggaggagtgc-caatttcat-tgctcgtc
SEQ ID NO:13    543 cgtctatgagagtat------aataaatagggttatcccaatgaccaata

SEQ ID NO:9     622 ggtgtcgg--tgaagacctgaccatgaccggcggtggctcccgcgat--c
SEQ ID NO:11    619 gggattgt--gaaagacgtggtcatgaccggcggtgtagcccagaac--t
SEQ ID NO:12    605 tctc-ctg--tgaagcgccgattctgtttactggtggcgttagtcattgc
SEQ ID NO:13    587 ggcttaaaattcaaaacatagtgtttagtggaggagttgctaaaaat--a

SEQ ID NO:9     668 ccggcgtcgtcgatgccgtatcgaaagaat------taggtattcctgtc
SEQ ID NO:11    665 atggcgtgagaggagcccct------ggaag------aaggccttggcgtg
SEQ ID NO:12    652 cagaagt-----ttgcccggatgctggaatctcacctgcgaatgccggta
SEQ ID NO:13    635 aggttttggttgagatgtttgagaaaaaat------tgaataaaaaacta

SEQ ID NO:9     712 agagtcgctctgcatccccaagcggtg------ggtgctctcggagctgc
SEQ ID NO:11    703 gaaatcaagacgtctcccctggctcagtacaacggtgccctgggtgccgc
SEQ ID NO:12    697 aatacccatcctgatgcgcaatttgct------ggcgcaattggcgcggc
SEQ ID NO:13    679 ctaattccaaaagaaccacagattgtt------tgctgtgttggagctat

SEQ ID NO:9     756 tttgattgctta-----tgataaaatcaagaaa-taa
SEQ ID NO:11    753 tctgtatgcgta-----t-aaaaaagcagccaaataa
SEQ ID NO:12    741 ggtaattggtcaacgagtgaggacacgccgatga---
SEQ ID NO:13    723 attggtt-------------taa--------------
```

Figure 12B

Figure 13

```
SEQ ID NO:10      1 vktvytlgidvgsssskaviledgkkivahavveigtgstgpervldevf
SEQ ID NO:14      1 ms-iytlgidvgstaskciilkdgkeivakslvavgtgtsgparsisevl
SEQ ID NO:15      1 mavaysigidsgstatkgilladg-vitrrflvpt---pfrpataiteaw
SEQ ID NO:16      1 ----milgidvgstttkmvlmeds-kiiwykiedigv--vieedillkmv

SEQ ID NO:10     51 kdtnlkiedmaniiatgygrfnvd-cakgevseitchakgalfecpgttt
SEQ ID NO:14     50 enahmkkedmaftlatgygrnslegiadkqmselschamgasfiwpnvht
SEQ ID NO:15     47 etlreglettpfltltgygrqlvd-fadkqvteischglgarflapatra
SEQ ID NO:16     44 keieqkyp-idkivatgygrhkvs-fadkivpevialgkganyffneadg

SEQ ID NO:10    100 ildiggqdvksiklngqglvmqfamndkcaagtgrfldvmskvleipmse
SEQ ID NO:14    100 vidiggqdvkvihve-ngtmtnfqmndkcaagtgrfldvmanilevkvsd
SEQ ID NO:15     96 vidiggqdskviqlddd gnlcdflmndkcaagtgrflevisrtlgtsveq
SEQ ID NO:16     92 vidiggqdtkvlkidkngkvvdfilsdkcaagtgkflekaldilkidkne

SEQ ID NO:10    150 mgdwyfkskhpaavsstctvfaesevisllsknvpkedivagvhqsiaak
SEQ ID NO:14    149 laelgakstkrvaisstctvfaesevisqlskgtdkidiiagihrsvasr
SEQ ID NO:15    146 l-dsitenvtphaitsmctvfaeseaislrsagvapeailagvlnamarr
SEQ ID NO:16    142 ink--yksdniakissmcavfaeseiisllskkvpkegilmgvyesiinr

SEQ ID NO:10    200 acalvrrvgvgedltmtgggsrdpgvvdavskelgipvrvalhpqavgal
SEQ ID NO:14    199 viglanrvgivkdvvmtggvaqnygvrgaleeglgveiktsplaqyngal
SEQ ID NO:15    195 sanfiarlsceapilftggvehcqkfarmleshlrmpvnthpdaqfagai
SEQ ID NO:16    190 vipmtnrlki-qnivfsggvaknkvlvemfekklnkkllipkepqivccv

SEQ ID NO:10    250 gaaliaydkikk--
SEQ ID NO:14    249 gaalyaykkaak--
SEQ ID NO:15    245 gaavig-qrvrtrr
SEQ ID NO:16    239 gailv---------
```

Figure 14

ATGAGTGAAGAAAAAACAGTAGATATTGAAAGCATGAGCTCCAAGGAAGCCCTTGGTTAC
TTCTTGCCGAAAGTCGATGAAGACGCACGTAAAGCGAAAAAAGAAGGCCGCCTCGTTTGC
TGGTCCGCTTCTGTCGCTCCTCCGGAATTCTGCACGGCTATGGACATCGCCATCGTCTAT
CCGGAAACTCACGCAGCTGGTATCGGTGCCCGTCACGGTGCTCCGGCCATGCTCGAAGTT
GCTGAAAACAAAGGTTACAACCAGGACATCTGTTCCTACTGCCGCGTCAACATGGGCTAC
ATGGAACTCCTCAAACAGCAGGCTCTGACAGGCGAAACGCCGGAAGTCCTCAAAAACTCC
CCGGCTTCTCCGATTCCCCTTCCGGATGTTGTCCTCACTTGCAACAACATCTGCAATACC
TTGCTCAAATGGTATGAAAACTTGGCTAAAGAATTGAACGTACCTCTCATCAACATCGAC
GTACCGTTCAACCATGAATTCCCTGTTACGAAACACGCTAAACAGTACATCGTCGGCGAA
TTCAAACATGCTATCAAACAGCTCGAAGACCTTTGCGGCCGTCCCTTCGACTATGACAAA
TTCTTCGAAGTACAGAAACAGACACAGCGCTCCATCGCTGCCTGGAACAAAATCGCTACG
TACTTCCAGTACAAACCGTCGCCGCTCAACGGCTTCGACCTCTTCAACTACATGGGCCTC
GCCGTTGCTGCCCGCTCCTTGAACTACTCGGAAATCACGTTCAACAAATTCCTCAAAGAA
TTGGACGAAAAAGTAGCTAATAAGAAATGGGCTTTCGGTGAAAACGAAAAATCCCGTGTT
ACTTGGGAAGGTATCGCTGTCTGGATCGCTCTCGGCCACACCTTCAAAGAACTCAAAGGT
CAGGGCGCTCTCATGACTGGTTCCGCTTATCCTGGCATGTGGGACGTTTCCTACGAACCG
GGCGACCTCGAATCCATGGCAGAAGCTTATTCCCGTACATACATCAACTGCTGCCTCGAA
CAGCGCGGTGCTGTTCTTGAAAAAGTTGTCCGCGATGGCAAATGCGACGGCTTGATCATG
CACCAGAACCGTTCCTGCAAGAACATGAGCCTCCTCAACAACGAAGGCGGCCAGCGCATC
CAGAAGAACCTCGGCGTACCGTACGTCATCTTCGACGGCGACCAGACCGATGCTCGTAAC
TTCTCGGAAGCACAGTTCGATACCCGCGTAGAAGCTTTGGCAGAAATGATGGCAGACAAA
AAAGCCAATGAAGGAGGAAACCACTAA (SEQ ID NO:17)

Figure 15

MSEEKTVDIESMSSKEALGYFLPKVDEDARKAKKEGRLVCWSASVAPPEFCTAMDIAIVY
PETHAAGIGARHGAPAMLEVAENKGYNQDICSYCRVNMGYMELLKQQALTGETPEVLKNS
PASPIPLPDVVLTCNNICNTLLKWYENLAKELNVPLINIDVPFNHEFPVTKHAKQYIVGE
FKHAIKQLEDLCGRPFDYDKFFEVQKQTQRSIAAWNKIATYFQYKPSPLNGFDLFNYMGL
AVAARSLNYSEITFNKFLKELDEKVANKKWAFGENEKSRVTWEGIAVWIALGHTFKELKG
QGALMTGSAYPGMWDVSYEPGDLESMAEAYSRTYINCCLEQRGAVLEKVVRDGKCDGLIM
HQNRSCKNMSLLNNEGGQRIQKNLGVPYVIFDGDQTDARNFSEAQFDTRVEALAEMMADK
KANEGGNH (SEQ ID NO:18)

```
SEQ ID NO:17      1 atgagtgaagaaaaaacagtagatattgaaagcatgagctccaaggaagc
SEQ ID NO:19      1 atg------ccaaagacagta---------agccctggcgttcagg----
SEQ ID NO:20      1 ----atgatgaaattaaag--gcaattgaaaagttga--tgcaa------
SEQ ID NO:21      1 ------------atgtcacttgtcaccga----------tcta--cccgc

SEQ ID NO:17     51 cctt---ggttacttcttgccgaaa--gtcgatgaagacgca-------c
SEQ ID NO:19     32 -cat---tgagagatgtagttgaaaaggtttacagagaactg-------c
SEQ ID NO:20     37 ----------aaatt-----cgcca--gtagaaaagaacagc-------t
SEQ ID NO:21     27 cattttcgatcagttct--ctgaag--ctcgccagacaggctttctcacc

SEQ ID NO:17     89 gta-aagcgaaaa-aagaaggccgcctcgttt-gctggtccgcttctgtc
SEQ ID NO:19     71 ggg-aaccgaaag-aaagaggagaaaaagtag-gctggtcctcttc--ca
SEQ ID NO:20     63 atataagcaaaaagaagaaggtagaaaagttt---ttggaatgttctgtg
SEQ ID NO:21     73 gtc-atggatctc-aaggag--cgcggcattccgctggt-------tggc

SEQ ID NO:17    136 gctcctccggaattctgcacggctatggacatcgccatcgtc--tatccg
SEQ ID NO:19    116 agttcccctgcgaactggctgaatcttttcggctgcatgttgggtatccg
SEQ ID NO:20    110 cct--------atgttcca-----atagaaat---aat--tt--tagcag
SEQ ID NO:21    112 act---------tactgcacctttatg----ccgcaagag-----atccc

SEQ ID NO:17    184 gaaactca--cgcagctggtatcggtgcc---cgtcacggtg--------
SEQ ID NO:19    166 gaaaacca--ggctgctggtatcgctgccaaccgtgacggcgaagtgatg
SEQ ID NO:20    140 caaatgcaatcccagttggtttgtgtgga---ggtaaaaat---------
SEQ ID NO:21    144 ga-----t--ggcagc------cggtgcg---gtt----gtg--------

SEQ ID NO:17    221 ----------------------------------------ctccggccatgc
SEQ ID NO:19    214 tgccaggctgcagaagatatcggttatgacaacgatatctgcggctatgc
SEQ ID NO:20    178 ---------------------------------------------gacacaa
SEQ ID NO:21    166 -------------------------------------gtttcgctctgt

SEQ ID NO:17    233 tcgaagt-t----------gctg--------------------aaaa--
SEQ ID NO:19    264 ccgtatt-tccctggcttatgctgccgggttccggggtgccaacaaaatg
SEQ ID NO:20    185 tcccaat-a----------gcag--------------------a-----
SEQ ID NO:21    178 tccacctct----------gatg--------------------aaac--

SEQ ID NO:17    249 --caaaggttacaaccaggacatctgttcctactgccgcgtcaacatg--
SEQ ID NO:19    313 gacaaagatggcaactatgtcatcaacccccacagcggcaaacagatgaa
SEQ ID NO:20    198 ---ggaggat-ttgccaagaaacctatgcc--------cattaata----
SEQ ID NO:21    195 --ca---ttgaagaagcggagaaagat-----ctgccgcg-caacct---

SEQ ID NO:17    295 ----------ggctacatggaactc--ctcaaacagcag-----------
SEQ ID NO:19    363 agatgccaatggcaaaaaggtattcgacgcagatggcaaacccgtaatcg
SEQ ID NO:20    232 --------------------aaatc--atccta----tg-----------
SEQ ID NO:21    231 ------------ctgcccg---ctg--attaaa-agca------------

SEQ ID NO:17    322 ----------------------------------------------------
SEQ ID NO:19    413 atcccaagaccctgaaaccctttgccaccaccgacaacatctatgaaatc
SEQ ID NO:20    245 ----------------------------------------------------
SEQ ID NO:21    251 ----------------------------------------------------

SEQ ID NO:17    322 ---gctctgac---aggcgaaa----------cgccggaa-gtcctcaa
SEQ ID NO:19    463 gctgctctgccggaaggggaagaaaagacccgccgccagaatgccctgca
SEQ ID NO:20    245 ---gttttaa----------------------------gaa-ggca--aa
SEQ ID NO:21    251 ---gctacggc---ttcggcaa----------aaccg----------at
```

Figure 16A

```
SEQ ID NO:17    354 aaactccccggcttctccgattccccttccggatgttgtcctcacttgca
SEQ ID NO:19    513 caaatatcgtcagatgaccatgcccatgccggacttcgtgctgtgctgca
SEQ ID NO:20    261 aacctgccc--ttactttgaagcatct----gatatagttat-tggagaa
SEQ ID NO:21    274 aaatgcccctacttct-------acttttcggatctggtggtc---ggtg

SEQ ID NO:17    404 acaacatctgca-------ataccttgctcaaatggtatgaaaacttgg-
SEQ ID NO:19    563 acaacatctgca-------actgcatgaccaaatggtatgaagacattg-
SEQ ID NO:20    304 actacctgtgaaggaaagaagaagatgtttgagttgatggagagattggt
SEQ ID NO:21    314 aaaccacctgcg-------acggcaaaaagaaaatgtatgaatacatgg-

SEQ ID NO:17    446 -ctaaagaattgaac---gtacctctca----tcaacatcgacgtac--c
SEQ ID NO:19    605 -cccgtcggcacaac---attcctttga----tcatgatcgacgttc--c
SEQ ID NO:20    354 gccaatgcatataat---gcacctcccacacatgaaagatgaagatt--c
SEQ ID NO:21    356 -c---ggagtttaagcctgttcatgtga----tgca-attgcccaacagc

SEQ ID NO:17    486 gttca--accatgaattc---cctg--tta-cgaa----ac--acgctaa
SEQ ID NO:19    645 ttaca--ac---gaattcgaccatg--tcaacgaa----gccaacgtgaa
SEQ ID NO:20    399 tttga--a-----aatct---ggat--taa-agaagttgaa--aagctaa
SEQ ID NO:21    397 gttaaggacgatgcctcg---cgtgcgtta-tgga----a----------

SEQ ID NO:17    522 acagtacatcgtcg-----gcgaattcaaacatgctatca----aacagc
SEQ ID NO:19    684 a---tacatccggt-----cccagctggatacggccatcc----gtcaaa
SEQ ID NO:20    434 --aagaattggttgagaaagagactggaaataaaataacagaggaaaagt
SEQ ID NO:21    429 --agccgagatgct-----gcgcttgcaa----------a----aaacgg

SEQ ID NO:17    563 tcgaagacctttgcggccgtcccttcgactatgacaaattcttcgaagta
SEQ ID NO:19    722 tggaagaaatcaccggcaagaagttcgatgaagacaaattc------gaa
SEQ ID NO:20    482 taaaaga-------------------------gacagttgat--aaagta
SEQ ID NO:21    458 tagaagaacgttttgggcacgagattagcgaagatgctctgcgcgatgcc

SEQ ID NO:17    613 cagaaacagacacagcgctc-catcg--ctgcc------tggaacaaaat
SEQ ID NO:19    766 cag-tgctgccagaacgc-c-aaccgtactgccaaagcatggctgaaggt
SEQ ID NO:20    505 aataaagttagggag-----------------t------tgttttataaa
SEQ ID NO:21    508 attgcgctgaaaaaccgcgaacgtcg--cgcac------tgg---ctaat

SEQ ID NO:17    654 cgctacgtacttc--c--agtacaaaccgtcgccgctcaacggcttcgac
SEQ ID NO:19    813 ttgcgactacctg--c--agtacaaaccggctccgttcaacgggttcgac
SEQ ID NO:20    532 ctctatgaattga--ggaagaataaaccagctccaattaagggtttagat
SEQ ID NO:21    547 ttttatcatcttgggc--agttaaatcctccggcgcttagcggcagcgac

SEQ ID NO:17    700 ctcttcaactacatgggcctcgccg-ttgctgcccgctccttgaactact
SEQ ID NO:19    859 ctgttcaaccatatggctgacgtgg-ttaccgcccgtggccgtgtggaag
SEQ ID NO:20    580 gttttaaaattattccagtttgcctatttattggatattgatgacacaat
SEQ ID NO:21    595 attctga---aagtggtttacggcg-caaccttccggttcgataaagagg

SEQ ID NO:17    749 cggaaatcacgttcaacaaattcctcaaagaattggacgaaaaagtagc-
SEQ ID NO:19    908 ctgctgaagctttcgaactgctggccaaggaactggaacagcatgt----
SEQ ID NO:20    630 agggatt----ttagaggatttaattgaggagttagaggagagagtt---
SEQ ID NO:21    641 cg------------------ttgatcaatgaactggatgcaatgaccgcc

SEQ ID NO:17    798 -----taataagaaatgggctttcggtgaa------aacgaaaaatcccg
SEQ ID NO:19    954 --------gaaggaaggcaccaccaccgctcccttcaaagaacagcatcg
SEQ ID NO:20    673 -----aaaaaaggagaaggttatgaaggaa------agagaa--------
SEQ ID NO:21    673 cgcgttcgtcagcagtgggaagaag--gcc------agcgactggacccg

SEQ ID NO:17    837 tgttacttgggaaggta-tcgctgtctggatcgctctcggccacacc---
SEQ ID NO:19    996 tatcatgttcgaaggga-tcccctgctgg--ccgaaactgccgaacc---
SEQ ID NO:20    704 -------ttttaataac-tggctgtc-caatggttgctggaaacaataag
SEQ ID NO:21    715 cgt--ccgcgcattttaatcaccggctg---cccgattggcggcgc----
```

Figure 16B

```
SEQ ID NO:17    883 ----t--tcaaagaactca--aaggtcagggcgctctcatgactggttcc
SEQ ID NO:19   1040 ----tgttcaaaccgctga--aagccaacggcctgaacatcaccggcgtt
SEQ ID NO:20    745 attgt--tgaaattattgaggaagtt---ggaggagtagttgttggtgaa
SEQ ID NO:21    756 ------------agcaga--aaaagtggtgcgcgcgattgaagagaatg

SEQ ID NO:17    925 gcttat---cctggcatgtgggacgtttcctacgaacc--------ggg-
SEQ ID NO:19   1084 gtatatgctcctgctttcgggttcgtgtacaacaacct--------gga-
SEQ ID NO:20    790 g--aaa---gctgcactggaacaagattctttgaaaactttgttgaggg-
SEQ ID NO:21    791 gc---g---gctgggttgtcggttatgaaaactgcacc--------gggg

SEQ ID NO:17    963 ------cga---cctcg-aatccatggcagaa----gcttattcccgtac
SEQ ID NO:19   1125 ------cga---attgg---tcaaagcctact----gcaaagccccgaac
SEQ ID NO:20    834 ------ctatagcgtag-aggacattgcaaaa----agata----cttta
SEQ ID NO:21    827 cgaaagcga---ccgagcaatgcgtggcagaaacgggcgatgtctacgac

SEQ ID NO:17    999 atac------atcaactgctgcct----------cgaacagcgcggtgct
SEQ ID NO:19   1159 -tcc------gtca------gcat----------cgaacagggtgttgcc
SEQ ID NO:20    869 aaat------cccatgtgcttgtagatttaaaaacgatgagagagttgaa
SEQ ID NO:21    874 gcgctggcggataaatatctggc------------gattggctgctcct

SEQ ID NO:17   1033 gttcttgaaaaagttgtccgcgatggcaaatgcgacggc-ttgatcatgc
SEQ ID NO:19   1186 tggcgtgaaggcctgatccgcgacaacaaggttgacggc-gtactggttc
SEQ ID NO:20    913 aatataaagagattggttaaagagttggacgtcgatggagttgtttat--
SEQ ID NO:21    911 gtgtttcgccgaacgatcagcgcctgaaaatgc-tcagc-cagatggtgg

SEQ ID NO:17   1082 accagaacc-gttcctgcaagaacatgagcctcctcaacaacgaaggcg-
SEQ ID NO:19   1235 actacaacc-ggtcctgcaaaccctggagcggctacatgcctgaaatgc-
SEQ ID NO:20    961 ----tacac-tttgcagtattgccat----acatttaacatagagggagc
SEQ ID NO:21    959 aggaatatcaggtcgatggcgtagttga-----tgtgattttgcaggcgt

SEQ ID NO:17   1130 ---gccagcgcatc-cagaagaacctc--ggcgtaccgtacgtcatcttc
SEQ ID NO:19   1283 ---agcgtcgtttc-accaaagacatg--ggtatccccactgctggattc
SEQ ID NO:20   1002 taaggtagaggagg-cattaaaagaggagggcattccaattataagaatt
SEQ ID NO:21   1004 ---gccatacctacgcggtggaatcgc--tggcgattaaacgtcatgtgc

SEQ ID NO:17   1174 gacggcgaccagaccgatgctcgtaacttctcggaagca----------
SEQ ID NO:19   1327 gacggtgaccaggctgacccgagaaacttcaacgcggct----------
SEQ ID NO:20   1051 gaaactgactattctga-------aagtgatag--agag----------
SEQ ID NO:21   1049 gccagc-agcacaacattccttatatcgctattgaaacagactactccac

SEQ ID NO:17   1213 -------------cagttcgatacccgcgtagaagctttggcagaaatga
SEQ ID NO:19   1366 -------------cagtatgagacccgtgttcagggcttggtcgaagcca
SEQ ID NO:20   1081 -------------cagttaaaaacaaggttggaggcatttattgagatga
SEQ ID NO:21   1098 ctcggatgtcgggcagctcagtacccgtgtcgcggcctttattgagatgc

SEQ ID NO:17   1250 tggcagacaaaaaagccaatgaaggaggaaaccactaa
SEQ ID NO:19   1403 tggaag-caaatgatgaaaagaagg-ggaaataa----
SEQ ID NO:20   1118 t------------------ttaa---------------
SEQ ID NO:21   1148 tgtaa-----------------------------------
```

Figure 16C

Figure 17

```
SEQ ID NO:18      1 --mseektvdiesmsskealgyflpkvdedarkakkegrlvcwsasvapp
SEQ ID NO:22      1 ----mpktvs----pgvqalrdvvekvyrelrepkergekvgwssskfpc
SEQ ID NO:23      1 --mmklka--ieklmqkfa------srkeqlykqkeegrkvfgm------
SEQ ID NO:24      1 mslvtdlpaifdqfsearqtg-fltvmdlkergiplvg------------

SEQ ID NO:18     49 efctamdiaivypethaag---igarhgapamlevaenkgynqdicsycr
SEQ ID NO:22     43 elaesfrlhvgypenqaag---iaanrdgevmcqaaedigydndicgyar
SEQ ID NO:23     35 -fcayvpieiila-anaip---vglcggkndtipiae-edlprnlcplik
SEQ ID NO:24     38 tyctfmpqei----pmaagavvvslcstsdetieeae-kdlprnlcplik

SEQ ID NO:18     96 vnmgym-----------------------------------------------
SEQ ID NO:22     90 islayaagfrgankmdkdgnyvinphsgkqmkdangkkvfdadgkpvidp
SEQ ID NO:23     79 ssygf-----------------------------------------------
SEQ ID NO:24     83 ssygf-----------------------------------------------

SEQ ID NO:18    102 ellkqqaltgetpev---------------lknspaspiplpdvvltcnn
SEQ ID NO:22    140 ktlkpfattdniyeiaalpegeektrrqnalhkyrqmtmpmpdfvlccnn
SEQ ID NO:23     84 -------------------------------kkaktcpyfeasdiviget
SEQ ID NO:24     88 ------gktdkcpyf---------------y---------fsdlvvg-et

SEQ ID NO:18    137 icntllkwyenlakelnvplinidvpfnhefpvtkhakqyivgefkhaik
SEQ ID NO:22    190 icncmtkwyediarrhniplimidvpynefdhvneanvkyirsqldtair
SEQ ID NO:23    103 tcegkkkmfelm--erlvpmhimhlphmkd----edslkiwikeveklke
SEQ ID NO:24    107 tcdgkkkmyeymaefkpvhvmqlpnsvkdd-----asralwkaemlrlqk

SEQ ID NO:18    187 qledlcgrpfdydkffe---vqkqtqrsiaawnkiatyfqykpsplngfd
SEQ ID NO:22    240 qmeeitgkkfdedkfeq---ccqnanrtakawlkvcdylqykpapfngfd
SEQ ID NO:23    147 lveketgnkiteeklke---tvdkvnkvrelfyklyelrknkpapikgld
SEQ ID NO:24    152 tveerfgheisedalrdaialknrerralanfyhlg---qlnppalsgsd

SEQ ID NO:18    234 ---lfnymglavaarslnyseitfnkflkeldekvan--kkwafge--n-
SEQ ID NO:22    287 ---lfnhmadvvtargrveaaeafellakeleqhvke--gtttapf--k-
SEQ ID NO:23    194 vlklfqfaylldiddtigile----dlieeleerv-----kk---ge--gy
SEQ ID NO:24    199 ---ilk----vvygatfrfdk---ealineldamtarvrqqweegqrld-

SEQ ID NO:18    276 eksrvtwegiavwialghtfkelkgqgalmtg----say---pgmwdvsy
SEQ ID NO:22    329 eqhrimfegipcwpklpnlfkplkanglnitg----vvy---apafgfvy
SEQ ID NO:23    231 egkrilitgcpmvagnnkiveiieevggvvvg----eesctgtrffenfv
SEQ ID NO:24    238 prprilitgcpiggaaekvvraieenggwvvgyenctga---kateqcva

SEQ ID NO:18    319 epgdl-esmaeaysrtyinccl--eqrgavlekvvrdgkcdglimhqnrs
SEQ ID NO:22    372 --nnl-delvkayckapnsvsi--eqgvawreglirdnkvdgvlvhynrs
SEQ ID NO:23    277 egysv-ediakryfkipcacrfknderveniklvkeldvdgvvyytlqy
SEQ ID NO:24    285 etgdvydaladkylaigcscvspndqrlkmlsqmveeyqvdgvvdvilqa

SEQ ID NO:18    366 cknmsllnnegg--qriqknlgvpyvifdgdqtdarnfseaqfdtrveal
SEQ ID NO:22    417 ckpwsgympemq--rrftkdmgiptagfdgdqadprnfnaaqyetrvqgl
SEQ ID NO:23    326 cht---fniegakveealkeegipiirietdyses---dreqlktrleaf
SEQ ID NO:24    335 chtyaveslaik--rhvrqqhnipyiai---etdystsdvgqlstrvaaf

SEQ ID NO:18    414 aemmadkkaneggnh
SEQ ID NO:22    465 veameandekkgk--
SEQ ID NO:23    370 iemi----------
SEQ ID NO:24    380 ieml----------
```

Figure 18

ATGAGTCAGATCGACGAACTTATCAGCAAATTACAGGAAGTATCCAACCATCCCCAGAAG
ACGGTTTTGAATTATAAAAAACAGGGTAAAGGCCTCGTAGGCATGATGCCCTACTACGCT
CCGGAAGAAATCGTATATGCTGCAGGCTACCTCCCGGTAGGCATGTTCGGTTCCCAGAAC
CCGCAGATCTCCGCAGCTCGTACGTACCTTCCTCCGTTCGCTTGCTCCTTGATGCAGGCT
GACATGGAACTCCAGCTCAACGGCACCTATGACTGCCTCGACGCTGTTATCTTCTCCGTT
CCTTGCGACACTCTCCGCTGCATGAGCCAGAAATGGCACGGCAAAGCTCCGGTCATCGTC
TTCACACAGCCGCAGAACCGTAAGATCCGCCCGGCTGTCGATTTCCTCAAAGCTGAATAC
GAACATGTCCGTACGGAATTGGGACGTATCCTCAACGTAAAAATCTCCGACCTGGCTATC
CAGGAAGCTATCAAAGTATATAACGAAAACCGTCAGGTTATGCGTGAATTCTGCGACGTA
GCTGCTCAGTACCCGCAGATCTTCACTCCGATAAAACGTCATGACGTCATCAAAGCCCGC
TGGTTCATGGACAAAGCTGAACACACCGCTTTGGTCCGCGAACTCATCGACGCTGTCAAG
AAAGAACCGGTACAGCCGTGGAATGGCAAAAAAGTCATCCTCTCCGGTATCATGGCAGAA
CCGGATGAATTCCTCGATATCTTCAGCGAATTCAACATCGCTGTCGTCGCTGACGACCTC
GCTCAGGAATCCCGCCAGTTCCGTACAGACGTACCGTCCGGCATCGATCCCCTCGAACAG
CTCGCTCAGCAGTGGCAGGACTTCGATGGCTGCCCGCTCGCTTTGAACGAAGACAAACCG
CGTGGCCAGATGCTCATCGACATGACTAAGAAATACAATGCTGACGCCGTCGTCATCTGC
ATGATGCGTTTCTGCGATCCTGAAGAATTCGACTATCCGATTTACAAACCGGAATTTGAA
GCTGCTGGCGTTCGTTACACGGTCCTCGACCTCGACATCGAATCTCCGTCCCTCGAACAG
CTCCGCACCCGTATCCAGGCTTTCTCGGAAATCCTCTAA (SEQ ID NO:25)

Figure 19

MSQIDELISKLQEVSNHPQKTVLNYKKQGKGLVGMMPYYAPEEIVYAAGYLPVGMFGSQN
PQISAARTYLPPFACSLMQADMELQLNGTYDCLDAVIFSVPCDTLRCMSQKWHGKAPVIV
FTQPQNRKIRPAVDFLKAEYEHVRTELGRILNVKISDLAIQEAIKVYNENRQVMREFCDV
AAQYPQIFTPIKRHDVIKARWFMDKAEHTALVRELIDAVKKEPVQPWNGKKVILSGIMAE
PDEFLDIFSEFNIAVVADDLAQESRQFRTDVPSGIDPLEQLAQQWQDFDGCPLALNEDKP
RGQMLIDMTKKYNADAVVICMMRFCDPEEFDYPIYKPEFEAAGVRYTVLDLDIESPSLEQ
LRTRIQAFSEIL (SEQ ID NO:26)

```
SEQ ID NO:25      1 atgagtcagatcgacgaacttatcagcaaattacaggaagtatccaacca
SEQ ID NO:27      1 atggct---atcagtgcacttattgaagagttccaaaaagtat-ctgcca
SEQ ID NO:28      1 ----------atgatgaaattaaaggcaattgaaaagttgatgcaaaaat
SEQ ID NO:29      1 atgtcacttgtcaccgatctacccgccattttcgatcagtttctctgaagc

SEQ ID NO:25     51 tccccagaag---------ac-------ggttttg---aattataaaaaa
SEQ ID NO:27     47 gccc--gaag---------ac-------catgctggccaaatataaagcc
SEQ ID NO:28     41 tcgccagtag---------aaaagaacagctatat---aagcaaaaagaa
SEQ ID NO:29     51 tcgccagacaggctttctcac--------cgtcatg---gatctcaaggag

SEQ ID NO:25     82 cagggtaaaggcctcgtaggca--tgatgccctactacgctccggaagaa
SEQ ID NO:27     79 cagggcaaaaaagccatcggct--gcctgccgtactatgttccggaagaa
SEQ ID NO:28     79 gaaggtagaaaagtttttggaa--tgttctgtgcctatgttccaatagaa
SEQ ID NO:29     91 cgcggcattccgctggttggcacttactgcacctttatgc--cgcaagag

SEQ ID NO:25    130 atcgtatatgctgcaggctacctcccggtaggcatgt---tcggttccca
SEQ ID NO:27    127 ctggtctatgctgcaggcatggttcccatgggtgtat---ggggctgcaa
SEQ ID NO:28    127 ataattttagcagcaaatgcaatcccagttggtttgt---gtggaggtaa
SEQ ID NO:29    139 atcccgatggcagccgg------tgcggttgtggtttcgctctgttccac

SEQ ID NO:25    177 -------gaacccgcag-atctccgcagctcgtacgtaccttcctccgtt
SEQ ID NO:27    174 -------tggcaaacaggaagtccgttccaaggaa-tactgtgcttcctt
SEQ ID NO:28    174 -------aaatgacaca-atcccaatagcagaggaggatttgccaagaaa
SEQ ID NO:29    183 ctctgatgaaacc-----attgaagaagcggagaaagatctgccgcgcaa

SEQ ID NO:25    219 cgcttgctccttgatgcaggctgacatggaactccagctcaacggca---
SEQ ID NO:27    216 ctactgcaccattgcccagcagtctctggaaatgctgctggacggga---
SEQ ID NO:28    216 cctatgcccattaataaaatcatcctatggttttaag----aaggca---
SEQ ID NO:29    228 cctctgcccgctga-------ttaaaagcagctacggct--tcggcaaaa

SEQ ID NO:25    266 cctatgactgcctcgacgctgttatcttctcc----gttcct-tgcg---
SEQ ID NO:27    263 ccctggatgggttggacgggatcatca-ctcc----ggtactgtgtg---
SEQ ID NO:28    259 --aaaacctgcccttactttg-aagcatctgatatagttatt-ggag---
SEQ ID NO:29    269 ccgataaatgcccctac----ttctacttttc----ggatct-ggtggtc

SEQ ID NO:25    308 ----acactctccgctgcatgagccagaaat-------gg--------c-
SEQ ID NO:27    305 ----ataccctgcgtcccatgagccagaacttcaaagtgg---------cc
SEQ ID NO:28    302 ----aaact-------------acctgtgaa-------gg--------a-
SEQ ID NO:29    310 ggtgaaaccacctgcgacggcaaaaagaaaa-------tgtatgaatac-

SEQ ID NO:25    338 ----acggcaaagct----ccggtcatcg-tcttcacacagccgcagaac
SEQ ID NO:27    343 atgaaagacaagatg----ccggttattt-tcctggctcatccccaggtc
SEQ ID NO:28    319 ----aagaagaagat----gtttgagttgatggagagattggtgccaatg
SEQ ID NO:29    352 ----atggcggagtttaagcctgttcatg-tgatgcaattgcccaacagc

SEQ ID NO:25    379 cgtaaga-tccgcccggc------------tgtcgatttcctcaaag-ct
SEQ ID NO:27    388 cgtcagaatgccgccggc------------aagc-agttcacctatg-at
SEQ ID NO:28    361 catataa-tgcacctcccacacatgaaagatgaagattctttgaaaatct
SEQ ID NO:29    397 gttaagg-acgatgcctc------------gcgtgcgttatggaaag-cc

SEQ ID NO:25    415 gaat--acgaacatgtc---cgt-------acgg--aattgg----gacg
SEQ ID NO:27    424 gcct--acagcgaagt-----ga--------aaggccatctgg----aaga
SEQ ID NO:28    410 ggattaaagaagttgaaaagcta-------aaag--aattggttgagaaa
SEQ ID NO:29    433 ga------gatgctgcg---cttgcaaaaaacgg--tagaag----aacg
```

Figure 20A

```
SEQ ID NO:25    447 tatcctcaacgtaaaa--atctccgacctggctatccaggaagctatcaa
SEQ ID NO:27    456 aatctgcggccatgaa--atcaccaatgatgccatcctggatgccatcaa
SEQ ID NO:28    451 gagactggaaataaaataacagaggaaaagttaaaagagacagttgataa
SEQ ID NO:29    468 ttttgggcacg---ag--attagcgaagatgctctgcgcgatgccattgc

SEQ ID NO:25    495 agtatataacgaaaaccgtcaggttatgcgtgaattct---------gcg
SEQ ID NO:27    504 agtgtacaacaagagccgtgctgcccgccgcgaattct---------gca
SEQ ID NO:28    501 agtaaataaagtta---gggagttgtttttataaactct---------atg
SEQ ID NO:29    513 gctgaaaaaccgcgaacgtcgcgcactggctaatttttatcatcttgggc

SEQ ID NO:25    536 acgtagctgctcag-----tacccgcagatcttcactccgataaa--acg
SEQ ID NO:27    545 aactggc--caacg-----aacatcctgatctgatcccggcttccgtacg
SEQ ID NO:28    539 a-attgaggaagaa-----taaac-cag-------ctccaattaa--ggg
SEQ ID NO:29    563 agttaaatcctccggcgcttagcggcag--cgacattctgaaagt--ggt

SEQ ID NO:25    579 tcatgacgtcatc------------aaag----cccgctgg-----ttca
SEQ ID NO:27    588 ggccaccgtactg------------cgtg----ccgcttac-----ttca
SEQ ID NO:28    573 tttagatgtttta------------aaattattccagtttgcctatttat
SEQ ID NO:29    609 ttacggcgcaaccttccggttcgataaag----aggcgttg-----atca

SEQ ID NO:25    608 tggacaaagctgaacacaccgctttggtccgcgaactcatcgacgctgtc
SEQ ID NO:27    617 tgctgaaggatgaatacaccgaaaagctggaagaactgaacaagg-----
SEQ ID NO:28    611 tggatattgatgacacaatagggatttagaggatttaattgaggagtta
SEQ ID NO:29    650 atgaactggatgcaatgaccgc------ccgcg--ttcgtcagcagtggg

SEQ ID NO:25    658 aagaa-------ag--aaccggtacagccgtggaat-----ggcaaaaaa
SEQ ID NO:27    662 aactg-------gc--agctgctcctgccggcaagttcgacggccacaaa
SEQ ID NO:28    661 gaggagagagttaa--aaaaggagaaggttatgaa------ggaaagaga
SEQ ID NO:29    692 aagaa-------ggccagcgactggacccgcgtccg-----cgcatttta

SEQ ID NO:25    694 gtcatcctctccggt------atcatggcagaaccggatgaattcct---
SEQ ID NO:27    703 gtggttgtttccggc------atcatctacaacacgcccggcatcct---
SEQ ID NO:28    703 attttaataactggctgtccaatggttgctggaaacaataagattgt---
SEQ ID NO:29    730 atcaccggctgcccg------attggcggcgcagcagaaaaagtggtgcg

SEQ ID NO:25    735 cgatatcttcagcgaatt-caacatcgctgtcgtcgctgacgacctc-gc
SEQ ID NO:27    744 gaaagccatggatgacaa-caaactggccattgctgctgatgactgc-gc
SEQ ID NO:28    750 tgaaattattgaggaagt-tggaggagtagttgttggtgaagaaagctgc
SEQ ID NO:29    774 cgcgat-tgaagagaatggcggctgggttgtcggttatgaaaactgc-ac

SEQ ID NO:25    783 tcagga-atcccgccagttccgtacagacgtaccgtccggcatcgatccc
SEQ ID NO:27    792 ttatga-aagccgcagctttgccgtggatgctccggaagatctgga---c
SEQ ID NO:28    799 actgga-a-----caagattctttgaaaactttgttgagg--gctatagc
SEQ ID NO:29    822 cggggcgaaagcgaccgagcaatgc-gtggcagaaacggg---cgatgtc

SEQ ID NO:25    832 ctcgaacagctcgctcag------cagtgg------caggacttcgat-g
SEQ ID NO:27    838 aacggactgcatgctctggctgtacagttctccaaacagaagaacgat-g
SEQ ID NO:28    841 gtagaggacattgcaaa--------------------aaga-tacttt-a
SEQ ID NO:29    868 tacgacgcgctggcggat------aaatat------ctgg----cgattg

SEQ ID NO:25    869 ----gctgcccgctcgctttgaa----cgaagacaaaccgcg-tggccag
SEQ ID NO:27    887 ttctgctgtacgatcc---tgaatttgccaagaatacccgttctgaacac
SEQ ID NO:28    869 ----aaatcccatgtgcttgta------gatttaaaaacgat-gagagag
SEQ ID NO:29    902 ----gctgctc-ctgtgtttcgc----cga--acgatcagcg-cctgaaa

SEQ ID NO:25    910 atgctcatcgaca-----tgactaagaaatacaatgctgacgccgtcgtc
SEQ ID NO:27    934 gttggca---atc-----tggtaaaagaaagcggcgcagaaggactgatc
SEQ ID NO:28    908 ttgaaaatataaagagattggttaaagagttggacgtcgatggagttgtt
SEQ ID NO:29    940 atgctcagccaga-----tggtggaggaatatcaggtcgatggcgtagtt
```

Figure 20B

```
SEQ ID NO:25    955 atctgcatgatgcgtttctgcgatcctgaagaattcgactatc---cgat
SEQ ID NO:27    976 gtgttcatgatgcagttctgcgatccggaagaaatggaatatc---ctga
SEQ ID NO:28    958 tattacactttgcagtattgccatacatttaacatagagggag---ctaa
SEQ ID NO:29    985 gatgtgattttgcaggcgtgccatacctacgcggtggaatcgctggcgat

SEQ ID NO:25   1002 ttacaaaccggaatttgaagctgctgg----cgttcgttacacggtcctc
SEQ ID NO:27   1023 tctgaagaaggctctggatgcccacca----cattcctcatgtgaagatt
SEQ ID NO:28   1005 ggtagaggaggcattaaaagaggaggg----cattc------caattata
SEQ ID NO:29   1035 t----aaacgtcatgtgcgccagcagcacaacattccttatatcgctatt

SEQ ID NO:25   1048 gacctcgacatcgaatctccgtccctcgaa------cagctccgcacccg
SEQ ID NO:27   1069 ggtgtggaccagatgacccgggactttggt------caggcccagaccgc
SEQ ID NO:28   1045 agaattgaaactgactattctgaaagtgatagagagcagttaaaaacaag
SEQ ID NO:29   1081 gaaacagactactccacctcggatgtcggg------cagctcagtacccg

SEQ ID NO:25   1092 tatccaggctttctcggaaatcctctaa
SEQ ID NO:27   1113 tctggaagctttcgcagaaagcctgtaa
SEQ ID NO:28   1095 gttggaggcatttattgagatgatttaa
SEQ ID NO:29   1125 tgtcgcggcctttattgagatgctgtaa
```

Figure 20C

Figure 21

```
SEQ ID NO:26      1 msqideliskl qevsnhpqk---tvlnykkqgk glvgmmpyya peeeivya
SEQ ID NO:30      1 -maisalieef qkvsaspkt---mlakykaqgk kaigclpyyv peelvya
SEQ ID NO:31      1 mmkl-kaieklmqkfasrke---qlykqkeegr kvfgmfcayv pieiila
SEQ ID NO:32      1 mslvtdlpaifdqfsearqtgfltvmdlkergiplvgtyctfmpqeipma

SEQ ID NO:26     48 agylpvgmfgsqnpqisaartylppfacslmqadmelqlngt---ydc--
SEQ ID NO:30     47 agmvpmgvwgcngkqevrskeycasfyctiaqqslemlldgt---ldg--
SEQ ID NO:31     47 anaipvglcggkndtipiaeedlprnlcplikssygfkkaktcpyfea--
SEQ ID NO:32     51 agavvvslcstsdetieeaekdlprnlcplikss--ygfgkt---dkcpy

SEQ ID NO:26     93 ---ldavifsvpcdtlrcmsqkwh----gkapvivftqpqnrkirpavdf
SEQ ID NO:30     92 ---ldgiitpvlcdtlrpmsqnfkvamkdkmpviflahpqvrqnaagkqf
SEQ ID NO:31     95 ---sdivigettcegkkkmfelme----rlvpmhimhlp-hmkdedslki
SEQ ID NO:32     96 fyfsdlvvgettcdgkkkmyeyma----efkpvhvmqlpnsvkddasral

SEQ ID NO:26    136 lkaeyehvrtelgrilnvkisdlaiqeaikvynenrqvmrefcdvaaqyp
SEQ ID NO:30    139 tydaysevkghleeicgheitndaildaikvynksraarrefcklanehp
SEQ ID NO:31    137 wikeveklkelveketgnkiteeklketvdkvnkvrelfyklyelrknkp
SEQ ID NO:32    142 wkaemlrlqktveerfgheisedalrdaialknrerralanfyhlgqlnp

SEQ ID NO:26    186 qiftpikrhdvik----arwf---mdkaehtalvrelidavkk--epvqp
SEQ ID NO:30    189 dlipasvratvlr----aayf---mlkdeytekleelnkelaa--apagk
SEQ ID NO:31    187 ---apikgldvlk----lfqfaylldiddtigiledlieeleervkkgeg
SEQ ID NO:32    192 ---palsgsdilkvvygatfr---fdk---ealinel-damta--rvrqq

SEQ ID NO:26    227 wn-gkk------vilsg--imaepdefldifsefniavvaddlaqesrqf
SEQ ID NO:30    230 fd-ghk------vvvsg--iiyntpgilkamddnklaiaaddcayesrsf
SEQ ID NO:31    230 ye-gkr------ilitgcpmvagnnkiveiieevggvvvgeesctgtrff
SEQ ID NO:32    230 weegqrldprprilitgcpiggaaekvvraieenggwvvgyenctgakat

SEQ ID NO:26    268 rtdvpsgidp-leqlaqqwqdfdgcplalned---kprgqmlidmtkkyn
SEQ ID NO:30    271 avdapedldnglhalavqfskqkndvllydpefakntrsehvgnlvkesg
SEQ ID NO:31    273 enfv-egys--vediakryfkip-cacrfknd---e-rvenikrlvkeld
SEQ ID NO:32    280 eqcvaetgdv-ydaladkylai-gcscvspnd---q-rlkmlsqmveeyq

SEQ ID NO:26    314 adavvicmmrfcdpeefdypiykpef-eaagvrytvldldiespsleqlr
SEQ ID NO:30    321 aeglivfmmqfcdpeemeypdlkkal-dahhiphvkigvdqmtrdfgqaq
SEQ ID NO:31    315 vdgvvyytlqychtfniegakveeal-keegipiirietdysesdreqlk
SEQ ID NO:32    324 vdgvvdvilqachtyaveslaikrhvrqqhnipyiaietdystsdvgqls

SEQ ID NO:26    363 triqafseil
SEQ ID NO:30    370 taleafaesl
SEQ ID NO:31    364 trleafiemi
SEQ ID NO:32    374 trvaafieml
```

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   1  CGACGGCCCG GGCTGGTATC ATTCTAGTCA GTAATTCACC TTTGGAAAAT TTTCACAAAG
  61  GCAGTACGAC AGAAGCGTCG ATACATTCCA TTTAGCAGGA GGAAGTTACG GTAATGAGAA
 121  AAGTAGAAAT CATTACAGCT GAACAAGCAG CTCAGCTCGT AAAAGACAAC GACACGATTA
 181  CGTCTATCGG CTTTGTCAGC AGCGCCCATC CGGAAGCACT GACCAAAGCT TTGGAAAAAC
 241  GGTTCCTGGA CACGAACACC CCGCAGAACT TGACCTACAT CTATGCAGGC TCTCAGGGCA
 301  AACGCGATGG CCGTGCCGCT GAACATCTGG CACACACAGG CCTTTTGAAA CGCGCCATCA
 361  TCGGTCACTG GCAGACTGTA CCGGCTATCG GTAAACTGGC TGTCGAAAAC AAGATTGAAG
 421  CTTACAACTT CTCGCAGGGC ACGTTGGTCC ACTGGTTCCG CGCCTTGGCA GGTCATAAGC
 481  TCGGCGTCTT CACCGACATC GGTCTGGAAA CTTTCCTCGA TCCCCGTCAG CTCGGCGGCA
 541  AGCTCAATGA CGTAACCAAA GAAGACCTCG TCAAACTGAT CGAAGTCGAT GGTCATGAAC
 601  AGCTTTTCTA CCCGACCTTC CCGGTCAACG TAGCTTTCCT CCGCGGTACG TATGCTGATG
 661  AATCCGGCAA TATCACCATG GACGAAGAAA TCGGGCCTTT CGAAAGCACT TCCGTAGCCC
 721  AGGCCGTTCA CAACTGTGGC GGTAAAGTCG TCGTCCAGGT CAAAGACGTC GTCGCTCACG
 781  GCAGCCTCGA CCCGCGCATG GTCAAGATCC CTGGCATCTA TGTCGACTAC GTCGTCGTAG
 841  CAGCTCCGGA AGACCATCAG CAGACGTATG ACTGCGAATA CGATCCGTCC CTCAGCGGTG
 901  AACATCGTGC TCCTGAAGGC GCTACCGATG CAGCTCTCCC CATGAGCGCT AAGAAAATCA
 961  TCGGCCGCCG CGGCGCTTTG GAATTGACTG AAAACGCTGT CGTCAACCTC GGCGTCGGTG
1021  CTCCGGAATA CGTTGCTTCT GTTGCCGGTG AAGAAGGTAT CGCCGATACC ATTACCCTGA
1081  CCGTCGAAGG TGGCGCCATC GGTGGCGTAC CGCAGGGCGG TGCCCGCTTC GGTTCGTCCC
1141  GCAATGCCGA TGCCATCATC GACCACACCT ATCAGTTCGA CTTCTACGAT GGCGGCGGTC
1201  TGGACATCGC TTACCTCGGC CTGGCCCAGT GCGATGGCTC GGGCAACATC AACGTCAGCA
1261  AGTTCGGTAC TAACGTTGCC GGCTGCGGCG GTTTCCCCAA CATTTCCCAG CAGACACCGA
1321  ATGTTTACTT CTGCGGCACC TTCACGGCTG GCGGCTTGAA AATCGCTGTC GAAGACGGCA
1381  AAGTCAAGAT CCTCCAGGAA GGCAAAGCCA AGAAGTTCAT CAAAGCTGTC GACCAGATCA
1441  CTTTCAACGG TTCCTATGCA GCCCGCAACG GCAAACACGT TCTCTACATC ACAGAACGCT
1501  GCGTATTTGA ACTGACCAAA GAAGGCTTGA AACTCATCGA AGTCGCACCG GGCATCGATA
1561  TTGAAAAAGA TATCCTCGCT CACATGGACT TCAAGCCGAT CATTGATAAT CCGAAACTCA
1621  TGGATGCCCG CCTCTTCCAG GACGGTCCCA TGGGACTGAA AAAATAAATC TCTGCTGTAA
1681  AGGAGACTTT ACTATGAAAC CAATGAGACT ACATCACGTA GGCATTGTCC TGCCGACCTT
1741  AGAAAAAGCC CATGAATTCA TGCAGAATAA TGGACTTGAA ATCGACTATG CCGGCTATGT
1801  CGATGCTTAC CAGGCTGATC TCATTTTCAC TAAGTTTGGT GAATTTGCCA GCCCGATTGA
1861  AATGATTATC CCGCACTCCG GTGTGCTTAC CCAATTCAAT GGTGGCCGCG GCGGCATTGC
1921  CCACATCGCC TTCGAAGTGG ACGATGTCGA AGCTGTCCGC CAGGAAATGG AAGCAGATTG
1981  TCCGGGATGC ATGTTAGAAA AGAAAGCTGT CCAGGGTACG GACGACATTA TCGTCAACTT
2041  CCGCCGCCCG ACAACCAACC AGGGTATCCT CGTTGAATAT GTTCAGACGA CAGCACCTAT
2101  CACCGGCCGC GGCGAAAATC CTTTCGTTAA GAATCTCGGC CCGGAAAAAG GGAAGCTCAA
2161  CGAAACATGG CATCCCATGC GCCTGCACCA TATCGGCATC GTCTTGCCGA CCTTGGAAAA
2221  GGCCCATGAA TTCATCAAGA CCAATGGTCT GGAAGTGGAT TATTCCGGTT TCGTCGACGC
2281  CTACCATGCG GATCTCATTT TCACTAAAAA AGGTGAAAAC AGTACGCCTA TCGAATTCAT
2341  TATTCCCCGT GAAGGGGTCC TCAAAGATTT CAATCATGGC AGGGGAGGTA TCGCTCATAT
2401  CGCCTTTGAA GTGGATGATG TCGAAAAGGT ACGTCAGATT ATGGAAAGCC AGAAGCCTGG
2461  TTGCATGCTC GAAAAGAAAG CCGTCCGGGG AACGGACGAT ATCATCGTCA ACTTCCGCCG
2521  TCCCAGCACG GACGCCGGCA TCCTCGTCGA ATATGTCCAG ACCGTAGCTC CCATCAATCG
2581  CAGCAATCCC AACCCTTTTA ATGATTGATT TTTTATAAAG AAAGGTGAAA ACTGTGTATA
2641  CTCTCGGAAT CGACGTTGGT TCTTCTTCTT CCAAGGCAGT CATCCTGGAA GATGGCAAGA
2701  AGATCGTCGC CCATGCCGTC GTTGAAATCG GCACCGGTTC GACCGGTCCG GAACGCGTCC
2761  TGGACGAAGT CTTCAAAGAT ACCAACTTAA AAATTGAAGA CATGGCGAAC ATCATCGCCA
2821  CAGGCTATGG CCGTTTCAAT GTCGACTGCG CCAAAGGCGA AGTCAGCGAA ATCACGTGCC
2881  ATGCCAAAGG GGCCCTCTTT GAATGCCCCG GTACGACGAC CATCCTCGAT ATCGGCGGTC
2941  AGGACGTCAA GTCCATCAAA TTGAATGGCC AGGGCCTGGT CATGCAGTTT GCCATGAACG
3001  ACAAATGCGC CGCTGGTACG GGCCGTTTCC TCGACGTCAT GTCGAAGGTA CTGGAAATCC
3061  CCATGTCTGA AATGGGGGAC TGGTACTTCA AATCGAAGCA TCCCGCTGCC GTCAGCAGTA
3121  CCTGCACGGT TTTTGCTGAA TCGGAAGTCA TTTCCCTTCT TTCCAAGAAT GTCCCGAAAG
3181  AAGATATCGT AGCCGGTGTC CATCAGTCCA TCGCCGCCAA AGCCTGCGCT CTCGTGCGCC
3241  GCGTCGGTGT CGGTGAAGAC CTGACCATGA CCGGCGGTGG CTCCCGCGAT CCCGGCGTCG
3301  TCGATGCCGT ATCGAAAGAA TTAGGTATTC CTGTCAGAGT CGCTCTGCAT CCCCAAGCGG
3361  TGGGTGCTCT CGGAGCTGCT TTGATTGCTT ATGATAAAAT CAAGAAATAA GTCAAAGGAG
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Figure 22A

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3421  AGAACAAAAT CATGAGTGAA GAAAAAACAG TAGATATTGA AAGCATGAGC TCCAAGGAAG
3481  CCCTTGGTTA CTTCTTGCCG AAAGTCGATG AAGACGCACG TAAAGCGAAA AAAGAAGGCC
3541  GCCTCGTTTG CTGGTCCGCT TCTGTCGCTC CTCCGGAATT CTGCACGGCT ATGGACATCG
3601  CCATCGTCTA TCCGGAAACT CACGCAGCTG GTATCGGTGC CCGTCACGGT GCTCCGGCCA
3661  TGCTCGAAGT TGCTGAAAAC AAAGGTTACA ACCAGGACAT CTGTTCCTAC TGCCGCGTCA
3721  ACATGGGCTA CATGGAACTC CTCAAACAGC AGGCTCTGAC AGGCGAAACG CCGGAAGTCC
3781  TCAAAAACTC CCCGGCTTCT CCGATTCCCC TTCCGGATGT TGTCCTCACT TGCAACAACA
3841  TCTGCAATAC CTTGCTCAAA TGGTATGAAA ACTTGGCTAA AGAATTGAAC GTACCTCTCA
3901  TCAACATCGA CGTACCGTTC AACCATGAAT TCCCTGTTAC GAAACACGCT AAACAGTACA
3961  TCGTCGGCGA ATTCAAACAT GCTATCAAAC AGCTCGAAGA CCTTTGCGGC CGTCCCTTCG
4021  ACTATGACAA ATTCTTCGAA GTACAGAAAC AGACACAGCG CTCCATCGCT GCCTGGAACA
4081  AAATCGCTAC GTACTTCCAG TACAAACCGT CGCCGCTCAA CGGCTTCGAC CTCTTCAACT
4141  ACATGGGCCT CGCCGTTGCT GCCCGCTCCT TGAACTACTC GGAAATCACG TTCAACAAAT
4201  TCCTCAAAGA ATTGGACGAA AAAGTAGCTA ATAAGAAATG GGCTTTCGGT GAAAACGAAA
4261  AATCCCGTGT TACTTGGGAA GGTATCGCTG TCTGGATCGC TCTCGGCCAC ACCTTCAAAG
4321  AACTCAAAGG TCAGGGCGCT CTCATGACTG GTTCCGCTTA TCCTGGCATG TGGGACGTTT
4381  CCTACGAACC GGGCGACCTC GAATCCATGG CAGAAGCTTA TTCCGGTACA TACATCAACT
4441  GCTGCCTCGA ACAGCGCGGT GCTGTTCTTG AAAAAGTTGT CCGCGATGGC AAATGCGACG
4501  GCTTGATCAT GCACCAGAAC CGTTCCTGCA AGAACATGAG CCTCCTCAAC AACGAAGGCG
4561  GCCAGCGCAT CCAGAAGAAC CTCGGCGTAC CGTACGTCAT CTTCGACGGC GACCAGACCG
4621  ATGCTCGTAA CTTCTCGGAA GCACAGTTCG ATACCCGCGT AGAAGCTTTG GCAGAAATGA
4681  TGGCAGACAA AAAAGCCAAT GAAGGAGGAA ACCACTAATG AGTCAGATCG ACGAACTTAT
4741  CAGCAAATTA CAGGAAGTAT CCAACCATCC CCAGAAGACG GTTTTGAATT ATAAAAAACA
4801  GGGTAAAGGC CTCGTAGGCA TGATGCCCTA CTACGCTCCG GAAGAAATCG TATATGCTGC
4861  AGGCTACCTC CCGGTAGGCA TGTTCGGTTC CCAGAACCCG CAGATCTCCG CAGCTCGTAC
4921  GTACCTTCCT CCGTTCGCTT GCTCCTTGAT GCAGGCTGAC ATGGAACTCC AGCTCAACGG
4981  CACCTATGAC TGCCTCGACG CTGTTATCTT CTCCGTTCCT TGCGACACTC TCCGCTGCAT
5041  GAGCCAGAAA TGGCACGGCA AAGCTCCGGT CATCGTCTTC ACACAGCCGC AGAACCGTAA
5101  GATCCGCCCG GCTGTCGATT TCCTCAAAGC TGAATACGAA CATGTCCGTA CGGAATTGGG
5161  ACGTATCCTC AACGTAAAAA TCTCCGACCT GGCTATCCAG GAAGCTATCA AAGTATATAA
5221  CGAAAACCGT CAGGTTATGC GTGAATTCTG CGACGTAGCT GCTCAGTACC CGCAGATCTT
5281  CACTCCGATA AAACGTCATG ACGTCATCAA AGCCCGCTGG TTCATGGACA AAGCTGAACA
5341  CACCGCTTTG GTCCGCGAAC TCATCGACGC TGTCAAGAAA GAACCGGTAC AGCCGTGGAA
5401  TGGCAAAAAA GTCATCCTCT CCGGTATCAT GGCAGAACCG GATGAATTCC TCGATATCTT
5461  CAGCGAATTC AACATCGCTG TCGTCGCTGA CGACCTCGCT CAGGAATCCC GCCAGTTCCG
5521  TACAGACGTA CCGTCCGGCA TCGATCCCCT CGAACAGCTC GCTCAGCAGT GGCAGGACTT
5581  CGATGGCTGC CCGCTCGCTT TGAACGAAGA CAAACCGCGT GGCCAGATGC TCATCGACAT
5641  GACTAAGAAA TACAATGCTG ACGCCGTCGT CATCTGCATG ATGCGTTTCT GCGATCCTGA
5701  AGAATTCGAC TATCCGATTT ACAAACCGGA ATTTGAAGCT GCTGGCGTTC GTTACACGGT
5761  CCTCGACCTC GACATCGAAT CTCCGTCCCT CGAACAGCTC CGCACCCGTA TCCAGGCTTT
5821  CTCGGAAATC CTCTAAGAAT CGCCTGAATC ATCAAACATC TGGGCGGGAC TCCGAAAGGT
5881  GCCTGCTACA TGATACATTG CCTGTTTTCA GGCAGACAGA TTTGCAGCTT GCGGCCCCCA
5941  TTGTACGGGC TGCAAGCTGT CAATGATGCT TTAAAGACGG CTCTGCCGTT TTTAAATAAA
6001  AACATAAAAC CATATATAAT CTATTAGGAG GAAACTCAAT CATGGAATTC AAACTTTCTG
6061  AATTACAGCA AGATATCGCA AATCTCGCAA AAGATTTCGC AGAAAAAAAA TTAGCTCCCA
6121  CTGTCAAAGA GCGTGACGAA AAAGAAGTTT TCGATCGTGC TATCCTTGAC GAAGTGGGTA
6181  CTCTCGGCCT TCTCGGTATT CCCTGGGAAG AAGAAAACGG CGGCGTAGGC GCTGACTTCC
6241  TCAGCCTCGC AGTTGCTTGC GAAGAAGTAG CTAAAGTTAC CAGCCCGGGC CGTCG  (SEQ
ID NO:33)
```

Figure 22B

Figure 23

ATGAAACCAATGAGACTACATCACGTAGGCATTGTCCTGCCGACCTTAGAAAAAGCCCAT
GAATTCATGCAGAATAATGGACTTGAAATCGACTATGCCGGCTATGTCGATGCTTACCAG
GCTGATCTCATTTTCACTAAGTTTGGTGAATTTGCCAGCCCGATTGAAATGATTATCCCG
CACTCCGGTGTGCTTACCCAATTCAATGGTGGCCGCGGCGGCATTGCCCACATCGCCTTC
GAAGTGGACGATGTCGAAGCTGTCCGCCAGGAAATGGAAGCAGATTGTCCGGGATGCATG
TTAGAAAAGAAAGCTGTCCAGGGTACGGACGACATTATCGTCAACTTCCGCCGCCCGACA
ACCAACCAGGGTATCCTCGTTGAATATGTTCAGACGACAGCACCTATCACCGGCCGCGGC
GAAAATCCTTTCGTTAAGAATCTCGGCCCGGAAAAAGGGAAGCTCAACGAAACATGGCAT
CCCATGCGCCTGCACCATATCGGCATCGTCTTGCCGACCTTGGAAAAGGCCCATGAATTC
ATCAAGACCAATGGTCTGGAAGTGGATTATTCCGGTTTCGTCGACGCCTACCATGCGGAT
CTCATTTTCACTAAAAAAGGTGAAAACAGTACGCCTATCGAATTCATTATTCCCCGTGAA
GGGGTCCTCAAAGATTTCAATCATGGCAGGGGAGGTATCGCTCATATCGCCTTTGAAGTG
GATGATGTCGAAAAGGTACGTCAGATTATGGAAAGCCAGAAGCCTGGTTGCATGCTCGAA
AAGAAAGCCGTCCGGGGAACGGACGATATCATCGTCAACTTCCGCCGTCCCAGCACGGAC
GCCGGCATCCTCGTCGAATATGTCCAGACCGTAGCTCCCATCAATCGCAGCAATCCCAAC
CCTTTTAATGATTGA (SEQ ID NO:34)

Figure 24

MKPMRLHHVGIVLPTLEKAHEFMQNNGLEIDYAGYVDAYQADLIFTKFGEFASPIEMIIP
HSGVLTQFNGGRGGIAHIAFEVDDVEAVRQEMEADCPGCMLEKKAVQGTDDIIVNFRRPT
TNQGILVEYVQTTAPITGRGENPFVKNLGPEKGKLNETWHPMRLHHIGIVLPTLEKAHEF
IKTNGLEVDYSGFVDAYHADLIFTKKGENSTPIEFIIPREGVLKDFNHGRGGIAHIAFEV
DDVEKVRQIMESQKPGCMLEKKAVRGTDDIIVNFRRPSTDAGILVEYVQTVAPINRSNPN
PFND (SEQ ID NO:35)

Figure 25

ATGGAATTCAAACTTTCTGAATTACAGCAAGATATCGCAAATCTCGCAAAAGATTTCGCA
GAAAAAAAATTAGCTCCCACTGTCAAAGAGCGTGACGAAAAAGAAGTTTTCGATCGTGCT
ATCCTTGACGAAGTGGGTACTCTCGGCCTTCTCGGTATTCCCTGGGAAGAAGAAAACGGC
GGCGTAGGCGCTGACTTCCTCAGCCTCGCAGTTGCTTGCGAAGAAGTAGCTAAAGTTACC
AGCCCGGGCCGTCG (SEQ ID NO: 36)

Figure 26

MEFKLSELQQDIANLAKDFAEKKLAPTVKERDEKEVFDRAILDEVGTLGLLGIPWEEENG
GVGADFLSLAVACEEVAKVTSPGR (SEQ ID NO:37)

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   1  GTGAGCACAC ACTTGATAGC TGATGCCGTC AATGATCAGT TGTTCGTCTA TAGCAGGCTG
  61  AAAGGACATG GGTTTGGTCA CAGTCTGAGC AGTTGCAGGC AGTCAAACAC GTTCGTAACT
 121  ACGCTGTAGA TGATATAAGC AGTATACCAT CTTGCTACGC TCTCGTTGAT CAGGTTGAAT
 181  GCTTTGAGGA AGGTCAGGCG AATAGCCATG CCTCTTGTTT CCAGAACATG GCATGGGGAT
 241  GGATCGACGG TACCCTGTCG GATGCATGCT ATGCGTGGCA TTCATATCAT CAACCAGAAT
 301  TTGATCTTGA ACTACACAGC AATTCTGCGC GTTATGCAAG TGTCTTCGGT CAGATGGTGA
 361  ACAATTCTCA ATTGTTGAGG TCTTGACGAA TTGCGTTATA CACTGTAGGC TATAGTATGC
 421  ACCCCTTGTT ATCTATATCA CAACCGGTCT ATTAGCATTT GCGTCAAGGA GGATGGTCGA
 481  TGATCGACAC TGCGCCCCTT GCCCCACCAC GGGCGCCCCG CTCTAATCCG ATTCGGGATC
 541  GAGTTGATTG GGAAGCTCAG CGCGCTGCTG CGCTGGCAGA TCCCGGTGCC TTTCATGGCG
 601  CGATTGCCCG GACAGTTATC CACTGGTACG ACCCACAACA CCATTGCTGG ATTCGCTTCA
 661  ACGAGTCTAG TCAGCGTTGG GAAGGGCTGG ATGCCGCTAC CGGTGCCCCT GTAACGGTAG
 721  ACTATCCCGC CGATTATCAG CCCTGGCAAC AGGCGTTTGA TGATAGTGAA GCGCCGTTTT
 781  ACCGCTGGTT TAGTGGTGGG TTGACAAATG CCTGCTTTAA TGAAGTAGAC CGGCATGTCA
 841  TGATGGGCTA TGGCGACGAG GTGGCCTACT ACTTTGAAGG TGACCGCTGG GATAACTCGC
 901  TCAACAATGG TCGTGGTGGT CCGGTTGTCC AGGAGACAAT CACGCGGCGG CGCCTGTTGG
 961  TGGAGGTGGT GAAGGCTGCG CAGGTGTTGC GTGATCTGGG CCTGAAGAAG GGTGATCGGA
1021  TTGCTCTGAA TATGCCGAAT ATTATGCCGC AGATTTATTA TACGGAAGCG GCAAAACGAC
1081  TGGGTATTCT GTACACGCCG GTCTTCGGTG GCTTCTCGGA CAAGACTCTT TCCGACCGTA
1141  TTCACAATGC CGGTGCACGA GTGGTGATTA CCTCTGATGG TGCGTACCGC AACGCGCAGG
1201  TGGTGCCCTA CAAAGAAGCG TATACCGATC AGGCGCTCGA TAAGTATATT CCGGTTGAGA
1261  CGGCGCAGGC GATTGTTGCG CAGACCCTGG CCACCTTGCC CCTGACTGAG TCGCAGCGCC
1321  AGACGATCAT CACCGAAGTG GAGGCCGCAC TGGCCGGTGA GATTACGGTT GAGCGCTCGG
1381  ACGTGATGCG TGGGGTTGGT TCTGCCCTCG CAAAGCTCCG CGATCTTGAT GCAAGCGTGC
1441  AGGCAAAGGT GCGTACAGTA CTGGCGCAGG CGCTGGTCGA GTCGCCGCCG CGGGTTGAAG
1501  CTGTGGTGGT TGTGCGTCAT ACCGGTCAGG AGATTTTGTG GAACGAGGGG CGAGATCGCT
1561  GGAGTCACGA CTTGCTGGAT GCTGCGCTGG CGAAGATTCT GGCCAATGCG CGTGCTGCCG
1621  GCTTTGATGT GCACAGTGAG AATGATCTGC TCAATCTCCC CGATGACCAG CTTATCCGTG
1681  CGCTCTACGC CAGTATTCCC TGTGAACCGG TTGATGCTGA ATATCCGATG TTTATCATTT
1741  ACACATCGGG TAGCACCGGT AAGCCCAAGG GTGTGATCCA CGTTCACGGC GGTTATGTCG
1801  CCGGTGTGGT GCACACCTTG CGGGTCAGTT TTGACGCCGA GCCGGGTGAT ACGATATATG
1861  TGATCGCCGA TCCGGGGCTGG ATCACCGGTC AGAGCTATAT GCTCACAGCC ACAATGGCCG
1921  GTCGGCTGAC CGGGGTGATT GCCGAGGGAT CACCGCTCTT CCCCTCAGCC GGGCGTTATG
1981  CCAGCATCAT CGAGCGCTAT GGGGTGCAGA TCTTTAAGGC GGGTGTGACC TTCCTCAAGA
2041  CAGTGATGTC CAATCCGCAG AATGTTGAAG ATGTGCGACT CTATGATATG CACTCGCTGC
2101  GGGTTGCAAC CTTCTGCGCC GAGCCGGTCA GTCCGGCGGT GCAGCAGTTT GGTATGCAGA
2161  TCATGACCCC GCAGTATATC AATTCGTACT GGGCGACCGA GCACGGTGGA ATTGTCTGGA
2221  CGCATTTCTA CGGTAATCAG GACTTCCCGC TTCGTCCCGA TGCCCATACC TATCCCTTGC
2281  CCTGGGTGAT GGGTGATGTC TGGGTGGCCG AAACTGATGA GAGCGGGACG ACGCGCTATC
2341  GGGTCGCTGA TTTCGATGAG AAGGGCGAGA TTGTGATTAC CGCCCCGTAT CCCTACCTGA
2401  CCCGCACACT CTGGGGTGAT GTGCCCGGTT TCGAGGCGTA CCTGCGCGGT GAGATTCCGC
2461  TGCGGGCCTG GAAGGGTGAT GCCGAGCGTT TCGTCAAGAC CTACTGGCGA CGTGGGCCAA
2521  ACGGTGAATG GGGCTATATC CAGGGTGATT TTGCCATCAA GTACCCCGAT GGTAGCTTCA
2581  CGCTCCACGG ACGCCCTGAC GATGTGATCA ATGTGTCGGG CCACCGTATG GGCACCGAGG
2641  AGATTGAGGG TGCCATTTTG CGTGACCGCC AGATCACGCC CGACTCGCCC GTCGGTAATT
2701  GTATTGTGGT CGGTGCGCCG CACCGTGAGA AGGGTCTGAC CCCGGTTGCC TTCATTCAAC
2761  CTGCGCCTGG CCGTCATCTG ACCGGCGCCG ACCGGCGCCG TCTCGATGAG CTGGTGCGTA
2821  CCGAGAAGGG GGCGGTCAGT GTCCCAGAGG ATTACATCGA GGTCAGTGCC TTTCCCGAAA
2881  CCCGCAGCGG GAAGTATATG CGGCGCTTTT TGCGCAATAT GATGCTCGAT GAACCACTGG
2941  GTGATACGAC GACGTTGCGC AATCCTGAAG TGCTCGAAGA GATTGCAGCC AAGATCGCTG
3001  AGTGGAAACG CCGTCAGCGT ATGGCCGAAG AGCAGCAGAT CATCGAACGC TATCGCTACT
3061  TCCGGATCGA GTATCACCCA CCAACGGCCA GTGCGGGTAA ACTCGCGGTA GTGACGGTGA
3121  CAAATCCGCC GGTGAACGCA CTGAATGAGC GTGCGCTCGA TGAGTTGAAC ACAATTGTTG
3181  ACCACCTGGC CCGTCGTCAG GATGTTGCCG CAATTGTCTT CACCGGACAG GGCGCCAGGA
3241  GTTTTGTCGC CGGCGCTGAT ATTCGCCAGT TGCTCGAAGA GATTCATACG GTTGAAGAGG
3301  CAATGGCCCT GCCGAATAAC GCCCATCTTG CTTTCCGCAA GATTGAGCGT ATGAATAAGC
3361  CGTGTATCGC GGCGATCAAC GGTGTGGCGC TCGGTGGTGG TCTGGAATTC GCCATGGCCT
```

Figure 27A

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3421  GCCATTACCG GGTTGCCGAT GTCTATGCCG AATTCGGTCA GCCAGAGATT AATCTGCGCT
3481  TGCTACCTGG TTATGGTGGC ACGCAGCGCT TGCCGCGCCT GTTGTACAAG CGCAACAACG
3541  GCACCGGTCT GCTCCGAGCG CTGGAGATGA TTCTGGGTGG GCGTAGCGTA CCGGCTGATG
3601  AGGCGCTGAA GCTGGGTCTG ATCGATGCCA TTGCTACCGG CGATCAGGAC TCACTGTCGC
3661  TGGCATGCGC GTTAGCCCGT GCCGCAATCG GCGCCGATGG TCAGTTGATC GAGTCGGCTG
3721  CGGTGACCCA GGCTTTCCGC CATCGCCACG AGCAGCTTGA CGAGTGGCGC AAACCAGACC
3781  CGCGCTTTGC CGATGACGAA CTGCGCTCGA TTATCGCCCA TCCACGTATC GAGCGGATTA
3841  TCCGGCAGGC CCATACCGTT GGGCGCGATG CGGCAGTGCA TCGGGCACTG GATGCAATCC
3901  GCTATGGCAT TATCCACGGC TTCGAGGCCG GTCTGGAGCA CGAGGCGAAG CTCTTTGCCG
3961  AGGCAGTGGT TGACCCGAAC GGTGGCAAGC GTGGTATTCG CGAGTTCCTC GACCGCCAGA
4021  GTGCGCCGTT GCCAACCCGC CGACCATTGA TTACACCTGA ACAGGAGCAA CTCTTGCGCG
4081  ATCAGAAAGA ACTGTTGCCG GTTGGTTCAC CCTTCTTCCC CGGTGTTGAC CGGATTCCGA
4141  AGTGGCAGTA CGCGCAGGCG GTTATTCGTG ATCCGGACAC CGGTGCGGCG GCTCACGGCG
4201  ATCCCATCGT GGCTGAAAAG CAGATTATTG TGCCGGTGGA ACGCCCCCGC GCCAATCAGG
4261  CGCTGATCTA TGTTCTGGCC TCGGAGGTGA ACTTCAACGA TATCTGGGCG ATTACCGGTA
4321  TTCCGGTGTC ACGGTTTGAT GAGCACGACC GCGACTGGCA CGTTACCGGT TCAGGTGGCA
4381  TCGGCCTGAT CGTTGCGCTG GGTGAAGAGG CGCGACGCGA AGGCCGGCTG AAGGTGGGTG
4441  ATCTGGTGGC GATCTACTCC GGGCAGTCGG ATCTGCTCTC ACCGCTGATG GGCCTTGATC
4501  CGATGGCCGC CGATTTCGTC ATCCAGGGGA ACGACACGCC AGATGGATCG CATCAGCAAT
4561  TTATGCTGGC CCAGGCCCCG CAGTGTCTGC CCATCCCAAC CGATATGTCT ATCGAGGCAG
4621  CCGGCAGCTA CATCCTCAAT CTCGGTACGA TCTATCGCGC CCTCTTTACG ACGTTGCAAA
4681  TCAAGGCCGG ACGCACCATC TTTATCGAGG GTGCGGCGAC CGGTACCGGT CTGGACGCAG
4741  CGCGCTCGGC GGCCCGGAAT GGTCTGCGCG TAATTGGAAT GGTCAGTTCG TCGTCACGTG
4801  CGTCTACGCT GCTGGCTGCG GGTGCCCACG GTGCGATTAA CCGTAAAGAC CCGGAGGTTG
4861  CCGATTGTTT CACGCGCGTG CCCGAAGATC CATCAGCCTG GGCAGCCTGG GAAGCCGCCG
4921  GTCAGCCGTT GCTGGCGATG TTCCGGGCGC AGAACGACGG GCGACTGGCC GATTATGTGG
4981  TCTCGCACGC GGGCGAGACG GCCTTCCCGC GCAGTTTCCA GCTTCTCGGC GAGCCACGCG
5041  ATGGTCACAT TCCGACGCTC ACATTCTACG GTGCCACCAG TGGCTACCAC TTCACCTTCC
5101  TGGGTAAGCC AGGGTCAGCT TCGCCGACCG AGATGCTGCG GCGGGCCAAT CTCCGCGCCG
5161  GTGAGGCGGT GTTGATCTAC TACGGGGTTG GGAGCGATGA CCTGGTAGAT ACCGGCGGTC
5221  TGGAGGCTAT CGAGGCGGCG CGGCAAATGG GAGCGCGGAT CGTCGTCGTT ACCGTCAGCG
5281  ATGCGCAACG CGAGTTTGTC CTCTCGTTGG GCTTCGGGGC TGCCCTACGT GGTGTCGTCA
5341  GCCTGGCGGA ACTCAAACGG CGCTTCGGCG ATGAGTTTGA GTGGCCGCGC ACGATGCCGC
5401  CGTTGCCGAA CGCCCGCCAG GACCCGCAGG GTCTGAAAGA GGCTGTCCGC CGCTTCAACG
5461  ATCTGGTCTT CAAGCCGCTA GGAAGCGCGG TCGGTGTCTT CTTGCGGAGT GCCGACAATC
5521  CGCGTGGCTA CCCCGATCTG ATCATCGAGC GGGCTGCCCA CGATGCACTG GCGGTGAGCG
5581  CGATGCTGAT CAAGCCCTTC ACCGGACGGA TTGTCTACTT CGAGGACATT GGTGGGCGGC
5641  GTTACTCCTT CTTCGCACCG CAAATCTGGG TGCGCCAGCG CCGCATCTAC ATGCCGACGG
5701  CACAGATCTT TGGTACGCAC CTCTCAAATG CGTATGAAAT TCTGCGTCTG AATGATGAGA
5761  TCAGCGCCGG TCTGCTGACG ATTACCGAGC CGGCAGTGGT GCCGTGGGAT GAACTACCCG
5821  AAGCACATCA GGCGATGTGG GAAAATCGCC ACACGGCGGC CACTTATGTG GTGAATCATG
5881  CCTTACCACG TCTCGGCCTA AAGAACAGGG ACGAGCTGTA CGAGGCGTGG ACGGCCGGCG
5941  AGCGGTAGCG CGGATGGGTA TTGAACAGGT AACGGACGGA AGATCGAACC TTCCGTCCGT
6001  TATCTTTTGG CCGTCGAAGC GTGCTGAGCC GATTATCGTT GCCGTGGTTG TCCCGATGGG
6061  CAGACGCGCT CGAACCAGAT GATACCACCG ACGGCTATCG TCACCAAACC GGCGAAGACC
6121  AGGTAAGCCT CTGAAGGACG C (SEQ ID NO:38)
```

Figure 27B

Figure 28

```
   1  MIDTAPLAPP RAPRSNPIRD RVDWEAQRAA ALADPGAFHG AIARTVIHWY DPQHHCWIRF
  61  NESSQRWEGL DAATGAPVTV DYPADYQPWQ QAFDDSEAPF YRWFSGGLTN ACFNEVDRHV
 121  MMGYGDEVAY YFEGDRWDNS LNNGRGGPVV QETITRRRLL VEVVKAAQVL RDLGLKKGDR
 181  IALNMPNIMP QIYYTEAAKR LGILYTPVFG GFSDKTLSDR IHNAGARVVI TSDGAYRNAQ
 241  VVPYKEAYTD QALDKYIPVE TAQAIVAQTL ATLPLTESQR QTIITEVEAA LAGEITVERS
 301  DVMRGVGSAL AKLRDLDASV QAKVRTVLAQ ALVESPPRVE AVVVVRHTGQ EILWNEGRDR
 361  WSHDLLDAAL AKILANARAA GFDVHSENDL LNLPDDQLIR ALYASIPCEP VDAEYPMFII
 421  YTSGSTGKPK GVIHVHGGYV AGVVHTLRVS FDAEPGDTIY VIADPGWITG QSYMLTATMA
 481  GRLTGVIAEG SPLFPSAGRY ASIIERYGVQ IFKAGVTFLK TVMSNPQNVE DVRLYDMHSL
 541  RVATFCAEPV SPAVQQFGMQ IMTPQYINSY WATEHGGIVW THFYGNQDFP LRPDAHTYPL
 601  PWVMGDVWVA ETDESGTTRY RVADFDEKGE IVITAPYPYL TRTLWGDVPG FEAYLRGEIP
 661  LRAWKGDAER FVKTYWRRGP NGEWGYIQGD FAIKYPDGSF TLHGRPDDVI NVSGHRMGTE
 721  EIEGAILRDR QITPDSPVGN CIVVGAPHRE KGLTPVAFIQ PAPGRHLTGA DRRRLDELVR
 781  TEKGAVSVPE DYIEVSAFPE TRSGKYMRRF LRNMMLDEPL GDTTTLRNPE VLEEIAAKIA
 841  EWKRRQRMAE EQQIIERYRY FRIEYHPPTA SAGKLAVVTV TNPPVNALNE RALDELNTIV
 901  DHLARRQDVA AIVFTGQGAR SFVAGADIRQ LLEEIHTVEE AMALPNNAHL AFRKIERMNK
 961  PCIAAINGVA LGGGLEFAMA CHYRVADVYA EFGQPEINLR LLPGYGGTQR LPRLLYKRNN
1021  GTGLLRALEM ILGGRSVPAD EALKLGLIDA IATGDQDSLS LACALARAAI GADGQLIESA
1081  AVTQAFRHRH EQLDEWRKPD PRFADDELRS IIAHPRIERI IRQAHTVGRD AAVHRALDAI
1141  RYGIIHGFEA GLEHEAKLFA EAVVDPNGGK RGIREFLDRQ SAPLPTRRPL ITPEQEQLLR
1201  DQKELLPVGS PFFPGVDRIP KWQYAQAVIR DPDTGAAAHG DPIVAEKQII VPVERPRANQ
1261  ALIYVLASEV NFNDIWAITG IPVSRFDEHD RDWHVTGSGG IGLIVALGEE ARREGRLKVG
1321  DLVAIYSGQS DLLSPLMGLD PMAADFVIQG NDTPDGSHQQ FMLAQAPQCL PIPTDMSIEA
1381  AGSYILNLGT IYRALFTTLQ IKAGRTIFIE GAATGTGLDA ARSAARNGLR VIGMVSSSSR
1441  ASTLLAAGAH GAINRKDPEV ADCFTRVPED PSAWAAWEAA GQPLLAMFRA QNDGRLADYV
1501  VSHAGETAFP RSFQLLGEPR DGHIPTLTFY GATSGYHFTF LGKPGSASPT EMLRRANLRA
1561  GEAVLIYYGV GSDDLVDTGG LEAIEAARQM GARIVVVTVS DAQREFVLSL GFGAALRGVV
1621  SLAELKRRFG DEFEWPRTMP PLPNARQDPQ GLKEAVRRFN DLVFKPLGSA VGVFLRSADN
1681  PRGYPDLIIE RAAHDALAVS AMLIKPFTGR IVYFEDIGGR RYSFFAPQIW VRQRRIYMPT
1741  AQIFGTHLSN AYEILRLNDE ISAGLLTITE PAVVPWDELP EAHQAMWENR HTAATYVVNH
1801  ALPRLGLKNR DELYEAWTAG ER (SEQ ID NO:39)
```

Figure 29

ATGAGTGAAGAGTCTCTGGTTCTCAGCACAATTGAAGGCCCCATCGCCATCCTCACCCTC
AATCGCCCCCAGGCCCTCAATGCGCTCAGTCCGGCCTTGATTGATGACCTCATTCGCCAT
TTAGAAGCCTGCGATGCCGATGACACAATCCGCGTGATCATTATCACCGGCGCCGGACGG
GCATTTGCTGCCGGCGCTGATATCAAAGCGATGGCCAATGCCACGCCTATTGATATGCTC
ACCAGTGGCATGATTGCGCGCTGGGCACGCATCGCCGCGGTGCGCAAACCGGTGATTGCT
GCCGTGAATGGGTATGCGCTCGGTGGTGGTTGTGAATTGGCAATGATGTGCGACATCATC
ATCGCCAGTGAAAACGCGCAGTTCGGACAACCGGAAATCAATCTGGGCATCATTCCCGGT
GCTGGTGGCACCCAACGGCTGACCCGCGCCCTTGGCCCGTATCGCGCAATGGAATTGATC
CTGACCGGCGCGACCATCAGTGCTCAGGAAGCTCTCGCCCACGGCCTGGTGTGCCGGGTC
TGCCCGCCTGAAAGCCTGCTCGATGAAGCCCGTCGGATCGCGCAAACCATTGCCACCAAA
TCACCACTGGCTGTACAGTTGGCGAAAGAGGCAGTCCGTATGGCCGCCGAAACCACTGTG
CGCGAGGGGTTGGCTATCGAGCTGCGTAACTTCTATCTGCTGTTTGCCAGTGCTGACCAA
AAAGAGGGGATGCAGGCATTTATCGAGAAACGCGCTCCCAACTTCAGTGGTCGTTGA
(SEQ ID NO:40)

Figure 30

MSEESLVLSTIEGPIAILTLNRPQALNALSPALIDDLIRHLEACDADDTIRVIIITGAGR
AFAAGADIKAMANATPIDMLTSGMIARWARIAAVRKPVIAAVNGYALGGGCELAMMCDII
IASENAQFGQPEINLGIIPGAGGTQRLTRALGPYRAMELILTGATISAQEALAHGLVCRV
CPPESLLDEARRIAQTIATKSPLAVQLAKEAVRMAAETTVREGLAIELRNFYLLFASADQ
KEGMQAFIEKRAPNFSGR (SEQ ID NO:41)

Figure 31

GGCGTAATCCGACCGGCAGGTTAGGGTCTTCTACTGGGGTCAAGGCGCGTCTCCTTTTGG
TGGCGCGAGCAACCCGGCTTTTCCTGGCTTCAATGTACCATAGAGCGGTTACTTCGTGCA
ACGGGCGTGGTACAATCGAGAGCAACCTTTCGCAAAAGCTATCCAATCCTGCACACGTGC
ATCTGTTACAGGGTATTATTGTCGGCAAACGACAGTCCTGTCGTTTATGTACAAGGAGAT
CAACGTATGAGTGAAGAGTCTCTGGTTCTCAGCACAATTGAAGGCCCCATCGCCATCCTC
ACCCTCAATCGCCCCCAGGCCCTCAATGCGCTCAGTCCGGCCTTGATTGATGACCTCATT
CGCCATTTAGAAGCCTGCGATGCCGATGACACAATCCGCGTGATCATTATCACCGGCGCC
GGACGGGCATTTGCTGCCGGCGCTGATATCAAAGCGATGGCCAATGCCACGCCTATTGAT
ATGCTCACCAGTGGCATGATTGCGCGCTGGGCACGCATCGCCGCGGTGCGCAAACCGGTG
ATTGCTGCCGTGAATGGGTATGCGCTCGGTGGTGGTTGTGAATTGGCAATGATGTGCGAC
ATCATCATCGCCAGTGAAAACGCGCAGTTCGGACAACCGGAAATCAATCTGGGCATCATT
CCCGGTGCTGGTGGCACCCAACGGCTGACCCGCGCCCTTGGCCCGTATCGCGCAATGGAA
TTGATCCTGACCGGCGCGACCATCAGTGCTCAGGAAGCTCTCGCCCACGGCCTGGTGTGC
CGGGTCTGCCCGCCTGAAAGCCTGCTCGATGAAGCCCGTCGGATCGCGCAAACCATTGCC
ACCAAATCACCACTGGCTGTACAGTTGGCGAAAGAGGCAGTCCGTATGGCCGCCGAAACC
ACTGTGCGCGAGGGGTTGGCTATCGAGCTGCGTAACTTCTATCTGCTGTTTGCCAGTGCT
GACCAAAAAGAGGGGATGCAGGCATTTATCGAGAAACGCGCTCCCAACTTCAGTGGTCGT
TGATCACGCGCAGAACATGGCAGCAGGGGCAATACCTGCACGTACTGCCTCCTGCCGCCA
TACTACCAGATGATCGAGCAGTAAAGGGTAAATACTCTATCAATCTGGCCAGATAAGCGG
TTGGGTAACAACGCAATGCTCCAAAGGAGACGATCATGGACATACACGAGCGATTGCGAT
CTCTCGAACGCGAAAATGCT (SEQ ID NO:42)

```
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SEQ ID NO:43      1 ---------atgacgta----------cgaaa------------------
SEQ ID NO:44      1 atggccgccctgcgtgt----------cctgctgtcctgcgcccgcggcc
SEQ ID NO:45      1 atggcggccctgcgtgctctgctgcccagagc------------------

SEQ ID NO:40     14 ---------ct---------ctg---------gttctc-agcacaattgaa
SEQ ID NO:43     14 ---------cc---------atc---------ctggtcgagcgc----gat
SEQ ID NO:44     41 cgctgaggccc---------ccg---------gttcgc-tgtcccgcctgg
SEQ ID NO:45     33 ---------ctgcaactcgctgttgtccccagttcgc-tgcccagaattc

SEQ ID NO:40     37 ggccccatcgcc----------------------atcctcacc--------
SEQ ID NO:43     34 cagcgagttggc----------------------attatcacg--------
SEQ ID NO:44     73 cgtcccttcgcctcgggtgctaactttgagtacatcatcgcagaaaaaag
SEQ ID NO:45     73 cggcgcttcgcctcgggtgctaactttcagtacatcatcacg--------

SEQ ID NO:40     58 --------------------------------c------------------
SEQ ID NO:43     55 ---------------------------------c-----------------
SEQ ID NO:44    123 agggaagaataacaccgtggggttgatccaac------------------
SEQ ID NO:45    115 --------------------------------gaaaagaaaggaaagaata

SEQ ID NO:40     59 ----------------------tcaatcgcccccaggccctcaatgcgctc
SEQ ID NO:43     56 ----------------------tgaaccgtccccaggcactgaacgcgctc
SEQ ID NO:44    155 ----------------------tgaaccgccccaaggccctcaatgcactt
SEQ ID NO:45    134 gcagcgtggggctgatccagttgaaccgtcccaaagcactcaatgcactt

SEQ ID NO:40     88 agtccggccttgattgatgacctcattc--gccatttagaagcctgcgat
SEQ ID NO:43     85 a--acagccagg--tgatgaacgaggtc--acca--gcgctgcaaccgaa
SEQ ID NO:44    184 tgcgatggcctgattgacgagctcaaccaggccctgaaga--tcttcgag
SEQ ID NO:45    184 tgcaatggactgattgaggagctcaacc--aagcactggagacctttgag

SEQ ID NO:40    136 ---gccgatgacaca---atccgcgtgatcattatcaccggcgccggacg
SEQ ID NO:43    127 ctggacgatgacccggacattggggcgatcatcatcaccggttcggccaa
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SEQ ID NO:45    232 ---gaagatcccgct---gtgggcgccattgtgctcactggtggggagaa

SEQ ID NO:40    180 ggcatttgctgccggcgctgatatcaaagcgatggccaa-------tgcc
SEQ ID NO:43    177 agcgtttgccgccggagccgacatcaaagaaatggccga-------cctg
SEQ ID NO:44    276 ggcctttgcagctggagctgatatcaaggaaatgcagaacctgagtttcc
SEQ ID NO:45    276 ggcctttgcagccggagctgacatcaaggaaatgcagaa-------ccgg

SEQ ID NO:40    223 acgcctattgatatgctcaccagtggcatgattgcgcgc---tgggcacg
SEQ ID NO:43    220 acgttcgccgacgcgttcaccgccgacttcttcgccacc---tggggcaa
SEQ ID NO:44    326 aggactgtt-------actccagcaagttcttgaagcac---tggggcca
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SEQ ID NO:40    320 tcggtggtggttgtgaattggcaatgatgtgcgacatcatcatcgccagt
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SEQ ID NO:45    416 ttggtgggggctgtgaacttgccatgatgtgcgatatcatctatgctggt
```

Figure 32A

```
SEQ ID NO:40    370 gaaaacgcgcagttcggacaaccggaaatcaatctgggcatcattcccgg
SEQ ID NO:43    367 gacaccgcgaagttcggacagcccgagataaagctgggcgtgctgccagg
SEQ ID NO:44    466 gagaaggcccagtttgcacagccggagatcttaataggaaccatcccagg
SEQ ID NO:45    466 gagaaagcccagtttggacagccagaaatcctcctggggaccatcccagg

SEQ ID NO:40    420 tgctggtggcacccaacggctgacccgcgcccttggcccgtatcgcgcaa
SEQ ID NO:43    417 catgggcggctcccagcggctgacccgggctatcggcaaggctaaggcga
SEQ ID NO:44    516 tgcaggcggcacccagagactcacccgtgctgttgggaagtcgctggagc
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SEQ ID NO:43    467 tggacctcatcctgaccgggcgcaccatggacgccgccgaggc-cgagcg
SEQ ID NO:44    566 tggagatggtcctcaccggtgacgcgatctcagcccaggacgc-caagca
SEQ ID NO:45    566 tggagatggtcctcactggtgaccgaatttcagcacaggatgc-caagca

SEQ ID NO:40    520 ca-c-ggcctggtgtgccgggtctgcccgcctgaaagcctgctcgatgaa
SEQ ID NO:43    516 cagc-ggtctggtttcacgggtggtgccggccgacgacttgctgaccgaa
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SEQ ID NO:43    565 gccagggccactgccacgaccatttcgcagatgtcggcctcggcggcccg
SEQ ID NO:44    664 gccatccagtgtgcagaaaaaattgccagcaattctaaaattgtagtagc
SEQ ID NO:45    664 gccatccaatgtgcagaaaagatcgccaacaattccaagatcatagtagc

SEQ ID NO:40    618 gttggcgaaagaggcagtccgtatggccgccgaaaccactgtgcgcgagg
SEQ ID NO:43    615 gatggccaaggaggccgtcaaccgggctttcgaatccagtttgtccgagg
SEQ ID NO:44    714 gatggccaaagaatcagtgaatgcagcttttgaaatgacattaacagaag
SEQ ID NO:45    714 catggcgaaagaatctgtgaatgcagcctttgaaatgacgttaacagaag

SEQ ID NO:40    668 ggttggctatcgagctgcgtaacttctatctgctgtttgccagtgctgac
SEQ ID NO:43    665 ggctgctctacgaacgccggcttttccattcggctttcgcgaccgaagac
SEQ ID NO:44    764 gaagtaagttggagaagaaactcttttattcaacctttgccactgatgac
SEQ ID NO:45    764 gaaataagctggagaagaagctcttctattccacctttgccactgatgac

SEQ ID NO:40    718 caaaaagaggggatgcaggcatttatcgagaaacgcgctcccaacttcag
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SEQ ID NO:44    814 cggaaagaagggatgaccgcgtttgtggaaaagagaaaggccaacttcaa
SEQ ID NO:45    814 cggagagaagggatgtctgcctttgtggagaaaaggaaggccaacttcaa

SEQ ID NO:40    768 tggtcgttga
SEQ ID NO:43    765 ccaccgatga
SEQ ID NO:44    864 agaccagtga
SEQ ID NO:45    864 agaccactga
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Figure 32B

Figure 33

```
SEQ ID NO:41      1 -mseeslv-------lstiegp----------------------------
SEQ ID NO:46      1 -mtyetil-------ver-dqr----------------------------
SEQ ID NO:47      1 -maalrvl-------lscargplrppvrcpawrpfasganfeyiiaekrg
SEQ ID NO:48      1 maalrallpracnsllspvrcpefrrfasganfqyiitekkgknss----

SEQ ID NO:41     15 ----iailtlnrpqalnalspaliddlirhleacdaddtirviiitgagr
SEQ ID NO:46     14 ----vgiitlnrpqalnalnsqvmnevtsaateldddpdigaiiitgsak
SEQ ID NO:47     43 knntvgliqlnrpkalnalcdglidelnqalkifeedpavgaivltggdk
SEQ ID NO:48     47 ----vgliqlnrpkalnalcnglieelnqaletfeedpavgaivltggek

SEQ ID NO:41     61 afaagadikamanatpidmltsgmiarwariaavrkpviaavngyalggg
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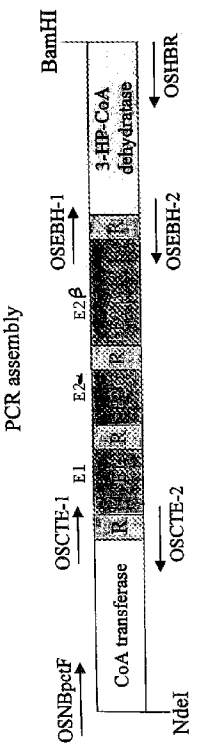
SEQ ID NO:47     93 afaagadikemqnlsfqdcysskflkhwdhltqvkkpviaavngyafggg
SEQ ID NO:48     93 afaagadikemqnrtfqdcysgkflshwdhitrikkpviaavngyalggg

SEQ ID NO:41    111 celammcdiiiasenaqfgqpeinlgiipgaggtqrltralgpyrameli
SEQ ID NO:46    110 celammcdvliaadtakfgqpeiklgvlpgmggsqrltraigkakamdli
SEQ ID NO:47    143 celammcdiiyagekaqfaqpeiligtipgaggtqrltravgkslamemv
SEQ ID NO:48    143 celammcdiiyagekaqfgqpeillgtipgaggtqrltravgkslamemv

SEQ ID NO:41    161 ltgatisaqealahglvcrvcppeslldearriaqtiatksplavqlake
SEQ ID NO:46    160 ltgrtmdaaeaersglvsrvvpaddlltearatattisqmsasaarmake
SEQ ID NO:47    193 ltgdrisaqdakqaglvskicpvetlveeaiqcaekiasnskivvamake
SEQ ID NO:48    193 ltgdrisaqdakqaglvskifpvetlveeaiqcaekiannskiivamake

