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Gilvocarcin Gene Cluster, Recombinant Production and use Thereof

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Rohr et al.

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(54) **GILVOCARCIN GENE CLUSTER, RECOMBINANT PRODUCTION AND USE THEREOF**

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(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**

C12N 15/00	(2006.01)
C12N 15/11	(2006.01)
C12N 15/52	(2006.01)
C12N 5/10	(2006.01)
C12N 1/21	(2006.01)

(52) **U.S. Cl.** **536/23.2**; 435/320.1; 435/477; 435/252.3; 536/23.1; 536/23.7

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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Primary Examiner—Maria Marvich
(74) *Attorney, Agent, or Firm*—McDermott Will & Emery LLP

(57) **ABSTRACT**

A nucleic acid molecule encoding the gilvocarcin V gene cluster and subunits thereof. Recombinant vectors and host cells comprising a nucleic acid compound encoding the gilvocarcin V gene cluster or subunits thereof. Host cells comprising recombinant vectors encoding the gilvocarcin polyketide synthase and gilvocarcin post-PKS modifying enzymes from *Streptomyces griseoflavus* can be used to produce gilvocarcin and functional gilvocarcin mutants, analogs and derivatives thereof with application as antibiotics, anticancer agents, immunosuppressants, antivirals, and neuroprotective agents.

7 Claims, 27 Drawing Sheets

Figure 1.

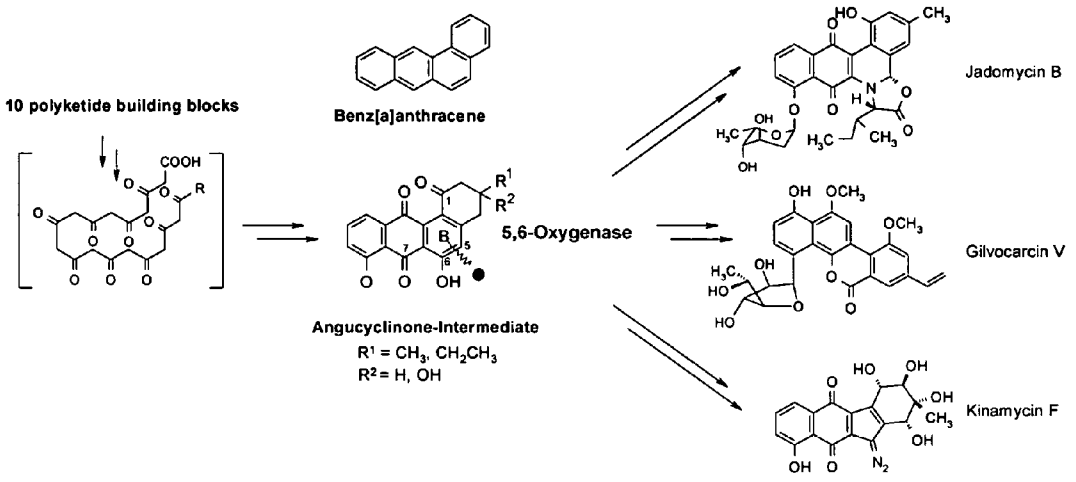

10 polyketide building blocks
Benz[a]anthracene
Angucyclinone-Intermediate
R1 = CH3, CH2CH3
R2 = H, OH
5,6-Oxygenase
Jadomycin B
Gilvocarcin V
Kinamycin F

Figure 2.

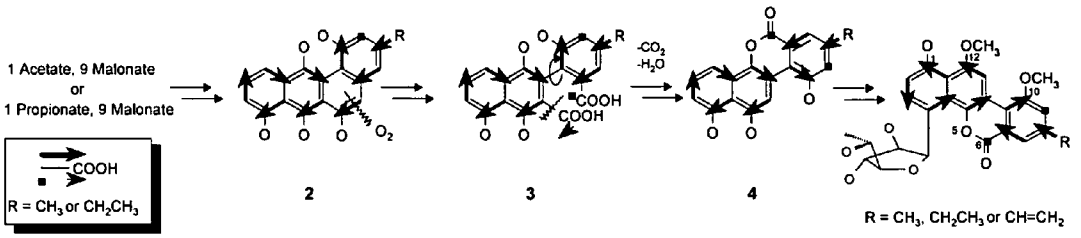
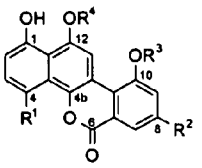
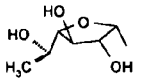

1 Acetate, 9 Malonate
or
1 Propionate, 9 Malonate
COOH
R = CH3 or CH2CH3
2
3
-CO2
-H2O
4
R = CH3, CH2CH3 or CH=CH2

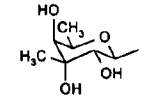
Figure 3.

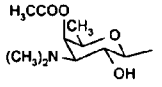


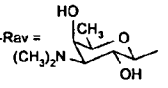
Gilvocarcin-Type Anticancer Drugs

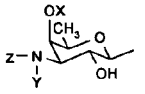
	R^1	R^2	R^3	R^4
Gilvocarcin M:	α-D-Fuc	CH_3	CH_3	CH_3
Gilvocarcin E:	α-D-Fuc	CH_2CH_3	CH_3	CH_3
Gilvocarcin V:	α-D-Fuc	$CH{=}CH_2$	CH_3	CH_3
Defucosyl-Gilvocarcin V:	H	$CH{=}CH_2$	CH_3	CH_3
Ravidomycin:	β-D-Rav	$CH{=}CH_2$	CH_3	CH_3
Deacetyl-Ravidomycin:	DeAc-β-D-Rav	$CH{=}CH_2$	CH_3	CH_3
Deacetyl-Ravidomycin M:	DeAc-β-D-Rav	CH_3	CH_3	CH_3
FE35A:	N,N-MeAc-DeAc-β-D-Rav'	$CH{=}CH_2$	CH_3	CH_3
FE35B:	N-Ac-β-D-Rav'	$CH{=}CH_2$	CH_3	CH_3
Chrysomycin A:	β-D-Vir	$CH{=}CH_2$	CH_3	CH_3
Chrysomycin B:	β-D-Vir	CH_3	CH_3	CH_3
BE-12406 A:	H	CH_3	CH_3	α-L-Rha
BE-12406 B:	H	CH_3	H	α-L-Rha

α-D-Fuc =  6-Deoxy-α-D-galacto-furanose (D-fucose)

β-D-Vir =  6-Deoxy-3-methyl-β-D-gulo-pyranose (D-virenose)

β-D-Rav =  4-Acetyl-3-amino-3,6-dideoxy-3-*N*,*N*-dimethylamino-β-D-galactopyranose (D-ravinose)

DeAc-β-D-Rav =  3-amino-3,6-dideoxy-3-*N*,*N*-dimethylamino-β-D-galactopyranose (deacetyl-D-ravinose)



N,N-MeAc-DeAc-β-D-Rav': X =H, Y =Ac, Z = CH_3

N-Ac-β-D-Rav': X = Ac, Y = Ac, Z = H

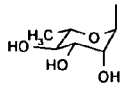
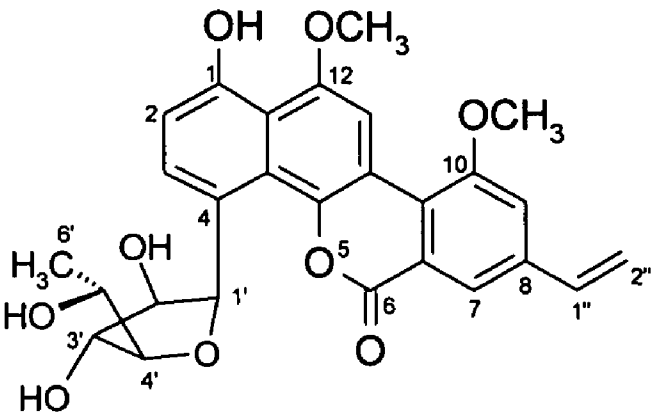
α-L-Rha =  6-Deoxy-α-L-manno-pyranose (L-rhamnose)

Figure 4.



Gilvocarcin V 1

Figure 5.

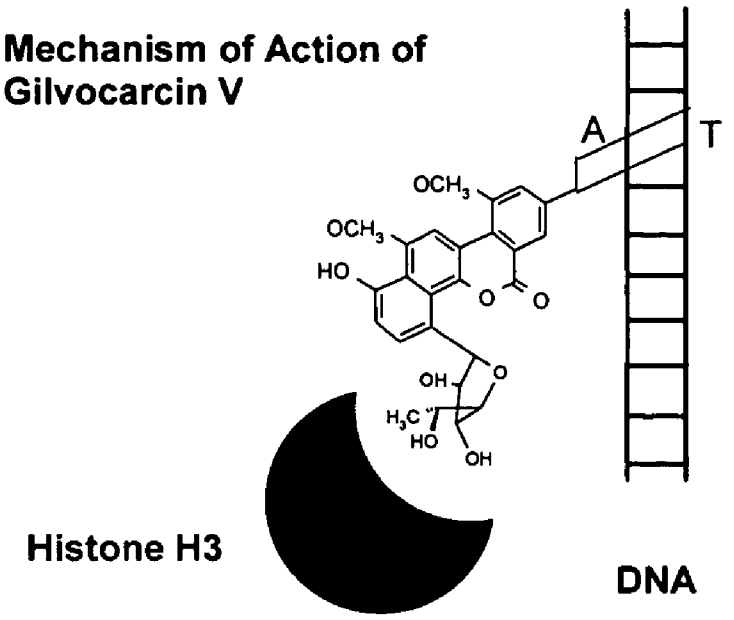
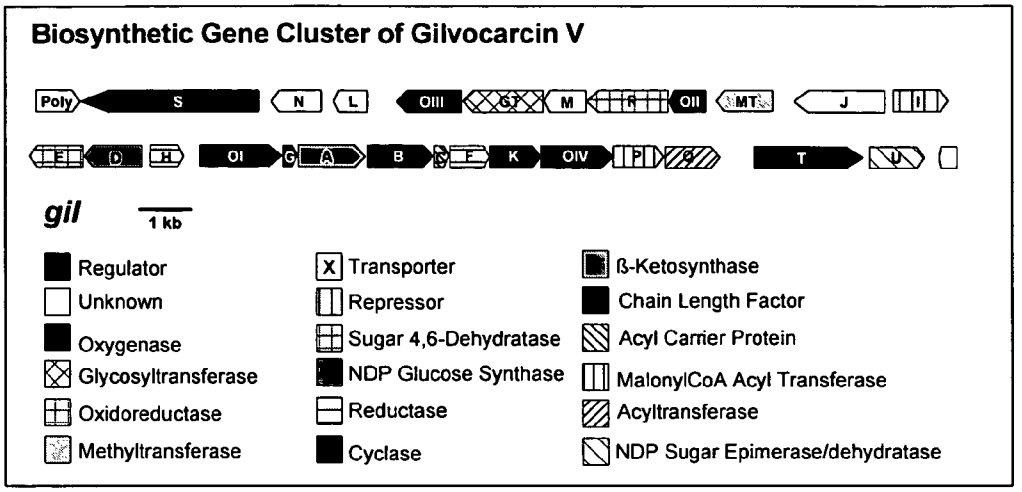

Mechanism of Action of
Gilvocarcin V
A
T
OCH3
OCH3
HO
O
O
OH
O
H3C
HO
OH
Histone H3
DNA

Figure 6.

A.


Biosynthetic Gene Cluster of Gilvocarcin V
Poly
S
N
L
OIII
GT
M
R
OII
MT
J
I
E
D
H
OI
G
A
B
C
F
K
OIV
P
Q
T
U
gil
1 kb
Regulator
Unknown
Oxygenase
Glycosyltransferase
Oxidoreductase
Methyltransferase
Transporter
Repressor
Sugar 4,6-Dehydratase
NDP Glucose Synthase
Reductase
Cyclase
ß-Ketosynthase
Chain Length Factor
Acyl Carrier Protein
MalonylCoA Acyl Transferase
Acyltransferase
NDP Sugar Epimerase/dehydratase

B.

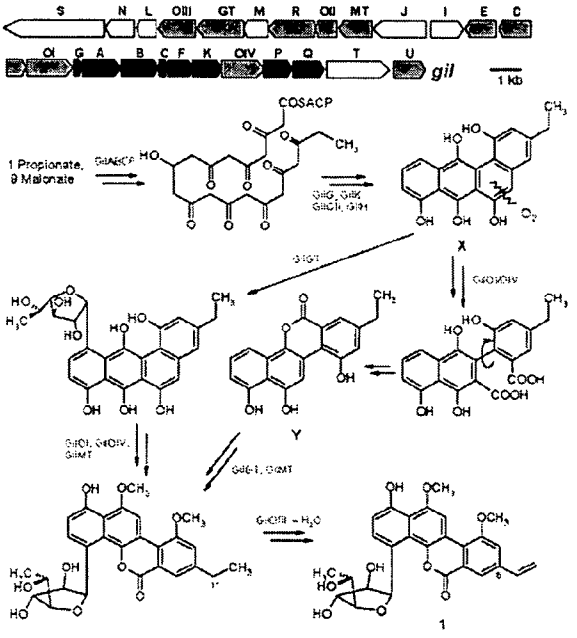

gil
1 kb
COSACP
1 Propionate,
9 Malonate
X
Y
1

Figure 7.

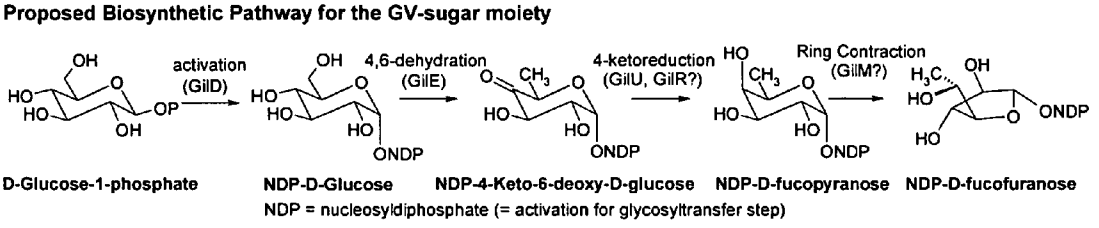

Proposed Biosynthetic Pathway for the GV-sugar moiety
activation (GilD)
4,6-dehydration (GilE)
4-ketoreduction (GilU, GilR?)
Ring Contraction (GilM?)
D-Glucose-1-phosphate
NDP-D-Glucose
NDP-4-Keto-6-deoxy-D-glucose
NDP-D-fucopyranose
NDP-D-fucofuranose
NDP = nucleosyldiphosphate (= activation for glycosyltransfer step)

Figure 8.

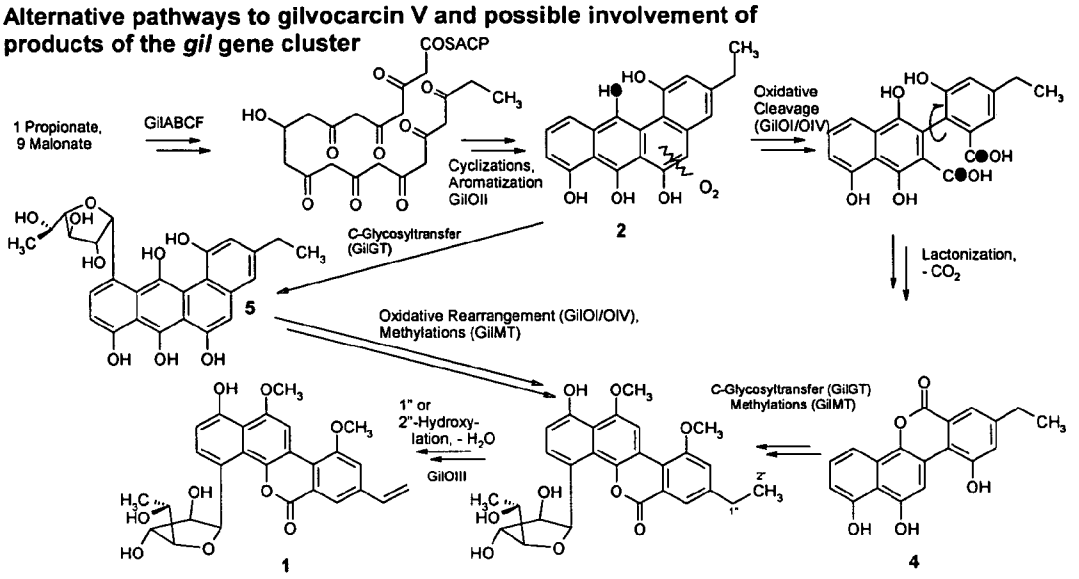

Alternative pathways to gilvocarcin V and possible involvement of products of the gil gene cluster
COSACP
1 Propionate, 9 Malonate
GilABCF
Cyclizations, Aromatization GilOII
Oxidative Cleavage (GilOI/OIV)
C-Glycosyltransfer (GilGT)
Lactonization, - CO2
Oxidative Rearrangement (GilOI/OIV), Methylations (GilMT)
1" or 2"-Hydroxy-lation, - H2O
GilOIII
C-Glycosyltransfer (GilGT) Methylations (GilMT)
1
2
4
5

Figure 9.
(SEQ ID NO:1)

PstI **polymerase ->**

```
   1  CTGCAGATCCCCAAGGTCATCCGCCGCGCCGCCGTCGCCGACCCCGGCTGGCGCCTCGTCGTCGC

  66  CGACGCCGACCAGATGGAACCGAGGGTGCTGGCGGCCATCTCCCGCGACCCCGGCCTGATGGAGG

 131  TGGCCGGCCGGGAGGGCGACCTCTACCAGTCGGTGTCCGACCGCGCCTTCTCCGGCGACCGCGCC

 196  CAGGCCAAGCTCGCCGTCCTCGGCGCCGTCTACGGCCAGACCTCCGGCGACGGCCTGAAGAACCT
                                                        SalI
 261  CGCCGCGCTCAGGCGCCGCTTCCCCAAGGCGGTGGCCTACGTCGACGAGGCCGCCCGCGCCGGCG

 326  AGGAGGGCCGTCTCGTACGGACCTGGCTGGGCCGCACCTGCCCGCCCGCCGTCCGCCCGACGGAC

 391  GACGCGGCGGAGGAGGCCGGCATCCCGCCCGCCCAGGAGGAGCCGGGCCCAGCGGCCCGACCGTG
                                        SmaI                    SmaI
 456  GGCCCCGGAGGCCGAGGCCCGCCCGTGGGTCCCGGGCTACGCCTCGACCGACGCCCGCGCCCGGG

 521  GCCGCTTCGCCCGCAACTTCGTGGTCCAGGGCAGCGCCGCCGACTGGGCCCTGCTGCTGCTCGCG

 586  GCGCTGCGGAGGACGCTGAGCGGCATGGCGGCCGAACTGGTCTTCTTCCAGCACGACGAGGTGAT

 651  CGTGCACTGCCCCGAGGAGGAGGCGGCGACGGTGGCGGAGGCGATCCGGCAGTCCGCCGACCTCG

 716  CCGGCCGGCTGACGTTCGGACCGACCCCCGTGCGCTTCCCGTTCACGACGGCGGTCGTGGAGTGC
                ******
 781  TACGCCGACGCCAAGTGATCAGCTCGCCGGCCGGGCACCGGCCACGAGTCCGCGCAACTCCTCCA

 846  CCACGGCCCGCTCGTCCGTGCCGTCCAGCGCGGCGAGCGCCGACCGCCACTGCTCGCGCGCCTCG

 911  CCCACCCGCCCCCGCTCGCGCAGCAGCAGACCGTACTGGTGGCGGGCCAGACCACCGGTGTAGCG

 976  GTCGGCTCGGGCGTCCGCGCGGCGCAGCAGTTCGGCGCACTCGGCGTCGGCCCGGCCGGCGTGCC

1041  CCAGCAGCCGCAGGGCGCGCACCAGGCCGAGCCGGGTCTGGGACTCTCCGTGCCAGTCGCCGTGC

1106  CCGGCGAGGATGCGCAGGCTCTCCTCGAAGTGCGGCAGGGCGGCGGCCGGTTCACCCAGCCGGAG

1171  GTGGGCGTAGCCGATGTTGCAGTGGGCGGAGTGCCGCACGATCACCGCTCCGATGCGGTCCCCGA

1236  TCACGAGCGAGCGCCGGTGCTGGTCGATCGCCGCGCGCGGATCGGTGTGCTCGTACAGGTTGCCG

1301  AGGTGGCTGAGGGTGACGGCCTCGCCGTAGGGGTCGGCCAACTGCCGCGAGTGGGTGAGGCTCTG

1366  CCGCAGTGCCCGCTCCGACTCCGCGTACCGGCCGAGCCCTTCGAGCAGCAGCCCCCGGTGGTTGA
            BamHI
1431  GGGCGCGCCGGATCCAGGAGACGGCTCCGAGCCGCCGCCAGATCTCCAGCGCGCGGTCGGTGAGG

1496  GCGAGGGCCTCGCCGGTGCGACCGGTCAGGAAGTGCAGCCCCGCCAGATCGCCCAGCGCGCACGC

1561  CTCGGCGGCCTCGTCCCCGAGCCGCCGCGCCACCCGCAGGGCCGCCCGCCCGAGCACCTCCAGCT

1626  CGGGGACCCGGCCGCCGCGCAGGAGGTAGGGGTGGAGCAGGCGGAGGAGTACGGCGATGTGGACG

1691  TCGTGACGGCCCCGGTCACCGCCCCCGCCGGTGCCCTCACCGGCGTACCGCCCGACGAGGGTGAC

1756  GATGTTCTCCAGCTCCCGGTCGCCCCAGGCGAAGGCCGCCCCCGCGTCGTCGAACGGGGGTACGG
```

Figure 9. cont.

```
                                  SmaI
1821  ACGCGACGTGAGCGGTGTGCCCGGGCGGCTGGGACGGGGTCGGGCGGCGGCGGTCGTCCTGGTCG

1886  GGGCCGGGTTCGACGATCGCGGTGAGGGCGCGTTCGGCGACGGCGGCGTACCAGCGCAGGGCGGT

1951  CTCGGCGGGGTGCTCGCGCGTGCCGGTCCGAGGGCCGGCGTCCTCGCGGGGTGCGGCGTCCTCGC

2016  GGGGTGCGGCATCCTCGCGGAGTACGGCCTCCTCGCGGGGTGCGGCGCCCTCGCGGGGTGCGGCG

2081  CCCTCGCGGAGTACGGCGCCCTCGCGGAGTACGGCGCCCTCGCGGAGTACGGCGCCCTCGCGGAG

2146  TACGGCATCCTCGCGGAGTACGGCATCCTCGCGGAGTACGGCGTCCTCGCGGAGTGCGGCGTCCT

2211  CGCGGGGCGCGGTCTGCGGACCCGCCGCCGCTCGGGCTCGTTCGCGGGCGAAGTCACGGACCAGG
                                                          SalI
2276  TCGTGGGGGACGTAGCGGCCGTACGCGGTCTCCTCCACGAGGGCCACGTCGACGAGCCGGTCCAG

2341  CGCGGCCTCCGCCCGGCGTTCGCCGGTGCCGGTGAGCCGGGCGAGCAGCGGCGCGCCGTAGGCGG

2406  GCAGGTCGAGCGCGCCGATGCGGCACAGGGCGAGGGCGGCGTCGCGGTCCGTCTCACGGTCGGAG

2471  ACGGTGAGCGCGTCGTGCGCGACGGCCAGCGAGCGGCGCACGCTGAGGTCGTCGTACTCCAGGTG

2536  CGGCAACCGGCTGTCGGTGGCGGAGAGCTGACCGGCGAGGTCGTCGGGCGTGAGGGCCCGGCGCG

2601  CGGCGAGCCGGGCCGCGACCACCCGCAGGGCCAGCGGAAGCCGGCCGGTGAGCGCGACGAGCGGG

2666  TGCCCGGCGCCGAGACCGTCCCGGCCGGAGACCGCCCGCAGCAGGGCGGCACTGTCCTCGTCGGA

2731  CAGCGGGCCGAGCGGGACACGGACGGCACCGTCGAGCGTGGTGAGCGGCGAACGGCTGGTGACGA
                                  NotI
2796  TCACCGCACAGCCGGGTCCGCCCGGCAGCAGCGGCCGCACCTGCGCGGCGTCCGCGGCGTCGTCC
                                                          NotI
2861  AGCACCAGGAGGGTGCGGGTGGGCGCGAGCAGCGAGCGCAGCAGGGCGGCGGCCGCGTCCGGCCG

2926  TTCGGGGACGGCGCAGGGCTCGGTGCCCAGGTCGCGCAGGAGAGCGCTGAGGGCCTGGGCGGGGG
                                                          SalI
2991  TGAGGGGGGTCATGCCGGGGGTCGTGCCGTGCAGGTTGACGTAGAGCTGACCGTCGACGAAACGT
                                                          KpnI
3056  TCCGCCAGTGTGTGTGCGACCTGGACGGCGAGCGCGCTCTTCCCCACACCAGCGGTACCGCTGAC

3121  GACGACGGCAGGGGGCCCGGCGGCGGGCCGGGGGGCACGGGTGAGCACACGGATCAGCTCGTCCC

3186  GCACCGCGTCCCGCCCGGTGAAGTGAGCAGGCGCGGGCGGCAGTTGCGCCGGCCTGGGCGGCACA

3251  CCGCCCCCTCCTGCGGCCTCCGCGCACCGACCGTGCTCCTGCCGCTCCCCCTGCCCCCGCAGCAC

3316  CTCCACGTGGGCCTCGCGCACCCCCGGCCCCGGTTCGACGCCGAGTTCGTCCGCCAGGCGGGCCC

3381  GCAGATCCCGGTGGACCACCAGCGCCTCCGCCTGACGGCCGGTGCGGTGCAGCGCGAGCATCAGC

3446  TGACGGTGGTACGACTCCCGCAGCGGATGTTCGGCGGCCAGTGCCGCCAGTTCGGGCACGAGATC

3511  GGCCAGGCGCTCGCCGCCCAGGGCCAGTTCGGCGTCGTACCGCCACTCCAGGAGCAGCAGCCGCG

3576  CCTCGCGCAGCCGCCGCACCAGGGCGTAGCCGCCCACTTCCGGGGGCATCCCGGCCAGCGGGTTC
```

Figure 9. cont.

```
                                                                    SmaI
3641  CCCCGCCACAGCGCGAGCGCGGCGGCGCACTCGCGCGCCACCCGCTCCCAGTCCCGGGCGGTGTG

3706  CGCGGCGCGGGCGGCTGCGGTGTGCGCGTCGAAGACCTGGACGTCCACCTCGCCCTCTCCCACCC
            EcoRV                                  SmaI
3771  GCAGCAGATATCCGGTGGGCACGGTGAGCAGCCGGCCCGGGTCGTCGAGCAGCCGCCGCAACCGG

3836  GCGACGTGATTGTGCAGCGAGGGCAGCGCGGAGACGGGCGGCGCACCGCCCCACAGGGCGTCCTT

3901  CAGCGCCTCGACGGACACGACCCGCCCGGCGTCGAGCAGCAACGCGACGAACAGCGCACGGAGTT
                                        SmaI
3966  TGGGACTTCCGGTCACCTGGACGGACCCGGGGGCGTCGGGCCCCTCGCCGTCGTACAGCACCGGT
                                              <- gilS
4031  GTTCCCAGCAGTCCGAACCGCAGTCCGCGCCGTGTCACGCCGCACCACTGCCTTCCGGGTGACCG

4096  AACGGACAACCAATGGCCTTATGTTAGCGATCCGTTGGCAAAGTCTGATGTGATCACTACATCGG
      BamHI  SmaI
4161  ATCCGGCCCGGGGGTGCGCGCGTAGAACGCGCAGCTCGGGGGAGTGTCGCCGCCGCGGCCCGGTC

4226  CGCACACGAGGGTTCCCGGTCGGACAGAGGTGAACGGCCGGGCCCTCGTTCTCTTCTGCCGCCGC
                      ***
4291  GTTCGCCCTTCGGGTCAGATGACGGGCGGCCGTCCGAGCCGGGTGAGCCGCCACACCGTCCGCCA

4356  GCGCATGGCCCGCCGCTCCCCGGCCGGCTCCCGCAGCCCCTCCGCGAAACCGCCGAACCAGGCGC

4421  GCAGCCCCTGGACCGACCGAGTCCGCAGCAGGGTGAGCAGGACCCACACCCCGAGGTGCACGGGG

4486  ATCAGCGCGAGCGGCAGCCGCCGCCGGGCCAGCCAGACCCGGTTGCGGGCGTTCACCCGGAAGTA

4551  GATCGCGTGCCGGGCCGGCGAGGTCTTGGGGTGCTGGAGCAGCAGGTCGGGCGCGTAGAGGATGC

4616  GCCACCCCGCGTCGGCGGCACGCCAGGCGAGGTCGGTCTCCTCGTGCGCGAAGAAGAACGCGCCG

4681  GGCCAGTCGCCGATCTCGTCGAGCATCGACATCCGCAGCGCGTGCCCGCCCCCGAGGAACCCGGT

4746  GACGTACCCGCCCTTCATCGGGTCCGCCTTTCCGAGCCGGGGCACGTGCCGCTGCTGCGTCTCCC

4811  CCAGCTCGTCGGCGATCCGGAAGCCGACGACGCCGAGCCGGTCGTCCGCCGCGTACAACTCCCCC

4876  ACCCGGCGCAGCACATCGGCGTCCACCAGCAGACCGTCGTCGTCCAGTTCGACGACGACGTCCAC
                                                          SmaI
4941  GTCCCCGAACTCCCGCAGCCGCTCGATCCCCACGTTCCGCCCGCCCGGGCAGCCGAGGTTCTCCG

5006  CCAGCTCGACGGTGGTGACCTCACCGGGCAGGGACAGCCGCCGGGCGAACTCGGGCAGCGGACAG

5071  CCGTTCCCCACGATCACGATCCGCGCGGGCGCCACGTCCTGCTTCGCCACGGACTCCAGCAGCGC
                                                                <- gilN
5136  GTCCACCTCGGCCGGCCGGTTCCCCATGGTCACGACGGCGACGGCGATCCTCGGCGTCCCCATGC
                                                                SphI
5201  CCTCACCCCACTCCACCCGGCTGCTCCGCTGACGGGCGATGCTAACCGTTCACGGTGAGGACGCA
      SphI
5266  TGCATGACACCCACCACCGCGGCGTACTCCGCCCCCGCCCCACACGAACAGGACACACGTGTCCC
                SmaI
5331  TGGTGCTCGCCCGGGGACGACCGGAGGGGCCCCGTGCGGCAGCCGCGGCCGGCTGCCGCACGGGG
                      ***
5396  GCCGGGAGCGGTCGCCGGTCAGCGGGTGGTGACGAAGGGGCTCTGGTGCGTGTGCGGGGTGGTGA
```