SEQ ID NO:41    211 avrmaaettvreglaielrnfyllfasadqkegmqafiekrapnfsgr
SEQ ID NO:46    210 avnrafesslseglyerrlfhsafatedqsegmaafiekrapqfthr
SEQ ID NO:47    243 svnaafemtltegsklekklfystfatddrkegmtafvekrkanfkdq
SEQ ID NO:48    243 svnaafemtltegnklekklfystfatddrregmsafvekrkanfkdh
```

Figure 34


PCR assembly
BamHI
OSNBpctF
OSCTE-1
E1
E2α
E2β
OSEBH-1
3-HP-CoA dehydratase
OSHBR
CoA transferase
OSCTE-2
OSEBH-2
NdeI

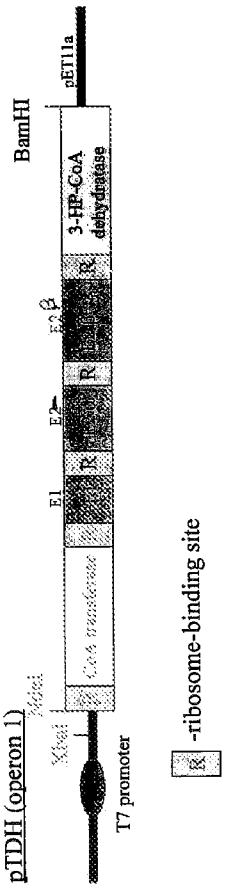

Cloning of PCR assembly in pET11a vector
pTDH (operon 1)
BamHI
pET11a
NdeI
XbaI
CoA transferase
E1
E2α
E2β
3-HP-CoA dehydratase
T7 promoter
-ribosome-binding site

Figure 35A

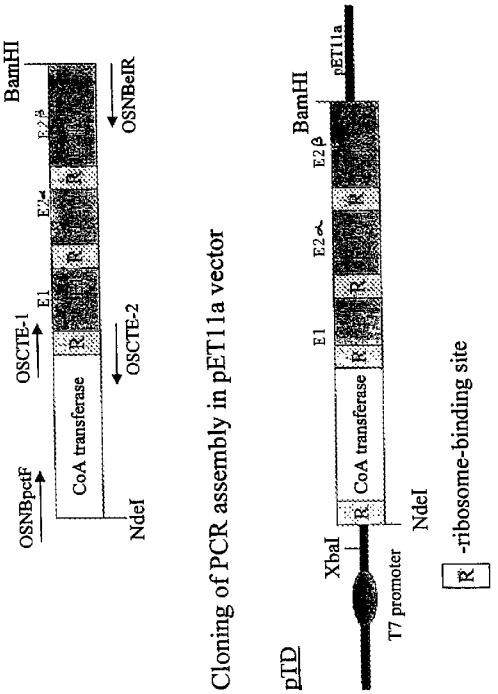

PCR assembly
OSNBpetF
OSCTE-1
CoA transferase
OSCTE-2
OSNBelR
NdeI
BamHI
E1
E2α
E2β
R
Cloning of PCR assembly in pET11a vector
pTD
XbaI
T7 promoter
NdeI
pET11a
R -ribosome-binding site

Figure 35B

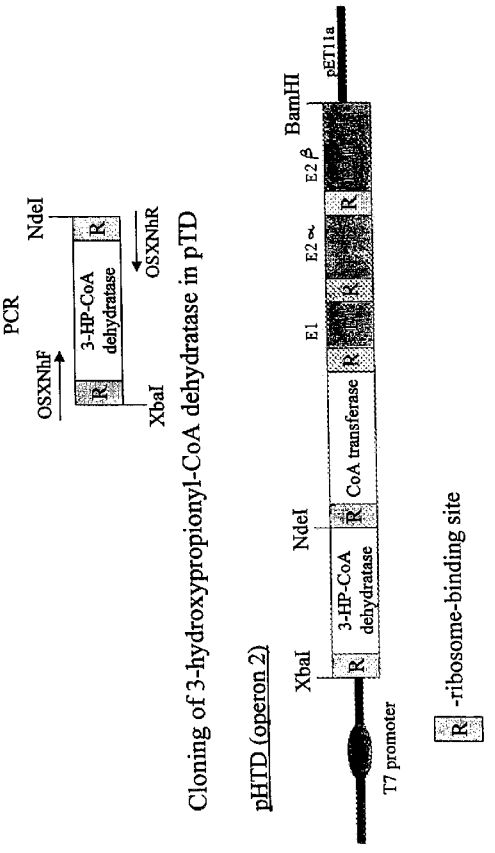

PCR
NdeI
OSXNhF
3-HP-CoA dehydratase
R
OSXNhR
XbaI
Cloning of 3-hydroxypropionyl-CoA dehydratase in pTD
pHTD (operon 2)
XbaI
NdeI
E1
E2 ∝
E2 β
BamHI
pET11a
CoA transferase
T7 promoter
-ribosome-binding site

Figure 36A

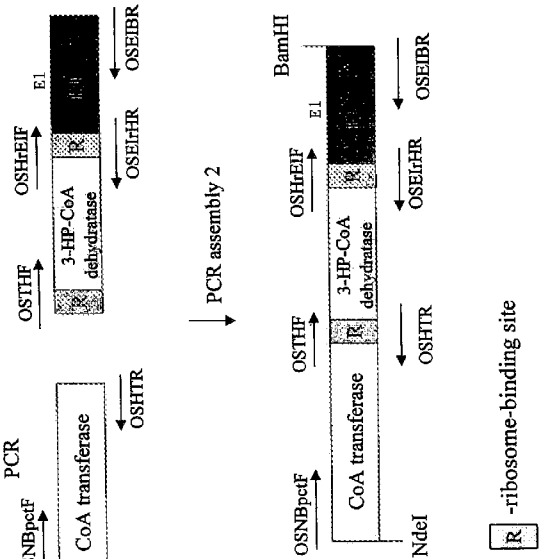

PCR assembly 1
PCR
OSNBpctF
CoA transferase
OSHTR
OSTHF
R
3-HP-CoA dehydratase
OSHrEIF
E1
OSEIrHR
OSEIBR
PCR assembly 2
BamHI
NdeI
-ribosome-binding site

Figure 36B

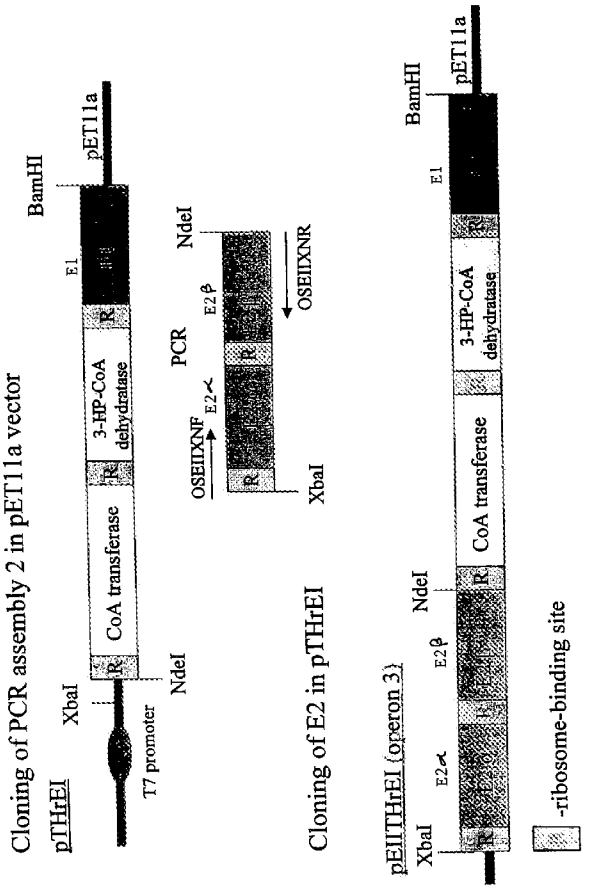

Cloning of PCR assembly 2 in pET11a vector
pTHrEI
XbaI
T7 promoter
CoA transferase
3-HP-CoA dehydratase
NdeI
BamHI
pET11a
E1
PCR
OSEIIXNF
E2α
E2β
OSEIIXNR
Cloning of E2 in pTHrEI
pEIITHrEI (operon 3)
-ribosome-binding site

Figure 37A

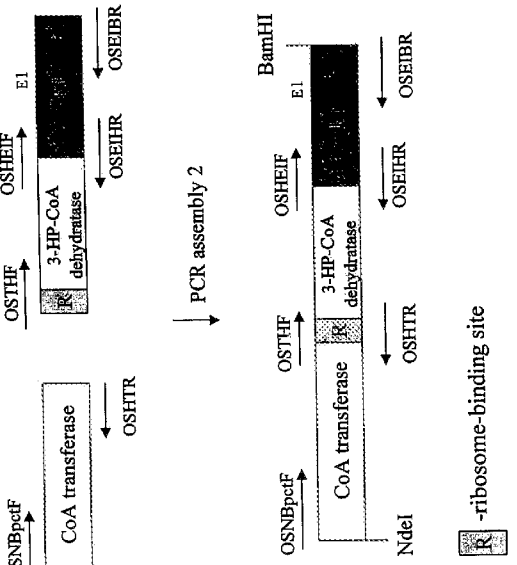

PCR
OSNBpctF
CoA transferase
OSHTR
PCR assembly 1
OSTHF
R
3-HP-CoA dehydratase
OSHEIF
E1
OSEIHR
OSEIBR
PCR assembly 2
BamHI
NdeI
R -ribosome-binding site

Figure 37B

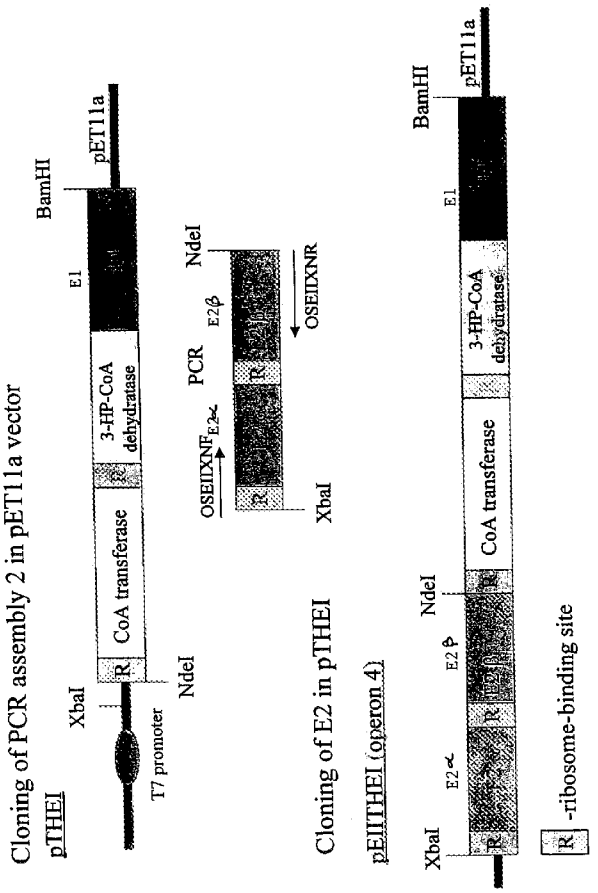

Cloning of PCR assembly 2 in pET11a vector
pTHEI
XbaI
T7 promoter
R
CoA transferase
3-HP-CoA dehydratase
NdeI
E1
BamHI
pET11a
OSEIIXNF
E2α
PCR
E2β
OSEIIXNR
XbaI
Cloning of E2 in pTHEI
pEIITHEI (operon 4)
-ribosome-binding site

Figure 38A

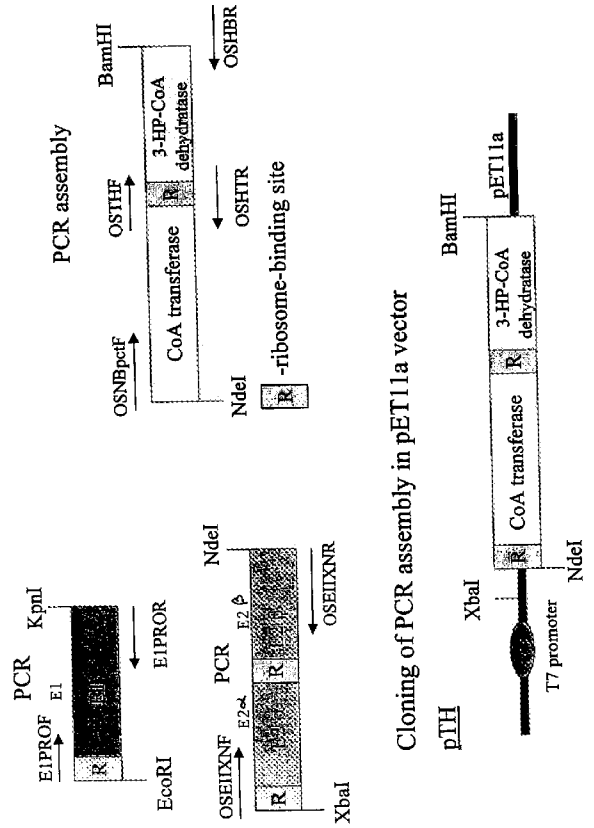
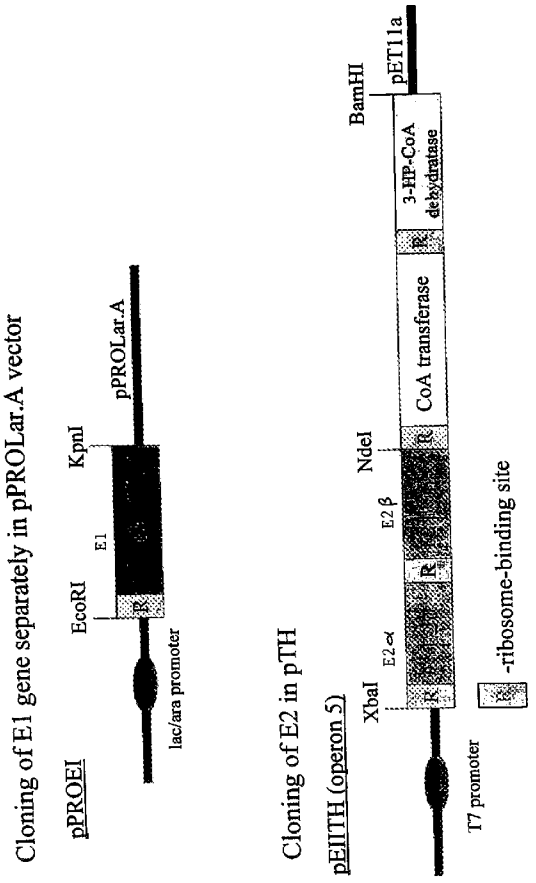

PCR assembly
OSNBpctF
OSTHF
BamHI
3-HP-CoA dehydratase
OSHBR
CoA transferase
OSHTR
NdeI
-ribosome-binding site
PCR
KpnI
E1
E1PROF
E1PROR
EcoRI
PCR
NdeI
E2
OSEIIXNF
OSEIIXNR
XbaI
Cloning of PCR assembly in pET11a vector
pTH
XbaI
BamHI
pET11a
3-HP-CoA dehydratase
CoA transferase
T7 promoter
NdeI

Figure 38B


Cloning of E1 gene separately in pPROLar.A vector
pPROEI
EcoRI
E1
KpnI
pPROLar.A
lac/ara promoter
Cloning of E2 in pTH
pEIITH (operon 5)
XbaI
E2-α
E2 β
NdeI
CoA transferase
BamHI
pET11a
3-HP-CoA dehydratase
T7 promoter
-ribosome-binding site

ATGATCGACACTGCGCCCCTTGCCCCACCACGGGCGCCCCGCTCTAATCCGATTCGGGAT
CGAGTTGATTGGGAAGCTCAGCGCGCTGCTGCGCTGGCAGATCCCGGTGCCTTTCATGGC
GCGATTGCCCGGACAGTTATCCACTGGTACGACCCACAACACCATTGCTGGATTCGCTTC
AACGAGTCTAGTCAGCGTTGGGAAGGGCTGGATGCCGCTACCGGTGCCCCTGTAACGGTA
GACTATCCCGCCGATTATCAGCCCTGGCAACAGGCGTTTGATGATAGTGAAGCGCCGTTT
TACCGCTGGTTTAGTGGTGGGTTGACAAATGCCTGCTTTAATGAAGTAGACCGGCATGTC
ATGATGGGCTATGGCGACGAGGTGGCCTACTACTTTGAAGGTGACCGCTGGGATAACTCG
CTCAACAATGGTCGTGGTGGTCCGGTTGTCCAGGAGACAATCACGCGGCGGCGCCTGTTG
GTGGAGGTGGTGAAGGCTGCGCAGGTGTTGCGTGATCTGGGCCTGAAGAAGGGTGATCGG
ATTGCTCTGAATATGCCGAATATTATGCCGCAGATTTATTATACGGAAGCGGCAAAACGA
CTGGGTATTCTGTACACGCCGGTCTTCGGTGGCTTCTCGGACAAGACTCTTTCCGACCGT
ATTCACAATGCCGGTGCACGAGTGGTGATTACCTCTGATGGTGCGTACCGCAACGCGCAG
GTGGTGCCCTACAAAGAAGCGTATACCGATCAGGCGCTCGATAAGTATATTCCGGTTGAG
ACGGCGCAGGCGATTGTTGCGCAGACCCTGGCCACCTTGCCCCTGACTGAGTCGCAGCGC
CAGACGATCATCACCGAAGTGGAGGCCGCACTGGCCGGTGAGATTACGGTTGAGCGCTCG
GACGTGATGCGTGGGGTTGGTTCTGCCCTCGCAAAGCTCCGCGATCTTGATGCAAGCGTG
CAGGCAAAGGTGCGTACAGTACTGGCGCAGGCGCTGGTCGAGTCGCCGCCGCGGGTTGAA
GCTGTGGTGGTTGTGCGTCATACCGGTCAGGAGATTTTGTGGAACGAGGGGCGAGATCGC
TGGAGTCACGACTTGCTGGATGCTGCGCTGGCGAAGATTCTGGCCAATGCGCGTGCTGCC
GGCTTTGATGTGCACAGTGAGAATGATCTGCTCAATCTCCCCGATGACCAGCTTATCCGT
GCGCTCTACGCCAGTATTCCCTGTGAACCGGTTGATGCTGAATATCCGATGTTTATCATT
TACACATCGGGTAGCACCGGTAAGCCCAAGGGTGTGATCCACGTTCACGGCGGTTATGTC
GCCGGTGTGGTGCACACCTTGCGGGTCAGTTTTGACGCCGAGCCGGGTGATACGATATAT
GTGATCGCCGATCCGGGCTGGATCACCGGTCAGAGCTATATGCTCACAGCCACAATGGCC
GGTCGGCTGACCGGGGTGATTGCCGAGGGATCACCGCTCTTCCCCTCAGCCGGGCGTTAT
GCCAGCATCATCGAGCGCTATGGGGTGCAGATCTTTAAGGCGGGTGTGACCTTCCTCAAG
ACAGTGATGTCCAATCCGCAGAATGTTGAAGATGTGCGACTCTATGATATGCACTCGCTG
CGGGTTGCAACCTTCTGCGCCGAGCCGGTCAGTCCGGCGGTGCAGCAGTTTGGTATGCAG
ATCATGACCCCGCAGTATATCAATTCGTACTGGGCGACCGAGCACGGTGGAATTGTCTGG
ACGCATTTCTACGGTAATCAGGACTTCCCGCTTCGTCCCGATGCCCATACCTATCCCTTG
CCCTGGGTGATGGGTGATGTCTGGGTGGCCGAAACTGATGAGAGCGGGACGACGCGCTAT
CGGGTCGCTGATTTCGATGAGAAGGGCGAGATTGTGATTACCGCCCCGTATCCCTACCTG
ACCCGCACACTCTGGGGTGATGTGCCCGGTTTCGAGGCGTACCTGCGCGGTGAGATTCCG
CTGCGGGCCTGGAAGGGTGATGCCGAGCGTTTCGTCAAGACCTACTGGCGACGTGGGCCA
AACGGTGAATGGGGCTATATCCAGGGTGATTTTGCCATCAAGTACCCCGATGGTAGCTTC
ACGCTCCACGGACGCCCTGACGATGTGATCAATGTGTCGGGCCACCGTATGGGCACCGAG
GAGATTGAGGGTGCCATTTTGCGTGACCGCCAGATCACGCCCGACTCGCCCGTCGGTAAT
TGTATTGTGGTCGGTGCGCCGCACCGTGAGAAGGGTCTGACCCCGGTTGCCTTCATTCAA
CCTGCGCCTGGCCGTCATCTGACCGGCGCCGACCGGCGCCGTCTCGATGAGCTGGTGCGT
ACCGAGAAGGGGGCGGTCAGTGTCCCAGAGGATTACATCGAGGTCAGTGCCTTTCCCGAA
ACCCGCAGCGGGAAGTATATGCGGCGCTTTTTGCGCAATATGATGCTCGATGAACCACTG
GGTGATACGACGACGTTGCGCAATCCTGAAGTGCTCGAAGAGATTGCAGCCAAGATCGCT
GAGTGGAAACGCCGTCAGCGTATGGCCGAAGAGCAGCAGATCATCGAACGCTATCGCTAC
TTCCGGATCGAGTATCACCCACCAACGGCCAGTGCGGGTAAACTCGCGGTAGTGACGGTG
ACAAATCCGCCGGTGAACGCACTGAATGAGCGTGCGCTCGATGAGTTGAACACAATTGTT
GACCACCTGGCCCGTCGTCAGGATGTTGCCGCAATTGTCTTCACCGGACAGGGCGCCAGG
AGTTTTGTCGCCGGCGCTGATATTCGCCAGTTGCTCGAAGAGATTCATACGGTTGAAGAG
GCAATGGCCCTGCCGAATAACGCCCATCTTGCTTTCCGCAAGATTGAGCGTATGAATAAG

Figure 39A

CCGTGTATCGCGGCGATCAACGGTGTGGCGCTCGGTGGTGGTCTGGAATTCGCCATGGCC
TGCCATTACCGGGTTGCCGATGTCTATGCCGAATTCGGTCAGCCAGAGATTAATCTGCGC
TTGCTACCTGGTTATGGTGGCACGCAGCGCTTGCCGCGCCTGTTGTACAAGCGCAACAAC
GGCACCGGTCTGCTCCGAGCGCTGGAGATGATTCTGGGTGGGCGTAGCGTACCGGCTGAT
GAGGCGCTGAAGCTGGGTCTGATCGATGCCATTGCTACCGGCGATCAGGACTCACTGTCG
CTGGCATGCGCGTTAGCCCGTGCCGCAATCGGCGCCGATGGTCAGTTGATCGAGTCGGCT
GCGGTGACCCAGGCTTTCCGCCATCGCCACGAGCAGCTTGACGAGTGGCGCAAACCAGAC
CCGCGCTTTGCCGATGACGAACTGCGCTCGATTATCGCCCATCCACGTATCGAGCGGATT
ATCCGGCAGGCCCATACCGTTGGGCGCGATGCGGCAGTGCATCGGGCACTGGATGCAATC
CGCTATGGCATTATCCACGGCTTCGAGGCCGGTCTGGAGCACGAGGCGAAGCTCTTTGCC
GAGGCAGTGGTTGACCCGAACGGTGGCAAGCGTGGTATTCGCGAGTTCCTCGACCGCCAG
AGTGCGCCGTTGCCAACCCGCCGACCATTGATTACACCTGAACAGGAGCAACTCTTGCGC
GATCAGAAAGAACTGTTGCCGGTTGGTTCACCCTTCTTCCCCGGTGTTGACCGGATTCCG
AAGTGGCAGTACGCGCAGGCGGTTATTCGTGATCCGGACACCGGTGCGGCGGCTCACGGC
GATCCCATCGTGGCTGAAAAGCAGATTATTGTGCCGGTGGAACGCCCCCGCGCCAATCAG
GCGCTGATCTATGTTCTGGCCTCGGAGGTGAACTTCAACGATATCTGGGCGATTACCGGT
ATTCCGGTGTCACGGTTTGATGAGCACGACCGCGACTGGCACGTTACCGGTTCAGGTGGC
ATCGGCCTGATCGTTGCGCTGGGTGAAGAGGCGCGACGCGAAGGCCGGCTGAAGGTGGGT
GATCTGGTGGCGATCTACTCCGGGCAGTCGGATCTGCTCTCACCGCTGATGGGCCTTGAT
CCGATGGCCGCCGATTTCGTCATCCAGGGGAACGACACGCCAGATGGATCGCATCAGCAA
TTTATGCTGGCCCAGGCCCCGCAGTGTCTGCCCATCCCAACCGATATGTCTATCGAGGCA
GCCGGCAGCTACATCCTCAATCTCGGTACGATCTATCGCGCCCTCTTTACGACGTTGCAA
ATCAAGGCCGGACGCACCATCTTTATCGAGGGTGCGGCGACCGGTACCGGTCTGGACGCA
GCGCGCTCGGCGGCCCGGAATGGTCTGCGCGTAATTGGAATGGTCAGTTCGTCGTCACGT
GCGTCTACGCTGCTGGCTGCGGGTGCCCACGGTGCGATTAACCGTAAAGACCCGGAGGTT
GCCGATTGTTTCACGCGCGTGCCCGAAGATCCATCAGCCTGGGCAGCCTGGGAAGCCGCC
GGTCAGCCGTTGCTGGCGATGTTCCGGGCGCAGAACGACGGGCGACTGGCCGATTATGTG
GTCTCGCACGCGGGCGAGACGGCCTTCCCGCGCAGTTTCCAGCTTCTCGGCGAGCCACGC
GATGGTCACATTCCGACGCTCACATTCTACGGTGCCACCAGTGGCTACCACTTCACCTTC
CTGGGTAAGCCAGGGTCAGCTTCGCCGACCGAGATGCTGCGGCGGGCCAATCTCCGCGCC
GGTGAGGCGGTGTTGATCTACTACGGGGTTGGGAGCGATGACCTGGTAGATACCGGCGGT
CTGGAGGCTATCGAGGCGGCGCGGCAAATGGGAGCGCGGATCGTCGTCGTTACCGTCAGC
GATGCGCAACGCGAGTTTGTCCTCTCGTTGGGCTTCGGGGCTGCCCTACGTGGTGTCGTC
AGCCTGGCGGAACTCAAACGGCGCTTCGGCGATGAGTTTGAGTGGCCGCGCACGATGCCG
CCGTTGCCGAACGCCCGCCAGGACCCGCAGGGTCTGAAAGAGGCTGTCCGCCGCTTCAAC
GATCTGGTCTTCAAGCCGCTAGGAAGCGCGGTCGGTGTCTTCTTGCGGAGTGCCGACAAT
CCGCGTGGCTACCCCGATCTGATCATCGAGCGGGCTGCCCACGATGCACTGGCGGTGAGC
GCGATGCTGATCAAGCCCTTCACCGGACGGATTGTCTACTTCGAGGACATTGGTGGGCGG
CGTTACTCCTTCTTCGCACCGCAAATCTGGGTGCGCCAGCGCCGCATCTACATGCCGACG
GCACAGATCTTTGGTACGCACCTCTCAAATGCGTATGAAATTCTGCGTCTGAATGATGAG
ATCAGCGCCGGTCTGCTGACGATTACCGAGCCGGCAGTGGTGCCGTGGGATGAACTACCC
GAAGCACATCAGGCGATGTGGGAAAATCGCCACACGGCGGCCACTTATGTGGTGAATCAT
GCCTTACCACGTCTCGGCCTAAAGAACAGGGACGAGCTGTACGAGGCGTGGACGGCCGGC
GAGCGGTAG (SEQ ID NO:129)

Figure 39B

```
SEQ ID NO:39      1 -------------------------midtaplappraprsnpirdrvdwe
SEQ ID NO:130     1 mglpeervrsgsgsrgqeeagaggrarswsp--ppevsrsahvpslqryr
SEQ ID NO:131     1 ----------------------------mslelkekeselpfdeqiind
                                                 PL PP   RS P

SEQ ID NO:39     26 aqraaaladpgafhgaiartvihwydpqhhcwirfnessqrwegldaatg
SEQ ID NO:130    49 elhrrsveeprefwgdiake-fywktpcpgpflryn--------------
SEQ ID NO:131    22 kwrs-------------kytpidayfkfhrqtvenlesf--wesv-----
                     R      P  F G IA T I WY P H    R NES   WE

SEQ ID NO:39     76 apvtvdypadyqpwqqafddseap-fyrwfsggltnacfnevdrhvm-mg
SEQ ID NO:130    84 -----------------fdvtkgkifiewmkgattnicynvldrnvhekk
SEQ ID NO:131    52 -akelew---fkpwdkvldasnpp-fykwfvggrlnlsylavdrhvk-tw
                          PW   FD S  P FY WF GG TN C N VDRHV

SEQ ID NO:39    124 ygdevayyfegdrwdnslnngrggpvvqetitrrrllvevvkaaqvlr-d
SEQ ID NO:130   117 lgdkvafywegne----------pgettqityhqllvqvcqfsnvlr-k
SEQ ID NO:131    96 rknklaiewegepvden-----gyptdrrkltyydlyrevnrvaymlkqn
                    GD VA Y EG   D       G P     IT   LLVEV   A VLR

SEQ ID NO:39    173 lglkkgdrialnmpnimpqiyyte-aakrlgilytpvfggfsdktlsdri
SEQ ID NO:130   155 qgiqkgdrvaiympmipelvvaml-acarigalhsivfagfsseslceri
SEQ ID NO:131   141 fgvkkgdkitlylp-mvpelpitmlaawrigaitsvvfsgfsadalaeri
                    G KKGDRIAL MP I P    T  AA R G L   VF GFS   L  RI

SEQ ID NO:39    222 hnagarvvitsdgayrnaqvvpykeaytdqal----dkyipvetaqaiva
SEQ ID NO:130   204 ldsscsllittdafyrgeklvnlkel-adealqkcqekgfpvrc--civv
SEQ ID NO:131   190 ndsqsrivitadgfwrrgrvvvrlkev-----------------------
                         R VIT DG YR   VV  KE   D AL     K  PV     IV

SEQ ID NO:39    268 qtlatlpltesqrqtiiteveaalageitversdvmrgvgsalaklrdld
SEQ ID NO:130   251 khlgrael-----------------------------gmgdsts------
SEQ ID NO:131   216 -------------------vdaal--------------------------
                      L    L           V AAL             G G

SEQ ID NO:39    318 asvqakvrtvlaqalvespprveavvvvrhtg-qeilwnegrdrwshdll
SEQ ID NO:130   266 ----------------qsppikrscpdv------qiswnqgidlwwhelm
SEQ ID NO:131   221 ----------------ekatgvesvivlprlglkdvpmtegrdywwnklm
                                   ESPP VE V VV   G   I WNEGRD W H L

SEQ ID NO:39    367 daalakilanaraagfdvhsendllnlpddqliralyasipcep--vdae
SEQ ID NO:130   294 qea-------------------------gde----------cepewcdae
SEQ ID NO:131   255 q-----------------------gippn-------ayiepep--vese
                     A                       P D       A I CEP  VDAE

SEQ ID NO:39    415 ypmfiiytsgstgkpkgvihvhggyvagvvhtlrvsfdaepgdtiyviad
SEQ ID NO:130   309 dplfilytsgstgkpkgvvhtvggymlyvattfkyvfdfhaedvfwctad
SEQ ID NO:131   272 hpsfilytsgttgkpkgivhdtggwavhvyatmkwvfdirdddifwctad
                    P FI YTSGSTGKPKGV H  GGY   V  T    FD    D     AD

SEQ ID NO:39    465 pgwitgqsymltatmagrltgviaegsplfpsagryasiierygvqifka
SEQ ID NO:130   359 igwitghsyvtygplangatsvlfegiptypdvnrlwsivdkykvtkfyt
SEQ ID NO:131   322 igwvtghsyvvlgpllmgateviyegapdypqpdrwwsiierygvtifyt
                    GWITG SY      A   T VI EG P  P   R  SIIERYGV IF
```

Figure 40A

```
SEQ ID NO:39    515 gvtflkttvmsnpqnvedvrlydmhslrvatfcaepvspavqqfgmqimtp
SEQ ID NO:130   409 aptairllmkfgd--epvtkhsraslqvlgtvgepinpeawlwyhrvvga
SEQ ID NO:131   372 sptairmfmryge--ewprkhdlstlriihsvgepinpeawrwayrvlgn
                      T     M       E VR  D  SLRV     EP  P

SEQ ID NO:39    565 q---yi---nsywatehggivwthfygnqdfplrpdahtyplpwvmgdvw
SEQ ID NO:130   457 qrcpiv---dtfwqtetgghmltplpgat--pmkpgsatfp----ffgva
SEQ ID NO:131   420 e---kvafgstwwmtetggivishapglylvpmkpgtngpplpgfevdv-
                    Q           W TE GGIV TH  G    P  P   T PLP    DV

SEQ ID NO:39    609 vaetdesgttryrvadfdekgeivitapypyltrtlwgdvpgfeaylrge
SEQ ID NO:130   498 pailnesg----eelegeaegylvfkqpwpgimrtvy-------------
SEQ ID NO:131   466 ---vdengnp----appgvkgylvikkpwpgmlhgiw-------------
                     A  DESG      A    KG  VI  P P   RT W

SEQ ID NO:39    659 iplrawkgdaerfvktywrrgpngewgyiqgdfaikypdgsftlhgrpdd
SEQ ID NO:130   531 -------gnherfettyfkkfpg---yyvtgdgcqrdqdgyywitgridd
SEQ ID NO:131   496 -------gdperyiktywsrfpg---mfyagdyaikdkdgyiwvlgrade
                          GD ERF KTYW R P     Y  GD AIK  DG     GR DD

SEQ ID NO:39    709 vinvsghrmgteeiegailrdrqitpdspvgncivvgaphrekgltpvaf
SEQ ID NO:130   571 mlnvsghllstaevesalve------heavaeaavvghphpvkgeclycf
SEQ ID NO:131   536 vikvaghrlgtyelesali------shpavaesavvgvpdaikgevpiaf
                    VINVSGHR GT E E A            V    VVG PH  KG  P AF

SEQ ID NO:39    759 iqpapgrhltgadrrrldelvrtekgavsvpedyie-vsafpetrsgkym
SEQ ID NO:130   615 vtlcdghtfspklteelkkqirekigpiatp-dyiqnapglpktrsgkim
SEQ ID NO:131   580 vvlkqgvapsdelrkelrehvrrtigpiaepaqiff-vtklpktrsgkim
                         G       R  L E VR   G    P DYI  V   P TRSGK M

SEQ ID NO:39    808 rrflrnmml-deplgdtttlrnpevleeiaakiaewkrrqrmaeeqqiie
SEQ ID NO:130   664 rrvlrkiaqndhdlgdmstvadpsvi-------------------------
SEQ ID NO:131   629 rrllkavat-gaplgdvtt-----------------------------
                    RR LR     D PLGD TT   P V

SEQ ID NO:39    857 ryryfrieyhpptasagklavvtvtnppvnalneraldelntivdhlarr
SEQ ID NO:130   690 ---------------------------------------------------
SEQ ID NO:131   647 ---------------------------------------------------


SEQ ID NO:39    907 qdvaaivftgqgarsfvagadirqlleeihtveeamalpnnahlafrkie
SEQ ID NO:130   690 ----------------------------------------shl------
SEQ ID NO:131   647 -------------------------ledetsveeak--------------
                                             LE    VEEA       HL

SEQ ID NO:39    957 rmnkpciaaingvalggglefamachyrvadvyaefgqpeinlrllpgyg
SEQ ID NO:130   693 ---------------------------------------------------
SEQ ID NO:131   658 ---------------------------------------------------


SEQ ID NO:39   1007 gtqrlprllykrnngtgllralemilggrsvpadealklglidaiatgdq
SEQ ID NO:130   693 ---------------------------------------------------
SEQ ID NO:131   658 -------------------raye----------------------------
                                      RA E

SEQ ID NO:39   1057 dslslacalaraaigadgqliesaavtqafrhrheqldewrkpdprfadd
SEQ ID NO:130   693 ----------------------------fshr-----------------
SEQ ID NO:131   662 ---------------------------------------------------
                                               F HR
```

Figure 40B

```
SEQ ID NO:39   1107 elrsiiahprieriirqahtvgrdaavhraldairygiihgfeaglehea
SEQ ID NO:130   697 --------------------------------------------------
SEQ ID NO:131   662 --------------------------------------------------

SEQ ID NO:39   1157 klfaeavvdpnggkrgirefldrqsaplptrrplitpeqeqllrdqkell
SEQ ID NO:130   697 --------------------------------------------------
SEQ ID NO:131   662 --------------------------------------------------

SEQ ID NO:39   1207 pvgspffpgvdripkwqyaqavirdpdtgaaahgdpivaekqiivpverp
SEQ ID NO:130   697 --------------------------------------------------
SEQ ID NO:131   662 --------------------------------------------------

SEQ ID NO:39   1257 ranqaliyvlasevnfndiwaitgipvsrfdehdrdwhvtgsggigliva
SEQ ID NO:130   697 --------------------------------------------------
SEQ ID NO:131   662 --------------------------------------------------

SEQ ID NO:39   1307 lgeearregrlkvgdlvaiysgqsdllsplmgldpmaadfviqgndtpdg
SEQ ID NO:130   697 --------------------------------------------------
SEQ ID NO:131   662 --------------------------------------------------

SEQ ID NO:39   1357 shqqfmlaqapqclpiptdmsieaagsyilnlgtiyralfttlqikagrt
SEQ ID NO:130   697 ------------cl---------s-----------------tiq------
SEQ ID NO:131   662 ------------------------------------------eika---
                                CL                          T QIKA

SEQ ID NO:39   1407 ifiegaatgtgldaarsaarnglrvigmvssssrastllaagahgainrk
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   666 --------------------------------------------------

SEQ ID NO:39   1457 dpevadcftrvpedpsawaaweaagqpllamfraqndgrladyvvshage
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   666 --------------------------------------------------

SEQ ID NO:39   1507 tafprsfqllgeprdghiptltfygatsgyhftflgkpgsasptemlrra
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   666 --------------------------------------------------

SEQ ID NO:39   1557 nlrageavliyygvgsddlvdtggleaieaarqmgarivvvtvsdaqref
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   666 --------------------------------------------------

SEQ ID NO:39   1607 vlslgfgaalrgvvslaelkrrfgdefewprtmpplpnarqdpqglkeav
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   666 --------------------------emart------------------
                                           E  RT

SEQ ID NO:39   1657 rrfndlvfkplgsavgvflrsadnprgypdliieraahdalavsamlikp
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   671 --------------------------------------------------
```

Figure 40C

```
SEQ ID NO:39   1707 ftgrivyfediggrrysffapqiwvrqrriymptaqifgthlsnayeilr
SEQ ID NO:130   702 --------------------------------------------------
SEQ ID NO:131   671 --------------------------------------------------

SEQ ID NO:39   1757 lndeisagllttitepavvpwdelpeahqamwenrhtaatyvvnhalprlg
SEQ ID NO:130   702 ----------------------------------------------------
SEQ ID NO:131   671 ----------------------------------------------------

SEQ ID NO:39   1807 lknrdelyeawtager
SEQ ID NO:130   702 ----------------
SEQ ID NO:131   671 ----------------
```

Figure 40D

```
SEQ ID NO:39      1 midtaplappraprsnpirdrvdweaqraaaladpgafhgaiartvihwy
SEQ ID NO:132     1 ------------------------------------------------
SEQ ID NO:133     1 ------------------------------------------------

SEQ ID NO:39     51 dpqhhcwirfnessqrwegldaatgapvtvdypadyqpwqqafddseapf
SEQ ID NO:132     1 ------------------------------------------------
SEQ ID NO:133     1 ------------------md----------------------------
                                      D

SEQ ID NO:39    101 yrwfsggltnacfnevdrhvmmgygdevayyfegdrwdnslnngrggpvv
SEQ ID NO:132     1 ------------------------------------melnn-------
SEQ ID NO:133     3 ------------fnnv--------------------------------
                               FN V                          LNN

SEQ ID NO:39    151 qetitrrrllvevvkaaqvlrdlglkkgdrialnmpnimpqiyyteaakr
SEQ ID NO:132     6 ------------------------------------------------
SEQ ID NO:133     7 ------------------------llnkddgial----------------
                                         L K D IAL

SEQ ID NO:39    201 lgilytpvfggfsdktlsdrihnagarvvitsdgayrnaqvvpykeaytd
SEQ ID NO:132     6 ------------------------------------------------
SEQ ID NO:133    17 ------------------------------------------------

SEQ ID NO:39    251 qaldkyipvetaqaivaqtlatlpltesqrqtiiteveaalageitvers
SEQ ID NO:132     6 ----------------------------------vileke----------
SEQ ID NO:133    17 ------------------------------------------------
                                                  I E E

SEQ ID NO:39    301 dvmrgvgsalaklrdldasvqakvrtvlaqalvespprveavvvvrhtgq
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    17 ------------------------------------------------

SEQ ID NO:39    351 eilwnegrdrwshdlldaalakilanaraagfdvhsendllnlpddqlir
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    17 ----------------------iiin------------------------
                                        I  N

SEQ ID NO:39    401 alyasipcepvdaeypmfiiytsgstgkpkgvihvhggyvagvvhtlrvs
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    21 ------------------------------------------------

SEQ ID NO:39    451 fdaepgdtiyviadpgwitgqsymltatmagrltgviaegsplfpsagry
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    21 ------------------------------------------------

SEQ ID NO:39    501 asiierygvqifkagvtflktvmsnpqnvedvrlydmhslrvatfcaepv
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    21 ------------------------------------------------
```

Figure 41A

```
SEQ ID NO:39    551 spavqqfgmqimtpqyinsywatehggivwthfygnqdfplrpdahtypl
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    21 ----------------------------------------rpka-----
                                                            RP A

SEQ ID NO:39    601 pwvmgdvwwaetdesgttryrvadfdekgeivitapypyltrtlwgdvpg
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    25 ------------------------------------------------

SEQ ID NO:39    651 feaylrgeiplrawkgdaerfvktywrrgpngewgyiqgdfaikypdgsf
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    25 ------------------------------------------------

SEQ ID NO:39    701 tlhgrpddvinvsghrmgteeiegailrdrqitpdspvgncivvgaphre
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    25 ------------------------------------------------

SEQ ID NO:39    751 kgltpvafiqpapgrhltgadrrrldelvrtekgavsvpedyievsafpe
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    25 ------------------------------------------------

SEQ ID NO:39    801 trsgkymrrflrnmmldeplgdtttlrnpevleeiaakiaewkrrqrmae
SEQ ID NO:132    12 ------------------------------------------------
SEQ ID NO:133    25 ------------------------------------------------

SEQ ID NO:39    851 eqqiieryryfrieyhpptasagklavvtvtnpp-vnalneraldelnti
SEQ ID NO:132    12 ----------------------gkvavvtinrpkalnalnsdtlkemdyv
SEQ ID NO:133    25 ----------------------------------lnalnyetlkeldsv
                                        GK AVVT   P   NALN   L EL

SEQ ID NO:39    900 vdhlarrqdvaaivftgqgarsfvagadirqlleeihtve-eamalpnna
SEQ ID NO:132    40 igeiendsevlaviltgageksfvagadisem-kemntiegrkfgilgnk
 EQ ID NO:133    40 ldivendkeikvliitgsgektfvagadiaemsn--mtpl-eakkfslyg
                     D       V A   TG G  SFVAGADI     E  T E EA     N

SEQ ID NO:39    949 hlafrkiermnkpciaaingvalggglefamachyrvadvyaefgqpein
SEQ ID NO:132    89 --vfrrlellekpviaavngfalgggceiamscdiriassnarfgqpevg
SEQ ID NO:133    87 qkvfrkiemlskpviaavngfalgggcelsmacdiriasknakfgqpevg
                       FRKIE   KP IAA NG ALGGG E AMAC  R A   A FGQPE

SEQ ID NO:39    999 lrllpgyggtqrlprllykrnngtgllralemilggrsvpadealklgli
SEQ ID NO:132   137 lgitpgfggtqrlsrlv-------gmgmakqliftaqnikadealriglv
SEQ ID NO:133   137 lgiipgfsgtqrlprli-------gtskakeliftgdminsdeaykigli
                    L   PG GGTQRLPRL        G   A E I  G    ADEALK GLI

SEQ ID NO:39   1049 daiatgdqdslslacalaraaigadgqliesaavtqafrhrheqldewrk
SEQ ID NO:132   180 n-----------------------------------------------
SEQ ID NO:133   180 skvv--------------------------------------------

SEQ ID NO:39   1099 pdprfaddelrsiiahprieriirqahtvgrdaavhraldairygiihgf
SEQ ID NO:132   181 ------------------------------------------------
SEQ ID NO:133   184 --------elsdli-------------------------------------
                           EL   I
```

Figure 41B

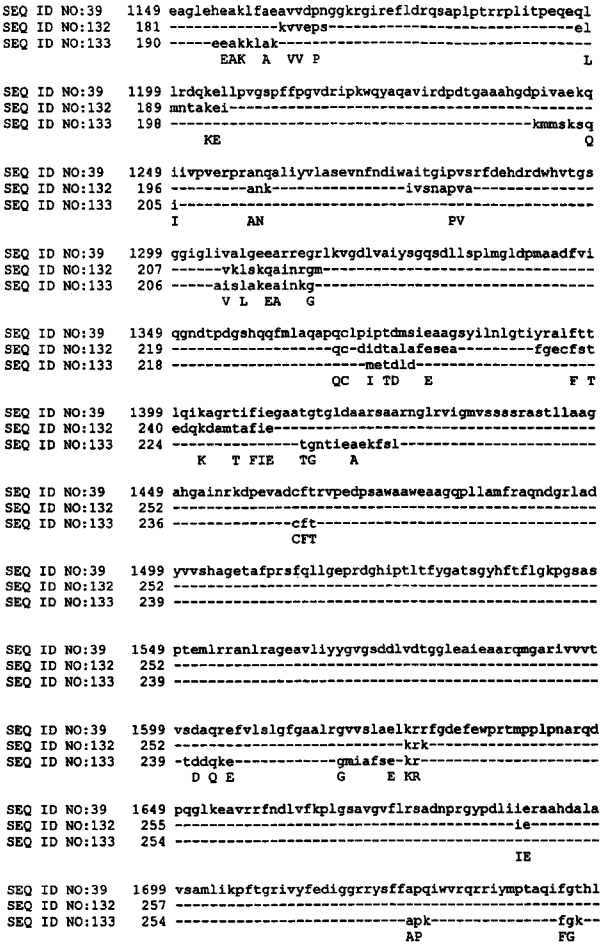

SEQ ID NO:39 1149 eagleheaklfaeavvdpnggkrgirefldrqsaplptrrplitpeqeql
SEQ ID NO:132 181 ----------kvveps----------el
SEQ ID NO:133 190 -----eeakklak----------
EAK A VV P L
SEQ ID NO:39 1199 lrdqkellpvgspffpgvdripkwqyaqavirdpdtgaaahgdpivaekq
SEQ ID NO:132 189 mntakei----------
SEQ ID NO:133 198 ----------kmmsksq
KE Q
SEQ ID NO:39 1249 iivpverpranqaliyvlasevnfndiwaitgipvsrfdehdrdwhvtgs
SEQ ID NO:132 196 ---------ank----------ivsnapva----------
SEQ ID NO:133 205 i----------
I AN PV
SEQ ID NO:39 1299 ggiglivalgeearregrlkvgdlvaiysgqsdllsplmgldpmaadfvi
SEQ ID NO:132 207 ------vklskqainrgm----------
SEQ ID NO:133 206 -----aislakeainkg----------
V L EA G
SEQ ID NO:39 1349 qgndtpdgshqqfmlaqapqclpiptdmsieaagsyilnlgtiyralftt
SEQ ID NO:132 219 ----------qc-didtalafesea---------fgecfst
SEQ ID NO:133 218 ----------metdld----------
QC I TD E F T
SEQ ID NO:39 1399 lqikagrtifiegaatgtgldaarsaarnglrvigmvssssrastllaag
SEQ ID NO:132 240 edqkdamtafie----------
SEQ ID NO:133 224 ----------tgntieaekfsl----------
K T FIE TG A
SEQ ID NO:39 1449 ahgainrkdpevadcftrvpedpsawaaweaagqpllamfraqndgrlad
SEQ ID NO:132 252 ----------
SEQ ID NO:133 236 ----------cft----------
CFT
SEQ ID NO:39 1499 yvvshagetafprsfqllgeprdghiptltfygatsgyhftflgkpgsas
SEQ ID NO:132 252 ----------
SEQ ID NO:133 239 ----------
SEQ ID NO:39 1549 ptemlrranlrageavliyygvgsddlvdtggleaieaarqmgarivvvt
SEQ ID NO:132 252 ----------
SEQ ID NO:133 239 ----------
SEQ ID NO:39 1599 vsdaqrefvlslgfgaalrgvvslaelkrrfgdefewprtmpplpnarqd
SEQ ID NO:132 252 ----------krk----------
SEQ ID NO:133 239 -tddqke----------gmiafse-kr----------
D Q E G E KR
SEQ ID NO:39 1649 pqglkeavrrfndlvfkplgsavgvflrsadnprgypdliieraahdala
SEQ ID NO:132 255 ----------ie---------
SEQ ID NO:133 254 ----------
IE
SEQ ID NO:39 1699 vsamlikpftgrivyfediggrrysffapqiwvrqrriymptaqifgthl
SEQ ID NO:132 257 ----------
SEQ ID NO:133 254 ----------apk----------fgk--
AP FG

Figure 41C

```
SEQ ID NO:39   1749 snayeilrlndeisaglltitepavvpwdelpeahqamwenrhtaatyvv
SEQ ID NO:132   257 ------------------------------------------------------------
SEQ ID NO:133   260 ------------------------------------------------------------

SEQ ID NO:39   1799 nhalprlglknrdelyeawtager
SEQ ID NO:132   257 -------gfknr------------
SEQ ID NO:133   260 -------------------------
                          G KNR
```

Figure 41D

```
SEQ ID NO:39      1 midtaplappraprsnpirdrvdweaqraaaladpgafhgaiartvihwy
SEQ ID NO:134     1 ------------------------------------------------------------
SEQ ID NO:135     1 ------------------------------------------------------------

SEQ ID NO:39     51 dpqhhcwirfnessqrwegldaatgapvtvdypadyqpwqqafddseapf
SEQ ID NO:134     1 --------------------maasaap-----------------------
SEQ ID NO:135     1 ------------------------------------------------------------
                                       AA  AP

SEQ ID NO:39    101 yrwfsggltnacfnevdrhvmmgygdevayyfegdrwdnslnngrggpvv
SEQ ID NO:134     8 ------------------------------------------------------------
SEQ ID NO:135     1 ------------------------------------------------------------

SEQ ID NO:39    151 qetitrrrllvevvkaaqvlrdlglkkgdrialnmpnimpqiyyteaakr
SEQ ID NO:134     8 ------------------------------------------------------------
SEQ ID NO:135     1 ------------------------------------------------------------

SEQ ID NO:39    201 lgilytpvfggfsdktlsdrihnagarvvitsdgayrnaqvvpykeaytd
SEQ ID NO:134     8 ----------------------------------------------------awtg
SEQ ID NO:135     1 ------------------------------------------------------------
                                                                   A T

SEQ ID NO:39    251 qaldkyipvetaqaivaqtlatlpltesqrqtiiteveaalageitvers
SEQ ID NO:134    12 q-----------------------------------------------taeak
SEQ ID NO:135     1 -------------mtiqtlettalkd-----------------------
                    Q               QTL T  L                    T E

SEQ ID NO:39    301 dvmrgvgsalaklrdldasvqakvrtvlaqalvespprveavvvvrhtgq
SEQ ID NO:134    18 d-----------------------------------------------------
SEQ ID NO:135    14 ------------------------------------------------------------
                    D

SEQ ID NO:39    351 eilwnegrdrwshdlldaalakilanaraagfdvhsendllnlpddqlir
SEQ ID NO:134    19 ------------------------------------------------------------
SEQ ID NO:135    14 ------------------------------------------------------------

SEQ ID NO:39    401 alyasipcepvdaeypmfiiytsgstgkpkgvihvhggyvagvvhtlrvs
SEQ ID NO:134    19 ------------------------------------------------------------
SEQ ID NO:135    14 ------------------------------------------------------------

SEQ ID NO:39    451 fdaepgdtiyviadpgwitgqsymltatmagrltgviaegsplfpsagry
SEQ ID NO:134    19 ------------------------------------------------------------
SEQ ID NO:135    14 ------------------------------------------------------------

SEQ ID NO:39    501 asiierygvqifkagvtflktvmsnpqnvedvrlydmhslrvatfcaepv
SEQ ID NO:134    19 ----------------------------------lyel-------------
SEQ ID NO:135    14 ----------------------------------lyei-------------
                                                      LY
```

Figure 42A

```
SEQ ID NO:39    551 spavqqfgmqimtpqyinsywatehggivwthfygnqdfplrpdahtypl
SEQ ID NO:134    23 ----------------------------------------------------
SEQ ID NO:135    18 ----------------------------------------------------

SEQ ID NO:39    601 pwvmgdvwvaetdesgttryrvadfdekgeivitapypyltrtlwgdvpg
SEQ ID NO:134    23 ----------------------------------------------------
SEQ ID NO:135    18 ----------------------------------------------------

SEQ ID NO:39    651 feaylrgeiplrawkgdaerfvktywrrgpngewgyiqgdfaikypdgsf
SEQ ID NO:134    23 ------geip------------------------------------------
SEQ ID NO:135    18 ------geip------------------------------------------
                        GEIP

SEQ ID NO:39    701 tlhgrpddvinvsghrmgteeiegailrdrqitpdspvgncivvgaphre
SEQ ID NO:134    27 ----------------------------------------------------
SEQ ID NO:135    22 ----------------------------------------------------

SEQ ID NO:39    751 kgltpvafiqpapgrhltgadrrrldelvrtekgavsvpedyievsafpe
SEQ ID NO:134    27 ----------------------------------------------------
SEQ ID NO:135    22 ------------pafhv--------------------------------pk
                              P  H                                   P

SEQ ID NO:39    801 trsgkymrrflrnmmldeplgdtttlrnpevleeiaakiaewkrrqrmae
SEQ ID NO:134    27 ------------------plg-----------hvpakmyawairr-----
SEQ ID NO:135    29 t-----------------------------------myawsirk-----
                    T                 PLG               AK   W  R

SEQ ID NO:39    851 eqqiieryryfrieyhpptasagklavvtvtnppvnalneraldelntiv
SEQ ID NO:134    43 -----erh--------------------------------------------
SEQ ID NO:135    38 ----------------------------------------------------
                        ER

SEQ ID NO:39    901 dhlarrqdvaaivftgqgarsfvagadirqlleeihtveeamalpnnahl
SEQ ID NO:134    46 ----------------------------------------------------
SEQ ID NO:135    38 ----------------------------------------------------

SEQ ID NO:39    951 afrkiermnkpciaaingvalggglefamachyrvadvyaefgqpeinlr
SEQ ID NO:134    46 ------------------------------------------gppe------
SEQ ID NO:135    38 -----erhgkp-----------------------------------------
                        ER  KP                                  G PE

SEQ ID NO:39   1001 llpgyggtqrlprllykrnngtgllralemilggrsvpadealklglida
SEQ ID NO:134    50 ----------------------------------------------------
SEQ ID NO:135    44 ----------------------------------------------------

SEQ ID NO:39   1051 iatgdqdslslacalaraaigadgqliesaavtqafrhrheqldewrkpd
SEQ ID NO:134    50 ----------------------------------------------------
SEQ ID NO:135    44 --------------------------------tqamq--------------
                                                    TQA

SEQ ID NO:39   1101 prfaddelrsiiahprieriirqahtvgrdaavhraldairygiihgfea
SEQ ID NO:134    50 ----------------------qsh---------------------------
SEQ ID NO:135    49 ----------------------------------------------------
                                      Q H
```

Figure 42B

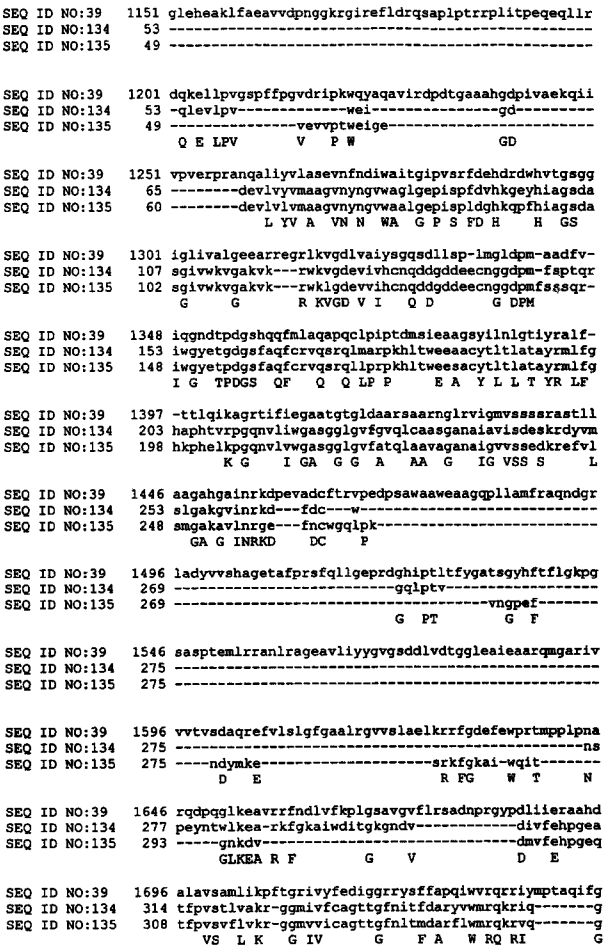

SEQ ID NO:39 1151 gleheaklfaeavvdpnggkrgirefldrqsaplptrrplitpeqeqllr
SEQ ID NO:134 53 ----------
SEQ ID NO:135 49 ----------
SEQ ID NO:39 1201 dqkellpvgspffpgvdripkwqyaqavirdpdtgaaahgdpivaekqii
SEQ ID NO:134 53 -qlevlpv----------wei----------gd---------
SEQ ID NO:135 49 ----------vevvptweige----------
Q E LPV V P W GD
SEQ ID NO:39 1251 vpverpranqaliyvlasevnfndiwaitgipvsrfdehdrdwhvtgsgg
SEQ ID NO:134 65 --------devlvyvmaagvnyngvwaglgepispfdvhkgeyhiagsda
SEQ ID NO:135 60 --------devlvlvmaagvnyngvwaalgepispldghkqpfhiagsda
L YV A VN N WA G P S FD H H GS
SEQ ID NO:39 1301 iglivalgeearregrlkvgdlvaiysgqsdllsp-lmgldpm-aadfv-
SEQ ID NO:134 107 sgivwkvgakvk---rwkvgdevivhcnqddgddeecnggdpm-fsptqr
SEQ ID NO:135 102 sgivwkvgakvk---rwklgdevvihcnqddgddeecnggdpmfs,ssqr-
G G R KVGD V I Q D G DPM
SEQ ID NO:39 1348 iqgndtpdgshqqfmlaqapqclpiptdmsieaagsyilnlgtiyralf-
SEQ ID NO:134 153 iwgyetgdgsfaqfcrvqsrqlmarpkhltweeaacytltlatayrmlfg
SEQ ID NO:135 148 iwgyetpdgsfaqfcrvqsrqllprpkhltweesacytltlatayrmlfg
I G TPDGS QF Q Q LP P E A Y L L T YR LF
SEQ ID NO:39 1397 -ttlqikagrtifiegaatgtgldaarsaarnglrvigmvssssrastll
SEQ ID NO:134 203 haphtvrpgqnvliwgasgglgvfgvqlcaasganaiavisdeskrdyvm
SEQ ID NO:135 198 hkphelkpgqnvlvwgasgglgvfatqlaavaganaigvvssedkrefvl
K G I GA G G A AA G IG VSS S L
SEQ ID NO:39 1446 aagahgainrkdpevadcftrvpedpsawaaweaagqpllamfraqndgr
SEQ ID NO:134 253 slgakgvinrkd---fdc---w----------
SEQ ID NO:135 248 smgakavlnrge---fncwgqlpk----------
GA G INRKD DC P
SEQ ID NO:39 1496 ladyvvshagetafprsfqllgeprdghiptltfygatsgyhftflgkpg
SEQ ID NO:134 269 ----------ggiptv----------
SEQ ID NO:135 269 ----------vngpef-------
G PT G F
SEQ ID NO:39 1546 sasptemlrranlrageavliyygvgsddlvdtggleaieaarqmgariv
SEQ ID NO:134 275 ----------
SEQ ID NO:135 275 ----------
SEQ ID NO:39 1596 vvtvsdaqrefvlslgfgaalrgvvslaelkrrfgdefewprtmpplpna
SEQ ID NO:134 275 ----------ns
SEQ ID NO:135 275 ----ndymke----------srkfgkai-wqit-------
D E R FG W T N
SEQ ID NO:39 1646 rqdpqglkeavrrfndlvfkplgsavgvflrsadnprgypdliieraahd
SEQ ID NO:134 277 peyntwlkea-rkfgkaiwditgkgndv----------divfehpgea
SEQ ID NO:135 293 -----gnkdv----------dmvfehpgeq
GLKEA R F G V D E
SEQ ID NO:39 1696 alavsamlikpftgrivyfediggrrysffapqiwvrqrriymptaqifg
SEQ ID NO:134 314 tfpvstlvakr-ggmivfcagttgfnitfdaryvwmrqkriq------g
SEQ ID NO:135 308 tfpvsvflvkr-ggmvvicagttgfnltmdarflwmrqkrvq------g
VS L K G IV G F A W RQ RI G

Figure 42C

```
SEQ ID NO:39   1746 thlsnayeilrlndeisaglltitepavvpwdelpeahqamwenrhtaat
SEQ ID NO:134   356 shfahlkqasaanqfvmdrrvdpcmsevfpwdkipaahtkmwknqhppgn
SEQ ID NO:135   350 shfanlmqasaanqlvidrrvdpclsevfpwdqipaahekmlanqhlpgn
                     H  N       N              V PWD  P AH  MW N H

SEQ ID NO:39   1796 yvvnhalprlglknrdelyeawtager
SEQ ID NO:134   406 mavlvnstraglrtvedvieagplkam
SEQ ID NO:135   400 mavlvcaqrpglrtfeevqelsgap--
                      V     R GL    E  EA  A
```

Figure 42D

Figure 43

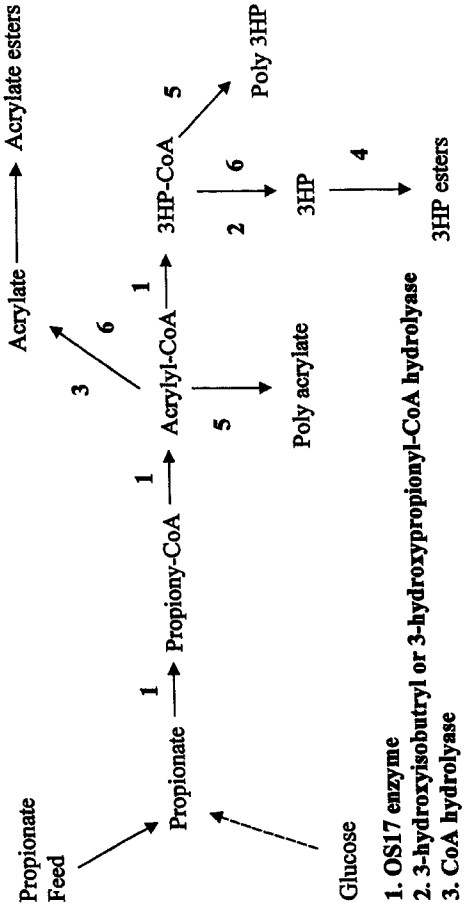

Propionate Feed
Propionate
Propiony-CoA
Acrylyl-CoA
Acrylate
Acrylate esters
3HP-CoA
Poly 3HP
Poly acrylate
3HP
3HP esters
Glucose
1
2
3
4
5
6
1. OS17 enzyme
2. 3-hydroxyisobutryl or 3-hydroxypropionyl-CoA hydrolyase
3. CoA hydrolyase
4. Lipase
5. Poly hydroxyacid synthase
6. CoA transferase

Figure 44

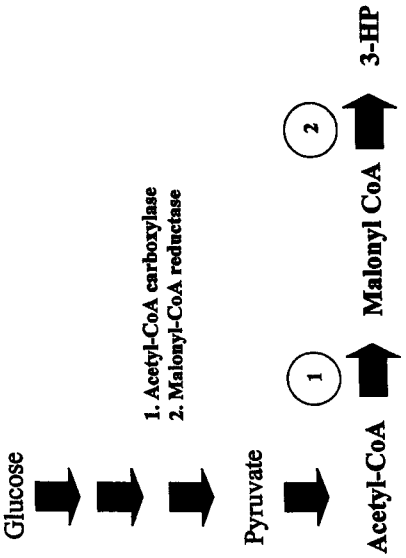
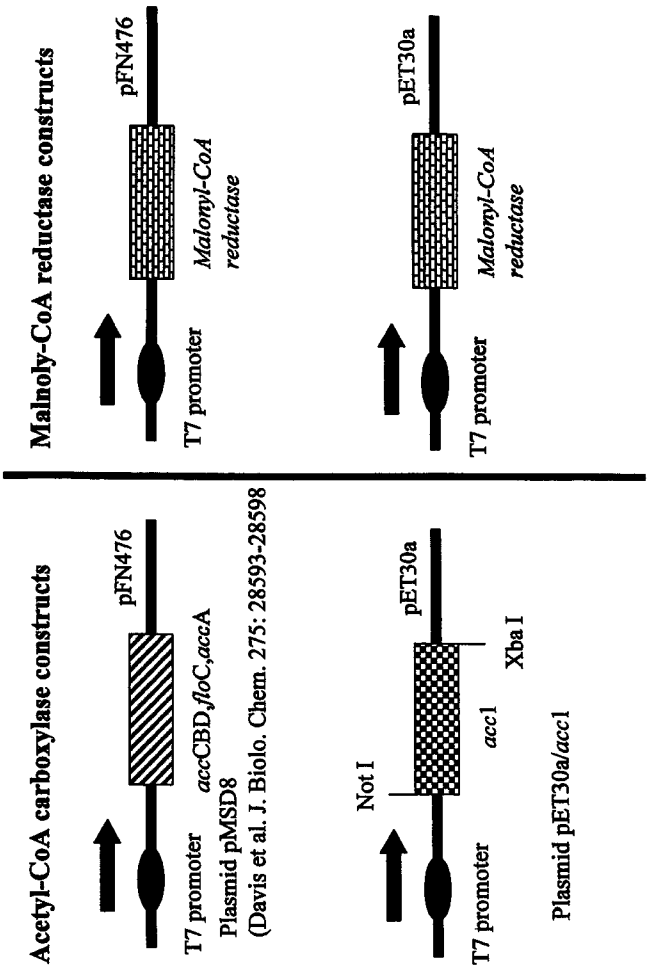

Glucose
1. Acetyl-CoA carboxylase
2. Malonyl-CoA reductase
Pyruvate
1
Acetyl-CoA
Malonyl CoA
2
3-HP

Figure 45


Malnoly-CoA reductase constructs
pFN476
T7 promoter
Malonyl-CoA reductase
pET30a
T7 promoter
Malonyl-CoA reductase
Acetyl-CoA carboxylase constructs
pFN476
T7 promoter
accCBD,floC,accA
Plasmid pMSD8
(Davis et al. J. Biolo. Chem. 275: 28593-28598
pET30a
Not I
T7 promoter
acc1
Xba I
Plasmid pET30a/acc1

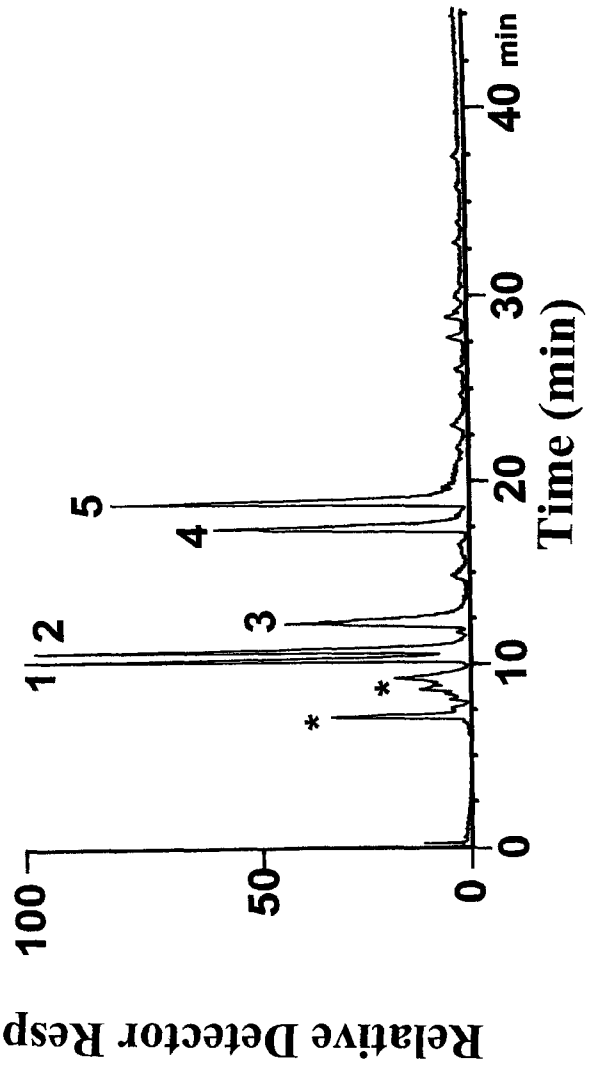

Relative Detector Response
100
50
0
0
10
20
30
40 min
Time (min)
1
2
3
4
5
*
*

Figure 46A

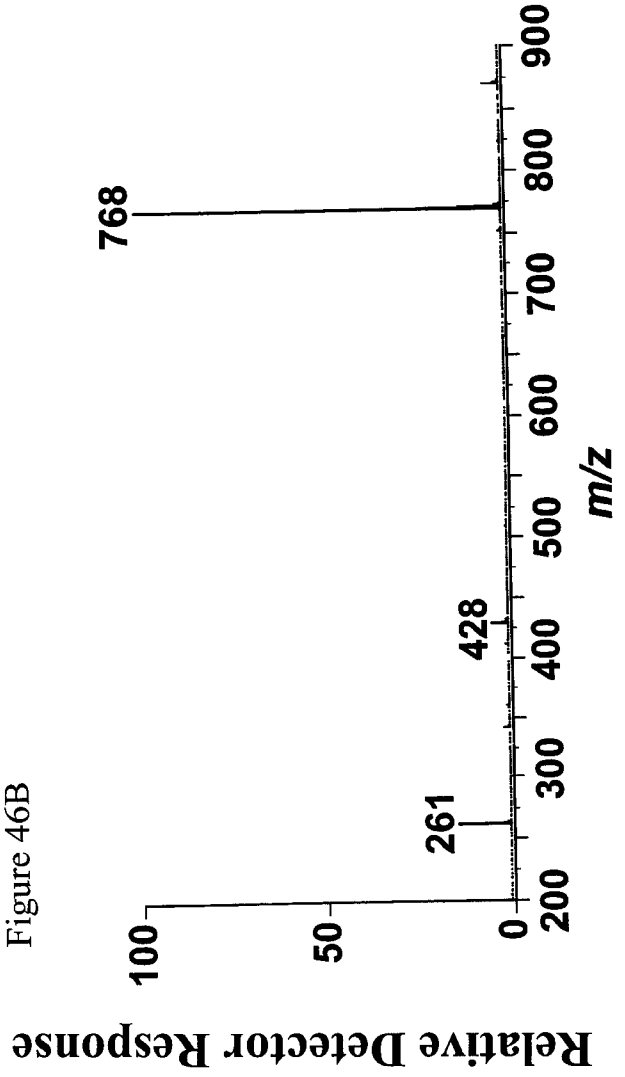

Relative Detector Response
100
50
0
200
300
400
500
600
700
800
900
m/z
261
428
768

Figure 46B

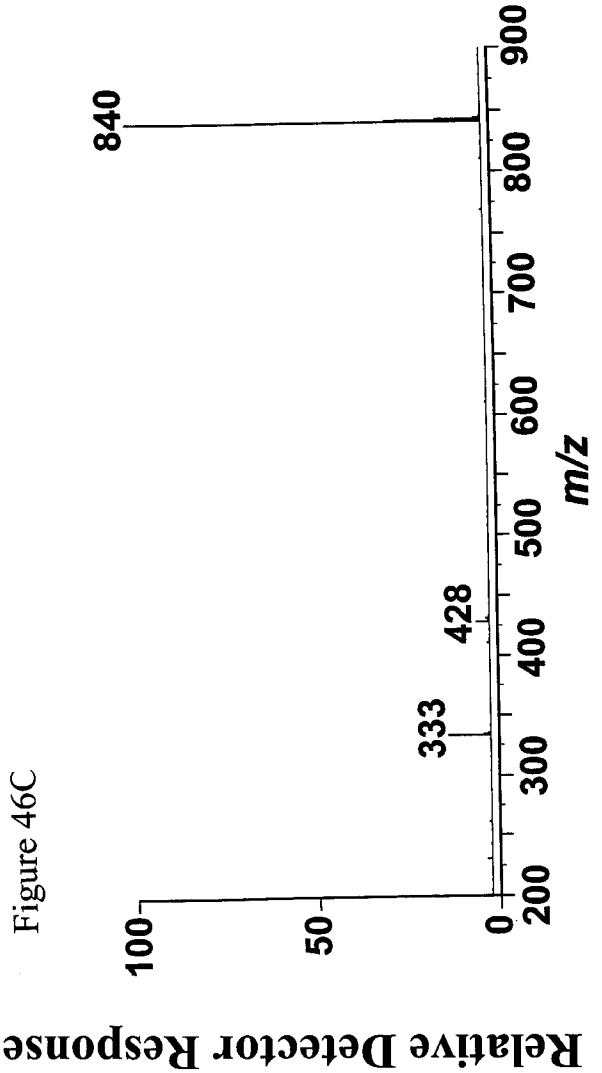

Relative Detector Response
100
50
0
200
300
400
500
600
700
800
900
m/z
333
428
840

Figure 46C

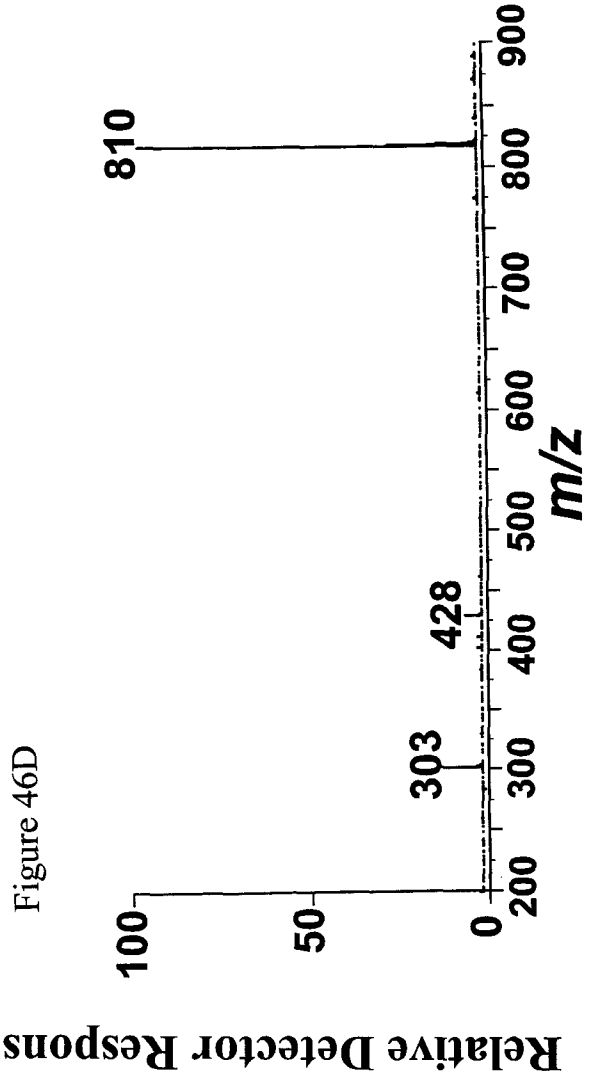

Relative Detector Response
100
50
0
200
300
400
500
600
700
800
900
m/z
303
428
810

Figure 46D

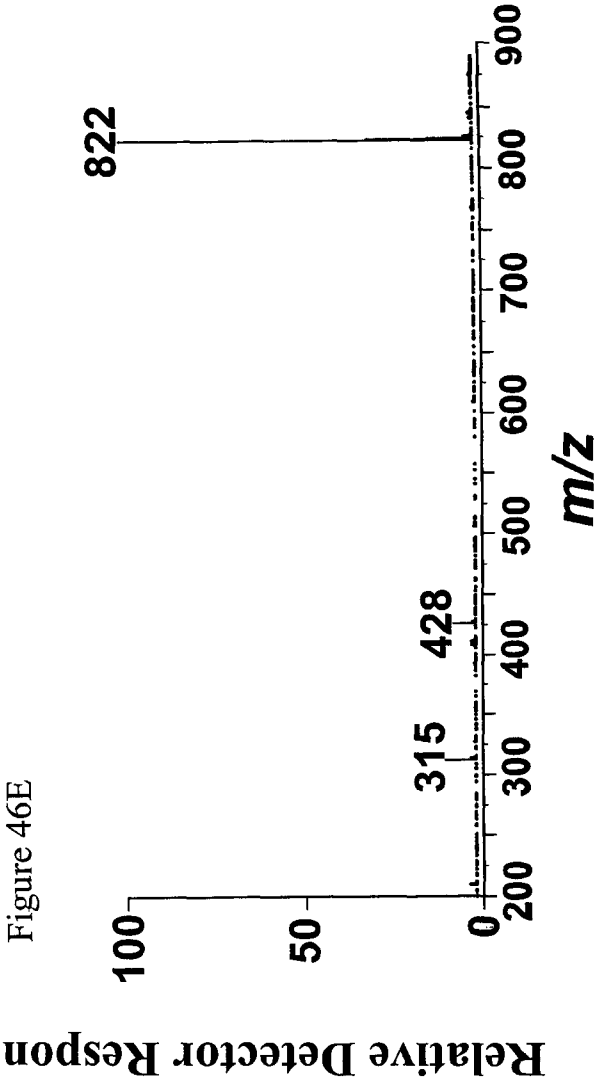

Relative Detector Response
100
50
0
200
300
400
500
600
700
800
900
m/z
315
428
822

Figure 46E

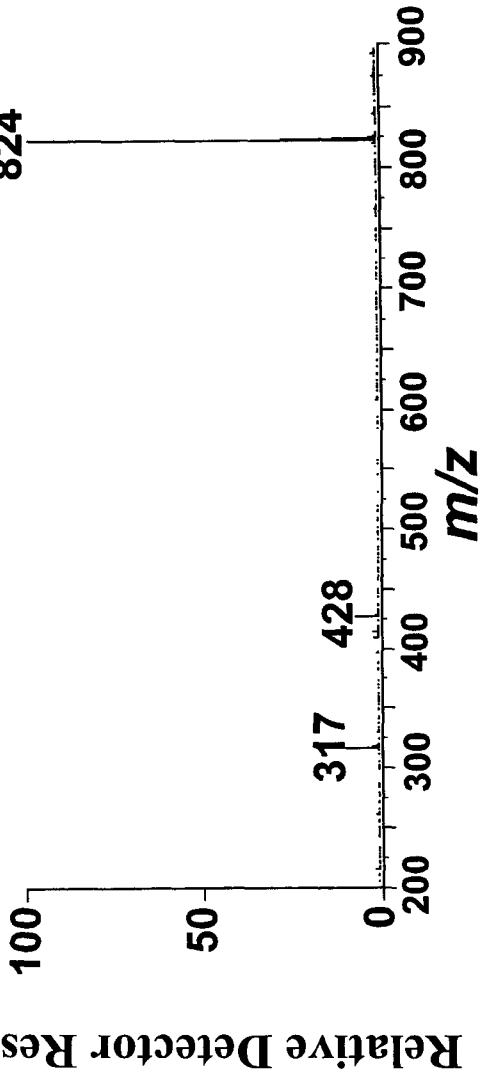

824
428
317
100
50
0
200
300
400
500
600
700
800
900
m/z
Relative Detector Response

Figure 46F

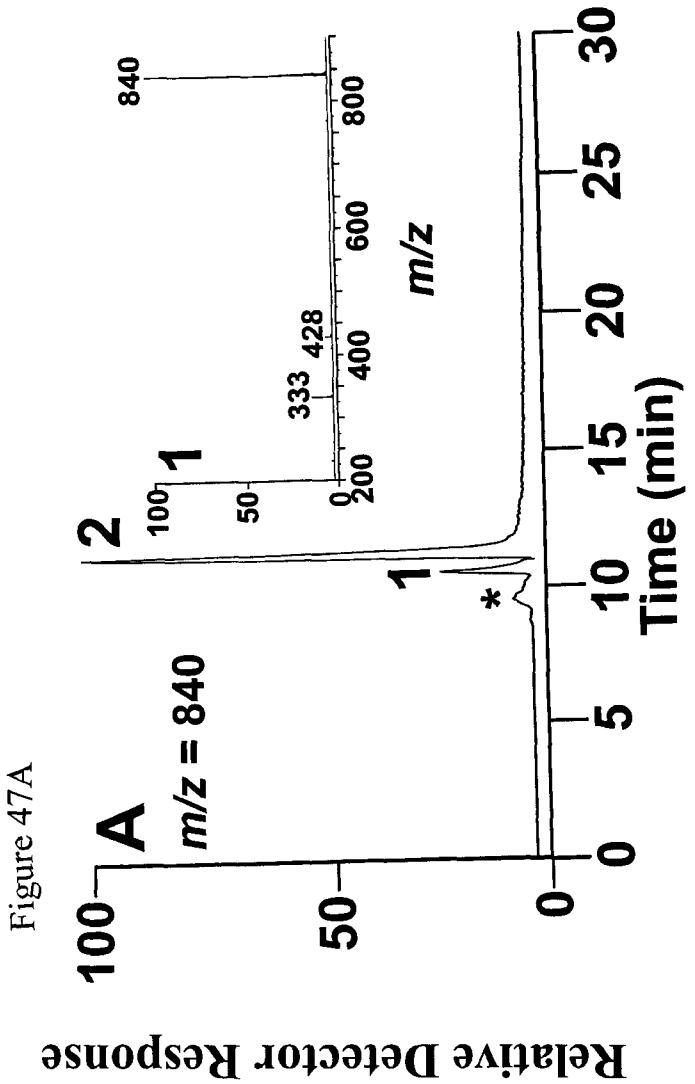

Relative Detector Response
A
m/z = 840
100
50
0
0
5
10
15
20
25
30
Time (min)
1
*
2
1
100
50
0
200
333
400
428
600
800
840
m/z

Figure 47A

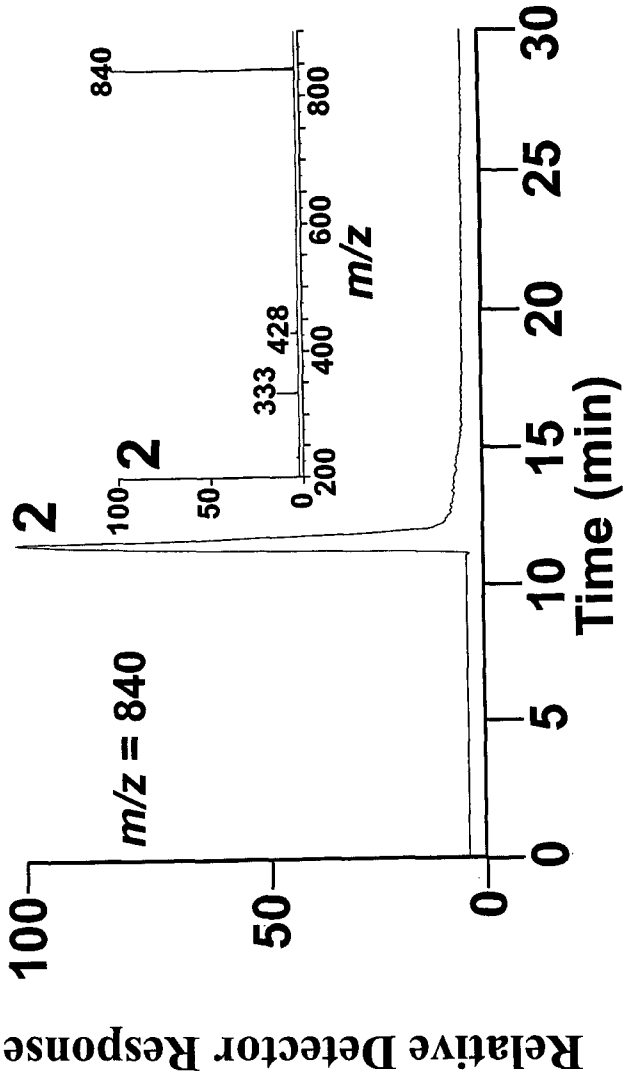

Relative Detector Response
100
50
0
m/z = 840
0
5
10
15
20
25
30
Time (min)
2
100
50
0
200
400
600
800
m/z
333
428
840
2

Figure 47B

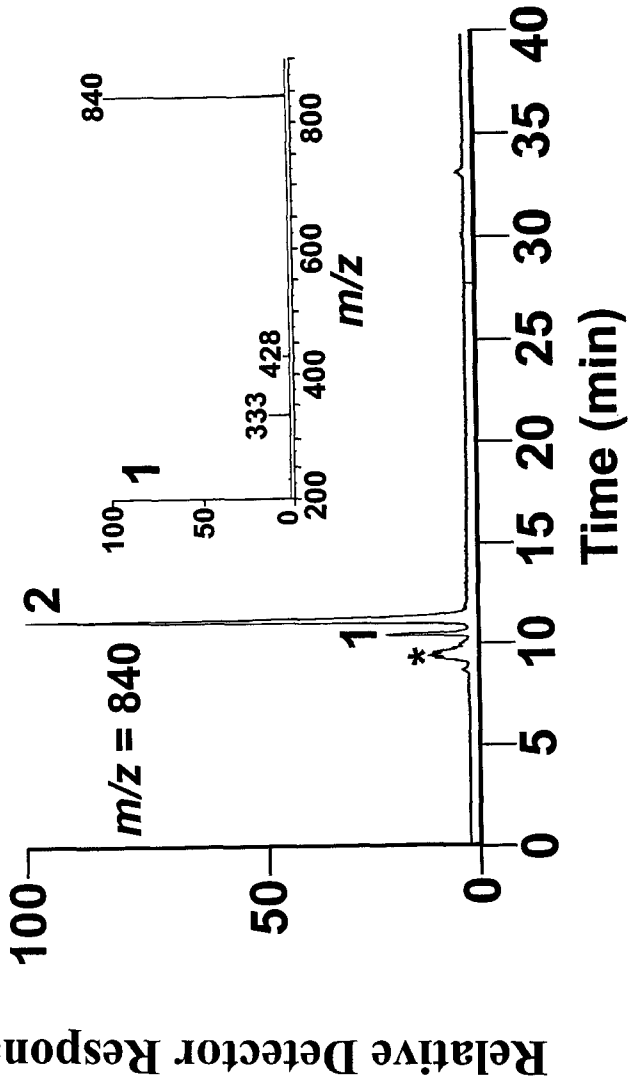

Relative Detector Response
Time (min)
m/z = 840
m/z
2
1
1
*
100
50
0
0
5
10
15
20
25
30
35
40
840
800
600
400
428
333
200
100
50
0

Figure 48A

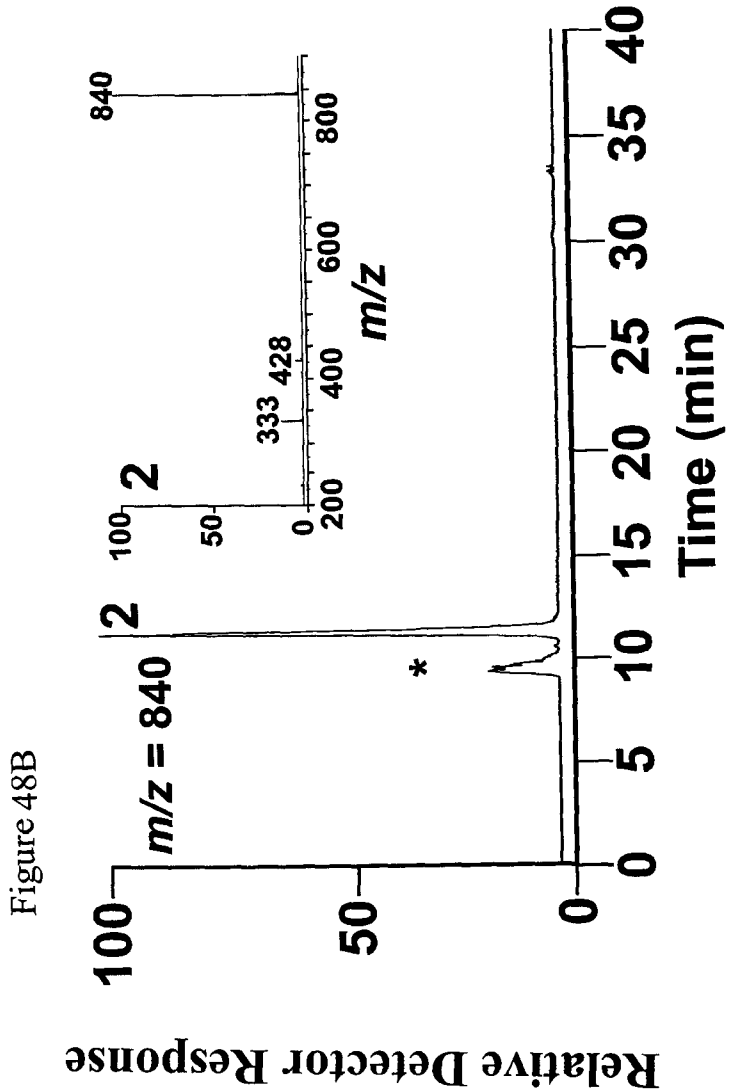

Relative Detector Response
100
50
0
m/z = 840
2
*
0 5 10 15 20 25 30 35 40
Time (min)
100
50
0
2
333 428
200 400 600 800
m/z
840

Figure 48B

ATGGCGACGGGGGAGTCCATGAGCGGAACAGGACGACTGGCAGGAAAGATTGCGTTAATT
ACCGGTGGCGCCGGCAATATCGGCAGTGAATTGACACGTCGCTTTCTCGCAGAGGGAGCG
ACGGTCATTATTAGTGGACGGAATCGGGCGAAGTTGACCGCACTGGCCGAACGGATGCAG
GCAGAGGCAGGAGTGCCGGCAAAGCGCATCGATCTCGAAGTCATGGATGGGAGTGATCCG
GTCGCGGTACGTGCCGGTATCGAAGCGATTGTGGCCCGTCACGGCCAGATCGACATTCTG
GTCAACAATGCAGGAAGTGCCGGTGCCCAGCGTCGTCTGGCCGAGATTCCACTCACTGAA
GCTGAATTAGGCCCTGGCGCCGAAGAGACGCTTCATGCCAGCATCGCCAATTTACTTGGT
ATGGGATGGCATCTGATGCGTATTGCGGCACCTCATATGCCGGTAGGAAGTGCGGTCATC
AATGTCTCGACCATCTTTTCACGGGCTGAGTACTACGGGCGGATTCCGTATGTCACCCCT
AAAGCTGCTCTTAATGCTCTATCTCAACTTGCTGCGCGTGAGTTAGGTGCACGTGGCATC
CGCGTTAATACGATCTTTCCCGGCCCGATTGAAAGTGATCGCATCCGTACAGTGTTCCAG
CGTATGGATCAGCTCAAGGGGCGGCCCGAAGGCGACACAGCGCACCATTTTTTGAACACC
ATGCGATTGTGTCGTGCCAACGACCAGGGCGCGCTTGAACGTCGGTTCCCCTCCGTCGGT
GATGTGGCAGACGCCGCTGTCTTTCTGGCCAGTGCCGAATCCGCCGCTCTCTCCGGTGAG
ACGATTGAGGTTACGCACGGAATGGAGTTGCCGGCCTGCAGTGAGACCAGCCTGCTGGCC
CGTACTGATCTGCGCACGATTGATGCCAGTGGCCGCACGACGCTCATCTGCGCCGGCGAC
CAGATTGAAGAGGTGATGGCGCTCACCGGTATGTTGCGTACCTGTGGGAGTGAAGTGATC
ATCGGCTTCCGTTCGGCTGCGGCGCTGGCCCAGTTCGAGCAGGCAGTCAATGAGAGTCGG
CGGCTGGCCGGCGCAGACTTTACGCCTCCCATTGCCTTGCCACTCGATCCACGCGATCCG
GCAACAATTGACGCTGTCTTCGATTGGGCCGGCGAGAATACCGGCGGGATTCATGCAGCG
GTGATTCTGCCTGCTACCAGTCACGAACCGGCACCGTGCGTGATTGAGGTTGATGATGAG
CGGGTGCTGAATTTTCTGGCCGATGAAATCACCGGGACAATTGTGATTGCCAGTCGCCTG
GCCCGTTACTGGCAGTCGCAACGGCTTACCCCCGGCGCACGTGCGCGTGGGCCGCGTGTC
ATTTTTCTCTCGAACGGTGCCGATCAAAATGGGAATGTTTACGGACGCATTCAAAGTGCC
GCTATCGGTCAGCTCATTCGTGTGTGGCGTCACGAGGCTGAACTTGACTATCAGCGTGCC
AGCGCCGCCGGTGATCATGTGCTGCCGCCGGTATGGGCCAATCAGATTGTGCGCTTCGCT
AACCGCAGCCTTGAAGGGTTAGAATTTGCCTGTGCCTGGACAGCTCAATTGCTCCATAGT
CAACGCCATATCAATGAGATTACCCTCAACATCCCTGCCAACATTAGCGCCACCACCGGC
GCACGCAGTGCATCGGTCGGATGGGCGGAAAGCCTGATCGGGTTGCATTTGGGGAAAGTT
GCCTTGATTACCGGTGGCAGCGCCGGTATTGGTGGGCAGATCGGGCGCCTCCTGGCTTTG
AGTGGCGCGCGCGTGATGCTGGCAGCCCGTGATCGGCATAAGCTCGAACAGATGCAGGCG
ATGATCCAATCTGAGCTGGCTGAGGTGGGGTATACCGATGTCGAAGATCGCGTCCACATT
GCACCGGGCTGCGATGTGAGTAGCGAAGCGCAGCTTGCGGATCTTGTTGAACGTACCCTG
TCAGCTTTTGGCACCGTCGATTATCTGATCAACAACGCCGGGATCGCCGGTGTCGAAGAG
ATGGTTATCGATATGCCAGTTGAGGGATGGCGCCATACCCTCTTCGCCAATCTGATCAGC
AACTACTCGTTGATGCGCAAACTGGCGCCGTTGATGAAAAAACAGGGTAGCGGTTACATC
CTTAACGTCTCATCATACTTTGGCGGTGAAAAAGATGCGGCCATTCCCTACCCCAACCGT
GCCGATTACGCCGTCTCGAAGGCTGGTCAGCGGGCAATGGCCGAAGTCTTTGCGCGCTTC
CTTGGCCCGGAGATACAGATCAATGCCATTGCGCCGGGTCCGGTCGAAGGTGATCGCTTG
CGCGGTACCGGTGAACGTCCCGGCCTCTTTGCCCGTCGGGCGCGGCTGATTTTGGAGAAC
AAGCGGCTGAATGAGCTTCACGCTGCTCTTATCGCGGCTGCGCGCACCGATGAGCGATCT
ATGCACGAACTGGTTGAACTGCTCTTACCCAATGATGTGGCCGCACTAGAGCAGAATCCC
GCAGCACCTACCGCGTTGCGTGAACTGGCACGACGTTTTCGCAGCGAAGGCGATCCGGCG
GCATCATCAAGCAGTGCGCTGCTGAACCGTTCAATTGCCGCTAAATTGCTGGCTCGTTTG
CATAATGGTGGCTATGTGTTGCCTGCCGACATCTTTGCAAACCTGCCAAACCCGCCCGAT
CCCTTCTTCACCCGAGCCCAGATTGATCGCGAGGCTCGCAAGGTTCGTGACGGCATCATG
GGGATGCTCTACCTGCAACGGATGCCGACTGAGTTTGATGTCGCAATGGCCACCGTCTAT
TACCTTGCCGACCGCAATGTCAGTGGTGAGACATTCCACCCATCAGGTGGTTTGCGTTAC

Figure 49A

GAACGCACCCCTACCGGTGGCGAACTCTTCGGCTTGCCCTCACCGGAACGGCTGGCGGAG
CTGGTCGGAAGCACGGTCTATCTGATAGGTGAACATCTGACTGAACACCTTAACCTGCTT
GCCCGTGCGTACCTCGAACGTTACGGGGCACGTCAGGTAGTGATGATTGTTGAGACAGAA
ACCGGGGCAGAGACAATGCGTCGCTTGCTCCACGATCACGTCGAGGCTGGTCGGCTGATG
ACTATTGTGGCCGGTGATCAGATCGAAGCCGCTATCGACCAGGCTATCACTCGCTACGGT
CCCCCAGGGCCGGTCGTCTGTACCCCCTTCCGGCCACTGCCGACGGTACCACTGGTCGGG
CGTAAAGACAGTGACTGGAGCACAGTGTTGAGTGAGGCTGAATTTGCCGAGTTGTGCGAA
CACCAGCTCACCCACCATTTCCGGGTAGCGCGCAAGATTGCCCTGAGTGATGGTGCCAGT
CTCGCGCTGGTCACTCCCGAAACTACGGCTACCTCAACTACCGAGCAATTTGCTCTGGCT
AACTTCATCAAAACGACCCTTCACGCTTTTACGGCTACGATTGGTGTCGAGAGCGAAAGA
ACTGCTCAGCGCATTCTGATCAATCAAGTCGATCTGACCCGGCGTGCGCGTGCCGAAGAG
CCGCGTGATCCGCACGAGCGTCAACAAGAACTGGAACGTTTTATCGAGGCAGTCTTGCTG
GTCACTGCACCACTCCCGCCTGAAGCCGATACCCGTTACGCCGGGCGGATTCATCGCGGA
CGGGCGATTACCGTGTAA (SEQ ID NO:140)

Figure 49B

Figure 50

MATGESMSGTGRLAGKIALITGGAGNIGSELTRRFLAEGATVIISGRNRAKLTALAERMQ
AEAGVPAKRIDLEVMDGSDPVAVRAGIEAIVARHGQIDILVNNAGSAGAQRRLAEIPLTE
AELGPGAEETLHASIANLLGMGWHLMRIAAPHMPVGSAVINVSTIFSRAEYYGRIPYVTP
KAALNALSQLAARELGARGIRVNTIFPGPIESDRIRTVFQRMDQLKGRPEGDTAHHFLNT
MRLCRANDQGALERRFPSVGDVADAAVFLASAESAALSGETIEVTHGMELPACSETSLLA
RTDLRTIDASGRTTLICAGDQIEEVMALTGMLRTCGSEVIIGFRSAAALAQFEQAVNESR
RLAGADFTPPIALPLDPRDPATIDAVFDWAGENTGGIHAAVILPATSHEPAPCVIEVDDE
RVLNFLADEITGTIVIASRLARYWQSQRLTPGARARGPRVIFLSNGADQNGNVYGRIQSA
AIGQLIRVWRHEAELDYQRASAAGDHVLPPVWANQIVRFANRSLEGLEFACAWTAQLLHS
QRHINEITLNIPANISATTGARSASVGWAESLIGLHLGKVALITGGSAGIGGQIGRLLAL
SGARVMLAARDRHKLEQMQAMIQSELAEVGYTDVEDRVHIAPGCDVSSEAQLADLVERTL
SAFGTVDYLINNAGIAGVEEMVIDMPVEGWRHTLFANLISNYSLMRKLAPLMKKQGSGYI
LNVSSYFGGEKDAAIPYPNRADYAVSKAGQRAMAEVFARFLGPEIQINAIAPGPVEGDRL
RGTGERPGLFARRARLILENKRLNELHAALIAAARTDERSMHELVELLLPNDVAALEQNP
AAPTALRELARRFRSEGDPAASSSSALLNRSIAAKLLARLHNGGYVLPADIFANLPNPPD
PFFTRAQIDREARKVRDGIMGMLYLQRMPTEFDVAMATVYYLADRNVSGETFHPSGGLRY
ERTPTGGELFGLPSPERLAELVGSTVYLIGEHLTEHLNLLARAYLERYGARQVVMIVETE
TGAETMRRLLHDHVEAGRLMTIVAGDQIEAAIDQAITRYGRPGPVVCTPFRPLPTVPLVG
RKDSDWSTVLSEAEFAELCEHQLTHHFRVARKIALSDGASLALVTPETTATSTTEQFALA
NFIKTTLHAFTATIGVESERTAQRILINQVDLTRRARAEEPRDPHERQQELERFIEAVLL
VTAPLPPEADTRYAGRIHRGRAITV (SEQ ID NO:141)

Figure 51

TCTTTCTGGCCAGTGCCGAATCCGCCGCTCTCTCCGGTGAGACGATTGAGGTTACGCACG
GAATGGAGTTGCCGGCCTGCAGTGAGACCAGCCTGCTGGCCCGTACTGATCTGCGCACGA
TTGATGCCAGTGGCCGCACGACGCTCATCTGCGCCGGCGACCAGATTGAAGAGGTGATGG
CGCTCACCGGTATGTTGCGTACCTGTGGGAGTGAAGTGATCATCGGCTTCCGTTCGGCTG
CGGCGCTGGCCCAGTTCGAGCAGGCAGTCAATGAGAGTCGGCGGCTGGCCGGCGCAGACT
TTACGCCTCCCATTGCCTTGCCACTCGATCCACGCG (SEQ ID NO:142)