Figure 9. cont.

```
5461   GTTCGTCGAGCATGGCGATCGCGAGGTCGCGGCGCGTCGTCCATGCCCGTCCGCCGGGAAGGTCG
                                 SmaI
5526   GCGGTGACCCGCAGCTCGTCGCCCGGGCGTGCCTCGTCGCTGAGCCGGGCGGGACGCATGACCGT

5591   CCACTCCAGGTCGCGGGCTCCGGTGAGGATGTCCTCCATGCGGCGCATGTCCGCGTACAGGGTCC
         SmaI                                                      SmaI
5656   GCCCGGGGCCGTTGCGCAGGATGCCGTAGACCGGCCGCTGCCATCGCACCCCGCCCCGGGTGACC

5721   GGATGTGTCAGGCCGGCGCTCACGACGACCAGGCGCCTGACGTCCGCCGCGCGCATCCCGTCCAC
              SmaI
5786   GACGGCCCGGGCGGACGCCGAGTAGACCGTGACCGGCCTCCAGGAGTACGGCGCTCCCAGGCAGG

5851   ACAGGACGGCGTCGGCGCCCTTGAACACGGACGTCATGTCCGCGACGTCCGTGACGTCCGCCGTT

5916   TCCACGGTCAGCCTCTCCCCCGGAGTGACCGAACCCGGACGCCGGACGACGGCGACGACGTCATG

5981   GCCGGCCGCACAGGCCAGGGCCGTCACCTGCCGTCCGGTCGGGCCGCTTGCACCGAGCACTGCTA
       <- gilL                                              SphI
6046   CTTTCATGCCGTCTCCAGACGATGCGCGGACGATGTACGGACGCATGCGGACGATGTGCGTCGTG

6111   CGGTGGTCGAGAAGGTGCCCCGGTCGGCGCCCTTCCCGAACGACGCCCCATCGTAGGAGTCGCGG
                 SmaI                                        SmaI
6176   CGCCCGGCCCCGGGTCCGAGGCCGTGGGCACCGTCGAGAACAGGCCGCCCGGGCGGGGCCGTTGC
                                                                   SmaI
6241   GGCGGGCGCCACGGAGCGGGAGCGCGGGCGGCACGCCGCCGGCTCCCGCCCGCGTTCCCGGGCGG

6306   CACGCCGCCACCCCTCAGGAGGAGCCCTGCCGCGCCGTCCGCGCGGACGTTGCGGGGGCCGTCCT

6371   GGCCAGTTCGAGGGCGCCCATCGGACAGGACCGGATCAACCGCTCCACCTGCCGGACATCGCCGA

6436   ACCCGTCGGCCGCCCCCGCTGAGACGGTGATCTGCTGCTCGGGGCTGCGGAAGACGGCCCTGGAC

6501   AACCCCTCGCACTGTTCGTGCAACTCGCACCGCCGGCCGTCGATCCTGACCACCGTCACGTCCTC
                ***
6566   GACGGGCTCAGGCAGTTCGTCCATGGAACGTCACATCCATCCTCTCGATCCCGCGGATGGTGTTG
                                                  SalI
6631   TGCAGCCTCCAGGTCGGCTCCCCCACCTCGATCCTGTCGACGTGACGGACCAGCGCCTCCAGGAG

6696   GCTCTGCCCCTCCAGGCGGGACAGGGCCTGACCGACGCAGGCGTGGATACCGTGCCCGAAACCGA

6761   CGTGCTGGGCCGCGCCCTGCCGGGCGACGTCGAAGGACTCCGGGTCCTTCCAGAACCGCTCGTCA

6826   CGGTTGGCCGAACCGAAGAGGAGGAGGACGCGCGATCCGCGGGGCAGCGCGGCTCCGCCCAGCTC

6891   CGTGTCCTCGGCGACGTAGCGGGTGAACCCGCGCAGCGGCGATTCCAGCCGGATGATCTCGTTGA

6956   AGGCCGACGAGACCAGGGAGGGGTCCTCCCGCAGACGGCGCCACTGGTCCTGGTGCGTGCCGAGC
              SphI
7021   AGCCACAGCATGCTGGAGAGCGCGCTGACGGACGTGTCCATGGACGGGGCGAGGAAGTCACCGAG

7086   CAGTCCGGGCAGCAGCTTGTCCTCGATCTTGCCCTCGCGGGCCTCCGAGACGAGTTCGGCACCCC

7151   AGCTCCCCGGACGCAGATTGCCGGGCTGTGACATCCGGTGGAGGAACTCGCCCATCTCGCCGAGC

7216   AGCGGCAGGCCGGCCCGCGTCCGGTCGTTCAGGGGACCGAAGGCGTTGAATCCGGCGCTGGCCCA
```

Figure 9. cont.

```
7281  CTCCAGGAGCCTTTCCTTCCCCTCGCCCTCCGGCCATCCCAGCAGATCGGGCACCACGGCCAGCG

7346  GGAAGGCGACCGCGAAGTCCTGGACGGCGTCGAACGACTTCCGGGCGACGAGGTCCCGGACAAGA

7411  CGGTCCGCCCAGGACGTGACGTACCCGTTGATGTCGGCCATCGCGCGGGGCTTCAGGTGGCGGGC

7476  CACGAGCCCCCGCACGTAGGCGTGGTACGGCGGGTCGCTGGTGAAGCTACTGCCCTTCTGCGCCT

7541  TGTTCAGGGTGTCGGTCAGACCGACGCCCTCGCCGGACACGAACGTGCCGTGGCGGTGCAGGGCC

7606  GCGTACACCTCGTCGTAGCGGGCGGCGCAGTGCACCTGGTGCGCCGTCAGGTACACCACCGGTGC

7671  CGCGTCGCGCAGCGCACCGTACAGGGGGTACGGGTCGGTTATCGACGCGTCCGTGTACGGATCGA
                                  <- gilOIII     ***
7736  GATCGAGGTGGGGGATCGTCGATGTGGAGATCACCGCTTCATCCTTTCCGCGCGAGGAGGTCTTC

7801  GATCAGCGGCACCATGCCCACGGCCGTCGGCGCGGTCGCGCCCTGCTCCGCCAGTCTCCGGGCCC
                    BamHI
7866  TGGAGGAGTAGGACGGATCCCCGAGCATCTCCTTGACCACCTGCGTGATCGCCTCCGGGGTGCCC

7931  TCCTCGCGGTAGAGCGTCCGCGCGCACCCGTAGTCCGTGAGGTGCTTCAGCCTCGGGACGAAGGC

7996  CTCGAAGGGGTTGAGGATCAGCTGCGGAGTCGCCGTGTTGATGGCGTTGATGGCCGTCAGTCCGC
                                                          BamHI
8061  CCGCCGGATGGATGATCACCTCACAGGTCGGGAGGATGGCCTCCAGCGGGATCCAGCCGGCGCGC
        SmaI              PstI
8126  ACCCGGGGGTACTTCTCCTGCAGCCGCTGCCCCTCCGCCTCGCCGATGGCCACGACGACCTCGAC
      XhoI  SalI
8191  CTCGAGCTCCAGCAGCCCTTCGACGATGGCCGAGATGCGGTCCATCGCGCCGGGGAAGGCGTACC
        HindIII
8256  GGAAGCTTCCCATCGTCAGGCACACGCGCCCGGCGTCCGGAGCCGTCAGCATCCACGGCTCGATC

8321  GCCCGCTGCATGTTGTGCGGGGTCCAGCGCATGAAGGTGCCGGTCGCCCCGACCAGGCCGGGCGG

8386  GCAGATGTCGATCTTCAGTGAGGGGTCGGGCAGTGCGTCCGAGCCGATCCTGGCCAGCTCGTCCG
            XhoI
8451  CCATCTCCTCGAGGAGGTACTCCTCGTAGCCGCCGACGTCGAACAGGTCCCAGGACTGCCGGACG

8516  AAGGGGATGCCGAGGAACCGGGCGGCGATCTCGGCACCGTGTCCCTGGCTCCCCGCGATCAGGAC

8581  GTCGGCGCCCCACGTCCGGGCGACGTCCACCAGGTCGTCGAAGACGTGGCTTCCCTGACGCCCGA

8646  ACCAGTGGCCCAGGTAGGGCATCTCCTGTTCGGGCCGGTGGGGGTACTCGATCGCCGGCTTGCCG
          NotI                                SmaI
8711  CCGGCGGCCGCCTTGATGCTCTCGGTCGTGTGCCCCCGGGCCACCGGGAGGGACGGCAGACCGAT

8776  GCCGGTGACGGCGCCGGACATCTCCTCGAAGGACGCCACCAGGACGTCGTGCCCGGACAACCGGA

8841  GTGCCGAGGCGAGGGGTCCGATGGCGAACGCGCTGGCCGGGCTCGTGCCCGCGGCGTAGAAGAGG

8906  GCCTTCACGCGGCGCCCTCCCGTGACCGGTCCCTTGTCGCGTTCCGCGAAGTAGGCCTCCTTCAG

8971  GCCCACGGTCTCGAAGTCGATGACGGCGAGGCCCGAGGTCAGCAGACGACTCGAACGCAGCTCCC

9036  GGACCAGGTCCAGGTAGTTGTCCATCTCGTCGAAGCCCAGGATGAAGAAACTCTCCGCCTCACGG
```

Figure 9. cont.

```
9101   CCGTACTTGATGGCGCGCACCGGTGTTATCTCCCCGCCCCGCTTCACGAAGGCGCCGATGCTGGG

9166   GAAGACGTGCTCGTGTTCGACCGTGATGCGCTCGTCGAGGGACAACTCGTACCACGGGGTGAGGT
                              <- gilGT
                                 ***
9231   AGTTGACGACAAGGACGGCGGCGAACTTCATGCCGGGCTCTCCGATCGGGTTGCCTGCGGACGCA
              SphI
9296   GTACGGCATGCATGTGCCAGATGTGGGAGACGACGTTCCCGGCTTCGTCCACCATCTGGGCGGCG
                                                      SalI
9361   AGGTTGTCGGTGGGGGCGTTCACCTCGAAGGTCCGCGGGTCGACCATCTGCGCGACGGCCTCCGG
                        KpnI
9426   CGGCAGCGAGCTGTGGTACCGCGTCGCCTCGATCACCTTGATGTCCCAGTCGGCGCCGAACGCCT
                                                SmaI
9491   CCCGCAACTCCGGTTCGGAGATGCGGCGCGGACCCGGGTAGTCGGGGGTCAGCTCCTTCGAGAAG
                                                         SacI
9556   GTGAACAGGTGCAGCTCGGCCCCCTCCTTGCACAGGGTCCGCAGGAGCTCCGTGTAGCGCGGCAC

9621   CTCCTCCTGCGGCAGCGTGTGGAAGAAGGCGCTGTCCAGGACGGCGTCGTACCGGACACCGGACT
                                          SalI                    SmaI
9686   CCGCGAGGCGGAAGGCGTCGGTCACCTGGAAGTCGACGCTCACGCCGTGGTCCCGGGCCTTGTCG
                                          SalI                    SmaI
9751   CGCCCGCACTGGACGGCGACCTCCGAGATGTCGACCGCGGAGACCCGCAGCCCCCGGGAAGCCAG

9816   GTAGAGCGCGTTGTCTCCGAGCCCGCAGCCCAGATCGAGCACGTGCCCGCGGAAGCCGCCGCGAT

9881   CACAGATCGCGCGTACGGCCGGCTGCGGGCCGCCGATGTTCCACGGCATGAGGGGGCCTGACTTC
                                                         <- gilM
9946   TCCCCGTCCTGGTAGAGCTTCTCGAAGGGGATCTTCTCCGTGCTGCCCGTTGGCATCGAGCGCCA
          ***
10011  CTCCTCTCAGAGTCCTATGGACATGCTGTGATGGAAGGTGTTCAAGGGGTCCCACGCGCGCTTCG

10076  CCGACCGCAGACGGGCGTAGTTGTCCTTGTAGTACAGGTGGTGCCAGGGCTCCCCGGAGCGGTTG
                                                            NotI
10141  CGGGCCGGGTCCAGGAGATCCGCGTCGGGGTAGTTGATGTAGCAGCCGTCCGTGCGGCCGCCGGT

10206  GACGGGCACCCCTCCCGTGCCGGCGAAGAACTCCTCGTAGAGCCCGCGCAGCCAGCCGAGGTGCA

10271  GCTCGTCCAGCTCCGCGTCCTGCCAGGCCGAGAACCAGGACGACTTCACGACGGAGTCCCGCTGG

10336  GGGACGGCGGCGTCCGACGGCCCCCGCCGGTTGATCTCTCCCCCGTAGCTGTTGAACATGACGTA

10401  CGAGGCCTGGCCGGGGTGGTCGGCGTGCAGGTGCCGGTGGAGCACCGAGAGCTGCTCGTCGGTGG

10466  GTGCCGCGCGGTGGTAGGCGGACTTGGAGGCGGAGCGGGCGCCCATGACGTCACCGCAGTCGGCC

10531  TGACTCATGTAGCGGGTTCCGGTGAGCCAGCTCATGACACCCCCTCGGGGGATGCCCACCACGCC

10596  GGTGCCCTCGGTCAGGGACGCGACGAACCGCGCGAGGATCTCGCCCTCGGGGTCCACGTCGGCGT
                        PstI
10661  CCTGCTGGACCATCAGCTGCAGGACGCCCGAGCTGACGTGGTTCACGAAGAAGGTGGCGAACAGC

10726  GAGGACTCCGGCGACCCCGGCTCGGAGTGGCGTTCATGCCACTCGAAGAAACGTCTCATCACAGT

10791  GACGAAGGACGTCTCGTCGATCATGGCCCAGGGGAACACCACCTTCTGGACGTGCAGTCGGCCGG

10856  CGGCGCGGGGCAGGCCGACGGGTTCCGTGGCGAGGTGCTCCGGGCTGCGGAACTCGTACGCCGTG
```

Figure 9. cont.

```
                                                                  SacI
10921  ACCACGCCGAAGTTGCCGCCGCCACCGCCGGTGTGTGCCCAGAAGAGCTCACCGAGATCGCCGGT
                                                              SalI
10986  GTCGTCGGCCCTCGCCGTCACGAGGCGAACGGTGCGGGACTCGTCGACGACGGCGACCTCCACCG
                 SalI
11051  CGTGCAGGTGGTCGACCACCAGCCCCAGCTGGCGCGACAGCGGCCCGTAACCACCTCCGGCGACC

11116  AGGCCGCCCATGCCGACCGCGGAGCAGGCCCCGAGCGGCAGGGCCGCGTTCCACCGGCGGAACAG
                       SalI
11181  GGCCTTCTGGACCTGGTCGACCGTCGCACCGGAACCGACGCGCACCCCGGCGCCGTCCGCGGCCG

11246  GGCCGATGGCATGGAGGTTGTGCAGGTCCAGGACGAGGTCCCGGCGCGGCGTGCCGACGAAGTCC
                                                                  PstI
11311  TGGCCGCAGTGACCGCCGGACCGGCAGGCGACCCCCCGCCCTTCCGTGACGGCCTTCTGCAGGGA

11376  GGCGACGACGTCGTCCGGCGTGGCGGGGAGGAAGAACTCCTCGGGCTCGACGACGAACCGGTGGT

11441  TGTCCGAGTGCGACAGTTCGATGTACCGCGGGTCCTCGCGGCCCACCGTGAACGGCGGTACGGAA
       <- gilR
          ***
11506  GCGGTCACGACGCGTACCCCTCGACCGACTGGGTGAACACGGTCTCGTAGGTGTTGGACTCCCGC

11571  GAGGTCAGCAGCGCCTTGCCGCGTGCGCGCATCTCCCGGTCGTGCGCCGGGCGTTCCTCCTCGGG

11636  CATGGCGTGGAACGCCTCGTAGGAGGCCGCGTCGTCCCACTGGGCGTAGTTGATGACCATGTCGC
                                          KpnI  SmaI
11701  CGTCGAGCGCCCTGAGGATGCTGTGGGACCGGTACCCGGGCACCTGCCGCATCCAGTCGTCCGGC
                          SacI
11766  TTCTCCAGCAGGGACACGAGCTCGCCCTGGTCCTTGGGGTCGCACCCCATCAGGACGATGACGGT

11831  CAGGTCCCCCCGGTCCGGGCCGATCTCCGTGCGGGCGGCACCGTCCTTCGTCTGGACGGACACCA

11896  CCTCGGTCTTCAGCAGGTGCACCGACGTGGTGAGTTCGGTGAAGTAGGGGACCGTGTTGTGCTTG

11961  AAGTTCTCCCCCTCGTACCGCTCCCTCAGGTCGTTGATCGACCGCCACTGTATGTAGTTGGCCGC
                                     SalI              SmaI
12026  ACAGGGCCTCGCCACCCCCGCGTGCAGGGTCGACGACCGCCATCCCGGGTAGTTCGCGTTCGCGA

12091  TGATGCCGCGCATGGCGTCCAGGAGGGTCGCCTGCTTCTCCGAGTCGGTGGTGTTGAACAGGTTG
                                          <- gilOII
12156  AACACGGTCAGGGAGCCGTTCTCGGGCTCGATGATCGGCATGAGGCCTTCCATGAGGTGTCGGAC

12221  GGCCCCGGAGGGCCGGCTGTGGCTGATGGGTTCTGACCTCGGTGACGACGAGGACGCGGGCGGCC

12286  GGCTCTCCGGGCCGCCCGGACCGCCTGTCGGGATGCCCGCCGCACACCCGGCCCGGCGGCCGGCG
       ***
12351  TCACCGGCTGCGGGGAGAGCCCGGTGTACCGGTCTCCCCCGAGACCTTCCTTCCTTCGACGACGG

12416  ACTGGAGTCCGGCGCGGCTGACCCCGACCAGCTCGATCCCGACCTCGGCCATCAGGAGCCGGAAC

12481  TCCTCCACGGTGCGCTGTTTGCCTTCGCACAGGAGGAGCATGTCCATGTCCATGAGGGAGATCGC

12546  CTTGTCCGCCGTGTCGTCCAGGCCCGCCACAGCCTCCACGACGGCGATGTGCGCCCCCTCGTGCA

12611  TGGCCTCCGCGATGTTCCTGAGGATGAGACGGGAACGGCCGTCGTCCCAGTCGTGCAGCACGTTG
```

Figure 9. cont.

```
12676  GAGATCATGAACAGGTCGCTGCCCGACGGAACGCCGTCGAAGAAGGAGCCGGACTTCACCGTGAC

12741  GCGGTGGGCCACCGCCGCGTCCGACAGCTCCGGCAGGGACCGCGACACGACGTCCGGCTGGTCGA

12806  AGAGGACGCCGGTCATCTCGGGGTGCTTGCGGAGCACGGCGGCCAGCAGCGCCCCCCTCCCCCCG

12871  CCCACGTCGGTGACACTGCTGAACCGGGAGAAGTCGAACGCCTCGATCACGGGCTGGATGACGCG
                                                              BamHI
12936  CTTGGACAGTTCGGTCATGGCCGAGTTGAAGACGGCCGCGGTGTCGGGATCCGACTCCATGAACT

13001  CCCACACGCCCGAACCGTGCATCGCGGCGAAGGGAGAGCGGCCGGTGCGCACCGCGGTGGCCAGG
                                  SalI
13066  TGGGCCCAGGTCGCGCGTTCGGCCGTCGACCCCGTCCACCGCGCGAAGTTGCGCATGGAGCCGTC

13131  GTCGGAGGCCAGGGCCCGGCCCAGCTCCGTCAGCTCCAGCGTGTCGTGCTCGCCCCTCCGCAGCA

13196  GCCCCACGGAGGCGCCGGCGCGGAAGAAGCGCTCCGCGGTGTCGTGCTCCAACCCCAGCCGGACC

13261  GCCAGTTGTGCGGGCGTCAGCCCGCCGGCGGCCAGGCCGTCGGCCACGCCCAGTTCCGCGAACGT

13326  GCTGACGACACCGGCGCGCCAGCCTCCCATCAGGAGGTCGAGGATCTGCACAGCGACGGGTGCCG
                                  <- gilMT
13391  ACGGGGGCAGCGATCCCGATGCAGTAATGGTCATGATGTCCCTTCGACTGGTGGACATGCGGGGA

13456  GGTGAGGCACCGCTGCAAGGGCAGTTCGCTCTCGAACACACTTTGTTCGTGATGCCTCACCGGCG
                                  SmaI
13521  CTAGGTATCCTCATGGGATGGTCCCGGGAAGCAGCGCCTGAACTGCGCAGAGTGCTGCCGGACGG

13586  GAGCGGATCGCCCGCCCGCACTCTGCGGCTGGTGGAACGGACGACCGTGCGGAGCCGGGCCGGCA

13651  CGGTCCGGTGGAAGGGCGGCCCTGCGGTGATCCGGGCGGCCCGCCCCCGTGCTCCCGGCGTCCCA

13716  CGCTCCGTAGGCGCGGGACGCCGGACACCTCATGATCCCGACGACGGCCCTGTGGGGTCCGGCCG
                    XhoI                ***
13781  GCGACGGGGCGCTCCTCGAGGAGCGCGGTGCTACTTCGCGTCCACGTCGGCTTCCTCGGCCCCGC

13846  GGGGAGCGTTCTCCCCTCCCCACTGCTGGGCGTCCCCCTTGGGCCGCGGGATGAGGAAGGTCGCC

13911  AGCATCCCGAGCGCCAGCAGCGCGGCGCAGGTGTAGCCGTTGATCTGGATCGCGTACGACATGGC
                          NotI
13976  GTCGCGGGCGGCGTCGGCGGCGGCCGCCGTGTCCGGGTTCTCACTCAGTCCGGAGATCATCGCGC
                                                              SalI
14041  CGGCGCTGTCGGAGACCGCCGACGACAGCTCGTTCGCCTCCGGCGCGGGCGTCCCCTGGTCGACG
                                                    SalI
14106  AGCCGGTCGGACAGGTCGGAGTCCAGTGCCGTGAAGAAGGTCGACGTCAGAATCGCGATGCCGAG
                                              BamHI
14171  CGCGGAACCGAGCTGGCGTGCGGCGCTCTGGATGCCGGATCCCTGGCCGGCGTGCCGCGGCGGCA

14236  CGTCCGCCAGGACGACGTTGGTGACCTGCGCCGTAGCGAACCCGACTCCCATGCCGTACAGGAAG

14301  AGGGCGATGCCGATGACCCACCACTGCGAGTCGCTGCTGGCCAGCAGACCGAACATCAGCAGACC
              XhoI
14366  GACGGCCTCGAGGACGAGGCCGATGCGCACCAGCCCGATGGGGCCGACGTTCTCGGCCATGCCGA

14431  AGCTCGCACCGCTCGCGAAGAAGCTGCCGACCGCGACGACGAAGACGATCAGTCCGGTCTGGAGC
```

Figure 9. cont.

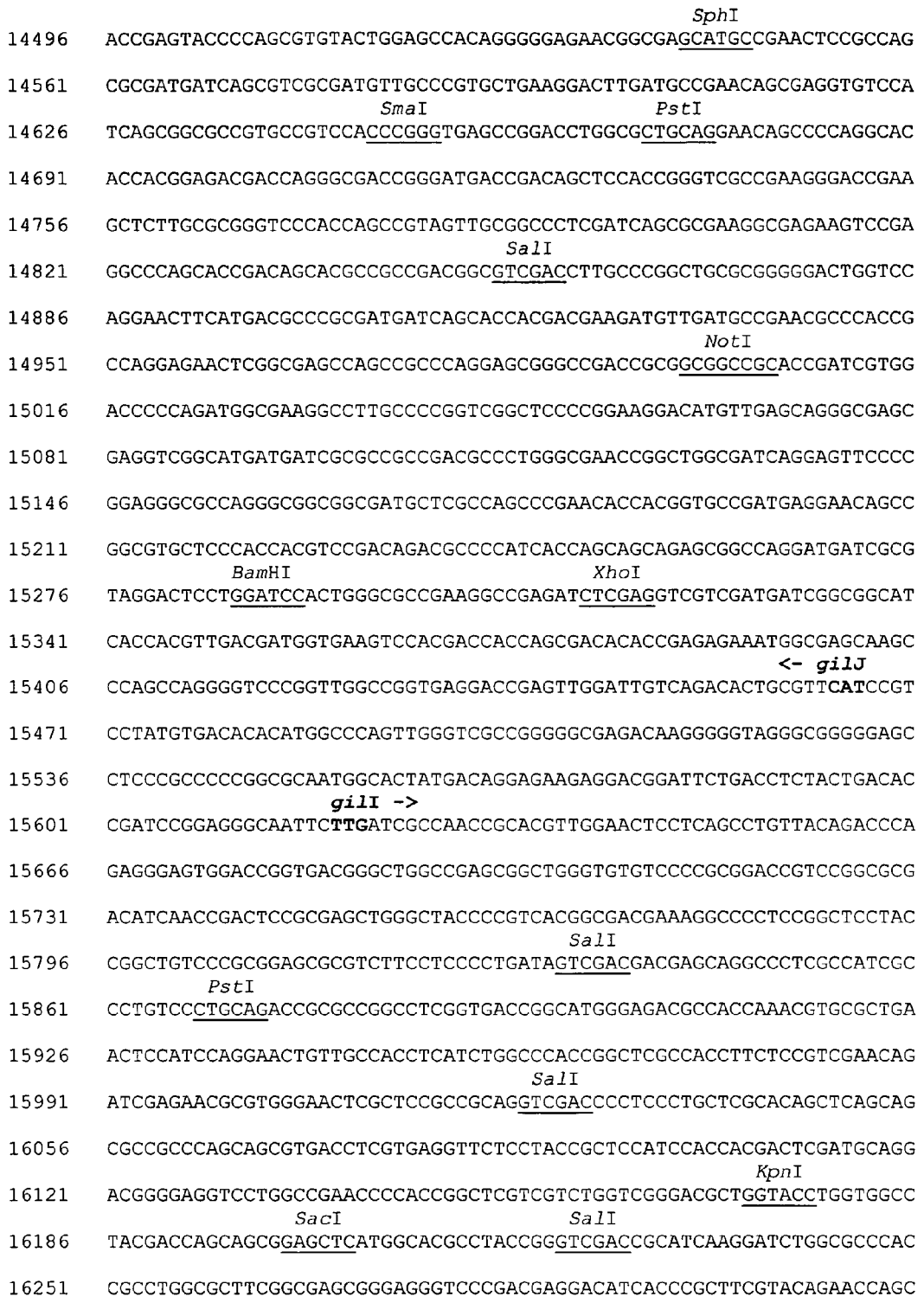


Figure 9. cont.

```
16316  CCGATCGCGGTGACACCCCGGACACCTGGCCCTGCTGGGGCACCGTCCTGATGGAGTGTCCCGCG
                              SmaI                              SalI
16381  TCGCTGGTGGCGAAGTGGGCCCCGGGAGTGGCGAGTTTCGAGGCGGTCGACGACAGGGTCACCCG
       BamHI
16446  GATCCAGATGGGCGCGTGGTCGTGGTCGGCGCTCATCGGCTTCCTGATCACCTTCAGTTGCCGCT

16511  TCACCGTCGAGGGTCCGCCCGAACTCGTCGCCGCGGCGCGGAGGGTGATGGGCCTCATCGACGTC

16576  GGGATTCCGTCGCACGACCCCCTCGCGGAGCCGTCGAGCCGCACACCCGGCCCCTCGGCCGGCCG
        ***                                                ***
16641  GTGACGCCGTGGCCGCGGCACCCCCGCACCGCGGGGAACCGCCGGGTCAGCGGACCGCCTGTCCC
                                                          KpnI
16706  AGCTCGGCGCGCTGCCGCAGCGGTTCCCACCAGTCCCGGTTCTCCCGGTACCAGGAGACGGTCTC

16771  CGCGAGGCCGGTGGCGAAATCCTTGCGGGGCTCGTAGCCCAGCTCCAGGGTGATCTTGGCGCAGT

16836  CCACCGAGTAGCGCCTGTCGTGTCCCTTGCGGTCGGCGACGTACTCCACCGACTCCCAGCCGGCC
                                                    SacI
16901  CCGCACGCCTCCAGGAGCAGTGACACCAGTTCCCTGTTGGTGAGCTCGGTGCCGCCGCCGATGTT

16966  GTAGACCTCGCCGGGTCTGCCCTTCGTGCGGACCAGTTCGATGCCCTGGACGTGGTCGTCGATGT
                                                                XhoI
17031  GCAGCCAGTCGCGGACGTTGAGCCCGTCCCCGTACAGCGGGACGGTGCGGCCCTCGAGGAGGCGC

17096  GTGATGAACAGCGGGATCAGCTTCTCGGGGAAGTGGTGATGGCCGTAGTTGTTGGAGCAGCGGGT

17161  CACCCGGACGTCGAGGCCGTGGGTGCGGTGGTGGGCGAGGGCGACGAGGTCGGCGGCGGCCTTCG

17226  AGGCCGCGTAGGGCGAACTGGGTGACAGCGGGTGCGTCTCCGGCCACGAACCGTAGGGGATCGAG
                                                    NotI
17291  CCGTACACCTCGTCCGTGGAGACCTGGACGAAGACGCCGCGGCCGCGTCCGTGGTGGCGCAGCGC

17356  CGCGTCGAGCAGGACCTGCGTTCCGCCCACGTTCGTGCGGACGAACTCGGCGCCCCCCAGGATCG

17421  ACCGGTCCACGTGCGACTCCGCGGCGAAGTGCACGATCTGGTCGTGCTCGGCGACCAGCCGGTCC
                                                    SmaI
17486  ACGACCGCGGCGTCGCAGACATCGCCCTGGACGAGGCGGAACCCGGGGTGGGCACGGACCGGGTC

17551  GAGGTTGGCCGGGTTGCCGGCATAGGTCAGTTTGTCGAGCACGGTGATGCGGACCCCGGCGGGCC

17616  CGTGCGGGCCGAGCAGGGTGCGGACGTAGTGGGAGCCGATGAAGCCGGCACCACCGGTGACGAGG
                <- gilE
       BamHI    ***
17681  ATCCTCGTGGCGGTCATGACGAGATACGCACCTCGCTGTGGTCCCCGAGGACCAGCCGGTGGGCC

17746  GCCGGCACACCGGCGGCGGGGGTCACCTCGACGTTCCGCCCGATCAGGGAACTCTCCACGCGGCG

17811  GACCCCCTGGACCGTGGCCCCGGCGAGGACGATGGAGAACTCGATCTCGCTGGACTCGATCCGGC

17876  AGTCCGCCGCTATCGACGTGGACGGGCCGACGTAGGAGCCGGTGATCCGCGTGTTGGCGCCGATC

17941  ACGGCCGGCCCCACGATCCGCGATCCGCGGATCTCGGCGCCCGCCTCGATCGTGACCTGGCCGAT
                      SalI          SalI
18006  GACCTCGCTGTCCGCGTCGACATAGCCGTCGACCCGGCCCTTGGCGCCCTCCAGCACGGCCCGGT

18071  TGACCTCCAGCATGTCGATGACGTTCCCGGTGTCCTTCCAGTAACCGGAAATGGTGGTGGAGCGG
```