```
SEQ ID NO:141      1 matgesmsgtgrlagkialitggagnigseltrrflaegatviisgrnra
SEQ ID NO:143      1 ----------mfankvvlvtggssgigaatveafvkegasvafvgrnqa
SEQ ID NO:144      1 ----------mrlegkvclitgaasgigkattllfaqegatviagdiske
SEQ ID NO:145      1 ------------------------------------------------
SEQ ID NO:146      1 --------------------------------mekf-----------
SEQ ID NO:147      1 ----------mrllhkrtlvtggsdgiglaiaeaflsegadvlivgrdaa

SEQ ID NO:141     51 kltalaermqa--e-agvpakridlevmdgsdpvavragieaivarhgqi
SEQ ID NO:143     40 klkevesrcqq--hganilaikadv-----skdeeakiivqqtvdkfgkl
SEQ ID NO:144     41 nldslvk--es--e--glp---------------------------gkv
SEQ ID NO:145      1 ------------------------------------------------
SEQ ID NO:146      5 ------------------------------------------------
SEQ ID NO:147     41 kleaarqklaalgq-aga----vetssadlatslgvatvveqvketgrpl

SEQ ID NO:141     98 dilvnnagsagaqrrlaeiplteaelgpgaeetlhasianllgmgwhlmr
SEQ ID NO:143     83 dvlvnnagil----rfasv--leptliqtfdetmntnlrpvv----lits
SEQ ID NO:144     57 d------------------------------------------------
SEQ ID NO:145      1 ------------------------------------------------
SEQ ID NO:146      5 ------------------------------------------------
SEQ ID NO:147     86 dipinnagvadl------vpfesv-----seaqfqhsfalnvaaaffltq

SEQ ID NO:141    148 iaaphm-pvgsavinvstifsr-aeyygrip--yvtpkaalnalsqlaar
SEQ ID NO:143    123 laiphliatkgsivnvssilstivripgims--ysvskaamdhftklaal
SEQ ID NO:144     58 ----------------------------p--yv-----lnv-------
SEQ ID NO:145      1 ------------------------------------------------
SEQ ID NO:146      5 ---php-p-------------------------------------------
SEQ ID NO:147    125 gllphf-gagasiinissyfar-kmipkrpssvyslskgalnsltrslaf

SEQ ID NO:141    194 elgargirvntifpgpiesdrirtvfqrmdqlkgrpegdtahhflntmrl
SEQ ID NO:143    171 elapsgvrvnsvnpgpv--------------------------------
SEQ ID NO:144     64 -----------------tdr-------------------------------
SEQ ID NO:145      1 ----------------------------mnpmdrqtegqepqh-------
SEQ ID NO:146      9 ------------------------------------------------
SEQ ID NO:147    173 elgprgirvnaiapgtvdt-------------------------------

SEQ ID NO:141    244 crandqgalerrfpsvgdvadaavflasaesaalsgetievthgmelpac
SEQ ID NO:143    188 ---------------ltdia--------------------------------
SEQ ID NO:144     67 ------------------------------------------------
SEQ ID NO:145     16 ------------------------------------------------
SEQ ID NO:146      9 ------------fpr-------------------------------------
SEQ ID NO:147    192 -------amrr----------------------------------------

SEQ ID NO:141    294 setsllartdlrtidasgrttlicagdqieevmaltgmlrtcgseviigf
SEQ ID NO:143    193 ------------------------------------------------
SEQ ID NO:144     67 --------------------------dqikev------------------
SEQ ID NO:145     16 ------------------------------------------------
SEQ ID NO:146     12 --------------------------qtqem-------------------
SEQ ID NO:147    196 -----------ktvd-----------------------------------

SEQ ID NO:141    344 rsaaalaqfeqavnesrrlagadftppialpldprdpatidavfdwagen
SEQ ID NO:143    193 --------------------agsgfspdll-------------------ed
SEQ ID NO:144     73 ------------------------------------------------
SEQ ID NO:145     16 ---------------------qdrqpgieskmnp----------------
SEQ ID NO:146     17 ------------------------------------pgttdrm-------
SEQ ID NO:147    200 ------------------------------------------------
```

Figure 52A

```
SEQ ID NO:141   394 tggihaavilpatshepapcvievddervlnfladeitgtiviasrlary
SEQ ID NO:143   205 tg----------ahtp---------------------------------
SEQ ID NO:144    73 ------------------------------------------------
SEQ ID NO:145    29 ---------lp--------------------------------------
SEQ ID NO:146    24 ---------------qplp------------------------------
SEQ ID NO:147   200 ------------------------------------------------

SEQ ID NO:141   444 wqsqrltpgarargprviflsngadqngnvygriqsaaiqqlirvwrhea
SEQ ID NO:143   211 ------------------------------------------------
SEQ ID NO:144    73 ------------------------------------------------
SEQ ID NO:145    31 -------------------lsededyrgs--gklk--------------
SEQ ID NO:146    28 ------------------------------------------------dhg
SEQ ID NO:147   200 ------------------------------------------------

SEQ ID NO:141   494 eldyqrasaagdhvlppvwanqivrfanrsleglefacawtaqllhsqrh
SEQ ID NO:143   211 ------------------------------------------------
SEQ ID NO:144    73 ------------------------------------------------
SEQ ID NO:145    45 ------------------------------------------------
SEQ ID NO:146    31 ensyqgsgrlkd-------------------------------------
SEQ ID NO:147   200 ------------------------------------------------

SEQ ID NO:141   544 ineitlnipanisattgarsasvgwaesliglhlgkvalitggsagiggq
SEQ ID NO:143   211 ----------------------------------lgkaa-----------
SEQ ID NO:144    73 ------------------------------------------------
SEQ ID NO:145    45 ----------------------------------gkvaiitggdsgigra
SEQ ID NO:146    43 ----------------------------------kraiitggdsgigra
SEQ ID NO:147   200 ------nlpa--------------------------------------

SEQ ID NO:141   594 igrllalsgarvmlaardrhk-leqmqamiqselaevgytdvedrvhiap
SEQ ID NO:143   216 ------------------------------qse-----------------
SEQ ID NO:144    73 ------------------------------------------------
SEQ ID NO:145    61 aaiafakegadisilyldehsdaeetrkrieke---------nvrcllip
SEQ ID NO:146    58 vaiayaregadvlisylsehd-----damatkalve----eagrkavlaa
SEQ ID NO:147   204 ------------------------------------------------

SEQ ID NO:141   643 gcdvsseaqladlvertlsafgtvdylinnagiagveemvidmpvegwrh
SEQ ID NO:143   219 --------eiadmi---------------------------------------
SEQ ID NO:144    73 -------------vekvvqkygridvlvnnagitr-dallvrmkeedwda
SEQ ID NO:145   102 g-dvgdenhceqavqqtvdhfgkldilvnnaaeqhpqdsilnisteqlek
SEQ ID NO:146    99 g-diqssdhcrrivetavrelggidilvnnaahqatfkniedisdeewel
SEQ ID NO:147   204 ----eakaelkayvers-------------------------------------

SEQ ID NO:141   693 tlfanlisnyslmrklaplmkkqgsgyilnvssyfggekdaaipypnrad
SEQ ID NO:143   225 ------------------------------------------------
SEQ ID NO:144   109 vinvnlkgvfnvtqmvvpymikqrngsivnvssvvg-----iygnpgqtn
SEQ ID NO:145   151 tfrtnifsmfhmtkkalphl--qegcaiinttsitayegdtal-----id
SEQ ID NO:146   148 tfrvnmhamfyltkaavphmkk-gsa-iintasi-----nadvpnpilla
SEQ ID NO:147   217 ------------------------------------------------

SEQ ID NO:141   743 yavskagqramaevfarfl-gpe-iqinaiapgpvegdrlrgtgerpglf
SEQ ID NO:143   225 ------------------------------------------------
SEQ ID NO:144   154 yaaskagvigmtktwakelagrn-irvnavapgfie--------------
SEQ ID NO:145   194 ysstkgaivsftrsmaksl-adkgirvnavapgpi---------------
SEQ ID NO:146   191 yattkgaihnfsaglaqml-aergirvnvvapgpi---------------
SEQ ID NO:147   217 yplgrigr-------------------------------------------
```

Figure 52B

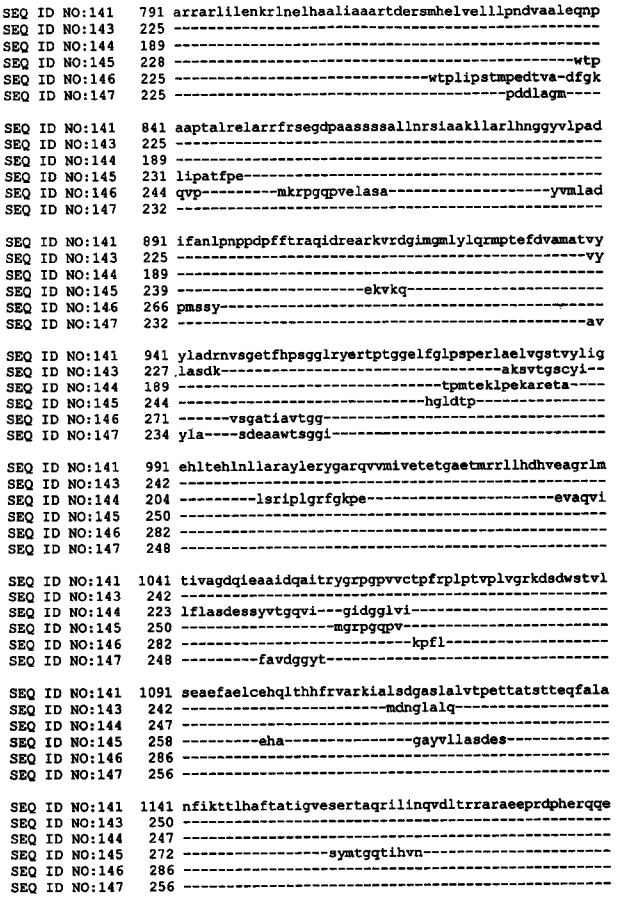

SEQ ID NO:141 791 arrarlilenkrlnelhaaliaaartdersmhelvelllpndvaaleqnp
SEQ ID NO:143 225 ----------
SEQ ID NO:144 189 ----------
SEQ ID NO:145 228 ----------wtp
SEQ ID NO:146 225 ----------wtplipstmpedtva-dfgk
SEQ ID NO:147 225 ----------pddlagm----
SEQ ID NO:141 841 aaptalrelarrfrsegdpaassssallnrsiaakllarlhnggyvlpad
SEQ ID NO:143 225 ----------
SEQ ID NO:144 189 ----------
SEQ ID NO:145 231 lipatfpe----------
SEQ ID NO:146 244 qvp---------mkrpgqpvelasa----------yvmlad
SEQ ID NO:147 232 ----------
SEQ ID NO:141 891 ifanlpnppdpfftraqidrearkvrdgimgmlylqrmptefdvamatvy
SEQ ID NO:143 225 ----------vy
SEQ ID NO:144 189 ----------
SEQ ID NO:145 239 ----------ekvkq----------
SEQ ID NO:146 266 pmssy----------
SEQ ID NO:147 232 ----------av
SEQ ID NO:141 941 yladrnvsgetfhpsgglryertptggelfglpsperlaelvgstvylig
SEQ ID NO:143 227 lasdk----------aksvtgscyi--
SEQ ID NO:144 189 ----------tpmteklpekareta----
SEQ ID NO:145 244 ----------hgldtp----------
SEQ ID NO:146 271 ------vsgatiavtgg----------
SEQ ID NO:147 234 yla----sdeaawtsggi----------
SEQ ID NO:141 991 ehltehlnllaraylerygarqvvmivetetgaetmrrllhdhveagrlm
SEQ ID NO:143 242 ----------
SEQ ID NO:144 204 ---------lsriplgrfgkpe----------evaqvi
SEQ ID NO:145 250 ----------
SEQ ID NO:146 282 ----------
SEQ ID NO:147 248 ----------
SEQ ID NO:141 1041 tivagdqieaaidqaitrygrpgpvvctpfrplptvplvgrkdsdwstvl
SEQ ID NO:143 242 ----------
SEQ ID NO:144 223 lflasdessyvtgqvi---gidgglvi----------
SEQ ID NO:145 250 ----------mgrpgqpv----------
SEQ ID NO:146 282 ----------kpfl----------
SEQ ID NO:147 248 ---------favdggyt----------
SEQ ID NO:141 1091 seaefaelcehqlthhfrvarkialsdgaslalvtpettatstteqfala
SEQ ID NO:143 242 ----------mdnglalq----------
SEQ ID NO:144 247 ----------
SEQ ID NO:145 258 ---------eha----------gayvllasdes----------
SEQ ID NO:146 286 ----------
SEQ ID NO:147 256 ----------
SEQ ID NO:141 1141 nfikttlhaftatigvesertaqrilinqvdltrraraeeprdpherqqe
SEQ ID NO:143 250 ----------
SEQ ID NO:144 247 ----------
SEQ ID NO:145 272 ----------symtgqtihvn----------
SEQ ID NO:146 286 ----------
SEQ ID NO:147 256 ----------

Figure 52C

```
SEQ ID NO:141  1191 lerfieavllvtaplppeadtryagrihrgraitv
SEQ ID NO:143   250 ------------------------------------
SEQ ID NO:144   247 ------------------------------------
SEQ ID NO:145   283 ----------------------------ggrfist
SEQ ID NO:146   286 ------------------------------------
SEQ ID NO:147   256 ----------------------ag----------
```

Figure 52D

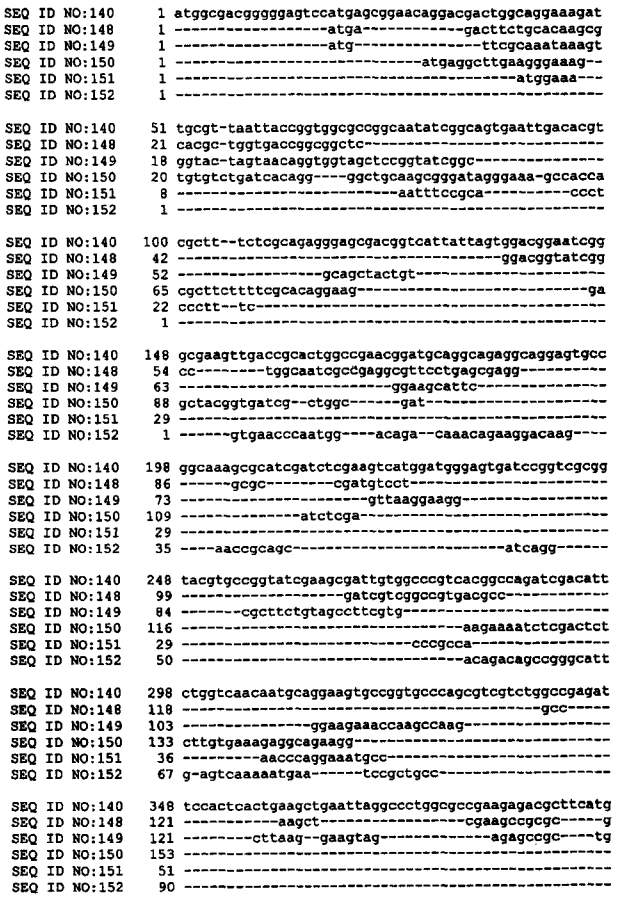

SEQ ID NO:140 1 atggcgacgggggagtccatgagcggaacaggacgactggcaggaaagat
SEQ ID NO:148 1 ------------------atga------------gacttctgcacaagcg
SEQ ID NO:149 1 ------------------atg---------------ttcgcaaataaagt
SEQ ID NO:150 1 ----------------------------atgaggcttgaagggaaag--
SEQ ID NO:151 1 --atggaaa---
SEQ ID NO:152 1 --
SEQ ID NO:140 51 tgcgt-taattaccggtggcgccggcaatatcggcagtgaattgacacgt
SEQ ID NO:148 21 cacgc-tggtgaccggcggctc----------------------------
SEQ ID NO:149 18 ggtac-tagtaacaggtggtagctccggtatcggc---------------
SEQ ID NO:150 20 tgtgtctgatcacagg----ggctgcaagcgggatagggaaa-gccacca
SEQ ID NO:151 8 -------------------------aatttccgca----------ccct
SEQ ID NO:152 1 --
SEQ ID NO:140 100 cgctt--tctcgcagagggagcgacggtcattattagtggacggaatcgg
SEQ ID NO:148 42 ---------------------------------------ggacggtatcgg
SEQ ID NO:149 52 ------------------gcagctactgt-----------------------
SEQ ID NO:150 65 cgcttctttttcgcacaggaag----------------------------ga
SEQ ID NO:151 22 ccctt--tc---
SEQ ID NO:152 1 --
SEQ ID NO:140 148 gcgaagttgaccgcactggccgaacggatgcaggcagaggcaggagtgcc
SEQ ID NO:148 54 cc--------tggcaatcgccgaggcgttcctgagcgagg----------
SEQ ID NO:149 63 -------------------------ggaagcattc---------------
SEQ ID NO:150 88 gctacggtgatcg--ctggc------gat---------------------
SEQ ID NO:151 29 --
SEQ ID NO:152 1 ------gtgaacccaatgg----acaga--caaacagaaggacaag----
SEQ ID NO:140 198 ggcaaagcgcatcgatctcgaagtcatggatgggagtgatccggtcgcgg
SEQ ID NO:148 86 ------gcgc---------cgatgtcct-----------------------
SEQ ID NO:149 73 ----------------------gttaaggaagg------------------
SEQ ID NO:150 109 --------------atctcga-----------------------------
SEQ ID NO:151 29 --
SEQ ID NO:152 35 ----aaccgcagc-------------------------atcagg------
SEQ ID NO:140 248 tacgtgccggtatcgaagcgattgtggcccgtcacggccagatcgacatt
SEQ ID NO:148 99 -------------------gatcgtcggccgtgacgcc------------
SEQ ID NO:149 84 -------cgctttctgtagccttcgtg-------------------------
SEQ ID NO:150 116 --------------------------------aagaaaatctcgactct
SEQ ID NO:151 29 ----------------------------cccgcca----------------
SEQ ID NO:152 50 ---------------------------------acagacagccgggcatt
SEQ ID NO:140 298 ctggtcaacaatgcaggaagtgccggtgcccagcgtcgtctggccgagat
SEQ ID NO:148 118 --gcc-----
SEQ ID NO:149 103 ----------------ggaagaaaccaagccaag-----------------
SEQ ID NO:150 133 cttgtgaaagaggcagaagg----------------------------
SEQ ID NO:151 36 ---------aacccaggaaatgcc--------------------------
SEQ ID NO:152 67 g-agtcaaaaatgaa------tccgctgcc--------------------
SEQ ID NO:140 348 tccactcactgaagctgaattaggccctggcgccgaagagacgcttcatg
SEQ ID NO:148 121 -----------aagct-----------------cgaagccgcgc-----g
SEQ ID NO:149 121 --------cttaag--gaagtag-------------agagccgc----tg
SEQ ID NO:150 153 --
SEQ ID NO:151 51 --
SEQ ID NO:152 90 --

Figure 53A

```
SEQ ID NO:140   398 ccagcatcgccaatttacttggtatgggatggcatctgatgcgtattgcg
SEQ ID NO:148   138 ccagaagc-----------------------------------------tggcg
SEQ ID NO:149   144 ccagcagc-----------------------------------------
SEQ ID NO:150   153 ----------------actt-------------------------------
SEQ ID NO:151    51 ------------------------------------------------cg
SEQ ID NO:152    90 -------------------------gctgtcagaggacgaggattatc

SEQ ID NO:140   448 gcacctcatatgccggtaggaagtgcggtcatcaatgtctcgaccatctt
SEQ ID NO:148   151 gc-----------------------------------tcttggcca----
SEQ ID NO:149   152 ---------------------------------atggagccaacatc--
SEQ ID NO:150   157 ------------ccgg--ggaag--------------------------
SEQ ID NO:151    53 gcac-------------------------------------------
SEQ ID NO:152   113 g----------------aggaa-----------------------

SEQ ID NO:140   498 ttcacgggctgagtactacgggcggattccgtatgtcacccctaaagctg
SEQ ID NO:148   162 -------------------ggc--------------------------
SEQ ID NO:149   166 --------ctggctatcaaag--------cagatgtctcc---aaag---
SEQ ID NO:150   166 ------------------------------------------------
SEQ ID NO:151    57 -------------tac--cgatcggatgc----------------agccg
SEQ ID NO:152   119 --------------------gcgg-----------------aaaactg

SEQ ID NO:140   548 ctcttaatgctctatctcaacttgctgcgcgtgagttaggtgcacgtggc
SEQ ID NO:148   165 ------------------------cggcgc--------ggtggagacgtc
SEQ ID NO:149   194 ----------------------------------------acgagga
SEQ ID NO:150   166 ------------------------------------------------
SEQ ID NO:151    76 c------tgcccgat---------------------------cacgggg-
SEQ ID NO:152   130 aaaggaa-----------aagttg--------------------------

SEQ ID NO:140   598 atccgcgttaatacgatctttcccggcccgattgaaagtgatcgcatccg
SEQ ID NO:148   183 gtccgc-------cgatcttgcc-----------------------------
SEQ ID NO:149   201 agc-------------------------------gaaaatcatcgta----
SEQ ID NO:150   166 ------gttgatccctacgtt----------ttgaacgtgaccg------
SEQ ID NO:151    92 ---------aaaac---------------------------------tcct
SEQ ID NO:152   143 -------------cgatcattactgg-------------------------

SEQ ID NO:140   648 tacagtgttccagcgtatggatcagctcaaggggcggcccgaaggcgaca
SEQ ID NO:148   199 ------------------------------------------------
SEQ ID NO:149   217 ------------------------------------------------
SEQ ID NO:150   194 -acag-------------ggatcagataaag---------gaag------
SEQ ID NO:151   101 accagggttcc----------------------ggacgcctgaag------
SEQ ID NO:152   156 ----------------------------------------aggcgaca

SEQ ID NO:140   698 cagcgcaccatttttttgaacaccatgcgattgtgtcgtgccaacgaccag
SEQ ID NO:148   199 ----------------------------------------------accag
SEQ ID NO:149   217 ---------------------------------------caacaa----
SEQ ID NO:150   215 -----------ttgtggaaaa-----------agtcgttcaaa-----ag
SEQ ID NO:151   124 ------------------------------------------gacaag
SEQ ID NO:152   164 ------------------------------------------------

SEQ ID NO:140   748 ggcgcgcttgaacgtcggttcccctccgtcggtgatgtggcagacgccgc
SEQ ID NO:148   204 ----------------------cct-------------------------
SEQ ID NO:149   223 ------------------------------------------------ac
SEQ ID NO:150   238 tacg---------gtcgaatc------------gatgt------------
SEQ ID NO:151   130 agagc------------catcatcaccggcgggga-----cagcggcatc
SEQ ID NO:152   164 ------------------------------------------------
```

Figure 53B

```
SEQ ID NO:140   798 tgtctttctggccagtgccgaatccgccgctctctccggtgagacgattg
SEQ ID NO:148   207 ------------cggtgtcgcaaccgtcg-tcgagcaggtgaaa------
SEQ ID NO:149   225 tgtc-----gacaagttc----------------------gggaagcttg
SEQ ID NO:150   255 ----------------------------------tctggtga--------
SEQ ID NO:151   163 gg-----cagggccgtggcga-----------tcgcc-------------
SEQ ID NO:152   164 --------------------------------------------------

SEQ ID NO:140   848 aggttacgcacggaatggagttgccggcctgcagtgagaccagcctgctg
SEQ ID NO:148   238 ----------------------------------gagaccggcc-----
SEQ ID NO:149   248 atgt--------------------------------------------------
SEQ ID NO:150   263 --------------------------------------------------
SEQ ID NO:151   184 ----tatgcgcgcgagggag----------------------------c
SEQ ID NO:152   164 ---------gcggaat----------------agggagagc---------

SEQ ID NO:140   898 gcccgtactgatctgcgcacgattgatgccagtggccgcacgacgctcat
SEQ ID NO:148   248 ---------------------------------ggccgctcgacattcct
SEQ ID NO:149   252 --------------------gcttgtt-----------aacaacgc-----
SEQ ID NO:150   263 ----------------------------------acaacgc----
SEQ ID NO:151   201 ggacgtccttatcagc-----------------------------tat
SEQ ID NO:152   180 --------------------------------------------------

SEQ ID NO:140   948 ctgcgccggcgaccagattgaagaggtgatggcgctcaccggtatgttgc
SEQ ID NO:148   265 at----------caacaatg-----------------ccggt-------
SEQ ID NO:149   267 --------------------------------------------------
SEQ ID NO:150   270 --------------------------------------------------
SEQ ID NO:151   220 ctgag-------cgagcatgacgacgcgatggccaccaaggct-------
SEQ ID NO:152   180 ----------------------------------------------------

SEQ ID NO:140   998 gtacctgtgggagtgaagtgatcatcggcttccgttcggctgcggcgctg
SEQ ID NO:148   280 ------------------------------------gtcgccgacctc
SEQ ID NO:149   267 -------tgggatt--------------ctacggttcg------------
SEQ ID NO:150   270 --------gggaat-----------------------------------
SEQ ID NO:151   256 ----ctggtggag-gaag-------------------------------
SEQ ID NO:152   180 --------------------------------------------------

SEQ ID NO:140  1048 gcccagttcgagcaggcagtcaatgagagtcggcggctggccggcgcaga
SEQ ID NO:148   292 gtgccgttcga-------------gagcgtcagcg------aggcgca--
SEQ ID NO:149   284 ----------------------cgagtgt---------tctggagccga
SEQ ID NO:150   276 --------------------------------------------------
SEQ ID NO:151   269 ---caggtcgc-aaggccgt---------------gcttgccgccggcga
SEQ ID NO:152   180 ----------agcag-------------------------------------

SEQ ID NO:140  1098 ctttacgcctcccattgccttgccactcgatccacgcgatccggcaacaa
SEQ ID NO:148   321 ------------------------------------gttccagcactcc
SEQ ID NO:149   302 cttta------------------------ataca-----------aactt
SEQ ID NO:150   276 ----------------------------------------------aacaa
SEQ ID NO:151   300 c----------------------------atccagtcg-tccg---acca
SEQ ID NO:152   185 -----------ctattgcctt-----------------------------

SEQ ID NO:140  1148 ttgacgctg--tcttcgattgggccggcgagaatacggcgggattcatg
SEQ ID NO:148   334 ttcgcgctc---------aatgtggcgg---------cggcg---------
SEQ ID NO:149   317 ttga--------------------------------------------------
SEQ ID NO:150   281 gggatgc-----------------------------------gcttcttg
SEQ ID NO:151   318 ttgccgcaggatcgtcgaaacggccgttcgggaactcggcggcat-----
SEQ ID NO:152   195 --------------------------------------------------
```

Figure 53C

```
SEQ ID NO:140  1196 cagcggtgattctgcctgctaccagtcacgaaccggcaccgtgcgtgatt
SEQ ID NO:148   358 ---------ttcttcct-------------------cacc----------
SEQ ID NO:149   321 ------tgaaact-------------------------------------
SEQ ID NO:150   296 -----------------------------------------------------
SEQ ID NO:151   363 -----------------------------------------------------
SEQ ID NO:152   195 ----------------tgcta-------------------------------

SEQ ID NO:140  1246 gaggttgatgatgagcgggtgctgaattttctggccgatgaaatcaccgg
SEQ ID NO:148   370 ---------caggggctgctgccgcattt----------------------
SEQ ID NO:149   328 ----------atgaac---------------------acgaatttac--g
SEQ ID NO:150   296 -----------tgag--------------------gatgaaa-------
SEQ ID NO:151   363 -----------------------------------------------c
SEQ ID NO:152   200 ------------aagaggggctga-------------------------

SEQ ID NO:140  1296 gacaattgtgattgccagtcgcctggcccgttactggcagtcgcaacggc
SEQ ID NO:148   390 -----------------------------------------------------
SEQ ID NO:149   345 tccagttgtcctcatcactagcctg-------------------------
SEQ ID NO:150   307 -----------------------------------------------------
SEQ ID NO:151   364 gaca-------------------------------------------------
SEQ ID NO:152   213 -----------------------------------------------------

SEQ ID NO:140  1346 ttacccccggcgcacgtgcgcgtgggccgcgtgtcatttttctctcgaac
SEQ ID NO:148   390 -------cggcgc-----------------------------------------c
SEQ ID NO:149   370 -----------------------------------------------------
SEQ ID NO:150   307 -----------------------------------------------------
SEQ ID NO:151   368 ----------------------------------------ttctcgtcaac
SEQ ID NO:152   213 --------------------------------tatctccattctat---ac

SEQ ID NO:140  1396 ggtgccgatcaaaatgggaatgtttacggacgcattcaaagtgccgctat
SEQ ID NO:148   397 ggtgc-----------------------------------------------at
SEQ ID NO:149   370 -------------------------------------------------gctat
SEQ ID NO:150   307 ------gaagaagactgggatg-----------------------------
SEQ ID NO:151   379 aatgc-------------------------------------------------
SEQ ID NO:152   229 ttagacgagca-------------ttcggacgca----------------

SEQ ID NO:140  1446 cggtcagctcattcgtgtgtggcgtcacgaggctgaacttgactatcagc
SEQ ID NO:148   404 cgatca----------------------------------------------
SEQ ID NO:149   375 ccctcatttgatt--------gctacaaaagggag---------------
SEQ ID NO:150   323 cggt-----------------------------------------aataaac
SEQ ID NO:151   384 -----------------------------------------------------
SEQ ID NO:152   250 ----------------------------gagg--------------aaac

SEQ ID NO:140  1496 gtgccagcgccgccggtgatcatgtgctgccgccggtatgggccaatcag
SEQ ID NO:148   410 -----------------------------------------------------
SEQ ID NO:149   402 -----------------------------------------------------
SEQ ID NO:150   334 gtg-----------------------------------------aatc--
SEQ ID NO:151   384 ---------------------------------------agcccatcag
SEQ ID NO:152   258 acgcaaacg--------gatc--------------------gaaaaggag

SEQ ID NO:140  1546 attgtgcgcttcgctaaccgcagccttgaagggttagaatttgcctgtgc
SEQ ID NO:148   410 -----------------------------------------------------
SEQ ID NO:149   402 -----------------------------------------------------
SEQ ID NO:150   341 -------------------------tgaagggt-----------------
SEQ ID NO:151   394 --------------------gcgaccttcaag-------------------
SEQ ID NO:152   280 aatgtccgctgc----------------------------ctgcttatcc
```

Figure 53D

```
SEQ ID NO:140  1596 ctggacagctcaattgctccatagtcaacgccatatcaatgagattaccc
SEQ ID NO:148   410 ------------------------------------------------
SEQ ID NO:149   402 ------------------catagttaacg---tatccagtata------
SEQ ID NO:150   349 ------------------gttttcaacg-------------------
SEQ ID NO:151   406 ------------------------------------------------
SEQ ID NO:152   302 cggga-------------------------------------------

SEQ ID NO:140  1646 tcaacatccctgccaacattagcgccaccaccggcgcacgcagtgcatcg
SEQ ID NO:148   410 tcaacatctcttcctattt--------------cgcccgca--------
SEQ ID NO:149   424 ---------ctgtctacaatag----------------------------
SEQ ID NO:150   359 ------------------------------------------------
SEQ ID NO:151   406 --aacatc---gaagacatcagcgac-----------------------
SEQ ID NO:152   307 ------------------------------------------------

SEQ ID NO:140  1696 gtcggatgggcggaaagcctgatcgggttgcatttggggaaagttgccttt
SEQ ID NO:148   437 ------------------------------------------------
SEQ ID NO:149   437 ------------------------------------------------
SEQ ID NO:150   359 ------------------------------------------------
SEQ ID NO:151   427 ----------------------------------gagga----------
SEQ ID NO:152   307 ----gatg-------------------------ttgggga----------

SEQ ID NO:140  1746 gattaccggtggcagcgccggtattggtgggcagatcgggcgcctcctgg
SEQ ID NO:148   437 ------------------------------------------------
SEQ ID NO:149   437 ------------------------------------------------
SEQ ID NO:150   359 ------------------------------------------------
SEQ ID NO:151   432 ------------------------gtggg-------------------
SEQ ID NO:152   318 ------------------------------------------------

SEQ ID NO:140  1796 ctttgagtggcgcgcgcgtgatgctggcagcccgtgatcggcataagctc
SEQ ID NO:148   437 ------------------------------------------------
SEQ ID NO:149   437 ----------------------------------------------taa----
SEQ ID NO:150   359 ------------------------------------------------
SEQ ID NO:151   437 --------------------agctgacattccg---------------c
SEQ ID NO:152   318 ------------------------------------------------

SEQ ID NO:140  1846 gaacagatgcaggcgatgatccaatctgagctggctgaggtggggtatac
SEQ ID NO:148   437 -------------agatgatcc-----------------------------
SEQ ID NO:149   440 ------------------------------------------gaatac
SEQ ID NO:150   359 --------------------------------tgactcagatgg-------
SEQ ID NO:151   451 gtcaacatgcacgccatgttc--------------------------tac
SEQ ID NO:152   318 -------------cga-gaaccattgtgaacaagctg-------------

SEQ ID NO:140  1896 cgatgtcgaagatcgcgtccacattgcaccgggctgcgatgtgagtagcg
SEQ ID NO:148   446 ----------------------------------------------cg
SEQ ID NO:149   446 c-----------------------------------------------
SEQ ID NO:150   371 ------------------------------------------------
SEQ ID NO:151   475 c--tgaccaag--------------gcagcgg-----------------
SEQ ID NO:152   341 ------------------------tgca------------------------

SEQ ID NO:140  1946 aagcgcagcttgcggatcttgttgaacgtaccctgtcagcttttggcacc
SEQ ID NO:148   448 aagcg-------------------------------gccatc------cagc
SEQ ID NO:149   447 ------------------------------------------------
SEQ ID NO:150   371 ------------------------------------------------
SEQ ID NO:151   491 ----------tgccgcacatgaagaa-----------------gggcagc
SEQ ID NO:152   345 ----gcaaacagtggacc----------------------attttggtaaa
```

Figure 53E

```
SEQ ID NO:140  1996 gtcgattatctga-tcaacaacgccgggatcgccggtgtcgaagagatgg
SEQ ID NO:148   463 gtctactccctgt-ccaagggcgc------------------------
SEQ ID NO:149   447 ------------------------------------------------
SEQ ID NO:150   371 ------------------------------------------------
SEQ ID NO:151   514 g---------cga-tcatcaacaccg------------------------
SEQ ID NO:152   370 ctcgat-atcttagtgaacaacgccg------------------------

SEQ ID NO:140  2045 ttatcgatatgccagttgagggatggcgccataccctcttcgccaatctg
SEQ ID NO:148   486 --------------gttga-------------------------------
SEQ ID NO:149   447 ------------------agggattatgtcatacagt-------------
SEQ ID NO:150   371 ------------------------------------------------
SEQ ID NO:151   530 --------cttcca-------------tcaatgccgacgttcccaatccg
SEQ ID NO:152   395 ------------------------------------------------ctg

SEQ ID NO:140  2095 atcagcaactactcgttgatgcgcaaactggcgccgttgatgaaaaaaca
SEQ ID NO:148   491 ----------actcgttga-------------------------------
SEQ ID NO:149   466 ------------------------------------------------
SEQ ID NO:150   371 ---------------------------tggtgccctacatgatcaaaca
SEQ ID NO:151   559 atc-----ctactcgcctatgcg--------------------accacca
SEQ ID NO:152   398 aacagcatc-----------------------------------ccca

SEQ ID NO:140  2145 gggtagcggttacatccttaacgtctcatcatactttggcggtgaaaaag
SEQ ID NO:148   500 ------------------------------------------------
SEQ ID NO:149   466 ------------------------------------------------
SEQ ID NO:150   393 gaggaacggttcgatcgtgaacgtctcctctgtcgttgg--------aat
SEQ ID NO:151   584 agggcgcg-----atc-------------cacaattt-------------
SEQ ID NO:152   411 ggacag------cattctcaataattcaaca--------------------

SEQ ID NO:140  2195 atgcggccattccctaccccaaccgtgccgattacgccgtctcgaaggct
SEQ ID NO:148   500 ---------------------------------------ccagatcgct
SEQ ID NO:149   466 ------------------------------------gtgtcaaaggct
SEQ ID NO:150   435 atacgggaat-------cctggtcagacgaattacgcggcgtcgaaggcg
SEQ ID NO:151   603 ----------------------cagcgccg--------gtctcg------
SEQ ID NO:152   436 ------------------------------------------------

SEQ ID NO:140  2245 ggtcagcgggcaatggccgaagtctttgcgcgcttccttggcccg---ga
SEQ ID NO:148   510 ggccttcgag--------------------------ctcggcccgcgcgg
SEQ ID NO:149   478 g-----------------------------------------------
SEQ ID NO:150   478 ggagtcataggaatgacc-aagacgt-----------------------
SEQ ID NO:151   617 ----cgcagatgctggccgaa--------------------cgcg---g-
SEQ ID NO:152   436 gaacagctggaa-------aaaacctttcgc--------------------

SEQ ID NO:140  2292 gatacagatcaatgccattgcgccgggtccggtcgaaggtgatcgcttgc
SEQ ID NO:148   534 catccgcgtcaacgccatcgcgcccggcacggtcga--------------
SEQ ID NO:149   479 ------------------------------------------------
SEQ ID NO:150   503 ------------------------------gggcgaaggaactcgct---
SEQ ID NO:151   639 gataagagtgaatgtcgtggccccgggcccgatc----------------
SEQ ID NO:152   460 -acaaatatttttccat-------------------------------

SEQ ID NO:140  2342 gcggtaccggtgaacgtcccggcctctttgcccgtcgggcgcggctgatt
SEQ ID NO:148   570 ------------------------------------------------
SEQ ID NO:149   479 ------------------------------------------------
SEQ ID NO:150   520 ------------------------------------------------
SEQ ID NO:151   673 ---------------------------------tggacgccgctg---
SEQ ID NO:152   477 ------------------------------------------------
```

Figure 53F

```
SEQ ID NO:140  2392 ttggagaacaagcggctgaatgagcttcacgctgctcttatcgcggctgc
SEQ ID NO:148   570 ------------------------------------------------------
SEQ ID NO:149   479 ------------------------------------------------------
SEQ ID NO:150   520 ----------------ggaagaaacatcagggtgaac--------gctgt
SEQ ID NO:151   685 ------------------atcccctccaccatgc---------------
SEQ ID NO:152   477 ----------------------gtttca--------------------

SEQ ID NO:140  2442 gcgcaccgatgagcgatctatgcacgaactggttgaactgctcttaccca
SEQ ID NO:148   570 ------------------------------------------------------
SEQ ID NO:149   479 ------ctatg---gatcacttcacaaaat--------------------
SEQ ID NO:150   546 g-gcacc-----------------------------------------cgga
SEQ ID NO:151   701 -----ccgagga-------------------------------------
SEQ ID NO:152   483 ------------------tatg-acgaa----------------------

SEQ ID NO:140  2492 atgatgtggccgcactagagcagaatcccgcagcacctaccgcgttgcgt
SEQ ID NO:148   570 ---------------------------------cacc-------------
SEQ ID NO:149   500 ------tggcagcgttggagctg---------gctccttctggcgtgcga
SEQ ID NO:150   556 ttcat----------------agaaaccccccatgac--------------
SEQ ID NO:151   708 ------------------------------------taccg--------
SEQ ID NO:152   492 -----------------------------------gaaagctttgcct

SEQ ID NO:140  2542 gaactggcacgacgttttcgcagcgaaggcgatccggcggcatcatcaag
SEQ ID NO:148   574 ---------------------------gccatgcggcg-------caag
SEQ ID NO:149   535 g------------------------------------------------------
SEQ ID NO:150   576 ----------------------cgaaaaacttccag-------aaaaag
SEQ ID NO:151   713 --------tcgccgatttcg-----------------------------
SEQ ID NO:152   505 cacctg-----------------------------------------caag

SEQ ID NO:140  2592 cagtgcgctgctgaaccgttcaattgccgctaaattgctggctcgtttgc
SEQ ID NO:148   589 --------------accgt-------------------------------
SEQ ID NO:149   536 -----------tgaac---tcagt-----------------------
SEQ ID NO:150   596 c--------------ccgtgaaacggcc-------------------
SEQ ID NO:151   725 ------------------------------------------------gc
SEQ ID NO:152   515 aggggtg-----------------tgccatta-----------------

SEQ ID NO:140  2642 ataatggtggctatgtgttgcctgccgacatctttgcaaacctgccaaac
SEQ ID NO:148   594 ------------------------cgac---------aacctgcc----
SEQ ID NO:149   546 ------------------------------------caaccctg------
SEQ ID NO:150   610 ---------------------------------ctttccaga----------
SEQ ID NO:151   727 aaacaggtgcctatg-----------------------------------
SEQ ID NO:152   530 ttaat-------------------acgacat-------------------

SEQ ID NO:140  2692 ccgcccgatcccttcttcacccgagcccagattgatcgcgaggctcgcaa
SEQ ID NO:148   606 ------------------------------------------------------
SEQ ID NO:149   554 ------gaccagttct-----------------------------------
SEQ ID NO:150   619 -------atacc-------------------------------gctgggaa
SEQ ID NO:151   742 ------------------------------------------------aa
SEQ ID NO:152   542 -----cgattaccgctt-----------------------------------

SEQ ID NO:140  2742 ggttcgtgacggcatcatggggatgctctacctgcaacggatgccgactg
SEQ ID NO:148   606 ------------------------------------------ggccga---
SEQ ID NO:149   564 ----------------------------tac-------------------
SEQ ID NO:150   632 ggtttgggaagccagaagagg-------------------------
SEQ ID NO:151   744 g------------------------------------------------
SEQ ID NO:152   554 ------------atgaaggggat-----------acgg----------
```

Figure 53G

```
SEQ ID NO:140  2792 agtttgatgtcgcaatggccaccgtctattaccttgccgaccgcaatgtc
SEQ ID NO:148   612 ----------------ggcca-----------aggccgaactgaaggcc
SEQ ID NO:149   567 ----tgatatcgc-------------------------------------
SEQ ID NO:150   653 -------tggcgca-----------------------------------
SEQ ID NO:151   745 ------------------------------------cgaccg-------
SEQ ID NO:152   569 ----------------------cgttaattgattattccagcacaaag--

SEQ ID NO:140  2842 agtggtgagaca-ttccacccatcaggtggtttgcgttacgaacgcaccc
SEQ ID NO:148   634 tatg-----------------------------------tcgaacgcagc-
SEQ ID NO:149   576 ---------------------------------------------------
SEQ ID NO:150   660 ---ggttatactcttcctcgcatcggacgagtcgagttacg---------
SEQ ID NO:151   751 ---------------------------------------------------
SEQ ID NO:152   595 ---ggtgcga-----------------ttgtttcctttacg---------

SEQ ID NO:140  2891 ctaccggtggcgaactcttcggcttgccctcaccggaacggctggcggag
SEQ ID NO:148   649 ----------------tatccgctgggccgcatcgg-ccgtccggacgac
SEQ ID NO:149   576 -------------------------------------------------ag
SEQ ID NO:150   698 ------------------------------tcaccggacagg---------
SEQ ID NO:151   751 --------------------ggccagccc--------------gtggaa
SEQ ID NO:152   616 cgttccatggcgaagtc----gcttgc-----------------------

SEQ ID NO:140  2941 ctggtcggaagcacggtctatctgataggtgaacatctgactgaacacct
SEQ ID NO:148   682 ctcgccggcatggcggtttatct---------------------------
SEQ ID NO:149   578 ctggt-------------------------------tctggct--------
SEQ ID NO:150   710 ----------------------tgatag-----------------------
SEQ ID NO:151   766 ctcg------cctcggcctatgtcat-----------------------
SEQ ID NO:152   639 -------------------------agataaa-----------------

SEQ ID NO:140  2991 taacctgcttgcccgtgcgtacctcgaacgttacggggcacgtcaggtag
SEQ ID NO:148   705 ---------------------------------------------------
SEQ ID NO:149   590 ---------------------------------------------------
SEQ ID NO:150   716 ---------------------------------------------------
SEQ ID NO:151   786 ---------------------------------------------------
SEQ ID NO:152   646 ------------------------------------ggca----------

SEQ ID NO:140  3041 tgatgattgttgagacagaaaccggggcagagacaatgcgtcgcttgctc
SEQ ID NO:148   705 ---------------------------------------------------
SEQ ID NO:149   590 ----------------------------------------------tttctc
SEQ ID NO:150   716 ---------------------------------------------------
SEQ ID NO:151   786 ---------------------------------------------------
SEQ ID NO:152   650 --------------------------tcagagtgaatgcg----------

SEQ ID NO:140  3091 cacgatcacgtcgaggctggtcggctgatgactattgtggccggtgatca
SEQ ID NO:148   705 ---------------------------------------------------
SEQ ID NO:149   596 c-------------------------------------------tgatct
SEQ ID NO:150   716 ---------------------------------------------------
SEQ ID NO:151   786 ---------------gctgg-------------------------------
SEQ ID NO:152   664 ---------------------------------gtggcgcccggt-----

SEQ ID NO:140  3141 gatcgaagccgctatcgaccaggctatcactcgctacggtcgcccagggc
SEQ ID NO:148   705 ---------agccagcgacgaggc-------------------------
SEQ ID NO:149   603 gcttgaag-----------------------------------------
SEQ ID NO:150   716 ---------------------------------------------------
SEQ ID NO:151   791 ---cggatccgatgtcga--------------gctac-------------
SEQ ID NO:152   676 --------ccgatttggacaccgct-------------------------
```

Figure 53H

```
SEQ ID NO:140  3191 cggtcgtctgtacccccttccggccactgccgacggtaccactggtcggg
SEQ ID NO:148   720 ----------------------------------------------------
SEQ ID NO:149   611 ----------------------------------------------------
SEQ ID NO:150   716 ----------------------------------------------------
SEQ ID NO:151   811 ----------------------------------------------------
SEQ ID NO:152   693 ---------------tattccgg-------cgacattccctgagg-----

SEQ ID NO:140  3241 cgtaaagacagtgactggagcacagtgttgagtgaggctgaatttgccga
SEQ ID NO:148   720 -----------ggcctgga----------------------------cga
SEQ ID NO:149   611 -----atacaggg-------------------------------------
SEQ ID NO:150   716 ----------------------------------------gaat-------
SEQ ID NO:151   811 ----------gtgtcaggcgca-----------------------------
SEQ ID NO:152   716 -----aaaaagtga-aacagcac------------ggcttggatacccca

SEQ ID NO:140  3291 gttgtgcgaacaccagctcacccaccatttccgggtagcgcgcaagattg
SEQ ID NO:148   731 gcggtgggatc-------------------------------------tttg
SEQ ID NO:149   619 ---------------gctcatacaccgt----------------------
SEQ ID NO:150   720 ----------------------------------------------------
SEQ ID NO:151   823 ------------------------------------------acgattg
SEQ ID NO:152   748 ---atgggaagaccgggacagcc----------ggttgagc---------

SEQ ID NO:140  3341 ccctgagtgatggtgc-cagtctcgcgctggtcactcccgaaactacggc
SEQ ID NO:148   746 ccgtg---gatggt-------------------------------------
SEQ ID NO:149   632 ---tggggaaagctgcgcagtct-----------------------------
SEQ ID NO:150   720 -------agatgg-------------------------------------
SEQ ID NO:151   830 ccgtga--------------------------------------------
SEQ ID NO:152   776 ------atgcaggcgc-ctatgttctgctggcgtctgacgaa--------

SEQ ID NO:140  3390 tacctcaactaccgagcaatttgctctggctaacttcatcaaaacgaccc
SEQ ID NO:148   757 ----------------------------------------------------
SEQ ID NO:149   652 -------------gaggagattgct-------------------------
SEQ ID NO:150   726 ----------------------------------------------------
SEQ ID NO:151   836 ----------------------------------------------------
SEQ ID NO:152   811 ------------------------tcttccta------------------

SEQ ID NO:140  3440 ttcacgcttttacggctacgattggtgtcgagagcgaaagaactgctcag
SEQ ID NO:148   757 -------------ggcta------------------------------
SEQ ID NO:149   664 --------------gatatgatt---------------------------
SEQ ID NO:150   726 ----------------------------------------------------
SEQ ID NO:151   836 ----------------------------------------------------
SEQ ID NO:152   819 ----------------tatga---------------------------cag

SEQ ID NO:140  3490 cgcattctgatcaatcaagtcgatctgacccggcgtgcgcgtgccgaaga
SEQ ID NO:148   762 ----------------------------------------------------
SEQ ID NO:149   673 -------------------gtgtatctg---------gctagtgataaagc
SEQ ID NO:150   726 ------------------------------------------------gg
SEQ ID NO:151   836 ----------------------------------------------------
SEQ ID NO:152   827 ggca----gaccattcatgt------------------------gaatg

SEQ ID NO:140  3540 gccgcgtgatccgcacgagcgtcaacaagaactggaacgttttatcgagg
SEQ ID NO:148   762 ----------------------------------------------------
SEQ ID NO:149   696 taagagtgtt--------------------acggggtcctgttat-----
SEQ ID NO:150   728 gcctcgtgat------------------------------------------
SEQ ID NO:151   836 ----------------------------------------------------
SEQ ID NO:152   848 gcggc-------------------------------cgttttat-----
```

Figure 53I

```
SEQ ID NO:140  3590 cagtcttgctggtcactgcaccactcccgcctgaagccgatacccgttac
SEQ ID NO:148   762 --------------------------------------------------
SEQ ID NO:149   721 -----atcatggacaatg---gactcgcgc--------------------
SEQ ID NO:150   738 ------------------------------ctga----------------
SEQ ID NO:151   836 --------------------------ccggcggcaagcc------------
SEQ ID NO:152   861 --------------------------------------------------

SEQ ID NO:140  3640 gccgggcggattcatcgcggacgggcgattaccgtgtaa
SEQ ID NO:148   762 --------------cacggccggatga-----------
SEQ ID NO:149   743 ----------tgca----------------------gtaa
SEQ ID NO:150   742 ---------------------------------------
SEQ ID NO:151   849 ----------------------------tttcctttga-
SEQ ID NO:152   861 ----------ttcaac-------------------gtaa
```

Figure 53J

Figure 54

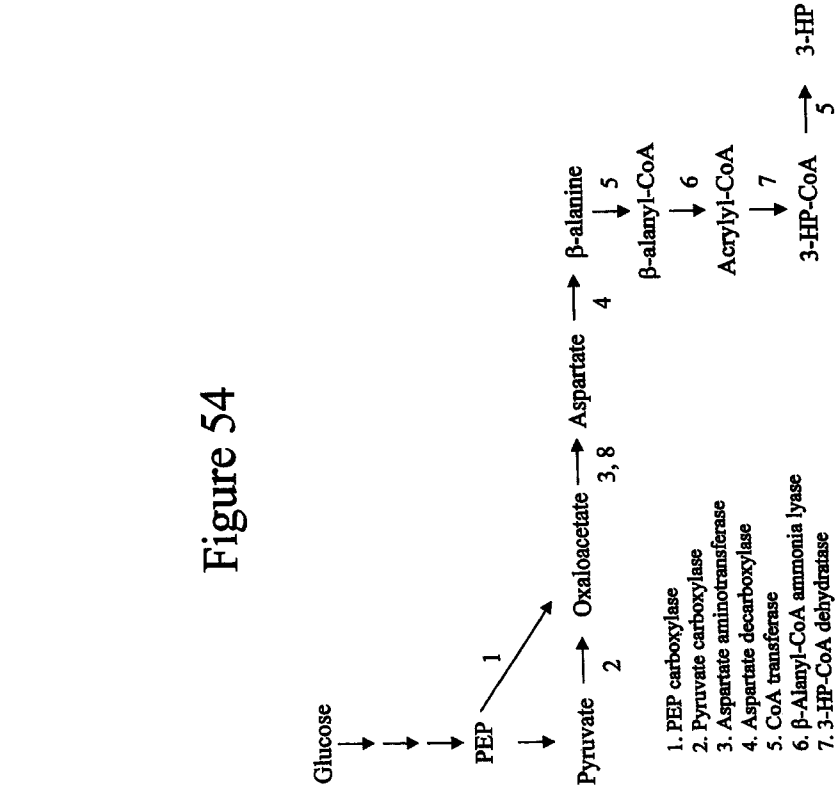

Glucose
PEP
1
Pyruvate
2
Oxaloacetate
3, 8
Aspartate
4
β-alanine
5
β-alanyl-CoA
6
Acrylyl-CoA
7
3-HP-CoA
5
3-HP
1. PEP carboxylase
2. Pyruvate carboxylase
3. Aspartate aminotransferase
4. Aspartate decarboxylase
5. CoA transferase
6. β-Alanyl-CoA ammonia lyase
7. 3-HP-CoA dehydratase
8. Glutamate dehydrogenase

Figure 55

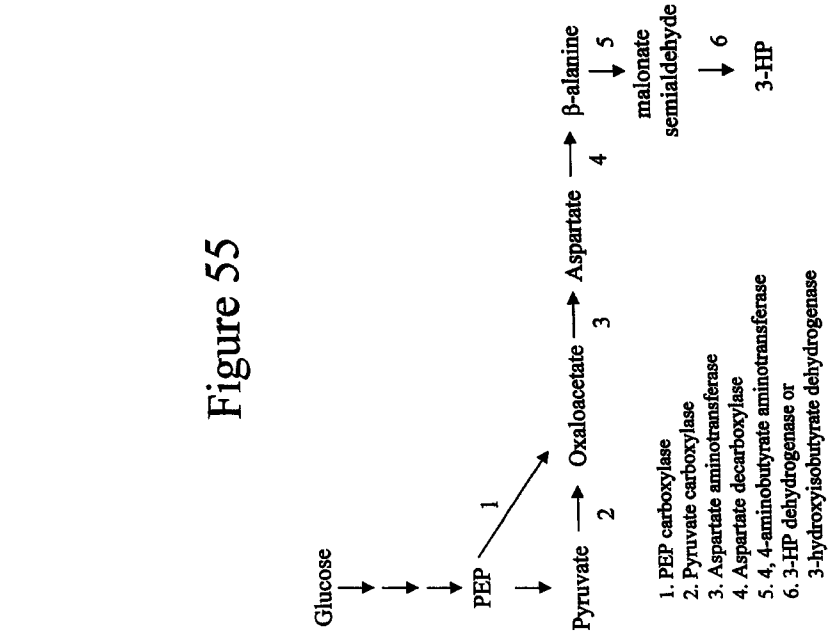

Glucose
PEP
1
Pyruvate
2
Oxaloacetate
3
Aspartate
4
β-alanine
5
malonate semialdehyde
6
3-HP
1. PEP carboxylase
2. Pyruvate carboxylase
3. Aspartate aminotransferase
4. Aspartate decarboxylase
5. 4, 4-aminobutyrate aminotransferase
6. 3-HP dehydrogenase or 3-hydroxyisobutyrate dehydrogenase

Figure 56

```
  1  MVGKKVVHHL MMSAKDAHYT GNLVNGARIV NQWGDVGTEL
 41  MVYVDGDISL FLGYKDIEFT APVYVGDFME YHGWIEKVGN
 81  QSYTCKFEAW KVATMVDITN PQDTRATACE PPVLCGRATG
121  SLFIAKKDQR GPQESSFKER KHPGE  (SEQ ID NO:160)
```

Figure 57

```
  1  MVGKKVVHHL MMSAKDAHYT GNLVNGARIV NQWGDVGTEL
 41  MVYVDGDISL FLGYKDIEFT APVYVGDFME YHGWIEKVGN
 81  QSYTCKFEAW KVAKMVDITN PQDTRATACE PPVLCGTATG
121  SLFIAKDNQR GPQESSFKDA KHPQ (SEQ ID NO:161)
```

Figure 58

```
  1  ATGGTAGGTA AAAAGGTTGT ACATCATTTA ATGATGAGCG
 41  CAAAAGATGC TCACTATACT GGAAACTTAG TAAACGGCGC
 81  TAGAATTGTG AATCAGTGGG GCGACGTTGG TACAGAATTA
121  ATGGTTTATG TTGATGGTGA CATAAGCTTA TTCTTGGGCT
161  ACAAAGATAT CGAATTCACA GCTCCTGTAT ATGTTGGTGA
201  CTTTATGGAA TACCACGGCT GGATTGAAAA AGTTGGTAAC
241  CAGTCCTATA CATGTAAATT TGAAGCATGG AAAGTTGCAA
281  CAATGGTTGA TATCACAAAT CCTCAGGATA CACGCGCAAC
321  AGCTTGTGAG CCTCCGGTAT TGTGCGGAAG AGCAACGGGT
361  AGTTTGTTCA TCGCAAAAAA AGATCAGAGA GGCCCTCAGG
401  AATCCTCTTT TAAAGAGAGA AAGCACCCCG GTGAATGA
(SEQ ID NO:162)
```

Figure 59

```
  1  ATGGTAGGTA AAAAGGTTGT ACATCATTTA ATGATGAGCG
 41  CAAAAGATGC TCACTATACT GGAAACTTAG TAAACGGCGC
 81  TAGAATTGTG AATCAGTGGG GCGACGTAGG TACAGAATTA
121  ATGGTTTATG TTGATGGTGA CATCAGCTTA TTCTTGGGCT
161  ACAAAGATAT CGAATTCACA GCTCCTGTAT ATGTTGGTGA
201  TTTTATGGAA TACCACGGCT GGATTGAAAA AGTTGGCAAC
241  CAGTCCTATA CATGTAAATT TGAAGCATGG AAAGTAGCAA
281  AGATGGTTGA TATCACAAAT CCACAGGATA CACGTGCAAC
321  AGCTTGTGAA CCTCCGGTAC TTTGTGGTAC TGCAACAGGC
361  AGCCTTTTCA TCGCAAAGGA TAATCAGAGA GGTCCTCAGG
401  AATCTTCCTT CAAGGATGCA AAGCACCCTC AATAA
(SEQ ID NO:163)
```

3-HYDROXYPROPIONIC ACID AND OTHER ORGANIC COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation of U.S. application Ser. No. 13/926,054, filed Jun. 25, 2013, which is a divisional of U.S. application Ser. No. 13/467,165 filed May 9, 2012, now U.S. Pat. No. 8,501,455, which is a divisional of U.S. application Ser. No. 13/294,820 filed Nov. 11, 2011, now U.S. Pat. No. 8,198,066, which is a divisional of U.S. application Ser. No. 12/127,700 filed May 27, 2008, now U.S. Pat. No. 8,076,120, which is a divisional of U.S. application Ser. No. 11/539,856 filed Oct. 9, 2006, now U.S. Pat. No. 7,638,316, which is a divisional of U.S. application Ser. No. 10/432,443 filed Oct. 20, 2003 now U.S. Pat. No. 7,186,541, which is the National Stage of International Application No. PCT/US01/43607, filed Nov. 20, 2001, which in turn claims the benefit of U.S. Provisional Application Nos. 60/252,123 filed Nov. 20, 2000, 60/285,478 filed Apr. 20, 2001, 60/306,727 filed Jul. 20, 2001, and 60/317,845 filed Sep. 7, 2001, all herein incorporated by reference.

FIELD OF THE INVENTION

The invention relates to enzymes and methods that can be used to produce organic acids and related products.

BACKGROUND

Organic chemicals such as organic acids, esters, and polyols can be used to synthesize plastic materials and other products. To meet the increasing demand for organic chemicals, more efficient and cost effective production methods are being developed which utilize raw materials based on carbohydrates rather than hydrocarbons. For example, certain bacteria have been used to produce large quantities of lactic acid used in the production of polylactic acid.

3-hydroxypropionic acid (3-HP) is an organic acid. Although several chemical synthesis routes have been described to produce 3-HP, only one biocatalytic route has been heretofore previously disclosed (WO 01/16346 to Suthers, et al.). 3-HP has utility for specialty synthesis and can be converted to commercially important intermediates by known art in the chemical industry, e.g., acrylic acid by dehydration, malonic acid by oxidation, esters by esterification reactions with alcohols, and reduction to 1,3 propanediol.

SUMMARY

The invention relates to methods and materials involved in producing 3-hydroxypropionic acid and other organic compounds (e.g., 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, esters of 3-HP, and malonic acid and its esters). Specifically, the invention provides nucleic acid molecules, polypeptides, host cells, and methods that can be used to produce 3-HP and other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, esters of 3-HP, and malonic acid and its esters. 3-HP has potential to be both biologically and commercially important. For example, the nutritional industry can use 3-HP as a food, feed additive or preservative, while the derivatives mentioned above can be produced from 3-HP. The nucleic acid molecules described herein can be used to engineer host cells with the ability to produce 3-HP as well as other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, and esters of 3-HP. The polypeptides described herein can be used in cell-free systems to make 3-HP as well as other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, and esters of 3-HP. The host cells described herein can be used in culture systems to produce large quantities of 3-HP as well as other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, and esters of 3-HP.

One aspect of the invention provides cells that have lactyl-CoA dehydratase activity and 3-hydroxypropionyl-CoA dehydratase activity, and methods of making products such as those described herein by culturing at least one of the cells that have lactyl-CoA dehydratase activity and 3-hydroxypropionyl-CoA dehydratase activity. In some embodiments, the cell can also contain an exogenous nucleic acid molecule that encodes one or more of the following polypeptides: a polypeptide having E1 activator activity; an E2α polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; an E2β polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; and a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity. Additionally, the cell can have CoA transferase activity, CoA synthetase activity, poly hydroxyacid synthase activity, 3-hydroxypropionyl-CoA hydrolase activity, 3-hydroxyisobutryl-CoA hydrolase activity, and/or lipase activity. Moreover, the cell can contain at least one exogenous nucleic acid molecule that expresses one or more polypeptides that have CoA transferase activity, 3-hydroxypropionyl-CoA hydrolase activity, 3-hydroxyisobutryl-CoA hydrolase activity, CoA synthetase activity, poly hydroxyacid synthase activity, and/or lipase activity.

In another embodiment of the invention, the cell that has lactyl-CoA dehydratase activity and 3-hydroxypropionyl-CoA dehydratase activity produces a product, for example, 3-HP, polymerized 3-HP, and/or an ester of 3-HP, such as methyl hydroxypropionate, ethyl hydroxypropionate, propyl hydroxypropionate, and/or butyl hydroxypropionate. Accordingly, the invention also provides methods of producing one or more of these products. These methods involve culturing the cell that has lactyl-CoA dehydratase activity and 3-hydroxypropionyl-CoA dehydratase activity under conditions that allow the product to be produced. These cells also can have CoA synthetase activity and/or poly hydroxyacid synthase activity.

Another aspect of the invention provides cells that have CoA synthetase activity, lactyl-CoA dehydratase activity, and poly hydroxyacid synthase activity. In some embodiments, these cells also can contain an exogenous nucleic acid molecule that encodes one or more of the following polypeptides: a polypeptide having E1 activator activity; an E2α polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; an E2β polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; a polypeptide having CoA synthetase activity; and a polypeptide having poly hydroxyacid synthase activity.

In another embodiment of the invention, the cell that has CoA synthetase activity, lactyl-CoA dehydratase activity, and poly hydroxyacid synthase activity can produce a product, for example, polymerized acrylate.

Another aspect of the invention provides a cell comprising CoA transferase activity, lactyl-CoA dehydratase activity, and lipase activity. In some embodiments, the cell also can contain an exogenous nucleic acid molecule that encodes one or more of the following polypeptides: a polypeptide having CoA transferase activity; a polypeptide having E1 activator

activity; an E2α polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; an E2β polypeptide that is a subunit of an enzyme having lactyl-CoA dehydratase activity; and a polypeptide having lipase activity. This cell can be used, among other things, to produce products such as esters of acrylate (e.g., methyl acrylate, ethyl acrylate, propyl acrylate, and butyl acrylate).

In some embodiments, 1,3 propanediol can be created from either 3-HP-CoA or 3-HP via the use of polypeptides having enzymatic activity. These polypeptides can be used either in vitro or in vivo. When converting 3-HP-CoA to 1,3 propanediol, polypeptides having oxidoreductase activity or reductase activity (e.g., enzymes from the 1.1.1.-class of enzymes) can be used. Alternatively, when creating 1,3 propanediol from 3-HP, a combination of (1) a polypeptide having aldehyde dehydrogenase activity (e.g., an enzyme from the 1.1.1.34 class) and (2) a polypeptide having alcohol dehydrogenase activity (e.g., an enzyme from the 1.1.1.32 class) can be used.

In some embodiments of the invention, products are produced in vitro (outside of a cell). In other embodiments of the invention, products are produced using a combination of in vitro and in vivo (within a cell) methods. In yet other embodiments of the invention, products are produced in vivo. For methods involving in vivo steps, the cells can be isolated cultured cells or whole organisms such as transgenic plants, non-human mammals, or single-celled organisms such as yeast and bacteria (e.g., *Lactobacillus, Lactococcus, Bacillus*, and *Escherichia* cells). Hereinafter such cells are referred to as production cells. Products produced by these production cells can be organic products such as 3-HP and/or the nucleic acid molecules and polypeptides described herein.

Another aspect of the invention provides polypeptides having an amino acid sequence that (1) is set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161, (2) is at least 10 contiguous amino acid residues of a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161, (3) has at least 65 percent sequence identity with at least 10 contiguous amino acid residues of a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161, (4) is a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 having conservative amino acid substitutions, or (5) has at least 65 percent sequence identity with a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. Accordingly, the invention also provides nucleic acid sequences that encode any of the polypeptides described herein as well as specific binding agents that bind to any of the polypeptides described herein. Likewise, the invention provides transformed cells that contain any of the nucleic acid sequences that encode any of the polypeptides described herein. These cells can be used to produce nucleic acid molecules, polypeptides, and organic compounds. The polypeptides can be used to catalyze the formation of organic compounds or can be used as antigens to create specific binding agents.

In yet another embodiment, the invention provides isolated nucleic acid molecules that contain at least one of the following nucleic acid sequences: (1) a nucleic acid sequence as set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163; (2) a nucleic acid sequence having at least 10 consecutive nucleotides from a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163; (3) a nucleic acid sequence that hybridizes under hybridization conditions (e.g., moderately or highly stringent hybridization conditions) to a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163; (4) a nucleic acid sequence having 65 percent sequence identity with at least 10 consecutive nucleotides from a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163; and (5) a nucleic acid sequence having at least 65 percent sequence identity with a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. Accordingly, the invention also provides a production cell that contains at least one exogenous nucleic acid having any of the nucleic acid sequences provided above. The production cell can be used to express polypeptides that have an enzymatic activity such as CoA transferase activity, lactyl-CoA dehydratase activity, CoA synthase activity, dehydratase activity, dehydrogenase activity, malonyl CoA reductase activity, β-alanine ammonia lyase activity, and/or 3-hydroxypropionyl-CoA dehydratase activity. Accordingly, the invention also provides methods of producing polypeptides encoded by the nucleic acid sequences described above.

The invention also provides several methods such as methods for making 3-HP from lactate, phosphoenolpyruvate (PEP), or pyruvate. In some embodiments, methods for making 3-HP from lactate, PEP, or pyruvate involve culturing a cell containing at least one exogenous nucleic acid under conditions that allow the cell to produce 3-HP. These methods can be practiced using the various types of production cells described herein. In some embodiments, the production cells can have one or more of the following activities: CoA transferase activity, 3-hydroxypropionyl-CoA hydrolase activity, 3-hydroxyisobutryl-CoA hydrolase activity, dehydratase activity, and/or malonyl CoA reductase activity.

In other embodiments, the methods involve making 3-HP wherein lactate is contacted with a first polypeptide having CoA transferase activity or CoA synthetase activity such that lactyl-CoA is formed, then contacting lactyl-CoA with a second polypeptide having lactyl-CoA dehydratase activity to form acrylyl-CoA, then contacting acrylyl-CoA with a third polypeptide having 3-hydroxypropionyl-CoA dehydratase activity to form 3-hydroxypropionic acid-CoA, and then contacting 3-hydroxypropionic acid-CoA with the first polypeptide to form 3-HP or with a fourth polypeptide having 3-hydroxypropionyl-CoA hydrolase activity or 3-hydroxyisobutryl-CoA hydrolase activity to form 3-HP.

Another aspect of the invention provides methods for making polymerized 3-HP. These methods involve making 3-hydroxypropionic acid-CoA as described above, and then contacting the 3-hydroxypropionic acid-CoA with a polypeptide having poly hydroxyacid synthase activity to form polymerized 3-HP.

In yet another embodiment of the invention, methods for making an ester of 3-HP are provided. These methods involve making 3-HP as described above, and then additionally contacting 3-HP with a fifth polypeptide having lipase activity to form an ester.

The invention also provides methods for making polymerized acrylate. These methods involve culturing a cell that has both CoA synthetase activity, lactyl-CoA dehydratase activity, and poly hydroxyacid synthase activity such that polymerized acrylate is made. Accordingly, the invention also provides methods of making polymerized acrylate wherein lactate is contacted with a first polypeptide having CoA synthetase activity to form lactyl-CoA, then contacting lactyl-CoA with a second polypeptide having lactyl-CoA dehydratase activity to form acrylyl-CoA, and then contacting acrylyl-CoA with a third polypeptide having poly hydroxyacid synthase activity to form polymerized acrylate.

The invention also provides methods of making an ester of acrylate. These methods involve culturing a cell that has CoA

transferase activity, lipase activity, and lactyl-CoA dehydratase activity under conditions that allow the cell to produce an ester.

In another embodiment, the invention provides methods for making an ester of acrylate, wherein acrylyl-CoA is formed as described above, and then acrylyl-CoA is contacted with a polypeptide having CoA transferase activity to form acrylate, and acrylate is contacted with a polypeptide having lipase activity to form the ester.

The invention also provides methods for making 3-HP. These methods involve culturing a cell containing at least one exogenous nucleic acid that encodes at least one polypeptide such that 3-HP is produced from acetyl-CoA or malonyl-CoA.

Alternative embodiments provide methods of making 3-HP, wherein acetyl-CoA is contacted with a first polypeptide having acetyl-CoA carboxylase activity to form malonyl-CoA, and malonyl-CoA is contacted with a second polypeptide having malonyl-CoA reductase activity to form 3-HP.

In other embodiments, malonyl-CoA can be contacted with a polypeptide having malonyl-CoA reductase activity so that 3-HP can be made.

In another embodiment, the invention provides a method for making 3-HP that uses a β-alanine intermediate. This method can be performed by contacting β-alanine CoA with a first polypeptide having β-alanyl-CoA ammonia lyase activity (such as a polypeptide having the amino acid sequence set forth in SEQ ID NO: 160 or 161) to form acrylyl-CoA, contacting acrylyl-CoA with a second polypeptide having 3-HP-CoA dehydratase activity to form 3-HP-CoA, and contacting 3-HP-CoA with a third polypeptide having glutamate dehydrogenase activity to make 3-HP.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. **1** is a diagram of a pathway for making 3-HP.

FIG. **2** is a diagram of a pathway for making polymerized 3-HP.

FIG. **3** is a diagram of a pathway for making esters of 3-HP.

FIG. **4** is a diagram of a pathway for making polymerized acrylic acid.

FIG. **5** is a diagram of a pathway for making esters of acrylate.

FIG. **6** is a listing of a nucleic acid sequence that encodes a polypeptide having CoA transferase activity (SEQ ID NO:1).

FIG. **7** is a listing of an amino acid sequence of a polypeptide having CoA transferase activity (SEQ ID NO:2).

FIGS. **8**A-**8**D show an alignment of the nucleic acid sequences set forth in SEQ ID NOs:1, 3, 4, and 5.

FIGS. **9**A-**9**B show an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 6, 7, and 8.

FIG. **10** is a listing of a nucleic acid sequence that encodes a polypeptide having E1 activator activity (SEQ ID NO:9).

FIG. **11** is a listing of an amino acid sequence of a polypeptide having E1 activator activity (SEQ ID NO:10).

FIGS. **12**A-**12**B show an alignment of the nucleic acid sequences set forth in SEQ ID NOs:9, 11, 12, and 13.

FIG. **13** is an alignment of the amino acid sequences set forth in SEQ ID NOs:10, 14, 15, and 16.

FIG. **14** is a listing of a nucleic acid sequence that encodes an E2α subunit of an enzyme having lactyl-CoA dehydratase activity (SEQ ID NO:17).

FIG. **15** is a listing of an amino acid sequence of an E2α subunit of an enzyme having lactyl-CoA dehydratase activity (SEQ ID NO:18).

FIGS. **16**A-**16**C show an alignment of the nucleic acid sequences set forth in SEQ ID NOs:17, 19, 20, and 21.

FIG. **17** is an alignment of the amino acid sequences set forth in SEQ ID NOs:18, 22, 23, and 24.

FIG. **18** is a listing of a nucleic acid sequence that encodes an E2β subunit of an enzyme having lactyl-CoA dehydratase activity (SEQ ID NO:25). The "G" at position 443 can be an "A"; and the "A" at position 571 can be a "G".

FIG. **19** is a listing of an amino acid sequence of an E2β subunit of an enzyme having lactyl-CoA dehydratase activity (SEQ ID NO:26).

FIGS. **20**A-**20**C show an alignment of the nucleic acid sequences set forth in SEQ ID NOs:25, 27, 28, and 29.

FIG. **21** is an alignment of the amino acid sequences set forth in SEQ ID NOs:26, 30, 31, and 32.

FIGS. **22**A-**22**B show a listing of a nucleic acid sequence of genomic DNA from *Megasphaera elsdenii* (SEQ ID NO:33).

FIG. **23** is a listing of a nucleic acid sequence that encodes a polypeptide from *Megasphaera elsdenii* (SEQ ID NO:34).

FIG. **24** is a listing of an amino acid sequence of a polypeptide from *Megasphaera elsdenii* (SEQ ID NO:35).

FIG. **25** is a listing of a nucleic acid sequence that encodes a polypeptide having enzymatic activity (SEQ ID NO:36).

FIG. **26** is a listing of an amino acid sequence of a polypeptide having enzymatic activity (SEQ ID NO:37).

FIGS. **27**A-**27**B show a listing of a nucleic acid sequence that contains non-coding as well as coding sequence of a polypeptide having CoA synthase, dehydratase, and dehydrogenase activity (SEQ ID NO:38). The start site for the coding sequence is at position 480, a ribosome binding site is at position 466-473, and the stop codon is at position 5946.

FIG. **28** is a listing of an amino acid sequence from a polypeptide having CoA synthase, dehydratase, and dehydrogenase activity (SEQ ID NO:39).

FIG. **29** is a listing of a nucleic acid sequence that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity (SEQ ID NO:40).

FIG. **30** is a listing of an amino acid sequence of a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity (SEQ ID NO:41).

FIG. **31** is a listing of a nucleic acid sequence that contains non-coding as well as coding sequence of a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity (SEQ ID NO:42).

FIGS. **32**A-**32**B show an alignment of the nucleic acid sequences set forth in SEQ ID NOs:40, 43, 44, and 45.

FIG. **33** is an alignment of the amino acid sequences set forth in SEQ ID NOs:41, 46, 47, and 48.

FIG. **34** is a diagram of the construction of a synthetic operon (pTDH) that encodes for polypeptides having CoA transferase activity, lactyl-CoA dehydratase activity (E1, E2α, and E2β), and 3-hydroxypropionyl-CoA dehydratase activity (3-HP-CoA dehydratase).

FIGS. **35**A and B is a diagram of the construction of a synthetic operon (pHTD) that encodes for polypeptides having CoA transferase activity, lactyl-CoA dehydratase activity (E1, E2α, and E2β), and 3-hydroxypropionyl-CoA dehydratase activity (3-HP-CoA dehydratase).

FIGS. **36**A and B is a diagram of the construction of a synthetic operon (pEIITHrEI) that encodes for polypeptides having CoA transferase activity, lactyl-CoA dehydratase activity (E1, E2α, and E2β), and 3-hydroxypropionyl-CoA dehydratase activity (3-HP-CoA dehydratase).

FIGS. **37**A and B is a diagram of the construction of a synthetic operon (pEIITHEI) that encodes for polypeptides having CoA transferase activity, lactyl-CoA dehydratase activity (E1, E2α, and E2β), and 3-hydroxypropionyl-CoA dehydratase activity (3-HP-CoA dehydratase).

FIGS. **38**A and B is a diagram of the construction of two plasmids, pEIITH and pPROEI. The pEIITH plasmid encodes polypeptides having CoA transferase activity, lactyl-CoA dehydratase activity (E2α and E2β), and 3-hydroxypropionyl-CoA dehydratase activity (3-HP-CoA dehydratase), and the pPROEI plasmid encodes a polypeptide having E1 activator activity.

FIGS. **39**A-**39**B show a listing of a nucleic acid sequence that encodes a polypeptide having CoA synthase, dehydratase, and dehydrogenase activity (SEQ ID NO:129).

FIGS. **40**A-**40**D show an alignment of the amino acid sequences set forth in SEQ ID NOs:39, 130, and 131. The uppercase amino acid residues represent positions where that amino acid residue is present in two or more sequences.

FIGS. **41**A-**41**D show an alignment of the amino acid sequences set forth in SEQ ID NOs:39, 132, and 133. The uppercase amino acid residues represent positions where that amino acid residue is present in two or more sequences.

FIGS. **42**A-**42**D show an alignment of the amino acid sequences set forth in SEQ ID NOs: 39, 134, and 135. The uppercase amino acid residues represent positions where that amino acid residue is present in two or more sequences.

FIG. **43** is a diagram of several pathways for making organic compounds using the multifunctional OS17 enzyme.

FIG. **44** is a diagram of a pathway for making 3-HP via acetyl-CoA and malonyl-CoA.

FIG. **45** is a diagram of pMSD8, pET30a/acc1, pFN476, and PET286 constructs.

FIGS. **46**A-**46**F show a total ion chromatogram and five mass spectrums of Coenzyme A thioesters. (A) is total ion chromatogram illustrating the separation of Coenzyme A and four CoA-organic thioesters: 1=Coenzyme A, 2=lactyl-CoA, 3=acetyl-CoA, 4=acrylyl-CoA, 5=propionyl-CoA. (B) is a mass spectrum of Coenzyme A. (C) is a mass spectrum of lactyl-CoA. (D) is a mass spectrum of acetyl-CoA. (E) is a mass spectrum of acrylyl-CoA. (F) is a mass spectrum of propionyl-CoA.

FIGS. **47**A-**47**B show ion chromatograms and mass spectrums. (A) is a total ion chromatogram of a mixture of lactyl-CoA and 3-HP-CoA. The insert is the mass spectrum recorded under peak 1. (B) is a total ion chromatogram of lactyl-CoA. The insert is the mass spectrum recorded under peak 2. In each panel, peak 1 is 3-HP-CoA, and peak 2 is lactyl-CoA. The peak labeled with an asterisk was confirmed not to be a CoA ester.

FIGS. **48**A-**48**B show ion chromatograms and mass spectrums. (A) is a total ion chromatogram of CoA esters derived from a broth produced by *E. coli* transfected with pEIITHrEI. The insert is the mass spectrum recorded under peak 1. (B) is a total ion chromatogram of CoA esters derived from a broth produced by control *E. coli* not transfected with pEIITHrEI. The insert is the mass spectrum recorded under peak 2. In each panel, peak 1 is 3-HP-CoA, and peak 2 is lactyl-CoA. The peaks labeled with an asterisk were confirmed not to be a CoA ester.

FIGS. **49**A-**49**B show a listing of a nucleic acid sequence that encodes a polypeptide having malonyl-CoA reductase activity (SEQ ID NO: 140).

FIG. **50** is a listing of an amino acid sequence of a polypeptide having malonyl-CoA reductase activity (SEQ ID NO:141).

FIG. **51** is a listing of a nucleic acid sequence that encodes a portion of a polypeptide having malonyl-CoA reductase activity (SEQ ID NO:142).

FIGS. **52**A-**52**D show is an alignment of the amino acid sequences set forth in SEQ ID NOs: 141, 143, 144, 145, 146, and 147.

FIGS. **53**A-**53**J show an alignment of the nucleic acid sequences set forth in SEQ ID NOs: 140, 148, 149, 150, 151, and 152.

FIG. **54** is a diagram of a pathway for making 3-HP via a β-alanine intermediate.

FIG. **55** is a diagram of a pathway for making 3-HP via a β-alanine intermediate.

FIG. **56** is a listing of an amino acid sequence of a polypeptide having β-alanyl-CoA ammonia lyase activity (SEQ ID NO:160).

FIG. **57** is a listing of an amino acid sequence of a polypeptide having β-alanyl-CoA ammonia lyase activity (SEQ ID NO:161).

FIG. **58** is a listing of a nucleic acid sequence that encodes a polypeptide having β-alanyl-CoA ammonia lyase activity (SEQ ID NO:162).

FIG. **59** is a listing of a nucleic acid sequence that can encode a polypeptide having β-alanyl-CoA ammonia lyase activity (SEQ ID NO:163).

DETAILED DESCRIPTION

I. Terms

Nucleic acid: The term "nucleic acid" as used herein encompasses both RNA and DNA including, without limitation, cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

Isolated: The term "isolated" as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term "isolated" as used herein with reference to nucleic acid also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

Exogenous: The term "exogenous" as used herein with reference to nucleic acid and a particular cell refers to any nucleic acid that does not originate from that particular cell as found in nature. Thus, non-naturally-occurring nucleic acid is considered to be exogenous to a cell once introduced into the cell. Nucleic acid that is naturally-occurring also can be exogenous to a particular cell. For example, an entire chromosome isolated from a cell of person X is an exogenous nucleic acid with respect to a cell of person Y once that chromosome is introduced into Y's cell.

Hybridization: The term "hybridization" as used herein refers to a method of testing for complementarity in the nucleotide sequence of two nucleic acid molecules, based on the ability of complementary single-stranded DNA and/or RNA to form a duplex molecule. Nucleic acid hybridization techniques can be used to obtain an isolated nucleic acid within the scope of the invention. Briefly, any nucleic acid having some homology to a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163 can be used as a probe to identify a similar nucleic acid by hybridization under conditions of moderate to high stringency. Once identified, the nucleic acid then can be purified, sequenced, and analyzed to determine whether it is within the scope of the invention as described herein.

Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The probe can be labeled with a biotin, digoxygenin, an enzyme, or a radioisotope such as ^{32}P. The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., (1989) Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Plainview, N.Y. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in SEQ ID NO: 1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, or 142 can be used to identify an identical or similar nucleic acid. In addition, probes longer or shorter than 20 nucleotides can be used.

The invention also provides isolated nucleic acid sequences that are at least about 12 bases in length (e.g., at least about 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, or 5000 bases in length) and hybridize, under hybridization conditions, to the sense or antisense strand of a nucleic acid having the sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. The hybridization conditions can be moderately or highly stringent hybridization conditions.

For the purpose of this invention, moderately stringent hybridization conditions mean the hybridization is performed at about 42° C. in a hybridization solution containing 25 mM KPO_4 (pH 7.4), 5×SSC, 5×Denhardt's solution, 50 μg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about 5×10^7 cpm/μg), while the washes are performed at about 50° C. with a wash solution containing 2×SSC and 0.1% sodium dodecyl sulfate.

Highly stringent hybridization conditions mean the hybridization is performed at about 42° C. in a hybridization solution containing 25 mM KPO_4 (pH 7.4), 5×SSC, 5×Denhardt's solution, 50 μg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about 5×10^7 cpm/μg), while the washes are performed at about 65° C. with a wash solution containing 0.2×SSC and 0.1% sodium dodecyl sulfate.

Purified: The term "purified" as used herein does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide or nucleic acid preparation can be one in which the subject polypeptide or nucleic acid, respectively, is at a higher concentration than the polypeptide or nucleic acid would be in its natural environment within an organism. For example, a polypeptide preparation can be considered purified if the polypeptide content in the preparation represents at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 98%, or 99% of the total protein content of the preparation.

Transformed: A "transformed" cell is a cell into which a nucleic acid molecule has been introduced by, for example, molecular biology techniques. As used herein, the term "transformation" encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell including, without limitation, transfection with a viral vector, conjugation, transformation with a plasmid vector, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Recombinant: A "recombinant" nucleic acid is one having (1) a sequence that is not naturally occurring in the organism in which it is expressed or (2) a sequence made by an artificial combination of two otherwise-separated, shorter sequences. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. "Recombinant" is also used to describe nucleic acid molecules that have been artificially manipulated, but contain the same regulatory sequences and coding regions that are found in the organism from which the nucleic acid was isolated.

Specific binding agent: A "specific binding agent" is an agent that is capable of specifically binding to any of the polypeptides described herein, and can include polyclonal antibodies, monoclonal antibodies (including humanized monoclonal antibodies), and fragments of monoclonal antibodies such as Fab, F(ab')2, and Fv fragments as well as any other agent capable of specifically binding to an epitope of such polypeptides.

Antibodies to the polypeptides provided herein (or fragments thereof) can be used to purify or identify such polypeptides. The amino acid and nucleic acid sequences provided

herein allow for the production of specific antibody-based binding agents that recognize the polypeptides described herein.

Monoclonal or polyclonal antibodies can be produced to the polypeptides, portions of the polypeptides, or variants thereof. Optimally, antibodies raised against one or more epitopes on a polypeptide antigen will specifically detect that polypeptide. That is, antibodies raised against one particular polypeptide would recognize and bind that particular polypeptide, and would not substantially recognize or bind to other polypeptides. The determination that an antibody specifically binds to a particular polypeptide is made by any one of a number of standard immunoassay methods; for instance, Western blotting (See, e.g., Sambrook et al. (ed.), Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

To determine that a given antibody preparation (such as a preparation produced in a mouse against a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2) specifically detects the appropriate polypeptide (e.g., a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2) by Western blotting, total cellular protein can be extracted from cells and separated by SDS-polyacrylamide gel electrophoresis. The separated total cellular protein can then be transferred to a membrane (e.g., nitrocellulose), and the antibody preparation incubated with the membrane. After washing the membrane to remove non-specifically bound antibodies, the presence of specifically bound antibodies can be detected using an appropriate secondary antibody (e.g., an anti-mouse antibody) conjugated to an enzyme such as alkaline phosphatase since application of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a densely blue-colored compound by immuno-localized alkaline phosphatase.

Substantially pure polypeptides suitable for use as an immunogen can be obtained from transfected cells, transformed cells, or wild-type cells. Polypeptide concentrations in the final preparation can be adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms per milliliter. In addition, polypeptides ranging in size from full-length polypeptides to polypeptides having as few as nine amino acid residues can be utilized as immunogens. Such polypeptides can be produced in cell culture, can be chemically synthesized using standard methods, or can be obtained by cleaving large polypeptides into smaller polypeptides that can be purified. Polypeptides having as few as nine amino acid residues in length can be immunogenic when presented to an immune system in the context of a Major Histocompatibility Complex (MHC) molecule such as an MHC class I or MHC class II molecule. Accordingly, polypeptides having at least 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 900, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or more consecutive amino acid residues of any amino acid sequence disclosed herein can be used as immunogens for producing antibodies.

Monoclonal antibodies to any of the polypeptides disclosed herein can be prepared from murine hybridomas according to the classic method of Kohler & Milstein (Nature 256:495 (1975)) or a derivative method thereof.

Polyclonal antiserum containing antibodies to the heterogeneous epitopes of any polypeptide disclosed herein can be prepared by immunizing suitable animals with the polypeptide (or fragment thereof), which can be unmodified or modified to enhance immunogenicity. An effective immunization protocol for rabbits can be found in Vaitukaitis et al. (*J. Clin. Endocrinol. Metab.* 33:988-991 (1971)).

Antibody fragments can be used in place of whole antibodies and can be readily expressed in prokaryotic host cells. Methods of making and using immunologically effective portions of monoclonal antibodies, also referred to as "antibody fragments," are well known and include those described in Better & Horowitz (*Methods Enzymol.* 178:476-496 (1989)), Glockshuber et al. (*Biochemistry* 29:1362-1367 (1990)), U.S. Pat. No. 5,648,237 ("Expression of Functional Antibody Fragments"), U.S. Pat. No. 4,946,778 ("Single Polypeptide Chain Binding Molecules"), U.S. Pat. No. 5,455,030 ("Immunotherapy Using Single Chain Polypeptide Binding Molecules"), and references cited therein.

Operably linked: A first nucleic acid sequence is "operably linked" with a second nucleic acid sequence whenever the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two polypeptide-coding regions, in the same reading frame.

Probes and primers: Nucleic acid probes and primers can be prepared readily based on the amino acid sequences and nucleic acid sequences provided herein. A "probe" includes an isolated nucleic acid containing a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed in, for example, Sambrook et al. (ed.), Molecular Cloning: A Laboratory Manual 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, and Ausubel et al. (ed.) Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York (with periodic updates), 1987.

"Primers" are typically nucleic acid molecules having ten or more nucleotides (e.g., nucleic acid molecules having between about 10 nucleotides and about 100 nucleotides). A primer can be annealed to a complementary target nucleic acid strand by nucleic acid hybridization to form a hybrid between the primer and the target nucleic acid strand, and then extended along the target nucleic acid strand by, for example, a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, for example, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Methods for preparing and using probes and primers are described, for example, in references such as Sambrook et al. (ed.), Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; Ausubel et al. (ed.), Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York (with periodic updates), 1987; and Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, .COPYRGT. 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). One of skill in the art will appreciate that the specificity of a particular probe or primer increases with the length, but that a probe or primer can range in size from a full-length sequence to sequences as short as five consecutive nucleotides. Thus, for example, a primer of 20 consecutive nucleotides can anneal to a target with a higher specificity than a corresponding primer of only

15 nucleotides. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise, for example, 10, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 2000, 2050, 2100, 2150, 2200, 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2600, 2650, 2700, 2750, 2800, 2850, 2900, 3000, 3050, 3100, 3150, 3200, 3250, 3300, 3350, 3400, 3450, 3500, 3550, 3600, 3650, 3700, 3750, 3800, 3850, 3900, 4000, 4050, 4100, 4150, 4200, 4250, 4300, 4350, 4400, 4450, 4500, 4550, 4600, 4650, 4700, 4750, 4800, 4850, 4900, 5000, 5050, 5100, 5150, 5200, 5250, 5300, 5350, 5400, 5450, or more consecutive nucleotides.

Percent sequence identity: The "percent sequence identity" between a particular nucleic acid or amino acid sequence and a sequence referenced by a particular sequence identification number is determined as follows. First, a nucleic acid or amino acid sequence is compared to the sequence set forth in a particular sequence identification number using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (www.fr.com) or the United States government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. To compare two nucleic acid sequences, the options are set as follows: -i is set to a file containing the first nucleic acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second nucleic acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastn; -o is set to any desired file name (e.g., C:\output.txt); -q is set to −1; -r is set to 2; and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two sequences: C:\Bl2seq c:\seq1.txt -j c:\seq2.txt -p blastn -o c:\output.txt -q −1 -r 2. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity is determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence (e.g., SEQ ID NO:1), or by an articulated length (e.g., 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100. For example, a nucleic acid sequence that has 1166 matches when aligned with the sequence set forth in SEQ ID NO:1 is 75.0 percent identical to the sequence set forth in SEQ ID NO:1 (i.e., 1166÷1554*100=75.0). It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 is rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 is rounded up to 75.2. It is also noted that the length value will always be an integer. In another example, a target sequence containing a 20-nucleotide region that aligns with 20 consecutive nucleotides from an identified sequence as follows contains a region that shares 75 percent sequence identity to that identified sequence (i.e., 15÷20*100=75).