Figure 9. cont.

```
                                        PstI
18136   ACGTCGTACCCGTGGTCGATGAGCCACTGCAGGGCGTCGGTGATCTCCAGCTCGCCGCGTGCGGA

18201   CGGCTCGATCCCCCGGACGGCCTCGTGCACCACGGAGGTGAACAGGAAGACGCCGACCAGGGCCA

18266   GGTCGCTGCGCGGAGCGGCCGGCTTCTCCTCCAGCGCCACCGCCTTCCCGTCGTCGTCGAGTTCG

18331   ACGACACCGAAGGCGCTGGGGTCGGCCACCTTGGTCAGGAGGATGTGCGTGTCGGGCCGGCTCTC

18396   GCGGAACCCGGCCACGAGATCCGCGATACCGCCCACGATGAAGTTGTCTCCGAGGTACATGACGA
                                  SmaI
18461   AGTCGTCGTCGCCGAGGAACTCCCGGGCGATCAGGACGGCATGGGCGAGACCGAGCGGCGCCGCC

18526   TGCCGTATGTAGGTGACGTCCAGACCGAAGGCGGAGCCGTCGCCGACCGCCTGCTGGATCTCGGC

18591   GGCCGTGTCCCCGACGACGATGCCGACCTCGGTGATGCCTGCCTCCGCGATCGCCTCCAACCCGT

18656   AGAAGAGCACGGGCTTGTTGGCTACGGGGACGAGCTGCTTGGCGTAGGAATGGGTGATGGGCCTC
                                                <- gilD
18721   AGGCGGGTTCCGGCCCCGCCGGACAGTACGAGAGCCTTCATGGCGGCGCAGTCTAGGCGGGCGGG

18786   GAAACATCTCAATCGGCCCGGCAGCGCACGGATGTCTGGAAACAACGGTCGGTAGAGGTCAGGAA
                                                                    SacI
18851   CTGACCTCTACCGCTCATAATCTGGCCGCTCCCCTCTCCCCGGAGATCAGCTTCGAGAGCTCGGT
                                        gilH ->
18916   CCCTACCGAAGGAGCGAAACAGATGATCAGGATCGCCGTCATCCTCGGAAGCACGCGTCCCGGCC

18981   GCCGCGGGGCCGTGGTGGCCCAATGGGTCGCCGAGGTCGCCGCGCGGCATCCCGCGGCGGTGATG
                                  SalI
19046   GGCGAGGCGGAGTTCGAGCTGGTCGACCTGGCGGAGTACGGCCTCCCGTTGCTCGACGAGCCCGT

19111   GCCGGCGATGTTCGGCCAGTACCAGAAGGAGGAGACCCGGCGGTGGGCCGCCGCCATCGGCTCGT

19176   TCGACGGATTCGTCTTCGTCACGCCGGAGTACAACCACTCGGTGCCCGCCGCGCTGAAGAACGCC

19241   ATCGACCACCTCTTCGCCGAGTGGACCGACAAGGCGGCCGGGTTCGTCAGCTACGGCGTGCACGG

19306   GGGAACCCGTGCCGTCGAGCACCTGCGGCTGGCCCTGGCCGAGGTGAAGGTGGCCGGGGTGCGCA

19371   GCCAGGTCGTCCTGTCGGTGTTCAACGACTTCGACTACACGGGATGCGACATGACGGACCCGACG

19436   GCCATGGGCCGGTTCACGCCGGGACCGCAGCAGGAGCAGACGGTGAACACGATGCTGGACGAGGT
                  SalI
19501   CGTCGCCTGGTCGACGGCGCTCAAGCCGCTGCGTACTGCTGCGACCGCTGAGGCGGACGGCCGGG
                  ***
19566   CCGTGTCGGTGTGACGCACCGGTCCGCCCGCCGGACCCCCTGGTGAACGTGCTGGTCACGGCCCC

19631   TCGTGCGTACGCTACGAGGGCCGTGACCAGCACGTTCGCCTGTACGGGCGAGCGTGGCCGCCACG
                                                                    SmaI
19696   CCGCGGGTGGGCGGCGGCAGCACGCCGGCCGGACCGATCGCCGAGTGGCTTGTTACGTGCCCGGG

19761   GCGACGGCGCCGGAGGGGGACGCGCCGAAAAAAACCGGTCAGTCGAGTTCCCCTTCGATAACACG
        EcoRV
19826   GATATCCCCCCGTCCTCACTTCGGGTGACCTACTTCGGCCGTGCGACTCCGAGCATCGTGAGCGG
         gilOI ->
19891   CATGACGTTGCACGCCGCAGAAGCCATACCGTCACACGTACCGGTTCTCGTCGTGGGAGCCGGCC
```

Figure 9. cont.

```
19956   CGACAGGTCTCATGCTCGGCGCCGAGCTGGCGCTCCACGGCAGCCGGCCGCTGGTGATCGACGCG
                                  SmaI
20021   CTGCCGAGCCCGAGCGGACAGTCCCGGGCCCTGGGCTTCACGGTGAGGACGCTGGAGATCTTCAA

20086   GCAGCGCGGCATCCTGGGCCGTTTCCAGGGACTCGCCCCGGTGCCCGGAGTCCATTTCGCCGGCC

20151   TCAGCATCAAGGGCGATCACCTCTCCAGCTCGATGCGCCCGGCCAACCAGTACCCGCAGTCCAAG

20216   ACCGAACAGGTCCTCGCCGCCTGGGCCGAGGAGCTGGGAGTACCGGTGCGGCGCCCGTGGACGCT
                                        KpnI
20281   GACGTCCATGGAGCCCACTGGCACCGGGTACCGCTGCGTGCTCAGCGGCCCGGCCGGGCAGCAGA
          SalI
20346   CCGTCGACGCCGACTACGTGGTCGGCTGCGACGGAGCGGGGAGCTTCGTCCGCGAGGCGATCGGC
        SphI
20411   ATGCCGACCAAGCGCACTCCCCCATCCGTACAGATGCTCCTCGGTGATCTGCGCGGATGCGGTCT

20476   GCCCGACGAACCCTTCGGGGTCAAGCACGAAAAGGGCATGGTCATGTCCGCACCGCTGGGCGACG
                                                    NotI
20541   GGACGGAACGCGTCATCGTCTGTGACTTCACCCAGCCGATGCGGCCGCAGGGCACTCCCGTCACG

20606   CACGACGAGATCAAGGCCGCCTACGAGCAGGTCGTCGGCAGCCCCCTGGCGGACGGCGAATGTCT
              SacI
20671   CTGGGCGAGCTCGTTCTCGGACGCGTCCTCCCTCGTGGAGTCCTACCGGTCCGGTCGTGCGCTGC

20736   TCGTCGGCGACACGGCGCACACCCATCTCCCCGCCGGCGGGCAGGGCATGAACGTCTCGATACAG

20801   GACGCGGTGAACGTCGGCTGGAAGCTCGCGCTGGTGAGCCAGGGCCGCGCGCCGGACACCCTGCT
                                  KpnI
20866   GGACACCTACCACGCCGAGCGGTACCCGGTCGGCAGGGAACTGCTGCTCAACACCGCCGCCCAGG

20931   GCCAGGTCTTCCTGCGCGGCCCGGAAGTGGACCCGCTGCGCGAGGTCCTGCGGCGACTGCTGAAC

20996   ATCCGGGAGGTGTCCGTCCTGCTGGCCGACGGAGTCAGCGGACTGGACATCCGCTACGACATGGG

21061   CCTCCCGGAAGCACCGCCACCCACGGGTGAACGGCTGCCGCCGGACGTGTTCCACGTCGTCGGGA

21126   CCGGCGGCGACGCCGTCGAGGAGTTGCGGCACGGCGCCGCTCTGCTGATCGTCCCGTCCCCCGAC

21191   AGCCCGGCGTCCTCGCTGGTCGCTCCGTGGCGGGACCAGGTGCGCGTCGTGCACGCGCGCCCCAC

21256   GGACCCGGACTGGGGCGGGGAGCCGGCCGCGTCGTCGCACTGGTTCGTACGACCGGACGGACACA
                        EcoRI
21321   TCGCGTGGGCGGGCACCGAATTCAGCGAGTTGAGCGCCTCACTGAGCCGCTGGCTCGGTCAGCCC
          ***                    gilG ->
21386   GCCGCGTAACCAGAGGAGGAAGAACCCTTGTTCAGCTCTCTCATCGTCGCCCGGATGGACACCGG

21451   CCACGCCGAAGCGGTGGCCGACGTCTTCGCCGGCTTCGACGCCACCGACATGCCCGCGCGGATGG

21516   GCACGCGGCGCCGCGAACTCTTCCGCTACCGCGGCCTCTACTTCCACCTCCAGGACTTCGAGACC

21581   CCCGACGGGACCGAAGCGGTCGAGGCGGCCAAGTCCGACCCGCGGTTCATCCGGGTGAGCAACGA

21646   CCTCAGGCCCTACATCGAGGCCTACGCCCCGGACTGGCAATCACCGAAGGACGCCATGGCAGAGC
                                  gilA ->
                                    ***
21711   GCTTCTATCACTGGAGTTCGAAACGATGAGCCGCAGGGTCTTCATCACCGGGGTCGGTGTCGTCG
```

Figure 9. cont.

```
21776  CGCCGGGAGCCGTCGGACGTGACCCCTTCTGGGAGCTGCTGACCCAAGGGCGCACGGCCACCCGC

21841  CGGCTCAGCCTCTGCGACCCGGAGCCCTTCCGGTCCCAGGTGGCCGCGGAGGCCGACTTCGACGC

21906  CGAGGCGGCGGGGCTGTCGGAGCGGCAGTCCGCGGAACTGGACCGGGCGGCGCAGTTCGCCCTGG

21971  TCGCCGCCCGTGAAGCGGTCGAGGACGCGGCATGGTCCGAGACATGTCCTCCCGAACGCGCCGGA
                                                  XhoI
22036  GTGATCGTGGGTTCGGCCGTCGGAGCCACGACCAAGCTCGAGGAGGTCTACCGGCAGCTCAGCCG

22101  TGACGGCTCCCTCTGGGACGTGGCCCCCGACTCCCCCGCCGAGCTGTACTCGTACTTCGTGCCCA
                                                                    SalI
22166  GCTCGTTCGCCTCCGGCATCGCACACGACCTCGGCGTCACGGGGCAGAGCGGCGTCGTGTCGACC

22231  GGGTGCACCTCCGGGATCGACTCCGTCGGCAACGCCTGGGAACTGATCCAGAGCGGCATCCTGGA

22296  CTCCGCCGTCTGCGGTGCCACCGACGCCCCCATCTCGCCCATCACCGTCGCCTGCTTCGACACGA

22361  TCAAGGCGACATCGACGTACAACGACACCCCGGAGAGCGCCTCACGGCCGTTCGACGCCACACGG

22426  GGCGGCTTCGTCCTCGGCGAGGGCAGCGCGATGTTCGTCCTCGAATCGGAGGAATCCGTCCACCG

22491  TCGCGGCGCACGCGTCTACGGCGAGATCCGCGGCTACGCGAGCCGCTGCAACGCCTACCACATGA

22556  CCGGTCTCAAGGCCGACGGACGCGAGCTGGCGGAGGCCGTCGTCTCCGCTCTCGGCCAGGCAGGC
         SmaI
22621  GTGGACCCGGGCCGGCTCGACTACGTCAACGCCCACGGCAGCGGCACGAAGCAGAACGACCGCCA

22686  CGAGACCGCCGCGCTGAAGTCGTCCCTCGGACCCGCCGCCCACGACGTGCCGATCAGTTCGATCA

22751  AGTCGATGATCGGCCATTCGCTGGGCGCCATCGGGTCGTTGGAGATCGCCGCCTGCGCCCTGGCG
                                                        BamHI
22816  CTGCGGGACGACGTGATCCCGCCGACGGCCAATCTCACCCGGCCGGATCCGGAACTCGATCTGGA

22881  CTACGTGCCGGTCCACGCGCGCAAGCAGCCGACCAACAGCGTGCTCACGACCGGAAGCGGCTTCG
                                                      gilB ->
                                                       ***
22946  GTGGGTTTCAGAGCGCCATGGTTCTCACGGACCCGGAGCATCACTCATGACCGCACACATCACCG

23011  GCATCGACATCGTCTCCCCGCTGGGCCTGTCCCGCGAGGAACACTGGAAGGCCCTCCTCGACGGA

23076  TGCAGCGGTCTGAGGGCGACGCAGTCGTTCGACTCCAGCAGGTACGACAACCCCATCAGCGGGGA

23141  GGTGCCCCACTTCGCCCCGGAGGGCCTGCCCAAGCGGCTGCTGCCGGCCACCGACCGGATGACCC
                                                  SalI              SalI
23206  AGATGTCGCTGGTCGCCGCGGCGGGGGCGTTCGACGACAGCGGTGTCGACACGAGCCGGGTCGAC

23271  CCCCTCGGAGTCGGTGTCATGACGGCGTCCACCGCGGGGGGTTACGCGTTCGGGCAGAAGGAGCT
       PstI
23336  GCAGAACCTGTGGTCCAAGGGGCCCAGGTACGTCAGCACCCATCAGTCCTACGCCTGGTTCTACG

23401  CGGTCAACACCGGTCAGATCTCCATCCGGCACGGCTGCCAGGGCCACAGCGGAGTGATCGTCGCG

23466  GACGACGCCGGCGGGCTCGACGCGATCTCCTTCGCCGCCCGCCGTCTGGCGCGCGGCAACCGCGT

23531  CATGCTCACCGGGTCGGTGGACAGCACGATGTGTCCCTGGGGGCGGGTCGCGCACACCTCGACCG
```

Figure 9. cont.

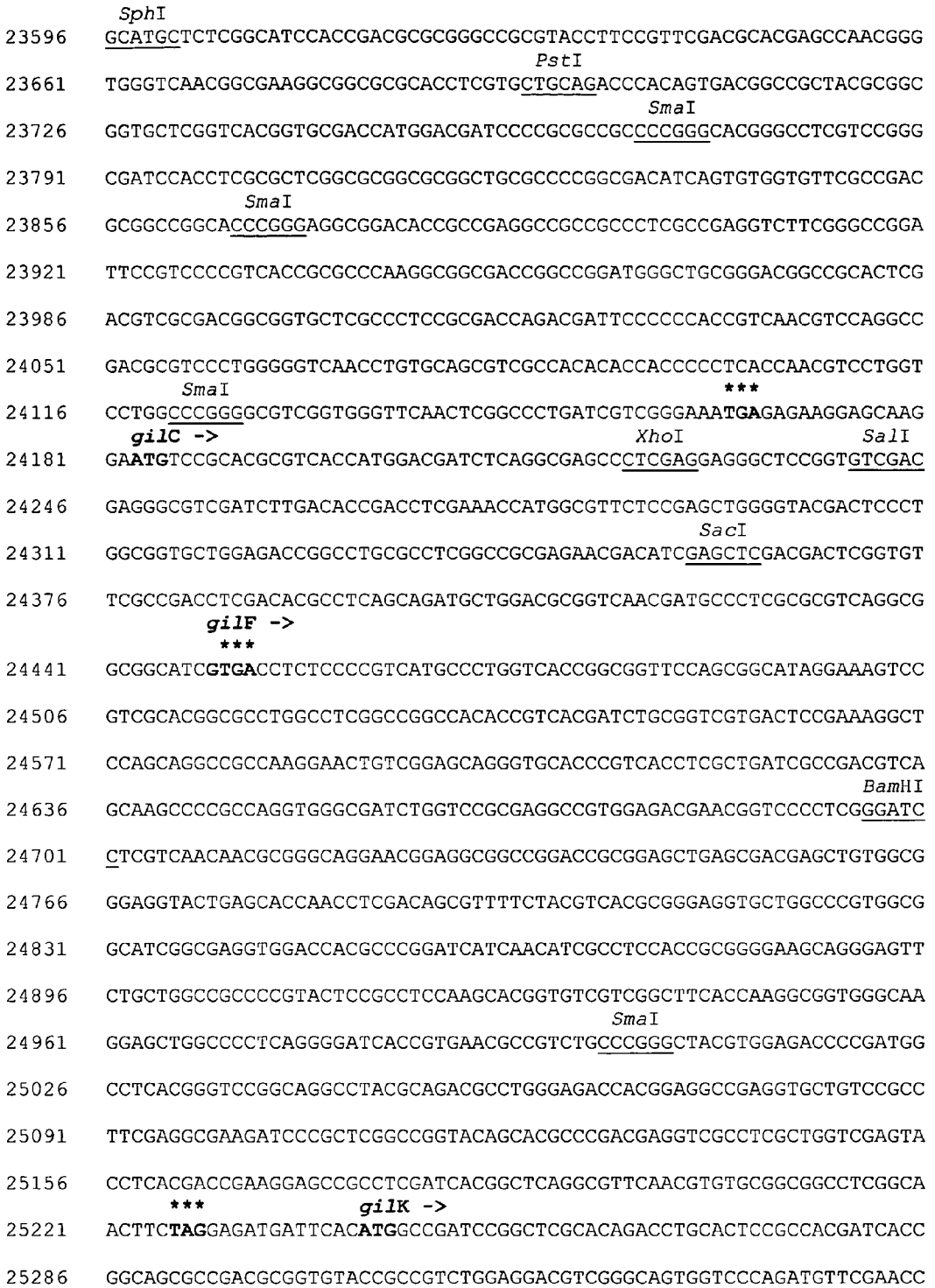


Figure 9. cont.

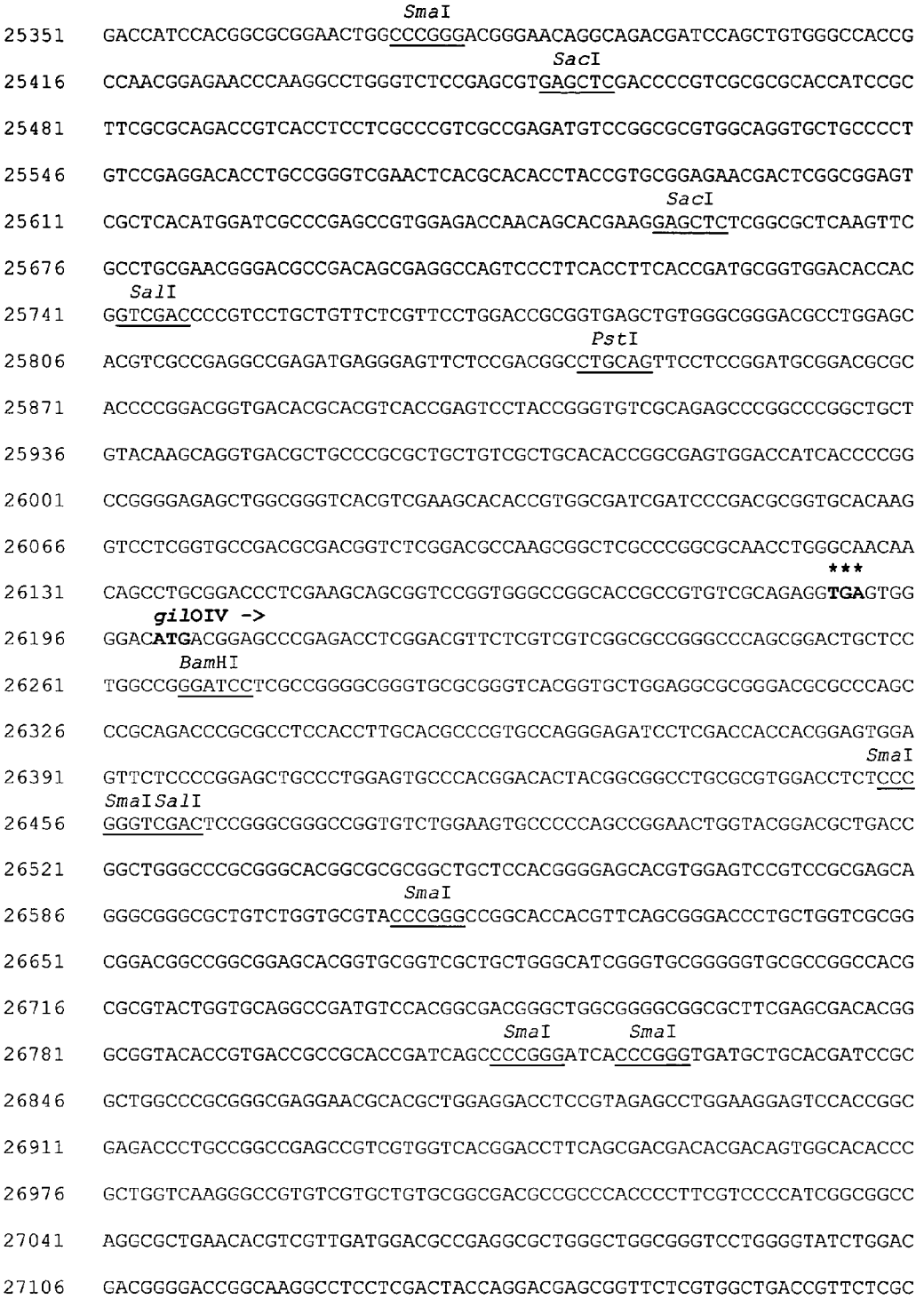


Figure 9. cont.

```
27171   GGGGAGACTGCGCGCCCAGGCACGTCTGCTGTTCGACACCGACGCGGCGGCCACGGAACGCAAGG

27236   CGCTGGTCGCCGCGAGACTGGCCGGGGACGCGGACTACCGGCGCAGGATCGCCGACGCCCTGGCC
            SalI
27301   GGTGTCGACGTGTGCTACCTGACGCCCGGCGGCGCGGTCCGCCGGCGTCTGTCCCCGGCCCGGCT

27366   CCGGGAGACCGGAGTGAACCCCGGCGCCCGCCGCGTGCAGCGGGCGCTCGTCCCCGACGACGGAA
                        BamHI                   KpnI
27431   CGCGCACGGACGCCTGGATCCGTCCCGATCACCACTGGTACCCGGTGGCCCGCGACGGGGCCCGG
                                                                  gilP ->
                                                                   ***
27496   CAGGACTGGGACGACGCGGTGCGCCTCCACGACGACTTGGAACCCGAGGTGACGCGGTGAGAGCG
                                                                      KpnI
27561   TTCCTGTTCCCCGGTCAGGGGACCCAGAAGATCGGCATGGGCACCTACCTGCGAGAACGGTACCC

27626   CCACCTGATCGCGCCGTTGTGGCGGGAGGCGGACGACGTCCTGGGTTTCCCCCTCACCCGCCTCT

27691   GCGAGGAAGGCCCCGGCGAGAAGCTCCGCCACATGCCGGTCACCCAGCCCGCCGTCTTCCTGTGC

27756   AGTTACGCCGCGCTCGTCGCCGCGCAGGCGAACGGCGCGGAGCCGGACGTCATCGCGGGCCACAG

27821   TCTGGGCGAGTACTCGGCGCTGGCGGCGGCCGGCGTCCTCACCTGGCAGGAGGTCCTTCAGCTCG

27886   TCCACCGCCGCGGTCAGCTCATGGCGGAGGTGCAGCACAAGGTGGACGGGAAGATGGCGGCCGTC

27951   ATCGGTCTCGCCATCGGGCAGGTCGAGGAGATCTGCGAGCAGGTGCGGTCCGAGACCGGTGAGGT

28016   GGTCGAGGTGGCCAACCACAACGAGCCCCTCCAGGTCGTCGTCTCCGGCCAGTGCGCTGCCATAG

28081   ACCTCCTGGTCCAGCGCGTCGCGACGGCGACCGACGTCCGCACGTCCGTCCTGAGGATCGGTGGC

28146   CCGGCCCACTCCAGTCTCATGGGCAGCGTCGCGGGGGACTTCGTGGAGTACCTCCGGCGCTTCGA
                                                  SalI
28211   CTTCTGCACGCCCAAGACGATGCTGATCTCCGGGTCGACCGCCGAGCCCTACGCGAGTGCGGAGG

28276   AGATCAGGCACCAGCTCGGCAGGCAGCTGGTGCACCGGGTGCGGTGGGTGGACGTGATGGCGCAG
          XhoI
28341   CTCGAGAGGCTGGGGGTCGCACAGACCTGGGAGCTGGGGCCGGGCAAGGTCCTCTCGGGATTCGT

28406   ACAGCGGTCGCTGCCTCAGGTGCGGACGTACCGCGCGAATGATCTGCCGTCCTTCCTGGCCGGCG
                    ***                     gilQ ->
28471   TGACGGGCTGGTGAGCCGGTGAAGCACGCAGTGCCGCATCAGGCAACCGGCGCCGCTCCCGACGG

28536   AGGGGGGTCCGCGCCCCGGTCCCTCGTGCTGATGCTCCCCGGCCAGGGGTCGCAGTTCGCTGCCA

28601   TGGGAGTCCCGCTCTACGAGTCCGACGCCCGGTTCAGGAAGGCGCTCGACGACTTCTTCGACGCG

28666   TTCGGCACCGGTGCCGAGCGGCTCCGGCGCGAGTGGCTGCACGGTTCGGCCCAGGGCATCGAACG

28731   TGGGTCCTTCGCGCAGCCGATGCTGTTCGGCCTCGACTACGCGGCGGGCGCGGTGTGGCTGGAGG
        SacI        SalI
28796   AGCTCAAGGGTGTCGACGTGACGCTGGTCGGTCACAGCGTGGGCGAGCTGGCGGCGGCCACCCTC
                          XhoI
28861   GCGGGGGCCTTCGACCTCGAGCTGGCGGGGGCACTCCTGGCCGAGCGGGCCCGGCTCCTCGACGC
              SmaI
28926   CGCCCCCCGGGGAGGGATGATCGCGTGCCGCGCGACGGAGGAGTCGCTGCGGGAGCATCTCGACG
```