```
                    1                   20
Target Sequence:    AGGTCGTGTACTGTCAGTCA
                    | || ||| |||| |||| |
Identified Sequence: ACGTGGTGAACTGCCAGTGA
```

Conservative substitution: The term "conservative substitution" as used herein refers to any of the amino acid substitutions set forth in Table 1. Typically, conservative substitutions have little to no impact on the activity of a polypeptide. A polypeptide can be produced to contain one or more conservative substitutions by manipulating the nucleotide sequence that encodes that polypeptide using, for example, standard procedures such as site-directed mutagenesis or PCR.

TABLE 1

Original Residue	Conservative Substitution(s)
Ala	ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

II. Metabolic Pathways

The invention provides methods and materials related to producing 3-HP as well as other organic compounds (e.g., 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, and esters of 3-HP). Specifically, the invention provides isolated nucleic acids, polypeptides, host cells, and methods and materials for producing 3-HP as well as other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, polymerized 3-HP, and esters of 3-HP.

Accordingly, the invention provides several metabolic pathways that can be used to produce organic compounds from PEP (FIGS. **1-5**, **43-44**, **54**, and **55**). As depicted in FIG. **1**, lactate can be converted into lactyl-CoA by a polypeptide having CoA transferase activity (EC 2.8.3.1); the resulting lactyl-CoA can be converted into acrylyl-CoA by a polypep-

tide (or multiple polypeptide complex such as an activated E2α and E2β complex) having lactyl-CoA dehydratase activity (EC 4.2.1.54); the resulting acrylyl-CoA can be converted into 3-hydroxypropionyl-CoA (3-HP-CoA) by a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity (EC 4.2.1.-); and the resulting 3-HP-CoA can be converted into 3-HP by a polypeptide having CoA transferase activity, a polypeptide having 3-hydroxypropionyl-CoA hydrolase activity (EC 3.1.2.-), or a polypeptide having 3-hydroxyisobutryl-CoA hydrolase activity (EC 3.1.2.4).

Polypeptides having CoA transferase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Megasphaera elsdenii, Clostridium propionicum, Clostridium kluyveri*, and *Escherichia coli*. For example, nucleic acid that encodes a polypeptide having CoA transferase activity can be obtained from *Megasphaera elsdenii* as described in Example 1 and can have a sequence as set forth in SEQ ID NO: 1. In addition, polypeptides having CoA transferase activity as well as nucleic acid encoding such polypeptides can be obtained as described herein. For example, the variations to SEQ ID NO: 1 provided herein can be used to encode a polypeptide having CoA transferase activity.

Polypeptides (or the polypeptides of a multiple polypeptide complex such as an activated E2α and E2β complex) having lactyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Megasphaera elsdenii* and *Clostridium propionicum*. For example, nucleic acid encoding an E1 activator, an E2α subunit, and an E2β subunit that can form a multiple polypeptide complex having lactyl-CoA dehydratase activity can be obtained from *Megasphaera elsdenii* as described in Example 2. The nucleic acid encoding the E1 activator can contain a sequence as set forth in SEQ ID NO: 9; the nucleic acid encoding the E2α subunit can contain a sequence as set forth in SEQ ID NO: 17; and the nucleic acid encoding the E2β subunit can contain a sequence as set forth in SEQ ID NO: 25. In addition, polypeptides (or the polypeptides of a multiple polypeptide complex) having lactyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides can be obtained as described herein. For example, the variations to SEQ ID NO: 9, 17, and 25 provided herein can be used to encode the polypeptides of a multiple polypeptide complex having CoA transferase activity.

Polypeptides having 3-hydroxypropionyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Chloroflexus aurantiacus, Candida rugosa, Rhodosprillium rubrum*, and *Rhodobacter capsulates*. For example, nucleic acid that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity can be obtained from *Chloroflexus aurantiacus* as described in Example 3 and can have a sequence as set forth in SEQ ID NO: 40. In addition, polypeptides having 3-hydroxypropionyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides can be obtained as described herein. For example, the variations to SEQ ID NO: 40 provided herein can be used to encode a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity.

Polypeptides having 3-hydroxypropionyl-CoA hydrolase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Candida rugosa*. Polypeptides having 3-hydroxyisobutryl-CoA hydrolase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Pseudomonas fluorescens, rattus*, and *homo sapiens*. For example, nucleic acid that encodes a polypeptide having 3-hydroxyisobutryl-CoA hydrolase activity can be obtained from *homo sapiens* and can have a sequence as set forth in GenBank® accession number U66669.

The term "polypeptide having enzymatic activity" as used herein refers to any polypeptide that catalyzes a chemical reaction of other substances without itself being destroyed or altered upon completion of the reaction. Typically, a polypeptide having enzymatic activity catalyzes the formation of one or more products from one or more substrates. Such polypeptides can have any type of enzymatic activity including, without limitation, the enzymatic activity or enzymatic activities associated with enzymes such as dehydratases/hydratases, 3-hydroxypropionyl-CoA dehydratases/hydratases, CoA transferases, lactyl-CoA dehydratases, 3-hydroxypropionyl-CoA hydrolases, 3-hydroxyisobutryl-CoA hydrolases, poly hydroxyacid synthases, CoA synthetases, malonyl-CoA reductases, 3-alanine ammonia lyases, and lipases.

As depicted in FIG. **2**, lactate can be converted into lactyl-CoA by a polypeptide having CoA synthetase activity (EC 6.2.1.-); the resulting lactyl-CoA can be converted into acrylyl-CoA by a polypeptide (or multiple polypeptide complex) having lactyl-CoA dehydratase activity; the resulting acrylyl-CoA can be converted into 3-HP-CoA by a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity; and the resulting 3-HP-CoA can be converted into polymerized 3-HP by a polypeptide having poly hydroxyacid synthase activity (EC 2.3.1.-). Polypeptides having CoA synthetase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Escherichia coli, Rhodobacter sphaeroides, Saccharomyces cervisiae*, and *Salmonella enterica*. For example, nucleic acid that encodes a polypeptide having CoA synthetase activity can be obtained from *Escherichia coli* and can have a sequence as set forth in GenBank® accession number U00006. Polypeptides (or multiple polypeptide complexes) having lactyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides can be obtained as provided herein. Polypeptides having 3-hydroxypropionyl-CoA dehydratase activity as well as nucleic acid encoding such polypeptides also can be obtained as provided herein. Polypeptides having poly hydroxyacid synthase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Rhodobacter sphaeroides, Comamonas acidororans, Ralstonia eutropha*, and *Pseudomonas oleovorans*. For example, nucleic acid that encodes a polypeptide having poly hydroxyacid synthase activity can be obtained from *Rhodobacter sphaeroides* and can have a sequence as set forth in GenBank® accession number X97200.

As depicted in FIG. **3**, lactate can be converted into lactyl-CoA by a polypeptide having CoA transferase activity; the resulting lactyl-CoA can be converted into acrylyl-CoA by a polypeptide (or multiple polypeptide complex) having lactyl-CoA dehydratase activity; the resulting acrylyl-CoA can be converted into 3-HP-CoA by a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity; the resulting 3-HP-CoA can be converted into 3-HP by a polypeptide having CoA transferase activity, a polypeptide having 3-hydroxypropionyl-CoA hydrolase activity, or a polypeptide having 3-hydroxyisobutryl-CoA hydrolase activity; and the resulting 3-HP can be converted into an ester of 3-HP by a polypeptide having lipase activity (EC 3.1.1.-). Polypeptides having lipase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Candida rugosa, Candida tropicalis*, and *Candida*

albicans. For example, nucleic acid that encodes a polypeptide having lipase activity can be obtained from *Candida rugosa* and can have a sequence as set forth in GenBank® accession number A81171.

As depicted in FIG. **4**, lactate can be converted into lactyl-CoA by a polypeptide having CoA synthetase activity; the resulting lactyl-CoA can be converted into acrylyl-CoA by a polypeptide (or multiple polypeptide complex) having lactyl-CoA dehydratase activity; and the resulting acrylyl-CoA can be converted into polymerized acrylate by a polypeptide having poly hydroxyacid synthase activity.

As depicted in FIG. **5**, lactate can be converted into lactyl-CoA by a polypeptide having CoA transferase activity; the resulting lactyl-CoA can be converted into acrylyl-CoA by a polypeptide (or multiple polypeptide complex) having lactyl-CoA dehydratase activity; the resulting acrylyl-CoA can be converted into acrylate by a polypeptide having CoA transferase activity; and the resulting acrylate can be converted into an ester of acrylate by a polypeptide having lipase activity.

As depicted in FIG. **44**, acetyl-CoA can be converted into malonyl-CoA by a polypeptide having acetyl-CoA carboxylase activity, and the resulting malonyl-CoA can be converted into 3-HP by a polypeptide having malonyl-CoA reductase activity. Polypeptides having acetyl-CoA carboxylase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Escherichia coli* and *Chloroflexus aurantiacus*. For example, nucleic acid that encodes a polypeptide having acetyl-CoA carboxylase activity can be obtained from *Escherichia coli* and can have a sequence as set forth in GenBank® accession number M96394 or U18997. Polypeptides having malonyl-CoA reductase activity as well as nucleic acid encoding such polypeptides can be obtained from various species including, without limitation, *Chloroflexus aurantiacus, Sulfolobus metacillus*, and *Acidianus brierleyi*. For example, nucleic acid that encodes a polypeptide having malonyl-CoA reductase activity can be obtained as described herein and can have a sequence similar to the sequence set forth in SEQ ID NO: 140. In addition, polypeptides having malonyl-CoA reductase activity as well as nucleic acid encoding such polypeptides can be obtained as described herein. For example, the variations to SEQ ID NO: 140 provided herein can be used to encode a polypeptide having malonyl-CoA reductase activity.

Polypeptides having malonyl-CoA reductase activity can use NADPH as a co-factor. For example, the polypeptide having the amino acid sequence set forth in SEQ ID NO: 141 is a polypeptide having malonyl-CoA reductase activity that uses NADPH as a co-factor when converting malonyl-CoA into 3-HP. Likewise, polypeptides having malonyl-CoA reductase activity can use NADH as a co-factor. Such polypeptides can be obtained by converting a polypeptide that has malonyl-CoA reductase, activity and uses NADPH as a cofactor into a polypeptide that has malonyl-CoA reductase activity and uses NADH as a cofactor. Any method can be used to convert a polypeptide that uses NADPH as a cofactor into a polypeptide that uses NADH as a cofactor such as those described by others (Eppink et al., *J. Mol. Biol.*, 292(1):87-96 (1999), Hall and Tomsett, *Microbiology*, 146(Pt 6):1399-406 (2000), and Dohr et al., *Proc. Natl. Acad. Sci.*, 98(1):81-86 (2001)). For example, mutagenesis can be used to convert the polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO: 140 into a polypeptide that, when converting malonyl-CoA into 3-HP, uses NADH as a co-factor instead of NADPH.

As depicted in FIG. **43**, propionate can be converted into propionyl-CoA by a polypeptide having CoA synthetase activity such as the polypeptide having the sequence set forth in SEQ ID NO: 39; the resulting propionyl-CoA can be converted into acrylyl-CoA by a polypeptide having dehydrogenase activity such as the polypeptide having the sequence set forth in SEQ ID NO: 39; and the resulting acrylyl-CoA can be converted into (1) acrylate by a polypeptide having CoA transferase activity or CoA hydrolase activity, (2) 3-HP-CoA by a polypeptide having 3-HP dehydratase activity (also referred to as acrylyl-CoA hydratase or simply hydratase) such as the polypeptide having the sequence set forth in SEQ ID NO:39, or (3) polymerized acrylate by a polypeptide having poly hydroxyacid synthase activity. The resulting acrylate can be converted into an ester of acrylate by a polypeptide having lipase activity. The resulting 3-HP-CoA can be converted into (1) 3-HP by a polypeptide having CoA transferase activity, a polypeptide having 3-hydroxypropionyl-CoA hydrolase activity (EC 3.1.2.-), or a polypeptide having 3-hydroxyisobutyryl-CoA hydrolase activity (EC 3.1.2.4), or (2) polymerized 3-HP by a polypeptide having poly hydroxyacid synthase activity (EC 2.3.1.-).

As depicted in FIG. **54**, PEP can be converted into β-alanine. β-alanine can be converted into β-alanyl-CoA through the use of a polypeptide having CoA transferase activity. β-alanyl-CoA can then be converted into acrylyl-CoA through the use of a polypeptide having β-alanyl-CoA ammonia lyase activity. Acrylyl-CoA can then be converted into 3-HP-CoA through the use of a polypeptide having 3-HP-CoA dehydratase activity, and a polypeptide having glutamate dehydrogenase activity can be used to convert 3-HP-CoA into 3-HP.

As depicted in FIG. **55**, 3-HP can be made from β-alanine by first contacting β-alanine with a polypeptide having 4,4-aminobutyrate aminotransferase activity to create malonate semialdehyde. The malonate semialdehyde can be converted into 3-HP with a polypeptide having 3-HP dehydrogenase activity or a polypeptide having 3-hydroxyisobutyrate dehydrogenase activity.

III. Nucleic Acid Molecules and Polypeptides

The invention provides isolated nucleic acid that contains the entire nucleic acid sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. In addition, the invention provides isolated nucleic acid that contains a portion of the nucleic acid sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. For example, the invention provides isolated nucleic acid that contains a 15 nucleotide sequence identical to any 15 nucleotide sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163 including, without limitation, the sequence starting at nucleotide number 1 and ending at nucleotide number 15, the sequence starting at nucleotide number 2 and ending at nucleotide number 16, the sequence starting at nucleotide number 3 and ending at nucleotide number 17, and so forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleotide sequence that is greater than 15 nucleotides (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more nucleotides) in length and identical to any portion of the sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. For example, the invention provides isolated nucleic acid that contains a 25 nucleotide sequence identical to any 25 nucleotide sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163 including, without

limitation, the sequence starting at nucleotide number 1 and ending at nucleotide number 25, the sequence starting at nucleotide number 2 and ending at nucleotide number 26, the sequence starting at nucleotide number 3 and ending at nucleotide number 27, and so forth. Additional examples include, without limitation, isolated nucleic acids that contain a nucleotide sequence that is 50 or more nucleotides (e.g., 100, 150, 200, 250, 300, or more nucleotides) in length and identical to any portion of the sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. Such isolated nucleic acids can include, without limitation, those isolated nucleic acids containing a nucleic acid sequence represented in a single line of sequence depicted in FIG. **6**, **10**, **14**, **18**, **22**, **23**, **25**, **27**, **29**, **31**, **39**, **49**, or **51** since each line of sequence depicted in these figures, with the possible exception of the last line, provides a nucleotide sequence containing at least 50 bases.

In addition, the invention provides isolated nucleic acid that contains a variation of the nucleic acid sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. For example, the invention provides isolated nucleic acid containing a nucleic acid sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). Such isolated nucleic acid molecules can share at least 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99 percent sequence identity with a sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163.

The invention provides multiple examples of isolated nucleic acid that contains a variation of a nucleic acid sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. For example, FIGS. **8**A-**8**D provide the sequence set forth in SEQ ID NO:1 aligned with three other nucleic acid sequences. Examples of variations of the sequence set forth in SEQ ID NO:1 include, without limitation, any variation of the sequence set forth in SEQ ID NO:1 provided in FIGS. **8**A-**8**D. Such variations are provided in FIGS. **8**A-**8**D in that a comparison of the nucleotide (or lack thereof) at a particular position of the sequence set forth in SEQ ID NO:1 with the nucleotide (or lack thereof) at the same aligned position of any of the other three nucleic acid sequences depicted in FIGS. **8**A-**8**D (i.e., SEQ ID NOs:3, 4, and 5) provides a list of specific changes for the sequence set forth in SEQ ID NO:1. For example, the "a" at position 49 of SEQ ID NO:1 can be substituted with an "c" as indicated in FIGS. **8**A-**8**D. As also indicated in FIGS. **8**A-**8**D, the "a" at position 590 of SEQ ID NO:1 can be substituted with a "atgg"; an "aaac" can be inserted before the "g" at position 393 of SEQ ID NO:1; or the "gaa" at position 736 of SEQ ID NO:1 can be deleted. It will be appreciated that the sequence set forth in SEQ ID NO:1 can contain any number of variations as well as any combination of types of variations. For example, the sequence set forth in SEQ ID NO:1 can contain one variation provided in FIGS. **8**A-**8**D or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in FIGS. **8**A-**8**D. It is noted that the nucleic acid sequences provided by FIGS. **8**A-**8**D can encode polypeptides having CoA transferase activity. The invention also provides isolated nucleic acid that contains a variant of a portion of the sequence set forth in SEQ ID NO:1 as depicted in FIGS. **8**A-**8**D and described herein.

Likewise, FIGS. **12**A-**12**B provide variations of SEQ ID NO:9 and portions thereof; FIGS. **16**A-**16**C provide variations of SEQ ID NO:17 and portions thereof; FIGS. **20**A-**20**C provide variations of SEQ ID NO:25 and portions thereof; FIGS. **32**A-**32**B provide variations of SEQ ID NO:40 and portions thereof; and FIGS. **53**A-**53**J provide variations of SEQ ID NO:140.

The invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes the entire amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a portion of the amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 15 amino acid sequence identical to any 15 amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 25 amino acid sequence identical to any 25 amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, isolated nucleic acids that contain a nucleic acid sequence that encodes an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, or more amino acid residues) in length and identical to any portion of the sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. Such isolated nucleic acids can include, without limitation, those isolated nucleic acids containing a nucleic acid sequence that encodes an amino acid sequence represented in a single line of sequence depicted in FIG. **7**, **11**, **15**, **19**, **24**, **26**, **28**, **30**, or **50** since each line of sequence depicted in these figures, with the possible exception of the last line, provides an amino acid sequence containing at least 50 residues.

In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence having a variation of the amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). Such isolated nucleic acid molecules can contain a nucleic acid sequence encoding an amino acid sequence that shares at least 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99 percent sequence identity with a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161.

The invention provides multiple examples of isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence having a variation of an amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, FIGS. **9**A-**9**B provide the amino acid sequence set forth in SEQ ID NO:2 aligned with three other amino acid sequences. Examples of variations of the sequence set forth in SEQ ID NO:2 include, without limitation, any variation of the sequence set forth in SEQ ID NO:2 provided in FIGS. **9**A-**9**B. Such variations are provided in FIGS. **9**A-**9**B in that a comparison of the amino acid residue (or lack thereof) at a particular position of the sequence set forth in SEQ ID NO:2 with the amino acid residue (or lack thereof) at the same aligned position of any of the other three amino acid sequences of FIGS. **9**A-**9**B (i.e., SEQ ID NOs:6, 7, and 8) provides a list of specific changes for the sequence set forth in SEQ ID NO:2. For example, the "k" at position 17 of SEQ ID NO:2 can be substituted with a "p" or "h" as indicated in FIGS. **9**A-**9**B. As also indicated in FIGS. **9**A-**9**B, the "v" at position 125 of SEQ ID NO:2 can be substituted with an "i" or "f". It will be appreciated that the sequence set forth in SEQ ID NO:2 can contain any number of variations as well as any combination of types of variations. For example, the sequence set forth in SEQ ID NO:2 can contain one variation provided in FIGS. **9**A-**9**B or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in FIGS. **9**A-**9**B. It is noted that the amino acid sequences provided in FIGS. **9**A-**9**B can be polypeptides having CoA transferase activity.

The invention also provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence that contains a variant of a portion of the sequence set forth in SEQ ID NO:2 as depicted in FIGS. **9**A-**9**B and described herein.

Likewise, FIG. **13** provides variations of SEQ ID NO:10 and portions thereof; FIG. **17** provides variations of SEQ ID NO:18 and portions thereof; FIG. **21** provides variations of SEQ ID NO:26 and portions thereof; FIG. **33** provides variations of SEQ ID NO:41 and portions thereof; FIGS. **40**, **41**, and **42** provide variations of SEQ ID NO:39; and FIGS. **52**A-**2**D provide variations of SEQ ID NO:141 and portions thereof.

It is noted that codon preferences and codon usage tables for a particular species can be used to engineer isolated nucleic acid molecules that take advantage of the codon usage preferences of that particular species. For example, the isolated nucleic acid provided herein can be designed to have codons that are preferentially used by a particular organism of interest.

The invention also provides isolated nucleic acid that is at least about 12 bases in length (e.g., at least about 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, or 5000 bases in length) and hybridizes, under hybridization conditions, to the sense or antisense strand of a nucleic acid having the sequence set forth in SEQ ID NO:1, 9, 17, 25, 33, 34, 36, 38, 40, 42, 129, 140, 142, 162, or 163. The hybridization conditions can be moderately or highly stringent hybridization conditions.

The invention provides polypeptides that contain the entire amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. In addition, the invention provides polypeptides that contain a portion of the amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides polypeptides that contain a 15 amino acid sequence identical to any 15 amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides polypeptides that contain an amino acid sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides polypeptides that contain a 25 amino acid sequence identical to any 25 amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, polypeptides that contain an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, or more amino acid residues) in length and identical to any portion of the sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. Such polypeptides can include, without limitation, those polypeptides containing an amino acid sequence represented in a single line of sequence depicted in FIG. **7**, **11**, **15**, **19**, **24**, **26**, **28**, **30**, or **50** since each line of sequence depicted in these figures, with the possible exception of the last line, provides an amino acid sequence containing at least 50 residues.

In addition, the invention provides polypeptides that have an amino acid sequence having a variation of the amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, the invention provides polypeptides containing an amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). Such polypeptides can contain an amino acid sequence that shares at least 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99 percent sequence identity with a sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161.

The invention provides multiple examples of polypeptides containing an amino acid sequence having a variation of an amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161. For example, FIGS. **9**A-**9**B provides the amino acid sequence set forth in SEQ ID NO:2 aligned with three other amino acid sequences. Examples of variations of the sequence set forth in SEQ ID NO:2 include, without limitation, any variation of the sequence set forth in SEQ ID NO:2 provided in FIGS. **9**A-**9**B. Such variations are provided in FIGS. **9**A-**9**B in that a comparison of the amino acid residue (or lack thereof) at a particular position of the sequence set forth in SEQ ID NO:2 with the amino acid residue (or lack thereof) at the same aligned position of any of the other three amino acid sequences of FIGS. **9**A-**9**B (i.e., SEQ ID NOs:6, 7, and 8) provides a list of specific changes for the sequence set forth in SEQ ID NO:2. For example, the "k" at position 17 of SEQ ID NO:2 can be substituted with a "p" or "h" as indicated in FIGS. **9**A-**9**B. As also indicated in FIGS. **9**A-**9**B, the "v" at position 125 of SEQ ID NO:2 can be substituted with an "i" or "f". It will be appreciated that the sequence set forth in SEQ ID NO:2 can contain any number of

variations as well as any combination of types of variations. For example, the sequence set forth in SEQ ID NO:2 can contain one variation provided in FIGS. **9A-9B** or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in FIGS. **9A-9B**. It is noted that the amino acid sequences provided in FIGS. **9A-9B** can be polypeptides having CoA transferase activity.

The invention also provides polypeptides containing an amino acid sequence that contains a variant of a portion of the sequence set forth in SEQ ID NO:2 as depicted in FIGS. **9A-9B** and described herein.

Likewise, FIG. **13** provides variations of SEQ ID NO:10 and portions thereof; FIG. **17** provides variations of SEQ ID NO:18 and portions thereof; FIG. **21** provides variations of SEQ ID NO:26 and portions thereof; FIG. **33** provides variations of SEQ ID NO:41 and portions thereof; FIGS. **40**, **41**, and **42** provide variations of SEQ ID NO:39; and FIGS. **52A-52D** provide variations of SEQ ID NO:141 and portions thereof.

Polypeptides having a variant amino acid sequence can retain enzymatic activity. Such polypeptides can be produced by manipulating the nucleotide sequence encoding a polypeptide using standard procedures such as site-directed mutagenesis or PCR. One type of modification includes the substitution of one or more amino acid residues for amino acid residues having a similar biochemical property. For example, a polypeptide can have an amino acid sequence set forth in SEQ ID NO:2, 10, 18, 26, 35, 37, 39, 41, 141, 160, or 161 with one or more conservative substitutions.

More substantial changes can be obtained by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining: (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation; (b) the charge or hydrophobicity of the polypeptide at the target site; or (c) the bulk of the side chain. The substitutions that in general are expected to produce the greatest changes in polypeptide function are those in which: (a) a hydrophilic residue, e.g., serine or threonine, is substituted for (or by) a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, valine or alanine; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysine, arginine, or histidine, is substituted for (or by) an electronegative residue, e.g., glutamic acid or aspartic acid; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine. The effects of these amino acid substitutions (or other deletions or additions) can be assessed for polypeptides having enzymatic activity by analyzing the ability of the polypeptide to catalyze the conversion of the same substrate as the related native polypeptide to the same product as the related native polypeptide. Accordingly, polypeptides having 5, 10, 20, 30, 40, 50 or fewer conservative substitutions are provided by the invention.

Polypeptides and nucleic acid encoding polypeptide can be produced by standard DNA mutagenesis techniques, for example, M13 primer mutagenesis. Details of these techniques are provided in Sambrook et al. (ed.), Molecular Cloning: A Laboratory Manual 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, Ch. 15. Nucleic acid molecules can contain changes of a coding region to fit the codon usage bias of the particular organism into which the molecule is to be introduced.

Alternatively, the coding region can be altered by taking advantage of the degeneracy of the genetic code to alter the coding sequence in such a way that, while the nucleic acid sequence is substantially altered, it nevertheless encodes a polypeptide having an amino acid sequence identical or substantially similar to the native amino acid sequence. For example, the ninth amino acid residue of the sequence set forth in SEQ ID NO: 2 is alanine, which is encoded in the open reading frame by the nucleotide codon triplet GCT. Because of the degeneracy of the genetic code, three other nucleotide codon triplets—GCA, GCC, and GCG—also code for alanine. Thus, the nucleic acid sequence of the open reading frame can be changed at this position to any of these three codons without affecting the amino acid sequence of the encoded polypeptide or the characteristics of the polypeptide. Based upon the degeneracy of the genetic code, nucleic acid variants can be derived from a nucleic acid sequence disclosed herein using standard DNA mutagenesis techniques as described herein, or by synthesis of nucleic acid sequences. Thus, this invention also encompasses nucleic acid molecules that encode the same polypeptide but vary in nucleic acid sequence by virtue of the degeneracy of the genetic code.

IV. Methods of Making 3-HP and Other Organic Acids

Each step provided in the pathways depicted in FIGS. **1-5**, **43-44**, **54**, and **55** can be performed within a cell (in vivo) or outside a cell (in vitro, e.g., in a container or column). Additionally, the organic acid products can be generated through a combination of in vivo synthesis and in vitro synthesis. Moreover, the in vitro synthesis step, or steps, can be via chemical reaction or enzymatic reaction.

For example, a microorganism provided herein can be used to perform the steps provided in FIG. **1**, or an extract containing polypeptides having the indicated enzymatic activities can be used to perform the steps provided in FIG. **1**. In addition, chemical treatments can be used to perform the conversions provided in FIGS. **1-5**, **43-44**, **54**, and **55**. For example, acrylyl-CoA can be converted into acrylate by hydrolysis. Other chemical treatments include, without limitation, trans esterification to convert acrylate into an acrylate ester.

Carbon sources suitable as starting points for bioconversion include carbohydrates and synthetic intermediates. Examples of carbohydrates which cells are capable of metabolizing to pyruvate include sugars such as dextrose, triglycerides, and fatty acids.

Additionally, intermediate chemical products can be starting points. For example, acetic acid and carbon dioxide can be introduced into a fermentation broth. Acetyl-CoA, malonyl-CoA, and 3-HP can be sequentially produced using a polypeptide having CoA synthase activity, a polypeptide having acetyl-CoA carboxylase activity, and a polypeptide having malonyl-CoA reductase activity. Other useful intermediate chemical starting points can include propionic acid, acrylic acid, lactic acid, pyruvic acid, and β-alanine.

A. Expression of Polypeptides

The polypeptides described herein can be produced individually in a host cell or in combination in a host cell. Moreover, the polypeptides having a particular enzymatic activity can be a polypeptide that is either naturally-occurring or non-naturally-occurring. A naturally-occurring polypeptide is any polypeptide having an amino acid sequence as found in nature, including wild-type and polymorphic polypeptides. Such naturally-occurring polypeptides can be obtained from any species including, without limitation, animal (e.g., mammalian), plant, fungal, and bacterial species. A non-naturally-occurring polypeptide is any polypeptide having an amino acid sequence that is not found in nature. Thus, a non-natu-

rally-occurring polypeptide can be a mutated version of a naturally-occurring polypeptide, or an engineered polypeptide. For example, a non-naturally-occurring polypeptide having 3-hydroxypropionyl-CoA dehydratase activity can be a mutated version of a naturally-occurring polypeptide having 3-hydroxypropionyl-CoA dehydratase activity that retains at least some 3-hydroxypropionyl-CoA dehydratase activity. A polypeptide can be mutated by, for example, sequence additions, deletions, substitutions, or combinations thereof.

The invention provides genetically modified cells that can be used to perform one or more steps of the steps in the metabolic pathways described herein or the genetically modified cells can be used to produce the disclosed polypeptides for subsequent use in vitro. For example, an individual microorganism can contain exogenous nucleic acid such that each of the polypeptides necessary to perform the steps depicted in FIG. **1**, **2**, **3**, **4**, **5**, **43**, **44**, **54**, or **55** are expressed. It is important to note that such cells can contain any number of exogenous nucleic acid molecules. For example, a particular cell can contain six exogenous nucleic acid molecules with each one encoding one of the six polypeptides necessary to convert lactate into 3-HP as depicted in FIG. **1**, or a particular cell can endogenously produce polypeptides necessary to convert lactate into acrylyl-CoA while containing exogenous nucleic acid that encodes polypeptides necessary to convert acrylyl-CoA into 3-HP.

In addition, a single exogenous nucleic acid molecule can encode one or more than one polypeptide. For example, a single exogenous nucleic acid molecule can contain sequences that encode three different polypeptides. Further, the cells described herein can contain a single copy, or multiple copies (e.g., about 5, 10, 20, 35, 50, 75, 100 or 150 copies), of a particular exogenous nucleic acid molecule. For example, a particular cell can contain about 50 copies of the constructs depicted in FIG. **34**, **35**, **36**, **37**, **38**, or **45**. Again, the cells described herein can contain more than one particular exogenous nucleic acid molecule. For example, a particular cell can contain about 50 copies of exogenous nucleic acid molecule X as well as about 75 copies of exogenous nucleic acid molecule Y.

In another embodiment, a cell within the scope of the invention can contain an exogenous nucleic acid molecule that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity. Such cells can have any level of 3-hydroxypropionyl-CoA dehydratase activity. For example, a cell containing an exogenous nucleic acid molecule that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity can have 3-hydroxypropionyl-CoA dehydratase activity with a specific activity greater than about 1 mg 3-HP-CoA formed per gram dry cell weight per hour (e.g., greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 200, 250, 300, 350, 400, 500, or more mg 3-HP-CoA formed per gram dry cell weight per hour). Alternatively, a cell can have 3-hydroxypropionyl-CoA dehydratase activity such that a cell extract from 1×10^6 cells has a specific activity greater than about 1 μg 3-HP-CoA formed per mg total protein per 10 minutes (e.g., greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 200, 250, 300, 350, 400, 500, or more μg 3-HP-CoA formed per mg total protein per 10 minutes).

A nucleic acid molecule encoding a polypeptide having enzymatic activity can be identified and obtained using any method such as those described herein. For example, nucleic acid molecules that encode a polypeptide having enzymatic activity can be identified and obtained using common molecular cloning or chemical nucleic acid synthesis procedures and techniques, including PCR. In addition, standard nucleic acid sequencing techniques and software programs that translate nucleic acid sequences into amino acid sequences based on the genetic code can be used to determine whether or not a particular nucleic acid has any sequence homology with known enzymatic polypeptides. Sequence alignment software such as MEGALIGN® (DNASTAR, Madison, Wis., 1997) can be used to compare various sequences. In addition, nucleic acid molecules encoding known enzymatic polypeptides can be mutated using common molecular cloning techniques (e.g., site-directed mutagenesis). Possible mutations include, without limitation, deletions, insertions, and base substitutions, as well as combinations of deletions, insertions, and base substitutions. Further, nucleic acid and amino acid databases (e.g., GenBank®) can be used to identify a nucleic acid sequence that encodes a polypeptide having enzymatic activity. Briefly, any amino acid sequence having some homology to a polypeptide having enzymatic activity, or any nucleic acid sequence having some homology to a sequence encoding a polypeptide having enzymatic activity can be used as a query to search GenBank®. The identified polypeptides then can be analyzed to determine whether or not they exhibit enzymatic activity.

In addition, nucleic acid hybridization techniques can be used to identify and obtain a nucleic acid molecule that encodes a polypeptide having enzymatic activity. Briefly, any nucleic acid molecule that encodes a known enzymatic polypeptide, or fragment thereof, can be used as a probe to identify similar nucleic acid molecules by hybridization under conditions of moderate to high stringency. Such similar nucleic acid molecules then can be isolated, sequenced, and analyzed to determine whether the encoded polypeptide has enzymatic activity.

Expression cloning techniques also can be used to identify and obtain a nucleic acid molecule that encodes a polypeptide having enzymatic activity. For example, a substrate known to interact with a particular enzymatic polypeptide can be used to screen a phage display library containing that enzymatic polypeptide. Phage display libraries can be generated as described elsewhere (Burritt et al., *Anal. Biochem.* 238:1-13 (1990)), or can be obtained from commercial suppliers such as Novagen (Madison, Wis.).

Further, polypeptide sequencing techniques can be used to identify and obtain a nucleic acid molecule that encodes a polypeptide having enzymatic activity. For example, a purified polypeptide can be separated by gel electrophoresis, and its amino acid sequence determined by, for example, amino acid microsequencing techniques. Once determined, the amino acid sequence can be used to design degenerate oligonucleotide primers. Degenerate oligonucleotide primers can be used to obtain the nucleic acid encoding the polypeptide by PCR. Once obtained, the nucleic acid can be sequenced, cloned into an appropriate expression vector, and introduced into a microorganism.

Any method can be used to introduce an exogenous nucleic acid molecule into a cell. In fact, many methods for introducing nucleic acid into microorganisms such as bacteria and yeast are well known to those skilled in the art. For example, heat shock, lipofection, electroporation, conjugation, fusion of protoplasts, and biolistic delivery are common methods for introducing nucleic acid into bacteria and yeast cells. See, e.g., Ito et al., *J. Bacterol.* 153:163-168 (1983); Durrens et al., *Curr. Genet.* 18:7-12 (1990); and Becker and Guarente, *Methods in Enzymology* 194:182-187 (1991).

An exogenous nucleic acid molecule contained within a particular cell of the invention can be maintained within that cell in any form. For example, exogenous nucleic acid mol-

ecules can be integrated into the genome of the cell or maintained in an episomal state. In other words, a cell of the invention can be a stable or transient transformant. Again, a microorganism described herein can contain a single copy, or multiple copies (e.g., about 5, 10, 20, 35, 50, 75, 100 or 150 copies), of a particular exogenous nucleic acid molecule as described herein.

Methods for expressing an amino acid sequence from an exogenous nucleic acid molecule are well known to those skilled in the art. Such methods include, without limitation, constructing a nucleic acid such that a regulatory element promotes the expression of a nucleic acid sequence that encodes a polypeptide. Typically, regulatory elements are DNA sequences that regulate the expression of other DNA sequences at the level of transcription. Thus, regulatory elements include, without limitation, promoters, enhancers, and the like. Any type of promoter can be used to express an amino acid sequence from an exogenous nucleic acid molecule. Examples of promoters include, without limitation, constitutive promoters, tissue-specific promoters, and promoters responsive or unresponsive to a particular stimulus (e.g., light, oxygen, chemical concentration, and the like). Moreover, methods for expressing a polypeptide from an exogenous nucleic acid molecule in cells such as bacterial cells and yeast cells are well known to those skilled in the art. For example, nucleic acid constructs that are capable of expressing exogenous polypeptides within *E. coli* are well known. See, e.g., Sambrook et al., Molecular cloning: a laboratory manual, Cold Spring Harbour Laboratory Press, New York, USA, second edition (1989).

B. Production of Organic Acids and Related Products Via Host Cells

The nucleic acid and amino acid sequences provided herein can be used with cells to produce 3-HP and/or other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, esters of 3-HP, and polymerized 3-HP. Such cells can be from any species including those listed within the taxonomy web pages at the National Institute of Health sponsored by the United States government (www.ncbi.nlm.nih.gov). The cells can be eukaryotic or prokaryotic. For example, genetically modified cells of the invention can be mammalian cells (e.g., human, murine, and bovine cells), plant cells (e.g., corn, wheat, rice, and soybean cells), fungal cells (e.g., *Aspergillus* and *Rhizopus* cells), yeast cells, or bacterial cells (e.g., *Lactobacillus, Lactococcus, Bacillus, Escherichia*, and *Clostridium* cells). A cell of the invention also can be a microorganism. The term "microorganism" as used herein refers to any microscopic organism including, without limitation, bacteria, algae, fungi, and protozoa. Thus, *E. coli, S. cerevisiae, Kluveromyces lactis, Candida blankii, Candida rugosa*, and *Pichia postoris* are considered microorganisms and can be used as described herein.

Typically, a cell of the invention is genetically modified such that a particular organic compound is produced. In one embodiment, the invention provides cells that make 3-HP from PEP. Examples of biosynthetic pathways that can be used by cells to make 3-HP are shown in FIGS. **1-5**, **43-44**, **54**, and **55**.

Generally, cells that are genetically modified to synthesize a particular organic compound contain one or more exogenous nucleic acid molecules that encode polypeptides having specific enzymatic activities. For example, a microorganism can contain exogenous nucleic acid that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity. In this case, acrylyl-CoA can be converted into 3-hydroxypropionic acid-CoA which can lead to the production of 3-HP. It is noted that a cell can be given an exogenous nucleic acid molecule that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound not normally produced by that cell. Alternatively, a cell can be given an exogenous nucleic acid molecule that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound that is normally produced by that cell. In this case, the genetically modified cell can produce more of the compound, or can produce the compound more efficiently, than a similar cell not having the genetic modification.

In one embodiment, the invention provides a cell containing an exogenous nucleic acid molecule that encodes a polypeptide having enzymatic activity that leads to the formation of 3-HP. It is noted that the produced 3-HP can be secreted from the cell, eliminating the need to disrupt cell membranes to retrieve the organic compound. Typically, the cell of the invention produces 3-HP with the concentration being at least about 100 mg per L (e.g., at least about 1 g/L, 5 g/L, 10 g/L, 25 g/L, 50 g/L, 75 g/L, 80 g/L, 90 g/L, 100 g/L, or 120 g/L). When determining the yield of an organic compound such as 3-HP for a particular cell, any method can be used. See, e.g., *Applied Environmental Microbiology* 59(12): 4261-4265 (1993). Typically, a cell within the scope of the invention such as a microorganism catabolizes a hexose carbon source such as glucose. A cell, however, can catabolize a variety of carbon sources such as pentose sugars (e.g., ribose, arabinose, xylose, and lyxose), fatty acids, acetate, or glycerols. In other words, a cell within the scope of the invention can utilize a variety of carbon sources.

As described herein, a cell within the scope of the invention can contain an exogenous nucleic acid molecule that encodes a polypeptide having enzymatic activity that leads to the formation of 3-HP or other organic compounds such as 1,3-propanediol, acrylic acid, poly-acrylate, acrylate-esters, 3-HP-esters, and poly-3-HP. Methods of identifying cells that contain exogenous nucleic acid are well known to those skilled in the art. Such methods include, without limitation, PCR and nucleic acid hybridization techniques such as Northern and Southern analysis (see hybridization described herein). In some cases, immunohisto-chemistry and biochemical techniques can be used to determine if a cell contains a particular nucleic acid by detecting the expression of the polypeptide encoded by that particular nucleic acid molecule. For example, an antibody having specificity for a polypeptide can be used to determine whether or not a particular cell contains nucleic acid encoding that polypeptide. Further, biochemical techniques can be used to determine if a cell contains a particular nucleic acid molecule encoding a polypeptide having enzymatic activity by detecting an organic product produced as a result of the expression of the polypeptide having enzymatic activity. For example, detection of 3-HP after introduction of exogenous nucleic acid that encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity into a cell that does not normally express such a polypeptide can indicate that that cell not only contains the introduced exogenous nucleic acid molecule but also expresses the encoded polypeptide from that introduced exogenous nucleic acid molecule. Methods for detecting specific enzymatic activities or the presence of particular organic products are well known to those skilled in the art. For example, the presence of an organic compound such as 3-HP can be determined as described elsewhere. See, Sullivan and Clarke, *J. Assoc. Offic. Agr. Chemists,* 38:514-518 (1955).

C. Cells with Reduced Polypeptide Activity

The invention also provides genetically modified cells having reduced polypeptide activity. The term "reduced" as used

herein with respect to a cell and a particular polypeptide's activity refers to a lower level of activity than that measured in a comparable cell of the same species. For example, a particular microorganism lacking enzymatic activity X is considered to have reduced enzymatic activity X if a comparable microorganism has at least some enzymatic activity X. It is noted that a cell can have the activity of any type of polypeptide reduced including, without limitation, enzymes, transcription factors, transporters, receptors, signal molecules, and the like. For example, a cell can contain an exogenous nucleic acid molecule that disrupts a regulatory and/or coding sequence of a polypeptide having pyruvate decarboxylase activity or alcohol dehydrogenase activity. Disrupting pyruvate decarboxylase and/or alcohol dehydrogenase expression can lead to the accumulation of lactate as well as products produced from lactate such as 3-HP, 1,3-propanediol, acrylic acid, poly-acrylate, acrylate-esters, 3-HP-esters, and poly-3-HP. It is also noted that reduced polypeptide activities can be the result of lower polypeptide concentration, lower specific activity of a polypeptide, or combinations thereof. Many different methods can be used to make a cell having reduced polypeptide activity. For example, a cell can be engineered to have a disrupted regulatory sequence or polypeptide-encoding sequence using common mutagenesis or knock-out technology. See, e.g., Methods in Yeast Genetics (1997 edition), Adams, Gottschling, Kaiser, and Sterns, Cold Spring Harbor Press (1998). Alternatively, antisense technology can be used to reduce the activity of a particular polypeptide. For example, a cell can be engineered to contain a cDNA that encodes an antisense molecule that prevents a polypeptide from being translated. The term "antisense molecule" as used herein encompasses any nucleic acid molecule or nucleic acid analog (e.g., peptide nucleic acids) that contains a sequence that corresponds to the coding strand of an endogenous polypeptide. An antisense molecule also can have flanking sequences (e.g., regulatory sequences). Thus, antisense molecules can be ribozymes or antisense oligonucleotides. A ribozyme can have any general structure including, without limitation, hairpin, hammerhead, or axhead structures, provided the molecule cleaves RNA. Further, gene silencing can be used to reduce the activity of a particular polypeptide.

A cell having reduced activity of a polypeptide can be identified using any method. For example, enzyme activity assays such as those described herein can be used to identify cells having a reduced enzyme activity.

A polypeptide having (1) the amino acid sequence set forth in SEQ ID NO:39 (the OS17 polypeptide) or (2) an amino acid sequence sharing at least about 60 percent sequence identity with the amino acid sequence set forth in SEQ ID NO:39 can have three functional domains: a domain having CoA-synthetase activity, a domain having 3-HP-CoA dehydratase activity, and a domain having CoA-reductase activity. Such polypeptides can be selectively modified by mutating and/or deleting domains such that one or two of the enzymatic activities are reduced. Reducing the dehydratase activity of the OS17 polypeptide can cause acrylyl-CoA to be created from propionyl-CoA. The acrylyl-CoA then can be contacted with a polypeptide having CoA hydrolase activity to produce acrylate from propionate (FIG. **43**). Similarly, acrylyl-CoA can be created from 3-HP by using, for example, an OS17 polypeptide having reduced reductase activity.

D. Production of Organic Acids and Related Products Via In Vitro Techniques

In addition, purified polypeptides having enzymatic activity can be used alone or in combination with cells to produce 3-HP or other organic compounds such as 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, esters of 3-HP, and polymerized 3-HP. For example, a preparation containing a substantially pure polypeptide having 3-hydroxypropionyl-CoA dehydratase activity can be used to catalyze the formation of 3-HP-CoA, a precursor to 3-HP. Further, cell-free extracts containing a polypeptide having enzymatic activity can be used alone or in combination with purified polypeptides and/or cells to produce 3-HP. For example, a cell-free extract containing a polypeptide having CoA transferase activity can be used to form lactyl-CoA, while a microorganism containing polypeptides having the enzymatic activities necessary to catalyze the reactions needed to form 3-HP from lactyl-CoA can be used to produce 3-HP. Any method can be used to produce a cell-free extract. For example, osmotic shock, sonication, and/or a repeated freeze-thaw cycle followed by filtration and/or centrifugation can be used to produce a cell-free extract from intact cells.

It is noted that a cell, purified polypeptide, and/or cell-free extract can be used to produce 3-HP that is, in turn, treated chemically to produce another compound. For example, a microorganism can be used to produce 3-HP, while a chemical process is used to modify 3-HP into a derivative such as polymerized 3-HP or an ester of 3-HP. Likewise, a chemical process can be used to produce a particular compound that is, in turn, converted into 3-HP or other organic compound (e.g., 1,3-propanediol, acrylic acid, polymerized acrylate, esters of acrylate, esters of 3-HP, and polymerized 3-HP) using a cell, substantially pure polypeptide, and/or cell-free extract described herein. For example, a chemical process can be used to produce acrylyl-CoA, while a microorganism can be used to convert acrylyl-CoA into 3-HP.

E. Fermentation of Cells to Produce Organic Acids

Typically, 3-HP is produced by providing a production cell, such as a microorganism, and culturing the microorganism with culture medium such that 3-HP is produced. In general, the culture media and/or culture conditions can be such that the microorganisms grow to an adequate density and produce 3-HP efficiently. For large-scale production processes, any method can be used such as those described elsewhere (Manual of Industrial Microbiology and Biotechnology, 2nd Edition, Editors: A. L. Demain and J. E. Davies, ASM Press; and Principles of Fermentation Technology, P. F. Stanbury and A. Whitaker, Pergamon). Briefly, a large tank (e.g., a 100 gallon, 200 gallon, 500 gallon, or more tank) containing appropriate culture medium with, for example, a glucose carbon source is inoculated with a particular microorganism. After inoculation, the microorganisms are incubated to allow biomass to be produced. Once a desired biomass is reached, the broth containing the microorganisms can be transferred to a second tank. This second tank can be any size. For example, the second tank can be larger, smaller, or the same size as the first tank. Typically, the second tank is larger than the first such that additional culture medium can be added to the broth from the first tank. In addition, the culture medium within this second tank can be the same as, or different from, that used in the first tank. For example, the first tank can contain medium with xylose, while the second tank contains medium with glucose.

Once transferred, the microorganisms can be incubated to allow for the production of 3-HP. Once produced, any method can be used to isolate the 3-HP. For example, common separation techniques can be used to remove the biomass from the broth, and common isolation procedures (e.g., extraction, distillation, and ion-exchange procedures) can be used to obtain the 3-HP from the microorganism-free broth. In addition, 3-HP can be isolated while it is being produced, or it can be isolated from the broth after the product production phase has been terminated.

F. Products Created from the Disclosed Biosynthetic Routes

The organic compounds produced from any of the steps provided in FIGS. **1-5**, **43-44**, **54**, and **55** can be chemically converted into other organic compounds. For example, 3-HP can be hydrogenated to form 1,3 propanediol, a valuable polyester monomer. Hydrogenating an organic acid such as 3-HP can be performed using any method such as those used to hydrogenate succinic acid and/or lactic acid. For example, 3-HP can be hydrogenated using a metal catalyst. In another example, 3-HP can be dehydrated to form acrylic acid. Any method can be used to perform a dehydration reaction. For example, 3-HP can be heated in the presence of a catalyst (e.g., a metal or mineral acid catalyst) to form acrylic acid. Propanediol also can be created using polypeptides having oxidoreductase activity (e.g., enzymes in the 1.1.1.-class of enzymes) in vitro or in vivo.

V. Overview of Methodology Used to Create Biosynthetic Pathways that Make 3-HP from PEP

The invention provides methods of making 3-HP and related products from PEP via the use of biosynthetic pathways. Illustrative examples include methods involving the production of 3-HP via a lactate intermediate, a malonyl-CoA intermediate, and a β-alanine intermediate.

A. Biosynthetic Pathway for Making 3-HP Through a Lactic Acid Intermediate

A biosynthetic pathway that allows for the production of 3-HP from PEP was constructed (FIG. **1**). This pathway involved using several polypeptides that were cloned and expressed as described herein. *M. elsdenii* cells (ATCC 17753) were used as a source of genomic DNA. Primers were used to identify and clone a nucleic acid sequence encoding a polypeptide having CoA transferase activity (SEQ ID NO: 1). The polypeptide was subsequently tested for enzymatic activity and found to have CoA transferase activity.

Similarly, PCR primers were used to identify nucleic acid sequences from *M. elsdenii* genomic DNA that encoded an E1 activator, E2α, and E2β polypeptides (SEQ ID NOs: 9, 17, and 25, respectively). These polypeptides were subsequently shown to have lactyl-CoA dehydratase activity.

Chloroflexus aurantiacus cells (ATCC 29365) were used as a source of genomic DNA. Initial cloning led to the identification of nucleic acid sequences: OS17 (SEQ ID NO: 129) and OS19 (SEQ ID NO: 40). Subsequence assays revealed that OS17 encodes a polypeptide having CoA synthase activity, dehydratase activity, and dehydrogenase activity (propionyl-CoA synthetase). Subsequence assays also revealed that OS19 encodes a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity (also referred to as acrylyl-CoA hydratase activity).

Several operons were constructed for use in *E. coli*. These operons allow for the production of 3-HP in bacterial cells. Additional experiments allowed for the expression of these polypeptide in yeast, which can be used to produce 3-HP.

B. Biosynthetic Pathway for Making 3-HP Through a Malonyl-CoA Intermediate

Another pathway leading to the production of 3-HP from PEP was constructed. This pathway used a polypeptide having acetyl CoA carboxylase activity that was isolated from *E. coli* (Example 9), and a polypeptide having malonyl-CoA reductase activity that was isolated from *Chloroflexus aurantiacus* (Example 10). The combination of these two polypeptides allows for the production of 3-HP from acetyl-CoA (FIG. **44**).

Nucleic acid encoding a polypeptide having malonyl-CoA reductase activity (SEQ ID NO:140) was cloned, sequenced, and expressed. The polypeptide having malonyl-CoA reductase activity was then used to make 3-HP.

C. Biosynthetic Pathways for Making 3-HP Through a β-Alanine Intermediate

In general, prokaryotes and eukaryotes metabolize glucose via the Embden-Meyerhof-Parnas pathway to PEP, a central metabolite in carbon metabolism. The PEP generated from glucose is either carboxylated to oxaloacetate or is converted to pyruvate. Carboxylation of PEP to oxaloacetate can be catalyzed by a polypeptide having PEP carboxylase activity, a polypeptide having PEP carboxykinase activity, or a polypeptide having PEP transcarboxylase activity. Pyruvate that is generated from PEP by a polypeptide having pyruvate kinase activity can also be converted to oxaloacetate by a polypeptide having pyruvate carboxylase activity.

Oxaloacetate generated either from PEP or pyruvate can act as a precursor for production of aspartic acid. This conversion can be carried out by a polypeptide having aspartate aminotransferase activity, which transfers an amino group from glutamate to oxaloacetate. Glutamate consumed in this reaction can be regenerated by the action of a polypeptide having glutamate dehydrogenase activity or by the action of a polypeptide having 4,4-aminobutyrate aminotransferase activity. The decarboxylation of aspartate to β-alanine is catalyzed by a polypeptide having aspartate decarboxylase activity. β-alanine produced through this biochemistry can be converted to 3-HP via two possible pathways. These pathways are provided in FIGS. **54** and **55**.

The steps involved in the production of β-alanine can be the same for both pathways. These steps can be accomplished by endogenous polypeptides in the host cells which convert PEP to β-alanine, or these steps can be accomplished with recombinant DNA technology using known polypeptides such as polypeptides having PEP-carboxykinase activity (4.1.1.32), aspartate aminotransferase activity (2.6.1.1), and aspartate alpha-decarboxylase activity (4.1.1.11).

As depicted in FIG. **54**, a polypeptide having CoA transferase activity (e.g., a polypeptide having a sequence set forth in SEQ ID NO:2) can be used to convert β-alanine to β-alanyl-CoA. β-alanyl-CoA can be converted to acrylyl-CoA via a polypeptide having β-alanyl-CoA ammonia lyase activity (e.g., a polypeptide having a sequence set forth in SEQ ID NO:160). Acrylyl-CoA can be converted to 3-HP-CoA using a polypeptide having 3-HP-CoA dehydratase activity (e.g., a polypeptide having a sequence set forth in SEQ ID NO:40). 3-HP-CoA can be converted into 3-HP via a polypeptide having CoA transferase activity (e.g., a polypeptide having a sequence set forth in SEQ ID NO:2).

As depicted in FIG. **55**, a polypeptide having 4,4-aminobutyrate aminotransferase activity (2.6.1.19) can be used to convert β-alanine into malonate semialdehyde. The malonate semialdehyde can be converted into 3-HP using either a polypeptide having 3-hydroxypropionate dehydrogenase activity (1.1.1.59) or a polypeptide having 3-hydroxyisobutyrate dehydrogenase activity.

EXAMPLES

Example 1

Cloning Nucleic Acid Molecules that Encode a Polypeptide Having CoA Transferase Activity

Genomic DNA was isolated from *Megasphaera elsdenii* cells (ATCC 17753) grown in 1053 Reinforced *Clostridium*

media under anaerobic conditions at 37° C. in roll tubes for 12-14 hours. Once grown, the cells were pelleted, washed with 5 mL of a 10 mM Tris solution, and repelleted. The pellet was resuspended in 1 mL of Gentra Cell Suspension Solution to which 14.2 mg of lysozyme and 4 μL of 20 mg/mL proteinase K solution was added. The cell suspension was incubated at 37° C. for 30 minutes. The genomic DNA was then isolated using a Gentra Genomic DNA Isolation Kit following the provided protocol. The precipitated genomic DNA was spooled and air-dried for 10 minutes. The genomic DNA was suspended in 500 μL of a 10 mM Tris solution and stored at 4° C.

Two degenerate forward (CoAF1 and CoAF2) and three degenerate reverse (CoAR1, CoAR2, and CoAR3) PCR primers were designed based on conserved acetoacetyl CoA transferase and propionate CoA transferase sequences (CoAF 1 5'-GAAWSCGGYSCNATYGGYGG-3', SEQ ID NO: 49; CoAF2 5'-TTYTGYG-GYRSBTTYACBGCWGG-3', SEQ ID NO: 50; CoAR1 5'-CCWGCVGTRAAV-SYRCCR-CARAA-3', SEQ ID NO: 51; CoAR2 5'-AARACDSM-RCGTTCVGTRA-TRTA-3', SEQ ID NO: 52; and CoAR3 5'-TCRAYRCCSGGWGCRAYTTC-3', SEQ ID NO: 53). The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.) and 1 ng of genomic DNA per μL reaction mix. PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 59° C., 4 cycles at 57° C., 4 cycles at 55° C., and 18 cycles at 52° C. Each cycle used an initial 30-second denaturing step at 94° C. and a 3 minute extension at 72° C. The program had an initial denaturing step for 2 minutes at 94° C. and a final extension step of 4 minutes at 72° C. Time allowed for annealing was 45 seconds. The amounts of PCR primer used in the reactions were increased 2-8 fold above typical PCR amounts depending on the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were made to identify PCR products resulting from single degenerate primers. Each PCR product (25 μL) was separated by electrophoresis using a 1% TAE (Tris-acetate-EDTA) agarose gel.

The CoAF1-CoAR2, CoAF1-CoAR3, CoAF2-CoAR2, and CoAF2-CoAR3 combinations produced a band of 423, 474, 177, and 228 bp, respectively. These bands matched the sizes based on other CoA transferase sequences. No band was visible from the individual primer control reactions. The CoAF1-CoAR3 fragment (474 bp) was isolated and purified using a Qiagen Gel Extraction Kit (Qiagen Inc., Valencia, Calif.). Four μL of the purified band was ligated into pCRII vector and transformed into TOP10 *E. coli* cells by heat-shock using a TOPO cloning procedure (Invitrogen, Carlsbad, Calif.). Transformations were plated on LB media containing 100 μg/mL of ampicillin (Amp) and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Single, white colonies were plated onto fresh media and screened in a PCR reaction using the CoAF1 and CoAR3 primers to confirm the presence of the insert.

Plasmid DNA obtained using a QiaPrep Spin Miniprep Kit (Qiagen, Inc) was quantified and used for DNA sequencing with M13R and M13F primers. Sequence analysis revealed that the CoAF1-CoAR3 fragment shared sequence similarity with acetoacetyl CoA transferase sequences.

Genome walking was performed to obtain the complete coding sequence. The following primers for genome walking in both upstream and downstream directions were designed using the portion of the 474 bp CoAF1-CoAR3 fragment sequence that was internal to the degenerate primers (COAGSP1F 5'-GAATGTTTACTTCTGCGG-CACCT-TCAC-3', SEQ ID NO:54; COAGSP2F 5'-GACCAGAT-CACTTTCAACG-GTTCCTATG-3', SEQ ID NO:55; COAGSP1R 5'-GCATAGGAACCGTTGAAA-GT-GATCTGG-3', SEQ ID NO:56; and COAGSP2R 5'-GTTAG-TACCGAACTTG-CTGACGTTGATG-3', SEQ ID NO:57). The COAGSP1F and COAGSP2F primers face downstream, while the COAGSP1R and COAGSP2R primers face upstream. In addition, the COAGSP2F and COAGSP2R primers are nested inside the COAGSP1F and COAGSP1R primers. Genome walking was performed using the Universal Genome Walking kit (ClonTech Laboratories, Inc., Palo Alto, Calif.) with the exception that additional libraries were generated with enzymes Nru I, Sca I, and Hinc II. First round PCR was conducted in a Perkin Elmer 2400 Thermocycler with 7 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 36 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. Second round PCR used 5 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 20 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. The first and second round product (20 μL) was separated by electrophoresis on a 1% TAE agarose gel. Amplification products were obtained with the Stu I library for the reverse direction. The second round product of 1.5 Kb from this library was gel purified, cloned, and sequenced. Sequence analysis revealed that the sequence derived from genome walking overlapped with the CoAF1-CoAR3 fragment and shared sequence similarity with other sequences such as acetoacetyl CoA transferase sequences (FIGS. **8-9**).

Nucleic acid encoding the CoA transferase (propionyl-CoA transferase or pct) from *Megasphaera elsdenii* was PCR amplified from chromosomal DNA using following PCR program: 25 cycles of 95° C. for 30 seconds to denature, 50° C. for 30 seconds to anneal, and 72° C. for 3 minutes for extension (plus 2 seconds per cycle). The primers used were designated PCT-1.114 (5'-ATGAGAAAAGTAGAAATCAT-TAC-3'; SEQ ID NO:58) and PCT-2.2045 (5'-GGCGGAAGTTGACGATAATG-3'; SEQ ID NO:59). The resulting PCR product (about 2 kb as judged by agarose gel electrophoresis) was purified using a Qiagen PCR purification kit (Qiagen Inc., Valencia, Calif.). The purified product was ligated to pETBlue-1 using the Perfectly Blunt cloning Kit (Novagen, Madison, Wis.). The ligation reaction was transformed into NovaBlue chemically competent cells (Novagen, Madison, Wis.) that were spread on LB agar plates supplemented with 50 μg/mL carbenicillin, 40 μg/mL IPTG, and 40 μg/mL X-Gal. White colonies were isolated and screened for the presence of inserts by restriction mapping. Isolates with the correct restriction pattern were sequenced from each end using the primers pETBlueUP and pETBlue-DOWN (Novagen) to confirm the sequence at the ligation points.

The plasmid was transformed into Tuner (DE3) pLacI chemically competent cells (Novagen, Madison, Wis.), and expression from the construct tested. Briefly, a culture was grown overnight to saturation and diluted 1:20 the following morning in fresh LB medium with the appropriate antibiotics. The culture was grown at 37° C. with aeration to an OD_{600} of about 0.6. The culture was induced with IPTG at a final concentration of 100 μM. The culture was incubated for an additional two hours at 37° C. with aeration. Aliquots were taken pre-induction and 2 hours post-induction for SDS-PAGE analysis. A band of the expected molecular weight (55,653 Daltons predicted from the sequence) was observed after IPTG treatment. This band was not observed in cells containing a plasmid lacking the nucleic acid encoding the transferase.

Cell free extracts were prepared to assess enzymatic activity. Briefly, the cells were harvested by centrifugation and disrupted by sonication. The sonicated cell suspension was centrifuged to remove cell debris, and the supernatant was used in the assays.

Transferase activity was measured in the following assay. The assay mixture used contained 100 mM potassium phosphate buffer (pH 7.0), 200 mM sodium acetate, 1 mM dithiobisnitrobenzoate (DTNB), 500 μM oxaloacetate, 25 μM CoA-ester substrate, and 3 μg/mL citrate synthase. If present, the CoA transferase transfers the CoA from the CoA ester to acetate to form acetyl-CoA. The added citrate synthase condenses oxaloacetate and acetyl-CoA to form citrate and free CoASH. The free CoASH complexes with DTNB, and the formation of this complex can be measured by a change in the optical density at 412 nm. The activity of the CoA transferase was measured using the following substrates: lactyl-CoA, propionyl-CoA, acrylyl-CoA, and 3-hydroxypropionyl-CoA. The units/mg of protein was calculated using the following formula:

$$(\Delta E/\text{min} * V_f * \text{dilution factor})/(V_S * 14.2) = \text{units/mL}$$

where ΔE/min is the change in absorbance per minute at 412 nm, V_f is the final volume of the reaction, and V_S is the volume of sample added. The total protein concentration of the cell free extract was about 1 mg/mL so the units/mL equals units/mg.

Cell free extracts from cells containing nucleic acid encoding the CoA transferase exhibited CoA transferase activity (Table 2). The observed CoA transferase activity was detected for the lactyl-CoA, propionyl-CoA, acrylyl-CoA, and 3-hydroxypropionyl-CoA substrates (Table 2). The highest CoA transferase activity was detected for lactyl-CoA and propionyl-CoA.

TABLE 2

Substrate	Units/mg
Lactyl-CoA	211
Propionyl-CoA	144
Acrylyl-CoA	118
3-Hydroxypropionyl-CoA	110

The following assay was performed to test whether the CoA transferase activity can use the same CoA substrate donors as recipients. Specifically, CoA transferase activity was assessed using a Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Voyager RP workstation (PerSeptive Biosystems). The following five reactions were analyzed:

1) acetate+lactyl-CoA→lactate+acetyl-CoA
2) acetate+propionyl-CoA→propionate+acetyl-CoA
3) lactate+acetyl-CoA→acetate+lactyl-CoA
4) lactate+acrylyl-CoA→acrylate+lactyl-CoA
5) 3-hydroxypropionate+lactyl-CoA→lactate+3-hydroxypropionyl-CoA

MALDI-TOF MS was used to measure simultaneously the appearance of the product CoA ester and the disappearance of the donor CoA ester. The assay buffer contained 50 mM potassium phosphate (pH 7.0), 1 mM CoA ester, and 100 mM respective acid salt. Protein from a cell free extract prepared as described above was added to a final concentration of 0.005 mg/mL. A control reaction was prepared from a cell free extract prepared from cells lacking the construct containing the CoA transferase-encoding nucleic acid. For each reaction, the cell free extract was added last to start the reaction. Reactions were allowed to proceed at room temperature and were stopped by adding 1 volume 10% trifluroacetic acid (TFA). The reaction mixtures were purified prior to MALDI-TOF MS analysis using Sep Pak Vac C_{18} 50 mg columns (Waters, Inc.). The columns were conditioned with 1 mL methanol and equilibrated with two washes of 1 mL 0.1% TFA. Each sample was applied to the column, and the flow through was discarded. The column was washed twice with 1 mL 0.1% TFA. The sample was eluted in 200 μL 40% acetonitrile, 0.1% TFA. The acetonitrile was removed by centrifugation in vacuo. Samples were prepared for MALDI-TOF MS analysis by mixing 1:1 with 110 mM sinapinic acid in 0.1% TFA, 67% acetonitrile. The samples were allowed to air dry.

In reaction #1, the control sample exhibited a main peak at a molecular weight corresponding to lactyl-CoA (MW 841). There was a minor peak at the molecular weight corresponding to acetyl-CoA (MW 811). This minor peak was determined to be the left-over acetyl-CoA from the synthesis of lactyl-CoA. The reaction #1 sample containing the cell extract from cells transfected with the CoA transferase-encoding plasmid exhibited complete conversion of lactyl-CoA to acetyl-CoA. No peak was observed for lactyl-CoA. This result indicates that the CoA transferase activity can transfer CoA from lactyl-CoA to acetate to form acetyl-CoA.

In reaction #2, the control sample exhibited a dominant peak at a molecular weight corresponding to propionyl-CoA (MW 825). The reaction #2 sample containing the cell extract from cells transfected with the CoA transferase-encoding plasmid exhibited a dominant peak at a molecular weight corresponding to acetyl-CoA (MW 811). No peak was observed for propionyl-CoA. This result indicates that the CoA transferase activity can transfer CoA from propionyl-CoA to acetate to form acetyl-CoA.

In reaction #3, the control sample exhibited a dominant peak at a molecular weight corresponding to acetyl-CoA (MW 811). The reaction #3 sample containing the cell extract from cells transfected with the CoA transferase-encoding plasmid exhibited a peak corresponding to lactyl-CoA (MW 841). The peak corresponding to acetyl-CoA did not disappear. In fact, the ratio of the size of the two peaks was about 1:1. The observed appearance of the peak corresponding to lactyl-CoA demonstrates that the CoA transferase activity catalyzes reaction #3.

In reaction #4, the control sample exhibited a dominant peak at a molecular weight corresponding to acrylyl-CoA (MW 823). The reaction #4 sample containing the cell extract from cells transfected with the CoA transferase-encoding plasmid exhibited a dominant peak corresponding to lactyl-CoA (MW 841). This result demonstrates that the CoA transferase activity catalyzes reaction #4.

In reaction #5, deuterated lactyl-CoA was used to detect the transfer of CoA from lactate to 3-hydroxypropionate since lactic acid and 3-HP have the same molecular weight as do their respective CoA esters. Using deuterated lactyl-CoA allowed for the differentiation between lactyl-CoA and 3-hydroxypropionate using MALDI-TOF MS. The control sample exhibited a diffuse group of peaks at molecular weights ranging from MW 841 to 845 due to the varying amounts of hydrogen atoms that were replaced with deuterium atoms. In addition, a significant peak was observed at a molecular weight corresponding to acetyl-CoA (MW 811). This peak was determined to be the left-over acetyl-CoA from the synthesis of lactyl-CoA. The reaction #5 sample containing the cell extract from cells transfected with the CoA transferase-encoding plasmid exhibited a dominant peak at a molecular weight corresponding to 3-hydroxypropionyl-

CoA (MW 841) as opposed to a group of peaks ranging from MW 841 to 845. This result demonstrates that the CoA transferase catalyzes reaction #5.

Example 2

Cloning Nucleic Acid Molecules that Encode a Multiple Polypeptide Complex Having Lactyl-CoA Dehydratase Activity

The following methods were used to clone an E1 activator polypeptide. Briefly, four degenerate forward and five degenerate reverse PCR primers were designed based on conserved sequences of E1 activator protein homologs (E1F1 5'-GCWACBGGY-TAYGGYCG-3', SEQ ID NO:60; E1F2 5'-GTYRTYGAYRTYGGYGGYCAGGA-3', SEQ ID NO:61; E1F3 5'-ATGAACGAYAARTGYGCWGCWGG-3', SEQ ID NO:62; E1F4 5'-TGYGCWGCWGGYACBGGY-CGYTT-3', SEQ ID NO:63; E1R1 5'-TCCT-GRCCRC-CRAYRTCRAYRAC-3', SEQ ID NO:64; E1R2 5'-CCWGCWGCRCAY-TTRTCGTTCAT-3', SEQ ID NO:65; E1R3 5'-AARCGRCCVGTRCCWGCWG-CRCA-3', SEQ ID NO:66; E1R4 5'-GCTTCGSWTTCRACRA-TGSW-3', SEQ ID NO:67; and E1R5 5'-GSWRATRACT-TCGCWTTCWGCRAA-3', SEQ ID NO:68).

The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.) and 1 ng of genomic DNA per reaction mix. PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 60° C., 4 cycles at 58° C., 4 cycles at 56° C., and 18 cycles at 54° C. Each cycle used an initial 30-second denaturing step at 94° C. and a 3 minute extension step at 72° C. The program had an initial denaturing step for 2 minutes at 94° C. and a final extension step of 4 minutes at 72° C. Time allowed for annealing was 45 seconds. The amounts of PCR primer used in the reactions were increased 2-10 fold above typical PCR amounts depending on the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were made to identify PCR product resulting from single degenerate primers. Each PCR product (25 μL) was separated by electrophoresis using a 1% TAE (Tris-acetate-EDTA) agarose gel.

The E1F2-E1R4, E1F2-E1R5, E1F3-E1R4, E1F3-E1R5, and E1F4-E1R4R2 combinations produced a band of 195, 207, 144, 156, and 144 bp, respectively. These bands matched the expected size based on E1 activator sequences from other species. No band was visible with individual primer control reactions. The E1F2-E1R5 fragment (207 bp) was isolated and purified using Qiagen Gel Extraction procedure (Qiagen Inc., Valencia, Calif.). The purified band (4 μL) was ligated into a pCRII vector that then was transformed into TOP10 *E. coli* cells by heat-shock using a TOPO cloning procedure (Invitrogen, Carlsbad, Calif.). Transformations were plated on LB media containing 100 μg/mL of ampicillin (Amp) and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Single, white colonies were plated onto fresh media and screened in a PCR reaction using the E1F2 and E1R5 primers to confirm the presence of the insert. Plasmid DNA was obtained from multiple colonies using a QiaPrep Spin Miniprep Kit (Qiagen, Inc). Once obtained, the plasmid DNA was quantified and used for DNA sequencing with M13R and M13F primers. Sequence analysis revealed a nucleic acid sequence encoding a polypeptide and revealed that the E1F2-E1R5 fragment shared sequence similarity with E1 activator sequences (FIGS. **12-13**).

Genome walking was performed to obtain the complete coding sequence of E2α and β subunits. Briefly, four primers for performing genome walking in both upstream and downstream directions were designed using the portion of the 207 bp E1F2-E1R5 fragment sequence that was internal to the E1F2 and E1R5 degenerate primers (E1GSP1F 5'-ACGT-CATGTCGAAGGTACTGGAAATCC-3', SEQ ID NO:69; E1GSP2F 5'-GGGACTGGTACTTCAAATCGAAGCATC-3', SEQ ID NO:70; E1GSP1R 5'-TGACGGCAGCGGGAT-GCTTCGATTTGA-3', SEQ ID NO:71; and E1GSP2R 5'-TCAGACATGGGGATTTCCAGTACCTTC-3', SEQ ID NO:72). The E1GSP1F and E1 GSP2F primers face downstream, while the E1 GSP1R and E1 GSP2R primers face upstream. In addition, the E1GSP2F and E1GSP2R primers are nested inside the E1GSP1F and E1GSP1R primers.

Genome walking was performed using the Universal Genome Walking Kit (ClonTech Laboratories, Inc., Palo Alto, Calif.) with the exception that additional libraries were generated with enzymes Nru I, Sca I, and Hinc II. First round PCR was performed in a Perkin Elmer 2400 Thermocycler with 7 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 36 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. Second round PCR used 5 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 20 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. The first and second round product (20 μL) was separated by electrophoresis using 1% TAE agarose gel. Amplification products were obtained with the Stu I library for both forward and reverse directions. The second round product of about 1.5 kb for forward direction and 3 kb fragment for reverse direction from the Stu I library were gel purified, cloned, and sequenced. Sequence analysis revealed that the sequence derived from genome walking overlapped with the E1F2-E1R5 fragment.

To obtain additional sequence, a second genome walk was performed using a first round primer (E1GSPF5 5'-CCGT-GTTACTTGGGAAGGTATCGCTGTCTG-3', SEQ ID NO:73) and a second round primer (E1 GSPF6 5'-GCCAAT-GAAGGAGGAAA-CCACTAATGAGTC-3', SEQ ID NO:74). The genome walk was performed using the NruI, ScaI, and HincII libraries. In addition, ClonTech's Advantage-Genomic Polymerase was used for the PCR. First round PCR was performed in a Perkin Elmer 2400 Thermocycler with an initial denaturing step at 94° C. for 2 minutes, 7 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 36 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. Second round PCR used 5 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 20 cycles of 2 seconds at 94° C. and 3 minutes at 65° C. with a final extension at 65° C. for 4 minutes. The first and second round product (20 μL) was separated by electrophoresis on a 1% agarose gel. An about 1.5 kb amplification product was obtained from second round PCR of the HincII library. This band was gel purified, cloned, and sequenced. Sequence analysis revealed that it overlapped with the previously obtained genome walk fragment. In addition, sequence analysis revealed a nucleic acid sequence encoding an E2α subunit that shares sequence similarities with other sequences (FIGS. **16-17**). Further, sequence analysis revealed a nucleic acid sequence encoding an E2β subunit that shares sequence similarities with other sequences (FIGS. **20-21**).

Additional PCR and sequence analysis revealed the order of polypeptide encoding sequences within the region containing the lactyl-CoA dehydratase-encoding sequences. Specifically, the E1GSP1F and COAGSP1R primer pair and the COAGSP1F and E1GSP1R primer pair were used to amplify

fragments that encode both the CoA transferase and E1 activator polypeptides. Briefly, *M. elsdenii* genome DNA (1 ng) was used as a template. The PCR was conducted in Perkin Elmer 2400 Thermocycler using Long Template Polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.). The PCR program used was as follows: 94° C. for 2 minutes; 29 cycles of 94° C. for 30 seconds, 61° C. for 45 seconds, and 72° C. for 6 minutes; and a final extension of 72° C. for 10 minutes. Both PCR products (20 μL) were separated on a 1% agarose gel. An amplification product (about 1.5 kb) was obtained using the COAGSP1F and E1GSP1R primer pair. This product was gel purified, cloned, and sequenced (FIGS. **22**A-**22**B).

The organization of the *M. elsdenii* operon containing the lactyl-CoA dehydratase-encoding sequences was determined to contain the following polypeptide-encoding sequences in the following order: CoA transferase (FIG. **6**), ORFX (FIG. **23**), E1 activator protein of lactyl-CoA dehydratase (FIG. **10**), E2α subunit of lactyl-CoA dehydratase (FIG. **14**), E2β subunit of lactyl-CoA dehydratase (FIG. **18**), and truncated CoA dehydrogenase (FIG. **25**).

The lactyl-CoA dehydratase (lactyl-CoA dehydratase or led) from *M. elsdenii* was PCR amplified from chromosomal DNA using the following program: 94° C. for 2 minutes; 7 cycles of 94° C. for 30 seconds, 47° C. for 45 seconds, and 72° C. for 3 minutes; 25 cycles of 94° C. for 30 seconds, 54° C. for 45 seconds, and 72° C. for 3 minutes; and 72° C. for 7 minutes. One primer pair was used (OSNBE1F 5'-GGGAATTCCATATG-AAAACTGTGTATACTCTC-3', SEQ ID NO:75 and OSNBE1R 5'-CGACGGAT-CCTTAGAGGATTTCCGAGAAAGC-3', SEQ ID NO:76). The amplified product (about 3.2 kb) was separated on 1% agarose gel, cut from the gel, and purified with a Qiagen Gel Extraction kit (Qiagen, Valencia, Calif.). The purified product was digested with Nde I and BamHI restriction enzymes and ligated into pET11a vector (Novagen) digested with the same enzymes. The ligation reaction was transformed into NovaBlue chemically competent cells (Novagen) that then were spread on LB agar plates supplemented with 50 μg/mL carbenicillin. Isolated individual colonies were screened for the presence of inserts by restriction mapping. Isolates with the correct restriction pattern were sequenced from each end using Novagen primers (T7 promoter primer #69348-3 and T7 terminator primer #69337-3) to confirm the sequence at the ligation points.

A plasmid having the correct insert was transformed into Tuner (DE3) pLacI chemically competent cells (Novagen, Madison, Wis.). Expression from this construct was tested as follows. A culture was grown overnight to saturation and diluted 1:20 the following morning in fresh LB medium with the appropriate antibiotics. The culture was grown at 37° C. with aeration to an OD_{600} of about 0.6. The culture was induced with IPTG at a final concentration of 100 μM. The culture was incubated for an additional two hours at 37° C. with aeration. Aliquots were taken pre-induction and 2 hours post-induction for SDS-PAGE analysis. Bands of the expected molecular weight (27,024 Daltons for the E1 subunit, 48,088 Daltons for the E2α subunit, and 42,517 Daltons for the E2β subunit—all predicted from the sequence) were observed. These bands were not observed in cells containing a plasmid lacking the nucleic acid encoding the three components of the lactyl-CoA dehydratase.

Cell free extracts were prepared by growing cells in a sealed serum bottle overnight at 37° C. Following overnight growth, the cultures were induced with 1 mM IPTG (added using anaerobic technique) and incubated an additional 2 hours at 37° C. The cells were harvested by centrifugation and disrupted by sonication under strict anaerobic conditions. The sonicated cell suspension was centrifuged to remove cell debris, and the supernatant was used in the assays. The buffer used for cell resuspension/sonication was 50 mM Tris-HCl (pH 7.5), 200 μM ATP, 7 mM $Mg(SO_4)$, 4 mM DTT, 1 mM dithionite, and 100 μM NADH.

Dehydratase activity was detected with MALDI-TOF MS. The assay was conducted in the same buffer as above with 1 mM lactyl-CoA or 1 mM acrylyl-CoA added and about 5 mg/mL cell free extract. Prior to MALDI-TOF MS analysis, samples were purified using Sep Pak Vac C_{18} columns (Waters, Inc.) as described in Example 1. The following two reactions were analyzed:

1) acrylyl-CoA→lactyl-CoA

2) lactyl-CoA→acrylyl-CoA

In reaction #1, the control sample exhibited a peak at a molecular weight corresponding to acrylyl-CoA (MW 823). The reaction #1 sample containing the cell extract from cells transfected with the dehydratase-encoding plasmid exhibited a major peak at a molecular weight corresponding to lactyl-CoA (MW 841). This result indicates that the dehydratase activity can convert acrylyl-CoA into lactyl-CoA.

To detect dehydratase activity on lactyl-CoA, reaction #2 was carried out in 80% D_2O. The control sample exhibited a peak at a molecular weight corresponding to lactyl-CoA (MW 841). The reaction #2 sample containing the cell extract from cells transfected with the dehydratase-encoding plasmid revealed a lactyl-CoA peak shifted to a deuterated form. This result indicates that the dehydratase enzyme is active on lactyl-CoA. In addition, the results from both reactions indicate that the dehydratase enzyme can catalyze the lactyl-CoA←→acrylyl-CoA reaction in both directions.

Example 3

Cloning Nucleic Acid Molecules that Encode a Polypeptide Having 3-Hydroxypropionyl CoA Dehydratase Activity

Genomic DNA was isolated from *Chloroflexus aurantiacus* cells (ATCC 29365). Briefly, *C. aurantiacus* cells in 920 *Chloroflexus* medium were grown in 50 mL cultures (Falcon 2070 polypropylene tubes) using an Innova 4230 Incubator, Shaker (New Brunswick Scientific; Edison, N.J.) at 50° C. with interior lights. Once grown, the cells were pelleted, washed with 5 mL of a 10 mM Tris solution, and re-pelleted. Genomic DNA was isolated from the pelleted cells using a Gentra Genomic "Puregene" DNA isolation kit (Gentra Systems; Minneapolis, Minn.). Briefly, the pelleted cells were resuspended in 1 mL Gentra Cell Suspension Solution to which 14.2 mg of lysozyme and 4 μL of 20 mg/mL proteinase K solution was added. The cell suspension was incubated at 37° C. for 30 minutes. The precipitated genomic DNA was recovered by centrifugation at 3500×g for 25 minutes and air-dried for 10 minutes. The genomic DNA was suspended in 300 μL of a 10 mM Tris solution and stored at 4° C.

The genomic DNA was used as a template in PCR amplification reactions with primers designed based on conserved domains of crotonase homologs and a *Chloroflexus aurantiacus* codon usage table. Briefly, two degenerate forward (CRF1 and CRF2) and three degenerate reverse (CRR1, CRR2, and CRR3) PCR primers were designed (CRF1 5'-AAYCGBCCVAARGCNCTSAAYGC-3', SEQ ID NO:77; CRF2: 5'-TTYGTBGCNGGYGCNGAYAT-3', SEQ ID NO:78; CRR1 5'-ATRTCNG-CRCCNGCVACRAA-3', SEQ ID NO:79; CRR2 5'-CCRCCRCCSAGNG-CRWARC-

CRTT-3', SEQ ID NO:80; and CRR3 5'-SSWNG-CRATVCGRATRTCRAC-3', SEQ ID NO:81).

These primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals; Indianapolis, Ind.) and 1 ng of the genomic DNA per μL reaction mix. The PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 61° C., 4 cycles at 59° C., 4 cycles at 57° C., 4 cycles at 55° C., and 16 cycles at 52° C. Each cycle used an initial 30-second denaturing step at 94° C. and a 3-minute extension step at 72° C. The program also had an initial denaturing step for 2 minutes at 94° C. and a final extension step of 4 minutes at 72° C. The time allowed for annealing was 45 seconds. The amounts of PCR primer used in the reaction were increased 4-12 fold above typical PCR amounts depending on the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were performed to identify amplification products resulting from single degenerate primers. Each PCR product (25 μL) was separated by gel electrophoresis using a 1% TAE (Tris-acetate-EDTA) agarose gel.

The CRF1-CRR1 and CRF2-CRR2 combinations produced a unique band of about 120 and about 150 bp, respectively. These bands matched the expected size based on crotonase genes from other species. No 120 bp or 150 bp band was observed from individual primer control reactions. Both fragments (i.e., the 120 bp and 150 bp bands) were isolated and purified using the Qiagen Gel Extraction kit (Qiagen Inc., Valencia, Calif.). Each purified fragment (4 μL) was ligated into pCRII vector that then was transformed into TOP10 *E. coli* cells by a heat-shock method using a TOPO cloning procedure (Invitrogen, Carlsbad, Calif.). Transformations were plated on LB media containing 100 μg/mL of ampicillin (Amp) and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Single, white colonies were plated onto fresh media and screened in a PCR reaction using the CRF1 and CRR1 primers and the CRF2 and CRR2 primers to confirm the presence of the desired insert. Plasmid DNA was obtained from multiple colonies with the desired insert using a QiaPrep Spin Miniprep Kit (Qiagen, Inc.). Once obtained, the DNA was quantified and used for DNA sequencing with M13R and M13F primers. Sequence analysis revealed the presence of two different clones from the PCR product of about 150 bp. Each shared sequence similarity with crotonase and hydratase sequences. The two clones were designated OS17 (157 bp PCR product) and OS19 (151 bp PCR product).

Genome walking was performed to obtain the complete coding sequence of OS17. Briefly, primers for conducting genome walking in both upstream and downstream directions were designed using the portion of the 157 bp CRF2-CRR2 fragment sequence that was internal to the CRF2 and CRR2 degenerate primers (OS17F1 5'-CGCTG-ATATTCGC-CAGTTGCTCGAAG-3 SEQ ID NO:82; OS17F2 5'-CCCATCTTG-CTTTCCGCAAGATTGAGC-3', SEQ ID NO:83; OS17F3 5'-CAATGGCCCTGCCGA-ATAACGC-CCATCT-3', SEQ ID NO:84; OS17R1 5'-CTTCGAG-CAACTGGCGAA-TATCAGCG-3', SEQ ID NO:85; OS17R2 5'-GCTCAATCTTGCGGAAAGCAAG-ATGGG-3', SEQ ID NO:86; and OS17R3 5'-AGATGGGCGTTAT-TCGGCAGGGCC-ATTG-3', SEQ ID NO:87). The OS17F1, OS17F3, and OS17F2 primers face downstream, while the OS17R2, OS17R3, and OS17R1 primers face upstream.

Genome walking was conducted using the Universal Genome Walking kit (ClonTech Laboratories, Inc., Palo Alto, Calif.) with the exception that additional libraries were generated with enzymes Nru I, Fsp I, and Hinc II. The first round PCR was conducted in a Perkin Elmer 2400 Thermocycler with 7 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 36 cycles of 2 seconds at 94° C. and 3 minutes at 66° C. with a final extension at 66° C. for 4 minutes. Second round PCR used 5 cycles of 2 seconds at 94° C. and 3 minutes at 72° C., and 20 cycles of 2 seconds at 94° C. and 3 minutes at 66° C. with a final extension at 66° C. for 4 minutes. The first and second round amplification product (5 μL) was separated by gel electrophoresis on a 1% TAE agarose gel. After the second round PCR, an amplification product of about 0.4 kb was obtained with the Fsp I library using the OS17R1 primer in the reverse direction, and an amplification product of about 0.6 kb was obtained with the Hinc II library using the OS17F2 primer in the forward direction. These PCR products were cloned and sequenced.

Sequence analysis revealed that the sequences derived from genome walking overlapped with the CRF2-CRR2 fragment and shared sequence similarity with crotonase and hydratase sequences.

A second genome walking was performed to obtain additional sequences. Six primers were designed for this second genome walk (OS17F4 5'-AAGCTGGG-TCTGATCGAT-GCCATTGCTACC-3', SEQ ID NO:88; OS17F5 5'-CTC-GATTATCG-CCCATCCACGTATCGAG-3', SEQ ID NO:89; OS17F6 5'-TGGATGCAATCCG-CTATGGCAT-TATCCACG-3', SEQ ID NO:90; OS17R4 5'-TCATTCAGT-GCG-TTCACCGGCGGATTTGTC-3', SEQ ID NO:91; OS17R5 5'-TCGATCCGGAAGT-AGCGATAGCGTTC-GATG-3', SEQ ID NO:92; and OS17R6 5'-CTTGGCTG-CAAT-CTCTTCGAGCACTTCAGG-3', SEQ ID NO:93). The OS17F4, OS17F5, and OS17F6 primers faced downstream, while the OS17R4, OS17R5, and OS17R6 primers faced upstream.

The second genome walk was performed using the same methods described for the first genome walk. After the second round of walking, an amplification product of about 2.3 kb was obtained with a Hinc II library using the OS17R5 primer in the reverse direction, and an amplification product of about 0.6 kb was obtained with a Pvu II library using the OS17F5 primer in the forward direction. The PCR products were cloned and sequenced. Sequence analysis revealed that the sequences derived from the second genome walking overlapped with the sequence obtained during the first genome walking. In addition, the sequence analysis revealed a sequence with 3572 bp.

A BLAST search revealed that the polypeptide encoded by this sequence shares sequence similarity with polypeptides having three different activities. Specifically, the beginning of the OS17 encoded-polypeptide shares sequence similarity with CoA-synthesases, the middle region of the OS17 encoded-polypeptide shares sequence similarity with enoyl-CoA hydratases, and the end region of the OS17 encoded-polypeptide shares sequence similarity with CoA-reductases.

A third genome walk was performed using four primers (OS17UP-6 5'-CATCAGAGGTAATCACCACTCGTGCA-3', SEQ ID NO:94; OS17UP-7 5'-AAGTAGTAGGCCAC-CTCGTCGCCATA-3', SEQ ID NO:95; OS17DN-1 5'-GC-CAATCAGGCGCTGATCTATGTTCT-3', SEQ ID NO:96; and OS17DN-2 5'-CTGATCTATGTTCTGGCCTCG-GAGGT-3', SEQ ID NO:97). The OS17UP-6 and OS17UP-7 primers face upstream, while the OS17DN-1 and OS17DN-2 primers face downstream. The third genome walk yielded an amplification product of about 1.2 kb with a Nru I library using the OS17UP-7 primer in the reverse direction. In addition, amplification products of about 4 kb and about 1.1 kb were obtained with a Hinc II and Fsp I library, respectively, using the OS17DN-2 primer in the forward direction.

Sequence analysis revealed a nucleic acid sequence encoding a polypeptide (FIGS. **27-28**). The complete OS17 gene had 5466 nucleotides and encoded a 1822 amino acid polypeptide. The calculated molecular weight of the OS17 polypeptide from the sequence was 201,346 (pI=5.71).

A BLAST search analysis revealed that the product of the OS17 nucleic acid has three different activities based on sequence similarity to (1) CoA-synthesases at the beginning of the OS17 sequence, (2) 3-HP dehydratases in the middle of the OS17 sequence, and (3) CoA-reductases at the end of the OS17 sequence. Thus, the OS17 clone appeared to encode a single enzyme capable of catalyzing three distinct reactions leading to the direct conversion of 3-hydroxypropionate to propionyl CoA: 3-HP→3-HP-CoA→acrylyl-CoA→propionyl-CoA.

The OS17 gene from *C. aurantiacus* was PCR amplified from chromosomal DNA using the following conditions: 94° C. for 3 minutes; 25 cycles of 94° C. for 30 seconds to denature, 54° C. for 30 seconds to anneal, and 68° C. for 6 minutes for extension; followed by 68° C. for 10 minutes for final extension. Two primers were used (OS17F 5'-GGGAATTCCATATGATCGACACTGCG-3', SEQ ID NO:136; and OS17R 5'-CGAAGGATCCAACGATAATCGGCTCAGCAC-3', SEQ ID NO:137). The resulting PCR product (~5.6 Kb) was purified using Qiagen PCR purification kit (Qiagen Inc., Valencia, Calif.). The purified product was digested with NdeI and BamHI restriction enzymes, heated at 80° C. for 20 minutes to inactivate the enzymes, purified using Qiagen PCR purification kit, and ligated into a pET11a vector (Novagen, Madison, Wis.) previously digested with NdeI and BamHI enzymes. The ligation reaction was transformed into NovaBlue chemically competent cells (Novagen, Madison, Wis.) that were spread on LB agar plates supplemented with 50 μg/mL carbenicillin. Individual transformants were screened by PCR amplification of the OS17 DNA with the OS17F and OS17R primers and conditions as described above directly from colonies cells. Clones that yielded the 5.6 Kb product were used for plasmid purification with Qiagen QiaPrep Spin Miniprep Kit (Qiagen, Inc). Resulting plasmids were transformed into *E. coli* BL21(DE3) cells, and OS17 polypeptide expression induced. The apparent molecular weight of the OS17 polypeptide according to SDS gel electrophoresis was about 190,000 Da.

To assay OS17 polypeptide function, a 100 mL culture of BL21-DE3/pET11a-OS17 cells was started using 1 mL of overnight grown culture as an inoculum. The culture was grown to an OD of 0.5-0.6 and was induced with 100 μM IPTG. After two and a half hours of induction, the cells were harvested by spinning at 8000 rpm in the floor centrifuge. The cells were washed with 10 mM Tris-HCl (pH 7.8) and passed twice through a French Press at a gauge pressure of 1000 psi. The cell debris was removed by centrifugation at 15,000 rpm. The activity of the OS17 polypeptide was measured spectrophotometrically, and the products formed during this enzymatic transformation were detected by LC/MS. The assay mix was as follows (*J. Bacteriol.*, 181:1088-1098 (1999)):

Reagent	Volume	Final Conc.
Tris-HCl (1000 mM, 7.8 pH)	10 μL	50 mM
$MgCl_2$ (100 mM)	10 μL	5 mM
ATP (30 mM)	20 μL	3 mM
KCl (100 mM)	20 μL	10 mM
CoASH (5 mM)	20 μL	0.5 mM
NAD(P)H	20 μL	0.5 mM

-continued

Reagent	Volume	Final Conc.
3-hydroxypropionate	2 μL	1 mM
Protein extract (7 mg/mL)	20 (40) μL	140 μg
DI water	78 (58) μL	
Total	200 μL	

The initial rate of reaction was measured by monitoring the disappearance of NAD(P)H at 340 nm. The activity of the OS17 polypeptide was measured using 3-HP as the substrate. The units/mL of total protein was calculated using the formula set forth in Example 1. The activity of the expressed OS17 polypeptide was calculated to be 0.061 U/mL of total protein. The reaction products were purified using a Sep Pak Vac column (Waters). The column was conditioned with 1 mL methanol and washed two times with 0.5 mL 0.1% TFA. The sample was then applied to the column, and the column was washed two more times with 0.5 mL 0.1% TFA. The sample was eluted with 200 μL of 40% acetonitrile, 0.1% TFA. The acetonitrile was removed from the sample by vacuum centrifugation. The reaction products were analyzed by LC/MS.

Analyses of thioesters namely propionyl CoA, acrylyl CoA, and 3 HP CoA from the above reaction were carried out using a Waters/Micromass ZQ LC/MS instrument which had a Waters 2690 liquid chromatograph with a Waters 996 Photo-Diode Array (PDA) placed in series between the chromatograph and the single quadropole mass spectrometer. LC separations were made using a 4.6×150 mm YMC ods-AQ (3 μm particles, 120 Å pores) reversed-phase chromatography column at room temperature. CoA esters were eluted in Buffer A (25 mM ammonium acetate, 0.5% acetic acid) with a linear gradient of buffer B (acetonitrile, 0.5% acetic acid). A flow rate of 0.25 mL/minute was used, and photodiode array UV absorbance was monitored from 200 to 400 nm. All parameters of the electrospray MS system were optimized and selected based on generation of protonated molecular ions ($[M+H]^+$) of the analytes of interest and production of characteristic fragment ions. The following instrumental parameters were used for ESI-MS detection of CoA and organic acid-CoA thioesters in the positive ion mode; Extractor: 1 V; RF lens: 0 V; Source temperature: 100° C.; Desolvation temperature: 300° C.; Desolvation gas: 500 L/hour; Cone gas: 40 L/hour; Low mass resolution: 13.0; High mass resolution: 14.5; Ion energy: 0.5; Multiplier: 650. Uncertainties for mass charge ratios (m/z) and molecular masses are ±0.01%.

The enzyme assay mix from strains expressing the OS17 polypeptide exhibited peaks for propionyl CoA, acrylyl CoA, and 3-HP CoA with the propionyl CoA peak being the dominant peak. These peaks were missing in the enzyme assay mix obtained from the control strain, which carried vector pET11a without an insert. These results indicate that the OS17 polypeptide has CoA synthetase activity, CoA hydratase activity, and dehydrogenase activity.

Genome walking also was performed to obtain the complete coding sequence of OS19. Briefly, primers for conducting genome walking in both upstream and downstream directions were designed using the portion of the 151 bp CRF2-CRR2 fragment sequence that was internal to the CRF2 and CRR2 degenerate primers (OS19F1 5'-GGCTGATATCAAAGCGATGGCCAATGC-3', SEQ ID NO:98; OS19F2 5'-CCAC-GCCTATTGATATGCTCACCAGTG-3', SEQ ID NO:99; OS19F3 5'-GCAAACCGG-TGATTGCTGCCGTGAATGG-3', SEQ ID NO:100; OS19R1 5'-GCATTGGC-

CAT-CGCTTTGATATCAGCC-3', SEQ ID NO:101; OS19R2 5'-CACTGGTGAGCATATC-AATAGGCGTGG-3', SEQ ID NO:102; and OS19R3 5'-CCATTCACGGCAG-CAA-TCACCGGTTTGC-3', SEQ ID NO:103). The OS19F1, OS19F2, and OS19F3 primers face downstream, while the OS19R1, OS19R2, and OS19R3 primers face upstream.

An amplification product of about 0.25 kb was obtained with the Fsp I library using the OS19R1 primer, while an amplification product of about 0.65 kb was obtained with the Pvu II library using the OS19R1 primer. In addition, an amplification product of about 0.4 kb was obtained with the Pvu II library using the OS19F3 primer. The PCR products were cloned and sequenced. Sequence analysis revealed that the sequences derived from genome walking overlapped with the CRF2-CRR2 fragment and shared sequence similarity with crotonase and hydratase sequences. The obtained sequences accounted for most of the coding sequence including the start codon.

A second genome walk was performed to obtain additional sequence using two primers (OS19F7 5'-TCATCATCGC-CAGTGAAAACGCGCAGTTCG-3', SEQ ID NO:104 and OS19F8 5'-GGATCGCGCAAACCATTGCCACCAAAT-CAC-3', SEQ ID NO:105). The OS19F7 and OS19F8 primers face downstream.

An amplification product (about 0.7 kb) obtained from the Pvu II library was cloned and sequenced. Sequence analysis revealed that the sequence derived from the second genome walk overlapped with the sequence obtained from the first genome walk and contained the stop codon. The full-length OS19 clone was found to share sequence similarity with other sequences such as crotonase and enoyl-CoA hydratase sequences (FIGS. **32-33**).

The OS19 clone was found to encode a polypeptide having 3-hydroxypropionyl-CoA dehydratase activity also referred to as acrylyl-CoA hydratase activity. The nucleic acid encoding the OS19 dehydratase from *C. aurantiacus* was PCR amplified from chromosomal DNA using the following conditions: 94° C. for 3 minutes; 25 cycles of 94° C. for 30 seconds to denature, 56° C. for 30 seconds to anneal, and 68° C. for 1 minute for extension; and 68° C. for 5 minutes for final extension. Two primers were used (OSACH3 5'-AT-GAGTGAAGAGTCTCTGGTTCTCAGC-3', SEQ ID NO:106 and OSACH2 5'-AGATCGCAATCGCTCGTGTAT-GTC-3', SEQ ID NO:107).

The resulting PCR product (about 1.2 kb) was separated by agarose gel electrophoresis and purified using Qiagen PCR purification kit (Qiagen Inc.; Valencia, Calif.). The purified product was ligated into pETBlue-1 using the Perfectly Blunt cloning Kit (Novagen; Madison, Wis.). The ligation reaction was transformed into NovaBlue chemically competent cells (Novagen, Madison, Wis.) that then were spread on LB agar plates supplemented with 50 μg/mL carbenicillin, 40 μg/mL IPTG, and 40 μg/mL X-Gal. White colonies were isolated and screened for the presence of inserts by restriction mapping. Isolates with the correct restriction pattern were sequenced from each end using the primer pETBlueUP and pETBlue-DOWN (Novagen) to confirm the sequence at the ligation points.

The plasmid containing the OS19 dehydratase-encoding sequence was transformed into Tuner (DE3) pLacI chemically competent cells (Novagen, Madison, Wis.), and expression from the construct tested. Briefly, a culture was grown overnight to saturation and diluted 1:20 the following morning in fresh LB medium with the appropriate antibiotics. The culture was grown at 37° C. and 250 rpm to an OD_{600} of about 0.6. At this point, the culture was induced with IPTG at a final concentration of 1 mM. The culture was incubated for an additional two hours at 37° C. and 250 rpm. Aliquots were taken pre-induction and 2 hours post-induction for SDS-PAGE analysis. A band of the expected molecular weight (27,336 Daltons predicted from the sequence) was observed. This band was not observed in cells containing a plasmid lacking the nucleic acid encoding the hydratase.

Cell free extracts were prepared by growing cells as described above. The cells were harvested by centrifugation and disrupted by sonication. The sonicated cell suspension was centrifuged to remove cell debris, and the supernatant was used in the assays. The ability of the 3-hydroxypropionyl-CoA dehydratase to perform the following three reactions was measured using MALDI-TOF MS:

1) acrylyl-CoA→3-hydroxypropionyl-CoA
2) 3-hydroxypropionyl-CoA→acrylyl-CoA
3) crotonyl-CoA→3-hydroxybutyryl-CoA

The assay mixture contained 50 mM Tris-HCl (pH 7.5), 1 mM CoA ester, and about 1 μg cell free extract. Reactions were allowed to proceed at room temperature and were stopped by adding 1 volume 10% trifluroacetic acid (TFA). The reaction mixtures were purified prior to MALDI-TOF MS analysis using Sep Pak Vac C_{18} 50 mg columns (Waters, Inc.). The columns were conditioned with 1 mL methanol and then equilibrated with two washes of 1 mL 0.1% TFA. The sample was applied to the column, and the flow through was discarded. The column was washed twice with 1 mL 0.1% TFA. The sample was eluted in 200 μL 40% acetonitrile, 0.1% TFA. The acetonitrile was removed by centrifugation in vacuo. Samples were prepared for MALDI-TOF MS analysis by mixing 1:1 with 110 mM sinapinic acid in 0.1% TFA, 67% acetonitrile. The samples were allowed to air dry.

The conversion of acrylyl-CoA into 3-hydroxypropionyl-CoA catalyzed by the 3-hydroxypropionyl-CoA dehydratase was detected using the MALDI-TOF MS technique. In reaction #1, the control sample exhibited a dominant peak at a molecular weight corresponding to acrylyl-CoA (MW 823). The reaction #1 sample containing the cell extract from cells transfected with the 3-hydroxypropionyl-CoA dehydratase-encoding plasmid exhibited a dominant peak corresponding to 3-hydroxypropionyl-CoA (MW 841). This result demonstrates that the 3-hydroxypropionyl-CoA dehydratase activity catalyzes reaction #1.

To detect the conversion of 3-hydroxypropionyl-CoA into acrylyl-CoA, reaction #2 was carried out in 80% D_2O. The reaction #2 sample containing the cell extract from cells transfected with the 3-hydroxypropionyl-CoA dehydratase-encoding plasmid revealed incorporation of deuterium in the 3-hydroxypropionyl-CoA molecule. This result indicates that the 3-hydroxypropionyl-CoA dehydratase enzyme catalyzes reaction #2. In addition, the results from both #1 and #2 reactions indicate that the 3-hydroxypropionyl-CoA dehydratase enzyme can catalyze the 3-hydroxypropionyl -CoA←→acrylyl-CoA reaction in both directions. It is noted that for both the #1 and #2 reactions, a peak was observed at MW 811, due to leftover acetyl-CoA from the synthesis of 3-hydroxypropionyl-CoA from 3-hydroxypropionate and acetyl-CoA.