Figure 9. cont.

```
28991   CCCTGGGCGGACGCGCCGTCATCGCGGCGGAGAACGCGGACAACCAGTGCGTCGTGAGCTGTGCC
                                        KpnI
29056   GAGGAAGACCTCCCGGACACGATGCGGTACCTCGGCTCGCACGGTGTGACGTGCCTGCGCGTCGC

29121   CTCGACCGAACCGTTCCACTCCCCCCTCCTCGCCCCCGCCGCCGCCCGGTTCGAGGAGTTCCTGG

29186   CCCGGCGCGGTCATCGTCTGTCCACGACGGAACTGCCCATGGTCTCGGCCTACTCGGCGCGGAGG

29251   ATCAGCGGCCGGGAGATCATGCCCGCCTCGTTCTGGACGCGTCAGATGGCTGAGAAGGTGCGTTT

29316   CTGGGAGGCGCTCCGCCACAACTTCGACTCCGGTCCCCGCACGTTCGTGGAAATCGGCCCAGGGA

29381   CCGTCCTCTCCCTGGCCGCACGTCGGCTGCCGTCCGTACGGGCCCGGCGTTCCACGGTGATCTCC

29446   ACGATGCCGCGTCATCGGCCCCACCCGGAGCACTGGGAATCGGCCATACATGAGGTCGCCGAGGA
        EcoRI  ***                                                    SmaI
29511   ATTCTGTTGACCATTGCACTACGTGCAACGCGCAAGGCCGGCCATGGGTGTCCCCCGAGTTCCCG
                                                          XhoI
29576   GGAGGCACCCATGGCCTTGTCGGTGAAATGTTCAACCAAATGAACCACCTCTCGAGGGCGCCCCG

29641   GATCAAAGATGTTCACCGATTTGCATAGTCGAAAAAATACGGACAGCAACGGAAGCGGAGTGTTA

29706   TCCTGCAATCTGCACGCAACGGGGGAAACGGGGGAGGATTCCAAGTGCAGGACCCGGTGGACCGG

29771   ACCTCGGGGAACTCCAGAGGCGCAAGCACCGTCGGACCCGAGCAGTCCCGCAAAGACGCGGGGCA
                                    KpnI
29836   CTCCTGTCGCCGAAGAGGGCCCGGTACCGTCACGCAGCAGTTTTCGCCGACCCTCCACCATTTCT

29901   TCACCGTCACGAGATCTCTTCGGGCCGACGGGGACCACACGGGCAGAGACTGAAGGACCGGCGCT

29966   CGACCTCGCCGGCTCCCGCCCCTCCACCCCTTCCCCGCGTTGCCCCTGACCGCATGGGCGGCATC

30031   TCGGTGCCGCGTTTCTTCCGCGCCTGGCGCGAGGGGAGATCTCCCATCAAGGGGGGCCTTTCAAG
              XhoI
30096   GCCCTCTCGCCTCGAGGGCACCGCACGCCGAAGACCGATCACAAAAGTATCCGAACGGCTCCGAC
                                                        gilt ->
30161   CGAGGTCATATCTGAGACTGATCGAATATCCAACGGGGAGATGTGATGGGTTTCATCCGGTTTGA

30226   CGTTCTGGGCCCGCTCAGGGTCCGGTGCGACGACACCCTGCTTCAATTGACCGGGCGCAAGTACC

30291   GCACCGTGGTTTCGTATCTCGCTCTTCAACCCGAGTATTCGGTGGCGATAGAGGACCTCGTCCGA

30356   GCCGCTTGGAGCGACAAGCGCCCGTCCAGCGCGCACCACCAGGTCCGTAAGATGGTCTCCGCACT

30421   CCGGACCAGCCTGGACCAGGACTGGGACCTGGTGGCGACGTCCCAGGACGGCTACATGCTGAAGT
                                    EcoRI
30486   TGCCGCCCAAGCAGTCCGACGTATCCGAATTCTGCCGCCTCTTCGACCAGGTGATGTCGGGTCCC

30551   CTGACGAGCGACGACGACCTGTCGGCCGCGTATTCGGCGCTGGCGCTCTGGCGCGGACGCCCTTG

30616   CGAAGGGTCCGAGCCCCATGGGCAGGAGCGCCGGATCTCTCAATTGGTGGAACAGCACCGCGTCC

30681   TCTTGAACAAGACCGTTCAGGGATTCGGCGACAGGGGCAGGTCCGATGAACTCGCCTCGATACTG

30746   CACGTCGCATCGAAGATTCACGGACAGCCGGTCACCGCTCGCTCCGGTGTCGCCGTTCCCGCGCC
```

Figure 9. cont.

```
                                                                  SalI
30811   CGCTGTTTCGTACGCGGGCACGACACAAGTCCCCGAACCTTCGGGGTCGACCACCCCTCCCCCAC
                                                          PstI          NotI
30876   GCCCCGGCTCCCCCGTCGGTCCGCGCTGCCTGCCACGGGATCTGCAGGACTTCGGCGGCCGCGAA
                        PstI
30941   CGCGAAATCAATGAGCTGCAGAAACTGTTGACCGCGGAAGGACCCCACCCACAGTTGGTGGCGAC

31006   CGTTCACGGAATGAGCGGCGTGGGTAAAACCGCCGTCGCCGTCCGCCTGGCGCACAGACTAGCCC

31071   ATCACTATCCGGACGGCCAGCTCTTTGTATCCCTGGACGGCTTTTCTTCGGCCTCCACCGCCACC

31136   GTGTCGAATGCGCTGGGAATACTCCTCAGACAGAAAGGCCTGGCGGACGAGGACATTTCACCTTC

31201   GGAAGACGGCCGCCTCGCACAATGGCGGACCATCACCGCCGGACAGAAGCTGCTCGTCGTGCTCG
                                                          XhoI
31266   ACGACGTGTGCGACATCGAGCAAGTAGAACCCCTCATCCCGCCCTCGAGCGAAAGCGCCTGCATC

31331   ATCACGTCGCGCATCATCCTCAATGGCATCGACGGCGCTCATCACATCTCACTCGAAGTACCGGA

31396   CGAGGACGAATGTCTGGAGATACTCAGTTGCATGATCGGCAGACGCTTCGACGACGAGGAGACGA

31461   AGGACGCCCGCGCGCTGATCCAGCAGTGCGCCAATCTGCCGCTGGCACTCCGTCTCGCCGCCGCC
          EcoRV
31526   CGGATATCGACGCGCGACTTCCTGAACCTCCGGGAACTCAGTGAGCAACTGTCGTCCTCGGCTTC

31591   CATCTTCAGTGAACTGGAAGTTCCCGGCCGTAGTCTGGTCGGCCGGCTCATGACGTCCTTCACGT
                                  KpnI
31656   GCCTGGAGGACTTCGATCACGACCGGTACCTCCGATTATCGCTGCTCCCCTGCCCCGAGATCGAT
                                                                  PstI
31721   GAAACGTCGGTCGCGGCCGTGCTGGGCGTATCCACCGACTGGGCACGGCGTGCCTGCAGGCGCTT
                                                                  PstI
31786   CGCAGACCGCGCGTTGCTGCAACGCACACGATGCGGTACGTACCGGATGCACCCGCTGCTGCTGC

31851   AGGCGGCACAGCTGGAAGCGCAGAAGACCATCCCGTTCGAGGAGCAACGCCGGCTCGTCCGCGCC
                                                                  SmaI
31916   GCTTTCCTCCATTACAAGGCGTCGAACGGCCTCGTGGGAGCCAGCCGCATCAGCCCTTCCCGGGT

31981   TCCTGACGGACACGTGGTACTGAGGACCCTCACGCAGTCCGCGAAGCTGGCCGCGCGGCTCGGCC

32046   TCCAGGAGGAGTTGGCCGATCTGTACACCGCCTGGAAGGAACTGCTCCCCCTCGTGCTGGACCGC

32111   CGGCAGCAGGAGGCGGTCGGGCGACGCGTACTCGCCGTTTCACAGCACCTGGACCGGCCCGCGTG
                                                                  *
32176   CGAGGGAGCACCCCACCGGAGGCGTCCGCGGCAGGCACGGGACATGCTGCCCGAGGGGCAGCGGT
        **
32241   GAACGAGGGCCGGCCGGAGGAAGGCAGTCGGACGATGACGACCGTTTGTCCGTACATCGGGAGAC
                                                                  SmaI
32306   CGGGGTGCCACGTGAAACATGTGACCCGGTCACCGGATGTCCGATCGCAGCCGCACCCGGGGGCG
                gilU ->
32371   AACTGACCATGGACCGCGTTCTTCCGTATGCAGCCGGCAGTGAGGCACTCCTGTCGTCGAGAGAG

32436   CACGGCCCCACGGTGAGCGAGAGAACCGTCTCGGCGCAGGAGATCGTCGTGGGCGGCGGGGGTCT

32501   GCTGGGGAGACACATCCTCGGCGTGCTGGGCAATCGGCTCAGCCGGCGGGTACGCATCCCGTGGG

32566   ACGACCACGGCCGCGCCTGTGAGCAGCTCTACGCGCTGGGCAGGGACCTGGCTCAGCAGCCGGCG
```

Figure 9. cont.

```
32631   CGCTGGAACCTGTACTGGTGCGCGGGACTGGCCGTCTTCCACACCCCCGCCGAGCAGGTGGAGCG

32696   AGAACGCCTCCAGGTCAGCCTCCTCCTGGCGGGCATCAACGACGGGCTCGAACGCTCGGGGGGCC

32761   CCACCGGCGGCGCGTTGTTCCTGGCCTCCTCAGCCGGCGGCGCGTTCGCGGGCTCGGAACACCCG

32826   CCGTTCACCGAGTTCTCCCCGCCCGTGCCCACGAACCCGTACGGCGCGTCCAAACTCGCCGTCGA

32891   GGAGGAGGCCGAGGTTCTGGCGCGCCGTTGGCGACTGCCCACGGTGTCGGGTCGGATCACGAACC

32956   TGTACGGCCCCGGCCAGAACCTCGACAAGAACCAGGGCCTGGTCAGTGCCCTCGTCAAAGCGCAG

33021   CTGACCGGTGAACCCCTGCGGCTGCGGGCCGCCCTGGAGACCACGCGCGACTACATCTACGCACG

33086   GGACTGCGCCCGGATGGTCGTCTCGGCGATGGAGACCGTACGGTCCCGCACCCGCGGCACGGACC

33151   CCCATGTCCGCAAGATATTCAGCAGCGGACGCCGTCTGCGGATCGACGAACTGCTCCGGATCGCC
                              KpnI
33216   GAGCGCCTCTTCGACCGGCCGGTACCCGTCGTCCACGAGCCGGTGGCGGGAGGGGCGAACGTCAA
                        SmaI
33281   CCTCTCGGTCGAGTCCCGGGTATGGGCGGACCTCGAATCGTCCCCCTTCCTCAGCATCGAGGAAG
                                        KpnI                    ***
33346   GGATGCGCGCCGTCCGCTCCGACCTCAGGTACCGACTCGGGCACGGGTGAGCGACACGAACGACA

33411   AAAGACCCAGGCCGCACATCAGCGCGGCCTGGGTCCGATGAGCCGCCTTCGGGATTCGAACCCGA

33476   GACCTACGCATTACGAGTGCGTTGCTCTGGCCATCTGAGCTAAGGCGGCGTGCTGGGTGCACCCA

33541   TGGTGCGATCAGCGACGTCGGTAAGTCTACACAGTTTCGAGGGGTGGTTCGTACCGGCCCCGGAG
                                              ***
33606   CGGGCCGGGGCTGCCTCCGGTGGGCGGGGCGGCTACGAGCAGCGTGTGCCGTCGGCGGGCGGGGT
                                           SalI
33671   GCCGTCGAGGAGGTAGGTGTTGATGGCGGAGTCGACGCAGGCGCTGCCGCGGCCGTACGGGGTGT

33736   GGCCGTCGCCGTCGTAGGTGAGGAGGCGGGCCGTGGCGAGCTGGCCGGCCAGACCCTCGGCCCAG
          <- proteinase  SmaI
33801   CGGTACGGGGTGGCCGGGTCCCGGG
```

GILVOCARCIN GENE CLUSTER, RECOMBINANT PRODUCTION AND USE THEREOF

This application claims the benefit of U.S. Provisional Application No. 60/477,957, filed on Jun. 13, 2003, which is incorporated herein by reference.

FIELD OF INVENTION

The present invention relates generally to polyketides and polyketide biosynthesis. In particular, the invention pertains to the nucleic acids encoding gilvocarcin polyketide synthase and the tailoring enzymes of the gilvocarcin biosynthesis, and to recombinant vectors and host cells containing such genes, and to the recombinant production of gilvocarcins and uses thereof.

BACKGROUND OF INVENTION

Polyketides represent a large family of diverse compounds synthesized from 2-carbon units through a series of condensations and subsequent modifications. Avermectin, candicidin, epothilone, erythromycin, FK-506, FK-520, narbomycin, oleandomycin, picromycin, rapamycin, spincoyn, tetracycline, and tylosin are examples of such compounds. Polyketides occur in many types of organisms, including fungi and mycelial bacteria, in particular, the actinomycetes. Polyketides can be divided into macrocyclic/polyether-type compounds, biosynthetically encoded by type-1 polyketide synthases (PKSs), and into multicyclic, aromatic compounds, whose core structures are encoded by type-2 PKSs. Type-1 PKSs are "complex" or "modular" PKSs which include assemblies of several large multifunctional proteins carrying, between them, a set of separate active sites for each step of carbon chain assembly and modification. As such, structural diversity occurs in this class from variations in the number and type of active sites in the PKSs. This class of PKSs displays a one-to-one correlation between the number and clustering of active sites in the primary sequence of the PKS and the structure of the polyketide backbone. The second class of PKSs, called Type-2 PKSs, is represented by the synthases for aromatic compounds. Type-2 PKSs have a single set of iteratively used active sites.

Angucycline group antibiotics, which are arranged by a type-2 PKS are structurally characterized by their angular, polyketide-derived benz[a]anthracene-derived backbone (angucyclinone), which is often further decorated with sugar moieties (angucyclines). Angucyclines/angucyclinones form the largest and structurally most diverse sub-group of the multicyclic, aromatic polyketides. Knobler, R. M., Radlwimmer, F. B. and Lane, M. J. *Nucleic Acid Res.* 20:4553-4557 (1992); Matsumoto, A. and Hanawalt, P. C. *Cancer Res.* 60:3921-3926 (2000). Yamashita, N., Shin-Ya, K., Furihata, K., Hayakawa, Y. and Seto, H. *J. Antibiot.* 51: 1105-1108 (1998); Nakashima, T. et al. U.S. Pat. No. 6,030,951. A very interesting set of natural products with respect to their biosyntheses as well as their biological activities derive from this angucycline/angucyclinone group. However, they are not easily recognizable as such, since their polyketide-derived skeleton is rearranged in a series of steps, initiated by oxidative biosynthetic processes. The gilvocarcin-type anticancer antibiotics (Morimoto, M., Okubo, S., Tomita, F. and Marumo, H. *J. Antibiot.* 34:701-707 (1981); Breiding-Mack, S. and Zeeck, A. *J. Antibiot.* 40:953-960 (1987); Yamashita, Y. and Nakano, H. *Nucleic Acids Res. Symp. Ser.* 20:65-67 (1988); Elespuru, R. K. and Gonda, S. K. *Science.* 223:69-71

(1984)) and the jadomycins (Oyola, R., Arce, R., Alegria, A. E. and Garcia, C. *Photophysical properties of gilvocarcins v and m and their binding constant to calf thymus DNA. Photochem. Photobiol.* 65:802-810 (1997)) are examples of such 'rearranged angucyclines'. Both of them, and the kinamycins (Takahashi, K. and Tomita, F. *J. Antibiot.* 36:1531-1535 (1983)), have in common biosynthetic rearrangement cascades that begin with an oxidative cleavage of the 5,6-bond of an angucyclinone intermediate (FIG. **1**).

Given the difficulty in producing polyketide compounds by traditional chemical methodology, and the typically low expression of polyketides in wild-type cells that produce them naturally, there has been considerable interest in finding improved or alternate means to produce polyketide compounds. This interest has resulted in the cloning, analysis, and manipulation by recombinant DNA technology of genes that encode PKS enzymes.

Gilvocarcin-Type Natural Aryl-C-Glycoside Antibiotics

The benzo[d]naphtho[1,2-b]pyran-6-one C-glycoside antibiotics, often referred to as gilvocarcin-type aryl-C-glycosides, were discovered in Japan in the early 1980s. Studies have shown that these molecules are decaketides, and that they originate either from one acetate starter and nine malonate extender units or from one propionate starter and nine malonate extender units, depending on the 8-side chain. The incorporation pattern suggests the key intermediate to be an angucyclinone, such as 2 in FIG. **2**., which then rearranges to form the coumarin frame. Krohn, K. and Rohr, J. Angucyclines: *Total Syntheses, New Structures, and Biosynthetic Studies of an Emerging New Class of Antibiotics. Topics Curr. Chem.* 188, 127-195 (1997); Takahashi, K. & Tomita, F. *Gilvocarcins, New Antitumor Antibiotics. 4. Mode of action" J.Antibiot.* 35: 1038-1041 (1982); Carter, G. T., Fantini, A. A., James, J. C., Borders, D. B. & White, R. J. *Biosynthesis of Ravidomycin.* Use of 13C-13C Double Quantum NMR to Follow Precursor Incorporation. Tetrahedron Lett. 25, 255-258 (1984); Carter, G. T., Fantini, A. A., James, J. C., Borders, D. B. & White, R. J. *Biosynthesis of Chrysomycins A and B. Origin of the Chromophore. J. Antibiot.* 38, 242-248 (1985). Gilvocarcins are biosynthesized by a type-II polyketide synthase (PKS) and the necessary post-PKS tailoring enzymes. Among these, the key enzyme responsible for the tremendous structural change from the suppossed angucyclinone intermediate (e.g., 2 in FIG. **2**.) to the unique tetracyclic lactone structure of the gilvocarcins is proposed to be a C—C-bond cleaving oxygenase. Other key post-PKS tailoring steps with respect to important structural features of gilvocarcin V are the oxygenation/dehydration reactions necessary for the formation of the vinyl side chain, and the C-glycosyltransfer step, through which the 6-deoxy-D-fuco-hexofuranose moiety is attached.

This distinct family of antitumor antibiotics shows excellent antitumor activity and remarkably low toxicity, and therefore has remained to be attractive for synthetic organic chemistry as well as for biological activity studies since their discovery. The group consists of the gilvocarcins (syn. toromycins, anandimycins), ravidomycins, the ravidomycin analogues FE35A and B, the chrysomycins (syn. virenomycin, albacarcins; including recent derivatives possessing branched ketofuranose and ketopyranose sugar moieties), and BE-12406 A and B (FIG. **3**.). Hirayama, N., Takahashi, K.; Shirahata, K., Ohashi, Y., Sasada, Y. Bull. *Chem. Soc. Jap.* 54:1338-1342 (1981); Krohn, K. et al. *J. Topics Curr. Chem.* 188:127-195 (1997); Hosoya, T., Takashiro, E., Matsumoto, T., Suzuki, K. *J. Am. Chem. Soc.* 116:1004-1015 (1994); Knobler, R. M. et al. *Nucleic Acid Res.* 20:4553-4557 (1992);

Matsumoto, A. et al. *Cancer Res.* 60:3921-3926 (2000); Yamashita, N. et al. *Antibiot.* 51:1105-1108 (1998); Nakashima, T. et al. U.S. Pat. No. 6,030,951; Morimoto, M. et al. *Antibiot.* 34:701-707 (1981).

Gilvocarcin V (GV) (FIG. **4**.), the principal product of *Streptomyces griseoflavus* Gö 3592 and of various other *Streptomyces* strains, is the most important member of the gilvocarcin-type aryl-C-glycosides, because of its potent bactericidal, virucidal, cytotoxic and antitumor activities. GV is one of the strongest antitumor compounds among these drugs, requiring only low concentrations and maintaining a low in vivo toxicity. The exact molecular mechanisms responsible for the in vivo mode of action of GV are still widely unknown. However, it was found that GV exhibits a strong tendency to intercalate with DNA. Both equilibrium DNA binding and UV light-induced DNA adduct formation was found, causing also topoisomerase II inhibition. Knobler, R. M. et al. *Nucleic Acid Res.* 20:4553-4557 (1992). The vinyl group is essential for the antitumor activity, since the minor congeners gilvocarcins M and E, in which the vinyl group is replaced by a methyl group and an ethyl group, respectively, are significantly less effective. Yamashita, Y. et al. *Nucleic Acids Res. Symp. Ser.* 20: 65-67 (1988); Elespuru, R. K. et al. *Science.* 223:69-71 (1984); Oyola, R. et al. Photochem. Photobiol. 65:802-810 (1997). Photobiological studies showed that the vinyl group undergoes a [2+2] cycloaddition with DNA thymine residues under photoirradiation. Moreover, it was shown recently that Givocarcin V promotes protein-DNA cross-linking when photo-activated by near-UV light, and histone H3, which plays an important role in DNA replication and transcription, was identified as one of the selectively cross-linked proteins (FIG. **5**.). This cross-linking with histone H3, believed to be part of the unique molecular mechanisms of the potent antitumor activity of gilvocarcin V, might contribute to the better and more specific activity of GV compared to other intercalating antitumor drugs. Matsumoto, A. et al. *Cancer Res.,* 60:3921-3926 (2000).[3b]

The molecular architecture of gilvocarcin V in conjunction with its biological activity makes GV an excellent target for the study of its biosynthesis and the development of novel, improved anticancer, immunosuppressant, antibiotic, antiviral and neuroprotective drugs through combinatorial biosynthesis.

SUMMARY OF INVENTION

In one aspect, the present invention provides isolated nucleic acid compounds comprising a sequence identical or complementary to all or part of a coding sequence for the gilvocarcin V biosynthetic gene cluster from *Streptomyces griseoflavus* (SEQ ID NO:1). Preferably, a part of said coding sequence is one or moreopen reading frame (ORF) selected from the group consisting of ORF1, ORF2, ORF3, ORF4, ORF5, ORF6, ORF7, ORF8, ORF9, ORF10, ORF11, ORF12, ORF13, ORF14, ORF15, ORF16, ORF17, ORF18, ORF19, ORF20, ORF21, ORF22, ORF23, ORF24, ORF25 and ORF26.

In one embodiment, the present invention provides an isolated nucleic acid strand that encodes a gilvocarcin gene cluster or subunit thereof comprising a nucleotide sequence identical or complementary to, or an amino acid sequence encoded by a nucleotide sequence identical or complementary to, all or part of a coding sequence for gilvocarcin V biosynthetic gene cluster from *Streptomyces griseoflavus* (SEQ ID NO:1). Preferably, the gene cluster encodes a functional PKS or a functional arrangement of the PKS and selected post-PKS tailoring enzymes. The gene cluster may be derived from a single species or may be hybrid in nature. In certain embodiments, the gene cluster is a replacement gene cluster. The replacement gene cluster may be a hybrid, mutant, analog or derivative thereof.

In another embodiment, the invention provides an isolated nucleic acid that encodes three or more open reading frames (ORFs) comprising a sequence identical or complementary to all or part of a coding sequence for enzymes performing the biosynthesis of gilvocarcin V from *Streptomyces griseoflavus* (SEQ ID NO:1). Preferably, the ORFs encode a functional PKS or a functional arrangement of the PKS and selected post-PKS tailoring enzymes. In certain embodiments, an ORF may be derived from a single species or may be hybrid in nature. In certain embodiments at least one of the ORFs is derived from the gilvocarcin V gene cluster. In other embodiments, at least one ORF is derived from a non-gilvocarcin V producing *Streptomyces* strain, or is hybrid in nature. In yet other embodiments, at least one ORF is a mutant, analog or derivative of the native coding sequence.

In still another embodiment, the present invention provides isolated nucleic acid compounds comprising three or more genes of the coding sequence for the biosynthesis of gilvocarcin from *Streptomyces griseoflavus*. Preferably, the mixture of genes encode a functional PKS or a functional arrangement of the PKS and selected post-PKS tailoring enzymes. In certain embodiments, a gene may be derived from a single species or may be hybrid in nature. In certain embodiments at least one gene is derived from the gilvocarcin V biosynthetic gene cluster. In other embodiments, at least one gene is derived from a non-gilvocarcin V producing *Streptomyces* strain, or is hybrid in nature. Non-limiting exemplary non-gilvocarcin V biosynthetic genes are preferably subunits of the gilvocarcin M, gilvocarcin E, defucosyl-gilvocarcin V, ravidomycin, deacetyl-ravidomycin, FE35A, FE35B, chrysomycin A, chrysomycin B, BE-12406 A, or BE-12406 B gene cluster. In yet other embodiments, at least one gene may be a mutant, analog or derivative of the native coding sequence. It is also preferred that the encoded activity of the gene is, for example and without limitation, a ketosynthase activity, a chain lengthening activity, an acyltransferase activity, an acyl carrier protein activity, an oxygenase activity, a reductase activity, an oxidoreductase activity, a cyclase activity, a glycosyltransferase activity, a methyltransferase activity, an activity encoded by any gene belonging to the biosynthesis or modification of a sugar moiety, a regulatory activity, a repressor activity, or a transporter activity.

In another aspect, the present invention provides recombinant expression vectors encoding a gilvocarcin gene cluster, hybrids, mutants, analogs or derivatives thereof. In certain embodiments, vectors encode one or more subunit of gilvocarcin gene cluster, hybrids, mutants, analogs or derivatives thereof.

In another aspect, the present invention provides a host cell transformed with a recombinant expression vector described herein.

In still another aspect, the invention provides a method of preparing gilvocarcin V, said method comprising transforming a host cell with a recombinant DNA vector that encodes a gilvocarcin V gene cluster or subunit thereof, and culturing said host cell under conditions such that gilvocarcin is produced and/or gilvocarcin analogs are produced. In one embodiment, the method is practiced with an *E. coli* host cell. In certain other embodiments, the method is practiced with a *Streptomyces* host cell. The gene cluster may be a replacement gene cluster and preferably a functional gene cluster. In certain embodiments, the invention provides methods for preparing new polketide-type compounds, preferably, gilvocar-

cin V-type polyketides. The gilvocarcin V-type polyketide produced may be gilvocarcin V or gilvocarcin hybrids, mutants, analogs or derivatives thereof. Such polyketides are useful as antibiotics, antitumor agents, and immunosuppressants, and for a wide variety of other pharmacological purposes.

These and other embodiments and aspects of the invention will be more fully understood after consideration of the attached Drawings and their brief description below, together with the detailed description, example, and claims that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. schematically illustrates formation of jadomycins, gilvocarcins and kinamycins.

FIG. **2**. schematically illustrates intermediates in biosynthesis of gilvocarcin.

FIG. **3**. provides the structure of gilvocarcin-type anticancer drugs.

FIG. **4**. provides the structure of gilvocarcin V.

FIG. **5**. illustrates the hypothesized mechanism of action of gilvocarcin V.

FIG. **6**. A shows the gilvocarcin gene cluster.

FIG. **6**. B shows a simplified gilvocarcin gene cluster, in which the polyketide synthase and associated genes are depicted in black, the genes encoding the tailoring enzymes are in pink (i.e. gray, if printed in black/white), and the regulatory, resistance and so far unknown genes are shown in white. Shown are two alternative pathways towards gilvocarcin V.

FIG. **7**. illustrates proposed biosynthetic pathway for gilvocarcin V sugar moiety.

FIG. **8**. illustrates the hypothesized alternative pathways to gilvocarcin V and possible involvement of products of the gilvocarcin gene cluster.

FIG. **9**. is the nucleotide sequence of the Gilvocarcin V gene cluster which sets out ORF1-26 (SEQ ID NO:1).

DETAILED DESCRIPTION

Given the valuable pharmaceutical properties of gilvocarcin-type aryl-C-glycosides, there is a need for methods and reagents for producing large quantities of gilvocarcin-type aryl-C-glycosides, for producing gilvocarcin-type aryl-C-glycosides in host cells that do not produce gilvocarcin-type aryl-C-glycosides naturally, and for producing novel gilvocarcin-type aryl-C-glycosides compounds not found in nature. The present invention provides the protein encoding nucleic acids, methods and reagents that produce gilvocarcins, with particular application to methods and reagents for producing the gilvocarcin-type aryl-C-glycosides known as gilvocarcin V ("GV") and its analogs and derivatives and novel compounds related through structure or genetics to gilvocarcin V.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al. Molecular Cloning: A Laboratory Manual (Current Edition); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., Current Edition); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., Current Edition); Transcription and Translation (B. Hames & S. Higgins, eds., Current Edition), and Practical Streptomyces Genetics (T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater, D. A. Hopwood, Norwich, UK: The John Innes Foundation; current edition).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, reference to "a polyketide" includes mixtures of polyketides, reference to "a polyketide synthase" includes mixtures of polyketide synthases, and the like.

Definitions

As used herein the term "coding sequence" or a sequence which "encodes" a particular protein, is a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eucaryotic mRNA, genomic DNA sequences from procaryotic or eucaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

As used herein the term DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

As used herein the term "functional PKS" refers to a set of genes (e.g., three or more) or subunits of a biosynthesis gene cluster, which catalyzes the synthesis of a polyketide, including without limitation a "minimal PKS".

As used herein the term "gene" refers to a segment of DNA or its complement that is involved in producing a polypeptide chain, including regions preceding (leader) and following (trailer) the coding sequence as well as intervening sequences (introns) between individual coding sequence (exons). A "gilvocarcin V gene" refers to any of the ORFs of SEQ ID NO:1.

As used herein the term "gene cluster" refers to a set of (e.g., three or more) closely related genes that code for the same or similar proteins and which are usually grouped together on the same chromosome. A "gilvocarcin V gene cluster" refers to the set of genes encoded by SEQ ID NO:1.

As used herein the term "genetically engineered host cell" is meant a host cell where the native gene cluster or subunits thereof has/have been deleted using recombinant DNA techniques. Thus, the term would not encompass mutational events occurring in nature. A "host cell" is a cell derived from a procaryotic microorganism or a eucaryotic cell line cultured as a unicellular entity, which can be, or has been, used as a recipient for recombinant vectors bearing the PKS gene clusters of the invention. The term includes the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell, which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide

sequence encoding desired biosynthetic enzymes, are included in the definition, and are covered by the above terms.

As used herein the term "gilvocarcin V analog" refers to a compound or molecule that resembles gilvocarcin V and that contains one or more structural differences relative to gilvocarcin V. Preferably, the gilvocarcin analog has gilvocarcin-type activity although a gilvocarcin analog may have enhanced or the same activity as products of the gilvocarcin V gene cluster. For example, the degree of saturation of at least one bond in the gilvocarcin structure can be changed (e.g., a single bond can be changed to a double or triple bond, or the converse), a bond can be removed, one or more carbon, oxygen or hydrogen atoms can be replaced with a different atom or a chemical moiety (e.g., a halogen, oxygen, nitrogen, sulfur, hydroxy, methoxy, alkyl, aryl, cycloalkyl, heterocycle, amine, amide, ketone, aldehyde, etc.) and the like. In addition, the C-glycosidically-linked sugar moiety can be changed, modified or replaced by other sugar moieties including deoxysugars, amino sugars, keto sugars, halogenated sugars etc., which may be connected as C-, O-, N- or S-glycosides at any possible position of the gilvocarcin molecule. Also other peripheral groups, such as OH groups, methyl groups, O-methyl groups, halogene atoms etc. can be added, modified or removed. Other types of derivatives of gilvocarcin that would be encompassed by the term "gilvocarcin analog" are known in the art.