The assays assessing conversion of crotonyl-CoA into 3-hydroxybutyryl-CoA also were carried out in 80% D_2O. In reaction #3, the control sample exhibited a dominant peak at a molecular weight corresponding to crotonyl-CoA (MW 837). This result indicated that the crotonyl-CoA was not converted into other products. The reaction #3 sample containing the cell extract from cells transfected with the 3-hydroxypropionyl-CoA dehydratase-encoding plasmid exhibited a diffuse group of peaks corresponding to deuterated

3-hydroxybutyryl-CoA (MW 855 to MW 857). This result demonstrates that the 3-hydroxypropionyl-CoA dehydratase activity catalyzes reaction #3.

A series of control reactions were performed to confirm the specificity of the 3-hydroxypropionyl-CoA dehydratase. Lactyl-CoA (1 mM) was added to the reaction mixture containing 100 mM Tris (pH 7.0) both in the presence and the absence of the 3-hydroxypropionyl-CoA dehydratase. In both cases, the dominant peak observed had a molecular weight corresponding to lactyl-CoA (MW 841). This result indicates that lactyl-CoA is not affected by the presence of 3-hydroxypropionyl-CoA dehydratase activity even in the presence of D_2O meaning that the 3-hydroxypropionyl-CoA dehydratase enzyme does not attach a hydroxyl group at the alpha carbon position. The presence of 3-hydroxypropionyl-CoA in an 80% D_2O reaction mixture resulted in a shift upon addition of the 3-hydroxypropionyl-CoA dehydratase activity. In the absence of 3-hydroxypropionyl-CoA dehydratase activity, a peak corresponding to 3-hydroxypropionyl-CoA was observed in addition to a peak of MW 811. The MW 811 peak was due to leftover acetyl-CoA from the synthesis of 3-hydroxypropionyl-CoA. In the presence of 3-hydroxypropionyl-CoA dehydratase activity, a peak corresponding to deuterated 3-hydroxypropionyl-CoA was observed (MW 842) due to exchange of a hydroxyl group during the conversion of 3-hydroxypropionyl-CoA to acrylyl-CoA and vice-versa. These control reactions demonstrate that the 3-hydroxypropionyl-CoA dehydratase enzyme is active on 3-hydroxypropionyl-CoA and not active on lactyl-CoA. In addition, these results demonstrate that the product of the acrylyl-CoA reaction is 3-hydroxypropionyl-CoA not lactyl-CoA.

Example 4

Construction of Operon #1

The following operon was constructed and can be used to produce 3-HP in *E. coli* (FIG. **34**). Briefly, the operon was cloned into a pET-11a expression vector under the control of a T7 promoter (Novagen, Madison, Wis.). The pET-11a expression vector is a 5677 bp plasmid that uses the ATG sequence of an Nde/restriction site as a start codon for inserted downstream sequences.

Nucleic acid molecules encoding a CoA transferase and a lactyl-CoA dehydratase were amplified from *Megasphaera elsdenii* genomic DNA by PCR. Two primers were used to amplify the CoA transferase-encoding sequence (OSNBpctF 5'-GGGAATTCC-ATATGAGAAAAGTAGAAATCATTA-CAGCTG-3', SEQ ID NO:108 and OSCTE-2 5'-GAGAG-TATACACAGTTTTCACCTCCTTTACAGCAGAGAT-3', SEQ ID NO:109), and two primers were used to amplify the lactyl-CoA dehydratase-encoding sequence (OSCTE-1 5'-ATCTCTGCTGTAAAGGAGGTGAAAACT-GTGTATACT-CTC-3', SEQ ID NO:110 and OSEBH-2 5'-ACGTTGATCTCCTTGTACATT-AGAGGATTTC-CGAGAAAGC-3', SEQ ID NO:111). A nucleic acid molecule encoding a 3-hydroxypropionyl-CoA dehydratase was amplified from *Chloroflexus aurantiacus* genomic DNA by PCR using two primers (OSEBH-1 5'-GCTTTCTCGG-AAATCCTCTAATGTACAAGGAGATCAACGT-3', SEQ ID NO:112 and OSHBR 5'-CGACGGATCCTCAACGAC-CACTGAAGTTGG-3', SEQ ID NO:113).

PCR was conducted in a Perkin Elmer 2400 Thermocycler using 100 ng of genomic DNA and a mix of rTth polymerase (Applied Biosystems; Foster City, Calif.) and Pfu Turbo polymerase (Stratagene; La Jolla, Calif.) in 8:1 ratio. The polymerase mix ensured higher fidelity of the PCR reaction. The following PCR conditions were used: initial denaturation step of 94° C. for 2 minutes; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2 minutes; and a final extension at 68° C. for 5 minutes. The obtained PCR products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Inc.; Valencia, Calif.).

The CoA transferase, lactyl-CoA dehydratase (E1, E2α subunit, and E2β subunit), and 3-hydroxypropionyl-CoA dehydratase PCR products were assembled using PCR. The OSCTE-1 and OSCTE-2 primers as well as the OSEBH-1 and OSEBH-2 primers were complementary to each other. Thus, the complementary DNA ends could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSNBpctF and OSHBR) were added to the assembly PCR mixture, which contained 100 ng of each PCR product (i.e., the PCR products from the CoA-transferase, lactyl-CoA dehydratase, and 3-hydroxypropionyl-CoA dehydratase reactions) as well as the rTth polymerase/Pfu Turbo polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 25 cycles of 94° C. for 30 seconds, 55° C. for 30 seconds, and 68° C. for 6 minutes; and a final extension at 68° C. for 7 minutes. The assembled PCR product was gel purified and digested with restriction enzymes (NdeI and BamHI). The sites for these restriction enzymes were introduced into the assembled PCR product using the OSNBpctF (NdeI) and OSHBR (BamHI) primers. The digested PCR product was heated at 80° C. for 30 minutes to inactivate the restriction enzymes and used directly for ligation into pET-11a vector.

The pET-11a vector was digested with NdeI and BamHI restriction enzymes, gel purified using a Qiagen Gel Extraction kit, treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.) and used in a ligation reaction with the assembled PCR product. The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into NovaBlue chemically competent cells (Novagen; Madison, Wis.) using a heat-shock method. Once heat shocked, the cells were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.) and analyzed by digestion with NdeI and BamHI restriction enzymes.

Example 5

Construction of Operon #2

The following operon was constructed and can be used to produce 3-HP in *E. coli* (FIGS. **35**A and B). Nucleic acid molecules encoding a CoA transferase and a lactyl-CoA dehydratase were amplified from *Megasphaera elsdenii* genomic DNA by PCR. Two primers were used to amplify the CoA transferase-encoding sequence (OSNBpctF and OSCTE-2), and two primers were used to amplify the lactyl-CoA dehydratase-encoding sequence (OSCTE-1 and OSNBe1R 5'-CGACGGATCCTTAGAGGATTT-CCGAGAAAGC-3', SEQ ID NO:114). A nucleic acid molecule encoding a 3-hydroxypropionyl-CoA dehydratase was amplified from *Chloroflexus aurantiacus* genomic DNA by PCR using two primers (OSXNhF 5'-GGTGTCT-AGAGA-CAGTCCTGTCGTTTATGTAGAAGGAG-3', SEQ ID

NO:115 and OSXNhR 5'-GGGAATTCCATATGCGTAACT-TCCTCCTGCTATCAACGACCACTGAA-GTTGG-3', SEQ ID NO:116).

PCR was conducted in a Perkin Elmer 2400 Thermocycler using 100 ng of genomic DNA and a mix of rTth polymerase (Applied Biosystems; Foster City, Calif.) and Pfu Turbo polymerase (Stratagene; La Jolla, Calif.) in 8:1 ratio. The polymerase mix ensured higher fidelity of the PCR reaction. The following PCR conditions were used: initial denaturation step of 94° C. for 2 minutes; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2 minutes; and a final extension at 68° C. for 5 minutes. The obtained PCR products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Inc.; Valencia, Calif.).

The CoA transferase and lactyl-CoA dehydratase (E1, E2α subunit, and E2β subunit) PCR products were assembled using PCR. The OSCTE-1 and OSCTE-2 primers were complementary to each other. Thus, the 22 nucleotides at the end of the CoA transferase sequence and the 22 nucleotides at the beginning of the lactyl-CoA dehydratase could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSNBpctF and OSNBe1R) were added to the assembly PCR mixture, which contained 100 ng of the CoA transferase PCR product, 100 ng of lactyl-CoA dehydratase PCR product, and the rTth polymerase/Pfu Turbo polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 5 minutes; and a final extension at 68° C. for 6 minutes.

The assembled PCR product was gel purified and digested with restriction enzymes (NdeI and BamHI). The sites for these restriction enzymes were introduced into the assembled PCR product using the OSNBpctF (NdeI) and OSNBe1R (BamHI) primers. The digested PCR product was heated at 80° C. for 30 minutes to inactivate the restriction enzymes and used directly for ligation into a pET-11a vector.

The pET-11a vector was digested with NdeI and BamHI restriction enzymes, gel purified using a Qiagen Gel Extraction kit, treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.) and used in a ligation reaction with the assembled PCR product. The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into NovaBlue chemically competent cells (Novagen; Madison, Wis.) using a heat-shock method. Once heat shocked, the cells were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.) and analyzed by digestion with NdeI and BamHI restriction enzymes. The digest revealed that the DNA fragment containing CoA transferase-encoding and lactyl-CoA dehydratase-encoding sequences was cloned into the pET-11a vector.

The plasmid carrying the CoA transferase-encoding and lactyl-CoA dehydratase-encoding sequences (pTD) was digested with XbaI and NdeI restriction enzymes, gel purified, and used for cloning the 3-hydroxypropionyl-CoA dehydratase-encoding product upstream of the CoA transferase-encoding sequence. Since this XbaI and NdeI digest eliminated a ribosome-binding site (RBS) from the pET-11a vector, a new homologous RBS was cloned into the plasmid together with the 3-hydroxypropionyl-CoA dehydratase-encoding product. Briefly, the 3-hydroxypropionyl-CoA dehydratase-encoding PCR product was digested with XbaI and NdeI restriction enzymes, heated at 65° C. for 30 minutes to inactivate the restriction enzymes, and ligated into pTD. The ligation mixture was transformed into chemically competent NovaBlue cells (Novagen) that then were plated on LB plates supplemented with 50 μg/mL carbenicillin.

Individual colonies were selected, and the plasmid DNA obtained using a Qiagen Spin Miniprep Kit. The obtained plasmids were digested with XbaI and NdeI restriction enzymes and analyzed by gel electrophoresis. pTD plasmids containing the inserted 3-hydroxypropionyl-CoA dehydratase-encoding PCR product were named pHTD. While expression of the lactyl-CoA hydratase, CoA transferase, and 3-hydroxypropionyl-CoA dehydratase sequences from pHTD was directed by a single T7 promoter, each coding sequence had an individual RBS upstream of their start codon.

To ensure the correct assembly and cloning of the lactyl-CoA hydratase, CoA transferase, and 3-hydroxypropionyl-CoA dehydratase sequences into one operon, both ends of the operon and all junctions between the coding sequences were sequenced. This DNA analysis revealed that the operon was assembled correctly.

The pHTD plasmid was transformed into BL21(DE3) cells to study the expression of the encoded sequences.

Example 6

Construction of Operons #3 and #4

Operon #3 (FIGS. **36**A and B) and operon #4 (FIGS. **37**A and B) each position the E1 activator at the end of the operon. Operon #3 contains a RBS between the 3-hydroxypropionyl-CoA dehydratase-encoding sequence and the E1 activator-encoding sequence. In operon #4, however, the stop codon of the 3-hydroxypropionyl-CoA dehydratase-encoding sequence is fused with the start codon of the E1 activator-encoding sequence as follows: TAGTG. The absence of the RBS in operon #4 can decrease the level of E1 activator expression.

To construct operon #3, nucleic acid molecules encoding a CoA transferase and a lactyl-CoA dehydratase were amplified from *Megasphaera elsdenii* genomic DNA by PCR. Two primers were used to amplify the CoA transferase-encoding sequence (OSNBpctF and OSHTR 5'-ACGTTGATCTCCT-TCTACATTATTTTTCAGT-CCCATG-3', SEQ ID NO:117), two primers were used to amplify the E2α and β subunits of the lactyl-CoA dehydratase-encoding sequence (OSEIIXNF 5'-GGTGTCTAGAGTCAAAGGAGAGAA-CAAAATCATGAGTG-3', SEQ ID NO:118 and OSEIIXNR 5'-GGGAATTCCATATGCGTAACTTCCTCCT-GCTATTAGAGGA-TTTCCGAGAAAGC-3', SEQ ID NO:119), and two primers were used to amplify the E1 activator of the lactyl-CoA dehydratase-encoding sequence (OSHrE1F 5'-TCAGTG-GTCGTTGATCACGCTATAAA-GAAAGGTGAAAACTGTGTATACTCTC-3', SEQ ID NO:120 and OSEIBR 5'-CGACGGATCCCTTCCTTG-GAGCTCATGCTTTC-3', SEQ ID NO:121). A nucleic acid molecule encoding a 3-hydroxypropionyl-CoA dehydratase was amplified from *Chloroflexus aurantiacus* genomic DNA by PCR using two primers (OSTHF 5'-CATGGGACT-GAAAAAATAATGTAGAAGGAGAT-CAACGT-3', SEQ ID NO:122 and OSEIrHR 5'-GAGAGTATACA-CAGTTTTCA-CCTTTCTTTATAGCGTGATCAACGAC-CACTGA-3', SEQ ID NO:123).

PCR was conducted in a Perkin Elmer 2400 Thermocycler using 100 ng of genomic DNA and a mix of rTth polymerase (Applied Biosystems; Foster City, Calif.) and Pfu Turbo polymerase (Stratagene; La Jolla, Calif.) in 8:1 ratio. The poly-

merase mix ensured higher fidelity of the PCR reaction. The following PCR conditions were used: initial denaturation step of 94° C. for 2 minutes; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2 minutes; and a final extension at 68° C. for 5 minutes. The obtained PCR products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Inc.; Valencia, Calif.).

The 3-hydroxypropionyl-CoA dehydratase and E1 activator PCR products were assembled using PCR. The OSHrE1F and OSEIrHR primers were complementary to each other. Thus, the primers could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSTHF and OSEIBR) were added to the assembly PCR mixture, which contained 100 ng of the 3-hydroxypropionyl-CoA dehydratase PCR product, 100 ng of E1 activator PCR product, and the rTth polymerase/Pfu Turbo polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 1.5 minutes; and a final extension at 68° C. for 5 minutes.

The assembled PCR product was gel purified and used in a second assembly PCR with gel purified CoA transferase PCR product. The OSTHF and OSHTR primers were complementary to each other. Thus, the complementary DNA ends could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSNBpctF and OSEIBR) were added to the second assembly PCR mixture, which contained 100 ng of the purified 3-hydroxypropionyl-CoA dehydratase/EI PCR assembly, 100 ng of the purified CoA transferase PCR product, and the polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 3 minutes; and a final extension at 68° C. for 5 minutes.

The assembled PCR product was gel purified and digested with NdeI and BamHI restriction enzymes. The sites for these restriction enzymes were introduced into the assembled PCR products with the OSNBpctF (NdeI) and OSEIBR (BamHI) primers. The digested PCR product was heated at 80° C. for 30 minutes to inactivate the restriction enzymes and used directly for ligation into a pET11a vector.

The pET-11a vector was digested with NdeI and BamHI restriction enzymes, gel purified using a Qiagen Gel Extraction kit, treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.) and used in a ligation reaction with the assembled PCR product. The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into NovaBlue chemically competent cells (Novagen; Madison, Wis.) using a heat-shock method. Once heat shocked, the cells were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc.; Valencia, Calif.). The resulting plasmids carrying the CoA transferase, 3-hydroxypropionyl-CoA dehydratase, and EI activator sequences (pTHrEI) were digested with XbaI and NdeI, purified using gel electrophoresis and a Qiagen Gel Extraction kit, and used as a vector for cloning of the E2α subunit/E2β subunit PCR product.

The E2α subunit/E2β subunit PCR product was digested with the same enzymes and ligated into the pTHrEI vector. The ligation reaction was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The ligation mixture was transformed into chemically competent NovaBlue cells (Novagen) that then were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.) and digested with XbaI and NdeI restriction enzymes for gel electrophoresis analysis. The resulting plasmids carrying the constructed operon #3 (pEIITHrEI) were transformed into BL21(DE3) cells to study the expression of the cloned sequences. Electrospray mass spectrometry assay confirmed that extracts from these cells have CoA transferase activity and 3-hydroxypropionyl-CoA dehydratase activity. Similar assays are used to confirm that extracts from these cells also have lactyl-CoA dehydratase activity.

To construct operon #4, nucleic acid molecules encoding a CoA transferase and a lactyl-CoA dehydratase were amplified from *Megasphaera elsdenii* genomic DNA by PCR. Two primers were used to amplify the CoA transferase-encoding sequence (OSNBpctF and OSHTR), two primers were used to amplify the E2α and 13 subunits of the lactyl-CoA dehydratase-encoding sequence (OSEIIXNF and OSEIIXNR), and two primers were used to amplify the E1 activator of the lactyl-CoA dehydratase-encoding sequence (OSHEIF 5'-CCAACTTCAGTGGTCGTTAGTGAAAACTGTGTATACTCTC-3', SEQ ID NO:124 and OSEIBR). A nucleic acid molecule encoding a 3-hydroxypropionyl-CoA dehydratase was amplified from *Chloroflexus aurantiacus* genomic DNA by PCR using two primers (OSTHF and OSEIHR 5'-GAGAGTATACACAGTTTTCACTAACGACCACTGAAGTTGG-3', SEQ ID NO:125).

PCR was conducted in a Perkin Elmer 2400 Thermocycler using 100 ng of genomic DNA and a mix of rTth polymerase (Applied Biosystems; Foster City, Calif.) and Pfu Turbo polymerase (Stratagene; La Jolla, Calif.) in 8:1 ratio. The polymerase mix ensured higher fidelity of the PCR reaction. The following PCR conditions were used: initial denaturation step of 94° C. for 2 minutes; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2 minutes; and a final extension at 68° C. for 5 minutes. The obtained PCR products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Inc.; Valencia, Calif.).

The 3-hydroxypropionyl-CoA dehydratase and E1 activator PCR products were assembled using PCR. The OSHEIF and OSEIHR primers were complementary to each other. Thus, the primers could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSTHF and OSE1BR) were added to the assembly PCR mixture, which contained 100 ng of the 3-hydroxypropionyl-CoA dehydratase PCR product, 100 ng of E1 activator PCR product, and the rTth polymerase/Pfu Turbo polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 1.5 minutes; and a final extension at 68° C. for 5 minutes.

The assembled PCR product was gel purified and used in a second assembly PCR with gel purified CoA transferase PCR product. The OSTHF and OSHTR primers were complementary to each other. Thus, the complementary DNA ends could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSNBpctF and OSEIBR) were added to the second assembly PCR mixture, which contained 100 ng of the purified 3-hydroxypropionyl-CoA dehydratase/EI PCR assembly, 100 ng of the purified CoA transferase PCR product, and the polymerase mix described above. The following PCR conditions were used to assemble the products:

94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 3 minutes; and a final extension at 68° C. for 5 minutes.

The assembled PCR product was gel purified and digested with NdeI and BamHI restriction enzymes. The sites for these restriction enzymes were introduced into the assembled PCR products with the OSNBpctF (NdeI) and OSEIBR (BamHI) primers. The digested PCR product was heated at 80° C. for 30 minutes to inactivate the restriction enzymes and used directly for ligation into a pET11a vector.

The pET-11a vector was digested with NdeI and BamHI restriction enzymes, gel purified using a Qiagen Gel Extraction kit, treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.) and used in a ligation reaction with the assembled PCR product. The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into NovaBlue chemically competent cells (Novagen; Madison, Wis.) using a heat-shock method. Once shocked, the cells were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.). The resulting plasmids carrying the CoA transferase, 3-hydroxypropionyl-CoA dehydratase, and E1 activator sequences (pTHEI) were digested with XbaI and NdeI, purified using gel electrophoresis and a Qiagen Gel Extraction kit, and used as a vector for cloning of the E2α subunit/E2β subunit PCR product.

The E2α subunit/E2β subunit PCR product was digested with the same enzymes and ligated into the pTHE1 vector. The ligation reaction was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals, Indianapolis, Ind.). The ligation mixture was transformed into chemically competent NovaBlue cells (Novagen) that then were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.) and digested with XbaI and NdeI restriction enzymes for gel electrophoresis analysis. The resulting plasmids carrying the constructed operon #4 (pEIITHEI) were transformed into BL21(DE3) cells to study the expression of the cloned sequences. Electrospray mass spectrometry assays confirmed that extracts from these cells have CoA transferase activity and 3-hydroxypropionyl-CoA dehydratase activity. Similar assays are used to confirm that extracts from these cells also have lactyl-CoA dehydratase activity.

E. coli plasmid pEIITHrEI carrying a synthetic 3-HP operon was digested with NruI, XbaI and BamHI restriction enzymes, XbaI-BamHI DNA fragment was gel purified with Qiagen Gel Extraction Kit (Qiagen, Inc., Valencia Calif.) and used for further cloning into Bacillu vector pWH1520 (MoBiTec BmBH, Gottingen, Germany). Vector pWH1520 was digested with SpeI and BamHI restriction enzymes and gel purified with Qiagen Gel Extraction Kit. The XbaI-BamHI fragment carrying 3-HP operon was ligated into WH1520 vector at 16° C. overnight using T4 ligase. The ligation mixture was transformed into chemically competent TOP10 cells and plated on LB plates supplemented with 50 μg/ml carbenicillin. One clone named *B. megaterium* (pBPO26) was used for assays of CoA-transferase and CoA-hydratase activities. The assays were performed as described above for *E. Coli*. The enzymatic activity was 5 U/mg and 13 U/mg respectively.

Example 7

Construction of a Two Plasmid System

The following constructs were constructed and can be used to produce 3-HP in *E. coli* (FIGS. **38**A and B). Nucleic acid molecules encoding a CoA transferase and a lactyl-CoA dehydratase were amplified from *Megasphaera elsdenii* genomic DNA by PCR. Two primers were used to amplify the CoA transferase-encoding sequence (OSNBpctF and OSHTR), two primers were used to amplify the E2α and (3 subunits of the lactyl-CoA dehydratase-encoding sequence (OSEIIXNF and OSEIIXNR), and two primers were used to amplify the E1 activator of the lactyl-CoA dehydratase-encoding sequence (E1PROF 5'-GTCGCAGAATTCCCATCAATCGCAGCAATCCCAAC-3', SEQ ID NO:126 and E1PROR 5'-TAACATGGTACCGACAGAAGCGGACCAGCA-AACGA-3', SEQ ID NO:127). A nucleic acid molecule encoding a 3-hydroxypropionyl-CoA dehydratase was amplified from *Chloroflexus aurantiacus* genomic DNA by PCR using two primers (OSTHF and OSHBR 5'-CGACGGATCCTCAACGACCA-CTGAAGTTGG-3', SEQ ID NO:128).

PCR was conducted in a Perkin Elmer 2400 Thermocycler using 100 ng of genomic DNA and a mix of rTth polymerase (Applied Biosystems; Foster City, Calif.) and Pfu Turbo polymerase (Stratagene; La Jolla, Calif.) in 8:1 ratio. The polymerase mix ensured higher fidelity of the PCR reaction. The following PCR conditions were used: initial denaturation step of 94° C. for 2 minutes; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2 minutes; and a final extension at 68° C. for 5 minutes. The obtained PCR products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Inc.; Valencia, Calif.).

The CoA transferase PCR product and the 3-hydroxypropionyl-CoA dehydratase PCR product were assembled using PCR. The OSTHF and OSHTR primers were complementary to each other. Thus, the complementary DNA ends could anneal to each other during the PCR reaction extending the DNA in both directions. To ensure the efficiency of the assembly, two end primers (OSNBpctF and OSHBR) were added to the assembly PCR mixture, which contained 100 ng of the purified CoA transferase PCR product, 100 ng of the purified 3-hydroxypropionyl-CoA dehydratase PCR product, and the polymerase mix described above. The following PCR conditions were used to assemble the products: 94° C. for 1 minute; 20 cycles of 94° C. for 30 seconds, 54° C. for 30 seconds, and 68° C. for 2.5 minutes; and a final extension at 68° C. for 5 minutes.

The assembled PCR product was gel purified and digested with NdeI and BamHI restriction enzymes. The sites for these restriction enzymes were introduced into the assembled PCR products with the OSNBpctF (NdeI) and OSHBR (BamHI) primers. The digested PCR product was heated at 80° C. for 30 minutes to inactivate the restriction enzymes and used directly for ligation into a pET11a vector.

The pET-11a vector was digested with NdeI and BamHI restriction enzymes, gel purified using a Qiagen Gel Extraction kit, treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.) and used in a ligation reaction with the assembled PCR product. The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into NovaBlue chemically competent cells (Novagen; Madison, Wis.) using a heat-shock method. Once shocked, the cells were plated on LB plates supplemented with 50 μg/mL carbenicillin. The

plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc.; Valencia, Calif.) and digested with NdeI and BamHI restriction enzymes for gel electrophoresis analysis. The resulting plasmids carrying the CoA transferase and 3-hydroxypropionyl-CoA dehydratase (pTH) were digested with XbaI and NdeI, purified using gel electrophoresis and a Qiagen Gel Extraction kit, and used as a vector for cloning of the E2α subunit/E2β subunit PCR product.

The E2α subunit/E2β subunit PCR product digested with the same enzymes was ligated into the pTH vector. The ligation reaction was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The ligation mixture was transformed into chemically competent NovaBlue cells (Novagen) that then were plated on LB plates supplemented with 50 μg/mL carbenicillin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc.; Valencia, Calif.) and digested with XbaI and NdeI restriction enzymes for gel electrophoresis analysis. The resulting plasmids carrying the E2α and β subunits of the lactyl-CoA dehydratase, the CoA transferase, and the 3-hydroxypropionyl-CoA dehydratase (pEIITH) were transformed into BL21(DE3) cells to study the expression of the cloned sequences.

The gel purified E1 activator PCR product was digested with EcoRI and KpnI restriction enzymes, heated at 65° C. for 30 minutes, and ligated into a vector (pPROLar.A) that was digested with EcoRI and KpnI restriction enzymes, gel purified using Qiagen Gel Extraction kit, and treated with shrimp alkaline phosphatase (Roche Molecular Biochemicals; Indianapolis, Ind.). The ligation was performed at 16° C. overnight using T4 ligase (Roche Molecular Biochemicals; Indianapolis, Ind.). The resulting ligation reaction was transformed into DH10B electro-competent cells (Gibco Life Technologies; Gaithersburg, Md.) using electroporation. Once electroporated, the cells were plated on LB plates supplemented with 25 μg/mL kanamycin. The plasmid DNA was purified from individual colonies using a QiaPrep Spin Miniprep Kit (Qiagen Inc., Valencia, Calif.) and digested with EcoRI and KpnI restriction enzymes for gel electrophoresis analysis. The resulting plasmids carrying the E1 activator (pPROEI) are transformed into BL21(DE3) cells to study the expression of the cloned sequence.

The pPROEI and pEIITH plasmids are compatible plasmids that can be used in the same bacterial host cell. In addition, the expression from the pPROEI and pEIITH plasmids can be induced at different levels using IPTG and arabinose, allowing for the fine-tuning of the expression of the cloned sequences.

Example 8

Production of 3-HP

3-HP was produced using recombinant *E. coli* in a small-scale batch fermentation reaction. The construction of strain ALS848 (also designated as TA3476 (*J. Bacteriol.,* 143: 1081-1085(1980))) that carried inducible T7 RNA polymerase was performed using λDE3 lysogenization kit (Novagen, Madison, Wis.) according to the manufacturer's instructions. The constructed strain was designated ALS484 (DE3). Strain ALS484(DE3) was transformed with pEIITHrEI plasmid using standard electroporation techniques. The transformants were selected on LB/carbenicillin (50 μg/mL) plates. A single colony was used to inoculate 4 mL culture in a 15 mL culture tube. Strain ALS484(DE3) strain carrying vector pET11a was used as a control. The cells were grown at 37° C. and 250 rpm in an Innova 4230 Incubator Shaker (New Brunswick Scientific, Edison, N.J.) for eight to nine hours. This culture (3 mL) was used to start an anaerobic fermentation. Two 100 mL anaerobic cultures of ALS(DE3)/pET11a and ALS(DE3)pEIITHrEI were grown in serum bottles using LB media supplemented with 0.4% glucose, 50 μg/mL carbenicillin, and 100 mM MOPS. The cultures were grown overnight at 37° C. without shaking. The overnight grown cultures were sub-cultured in serum bottles using fresh LB media supplemented with 0.4% glucose, 50 μg/mL carbenicillin, and 100 mM MOPS. The starting OD(600) of these cultures was adjusted to 0.3. These serum bottles were incubated at 37° C. without shaking. After one hour of incubation, the cultures were induced with 100 μM IPTG. A 3 mL sample was taken from each of the serum bottles at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 24 hours. The samples were transferred into two properly labeled 2 mL microcentrifuge tubes, each containing 1.5 mL sample. The samples were spun down in a microcentrifuge centrifuge at 14000 g for 3 minutes. The supernatant was passed through a 0.45μ syringe filter, and the resulting filtrate was stored at −20° C. until further analysis. The formation of fermentation products, mainly lactate and 3-hydroxypropionate, was measured by detecting derivatized CoA esters of lactate and 3-HP using LC/MS.

The following methods were performed to convert lactate and 3-HP into their respective CoA esters. Briefly, the filtrates were mixed with CoA-reaction buffer (200 mM potassium phosphate buffer, 2 mM acetyl-CoA, and 0.1 mg/mL purified transferase) in 1:1 ratio. The reaction was allowed to proceed for 20 minutes at room temperature. The reaction was stopped by adding 1 volume of 10% TFA. The sample was purified using Sep Pak Vac columns (Waters). The column was conditioned with methanol and washed two times with 0.1% TFA. The sample was then applied to the column, and the column was washed two more times with 0.1% TFA. The sample was eluted with 40% acetonitrile, 0.1% TFA. The acetonitrile was removed from the sample by vacuum centrifugation. The samples were then analyzed by LC/MS.

Analysis of the standard CoA/CoA thioester mixtures and the CoA thioester mixtures derived from fermentation broths were carried out using a Waters/Micromass ZQ LC/MS instrument which had a Waters 2690 liquid chromatograph with a Waters 996 Photo-Diode Array (PDA) absorbance monitor placed in series between the chromatograph and the single quadrupole mass spectrometer. LC separations were made using a 4.6×150 mm YMC ODS-AQ (3 μm particles, 120 Å pores) reversed-phase chromatography column at room temperature. Two gradient elution systems were developed using different mobile phases for the separation of the CoA esters. These two systems are summarized in Table 3. Elution system 1 was developed to provide the most rapid and efficient separation of the five-component CoA/CoA thioester mixture (CoA, acetyl-CoA, lactyl-CoA, acrylyl-CoA, and propionyl-CoA), while elution system 2 was developed to provide baseline separation of the structurally isomeric esters lactyl-CoA and 3HP-CoA in addition to separation of the remaining esters listed above. In all cases, the flow rate was 0.250 mL/minute, and photodiode array UV absorbance was monitored from 200 nm to 400 nm. All parameters of the electrospray MS system were optimized and selected based on generation of protonated molecular ions ($[M+H]^+$) of the analytes of interest and production of characteristic fragment ions. The following instrumental parameters were used for ESI-MS detection of CoA and organic acid-CoA thioesters in the positive ion mode: Capillary: 4.0 V; Cone: 56 V; Extractor: 1 V; RF lens: 0 V; Source

temperature: 100° C.; Desolvation temperature: 300° C.; Desolvation gas: 500 L/hour; Cone gas: 40 L/hour; Low mass resolution: 13.0; High mass resolution: 14.5; Ion energy: 0.5; Multiplier: 650. Uncertainties for reported mass/charge ratios (m/z) and molecular masses are ±0.01%. Table 3 provides a summary of gradient elution systems for the separation of organic acid-Coenzyme A thioesters.

TABLE 3

			Gradient	
System	Buffer A	Buffer B	Time	Percent B
1	25 mM ammonium acetate 0.5% acetic acid	ACN 0.5% acetic acid	0 40 42 47 50	10 40 100 100 10
2	25 mM ammonium acetate 10 mM TEA 0.5% acetic acid	ACN 0.5% acetic acid	0 10 45 50 53 54	10 10 60 100 100 10

The following methods were used to separate the derivatized 3-hydroxypropionyl-CoA, which was formed from 3-HP, from 2-hydroxypropionyl-CoA (i.e., lactyl-CoA), which was formed from lactate. Because these structural isomers have identical masses and mass spectral fragmentation behavior, the separation and identification of these analytes in a mixture depends on their chromatographic separation. While elution system 1 provided excellent separation of the CoA thioesters tested (FIGS. **46**A-**46**F), it was unable to resolve 3-HP-CoA and lactyl-CoA. An alternative LC elution system was developed using ammonium acetate and triethylamine (system 2; Table 3).

The ability of system 2 to separate 3-HP-CoA and lactyl-CoA was tested on a mixture of these two compounds. Comparing the results from a mixture of 3-HP-CoA and lactyl-CoA (FIG. **47** A) to the results from lactyl-CoA only (FIG. **47** B) revealed that system 2 can separate 3-HP-CoA and lactyl-CoA. The mass spectrum recorded under peak 1 (FIG. **47**A insert) was used to identify peak 1 as being a hydroxypropionyl-CoA thioester when compared to FIG. **46**C. In addition, comparison of FIGS. **47**A and **47**B as well as the mass spectra results corresponding to each peak revealed that peak 1 corresponds to 3-HP-CoA and peak 2 corresponds to lactyl-CoA.

System 2 was used to confirm that *E. coli* transfected with pEIITHrEI produced 3-HP in that 3-HP-CoA was detected. Specifically, an ion chromatogram for m/z=840 in the analysis of a CoA transferase-treated fermentation broth aliquot collected from a culture of *E. coli* containing pEIITHrEI revealed the presence of 3-HP-CoA (FIG. **48**A). The CoA transferase-treated fermentation broth aliquot collected from a culture of *E. coli* lacking pEIITHrEI did not exhibit the peak corresponding to 3-HP-CoA (FIG. **48**B). Thus, these results indicate that the pEIITHrEI plasmid directs the expression of polypeptides having propionyl-CoA transferase activity, lactyl-CoA dehydratase activity, and acrylyl-CoA hydratase activity. These results also indicate that expression of these polypeptides leads to the formation of 3-HP.

Example 9

Cloning Nucleic Acid Molecules that Encode a Polypeptide Having Acetyl CoA Carboxylase Activity

Polypeptides having acetyl-CoA carboxylase activity catalyze the first committed step of the fatty acid synthesis by carboxylation of acetyl-CoA to malonyl-CoA. Polypeptides having acetyl-CoA carboxylase activity are also responsible for providing malonyl-CoA for the biosynthesis of very-long-chain fatty acids required for proper cell function. Polypeptides having acetyl-CoA carboxylase activity can be biotin dependent enzymes in which the cofactor biotin is post-translationally attached to a specific lysine residue. The reaction catalyzed by such polypeptides consists of two discrete half reactions. In the first half reaction, biotin is carboxylated by biocarbonate in an ATP-dependent reaction to form carboxybiotin. In the second half reaction, the carboxyl group is transferred to acetyl-CoA to form malonyl-CoA.

Prokaryotic and eukaryotic polypeptides having acetyl-CoA carboxylase activity exist. The prokaryotic polypeptide is a multi-subunit enzyme (four subunits), where each of the subunits is encoded by a different nucleic acid sequence. For example, in *E. coli*, the following genes encode for the four subunits of acetyl-CoA carboxylase:

accA: Acetyl-coenzyme a carboxylase carboxyl transferase subunit alpha (GenBank® accession number M96394)

accB: Biotin carboxyl carrier protein (GenBank® accession number U18997)

accC: Biotin carboxylase (GenBank® accession number U18997)

accD: Acetyl-coenzyme a carboxylase carboxyl transferase subunit beta (GenBank® accession number M68934)

The eukaryotic polypeptide is a high molecular weight multi-functional enzyme encoded by a single gene. For example, in *Saccharomyces cerevisiae*, the acetyl-CoA carboxylase can have the sequence set forth in GenBank® accession number M92156.

The prokaryotic type acetyl-CoA carboxylase from *E. coli* was overexpressed using T7 promoter vector pFN476 as described elsewhere (Davis et al. *J. Biol. Chem.*, 275:28593-28598 (2000)). The eukaryotic type acetyl-CoA carboxylase gene was amplified from *Saccharomyces cerevisiae* genomic DNA. Two primers were designed to amplify the acc1 gene from *S. cerevisiae* (acc1F 5'-atagGCGGCCGC AGGAATGCTGTATGAGCGAAGAAAGCTTATT C-3', SEQ ID NO: 138 where the bold is homologous sequence, the italics is a Not I site, the underline is a RBS, and the lowercase is extra; and acc1R 5'-atgctcgcatCTCGAGTAG-CTAAATTAAATTACATCAATAGTA-3', SEQ ID NO: 139 where the bold is homologous sequence, the italics is a Xho I site, and the lowercase is extra). The following PCR mix is used to amplify acc1 gene 10× pfu buffer (10 μL), dNTP (10 mM; 2 μL), cDNA (2 μL), acc1F (100 μM; 1 μL), acc1R (100 μM; 1 pfu enzyme (2.5 units/μL; 2 μL), and DI water (82 μL). The following protocol was used to amplify the acc1 gene. After performing PCR, the PCR product was separated on a gel, and the band corresponding to acc1 nucleic acid (about 6.7 Kb) was gel isolated using Qiagen gel isolation kit. The PCR fragment is digested with Not I and Xho I (New England BioLab) restriction enzymes. The digested PCR fragment is then ligated to pET30a which was restricted with Not I and Xho I and dephosphorylated with SAP enzyme. The *E. coli* strain DH10B was transformed with 1 μL of the ligation mix, and the cells were recovered in 1 mL of SOC media. The transformed cells were selected on LB/kanamycin (50 μg/μL) plates. Eight single colonies are selected, and PCR was used to screen for the correct insert. The plasmid having correct insert was isolated using Qiagen Spin Mini prep kit.

To obtain a polypeptide having acetyl-CoA carboxylase activity, the plasmid pMSD8 or pET30a/acc1 overexpressing *E. coli* or *S. cerevisiae* acetyl-CoA carboxylase was transformed into Tuner pLacI chemically competent cells (Novagen, Madison, Wis.). The transformed cells were

selected on LB/chloramphenicol (25 μg/mL) plus carbenicillin (50 μg/mL) or kanamycin (50 μg/mL).

A crude extract of this strain can be prepared in the following manner. An overnight culture of Tuner pLacI with pMSD8 is subcultured into 200 mL (in one liter baffle culture flask) of fresh M9 media supplemented with 0.4% glucose, 1 μg/mL thiamine, 0.1% casamino acids, and 50 μg/mL carbenicillin or 50 μg/mL kanamycin and 25 μg/mL chloramphenicol. The culture is grown at 37° C. in a shaker with 250 rpm agitation until it reaches an optical density at 600 nm of about 0.6. IPTG is then added to a final concentration of 100 μM. The culture is then incubated for an additional 3 hours with shaking speed of 250 rpm at 37° C. Cells are harvested by centrifugation at 8000×g and are washed one time with 0.85% NaCl. The cell pellet was resuspended in a minimal volume of 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM KCl, 2 mM DTT, and 5% glycerol. The cells are lysed by passing them two times through a French Pressure cell at 1000 psig pressure. The cell debris was removed by centrifugation for 20 minutes at 30,000×g.

The enzyme can be assayed using a method from Davis et al. (*J. Biol. Chem.,* 275:28593-28598 (2000)).

Example 10

Cloning a Nucleic Acid Molecule that Encodes a Polypeptide Having Malonyl-CoA Reductase Activity from *Chloroflexus aurantiacus*

A polypeptide having malonyl-CoA reductase activity was partially purified from *Chloroflexus aurantiacus* and used to obtain amino acid micro-sequencing results. The amino acid sequencing results were used to identify and clone the nucleic acid that encodes a polypeptide having malonyl-CoA reductase activity.

Biomass required for protein purification was grown in B. Braun BIOSTAT B fermenters (B. Braun Biotech International GmbH, Melsungen, Germany). A glass vessel fitted with a water jacket for heating was used to grow the required biomass. The glass vessel was connected to its own control unit. The liquid working volume was 4 L, and the fermenter was operated at 55° C. with 75 rpm of agitation. Carbon dioxide was occasionally bubbled through the headspace of the fermenter to maintain anaerobic conditions. The pH of the cultures was monitored using a standard pH probe and was maintained between 8.0 and 8.3. The inoculum for the fermenters was grown in two 250 mL bottles in an Innova 4230 Incubator, Shaker (New Brunswick Scientific, Edison, N.J.) at 55° C. with interior lights. The fermenters were illuminated by three 65 W plant light reflector lamps (General Electric, Cleveland, Ohio). Each lamp was placed approximately 50 cm away from the glass vessel. The media used for the inoculum and the fermenter culture was as follows per liter: 0.07 g EDTA, 1 mL micronutrient solution, 1 mL $FeCl_3$ solution, 0.06 g $CaSO_4.2H_2O$, 0.1 g $MgSO_4.7H_2O$, 0.008 g NaCl, 0.075 g KCl, 0.103 g KNO_3, 0.68 g $NaNO_3$, 0.111 g Na_2HPO_4, 0.2 g NH_4Cl, 1 g yeast extract, 2.5 g casamino acid, 0.5 g Glycyl-Glycine, and 900 mL DI water. The micronutrient solution contained the following per liter: 0.5 mL H_2SO_4 (conc.), 2.28 g $MnSO_4.7H_2O$, 0.5 g $ZnSO_4.7H_2O$, 0.5 g H_3BO_3, 0.025 g $CuSO_4.2H_2O$, 0.025 g $Na_2MoO_4.2H_2O$, and 0.045 g $CoCl_2.6H_2O$. The $FeCl_3$ solution contained 0.2905 g $FeCl_3$ per liter. After adjusting the pH of the media to 8.2 to 8.4, 0.75 g/L $Na_2S.9H_2O$ was added, the pH was readjusted to 8.2 to 8.4, and the media was filter-sterilized through a 0.22μ filter.

The fermenter was inoculated with 500 mL of grown culture. The fermentation was stopped, and the biomass was harvested after the cell density was about 0.5 to 0.6 units at 600 nm.

The cells were harvested by centrifugation at 5000×g (Beckman JLA 8.1000 rotor) at 4° C., washed with 1 volume of ice cold 0.85% NaCl, and centrifuged again. The cell pellet was resuspended in 30 mL of ice cold 100 mM Tris-HCl (pH 7.8) buffer that was supplemented with 2 mM DTT, 5 mM $MgCl_2$, 0.4 mM PEFABLOC (Roche Molecular Biochemicals, Indianapolis, Ind.), 1% streptomycin sulfate, and 2 tablets of Complete EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, Ind.). The cell suspension was lysed by passing the suspension, three times, through a 50 mL French Pressure Cell operated at 1600 psi (gauge reading). Cell debris was removed by centrifugation at 30,000×g (Beckman JA 25.50 rotor). The crude extract was filtered prior to chromatography using a 0.2 μm HT Tuffryn membrane syringe filter (Pall Corp., Ann Arbor, Mich.). The protein concentration of the crude extract was 29 mg/mL, which was determined using the BioRad Protein Assay according to the manufacturer's microassay protocol. Bovine gamma globulin was used for the standard curve determination. This assay was based on the Bradford dye-binding procedure (Bradford, *Anal. Biochem.,* 72:248 (1976)).

Before starting the protein purification, the following assay was used to determine the activity of malonyl-CoA reductase in the crude extract. A 50 μL aliquot of the cell extract (29 mg/mL) was added to 10 μL 1M Tris-HCl (final concentration in assay 100 mM), 10 μL 10 mM malonyl CoA (final concentration in assay 1 mM), 5.5 μL 5.5 mM NADPH (final concentration in assay 0.3 mM), and 24.5 μL DI water in a 96 well UV transparent plate (Corning, N.Y.). The enzyme activity was measured at 45° C. using SpectraMAX Plus 96 well plate reader (Molecular devices Sunnyvale, Calif.). The activity of malonyl-CoA reductase was monitored by measuring the disappearance of NADPH at 340 nm wavelength. The crude extract exhibited malonyl-CoA reductase activity.

The 5 mL (total 145 mg) protein cell extract was diluted with 20 mL buffer A (20 mM ethanolamine (pH 9.0), 5 mM $MgCl_2$, 2 mM DTT). The chromatographic protein purification was conducted using a BioLogic protein purification system (BioRad Hercules, Calif.). The 25 mL of cell suspension was loaded onto a UNO Q-6 ion-exchange column that had been equilibrated with buffer A at a rate of 1 mL/minute. After sample loading, the column was washed with 2.5 times column volume of buffer A at a rate of 2 mL/minute. The proteins were eluted with a linear gradient of NaCl in buffer A from 0-0.33 M in 25 Column volume. During the entire chromatographic separation, three mL fractions were collected. The collection tubes contained 50 μL of Tris-HCl (pH 6.5) so that the pH of the eluted sample dropped to about pH 7. Major chromatographic peaks were detected in the region that corresponded to fractions 18 to 21 and 23 to 30. A 200 μL sample was taken from these fractions and concentrated in a microcentrifuge at 4° C. using a Microcon YM-10 column (Millipore Corp., Bedford, Mass.) as per manufacturer's instructions. To each of the concentrated fractions, buffer A-Tris (100 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 2 mM DTT) was added to bring the total volume to 100 μL. Each of these fractions was tested for the malonyl-CoA reductase activity using the spectrophotometric assay described above. The majority of specific malonyl CoA activity was found in fractions 18 to 21. These fractions were pooled together, and the pooled sample was desalted using PD-10 column (Amersham Pharmacia Piscataway, N.J.) as per manufacturer's instructions.

The 10.5 mL of desalted protein extract was diluted with buffer A-Tris to a volume of 25 mL. This desalted diluted sample was applied to a 1 mL HiTrap Blue column (Amersham Pharmacia Piscataway, N.J.) which was equilibrated with buffer A-Tris. The sample was loaded at a rate of 0.1 mL/minute. Unbound proteins were washed with 2.5 CV buffer A-Tris. The protein was eluted with 100 Mm Tris (pH 7.8), 5 mM $MgCl_2$, 2 mM DTT, 2 mM NADPH, and 1 M NaCl. During this separation process, one mL fractions were collected. A 200 μL sample was drawn from fractions 49 to 54 and concentrated. Buffer A-Tris was added to each of the concentrated fractions to bring the total volume to 100 μL. Fractions were assayed for enzyme activity as described above. The highest specific activity was observed in fraction 51. The entire fraction 51 was concentrated as described above, and the concentrated sample was separated on an SDS-PAGE gel.

Electrophoresis was carried out using a Bio-Rad Protean II minigel system and pre-cast SDS-PAGE gels (4-15%), or a Protean II XI system and 16 cm×20 cm×1 mm SDS-PAGE gels (10%) cast as per the manufacturer's protocol. The gels were run according to the manufacturer's instructions with a running buffer of 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 0.1% SDS.

A gel thickness of 1 mm was used to run samples from fraction 51. Protein from fraction 51 was loaded onto 10% SDS-PAGE (3 lanes, each containing 75 μg of total protein). The gels were stained briefly with Coomassie blue (Bio-Rad, Hercules, Calif.) and then destained to a clear background with a 10% acetic acid and 20% methanol solution. The staining revealed a band of about 130 to 140 KDa.

The protein band of about 130-140 KDa was excised with no excess unstained gel present. An equal area gel without protein was excised as a negative control. The gel slices were placed in uncolored microcentrifuge tubes, prewashed with 50% acetonitrile in HPLC-grade water, washed twice with 50% acetonitrile, and shipped on dry ice to Harvard Microchemistry Sequencing Facility, Cambridge, Mass.

After in-situ enzymatic digestion of the polypeptide sample with trypsin, the resulting polypeptides were separated by micro-capillary reverse-phase HPLC. The HPLC was directly coupled to the nano-electrospray ionization source of a Finnigan LCQ quadrupole ion trap mass spectrometer (μLC/MS/MS). Individual sequence spectra (MS/MS) were acquired on-line at high sensitivity for the multiple polypeptides separated during the chromatographic run. The MS/MS spectra of the polypeptides were correlated with known sequences using the algorithm Sequest developed at the University of Washington (Eng et al., *J. Am. Soc. Mass Spectrom.,* 5:976 (1994)) and programs developed at Harvard (Chittum et al., *Biochemistry,* 37:10866 (1998)). The results were reviewed for consensus with known proteins and for manual confirmation of fidelity.

A similar purification procedure was used to obtain another sample (protein 1 sample) that was subjected to the same analysis that was used to evaluate the fraction 51 sample.

The polypeptide sequence results indicated that the polypeptides obtained from both the fraction 51 sample and the protein 1 sample had similarity to the six (764, 799, 859, 923, 1090, 1024) contigs sequenced from the *C. aurantiacus* genome and presented on the Joint Genome Institute's web site (http://www.jgi.doe.gov/). The 764 contig was the most prominent of the six with about 40 peptide sequences showing similarity. BLASTX analysis of each of these contigs on the GenBank web site (http://www.ncbi.nlm.nih.gov/BLAST/) indicated that the DNA sequence of the 764 contig (4201 bases) encoded for polypeptides that had a dehydrogenase/reductase type activity. Close inspection of the 764 contig, however, revealed that this contig did not have an appropriate ORF that would encode for a 130-140 KDa polypeptide.

BLASTX analysis also was conducted using the other five contigs. The results of this analysis were as follows. The 799 contig (3173 bases) appeared to encode polypeptides having phosphate and dehydrogenase type activities. The 859 contig (5865 bases) appeared to encode polypeptides having synthetase type activities. The 923 contig (5660 bases) appeared to encode polypeptides having elongation factor and synthetase type activities. The 1090 contig (15201 bases) appeared to encode polypeptides having dehydrogenase/reductase and cytochrome and sigma factor activities. The 1024 contig (12276 bases) appeared to encode polypeptides having dehydrogenase and decarboxylase and synthetase type activities. Thus, the 859 and 923 contigs were eliminated from any further analysis.

The results from the BLASTX analysis also revealed that the dehydrogenase found in the 1024 contig was most likely an inositol monophosphate dehydrogenase. Thus, the 1024 contig was eliminated as a possible candidate that might encode for a polypeptide having malonyl-CoA reductase activity. The 799 contig also was eliminated since this contig is part of the OS17 polypeptide described above.

This narrowed down the search to 2 contigs, the 764 and 1090 contigs. Since the contigs were identified using the same protein sample and the dehydrogenase activities found in these contigs gave very similar BLASTX results, it was hypothesized that they are part of the same polypeptide. Additional evidence supporting this hypothesis was obtained from the discovery that the 764 and 1090 contigs are adjacent to each other in the *C. aurantiacus* genome as revealed by an analysis of scaffold data provided by the Joint Genome Institute. Sequence similarity and assembly analysis, however, revealed no overlapping sequence between these two contigs, possibly due to the presence of gaps in the genome sequencing.

The polypeptide sequences that belonged to the 764 and 1090 contigs were mapped. Based on this analysis, an appropriate coding frame and potential start and stop codons were identified. The following PCR primers were designed to PCR amplify a fragment that encoded for a polypeptide having malonyl-CoA reductase activity: PRO140F 5'-ATGGCGACGGGCGAGTCCATGAG-3', SEQ ID NO:153; PRO140R 5'-GGACACGAAGAACAGGGCGACAC-3', SEQ ID NO:154; and PRO140UP 5'-GAACTGTCTGGAGTAAGGCTGTC-3', SEQ ID NO:155. The PRO140F primer was designed based on the sequence of the 1090 contig and corresponds to the start of the potential start codon. The twelfth base was changed from G to C to avoid primer-dimer formation. This change does not change the amino acid that was encoded by the codon. The PRO140R primer was designed based on sequence of the 764 contig and corresponds to a region located about 1 kB downstream from the potential stop codon. The PRO140UPF primer was designed based on sequence of the 1090 contig and corresponds to a region located about 300 bases upstream of potential start codon.

Genomic *C. aurantiacus* DNA was obtained. Briefly, *C. aurantiacus* was grown in 50 mL cultures for 3 to 4 days. Cells were pelleted and washed with 5 mL of a 10 mM Tris solution. The genomic DNA was then isolated using the gram positive bacteria protocol provided with Gentra Genomic "Puregene" DNA isolation kit (Gentra Systems, Minneapolis, Minn.). The cell pellet was resuspended in 1 mL Gentra Cell Suspension Solution to which 14.2 mg of lysozyme and

4 μL of 20 mg/mL proteinase K solution was added. The cell suspension was incubated at 37° C. for 30 minutes. The precipitated genomic DNA was recovered by centrifugation at 3500 g for 25 minutes and air-dried for 10 minutes. The genomic DNA was suspended in an appropriate amount of a 10 mM Tris solution and stored at 4° C.

Two PCR reactions were set-up using *C. aurantiacus* genomic DNA as template as follows:

		PCR program
PCR Reaction #1		
3.3 X rTH polymerase Buffer	30 μL	94° C. 2 minutes
Mg(OAC) (25 mM)	4 μL	29 cycles of:
dNTP Mix (10 mM)	3 μL	94° C. 30 seconds
PRO140F (100 μM)	2 μL	63° C. 45 seconds
PRO140R (100 μM)	2 μL	68° C. 4.5 minutes
Genomic DNA (100 ng/mL)	1 μL	68° C. 7 minutes
rTH polymerase (2 U/μL)	2 μL	4° C. Until further use
pfu polymerase (2.5 U/μL)	0.25 μL	
DI water	55.75 μL	
Total	100 μL	
PCR Reaction #2		
3.3 X rTH polymerase Buffer	30 μL	94° C. 2 minutes
Mg(OAC) (25 mM)	4 μL	29 cycles of:
dNTP Mix (10 mM)	3 μL	94° C. 30 seconds
PRO140UPF (100 μM)	2 μL	60° C. 45 seconds
PRO140R (100 μM)	2 μL	68° C. 4.5 minutes
Genomic DNA (100 ng/mL)	1 μL	68° C. 7 minutes
rTH polymerase (2 U/μL)	2 μL	4° C. Until further use
pfu polymerase 2.5 U/μL)	0.25 μL	
DI water	55.75 μL	
Total	100 μL	

The products from both PCR reactions were separated on a 0.8% TAE gel. Both PCR reactions produced a product of 4.7 to 5 Kb in size. This approximately matched the expected size of a nucleic acid molecule that could encode a polypeptide having malonyl-CoA reductase activity.

Both PCR products were sequenced using sequencing primers (1090Fseq 5'-GATTCCGTATGTCACCCCTA-3', SEQ ID NO:156; and 764Rseq 5'-CAGGCGACTGGCAATCACAA-3', SEQ ID NO:157). The sequence analysis revealed a gap between the 764 and 1090 contigs. The nucleic acid sequence between the sequences from the 764 and 1090 contigs was greater than 300 base pairs in length (FIG. **51**). In addition, the sequence analysis revealed an ORF of 3678 bases that showed similarities to dehydrogenase/reductase type enzymes (FIGS. **52**A-**52**D). The amino acid sequence encoded by this ORF is 1225 amino acids in length (FIG. **50**). Also, BLASTP analysis of the amino acid sequence encoded by this ORF revealed two short chain dehydrogenase domains (adh type). These results are consistent with a polypeptide having malonyl-CoA reductase activity since such an enzyme involves two reduction steps for the conversion of malonyl CoA to 3-HP. Further, the computed MW of the polypeptide was determined to be about 134 KDa.

PCR was conducted using the PRO140F/PRO140R primer pair, *C. aurantiacus* genomic DNA, and the protocol described above as PCR reaction #1. After the PCR was completed, 0.25 U of Taq polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.) was added to the PCR mix, which was then incubated at 72° C. for 10 minutes. The PCR product was column purified using Qiagen PCR purification kit (Qiagen Inc., Valencia, Calif.). The purified PCR product was then TOPO cloned into expression vector pCRT7/CT as per manufacturer's instructions (Invitrogen, Carlsbad, Calif.). TOP10 F' chemical competent cells were transformed with the TOPO ligation mix as per manufacturer's instructions (Invitrogen, Carlsbad, Calif.). The cells were recovered for half an hour, and the transformants were selected on LB/ampicillin (100 μg/mL) plates. Twenty single colonies were selected, and the plasmid DNA was isolated using Qiagen spin Mini prep kit (Qiagen Inc., Valencia, Calif.).

Each of these twenty clones were tested for correct orientation and right insert size by PCR. Briefly, plasmid DNA was used as a template, and the following two primers were used in the PCR amplification: PCRT7 5'-GAGACCACAACGGTTTCCCTCTA-3', SEQ ID NO:158; and PRO140R 5'-GGACACGAAGAACAGGGCGACAC-3', SEQ ID NO:159. The following PCR reaction mix and program was used:

PCR Reaction		PCR program
3.3 X rTH polymerase Buffer	7.5 μL	94° C. 2 minutes
Mg(OAC) (25 mM)	1 μL	25 cycles of:
dNTP Mix (10 mM)	0.5 μL	94° C. 30 seconds
PCRT7 (100 μM)	0.125 μL	55° C. 45 seconds
PRO140R (100 μM)	0.125 μL	68° C. 4 minutes
Plasmid DNA	0.5 μL	68° C. 7 minutes
rTH polymerase (2 U/μL)	0.5 μL	4° C. Until further use
DI water	14.75 μL	
Total	25 μL	

Out of twenty clones tested, only one clone exhibited the correct insert (Clone # P-10). Chemical competent cells of BL21(DE3)pLysS (Invitrogen, Carlsbad, Calif.) were transformed with 2 μL of the P-10 plasmid DNA as per the manufacturer's instructions. The cells were recovered at 37° C. for 30 minutes and were plated on LB ampicillin (100 μg/mL) and chloramphenicol (25 μg/mL).

A 20 mL culture of BL21(DE3)pLysS/P-10 and a 20 mL control culture of BL21(DE3)pLysS was incubated overnight. Using the overnight cultures as an inoculum, two 100 mL BL21(DE3)pLysS/P-10 clone cultures and two control strain cultures (BL21(DE3)pLysS) were started. All the cultures were induced with IPTG when they reached an OD of about 0.5 at 600 nm. The control strain culture was induced with 10 μM IPTG or 100 μM IPTG, while one of the BL21 (DE3)pLysS/P-10 clone cultures was induced with 10 μM IPTG and the other with 100 μM IPTG. The cultures were grown for 2.5 hours after induction. Aliquots were taken from each of the culture flasks before and after 2.5 hours of induction and separated using 4-15% SDS-PAGE to analyze polypeptide expression. In the induced BL21(DE3)pLysS/P-10 samples, a band corresponding to a polypeptide having a molecular weight of about 135 KDa was observed. This band was absent in the control strain samples and in samples taken before IPTG induction.

To assess malonyl-CoA reductase activity, BL21(DE3) pLysS/P-10 and BL21(DE3)pLysS cells were cultured and then harvested by centrifugation at 8,000×g (Rotor JA 16.250, Beckman Coulter, Fullerton, Calif.). Once harvested, the cells were washed once with an equal volume of a 0.85% NaCl solution. The cell pellets were resuspended into 100 mM Tris-HCl buffer that was supplemented with 5 mM Mg_2Cl and 2 mM DTT. The cells were disrupted by passing twice through a French Press Cell at 1,000 psi pressure (Gauge value). The cell debris was removed by centrifugation at 30,000×g (Rotor JA 25.50, Beckman Coulter, Fullerton, Calif.). The cell extract was maintained at 4° C. or on ice until further use.

Activity of malonyl-CoA reductase was measured at 37° C. for both the control cells and the IPTG-induced cells. The activity of malonyl-CoA reductase was monitored by observing the disappearance of added NADPH as described above. No activity was found in the cell extract of the control strain, while the cell extract from the IPTG-induced BL21(DE3) pLysS/P-10 cells displayed malonyl-CoA reductase activity with a specific activity calculated to be about 0.0942 μmole/minute/mg of total protein.

Malonyl-CoA reductase activity also was measured by analyzing 3-HP formation from malonyl CoA using the following reaction conducted at 37° C.:

	Volume	Final conc.
Tris HCl (1M)	10 μL	100 mM
Malonyl CoA (10 mM)	40 μL	4 mM
NADPH (10 mM)	30 μL	3 mM
Cell extract	20 μL	
Total	100 μL	

The reaction was carried out at 37° C. for 30 minutes. In the control reaction, a cell extract from BL21(DE3)pLysS was added to a final concentration of 322 mg total protein. In the experimental reaction mix, a cell extract from BL21(DE3) pLysS/P-10 was added to a final concentration of 226 mg of total protein. The reaction mixtures were frozen at −20° C. until further analysis.

Chromatographic separation of the components in the reaction mixtures was performed using a HPX-87H (7.8×300 mm) organic acid HPLC column (BioRad Laboratories, Hercules, Calif.). The column was maintained at 60° C. Mobile phase composition was HPLC grade water pH to 2.5 using triflouroacetic acid (TFA) and was delivered at a flow rate of 0.6 mL/minute.

Detection of 3-HP in the reaction samples was accomplished using a Waters/Micromass ZQ LC/MS instrument consisting of a Waters 2690 liquid chromatograph (Waters Corp., Milford, Mass.) with a Waters 996 Photo-diode Array (PDA) absorbance monitor placed in series between the chromatograph and the single quadrupole mass spectrometer. The ionization source was an Atmospheric Pressure Chemical Ionization (APCI) ionization source. All parameters of the APCI-MS system were optimized and selected based on the generation of the protonated molecular ion $([M+H])^+$ of 3-HP. The following parameters were used to detect 3-HP in the positive ion mode: Corona: 10 μA; Cone: 20V; Extractor: 2V; RF lens: 0.2V; Source temperature: 100° C.; APCI Probe temperature: 300° C.; Desolvation gas: 500 L/hour; Cone gas: 50 L/hour; Low mass resolution: 15; High mass resolution: 15; Ion energy: 1.0; Multiplier: 650. Data was collected in Selected Ion Reporting (SIR) mode set at m/z=90.9.

Both the control reaction sample and the experimental reaction sample were probed for presence of 3-HP using the HPLC-mass spectroscopy technique. In the control samples, no 3-HP peak was observed, while the experimental sample exhibited a peak that matched the retention and the mass of 3-HP.

Example 11

Constructing Recombinant Cells that Produce 3-HP

A pathway to make 3-hydroxypropionate directly from glucose via acetyl CoA is presented in FIG. **44**. Most organisms such as *E. coli, Bacillus*, and yeast produce acetyl CoA from glucose via glycolysis and the action of pyruvate dehydrogenase. In order to divert the acetyl CoA generated from glucose, it is desirable to overexpress two genes, one encoding for acetyl CoA carboxylase and the other encoding malonyl-CoA reductase. As an example, these genes are expressed in *E. coli* through a T7 promoter using vectors pET30a and pFN476. The vector pET30a has a pBR ori and kanamycin resistance, while pFN476 has pSC101 ori and uses carbenicillin resistance for selection. Because these two vectors have compatible ori and different markers they can be maintained in *E. coli* at the same time. Hence, the constructs used to engineer *E. coli* for direct production of 3-hydroxypropionate from glucose are pMSD8 (pFN476/accABCD) (Davis et al., *J. Biol. Chem.*, 275:28593-28598, 2000) and pET30a/malonyl-CoA reductase or pET30a/acc1 and pFN476/malonyl-CoA reductase. The constructs are depicted in FIG. **45**.

To test the production of 3-hydroxypropionate from glucose, *E. coli* strain Tuner pLacI carrying plasmid pMSD8 (pFN476/accABCD) and pET30a/malonyl-CoA reductase or pET30a/acc1 and pFN476/malonyl-CoA reductase are grown in a B. Braun BIOSTAT B fermenter. A glass vessel fitted with a water jacket for heating is used to conduct this experiment. The fermenter working volume is 1.5 L and is operated at 37° C. The fermenter is continuously supplied with oxygen by bubbling sterile air through it at a rate of 1 vvm. The agitation is cascaded to the dissolve oxygen concentration which is maintained at 40% DO. The pH of the liquid media is maintained at 7 using 2 N NaOH. The *E. coli* strain is grown in M9 media supplemented with 1% glucose, 1 μg/mL thiamine, 0.1% casamino acids, 10 μg/mL biotin, 50 μg/mL carbenicillin, 50 μg/mL kanamycin, and 25 μg/mL chloramphenicol. The expression of the genes is induced when the cell density reached 0.5 OD(600 nm) by adding 100 μM IPTG. After induction, samples of 2 mL volume are taken at 1, 2, 3, 4, and 8 hours. In addition, at 3 hours after induction, a 200 mL sample is taken to make a cell extract. The 2 mL samples are spun, and the supernatant is used to analyze products using LC/MS technique. The supernatant is stored at −20° C. until further analysis.

The extract is prepared by spinning the 200 mL of cell suspension at 8000 g and washing the cell pellet with 50 mL of 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM KCl, 2 mM DTT, and 5% glycerol. The cell suspension is spun again at 8000 g, and the pellet is resuspended into 5 mL of 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM KCl, 2 mM DTT, and 5% glycerol. The cells are disrupted by passing twice through a French Press at 1000 psig. The cell debris is removed by centrifugation for 20 minutes at 30,000 g. All the operations are conducted at 4° C. To demonstrate in vitro formation of 3-hydroxypropionate using this recombinant cell extract, the following reaction of 200 μL is conducted at 37° C. The reaction mix is as follows: Tris HCl (pH 8.0; 100 mM), ATP (1 mM), $MgCl_2$ (5 mM), KCl (100 mM), DTT (5 mM), $NaHCO_3$ (40 mM), NADPH (0.5 mM), acetyl CoA (0.5 mM), and cell extract (0.2 mg). The reaction is stopped after 15 minutes by adding 1 volume of 10% trifluroacetic acid (TFA). The products of this reaction are detected using an LC/MS technique.

The detection and analysis for the presence of 3-hydroxypropionate in the supernatant and the in vitro reaction mixture is carried out using a Waters/Micromass ZQ LC/MS instrument. This instrument consists of a Waters 2690 liquid chromatograph with a Waters 2410 refractive index detector placed in series between the chromatograph and the single quadrupole mass spectrometer. LC separations are made using a Bio-Rad Aminex 87-H ion-exchange column at 45° C. Sugars, alcohol, and organic acid products are eluted with

0.015% TFA buffer. For elution, the buffer is passed at a flow rate of 0.6 mL/minute. For detection and quantification of 3-hydroxypropionate, a sample obtained from TCI, America (Portland, Oreg.) is used as a standard.

Example 12

Cloning of Propionyl-CoA Transferase, Lactyl-CoA Dehydratase (LDH), and a Hydratase (OS19) for Expression in *Saccharomyces cerevisiae*

The pESC Yeast Epitope Tagging Vector System (Stratagene, La Jolla, Calif.) was used in cloning the genes involved in 3-hydroxypropionic acid production via lactic acid. The pESC vectors each contain GAL1 and GAL10 promoters in opposing directions, allowing the expression of two genes from each vector. The GAL1 and GAL10 promoters are repressed by glucose and induced by galactose. Each of the four available pESC vectors has a different yeast-selectable marker (HIS3, TRP1, LEU2, URA3) so multiple plasmids can be maintained in a single strain. Each cloning region has a polylinker site for gene insertion, a transcription terminator, and an epitope coding sequence for C-terminal or N-terminal epitope tagging of expressed proteins. The pESC vectors also have a ColE1 origin of replication and an ampicillin resistance gene to allow replication and selection in *E. coli*. The following vector/promoter/nucleic acid combinations were constructed:

Vector	Promoter	Polypeptide	Source of nucleic acid
pESC-Trp	GAL1	OS19 hydratase	*Chloroflexus aurantiacus*
	GAL10	E1	*Megasphaera elsdenii*
pESC-Leu	GAL1	E2α	*Megasphaera elsdenii*
	GAL10	E2β	*Megasphaera elsdenii*
pESC-His	GAL1	D-LDH	*Escherishia coli*
	GAL10	PCT	*Megasphaera elsdenii*

The primers used were as follows:

OS19APAF: (SEQ ID NO: 164)
5'-ATAGGGCCCAGGAGATCAAACCATGGGTGAAGAGTCT-CTGGTTC-3'

OS19SALR: (SEQ ID NO: 165)
5'-CCTCTGCTACAGTCGACACAACGACCACTGAAGTTG-GGAG-3'

OS19KPNR: (SEQ ID NO: 166)
5'-AGTCTGCTATCGGTACCTCAACGACCACTGAAGTTG-GGAG-3'

EINOTF: (SEQ ID NO: 167)
5'-ATAGCGGCCGCATAATGGATACTCTCGGAATCGACG-TTGG-3'

EICLAR: (SEQ ID NO: 168)
5'-CCCCATCGATACATATTTCTTGATTTTATCATAAGCA-ATC-3'

EIIαAPAF: (SEQ ID NO: 169)
5'-CCAGGGCCCATAATGGGTGAAGAAAAAACAGTAGA-TATTG-3'

-continued

EIIαSALR: (SEQ ID NO: 170)
5'-GGTAGACTTGTCGACGTAGTGGTTTCCTCCTTCATT-GG-3'

EIIβNOTF: (SEQ ID NO: 171)
5'-ATAGCGGCCGCATAATGGGTCAGATCGACGAACTTA-TCAG-3'

EIIβSPER: (SEQ ID NO: 172)
5'-AGGTTCAACTAGTTCGTAGAGGATTTCCGAGAAAGC-CTG-3'

LDHAPAF: (SEQ ID NO: 173)
5'-CTAGGGCCCATAATGGAACTCGCCGTTTATAG-CAC-3'

LDHXHOR: (SEQ ID NO: 174)
5'-ACTTCTCGAGTTAAACCAGTTCGTTCGGGCA-GGT-3'

PCTSPEF: (SEQ ID NO: 175)
5'-GGGACTAGTATAATGGGAAAAGTAGAAATCAT-TACAG-3'

PCTPACR: (SEQ ID NO: 176)
5'-CGGCTTAATTAACAGCAGAGATTTATTTTTTCA-GTCC-3'

All restriction enzymes were obtained from New England Biolabs, Beverly, Mass. All plasmid DNA preparations were done using QIAprep Spin Miniprep Kits, and all gel purifications were done using QIAquick Gel Extraction Kits (Qiagen, Valencia, Calif.).

A. Construction of the pESC-Trp/OS19 Hydratase Vector

Two constructs in pESC-Trp were made for the OS19 nucleic acid from *C. aurantiacus*. One of these constructs utilized the Apa I and Sal I restriction sites of the GAL1 multiple cloning site and was designed to include the c-myc epitope. The second construct utilized the Apa I and Kpn I sites and thus did not include the c-myc epitope sequence.

Six μg of pESC-Trp vector DNA was digested with the restriction enzyme Apa I and the digest was purified using a QIAquick PCR Purification Column. Three μg of the Apa I-digested vector DNA was then digested with the restriction enzyme Kpn I, and 3 μg was digested with Sal I. The double-digested vector DNAs were separated on a 1% TAE-agarose gel, purified, dephosphorylated with shrimp alkaline phosphatase (Roche Biochemical Products, Indianapolis, Ind.), and purified with a QIAquick PCR Purification Column.

The nucleic acid encoding the *Chloroflexus aurantiacus* polypeptide having hydratase activity (OS19) was amplified from genomic DNA using the PCR primer pair OS19APAF and OS19SALR and the primer pair OS19APAF and OS19KPNR. OS19APAF was designed to introduce an Apa I restriction site and a translation initiation site (ACCATGG) at the beginning of the amplified fragment. The OS19KPNR primer was designed to introduce a Kpn I restriction site at the end of the amplified fragment and to contain the translational stop codon for the hydratase gene. OS19SALR introduces a Sal I site at the end of the amplified fragment and has an altered stop codon so that translation continues in-frame through the vector c-myc epitope. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *C. aurantiacus* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase (Roche) in a final

volume of 100 μL. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 57° C. for 1 minute, and 72° C. for 2.25 minutes; 24 cycles of 94° C. for 30 seconds, 62° C. for 1 minute, and 72° C. for 2.25 minutes; and a final extension for 7 minutes at 72° C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. A 0.8 Kb fragment was excised from the gel and purified for each primer pair. The purified fragments were digested with Kpn I or Sal I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Apa I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

50-60 ng of the digested PCR product containing the nucleic acid encoding the *C. aurantiacus* polypeptide having hydratase activity (OS19) and 50 ng of the prepared pESC-Trp vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto LB plates containing 100 μg/mL of carbenicillin (LBC). Individual colonies were screened using colony PCR with the appropriate PCR primers. Individual colonies were suspended in about 25 μL of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 pt of the heated cells was used in a 25 μL PCR reaction. The PCR mix contained the following: 1× Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the nucleic acid from genomic DNA.

Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR. A construct with a confirmed sequence was transformed into *S. cerevisiae* strain YPH500 using a Frozen-EZ Yeast Transformation II™ Kit (Zymo Research, Orange, Calif.). Transformation reactions were plated on SC-Trp media (see Stratagene pESC Vector Instruction Manual for media recipes). Individual yeast colonies were screened for the presence of the OS19 nucleic acid by colony PCR. Colonies were suspended in 20 μL of Y-Lysis Buffer (Zymo Research) containing 5 units of zymolase and heated at 37° C. for 10 minutes. Three μL of this suspension was then used in a 25 μL PCR reaction using the PCR reaction mixture and program described for the colony screen of the *E. coli* transformants. The pESC-Trp vector was also transformed into YPH500 for use as a hydratase assay control and transformants were screened by PCR using GAL1 and GAL10 primers.

B. Construction of the pESC-Trp/OS19/EI Hydratase Vector

Plasmid DNA of a pESC-Trp/OS19 construct (Apa I-Sal I sites) with confirmed sequence and positive assay results was used for insertion of the nucleic acid for the *M. elsdenii* E1 activator polypeptide downstream of the GAL10 promoter. Three μg of plasmid DNA was digested with the restriction enzyme Cla I, and the digest was purified using a QIAquick PCR Purification Column. The vector DNA was then digested with the restriction enzyme Not I, and the digest was inactivated by heating to 65° C. for 20 minutes. The double-digested vector DNA was dephosphorylated with shrimp alkaline phosphatase (Roche), separated on a 1% TAE-agarose gel, and gel purified.

The nucleic acid encoding the *M. elsdenii* E1 activator polypeptide was amplified from genomic DNA using the PCR primer pair EINOTF and EICLAR. EINOTF was designed to introduce a Not I restriction site and a translation initiation site at the beginning of the amplified fragment. The EICLAR primer was designed to introduce a Cla I restriction site at the end of the amplified fragment and to contain an altered translational stop codon to allow in-frame translation of the FLAG epitope. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *M. elsdenii* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase in a final volume of 100 μL. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 55° C. for 45 seconds, and 72° C. for 3 minutes; 24 cycles of 94° C. for 30 seconds, 62° C. for 45 seconds, and 72° C. for 3 minutes; and a final extension for 7 minutes at 72° C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel, and a 0.8 Kb fragment was excised and purified. The purified fragment was digested with Cla I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Not I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

60 ng of the digested PCR product containing the nucleic acid for the *M. elsdenii* E1 activator polypeptide and 70 ng of the prepared pESC-Trp/OS19 hydratase vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10™ cells. The electroporated cells were plated onto LBC media. Individual colonies were screened using colony PCR with the EINOTF and EICLAR primers. Individual colonies were suspended in about 25 μL of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR mix contained the following: 1× Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the gene from genomic DNA. Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR.

C. Construction of the pESC-Leu/EIIα/EIIβ Vector

Three μg of DNA of the vector pESC-Leu was digested with the restriction enzyme Apa I, and the digest was purified using a QIAquick PCR Purification Column. The vector DNA was then digested with the restriction enzyme Sal I, and the digest was inactivated by heating to 65° C. for 20 minutes. The double-digested vector DNA was dephosphorylated with shrimp alkaline phosphatase (Roche), separated on a 1% TAE-agarose gel, and gel purified.

The nucleic acid encoding the *M. elsdenii* E2α polypeptide was amplified from genomic DNA using the PCR primer pair EIIαAPAF and EIIαSALR. EIIαAPAF was designed to introduce an Apa I restriction site and a translation initiation site at the beginning of the amplified fragment. The EIIαSALR primer was designed to introduce a Sal I restriction site at the end of the amplified fragment and to contain an altered translational stop codon to allow in-frame translation of the c-myc epitope. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *M. elsdenii* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase in a final volume of 100 μL. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 55° C. for 1 minute, and 72° C. for 3 minutes; 24 cycles of 94° C. for 30

seconds, 62° C. for 1 minute, and 72° C. for 3 minutes; and a final extension for 7 minutes at 72° C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel, and a 1.3 Kb fragment was excised and purified. The purified fragment was digested with Apa I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Sal I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

80 ng of the digested PCR product containing the nucleic acid encoding the *M. elsdenii* E2α polypeptide and 80 ng of the prepared pESC-Leu vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto LBC media. Individual colonies were screened using colony PCR with the EIIαAPAF and EIIαSALR primers. Individual colonies were suspended in about 25 μl of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR mix contained the following: 1× Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the gene from genomic DNA. Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR.

Plasmid DNA of a pESC-Leu/EIIα vector with confirmed sequence was used for insertion of the nucleic acid encoding the *M. elsdenii* E2β polypeptide. Three μg of plasmid DNA was digested with the restriction enzyme Spe I, and the digest was purified using a QIAquick PCR Purification Column. The vector DNA was then digested with the restriction enzyme Not I and gel purified from a 1% TAE-agarose gel. The double-digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase (Roche) and purified with a QIAquick PCR Purification Column.

The nucleic acid encoding the *M. elsdenii* E2β polypeptide was amplified from genomic DNA using the PCR primer pair EIIβNOTF and EIIβSPER. The EIIβNOTF primer was designed to introduce a Not I restriction site and a translation initiation site at the beginning of the amplified fragment. The EIIβSPER primer was designed to introduce an Spe I restriction site at the end of the amplified fragment and to contain an altered translational stop codon to allow for in-frame translation of the FLAG epitope. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *M. elsdenii* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase in a final volume of 100 μL. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 55° C. for 45 seconds, and 72° C. for 3 minutes; 24 cycles of 94° C. for 30 seconds, 62° C. for 45 seconds, and 72° C. for 3 minutes; and a final extension for 7 minutes at 72° C. The amplification product was separated by gel electrophoresis using a 1% TAE-agarose gel, and a 1.1 Kb fragment was excised and purified. The purified fragment was digested with Spe I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Not I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

38 ng of the digested PCR product containing the nucleic acid encoding the *M. elsdenii* E2β polypeptide and 50 ng of the prepared pESC-Leu/EIIα vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto LBC plates. Individual colonies were screened using colony PCR with the EIIβNOTF and EIIβSPER primers. Individual colonies were suspended in about 25 μL of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 μL of the heated cells was used in a 25 μL PCR reaction. The PCR mix contained the following: 1× Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the gene from genomic DNA.

Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR. A pESC-Leu/EIIα/EIIβ construct with a confirmed sequence was co-transformed along with the pESC-Trp/OS19/EI vector into *S. cerevisiae* strain YPH500 using a Frozen-EZ Yeast Transformation II™ Kit (Zymo Research, Orange, Calif.). Transformation reactions were plated on SC-Trp-Leu media. Individual yeast colonies were screened for the presence of the OS19, E1, E2α, and E2β nucleic acid by colony PCR. Colonies were suspended in 20 μL of Y-Lysis Buffer (Zymo Research) containing 5 units of zymolase and heated at 37° C. for 10 minutes. Three μL of this suspension was then used in a 25 μL PCR reaction using the PCR reaction mixtures and programs described for the colony screens of the *E. coli* transformants. The pESC-Trp/OS19 and pESC-Leu vectors were also co-transformed into YPH500 for use as a lactyl-CoA dehydratase assay control. These transformants were colony screened using the GAL1 and GAL10 primers (Instruction manual, pESC Yeast Epitope Tagging Vectors, Stratagene).

D. Construction of the pESC-His/D-LDH/PCT Vector

Three μg of DNA of the vector pESC-His was digested with the restriction enzyme Xho I, and the digest was purified using a QIAquick PCR Purification Column. The vector DNA was then digested with the restriction enzyme Apa I and gel purified from a 1% TAE-agarose gel. The double-digested vector DNA was dephosphorylated with shrimp alkaline phosphatase (Roche) and purified using a QIAquick PCR Purification Column.

The *E. coli* D-LDH gene was amplified from genomic DNA of strain DH10B using the PCR primer pair LDHAPAF and LDHXHOR. LDHAPAF was designed to introduce an Apa I restriction site and a translation initiation site at the beginning of the amplified fragment. The LDHXHOR primer was designed to introduce an Xho I restriction site at the end of the amplified fragment and to contain the translational stop codon for the D-LDH gene. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *E. coli* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase in a final volume of 100 pt. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 59° C. for 45 seconds, and 72° C. for 2 minutes; 24 cycles of 94° C. for 30 seconds, 64° C. for 45 seconds, and 72° C. for 2 minutes; and a final extension for 7 minutes at 72° C. The amplification product was separated by gel electrophoresis using a 1% TAE-agarose gel, and a 1.0 Kb fragment was excised and purified. The purified fragment was digested with Apa I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Xho I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

80 ng of the digested PCR product containing the *E. coli* D-LDH gene and 80 ng of the prepared pESC-His vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto LBC media. Individual colonies were screened using colony PCR with the LDHAPAF and LDHXHOR primers. Individual colonies were suspended in about 25 μL of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR mix contained the following: 1×Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the gene from genomic DNA. Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR.

Plasmid DNA of a pESC-His/D-LDH construct with a confirmed sequence was used for insertion of the nucleic acid encoding the *M. elsdenii* PCT polypeptide. Three μg of plasmid DNA was digested with the restriction enzyme Pac I, and the digest was purified using a QIAquick PCR Purification Column. The vector DNA was then digested with the restriction enzyme Spe I and gel purified from a 1% TAE-agarose gel. The double-digested vector DNA was dephosphorylated with shrimp alkaline phosphatase (Roche) and purified with a QIAquick PCR Purification Column.

The nucleic acid encoding the *M. elsdenii* PCT polypeptide was amplified from genomic DNA using the PCR primer pair PCTSPEF and PCTPACR. PCTSPEF was designed to introduce an Spe I restriction site and a translation initiation site at the beginning of the amplified fragment. The PCTPACR primer was designed to introduce a Pac I restriction site at the end of the amplified fragment and to contain the translational stop codon for the PCT gene. The PCR mix contained the following: 1× Expand PCR buffer, 100 ng *M. elsdenii* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, and 5.25 units of Expand DNA Polymerase in a final volume of 100 μL. The PCR reaction was performed in an MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 1 minute; 8 cycles of 94° C. for 30 seconds, 56° C. for 45 seconds, and 72° C. for 2.5 minutes; 24 cycles of 94° C. for 30 seconds, 64° C. for 45 seconds, and 72° C. for 2.5 minutes; and a final extension for 7 minutes at 72° C. The amplification product was separated by gel electrophoresis using a 1% TAE-agarose gel, and a 1.55 Kb fragment was excised and purified. The purified fragment was digested with Pac I restriction enzyme, purified with a QIAquick PCR Purification Column, digested with Spe I restriction enzyme, purified again with a QIAquick PCR Purification Column, and quantified on a minigel.

95 ng of the digested PCR product containing the nucleic acid encoding the *M. elsdenii* PCT polypeptide and 75 ng of the prepared pESC-His/D-LDH vector were ligated using T4 DNA ligase at 16° C. for 16 hours. One 4, of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto LBC plates. Individual colonies were screened using colony PCR with the PCTSPEF and PCTPACR primers. Individual colonies were suspended in about 25 μL of 10 mM Tris, and 2 μL of the suspension was plated on LBC media. The remnant suspension was heated for 10 minutes at 95° C. to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR mix contained the following: 1× Taq buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR program used was the same as described above for amplification of the gene from genomic DNA.

Plasmid DNA was isolated from cultures of colonies having the desired insert and was sequenced to confirm the lack of nucleotide errors from PCR. A construct with a confirmed sequence was transformed into *S. cerevisiae* strain YPH500 using a Frozen-EZ Yeast Transformation II™ Kit (Zymo Research, Orange, Calif.). Transformation reactions were plated on SC-His media. Individual yeast colonies were screened for the presence of the D-LDH and PCT genes by colony PCR. Colonies were suspended in 20 μL of Y-Lysis Buffer (Zymo Research) containing 5 units of zymolase and heated at 37° C. for 10 minutes. Three μL of this suspension was then used in a 25 μL PCR reaction using the PCR reaction mixture and program described for the colony screen of the *E. coli* transformants. The pESC-His vector was also transformed into YPH500 for use as an assay control, and transformants were screened by PCR using GAL1 and GAL10 primers.

Example 13

Expression of Enzymes in *S. cerevisiae*

A. Hydratase Activity in Transformed Yeast

Individual colonies carrying the pESC-Trp/OS19 construct or the pESC-Trp vector (negative control) were used to inoculate 5 mL cultures of SC-Trp media containing 2% glucose. These cultures were grown for 16 hours at 30° C. and used to inoculate 35 mL of the same media. The subcultures were grown for 7 hours at 30° C., and their OD_{600}s were determined. A volume of cells giving an OD×volume equal to 40 was pelleted, washed with SC-Trp media with no carbon source, and repelleted. The cells were suspended in 5 mL of SC-Trp media containing 2% galactose and used to inoculate a total volume of 100 mL of this media. Cultures were grown for 17.5 hours at 30° C. and 250 rpm. Cells were then pelleted, rinsed in 0.85% NaCl, and repelleted. Cell pellets (70 mg) were suspended in 140 μL of 50 mM TrisHCl, pH 7.5, and an equal volume (pellet plus buffer) of pre-rinsed glass beads (Sigma, 150-212 microns) was added. This mixture was vortexed for 30 seconds and placed on ice for 1 minute, and the vortexing/cooling cycle was repeated 8 additional times. The cells were then centrifuged for 6 minutes at 5,000 g, and the supernatant was removed to a fresh tube. The beads/pellet were washed twice with 250 μL of buffer, centrifuged, and the supernatants joined with the first supernatant.

An *E. coli* strain carrying the pETBlue-1/OS19 construct, described previously, was used as a positive control for hydratase assays. A culture of this strain was grown to saturation overnight and diluted 1:20 the following morning in fresh LBC media. The culture was grown at 37° C. and 250 rpm to an OD_{600} of 0.6, at which point it was induced with IPTG at a final concentration of 1 mM. The culture was incubated for an additional two hours at 37° C. and 250 rpm. Cells were pelleted, washed with 0.85% NaCl, and repelleted. Cells were disrupted using BugBuster™ Protein Extraction Reagent and Benzonase® (Novagen) as per manufacturer's instructions with a 20 minute incubation at room temperature. After centrifugation at 16,000 g and 4° C., the supernatant was transferred to a new tube and used in the activity assay.

Total protein content of cell extracts from *S. cerevisiae* described above were quantified using a microplate Bio-Rad Protein Assay (Bio-Rad, Hercules, Calif.). The OS19 constructs (both Apa I-Sal I and Apa I-Kpn I sites) in YPH500, the pESC-Trp negative control in YPH500, and the pETBlue-1/

OS19 construct in *E. coli* were tested for their ability to convert acrylyl-CoA to 3-hydroxypropionyl-CoA. The assay was conducted as previously described for the pETBlue-1/OS19 constructs in the *E. coli* Tuner strain. When cell extract of the negative control strain was added to the reaction mixture containing acrylyl-CoA, one dominant peak of MW 823 was exhibited. This peak corresponds to acrylyl-CoA and indicates that acrylyl-CoA was not converted to any other product. When cell extract of the strain carrying a pESC-Trp/OS19 construct (either Apa I-Sal I or Apa I-Kpn I sites) was added to the reaction mix, the dominant peak shifted to MW 841, which corresponds to 3-hydroxypropionyl-CoA. The reaction mix from the *E. coli* control also showed the MW 841 peak. A time course study was conducted for the pESC-Trp/OS19(Apa I-Sal I) construct, which measured the appearance of the MW 841 and MW 823 peaks after 0, 1, 3, 7, 15, 30, and 60 minutes of reaction time. An increase in the 3-hydroxypropionyl-CoA peak was seen over time with the cell extracts from both this construct and the *E. coli* control, whereas cell extract from the YPH500 strain with vector only showed a dominant acrylyl-CoA peak.

B. Propionyl CoA-Transferase Activity in Transformed Yeast

Individual colonies of *S. cerevisiae* strain YPH500 carrying the pESC-His/D-LDH or pESC-His/D-LDH/PCT construct or the pESC-His vector with no insert (negative control) were used to inoculate 5 mL cultures of SC-His media containing 2% glucose. These cultures were grown for 16 hours at 30° C. and 250 rpm and were then used to inoculate 35 mL of the same media. The subcultures were grown for 7 hours at 30° C., and their OD_{600}s were determined. For each strain, a volume of cells giving an OD×volume equal to 40 was pelleted, washed with SC-His media with no carbon source, and repelleted. The cells were suspended in 5 mL of SC-His media containing 2% galactose and used to inoculate a total volume of 100 mL of this media. Cultures were grown for 16.75 hours at 30° C. and 250 rpm. Cells were then pelleted, rinsed in 0.85% NaCl, and repelleted. Cell pellets (70 mg) were suspended in 140 μL of 100 mM potassium phosphate buffer, pH 7.5, and an equal volume (pellet plus buffer) of pre-rinsed glass beads (Sigma, 150-212 microns) was added. This mixture was vortexed for 30 seconds and placed on ice for 1 minute, and the vortexing/cooling cycle was repeated 8 additional times. The cells were then centrifuged for 6 minutes at 5,000 g, and the supernatant was removed to a fresh tube. The beads/pellet were washed twice with 250 μL of buffer and centrifuged, and the supernatants joined with the first supernatant.

An *E. coli* strain carrying the pETBlue-1/PCT construct, described previously, was used as a positive control for propionyl CoA transferase assays. A culture of this strain was grown to saturation overnight and diluted 1:20 the following morning in fresh LB media containing 100 μg/mL of carbenicillin. The culture was grown at 37° C. and 250 rpm to an OD_{600} of 0.6, at which point it was induced with IPTG at a final concentration of 1 mM. The culture was incubated for an additional two hours at 37° C. and 250 rpm. Cells were pelleted, washed with 0.85% NaCl, and repelleted. Cells were disrupted using BugBuster™ Protein Extraction Reagent and Benzonase® (Novagen) as per manufacturer's instructions with a 20 minute incubation at room temperature. After centrifugation at 16,000 g and 4° C., the supernatant was transferred to a new tube and used in the activity assay.

Total protein content of cell extracts was quantified using a microplate Bio-Rad Protein Assay (Bio-Rad, Hercules, Calif.). The D-LDH and D-LDH/PCT constructs in *S. cerevisiae* strain YPH500, the pESC-His negative control in YPH500, and the pETBlue-1/PCT construct in *E. coli* were tested for their ability to catalyze the conversion of propionyl-CoA and acetate to acetyl-CoA and propionate. The assay mixture used was that previously described for the pETBlue-1/PCT constructs in the *E. coli* Tuner strain.

When 1 μg of total cell extract protein of the negative control strain or the YPH500/pESC-His/D-LDH strain was added to the reaction mixture, no increase in absorbance (0.00 to 0.00) was seen over 11 minutes. Increases in absorbance from 0.00 to 0.04 and from 0.00 to 0.06 were seen, respectively, with 1 μg of cell extract protein from the YPH500/pESC-His/D-LDH/PCT strain and the *E. coli*/PCT strain. With 2 mg of total cell extract protein, the negative control strain and the YPH500/pESC-His/D-LDH strain showed an increase in absorbance from 0.00 to 0.01 over 11 minutes, whereas increases from 0.00 to 0.10 and 0.00 to 0.08 were seen, respectively, with the YPH500/pESC-His/D-LDH/PCT strain and the *E. coli*/PCT strain.

C. Lactyl-CoA Dehydratase Activity in Transformed Yeast

Individual colonies of *S. cerevisiae* strain YPH500 carrying the pESC-His/D-LDH or pESC-His/D-LDH/PCT construct or the pESC-His vector with no insert (negative control) were used to inoculate 5 mL cultures of SC-His media containing 4% glucose. These cultures were grown for 23 hours at 30° C. and used to inoculate 35 mL of SC-His media containing 2% raffinose. The subcultures were grown for 8 hours at 30° C., and their OD_{600}s were determined. For each strain, a volume of cells giving an OD×volume equal to 40 was pelleted, resuspended in 10 mL of SC-His media containing 2% galactose, and used to inoculate a total volume of 100 mL of this media. Cultures were grown for 17 hours at 30° C. and 250 rpm. Cells were then pelleted, rinsed in 0.85% NaCl, and repelleted. Cell pellets (190 mg) were suspended in 380 μL of 100 mM potassium phosphate buffer, pH 7.5, and an equal volume (pellet plus buffer) of pre-rinsed glass beads (Sigma, 150-212 microns) was added. This mixture was vortexed for 30 seconds and placed on ice for 1 minute, and the vortexing/cooling cycle was repeated 7 additional times. The cells were then centrifuged for 6 minutes at 5,000 g and the supernatant was removed to a fresh tube. The beads/pellet were washed twice with 300 μL of buffer and centrifuged, and the supernatants joined with the first supernatant.

An anaerobically-grown culture of *E. coli* strain DH10B was used as a positive control for D-LDH assays. A culture of this strain was grown to saturation overnight and diluted 1:20 the following morning in fresh LB media. The culture was grown anaerobically at 37° C. for 7.5 hours. Cells were pelleted, washed with 0.85% NaCl, and repelleted. Cells were disrupted using BugBuster™ Protein Extraction Reagent and Benzonase® (Novagen) as per manufacturer's instructions with a 20-minute incubation at room temperature. After centrifugation at 16,000 g and 4° C., the supernatant was transferred to a new tube and used in the activity assay.

Total protein content of cell extracts was quantified using a microplate Bio-Rad Protein Assay (Bio-Rad, Hercules, Calif.). The D-LDH and D-LDH/PCT constructs in YPH500, the pESC-His negative control in YPH500, and the anaerobically-grown *E. coli* strain were tested for their ability to catalyze the conversion of pyruvate to lactate by assaying the concurrent oxidation of NADH to NAD. The assay mixture contained 100 mM potassium phosphate buffer, pH 7.5, 0.2 mM NADH, and 0.5-1.0 μg of cell extract. The reaction was started by the addition of sodium pyruvate to a final concentration of 5 mM, and the decrease in absorbance at 340 nm was measured over 10 minutes. When 0.5 μg of total cell extract protein of the negative control strain was added to the reaction mixture, a decrease in absorbance from −0.01 to −0.02 was seen over 200 seconds. A decrease in absorbance

from −0.21 to −0.47 and −0.20 to −0.47 over 200 seconds was seen, respectively, for cell extract from the YPH500/pESC-His/D-LDH or YPH500/pESC-His/D-LDH/PCT strains. 0.5 μL (7.85 μg) of cell extract from the anaerobically-grown *E. coli* strain showed a decrease in absorbance very similar to that for 1 μg of cell extract of the YPH500/pESC-His/D-LDH/PCT strain. When 4 μg of cell extract was used, the YPH500/pESC-His/D-LDH/PCT strain showed a decrease in absorbance from −0.18 to −0.60 over 10 minutes, whereas the negative control strain showed no decrease in absorbance (−0.08 to −0.08).

D. Demonstration of 3-HP Production in *S. cerevisiae*

The pESC-Trp/OS19/EI pESC-Leu/EIIα/EIIβ, and pESC-His/D-LDH/PCT constructs were transformed into a single strain of *S. cerevisiae* YPH500 using a Frozen-EZ Yeast Transformation II™ Kit (Zymo Research, Orange, Calif.). A negative control strain was also developed by transformation of the pESC-Trp, pESC-Leu, and pESC-His vectors into a single YPH500 strain. Transformation reactions were plated on SC-Trp-Leu-His media. Individual yeast colonies were screened by colony PCR for the presence or absence of nucleic acid corresponding to each construct.

The strain carrying all six genes and the negative control strain were grown in 5 mL of SC-Trp-Leu-His media containing 2% glucose. These cultures were grown for 31 hours at 30° C., and 2 mL was used to inoculate 50 mL of the same media. The subcultures were grown for 19 hours at 30° C., and their OD600s were determined. For each strain, a volume of cells giving an OD×volume equal to 100 was pelleted, washed with SC-Trp-Leu-His media with no carbon source, and repelleted. The cells were suspended in 10 mL of SC-Trp-Leu-His media containing 2% galactose and 2% raffinose and used to inoculate a total volume of 250 mL of this media. The cultures were grown in bottles at 30° C. with no shaking, and samples were taken at 0, 4.5, 20, 28.5, 45, and 70 hours. Samples were spun down to remove cells and the supernatant was filtered using 0.45 micron Acrodisc Syringe Filters (Pall Gelman Laboratory, Ann Arbor, Mich.).

100 microliters of the filtered broth was used to derive CoA esters of any lactate or 3-HP in the broth using 6 micrograms of purified propionyl-CoA transferase, 50 mM potassium phosphate buffer (pH 7.0), and 1 mM acetyl-CoA. The reaction was allowed to proceed at room temperature for 15 minutes and was stopped by adding 1 volume 10% trifluoroacetic acid. The reaction mixtures were purified using Sep Pak C18 columns as previously described and analyzed by LC/MS.

Example 14 Constructing a Biosynthetic Pathway that Produces Organic Acids from β-Alanine

One possible pathway to 3-HP from β-alanine involves the use of a polypeptide having CoA transferase activity (e.g., an enzyme from a class of enzymes that transfers a CoA group from one metabolite to the other). As shown in FIG. **54**, β-alanine can be converted to β-alanyl-CoA using a polypeptide having CoA transferase activity and CoA donors such as acetyl-CoA or propionyl-CoA. Alternatively, β-alanyl-CoA can be generated by the action of a polypeptide having CoA synthetase activity. The β-alanyl-CoA can be deaminated to form acrylyl-CoA by a polypeptide having β-alanyl-CoA ammonia lyase activity. The hydration of acrylyl-CoA at the β position to yield 3-HP-CoA can be carried out by a polypeptide having 3-HP-CoA dehydratase activity. The 3-HP-CoA can act as a CoA donor for β-alanine, a reaction that can be catalyzed by a polypeptide having CoA transferase activity, thus yielding 3-HP as a product. Alternatively, 3-HP-CoA can be hydrolyzed to yield 3-HP by a polypeptide having specific CoA hydrolase activity.

Methods for isolating, sequencing, expressing, and testing the activity of a polypeptide having CoA transferase activity are described herein.

A. Isolation of a Polypeptide Having β-Alanyl-CoA Ammonia Lyase Activity

Polypeptides having β-alanyl-CoA ammonia lyase activity can catalyze the conversion of β-alanyl-CoA into acrylyl-CoA. The activity of such polypeptides has been described by Vagelos et al. (*J. Biol. Chem.,* 234:490-497 (1959)) in *Clostridium propionicum*. This polypeptide can be used as part of the acrylate pathway in *Clostridium propionicum* to produce propionic acid.

C. propionicum was grown at 37° C. in an anoxic medium containing 0.2% yeast extract, 0.2% trypticase peptone, 0.05% cysteine, 0.5% b-alanine, 0.4% VRB-salts, 5 mM potassium phosphate, pH 7.0. The cells were harvested after 12 hours and washed twice with 50 mM potassium phosphate (Kpi), pH 7.0. About 2 g of wet packed cells were re-suspended in 40 mL of Kpi pH 7.0, 1 mM $MgCl_2$, 1 mM EDTA, and 1 mM DTT (Buffer A), and homogenized by sonication at about 85-100 W power using a 3 mm tip (Branson sonifier 250). Cell debris was removed by centrifugation at 100,000 g for 45 minutes in a Centricon T-1080 Ultra centrifuge, and the cell free extract (~110 U/mg activity) was subjected to anion exchange chromatography on Source-15Q-material. The Source-15Q column was loaded with 32 mL of cell free extract. The column was developed by a linear gradient of 0 M to 0.5 M NaCl within 10 column volumes. The polypeptide eluted between 70-110 mM NaCl.

The solution was adjusted to a final concentration of 1 M $(NH_4)_2SO_4$ and applied onto a Resource-Phe column equilibrated with 1 M $(NH_4)_2SO_4$ in buffer A. The polypeptide did not bind to this column.

The final preparation was obtained after concentration in an Amicon chamber (filter cut-off 30 kDa). The functional polypeptide is composed of four polypeptide sub-units, each having a molecular mass of 16 kDa. The polypeptide had a final specific activity of 1033 U/mg in the standard assay (see below).

The polypeptide sample after every purification step was separated on a 15% SDS-PAGE gel. The gel was stained with 0.1% Coomassie R 250, and the destaining was achieved by using 7.1% acetic acid/5% ethanol solution.

The polypeptide was desalted by RP-HPLC and subjected to N-terminal sequencing by gas phase Edman degradation. The results of this analysis yielded a 35 amino acid N-terminal sequence of the polypeptide. The sequence was as follows:

(SEQ ID NO: 177)
MV-GKKVVHHLMMSAKDAHYTGNLVNGARIVNQWGD.

B. Amplification of a Gene Fragment

The 35 amino acid sequence of the polypeptide having β-alanine-CoA ammonia lyase activity was used to design primers with which to amplify the corresponding DNA from genome of *C. propionicum*. Genomic DNA from *C. propionicum* was isolated using the Gentra Genomic DNA isolation Kit (Gentra Systems, Minneapolis) following the genomic DNA protocol for gram-positive bacteria. A codon usage table for *Clostridium propionicum* was used to back translate

the seven amino acids on either end of the amino acid sequence to obtain 20-nucleotide degenerate primers:

ACLF:
(SEQ ID NO: 178)
5'-ATGGTWGGYAARAARGTWGT-3'

ACLR:
(SEQ ID NO: 179)
5'-TCRCCCCAYTGRTTWACRAT-3'

The primers were used in a 50 μL PCR reaction containing 1× Taq PCR buffer, 0.6 μM each primer, 0.2 mM each dNTP, 2 units of Taq DNA polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.), 2.5% (v/v) DMSO, and 100 ng of genomic DNA. PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 58° C., 4 cycles at 56° C., 4 cycles at 54° C., and 24 cycles at 52° C. Each cycle used an initial 30 second denaturing step at 94° C. and a 1.25 minute extension at 72° C., and the program had an initial denaturation step at 94° C. for 2 minutes and final extension at 72° C. for 5 minutes. The amounts of PCR primer used in the reaction were increased three-fold above typical PCR amounts due to the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were made to identify PCR product resulting from single degenerate primers. Twenty μL of each PCR product was separated on a 2.0% TAE (Tris-acetate-EDTA)-agarose gel.

A band of about 100 bp was produced by the reaction containing both the forward and reverse primers, but was not present in the individual forward and reverse primer control reactions. This fragment was excised and purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, Calif.). Four microliters of the purified band was ligated into pCRII-TOPO vector and transformed by a heat-shock method into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, Calif.). Transformations were plated on LB media containing 50 μg/mL of kanamycin and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Individual, white colonies were resuspended in 25 μL of 10 mM Tris and heated for 10 minutes at 95° C. to break open the bacterial cells. Two microliters of the heated cells were used in a 25 μL PCR reaction using M13R and M13F universal primers homologous to the pCRII-TOPO vector. The PCR mix contained the following: 1× Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94° C. for 2 minutes; 30 cycles of 94° C. for 30 seconds, 52° C. for 1 minute, and 72° C. for 1.25 minutes; and a final extension for 7 minutes at 72° C. Plasmid DNA was obtained (QIAprep Spin Miniprep Kit, Qiagen) from cultures of colonies showing the desired insert and was used for DNA sequencing with M13R universal primer. The following nucleic acid sequence was internal to the degenerate primers and corresponds to a portion of the 35 amino acid residue sequence:

(SEQ ID NO: 180)
5'-ACATCATTTAATGATGAGCGCAAAAGATGCTCACTAT-ACTGGAAACTTAGTAAACGGCGCTAGA-3'.

C. Genome Walking to Obtain the Complete Coding Sequence

Primers for conducting genome walking in both upstream and downstream directions were designed using the portion of the nucleic acid sequence that was internal to the degenerate primers. The primer sequences were as follows:

ACLGSP1F:
(SEQ ID NO: 181)
5'-GTACATCATTTAATGATGAGCGCAAAAGATG-3'

ACLGSP2F:
(SEQ ID NO: 182)
5'-GATGCTCACTATACTGGAAACTTAGTAAAC-3'

ACLGSP1R:
(SEQ ID NO: 183)
5'-ATTCTAGCGCCGTTTACTAAGTTTCCAG-3'

ACLGSP2R:
(SEQ ID NO: 184)
5'-CCAGTATAGTGAGCATCTTTTGCGCTCATC-3'

GSP1F and GSP2F are primers facing downstream, GSP1R and GSP2R are primers facing upstream, and GSP2F and GSP2R are primers nested inside GSP1F and GSP1R, respectively. Genome walking libraries were constructed according to the manual for CLONTECH's Universal Genome Walking Kit (CLONTECH Laboratories, Palo Alto, Calif.), with the exception that the restriction enzymes Ssp I and Hinc II were used in addition to Dra I, EcoR V, and Pvu II. PCR was conducted in a Perkin Elmer 9700 Thermocycler using the following reaction mix: 1×XL Buffer II, 0.2 mM each dNTP, 1.25 mM $Mg(OAc)_2$, 0.2 μM each primer, 2 units of rTth DNA polymerase XL (Applied Biosystems, Foster City, Calif.), and 1 μL of library per 50 μL reaction. First round PCR used an initial denaturation at 94° C. for 5 seconds; 7 cycles consisting of 2 sec at 94° C. and 3 min at 70° C.; 32 cycles consisting of 2 sec at 94° C. and 3 min at 64° C.; and a final extension at 64° C. for 4 min. Second round PCR used an initial denaturation at 94° C. for 15 seconds; 5 cycles consisting of 5 sec at 94° C. and 3 min at 70° C.; 26 cycles consisting of 5 sec at 94° C. and 3 min at 64° C.; and a final extension at 66° C. for 7 min. Twenty μL of each first and second round product was run on a 1.0% TAE-agarose gel. In the second round PCR for the forward reactions, a 1.4 Kb band was obtained for Dra I, a 1.5 Kb band for Hinc II, a 4.0 Kb band for Pvu II, and 2.0 and 2.6 Kb bands were obtained for Ssp I. In the second round PCR for the reverse reactions, a 1.5 Kb band was obtained for Dra I, a 0.8 Kb band for EcoR V, a 2.0 Kb band for Hinc II, a 2.9 Kb band for Pvu II, and a 1.5 Kb band was obtained for Ssp I. Several of these fragments were gel purified, cloned, and sequenced.

The coding sequence of the polypeptide having β-alanyl-CoA ammonia lyase activity is set forth in SEQ ID NO:162. This coding sequence encodes the amino acid sequence set forth in SEQ ID NO:160. The coding sequence was cloned and expressed in bacterial cells. A polypeptide with the expected size was isolated and tested for enzymatic activity.

The isolation of a nucleic acid molecule encoding a polypeptide having 3-HP-CoA dehydratase activity (e.g., the seventh enzymatic activity in FIG. **54**, which can be accomplished with a polypeptide having the amino acid sequence set forth in SEQ ID NO:41) is described herein. This polypeptide in combination with a polypeptide having CoA transferase activity (e.g., a polypeptide having the amino acid sequence set forth in SEQ ID NO:2) and a polypeptide having β-alanyl-CoA ammonia lyase activity (e.g., a polypeptide having the amino acid sequence set forth in SEQ ID NO: 160) provides one method of making 3-HP from β-alanine.

Example 15

Constructing a Biosynthetic Pathway that Produces Organic Acids from β-Alanine

In another pathway, β-alanine generated from aspartate can be deaminated by a polypeptide having 4,4-aminobutyrate aminotransferase activity (FIG. **55**). This reaction also can regenerate glutamate that is consumed in the formation of aspartate. The deamination of β-alanine can yield malonate semialdehyde, which can be further reduced to 3-HP by a polypeptide having 3-hydroxypropionate dehydrogenase activity or a polypeptide having 3-hydroxyisobutyrate dehydrogenase activity. Such polypeptides can be obtained as follows.

a. Cloning gabT (4-Aminobutyrate Aminotransferase) from *C. acetobutycilicum*

The following PCR primers were designed based on a published sequence for a gabT gene from *Clostridium acetobutycilicum* (GenBank# AE007654):