As used herein the term "gilvocarcin V derivative" refers to a polyketide compound or molecule, that may be produced from gilvocarcin in one or more steps or with few chemical or moiety modifications.

As used herein the term "gilvocarcin V-type polyketide" refers to a compound or molecule that is encoded by at least one native gilvocarcin V gene or a hybrid, mutant, analog or derivative thereof.

As used herein the term "minimal PKS" refers to those minimum number of PKS genes or subunits of a biosynthesis polyketide gene cluster required for biosynthesis of a polyketide, such as gilvocarcin. For example, in *Streptomyces griseoflavus* the required genes to encode the minimal PKS are ketosynthase I (KSI) and ketosynthase II (KSII, also known as chain length factor CLF) and an acyl carrier protein (ACP). Thus, these three genes, without the other components of the native clusters, can be included in one or more recombinant vectors, to constitute a "minimal" replacement PKS gene cluster.

As used herein the term "mutant" refers to a nucleic acid compound, protein, molecule, vector or cell resulting from mutation of the native wild type coding sequence or subunits thereof.

As used herein the term "mutation" refers to any change that alters a native coding sequence either by displacement, addition, deletion, insertion, cross-linking, or other destruction or substitution of one or more nucleotides of the native coding sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are also known to those skilled in the art.

As used herein the term "nucleic acid" sequence can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methyl-cytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxy-aminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine. A transcription termination sequence will usually be located 3' to the coding sequence.

As used here the term "open reading frame" or "ORF" refers to a region of a nucleic acid molecule that contains a series of triplet bases coding for amino acids without any termination codons. An "open reading frame" does include any start codons.

As used herein, the term "polyketide-type compound" refers to a compound or molecule that is encoded by at least one native polyketide subunit, or hybrid, mutant, analog, or derivative thereof, including for example, without limitation, gilvocarcin V-type polyketides.

As used herein, the term "post-PKS enzyme" or "post-PKS modifying enzyme" or "post-PKS tailoring enzyme" refers to a protein or enzyme that is involved in modifications of a polyketide after a polyketide is synthesized by polyketide synthase. Exemplary, post-PKS enzymes involved in gilvocarcin synthesis include, without limitation, a C—C-bond cleaving oxygenase involved in forming the tetraxyclic lactone structure, oxygenase/dehydratase involved in forming the vinyl side chain, and C-glycosyltransferase involved in attaching the 6-deoxy-D-fuco-hexofuranose moiety.

As used herein, the term "post-PKS modifying step" or "post-PKS tailoring step" refers to an action or actions taken by a protein or enzyme to modify a polyketide after it has been synthesized by polyketide synthase. Exemplary post-PKS tailoring steps involved in gilvocarcin synthesis include, without limitation, the formation of the tetracyclic lactone structure by a C—C-bond cleaving oxygenase, oxygenation/dehydration reactions for the formation of the vinyl side chain, and a C-glycosyltransfer step involved in attaching a 6-deoxy-D-fuco-hexofuranose moiety.

As used herein the term "replacement gene cluster" means any set of genes and/or genes encoding tailoring steps capable of producing a "functional PKS" when under the direction of one or more compatible control elements, as defined above, in a host cell transformed therewith. The term "replacement gene cluster" encompasses three or more genes encoding the various proteins necessary to catalyze the production of a polyketide. A "replacement gene cluster" need not include all of the genes found in the corresponding cluster in nature. Rather, the gene cluster need only encode the necessary components to catalyze the production of an active polyketide. Thus, if the gene cluster includes, for example, eight genes in its native state and only three of these genes are necessary to provide an active polyketide, only these three genes need be present. Furthermore, a replacement gene cluster can include genes derived from a single species, or may be hybrid in nature with, e.g., a gene derived from a cluster for the synthesis of a particular polyketide replaced with a corresponding gene from a cluster for the synthesis of another polyketide. Hybrid clusters can include genes derived from both Type I and Type II PKSs. As explained above, Type I PKSs include several large multifunctional proteins carrying, between them, a set of separate active sites for each step of

carbon chain assembly and modification. Type II PKSs, on the other hand, have a single set of iteratively used active sites.[43] The genes included in the replacement gene cluster need not be the native genes, but can be mutants or analogs thereof. Mutants or analogs may be prepared by the deletion, insertion or substitution of one or more nucleotides of the coding sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are described in the literature. See e.g., Sambrook, et al. Molecular Cloning: A Laboratory Manual (Current Edition); DNA Cloning: A Practical Approach, Vol. I & II (D. Glover, ed.) and Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., Current Edition).

The term, "replacement gene cluster" may also contain genes coding for modifications to the core polyketide catalyzed by a PKS, including, for example, genes encoding hydroxylases, methylases or other alkylases, oxidases, reductases, glycotransferases, lyases, ester or amide synthases, and various hydrolases such as esterases and amidases. The genes included in the replacement gene cluster need not be on the same plasmid or if present on the same plasmid, can be controlled by the same or different control sequences.

As used herein, the term "subunit" refers to a part of a complete gene cluster including, for example, a module, domain, gene, or open reading frame, and parts thereof. A "subunit" may comprise for example, a gene or genes derived from a single species or may be hybrid in nature (e.g., a gene derived from a cluster for the synthesis of a particular polyketide replaced with a corresponding gene from a cluster for the synthesis of another polyketide.). A "subunit" may comprise mutants, analogs or derivatives of the native gene(s). Mutants, analogs or derivatives thereof may be prepared by techniques known to those of skill in the art, including, without limitation, the displacement, addition, deletion, insertion, cross-linking, or other destruction or substitution of one or more nucleotides of the coding sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are also known in to those skilled in the art.

Cloning and Identification of the Gilvocarcin Biosynthetic Gene Cluster

Central to the present invention is the identification and cloning of the gilvocarcin ("gil") gene cluster (SEQ ID NO:1). Identification of the gilvocarcin gene cluster was achieved by generating a *S. griseoflavus* genomic cosmid library using the *Streptomyces-E. coli* shuttle vector pOJ446. For the generation of a genomic cosmid library, isolation and subsequent random fragmentation of high molecular weight genomic DNA was performed, followed by ligation of these fragments to vector arms containing cos sequences, packaging into λ particles and transduction into a suitable *E. coli* host strain. DNA fragments of the NDP-glucose-4,6-dehydratase (an enzyme catalyzing a key step in 6-deoxysugar biosynthesis) (See Decker, H.; Gaisser, S.; Pelzer, S.; Schneider, P.; Westrich, L.; Wohlleben, W.; Bechthold, A. *FEMS Microbiol. Lett.* 141:195-201 (1996)) and also the actI PKS (See Hopwood, D. A. *Chem. Rev.* 97:2465-2497 (1997)) genes, highly conserved among *Streptomyces*, were used to probe the cosmid library. Cosmid DNA isolated from clones hybridizing with both probes was analyzed by restriction mapping and Southern blot experiments. Hybridization using both probes with one of the cosmids increased the likelihood that the cosmid would contain the entire gilvocarcin cluster. One of the cosmids, cos-G9B3, was transformed into *S. lividans* TK24, where it stimulated the production of gilvocarcins V and M in the same quantities as the wild-type strain (20-30mg/L of (1) in FIG. **6**.B), proving that it most likely contains the entire gene cluster of gilvocarcin biosynthesis.

Subcloning of cos-G9B3-DNA fragments into pUC19 or pBluescript II SK(+) followed by sequencing revealed the entire gilvocarcin gene cluster (FIG. **6**.A). The cluster spans a 32.9 kB region and consists of 26 ORFs identified as follows. ORF1 is gilS, encoded on the complement to SEQ ID NO:1, and represented on SEQ ID NO:1 as nucleotides (nt) 802-4068 read in the 3' to 5' direction. ORF2 is gilN, encoded on the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 4308-5198 read in the 3' to 5' direction. ORF3 is gilL, encoded on the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 5417-6052 read in the 3' to 5' direction. ORF4 is gilOIII, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 6576-7769 read in the 3' to 5' direction. ORF5 is gilGT, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 7777-9261 read in the 3' to 5' direction. ORF6 is gilM, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 9261-10001 read in the 3' to 5' direction. ORF7 is gilR, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 10020-11513 read in the 3' to 5' direction. ORF8 is gilOII, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 11513-12196 read in the 3' to 5' direction. ORF9 is gilMT, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 12354-13424 read in the 3' to 5' direction. ORF10 is gilJ, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 13814-15466 read in the 3' to 5' direction. ORF11 is gilI, nt 15619-16641 of SEQ ID NO:1 read in the 5' to 3'direction. SEQ ID NO:2 is the amino acid sequence of ORF11. ORF12 is gilE, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 16690-17697 read in the 3' to 5'direction. ORF13 is gilD, encoded by the complement to SEQ ID NO:1 and represented on SEQ ID NO:1 as nt 17697-18761 read in the 3' to 5' direction. ORF14 is gilH, nt 18938-19576 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:3 is the amino acid sequence of ORF14. ORF15 is gilOI, nt 19892-21391 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:4 is the amino acid sequence of ORF15. ORF16 is gilG, nt 21413-21736 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:13 is the amino acid sequence of ORF16. ORF17 is gilA, nt 21736-22992 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:5 is the amino acid sequence of ORF17. ORF18 is gilB, nt 22992-24164 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:14 is the amino acid sequence of ORF18. ORF19 is gilC, nt 24183-24449 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:6 is the amino acid sequence of ORF19. ORF20 is gilF, nt 24449-25225 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:15 is the amino acid sequence of ORF20. ORF21 is gilK, nt 25241-26188 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:7 is the amino acid sequence of ORF21. ORF22 is gilOIV, nt 26200-27552 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:8 is the amino acid sequence of ORF22. ORF23 is gilP, nt 27552-28481 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:16 is the amino acid of ORF23. ORF24 is gilQ, nt 28501-29517 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:9 is the amino acid sequence of ORF24. ORF25 is gilT, nt 30206-32239 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:10 is the amino acid sequence of ORF25. ORF26 is gilU, nt 32379-33392 of SEQ ID NO:1 read in the 5' to 3' direction. SEQ ID NO:11 is the amino acid sequence of ORF26.

The database analysis yielded genes encoding the type II PKS and associated enzymes (gil G, A, B, C, F, K, P and Q),

several genes encoding proteins involved in post-PKS tailoring steps (gil OIII, GT, R, OII, MT, E, D, H, OI, OIV, and U), and genes of regulation and self resistance enzymes (gil S, J, I and T), this group also includes three genes coding for proteins of unknown function (gil L, M and N).

Methods

Cosmid Library Generation

A cosmid library of the gilvocarcin V producer *Streptomyces griseoflavus* Gö3592 using pOJ446 as a host was constructed using standard methods. Transduction into *E. coli* XL 1-Blue MRF' yielded several thousand colonies, which were pooled and used as a master culture. An initial library evaluation of cosmid DNA from several randomly chosen transductants by restriction enzyme analysis revealed whether these clones have inserts and if so, the size of the inserts. It was expected that the inserts should ideally have the size of 30 to 40 kb, since from comparison with known, related molecules, this was the expected size of the entire gene cluster of the gilvocarcin pathway. As such, we were able to generate a cosmid containing the complete gilvocarcin gene cluster.

The high molecular weight donor DNA was isolated according to the following standard methods. CRM medium supplemented with 0.5% (w/v) glycine was inoculated with *S. griseoflavus* Gö3592 spores and incubated for 24 hours or until the culture reached nearly stationary phase. Cells were harvested by centrifugation and resuspended in lysis buffer containing 1 mg/mL lysozyme. After incubation for 15-60 min at 37° C., a 10% SDS solution and proteinase K (0.5 mg/mL) was added to lyse the cells and the lysed cells were incubated at 55° C. for two hours. The resulting viscous solution was extracted with 5 M NaCl solution and phenol/chloroform 1:1 and centrifuged. The aqueous phase was transferred to a fresh tube and again extracted with chloroform, then the DNA in the water phase was precipitated by adding 0.6 vol. of isopropanol and spooled onto a sealed Pasteur pipette. After rinsing with 70% ethanol, air drying and dissolving in TE-buffer, the concentration and purity of the DNA was estimated by measuring the optical density of the solution at 260 and 280 nm. DNA size was checked on a 0.3% agarose gel by conventional electrophoresis. For cosmid cloning, the DNA should be ≧150 kB.

The partial digestion and dephosphorylation of the donor DNA was performed as follows by standard methods. The restriction enzyme Sau3AI was used for the partial digestion, since it recognizes a 4-base-pair-sequence thus ensuring random fragmentation and generation of cohesive ends that can be ligated into the BamHI site of vector pOJ446. Bierman, M. et al. *Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp. Gene* 116: 43-49 (1992). The amount of enzyme and incubation time to digest the DNA to the point where its average size is approximately in the size range of 30-40 kB was determined empirically by an enzyme dilution series and then scaled up to digest 200-300 μg of chromosomal DNA. A subsequent dephosphorylation step with calf intestinal phosphatase (CIP) prevented segments of different regions of chromosomal DNA from ligating to one another and forming a recombinant vector containing noncontiguous segments of the genome. The extent of dephosphorylation was determined using a small batch of dephosphorylated DNA in a ligase reaction and subsequent gel electrophoresis of the ligated and unligated DNA samples. The complete dephosphorylation showed no differences between ligated and unligated DNA.

Cosmid pOJ446 is a low copy shuttle vector containing three cos sequences and an apramycin resistance gene. It also carries the origin of replication from *E. coli* and *Streptomyces* allowing an easy transfer between these two species. To prepare the two cos arms necessary for the in vitro packaging reaction, cosmid pOJ446 was cut at the unique HpaI site situated between two cos sites. Afterwards, the resulting ends were dephosphorylated to prevent re-ligation. The linearized vector was cut with BamHI to yield two vector arms each containing a cos site and a ligatable end. Finally, phenol/chloroform extraction and ethanol precipitation was used to purify the DNA.

Ligation and packaging was performed according to the following standard methods. Different ratios of donor to vector DNA were ligated with T4 DNA ligase for 16 hours at 16° C. To favor the formation of cosmid-insert concatemers and not circular DNA the ligation was carried out at DNA concentrations of about 1 μg/μL or greater. The ligation was monitored by agarose gel electrophoresis of ligation mixture, using unligated DNA samples as a control. Successful ligation showed a significant shift of the chromosomal smear to a higher molecular weight and the disappearance of the vector bands. The in vitro packaging reaction was done using Gigapack III XL packaging extracts (Stratagene), a kit which contains all required enzymes necessary to pack concatemeric DNA into preformed λ phage particles. The packaging was carried out as described in the Stratagene manual.

Transduction into *E. coli* XL 1-Blue MRF' was performed by the following standard methods. LB medium supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose was inoculated with a single colony of *E. coli* XL 1-Blue MRF' and grown at 37° C. for 4-6 hours. After cell harvesting by centrifugation, the cells were diluted to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$ solution. Two hundred μL of cell solution was then infected with in vitro packaged phage and incubated for 30 min at room temperature. Additional LB broth was added and after incubation for 1 hour, 100 μL aliquots were plated on LB plates containing 100 μg/mL apramycin and incubated at 37° C. overnight. To evaluate the quality of the constructed library, randomly picked colonies were proliferated in LB medium supplemented with 100 μg/mL apramycin. Cosmid DNA was then isolated according to standard isolation procedures and used for restriction enzyme digests.

Labeling, Synthesis of Hybridization Probes and Colony Hybridization

Gene probes were labeled by PCR using the digoxigenin (DIG) system (Roche). The 4,6-dehydratase gene was amplified by PCR from genomic *S. griseoflavus* DNA using a method developed by Bechthold et al. Decker, H. et al. *A general approach for cloning and characterizing dndp-glucose dehydratase genes from actinomycetes. FEMS Microbiology Letters* 141:195-201 (1996) The PKS probe was prepared from plasmid pIJ2345, which contains parts of the actinorhodin minimal PKS (actI) of *S. coelicolor*. Malpartida, F. and Hopwood, D. *Physical and genetic characterisation of the gene cluster for the antibiotic actinorhodin in Streptomyces coelicolo A3*(2). *Mol. Gen. Genet* 205:66-73 (1986). Labeled DNA probes were purified by gel electrophoresis and labeling efficiency was estimated in a spot test with a DIG-labeled control.

Colony hybridization was carried out as described in the DIG user manual (Roche, online) for membrane hybridization. Roche, Molecular &. Biochemicals. http://biochem.roche.com/prodinfo_fst.htm?/prod_inf/manuals/dig_man/dig._joc.htm. Briefly, colonies were grown overnight on LB agar containing 100 μg/mL apramycin. Nylon membranes (Roche) were placed on the agar plates and punched to mark

the orientation. After a short incubation time, the membranes were removed and successively blotted for 15 minutes on Whatmann 3MM paper soaked in denaturation, neutralization and 2×SSC solutions. UV-light was used to cross-link the transferred DNA to the membrane. Membranes were then pre-hybridized for 2 hours at 42° C. in DIG Easy Hyb hybridization solution. Probes were denatured by boiling to produce single-stranded DNA and added to start the hybridization process. Probe concentration, hybridization time and temperature, and the stringency of subsequent washing steps with SSC solution were determined empirically for each probe (e.g., 2× at 45° C. with 2 mol SSC solution for 15 min., then 2× at 68° C. with 0.1 mol SSC solution for 30 min). To reduce nonspecific binding of the anti-digoxigenin-AP conjugate, the membrane was treated with blocking buffer for 30-60 min before the antibody solution was added. After removal of unbound antibodies, nitroblue tetrazolium salt (NBT) and 5-bromo-4-chloro-3-indolyl phosphate toluidinum salt (BCIP), the colorimetric substrates for AP, were added to initiate the color reaction.

Cosmid DNA isolated from clones hybridizing with both probes were analyzed by restriction mapping and Southern blot experiments. In order to confirm that the cosmid DNA indeed contained genes of the gilvocarcin gene cluster, the corresponding cosmids were introduced into *S. lividans* TK24 or *S. albus* by protoplast fusion. Hopwood, D. A. *Genetic contributions to understanding polyketide synthases. Chem. Rev.* 97:2465-2497 (1997); Hopwood, D. A. et al. *Genetic Manipulation of Streptomyces. A Laboratory Manual* (The John Innes Foundation, Norwich, UK) (1985); Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. and Hopwood, D. A. *Practical Streptomyces Genetics* (The John Innes Foundation, Norwich, UK) (2000). The resulting recombinant strains were then screened for gilvocarcin resistance and the production of new metabolites. Resistance against gilvocarcin V and/or a production of gilvocarcins or biosynthetic intermediates with the gilvocarcin chromophore, were easily detected on TLC with use of the unique yellow fluorescence (UV light, 366 nm), which proved the presence of genes of the gilvocarcin pathway.

Nucleotide Sequence Analysis

The nucleotide sequence of isolated clones was determined using conventional methodology. Automated thermocycle sequencing of pUC19 or pBluescript II SK(+)-based subclones using taq DNA polymerase and fluorescent dye-labeled terminators was carried out at the UK biotechnology resource service laboratory on an ABI 377 and 310 DNA sequencers. Both, standard (M13 forward and reverse, T7, or T3) and custom made primers (18-21 nucleotides) were used.

Functional Assignment of the Gilvocarcin Biosynthetic Gene Cluster

The genes encoding the PKS and associated enzymes are in a type II PKS arrangement, in which the minimal PKS gene cluster, encoded by gilABC (consisting of ketosynthase (KS) α, KSβ, and the acyl carrier protein (ACP)), is flanked by the PKS-associated ketoreductase (KR, encoded by gilF) and two cyclases (encoded by gilG and gilK). Unexpectedly, the genes gilP and gilQ, which encode a malonyl CoA:ACP transacylase (MAT) and an acyl transferase (AT), respectively, were found to be located further downstream of gilABC. Although essential for polyketide biosynthesis, MATs are usually not found in type-II PKS gene clusters, and are often 'recruited' from the fatty acid synthase. Summers, R. G., Ali, A., Shen, B., Wessel, W. A. and Hutchinson, C. R. *Biochemistry* 34:9389-9402 (1995). The gilvocarcin gene cluster disclosed herein is the third example in which such a gene was located, but the first one where the cluster is associated with a known structure. Novakova, R.; Bistakova, J.; Homerova, D.; Rezuchova, B.; Kormanec, J. *Gene* 297:197-208 (2002). GilQ resembles AT proteins found in producers of aromatic polyketides with starter units other than acetate, such as doxorubicin, enterocin, etc. Hutchinson, C. R. *Chem. Rev.* 97:2525-2536 (1997); Moore, B. S. and Hertweck, C. *Nat. Prod. Rep*). 19:70-99 (2002); Marti, T., Hu, Z., Pohl, N. L., Shah, A. N. and Khosla, C. *J. Biol. Chem.* 275:33443-33448 (2000). Therefore, gilQ might play a role in the choice of the starter unit (propionate vs. acetate for the production of gilvocarcin V and gilvocarcin M, respectively).

The following genes are suspected to be responsible for the post-PKS tailoring steps including the above mentioned biosynthetic steps towards the key structural features of GV. Four oxygenase-encoding genes were found (gilOI, gilOII, gilOIII and gilOIV). Genes gilOI and gilOIV encode FAD-dependent oxygenases assumed to catalyze the oxidative rearrangement of a putative angucyclinone-precursor to the unique coumarin-based aromatic core of the gilvocarcins. The corresponding enzymes gilOI and gilOIV are closely related to jadF and jadH (gilOI: 37% aa-identity to jadF and 41% aa-identity to jadH; gilOIV: 37% aa-identity to jadF and 29% aa-identity to jadH), which probably catalyze a similar rearrangement in the jadomycin pathway.

The other two oxygenases, gilOII and gilOIII, are most likely responsible for the anthrone oxidation leading to the angucyclinone intermediate X (gilOII), and for the generation of the 8-vinyl group. For the latter, we assume a hydroxylation in 1"-position through gilOIII followed by dehydration, since gilOIII is a P-450 hydroxylase predestined for such a reaction. Other oxidoreductase encoding genes are gilH (encoding a KR presumably involved in the hydroquinone generation) and gilR (encoding an oxidoreductase of unclear function).

The C-glycosidically linked D-fucofuranose is a unique deoxysugar not found in any other polyketide, and whose biosynthesis requires only a few enzymes (FIG. **7**). Hosoya T. et al. *J. Am. Chem. Soc.* 116:1004-1015 (1994). This is confirmed by the presence of only a few typical deoxysugar biosynthesis genes in the gil cluster. Two of these crucial genes, gilD and gilE, encode NDP-glucose synthase and 4,6-dehydratase, respectively. A third gene possibly involved in the D-fucose biosynthesis is gilU located at the end of the gil cluster. GilU, apparently an epimerase/dehydratase, or oxidoreductase gilR, might function as 4-KR, while it is unclear how the contraction from the pyranose to the furanose is catalyzed. Without intending to be bound by theory, gilM is a possible candidate to encode the enzyme responsible for shrinking the pyranose to furanose.

GilGT encodes the glycosyl transferase (GT) responsible for the unusual p-OH activated C-glycosylation. Synthetic model studies suggest that the favored mechanism for C-glycosyltransfer is an initial O-glycosylation followed by a Fries-like rearrangement. See e.g., Hosoya, T. et al. *J. Am. Chem. Soc.* 116:1004-1015 (1994); Palmacci, E. R. and Seeberger, P. H. *Org. Lett.* 3:1547-1550 (2001). Although principally possible, it is difficult to imagine in an enzymatic environment that such a rearrangement to the p-position occurs. GilGT resembles mostly lanGT2 and urdGT2 both of which transfer D-olivoses to angucyclinone acceptor molecules, the latter being also a C-GT. Künzel, E., Faust, B., Oelkers, C., Weissbach, U., Bearden D. W., Weitnauer, G., Westrich, L., Bechthold, A. and Rohr, J. *Inactivation of the urdGT2 Gene, Which Encodes a Glycosyltransferase Responsible for the C-Glycosyltransfer of Activated D-Olivose, Leads to Formation of the Novel Urdamycins* I, J, and K

J. Am. Chem. Soc. 121:11058-11062 (1999). However, in contrast to gilGT, urdGT2 places its sugar moiety ortho to a phenolic OH-group. An interesting novelty of gilGT is its unusual size, due to its N-terminal part being approximately 120 amino acids longer than any other polyketide GT found so far. The BLAST analysis of the deduced aa-sequence of gilGT (495 aa, MW 53846 g/Mol) revealed 38% aa-identity with lanGT2 from the landomycin producer *S. cyanogenus,* 31% identity with urdGT2 from *S. fradiae* Tü2717, and 25% identity with jadS, the O-GT of the jadomycin pathway from *S. venezuelae*. Without intending to be bound by theory, the similarity of gilGT with lanGT2, urdGT and jadS may point to an angucyclinone-shaped acceptor substrate for gilGT, since lanGT2, urdGT2 and jadS transfer their sugar substrates to an angucyclinone-type aglycon. Künzel, E. et al. *J. Am. Chem. Soc.* 121:11058-11062 (1999); Bechthold, A. and Rohr, *J. Bioorganic Chemistry* (eds. Diederichsen, U., Lindhorst, T. K., Westermann, B. & Wessjohann, L. A.) 313-321 (Wiley-VCH, Weinheim, 1999); Kirschning, A., Bechthold, A. and Rohr, J. *Chemical and Biochemical Aspects of Deoxysugars and Deoxysugar Oligosaccharides. Topics Curr. Chem.* 188:1-84 (1997); Wohlert, S.-E., Bechthold, A., Beninga, C., Henkel, T., Holzenkämpfer, M., Kirschning, A., Oelkers, C., Ries, M., Weber, S., Weissbach, U., Westrich, L. & Rohr, J. *Bioorganic Chemistry* (eds. Diederichsen, U., Lindhorst, T. K., Westermann, B. & Wessjohann, L. A.) 305-312 (Wiley-VCH, Weinheim, N.Y., Chichester, Brisbane, Singapore, Toronto, 1999); Hoffmeister, D., Ichinose, K., Domann, S., Faust, B., Trefzer, A., Dräger, G., Kirschning, A., Fischer, C., Künzel, E., Bearden, D. W., Rohr, J. and Bechthold, A. *The NDP-Sugar Co-Substrate Concentration and the Enzyme Expression Level Influence the Substrate Specificity of Glycosyltransferases: Cloning and Characterization of Deoxysugar Biosynthetic Genes of the Urdamycin Biosynthetic Gene Cluster. Chem. Biol.* 7:821-831 (2000); Trefzer, A., Hoffmeister, D., Künzel, E., Stockert, S., Weitnauer, G., Westrich, L., Rix, U., Fuchser, J., Bindseil, K. U., Rohr, J. and Bechthold, A. *Function of Glycosyltransferase Genes Involved in Urdamycin a Biosynthesis. Chem. Biol.* 7:133-142 (2000); Trefzer, A., Fischer, C., Stockert, S., Westrich, L., Künzel, E., Girreser, U., Rohr, J. and Bechthold, A. *Elucidation of the Function of Two Glycosyltransferase Genes (aanGT1 and lanGT4) Involved in Landomycin Biosynthesis and Generation of New Oligosaccharide Antibiotics. Chem. Biol.* 8:1239-1252 (2001); Krohn, K. et al, *Topics Curr. Chem.* 188:127-195 (1997); Westrich, L., Domann, S., Faust, B., Bedford, D., Hopwood, D. A. and Bechthold, A. *Cloning and Characterization of a Gene Cluster from Streptomyces cyanogenus S136 Probably Involved in Landomycin Biosynthesis. FEMS Microbiol. Lett.* 170:381-387 (1999).

Methods

ORF assignments were accomplished using the GCG software package (University of Wisconsin) and the NCBI database. When applying the GCG software on *Streptomyces*, assignment priority will be given to ORFs with consistently high G/C %. Preliminary gene assignments were then derived from the translated amino acid sequence similarity of translated genes of known function using the BLAST (Basic Local Alignment Search Tool) program, and standard protein sequence data bases (Genbank, EMBO, Swiss Prot). Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. *Basic Local Alignment Research Tool. J. Mol. Biol.* 215: 403-410 (1990); Altschul, S. F. and Lipman, D. J. *Protein Data Base Searches for Multiple Alignments. Proc. Natl. Acad. Sci. USA* 87: 5509-5513 (1990); Altschul, S. F. et al. *Gapped BLAST and Psi-BLAST—A New Generation of Protein Database Search Programs. Nucl. Acid Res.* 25:3389-3402 (1997).

Host-Vector System

Identification and cloning of the gilvocarcin V gene cluster led to the discovery of a host-vector system for the efficient recombinant production of both novel and known polyketides. The coding sequences which collectively encode a gilvocarcin V gene cluster or hybrids, mutants, analogs or derivatives thereof, can be inserted into one or more expression vectors, using methods known to those of skill in the art. The replacement gene cluster need not correspond to the complete native gilvocarcin gene cluster but need only encode a minimal PKS gene cluster to catalyze the production of a polyketide.

The recombinant vector(s) of the present invention includes replacement gene clusters derived from a single gene cluster, or may comprise hybrid replacement gene clusters with, e.g., a gene of one cluster replaced by the corresponding gene from another gene cluster. For example, acyl carrier proteins (ACPs) or certain deoxysugar genes are readily interchangeable among different synthases without an effect on the product structure. Furthermore, a given ketosynthase (KS) or ketoreductase (KR) may recognize or reduce polyketide chains of different chain lengths. Accordingly, these genes may be freely interchangeable in the constructs described herein. Thus, the replacement clusters of the present invention can be derived from any combination of PKS gene sets, which ultimately function to produce an identifiable polyketide.

Expression vectors also include control sequences operably linked to the desired PKS coding sequence. Suitable expression systems for use with the present invention include systems, which function in eucaryotic and procaryotic host cells. However, procaryotic systems are preferred, and in particular, systems compatible with Streptomyces species are of particular interest. Control elements for use in such systems include promoters, optionally containing operator sequences, and ribosome binding sites. Particularly useful promoters include control sequences derived from PKS gene clusters, such as one or more act promoters. However, other bacterial promoters, such as those derived from sugar metabolizing enzymes, such as galactose, lactose (lac) and maltose, will also find use in the present constructs. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (trp), the beta-lactamase (bla) promoter system, bacteriophage lambda PL, and T5. In addition, synthetic promoters, such as the tac promoter, which do not occur in nature also function in bacterial host cells.

Other regulatory sequences may also be desirable which allow for regulation of expression of the replacement PKS gene cluster relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

Selectable markers can also be included in the recombinant expression vectors. A variety of markers are known which are useful in selecting for transformed cell lines and generally comprise a gene whose expression confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers include, for example, genes which confer antibiotic resistance or sensi-

tivity to the plasmid. Alternatively, several polyketides are naturally colored and this characteristic provides a built-in marker for selecting cells successfully transformed by the present constructs.

The various subunits of gene clusters of interest can be cloned into one or more recombinant vectors as individual cassettes, with separate control elements, or under the control of, e.g., a single promoter. These subunits can include flanking restriction sites to allow for the easy deletion and insertion of other subunits so that hybrid gene clusters can be generated. The design of such unique restriction sites is known to those of skill in the art and can be accomplished using the techniques described above, such as site-directed mutagenesis and PCR.

Further, the vectors, which collectively encode a replacement gene cluster can be inserted in to one or more host cell, using methods known to those of skill in the art. As such, the present invention also provides host cells which have their naturally occurring gene substantially deleted, transformed with vectors encoding a replacement gene cluster or parts thereof, for the production of active polyketides. The invention provides for the production of significant quantities of product at an appropriate stage of the growth cycle. The polyketides so produced can be used as therapeutic agents, to treat a number of disorders, depending on the type of polyketide, like immunosuppressants, anti-tumor agents, as well as for the treatment of viral, bacterial and parasitic infections. The ability to recombinantly produce polyketides also provides a powerful tool for characterizing biosynthetic enzymes and the mechanism of their actions.

More particularly, host cells for the recombinant production of the subject polyketides can be derived from any organism with the capability of harboring a recombinant PKS gene cluster. Thus, the genetically engineered host cells of the present invention can be derived from either procaryotic or eucaryotic organisms. Preferably, the host may be *E. coli*. However, more preferred host cells are those constructed from the actinomycetes (act), a class of mycelial bacteria which are abundant producers of a number of polyketides. A particularly preferred genus for use with the present system is *Streptomyces*. Thus, for example, *S. ambofaciens, S. argillaceus, S. avermitilis, S. azureus, S. cinnamonensis, S. coelicolor, S. curacoi, S. cyanogenus, S. erythraeus, S. fradiae, S. galilaeus, S. glaucescens, S. globisporus, S. griseus, S. hygroscopicus, S. lividans, S. parvulus, S. peucetius, S. rimosus, S. roseofulvus, S. thermotolerans, S. venezuelae, S. violaceoruber*, among others, will provide convenient host cells for the subject invention. See e.g., Hopwood, D. A. and Sherman, D. H. Ann. Rev. *Genet.* 24:37-66 (1990); O'Hagan, D. *The Polyketide Metabolites* (Ellis Horwood Limited, 1991).

The above-described host cells are genetically engineered by deleting the naturally occurring PKS genes or genes encoding post-PKS tailoring enzymes therefrom, using standard techniques, such as by homologous or heterologous recombination. See e.g., Khosla, C. et al. *Molec. Microbiol.* 6:3237 (1992) One or more recombinant vector, collectively encoding a replacement gene cluster of the present invention, is then introduced into a host cell. The vector(s) can include native or hybrid combinations of gilvocarcin gene cluster subunits, or mutants, analogs, or derivatives thereof. Methods for introducing the recombinant vectors of the present invention into suitable host cells are known to those of skill in the art and typically include the use of $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Once the genes or gene clusters are expressed, the polyketide producing colonies can be identified and isolated using known techniques. The produced polyketides can then be further characterized, e.g. by NMR and mass spectroscopy.

Generation of New Gilvocarcin-Type Compound Producing Hybrids, Mutants, Analogs and Derivatives of *Streptomyces griseoflavus*

The generation of new gilvocarcin-type drugs, and gilvocarcin analogs and derivatives thereof can be produced by known methods in the art. Native gene sequences or parts thereof can be used alone or in combination with non-native gene sequences or parts thereof to produce analogs or hybrids of *Streptomyces griseoflavus*. For example, the replacement gene or gene cluster or subunits thereof of interest can be obtained from an organism that expresses the same, using recombinant methods, such as by screening cDNA or genomic libraries, derived from cells expressing the gene of interest, or by deriving the gene from a vector known to include the same. The gene can then be isolated and combined with other desired genes, using standard techniques. If the gene in question is already present in a suitable expression vector, it can be combined in situ, with, e.g., other PKS subunits or genes encoding tailoring enzymes such as deoxysugar genes, as desired. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. Edge. *Nature* 292:756 (1981); Nambair et al. *Science* 223:1299 (1984); Jay et al. *J. Biol. Chem.* 259:6311 (1984).

The replacement gene clusters of the present invention are derived from a single gene cluster, or may comprise hybrid replacement gene clusters with, e.g., a gene for one cluster replaced by the corresponding gene from another gene cluster. Non-limiting exemplary non-gilvocarcin V biosynthetic genes may be subunits of the gilvocarcin M, gilvocarcin E, defucosyl-gilvocarcin V, ravidomycin, deacetyl-ravidomycin, FE35A, FE35B, chrysomycin A, chrysomycin B, BE-12406 A, or BE-12406 B gene cluster. For example, deoxysugar pathways have common enzymes, which start the pathway, and which then can be complemented with various genes known from various sugar pathways in order to create novel or altered sugar moieties. Accordingly, these genes are freely interchangeable in the constructs described herein. Thus, the replacement clusters of the present invention can be derived from any combination of gene sets, which ultimately function to produce an identifiable new polyketide-type compound.

Examples of hybrid replacement gilvocarcin gene clusters include clusters with genes derived from two or more of the act gene clusters, such as granaticin (gra), gilvocarcin (gil), urdamycin (urd), landomycin (lan), mithramycin (mtm), tetracenomycin (tcm), oxytetracycline (otc), tetracycline (tet), erythromycin (ery), oleandomycin (ole), griseusin, nanaomycin, medermycin, daunorubicin, tylosin, carbomycin, spiramycin, avermectin, monensin, nonactin, curamycin, rifamycin and candicidin synthase gene clusters, among others.

Mutations can be made to the native gene sequences and such mutants used in place of the native sequence, so long as the mutants are able to function with other genes to collectively catalyze the synthesis of an identifiable polyketide. Such mutations can be made to the native sequences using conventional techniques such as by preparing synthetic oli-

gonucleotides including the mutations and inserting the mutated sequence into the gene encoding a PKS subunit using restriction endonuclease digestion. See e.g., Kunkel, T. A. *Proc. Natl. Acad. Sci. USA* 82:448(1985); Geisselsoder et al. *BioTechniques* 5:786 1987). Alternatively, the mutations can be effected using a mismatched primer (generally 10-20 nucleotides in length) which hybridizes to the native nucleotide sequence (generally cDNA corresponding to the RNA sequence), at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located. Zoller and Smith, *Methods Enzyvnol.* 100:468 (1983). Primer extension is effected using DNA polymerase, the product cloned and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations. See e.g., Dalbie-McFarland et al. *Proc. Natl. Acad. Sci USA* 79:6409 (1982).

In summary, this mixing and matching on biosynthetic genes, also called combinatorial biosynthesis is a new method of drug derivatization and SAR (structure-activity-relationship) assessment, which not only generates new drug analogs and derivatives but also the bacterial mutant strains for the biotechnological production of the new drugs.

Gene recombination is a well-known method in the art used for generating new gilvocarcin-type drug analogs and derivatives and new bacterial mutants. For example, gene recombination involves transforming a host cell, including the GV producer *Streptomyces griseoflavus* Gö 3592 or the newly generated GV-producer *S. lividans* TK24 (cos-G9B3) or other strains, with a recombinant vector encoding specific foreign genes. For the plasmid constructions, *Streptomyces-E. coli* shuttle vectors containing the strong constitutive ermE* promoter, which allows an overexpression of the inserted genes can be used. The recombinant vectors may be transferred into a host cell by either protoplast transformation or conjugal plasmid transfer. Non-limiting exemplary suitable genes and plasmids known in the art include the oxygenase genes jadF,G,H, and the GT-encoding genes jadS, urdGT2, lanGT2 and various other plasmids with deoxysugar biosynthesis genes.

Gene disruption, a method of generating a knockout or minus-mutants, can also be used to generate mutations for inclusion in a replacement PKS gene cluster. Knockouts are made by standard methods well established in the art, namely, insertional inactivation and in-frame gene deletion. See, e.g., Künzel, E.; Faust, B.; Oelkers, C.; Weissbach, U.; Bearden D. W.; Weitnauer, G.; Westrich, L., Bechthold, A. and Rohr, J. *J. Am. Chem. Soc.* 121:11058-11062 (1999); Westrich, L.; Domann, S.; Faust, B.; Bedford, D.; Hopwood, D. A. and Bechthold, A. *FEMS Microbiol. Lett.* 170, 381-387 (1999); Remsing, L. L., Garcia-Bernardo, J., Gonzalez, A., Kunzel, E., Rix, U., Brana, A. F., Bearden, D. W., Mendez, C., Salas, J. A. and Rohr, J. *Ketopremithramycins and Ketomithramycins, Four New Aureolic Acid-Type Compounds Obtained Upon Inactivation of Two Genes Involved in the Biosynthesis of the Deoxysugar Moieties of the Antitumor Drug Mithramycin by Streptomyces argillaceus, Reveal Novel Insights into Post-Pks Tailoring Steps of the Mithramycin Biosynthetic Pathway. J. Am. Chem. Soc.* 124:1606-1614 (2002).

Generally, insertional inactivation is obtained by subcloning a gene fragment from a strain containing the gene of interest into a suitable plasmid (e.g. pBSKT, pBluescript or pUC18-derivatives etc.), inserting an apramycin or other resistance cassette into the plasmid and transformation of this plasmid construct into the same strain from which the gene of interest came. Selection for both thiostreptone resistance (in the plasmid) and for apramycin or other suitable antibiotic resistance then indicates the knockout of the target gene. A stable double-crossover mutant is achieved by replacing the wild-type region by the in vitro-altered one. Remsing, L. et al. *J. Am. Chem. Soc.* 124:1606-1614 (2002).

Another method to inactivate genes is the Donnenberg method. See e.g., Donnenberg, M. S. and Kaper, J. B. *Construction of an Eae Deletion Mutant of Enteropathogenic Escherichia Coli by Using a Positive-Selection Suicide Vector. Infect. Immun.* 59: 4310-4317 (1991); Donnenberg, M. S. and Kaper, J. B. *Enteropathogenic Escherichia coli. Infect. Immun.* 60:3953-3961 (1992); Donnenberg-laboratory webpage: http://medschool.umaryland.edu/infemsd/resources.htm. In this method, suitable fragments for gene inactivation are cloned into pCVD442 and introduced into *E. coli* SM10-λ. The *E. coli* SM10-λ strain supplies the tra genes for conjugation of the pCVD442 inactivation construct into *E. coli* RR1 cells containing cos-G9B3. Cells growing under selection for apramycin, ampicillin and streptomycin (for streptomycin sensitive *E. coli* SM10-λ donor cells) should have the pCVD442 derivative integrated into cos-G9B3 via a single cross over event. Growing the cells without the addition of ampicillin and plating them out on agar plates containing 5% (w/v) sucrose should give colonies containing either the original cos-G9B3 or a mutated version of cos-G9B3 with the desired deletion. Restriction enzyme analysis is used to differentiate between these two colonies.

Also, the recently developed REDIRECT© technology can be used, which allows a fast insertion of an antibiotic resistance marker into the gene of interest through PCR. See e.g., Gust, B., Kieser, T. and Chater, K. F. *Redirect Technology: PCR Targeting System in Streptomyces coelicolor*. The John Innes Center; www.plantbioscience.com (2002).

As an alternative method for generating knockouts, in-frame gene deletion can be used to inactivate unwanted biosynthetic genes. Kulowski, K. et al. *Functional characterization of the jadI gene as a cyclase forming angucyclinones. Journal of the American Chemical Society* 121:1786-1794 (1999). Such methods are known to those skilled in the art.

EXAMPLES

The generation of the specific gene minus mutants such as, inter alia, gilGT, gilOI, gilOIV and gilMT are useful for determining the biosynthetic steps of gilvocarcin V and lead to the creation of gilvocarcin-type drug mutants and the discovery of new gilvocarcin-type drug analogs and derivatives thereof.

Inactivation of GilGT

For generation of a gilGT-minus-mutant, an in-frame deletion of a 267 base-pair (bp) segment flanked by two XhoI restriction sites within the gilGT gene is anticipated. For this, a SphI fragment of cos-G9B3 carrying gilGT is cloned into the same site of pUC19. Digestion with XhoI and religation of the vector removes the 267 bp-fragment. The resulting shorter gene fragment is then rescued as an EcoRI-HindIII fragment and cloned into a suitable *Streptomyces* suicide plasmid (e.g. pBSKT or pHZ1358; both carrying the tsr thiostrepton resistance gene). The resulting plasmid is then introduced into the *S. griseoflavus* Gö3592 wild type strain either by protoplast transformation or by conjugal transfer from *E. coli* ET12567 (pUB307). Selection for thiostrepton resistance should yield *S. griseoflavus* mutant strains with the entire vector integrated

into the chromosome by a single crossover event. These mutants are used to generate a stable double crossover mutant by allowing them to grow without selective pressure and subsequent screening for thiostrepton sensitive mutants. A successful second crossover event, yielding the gilGT-minus mutant strain, is verified by southern hybridization experiments. Without intending to be bound by theory, it is expected that the gilGT-minus mutant will accumulate the acceptor substrate of gilGT, namely either angucyclinone 2 or defucogilvocarvin E 4 (Scheme 1 of FIG. **8**).

To confirm that the resulting product(s) of the inactivation gilGT-mutants are really only an effect of the respective gene inactivation, the gilGT-mutants will be complemented with gilGT. For these experiments, gene fragments containing the gilGT gene will be ligated into an expression vector containing an antibiotic resistance marker, such as pEM4, pWHM3 or pUWL201-1 (all thiostreptone), and the minus-mutants will be transformed with the resulting plasmid. Growth in a medium containing thiostreptone will yield the complementation strain, whose product spectrum will be analyzed and compared to the wild-type strain. We expect an essentially identical product spectrum as from the wild-type strain. It is hypothesized that the inactivation of gilGT will help to clarify the sequence of events of the gilvocarcin biosynthesis, and will provide insights regarding the gilGT acceptor substrate, which is important for the generation of new gilvocarcin-type analogs.

Inactivation of GilOI

Without intending to be bound by theory, it is hypothesized that that gilOI/gilOIV encode the enzymes catalyzing a C—C-bond cleaving step for gilvocarcin biosynthesis. As with gilGT, in-frame deletion is possible for gilOI, due to the two suitable KpnI sites found in gilOI, which should allow the deletion of a 578-bp fragment. Inactivation of gilOI follows the general experimental procedure outlined above for the gilGT inactivation and confirmation complementation. Without intending to be bound by theory, we expect due to the inactivation of gilOI/gilOIV an accumulation of either angucyclinone (2) or a glycosylated intermediate, such as (5) as set out in Scheme 1 of FIG. **8**.

Mutation of GilGT.

The sequence comparison of gilGT with other glycosyltransferase encoding genes, in particular with urdGT2, showed that gilGT is about 300 base pairs longer at the beginning of the ORF, i.e. gilGT translates into a protein, which contains roughly 100 extra amino acids at its N-terminal end. The mutation, in which this extra portion of gilGT is removed, will be achieved by overexpression of a 300-bp shorter version of gilGT gene into the gilGT-mutant.

It is hypothesized that if the unique portion of gilGT is responsible for forcing the activated D-fucose from the pyranose into the furanose configuration, then the mutation experiment might yield a gilvocarcin bearing a fucopyranose moiety instead of the fucofuranose moiety, presuming that the remaining portion of the GT remains functional.

Synthetic studies suggest that such C-glycosides arise from O-glycoside intermediates via Fries-like rearrangement. Therefore, the glycosylation sequence probably leads first to the O-glycoside, and then the sugar moiety migrates to the neighbor carbon atom. For most C-glycosides, like the C-glycosidic D-olivose in urdamycin A, this is an ortho-shift. However, gilvocarcin V does not possess an oxygen atom in ortho-position, and since the Fries-rearrangement allows both, an o- and p-shift the C-glycosylation must proceed via the p-OH group. We hypothesize that the unique extra segment of gilGT might encode larger binding sites enabling this more complicated p-Fries rearrangement (e.g. through suiting two donor and two acceptor substrates). Therefore, the anticipated mutation gilGT might yield a molecule bearing an O-glycosidically linked sugar at 1-position instead of the usual C-glycosidically linked sugar at 4-position.

Complementation of the GilGT-Minus Mutant with Foreign GT-genes (UrdGT2, LanGT2 or JadS)

If the acceptor substrate of gilGT is angucyclinone (2) then the gilGT-minus-mutant (see above) is likely to be successfully complemented with urdGT2, which is a gene encoding the C-glycosyltransferase of the urdamycin pathway, for which a similar acceptor substrate is discussed. As the result of this complementation experiment, we expect an ortho-C-glycosylated product. This can be either an angucycline, or a novel gilvocarcin-type molecule depending on the substrate flexibility of the downstream enzymes of gilvocarcin biosynthesis. To accomplish this complementation experiment, the urdGT2 genes are inserted into plasmid pEM4, a pHWM3-derived overexpression vector. UrdGT2 is known to possess very broad substrate specificity to both the acceptor as well as the NDP (nucleosyldiphosphate)-activated sugar donor substrate. In case it should be unable to handle activated D-fucose (or one of its biosynthetic intermediates) provided by *S. griseoflavus* Gö3592, the resulting mutant strain *S. griseoflavus* gilGT-minus (urdGT2) can be complemented with designed plasmids (pLN2 derivatives) (See e.g., Rodriguez, L., Aguirrezabalaga, I., Allende, N., Brana, A. F., Mendez, C. & Salas, J. A. *Engineering Deoxysugar Biosynthetic Pathways from Antibiotic-Producing Microorganisms: A Tool to Produce Novel Glycosylated Bioactive Compounds. Chem. Biol.* 9:721-729 (2002)) providing NDP (nucleosyldiphosphate)-D-olivose, NDP-D-mycarose or NDP-D-rhodinose, which are known sugar donor substrates of urdGT2. These plasmids (e.g., pLNR for NDP-D-olivose) are available from our collaboration with J. A. Salas et al., or can be designed. For example, to achieve D-rhodinose, urdR in pLNR (Generates D-Olivose) has to be replaced by mtmU (Generating an Axial 4-OH) and complemented with urdQ; for D-mycarose, pLNR needs to be complemented with mtmC.

Complementation experiments using lanGT2 and jadS can be carried out following the same procedure. Here, O-glycosidically bound sugars are expected, since lanGT2 and jadS yield O-glycosides.

Complementation of the Gil Gene Cluster with Other Suitable Deoxysugar Biosynthesis Genes

As illustrated in Scheme 2 of FIG. **7**, there are only a few genes necessary to encode the biosynthesis of the deoxysugar moiety of gilvocarcin V. These genes can be complemented with other known genes of sugar pathways to generate various new gilvocarcin-type drugs with alternated sugar moieties. Basically, the gil gene cluster contains all elements to achieve activation (necessary for the glycosyltransfer) and 6-deoxygenation (a common step of all deoxysugar pathways, catalyzed by a 4,6-dehydratase). The gil cluster does not contain genes of branching elements (such as C-methyltransferases), amination elements (transaminases) and further deoxygenating enzymes. Only a few genes are needed to alter the D-fucofuranose moiety of GV sugar into an amino sugar (with or without methyl groups), into a branched sugar, or into a more deoxygenated sugar. Many of these genes, e.g. those encoding the 3-deoxygantion or 2-deoxygenation, are known from many pathways. Without intending to be bound by theory, both, pyranose or furanose GV moieties are expected.

Inactivation Experiments to Generate Increased Hydrophilicity.

Gene inactivation experiments can be used to generate less lipophilic gilvocarcin-type drugs: (i) inactivation of gilMT (presumably the methyltransferase responsible for the introduction of both O-methyl groups in 10- and 12-position), (ii) inactivation of gilE (the 4,6-dehydratase catalyzing the first

deoxygenation step in deoxysugar biosynthesis), and (iii) inactivation of gilU (or gilR, one of which is probably the ketoreductase of the sugar pathway). The experimental procedure will be analogous to the above-described examples. Without intending to be bound by theory, we expect that the first experiment should yield unmethylated GV, the second might yield a D-glucose analog of GV, and the third might yield a GV-analog with a keto sugar, which often is found as the hydrate form. All anticipated derivatives should be significantly more hydrophilic than the parent drug. Also, combinations of these mutations, e.g. a gilMT/gilE-double mutant can be envisaged, if the previous experiments are successful.

Determining Gilvocarcin Therapeutic Indications

The gilvocarcins, its analogs and derivatives thereof, of the present invention are useful as antibiotics, antitumor agents, immunosuppressants, antivirals and neuroprotective agents. Considering the fact that gilvocarcins are quite lipophilic compounds making them good candidates to pass the blood-brain barrier (BBB), brain tumors may be treatable by targeted submission of light (e.g., through fiber optics) after gilvocarcin chemotherapy. This would cause only few systemic side effects due to the absence of light elsewhere. This is attractive, since brain tumor surgery is often impossible. Targeted submission of light through fiber optics after gilvocarcin chemotherapy may be also an attractive treatment of prostate cancer. A more selective systemic treatment of leukemia might be possible with photoactivatable drugs like gilvocarcin V, since blood can be channeled outside the human body, where it is light exposed, while no major side effects will occur inside the body due to the exclusion of light. Also treatment of proliferative eye diseases, such as glaucoma, may be another future application of gilvocarcin-type anticancer drugs.

To determine anticancer therapeutic uses of gilvocarcin, its analogs and derivatives thereof, a compound derived from the host-vector recombinant production system is assayed as drugs against selected cancers or diseases in vitro and in vivo. Initially gilvocarcin and gilvocarcin analogs and derivatives are screened against selected human skin, brain, leukemia and prostate cancer cell lines, e.g., UACC-62, MALME-3M, SK-MEL-5 (melanoma), SF-268, SNB-75, U251 (brain tumors), CCRF-CEM, K-562, MOLT-4 (leukemia), and PC-3, DU-145 (prostate). To gain initial toxicity data on non-cancerous cells, similar experiments can be performed using normal epithelial and fibroblast cell cultures, purchased from ATCC or Clonetics.

The MTT assay, which measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase to an insoluble, colored, formazan product, is performed according to described standard procedures.

The SRB assay is a rapid and sensitive method to measure drug-induced cytotoxicity. It measures the uptake of sulforhodamin B (SRB), which is dependent on the cellular protein quantities, and is performed in 96-well microtiter plates according to the protocol published by Boyd et al. (NCI). See also, Skehan, P. et al. *New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute.* 82:1107-12 (1990).

Although illustrative embodiments of the present invention have been described in detail, it is to be understood that the present invention is not limited to those precise embodiments, and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope and spirit of the invention as defined by the appended claims.

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Thr Gly Met Gly Asp Ala Thr Lys Arg Ala Leu Asn Ser Ile Gln Glu
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Phe Ser Tyr Arg Ser Ile His His Asp Ser Met Gln Asp Gly Glu Val
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Val Ala Tyr Asp Gln Gln Arg Ser Ser Trp His Ala Tyr Arg Val Asp
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Cys Pro Ala Ser Leu Val Ala Lys Trp Ala Pro Gly Val Ala Ser Phe
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ccgcgtgttg gcgccgatca cggccggccc cacgatccgc gatccgcgga tctcggcgcc  17981

cgcctcgatc gtgacctggc cgatgacctc gctgtccgcg tcgacatagc cgtcgacccg  18041

gcccttggcg ccctccagca cggcccggtt gacctccagc atgtcgatga cgttcccggt  18101
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gtccttccag taaccggaaa tggtggtgga gcggacgtcg tacccgtggt cgatgagcca  18161

ctgcagggcg tcggtgatct ccagctcgcc gcgtgcggac ggctcgatcc cccggacggc  18221

ctcgtgcacc acggaggtga acaggaagac gccgaccagg gccaggtcgc tgcgcggagc  18281

ggccggcttc tcctccagcg ccaccgcctt cccgtcgtcg tcgagttcga cgacaccgaa  18341

ggcgctgggg tcggccacct tggtcaggag gatgtgcgtg tcgggccggc tctcgcggaa  18401

cccggccacg agatccgcga taccgcccac gatgaagttg tctccgaggt acatgacgaa  18461

gtcgtcgtcg ccgaggaact cccgggcgat caggacggca tgggcgagac cgagcggcgc  18521

cgcctgccgt atgtaggtga cgtccagacc gaaggcggag ccgtcgccga ccgcctgctg  18581

gatctcggcg gccgtgtccc cgacgacgat gccgacctcg gtgatgcctg cctccgcgat  18641

cgcctccaac ccgtagaaga gcacgggctt gttggctacg gggacgagct gcttggcgta  18701

ggaatgggtg atgggcctca ggcgggttcc ggccccgccg gacagtacga gagccttcat  18761

ggcggcgcag tctaggcggg cggggaaaca tctcaatcgg cccggcagcg cacggatgtc  18821

tggaaacaac ggtcggtaga ggtcaggaac tgacctctac cgctcataat ctggccgctc  18881

ccctctcccc ggagatcagc ttcgagagct cggtccctac cgaaggagcg aaacag atg  18940
                                                                Met

atc agg atc gcc gtc atc ctc gga agc acg cgt ccc ggc cgc cgc ggg    18988
Ile Arg Ile Ala Val Ile Leu Gly Ser Thr Arg Pro Gly Arg Arg Gly
        345                 350                 355

gcc gtg gtg gcc caa tgg gtc gcc gag gtc gcc gcg cgg cat ccc gcg    19036
Ala Val Val Ala Gln Trp Val Ala Glu Val Ala Ala Arg His Pro Ala
    360                 365                 370

gcg gtg atg ggc gag gcg gag ttc gag ctg gtc gac ctg gcg gag tac    19084
Ala Val Met Gly Glu Ala Glu Phe Glu Leu Val Asp Leu Ala Glu Tyr
375                 380                 385                 390

ggc ctc ccg ttg ctc gac gag ccc gtg ccg gcg atg ttc ggc cag tac    19132
Gly Leu Pro Leu Leu Asp Glu Pro Val Pro Ala Met Phe Gly Gln Tyr
                395                 400                 405

cag aag gag gag acc cgg cgg tgg gcc gcc gcc atc ggc tcg ttc gac    19180
Gln Lys Glu Glu Thr Arg Arg Trp Ala Ala Ala Ile Gly Ser Phe Asp
            410                 415                 420

gga ttc gtc ttc gtc acg ccg gag tac aac cac tcg gtg ccc gcc gcg    19228
Gly Phe Val Phe Val Thr Pro Glu Tyr Asn His Ser Val Pro Ala Ala
        425                 430                 435

ctg aag aac gcc atc gac cac ctc ttc gcc gag tgg acc gac aag gcg    19276
Leu Lys Asn Ala Ile Asp His Leu Phe Ala Glu Trp Thr Asp Lys Ala
    440                 445                 450

gcc ggg ttc gtc agc tac ggc gtg cac ggg gga acc cgt gcc gtc gag    19324
Ala Gly Phe Val Ser Tyr Gly Val His Gly Gly Thr Arg Ala Val Glu
455                 460                 465                 470

cac ctg cgg ctg gcc ctg gcc gag gtg aag gtg gcc ggg gtg cgc agc    19372
His Leu Arg Leu Ala Leu Ala Glu Val Lys Val Ala Gly Val Arg Ser
                475                 480                 485

cag gtc gtc ctg tcg gtg ttc aac gac ttc gac tac acg gga tgc gac    19420
Gln Val Val Leu Ser Val Phe Asn Asp Phe Asp Tyr Thr Gly Cys Asp
            490                 495                 500

atg acg gac ccg acg gcc atg ggc cgg ttc acg ccg gga ccg cag cag    19468
Met Thr Asp Pro Thr Ala Met Gly Arg Phe Thr Pro Gly Pro Gln Gln
        505                 510                 515

gag cag acg gtg aac acg atg ctg gac gag gtc gtc gcc tgg tcg acg    19516
Glu Gln Thr Val Asn Thr Met Leu Asp Glu Val Val Ala Trp Ser Thr
    520                 525                 530
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gcg ctc aag ccg ctg cgt act gct gcg acc gct gag gcg gac ggc cgg    19564
Ala Leu Lys Pro Leu Arg Thr Ala Ala Thr Ala Glu Ala Asp Gly Arg
535                 540                 545                 550

gcc gtg tcg gtg tgacgcaccg gtccgcccgc cggacccccc ggtgaacgtg        19616
Ala Val Ser Val