```
Cac aba nco sen:
                                    (SEQ ID NO: 185)
5'-GAGCCATGGAAGAAATAAATGCTAAAG-3'

Cac aba bam anti:
                                    (SEQ ID NO: 186)
5'-AGAGGATGGCTTTTTAAATCGCTATTC-3'
```

The primers introduced a NcoI site at the 5' end and a BamH I site at the 3' end. A PCR reaction was set up using chromosomal DNA from *C. acetobutylicum* as the template.

H2O	80.75	μL	PCR Program
Taq Plus Long 10x Buffer	10	μL	94° C. 5 minutes
dNTP mix (10 mM)	3	μL	25 cycles of:
Cac aba nco sen (20 mM)	2	μL	94° C. 30 seconds
Cac aba bam anti (20 mM)	2	μL	50° C. 30 seconds
C. acetobutylicum DNA (~100 ng)	1	μL	72° C. 80 seconds + 2
Taq Plus Long (5 U/mL)	1	μL	seconds/cycle
Pfu (2.5 U/mL)	0.25	μL	68° C. 7 minutes
	1	cycle of:	4° C. until use

Upon agarose gel analysis a single band was observed of ~1.3 Kb in size. This fragment was purified using QIAquick PCR purification kit (Qiagen, Valencia, Calif.) and cloned into pCRII TOPO using the TOPO Zero Blunt PCR cloning kit (Invitrogen, Carlsbad, Calif.). 1 μL of the pCRII TOPO ligation mix was used to transform chemically competent TOP10 *E. coli* cells. The cells were grown for 1 hour in SOC media, and the transformants were selected on LB/kanamycin (50 μg/mL) plates. Single colonies of the transformants grown overnight in LB/kanamycin media, and the plasmid DNA was extracted using a Mini prep kit (Qiagen, Valencia, Calif.). The super-coiled plasmid DNA was separated on a 1% agarose gel and digested, and the colonies with insert were selected. The insert was sequenced to confirm the sequence and its quality.

The plasmid having the correct insert was digested with restriction enzyme Nco I and BamH I. The digested insert was gel isolated and ligated to pET28b expression vector that was also restricted with Nco I and BamH I enzymes. 1 μl of ligation mix was used to transform chemically competent TOP10 *E. coli* cells. The cells were recovered for 1 hour in SOC media, and the transformants were selected on LB/kanamycin (50 μg/mL) plates. The super-coiled plasmid DNA was separated on a 1% agarose gel and digested, and the colonies with insert were selected. The plasmid with the insert was isolated using a Mini prep kit (Qiagen, Valencia, Calif.), and 1 μL of this plasmid DNA was used to transform electrocompetent BL21(DE3) (Novagen, Madison, Wis.). These cells were used to check the expression of a polypeptide having 4-aminobutyrate aminotransferase activity.

B. Cloning mmsB (3-Hydroxyisobutyrate Dehydrogenase) from *P. aeruginosa*

The following PCR primers were designed based on a published sequence for a mmsB gene from *Pseudomona_aeruginosa* (GenBank# M84911):

```
Ppu hid nde sen:
                                    (SEQ ID NO: 187)
5'-ATACATATGACCGACCGACATCGCATT-3'

Ppu hid sal anti:
                                    (SEQ ID NO: 188)
5'-ATAGTCGACGGGTCAGTCCTTGCCGCG-3'
```

The primers introduced a Nde I site at the 5' end and a BamH I site at the 3' end.

H_2O	80.75	μL	PCR Program
Taq Plus Long 10x Buffer	10	μL	94° C. 5 minutes
dNTP mix (10mM)	3	μL	25 cycles of:
			94° C. 30 seconds
			55° C. 30 seconds
			72° C. 90 seconds + 2
			seconds/cycle
Ppu hid nde sen (20 μM)	2	μL	68° C. 7 minutes
Ppu hid sal anti (20 μM)	2	μL	4° C. until use
C. acetobutylicum DNA (~100 ng)	1	μL	
Taq Plus Long (Stratagene, La Jolla, CA)	1	μL	
Pfu (Stratagene, La Jolla, CA)	0.25	μL	

A PCR reaction was set up using chromosomal DNA from *P. aeruginosa* as the template. Chromosomal DNA was obtained from ATCC (Manassas, Va.) *P. aeruginosa* 17933D.

Upon agarose gel analysis, a single band was observed of ~1.6 Kb in size. This fragment was purified using QIAquick PCR purification kit (Qiagen, Valencia, Calif.) and cloned into pCRII TOPO using the TOPO Zero Blunt PCR cloning kit (Invitrogen, Carlsbad, Calif.). 1 μL of the pCRII TOPO ligation mix was used to transform chemically competent TOP10 *E. coli* cells. The cells were recovered for 1 hour in SOC media, and the transformants were selected on LB/kanamycin (50 μg/mL) plates. Single colonies of the transformants grown overnight in LB/kanamycin media, and the plasmid DNA was extracted using a Mini prep kit (Qiagen, Valencia, Calif.). The super-coiled plasmid DNA was separated on a 1% agarose gel and digested, and the colonies with insert were selected. The insert was sequenced to confirm the sequence and its quality.

The plasmid having the correct insert was digested with restriction enzyme Nde I and BamHI. The digested insert was gel isolated and ligated to pET30a expression vector that was also restricted with Nde I and BamH I enzymes. 1 μL of ligation mix was used to transform chemically competent TOP10 *E. coli* cells. The cells were recovered for 1 hour in SOC media, and the transformants were selected on LB/kanamycin (50 μg/mL) plates. The super-coiled plasmid DNA was separated on a 1% agarose gel and digested, and the colonies with insert were selected. The plasmid with the insert was isolated using a Mini prep kit (Qiagen, Valencia, Calif.), and 1 μl of this plasmid DNA was used to transform electrocompetent BL21(DE3) (Novagen, Madison, Wis.). These cells were used to check the expression of a polypeptide having 3-hydroxyisobutyrate dehydrogenase activity.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

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<213> ORGANISM: Megasphaera elsdenii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1551)

<400> SEQUENCE: 1

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1               5                   10                  15

aaa gac aac gac acg att acg tct atc ggc ttt gtc agc agc gcc cat       96
Lys Asp Asn Asp Thr Ile Thr Ser Ile Gly Phe Val Ser Ser Ala His
            20                  25                  30

ccg gaa gca ctg acc aaa gct ttg gaa aaa cgg ttc ctg gac acg aac      144
Pro Glu Ala Leu Thr Lys Ala Leu Glu Lys Arg Phe Leu Asp Thr Asn
        35                  40                  45

acc ccg cag aac ttg acc tac atc tat gca ggc tct cag ggc aaa cgc      192
Thr Pro Gln Asn Leu Thr Tyr Ile Tyr Ala Gly Ser Gln Gly Lys Arg
    50                  55                  60

gat ggc cgt gcc gct gaa cat ctg gca cac aca ggc ctt ttg aaa cgc      240
Asp Gly Arg Ala Ala Glu His Leu Ala His Thr Gly Leu Leu Lys Arg
65                  70                  75                  80

gcc atc atc ggt cac tgg cag act gta ccg gct atc ggt aaa ctg gct      288
Ala Ile Ile Gly His Trp Gln Thr Val Pro Ala Ile Gly Lys Leu Ala
                85                  90                  95

gtc gaa aac aag att gaa gct tac aac ttc tcg cag ggc acg ttg gtc      336
Val Glu Asn Lys Ile Glu Ala Tyr Asn Phe Ser Gln Gly Thr Leu Val
            100                 105                 110

cac tgg ttc cgc gcc ttg gca ggt cat aag ctc ggc gtc ttc acc gac      384
His Trp Phe Arg Ala Leu Ala Gly His Lys Leu Gly Val Phe Thr Asp
        115                 120                 125

atc ggt ctg gaa act ttc ctc gat ccc cgt cag ctc ggc ggc aag ctc      432
Ile Gly Leu Glu Thr Phe Leu Asp Pro Arg Gln Leu Gly Gly Lys Leu
    130                 135                 140

aat gac gta acc aaa gaa gac ctc gtc aaa ctg atc gaa gtc gat ggt      480
Asn Asp Val Thr Lys Glu Asp Leu Val Lys Leu Ile Glu Val Asp Gly
145                 150                 155                 160

cat gaa cag ctt ttc tac ccg acc ttc ccg gtc aac gta gct ttc ctc      528
His Glu Gln Leu Phe Tyr Pro Thr Phe Pro Val Asn Val Ala Phe Leu
                165                 170                 175

cgc ggt acg tat gct gat gaa tcc ggc aat atc acc atg gac gaa gaa      576
Arg Gly Thr Tyr Ala Asp Glu Ser Gly Asn Ile Thr Met Asp Glu Glu
            180                 185                 190

atc ggg cct ttc gaa agc act tcc gta gcc cag gcc gtt cac aac tgt      624
Ile Gly Pro Phe Glu Ser Thr Ser Val Ala Gln Ala Val His Asn Cys
        195                 200                 205

ggc ggt aaa gtc gtc gtc cag gtc aaa gac gtc gtc gct cac ggc agc      672
Gly Gly Lys Val Val Val Gln Val Lys Asp Val Val Ala His Gly Ser
    210                 215                 220

ctc gac ccg cgc atg gtc aag atc cct ggc atc tat gtc gac tac gtc      720
Leu Asp Pro Arg Met Val Lys Ile Pro Gly Ile Tyr Val Asp Tyr Val
225                 230                 235                 240
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gtc gta gca gct ccg gaa gac cat cag cag acg tat gac tgc gaa tac      768
Val Val Ala Ala Pro Glu Asp His Gln Gln Thr Tyr Asp Cys Glu Tyr
                245                 250                 255

gat ccg tcc ctc agc ggt gaa cat cgt gct cct gaa ggc gct acc gat      816
Asp Pro Ser Leu Ser Gly Glu His Arg Ala Pro Glu Gly Ala Thr Asp
            260                 265                 270

gca gct ctc ccc atg agc gct aag aaa atc atc ggc cgc cgc ggc gct      864
Ala Ala Leu Pro Met Ser Ala Lys Lys Ile Ile Gly Arg Arg Gly Ala
        275                 280                 285

ttg gaa ttg act gaa aac gct gtc gtc aac ctc ggc gtc ggt gct ccg      912
Leu Glu Leu Thr Glu Asn Ala Val Val Asn Leu Gly Val Gly Ala Pro
    290                 295                 300

gaa tac gtt gct tct gtt gcc ggt gaa gaa ggt atc gcc gat acc att      960
Glu Tyr Val Ala Ser Val Ala Gly Glu Glu Gly Ile Ala Asp Thr Ile
305                 310                 315                 320

acc ctg acc gtc gaa ggt ggc gcc atc ggt ggc gta ccg cag ggc ggt     1008
Thr Leu Thr Val Glu Gly Gly Ala Ile Gly Gly Val Pro Gln Gly Gly
                325                 330                 335

gcc cgc ttc ggt tcg tcc cgc aat gcc gat gcc atc atc gac cac acc     1056
Ala Arg Phe Gly Ser Ser Arg Asn Ala Asp Ala Ile Ile Asp His Thr
            340                 345                 350

tat cag ttc gac ttc tac gat ggc ggc ggt ctg gac atc gct tac ctc     1104
Tyr Gln Phe Asp Phe Tyr Asp Gly Gly Gly Leu Asp Ile Ala Tyr Leu
        355                 360                 365

ggc ctg gcc cag tgc gat ggc tcg ggc aac atc aac gtc agc aag ttc     1152
Gly Leu Ala Gln Cys Asp Gly Ser Gly Asn Ile Asn Val Ser Lys Phe
    370                 375                 380

ggt act aac gtt gcc ggc tgc ggc ggt ttc ccc aac att tcc cag cag     1200
Gly Thr Asn Val Ala Gly Cys Gly Gly Phe Pro Asn Ile Ser Gln Gln
385                 390                 395                 400

aca ccg aat gtt tac ttc tgc ggc acc ttc acg gct ggc ggc ttg aaa     1248
Thr Pro Asn Val Tyr Phe Cys Gly Thr Phe Thr Ala Gly Gly Leu Lys
                405                 410                 415

atc gct gtc gaa gac ggc aaa gtc aag atc ctc cag gaa ggc aaa gcc     1296
Ile Ala Val Glu Asp Gly Lys Val Lys Ile Leu Gln Glu Gly Lys Ala
            420                 425                 430

aag aag ttc atc aaa gct gtc gac cag atc act ttc aac ggt tcc tat     1344
Lys Lys Phe Ile Lys Ala Val Asp Gln Ile Thr Phe Asn Gly Ser Tyr
        435                 440                 445

gca gcc cgc aac ggc aaa cac gtt ctc tac atc aca gaa cgc tgc gta     1392
Ala Ala Arg Asn Gly Lys His Val Leu Tyr Ile Thr Glu Arg Cys Val
    450                 455                 460

ttt gaa ctg acc aaa gaa ggc ttg aaa ctc atc gaa gtc gca ccg ggc     1440
Phe Glu Leu Thr Lys Glu Gly Leu Lys Leu Ile Glu Val Ala Pro Gly
465                 470                 475                 480

atc gat att gaa aaa gat atc ctc gct cac atg gac ttc aag ccg atc     1488
Ile Asp Ile Glu Lys Asp Ile Leu Ala His Met Asp Phe Lys Pro Ile
                485                 490                 495

att gat aat ccg aaa ctc atg gat gcc cgc ctc ttc cag gac ggt ccc     1536
Ile Asp Asn Pro Lys Leu Met Asp Ala Arg Leu Phe Gln Asp Gly Pro
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Met Gly Leu Lys Lys
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<212> TYPE: PRT
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 2

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Lys Asp Asn Asp Thr Ile Thr Ser Ile Gly Phe Val Ser Ser Ala His
            20                  25                  30

Pro Glu Ala Leu Thr Lys Ala Leu Glu Lys Arg Phe Leu Asp Thr Asn
        35                  40                  45

Thr Pro Gln Asn Leu Thr Tyr Ile Tyr Ala Gly Ser Gln Gly Lys Arg
    50                  55                  60

Asp Gly Arg Ala Ala Glu His Leu Ala His Thr Gly Leu Leu Lys Arg
65                  70                  75                  80

Ala Ile Ile Gly His Trp Gln Thr Val Pro Ala Ile Gly Lys Leu Ala
                85                  90                  95

Val Glu Asn Lys Ile Glu Ala Tyr Asn Phe Ser Gln Gly Thr Leu Val
            100                 105                 110

His Trp Phe Arg Ala Leu Ala Gly His Lys Leu Gly Val Phe Thr Asp
        115                 120                 125

Ile Gly Leu Glu Thr Phe Leu Asp Pro Arg Gln Leu Gly Gly Lys Leu
    130                 135                 140

Asn Asp Val Thr Lys Glu Asp Leu Val Lys Leu Ile Glu Val Asp Gly
145                 150                 155                 160

His Glu Gln Leu Phe Tyr Pro Thr Phe Pro Val Asn Val Ala Phe Leu
                165                 170                 175

Arg Gly Thr Tyr Ala Asp Glu Ser Gly Asn Ile Thr Met Asp Glu Glu
            180                 185                 190

Ile Gly Pro Phe Glu Ser Thr Ser Val Ala Gln Ala Val His Asn Cys
        195                 200                 205

Gly Gly Lys Val Val Val Gln Val Lys Asp Val Val Ala His Gly Ser
    210                 215                 220

Leu Asp Pro Arg Met Val Lys Ile Pro Gly Ile Tyr Val Asp Tyr Val
225                 230                 235                 240

Val Val Ala Ala Pro Glu Asp His Gln Gln Thr Tyr Asp Cys Glu Tyr
                245                 250                 255

Asp Pro Ser Leu Ser Gly Glu His Arg Ala Pro Glu Gly Ala Thr Asp
            260                 265                 270

Ala Ala Leu Pro Met Ser Ala Lys Lys Ile Ile Gly Arg Arg Gly Ala
        275                 280                 285

Leu Glu Leu Thr Glu Asn Ala Val Val Asn Leu Gly Val Gly Ala Pro
    290                 295                 300

Glu Tyr Val Ala Ser Val Ala Gly Glu Glu Gly Ile Ala Asp Thr Ile
305                 310                 315                 320

Thr Leu Thr Val Glu Gly Gly Ala Ile Gly Gly Val Pro Gln Gly Gly
                325                 330                 335

Ala Arg Phe Gly Ser Ser Arg Asn Ala Asp Ala Ile Ile Asp His Thr
            340                 345                 350

Tyr Gln Phe Asp Phe Tyr Asp Gly Gly Gly Leu Asp Ile Ala Tyr Leu
        355                 360                 365

Gly Leu Ala Gln Cys Asp Gly Ser Gly Asn Ile Asn Val Ser Lys Phe
    370                 375                 380

Gly Thr Asn Val Ala Gly Cys Gly Gly Phe Pro Asn Ile Ser Gln Gln
385                 390                 395                 400

Thr Pro Asn Val Tyr Phe Cys Gly Thr Phe Thr Ala Gly Gly Leu Lys
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Ile Ala Val Glu Asp Gly Lys Val Lys Ile Leu Gln Glu Gly Lys Ala
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Lys Lys Phe Ile Lys Ala Val Asp Gln Ile Thr Phe Asn Gly Ser Tyr
        435                 440                 445

Ala Ala Arg Asn Gly Lys His Val Leu Tyr Ile Thr Glu Arg Cys Val
    450                 455                 460

Phe Glu Leu Thr Lys Glu Gly Leu Lys Leu Ile Glu Val Ala Pro Gly
465                 470                 475                 480

Ile Asp Ile Glu Lys Asp Ile Leu Ala His Met Asp Phe Lys Pro Ile
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<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

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aaatataaac agactcaaac accacgtaat ttatcgatta ttagtccaac agggcttggc    180

gatcgcgccg accgtggtat tagtcctctg gcgcaagaag gtctggtgaa atgggcatta    240

tgtggtcact ggggacaatc gccgcgtatt tctgaactcg cagaacaaaa taaaattatt    300

gcttataact acccacaagg tgtacttaca caaaccttac gcgccgccgc agcccaccag    360

cctggtatta ttagtgatat tggcatcggg acatttgtcg atccacgcca gcaaggcggc    420

aaactgaatg aagtcactaa agaagacctg attaaactgg tcgagtttga taacaaagaa    480

tatctctatt acaaagcgat tgcgccagat attgccttca ttcgcgctac cacctgcgac    540

agtgaaggct acgccacttt tgaagatgag gtgatgtatc tcgacgcatt ggttattgcc    600

caggcggtgc acaataacgg cggtattgtg atgatgcagg tgcagaaaat ggttaagaaa    660

gccacgctgc atcctaaatc tgtccgtatt ccgggttatc tggtggatat tgtggtggtc    720

gatccggatc aaacccaact gtatggcggt gcaccggtta accgctttat ttctggtgac    780

ttcacccttg atgacagtac caaacttagc ctgcccctaa accaacgtaa attagttgcg    840

cggcgcgcat tattcgaaat gcgtaaaggc gcggtgggga atgtcggcgt cggtattgct    900

gacggcattg gcctggtcgc ccgagaagaa ggttgtgctg atgactttat tctgacggta    960

gaaacaggtc cgattggcgg aattacttca caggggatcg cctttggcgc gaacgtgaat   1020

acccgtgcca ttctggatat gacgtcccag tttgattttt atcacggtgg cggtctggat   1080

gtttgttatt tgagttttgc tgaagtcgac cagcacggta acgtcggcgt gcataaattc   1140

aatggtaaaa tcatgggcac cggtggattt attgatatca gtgccacttc gaagaaaatc   1200

attttctgcg gcacattaac tgcgggcagt ttaaaaacag aaattaccga cggcaaatta   1260

aatatcgtcc aggaaggacg ggtgaagaaa tttattcggg aactaccgga aattactttc   1320

agcggaaaaa tcgctctcga gcgagggctg gatgttcgtt atatcactga gcgcgcagta   1380

ttcacgctga aagaagacgg cctgcattta atcgaaatcg cccctggcgt cgatttacaa   1440

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<212> TYPE: DNA
<213> ORGANISM: Caenorhabditis elegans

<400> SEQUENCE: 4

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aactctgccg aagaagccgt gaaggatatt ccagataatg caaagctttt agttggcggc 180

ttcggactat gcggaatccc agaaaatctc atccaagcta tcacaaaaac tggtcaaaaa 240

ggtcttacat gtgtatcaaa caatgcggga gttgataatt ggggacttgg cttgctcctt 300

caaactcgac aaatcaagaa aatgatctca tcgtacgtcg gtgaaaacgg agaatttgct 360

cgacaatatc ttagcggaga gctcgagttg gaattcacac cacaaggaac actcgccgaa 420

cgaattcgtg cagctggtgc cggtgttccc gcattctaca caccaacagg atacggtacc 480

cagattcaag aaggaggtgc tccgattaag tacagtaaaa ctgaaaaagg aaagattgaa 540

gttgcaagta aagcgaaaga aacacgacaa ttcaatggaa ttaattatgt aatggaagag 600

gctatttggg gagattttgc attgatcaag gcgtggagag cagatactct tggaaatatt 660

caattcagac atgctgctgg aaatttcaat aatccaatgt gcaaagcctc taaatgcacc 720

atcgtcgaag tagaggaaat cgtcgaaccg ggagtaattg ctccaaacga tgtgcacatt 780

ccatcaatct attgtcatcg tctagttttg ggaaagaact acaaaaaacc aatcgaacgg 840

ccaatgttcg cacacgaagg accaataaaa ccatctacat cggctgctgg aaaatcgaga 900

gaaatcattg cagcacgtgc agctttggag ttcacagatg gaatgtacgc caatttgggt 960

atcgggattc cgactttggc gccaaattat ataccaaatg gatttactgt tcatttgcaa 1020

agtgagaatg gtattattgg agtgggacca tatccaagaa aaggaacaga agacgccgat 1080

ctcattaatg ctggaaaaga gccaattact cttctcaaag gagcttcaat tgttggttct 1140

gatgaatcat tcgcaatgat tcgtggttct catatggata ttactgtgct cggtgcactt 1200

cagtgctcac agtttggaga tttagcgaat tggatgattc cgggaaaatt ggtgaaagga 1260

atgggcggtg caatggatct tgtctctgct cccggagccc gtgtgatcgt tgtaatggag 1320

catgtatcga agaacggaga gccaaaaatt ctagagcact gcgaacttcc tctgaccggc 1380

aaaggagtaa tttcccgaat cattactgat atggcagttt tcgacgtgga cacaaagaac 1440

ggattgacat tgatcgaagt caggaaggat cttactgtag atgatatcaa gaaactcacc 1500

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ggataa 1566

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<212> TYPE: DNA
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attacacttc aatcagaaaa tggctttctt ggtttaactg catttgaccc agaaaatgct 180

aattcaaact tagtaaatgc tggtggtcag ccttgtggaa ttaaaaaagg cggctctact 240

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ggtggacttg aagttgatca agaagcaaat ctcgctaact ggatggtgcc tggcaaaatg    360

gtaccaggaa tgggcggagc aatggactta gtgactggtg caaaaaaagt gattattggc    420

atggaacatt gtgccaagtc aggttcctca aaaattctaa agaaatgtac attaccgctc    480

acagcaagta aaaaagttgc catggtggtt accgaattgg cagtatttaa cttcattgaa    540

ggcagattag ttctaaaaga acatgctcct catgtggatt tagaaacaat taaagccaaa    600

acagaagccg atttcattgt tgccgatgat ttcaaagaaa tgcaaatcag ccagaaagga    660

cttgaattat ga                                                        672


<210> SEQ ID NO 6
<211> LENGTH: 519
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 6

Met Pro Val Leu Ser Ala Gln Glu Ala Val Asn Tyr Ile Pro Asp Glu
1               5                   10                  15

Ala Thr Leu Cys Val Leu Gly Ala Gly Gly Gly Ile Leu Glu Ala Thr
            20                  25                  30

Thr Leu Ile Thr Ala Leu Ala Asp Lys Tyr Lys Gln Thr Gln Thr Pro
        35                  40                  45

Arg Asn Leu Ser Ile Ile Ser Pro Thr Gly Leu Gly Asp Arg Ala Asp
    50                  55                  60

Arg Gly Ile Ser Pro Leu Ala Gln Glu Gly Leu Val Lys Trp Ala Leu
65                  70                  75                  80

Cys Gly His Trp Gly Gln Ser Pro Arg Ile Ser Glu Leu Ala Glu Gln
                85                  90                  95

Asn Lys Ile Ile Ala Tyr Asn Tyr Pro Gln Gly Val Leu Thr Gln Thr
            100                 105                 110

Leu Arg Ala Ala Ala Ala His Gln Pro Gly Ile Ile Ser Asp Ile Gly
        115                 120                 125

Ile Gly Thr Phe Val Asp Pro Arg Gln Gln Gly Gly Lys Leu Asn Glu
    130                 135                 140

Val Thr Lys Glu Asp Leu Ile Lys Leu Val Glu Phe Asp Asn Lys Glu
145                 150                 155                 160

Tyr Leu Tyr Tyr Lys Ala Ile Ala Pro Asp Ile Ala Phe Ile Arg Ala
                165                 170                 175

Thr Thr Cys Asp Ser Glu Gly Tyr Ala Thr Phe Glu Asp Glu Val Met
            180                 185                 190

Tyr Leu Asp Ala Leu Val Ile Ala Gln Ala Val His Asn Asn Gly Gly
        195                 200                 205

Ile Val Met Met Gln Val Gln Lys Met Val Lys Lys Ala Thr Leu His
    210                 215                 220

Pro Lys Ser Val Arg Ile Pro Gly Tyr Leu Val Asp Ile Val Val Val
225                 230                 235                 240

Asp Pro Asp Gln Thr Gln Leu Tyr Gly Gly Ala Pro Val Asn Arg Phe
                245                 250                 255

Ile Ser Gly Asp Phe Thr Leu Asp Asp Ser Thr Lys Leu Ser Leu Pro
            260                 265                 270

Leu Asn Gln Arg Lys Leu Val Ala Arg Arg Ala Leu Phe Glu Met Arg
        275                 280                 285
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Lys Gly Ala Val Gly Asn Val Gly Val Gly Ile Ala Asp Gly Ile Gly
    290                 295                 300

Leu Val Ala Arg Glu Glu Gly Cys Ala Asp Asp Phe Ile Leu Thr Val
305                 310                 315                 320

Glu Thr Gly Pro Ile Gly Gly Ile Thr Ser Gln Gly Ile Ala Phe Gly
                325                 330                 335

Ala Asn Val Asn Thr Arg Ala Ile Leu Asp Met Thr Ser Gln Phe Asp
            340                 345                 350

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

Val Asp Gln His Gly Asn Val Gly Val His Lys Phe Asn Gly Lys Ile
    370                 375                 380

Met Gly Thr Gly Gly Phe Ile Asp Ile Ser Ala Thr Ser Lys Lys Ile
385                 390                 395                 400

Ile Phe Cys Gly Thr Leu Thr Ala Gly Ser Leu Lys Thr Glu Ile Thr
                405                 410                 415

Asp Gly Lys Leu Asn Ile Val Gln Glu Gly Arg Val Lys Lys Phe Ile
            420                 425                 430

Arg Glu Leu Pro Glu Ile Thr Phe Ser Gly Lys Ile Ala Leu Glu Arg
        435                 440                 445

Gly Leu Asp Val Arg Tyr Ile Thr Glu Arg Ala Val Phe Thr Leu Lys
    450                 455                 460

Glu Asp Gly Leu His Leu Ile Glu Ile Ala Pro Gly Val Asp Leu Gln
465                 470                 475                 480

Lys Asp Ile Leu Asp Lys Met Asp Phe Thr Pro Val Ile Ser Pro Glu
                485                 490                 495

Leu Lys Leu Met Asp Glu Arg Leu Phe Ile Asp Ala Ala Met Gly Phe
            500                 505                 510

Val Leu Pro Glu Ala Ala His
        515


<210> SEQ ID NO 7
<211> LENGTH: 521
<212> TYPE: PRT
<213> ORGANISM: Caenorhabditis elegans

<400> SEQUENCE: 7

Met Pro Ile Leu Ser Lys Ile Trp Ala Ala Pro Ala Ala Gly Ile Leu
1               5                   10                  15

Arg Lys Thr Pro Arg Asn Ala His Gln Met Arg Leu Ile Ser Met Thr
            20                  25                  30

Ser Ser Met Lys Ala Lys Val Phe Asn Ser Ala Glu Glu Ala Val Lys
        35                  40                  45

Asp Ile Pro Asp Asn Ala Lys Leu Leu Val Gly Gly Phe Gly Leu Cys
    50                  55                  60

Gly Ile Pro Glu Asn Leu Ile Gln Ala Ile Thr Lys Thr Gly Gln Lys
65                  70                  75                  80

Gly Leu Thr Cys Val Ser Asn Asn Ala Gly Val Asp Asn Trp Gly Leu
                85                  90                  95

Gly Leu Leu Leu Gln Thr Arg Gln Ile Lys Lys Met Ile Ser Ser Tyr
            100                 105                 110

Val Gly Glu Asn Gly Glu Phe Ala Arg Gln Tyr Leu Ser Gly Glu Leu
        115                 120                 125

Glu Leu Glu Phe Thr Pro Gln Gly Thr Leu Ala Glu Arg Ile Arg Ala
    130                 135                 140
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Ala Gly Ala Gly Val Pro Ala Phe Tyr Thr Pro Thr Gly Tyr Gly Thr
145                 150                 155                 160

Gln Ile Gln Glu Gly Gly Ala Pro Ile Lys Tyr Ser Lys Thr Glu Lys
                165                 170                 175

Gly Lys Ile Glu Val Ala Ser Lys Ala Lys Glu Thr Arg Gln Phe Asn
            180                 185                 190

Gly Ile Asn Tyr Val Met Glu Glu Ala Ile Trp Gly Asp Phe Ala Leu
        195                 200                 205

Ile Lys Ala Trp Arg Ala Asp Thr Leu Gly Asn Ile Gln Phe Arg His
    210                 215                 220

Ala Ala Gly Asn Phe Asn Asn Pro Met Cys Lys Ala Ser Lys Cys Thr
225                 230                 235                 240

Ile Val Glu Val Glu Glu Ile Val Glu Pro Gly Val Ile Ala Pro Asn
                245                 250                 255

Asp Val His Ile Pro Ser Ile Tyr Cys His Arg Leu Val Leu Gly Lys
            260                 265                 270

Asn Tyr Lys Lys Pro Ile Glu Arg Pro Met Phe Ala His Glu Gly Pro
        275                 280                 285

Ile Lys Pro Ser Thr Ser Ala Ala Gly Lys Ser Arg Glu Ile Ile Ala
    290                 295                 300

Ala Arg Ala Ala Leu Glu Phe Thr Asp Gly Met Tyr Ala Asn Leu Gly
305                 310                 315                 320

Ile Gly Ile Pro Thr Leu Ala Pro Asn Tyr Ile Pro Asn Gly Phe Thr
                325                 330                 335

Val His Leu Gln Ser Glu Asn Gly Ile Ile Gly Val Gly Pro Tyr Pro
            340                 345                 350

Arg Lys Gly Thr Glu Asp Ala Asp Leu Ile Asn Ala Gly Lys Glu Pro
        355                 360                 365

Ile Thr Leu Leu Lys Gly Ala Ser Ile Val Gly Ser Asp Glu Ser Phe
    370                 375                 380

Ala Met Ile Arg Gly Ser His Met Asp Ile Thr Val Leu Gly Ala Leu
385                 390                 395                 400

Gln Cys Ser Gln Phe Gly Asp Leu Ala Asn Trp Met Ile Pro Gly Lys
                405                 410                 415

Leu Val Lys Gly Met Gly Gly Ala Met Asp Leu Val Ser Ala Pro Gly
            420                 425                 430

Ala Arg Val Ile Val Val Met Glu His Val Ser Lys Asn Gly Glu Pro
        435                 440                 445

Lys Ile Leu Glu His Cys Glu Leu Pro Leu Thr Gly Lys Gly Val Ile
    450                 455                 460

Ser Arg Ile Ile Thr Asp Met Ala Val Phe Asp Val Asp Thr Lys Asn
465                 470                 475                 480

Gly Leu Thr Leu Ile Glu Val Arg Lys Asp Leu Thr Val Asp Asp Ile
                485                 490                 495

Lys Lys Leu Thr Ala Cys Lys Phe Glu Ile Ser Glu Asn Leu Lys Pro
            500                 505                 510

Met Gly Gln Ala Pro Leu Asn Gln Gly
        515                 520


<210> SEQ ID NO 8
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Haemophilus influenzae
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<400> SEQUENCE: 8

Met Asn Ala Lys Glu Leu Ile Ala Arg Arg Ile Ala Met Glu Leu His
1 5 10 15

Asp Gly Asp Ile Val Asn Leu Gly Ile Gly Leu Pro Thr Gln Val Val
20 25 30

Asn Tyr Leu Pro Asp Asn Val Asn Ile Thr Leu Gln Ser Glu Asn Gly
35 40 45

Phe Leu Gly Leu Thr Ala Phe Asp Pro Glu Asn Ala Asn Ser Asn Leu
50 55 60

Val Asn Ala Gly Gly Gln Pro Cys Gly Ile Lys Lys Gly Gly Ser Thr
65 70 75 80

Phe Asp Ser Ala Phe Ser Phe Ala Leu Ile Arg Gly Gly His Val Asp
85 90 95

Ala Cys Val Leu Gly Gly Leu Glu Val Asp Gln Glu Ala Asn Leu Ala
100 105 110

Asn Trp Met Val Pro Gly Lys Met Val Pro Gly Met Gly Gly Ala Met
115 120 125

Asp Leu Val Thr Gly Ala Lys Lys Val Ile Ile Gly Met Glu His Cys
130 135 140

Ala Lys Ser Gly Ser Ser Lys Ile Leu Lys Lys Cys Thr Leu Pro Leu
145 150 155 160

Thr Ala Ser Lys Lys Val Ala Met Val Val Thr Glu Leu Ala Val Phe
165 170 175

Asn Phe Ile Glu Gly Arg Leu Val Leu Lys Glu His Ala Pro His Val
180 185 190

Asp Leu Glu Thr Ile Lys Ala Lys Thr Glu Ala Asp Phe Ile Val Ala
195 200 205

Asp Asp Phe Lys Glu Met Gln Ile Ser Gln Lys Gly Leu Glu Leu
210 215 220

<210> SEQ ID NO 9
<211> LENGTH: 786
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(783)

<400> SEQUENCE: 9

gtg aaa act gtg tat act ctc gga atc gac gtt ggt tct tct tct tcc 48
Val Lys Thr Val Tyr Thr Leu Gly Ile Asp Val Gly Ser Ser Ser Ser
1 5 10 15

aag gca gtc atc ctg gaa gat ggc aag aag atc gtc gcc cat gcc gtc 96
Lys Ala Val Ile Leu Glu Asp Gly Lys Lys Ile Val Ala His Ala Val
20 25 30

gtt gaa atc ggc acc ggt tcg acc ggt ccg gaa cgc gtc ctg gac gaa 144
Val Glu Ile Gly Thr Gly Ser Thr Gly Pro Glu Arg Val Leu Asp Glu
35 40 45

gtc ttc aaa gat acc aac tta aaa att gaa gac atg gcg aac atc atc 192
Val Phe Lys Asp Thr Asn Leu Lys Ile Glu Asp Met Ala Asn Ile Ile
50 55 60

gcc aca ggc tat ggc cgt ttc aat gtc gac tgc gcc aaa ggc gaa gtc 240
Ala Thr Gly Tyr Gly Arg Phe Asn Val Asp Cys Ala Lys Gly Glu Val
65 70 75 80

agc gaa atc acg tgc cat gcc aaa ggg gcc ctc ttt gaa tgc ccc ggt 288
Ser Glu Ile Thr Cys His Ala Lys Gly Ala Leu Phe Glu Cys Pro Gly
85 90 95

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acg acg acc atc ctc gat atc ggc ggt cag gac gtc aag tcc atc aaa      336
Thr Thr Thr Ile Leu Asp Ile Gly Gly Gln Asp Val Lys Ser Ile Lys
            100                 105                 110

ttg aat ggc cag ggc ctg gtc atg cag ttt gcc atg aac gac aaa tgc      384
Leu Asn Gly Gln Gly Leu Val Met Gln Phe Ala Met Asn Asp Lys Cys
        115                 120                 125

gcc gct ggt acg ggc cgt ttc ctc gac gtc atg tcg aag gta ctg gaa      432
Ala Ala Gly Thr Gly Arg Phe Leu Asp Val Met Ser Lys Val Leu Glu
    130                 135                 140

atc ccc atg tct gaa atg ggg gac tgg tac ttc aaa tcg aag cat ccc      480
Ile Pro Met Ser Glu Met Gly Asp Trp Tyr Phe Lys Ser Lys His Pro
145                 150                 155                 160

gct gcc gtc agc agt acc tgc acg gtt ttt gct gaa tcg gaa gtc att      528
Ala Ala Val Ser Ser Thr Cys Thr Val Phe Ala Glu Ser Glu Val Ile
                165                 170                 175

tcc ctt ctt tcc aag aat gtc ccg aaa gaa gat atc gta gcc ggt gtc      576
Ser Leu Leu Ser Lys Asn Val Pro Lys Glu Asp Ile Val Ala Gly Val
            180                 185                 190

cat cag tcc atc gcc gcc aaa gcc tgc gct ctc gtg cgc cgc gtc ggt      624
His Gln Ser Ile Ala Ala Lys Ala Cys Ala Leu Val Arg Arg Val Gly
        195                 200                 205

gtc ggt gaa gac ctg acc atg acc ggc ggt ggc tcc cgc gat ccc ggc      672
Val Gly Glu Asp Leu Thr Met Thr Gly Gly Gly Ser Arg Asp Pro Gly
    210                 215                 220

gtc gtc gat gcc gta tcg aaa gaa tta ggt att cct gtc aga gtc gct      720
Val Val Asp Ala Val Ser Lys Glu Leu Gly Ile Pro Val Arg Val Ala
225                 230                 235                 240

ctg cat ccc caa gcg gtg ggt gct ctc gga gct gct ttg att gct tat      768
Leu His Pro Gln Ala Val Gly Ala Leu Gly Ala Ala Leu Ile Ala Tyr
                245                 250                 255

gat aaa atc aag aaa taa                                             786
Asp Lys Ile Lys Lys
            260
```

<210> SEQ ID NO 10
<211> LENGTH: 261
<212> TYPE: PRT
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 10

```
Val Lys Thr Val Tyr Thr Leu Gly Ile Asp Val Gly Ser Ser Ser Ser
1               5                   10                  15

Lys Ala Val Ile Leu Glu Asp Gly Lys Lys Ile Val Ala His Ala Val
            20                  25                  30

Val Glu Ile Gly Thr Gly Ser Thr Gly Pro Glu Arg Val Leu Asp Glu
        35                  40                  45

Val Phe Lys Asp Thr Asn Leu Lys Ile Glu Asp Met Ala Asn Ile Ile
    50                  55                  60

Ala Thr Gly Tyr Gly Arg Phe Asn Val Asp Cys Ala Lys Gly Glu Val
65                  70                  75                  80

Ser Glu Ile Thr Cys His Ala Lys Gly Ala Leu Phe Glu Cys Pro Gly
                85                  90                  95

Thr Thr Thr Ile Leu Asp Ile Gly Gly Gln Asp Val Lys Ser Ile Lys
            100                 105                 110

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

Ala Ala Gly Thr Gly Arg Phe Leu Asp Val Met Ser Lys Val Leu Glu
    130                 135                 140
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Ile Pro Met Ser Glu Met Gly Asp Trp Tyr Phe Lys Ser Lys His Pro
145                 150                 155                 160

Ala Ala Val Ser Ser Thr Cys Thr Val Phe Ala Glu Ser Glu Val Ile
                165                 170                 175

Ser Leu Leu Ser Lys Asn Val Pro Lys Glu Asp Ile Val Ala Gly Val
            180                 185                 190

His Gln Ser Ile Ala Ala Lys Ala Cys Ala Leu Val Arg Arg Val Gly
        195                 200                 205

Val Gly Glu Asp Leu Thr Met Thr Gly Gly Gly Ser Arg Asp Pro Gly
    210                 215                 220

Val Val Asp Ala Val Ser Lys Glu Leu Gly Ile Pro Val Arg Val Ala
225                 230                 235                 240

Leu His Pro Gln Ala Val Gly Ala Leu Gly Ala Ala Leu Ile Ala Tyr
                245                 250                 255

Asp Lys Ile Lys Lys
            260


<210> SEQ ID NO 11
<211> LENGTH: 783
<212> TYPE: DNA
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 11

atgagtatct ataccttggg aatcgatgtt ggatctactg catccaagtg cattatcctg     60

aaagatggaa aagaaatcgt ggcgaaatcc ctggtagccg tggggaccgg aacttccggt    120

cccgcacggt ctatttcgga agtcctggaa aatgcccaca tgaaaaaaga agacatggcc    180

tttaccctgg ctaccggcta cggacgcaat tcgctggaag gcattgccga caagcagatg    240

agcgaactga gctgccatgc catgggcgcc agctttatct ggcccaacgt ccataccgtc    300

atcgatatcg gcgggcagga tgtgaaggtc atccatgtgg aaaacgggac catgaccaat    360

ttccagatga atgataaatg cgctgccggg actggccgtt tcctggatgt tatggccaat    420

atcctggaag tgaaggtttc cgacctggct gagctgggag ccaaatccac caaacgggtg    480

gctatcagct ccacctgtac tgtgtttgca gaaagtgaag tcatcagcca gctgtccaaa    540

ggaaccgaca agatcgacat cattgccggg atccatcgtt ctgtagccag ccgggtcatt    600

ggtcttgcca atcgggtggg gattgtgaaa gacgtggtca tgaccggcgg tgtagcccag    660

aactatggcg tgagaggagc cctggaagaa ggccttggcg tggaaatcaa gacgtctccc    720

ctggctcagt acaacggtgc cctgggtgcc gctctgtatg cgtataaaaa agcagccaaa    780

taa                                                                  783


<210> SEQ ID NO 12
<211> LENGTH: 774
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 12

gtggcagtgg catattcgat tggcattgat tccggctcaa ccgccaccaa agggatctta     60

ctggcagacg gcgtgattac gcgccgtttc ctcgttccaa ccccctttcg cccggcaaca    120

gcaattactg aagcctggga aactctgcgc gaagggttag agacaacgcc gtttctgacg    180

ctcaccggct acgggcggca actggtggat tttgccgata aacaggtaac ggaaatctcc    240

tgtcacgggc tgggcgcacg gtttcttgcg ccagcaacgc gcgcggtaat cgacatcggt    300

ggtcaggaca gcaaagtgat tcagcttgat gatgacggta acctgtgcga tttcctgatg    360
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aatgacaaat gcgcggcggg caccgggcgt ttcctggagg tgatctcgcg cacgcttggc 420

accagcgtcg agcaactcga cagcattacc gaaaatgtca cgccgcacgc catcacgagt 480

atgtgcacag tgtttgctga atcagaagcg atcagcctgc gctcagcggg cgtcgcgcca 540

gaagcgattc tcgcaggagt gattaacgcg atggcgcgga ggagtgccaa tttcattgct 600

cgtctctcct gtgaagcgcc gattctgttt actggtggcg ttagtcattg ccagaagttt 660

gcccggatgc tggaatctca cctgcgaatg ccggtaaata cccatcctga tgcgcaattt 720

gctggcgcaa ttggcgcggc ggtaattggt caacgagtga ggacacgccg atga 774

<210> SEQ ID NO 13
<211> LENGTH: 732
<212> TYPE: DNA
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 13

atgattttag ggatagatgt tggatctaca acaacgaaga tggttctaat ggaagatagc 60

aagataattt ggtataagat agaggatatt ggagttgtta ttgaggaaga tattttatta 120

aaaatggtta aggagattga acaaaaatat ccaatagata aaatcgttgc aactggatat 180

ggaaggcata aggttagttt tgcagataag atagttccag aagttattgc attgggaaaa 240

ggagctaact atttctttaa cgaggcagat ggagttatag acattggagg gcaagataca 300

aaggtcttaa agattgataa aaacggaaaa gttgttgatt ttatcctatc agataaatgt 360

gccgctggaa ctggaaaatt cttagaaaag gcattagata ttttaaaaat tgataaaaat 420

gagataaata aatacaaatc agataatatc gctaaaatat cttcaatgtg tgctgtcttt 480

gctgaaagtg agataataag cttactatca aaaaaagttc caaaggaagg cattttaatg 540

ggcgtctatg agagtataat aaatagggtt atcccaatga ccaataggct taaaattcaa 600

aacatagtgt ttagtggagg agttgctaaa aataaggttt tggttgagat gtttgagaaa 660

aaattgaata aaaaactact aattccaaaa gaaccacaga ttgtttgctg tgttggagct 720

atattggttt aa 732

<210> SEQ ID NO 14
<211> LENGTH: 260
<212> TYPE: PRT
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 14

Met Ser Ile Tyr Thr Leu Gly Ile Asp Val Gly Ser Thr Ala Ser Lys
1 5 10 15

Cys Ile Ile Leu Lys Asp Gly Lys Glu Ile Val Ala Lys Ser Leu Val
20 25 30

Ala Val Gly Thr Gly Thr Ser Gly Pro Ala Arg Ser Ile Ser Glu Val
35 40 45

Leu Glu Asn Ala His Met Lys Lys Glu Asp Met Ala Phe Thr Leu Ala
50 55 60

Thr Gly Tyr Gly Arg Asn Ser Leu Glu Gly Ile Ala Asp Lys Gln Met
65 70 75 80

Ser Glu Leu Ser Cys His Ala Met Gly Ala Ser Phe Ile Trp Pro Asn
85 90 95

Val His Thr Val Ile Asp Ile Gly Gly Gln Asp Val Lys Val Ile His
100 105 110

Val Glu Asn Gly Thr Met Thr Asn Phe Gln Met Asn Asp Lys Cys Ala

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        115                 120                 125

Ala Gly Thr Gly Arg Phe Leu Asp Val Met Ala Asn Ile Leu Glu Val
    130                 135                 140

Lys Val Ser Asp Leu Ala Glu Leu Gly Ala Lys Ser Thr Lys Arg Val
145                 150                 155                 160

Ala Ile Ser Ser Thr Cys Thr Val Phe Ala Glu Ser Glu Val Ile Ser
                165                 170                 175

Gln Leu Ser Lys Gly Thr Asp Lys Ile Asp Ile Ile Ala Gly Ile His
            180                 185                 190

Arg Ser Val Ala Ser Arg Val Ile Gly Leu Ala Asn Arg Val Gly Ile
        195                 200                 205

Val Lys Asp Val Val Met Thr Gly Gly Val Ala Gln Asn Tyr Gly Val
    210                 215                 220

Arg Gly Ala Leu Glu Glu Gly Leu Gly Val Glu Ile Lys Thr Ser Pro
225                 230                 235                 240

Leu Ala Gln Tyr Asn Gly Ala Leu Gly Ala Ala Leu Tyr Ala Tyr Lys
                245                 250                 255

Lys Ala Ala Lys
            260


<210> SEQ ID NO 15
<211> LENGTH: 257
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 15

Met Ala Val Ala Tyr Ser Ile Gly Ile Asp Ser Gly Ser Thr Ala Thr
1               5                   10                  15

Lys Gly Ile Leu Leu Ala Asp Gly Val Ile Thr Arg Arg Phe Leu Val
            20                  25                  30

Pro Thr Pro Phe Arg Pro Ala Thr Ala Ile Thr Glu Ala Trp Glu Thr
        35                  40                  45

Leu Arg Glu Gly Leu Glu Thr Thr Pro Phe Leu Thr Leu Thr Gly Tyr
    50                  55                  60

Gly Arg Gln Leu Val Asp Phe Ala Asp Lys Gln Val Thr Glu Ile Ser
65                  70                  75                  80

Cys His Gly Leu Gly Ala Arg Phe Leu Ala Pro Ala Thr Arg Ala Val
                85                  90                  95

Ile Asp Ile Gly Gly Gln Asp Ser Lys Val Ile Gln Leu Asp Asp Asp
            100                 105                 110

Gly Asn Leu Cys Asp Phe Leu Met Asn Asp Lys Cys Ala Ala Gly Thr
        115                 120                 125

Gly Arg Phe Leu Glu Val Ile Ser Arg Thr Leu Gly Thr Ser Val Glu
    130                 135                 140

Gln Leu Asp Ser Ile Thr Glu Asn Val Thr Pro His Ala Ile Thr Ser
145                 150                 155                 160

Met Cys Thr Val Phe Ala Glu Ser Glu Ala Ile Ser Leu Arg Ser Ala
                165                 170                 175

Gly Val Ala Pro Glu Ala Ile Leu Ala Gly Val Ile Asn Ala Met Ala
            180                 185                 190

Arg Arg Ser Ala Asn Phe Ile Ala Arg Leu Ser Cys Glu Ala Pro Ile
        195                 200                 205

Leu Phe Thr Gly Gly Val Ser His Cys Gln Lys Phe Ala Arg Met Leu
    210                 215                 220
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```
Glu Ser His Leu Arg Met Pro Val Asn Thr His Pro Asp Ala Gln Phe
225                 230                 235                 240

Ala Gly Ala Ile Gly Ala Ala Val Ile Gly Gln Arg Val Arg Thr Arg
                245                 250                 255

Arg


<210> SEQ ID NO 16
<211> LENGTH: 243
<212> TYPE: PRT
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 16

Met Ile Leu Gly Ile Asp Val Gly Ser Thr Thr Thr Lys Met Val Leu
1               5                   10                  15

Met Glu Asp Ser Lys Ile Ile Trp Tyr Lys Ile Glu Asp Ile Gly Val
            20                  25                  30

Val Ile Glu Glu Asp Ile Leu Leu Lys Met Val Lys Glu Ile Glu Gln
        35                  40                  45

Lys Tyr Pro Ile Asp Lys Ile Val Ala Thr Gly Tyr Gly Arg His Lys
    50                  55                  60

Val Ser Phe Ala Asp Lys Ile Val Pro Glu Val Ile Ala Leu Gly Lys
65                  70                  75                  80

Gly Ala Asn Tyr Phe Phe Asn Glu Ala Asp Gly Val Ile Asp Ile Gly
                85                  90                  95

Gly Gln Asp Thr Lys Val Leu Lys Ile Asp Lys Asn Gly Lys Val Val
            100                 105                 110

Asp Phe Ile Leu Ser Asp Lys Cys Ala Ala Gly Thr Gly Lys Phe Leu
        115                 120                 125

Glu Lys Ala Leu Asp Ile Leu Lys Ile Asp Lys Asn Glu Ile Asn Lys
    130                 135                 140

Tyr Lys Ser Asp Asn Ile Ala Lys Ile Ser Ser Met Cys Ala Val Phe
145                 150                 155                 160

Ala Glu Ser Glu Ile Ile Ser Leu Leu Ser Lys Lys Val Pro Lys Glu
                165                 170                 175

Gly Ile Leu Met Gly Val Tyr Glu Ser Ile Ile Asn Arg Val Ile Pro
            180                 185                 190

Met Thr Asn Arg Leu Lys Ile Gln Asn Ile Val Phe Ser Gly Gly Val
        195                 200                 205

Ala Lys Asn Lys Val Leu Val Glu Met Phe Glu Lys Lys Leu Asn Lys
    210                 215                 220

Lys Leu Leu Ile Pro Lys Glu Pro Gln Ile Val Cys Cys Val Gly Ala
225                 230                 235                 240

Ile Leu Val


<210> SEQ ID NO 17
<211> LENGTH: 1287
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1284)

<400> SEQUENCE: 17

atg agt gaa gaa aaa aca gta gat att gaa agc atg agc tcc aag gaa       48
Met Ser Glu Glu Lys Thr Val Asp Ile Glu Ser Met Ser Ser Lys Glu
1               5                   10                  15

gcc ctt ggt tac ttc ttg ccg aaa gtc gat gaa gac gca cgt aaa gcg       96
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Ala Leu Gly Tyr Phe Leu Pro Lys Val Asp Glu Asp Ala Arg Lys Ala
           20                  25                  30

aaa aaa gaa ggc cgc ctc gtt tgc tgg tcc gct tct gtc gct cct ccg      144
Lys Lys Glu Gly Arg Leu Val Cys Trp Ser Ala Ser Val Ala Pro Pro
        35                  40                  45

gaa ttc tgc acg gct atg gac atc gcc atc gtc tat ccg gaa act cac      192
Glu Phe Cys Thr Ala Met Asp Ile Ala Ile Val Tyr Pro Glu Thr His
    50                  55                  60

gca gct ggt atc ggt gcc cgt cac ggt gct ccg gcc atg ctc gaa gtt      240
Ala Ala Gly Ile Gly Ala Arg His Gly Ala Pro Ala Met Leu Glu Val
65                  70                  75                  80

gct gaa aac aaa ggt tac aac cag gac atc tgt tcc tac tgc cgc gtc      288
Ala Glu Asn Lys Gly Tyr Asn Gln Asp Ile Cys Ser Tyr Cys Arg Val
                85                  90                  95

aac atg ggc tac atg gaa ctc ctc aaa cag cag gct ctg aca ggc gaa      336
Asn Met Gly Tyr Met Glu Leu Leu Lys Gln Gln Ala Leu Thr Gly Glu
            100                 105                 110

acg ccg gaa gtc ctc aaa aac tcc ccg gct tct ccg att ccc ctt ccg      384
Thr Pro Glu Val Leu Lys Asn Ser Pro Ala Ser Pro Ile Pro Leu Pro
        115                 120                 125

gat gtt gtc ctc act tgc aac aac atc tgc aat acc ttg ctc aaa tgg      432
Asp Val Val Leu Thr Cys Asn Asn Ile Cys Asn Thr Leu Leu Lys Trp
    130                 135                 140

tat gaa aac ttg gct aaa gaa ttg aac gta cct ctc atc aac atc gac      480
Tyr Glu Asn Leu Ala Lys Glu Leu Asn Val Pro Leu Ile Asn Ile Asp
145                 150                 155                 160

gta ccg ttc aac cat gaa ttc cct gtt acg aaa cac gct aaa cag tac      528
Val Pro Phe Asn His Glu Phe Pro Val Thr Lys His Ala Lys Gln Tyr
                165                 170                 175

atc gtc ggc gaa ttc aaa cat gct atc aaa cag ctc gaa gac ctt tgc      576
Ile Val Gly Glu Phe Lys His Ala Ile Lys Gln Leu Glu Asp Leu Cys
            180                 185                 190

ggc cgt ccc ttc gac tat gac aaa ttc ttc gaa gta cag aaa cag aca      624
Gly Arg Pro Phe Asp Tyr Asp Lys Phe Phe Glu Val Gln Lys Gln Thr
        195                 200                 205

cag cgc tcc atc gct gcc tgg aac aaa atc gct acg tac ttc cag tac      672
Gln Arg Ser Ile Ala Ala Trp Asn Lys Ile Ala Thr Tyr Phe Gln Tyr
    210                 215                 220

aaa ccg tcg ccg ctc aac ggc ttc gac ctc ttc aac tac atg ggc ctc      720
Lys Pro Ser Pro Leu Asn Gly Phe Asp Leu Phe Asn Tyr Met Gly Leu
225                 230                 235                 240

gcc gtt gct gcc cgc tcc ttg aac tac tcg gaa atc acg ttc aac aaa      768
Ala Val Ala Ala Arg Ser Leu Asn Tyr Ser Glu Ile Thr Phe Asn Lys
                245                 250                 255

ttc ctc aaa gaa ttg gac gaa aaa gta gct aat aag aaa tgg gct ttc      816
Phe Leu Lys Glu Leu Asp Glu Lys Val Ala Asn Lys Lys Trp Ala Phe
            260                 265                 270

ggt gaa aac gaa aaa tcc cgt gtt act tgg gaa ggt atc gct gtc tgg      864
Gly Glu Asn Glu Lys Ser Arg Val Thr Trp Glu Gly Ile Ala Val Trp
        275                 280                 285

atc gct ctc ggc cac acc ttc aaa gaa ctc aaa ggt cag ggc gct ctc      912
Ile Ala Leu Gly His Thr Phe Lys Glu Leu Lys Gly Gln Gly Ala Leu
    290                 295                 300

atg act ggt tcc gct tat cct ggc atg tgg gac gtt tcc tac gaa ccg      960
Met Thr Gly Ser Ala Tyr Pro Gly Met Trp Asp Val Ser Tyr Glu Pro
305                 310                 315                 320

ggc gac ctc gaa tcc atg gca gaa gct tat tcc cgt aca tac atc aac     1008
Gly Asp Leu Glu Ser Met Ala Glu Ala Tyr Ser Arg Thr Tyr Ile Asn
                325                 330                 335
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tgc tgc ctc gaa cag cgc ggt gct gtt ctt gaa aaa gtt gtc cgc gat     1056
Cys Cys Leu Glu Gln Arg Gly Ala Val Leu Glu Lys Val Val Arg Asp
            340                 345                 350

ggc aaa tgc gac ggc ttg atc atg cac cag aac cgt tcc tgc aag aac     1104
Gly Lys Cys Asp Gly Leu Ile Met His Gln Asn Arg Ser Cys Lys Asn
        355                 360                 365

atg agc ctc ctc aac aac gaa ggc ggc cag cgc atc cag aag aac ctc     1152
Met Ser Leu Leu Asn Asn Glu Gly Gly Gln Arg Ile Gln Lys Asn Leu
    370                 375                 380

ggc gta ccg tac gtc atc ttc gac ggc gac cag acc gat gct cgt aac     1200
Gly Val Pro Tyr Val Ile Phe Asp Gly Asp Gln Thr Asp Ala Arg Asn
385                 390                 395                 400

ttc tcg gaa gca cag ttc gat acc cgc gta gaa gct ttg gca gaa atg     1248
Phe Ser Glu Ala Gln Phe Asp Thr Arg Val Glu Ala Leu Ala Glu Met
                405                 410                 415

atg gca gac aaa aaa gcc aat gaa gga gga aac cac taa                 1287
Met Ala Asp Lys Lys Ala Asn Glu Gly Gly Asn His
            420                 425


<210> SEQ ID NO 18
<211> LENGTH: 428
<212> TYPE: PRT
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 18

Met Ser Glu Glu Lys Thr Val Asp Ile Glu Ser Met Ser Ser Lys Glu
1               5                   10                  15

Ala Leu Gly Tyr Phe Leu Pro Lys Val Asp Glu Asp Ala Arg Lys Ala
            20                  25                  30

Lys Lys Glu Gly Arg Leu Val Cys Trp Ser Ala Ser Val Ala Pro Pro
        35                  40                  45

Glu Phe Cys Thr Ala Met Asp Ile Ala Ile Val Tyr Pro Glu Thr His
    50                  55                  60

Ala Ala Gly Ile Gly Ala Arg His Gly Ala Pro Ala Met Leu Glu Val
65                  70                  75                  80

Ala Glu Asn Lys Gly Tyr Asn Gln Asp Ile Cys Ser Tyr Cys Arg Val
                85                  90                  95

Asn Met Gly Tyr Met Glu Leu Leu Lys Gln Gln Ala Leu Thr Gly Glu
            100                 105                 110

Thr Pro Glu Val Leu Lys Asn Ser Pro Ala Ser Pro Ile Pro Leu Pro
        115                 120                 125

Asp Val Val Leu Thr Cys Asn Asn Ile Cys Asn Thr Leu Leu Lys Trp
    130                 135                 140

Tyr Glu Asn Leu Ala Lys Glu Leu Asn Val Pro Leu Ile Asn Ile Asp
145                 150                 155                 160

Val Pro Phe Asn His Glu Phe Pro Val Thr Lys His Ala Lys Gln Tyr
                165                 170                 175

Ile Val Gly Glu Phe Lys His Ala Ile Lys Gln Leu Glu Asp Leu Cys
            180                 185                 190

Gly Arg Pro Phe Asp Tyr Asp Lys Phe Phe Glu Val Gln Lys Gln Thr
        195                 200                 205

Gln Arg Ser Ile Ala Ala Trp Asn Lys Ile Ala Thr Tyr Phe Gln Tyr
    210                 215                 220

Lys Pro Ser Pro Leu Asn Gly Phe Asp Leu Phe Asn Tyr Met Gly Leu
225                 230                 235                 240

Ala Val Ala Ala Arg Ser Leu Asn Tyr Ser Glu Ile Thr Phe Asn Lys
                245                 250                 255
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Phe Leu Lys Glu Leu Asp Glu Lys Val Ala Asn Lys Lys Trp Ala Phe
            260                 265                 270

Gly Glu Asn Glu Lys Ser Arg Val Thr Trp Glu Gly Ile Ala Val Trp
        275                 280                 285

Ile Ala Leu Gly His Thr Phe Lys Glu Leu Lys Gly Gln Gly Ala Leu
    290                 295                 300

Met Thr Gly Ser Ala Tyr Pro Gly Met Trp Asp Val Ser Tyr Glu Pro
305                 310                 315                 320

Gly Asp Leu Glu Ser Met Ala Glu Ala Tyr Ser Arg Thr Tyr Ile Asn
                325                 330                 335

Cys Cys Leu Glu Gln Arg Gly Ala Val Leu Glu Lys Val Val Arg Asp
            340                 345                 350

Gly Lys Cys Asp Gly Leu Ile Met His Gln Asn Arg Ser Cys Lys Asn
        355                 360                 365

Met Ser Leu Leu Asn Asn Glu Gly Gly Gln Arg Ile Gln Lys Asn Leu
    370                 375                 380

Gly Val Pro Tyr Val Ile Phe Asp Gly Asp Gln Thr Asp Ala Arg Asn
385                 390                 395                 400

Phe Ser Glu Ala Gln Phe Asp Thr Arg Val Glu Ala Leu Ala Glu Met
                405                 410                 415

Met Ala Asp Lys Lys Ala Asn Glu Gly Gly Asn His
            420                 425


<210> SEQ ID NO 19
<211> LENGTH: 1434
<212> TYPE: DNA
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 19

atgccaaaga cagtaagccc tggcgttcag gcattgagag atgtagttga aaaggtttac     60

agagaactgc gggaaccgaa agaaagagga gaaaaagtag gctggtcctc ttccaagttc    120

ccctgcgaac tggctgaatc ttttcggctg catgttgggt atccggaaaa ccaggctgct    180

ggtatcgctg ccaaccgtga cggcgaagtg atgtgccagg ctgcagaaga tatcggttat    240

gacaacgata tctgcggcta tgcccgtatt tccctggctt atgctgccgg gttccggggt    300

gccaacaaaa tggacaaaga tggcaactat gtcatcaacc cccacagcgg caaacagatg    360

aaagatgcca atggcaaaaa ggtattcgac gcagatggca aacccgtaat cgatcccaag    420

accctgaaac cctttgccac caccgacaac atctatgaaa tcgctgctct gccggaaggg    480

gaagaaaaga cccgccgcca gaatgccctg cacaaatatc gtcagatgac catgcccatg    540

ccggacttcg tgctgtgctg caacaacatc tgcaactgca tgaccaaatg gtatgaagac    600

attgcccgtc ggcacaacat tcctttgatc atgatcgacg ttccttacaa cgaattcgac    660

catgtcaacg aagccaacgt gaaatacatc cggtcccagc tggatacggc catccgtcaa    720

atggaagaaa tcaccggcaa gaagttcgat gaagacaaat tcgaacagtg ctgccagaac    780

gccaaccgta ctgccaaagc atggctgaag gtttgcgact acctgcagta caaaccggct    840

ccgttcaacg ggttcgacct gttcaaccat atggctgacg tggttaccgc ccgtggccgt    900

gtggaagctg ctgaagcttt cgaactgctg gccaaggaac tggaacagca tgtgaaggaa    960

ggcaccacca ccgctccctt caaagaacag catcgtatca tgttcgaagg gatcccctgc   1020

tggccgaaac tgccgaacct gttcaaaccg ctgaaagcca acggcctgaa catcaccggc   1080

gttgtatatg ctcctgcttt cgggttcgtg tacaacaacc tggacgaatt ggtcaaagcc   1140
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tactgcaaag ccccgaactc cgtcagcatc gaacagggtg ttgcctggcg tgaaggcctg 1200

atccgcgaca acaaggttga cggcgtactg gttcactaca accggtcctg caaaccctgg 1260

agcggctaca tgcctgaaat gcagcgtcgt ttcaccaaag acatgggtat ccccactgct 1320

ggattcgacg gtgaccaggc tgacccgaga aacttcaacg cggctcagta tgagacccgt 1380

gttcagggct tggtcgaagc catggaagca aatgatgaaa agaaggggaa ataa 1434

<210> SEQ ID NO 20
<211> LENGTH: 1122
<212> TYPE: DNA
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 20

atgatgaaat taaaggcaat tgaaaagttg atgcaaaaat tcgccagtag aaaagaacag 60

ctatataagc aaaaagaaga aggtagaaaa gtttttggaa tgttctgtgc ctatgttcca 120

atagaaataa ttttagcagc aaatgcaatc ccagttggtt tgtgtggagg taaaaatgac 180

acaatcccaa tagcagagga ggatttgcca agaaacctat gcccattaat aaaatcatcc 240

tatggtttta agaaggcaaa aacctgccct tactttgaag catctgatat agttattgga 300

gaaactacct gtgaaggaaa gaagaagatg tttgagttga tggagagatt ggtgccaatg 360

catataatgc acctcccaca catgaaagat gaagattctt tgaaaatctg gattaaagaa 420

gttgaaaagc taaaagaatt ggttgagaaa gagactggaa ataaaataac agaggaaaag 480

ttaaaagaga cagttgataa agtaaataaa gttagggagt tgttttataa actctatgaa 540

ttgaggaaga ataaaccagc tccaattaag ggtttagatg ttttaaaatt attccagttt 600

gcctatttat tggatattga tgacacaata gggattttag aggatttaat tgaggagtta 660

gaggagagag ttaaaaaagg agaaggttat gaaggaaaga gaattttaat aactggctgt 720

ccaatggttg ctggaaacaa taagattgtt gaaattattg aggaagttgg aggagtagtt 780

gttggtgaag aaagctgcac tggaacaaga ttctttgaaa actttgttga gggctatagc 840

gtagaggaca ttgcaaaaag atactttaaa atcccatgtg cttgtagatt taaaaacgat 900

gagagagttg aaaatataaa gagattggtt aaagagttgg acgtcgatgg agttgtttat 960

tacactttgc agtattgcca tacatttaac atagagggag ctaaggtaga ggaggcatta 1020

aaagaggagg gcattccaat tataagaatt gaaactgact attctgaaag tgatagagag 1080

cagttaaaaa caaggttgga ggcatttatt gagatgattt aa 1122

<210> SEQ ID NO 21
<211> LENGTH: 1152
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 21

atgtcacttg tcaccgatct acccgccatt ttcgatcagt tctctgaagc tcgccagaca 60

ggctttctca ccgtcatgga tctcaaggag cgcggcattc cgctggttgg cacttactgc 120

acctttatgc cgcaagagat cccgatggca gccggtgcgg ttgtggtttc gctctgttcc 180

acctctgatg aaaccattga agaagcggag aaagatctgc cgcgcaacct ctgcccgctg 240

attaaaagca gctacggctt cggcaaaacc gataaatgcc cctacttcta cttttcggat 300

ctggtggtcg gtgaaaccac ctgcgacggc aaaaagaaaa tgtatgaata catggcggag 360

tttaagcctg ttcatgtgat gcaattgccc aacagcgtta aggacgatgc ctcgcgtgcg 420

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ttatggaaag ccgagatgct gcgcttgcaa aaaacggtag aagaacgttt tgggcacgag    480

attagcgaag atgctctgcg cgatgccatt gcgctgaaaa accgcgaacg tcgcgcactg    540

gctaattttt atcatcttgg gcagttaaat cctccggcgc ttagcggcag cgacattctg    600

aaagtggttt acggcgcaac cttccggttc gataaagagg cgttgatcaa tgaactggat    660

gcaatgaccg cccgcgttcg tcagcagtgg gaagaaggcc agcgactgga cccgcgtccg    720

cgcattttaa tcaccggctg cccgattggc ggcgcagcag aaaaagtggt gcgcgcgatt    780

gaagagaatg gcggctgggt tgtcggttat gaaaactgca ccggggcgaa agcgaccgag    840

caatgcgtgg cagaaacggg cgatgtctac gacgcgctgg cggataaata tctggcgatt    900

ggctgctcct gtgtttcgcc gaacgatcag cgcctgaaaa tgctcagcca gatggtggag    960

gaatatcagg tcgatggcgt agttgatgtg attttgcagg cgtgccatac ctacgcggtg   1020

gaatcgctgg cgattaaacg tcatgtgcgc cagcagcaca acattcctta tatcgctatt   1080

gaaacagact actccacctc ggatgtcggg cagctcagta cccgtgtcgc ggcctttatt   1140

gagatgctgt aa                                                       1152
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<210> SEQ ID NO 22
<211> LENGTH: 477
<212> TYPE: PRT
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 22

```
Met Pro Lys Thr Val Ser Pro Gly Val Gln Ala Leu Arg Asp Val Val
1               5                   10                  15

Glu Lys Val Tyr Arg Glu Leu Arg Glu Pro Lys Glu Arg Gly Glu Lys
            20                  25                  30

Val Gly Trp Ser Ser Ser Lys Phe Pro Cys Glu Leu Ala Glu Ser Phe
        35                  40                  45

Arg Leu His Val Gly Tyr Pro Glu Asn Gln Ala Ala Gly Ile Ala Ala
    50                  55                  60

Asn Arg Asp Gly Glu Val Met Cys Gln Ala Ala Glu Asp Ile Gly Tyr
65                  70                  75                  80

Asp Asn Asp Ile Cys Gly Tyr Ala Arg Ile Ser Leu Ala Tyr Ala Ala
                85                  90                  95

Gly Phe Arg Gly Ala Asn Lys Met Asp Lys Asp Gly Asn Tyr Val Ile
            100                 105                 110

Asn Pro His Ser Gly Lys Gln Met Lys Asp Ala Asn Gly Lys Lys Val
        115                 120                 125

Phe Asp Ala Asp Gly Lys Pro Val Ile Asp Pro Lys Thr Leu Lys Pro
    130                 135                 140

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

Glu Glu Lys Thr Arg Arg Gln Asn Ala Leu His Lys Tyr Arg Gln Met
                165                 170                 175

Thr Met Pro Met Pro Asp Phe Val Leu Cys Cys Asn Asn Ile Cys Asn
            180                 185                 190

Cys Met Thr Lys Trp Tyr Glu Asp Ile Ala Arg Arg His Asn Ile Pro
        195                 200                 205

Leu Ile Met Ile Asp Val Pro Tyr Asn Glu Phe Asp His Val Asn Glu
    210                 215                 220

Ala Asn Val Lys Tyr Ile Arg Ser Gln Leu Asp Thr Ala Ile Arg Gln
225                 230                 235                 240
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Met Glu Glu Ile Thr Gly Lys Lys Phe Asp Glu Asp Lys Phe Glu Gln
                245                 250                 255

Cys Cys Gln Asn Ala Asn Arg Thr Ala Lys Ala Trp Leu Lys Val Cys
            260                 265                 270

Asp Tyr Leu Gln Tyr Lys Pro Ala Pro Phe Asn Gly Phe Asp Leu Phe
        275                 280                 285

Asn His Met Ala Asp Val Val Thr Ala Arg Gly Arg Val Glu Ala Ala
    290                 295                 300

Glu Ala Phe Glu Leu Leu Ala Lys Glu Leu Glu Gln His Val Lys Glu
305                 310                 315                 320

Gly Thr Thr Thr Ala Pro Phe Lys Glu Gln His Arg Ile Met Phe Glu
                325                 330                 335

Gly Ile Pro Cys Trp Pro Lys Leu Pro Asn Leu Phe Lys Pro Leu Lys
            340                 345                 350

Ala Asn Gly Leu Asn Ile Thr Gly Val Val Tyr Ala Pro Ala Phe Gly
        355                 360                 365

Phe Val Tyr Asn Asn Leu Asp Glu Leu Val Lys Ala Tyr Cys Lys Ala
    370                 375                 380

Pro Asn Ser Val Ser Ile Glu Gln Gly Val Ala Trp Arg Glu Gly Leu
385                 390                 395                 400

Ile Arg Asp Asn Lys Val Asp Gly Val Leu Val His Tyr Asn Arg Ser
                405                 410                 415

Cys Lys Pro Trp Ser Gly Tyr Met Pro Glu Met Gln Arg Arg Phe Thr
            420                 425                 430

Lys Asp Met Gly Ile Pro Thr Ala Gly Phe Asp Gly Asp Gln Ala Asp
        435                 440                 445

Pro Arg Asn Phe Asn Ala Ala Gln Tyr Glu Thr Arg Val Gln Gly Leu
    450                 455                 460

Val Glu Ala Met Glu Ala Asn Asp Glu Lys Lys Gly Lys
465                 470                 475


<210> SEQ ID NO 23
<211> LENGTH: 373
<212> TYPE: PRT
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 23

Met Met Lys Leu Lys Ala Ile Glu Lys Leu Met Gln Lys Phe Ala Ser
1               5                   10                  15

Arg Lys Glu Gln Leu Tyr Lys Gln Lys Glu Glu Gly Arg Lys Val Phe
            20                  25                  30

Gly Met Phe Cys Ala Tyr Val Pro Ile Glu Ile Ile Leu Ala Ala Asn
        35                  40                  45

Ala Ile Pro Val Gly Leu Cys Gly Gly Lys Asn Asp Thr Ile Pro Ile
    50                  55                  60

Ala Glu Glu Asp Leu Pro Arg Asn Leu Cys Pro Leu Ile Lys Ser Ser
65                  70                  75                  80

Tyr Gly Phe Lys Lys Ala Lys Thr Cys Pro Tyr Phe Glu Ala Ser Asp
                85                  90                  95

Ile Val Ile Gly Glu Thr Thr Cys Glu Gly Lys Lys Lys Met Phe Glu
            100                 105                 110

Leu Met Glu Arg Leu Val Pro Met His Ile Met His Leu Pro His Met
        115                 120                 125

Lys Asp Glu Asp Ser Leu Lys Ile Trp Ile Lys Glu Val Glu Lys Leu
    130                 135                 140
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Lys Glu Leu Val Glu Lys Glu Thr Gly Asn Lys Ile Thr Glu Glu Lys
145                 150                 155                 160

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

Lys Leu Tyr Glu Leu Arg Lys Asn Lys Pro Ala Pro Ile Lys Gly Leu
            180                 185                 190

Asp Val Leu Lys Leu Phe Gln Phe Ala Tyr Leu Leu Asp Ile Asp Asp
        195                 200                 205

Thr Ile Gly Ile Leu Glu Asp Leu Ile Glu Glu Leu Glu Glu Arg Val
    210                 215                 220

Lys Lys Gly Glu Gly Tyr Glu Gly Lys Arg Ile Leu Ile Thr Gly Cys
225                 230                 235                 240

Pro Met Val Ala Gly Asn Asn Lys Ile Val Glu Ile Ile Glu Glu Val
                245                 250                 255

Gly Gly Val Val Val Gly Glu Glu Ser Cys Thr Gly Thr Arg Phe Phe
            260                 265                 270

Glu Asn Phe Val Glu Gly Tyr Ser Val Glu Asp Ile Ala Lys Arg Tyr
        275                 280                 285

Phe Lys Ile Pro Cys Ala Cys Arg Phe Lys Asn Asp Glu Arg Val Glu
    290                 295                 300

Asn Ile Lys Arg Leu Val Lys Glu Leu Asp Val Asp Gly Val Val Tyr
305                 310                 315                 320

Tyr Thr Leu Gln Tyr Cys His Thr Phe Asn Ile Glu Gly Ala Lys Val
                325                 330                 335

Glu Glu Ala Leu Lys Glu Glu Gly Ile Pro Ile Ile Arg Ile Glu Thr
            340                 345                 350

Asp Tyr Ser Glu Ser Asp Arg Glu Gln Leu Lys Thr Arg Leu Glu Ala
        355                 360                 365

Phe Ile Glu Met Ile
    370


<210> SEQ ID NO 24
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 24

Met Ser Leu Val Thr Asp Leu Pro Ala Ile Phe Asp Gln Phe Ser Glu
1               5                   10                  15

Ala Arg Gln Thr Gly Phe Leu Thr Val Met Asp Leu Lys Glu Arg Gly
            20                  25                  30

Ile Pro Leu Val Gly Thr Tyr Cys Thr Phe Met Pro Gln Glu Ile Pro
        35                  40                  45

Met Ala Ala Gly Ala Val Val Val Ser Leu Cys Ser Thr Ser Asp Glu
    50                  55                  60

Thr Ile Glu Glu Ala Glu Lys Asp Leu Pro Arg Asn Leu Cys Pro Leu
65                  70                  75                  80

Ile Lys Ser Ser Tyr Gly Phe Gly Lys Thr Asp Lys Cys Pro Tyr Phe
                85                  90                  95

Tyr Phe Ser Asp Leu Val Val Gly Glu Thr Thr Cys Asp Gly Lys Lys
            100                 105                 110

Lys Met Tyr Glu Tyr Met Ala Glu Phe Lys Pro Val His Val Met Gln
        115                 120                 125

Leu Pro Asn Ser Val Lys Asp Asp Ala Ser Arg Ala Leu Trp Lys Ala
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    130                 135                 140

Glu Met Leu Arg Leu Gln Lys Thr Val Glu Glu Arg Phe Gly His Glu
145                 150                 155                 160

Ile Ser Glu Asp Ala Leu Arg Asp Ala Ile Ala Leu Lys Asn Arg Glu
                165                 170                 175

Arg Arg Ala Leu Ala Asn Phe Tyr His Leu Gly Gln Leu Asn Pro Pro
            180                 185                 190

Ala Leu Ser Gly Ser Asp Ile Leu Lys Val Val Tyr Gly Ala Thr Phe
        195                 200                 205

Arg Phe Asp Lys Glu Ala Leu Ile Asn Glu Leu Asp Ala Met Thr Ala
    210                 215                 220

Arg Val Arg Gln Gln Trp Glu Glu Gly Gln Arg Leu Asp Pro Arg Pro
225                 230                 235                 240

Arg Ile Leu Ile Thr Gly Cys Pro Ile Gly Gly Ala Ala Glu Lys Val
                245                 250                 255

Val Arg Ala Ile Glu Glu Asn Gly Gly Trp Val Val Gly Tyr Glu Asn
            260                 265                 270

Cys Thr Gly Ala Lys Ala Thr Glu Gln Cys Val Ala Glu Thr Gly Asp
        275                 280                 285

Val Tyr Asp Ala Leu Ala Asp Lys Tyr Leu Ala Ile Gly Cys Ser Cys
    290                 295                 300

Val Ser Pro Asn Asp Gln Arg Leu Lys Met Leu Ser Gln Met Val Glu
305                 310                 315                 320

Glu Tyr Gln Val Asp Gly Val Val Asp Val Ile Leu Gln Ala Cys His
                325                 330                 335

Thr Tyr Ala Val Glu Ser Leu Ala Ile Lys Arg His Val Arg Gln Gln
            340                 345                 350

His Asn Ile Pro Tyr Ile Ala Ile Glu Thr Asp Tyr Ser Thr Ser Asp
        355                 360                 365

Val Gly Gln Leu Ser Thr Arg Val Ala Ala Phe Ile Glu Met Leu
    370                 375                 380


<210> SEQ ID NO 25
<211> LENGTH: 1119
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1116)

<400> SEQUENCE: 25

atg agt cag atc gac gaa ctt atc agc aaa tta cag gaa gta tcc aac       48
Met Ser Gln Ile Asp Glu Leu Ile Ser Lys Leu Gln Glu Val Ser Asn
1               5                   10                  15

cat ccc cag aag acg gtt ttg aat tat aaa aaa cag ggt aaa ggc ctc       96
His Pro Gln Lys Thr Val Leu Asn Tyr Lys Lys Gln Gly Lys Gly Leu
            20                  25                  30

gta ggc atg atg ccc tac tac gct ccg gaa gaa atc gta tat gct gca      144
Val Gly Met Met Pro Tyr Tyr Ala Pro Glu Glu Ile Val Tyr Ala Ala
        35                  40                  45

ggc tac ctc ccg gta ggc atg ttc ggt tcc cag aac ccg cag atc tcc      192
Gly Tyr Leu Pro Val Gly Met Phe Gly Ser Gln Asn Pro Gln Ile Ser
    50                  55                  60

gca gct cgt acg tac ctt cct ccg ttc gct tgc tcc ttg atg cag gct      240
Ala Ala Arg Thr Tyr Leu Pro Pro Phe Ala Cys Ser Leu Met Gln Ala
65                  70                  75                  80

gac atg gaa ctc cag ctc aac ggc acc tat gac tgc ctc gac gct gtt      288
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Asp Met Glu Leu Gln Leu Asn Gly Thr Tyr Asp Cys Leu Asp Ala Val
                85                  90                  95

atc ttc tcc gtt cct tgc gac act ctc cgc tgc atg agc cag aaa tgg      336
Ile Phe Ser Val Pro Cys Asp Thr Leu Arg Cys Met Ser Gln Lys Trp
            100                 105                 110

cac ggc aaa gct ccg gtc atc gtc ttc aca cag ccg cag aac cgt aag      384
His Gly Lys Ala Pro Val Ile Val Phe Thr Gln Pro Gln Asn Arg Lys
        115                 120                 125

atc cgc ccg gct gtc gat ttc ctc aaa gct gaa tac gaa cat gtc cgt      432
Ile Arg Pro Ala Val Asp Phe Leu Lys Ala Glu Tyr Glu His Val Arg
    130                 135                 140

acg gaa ttg gga cgt atc ctc aac gta aaa atc tcc gac ctg gct atc      480
Thr Glu Leu Gly Arg Ile Leu Asn Val Lys Ile Ser Asp Leu Ala Ile
145                 150                 155                 160

cag gaa gct atc aaa gta tat aac gaa aac cgt cag gtt atg cgt gaa      528
Gln Glu Ala Ile Lys Val Tyr Asn Glu Asn Arg Gln Val Met Arg Glu
                165                 170                 175

ttc tgc gac gta gct gct cag tac ccg cag atc ttc act ccg ata aaa      576
Phe Cys Asp Val Ala Ala Gln Tyr Pro Gln Ile Phe Thr Pro Ile Lys
            180                 185                 190

cgt cat gac gtc atc aaa gcc cgc tgg ttc atg gac aaa gct gaa cac      624
Arg His Asp Val Ile Lys Ala Arg Trp Phe Met Asp Lys Ala Glu His
        195                 200                 205

acc gct ttg gtc cgc gaa ctc atc gac gct gtc aag aaa gaa ccg gta      672
Thr Ala Leu Val Arg Glu Leu Ile Asp Ala Val Lys Lys Glu Pro Val
    210                 215                 220

cag ccg tgg aat ggc aaa aaa gtc atc ctc tcc ggt atc atg gca gaa      720
Gln Pro Trp Asn Gly Lys Lys Val Ile Leu Ser Gly Ile Met Ala Glu
225                 230                 235                 240

ccg gat gaa ttc ctc gat atc ttc agc gaa ttc aac atc gct gtc gtc      768
Pro Asp Glu Phe Leu Asp Ile Phe Ser Glu Phe Asn Ile Ala Val Val
                245                 250                 255

gct gac gac ctc gct cag gaa tcc cgc cag ttc cgt aca gac gta ccg      816
Ala Asp Asp Leu Ala Gln Glu Ser Arg Gln Phe Arg Thr Asp Val Pro
            260                 265                 270

tcc ggc atc gat ccc ctc gaa cag ctc gct cag cag tgg cag gac ttc      864
Ser Gly Ile Asp Pro Leu Glu Gln Leu Ala Gln Gln Trp Gln Asp Phe
        275                 280                 285

gat ggc tgc ccg ctc gct ttg aac gaa gac aaa ccg cgt ggc cag atg      912
Asp Gly Cys Pro Leu Ala Leu Asn Glu Asp Lys Pro Arg Gly Gln Met
    290                 295                 300

ctc atc gac atg act aag aaa tac aat gct gac gcc gtc gtc atc tgc      960
Leu Ile Asp Met Thr Lys Lys Tyr Asn Ala Asp Ala Val Val Ile Cys
305                 310                 315                 320

atg atg cgt ttc tgc gat cct gaa gaa ttc gac tat ccg att tac aaa     1008
Met Met Arg Phe Cys Asp Pro Glu Glu Phe Asp Tyr Pro Ile Tyr Lys
                325                 330                 335

ccg gaa ttt gaa gct gct ggc gtt cgt tac acg gtc ctc gac ctc gac     1056
Pro Glu Phe Glu Ala Ala Gly Val Arg Tyr Thr Val Leu Asp Leu Asp
            340                 345                 350

atc gaa tct ccg tcc ctc gaa cag ctc cgc acc cgt atc cag gct ttc     1104
Ile Glu Ser Pro Ser Leu Glu Gln Leu Arg Thr Arg Ile Gln Ala Phe
        355                 360                 365

tcg gaa atc ctc taa                                                1119
Ser Glu Ile Leu
    370


<210> SEQ ID NO 26
<211> LENGTH: 372
<212> TYPE: PRT
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<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 26

Met Ser Gln Ile Asp Glu Leu Ile Ser Lys Leu Gln Glu Val Ser Asn
1 5 10 15

His Pro Gln Lys Thr Val Leu Asn Tyr Lys Lys Gln Gly Lys Gly Leu
20 25 30

Val Gly Met Met Pro Tyr Tyr Ala Pro Glu Glu Ile Val Tyr Ala Ala
35 40 45

Gly Tyr Leu Pro Val Gly Met Phe Gly Ser Gln Asn Pro Gln Ile Ser
50 55 60

Ala Ala Arg Thr Tyr Leu Pro Pro Phe Ala Cys Ser Leu Met Gln Ala
65 70 75 80

Asp Met Glu Leu Gln Leu Asn Gly Thr Tyr Asp Cys Leu Asp Ala Val
85 90 95

Ile Phe Ser Val Pro Cys Asp Thr Leu Arg Cys Met Ser Gln Lys Trp
100 105 110

His Gly Lys Ala Pro Val Ile Val Phe Thr Gln Pro Gln Asn Arg Lys
115 120 125

Ile Arg Pro Ala Val Asp Phe Leu Lys Ala Glu Tyr Glu His Val Arg
130 135 140

Thr Glu Leu Gly Arg Ile Leu Asn Val Lys Ile Ser Asp Leu Ala Ile
145 150 155 160

Gln Glu Ala Ile Lys Val Tyr Asn Glu Asn Arg Gln Val Met Arg Glu
165 170 175

Phe Cys Asp Val Ala Ala Gln Tyr Pro Gln Ile Phe Thr Pro Ile Lys
180 185 190

Arg His Asp Val Ile Lys Ala Arg Trp Phe Met Asp Lys Ala Glu His
195 200 205

Thr Ala Leu Val Arg Glu Leu Ile Asp Ala Val Lys Lys Glu Pro Val
210 215 220

Gln Pro Trp Asn Gly Lys Lys Val Ile Leu Ser Gly Ile Met Ala Glu
225 230 235 240

Pro Asp Glu Phe Leu Asp Ile Phe Ser Glu Phe Asn Ile Ala Val Val
245 250 255

Ala Asp Asp Leu Ala Gln Glu Ser Arg Gln Phe Arg Thr Asp Val Pro
260 265 270

Ser Gly Ile Asp Pro Leu Glu Gln Leu Ala Gln Gln Trp Gln Asp Phe
275 280 285

Asp Gly Cys Pro Leu Ala Leu Asn Glu Asp Lys Pro Arg Gly Gln Met
290 295 300

Leu Ile Asp Met Thr Lys Lys Tyr Asn Ala Asp Ala Val Val Ile Cys
305 310 315 320

Met Met Arg Phe Cys Asp Pro Glu Glu Phe Asp Tyr Pro Ile Tyr Lys
325 330 335

Pro Glu Phe Glu Ala Ala Gly Val Arg Tyr Thr Val Leu Asp Leu Asp
340 345 350

Ile Glu Ser Pro Ser Leu Glu Gln Leu Arg Thr Arg Ile Gln Ala Phe
355 360 365

Ser Glu Ile Leu
370

<210> SEQ ID NO 27
<211> LENGTH: 1140

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<212> TYPE: DNA
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 27

atggctatca gtgcacttat tgaagagttc caaaaagtat ctgccagccc gaagaccatg 60

ctggccaaat ataaagccca gggcaaaaaa gccatcggct gcctgccgta ctatgttccg 120

gaagaactgg tctatgctgc aggcatggtt cccatgggtg tatggggctg caatggcaaa 180

caggaagtcc gttccaagga atactgtgct tccttctact gcaccattgc ccagcagtct 240

ctggaaatgc tgctggacgg gaccctggat gggttggacg ggatcatcac tccggtactg 300

tgtgataccc tgcgtcccat gagccagaac ttcaaagtgg ccatgaaaga caagatgccg 360

gttattttcc tggctcatcc ccaggtccgt cagaatgccg ccggcaagca gttcacctat 420

gatgcctaca gcgaagtgaa aggccatctg gaagaaatct gcggccatga aatcaccaat 480

gatgccatcc tggatgccat caaagtgtac aacaagagcc gtgctgcccg ccgcgaattc 540

tgcaaactgg ccaacgaaca tcctgatctg atcccggctt ccgtacgggc caccgtactg 600

cgtgccgctt acttcatgct gaaggatgaa tacaccgaaa agctggaaga actgaacaag 660

gaactggcag ctgctcctgc cggcaagttc gacggccaca aagtggttgt ttccggcatc 720

atctacaaca cgcccggcat cctgaaagcc atggatgaca acaaactggc cattgctgct 780

gatgactgcg cttatgaaag ccgcagcttt gccgtggatg ctccggaaga tctggacaac 840

ggactgcatg ctctggctgt acagttctcc aaacagaaga acgatgttct gctgtacgat 900

cctgaatttg ccaagaatac ccgttctgaa cacgttggca atctggtaaa agaaagcggc 960

gcagaaggac tgatcgtgtt catgatgcag ttctgcgatc cggaagaaat ggaatatcct 1020

gatctgaaga aggctctgga tgcccaccac attcctcatg tgaagattgg tgtggaccag 1080

atgacccggg actttggtca ggcccagacc gctctggaag ctttcgcaga aagcctgtaa 1140

<210> SEQ ID NO 28
<211> LENGTH: 1122
<212> TYPE: DNA
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 28

atgatgaaat taaaggcaat tgaaaagttg atgcaaaaat tcgccagtag aaaagaacag 60

ctatataagc aaaaagaaga aggtagaaaa gtttttggaa tgttctgtgc ctatgttcca 120

atagaaataa ttttagcagc aaatgcaatc ccagttggtt tgtgtggagg taaaaatgac 180

acaatcccaa tagcagagga ggatttgcca agaaacctat gcccattaat aaaatcatcc 240

tatggtttta agaaggcaaa aacctgccct tactttgaag catctgatat agttattgga 300

gaaactacct gtgaaggaaa gaagaagatg tttgagttga tggagagatt ggtgccaatg 360

catataatgc acctcccaca catgaaagat gaagattctt tgaaaatctg gattaaagaa 420

gttgaaaagc taaaagaatt ggttgagaaa gagactggaa ataaaataac agaggaaaag 480

ttaaaagaga cagttgataa agtaaataaa gttagggagt tgttttataa actctatgaa 540

ttgaggaaga ataaaccagc tccaattaag ggtttagatg ttttaaaatt attccagttt 600

gcctatttat tggatattga tgacacaata gggattttag aggatttaat tgaggagtta 660

gaggagagag ttaaaaaagg agaaggttat gaaggaaaga gaattttaat aactggctgt 720

ccaatggttg ctggaaacaa taagattgtt gaaattattg aggaagttgg aggagtagtt 780

gttggtgaag aaagctgcac tggaacaaga ttctttgaaa actttgttga gggctatagc 840

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gtagaggaca ttgcaaaaag atactttaaa atcccatgtg cttgtagatt taaaaacgat 900

gagagagttg aaaatataaa gagattggtt aaagagttgg acgtcgatgg agttgtttat 960

tacactttgc agtattgcca tacatttaac atagagggag ctaaggtaga ggaggcatta 1020

aaagaggagg gcattccaat tataagaatt gaaactgact attctgaaag tgatagagag 1080

cagttaaaaa caaggttgga ggcatttatt gagatgattt aa 1122

<210> SEQ ID NO 29
<211> LENGTH: 1152
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 29

atgtcacttg tcaccgatct acccgccatt ttcgatcagt tctctgaagc tcgccagaca 60

ggctttctca ccgtcatgga tctcaaggag cgcggcattc cgctggttgg cacttactgc 120

acctttatgc cgcaagagat cccgatggca gccggtgcgg ttgtggtttc gctctgttcc 180

acctctgatg aaaccattga agaagcggag aaagatctgc cgcgcaacct ctgcccgctg 240

attaaaagca gctacggctt cggcaaaacc gataaatgcc cctacttcta cttttcggat 300

ctggtggtcg gtgaaaccac ctgcgacggc aaaaagaaaa tgtatgaata catggcggag 360

tttaagcctg ttcatgtgat gcaattgccc aacagcgtta aggacgatgc ctcgcgtgcg 420

ttatggaaag ccgagatgct gcgcttgcaa aaaacggtag aagaacgttt tgggcacgag 480

attagcgaag atgctctgcg cgatgccatt gcgctgaaaa accgcgaacg tcgcgcactg 540

gctaattttt atcatcttgg gcagttaaat cctccggcgc ttagcggcag cgacattctg 600

aaagtggttt acggcgcaac cttccggttc gataaagagg cgttgatcaa tgaactggat 660

gcaatgaccg cccgcgttcg tcagcagtgg gaagaaggcc agcgactgga cccgcgtccg 720

cgcattttaa tcaccggctg cccgattggc ggcgcagcag aaaaagtggt gcgcgcgatt 780

gaagagaatg gcggctgggt tgtcggttat gaaaactgca ccggggcgaa agcgaccgag 840

caatgcgtgg cagaaacggg cgatgtctac gacgcgctgg cggataaata tctggcgatt 900

ggctgctcct gtgtttcgcc gaacgatcag cgcctgaaaa tgctcagcca gatggtggag 960

gaatatcagg tcgatggcgt agttgatgtg attttgcagg cgtgccatac ctacgcggtg 1020

gaatcgctgg cgattaaacg tcatgtgcgc cagcagcaca acattcctta tatcgctatt 1080

gaaacagact actccacctc ggatgtcggg cagctcagta cccgtgtcgc ggcctttatt 1140

gagatgctgt aa 1152

<210> SEQ ID NO 30
<211> LENGTH: 379
<212> TYPE: PRT
<213> ORGANISM: Acidaminococcus fermentans