ctggtcacgg cccctcgtgc gtacgctacg agggccgtga ccagcacgtt cgcctgtacg  19676

ggcgagcgtg gccgccacgc cgcgggtggg cggcggcagc acgccggccg gaccgatcgc  19736

cgagtggctt gttacgtgcc cggggcgacg gcgccggagg gggacgcgcc gaaaaaaacc  19796

ggtcagtcga gttccccttc gataacacgg atatcccccc gtcctcactt cgggtgacct  19856

acttcggccg tgcgactccg agcatcgtga gcggc atg acg ttg cac gcc gca     19909
                                     Met Thr Leu His Ala Ala
                                     555                 560

gaa gcc ata ccg tca cac gta ccg gtt ctc gtc gtg gga gcc ggc ccg    19957
Glu Ala Ile Pro Ser His Val Pro Val Leu Val Val Gly Ala Gly Pro
                565                 570                 575

aca ggt ctc atg ctc ggc gcc gag ctg gcg ctc cac ggc agc cgg ccg    20005
Thr Gly Leu Met Leu Gly Ala Glu Leu Ala Leu His Gly Ser Arg Pro
            580                 585                 590

ctg gtg atc gac gcg ctg ccg agc ccg agc gga cag tcc cgg gcc ctg    20053
Leu Val Ile Asp Ala Leu Pro Ser Pro Ser Gly Gln Ser Arg Ala Leu
        595                 600                 605

ggc ttc acg gtg agg acg ctg gag atc ttc aag cag cgc ggc atc ctg    20101
Gly Phe Thr Val Arg Thr Leu Glu Ile Phe Lys Gln Arg Gly Ile Leu
    610                 615                 620

ggc cgt ttc cag gga ctc gcc ccg gtg ccc gga gtc cat ttc gcc ggc    20149
Gly Arg Phe Gln Gly Leu Ala Pro Val Pro Gly Val His Phe Ala Gly
625                 630                 635                 640

ctc agc atc aag ggc gat cac ctc tcc agc tcg atg cgc ccg gcc aac    20197
Leu Ser Ile Lys Gly Asp His Leu Ser Ser Ser Met Arg Pro Ala Asn
                645                 650                 655

cag tac ccg cag tcc aag acc gaa cag gtc ctc gcc gcc tgg gcc gag    20245
Gln Tyr Pro Gln Ser Lys Thr Glu Gln Val Leu Ala Ala Trp Ala Glu
            660                 665                 670

gag ctg gga gta ccg gtg cgg cgc ccg tgg acg ctg acg tcc atg gag    20293
Glu Leu Gly Val Pro Val Arg Arg Pro Trp Thr Leu Thr Ser Met Glu
        675                 680                 685

ccc act ggc acc ggg tac cgc tgc gtg ctc agc ggc ccg gcc ggg cag    20341
Pro Thr Gly Thr Gly Tyr Arg Cys Val Leu Ser Gly Pro Ala Gly Gln
    690                 695                 700

cag acc gtc gac gcc gac tac gtg gtc ggc tgc gac gga gcg ggg agc    20389
Gln Thr Val Asp Ala Asp Tyr Val Val Gly Cys Asp Gly Ala Gly Ser
705                 710                 715                 720

ttc gtc cgc gag gcg atc ggc atg ccg acc aag cgc act ccc cca tcc    20437
Phe Val Arg Glu Ala Ile Gly Met Pro Thr Lys Arg Thr Pro Pro Ser
                725                 730                 735

gta cag atg ctc ctc ggt gat ctg cgc gga tgc ggt ctg ccc gac gaa    20485
Val Gln Met Leu Leu Gly Asp Leu Arg Gly Cys Gly Leu Pro Asp Glu
            740                 745                 750

ccc ttc ggg gtc aag cac gaa aag ggc atg gtc atg tcc gca ccg ctg    20533
Pro Phe Gly Val Lys His Glu Lys Gly Met Val Met Ser Ala Pro Leu
        755                 760                 765

ggc gac ggg acg gaa cgc gtc atc gtc tgt gac ttc acc cag ccg atg    20581
Gly Asp Gly Thr Glu Arg Val Ile Val Cys Asp Phe Thr Gln Pro Met
    770                 775                 780

cgg ccg cag ggc act ccc gtc acg cac gac gag atc aag gcc gcc tac    20629
Arg Pro Gln Gly Thr Pro Val Thr His Asp Glu Ile Lys Ala Ala Tyr
785                 790                 795                 800
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gag cag gtc gtc ggc agc ccc ctg gcg gac ggc gaa tgt ctc tgg gcg    20677
Glu Gln Val Val Gly Ser Pro Leu Ala Asp Gly Glu Cys Leu Trp Ala
                805                 810                 815

agc tcg ttc tcg gac gcg tcc tcc ctc gtg gag tcc tac cgg tcc ggt    20725
Ser Ser Phe Ser Asp Ala Ser Ser Leu Val Glu Ser Tyr Arg Ser Gly
            820                 825                 830

cgt gcg ctg ctc gtc ggc gac acg gcg cac acc cat ctc ccc gcc ggc    20773
Arg Ala Leu Leu Val Gly Asp Thr Ala His Thr His Leu Pro Ala Gly
        835                 840                 845

ggg cag ggc atg aac gtc tcg ata cag gac gcg gtg aac gtc ggc tgg    20821
Gly Gln Gly Met Asn Val Ser Ile Gln Asp Ala Val Asn Val Gly Trp
    850                 855                 860

aag ctc gcg ctg gtg agc cag ggc cgc gcg ccg gac acc ctg ctg gac    20869
Lys Leu Ala Leu Val Ser Gln Gly Arg Ala Pro Asp Thr Leu Leu Asp
865                 870                 875                 880

acc tac cac gcc gag cgg tac ccg gtc ggc agg gaa ctg ctg ctc aac    20917
Thr Tyr His Ala Glu Arg Tyr Pro Val Gly Arg Glu Leu Leu Leu Asn
                885                 890                 895

acc gcc gcc cag ggc cag gtc ttc ctg cgc ggc ccg gaa gtg gac ccg    20965
Thr Ala Ala Gln Gly Gln Val Phe Leu Arg Gly Pro Glu Val Asp Pro
            900                 905                 910

ctg cgc gag gtc ctg cgg cga ctg ctg aac atc cgg gag gtg tcc gtc    21013
Leu Arg Glu Val Leu Arg Arg Leu Leu Asn Ile Arg Glu Val Ser Val
        915                 920                 925

ctg ctg gcc gac gga gtc agc gga ctg gac atc cgc tac gac atg ggc    21061
Leu Leu Ala Asp Gly Val Ser Gly Leu Asp Ile Arg Tyr Asp Met Gly
    930                 935                 940

ctc ccg gaa gca ccg cca ccc acg ggt gaa cgg ctg ccg ccg gac gtg    21109
Leu Pro Glu Ala Pro Pro Pro Thr Gly Glu Arg Leu Pro Pro Asp Val
945                 950                 955                 960

ttc cac gtc gtc ggg acc ggc ggc gac gcc gtc gag gag ttg cgg cac    21157
Phe His Val Val Gly Thr Gly Gly Asp Ala Val Glu Glu Leu Arg His
                965                 970                 975

ggc gcc gct ctg ctg atc gtc ccg tcc ccc gac agc ccg gcg tcc tcg    21205
Gly Ala Ala Leu Leu Ile Val Pro Ser Pro Asp Ser Pro Ala Ser Ser
            980                 985                 990

ctg gtc gct ccg tgg cgg gac cag  gtg cgc gtc gtg cac  gcg cgc ccc  21253
Leu Val Ala Pro Trp Arg Asp Gln  Val Arg Val Val His  Ala Arg Pro
        995                 1000                1005

acg gac  ccg gac tgg ggc ggg  gag ccg gcc gcg tcg  tcg cac tgg     21298
Thr Asp  Pro Asp Trp Gly Gly  Glu Pro Ala Ala Ser  Ser His Trp
    1010                1015                1020

ttc gta  cga ccg gac gga cac  atc gcg tgg gcg ggc  acc gaa ttc     21343
Phe Val  Arg Pro Asp Gly His  Ile Ala Trp Ala Gly  Thr Glu Phe
    1025                1030                1035

agc gag  ttg agc gcc tca ctg  agc cgc tgg ctc ggt  cag ccc gcc     21388
Ser Glu  Leu Ser Ala Ser Leu  Ser Arg Trp Leu Gly  Gln Pro Ala
    1040                1045                1050

gcg taaccagagg aggaagaacc cttgttcagc tctctcatcg tcgcccggat         21441
Ala

ggacaccggc cacgccgaag cggtggccga cgtcttcgcc ggcttcgacg ccaccgacat  21501

gcccgcgcgg atgggcacgc ggcgccgcga actcttccgc taccgcggcc tctacttcca  21561

cctccaggac ttcgagaccc ccgacgggac cgaagcggtc gaggcggcca agtccgaccc  21621

gcggttcatc cgggtgagca acgacctcag gccctacatc gaggcctacg ccccggactg  21681

gcaatcaccg aaggacgcca tggcagagcg cttctatcac tggagttcga aacg atg    21738
                                                          Met
                                                          1055
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agc cgc agg gtc ttc  atc acc ggg gtc ggt  gtc gtc gcg ccg gga      21783
Ser Arg Arg Val Phe  Ile Thr Gly Val Gly  Val Val Ala Pro Gly
                1060                1065                1070

gcc gtc gga cgt gac  ccc ttc tgg gag ctg  ctg acc caa ggg cgc      21828
Ala Val Gly Arg Asp  Pro Phe Trp Glu Leu  Leu Thr Gln Gly Arg
                1075                1080                1085

acg gcc acc cgc cgg  ctc agc ctc tgc gac  ccg gag ccc ttc cgg      21873
Thr Ala Thr Arg Arg  Leu Ser Leu Cys Asp  Pro Glu Pro Phe Arg
                1090                1095                1100

tcc cag gtg gcc gcg  gag gcc gac ttc gac  gcc gag gcg gcg ggg      21918
Ser Gln Val Ala Ala  Glu Ala Asp Phe Asp  Ala Glu Ala Ala Gly
                1105                1110                1115

ctg tcg gag cgg cag  tcc gcg gaa ctg gac  cgg gcg gcg cag ttc      21963
Leu Ser Glu Arg Gln  Ser Ala Glu Leu Asp  Arg Ala Ala Gln Phe
                1120                1125                1130

gcc ctg gtc gcc gcc  cgt gaa gcg gtc gag  gac gcg gca tgg tcc      22008
Ala Leu Val Ala Ala  Arg Glu Ala Val Glu  Asp Ala Ala Trp Ser
                1135                1140                1145

gag aca tgt cct ccc  gaa cgc gcc gga gtg  atc gtg ggt tcg gcc      22053
Glu Thr Cys Pro Pro  Glu Arg Ala Gly Val  Ile Val Gly Ser Ala
                1150                1155                1160

gtc gga gcc acg acc  aag ctc gag gag gtc  tac cgg cag ctc agc      22098
Val Gly Ala Thr Thr  Lys Leu Glu Glu Val  Tyr Arg Gln Leu Ser
                1165                1170                1175

cgt gac ggc tcc ctc  tgg gac gtg gcc ccc  gac tcc ccc gcc gag      22143
Arg Asp Gly Ser Leu  Trp Asp Val Ala Pro  Asp Ser Pro Ala Glu
                1180                1185                1190

ctg tac tcg tac ttc  gtg ccc agc tcg ttc  gcc tcc ggc atc gca      22188
Leu Tyr Ser Tyr Phe  Val Pro Ser Ser Phe  Ala Ser Gly Ile Ala
                1195                1200                1205

cac gac ctc ggc gtc  acg ggg cag agc ggc  gtc gtg tcg acc ggg      22233
His Asp Leu Gly Val  Thr Gly Gln Ser Gly  Val Val Ser Thr Gly
                1210                1215                1220

tgc acc tcc ggg atc  gac tcc gtc ggc aac  gcc tgg gaa ctg atc      22278
Cys Thr Ser Gly Ile  Asp Ser Val Gly Asn  Ala Trp Glu Leu Ile
                1225                1230                1235

cag agc ggc atc ctg  gac tcc gcc gtc tgc  ggt gcc acc gac gcc      22323
Gln Ser Gly Ile Leu  Asp Ser Ala Val Cys  Gly Ala Thr Asp Ala
                1240                1245                1250

ccc atc tcg ccc atc  acc gtc gcc tgc ttc  gac acg atc aag gcg      22368
Pro Ile Ser Pro Ile  Thr Val Ala Cys Phe  Asp Thr Ile Lys Ala
                1255                1260                1265

aca tcg acg tac aac  gac acc ccg gag agc  gcc tca cgg ccg ttc      22413
Thr Ser Thr Tyr Asn  Asp Thr Pro Glu Ser  Ala Ser Arg Pro Phe
                1270                1275                1280

gac gcc aca cgg ggc  ggc ttc gtc ctc ggc  gag ggc agc gcg atg      22458
Asp Ala Thr Arg Gly  Gly Phe Val Leu Gly  Glu Gly Ser Ala Met
                1285                1290                1295

ttc gtc ctc gaa tcg  gag gaa tcc gtc cac  cgt cgc ggc gca cgc      22503
Phe Val Leu Glu Ser  Glu Glu Ser Val His  Arg Arg Gly Ala Arg
                1300                1305                1310

gtc tac ggc gag atc  cgc ggc tac gcg agc  cgc tgc aac gcc tac      22548
Val Tyr Gly Glu Ile  Arg Gly Tyr Ala Ser  Arg Cys Asn Ala Tyr
                1315                1320                1325

cac atg acc ggt ctc  aag gcc gac gga cgc  gag ctg gcg gag gcc      22593
His Met Thr Gly Leu  Lys Ala Asp Gly Arg  Glu Leu Ala Glu Ala
                1330                1335                1340

gtc gtc tcc gct ctc  ggc cag gca ggc gtg  gac ccg ggc cgg ctc      22638
Val Val Ser Ala Leu  Gly Gln Ala Gly Val  Asp Pro Gly Arg Leu
                1345                1350                1355
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gac tac gtc aac gcc  cac ggc agc ggc acg  aag cag aac gac cgc      22683
Asp Tyr Val Asn Ala  His Gly Ser Gly Thr  Lys Gln Asn Asp Arg
                1360                1365                1370

cac gag acc gcc gcg  ctg aag tcg tcc ctc  gga ccc gcc gcc cac      22728
His Glu Thr Ala Ala  Leu Lys Ser Ser Leu  Gly Pro Ala Ala His
                1375                1380                1385

gac gtg ccg atc agt  tcg atc aag tcg atg  atc ggc cat tcg ctg      22773
Asp Val Pro Ile Ser  Ser Ile Lys Ser Met  Ile Gly His Ser Leu
                1390                1395                1400

ggc gcc atc ggg tcg  ttg gag atc gcc gcc  tgc gcc ctg gcg ctg      22818
Gly Ala Ile Gly Ser  Leu Glu Ile Ala Ala  Cys Ala Leu Ala Leu
                1405                1410                1415

cgg gac gac gtg atc  ccg ccg acg gcc aat  ctc acc cgg ccg gat      22863
Arg Asp Asp Val Ile  Pro Pro Thr Ala Asn  Leu Thr Arg Pro Asp
                1420                1425                1430

ccg gaa ctc gat ctg  gac tac gtg ccg gtc  cac gcg cgc aag cag      22908
Pro Glu Leu Asp Leu  Asp Tyr Val Pro Val  His Ala Arg Lys Gln
                1435                1440                1445

ccg acc aac agc gtg  ctc acg acc gga agc  ggc ttc ggt ggg ttt      22953
Pro Thr Asn Ser Val  Leu Thr Thr Gly Ser  Gly Phe Gly Gly Phe
                1450                1455                1460

cag agc gcc atg gtt  ctc acg gac ccg gag  cat cac tca tgaccgcaca   23002
Gln Ser Ala Met Val  Leu Thr Asp Pro Glu  His His Ser
                1465                1470

catcaccggc atcgacatcg tctccccgct gggcctgtcc cgcgaggaac actggaaggc  23062

cctcctcgac ggatgcagcg gtctgagggc gacgcagtcg ttcgactcca gcaggtacga  23122

caaccccatc agcggggagg tgccccactt cgccccggag ggcctgccca agcggctgct  23182

gccggccacc gaccggatga cccagatgtc gctggtcgcc gcggcggggg cgttcgacga  23242

cagcggtgtc gacacgagcc gggtcgaccc cctcggagtc ggtgtcatga cggcgtccac  23302

cgcggggggt tacgcgttcg ggcagaagga gctgcagaac ctgtggtcca aggggcccag  23362

gtacgtcagc acccatcagt cctacgcctg gttctacgcg gtcaacaccg gtcagatctc  23422

catccggcac ggctgccagg gccacagcgg agtgatcgtc gcggacgacg ccggcgggct  23482

cgacgcgatc tccttcgccg cccgccgtct ggcgcgcggc aaccgcgtca tgctcaccgg  23542

gtcggtggac agcacgatgt gtccctgggg gcgggtcgcg cacacctcga ccggcatgct  23602

ctcggcatcc accgacgcgc gggccgcgta ccttccgttc gacgcacgag ccaacgggtg  23662

ggtcaacggc gaaggcggcg cgcacctcgt gctgcagacc cacagtgacg gccgctacgc  23722

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cgtccgggcg atccacctcg cgctcggcgc ggcgcggctg cgccccggcg acatcagtgt  23842

ggtgttcgcc gacgcggccg gcacccggga ggcggacacc gccgaggccg ccgccctcgc  23902

cgaggtcttc gggccggatt ccgtccccgt caccgcgccc aaggcggcga ccggccggat  23962

gggctgcggg acggccgcac tcgacgtcgc gacggcggtg ctcgccctcc gcgaccagac  24022

gattcccccc accgtcaacg tccaggccga cgcgtccctg ggggtcaacc tgtgcagcgt  24082

cgccacacac cacccctca ccaacgtcct ggtcctggcc cggggcgtcg gtgggttcaa  24142

ctcggccctg atcgtcggga aatgagagaa ggagcaagga atg tcc  gca cgc gtc   24197
                                        Met Ser  Ala Arg Val
                                            1475

acc atg  gac gat ctc agg cga  gcc ctc gag gag ggc  tcc ggt gtc     24242
Thr Met  Asp Asp Leu Arg Arg  Ala Leu Glu Glu Gly  Ser Gly Val
    1480                1485                1490
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gac gag  ggc gtc gat ctt gac  acc gac ctc gaa acc  atg gcg ttc     24287
Asp Glu  Gly Val Asp Leu Asp  Thr Asp Leu Glu Thr  Met Ala Phe
    1495                 1500                 1505

tcc gag  ctg ggg tac gac tcc  ctg gcg gtg ctg gag  acc ggc ctg     24332
Ser Glu  Leu Gly Tyr Asp Ser  Leu Ala Val Leu Glu  Thr Gly Leu
    1510                 1515                 1520

cgc ctc  ggc cgc gag aac gac  atc gag ctc gac gac  tcg gtg ttc     24377
Arg Leu  Gly Arg Glu Asn Asp  Ile Glu Leu Asp Asp  Ser Val Phe
    1525                 1530                 1535

gcc gac  ctc gac acg cct cag  cag atg ctg gac gcg  gtc aac gat     24422
Ala Asp  Leu Asp Thr Pro Gln  Gln Met Leu Asp Ala  Val Asn Asp
    1540                 1545                 1550

gcc ctc  gcg cgt cag gcg gcg  gca tcg tgacctctcc ccgtcatgcc       24469
Ala Leu  Ala Arg Gln Ala Ala  Ala Ser
    1555                 1560

ctggtcaccg gcggttccag cggcatagga aagtccgtcg cacggcgcct ggcctcggcc  24529

ggccacaccg tcacgatctg cggtcgtgac tccgaaaggc tccagcaggc cgccaaggaa  24589

ctgtcggagc agggtgcacc cgtcacctcg ctgatcgccg acgtcagcaa gccccgccag  24649

gtgggcgatc tggtccgcga ggccgtggag acgaacggtc ccctcgggat cctcgtcaac  24709

aacgcgggca ggaacggagg cggccggacc gcggagctga gcgacgagct gtggcgggag  24769

gtactgagca ccaacctcga cagcgttttc tacgtcacgc gggaggtgct ggcccgtggc  24829

ggcatcggcg aggtggacca cgcccggatc atcaacatcg cctccaccgc ggggaagcag  24889

ggagttctgc tggccgcccc gtactccgcc tccaagcacg gtgtcgtcgg cttcaccaag  24949

gcggtgggca aggagctggc ccctcagggg atcaccgtga acgccgtctg cccgggctac  25009

gtggagaccc cgatggcctc acgggtccgg caggcctacg cagacgcctg ggagaccacg  25069

gaggccgagg tgctgtccgc cttcgaggcg aagatcccgc tcggccggta cagcacgccc  25129

gacgaggtcg cctcgctggt cgagtacctc acgaccgaag gagccgcctc gatcacggct  25189

caggcgttca acgtgtgcgg cggcctcggc aacttctagg agatgattca c atg gcc   25246
                                                      Met Ala

gat  ccg gct cgc aca gac  ctg cac tcc gcc acg  atc acc ggc agc     25291
Asp  Pro Ala Arg Thr Asp  Leu His Ser Ala Thr  Ile Thr Gly Ser
1565                 1570                 1575

gcc  gac gcg gtg tac cgc  cgt ctg gag gac gtc  ggg cag tgg tcc     25336
Ala  Asp Ala Val Tyr Arg  Arg Leu Glu Asp Val  Gly Gln Trp Ser
1580                 1585                 1590

cag  atg ttc gaa ccg acc  atc cac ggc gcg gaa  ctg gcc cgg gac     25381
Gln  Met Phe Glu Pro Thr  Ile His Gly Ala Glu  Leu Ala Arg Asp
1595                 1600                 1605

ggg  aac agg cag acg atc  cag ctg tgg gcc acc  gcc aac gga gaa     25426
Gly  Asn Arg Gln Thr Ile  Gln Leu Trp Ala Thr  Ala Asn Gly Glu
1610                 1615                 1620

ccc  aag gcc tgg gtc tcc  gag cgt gag ctc gac  ccc gtc gcg cgc     25471
Pro  Lys Ala Trp Val Ser  Glu Arg Glu Leu Asp  Pro Val Ala Arg
1625                 1630                 1635

acc  atc cgc ttc gcg cag  acc gtc acc tcc tcg  ccc gtc gcc gag     25516
Thr  Ile Arg Phe Ala Gln  Thr Val Thr Ser Ser  Pro Val Ala Glu
1640                 1645                 1650

atg  tcc ggc gcg tgg cag  gtg ctg ccc ctg tcc  gag gac acc tgc     25561
Met  Ser Gly Ala Trp Gln  Val Leu Pro Leu Ser  Glu Asp Thr Cys
1655                 1660                 1665

cgg  gtc gaa ctc acg cac  acc tac cgt gcg gag  aac gac tcg gcg     25606
Arg  Val Glu Leu Thr His  Thr Tyr Arg Ala Glu  Asn Asp Ser Ala
1670                 1675                 1680
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gag  tcg ctc aca tgg atc  gcc cga gcc gtg gag  acc aac agc acg     25651
Glu  Ser Leu Thr Trp Ile  Ala Arg Ala Val Glu  Thr Asn Ser Thr
1685                1690                1695

aag  gag ctc tcg gcg ctc  aag ttc gcc tgc gaa  cgg gac gcc gac     25696
Lys  Glu Leu Ser Ala Leu  Lys Phe Ala Cys Glu  Arg Asp Ala Asp
1700                1705                1710

agc  gag gcc agt ccc ttc  acc ttc acc gat gcg  gtg gac acc acg     25741
Ser  Glu Ala Ser Pro Phe  Thr Phe Thr Asp Ala  Val Asp Thr Thr
1715                1720                1725

gtc  gac ccc gtc ctg ctg  ttc tcg ttc ctg gac  cgc ggt gag ctg     25786
Val  Asp Pro Val Leu Leu  Phe Ser Phe Leu Asp  Arg Gly Glu Leu
1730                1735                1740

tgg  gcg gga cgc ctg gag  cac gtc gcc gag gcc  gag atg agg gag     25831
Trp  Ala Gly Arg Leu Glu  His Val Ala Glu Ala  Glu Met Arg Glu
1745                1750                1755

ttc  tcc gac ggc ctg cag  ttc ctc cgg atg cgg  acg cgc acc ccg     25876
Phe  Ser Asp Gly Leu Gln  Phe Leu Arg Met Arg  Thr Arg Thr Pro
1760                1765                1770

gac  ggt gac acg cac gtc  acc gag tcc tac cgg  gtg tcg cag agc     25921
Asp  Gly Asp Thr His Val  Thr Glu Ser Tyr Arg  Val Ser Gln Ser
1775                1780                1785

ccg  gcc cgg ctg ctg tac  aag cag gtg acg ctg  ccc gcg ctg ctg     25966
Pro  Ala Arg Leu Leu Tyr  Lys Gln Val Thr Leu  Pro Ala Leu Leu
1790                1795                1800

tcg  ctg cac acc ggc gag  tgg acc atc acc ccg  gcc ggg gag agc     26011
Ser  Leu His Thr Gly Glu  Trp Thr Ile Thr Pro  Ala Gly Glu Ser
1805                1810                1815

tgg  cgg gtc acg tcg aag  cac acc gtg gcg atc  gat ccc gac gcg     26056
Trp  Arg Val Thr Ser Lys  His Thr Val Ala Ile  Asp Pro Asp Ala
1820                1825                1830

gtg  cac aag gtc ctc ggt  gcc gac gcg acg gtc  tcg gac gcc aag     26101
Val  His Lys Val Leu Gly  Ala Asp Ala Thr Val  Ser Asp Ala Lys
1835                1840                1845

cgg  ctc gcc cgg cgc aac  ctg ggc aac aac agc  ctg cgg acc ctc     26146
Arg  Leu Ala Arg Arg Asn  Leu Gly Asn Asn Ser  Leu Arg Thr Leu
1850                1855                1860

gaa  gca gcg gtc cgg tgg  gcc ggc acc gcc gtg  tcg cag agg         26188
Glu  Ala Ala Val Arg Trp  Ala Gly Thr Ala Val  Ser Gln Arg
1865                1870                1875

tgagtgggga c atg acg  gag ccc gag acc tcg  gac gtt ctc gtc gtc     26235

             Met Thr  Glu Pro Glu Thr Ser  Asp Val Leu Val Val
                 1880                1885                1890

ggc gcc ggg ccc agc  gga ctg ctc ctg gcc  ggg atc ctc gcc ggg     26280
Gly Ala Gly Pro Ser  Gly Leu Leu Leu Ala  Gly Ile Leu Ala Gly
                1895                1900                1905

gcg ggt gcg cgg gtc  acg gtg ctg gag gcg  cgg gac gcg ccc agc     26325
Ala Gly Ala Arg Val  Thr Val Leu Glu Ala  Arg Asp Ala Pro Ser
                1910                1915                1920

ccg cag acc cgc gcc  tcc acc ttg cac gcc  cgt gcc agg gag atc     26370
Pro Gln Thr Arg Ala  Ser Thr Leu His Ala  Arg Ala Arg Glu Ile
                1925                1930                1935

ctc gac cac cac gga  gtg gag ttc tcc ccg  gag ctg ccc tgg agt     26415
Leu Asp His His Gly  Val Glu Phe Ser Pro  Glu Leu Pro Trp Ser
                1940                1945                1950

gcc cac gga cac tac  ggc ggc ctg cgc gtg  gac ctc tcc cgg gtc     26460
Ala His Gly His Tyr  Gly Gly Leu Arg Val  Asp Leu Ser Arg Val
                1955                1960                1965
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gac tcc ggg cgg gcc  ggt gtc tgg aag tgc  ccc cag ccg gaa ctg      26505
Asp Ser Gly Arg Ala  Gly Val Trp Lys Cys  Pro Gln Pro Glu Leu
                1970                 1975                 1980

gta cgg acg ctg acc  ggc tgg gcc cgc ggg  cac ggc gcg cgg ctg      26550
Val Arg Thr Leu Thr  Gly Trp Ala Arg Gly  His Gly Ala Arg Leu
                1985                 1990                 1995

ctc cac ggg gag cac  gtg gag tcc gtc cgc  gag cag ggc ggg cgc      26595
Leu His Gly Glu His  Val Glu Ser Val Arg  Glu Gln Gly Gly Arg
                2000                 2005                 2010

tgt ctg gtg cgt acc  cgg gcc ggc acc acg  ttc agc ggg acc ctg      26640
Cys Leu Val Arg Thr  Arg Ala Gly Thr Thr  Phe Ser Gly Thr Leu
                2015                 2020                 2025

ctg gtc gcg gcg gac  ggc cgg cgg agc acg  gtg cgg tcg ctg ctg      26685
Leu Val Ala Ala Asp  Gly Arg Arg Ser Thr  Val Arg Ser Leu Leu
                2030                 2035                 2040

ggc atc ggg tgc ggg  ggt gcg ccg gcc acg  cgc gta ctg gtg cag      26730
Gly Ile Gly Cys Gly  Gly Ala Pro Ala Thr  Arg Val Leu Val Gln
                2045                 2050                 2055

gcc gat gtc cac ggc  gac ggg ctg gcg ggg  cgg cgc ttc gag cga      26775
Ala Asp Val His Gly  Asp Gly Leu Ala Gly  Arg Arg Phe Glu Arg
                2060                 2065                 2070

cac ggg cgg tac acc  gtg acc gcc gca ccg  atc agc ccc ggg atc      26820
His Gly Arg Tyr Thr  Val Thr Ala Ala Pro  Ile Ser Pro Gly Ile
                2075                 2080                 2085

acc cgg gtg atg ctg  cac gat ccg cgc tgg  ccc gcg ggc gag gaa      26865
Thr Arg Val Met Leu  His Asp Pro Arg Trp  Pro Ala Gly Glu Glu
                2090                 2095                 2100