<400> SEQUENCE: 30

Met Ala Ile Ser Ala Leu Ile Glu Glu Phe Gln Lys Val Ser Ala Ser
1 5 10 15

Pro Lys Thr Met Leu Ala Lys Tyr Lys Ala Gln Gly Lys Lys Ala Ile
20 25 30

Gly Cys Leu Pro Tyr Tyr Val Pro Glu Glu Leu Val Tyr Ala Ala Gly
35 40 45

Met Val Pro Met Gly Val Trp Gly Cys Asn Gly Lys Gln Glu Val Arg
50 55 60

Ser Lys Glu Tyr Cys Ala Ser Phe Tyr Cys Thr Ile Ala Gln Gln Ser

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65                  70                  75                  80

Leu Glu Met Leu Leu Asp Gly Thr Leu Asp Gly Leu Asp Gly Ile Ile
                85                  90                  95

Thr Pro Val Leu Cys Asp Thr Leu Arg Pro Met Ser Gln Asn Phe Lys
            100                 105                 110

Val Ala Met Lys Asp Lys Met Pro Val Ile Phe Leu Ala His Pro Gln
        115                 120                 125

Val Arg Gln Asn Ala Ala Gly Lys Gln Phe Thr Tyr Asp Ala Tyr Ser
    130                 135                 140

Glu Val Lys Gly His Leu Glu Glu Ile Cys Gly His Glu Ile Thr Asn
145                 150                 155                 160

Asp Ala Ile Leu Asp Ala Ile Lys Val Tyr Asn Lys Ser Arg Ala Ala
                165                 170                 175

Arg Arg Glu Phe Cys Lys Leu Ala Asn Glu His Pro Asp Leu Ile Pro
            180                 185                 190

Ala Ser Val Arg Ala Thr Val Leu Arg Ala Ala Tyr Phe Met Leu Lys
        195                 200                 205

Asp Glu Tyr Thr Glu Lys Leu Glu Glu Leu Asn Lys Glu Leu Ala Ala
    210                 215                 220

Ala Pro Ala Gly Lys Phe Asp Gly His Lys Val Val Val Ser Gly Ile
225                 230                 235                 240

Ile Tyr Asn Thr Pro Gly Ile Leu Lys Ala Met Asp Asp Asn Lys Leu
                245                 250                 255

Ala Ile Ala Ala Asp Asp Cys Ala Tyr Glu Ser Arg Ser Phe Ala Val
            260                 265                 270

Asp Ala Pro Glu Asp Leu Asp Asn Gly Leu His Ala Leu Ala Val Gln
        275                 280                 285

Phe Ser Lys Gln Lys Asn Asp Val Leu Leu Tyr Asp Pro Glu Phe Ala
    290                 295                 300

Lys Asn Thr Arg Ser Glu His Val Gly Asn Leu Val Lys Glu Ser Gly
305                 310                 315                 320

Ala Glu Gly Leu Ile Val Phe Met Met Gln Phe Cys Asp Pro Glu Glu
                325                 330                 335

Met Glu Tyr Pro Asp Leu Lys Lys Ala Leu Asp Ala His His Ile Pro
            340                 345                 350

His Val Lys Ile Gly Val Asp Gln Met Thr Arg Asp Phe Gly Gln Ala
        355                 360                 365

Gln Thr Ala Leu Glu Ala Phe Ala Glu Ser Leu
    370                 375


<210> SEQ ID NO 31
<211> LENGTH: 373
<212> TYPE: PRT
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 31

Met Met Lys Leu Lys Ala Ile Glu Lys Leu Met Gln Lys Phe Ala Ser
1               5                   10                  15

Arg Lys Glu Gln Leu Tyr Lys Gln Lys Glu Glu Gly Arg Lys Val Phe
            20                  25                  30

Gly Met Phe Cys Ala Tyr Val Pro Ile Glu Ile Ile Leu Ala Ala Asn
        35                  40                  45

Ala Ile Pro Val Gly Leu Cys Gly Gly Lys Asn Asp Thr Ile Pro Ile
    50                  55                  60
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Ala Glu Glu Asp Leu Pro Arg Asn Leu Cys Pro Leu Ile Lys Ser Ser
65                  70                  75                  80

Tyr Gly Phe Lys Lys Ala Lys Thr Cys Pro Tyr Phe Glu Ala Ser Asp
                85                  90                  95

Ile Val Ile Gly Glu Thr Thr Cys Glu Gly Lys Lys Lys Met Phe Glu
            100                 105                 110

Leu Met Glu Arg Leu Val Pro Met His Ile Met His Leu Pro His Met
        115                 120                 125

Lys Asp Glu Asp Ser Leu Lys Ile Trp Ile Lys Glu Val Glu Lys Leu
    130                 135                 140

Lys Glu Leu Val Glu Lys Glu Thr Gly Asn Lys Ile Thr Glu Glu Lys
145                 150                 155                 160

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

Lys Leu Tyr Glu Leu Arg Lys Asn Lys Pro Ala Pro Ile Lys Gly Leu
            180                 185                 190

Asp Val Leu Lys Leu Phe Gln Phe Ala Tyr Leu Leu Asp Ile Asp Asp
        195                 200                 205

Thr Ile Gly Ile Leu Glu Asp Leu Ile Glu Glu Leu Glu Glu Arg Val
    210                 215                 220

Lys Lys Gly Glu Gly Tyr Glu Gly Lys Arg Ile Leu Ile Thr Gly Cys
225                 230                 235                 240

Pro Met Val Ala Gly Asn Asn Lys Ile Val Glu Ile Ile Glu Glu Val
                245                 250                 255

Gly Gly Val Val Val Gly Glu Glu Ser Cys Thr Gly Thr Arg Phe Phe
            260                 265                 270

Glu Asn Phe Val Glu Gly Tyr Ser Val Glu Asp Ile Ala Lys Arg Tyr
        275                 280                 285

Phe Lys Ile Pro Cys Ala Cys Arg Phe Lys Asn Asp Glu Arg Val Glu
    290                 295                 300

Asn Ile Lys Arg Leu Val Lys Glu Leu Asp Val Asp Gly Val Val Tyr
305                 310                 315                 320

Tyr Thr Leu Gln Tyr Cys His Thr Phe Asn Ile Glu Gly Ala Lys Val
                325                 330                 335

Glu Glu Ala Leu Lys Glu Glu Gly Ile Pro Ile Ile Arg Ile Glu Thr
            340                 345                 350

Asp Tyr Ser Glu Ser Asp Arg Glu Gln Leu Lys Thr Arg Leu Glu Ala
        355                 360                 365

Phe Ile Glu Met Ile
    370


<210> SEQ ID NO 32
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Methanocaldococcus jannaschii

<400> SEQUENCE: 32

Met Ser Leu Val Thr Asp Leu Pro Ala Ile Phe Asp Gln Phe Ser Glu
1               5                   10                  15

Ala Arg Gln Thr Gly Phe Leu Thr Val Met Asp Leu Lys Glu Arg Gly
            20                  25                  30

Ile Pro Leu Val Gly Thr Tyr Cys Thr Phe Met Pro Gln Glu Ile Pro
        35                  40                  45

Met Ala Ala Gly Ala Val Val Val Ser Leu Cys Ser Thr Ser Asp Glu
    50                  55                  60
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Thr Ile Glu Glu Ala Glu Lys Asp Leu Pro Arg Asn Leu Cys Pro Leu
65                  70                  75                  80

Ile Lys Ser Ser Tyr Gly Phe Gly Lys Thr Asp Lys Cys Pro Tyr Phe
                85                  90                  95

Tyr Phe Ser Asp Leu Val Val Gly Glu Thr Thr Cys Asp Gly Lys Lys
            100                 105                 110

Lys Met Tyr Glu Tyr Met Ala Glu Phe Lys Pro Val His Val Met Gln
        115                 120                 125

Leu Pro Asn Ser Val Lys Asp Asp Ala Ser Arg Ala Leu Trp Lys Ala
    130                 135                 140

Glu Met Leu Arg Leu Gln Lys Thr Val Glu Glu Arg Phe Gly His Glu
145                 150                 155                 160

Ile Ser Glu Asp Ala Leu Arg Asp Ala Ile Ala Leu Lys Asn Arg Glu
                165                 170                 175

Arg Arg Ala Leu Ala Asn Phe Tyr His Leu Gly Gln Leu Asn Pro Pro
            180                 185                 190

Ala Leu Ser Gly Ser Asp Ile Leu Lys Val Val Tyr Gly Ala Thr Phe
        195                 200                 205

Arg Phe Asp Lys Glu Ala Leu Ile Asn Glu Leu Asp Ala Met Thr Ala
    210                 215                 220

Arg Val Arg Gln Gln Trp Glu Glu Gly Gln Arg Leu Asp Pro Arg Pro
225                 230                 235                 240

Arg Ile Leu Ile Thr Gly Cys Pro Ile Gly Gly Ala Ala Glu Lys Val
                245                 250                 255

Val Arg Ala Ile Glu Glu Asn Gly Gly Trp Val Val Gly Tyr Glu Asn
            260                 265                 270

Cys Thr Gly Ala Lys Ala Thr Glu Gln Cys Val Ala Glu Thr Gly Asp
        275                 280                 285

Val Tyr Asp Ala Leu Ala Asp Lys Tyr Leu Ala Ile Gly Cys Ser Cys
    290                 295                 300

Val Ser Pro Asn Asp Gln Arg Leu Lys Met Leu Ser Gln Met Val Glu
305                 310                 315                 320

Glu Tyr Gln Val Asp Gly Val Val Asp Val Ile Leu Gln Ala Cys His
                325                 330                 335

Thr Tyr Ala Val Glu Ser Leu Ala Ile Lys Arg His Val Arg Gln Gln
            340                 345                 350

His Asn Ile Pro Tyr Ile Ala Ile Glu Thr Asp Tyr Ser Thr Ser Asp
        355                 360                 365

Val Gly Gln Leu Ser Thr Arg Val Ala Ala Phe Ile Glu Met Leu
    370                 375                 380


<210> SEQ ID NO 33
<211> LENGTH: 6295
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 33

cgacggcccg ggctggtatc attctagtca gtaattcacc tttggaaaat tttcacaaag     60

gcagtacgac agaagcgtcg atacattcca tttagcagga ggaagttacg gtaatgagaa    120

aagtagaaat cattacagct gaacaagcag ctcagctcgt aaaagacaac gacacgatta    180

cgtctatcgg ctttgtcagc agcgcccatc cggaagcact gaccaaagct ttggaaaaac    240

ggttcctgga cacgaacacc ccgcagaact tgacctacat ctatgcaggc tctcagggca    300
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aacgcgatgg ccgtgccgct gaacatctgg cacacacagg ccttttgaaa cgcgccatca    360

tcggtcactg gcagactgta ccggctatcg gtaaactggc tgtcgaaaac aagattgaag    420

cttacaactt ctcgcagggc acgttggtcc actggttccg cgccttggca ggtcataagc    480

tcggcgtctt caccgacatc ggtctggaaa ctttcctcga tccccgtcag ctcggcggca    540

agctcaatga cgtaaccaaa gaagacctcg tcaaactgat cgaagtcgat ggtcatgaac    600

agcttttcta cccgaccttc ccggtcaacg tagctttcct ccgcggtacg tatgctgatg    660

aatccggcaa tatcaccatg gacgaagaaa tcgggccttt cgaaagcact tccgtagccc    720

aggccgttca caactgtggc ggtaaagtcg tcgtccaggt caaagacgtc gtcgctcacg    780

gcagcctcga cccgcgcatg gtcaagatcc ctggcatcta tgtcgactac gtcgtcgtag    840

cagctccgga agaccatcag cagacgtatg actgcgaata cgatccgtcc ctcagcggtg    900

aacatcgtgc tcctgaaggc gctaccgatg cagctctccc catgagcgct aagaaaatca    960

tcggccgccg cggcgctttg gaattgactg aaaacgctgt cgtcaacctc ggcgtcggtg   1020

ctccggaata cgttgcttct gttgccggtg aagaaggtat cgccgatacc attaccctga   1080

ccgtcgaagg tggcgccatc ggtggcgtac cgcagggcgg tgcccgcttc ggttcgtccc   1140

gcaatgccga tgccatcatc gaccacacct atcagttcga cttctacgat ggcggcggtc   1200

tggacatcgc ttacctcggc ctggcccagt gcgatggctc gggcaacatc aacgtcagca   1260

agttcggtac taacgttgcc ggctgcggcg gtttccccaa catttcccag cagacaccga   1320

atgtttactt ctgcggcacc ttcacggctg gcggcttgaa aatcgctgtc gaagacggca   1380

aagtcaagat cctccaggaa ggcaaagcca agaagttcat caaagctgtc gaccagatca   1440

ctttcaacgg ttcctatgca gcccgcaacg gcaaacacgt tctctacatc acagaacgct   1500

gcgtatttga actgaccaaa gaaggcttga aactcatcga agtcgcaccg ggcatcgata   1560

ttgaaaaaga tatcctcgct cacatggact tcaagccgat cattgataat ccgaaactca   1620

tggatgcccg cctcttccag gacggtccca tgggactgaa aaaataaatc tctgctgtaa   1680

aggagacttt actatgaaac caatgagact acatcacgta ggcattgtcc tgccgacctt   1740

agaaaaagcc catgaattca tgcagaataa tggacttgaa atcgactatg ccggctatgt   1800

cgatgcttac caggctgatc tcattttcac taagtttggt gaatttgcca gcccgattga   1860

aatgattatc ccgcactccg gtgtgcttac ccaattcaat ggtggccgcg gcggcattgc   1920

ccacatcgcc ttcgaagtgg acgatgtcga agctgtccgc caggaaatgg aagcagattg   1980

tccgggatgc atgttagaaa agaaagctgt ccagggtacg gacgacatta tcgtcaactt   2040

ccgccgcccg acaaccaacc agggtatcct cgttgaatat gttcagacga cagcacctat   2100

caccggccgc ggcgaaaatc ctttcgttaa gaatctcggc ccggaaaaag ggaagctcaa   2160

cgaaacatgg catcccatgc gcctgcacca tatcggcatc gtcttgccga ccttggaaaa   2220

ggcccatgaa ttcatcaaga ccaatggtct ggaagtggat tattccggtt tcgtcgacgc   2280

ctaccatgcg gatctcattt tcactaaaaa aggtgaaaac agtacgccta tcgaattcat   2340

tattccccgt gaaggggtcc tcaaagattt caatcatggc aggggaggta tcgctcatat   2400

cgcctttgaa gtggatgatg tcgaaaaggt acgtcagatt atggaaagcc agaagcctgg   2460

ttgcatgctc gaaaagaaag ccgtccgggg aacggacgat atcatcgtca acttccgccg   2520

tcccagcacg gacgccggca tcctcgtcga atatgtccag accgtagctc ccatcaatcg   2580

cagcaatccc aacccttta atgattgatt ttttataaag aaaggtgaaa actgtgtata   2640

ctctcggaat cgacgttggt tcttcttctt ccaaggcagt catcctggaa gatggcaaga   2700
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agatcgtcgc ccatgccgtc gttgaaatcg gcaccggttc gaccggtccg gaacgcgtcc 2760

tggacgaagt cttcaaagat accaacttaa aaattgaaga catggcgaac atcatcgcca 2820

caggctatgg ccgtttcaat gtcgactgcg ccaaaggcga agtcagcgaa atcacgtgcc 2880

atgccaaagg ggccctcttt gaatgccccg gtacgacgac catcctcgat atcggcggtc 2940

aggacgtcaa gtccatcaaa ttgaatggcc agggcctggt catgcagttt gccatgaacg 3000

acaaatgcgc cgctggtacg ggccgtttcc tcgacgtcat gtcgaaggta ctggaaatcc 3060

ccatgtctga aatgggggac tggtacttca aatcgaagca tcccgctgcc gtcagcagta 3120

cctgcacggt ttttgctgaa tcggaagtca tttcccttct ttccaagaat gtcccgaaag 3180

aagatatcgt agccggtgtc catcagtcca tcgccgccaa agcctgcgct ctcgtgcgcc 3240

gcgtcggtgt cggtgaagac ctgaccatga ccggcggtgg ctcccgcgat cccggcgtcg 3300

tcgatgccgt atcgaaagaa ttaggtattc ctgtcagagt cgctctgcat ccccaagcgg 3360

tgggtgctct cggagctgct ttgattgctt atgataaaat caagaaataa gtcaaaggag 3420

agaacaaaat catgagtgaa gaaaaaacag tagatattga aagcatgagc tccaaggaag 3480

cccttggtta cttcttgccg aaagtcgatg aagacgcacg taaagcgaaa aaagaaggcc 3540

gcctcgtttg ctggtccgct tctgtcgctc ctccggaatt ctgcacggct atggacatcg 3600

ccatcgtcta tccggaaact cacgcagctg gtatcggtgc ccgtcacggt gctccggcca 3660

tgctcgaagt tgctgaaaac aaaggttaca accaggacat ctgttcctac tgccgcgtca 3720

acatgggcta catggaactc ctcaaacagc aggctctgac aggcgaaacg ccggaagtcc 3780

tcaaaaactc cccggcttct ccgattcccc ttccggatgt tgtcctcact tgcaacaaca 3840

tctgcaatac cttgctcaaa tggtatgaaa acttggctaa agaattgaac gtacctctca 3900

tcaacatcga cgtaccgttc aaccatgaat tccctgttac gaaacacgct aaacagtaca 3960

tcgtcggcga attcaaacat gctatcaaac agctcgaaga cctttgcggc cgtcccttcg 4020

actatgacaa attcttcgaa gtacagaaac agacacagcg ctccatcgct gcctggaaca 4080

aaatcgctac gtacttccag tacaaaccgt cgccgctcaa cggcttcgac ctcttcaact 4140

acatgggcct cgccgttgct gcccgctcct tgaactactc ggaaatcacg ttcaacaaat 4200

tcctcaaaga attggacgaa aaagtagcta ataagaaatg ggctttcggt gaaaacgaaa 4260

aatcccgtgt tacttgggaa ggtatcgctg tctggatcgc tctcggccac accttcaaag 4320

aactcaaagg tcagggcgct ctcatgactg gttccgctta tcctggcatg tgggacgttt 4380

cctacgaacc gggcgacctc gaatccatgg cagaagctta ttcccgtaca tacatcaact 4440

gctgcctcga acagcgcggt gctgttcttg aaaaagttgt ccgcgatggc aaatgcgacg 4500

gcttgatcat gcaccagaac cgttcctgca agaacatgag cctcctcaac aacgaaggcg 4560

gccagcgcat ccagaagaac ctcggcgtac cgtacgtcat cttcgacggc gaccagaccg 4620

atgctcgtaa cttctcggaa gcacagttcg atacccgcgt agaagctttg gcagaaatga 4680

tggcagacaa aaaagccaat gaaggaggaa accactaatg agtcagatcg acgaacttat 4740

cagcaaatta caggaagtat ccaaccatcc ccagaagacg gttttgaatt ataaaaaaca 4800

gggtaaaggc ctcgtaggca tgatgcccta ctacgctccg gaagaaatcg tatatgctgc 4860

aggctacctc ccggtaggca tgttcggttc ccagaacccg cagatctccg cagctcgtac 4920

gtaccttcct ccgttcgctt gctccttgat gcaggctgac atggaactcc agctcaacgg 4980

cacctatgac tgcctcgacg ctgttatctt ctccgttcct tgcgacactc tccgctgcat 5040

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gagccagaaa tggcacggca aagctccggt catcgtcttc acacagccgc agaaccgtaa 5100

gatccgcccg gctgtcgatt tcctcaaagc tgaatacgaa catgtccgta cggaattggg 5160

acgtatcctc aacgtaaaaa tctccgacct ggctatccag gaagctatca aagtatataa 5220

cgaaaaccgt caggttatgc gtgaattctg cgacgtagct gctcagtacc cgcagatctt 5280

cactccgata aaacgtcatg acgtcatcaa agcccgctgg ttcatggaca aagctgaaca 5340

caccgctttg gtccgcgaac tcatcgacgc tgtcaagaaa gaaccggtac agccgtggaa 5400

tggcaaaaaa gtcatcctct ccggtatcat ggcagaaccg gatgaattcc tcgatatctt 5460

cagcgaattc aacatcgctg tcgtcgctga cgacctcgct caggaatccc gccagttccg 5520

tacagacgta ccgtccggca tcgatcccct cgaacagctc gctcagcagt ggcaggactt 5580

cgatggctgc ccgctcgctt tgaacgaaga caaaccgcgt ggccagatgc tcatcgacat 5640

gactaagaaa tacaatgctg acgccgtcgt catctgcatg atgcgtttct gcgatcctga 5700

agaattcgac tatccgattt acaaaccgga atttgaagct gctggcgttc gttacacggt 5760

cctcgacctc gacatcgaat ctccgtccct cgaacagctc cgcacccgta tccaggcttt 5820

ctcggaaatc ctctaagaat cgcctgaatc atcaaacatc tgggcgggac tccgaaaggt 5880

gcctgctaca tgatacattg cctgttttca ggcagacaga tttgcagctt gcggccccca 5940

ttgtacgggc tgcaagctgt caatgatgct ttaaagacgg ctctgccgtt tttaaataaa 6000

aacataaaac catatataat ctattaggag gaaactcaat catggaattc aaactttctg 6060

aattacagca agatatcgca aatctcgcaa aagatttcgc agaaaaaaaa ttagctccca 6120

ctgtcaaaga gcgtgacgaa aaagaagttt tcgatcgtgc tatccttgac gaagtgggta 6180

ctctcggcct tctcggtatt ccctgggaag aagaaaacgg cggcgtaggc gctgacttcc 6240

tcagcctcgc agttgcttgc gaagaagtag ctaaagttac cagcccgggc cgtcg 6295

<210> SEQ ID NO 34
<211> LENGTH: 915
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 34

atgaaaccaa tgagactaca tcacgtaggc attgtcctgc cgaccttaga aaaagcccat 60

gaattcatgc agaataatgg acttgaaatc gactatgccg gctatgtcga tgcttaccag 120

gctgatctca ttttcactaa gtttggtgaa tttgccagcc cgattgaaat gattatcccg 180

cactccggtg tgcttaccca attcaatggt ggccgcggcg gcattgccca catcgccttc 240

gaagtggacg atgtcgaagc tgtccgccag gaaatggaag cagattgtcc gggatgcatg 300

ttagaaaaga aagctgtcca gggtacggac gacattatcg tcaacttccg ccgcccgaca 360

accaaccagg gtatcctcgt tgaatatgtt cagacgacag cacctatcac cggccgcggc 420

gaaaatcctt tcgttaagaa tctcggcccg gaaaaaggga agctcaacga aacatggcat 480

cccatgcgcc tgcaccatat cggcatcgtc ttgccgacct tggaaaaggc ccatgaattc 540

atcaagacca atggtctgga agtggattat tccggtttcg tcgacgccta ccatgcggat 600

ctcattttca ctaaaaaagg tgaaaacagt acgcctatcg aattcattat tccccgtgaa 660

ggggtcctca aagatttcaa tcatggcagg ggaggtatcg ctcatatcgc ctttgaagtg 720

gatgatgtcg aaaaggtacg tcagattatg gaaagccaga agcctggttg catgctcgaa 780

aagaaagccg tccggggaac ggacgatatc atcgtcaact tccgccgtcc cagcacggac 840

gccggcatcc tcgtcgaata tgtccagacc gtagctccca tcaatcgcag caatcccaac 900

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cctttaatg attga 915

<210> SEQ ID NO 35
<211> LENGTH: 304
<212> TYPE: PRT
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 35

Met Lys Pro Met Arg Leu His His Val Gly Ile Val Leu Pro Thr Leu
1 5 10 15

Glu Lys Ala His Glu Phe Met Gln Asn Asn Gly Leu Glu Ile Asp Tyr
20 25 30

Ala Gly Tyr Val Asp Ala Tyr Gln Ala Asp Leu Ile Phe Thr Lys Phe
35 40 45

Gly Glu Phe Ala Ser Pro Ile Glu Met Ile Ile Pro His Ser Gly Val
50 55 60

Leu Thr Gln Phe Asn Gly Gly Arg Gly Gly Ile Ala His Ile Ala Phe
65 70 75 80

Glu Val Asp Asp Val Glu Ala Val Arg Gln Glu Met Glu Ala Asp Cys
85 90 95

Pro Gly Cys Met Leu Glu Lys Lys Ala Val Gln Gly Thr Asp Asp Ile
100 105 110

Ile Val Asn Phe Arg Arg Pro Thr Thr Asn Gln Gly Ile Leu Val Glu
115 120 125

Tyr Val Gln Thr Thr Ala Pro Ile Thr Gly Arg Gly Glu Asn Pro Phe
130 135 140

Val Lys Asn Leu Gly Pro Glu Lys Gly Lys Leu Asn Glu Thr Trp His
145 150 155 160

Pro Met Arg Leu His His Ile Gly Ile Val Leu Pro Thr Leu Glu Lys
165 170 175

Ala His Glu Phe Ile Lys Thr Asn Gly Leu Glu Val Asp Tyr Ser Gly
180 185 190

Phe Val Asp Ala Tyr His Ala Asp Leu Ile Phe Thr Lys Lys Gly Glu
195 200 205

Asn Ser Thr Pro Ile Glu Phe Ile Ile Pro Arg Glu Gly Val Leu Lys
210 215 220

Asp Phe Asn His Gly Arg Gly Gly Ile Ala His Ile Ala Phe Glu Val
225 230 235 240

Asp Asp Val Glu Lys Val Arg Gln Ile Met Glu Ser Gln Lys Pro Gly
245 250 255

Cys Met Leu Glu Lys Lys Ala Val Arg Gly Thr Asp Asp Ile Ile Val
260 265 270

Asn Phe Arg Arg Pro Ser Thr Asp Ala Gly Ile Leu Val Glu Tyr Val
275 280 285

Gln Thr Val Ala Pro Ile Asn Arg Ser Asn Pro Asn Pro Phe Asn Asp
290 295 300

<210> SEQ ID NO 36
<211> LENGTH: 254
<212> TYPE: DNA
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 36

atggaattca aactttctga ttacagcaa gatatcgcaa atctcgcaaa agatttcgca 60

gaaaaaaaat tagctcccac tgtcaaagag cgtgacgaaa aagaagtttt cgatcgtgct 120

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atccttgacg aagtgggtac tctcggcctt ctcggtattc cctgggaaga agaaaacggc    180

ggcgtaggcg ctgacttcct cagcctcgca gttgcttgcg aagaagtagc taaagttacc    240

agcccgggcc gtcg                                                      254


<210> SEQ ID NO 37
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Megasphaera elsdenii

<400> SEQUENCE: 37

Met Glu Phe Lys Leu Ser Glu Leu Gln Gln Asp Ile Ala Asn Leu Ala
1               5                   10                  15

Lys Asp Phe Ala Glu Lys Lys Leu Ala Pro Thr Val Lys Glu Arg Asp
            20                  25                  30

Glu Lys Glu Val Phe Asp Arg Ala Ile Leu Asp Glu Val Gly Thr Leu
        35                  40                  45

Gly Leu Leu Gly Ile Pro Trp Glu Glu Glu Asn Gly Gly Val Gly Ala
    50                  55                  60

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

Ser Pro Gly Arg


<210> SEQ ID NO 38
<211> LENGTH: 6141
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (480)..(5945)

<400> SEQUENCE: 38

gtgagcacac acttgatagc tgatgccgtc aatgatcagt tgttcgtcta tagcaggctg     60

aaaggacatg ggtttggtca cagtctgagc agttgcaggc agtcaaacac gttcgtaact    120

acgctgtaga tgatataagc agtataccat cttgctacgc tctcgttgat caggttgaat    180

gctttgagga aggtcaggcg aatagccatg cctcttgttt ccagaacatg gcatggggat    240

ggatcgacgg taccctgtcg gatgcatgct atgcgtggca ttcatatcat caaccagaat    300

ttgatcttga actacacagc aattctgcgc gttatgcaag tgtcttcggt cagatggtga    360

acaattctca attgttgagg tcttgacgaa ttgcgttata cactgtaggc tatagtatgc    420

acccctgtt atctatatca caaccggtct attagcattt gcgtcaagga ggatggtcg      479

atg atc gac act gcg ccc ctt gcc cca cca cgg gcg ccc cgc tct aat      527
Met Ile Asp Thr Ala Pro Leu Ala Pro Pro Arg Ala Pro Arg Ser Asn
1               5                   10                  15

ccg att cgg gat cga gtt gat tgg gaa gct cag cgc gct gct gcg ctg      575
Pro Ile Arg Asp Arg Val Asp Trp Glu Ala Gln Arg Ala Ala Ala Leu
            20                  25                  30

gca gat ccc ggt gcc ttt cat ggc gcg att gcc cgg aca gtt atc cac      623
Ala Asp Pro Gly Ala Phe His Gly Ala Ile Ala Arg Thr Val Ile His
        35                  40                  45

tgg tac gac cca caa cac cat tgc tgg att cgc ttc aac gag tct agt      671
Trp Tyr Asp Pro Gln His His Cys Trp Ile Arg Phe Asn Glu Ser Ser
    50                  55                  60

cag cgt tgg gaa ggg ctg gat gcc gct acc ggt gcc cct gta acg gta      719
Gln Arg Trp Glu Gly Leu Asp Ala Ala Thr Gly Ala Pro Val Thr Val
65                  70                  75                  80
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gac tat ccc gcc gat tat cag ccc tgg caa cag gcg ttt gat gat agt      767
Asp Tyr Pro Ala Asp Tyr Gln Pro Trp Gln Gln Ala Phe Asp Asp Ser
                85                  90                  95

gaa gcg ccg ttt tac cgc tgg ttt agt ggt ggg ttg aca aat gcc tgc      815
Glu Ala Pro Phe Tyr Arg Trp Phe Ser Gly Gly Leu Thr Asn Ala Cys
            100                 105                 110

ttt aat gaa gta gac cgg cat gtc atg atg ggc tat ggc gac gag gtg      863
Phe Asn Glu Val Asp Arg His Val Met Met Gly Tyr Gly Asp Glu Val
        115                 120                 125

gcc tac tac ttt gaa ggt gac cgc tgg gat aac tcg ctc aac aat ggt      911
Ala Tyr Tyr Phe Glu Gly Asp Arg Trp Asp Asn Ser Leu Asn Asn Gly
    130                 135                 140

cgt ggt ggt ccg gtt gtc cag gag aca atc acg cgg cgg cgc ctg ttg      959
Arg Gly Gly Pro Val Val Gln Glu Thr Ile Thr Arg Arg Arg Leu Leu
145                 150                 155                 160

gtg gag gtg gtg aag gct gcg cag gtg ttg cgt gat ctg ggc ctg aag     1007
Val Glu Val Val Lys Ala Ala Gln Val Leu Arg Asp Leu Gly Leu Lys
                165                 170                 175

aag ggt gat cgg att gct ctg aat atg ccg aat att atg ccg cag att     1055
Lys Gly Asp Arg Ile Ala Leu Asn Met Pro Asn Ile Met Pro Gln Ile
            180                 185                 190

tat tat acg gaa gcg gca aaa cga ctg ggt att ctg tac acg ccg gtc     1103
Tyr Tyr Thr Glu Ala Ala Lys Arg Leu Gly Ile Leu Tyr Thr Pro Val
        195                 200                 205

ttc ggt ggc ttc tcg gac aag act ctt tcc gac cgt att cac aat gcc     1151
Phe Gly Gly Phe Ser Asp Lys Thr Leu Ser Asp Arg Ile His Asn Ala
    210                 215                 220

ggt gca cga gtg gtg att acc tct gat ggt gcg tac cgc aac gcg cag     1199
Gly Ala Arg Val Val Ile Thr Ser Asp Gly Ala Tyr Arg Asn Ala Gln
225                 230                 235                 240

gtg gtg ccc tac aaa gaa gcg tat acc gat cag gcg ctc gat aag tat     1247
Val Val Pro Tyr Lys Glu Ala Tyr Thr Asp Gln Ala Leu Asp Lys Tyr
                245                 250                 255

att ccg gtt gag acg gcg cag gcg att gtt gcg cag acc ctg gcc acc     1295
Ile Pro Val Glu Thr Ala Gln Ala Ile Val Ala Gln Thr Leu Ala Thr
            260                 265                 270

ttg ccc ctg act gag tcg cag cgc cag acg atc atc acc gaa gtg gag     1343
Leu Pro Leu Thr Glu Ser Gln Arg Gln Thr Ile Ile Thr Glu Val Glu
        275                 280                 285

gcc gca ctg gcc ggt gag att acg gtt gag cgc tcg gac gtg atg cgt     1391
Ala Ala Leu Ala Gly Glu Ile Thr Val Glu Arg Ser Asp Val Met Arg
    290                 295                 300

ggg gtt ggt tct gcc ctc gca aag ctc cgc gat ctt gat gca agc gtg     1439
Gly Val Gly Ser Ala Leu Ala Lys Leu Arg Asp Leu Asp Ala Ser Val
305                 310                 315                 320

cag gca aag gtg cgt aca gta ctg gcg cag gcg ctg gtc gag tcg ccg     1487
Gln Ala Lys Val Arg Thr Val Leu Ala Gln Ala Leu Val Glu Ser Pro
                325                 330                 335

ccg cgg gtt gaa gct gtg gtg gtt gtg cgt cat acc ggt cag gag att     1535
Pro Arg Val Glu Ala Val Val Val Val Arg His Thr Gly Gln Glu Ile
            340                 345                 350

ttg tgg aac gag ggg cga gat cgc tgg agt cac gac ttg ctg gat gct     1583
Leu Trp Asn Glu Gly Arg Asp Arg Trp Ser His Asp Leu Leu Asp Ala
        355                 360                 365

gcg ctg gcg aag att ctg gcc aat gcg cgt gct gcc ggc ttt gat gtg     1631
Ala Leu Ala Lys Ile Leu Ala Asn Ala Arg Ala Ala Gly Phe Asp Val
    370                 375                 380

cac agt gag aat gat ctg ctc aat ctc ccc gat gac cag ctt atc cgt     1679
His Ser Glu Asn Asp Leu Leu Asn Leu Pro Asp Asp Gln Leu Ile Arg
385                 390                 395                 400
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gcg ctc tac gcc agt att ccc tgt gaa ccg gtt gat gct gaa tat ccg     1727
Ala Leu Tyr Ala Ser Ile Pro Cys Glu Pro Val Asp Ala Glu Tyr Pro
                405                 410                 415

atg ttt atc att tac aca tcg ggt agc acc ggt aag ccc aag ggt gtg     1775
Met Phe Ile Ile Tyr Thr Ser Gly Ser Thr Gly Lys Pro Lys Gly Val
            420                 425                 430

atc cac gtt cac ggc ggt tat gtc gcc ggt gtg gtg cac acc ttg cgg     1823
Ile His Val His Gly Gly Tyr Val Ala Gly Val Val His Thr Leu Arg
        435                 440                 445

gtc agt ttt gac gcc gag ccg ggt gat acg ata tat gtg atc gcc gat     1871
Val Ser Phe Asp Ala Glu Pro Gly Asp Thr Ile Tyr Val Ile Ala Asp
    450                 455                 460

ccg ggc tgg atc acc ggt cag agc tat atg ctc aca gcc aca atg gcc     1919
Pro Gly Trp Ile Thr Gly Gln Ser Tyr Met Leu Thr Ala Thr Met Ala
465                 470                 475                 480

ggt cgg ctg acc ggg gtg att gcc gag gga tca ccg ctc ttc ccc tca     1967
Gly Arg Leu Thr Gly Val Ile Ala Glu Gly Ser Pro Leu Phe Pro Ser
                485                 490                 495

gcc ggg cgt tat gcc agc atc atc gag cgc tat ggg gtg cag atc ttt     2015
Ala Gly Arg Tyr Ala Ser Ile Ile Glu Arg Tyr Gly Val Gln Ile Phe
            500                 505                 510

aag gcg ggt gtg acc ttc ctc aag aca gtg atg tcc aat ccg cag aat     2063
Lys Ala Gly Val Thr Phe Leu Lys Thr Val Met Ser Asn Pro Gln Asn
        515                 520                 525

gtt gaa gat gtg cga ctc tat gat atg cac tcg ctg cgg gtt gca acc     2111
Val Glu Asp Val Arg Leu Tyr Asp Met His Ser Leu Arg Val Ala Thr
    530                 535                 540

ttc tgc gcc gag ccg gtc agt ccg gcg gtg cag cag ttt ggt atg cag     2159
Phe Cys Ala Glu Pro Val Ser Pro Ala Val Gln Gln Phe Gly Met Gln
545                 550                 555                 560

atc atg acc ccg cag tat atc aat tcg tac tgg gcg acc gag cac ggt     2207
Ile Met Thr Pro Gln Tyr Ile Asn Ser Tyr Trp Ala Thr Glu His Gly
                565                 570                 575

gga att gtc tgg acg cat ttc tac ggt aat cag gac ttc ccg ctt cgt     2255
Gly Ile Val Trp Thr His Phe Tyr Gly Asn Gln Asp Phe Pro Leu Arg
            580                 585                 590

ccc gat gcc cat acc tat ccc ttg ccc tgg gtg atg ggt gat gtc tgg     2303
Pro Asp Ala His Thr Tyr Pro Leu Pro Trp Val Met Gly Asp Val Trp
        595                 600                 605

gtg gcc gaa act gat gag agc ggg acg acg cgc tat cgg gtc gct gat     2351
Val Ala Glu Thr Asp Glu Ser Gly Thr Thr Arg Tyr Arg Val Ala Asp
    610                 615                 620

ttc gat gag aag ggc gag att gtg att acc gcc ccg tat ccc tac ctg     2399
Phe Asp Glu Lys Gly Glu Ile Val Ile Thr Ala Pro Tyr Pro Tyr Leu
625                 630                 635                 640

acc cgc aca ctc tgg ggt gat gtg ccc ggt ttc gag gcg tac ctg cgc     2447
Thr Arg Thr Leu Trp Gly Asp Val Pro Gly Phe Glu Ala Tyr Leu Arg
                645                 650                 655

ggt gag att ccg ctg cgg gcc tgg aag ggt gat gcc gag cgt ttc gtc     2495
Gly Glu Ile Pro Leu Arg Ala Trp Lys Gly Asp Ala Glu Arg Phe Val
            660                 665                 670

aag acc tac tgg cga cgt ggg cca aac ggt gaa tgg ggc tat atc cag     2543
Lys Thr Tyr Trp Arg Arg Gly Pro Asn Gly Glu Trp Gly Tyr Ile Gln
        675                 680                 685

ggt gat ttt gcc atc aag tac ccc gat ggt agc ttc acg ctc cac gga     2591
Gly Asp Phe Ala Ile Lys Tyr Pro Asp Gly Ser Phe Thr Leu His Gly
    690                 695                 700

cgc cct gac gat gtg atc aat gtg tcg ggc cac cgt atg ggc acc gag     2639
Arg Pro Asp Asp Val Ile Asn Val Ser Gly His Arg Met Gly Thr Glu
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705                 710                 715                 720

gag att gag ggt gcc att ttg cgt gac cgc cag atc acg ccc gac tcg     2687
Glu Ile Glu Gly Ala Ile Leu Arg Asp Arg Gln Ile Thr Pro Asp Ser
                725                 730                 735

ccc gtc ggt aat tgt att gtg gtc ggt gcg ccg cac cgt gag aag ggt     2735
Pro Val Gly Asn Cys Ile Val Val Gly Ala Pro His Arg Glu Lys Gly
            740                 745                 750

ctg acc ccg gtt gcc ttc att caa cct gcg cct ggc cgt cat ctg acc     2783
Leu Thr Pro Val Ala Phe Ile Gln Pro Ala Pro Gly Arg His Leu Thr
        755                 760                 765

ggc gcc gac cgg cgc cgt ctc gat gag ctg gtg cgt acc gag aag ggg     2831
Gly Ala Asp Arg Arg Arg Leu Asp Glu Leu Val Arg Thr Glu Lys Gly
    770                 775                 780

gcg gtc agt gtc cca gag gat tac atc gag gtc agt gcc ttt ccc gaa     2879
Ala Val Ser Val Pro Glu Asp Tyr Ile Glu Val Ser Ala Phe Pro Glu
785                 790                 795                 800

acc cgc agc ggg aag tat atg cgg cgc ttt ttg cgc aat atg atg ctc     2927
Thr Arg Ser Gly Lys Tyr Met Arg Arg Phe Leu Arg Asn Met Met Leu
                805                 810                 815

gat gaa cca ctg ggt gat acg acg acg ttg cgc aat cct gaa gtg ctc     2975
Asp Glu Pro Leu Gly Asp Thr Thr Thr Leu Arg Asn Pro Glu Val Leu
            820                 825                 830

gaa gag att gca gcc aag atc gct gag tgg aaa cgc cgt cag cgt atg     3023
Glu Glu Ile Ala Ala Lys Ile Ala Glu Trp Lys Arg Arg Gln Arg Met
        835                 840                 845

gcc gaa gag cag cag atc atc gaa cgc tat cgc tac ttc cgg atc gag     3071
Ala Glu Glu Gln Gln Ile Ile Glu Arg Tyr Arg Tyr Phe Arg Ile Glu
    850                 855                 860

tat cac cca cca acg gcc agt gcg ggt aaa ctc gcg gta gtg acg gtg     3119
Tyr His Pro Pro Thr Ala Ser Ala Gly Lys Leu Ala Val Val Thr Val
865                 870                 875                 880

aca aat ccg ccg gtg aac gca ctg aat gag cgt gcg ctc gat gag ttg     3167
Thr Asn Pro Pro Val Asn Ala Leu Asn Glu Arg Ala Leu Asp Glu Leu
                885                 890                 895

aac aca att gtt gac cac ctg gcc cgt cgt cag gat gtt gcc gca att     3215
Asn Thr Ile Val Asp His Leu Ala Arg Arg Gln Asp Val Ala Ala Ile
            900                 905                 910

gtc ttc acc gga cag ggc gcc agg agt ttt gtc gcc ggc gct gat att     3263
Val Phe Thr Gly Gln Gly Ala Arg Ser Phe Val Ala Gly Ala Asp Ile
        915                 920                 925

cgc cag ttg ctc gaa gag att cat acg gtt gaa gag gca atg gcc ctg     3311
Arg Gln Leu Leu Glu Glu Ile His Thr Val Glu Glu Ala Met Ala Leu
    930                 935                 940

ccg aat aac gcc cat ctt gct ttc cgc aag att gag cgt atg aat aag     3359
Pro Asn Asn Ala His Leu Ala Phe Arg Lys Ile Glu Arg Met Asn Lys
945                 950                 955                 960

ccg tgt atc gcg gcg atc aac ggt gtg gcg ctc ggt ggt ggt ctg gaa     3407
Pro Cys Ile Ala Ala Ile Asn Gly Val Ala Leu Gly Gly Gly Leu Glu
                965                 970                 975

ttc gcc atg gcc tgc cat tac cgg gtt gcc gat gtc tat gcc gaa ttc     3455
Phe Ala Met Ala Cys His Tyr Arg Val Ala Asp Val Tyr Ala Glu Phe
            980                 985                 990

ggt cag cca gag att aat ctg cgc  ttg cta cct ggt tat  ggt ggc acg   3503
Gly Gln Pro Glu Ile Asn Leu Arg  Leu Leu Pro Gly Tyr  Gly Gly Thr
        995                 1000                1005

cag cgc  ttg ccg cgc ctg ttg  tac aag cgc aac aac  ggc acc ggt      3548
Gln Arg  Leu Pro Arg Leu Leu  Tyr Lys Arg Asn Asn  Gly Thr Gly
    1010                1015                1020

ctg ctc  cga gcg ctg gag atg  att ctg ggt ggg cgt  agc gta ccg      3593
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Leu Leu  Arg Ala Leu Glu Met  Ile Leu Gly Gly Arg  Ser Val Pro
    1025                1030                1035

gct gat  gag gcg ctg aag ctg  ggt ctg atc gat gcc  att gct acc      3638
Ala Asp  Glu Ala Leu Lys Leu  Gly Leu Ile Asp Ala  Ile Ala Thr
    1040                1045                1050

ggc gat  cag gac tca ctg tcg  ctg gca tgc gcg tta  gcc cgt gcc      3683
Gly Asp  Gln Asp Ser Leu Ser  Leu Ala Cys Ala Leu  Ala Arg Ala
    1055                1060                1065

gca atc  ggc gcc gat ggt cag  ttg atc gag tcg gct  gcg gtg acc      3728
Ala Ile  Gly Ala Asp Gly Gln  Leu Ile Glu Ser Ala  Ala Val Thr
    1070                1075                1080

cag gct  ttc cgc cat cgc cac  gag cag ctt gac gag  tgg cgc aaa      3773
Gln Ala  Phe Arg His Arg His  Glu Gln Leu Asp Glu  Trp Arg Lys
    1085                1090                1095

cca gac  ccg cgc ttt gcc gat  gac gaa ctg cgc tcg  att atc gcc      3818
Pro Asp  Pro Arg Phe Ala Asp  Asp Glu Leu Arg Ser  Ile Ile Ala
    1100                1105                1110

cat cca  cgt atc gag cgg att  atc cgg cag gcc cat  acc gtt ggg      3863
His Pro  Arg Ile Glu Arg Ile  Ile Arg Gln Ala His  Thr Val Gly
    1115                1120                1125

cgc gat  gcg gca gtg cat cgg  gca ctg gat gca atc  cgc tat ggc      3908
Arg Asp  Ala Ala Val His Arg  Ala Leu Asp Ala Ile  Arg Tyr Gly
    1130                1135                1140

att atc  cac ggc ttc gag gcc  ggt ctg gag cac gag  gcg aag ctc      3953
Ile Ile  His Gly Phe Glu Ala  Gly Leu Glu His Glu  Ala Lys Leu
    1145                1150                1155

ttt gcc  gag gca gtg gtt gac  ccg aac ggt ggc aag  cgt ggt att      3998
Phe Ala  Glu Ala Val Val Asp  Pro Asn Gly Gly Lys  Arg Gly Ile
    1160                1165                1170

cgc gag  ttc ctc gac cgc cag  agt gcg ccg ttg cca  acc cgc cga      4043
Arg Glu  Phe Leu Asp Arg Gln  Ser Ala Pro Leu Pro  Thr Arg Arg
    1175                1180                1185

cca ttg  att aca cct gaa cag  gag caa ctc ttg cgc  gat cag aaa      4088
Pro Leu  Ile Thr Pro Glu Gln  Glu Gln Leu Leu Arg  Asp Gln Lys
    1190                1195                1200

gaa ctg  ttg ccg gtt ggt tca  ccc ttc ttc ccc ggt  gtt gac cgg      4133
Glu Leu  Leu Pro Val Gly Ser  Pro Phe Phe Pro Gly  Val Asp Arg
    1205                1210                1215

att ccg  aag tgg cag tac gcg  cag gcg gtt att cgt  gat ccg gac      4178
Ile Pro  Lys Trp Gln Tyr Ala  Gln Ala Val Ile Arg  Asp Pro Asp
    1220                1225                1230

acc ggt  gcg gcg gct cac ggc  gat ccc atc gtg gct  gaa aag cag      4223
Thr Gly  Ala Ala Ala His Gly  Asp Pro Ile Val Ala  Glu Lys Gln
    1235                1240                1245

att att  gtg ccg gtg gaa cgc  ccc cgc gcc aat cag  gcg ctg atc      4268
Ile Ile  Val Pro Val Glu Arg  Pro Arg Ala Asn Gln  Ala Leu Ile
    1250                1255                1260

tat gtt  ctg gcc tcg gag gtg  aac ttc aac gat atc  tgg gcg att      4313
Tyr Val  Leu Ala Ser Glu Val  Asn Phe Asn Asp Ile  Trp Ala Ile
    1265                1270                1275

acc ggt  att ccg gtg tca cgg  ttt gat gag cac gac  cgc gac tgg      4358
Thr Gly  Ile Pro Val Ser Arg  Phe Asp Glu His Asp  Arg Asp Trp
    1280                1285                1290

cac gtt  acc ggt tca ggt ggc  atc ggc ctg atc gtt  gcg ctg ggt      4403
His Val  Thr Gly Ser Gly Gly  Ile Gly Leu Ile Val  Ala Leu Gly
    1295                1300                1305

gaa gag  gcg cga cgc gaa ggc  cgg ctg aag gtg ggt  gat ctg gtg      4448
Glu Glu  Ala Arg Arg Glu Gly  Arg Leu Lys Val Gly  Asp Leu Val
    1310                1315                1320
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gcg atc  tac tcc ggg cag tcg  gat ctg ctc tca ccg  ctg atg ggc      4493
Ala Ile  Tyr Ser Gly Gln Ser  Asp Leu Leu Ser Pro  Leu Met Gly
    1325                1330                1335

ctt gat  ccg atg gcc gcc gat  ttc gtc atc cag ggg  aac gac acg      4538
Leu Asp  Pro Met Ala Ala Asp  Phe Val Ile Gln Gly  Asn Asp Thr
    1340                1345                1350

cca gat  gga tcg cat cag caa  ttt atg ctg gcc cag  gcc ccg cag      4583
Pro Asp  Gly Ser His Gln Gln  Phe Met Leu Ala Gln  Ala Pro Gln
    1355                1360                1365

tgt ctg  ccc atc cca acc gat  atg tct atc gag gca  gcc ggc agc      4628
Cys Leu  Pro Ile Pro Thr Asp  Met Ser Ile Glu Ala  Ala Gly Ser
    1370                1375                1380

tac atc  ctc aat ctc ggt acg  atc tat cgc gcc ctc  ttt acg acg      4673
Tyr Ile  Leu Asn Leu Gly Thr  Ile Tyr Arg Ala Leu  Phe Thr Thr
    1385                1390                1395

ttg caa  atc aag gcc gga cgc  acc atc ttt atc gag  ggt gcg gcg      4718
Leu Gln  Ile Lys Ala Gly Arg  Thr Ile Phe Ile Glu  Gly Ala Ala
    1400                1405                1410

acc ggt  acc ggt ctg gac gca  gcg cgc tcg gcg gcc  cgg aat ggt      4763
Thr Gly  Thr Gly Leu Asp Ala  Ala Arg Ser Ala Ala  Arg Asn Gly
    1415                1420                1425

ctg cgc  gta att gga atg gtc  agt tcg tcg tca cgt  gcg tct acg      4808
Leu Arg  Val Ile Gly Met Val  Ser Ser Ser Ser Arg  Ala Ser Thr
    1430                1435                1440

ctg ctg  gct gcg ggt gcc cac  ggt gcg att aac cgt  aaa gac ccg      4853
Leu Leu  Ala Ala Gly Ala His  Gly Ala Ile Asn Arg  Lys Asp Pro
    1445                1450                1455

gag gtt  gcc gat tgt ttc acg  cgc gtg ccc gaa gat  cca tca gcc      4898
Glu Val  Ala Asp Cys Phe Thr  Arg Val Pro Glu Asp  Pro Ser Ala
    1460                1465                1470

tgg gca  gcc tgg gaa gcc gcc  ggt cag ccg ttg ctg  gcg atg ttc      4943
Trp Ala  Ala Trp Glu Ala Ala  Gly Gln Pro Leu Leu  Ala Met Phe
    1475                1480                1485

cgg gcg  cag aac gac ggg cga  ctg gcc gat tat gtg  gtc tcg cac      4988
Arg Ala  Gln Asn Asp Gly Arg  Leu Ala Asp Tyr Val  Val Ser His
    1490                1495                1500

gcg ggc  gag acg gcc ttc ccg  cgc agt ttc cag ctt  ctc ggc gag      5033
Ala Gly  Glu Thr Ala Phe Pro  Arg Ser Phe Gln Leu  Leu Gly Glu
    1505                1510                1515

cca cgc  gat ggt cac att ccg  acg ctc aca ttc tac  ggt gcc acc      5078
Pro Arg  Asp Gly His Ile Pro  Thr Leu Thr Phe Tyr  Gly Ala Thr
    1520                1525                1530

agt ggc  tac cac ttc acc ttc  ctg ggt aag cca ggg  tca gct tcg      5123
Ser Gly  Tyr His Phe Thr Phe  Leu Gly Lys Pro Gly  Ser Ala Ser
    1535                1540                1545

ccg acc  gag atg ctg cgg cgg  gcc aat ctc cgc gcc  ggt gag gcg      5168
Pro Thr  Glu Met Leu Arg Arg  Ala Asn Leu Arg Ala  Gly Glu Ala
    1550                1555                1560

gtg ttg  atc tac tac ggg gtt  ggg agc gat gac ctg  gta gat acc      5213
Val Leu  Ile Tyr Tyr Gly Val  Gly Ser Asp Asp Leu  Val Asp Thr
    1565                1570                1575

ggc ggt  ctg gag gct atc gag  gcg gcg cgg caa atg  gga gcg cgg      5258
Gly Gly  Leu Glu Ala Ile Glu  Ala Ala Arg Gln Met  Gly Ala Arg
    1580                1585                1590

atc gtc  gtc gtt acc gtc agc  gat gcg caa cgc gag  ttt gtc ctc      5303
Ile Val  Val Val Thr Val Ser  Asp Ala Gln Arg Glu  Phe Val Leu
    1595                1600                1605

tcg ttg  ggc ttc ggg gct gcc  cta cgt ggt gtc gtc  agc ctg gcg      5348
Ser Leu  Gly Phe Gly Ala Ala  Leu Arg Gly Val Val  Ser Leu Ala
    1610                1615                1620
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gaa ctc  aaa cgg cgc ttc ggc  gat gag ttt gag tgg  ccg cgc acg      5393
Glu Leu  Lys Arg Arg Phe Gly  Asp Glu Phe Glu Trp  Pro Arg Thr
    1625                 1630                 1635

atg ccg  ccg ttg ccg aac gcc  cgc cag gac ccg cag  ggt ctg aaa      5438
Met Pro  Pro Leu Pro Asn Ala  Arg Gln Asp Pro Gln  Gly Leu Lys
    1640                 1645                 1650

gag gct  gtc cgc cgc ttc aac  gat ctg gtc ttc aag  ccg cta gga      5483
Glu Ala  Val Arg Arg Phe Asn  Asp Leu Val Phe Lys  Pro Leu Gly
    1655                 1660                 1665

agc gcg  gtc ggt gtc ttc ttg  cgg agt gcc gac aat  ccg cgt ggc      5528
Ser Ala  Val Gly Val Phe Leu  Arg Ser Ala Asp Asn  Pro Arg Gly
    1670                 1675                 1680

tac ccc  gat ctg atc atc gag  cgg gct gcc cac gat  gca ctg gcg      5573
Tyr Pro  Asp Leu Ile Ile Glu  Arg Ala Ala His Asp  Ala Leu Ala
    1685                 1690                 1695

gtg agc  gcg atg ctg atc aag  ccc ttc acc gga cgg  att gtc tac      5618
Val Ser  Ala Met Leu Ile Lys  Pro Phe Thr Gly Arg  Ile Val Tyr
    1700                 1705                 1710

ttc gag  gac att ggt ggg cgg  cgt tac tcc ttc ttc  gca ccg caa      5663
Phe Glu  Asp Ile Gly Gly Arg  Arg Tyr Ser Phe Phe  Ala Pro Gln
    1715                 1720                 1725

atc tgg  gtg cgc cag cgc cgc  atc tac atg ccg acg  gca cag atc      5708
Ile Trp  Val Arg Gln Arg Arg  Ile Tyr Met Pro Thr  Ala Gln Ile
    1730                 1735                 1740

ttt ggt  acg cac ctc tca aat  gcg tat gaa att ctg  cgt ctg aat      5753
Phe Gly  Thr His Leu Ser Asn  Ala Tyr Glu Ile Leu  Arg Leu Asn
    1745                 1750                 1755

gat gag  atc agc gcc ggt ctg  ctg acg att acc gag  ccg gca gtg      5798
Asp Glu  Ile Ser Ala Gly Leu  Leu Thr Ile Thr Glu  Pro Ala Val
    1760                 1765                 1770

gtg ccg  tgg gat gaa cta ccc  gaa gca cat cag gcg  atg tgg gaa      5843
Val Pro  Trp Asp Glu Leu Pro  Glu Ala His Gln Ala  Met Trp Glu
    1775                 1780                 1785

aat cgc  cac acg gcg gcc act  tat gtg gtg aat cat  gcc tta cca      5888
Asn Arg  His Thr Ala Ala Thr  Tyr Val Val Asn His  Ala Leu Pro
    1790                 1795                 1800

cgt ctc  ggc cta aag aac agg  gac gag ctg tac gag  gcg tgg acg      5933
Arg Leu  Gly Leu Lys Asn Arg  Asp Glu Leu Tyr Glu  Ala Trp Thr
    1805                 1810                 1815

gcc ggc  gag cgg tagcgcggat gggtattgaa caggtaacgg acggaagatc       5985
Ala Gly  Glu Arg
    1820

gaaccttccg tccgttatct tttggccgtc gaagcgtgct gagccgatta tcgttgccgt   6045

ggttgtcccg atgggcagac gcgctcgaac cagatgatac caccgacggc tatcgtcacc   6105

aaaccggcga agaccaggta agcctctgaa ggacgc                             6141


<210> SEQ ID NO 39
<211> LENGTH: 1822
<212> TYPE: PRT
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 39

Met Ile Asp Thr Ala Pro Leu Ala Pro Pro Arg Ala Pro Arg Ser Asn
1               5                  10                  15

Pro Ile Arg Asp Arg Val Asp Trp Glu Ala Gln Arg Ala Ala Ala Leu
            20                  25                  30

Ala Asp Pro Gly Ala Phe His Gly Ala Ile Ala Arg Thr Val Ile His
        35                  40                  45
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Trp Tyr Asp Pro Gln His His Cys Trp Ile Arg Phe Asn Glu Ser Ser
    50                  55                  60

Gln Arg Trp Glu Gly Leu Asp Ala Ala Thr Gly Ala Pro Val Thr Val
65                  70                  75                  80

Asp Tyr Pro Ala Asp Tyr Gln Pro Trp Gln Gln Ala Phe Asp Asp Ser
                85                  90                  95

Glu Ala Pro Phe Tyr Arg Trp Phe Ser Gly Gly Leu Thr Asn Ala Cys
            100                 105                 110

Phe Asn Glu Val Asp Arg His Val Met Met Gly Tyr Gly Asp Glu Val
        115                 120                 125

Ala Tyr Tyr Phe Glu Gly Asp Arg Trp Asp Asn Ser Leu Asn Asn Gly
    130                 135                 140

Arg Gly Gly Pro Val Val Gln Glu Thr Ile Thr Arg Arg Arg Leu Leu
145                 150                 155                 160

Val Glu Val Val Lys Ala Ala Gln Val Leu Arg Asp Leu Gly Leu Lys
                165                 170                 175

Lys Gly Asp Arg Ile Ala Leu Asn Met Pro Asn Ile Met Pro Gln Ile
            180                 185                 190

Tyr Tyr Thr Glu Ala Ala Lys Arg Leu Gly Ile Leu Tyr Thr Pro Val
        195                 200                 205

Phe Gly Gly Phe Ser Asp Lys Thr Leu Ser Asp Arg Ile His Asn Ala
    210                 215                 220

Gly Ala Arg Val Val Ile Thr Ser Asp Gly Ala Tyr Arg Asn Ala Gln
225                 230                 235                 240

Val Val Pro Tyr Lys Glu Ala Tyr Thr Asp Gln Ala Leu Asp Lys Tyr
                245                 250                 255

Ile Pro Val Glu Thr Ala Gln Ala Ile Val Ala Gln Thr Leu Ala Thr
            260                 265                 270

Leu Pro Leu Thr Glu Ser Gln Arg Gln Thr Ile Ile Thr Glu Val Glu
        275                 280                 285

Ala Ala Leu Ala Gly Glu Ile Thr Val Glu Arg Ser Asp Val Met Arg
    290                 295                 300

Gly Val Gly Ser Ala Leu Ala Lys Leu Arg Asp Leu Asp Ala Ser Val
305                 310                 315                 320

Gln Ala Lys Val Arg Thr Val Leu Ala Gln Ala Leu Val Glu Ser Pro
                325                 330                 335

Pro Arg Val Glu Ala Val Val Val Val Arg His Thr Gly Gln Glu Ile
            340                 345                 350

Leu Trp Asn Glu Gly Arg Asp Arg Trp Ser His Asp Leu Leu Asp Ala
        355                 360                 365

Ala Leu Ala Lys Ile Leu Ala Asn Ala Arg Ala Ala Gly Phe Asp Val
    370                 375                 380

His Ser Glu Asn Asp Leu Leu Asn Leu Pro Asp Asp Gln Leu Ile Arg
385                 390                 395                 400

Ala Leu Tyr Ala Ser Ile Pro Cys Glu Pro Val Asp Ala Glu Tyr Pro
                405                 410                 415

Met Phe Ile Ile Tyr Thr Ser Gly Ser Thr Gly Lys Pro Lys Gly Val
            420                 425                 430

Ile His Val His Gly Gly Tyr Val Ala Gly Val Val His Thr Leu Arg
        435                 440                 445

Val Ser Phe Asp Ala Glu Pro Gly Asp Thr Ile Tyr Val Ile Ala Asp
    450                 455                 460
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Pro Gly Trp Ile Thr Gly Gln Ser Tyr Met Leu Thr Ala Thr Met Ala
465                 470                 475                 480

Gly Arg Leu Thr Gly Val Ile Ala Glu Gly Ser Pro Leu Phe Pro Ser
                485                 490                 495

Ala Gly Arg Tyr Ala Ser Ile Ile Glu Arg Tyr Gly Val Gln Ile Phe
            500                 505                 510

Lys Ala Gly Val Thr Phe Leu Lys Thr Val Met Ser Asn Pro Gln Asn
        515                 520                 525

Val Glu Asp Val Arg Leu Tyr Asp Met His Ser Leu Arg Val Ala Thr
    530                 535                 540

Phe Cys Ala Glu Pro Val Ser Pro Ala Val Gln Gln Phe Gly Met Gln
545                 550                 555                 560

Ile Met Thr Pro Gln Tyr Ile Asn Ser Tyr Trp Ala Thr Glu His Gly
                565                 570                 575

Gly Ile Val Trp Thr His Phe Tyr Gly Asn Gln Asp Phe Pro Leu Arg
            580                 585                 590

Pro Asp Ala His Thr Tyr Pro Leu Pro Trp Val Met Gly Asp Val Trp
        595                 600                 605

Val Ala Glu Thr Asp Glu Ser Gly Thr Thr Arg Tyr Arg Val Ala Asp
    610                 615                 620

Phe Asp Glu Lys Gly Glu Ile Val Ile Thr Ala Pro Tyr Pro Tyr Leu
625                 630                 635                 640

Thr Arg Thr Leu Trp Gly Asp Val Pro Gly Phe Glu Ala Tyr Leu Arg
                645                 650                 655

Gly Glu Ile Pro Leu Arg Ala Trp Lys Gly Asp Ala Glu Arg Phe Val
            660                 665                 670

Lys Thr Tyr Trp Arg Arg Gly Pro Asn Gly Glu Trp Gly Tyr Ile Gln
        675                 680                 685

Gly Asp Phe Ala Ile Lys Tyr Pro Asp Gly Ser Phe Thr Leu His Gly
    690                 695                 700

Arg Pro Asp Asp Val Ile Asn Val Ser Gly His Arg Met Gly Thr Glu
705                 710                 715                 720

Glu Ile Glu Gly Ala Ile Leu Arg Asp Arg Gln Ile Thr Pro Asp Ser
                725                 730                 735

Pro Val Gly Asn Cys Ile Val Val Gly Ala Pro His Arg Glu Lys Gly
            740                 745                 750

Leu Thr Pro Val Ala Phe Ile Gln Pro Ala Pro Gly Arg His Leu Thr
        755                 760                 765

Gly Ala Asp Arg Arg Arg Leu Asp Glu Leu Val Arg Thr Glu Lys Gly
    770                 775                 780

Ala Val Ser Val Pro Glu Asp Tyr Ile Glu Val Ser Ala Phe Pro Glu
785                 790                 795                 800

Thr Arg Ser Gly Lys Tyr Met Arg Arg Phe Leu Arg Asn Met Met Leu
                805                 810                 815

Asp Glu Pro Leu Gly Asp Thr Thr Thr Leu Arg Asn Pro Glu Val Leu
            820                 825                 830

Glu Glu Ile Ala Ala Lys Ile Ala Glu Trp Lys Arg Arg Gln Arg Met
        835                 840                 845

Ala Glu Glu Gln Gln Ile Ile Glu Arg Tyr Arg Tyr Phe Arg Ile Glu
    850                 855                 860

Tyr His Pro Pro Thr Ala Ser Ala Gly Lys Leu Ala Val Val Thr Val
865                 870                 875                 880

Thr Asn Pro Pro Val Asn Ala Leu Asn Glu Arg Ala Leu Asp Glu Leu
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               885                 890                 895

Asn Thr Ile Val Asp His Leu Ala Arg Arg Gln Asp Val Ala Ala Ile
            900                 905                 910

Val Phe Thr Gly Gln Gly Ala Arg Ser Phe Val Ala Gly Ala Asp Ile
        915                 920                 925

Arg Gln Leu Leu Glu Glu Ile His Thr Val Glu Glu Ala Met Ala Leu
    930                 935                 940

Pro Asn Asn Ala His Leu Ala Phe Arg Lys Ile Glu Arg Met Asn Lys
945                 950                 955                 960

Pro Cys Ile Ala Ala Ile Asn Gly Val Ala Leu Gly Gly Gly Leu Glu
               965                 970                 975

Phe Ala Met Ala Cys His Tyr Arg Val Ala Asp Val Tyr Ala Glu Phe
            980                 985                 990

Gly Gln Pro Glu Ile Asn Leu Arg  Leu Leu Pro Gly Tyr  Gly Gly Thr
        995                 1000                1005

Gln Arg  Leu Pro Arg Leu Leu  Tyr Lys Arg Asn Asn  Gly Thr Gly
    1010                1015                1020

Leu Leu  Arg Ala Leu Glu Met  Ile Leu Gly Gly Arg  Ser Val Pro
    1025                1030                1035

Ala Asp  Glu Ala Leu Lys Leu  Gly Leu Ile Asp Ala  Ile Ala Thr
    1040                1045                1050

Gly Asp  Gln Asp Ser Leu Ser  Leu Ala Cys Ala Leu  Ala Arg Ala
    1055                1060                1065

Ala Ile  Gly Ala Asp Gly Gln  Leu Ile Glu Ser Ala  Ala Val Thr
    1070                1075                1080

Gln Ala  Phe Arg His Arg His  Glu Gln Leu Asp Glu  Trp Arg Lys
    1085                1090                1095

Pro Asp  Pro Arg Phe Ala Asp  Asp Glu Leu Arg Ser  Ile Ile Ala
    1100                1105                1110

His Pro  Arg Ile Glu Arg Ile  Ile Arg Gln Ala His  Thr Val Gly
    1115                1120                1125

Arg Asp  Ala Ala Val His Arg  Ala Leu Asp Ala Ile  Arg Tyr Gly
    1130                1135                1140

Ile Ile  His Gly Phe Glu Ala  Gly Leu Glu His Glu  Ala Lys Leu
    1145                1150                1155

Phe Ala  Glu Ala Val Val Asp  Pro Asn Gly Gly Lys  Arg Gly Ile
    1160                1165                1170

Arg Glu  Phe Leu Asp Arg Gln  Ser Ala Pro Leu Pro  Thr Arg Arg
    1175                1180                1185

Pro Leu  Ile Thr Pro Glu Gln  Glu Gln Leu Leu Arg  Asp Gln Lys
    1190                1195                1200

Glu Leu  Leu Pro Val Gly Ser  Pro Phe Phe Pro Gly  Val Asp Arg
    1205                1210                1215

Ile Pro  Lys Trp Gln Tyr Ala  Gln Ala Val Ile Arg  Asp Pro Asp
    1220                1225                1230

Thr Gly  Ala Ala Ala His Gly  Asp Pro Ile Val Ala  Glu Lys Gln
    1235                1240                1245

Ile Ile  Val Pro Val Glu Arg  Pro Arg Ala Asn Gln  Ala Leu Ile
    1250                1255                1260

Tyr Val  Leu Ala Ser Glu Val  Asn Phe Asn Asp Ile  Trp Ala Ile
    1265                1270                1275

Thr Gly  Ile Pro Val Ser Arg  Phe Asp Glu His Asp  Arg Asp Trp
    1280                1285                1290
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His Val  Thr Gly Ser Gly Gly  Ile Gly Leu Ile Val  Ala Leu Gly
    1295                1300                1305

Glu Glu  Ala Arg Arg Glu Gly  Arg Leu Lys Val Gly  Asp Leu Val
    1310                1315                1320

Ala Ile  Tyr Ser Gly Gln Ser  Asp Leu Leu Ser Pro  Leu Met Gly
    1325                1330                1335

Leu Asp  Pro Met Ala Ala Asp  Phe Val Ile Gln Gly  Asn Asp Thr
    1340                1345                1350

Pro Asp  Gly Ser His Gln Gln  Phe Met Leu Ala Gln  Ala Pro Gln
    1355                1360                1365

Cys Leu  Pro Ile Pro Thr Asp  Met Ser Ile Glu Ala  Ala Gly Ser
    1370                1375                1380

Tyr Ile  Leu Asn Leu Gly Thr  Ile Tyr Arg Ala Leu  Phe Thr Thr
    1385                1390                1395

Leu Gln  Ile Lys Ala Gly Arg  Thr Ile Phe Ile Glu  Gly Ala Ala
    1400                1405                1410

Thr Gly  Thr Gly Leu Asp Ala  Ala Arg Ser Ala Ala  Arg Asn Gly
    1415                1420                1425

Leu Arg  Val Ile Gly Met Val  Ser Ser Ser Ser Arg  Ala Ser Thr
    1430                1435                1440

Leu Leu  Ala Ala Gly Ala His  Gly Ala Ile Asn Arg  Lys Asp Pro
    1445                1450                1455

Glu Val  Ala Asp Cys Phe Thr  Arg Val Pro Glu Asp  Pro Ser Ala
    1460                1465                1470

Trp Ala  Ala Trp Glu Ala Ala  Gly Gln Pro Leu Leu  Ala Met Phe
    1475                1480                1485

Arg Ala  Gln Asn Asp Gly Arg  Leu Ala Asp Tyr Val  Val Ser His
    1490                1495                1500

Ala Gly  Glu Thr Ala Phe Pro  Arg Ser Phe Gln Leu  Leu Gly Glu
    1505                1510                1515

Pro Arg  Asp Gly His Ile Pro  Thr Leu Thr Phe Tyr  Gly Ala Thr
    1520                1525                1530

Ser Gly  Tyr His Phe Thr Phe  Leu Gly Lys Pro Gly  Ser Ala Ser
    1535                1540                1545

Pro Thr  Glu Met Leu Arg Arg  Ala Asn Leu Arg Ala  Gly Glu Ala
    1550                1555                1560

Val Leu  Ile Tyr Tyr Gly Val  Gly Ser Asp Asp Leu  Val Asp Thr
    1565                1570                1575

Gly Gly  Leu Glu Ala Ile Glu  Ala Ala Arg Gln Met  Gly Ala Arg
    1580                1585                1590

Ile Val  Val Val Thr Val Ser  Asp Ala Gln Arg Glu  Phe Val Leu
    1595                1600                1605

Ser Leu  Gly Phe Gly Ala Ala  Leu Arg Gly Val Val  Ser Leu Ala
    1610                1615                1620

Glu Leu  Lys Arg Arg Phe Gly  Asp Glu Phe Glu Trp  Pro Arg Thr
    1625                1630                1635

Met Pro  Pro Leu Pro Asn Ala  Arg Gln Asp Pro Gln  Gly Leu Lys
    1640                1645                1650

Glu Ala  Val Arg Arg Phe Asn  Asp Leu Val Phe Lys  Pro Leu Gly
    1655                1660                1665

Ser Ala  Val Gly Val Phe Leu  Arg Ser Ala Asp Asn  Pro Arg Gly
    1670                1675                1680
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Tyr Pro  Asp Leu Ile Ile Glu  Arg Ala Ala His Asp  Ala Leu Ala
    1685                1690                1695

Val Ser  Ala Met Leu Ile Lys  Pro Phe Thr Gly Arg  Ile Val Tyr
    1700                1705                1710

Phe Glu  Asp Ile Gly Gly Arg  Arg Tyr Ser Phe Phe  Ala Pro Gln
    1715                1720                1725

Ile Trp  Val Arg Gln Arg Arg  Ile Tyr Met Pro Thr  Ala Gln Ile
    1730                1735                1740

Phe Gly  Thr His Leu Ser Asn  Ala Tyr Glu Ile Leu  Arg Leu Asn
    1745                1750                1755

Asp Glu  Ile Ser Ala Gly Leu  Leu Thr Ile Thr Glu  Pro Ala Val
    1760                1765                1770

Val Pro  Trp Asp Glu Leu Pro  Glu Ala His Gln Ala  Met Trp Glu
    1775                1780                1785

Asn Arg  His Thr Ala Ala Thr  Tyr Val Val Asn His  Ala Leu Pro
    1790                1795                1800

Arg Leu  Gly Leu Lys Asn Arg  Asp Glu Leu Tyr Glu  Ala Trp Thr
    1805                1810                1815

Ala Gly  Glu Arg
    1820


<210> SEQ ID NO 40
<211> LENGTH: 777
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(774)

<400> SEQUENCE: 40

atg agt gaa gag tct ctg gtt ctc agc aca att gaa ggc ccc atc gcc       48
Met Ser Glu Glu Ser Leu Val Leu Ser Thr Ile Glu Gly Pro Ile Ala
1               5                  10                  15

atc ctc acc ctc aat cgc ccc cag gcc ctc aat gcg ctc agt ccg gcc       96
Ile Leu Thr Leu Asn Arg Pro Gln Ala Leu Asn Ala Leu Ser Pro Ala
            20                  25                  30

ttg att gat gac ctc att cgc cat tta gaa gcc tgc gat gcc gat gac      144
Leu Ile Asp Asp Leu Ile Arg His Leu Glu Ala Cys Asp Ala Asp Asp
        35                  40                  45

aca atc cgc gtg atc att atc acc ggc gcc gga cgg gca ttt gct gcc      192
Thr Ile Arg Val Ile Ile Ile Thr Gly Ala Gly Arg Ala Phe Ala Ala
    50                  55                  60

ggc gct gat atc aaa gcg atg gcc aat gcc acg cct att gat atg ctc      240
Gly Ala Asp Ile Lys Ala Met Ala Asn Ala Thr Pro Ile Asp Met Leu
65                  70                  75                  80

acc agt ggc atg att gcg cgc tgg gca cgc atc gcc gcg gtg cgc aaa      288
Thr Ser Gly Met Ile Ala Arg Trp Ala Arg Ile Ala Ala Val Arg Lys
                85                  90                  95

ccg gtg att gct gcc gtg aat ggg tat gcg ctc ggt ggt ggt tgt gaa      336
Pro Val Ile Ala Ala Val Asn Gly Tyr Ala Leu Gly Gly Gly Cys Glu
            100                 105                 110

ttg gca atg atg tgc gac atc atc atc gcc agt gaa aac gcg cag ttc      384
Leu Ala Met Met Cys Asp Ile Ile Ile Ala Ser Glu Asn Ala Gln Phe
        115                 120                 125

gga caa ccg gaa atc aat ctg ggc atc att ccc ggt gct ggt ggc acc      432
Gly Gln Pro Glu Ile Asn Leu Gly Ile Ile Pro Gly Ala Gly Gly Thr
    130                 135                 140

caa cgg ctg acc cgc gcc ctt ggc ccg tat cgc gca atg gaa ttg atc      480
Gln Arg Leu Thr Arg Ala Leu Gly Pro Tyr Arg Ala Met Glu Leu Ile
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145                 150                 155                 160

ctg acc ggc gcg acc atc agt gct cag gaa gct ctc gcc cac ggc ctg      528
Leu Thr Gly Ala Thr Ile Ser Ala Gln Glu Ala Leu Ala His Gly Leu
                165                 170                 175

gtg tgc cgg gtc tgc ccg cct gaa agc ctg ctc gat gaa gcc cgt cgg      576
Val Cys Arg Val Cys Pro Pro Glu Ser Leu Leu Asp Glu Ala Arg Arg
            180                 185                 190

atc gcg caa acc att gcc acc aaa tca cca ctg gct gta cag ttg gcg      624
Ile Ala Gln Thr Ile Ala Thr Lys Ser Pro Leu Ala Val Gln Leu Ala
        195                 200                 205

aaa gag gca gtc cgt atg gcc gcc gaa acc act gtg cgc gag ggg ttg      672
Lys Glu Ala Val Arg Met Ala Ala Glu Thr Thr Val Arg Glu Gly Leu
    210                 215                 220

gct atc gag ctg cgt aac ttc tat ctg ctg ttt gcc agt gct gac caa      720
Ala Ile Glu Leu Arg Asn Phe Tyr Leu Leu Phe Ala Ser Ala Asp Gln
225                 230                 235                 240

aaa gag ggg atg cag gca ttt atc gag aaa cgc gct ccc aac ttc agt      768
Lys Glu Gly Met Gln Ala Phe Ile Glu Lys Arg Ala Pro Asn Phe Ser
                245                 250                 255

ggt cgt tga                                                         777
Gly Arg


<210> SEQ ID NO 41
<211> LENGTH: 258
<212> TYPE: PRT
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 41

Met Ser Glu Glu Ser Leu Val Leu Ser Thr Ile Glu Gly Pro Ile Ala
1               5                   10                  15

Ile Leu Thr Leu Asn Arg Pro Gln Ala Leu Asn Ala Leu Ser Pro Ala
            20                  25                  30

Leu Ile Asp Asp Leu Ile Arg His Leu Glu Ala Cys Asp Ala Asp Asp
        35                  40                  45

Thr Ile Arg Val Ile Ile Ile Thr Gly Ala Gly Arg Ala Phe Ala Ala
    50                  55                  60

Gly Ala Asp Ile Lys Ala Met Ala Asn Ala Thr Pro Ile Asp Met Leu
65                  70                  75                  80

Thr Ser Gly Met Ile Ala Arg Trp Ala Arg Ile Ala Ala Val Arg Lys
                85                  90                  95

Pro Val Ile Ala Ala Val Asn Gly Tyr Ala Leu Gly Gly Gly Cys Glu
            100                 105                 110

Leu Ala Met Met Cys Asp Ile Ile Ile Ala Ser Glu Asn Ala Gln Phe
        115                 120                 125

Gly Gln Pro Glu Ile Asn Leu Gly Ile Ile Pro Gly Ala Gly Gly Thr
    130                 135                 140

Gln Arg Leu Thr Arg Ala Leu Gly Pro Tyr Arg Ala Met Glu Leu Ile
145                 150                 155                 160

Leu Thr Gly Ala Thr Ile Ser Ala Gln Glu Ala Leu Ala His Gly Leu
                165                 170                 175

Val Cys Arg Val Cys Pro Pro Glu Ser Leu Leu Asp Glu Ala Arg Arg
            180                 185                 190

Ile Ala Gln Thr Ile Ala Thr Lys Ser Pro Leu Ala Val Gln Leu Ala
        195                 200                 205

Lys Glu Ala Val Arg Met Ala Ala Glu Thr Thr Val Arg Glu Gly Leu
    210                 215                 220
```

-continued

Ala Ile Glu Leu Arg Asn Phe Tyr Leu Leu Phe Ala Ser Ala Asp Gln
225 230 235 240

Lys Glu Gly Met Gln Ala Phe Ile Glu Lys Arg Ala Pro Asn Phe Ser
245 250 255

Gly Arg

<210> SEQ ID NO 42
<211> LENGTH: 1220
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 42

ggcgtaatcc gaccggcagg ttagggtctt ctactggggt caaggcgcgt ctccttttgg 60

tggcgcgagc aacccggctt ttcctggctt caatgtacca tagagcggtt acttcgtgca 120

acgggcgtgg tacaatcgag agcaaccttt cgcaaaagct atccaatcct gcacacgtgc 180

atctgttaca gggtattatt gtcggcaaac gacagtcctg tcgtttatgt acaaggagat 240

caacgtatga gtgaagagtc tctggttctc agcacaattg aaggccccat cgccatcctc 300

accctcaatc gcccccaggc cctcaatgcg ctcagtccgg ccttgattga tgacctcatt 360

cgccatttag aagcctgcga tgccgatgac acaatccgcg tgatcattat caccggcgcc 420

ggacgggcat ttgctgccgg cgctgatatc aaagcgatgg ccaatgccac gcctattgat 480

atgctcacca gtggcatgat tgcgcgctgg gcacgcatcg ccgcggtgcg caaaccggtg 540

attgctgccg tgaatgggta tgcgctcggt ggtggttgtg aattggcaat gatgtgcgac 600

atcatcatcg ccagtgaaaa cgcgcagttc ggacaaccgg aaatcaatct gggcatcatt 660

cccggtgctg gtggcaccca acggctgacc cgcgcccttg gcccgtatcg cgcaatggaa 720

ttgatcctga ccggcgcgac catcagtgct caggaagctc tcgcccacgg cctggtgtgc 780

cgggtctgcc cgcctgaaag cctgctcgat gaagcccgtc ggatcgcgca aaccattgcc 840

accaaatcac cactggctgt acagttggcg aaagaggcag tccgtatggc cgccgaaacc 900

actgtgcgcg aggggttggc tatcgagctg cgtaacttct atctgctgtt tgccagtgct 960

gaccaaaaag aggggatgca ggcatttatc gagaaacgcg ctcccaactt cagtggtcgt 1020

tgatcacgcg cagaacatgg cagcaggggc aatacctgca cgtactgcct cctgccgcca 1080

tactaccaga tgatcgagca gtaaagggta aatactctat caatctggcc agataagcgg 1140

ttgggtaaca acgcaatgct ccaaaggaga cgatcatgga catacacgag cgattgcgat 1200

ctctcgaacg cgaaaatgct 1220

<210> SEQ ID NO 43
<211> LENGTH: 774
<212> TYPE: DNA
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 43

atgacgtacg aaaccatcct ggtcgagcgc gatcagcgag ttggcattat cacgctgaac 60

cgtccccagg cactgaacgc gctcaacagc caggtgatga acgaggtcac cagcgctgca 120

accgaactgg acgatgaccc ggacattggg gcgatcatca tcaccggttc ggccaaagcg 180

tttgccgccg gagccgacat caaagaaatg gccgacctga cgttcgccga cgcgttcacc 240

gccgacttct tcgccacctg gggcaagctg gccgccgtgc gcaccccgac gatcgccgcg 300

gtggcgggat acgcgctcgg cggtggctgc gagctggcga tgatgtgcga cgtgctgatc 360

gccgccgaca ccgcgaagtt cggacagccc gagataaagc tgggcgtgct gccaggcatg 420

ggcggctccc agcggctgac ccgggctatc ggcaaggcta aggcgatgga cctcatcctg 480

accgggcgca ccatggacgc cgccgaggcc gagcgcagcg gtctggtttc acgggtggtg 540

ccggccgacg acttgctgac cgaagccagg gccactgcca cgaccatttc gcagatgtcg 600

gcctcggcgg cccggatggc caaggaggcc gtcaaccggg ctttcgaatc cagtttgtcc 660

gaggggctgc tctacgaacg ccggcttttc cattcggctt tcgcgaccga agaccaatcc 720

gaaggtatgg cagcgttcat cgagaaacgc gctccccagt tcacccaccg atga 774

<210> SEQ ID NO 44
<211> LENGTH: 873
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

atggccgccc tgcgtgtcct gctgtcctgc gcccgcggcc cgctgaggcc cccggttcgc 60

tgtcccgcct ggcgtccctt cgcctcgggt gctaactttg agtacatcat cgcagaaaaa 120

agagggaaga ataacaccgt ggggttgatc caactgaacc gccccaaggc cctcaatgca 180

ctttgcgatg gcctgattga cgagctcaac caggccctga agatcttcga ggaggacccg 240

gccgttgggg gcattgtcct caccggcggg gataaggcct ttgcagctgg agctgatatc 300

aaggaaatgc agaacctgag tttccaggac tgttactcca gcaagttctt gaagcactgg 360

ggccacctca cccaggtcaa gaagccagtc atcgctgctg tcaatggcta tccgtttggc 420

gggggctgtg agcttgccat gatgtgtgat atcatctatg ccggtgagaa ggcccagttt 480

gcacagccgg agatcttaat aggaaccatc ccaggtgcag gcggcaccca gagactcacc 540

cgtgctgttg ggaagtcgct ggagctggag atggtcctca ccggtgacgc gatctcagcc 600

caggacgcca agcaagcagg tcttgtcagc aagatttgtc ctgttgagac actggtggaa 660

gaagccatcc agtgtgcaga aaaaattgcc agcaattcta aaattgtagt agcgatggcc 720

aaagaatcag tgaatgcagc ttttgaaatg acattaacag aaggaagtaa gttggagaag 780

aaactctttt attcaacctt tgccactgat gaccggaaag aagggatgac cgcgtttgtg 840

gaaaagagaa aggccaactt caaagaccag tga 873

<210> SEQ ID NO 45
<211> LENGTH: 873
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 45

atggcggccc tgcgtgctct gctgcccaga gcctgcaact cgctgttgtc cccagttcgc 60

tgcccagaat tccggcgctt cgcctcgggt gctaactttc agtacatcat cacggaaaag 120

aaaggaaaga atagcagcgt ggggctgatc cagttgaacc gtcccaaagc actcaatgca 180

ctttgcaatg gactgattga ggagctcaac caagcactgg agacctttga ggaagatccc 240

gctgtgggcg ccattgtgct cactggtggg gagaaggcct ttgcagccgg agctgacatc 300

aaggaaatgc agaaccggac atttcaggac tgttactcag gcaagttcct gagccactgg 360

gaccatatca cccggatcaa gaaaccggtc atcgcggctg tcaatggcta tgctcttggt 420

gggggctgtg aacttgccat gatgtgcgat atcatctatg ctggtgagaa agcccagttt 480

ggacagccag aaatcctcct ggggaccatc ccaggtgcag ggggcactca gagactcacc 540

cgagcagtcg gcaaatcact agcaatggag atggtcctca ctggtgaccg aatttcagca 600

-continued

caggatgcca agcaagcagg tcttgtaagc aagatttttc ccgttgaaac actggttgaa 660

gaggccatcc aatgtgcaga aaagatcgcc aacaattcca agatcatagt agccatggcg 720

aaagaatctg tgaatgcagc ctttgaaatg acgttaacag aaggaaataa gctggagaag 780

aagctcttct attccacctt tgccactgat gaccggagag aagggatgtc tgcctttgtg 840

gagaaaagga aggccaactt caaagaccac tga 873

<210> SEQ ID NO 46
<211> LENGTH: 257
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 46

Met Thr Tyr Glu Thr Ile Leu Val Glu Arg Asp Gln Arg Val Gly Ile
1 5 10 15

Ile Thr Leu Asn Arg Pro Gln Ala Leu Asn Ala Leu Asn Ser Gln Val
20 25 30

Met Asn Glu Val Thr Ser Ala Ala Thr Glu Leu Asp Asp Asp Pro Asp
35 40 45

Ile Gly Ala Ile Ile Ile Thr Gly Ser Ala Lys Ala Phe Ala Ala Gly
50 55 60

Ala Asp Ile Lys Glu Met Ala Asp Leu Thr Phe Ala Asp Ala Phe Thr
65 70 75 80

Ala Asp Phe Phe Ala Thr Trp Gly Lys Leu Ala Ala Val Arg Thr Pro
85 90 95

Thr Ile Ala Ala Val Ala Gly Tyr Ala Leu Gly Gly Gly Cys Glu Leu
100 105 110

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

Gln Pro Glu Ile Lys Leu Gly Val Leu Pro Gly Met Gly Gly Ser Gln
130 135 140

Arg Leu Thr Arg Ala Ile Gly Lys Ala Lys Ala Met Asp Leu Ile Leu
145 150 155 160

Thr Gly Arg Thr Met Asp Ala Ala Glu Ala Glu Arg Ser Gly Leu Val
165 170 175

Ser Arg Val Val Pro Ala Asp Asp Leu Leu Thr Glu Ala Arg Ala Thr
180 185 190

Ala Thr Thr Ile Ser Gln Met Ser Ala Ser Ala Ala Arg Met Ala Lys
195 200 205

Glu Ala Val Asn Arg Ala Phe Glu Ser Ser Leu Ser Glu Gly Leu Leu
210 215 220

Tyr Glu Arg Arg Leu Phe His Ser Ala Phe Ala Thr Glu Asp Gln Ser
225 230 235 240

Glu Gly Met Ala Ala Phe Ile Glu Lys Arg Ala Pro Gln Phe Thr His
245 250 255

Arg

<210> SEQ ID NO 47
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Met Ala Ala Leu Arg Val Leu Leu Ser Cys Ala Arg Gly Pro Leu Arg
1 5 10 15

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Pro Pro Val Arg Cys Pro Ala Trp Arg Pro Phe Ala Ser Gly Ala Asn
            20                  25                  30

Phe Glu Tyr Ile Ile Ala Glu Lys Arg Gly Lys Asn Asn Thr Val Gly
        35                  40                  45

Leu Ile Gln Leu Asn Arg Pro Lys Ala Leu Asn Ala Leu Cys Asp Gly
    50                  55                  60

Leu Ile Asp Glu Leu Asn Gln Ala Leu Lys Ile Phe Glu Glu Asp Pro
65                  70                  75                  80

Ala Val Gly Ala Ile Val Leu Thr Gly Gly Asp Lys Ala Phe Ala Ala
                85                  90                  95

Gly Ala Asp Ile Lys Glu Met Gln Asn Leu Ser Phe Gln Asp Cys Tyr
            100                 105                 110

Ser Ser Lys Phe Leu Lys His Trp Asp His Leu Thr Gln Val Lys Lys
        115                 120                 125

Pro Val Ile Ala Ala Val Asn Gly Tyr Ala Phe Gly Gly Gly Cys Glu
    130                 135                 140

Leu Ala Met Met Cys Asp Ile Ile Tyr Ala Gly Glu Lys Ala Gln Phe
145                 150                 155                 160

Ala Gln Pro Glu Ile Leu Ile Gly Thr Ile Pro Gly Ala Gly Gly Thr
                165                 170                 175

Gln Arg Leu Thr Arg Ala Val Gly Lys Ser Leu Ala Met Glu Met Val
            180                 185                 190

Leu Thr Gly Asp Arg Ile Ser Ala Gln Asp Ala Lys Gln Ala Gly Leu
        195                 200                 205

Val Ser Lys Ile Cys Pro Val Glu Thr Leu Val Glu Glu Ala Ile Gln
    210                 215                 220

Cys Ala Glu Lys Ile Ala Ser Asn Ser Lys Ile Val Val Ala Met Ala
225                 230                 235                 240

Lys Glu Ser Val Asn Ala Ala Phe Glu Met Thr Leu Thr Glu Gly Ser
                245                 250                 255

Lys Leu Glu Lys Lys Leu Phe Tyr Ser Thr Phe Ala Thr Asp Asp Arg
            260                 265                 270

Lys Glu Gly Met Thr Ala Phe Val Glu Lys Arg Lys Ala Asn Phe Lys
        275                 280                 285

Asp Gln
    290


<210> SEQ ID NO 48
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 48

Met Ala Ala Leu Arg Ala Leu Leu Pro Arg Ala Cys Asn Ser Leu Leu
1               5                   10                  15

Ser Pro Val Arg Cys Pro Glu Phe Arg Arg Phe Ala Ser Gly Ala Asn
            20                  25                  30

Phe Gln Tyr Ile Ile Thr Glu Lys Lys Gly Lys Asn Ser Ser Val Gly
        35                  40                  45

Leu Ile Gln Leu Asn Arg Pro Lys Ala Leu Asn Ala Leu Cys Asn Gly
    50                  55                  60

Leu Ile Glu Glu Leu Asn Gln Ala Leu Glu Thr Phe Glu Glu Asp Pro
65                  70                  75                  80

Ala Val Gly Ala Ile Val Leu Thr Gly Gly Glu Lys Ala Phe Ala Ala
                85                  90                  95
```

```
Gly Ala Asp Ile Lys Glu Met Gln Asn Arg Thr Phe Gln Asp Cys Tyr
            100                 105                 110

Ser Gly Lys Phe Leu Ser His Trp Asp His Ile Thr Arg Ile Lys Lys
        115                 120                 125

Pro Val Ile Ala Ala Val Asn Gly Tyr Ala Leu Gly Gly Gly Cys Glu
    130                 135                 140

Leu Ala Met Met Cys Asp Ile Ile Tyr Ala Gly Glu Lys Ala Gln Phe
145                 150                 155                 160

Gly Gln Pro Glu Ile Leu Leu Gly Thr Ile Pro Gly Ala Gly Gly Thr
                165                 170                 175

Gln Arg Leu Thr Arg Ala Val Gly Lys Ser Leu Ala Met Glu Met Val
            180                 185                 190

Leu Thr Gly Asp Arg Ile Ser Ala Gln Asp Ala Lys Gln Ala Gly Leu
        195                 200                 205

Val Ser Lys Ile Phe Pro Val Glu Thr Leu Val Glu Glu Ala Ile Gln
    210                 215                 220

Cys Ala Glu Lys Ile Ala Asn Asn Ser Lys Ile Ile Val Ala Met Ala
225                 230                 235                 240

Lys Glu Ser Val Asn Ala Ala Phe Glu Met Thr Leu Thr Glu Gly Asn
                245                 250                 255

Lys Leu Glu Lys Lys Leu Phe Tyr Ser Thr Phe Ala Thr Asp Asp Arg
            260                 265                 270

Arg Glu Gly Met Ser Ala Phe Val Glu Lys Arg Lys Ala Asn Phe Lys
        275                 280                 285

Asp His
    290
```

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: n is any nucleotide; w is a or t; y is t or c; s is g or c

<400> SEQUENCE: 49

gaawscggys cnatyggygg 20

<210> SEQ ID NO 50
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 50

ttytgyggyr sbttyacbgc wgg 23

<210> SEQ ID NO 51
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 51

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ccwgcvgtra avsyrccrca raa 23

<210> SEQ ID NO 52
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 52

aaracdsmrc gttcvgtrat rta 23

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 53

tcrayrccsg gwgcrayttc 20

<210> SEQ ID NO 54
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 54

gaatgtttac ttctgcggca ccttcac 27

<210> SEQ ID NO 55
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 55

gaccagatca ctttcaacgg ttcctatg 28

<210> SEQ ID NO 56
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 56

gcataggaac cgttgaaagt gatctgg 27

<210> SEQ ID NO 57
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 57

gttagtaccg aacttgctga cgttgatg 28

<210> SEQ ID NO 58
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 58

atgagaaaag tagaaatcat tac 23

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 59

ggcggaagtt gacgataatg 20

<210> SEQ ID NO 60
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 60

gcwacbggyt ayggycg 17

<210> SEQ ID NO 61
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 61

gtyrtygayr tyggyggyca gga 23

<210> SEQ ID NO 62
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 62

atgaacgaya artgygcwgc wgg 23

<210> SEQ ID NO 63
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 63

tgygcwgcwg gyacbggycg ytt 23

<210> SEQ ID NO 64
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 64

tcctgrccrc crayrtcray rac 23

<210> SEQ ID NO 65
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 65

ccwgcwgcrc ayttrtcgtt cat 23

<210> SEQ ID NO 66
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 66

aarcgrccvg trccwgcwgc rca 23

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 67

gcttcgswtt cracratgsw 20

<210> SEQ ID NO 68
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 68

gswratract tcgcwttcwg craa 24

<210> SEQ ID NO 69
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 69

acgtcatgtc gaaggtactg gaaatcc 27

<210> SEQ ID NO 70
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 70

gggactggta cttcaaatcg aagcatc 27

<210> SEQ ID NO 71
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 71

tgacggcagc gggatgcttc gatttga 27

<210> SEQ ID NO 72
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 72

tcagacatgg ggatttccag taccttc 27

<210> SEQ ID NO 73
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 73

ccgtgttact tgggaaggta tcgctgtctg 30

<210> SEQ ID NO 74
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 74

gccaatgaag gaggaaacca ctaatgagtc 30

<210> SEQ ID NO 75
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 75

gggaattcca tatgaaaact gtgtatactc tc 32

<210> SEQ ID NO 76
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 76

cgacggatcc ttagaggatt tccgagaaag c 31

<210> SEQ ID NO 77
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: n is any nucleotide; y is t or c; b is g, c or t; v is a, g, or c; s is g or c

<400> SEQUENCE: 77

aaycgbccva argcnctsaa ygc 23

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: n is any nucleotide; y is t or c; b is c, g, or t

<400> SEQUENCE: 78

ttygtbgcng gygcngayat 20

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: n is any nucleotide; r is g or a; v is a, g, or c.

<400> SEQUENCE: 79

atrtcngcrc cngcvacraa 20

<210> SEQ ID NO 80
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: n is any nucleotide; r is g or a; s is g or c; w is a or t.

<400> SEQUENCE: 80

ccrccrccsa gngcrwarcc rtt 23

<210> SEQ ID NO 81
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: n is any nucleotide; r is g or a; s is g or c; w is a or t; v is a, g or c.

<400> SEQUENCE: 81

sswngcratv cgratrtcra c 21

<210> SEQ ID NO 82
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 82

cgctgatatt cgccagttgc tcgaag 26

<210> SEQ ID NO 83
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 83

cccatcttgc tttccgcaag attgagc 27

<210> SEQ ID NO 84
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 84

caatggccct gccgaataac gcccatct 28

<210> SEQ ID NO 85
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 85

cttcgagcaa ctggcgaata tcagcg 26

<210> SEQ ID NO 86
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 86

gctcaatctt gcggaaagca agatggg 27

<210> SEQ ID NO 87
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 87

agatgggcgt tattcggcag ggccattg 28

<210> SEQ ID NO 88
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 88

aagctgggtc tgatcgatgc cattgctacc 30

<210> SEQ ID NO 89
<211> LENGTH: 28

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 89

ctcgattatc gcccatccac gtatcgag 28

<210> SEQ ID NO 90
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 90

tggatgcaat ccgctatggc attatccacg 30

<210> SEQ ID NO 91
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 91

tcattcagtg cgttcaccgg cggatttgtc 30

<210> SEQ ID NO 92
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 92

tcgatccgga agtagcgata gcgttcgatg 30

<210> SEQ ID NO 93
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 93

cttggctgca atctcttcga gcacttcagg 30

<210> SEQ ID NO 94
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 94

catcagaggt aatcaccact cgtgca 26

<210> SEQ ID NO 95
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 95

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aagtagtagg ccacctcgtc gccata 26

<210> SEQ ID NO 96
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 96

gccaatcagg cgctgatcta tgttct 26

<210> SEQ ID NO 97
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 97

ctgatctatg ttctggcctc ggaggt 26

<210> SEQ ID NO 98
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 98

ggctgatatc aaagcgatgg ccaatgc 27

<210> SEQ ID NO 99
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 99

ccacgcctat tgatatgctc accagtg 27

<210> SEQ ID NO 100
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 100

gcaaaccggt gattgctgcc gtgaatgg 28

<210> SEQ ID NO 101
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 101

gcattggcca tcgctttgat atcagcc 27

<210> SEQ ID NO 102
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 102

cactggtgag catatcaata ggcgtgg 27

<210> SEQ ID NO 103
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 103

ccattcacgg cagcaatcac cggtttgc 28

<210> SEQ ID NO 104
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 104

tcatcatcgc cagtgaaaac gcgcagttcg 30

<210> SEQ ID NO 105
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 105

ggatcgcgca aaccattgcc accaaatcac 30

<210> SEQ ID NO 106
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 106

atgagtgaag agtctctggt tctcagc 27

<210> SEQ ID NO 107
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 107

agatcgcaat cgctcgtgta tgtc 24

<210> SEQ ID NO 108
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 108

gggaattcca tatgagaaaa gtagaaatca ttacagctg 39

<210> SEQ ID NO 109
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 109

gagagtatac acagttttca cctcctttac agcagagat 39

<210> SEQ ID NO 110
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 110

atctctgctg taaaggaggt gaaaactgtg tatactctc 39

<210> SEQ ID NO 111
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 111

acgttgatct ccttgtacat tagaggattt ccgagaaagc 40

<210> SEQ ID NO 112
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 112

gctttctcgg aaatcctcta atgtacaagg agatcaacgt 40

<210> SEQ ID NO 113
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 113

cgacggatcc tcaacgacca ctgaagttgg 30

<210> SEQ ID NO 114
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 114

cgacggatcc ttagaggatt tccgagaaag c 31

<210> SEQ ID NO 115
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 115

ggtgtctaga gacagtcctg tcgtttatgt agaaggag 38

<210> SEQ ID NO 116
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 116

gggaattcca tatgcgtaac ttcctcctgc tatcaacgac cactgaagtt gg 52

<210> SEQ ID NO 117
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 117

acgttgatct ccttctacat tatttttca gtcccatg 38

<210> SEQ ID NO 118
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 118

ggtgtctaga gtcaaaggag agaacaaaat catgagtg 38

<210> SEQ ID NO 119
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 119

gggaattcca tatgcgtaac ttcctcctgc tattagagga tttccgagaa agc 53

<210> SEQ ID NO 120
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 120

tcagtggtcg ttgatcacgc tataaagaaa ggtgaaaact gtgtatactc tc 52

<210> SEQ ID NO 121
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 121

cgacggatcc cttccttgga gctcatgctt tc 32

<210> SEQ ID NO 122

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<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 122

catgggactg aaaaaataat gtagaaggag atcaacgt 38

<210> SEQ ID NO 123
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 123

gagagtatac acagttttca cctttcttta tagcgtgatc aacgaccact ga 52

<210> SEQ ID NO 124
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 124

ccaacttcag tggtcgttag tgaaaactgt gtatactctc 40

<210> SEQ ID NO 125
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 125

gagagtatac acagttttca ctaacgacca ctgaagttgg 40

<210> SEQ ID NO 126
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 126

gtcgcagaat tcccatcaat cgcagcaatc ccaac 35

<210> SEQ ID NO 127
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 127

taacatggta ccgacagaag cggaccagca aacga 35

<210> SEQ ID NO 128
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 128

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<210> SEQ ID NO 129
<211> LENGTH: 5469
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 129

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gactatcccg ccgattatca gccctggcaa caggcgtttg atgatagtga agcgccgttt 300

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tacacatcgg gtagcaccgg taagcccaag ggtgtgatcc acgttcacgg cggttatgtc 1320

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<210> SEQ ID NO 130
<211> LENGTH: 701
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 130

Met Gly Leu Pro Glu Glu Arg Val Arg Ser Gly Ser Gly Ser Arg Gly
1 5 10 15

Gln Glu Glu Ala Gly Ala Gly Gly Arg Ala Arg Ser Trp Ser Pro Pro
20 25 30

Pro Glu Val Ser Arg Ser Ala His Val Pro Ser Leu Gln Arg Tyr Arg
35 40 45

Glu Leu His Arg Arg Ser Val Glu Glu Pro Arg Glu Phe Trp Gly Asp
50 55 60

Ile Ala Lys Glu Phe Tyr Trp Lys Thr Pro Cys Pro Gly Pro Phe Leu
65 70 75 80

Arg Tyr Asn Phe Asp Val Thr Lys Gly Lys Ile Phe Ile Glu Trp Met
85 90 95

Lys Gly Ala Thr Thr Asn Ile Cys Tyr Asn Val Leu Asp Arg Asn Val
100 105 110

His Glu Lys Lys Leu Gly Asp Lys Val Ala Phe Tyr Trp Glu Gly Asn
115 120 125

Glu Pro Gly Glu Thr Thr Gln Ile Thr Tyr His Gln Leu Leu Val Gln
130 135 140

Val Cys Gln Phe Ser Asn Val Leu Arg Lys Gln Gly Ile Gln Lys Gly
145 150 155 160

Asp Arg Val Ala Ile Tyr Met Pro Met Ile Pro Glu Leu Val Val Ala
165 170 175

Met Leu Ala Cys Ala Arg Ile Gly Ala Leu His Ser Ile Val Phe Ala

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            180                 185                 190

Gly Phe Ser Ser Glu Ser Leu Cys Glu Arg Ile Leu Asp Ser Ser Cys
        195                 200                 205

Ser Leu Leu Ile Thr Thr Asp Ala Phe Tyr Arg Gly Glu Lys Leu Val
    210                 215                 220

Asn Leu Lys Glu Leu Ala Asp Glu Ala Leu Gln Lys Cys Gln Glu Lys
225                 230                 235                 240

Gly Phe Pro Val Arg Cys Cys Ile Val Val Lys His Leu Gly Arg Ala
                245                 250                 255

Glu Leu Gly Met Gly Asp Ser Thr Ser Gln Ser Pro Pro Ile Lys Arg
            260                 265                 270

Ser Cys Pro Asp Val Gln Ile Ser Trp Asn Gln Gly Ile Asp Leu Trp
        275                 280                 285

Trp His Glu Leu Met Gln Glu Ala Gly Asp Glu Cys Glu Pro Glu Trp
    290                 295                 300

Cys Asp Ala Glu Asp Pro Leu Phe Ile Leu Tyr Thr Ser Gly Ser Thr
305                 310                 315                 320

Gly Lys Pro Lys Gly Val Val His Thr Val Gly Gly Tyr Met Leu Tyr
                325                 330                 335

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

Phe Trp Cys Thr Ala Asp Ile Gly Trp Ile Thr Gly His Ser Tyr Val
        355                 360                 365

Thr Tyr Gly Pro Leu Ala Asn Gly Ala Thr Ser Val Leu Phe Glu Gly
    370                 375                 380

Ile Pro Thr Tyr Pro Asp Val Asn Arg Leu Trp Ser Ile Val Asp Lys
385                 390                 395                 400

Tyr Lys Val Thr Lys Phe Tyr Thr Ala Pro Thr Ala Ile Arg Leu Leu
                405                 410                 415

Met Lys Phe Gly Asp Glu Pro Val Thr Lys His Ser Arg Ala Ser Leu
            420                 425                 430

Gln Val Leu Gly Thr Val Gly Glu Pro Ile Asn Pro Glu Ala Trp Leu
        435                 440                 445

Trp Tyr His Arg Val Val Gly Ala Gln Arg Cys Pro Ile Val Asp Thr
    450                 455                 460

Phe Trp Gln Thr Glu Thr Gly Gly His Met Leu Thr Pro Leu Pro Gly
465                 470                 475                 480

Ala Thr Pro Met Lys Pro Gly Ser Ala Thr Phe Pro Phe Phe Gly Val
                485                 490                 495

Ala Pro Ala Ile Leu Asn Glu Ser Gly Glu Glu Leu Glu Gly Glu Ala
            500                 505                 510

Glu Gly Tyr Leu Val Phe Lys Gln Pro Trp Pro Gly Ile Met Arg Thr
        515                 520                 525

Val Tyr Gly Asn His Glu Arg Phe Glu Thr Thr Tyr Phe Lys Lys Phe
    530                 535                 540

Pro Gly Tyr Tyr Val Thr Gly Asp Gly Cys Gln Arg Asp Gln Asp Gly
545                 550                 555                 560

Tyr Tyr Trp Ile Thr Gly Arg Ile Asp Asp Met Leu Asn Val Ser Gly
                565                 570                 575

His Leu Leu Ser Thr Ala Glu Val Glu Ser Ala Leu Val Glu His Glu
            580                 585                 590

Ala Val Ala Glu Ala Ala Val Val Gly His Pro His Pro Val Lys Gly
        595                 600                 605
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Glu Cys Leu Tyr Cys Phe Val Thr Leu Cys Asp Gly His Thr Phe Ser
    610                 615                 620

Pro Lys Leu Thr Glu Glu Leu Lys Lys Gln Ile Arg Glu Lys Ile Gly
625                 630                 635                 640

Pro Ile Ala Thr Pro Asp Tyr Ile Gln Asn Ala Pro Gly Leu Pro Lys
                645                 650                 655

Thr Arg Ser Gly Lys Ile Met Arg Arg Val Leu Arg Lys Ile Ala Gln
            660                 665                 670

Asn Asp His Asp Leu Gly Asp Met Ser Thr Val Ala Asp Pro Ser Val
        675                 680                 685

Ile Ser His Leu Phe Ser His Arg Cys Leu Thr Ile Gln
    690                 695                 700


<210> SEQ ID NO 131
<211> LENGTH: 670
<212> TYPE: PRT
<213> ORGANISM: Pyrobaculum aerophilum

<400> SEQUENCE: 131

Met Ser Leu Glu Leu Lys Glu Lys Glu Ser Glu Leu Pro Phe Asp Glu
1               5                   10                  15

Gln Ile Ile Asn Asp Lys Trp Arg Ser Lys Tyr Thr Pro Ile Asp Ala
            20                  25                  30

Tyr Phe Lys Phe His Arg Gln Thr Val Glu Asn Leu Glu Ser Phe Trp
        35                  40                  45

Glu Ser Val Ala Lys Glu Leu Glu Trp Phe Lys Pro Trp Asp Lys Val
    50                  55                  60

Leu Asp Ala Ser Asn Pro Pro Phe Tyr Lys Trp Phe Val Gly Gly Arg
65                  70                  75                  80

Leu Asn Leu Ser Tyr Leu Ala Val Asp Arg His Val Lys Thr Trp Arg
                85                  90                  95

Lys Asn Lys Leu Ala Ile Glu Trp Glu Gly Glu Pro Val Asp Glu Asn
            100                 105                 110

Gly Tyr Pro Thr Asp Arg Arg Lys Leu Thr Tyr Tyr Asp Leu Tyr Arg
        115                 120                 125

Glu Val Asn Arg Val Ala Tyr Met Leu Lys Gln Asn Phe Gly Val Lys
    130                 135                 140

Lys Gly Asp Lys Ile Thr Leu Tyr Leu Pro Met Val Pro Glu Leu Pro
145                 150                 155                 160

Ile Thr Met Leu Ala Ala Trp Arg Ile Gly Ala Ile Thr Ser Val Val
                165                 170                 175

Phe Ser Gly Phe Ser Ala Asp Ala Leu Ala Glu Arg Ile Asn Asp Ser
            180                 185                 190

Gln Ser Arg Ile Val Ile Thr Ala Asp Gly Phe Trp Arg Arg Gly Arg
        195                 200                 205

Val Val Arg Leu Lys Glu Val Val Asp Ala Ala Leu Glu Lys Ala Thr
    210                 215                 220

Gly Val Glu Ser Val Ile Val Leu Pro Arg Leu Gly Leu Lys Asp Val
225                 230                 235                 240

Pro Met Thr Glu Gly Arg Asp Tyr Trp Trp Asn Lys Leu Met Gln Gly
                245                 250                 255

Ile Pro Pro Asn Ala Tyr Ile Glu Pro Glu Pro Val Glu Ser Glu His
            260                 265                 270

Pro Ser Phe Ile Leu Tyr Thr Ser Gly Thr Thr Gly Lys Pro Lys Gly
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        275                 280                 285

Ile Val His Asp Thr Gly Gly Trp Ala Val His Val Tyr Ala Thr Met
    290                 295                 300

Lys Trp Val Phe Asp Ile Arg Asp Asp Asp Ile Phe Trp Cys Thr Ala
305                 310                 315                 320

Asp Ile Gly Trp Val Thr Gly His Ser Tyr Val Val Leu Gly Pro Leu
                325                 330                 335

Leu Met Gly Ala Thr Glu Val Ile Tyr Glu Gly Ala Pro Asp Tyr Pro
            340                 345                 350

Gln Pro Asp Arg Trp Trp Ser Ile Ile Glu Arg Tyr Gly Val Thr Ile
        355                 360                 365

Phe Tyr Thr Ser Pro Thr Ala Ile Arg Met Phe Met Arg Tyr Gly Glu
    370                 375                 380

Glu Trp Pro Arg Lys His Asp Leu Ser Thr Leu Arg Ile Ile His Ser
385                 390                 395                 400

Val Gly Glu Pro Ile Asn Pro Glu Ala Trp Arg Trp Ala Tyr Arg Val
                405                 410                 415

Leu Gly Asn Glu Lys Val Ala Phe Gly Ser Thr Trp Trp Met Thr Glu
            420                 425                 430

Thr Gly Gly Ile Val Ile Ser His Ala Pro Gly Leu Tyr Leu Val Pro
        435                 440                 445

Met Lys Pro Gly Thr Asn Gly Pro Pro Leu Pro Gly Phe Glu Val Asp
    450                 455                 460

Val Val Asp Glu Asn Gly Asn Pro Ala Pro Pro Gly Val Lys Gly Tyr
465                 470                 475                 480

Leu Val Ile Lys Lys Pro Trp Pro Gly Met Leu His Gly Ile Trp Gly
                485                 490                 495

Asp Pro Glu Arg Tyr Ile Lys Thr Tyr Trp Ser Arg Phe Pro Gly Met
            500                 505                 510

Phe Tyr Ala Gly Asp Tyr Ala Ile Lys Asp Lys Asp Gly Tyr Ile Trp
        515                 520                 525

Val Leu Gly Arg Ala Asp Glu Val Ile Lys Val Ala Gly His Arg Leu
    530                 535                 540

Gly Thr Tyr Glu Leu Glu Ser Ala Leu Ile Ser His Pro Ala Val Ala
545                 550                 555                 560

Glu Ser Ala Val Val Gly Val Pro Asp Ala Ile Lys Gly Glu Val Pro
                565                 570                 575

Ile Ala Phe Val Val Leu Lys Gln Gly Val Ala Pro Ser Asp Glu Leu
            580                 585                 590

Arg Lys Glu Leu Arg Glu His Val Arg Arg Thr Ile Gly Pro Ile Ala
        595                 600                 605

Glu Pro Ala Gln Ile Phe Phe Val Thr Lys Leu Pro Lys Thr Arg Ser
    610                 615                 620

Gly Lys Ile Met Arg Arg Leu Leu Lys Ala Val Ala Thr Gly Ala Pro
625                 630                 635                 640

Leu Gly Asp Val Thr Thr Leu Glu Asp Glu Thr Ser Val Glu Glu Ala
                645                 650                 655

Lys Arg Ala Tyr Glu Glu Ile Lys Ala Glu Met Ala Arg Thr
            660                 665                 670
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<210> SEQ ID NO 132
<211> LENGTH: 261
<212> TYPE: PRT
<213> ORGANISM: Clostridium acetobutylicum

<400> SEQUENCE: 132

Met Glu Leu Asn Asn Val Ile Leu Glu Lys Glu Gly Lys Val Ala Val
1 5 10 15

Val Thr Ile Asn Arg Pro Lys Ala Leu Asn Ala Leu Asn Ser Asp Thr
20 25 30

Leu Lys Glu Met Asp Tyr Val Ile Gly Glu Ile Glu Asn Asp Ser Glu
35 40 45

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

Gly Ala Asp Ile Ser Glu Met Lys Glu Met Asn Thr Ile Glu Gly Arg
65 70 75 80

Lys Phe Gly Ile Leu Gly Asn Lys Val Phe Arg Arg Leu Glu Leu Leu
85 90 95

Glu Lys Pro Val Ile Ala Ala Val Asn Gly Phe Ala Leu Gly Gly Gly
100 105 110

Cys Glu Ile Ala Met Ser Cys Asp Ile Arg Ile Ala Ser Ser Asn Ala
115 120 125

Arg Phe Gly Gln Pro Glu Val Gly Leu Gly Ile Thr Pro Gly Phe Gly
130 135 140

Gly Thr Gln Arg Leu Ser Arg Leu Val Gly Met Gly Met Ala Lys Gln
145 150 155 160

Leu Ile Phe Thr Ala Gln Asn Ile Lys Ala Asp Glu Ala Leu Arg Ile
165 170 175

Gly Leu Val Asn Lys Val Val Glu Pro Ser Glu Leu Met Asn Thr Ala
180 185 190

Lys Glu Ile Ala Asn Lys Ile Val Ser Asn Ala Pro Val Ala Val Lys
195 200 205

Leu Ser Lys Gln Ala Ile Asn Arg Gly Met Gln Cys Asp Ile Asp Thr
210 215 220

Ala Leu Ala Phe Glu Ser Glu Ala Phe Gly Glu Cys Phe Ser Thr Glu
225 230 235 240

Asp Gln Lys Asp Ala Met Thr Ala Phe Ile Glu Lys Arg Lys Ile Glu
245 250 255

Gly Phe Lys Asn Arg
260

<210> SEQ ID NO 133
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Thermoanaerobacterium thermosaccharolyticum

<400> SEQUENCE: 133

Met Asp Phe Asn Asn Val Leu Leu Asn Lys Asp Asp Gly Ile Ala Leu
1 5 10 15

Ile Ile Ile Asn Arg Pro Lys Ala Leu Asn Ala Leu Asn Tyr Glu Thr
20 25 30

Leu Lys Glu Leu Asp Ser Val Leu Asp Ile Val Glu Asn Asp Lys Glu
35 40 45

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

Gly Ala Asp Ile Ala Glu Met Ser Asn Met Thr Pro Leu Glu Ala Lys
65 70 75 80

Lys Phe Ser Leu Tyr Gly Gln Lys Val Phe Arg Lys Ile Glu Met Leu
85 90 95

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Ser Lys Pro Val Ile Ala Ala Val Asn Gly Phe Ala Leu Gly Gly Gly
            100                 105                 110

Cys Glu Leu Ser Met Ala Cys Asp Ile Arg Ile Ala Ser Lys Asn Ala
        115                 120                 125

Lys Phe Gly Gln Pro Glu Val Gly Leu Gly Ile Ile Pro Gly Phe Ser
    130                 135                 140

Gly Thr Gln Arg Leu Pro Arg Leu Ile Gly Thr Ser Lys Ala Lys Glu
145                 150                 155                 160

Leu Ile Phe Thr Gly Asp Met Ile Asn Ser Asp Glu Ala Tyr Lys Ile
                165                 170                 175

Gly Leu Ile Ser Lys Val Val Glu Leu Ser Asp Leu Ile Glu Glu Ala
            180                 185                 190

Lys Lys Leu Ala Lys Lys Met Met Ser Lys Ser Gln Ile Ala Ile Ser
        195                 200                 205

Leu Ala Lys Glu Ala Ile Asn Lys Gly Met Glu Thr Asp Leu Asp Thr
    210                 215                 220

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

Asp Gln Lys Glu Gly Met Ile Ala Phe Ser Glu Lys Arg Ala Pro Lys
                245                 250                 255

Phe Gly Lys


<210> SEQ ID NO 134
<211> LENGTH: 432
<212> TYPE: PRT
<213> ORGANISM: Methylobacterium extorquens

<400> SEQUENCE: 134

Met Ala Ala Ser Ala Ala Pro Ala Trp Thr Gly Gln Thr Ala Glu Ala
1               5                   10                  15

Lys Asp Leu Tyr Glu Leu Gly Glu Ile Pro Pro Leu Gly His Val Pro
            20                  25                  30

Ala Lys Met Tyr Ala Trp Ala Ile Arg Arg Glu Arg His Gly Pro Pro
        35                  40                  45

Glu Gln Ser His Gln Leu Glu Val Leu Pro Val Trp Glu Ile Gly Asp
    50                  55                  60

Asp Glu Val Leu Val Tyr Val Met Ala Ala Gly Val Asn Tyr Asn Gly
65                  70                  75                  80

Val Trp Ala Gly Leu Gly Glu Pro Ile Ser Pro Phe Asp Val His Lys
                85                  90                  95

Gly Glu Tyr His Ile Ala Gly Ser Asp Ala Ser Gly Ile Val Trp Lys
            100                 105                 110

Val Gly Ala Lys Val Lys Arg Trp Lys Val Gly Asp Glu Val Ile Val
        115                 120                 125

His Cys Asn Gln Asp Asp Gly Asp Asp Glu Glu Cys Asn Gly Gly Asp
    130                 135                 140

Pro Met Phe Ser Pro Thr Gln Arg Ile Trp Gly Tyr Glu Thr Gly Asp
145                 150                 155                 160

Gly Ser Phe Ala Gln Phe Cys Arg Val Gln Ser Arg Gln Leu Met Ala
                165                 170                 175

Arg Pro Lys His Leu Thr Trp Glu Glu Ala Ala Cys Tyr Thr Leu Thr
            180                 185                 190

Leu Ala Thr Ala Tyr Arg Met Leu Phe Gly His Ala Pro His Thr Val
        195                 200                 205
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Arg Pro Gly Gln Asn Val Leu Ile Trp Gly Ala Ser Gly Gly Leu Gly
    210                 215                 220

Val Phe Gly Val Gln Leu Cys Ala Ala Ser Gly Ala Asn Ala Ile Ala
225                 230                 235                 240

Val Ile Ser Asp Glu Ser Lys Arg Asp Tyr Val Met Ser Leu Gly Ala
                245                 250                 255

Lys Gly Val Ile Asn Arg Lys Asp Phe Asp Cys Trp Gly Gln Leu Pro
            260                 265                 270

Thr Val Asn Ser Pro Glu Tyr Asn Thr Trp Leu Lys Glu Ala Arg Lys
        275                 280                 285

Phe Gly Lys Ala Ile Trp Asp Ile Thr Gly Lys Gly Asn Asp Val Asp
    290                 295                 300

Ile Val Phe Glu His Pro Gly Glu Ala Thr Phe Pro Val Ser Thr Leu
305                 310                 315                 320

Val Ala Lys Arg Gly Gly Met Ile Val Phe Cys Ala Gly Thr Thr Gly
                325                 330                 335

Phe Asn Ile Thr Phe Asp Ala Arg Tyr Val Trp Met Arg Gln Lys Arg
            340                 345                 350

Ile Gln Gly Ser His Phe Ala His Leu Lys Gln Ala Ser Ala Ala Asn
        355                 360                 365

Gln Phe Val Met Asp Arg Arg Val Asp Pro Cys Met Ser Glu Val Phe
    370                 375                 380

Pro Trp Asp Lys Ile Pro Ala Ala His Thr Lys Met Trp Lys Asn Gln
385                 390                 395                 400

His Pro Pro Gly Asn Met Ala Val Leu Val Asn Ser Thr Arg Ala Gly
                405                 410                 415

Leu Arg Thr Val Glu Asp Val Ile Glu Ala Gly Pro Leu Lys Ala Met
            420                 425                 430


<210> SEQ ID NO 135
<211> LENGTH: 424
<212> TYPE: PRT
<213> ORGANISM: Caulobacter crescentus

<400> SEQUENCE: 135

Met Thr Ile Gln Thr Leu Glu Thr Thr Ala Leu Lys Asp Leu Tyr Glu
1               5                   10                  15

Ile Gly Glu Ile Pro Pro Ala Phe His Val Pro Lys Thr Met Tyr Ala
            20                  25                  30

Trp Ser Ile Arg Lys Glu Arg His Gly Lys Pro Thr Gln Ala Met Gln
        35                  40                  45

Val Glu Val Val Pro Thr Trp Glu Ile Gly Glu Asp Glu Val Leu Val
    50                  55                  60

Leu Val Met Ala Ala Gly Val Asn Tyr Asn Gly Val Trp Ala Ala Leu
65                  70                  75                  80

Gly Glu Pro Ile Ser Pro Leu Asp Gly His Lys Gln Pro Phe His Ile
                85                  90                  95

Ala Gly Ser Asp Ala Ser Gly Ile Val Trp Lys Val Gly Ala Lys Val
            100                 105                 110

Lys Arg Trp Lys Leu Gly Asp Glu Val Val Ile His Cys Asn Gln Asp
        115                 120                 125

Asp Gly Asp Asp Glu Glu Cys Asn Gly Gly Asp Pro Met Phe Ser Ser
    130                 135                 140

Ser Gln Arg Ile Trp Gly Tyr Glu Thr Pro Asp Gly Ser Phe Ala Gln
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145                 150                 155                 160

Phe Cys Arg Val Gln Ser Arg Gln Leu Leu Pro Arg Pro Lys His Leu
                165                 170                 175

Thr Trp Glu Glu Ser Ala Cys Tyr Thr Leu Thr Leu Ala Thr Ala Tyr
            180                 185                 190

Arg Met Leu Phe Gly His Lys Pro His Glu Leu Lys Pro Gly Gln Asn
        195                 200                 205

Val Leu Val Trp Gly Ala Ser Gly Gly Leu Gly Val Phe Ala Thr Gln
    210                 215                 220

Leu Ala Ala Val Ala Gly Ala Asn Ala Ile Gly Val Val Ser Ser Glu
225                 230                 235                 240

Asp Lys Arg Glu Phe Val Leu Ser Met Gly Ala Lys Ala Val Leu Asn
                245                 250                 255

Arg Gly Glu Phe Asn Cys Trp Gly Gln Leu Pro Lys Val Asn Gly Pro
            260                 265                 270

Glu Phe Asn Asp Tyr Met Lys Glu Ser Arg Lys Phe Gly Lys Ala Ile
        275                 280                 285

Trp Gln Ile Thr Gly Asn Lys Asp Val Asp Met Val Phe Glu His Pro
    290                 295                 300

Gly Glu Gln Thr Phe Pro Val Ser Val Phe Leu Val Lys Arg Gly Gly
305                 310                 315                 320

Met Val Val Ile Cys Ala Gly Thr Thr Gly Phe Asn Leu Thr Met Asp
                325                 330                 335

Ala Arg Phe Leu Trp Met Arg Gln Lys Arg Val Gln Gly Ser His Phe
            340                 345                 350

Ala Asn Leu Met Gln Ala Ser Ala Ala Asn Gln Leu Val Ile Asp Arg
        355                 360                 365

Arg Val Asp Pro Cys Leu Ser Glu Val Phe Pro Trp Asp Gln Ile Pro
    370                 375                 380

Ala Ala His Glu Lys Met Leu Ala Asn Gln His Leu Pro Gly Asn Met
385                 390                 395                 400

Ala Val Leu Val Cys Ala Gln Arg Pro Gly Leu Arg Thr Phe Glu Glu
                405                 410                 415

Val Gln Glu Leu Ser Gly Ala Pro
            420
```

<210> SEQ ID NO 136
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 136

gggaattcca tatgatcgac actgcg 26

<210> SEQ ID NO 137
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 137

cgaaggatcc aacgataatc ggctcagcac 30

<210> SEQ ID NO 138

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<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 138

ataggcggcc gcaggaatgc tgtatgagcg aagaaagctt attc 44

<210> SEQ ID NO 139
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 139

atgctcgcat ctcgagtagc taaattaaat tacatcaata gta 43

<210> SEQ ID NO 140
<211> LENGTH: 3678
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(3675)

<400> SEQUENCE: 140

```
atg gcg acg ggg gag tcc atg agc gga aca gga cga ctg gca gga aag       48
Met Ala Thr Gly Glu Ser Met Ser Gly Thr Gly Arg Leu Ala Gly Lys
1               5                  10                  15

att gcg tta att acc ggt ggc gcc ggc aat atc ggc agt gaa ttg aca       96
Ile Ala Leu Ile Thr Gly Gly Ala Gly Asn Ile Gly Ser Glu Leu Thr
            20                  25                  30

cgt cgc ttt ctc gca gag gga gcg acg gtc att att agt gga cgg aat      144
Arg Arg Phe Leu Ala Glu Gly Ala Thr Val Ile Ile Ser Gly Arg Asn
        35                  40                  45

cgg gcg aag ttg acc gca ctg gcc gaa cgg atg cag gca gag gca gga      192
Arg Ala Lys Leu Thr Ala Leu Ala Glu Arg Met Gln Ala Glu Ala Gly
    50                  55                  60

gtg ccg gca aag cgc atc gat ctc gaa gtc atg gat ggg agt gat ccg      240
Val Pro Ala Lys Arg Ile Asp Leu Glu Val Met Asp Gly Ser Asp Pro
65                  70                  75                  80

gtc gcg gta cgt gcc ggt atc gaa gcg att gtg gcc cgt cac ggc cag      288
Val Ala Val Arg Ala Gly Ile Glu Ala Ile Val Ala Arg His Gly Gln
                85                  90                  95

atc gac att ctg gtc aac aat gca gga agt gcc ggt gcc cag cgt cgt      336
Ile Asp Ile Leu Val Asn Asn Ala Gly Ser Ala Gly Ala Gln Arg Arg
            100                 105                 110

ctg gcc gag att cca ctc act gaa gct gaa tta ggc cct ggc gcc gaa      384
Leu Ala Glu Ile Pro Leu Thr Glu Ala Glu Leu Gly Pro Gly Ala Glu
        115                 120                 125

gag acg ctt cat gcc agc atc gcc aat tta ctt ggt atg gga tgg cat      432
Glu Thr Leu His Ala Ser Ile Ala Asn Leu Leu Gly Met Gly Trp His
    130                 135                 140

ctg atg cgt att gcg gca cct cat atg ccg gta gga agt gcg gtc atc      480
Leu Met Arg Ile Ala Ala Pro His Met Pro Val Gly Ser Ala Val Ile
145                 150                 155                 160

aat gtc tcg acc atc ttt tca cgg gct gag tac tac ggg cgg att ccg      528
Asn Val Ser Thr Ile Phe Ser Arg Ala Glu Tyr Tyr Gly Arg Ile Pro
                165                 170                 175

tat gtc acc cct aaa gct gct ctt aat gct cta tct caa ctt gct gcg      576
Tyr Val Thr Pro Lys Ala Ala Leu Asn Ala Leu Ser Gln Leu Ala Ala
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           180                 185                 190

cgt gag tta ggt gca cgt ggc atc cgc gtt aat acg atc ttt ccc ggc      624
Arg Glu Leu Gly Ala Arg Gly Ile Arg Val Asn Thr Ile Phe Pro Gly
        195                 200                 205

ccg att gaa agt gat cgc atc cgt aca gtg ttc cag cgt atg gat cag      672
Pro Ile Glu Ser Asp Arg Ile Arg Thr Val Phe Gln Arg Met Asp Gln
    210                 215                 220

ctc aag ggg cgg ccc gaa ggc gac aca gcg cac cat ttt ttg aac acc      720
Leu Lys Gly Arg Pro Glu Gly Asp Thr Ala His His Phe Leu Asn Thr
225                 230                 235                 240

atg cga ttg tgt cgt gcc aac gac cag ggc gcg ctt gaa cgt cgg ttc      768
Met Arg Leu Cys Arg Ala Asn Asp Gln Gly Ala Leu Glu Arg Arg Phe
                245                 250                 255

ccc tcc gtc ggt gat gtg gca gac gcc gct gtc ttt ctg gcc agt gcc      816
Pro Ser Val Gly Asp Val Ala Asp Ala Ala Val Phe Leu Ala Ser Ala
            260                 265                 270

gaa tcc gcc gct ctc tcc ggt gag acg att gag gtt acg cac gga atg      864
Glu Ser Ala Ala Leu Ser Gly Glu Thr Ile Glu Val Thr His Gly Met
        275                 280                 285

gag ttg ccg gcc tgc agt gag acc agc ctg ctg gcc cgt act gat ctg      912
Glu Leu Pro Ala Cys Ser Glu Thr Ser Leu Leu Ala Arg Thr Asp Leu
    290                 295                 300

cgc acg att gat gcc agt ggc cgc acg acg ctc atc tgc gcc ggc gac      960
Arg Thr Ile Asp Ala Ser Gly Arg Thr Thr Leu Ile Cys Ala Gly Asp
305                 310                 315                 320

cag att gaa gag gtg atg gcg ctc acc ggt atg ttg cgt acc tgt ggg     1008
Gln Ile Glu Glu Val Met Ala Leu Thr Gly Met Leu Arg Thr Cys Gly
                325                 330                 335

agt gaa gtg atc atc ggc ttc cgt tcg gct gcg gcg ctg gcc cag ttc     1056
Ser Glu Val Ile Ile Gly Phe Arg Ser Ala Ala Ala Leu Ala Gln Phe
            340                 345                 350

gag cag gca gtc aat gag agt cgg cgg ctg gcc ggc gca gac ttt acg     1104
Glu Gln Ala Val Asn Glu Ser Arg Arg Leu Ala Gly Ala Asp Phe Thr
        355                 360                 365

cct ccc att gcc ttg cca ctc gat cca cgc gat ccg gca aca att gac     1152
Pro Pro Ile Ala Leu Pro Leu Asp Pro Arg Asp Pro Ala Thr Ile Asp
    370                 375                 380

gct gtc ttc gat tgg gcc ggc gag aat acc ggc ggg att cat gca gcg     1200
Ala Val Phe Asp Trp Ala Gly Glu Asn Thr Gly Gly Ile His Ala Ala
385                 390                 395                 400

gtg att ctg cct gct acc agt cac gaa ccg gca ccg tgc gtg att gag     1248
Val Ile Leu Pro Ala Thr Ser His Glu Pro Ala Pro Cys Val Ile Glu
                405                 410                 415

gtt gat gat gag cgg gtg ctg aat ttt ctg gcc gat gaa atc acc ggg     1296
Val Asp Asp Glu Arg Val Leu Asn Phe Leu Ala Asp Glu Ile Thr Gly
            420                 425                 430

aca att gtg att gcc agt cgc ctg gcc cgt tac tgg cag tcg caa cgg     1344
Thr Ile Val Ile Ala Ser Arg Leu Ala Arg Tyr Trp Gln Ser Gln Arg
        435                 440                 445

ctt acc ccc ggc gca cgt gcg cgt ggg ccg cgt gtc att ttt ctc tcg     1392
Leu Thr Pro Gly Ala Arg Ala Arg Gly Pro Arg Val Ile Phe Leu Ser
    450                 455                 460

aac ggt gcc gat caa aat ggg aat gtt tac gga cgc att caa agt gcc     1440
Asn Gly Ala Asp Gln Asn Gly Asn Val Tyr Gly Arg Ile Gln Ser Ala
465                 470                 475                 480

gct atc ggt cag ctc att cgt gtg tgg cgt cac gag gct gaa ctt gac     1488
Ala Ile Gly Gln Leu Ile Arg Val Trp Arg His Glu Ala Glu Leu Asp
                485                 490                 495

tat cag cgt gcc agc gcc gcc ggt gat cat gtg ctg ccg ccg gta tgg     1536
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```
Tyr Gln Arg Ala Ser Ala Ala Gly Asp His Val Leu Pro Pro Val Trp
            500                 505                 510

gcc aat cag att gtg cgc ttc gct aac cgc agc ctt gaa ggg tta gaa     1584
Ala Asn Gln Ile Val Arg Phe Ala Asn Arg Ser Leu Glu Gly Leu Glu
        515                 520                 525

ttt gcc tgt gcc tgg aca gct caa ttg ctc cat agt caa cgc cat atc     1632
Phe Ala Cys Ala Trp Thr Ala Gln Leu Leu His Ser Gln Arg His Ile
    530                 535                 540

aat gag att acc ctc aac atc cct gcc aac att agc gcc acc acc ggc     1680
Asn Glu Ile Thr Leu Asn Ile Pro Ala Asn Ile Ser Ala Thr Thr Gly
545                 550                 555                 560

gca cgc agt gca tcg gtc gga tgg gcg gaa agc ctg atc ggg ttg cat     1728
Ala Arg Ser Ala Ser Val Gly Trp Ala Glu Ser Leu Ile Gly Leu His
                565                 570                 575

ttg ggg aaa gtt gcc ttg att acc ggt ggc agc gcc ggt att ggt ggg     1776
Leu Gly Lys Val Ala Leu Ile Thr Gly Gly Ser Ala Gly Ile Gly Gly
            580                 585                 590

cag atc ggg cgc ctc ctg gct ttg agt ggc gcg cgc gtg atg ctg gca     1824
Gln Ile Gly Arg Leu Leu Ala Leu Ser Gly Ala Arg Val Met Leu Ala
        595                 600                 605

gcc cgt gat cgg cat aag ctc gaa cag atg cag gcg atg atc caa tct     1872
Ala Arg Asp Arg His Lys Leu Glu Gln Met Gln Ala Met Ile Gln Ser
    610                 615                 620

gag ctg gct gag gtg ggg tat acc gat gtc gaa gat cgc gtc cac att     1920
Glu Leu Ala Glu Val Gly Tyr Thr Asp Val Glu Asp Arg Val His Ile
625                 630                 635                 640

gca ccg ggc tgc gat gtg agt agc gaa gcg cag ctt gcg gat ctt gtt     1968
Ala Pro Gly Cys Asp Val Ser Ser Glu Ala Gln Leu Ala Asp Leu Val
                645                 650                 655

gaa cgt acc ctg tca gct ttt ggc acc gtc gat tat ctg atc aac aac     2016
Glu Arg Thr Leu Ser Ala Phe Gly Thr Val Asp Tyr Leu Ile Asn Asn
            660                 665                 670

gcc ggg atc gcc ggt gtc gaa gag atg gtt atc gat atg cca gtt gag     2064
Ala Gly Ile Ala Gly Val Glu Glu Met Val Ile Asp Met Pro Val Glu
        675                 680                 685

gga tgg cgc cat acc ctc ttc gcc aat ctg atc agc aac tac tcg ttg     2112
Gly Trp Arg His Thr Leu Phe Ala Asn Leu Ile Ser Asn Tyr Ser Leu
    690                 695                 700

atg cgc aaa ctg gcg ccg ttg atg aaa aaa cag ggt agc ggt tac atc     2160
Met Arg Lys Leu Ala Pro Leu Met Lys Lys Gln Gly Ser Gly Tyr Ile
705                 710                 715                 720

ctt aac gtc tca tca tac ttt ggc ggt gaa aaa gat gcg gcc att ccc     2208
Leu Asn Val Ser Ser Tyr Phe Gly Gly Glu Lys Asp Ala Ala Ile Pro
                725                 730                 735

tac ccc aac cgt gcc gat tac gcc gtc tcg aag gct ggt cag cgg gca     2256
Tyr Pro Asn Arg Ala Asp Tyr Ala Val Ser Lys Ala Gly Gln Arg Ala
            740                 745                 750

atg gcc gaa gtc ttt gcg cgc ttc ctt ggc ccg gag ata cag atc aat     2304
Met Ala Glu Val Phe Ala Arg Phe Leu Gly Pro Glu Ile Gln Ile Asn
        755                 760                 765

gcc att gcg ccg ggt ccg gtc gaa ggt gat cgc ttg cgc ggt acc ggt     2352
Ala Ile Ala Pro Gly Pro Val Glu Gly Asp Arg Leu Arg Gly Thr Gly
    770                 775                 780

gaa cgt ccc ggc ctc ttt gcc cgt cgg gcg cgg ctg att ttg gag aac     2400
Glu Arg Pro Gly Leu Phe Ala Arg Arg Ala Arg Leu Ile Leu Glu Asn
785                 790                 795                 800

aag cgg ctg aat gag ctt cac gct gct ctt atc gcg gct gcg cgc acc     2448
Lys Arg Leu Asn Glu Leu His Ala Ala Leu Ile Ala Ala Ala Arg Thr
                805                 810                 815
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gat gag cga tct atg cac gaa ctg gtt gaa ctg ctc tta ccc aat gat     2496
Asp Glu Arg Ser Met His Glu Leu Val Glu Leu Leu Leu Pro Asn Asp
            820                 825                 830

gtg gcc gca cta gag cag aat ccc gca gca cct acc gcg ttg cgt gaa     2544
Val Ala Ala Leu Glu Gln Asn Pro Ala Ala Pro Thr Ala Leu Arg Glu
        835                 840                 845

ctg gca cga cgt ttt cgc agc gaa ggc gat ccg gcg gca tca tca agc     2592
Leu Ala Arg Arg Phe Arg Ser Glu Gly Asp Pro Ala Ala Ser Ser Ser
    850                 855                 860

agt gcg ctg ctg aac cgt tca att gcc gct aaa ttg ctg gct cgt ttg     2640
Ser Ala Leu Leu Asn Arg Ser Ile Ala Ala Lys Leu Leu Ala Arg Leu
865                 870                 875                 880

cat aat ggt ggc tat gtg ttg cct gcc gac atc ttt gca aac ctg cca     2688
His Asn Gly Gly Tyr Val Leu Pro Ala Asp Ile Phe Ala Asn Leu Pro
                885                 890                 895

aac ccg ccc gat ccc ttc ttc acc cga gcc cag att gat cgc gag gct     2736
Asn Pro Pro Asp Pro Phe Phe Thr Arg Ala Gln Ile Asp Arg Glu Ala
            900                 905                 910

cgc aag gtt cgt gac ggc atc atg ggg atg ctc tac ctg caa cgg atg     2784
Arg Lys Val Arg Asp Gly Ile Met Gly Met Leu Tyr Leu Gln Arg Met
        915                 920                 925

ccg act gag ttt gat gtc gca atg gcc acc gtc tat tac ctt gcc gac     2832
Pro Thr Glu Phe Asp Val Ala Met Ala Thr Val Tyr Tyr Leu Ala Asp
    930                 935                 940

cgc aat gtc agt ggt gag aca ttc cac cca tca ggt ggt ttg cgt tac     2880
Arg Asn Val Ser Gly Glu Thr Phe His Pro Ser Gly Gly Leu Arg Tyr
945                 950                 955                 960

gaa cgc acc cct acc ggt ggc gaa ctc ttc ggc ttg ccc tca ccg gaa     2928
Glu Arg Thr Pro Thr Gly Gly Glu Leu Phe Gly Leu Pro Ser Pro Glu
                965                 970                 975

cgg ctg gcg gag ctg gtc gga agc acg gtc tat ctg ata ggt gaa cat     2976
Arg Leu Ala Glu Leu Val Gly Ser Thr Val Tyr Leu Ile Gly Glu His
            980                 985                 990

ctg act gaa cac ctt aac ctg ctt  gcc cgt gcg tac ctc  gaa cgt tac   3024
Leu Thr Glu His Leu Asn Leu Leu  Ala Arg Ala Tyr Leu  Glu Arg Tyr
        995                 1000                1005

ggg gca  cgt cag gta gtg atg  att gtt gag aca gaa  acc ggg gca      3069
Gly Ala  Arg Gln Val Val Met  Ile Val Glu Thr Glu  Thr Gly Ala
    1010                1015                1020

gag aca  atg cgt cgc ttg ctc  cac gat cac gtc gag  gct ggt cgg      3114
Glu Thr  Met Arg Arg Leu Leu  His Asp His Val Glu  Ala Gly Arg
    1025                1030                1035

ctg atg  act att gtg gcc ggt  gat cag atc gaa gcc  gct atc gac      3159
Leu Met  Thr Ile Val Ala Gly  Asp Gln Ile Glu Ala  Ala Ile Asp
    1040                1045                1050

cag gct  atc act cgc tac ggt  cgc cca ggg ccg gtc  gtc tgt acc      3204
Gln Ala  Ile Thr Arg Tyr Gly  Arg Pro Gly Pro Val  Val Cys Thr
    1055                1060                1065

ccc ttc  cgg cca ctg ccg acg  gta cca ctg gtc ggg  cgt aaa gac      3249
Pro Phe  Arg Pro Leu Pro Thr  Val Pro Leu Val Gly  Arg Lys Asp
    1070                1075                1080

agt gac  tgg agc aca gtg ttg  agt gag gct gaa ttt  gcc gag ttg      3294
Ser Asp  Trp Ser Thr Val Leu  Ser Glu Ala Glu Phe  Ala Glu Leu
    1085                1090                1095

tgc gaa  cac cag ctc acc cac  cat ttc cgg gta gcg  cgc aag att      3339
Cys Glu  His Gln Leu Thr His  His Phe Arg Val Ala  Arg Lys Ile
    1100                1105                1110

gcc ctg  agt gat ggt gcc agt  ctc gcg ctg gtc act  ccc gaa act      3384
Ala Leu  Ser Asp Gly Ala Ser  Leu Ala Leu Val Thr  Pro Glu Thr
    1115                1120                1125
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acg gct  acc tca act acc gag  caa ttt gct ctg gct  aac ttc atc      3429
Thr Ala  Thr Ser Thr Thr Glu  Gln Phe Ala Leu Ala  Asn Phe Ile
    1130                1135                1140

aaa acg  acc ctt cac gct ttt  acg gct acg att ggt  gtc gag agc      3474
Lys Thr  Thr Leu His Ala Phe  Thr Ala Thr Ile Gly  Val Glu Ser
    1145                1150                1155

gaa aga  act gct cag cgc att  ctg atc aat caa gtc  gat ctg acc      3519
Glu Arg  Thr Ala Gln Arg Ile  Leu Ile Asn Gln Val  Asp Leu Thr
    1160                1165                1170

cgg cgt  gcg cgt gcc gaa gag  ccg cgt gat ccg cac  gag cgt caa      3564
Arg Arg  Ala Arg Ala Glu Glu  Pro Arg Asp Pro His  Glu Arg Gln
    1175                1180                1185

caa gaa  ctg gaa cgt ttt atc  gag gca gtc ttg ctg  gtc act gca      3609
Gln Glu  Leu Glu Arg Phe Ile  Glu Ala Val Leu Leu  Val Thr Ala
    1190                1195                1200

cca ctc  ccg cct gaa gcc gat  acc cgt tac gcc ggg  cgg att cat      3654
Pro Leu  Pro Pro Glu Ala Asp  Thr Arg Tyr Ala Gly  Arg Ile His
    1205                1210                1215

cgc gga  cgg gcg att acc gtg  taa                                  3678
Arg Gly  Arg Ala Ile Thr Val
    1220                1225
```

<210> SEQ ID NO 141
<211> LENGTH: 1225
<212> TYPE: PRT
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 141

```
Met Ala Thr Gly Glu Ser Met Ser Gly Thr Gly Arg Leu Ala Gly Lys
1               5                   10                  15

Ile Ala Leu Ile Thr Gly Gly Ala Gly Asn Ile Gly Ser Glu Leu Thr
            20                  25                  30

Arg Arg Phe Leu Ala Glu Gly Ala Thr Val Ile Ile Ser Gly Arg Asn
        35                  40                  45

Arg Ala Lys Leu Thr Ala Leu Ala Glu Arg Met Gln Ala Glu Ala Gly
    50                  55                  60

Val Pro Ala Lys Arg Ile Asp Leu Glu Val Met Asp Gly Ser Asp Pro
65                  70                  75                  80

Val Ala Val Arg Ala Gly Ile Glu Ala Ile Val Ala Arg His Gly Gln
                85                  90                  95

Ile Asp Ile Leu Val Asn Asn Ala Gly Ser Ala Gly Ala Gln Arg Arg
            100                 105                 110

Leu Ala Glu Ile Pro Leu Thr Glu Ala Glu Leu Gly Pro Gly Ala Glu
        115                 120                 125

Glu Thr Leu His Ala Ser Ile Ala Asn Leu Leu Gly Met Gly Trp His
    130                 135                 140

Leu Met Arg Ile Ala Ala Pro His Met Pro Val Gly Ser Ala Val Ile
145                 150                 155                 160

Asn Val Ser Thr Ile Phe Ser Arg Ala Glu Tyr Tyr Gly Arg Ile Pro
                165                 170                 175

Tyr Val Thr Pro Lys Ala Ala Leu Asn Ala Leu Ser Gln Leu Ala Ala
            180                 185                 190

Arg Glu Leu Gly Ala Arg Gly Ile Arg Val Asn Thr Ile Phe Pro Gly
        195                 200                 205

Pro Ile Glu Ser Asp Arg Ile Arg Thr Val Phe Gln Arg Met Asp Gln
    210                 215                 220
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Leu Lys Gly Arg Pro Glu Gly Asp Thr Ala His His Phe Leu Asn Thr
225                 230                 235                 240

Met Arg Leu Cys Arg Ala Asn Asp Gln Gly Ala Leu Glu Arg Arg Phe
                245                 250                 255

Pro Ser Val Gly Asp Val Ala Asp Ala Ala Val Phe Leu Ala Ser Ala
            260                 265                 270

Glu Ser Ala Ala Leu Ser Gly Glu Thr Ile Glu Val Thr His Gly Met
        275                 280                 285

Glu Leu Pro Ala Cys Ser Glu Thr Ser Leu Leu Ala Arg Thr Asp Leu
    290                 295                 300

Arg Thr Ile Asp Ala Ser Gly Arg Thr Thr Leu Ile Cys Ala Gly Asp
305                 310                 315                 320

Gln Ile Glu Glu Val Met Ala Leu Thr Gly Met Leu Arg Thr Cys Gly
                325                 330                 335

Ser Glu Val Ile Ile Gly Phe Arg Ser Ala Ala Ala Leu Ala Gln Phe
            340                 345                 350

Glu Gln Ala Val Asn Glu Ser Arg Arg Leu Ala Gly Ala Asp Phe Thr
        355                 360                 365

Pro Pro Ile Ala Leu Pro Leu Asp Pro Arg Asp Pro Ala Thr Ile Asp
    370                 375                 380

Ala Val Phe Asp Trp Ala Gly Glu Asn Thr Gly Gly Ile His Ala Ala
385                 390                 395                 400

Val Ile Leu Pro Ala Thr Ser His Glu Pro Ala Pro Cys Val Ile Glu
                405                 410                 415

Val Asp Asp Glu Arg Val Leu Asn Phe Leu Ala Asp Glu Ile Thr Gly
            420                 425                 430

Thr Ile Val Ile Ala Ser Arg Leu Ala Arg Tyr Trp Gln Ser Gln Arg
        435                 440                 445

Leu Thr Pro Gly Ala Arg Ala Arg Gly Pro Arg Val Ile Phe Leu Ser
    450                 455                 460

Asn Gly Ala Asp Gln Asn Gly Asn Val Tyr Gly Arg Ile Gln Ser Ala
465                 470                 475                 480

Ala Ile Gly Gln Leu Ile Arg Val Trp Arg His Glu Ala Glu Leu Asp
                485                 490                 495

Tyr Gln Arg Ala Ser Ala Ala Gly Asp His Val Leu Pro Pro Val Trp
            500                 505                 510

Ala Asn Gln Ile Val Arg Phe Ala Asn Arg Ser Leu Glu Gly Leu Glu
        515                 520                 525

Phe Ala Cys Ala Trp Thr Ala Gln Leu Leu His Ser Gln Arg His Ile
    530                 535                 540

Asn Glu Ile Thr Leu Asn Ile Pro Ala Asn Ile Ser Ala Thr Thr Gly
545                 550                 555                 560

Ala Arg Ser Ala Ser Val Gly Trp Ala Glu Ser Leu Ile Gly Leu His
                565                 570                 575

Leu Gly Lys Val Ala Leu Ile Thr Gly Gly Ser Ala Gly Ile Gly Gly
            580                 585                 590

Gln Ile Gly Arg Leu Leu Ala Leu Ser Gly Ala Arg Val Met Leu Ala
        595                 600                 605

Ala Arg Asp Arg His Lys Leu Glu Gln Met Gln Ala Met Ile Gln Ser
    610                 615                 620

Glu Leu Ala Glu Val Gly Tyr Thr Asp Val Glu Asp Arg Val His Ile
625                 630                 635                 640

Ala Pro Gly Cys Asp Val Ser Ser Glu Ala Gln Leu Ala Asp Leu Val
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               645                 650                 655

Glu Arg Thr Leu Ser Ala Phe Gly Thr Val Asp Tyr Leu Ile Asn Asn
            660                 665                 670

Ala Gly Ile Ala Gly Val Glu Glu Met Val Ile Asp Met Pro Val Glu
        675                 680                 685

Gly Trp Arg His Thr Leu Phe Ala Asn Leu Ile Ser Asn Tyr Ser Leu
    690                 695                 700

Met Arg Lys Leu Ala Pro Leu Met Lys Lys Gln Gly Ser Gly Tyr Ile
705                 710                 715                 720

Leu Asn Val Ser Ser Tyr Phe Gly Gly Glu Lys Asp Ala Ala Ile Pro
               725                 730                 735

Tyr Pro Asn Arg Ala Asp Tyr Ala Val Ser Lys Ala Gly Gln Arg Ala
            740                 745                 750

Met Ala Glu Val Phe Ala Arg Phe Leu Gly Pro Glu Ile Gln Ile Asn
        755                 760                 765

Ala Ile Ala Pro Gly Pro Val Glu Gly Asp Arg Leu Arg Gly Thr Gly
    770                 775                 780

Glu Arg Pro Gly Leu Phe Ala Arg Arg Ala Arg Leu Ile Leu Glu Asn
785                 790                 795                 800

Lys Arg Leu Asn Glu Leu His Ala Ala Leu Ile Ala Ala Ala Arg Thr
               805                 810                 815

Asp Glu Arg Ser Met His Glu Leu Val Glu Leu Leu Leu Pro Asn Asp
            820                 825                 830

Val Ala Ala Leu Glu Gln Asn Pro Ala Ala Pro Thr Ala Leu Arg Glu
        835                 840                 845

Leu Ala Arg Arg Phe Arg Ser Glu Gly Asp Pro Ala Ala Ser Ser Ser
    850                 855                 860

Ser Ala Leu Leu Asn Arg Ser Ile Ala Ala Lys Leu Leu Ala Arg Leu
865                 870                 875                 880

His Asn Gly Gly Tyr Val Leu Pro Ala Asp Ile Phe Ala Asn Leu Pro
               885                 890                 895

Asn Pro Pro Asp Pro Phe Phe Thr Arg Ala Gln Ile Asp Arg Glu Ala
            900                 905                 910

Arg Lys Val Arg Asp Gly Ile Met Gly Met Leu Tyr Leu Gln Arg Met
        915                 920                 925

Pro Thr Glu Phe Asp Val Ala Met Ala Thr Val Tyr Tyr Leu Ala Asp
    930                 935                 940

Arg Asn Val Ser Gly Glu Thr Phe His Pro Ser Gly Gly Leu Arg Tyr
945                 950                 955                 960

Glu Arg Thr Pro Thr Gly Gly Glu Leu Phe Gly Leu Pro Ser Pro Glu
               965                 970                 975

Arg Leu Ala Glu Leu Val Gly Ser Thr Val Tyr Leu Ile Gly Glu His
            980                 985                 990

Leu Thr Glu His Leu Asn Leu Leu  Ala Arg Ala Tyr Leu  Glu Arg Tyr
        995                 1000                1005

Gly Ala  Arg Gln Val Val Met  Ile Val Glu Thr Glu  Thr Gly Ala
    1010                1015                1020

Glu Thr  Met Arg Arg Leu Leu  His Asp His Val Glu  Ala Gly Arg
    1025                1030                1035

Leu Met  Thr Ile Val Ala Gly  Asp Gln Ile Glu Ala  Ala Ile Asp
    1040                1045                1050

Gln Ala  Ile Thr Arg Tyr Gly  Arg Pro Gly Pro Val  Val Cys Thr
    1055                1060                1065
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Pro Phe  Arg Pro Leu Pro Thr  Val Pro Leu Val Gly  Arg Lys Asp
    1070                 1075                 1080

Ser Asp  Trp Ser Thr Val Leu  Ser Glu Ala Glu Phe  Ala Glu Leu
    1085                 1090                 1095

Cys Glu  His Gln Leu Thr His  His Phe Arg Val Ala  Arg Lys Ile
    1100                 1105                 1110

Ala Leu  Ser Asp Gly Ala Ser  Leu Ala Leu Val Thr  Pro Glu Thr
    1115                 1120                 1125

Thr Ala  Thr Ser Thr Thr Glu  Gln Phe Ala Leu Ala  Asn Phe Ile
    1130                 1135                 1140

Lys Thr  Thr Leu His Ala Phe  Thr Ala Thr Ile Gly  Val Glu Ser
    1145                 1150                 1155

Glu Arg  Thr Ala Gln Arg Ile  Leu Ile Asn Gln Val  Asp Leu Thr
    1160                 1165                 1170

Arg Arg  Ala Arg Ala Glu Glu  Pro Arg Asp Pro His  Glu Arg Gln
    1175                 1180                 1185

Gln Glu  Leu Glu Arg Phe Ile  Glu Ala Val Leu Leu  Val Thr Ala
    1190                 1195                 1200

Pro Leu  Pro Pro Glu Ala Asp  Thr Arg Tyr Ala Gly  Arg Ile His
    1205                 1210                 1215

Arg Gly  Arg Ala Ile Thr Val
    1220                 1225


<210> SEQ ID NO 142
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Chloroflexus aurantiacus

<400> SEQUENCE: 142

tctttctggc cagtgccgaa tccgccgctc tctccggtga gacgattgag gttacgcacg     60

gaatggagtt gccggcctgc agtgagacca gcctgctggc ccgtactgat ctgcgcacga    120

ttgatgccag tggccgcacg acgctcatct gcgccggcga ccagattgaa gaggtgatgg    180

cgctcaccgg tatgttgcgt acctgtggga gtgaagtgat catcggcttc cgttcggctg    240

cggcgctggc ccagttcgag caggcagtca atgagagtcg gcggctggcc ggcgcagact    300

ttacgcctcc cattgccttg ccactcgatc cacgcg                              336


<210> SEQ ID NO 143
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Spodoptera littoralis

<400> SEQUENCE: 143

Met Phe Ala Asn Lys Val Val Leu Val Thr Gly Gly Ser Ser Gly Ile
1               5                  10                  15

Gly Ala Ala Thr Val Glu Ala Phe Val Lys Glu Gly Ala Ser Val Ala
            20                  25                  30

Phe Val Gly Arg Asn Gln Ala Lys Leu Lys Glu Val Glu Ser Arg Cys
        35                  40                  45

Gln Gln His Gly Ala Asn Ile Leu Ala Ile Lys Ala Asp Val Ser Lys
    50                  55                  60

Asp Glu Glu Ala Lys Ile Ile Val Gln Gln Thr Val Asp Lys Phe Gly
65                  70                  75                  80

Lys Leu Asp Val Leu Val Asn Asn Ala Gly Ile Leu Arg Phe Ala Ser
                85                  90                  95
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Val Leu Glu Pro Thr Leu Ile Gln Thr Phe Asp Glu Thr Met Asn Thr
            100                 105                 110

Asn Leu Arg Pro Val Val Leu Ile Thr Ser Leu Ala Ile Pro His Leu
        115                 120                 125

Ile Ala Thr Lys Gly Ser Ile Val Asn Val Ser Ser Ile Leu Ser Thr
    130                 135                 140

Ile Val Arg Ile Pro Gly Ile Met Ser Tyr Ser Val Ser Lys Ala Ala
145                 150                 155                 160

Met Asp His Phe Thr Lys Leu Ala Ala Leu Glu Leu Ala Pro Ser Gly
                165                 170                 175

Val Arg Val Asn Ser Val Asn Pro Gly Pro Val Leu Thr Asp Ile Ala
            180                 185                 190

Ala Gly Ser Gly Phe Ser Pro Asp Leu Leu Glu Asp Thr Gly Ala His
        195                 200                 205

Thr Pro Leu Gly Lys Ala Ala Gln Ser Glu Glu Ile Ala Asp Met Ile
    210                 215                 220

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

Ile Met Asp Asn Gly Leu Ala Leu Gln
                245


<210> SEQ ID NO 144
<211> LENGTH: 246
<212> TYPE: PRT
<213> ORGANISM: Thermotoga maritima

<400> SEQUENCE: 144

Met Arg Leu Glu Gly Lys Val Cys Leu Ile Thr Gly Ala Ala Ser Gly
1               5                   10                  15

Ile Gly Lys Ala Thr Thr Leu Leu Phe Ala Gln Glu Gly Ala Thr Val
            20                  25                  30

Ile Ala Gly Asp Ile Ser Lys Glu Asn Leu Asp Ser Leu Val Lys Glu
        35                  40                  45

Ala Glu Gly Leu Pro Gly Lys Val Asp Pro Tyr Val Leu Asn Val Thr
    50                  55                  60

Asp Arg Asp Gln Ile Lys Glu Val Val Glu Lys Val Val Gln Lys Tyr
65                  70                  75                  80

Gly Arg Ile Asp Val Leu Val Asn Asn Ala Gly Ile Thr Arg Asp Ala
                85                  90                  95

Leu Leu Val Arg Met Lys Glu Glu Asp Trp Asp Ala Val Ile Asn Val
            100                 105                 110

Asn Leu Lys Gly Val Phe Asn Val Thr Gln Met Val Val Pro Tyr Met
        115                 120                 125

Ile Lys Gln Arg Asn Gly Ser Ile Val Asn Val Ser Ser Val Val Gly
    130                 135                 140

Ile Tyr Gly Asn Pro Gly Gln Thr Asn Tyr Ala Ala Ser Lys Ala Gly
145                 150                 155                 160

Val Ile Gly Met Thr Lys Thr Trp Ala Lys Glu Leu Ala Gly Arg Asn
                165                 170                 175

Ile Arg Val Asn Ala Val Ala Pro Gly Phe Ile Glu Thr Pro Met Thr
            180                 185                 190

Glu Lys Leu Pro Glu Lys Ala Arg Glu Thr Ala Leu Ser Arg Ile Pro
        195                 200                 205

Leu Gly Arg Phe Gly Lys Pro Glu Glu Val Ala Gln Val Ile Leu Phe
```

```
    210                 215                 220

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

Asp Gly Gly Leu Val Ile
                245


<210> SEQ ID NO 145
<211> LENGTH: 289
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 145

Met Asn Pro Met Asp Arg Gln Thr Glu Gly Gln Glu Pro Gln His Gln
1               5                   10                  15

Asp Arg Gln Pro Gly Ile Glu Ser Lys Met Asn Pro Leu Pro Leu Ser
            20                  25                  30

Glu Asp Glu Asp Tyr Arg Gly Ser Gly Lys Leu Lys Gly Lys Val Ala
        35                  40                  45

Ile Ile Thr Gly Gly Asp Ser Gly Ile Gly Arg Ala Ala Ala Ile Ala
    50                  55                  60

Phe Ala Lys Glu Gly Ala Asp Ile Ser Ile Leu Tyr Leu Asp Glu His
65                  70                  75                  80

Ser Asp Ala Glu Glu Thr Arg Lys Arg Ile Glu Lys Glu Asn Val Arg
                85                  90                  95

Cys Leu Leu Ile Pro Gly Asp Val Gly Asp Glu Asn His Cys Glu Gln
            100                 105                 110

Ala Val Gln Gln Thr Val Asp His Phe Gly Lys Leu Asp Ile Leu Val
        115                 120                 125

Asn Asn Ala Ala Glu Gln His Pro Gln Asp Ser Ile Leu Asn Ile Ser
    130                 135                 140

Thr Glu Gln Leu Glu Lys Thr Phe Arg Thr Asn Ile Phe Ser Met Phe
145                 150                 155                 160

His Met Thr Lys Lys Ala Leu Pro His Leu Gln Glu Gly Cys Ala Ile
                165                 170                 175

Ile Asn Thr Thr Ser Ile Thr Ala Tyr Glu Gly Asp Thr Ala Leu Ile
            180                 185                 190

Asp Tyr Ser Ser Thr Lys Gly Ala Ile Val Ser Phe Thr Arg Ser Met
        195                 200                 205

Ala Lys Ser Leu Ala Asp Lys Gly Ile Arg Val Asn Ala Val Ala Pro
    210                 215                 220

Gly Pro Ile Trp Thr Pro Leu Ile Pro Ala Thr Phe Pro Glu Glu Lys
225                 230                 235                 240

Val Lys Gln His Gly Leu Asp Thr Pro Met Gly Arg Pro Gly Gln Pro
                245                 250                 255

Val Glu His Ala Gly Ala Tyr Val Leu Leu Ala Ser Asp Glu Ser Ser
            260                 265                 270

Tyr Met Thr Gly Gln Thr Ile His Val Asn Gly Gly Arg Phe Ile Ser
        275                 280                 285

Thr


<210> SEQ ID NO 146
<211> LENGTH: 285
<212> TYPE: PRT
<213> ORGANISM: Sinorhizobium meliloti

<400> SEQUENCE: 146
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```
Met Glu Lys Phe Pro His Pro Pro Phe Pro Arg Gln Thr Gln Glu Met
1               5                   10                  15

Pro Gly Thr Thr Asp Arg Met Gln Pro Leu Pro Asp His Gly Glu Asn
            20                  25                  30

Ser Tyr Gln Gly Ser Gly Arg Leu Lys Asp Lys Arg Ala Ile Ile Thr
        35                  40                  45

Gly Gly Asp Ser Gly Ile Gly Arg Ala Val Ala Ile Ala Tyr Ala Arg
    50                  55                  60

Glu Gly Ala Asp Val Leu Ile Ser Tyr Leu Ser Glu His Asp Asp Ala
65                  70                  75                  80

Met Ala Thr Lys Ala Leu Val Glu Glu Ala Gly Arg Lys Ala Val Leu
                85                  90                  95

Ala Ala Gly Asp Ile Gln Ser Ser Asp His Cys Arg Arg Ile Val Glu
            100                 105                 110

Thr Ala Val Arg Glu Leu Gly Gly Ile Asp Ile Leu Val Asn Asn Ala
        115                 120                 125

Ala His Gln Ala Thr Phe Lys Asn Ile Glu Asp Ile Ser Asp Glu Glu
    130                 135                 140

Trp Glu Leu Thr Phe Arg Val Asn Met His Ala Met Phe Tyr Leu Thr
145                 150                 155                 160

Lys Ala Ala Val Pro His Met Lys Lys Gly Ser Ala Ile Ile Asn Thr
                165                 170                 175

Ala Ser Ile Asn Ala Asp Val Pro Asn Pro Ile Leu Leu Ala Tyr Ala
            180                 185                 190

Thr Thr Lys Gly Ala Ile His Asn Phe Ser Ala Gly Leu Ala Gln Met
        195                 200                 205

Leu Ala Glu Arg Gly Ile Arg Val Asn Val Val Ala Pro Gly Pro Ile
    210                 215                 220

Trp Thr Pro Leu Ile Pro Ser Thr Met Pro Glu Asp Thr Val Ala Asp
225                 230                 235                 240

Phe Gly Lys Gln Val Pro Met Lys Arg Pro Gly Gln Pro Val Glu Leu
                245                 250                 255

Ala Ser Ala Tyr Val Met Leu Ala Asp Pro Met Ser Ser Tyr Val Ser
            260                 265                 270

Gly Ala Thr Ile Ala Val Thr Gly Gly Lys Pro Phe Leu
        275                 280                 285


<210> SEQ ID NO 147
<211> LENGTH: 257
<212> TYPE: PRT
<213> ORGANISM: Mesorhizobium loti

<400> SEQUENCE: 147

Met Arg Leu Leu His Lys Arg Thr Leu Val Thr Gly Gly Ser Asp Gly
1               5                   10                  15

Ile Gly Leu Ala Ile Ala Glu Ala Phe Leu Ser Glu Gly Ala Asp Val
            20                  25                  30

Leu Ile Val Gly Arg Asp Ala Ala Lys Leu Glu Ala Ala Arg Gln Lys
        35                  40                  45

Leu Ala Ala Leu Gly Gln Ala Gly Ala Val Glu Thr Ser Ser Ala Asp
    50                  55                  60

Leu Ala Thr Ser Leu Gly Val Ala Thr Val Val Glu Gln Val Lys Glu
65                  70                  75                  80

Thr Gly Arg Pro Leu Asp Ile Pro Ile Asn Asn Ala Gly Val Ala Asp
```

-continued

```
                85                  90                  95

Leu Val Pro Phe Glu Ser Val Ser Glu Ala Gln Phe Gln His Ser Phe
            100                 105                 110

Ala Leu Asn Val Ala Ala Ala Phe Phe Leu Thr Gln Gly Leu Leu Pro
        115                 120                 125

His Phe Gly Ala Gly Ala Ser Ile Ile Asn Ile Ser Ser Tyr Phe Ala
    130                 135                 140

Arg Lys Met Ile Pro Lys Arg Pro Ser Ser Val Tyr Ser Leu Ser Lys
145                 150                 155                 160

Gly Ala Leu Asn Ser Leu Thr Arg Ser Leu Ala Phe Glu Leu Gly Pro
                165                 170                 175

Arg Gly Ile Arg Val Asn Ala Ile Ala Pro Gly Thr Val Asp Thr Ala
            180                 185                 190

Met Arg Arg Lys Thr Val Asp Asn Leu Pro Ala Glu Ala Lys Ala Glu
        195                 200                 205

Leu Lys Ala Tyr Val Glu Arg Ser Tyr Pro Leu Gly Arg Ile Gly Arg
    210                 215                 220

Pro Asp Asp Leu Ala Gly Met Ala Val Tyr Leu Ala Ser Asp Glu Ala
225                 230                 235                 240

Ala Trp Thr Ser Gly Gly Ile Phe Ala Val Asp Gly Gly Tyr Thr Ala
                245                 250                 255

Gly
```

<210> SEQ ID NO 148
<211> LENGTH: 774
<212> TYPE: DNA
<213> ORGANISM: Mesorhizobium loti

<400> SEQUENCE: 148

```
atgagacttc tgcacaagcg cacgctggtg accggcggct cggacggtat cggcctggca     60

atcgccgagg cgttcctgag cgagggcgcc gatgtcctga tcgtcggccg tgacgccgcc    120

aagctcgaag ccgcgcgcca gaagctggcg gctcttggcc aggccggcgc ggtggagacg    180

tcgtccgccg atcttgccac cagcctcggt gtcgcaaccg tcgtcgagca ggtgaaagag    240

accggccggc cgctcgacat tcctatcaac aatgccggtg tcgccgacct cgtgccgttc    300

gagagcgtca gcgaggcgca gttccagcac tccttcgcgc tcaatgtggc ggcggcgttc    360

ttcctcaccc aggggctgct gccgcatttc ggcgccggtg catcgatcat caacatctct    420

tcctatttcg cccgcaagat gatcccgaag cggccatcca gcgtctactc cctgtccaag    480

ggcgcgttga actcgttgac cagatcgctg gccttcgagc tcggcccgcg cggcatccgc    540

gtcaacgcca tcgcgcccgg cacggtcgac accgccatgc ggcgcaagac cgtcgacaac    600

ctgccggccg aggccaaggc cgaactgaag gcctatgtcg aacgcagcta tccgctgggc    660

cgcatcggcc gtccggacga cctcgccggc atggcggttt atctagccag cgacgaggcg    720

gcctggacga gcggtgggat ctttgccgtg gatggtggct acacggccgg atga          774
```

<210> SEQ ID NO 149
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Spodoptera littoralis

<400> SEQUENCE: 149

```
atgttcgcaa ataaagtggt actagtaaca ggtggtagct ccggtatcgg cgcagctact     60

gtggaagcat tcgttaagga aggcgcttct gtagccttcg tgggaagaaa ccaagccaag    120
```

cttaaggaag tagagagccg ctgccagcag catggagcca acatcctggc tatcaaagca 180

gatgtctcca aagacgagga agcgaaaatc atcgtacaac aaactgtcga caagttcggg 240

aagcttgatg tgcttgttaa caacgctggg attctacggt tcgcgagtgt tctggagccg 300

actttaatac aaacttttga tgaaactatg aacacgaatt tacgtccagt tgtcctcatc 360

actagcctgg ctatccctca tttgattgct acaaaaggga gcatagttaa cgtatccagt 420

atactgtcta caatagtaag aataccaggg attatgtcat acagtgtgtc aaaggctgct 480

atggatcact tcacaaaatt ggcagcgttg gagctggctc cttctggcgt gcgagtgaac 540

tcagtcaacc ctggaccagt tcttactgat atcgcagctg gttctggctt ttctcctgat 600

ctgcttgaag atacaggggc tcatacaccg ttggggaaag ctgcgcagtc tgaggagatt 660

gctgatatga ttgtgtatct ggctagtgat aaagctaaga gtgttacggg gtcctgttat 720

atcatggaca atggactcgc gctgcagtaa 750

<210> SEQ ID NO 150
<211> LENGTH: 741
<212> TYPE: DNA
<213> ORGANISM: Thermotoga maritima

<400> SEQUENCE: 150

atgaggcttg aagggaaagt gtgtctgatc acaggggctg caagcgggat agggaaagcc 60

accacgcttc ttttcgcaca ggaaggagct acggtgatcg ctggcgatat ctcgaaagaa 120

aatctcgact ctcttgtgaa agaggcagaa ggacttccgg ggaaggttga tccctacgtt 180

ttgaacgtga ccgacaggga tcagataaag gaagttgtgg aaaaagtcgt tcaaaagtac 240

ggtcgaatcg atgttctggt gaacaacgcg ggaataacaa gggatgcgct tcttgtgagg 300

atgaaagaag aagactggga tgcggtaata aacgtgaatc tgaagggtgt tttcaacgtg 360

actcagatgg tggtgcccta catgatcaaa cagaggaacg gttcgatcgt gaacgtctcc 420

tctgtcgttg gaatatacgg gaatcctggt cagacgaatt acgcggcgtc gaaggcggga 480

gtcataggaa tgaccaagac gtgggcgaag gaactcgctg gaagaaacat cagggtgaac 540

gctgtggcac ccggattcat agaaaccccc atgaccgaaa aacttccaga aaaagcccgt 600

gaaacggccc tttccagaat accgctggga aggtttggga agccagaaga ggtggcgcag 660

gttatactct tcctcgcatc ggacgagtcg agttacgtca ccggacaggt gataggaata 720

gatgggggcc tcgtgatctg a 741

<210> SEQ ID NO 151
<211> LENGTH: 858
<212> TYPE: DNA
<213> ORGANISM: Sinorhizobium meliloti

<400> SEQUENCE: 151

atggaaaaat ttccgcaccc tccctttccc cgccaaaccc aggaaatgcc cggcactacc 60

gatcggatgc agccgctgcc cgatcacggg gaaaactcct accagggttc cggacgcctg 120

aaggacaaga gagccatcat caccggcggg gacagcggca tcggcagggc cgtggcgatc 180

gcctatgcgc gcgagggagc ggacgtcctt atcagctatc tgagcgagca tgacgacgcg 240

atggccacca aggctctggt ggaggaagca ggtcgcaagg ccgtgcttgc cgccggcgac 300

atccagtcgt ccgaccattg ccgcaggatc gtcgaaacgg ccgttcggga actcggcggc 360

atcgacattc tcgtcaacaa tgcagcccat caggcgacct tcaagaacat cgaagacatc 420

-continued

agcgacgagg agtgggagct gacattccgc gtcaacatgc acgccatgtt ctacctgacc 480

aaggcagcgg tgccgcacat gaagaagggc agcgcgatca tcaacaccgc ttccatcaat 540

gccgacgttc ccaatccgat cctactcgcc tatgcgacca ccaagggcgc gatccacaat 600

ttcagcgccg gtctcgcgca gatgctggcc gaacgcggga taagagtgaa tgtcgtggcc 660

ccgggcccga tctggacgcc gctgatcccc tccaccatgc ccgaggatac cgtcgccgat 720

ttcggcaaac aggtgcctat gaagcgaccg ggccagcccg tggaactcgc ctcggcctat 780

gtcatgctgg cggatccgat gtcgagctac gtgtcaggcg caacgattgc cgtgaccggc 840

ggcaagcctt tcctttga 858

<210> SEQ ID NO 152
<211> LENGTH: 870
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 152

gtgaacccaa tggacagaca aacagaagga caagaaccgc agcatcagga cagacagccg 60

ggcattgagt caaaaatgaa tccgctgccg ctgtcagagg acgaggatta tcgaggaagc 120

ggaaaactga aaggaaaagt tgcgatcatt actggaggcg acagcggaat agggagagca 180

gcagctattg cctttgctaa agaggggget gatatctcca ttctatactt agacgagcat 240

tcggacgcag aggaaacacg caaacggatc gaaaaggaga atgtccgctg cctgcttatc 300

ccgggagatg ttggggacga gaaccattgt gaacaagctg tgcagcaaac agtggaccat 360

tttggtaaac tcgatatctt agtgaacaac gccgctgaac agcatcccca ggacagcatt 420

ctcaatattt caacagaaca gctggaaaaa acctttcgca caaatatttt ttccatgttt 480

catatgacga agaaagcttt gcctcacctg caagaggggt gtgccattat taatacgaca 540

tcgattaccg cttatgaagg ggatacggcg ttaattgatt attccagcac aaagggtgcg 600

attgtttcct ttacgcgttc catggcgaag tcgcttgcag ataaaggcat cagagtgaat 660

gcggtggcgc ccggtccgat ttggacaccg cttattccgg cgacattccc tgaggaaaaa 720

gtgaaacagc acggcttgga taccccaatg ggaagaccgg gacagccggt tgagcatgca 780

ggcgcctatg ttctgctggc gtctgacgaa tcttcctata tgacagggca gaccattcat 840

gtgaatggcg gccgttttat ttcaacgtaa 870

<210> SEQ ID NO 153
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 153

atggcgacgg gcgagtccat gag 23

<210> SEQ ID NO 154
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 154

ggacacgaag aacagggcga cac 23

<210> SEQ ID NO 155
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 155

gaactgtctg gagtaaggct gtc 23

<210> SEQ ID NO 156
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 156

gattccgtat gtcaccccta 20

<210> SEQ ID NO 157
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 157

caggcgactg gcaatcacaa 20

<210> SEQ ID NO 158
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 158

gagaccacaa cggtttccct cta 23

<210> SEQ ID NO 159
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 159

ggacacgaag aacagggcga cac 23

<210> SEQ ID NO 160
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Clostridium propionicum

<400> SEQUENCE: 160

Met Val Gly Lys Lys Val Val His His Leu Met Met Ser Ala Lys Asp
1 5 10 15

Ala His Tyr Thr Gly Asn Leu Val Asn Gly Ala Arg Ile Val Asn Gln
20 25 30

Trp Gly Asp Val Gly Thr Glu Leu Met Val Tyr Val Asp Gly Asp Ile
35 40 45

Ser Leu Phe Leu Gly Tyr Lys Asp Ile Glu Phe Thr Ala Pro Val Tyr
50 55 60

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```
Val Gly Asp Phe Met Glu Tyr His Gly Trp Ile Glu Lys Val Gly Asn
65                  70                  75                  80

Gln Ser Tyr Thr Cys Lys Phe Glu Ala Trp Lys Val Ala Thr Met Val
                85                  90                  95

Asp Ile Thr Asn Pro Gln Asp Thr Arg Ala Thr Ala Cys Glu Pro Pro
            100                 105                 110

Val Leu Cys Gly Arg Ala Thr Gly Ser Leu Phe Ile Ala Lys Lys Asp
        115                 120                 125

Gln Arg Gly Pro Gln Glu Ser Ser Phe Lys Glu Arg Lys His Pro Gly
    130                 135                 140

Glu
145
```

<210> SEQ ID NO 161
<211> LENGTH: 144
<212> TYPE: PRT
<213> ORGANISM: Clostridium propionicum

<400> SEQUENCE: 161

```
Met Val Gly Lys Lys Val Val His His Leu Met Met Ser Ala Lys Asp
1               5                   10                  15

Ala His Tyr Thr Gly Asn Leu Val Asn Gly Ala Arg Ile Val Asn Gln
            20                  25                  30

Trp Gly Asp Val Gly Thr Glu Leu Met Val Tyr Val Asp Gly Asp Ile
        35                  40                  45

Ser Leu Phe Leu Gly Tyr Lys Asp Ile Glu Phe Thr Ala Pro Val Tyr
    50                  55                  60

Val Gly Asp Phe Met Glu Tyr His Gly Trp Ile Glu Lys Val Gly Asn
65                  70                  75                  80

Gln Ser Tyr Thr Cys Lys Phe Glu Ala Trp Lys Val Ala Lys Met Val
                85                  90                  95

Asp Ile Thr Asn Pro Gln Asp Thr Arg Ala Thr Ala Cys Glu Pro Pro
            100                 105                 110

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

Gln Arg Gly Pro Gln Glu Ser Ser Phe Lys Asp Ala Lys His Pro Gln
    130                 135                 140
```

<210> SEQ ID NO 162
<211> LENGTH: 438
<212> TYPE: DNA
<213> ORGANISM: Clostridium propionicum

<400> SEQUENCE: 162

```
atggtaggta aaaaggttgt acatcattta atgatgagcg caaaagatgc tcactatact     60

ggaaacttag taaacggcgc tagaattgtg aatcagtggg gcgacgttgg tacagaatta    120

atggtttatg ttgatggtga cataagctta ttcttgggct acaaagatat cgaattcaca    180

gctcctgtat atgttggtga ctttatggaa taccacggct ggattgaaaa agttggtaac    240

cagtcctata catgtaaatt tgaagcatgg aaagttgcaa caatggttga tatcacaaat    300

cctcaggata cacgcgcaac agcttgtgag cctccggtat tgtgcggaag agcaacgggt    360

agtttgttca tcgcaaaaaa agatcagaga ggccctcagg aatcctcttt taaagagaga    420

aagcaccccg gtgaatga                                                  438
```

<210> SEQ ID NO 163

-continued

<211> LENGTH: 435
<212> TYPE: DNA
<213> ORGANISM: Clostridium propionicum

<400> SEQUENCE: 163

atggtaggta aaaaggttgt acatcattta atgatgagcg caaaagatgc tcactatact 60

ggaaacttag taaacggcgc tagaattgtg aatcagtggg gcgacgtagg tacagaatta 120

atggtttatg ttgatggtga catcagctta ttcttgggct acaaagatat cgaattcaca 180

gctcctgtat atgttggtga ttttatggaa taccacggct ggattgaaaa agttggcaac 240

cagtcctata catgtaaatt tgaagcatgg aaagtagcaa agatggttga tatcacaaat 300

ccacaggata cacgtgcaac agcttgtgaa cctccggtac tttgtggtac tgcaacaggc 360

agccttttca tcgcaaagga taatcagaga ggtcctcagg aatcttcctt caaggatgca 420

aagcaccctc aataa 435

<210> SEQ ID NO 164
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 164

atagggccca ggagatcaaa ccatgggtga agagtctctg gttc 44

<210> SEQ ID NO 165
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 165

cctctgctac agtcgacaca acgaccactg aagttgggag 40

<210> SEQ ID NO 166
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 166

agtctgctat cggtacctca acgaccactg aagttgggag 40

<210> SEQ ID NO 167
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 167

atagcggccg cataatggat actctcggaa tcgacgttgg 40

<210> SEQ ID NO 168
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 168

ccccatcgat acatatttct tgattttatc ataagcaatc 40

<210> SEQ ID NO 169
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 169

ccagggccca taatgggtga agaaaaaaca gtagatattg 40

<210> SEQ ID NO 170
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 170

ggtagacttg tcgacgtagt ggtttcctcc ttcattgg 38

<210> SEQ ID NO 171
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 171

atagcggccg cataatgggt cagatcgacg aacttatcag 40

<210> SEQ ID NO 172
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 172

aggttcaact agttcgtaga ggatttccga gaaagcctg 39

<210> SEQ ID NO 173
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 173

ctagggccca taatggaact cgccgtttat agcac 35

<210> SEQ ID NO 174
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 174

acttctcgag ttaaaccagt tcgttcgggc aggt 34

<210> SEQ ID NO 175
<211> LENGTH: 37
<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 175

gggactagta taatgggaaa agtagaaatc attacag 37

<210> SEQ ID NO 176
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 176

cggcttaatt aacagcagag atttattttt tcagtcc 37

<210> SEQ ID NO 177
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Clostridium propionicum

<400> SEQUENCE: 177

Met Val Gly Lys Lys Val Val His His Leu Met Met Ser Ala Lys Asp
1 5 10 15

Ala His Tyr Thr Gly Asn Leu Val Asn Gly Ala Arg Ile Val Asn Gln
20 25 30

Trp Gly Asp
35

<210> SEQ ID NO 178
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 178

atggtwggya araargtwgt 20

<210> SEQ ID NO 179
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 179

tcrccccayt grttwacrat 20

<210> SEQ ID NO 180
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 180

acatcattta atgatgagcg caaaagatgc tcactatact ggaaacttag taaacggcgc 60

taga 64

<210> SEQ ID NO 181
<211> LENGTH: 31
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 181

gtacatcatt taatgatgag cgcaaaagat g 31

<210> SEQ ID NO 182
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 182

gatgctcact atactggaaa cttagtaaac 30

<210> SEQ ID NO 183
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 183

attctagcgc cgtttactaa gtttccag 28

<210> SEQ ID NO 184
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 184

ccagtatagt gagcatcttt tgcgctcatc 30

<210> SEQ ID NO 185
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 185

gagccatgga agaaataaat gctaaag 27

<210> SEQ ID NO 186
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 186

agaggatggc tttttaaatc gctattc 27

<210> SEQ ID NO 187
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 187

atacatatga ccgaccgaca tcgcatt 27

<210> SEQ ID NO 188
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 188

atagtcgacg ggtcagtcct tgccgcg 27

<210> SEQ ID NO 189
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 189

atgagtctga atttccttga ttttgaacag ccgattgcag agctggaagc gaaaatcgat 60

tctctgactg cggttagccg tcaggatgag aaactggata ttaacatcga tgaagaagtg 120

catcgtctgc gtgaaaaaag cgtagaactg acacgtaaaa tcttcgccga tctcggtgca 180

tggcagattg cgcaactggc acgccatcca cagcgtcctt ataccctgga ttacgttcgc 240

ctggcatttg atgaatttga cgaactggct ggcgaccgcg cgtatgcaga cgataaagct 300

atcgtcggtg gtatcgcccg tctcgatggt cgtccggtga tgatcattgg tcatcaaaaa 360

ggtcgtgaaa ccaaagaaaa aattcgccgt aactttggta tgccagcgcc agaaggttac 420

cgcaaagcac tgcgtctgat gcaaatggct gaacgcttta agatgcctat catcaccttt 480

atcgacaccc cgggggctta tcctggcgtg ggcgcagaag agcgtggtca gtctgaagcc 540

attgcacgca acctgcgtga aatgtctcgc ctcggcgtac cggtagtttg tacggttatc 600

ggtgaaggtg gttctggcgg tgcgctggcg attggcgtgg gcgataaagt gaatatgctg 660

caatacagca cctattccgt tatctcgccg gaaggttgtg cgtccattct gtggaagagc 720

gccgacaaag cgccgctggc ggctgaagcg atgggtatca ttgctccgcg tctgaaagaa 780

ctgaaactga tcgactccat catcccggaa ccactgggtg gtgctcaccg taacccggaa 840

gcgatggcgg catcgttgaa agcgcaactg ctggcggatc tggccgatct cgacgtgtta 900

agcactgaag atttaaaaaa tcgtcgttat cagcgcctga tgagctacgg ttacgcgtaa 960

<210> SEQ ID NO 190
<211> LENGTH: 471
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 190

atggatattc gtaagattaa aaaactgatc gagctggttg aagaatcagg catctccgaa 60

ctggaaattt ctgaaggcga agagtcagta cgcattagcc gtgcagctcc tgccgcaagt 120

ttccctgtga tgcaacaagc ttacgctgca ccaatgatgc agcagccagc tcaatctaac 180

gcagccgctc cggcgaccgt tccttccatg gaagcgccag cagcagcgga aatcagtggt 240

cacatcgtac gttccccgat ggttggtact ttctaccgca ccccaagccc ggacgcaaaa 300

gcgttcatcg aagtgggtca gaaagtcaac gtgggcgata ccctgtgcat cgttgaagcc 360

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We claim:

1. A transformed yeast or bacterial cell for producing 3-hydroxypropionic acid (3-HP), wherein the transformed cell comprises a metabolic pathway in which metabolites are sequentially produced in a sequence of pyruvate, acetyl-CoA, and malonyl-CoA, and wherein the transformed cell comprises

(i) a disruption of a gene encoding an alcohol dehydrogenase,

(ii) a disruption of a gene encoding a pyruvate decarboxylase, or

(iii) a disruption of both the alcohol dehydrogenase gene and the pyruvate decarboxylase gene.

2. The transformed cell of claim 1, wherein the disruption comprises a deletion or knock out of the gene.

3. The transformed cell of claim 1, wherein the transformed cell is a yeast cell comprising a disruption of a gene encoding the pyruvate decarboxylase.

4. The transformed cell of claim 1, wherein the transformed cell is a bacterial cell or a yeast cell comprising a disruption of a gene encoding the alcohol dehydrogenase.

5. The transformed cell of claim 1, wherein disruption of the gene encoding the alcohol dehydrogenase, pyruvate decarboxylase, or both, increases production of 3-HP by the cell.

6. The transformed cell of claim 1, wherein the cell is a yeast, *Lactobacillus, Lactococcus, Bacillus*, or *Escherichia* cell.

7. The transformed cell of claim 1, wherein the cell produces at least 1 g/L 3-HP.

8. The transformed cell of claim 1, wherein the cell comprises at least one exogenous nucleic acid molecule that encodes at least one polypeptide that is capable of producing 3-HP from pyruvate.

9. The transformed cell of claim 8, wherein the at least one exogenous nucleic acid molecule encodes acetyl-CoA carboxylase, a protein that reduces malonyl-CoA, or combinations thereof.

10. The transformed cell of claim 8, wherein the transformed cell produces:

acetyl-CoA from pyruvate,

malonyl CoA from the acetyl-CoA,

and wherein the 3-HP is produced from the malonyl CoA.

11. The transformed cell of claim 10, wherein production of the 3-HP from the malonyl CoA comprises:

reduction of the malonyl-CoA into MSA; and

reduction of the MSA into 3-HP.

12. The transformed cell of claim 8, wherein one of the at least one exogenous nucleic acid molecules encodes an oxidoreductase polypeptide, and wherein the cell can convert the 3-HP into propanediol.

13. The transformed cell of claim 8, wherein one of the at least one exogenous nucleic acid molecules encodes a lipase, wherein the cell can convert the 3-HP into an ester of 3-HP.

14. A method of making 3-hydroxypropionic acid (3-HP), comprising:

culturing the transformed cell of claim 1 under conditions such that the 3-HP is produced by the cell.

15. A method of making 3-hydroxypropionic acid (3-HP), comprising:

culturing the transformed cell of claim 8 under conditions such that the 3-HP is produced by the cell.

16. The method of claim 15, further comprising:

isolating the 3-HP from the transformed cell; or

hydrogenating the 3-HP produced from the transformed cell to form 1,3 propanediol; or

dehydrating the 3-HP produced from the transformed cell to form acrylic acid; or

combinations thereof.

17. A transformed yeast or bacterial cell for producing 3-hydroxypropionic acid (3-HP), wherein the transformed cell comprises a metabolic pathway in which metabolites are sequentially produced in a sequence of pyruvate and/or PEP, oxaloacetate, asparate, beta-alanine, and malonate semialdehyde, and wherein the transformed cell comprises a disruption of a gene encoding an alcohol dehydrogenase, a pyruvate decarboxylase, or a deletion or knockout of both the gene encoding the alcohol dehydrogenase and the gene encoding the pyruvate decarboxylase.

18. The transformed cell of claim 17, wherein the transformed cell is a yeast cell comprising a disruption of a gene encoding the pyruvate decarboxylase.

19. The transformed cell of claim 17, wherein the transformed cell is a bacterial cell or a yeast cell comprising a disruption of a gene encoding the alcohol dehydrogenase.

20. The transformed cell of claim 17, wherein disruption of the gene encoding the alcohol dehydrogenase, pyruvate decarboxylase, or both, increases production of 3-HP by the cell.

21. The transformed cell of claim 17, wherein the cell is a yeast, *Lactobacillus, Lactococcus, Bacillus*, or *Escherichia* cell.

22. The transformed cell of claim 17, wherein the cell produces at least 1 g/L 3-HP.

23. The transformed cell of claim 17, wherein the cell comprises at least one exogenous nucleic acid molecule that encodes at least one polypeptide that is capable of producing 3-HP from PEP and/or pyruvate.

24. The transformed cell of claim 23, wherein the at least one exogenous nucleic acid molecule encodes aspartate decarboxylase.

25. The transformed cell of claim 23, wherein the at least one exogenous nucleic acid molecule encodes one or more of PEP carboxylase, pyruvate carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, 3-HP dehydrogenase, 3-hydroxyisobutyrate dehydrogenase.

26. The transformed cell of claim 23, wherein the at least one exogenous nucleic acid molecule encodes at least one polypeptide that is capable of producing 3-HP from PEP, and wherein the at least one exogenous nucleic acid molecule encodes PEP carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, 3-HP dehydrogenase, 3-hydroxyisobutyrate dehydrogenase, or combinations thereof.

27. The transformed cell of claim 26, wherein at least one exogenous nucleic acid molecule encodes PEP carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, and 3-HP dehydrogenase.

28. The transformed cell of claim 26, and wherein at least one exogenous nucleic acid molecule encodes PEP carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, and 3-hydroxyisobutyrate dehydrogenase.

29. The transformed cell of claim 23, wherein at least one exogenous nucleic acid molecule encodes at least one polypeptide that is capable of producing 3-HP from pyruvate, and wherein the at least one exogenous nucleic acid molecule encodes pyruvate carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, 3-HP dehydrogenase, 3-hydroxyisobutyrate dehydrogenase, or combinations thereof.

30. The transformed cell of claim 29, wherein at least one exogenous nucleic acid molecule encodes pyruvate carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, and 3-HP dehydrogenase.

31. The transformed cell of claim 29, wherein at least one exogenous nucleic acid molecule encodes pyruvate carboxylase, aspartate aminotransferase, aspartate decarboxylase, 4-aminobutyrate aminotransferase, and 3-hydroxyisobutyrate dehydrogenase.

32. The transformed cell of claim 23, wherein the transformed cell further produces:

oxaloacetate from PEP and/or pyruvate,

aspartate from the oxaloacetate,

β-alanine from the aspartate,

malonate semialdehyde from the β-alanine,

and wherein the 3-HP is produced from the malonate semialdehyde.

33. The transformed cell of claim 23, wherein one of the at least one exogenous nucleic acid molecules encodes an oxidoreductase polypeptide, and wherein the cell can convert the 3-HP into propanediol.

34. The transformed cell of claim 23, wherein one of the at least one exogenous nucleic acid molecules encodes a lipase, wherein the cell can convert the 3-HP into an ester of 3-HP.

35. A method of making 3-hydroxypropionic acid (3-HP), comprising:

culturing the transformed cell of claim 19 under conditions such that the 3-HP is produced by the cell.

36. A method of making 3-hydroxypropionic acid (3-HP), comprising:

culturing the transformed cell of claim 23 under conditions such that the 3-HP is produced by the cell.

37. The method of claim 23, further comprising:

isolating the 3-HP from the transformed cell; or

hydrogenating the 3-HP produced from the transformed cell to form 1,3 propanediol; or

dehydrating the 3-HP produced from the transformed cell to form acrylic acid; or

combinations thereof.

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