cgc acg ctg gag gac  ctc cgt aga gcc tgg  aag gag tcc acc ggc      26910
Arg Thr Leu Glu Asp  Leu Arg Arg Ala Trp  Lys Glu Ser Thr Gly
                2105                 2110                 2115

gag acc ctg ccg gcc  gag ccg tcg tgg tca  cgg acc ttc agc gac      26955
Glu Thr Leu Pro Ala  Glu Pro Ser Trp Ser  Arg Thr Phe Ser Asp
                2120                 2125                 2130

gac acg aca gtg gca  cac ccg ctg gtc aag  ggc cgt gtc gtg ctg      27000
Asp Thr Thr Val Ala  His Pro Leu Val Lys  Gly Arg Val Val Leu
                2135                 2140                 2145

tgc ggc gac gcc gcc  cac ccc ttc gtc ccc  atc ggc ggc cag gcg      27045
Cys Gly Asp Ala Ala  His Pro Phe Val Pro  Ile Gly Gly Gln Ala
                2150                 2155                 2160

ctg aac acg tcg ttg  atg gac gcc gag gcg  ctg ggc tgg cgg gtc      27090
Leu Asn Thr Ser Leu  Met Asp Ala Glu Ala  Leu Gly Trp Arg Val
                2165                 2170                 2175

ctg ggg tat ctg gac  gac ggg gac cgg caa  ggc ctc ctc gac tac      27135
Leu Gly Tyr Leu Asp  Asp Gly Asp Arg Gln  Gly Leu Leu Asp Tyr
                2180                 2185                 2190

cag gac gag cgg ttc  tcg tgg ctg acc gtt  ctc gcg ggg aga ctg      27180
Gln Asp Glu Arg Phe  Ser Trp Leu Thr Val  Leu Ala Gly Arg Leu
                2195                 2200                 2205

cgc gcc cag gca cgt  ctg ctg ttc gac acc  gac gcg gcg gcc acg      27225
Arg Ala Gln Ala Arg  Leu Leu Phe Asp Thr  Asp Ala Ala Ala Thr
                2210                 2215                 2220

gaa cgc aag gcg ctg  gtc gcc gcg aga ctg  gcc ggg gac gcg gac      27270
Glu Arg Lys Ala Leu  Val Ala Ala Arg Leu  Ala Gly Asp Ala Asp
                2225                 2230                 2235

tac cgg cgc agg atc  gcc gac gcc ctg gcc  ggt gtc gac gtg tgc      27315
Tyr Arg Arg Arg Ile  Ala Asp Ala Leu Ala  Gly Val Asp Val Cys
                2240                 2245                 2250

tac ctg acg ccc ggc  ggc gcg gtc cgc cgg  cgt ctg tcc ccg gcc      27360
Tyr Leu Thr Pro Gly  Gly Ala Val Arg Arg  Arg Leu Ser Pro Ala
                2255                 2260                 2265
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cgg ctc cgg gag acc  gga gtg aac ccc ggc  gcc cgc cgc gtg cag      27405
Arg Leu Arg Glu Thr  Gly Val Asn Pro Gly  Ala Arg Arg Val Gln
                2270                2275                2280

cgg gcg ctc gtc ccc  gac gac gga acg cgc  acg gac gcc tgg atc      27450
Arg Ala Leu Val Pro  Asp Asp Gly Thr Arg  Thr Asp Ala Trp Ile
                2285                2290                2295

cgt ccc gat cac cac  tgg tac ccg gtg gcc  cgc gac ggg gcc cgg      27495
Arg Pro Asp His His  Trp Tyr Pro Val Ala  Arg Asp Gly Ala Arg
                2300                2305                2310

cag gac tgg gac gac  gcg gtg cgc ctc cac  gac gac ttg gaa ccc      27540
Gln Asp Trp Asp Asp  Ala Val Arg Leu His  Asp Asp Leu Glu Pro
                2315                2320                2325

gag gtg acg cgg tgagagcgtt cctgttcccc ggtcagggga cccagaagat        27592
Glu Val Thr Arg
cggcatgggc acctacctgc gagaacggta cccccacctg atcgcgccgt tgtggcggga  27652

ggcggacgac gtcctgggtt tccccctcac ccgcctctgc gaggaaggcc ccggcgagaa  27712

gctccgccac atgccggtca cccagcccgc cgtcttcctg tgcagttacg ccgcgctcgt  27772

cgccgcgcag gcgaacggcg cggagccgga cgtcatcgcg ggccacagtc tgggcgagta  27832

ctcggcgctg gcggcggccg gcgtcctcac ctggcaggag gtccttcagc tcgtccaccg  27892

ccgcggtcag ctcatggcgg aggtgcagca caaggtggac gggaagatgg cggccgtcat  27952

cggtctcgcc atcgggcagg tcgaggagat ctgcgagcag gtgcggtccg agaccggtga  28012

ggtggtcgag gtggccaacc acaacgagcc cctccaggtc gtcgtctccg gccagtgcgc  28072

tgccatagac ctcctggtcc agcgcgtcgc gacggcgacc gacgtccgca cgtccgtcct  28132

gaggatcggt ggcccggccc actccagtct catgggcagc gtcgcggggg acttcgtgga  28192

gtacctccgg cgcttcgact tctgcacgcc caagacgatg ctgatctccg ggtcgaccgc  28252

cgagccctac gcgagtgcgg aggagatcag gcaccagctc ggcaggcagc tggtgcaccg  28312

ggtgcggtgg gtggacgtga tggcgcagct cgagaggctg ggggtcgcac agacctggga  28372

gctggggccg ggcaaggtcc tctcgggatt cgtacagcgg tcgctgcctc aggtgcggac  28432

gtaccgcgcg aatgatctgc cgtccttcct ggccggcgtg acgggctggt gagccggtga  28492

agcacgca gtg  ccg cat cag gca acc  ggc gcc gct ccc gac  gga ggg     28539
         Val  Pro His Gln Ala Thr  Gly Ala Ala Pro Asp  Gly Gly
         2330                2335                2340

ggg tcc gcg  ccc cgg tcc ctc gtg  ctg atg ctc ccc ggc  cag ggg     28584
Gly Ser Ala  Pro Arg Ser Leu Val  Leu Met Leu Pro Gly  Gln Gly
        2345                2350                2355

tcg cag ttc  gct gcc atg gga gtc  ccg ctc tac gag tcc  gac gcc     28629
Ser Gln Phe  Ala Ala Met Gly Val  Pro Leu Tyr Glu Ser  Asp Ala
        2360                2365                2370

cgg ttc agg  aag gcg ctc gac gac  ttc ttc gac gcg ttc  ggc acc     28674
Arg Phe Arg  Lys Ala Leu Asp Asp  Phe Phe Asp Ala Phe  Gly Thr
        2375                2380                2385

ggt gcc gag  cgg ctc cgg cgc gag  tgg ctg cac ggt tcg  gcc cag     28719
Gly Ala Glu  Arg Leu Arg Arg Glu  Trp Leu His Gly Ser  Ala Gln
        2390                2395                2400

ggc atc gaa  cgt ggg tcc ttc gcg  cag ccg atg ctg ttc  ggc ctc     28764
Gly Ile Glu  Arg Gly Ser Phe Ala  Gln Pro Met Leu Phe  Gly Leu
        2405                2410                2415

gac tac gcg  gcg ggc gcg gtg tgg  ctg gag gag ctc aag  ggt gtc     28809
Asp Tyr Ala  Ala Gly Ala Val Trp  Leu Glu Glu Leu Lys  Gly Val
        2420                2425                2430

gac gtg acg  ctg gtc ggt cac agc  gtg ggc gag ctg gcg  gcg gcc     28854
Asp Val Thr  Leu Val Gly His Ser  Val Gly Glu Leu Ala  Ala Ala
        2435                2440                2445
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acc ctc gcg  ggg gcc ttc gac ctc  gag ctg gcg ggg gca  ctc ctg     28899
Thr Leu Ala  Gly Ala Phe Asp Leu  Glu Leu Ala Gly Ala  Leu Leu
        2450                 2455                 2460

gcc gag cgg  gcc cgg ctc ctc gac  gcc gcc ccc cgg gga  ggg atg     28944
Ala Glu Arg  Ala Arg Leu Leu Asp  Ala Ala Pro Arg Gly  Gly Met
        2465                 2470                 2475

atc gcg tgc  cgc gcg acg gag gag  tcg ctg cgg gag cat  ctc gac     28989
Ile Ala Cys  Arg Ala Thr Glu Glu  Ser Leu Arg Glu His  Leu Asp
        2480                 2485                 2490

gcc ctg ggc  gga cgc gcc gtc atc  gcg gcg gag aac gcg  gac aac     29034
Ala Leu Gly  Gly Arg Ala Val Ile  Ala Ala Glu Asn Ala  Asp Asn
        2495                 2500                 2505

cag tgc gtc  gtg agc tgt gcc gag  gaa gac ctc ccg gac  acg atg     29079
Gln Cys Val  Val Ser Cys Ala Glu  Glu Asp Leu Pro Asp  Thr Met
        2510                 2515                 2520

cgg tac ctc  ggc tcg cac ggt gtg  acg tgc ctg cgc gtc  gcc tcg     29124
Arg Tyr Leu  Gly Ser His Gly Val  Thr Cys Leu Arg Val  Ala Ser
        2525                 2530                 2535

acc gaa ccg  ttc cac tcc ccc ctc  ctc gcc ccc gcc gcc  gcc cgg     29169
Thr Glu Pro  Phe His Ser Pro Leu  Leu Ala Pro Ala Ala  Ala Arg
        2540                 2545                 2550

ttc gag gag  ttc ctg gcc cgg cgc  ggt cat cgt ctg tcc  acg acg     29214
Phe Glu Glu  Phe Leu Ala Arg Arg  Gly His Arg Leu Ser  Thr Thr
        2555                 2560                 2565

gaa ctg ccc  atg gtc tcg gcc tac  tcg gcg cgg agg atc  agc ggc     29259
Glu Leu Pro  Met Val Ser Ala Tyr  Ser Ala Arg Arg Ile  Ser Gly
        2570                 2575                 2580

cgg gag atc  atg ccc gcc tcg ttc  tgg acg cgt cag atg  gct gag     29304
Arg Glu Ile  Met Pro Ala Ser Phe  Trp Thr Arg Gln Met  Ala Glu
        2585                 2590                 2595

aag gtg cgt  ttc tgg gag gcg ctc  cgc cac aac ttc gac  tcc ggt     29349
Lys Val Arg  Phe Trp Glu Ala Leu  Arg His Asn Phe Asp  Ser Gly
        2600                 2605                 2610

ccc cgc acg  ttc gtg gaa atc ggc  cca ggg acc gtc ctc  tcc ctg     29394
Pro Arg Thr  Phe Val Glu Ile Gly  Pro Gly Thr Val Leu  Ser Leu
        2615                 2620                 2625

gcc gca cgt  cgg ctg ccg tcc gta  cgg gcc cgg cgt tcc  acg gtg     29439
Ala Ala Arg  Arg Leu Pro Ser Val  Arg Ala Arg Arg Ser  Thr Val
        2630                 2635                 2640

atc tcc acg  atg ccg cgt cat cgg  ccc cac ccg gag cac  tgg gaa     29484
Ile Ser Thr  Met Pro Arg His Arg  Pro His Pro Glu His  Trp Glu
        2645                 2650                 2655

tcg gcc ata  cat gag gtc gcc gag  gaa ttc tgt tgaccattgc          29527
Ser Ala Ile  His Glu Val Ala Glu  Glu Phe Cys
        2660                 2665

actacgtgca acgcgcaagg ccggccatgg gtgtcccccg agttcccggg aggcacccat  29587

ggccttgtcg gtgaaatgtt caaccaaatg aaccacctct cgagggcgcc ccggatcaaa  29647

gatgttcacc gatttgcata gtcgaaaaaa tacggacagc aacggaagcg gagtgttatc  29707

ctgcaatctg cacgcaacgg gggaaacggg ggaggattcc aagtgcagga cccggtggac  29767

cggacctcgg ggaactccag aggcgcaagc accgtcggac ccgagcagtc ccgcaaagac  29827

gcggggcact cctgtcgccg aagagggccc ggtaccgtca cgcagcagtt ttcgccgacc  29887

ctccaccatt tcttcaccgt cacgagatct cttcgggccg acggggacca cacgggcaga  29947

gactgaagga ccggcgctcg acctcgccgg ctcccgcccc tccacccctt ccccgcgttg  30007

cccctgaccg catgggcggc atctcggtgc cgcgtttctt ccgcgcctgg cgcgagggga  30067
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gatctcccat caaggggggc ctttcaaggc cctctcgcct cgagggcacc gcacgccgaa  30127

gaccgatcac aaaagtatcc gaacggctcc gaccgaggtc atatctgaga ctgatcgaat  30187

atccaacggg gagatgtg atg ggt  ttc atc cgg ttt gac  gtt ctg ggc ccg  30238
                    Met Gly  Phe Ile Arg Phe Asp  Val Leu Gly Pro
                        2670                2675

ctc  agg gtc cgg tgc gac  gac acc ctg ctt caa  ttg acc ggg cgc     30283
Leu  Arg Val Arg Cys Asp  Asp Thr Leu Leu Gln  Leu Thr Gly Arg
2680                2685                2690

aag  tac cgc acc gtg gtt  tcg tat ctc gct ctt  caa ccc gag tat     30328
Lys  Tyr Arg Thr Val Val  Ser Tyr Leu Ala Leu  Gln Pro Glu Tyr
2695                2700                2705

tcg  gtg gcg ata gag gac  ctc gtc cga gcc gct  tgg agc gac aag     30373
Ser  Val Ala Ile Glu Asp  Leu Val Arg Ala Ala  Trp Ser Asp Lys
2710                2715                2720

cgc  ccg tcc agc gcg cac  cac cag gtc cgt aag  atg gtc tcc gca     30418
Arg  Pro Ser Ser Ala His  His Gln Val Arg Lys  Met Val Ser Ala
2725                2730                2735

ctc  cgg acc agc ctg gac  cag gac tgg gac ctg  gtg gcg acg tcc     30463
Leu  Arg Thr Ser Leu Asp  Gln Asp Trp Asp Leu  Val Ala Thr Ser
2740                2745                2750

cag  gac ggc tac atg ctg  aag ttg ccg ccc aag  cag tcc gac gta     30508
Gln  Asp Gly Tyr Met Leu  Lys Leu Pro Pro Lys  Gln Ser Asp Val
2755                2760                2765

tcc  gaa ttc tgc cgc ctc  ttc gac cag gtg atg  tcg ggt ccc ctg     30553
Ser  Glu Phe Cys Arg Leu  Phe Asp Gln Val Met  Ser Gly Pro Leu
2770                2775                2780

acg  agc gac gac gac ctg  tcg gcc gcg tat tcg  gcg ctg gcg ctc     30598
Thr  Ser Asp Asp Asp Leu  Ser Ala Ala Tyr Ser  Ala Leu Ala Leu
2785                2790                2795

tgg  cgc gga cgc cct tgc  gaa ggg tcc gag ccc  cat ggg cag gag     30643
Trp  Arg Gly Arg Pro Cys  Glu Gly Ser Glu Pro  His Gly Gln Glu
2800                2805                2810

cgc  cgg atc tct caa ttg  gtg gaa cag cac cgc  gtc ctc ttg aac     30688
Arg  Arg Ile Ser Gln Leu  Val Glu Gln His Arg  Val Leu Leu Asn
2815                2820                2825

aag  acc gtt cag gga ttc  ggc gac agg ggc agg  tcc gat gaa ctc     30733
Lys  Thr Val Gln Gly Phe  Gly Asp Arg Gly Arg  Ser Asp Glu Leu
2830                2835                2840

gcc  tcg ata ctg cac gtc  gca tcg aag att cac  gga cag ccg gtc     30778
Ala  Ser Ile Leu His Val  Ala Ser Lys Ile His  Gly Gln Pro Val
2845                2850                2855

acc  gct cgc tcc ggt gtc  gcc gtt ccc gcg ccc  gct gtt tcg tac     30823
Thr  Ala Arg Ser Gly Val  Ala Val Pro Ala Pro  Ala Val Ser Tyr
2860                2865                2870

gcg  ggc acg aca caa gtc  ccc gaa cct tcg ggg  tcg acc acc cct     30868
Ala  Gly Thr Thr Gln Val  Pro Glu Pro Ser Gly  Ser Thr Thr Pro
2875                2880                2885

ccc  cca cgc ccc ggc tcc  ccc gtc ggt ccg cgc  tgc ctg cca cgg     30913
Pro  Pro Arg Pro Gly Ser  Pro Val Gly Pro Arg  Cys Leu Pro Arg
2890                2895                2900

gat  ctg cag gac ttc ggc  ggc cgc gaa cgc gaa  atc aat gag ctg     30958
Asp  Leu Gln Asp Phe Gly  Gly Arg Glu Arg Glu  Ile Asn Glu Leu
2905                2910                2915

cag  aaa ctg ttg acc gcg  gaa gga ccc cac cca  cag ttg gtg gcg     31003
Gln  Lys Leu Leu Thr Ala  Glu Gly Pro His Pro  Gln Leu Val Ala
2920                2925                2930

acc  gtt cac gga atg agc  ggc gtg ggt aaa acc  gcc gtc gcc gtc     31048
Thr  Val His Gly Met Ser  Gly Val Gly Lys Thr  Ala Val Ala Val
2935                2940                2945
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cgc  ctg gcg cac aga cta  gcc cat cac tat ccg  gac ggc cag ctc     31093
Arg  Leu Ala His Arg Leu  Ala His His Tyr Pro  Asp Gly Gln Leu
2950                 2955                 2960

ttt  gta tcc ctg gac ggc  ttt tct tcg gcc tcc  acc gcc acc gtg     31138
Phe  Val Ser Leu Asp Gly  Phe Ser Ser Ala Ser  Thr Ala Thr Val
2965                 2970                 2975

tcg  aat gcg ctg gga ata  ctc ctc aga cag aaa  ggc ctg gcg gac     31183
Ser  Asn Ala Leu Gly Ile  Leu Leu Arg Gln Lys  Gly Leu Ala Asp
2980                 2985                 2990

gag  gac att tca cct tcg  gaa gac ggc cgc ctc  gca caa tgg cgg     31228
Glu  Asp Ile Ser Pro Ser  Glu Asp Gly Arg Leu  Ala Gln Trp Arg
2995                 3000                 3005

acc  atc acc gcc gga cag  aag ctg ctc gtc gtg  ctc gac gac gtg     31273
Thr  Ile Thr Ala Gly Gln  Lys Leu Leu Val Val  Leu Asp Asp Val
3010                 3015                 3020

tgc  gac atc gag caa gta  gaa ccc ctc atc ccg  ccc tcg agc gaa     31318
Cys  Asp Ile Glu Gln Val  Glu Pro Leu Ile Pro  Pro Ser Ser Glu
3025                 3030                 3035

agc  gcc tgc atc atc acg  tcg cgc atc atc ctc  aat ggc atc gac     31363
Ser  Ala Cys Ile Ile Thr  Ser Arg Ile Ile Leu  Asn Gly Ile Asp
3040                 3045                 3050

ggc  gct cat cac atc tca  ctc gaa gta ccg gac  gag gac gaa tgt     31408
Gly  Ala His His Ile Ser  Leu Glu Val Pro Asp  Glu Asp Glu Cys
3055                 3060                 3065

ctg  gag ata ctc agt tgc  atg atc ggc aga cgc  ttc gac gac gag     31453
Leu  Glu Ile Leu Ser Cys  Met Ile Gly Arg Arg  Phe Asp Asp Glu
3070                 3075                 3080

gag  acg aag gac gcc cgc  gcg ctg atc cag cag  tgc gcc aat ctg     31498
Glu  Thr Lys Asp Ala Arg  Ala Leu Ile Gln Gln  Cys Ala Asn Leu
3085                 3090                 3095

ccg  ctg gca ctc cgt ctc  gcc gcc gcc cgg ata  tcg acg cgc gac     31543
Pro  Leu Ala Leu Arg Leu  Ala Ala Ala Arg Ile  Ser Thr Arg Asp
3100                 3105                 3110

ttc  ctg aac ctc cgg gaa  ctc agt gag caa ctg  tcg tcc tcg gct     31588
Phe  Leu Asn Leu Arg Glu  Leu Ser Glu Gln Leu  Ser Ser Ser Ala
3115                 3120                 3125

tcc  atc ttc agt gaa ctg  gaa gtt ccc ggc cgt  agt ctg gtc ggc     31633
Ser  Ile Phe Ser Glu Leu  Glu Val Pro Gly Arg  Ser Leu Val Gly
3130                 3135                 3140

cgg  ctc atg acg tcc ttc  acg tgc ctg gag gac  ttc gat cac gac     31678
Arg  Leu Met Thr Ser Phe  Thr Cys Leu Glu Asp  Phe Asp His Asp
3145                 3150                 3155

cgg  tac ctc cga tta tcg  ctg ctc ccc tgc ccc  gag atc gat gaa     31723
Arg  Tyr Leu Arg Leu Ser  Leu Leu Pro Cys Pro  Glu Ile Asp Glu
3160                 3165                 3170

acg  tcg gtc gcg gcc gtg  ctg ggc gta tcc acc  gac tgg gca cgg     31768
Thr  Ser Val Ala Ala Val  Leu Gly Val Ser Thr  Asp Trp Ala Arg
3175                 3180                 3185

cgt  gcc tgc agg cgc ttc  gca gac cgc gcg ttg  ctg caa cgc aca     31813
Arg  Ala Cys Arg Arg Phe  Ala Asp Arg Ala Leu  Leu Gln Arg Thr
3190                 3195                 3200

cga  tgc ggt acg tac cgg  atg cac ccg ctg ctg  ctg cag gcg gca     31858
Arg  Cys Gly Thr Tyr Arg  Met His Pro Leu Leu  Leu Gln Ala Ala
3205                 3210                 3215

cag  ctg gaa gcg cag aag  acc atc ccg ttc gag  gag caa cgc cgg     31903
Gln  Leu Glu Ala Gln Lys  Thr Ile Pro Phe Glu  Glu Gln Arg Arg
3220                 3225                 3230

ctc  gtc cgc gcc gct ttc  ctc cat tac aag gcg  tcg aac ggc ctc     31948
Leu  Val Arg Ala Ala Phe  Leu His Tyr Lys Ala  Ser Asn Gly Leu
3235                 3240                 3245
```

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```
gtg  gga gcc agc cgc atc  agc cct tcc cgg gtt  cct gac gga cac     31993
Val  Gly Ala Ser Arg Ile  Ser Pro Ser Arg Val  Pro Asp Gly His
3250                 3255                 3260

gtg  gta ctg agg acc ctc  acg cag tcc gcg aag  ctg gcc gcg cgg     32038
Val  Val Leu Arg Thr Leu  Thr Gln Ser Ala Lys  Leu Ala Ala Arg
3265                 3270                 3275

ctc  ggc ctc cag gag gag  ttg gcc gat ctg tac  acc gcc tgg aag     32083
Leu  Gly Leu Gln Glu Glu  Leu Ala Asp Leu Tyr  Thr Ala Trp Lys
3280                 3285                 3290

gaa  ctg ctc ccc ctc gtg  ctg gac cgc cgg cag  cag gag gcg gtc     32128
Glu  Leu Leu Pro Leu Val  Leu Asp Arg Arg Gln  Gln Glu Ala Val
3295                 3300                 3305

ggg  cga cgc gta ctc gcc  gtt tca cag cac ctg  gac cgg ccc gcg     32173
Gly  Arg Arg Val Leu Ala  Val Ser Gln His Leu  Asp Arg Pro Ala
3310                 3315                 3320

tgc  gag gga gca ccc cac  cgg agg cgt ccg cgg  cag gca cgg gac     32218
Cys  Glu Gly Ala Pro His  Arg Arg Arg Pro Arg  Gln Ala Arg Asp
3325                 3330                 3335

atg  ctg ccc gag ggg cag  cgg tgaacgaggg ccggccggag gaaggcagtc     32269
Met  Leu Pro Glu Gly Gln  Arg
3340                 3345

ggacgatgac gaccgtttgt ccgtacatcg ggagaccggg gtgccacgtg aaacatgtga  32329

cccggtcacc ggatgtccga tcgcagccgc acccgggggc gaactgacc atg gac cgc  32387
                                                    Met Asp Arg

gtt  ctt ccg tat gca gcc  ggc agt gag gca ctc  ctg tcg tcg aga     32432
Val  Leu Pro Tyr Ala Ala  Gly Ser Glu Ala Leu  Leu Ser Ser Arg
3350                 3355                 3360

gag  cac ggc ccc acg gtg  agc gag aga acc gtc  tcg gcg cag gag     32477
Glu  His Gly Pro Thr Val  Ser Glu Arg Thr Val  Ser Ala Gln Glu
3365                 3370                 3375

atc  gtc gtg ggc ggc ggg  ggt ctg ctg ggg aga  cac atc ctc ggc     32522
Ile  Val Val Gly Gly Gly  Gly Leu Leu Gly Arg  His Ile Leu Gly
3380                 3385                 3390

gtg  ctg ggc aat cgg ctc  agc cgg cgg gta cgc  atc ccg tgg gac     32567
Val  Leu Gly Asn Arg Leu  Ser Arg Arg Val Arg  Ile Pro Trp Asp
3395                 3400                 3405

gac  cac ggc cgc gcc tgt  gag cag ctc tac gcg  ctg ggc agg gac     32612
Asp  His Gly Arg Ala Cys  Glu Gln Leu Tyr Ala  Leu Gly Arg Asp
3410                 3415                 3420

ctg  gct cag cag ccg gcg  cgc tgg aac ctg tac  tgg tgc gcg gga     32657
Leu  Ala Gln Gln Pro Ala  Arg Trp Asn Leu Tyr  Trp Cys Ala Gly
3425                 3430                 3435

ctg  gcc gtc ttc cac acc  ccc gcc gag cag gtg  gag cga gaa cgc     32702
Leu  Ala Val Phe His Thr  Pro Ala Glu Gln Val  Glu Arg Glu Arg
3440                 3445                 3450

ctc  cag gtc agc ctc ctc  ctg gcg ggc atc aac  gac ggg ctc gaa     32747
Leu  Gln Val Ser Leu Leu  Leu Ala Gly Ile Asn  Asp Gly Leu Glu
3455                 3460                 3465

cgc  tcg ggg ggc ccc acc  ggc ggc gcg ttg ttc  ctg gcc tcc tca     32792
Arg  Ser Gly Gly Pro Thr  Gly Gly Ala Leu Phe  Leu Ala Ser Ser
3470                 3475                 3480

gcc  ggc ggc gcg ttc gcg  ggc tcg gaa cac ccg  ccg ttc acc gag     32837
Ala  Gly Gly Ala Phe Ala  Gly Ser Glu His Pro  Pro Phe Thr Glu
3485                 3490                 3495

ttc  tcc ccg ccc gtg ccc  acg aac ccg tac ggc  gcg tcc aaa ctc     32882
Phe  Ser Pro Pro Val Pro  Thr Asn Pro Tyr Gly  Ala Ser Lys Leu
3500                 3505                 3510
```

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```
gcc  gtc gag gag gag gcc  gag gtt ctg gcg cgc  cgt tgg cga ctg     32927
Ala  Val Glu Glu Glu Ala  Glu Val Leu Ala Arg  Arg Trp Arg Leu
3515                 3520                 3525

ccc  acg gtg tcg ggt cgg  atc acg aac ctg tac  ggc ccc ggc cag     32972
Pro  Thr Val Ser Gly Arg  Ile Thr Asn Leu Tyr  Gly Pro Gly Gln
3530                 3535                 3540

aac  ctc gac aag aac cag  ggc ctg gtc agt gcc  ctc gtc aaa gcg     33017
Asn  Leu Asp Lys Asn Gln  Gly Leu Val Ser Ala  Leu Val Lys Ala
3545                 3550                 3555

cag  ctg acc ggt gaa ccc  ctg cgg ctg cgg gcc  gcc ctg gag acc     33062
Gln  Leu Thr Gly Glu Pro  Leu Arg Leu Arg Ala  Ala Leu Glu Thr
3560                 3565                 3570

acg  cgc gac tac atc tac  gca cgg gac tgc gcc  cgg atg gtc gtc     33107
Thr  Arg Asp Tyr Ile Tyr  Ala Arg Asp Cys Ala  Arg Met Val Val
3575                 3580                 3585

tcg  gcg atg gag acc gta  cgg tcc cgc acc cgc  ggc acg gac ccc     33152
Ser  Ala Met Glu Thr Val  Arg Ser Arg Thr Arg  Gly Thr Asp Pro
3590                 3595                 3600

cat  gtc cgc aag ata ttc  agc agc gga cgc cgt  ctg cgg atc gac     33197
His  Val Arg Lys Ile Phe  Ser Ser Gly Arg Arg  Leu Arg Ile Asp
3605                 3610                 3615

gaa  ctg ctc cgg atc gcc  gag cgc ctc ttc gac  cgg ccg gta ccc     33242
Glu  Leu Leu Arg Ile Ala  Glu Arg Leu Phe Asp  Arg Pro Val Pro
3620                 3625                 3630

gtc  gtc cac gag ccg gtg  gcg gga ggg gcg aac  gtc aac ctc tcg     33287
Val  Val His Glu Pro Val  Ala Gly Gly Ala Asn  Val Asn Leu Ser
3635                 3640                 3645

gtc  gag tcc cgg gta tgg  gcg gac ctc gaa tcg  tcc ccc ttc ctc     33332
Val  Glu Ser Arg Val Trp  Ala Asp Leu Glu Ser  Ser Pro Phe Leu
3650                 3655                 3660

agc  atc gag gaa ggg atg  cgc gcc gtc cgc tcc  gac ctc agg tac     33377
Ser  Ile Glu Glu Gly Met  Arg Ala Val Arg Ser  Asp Leu Arg Tyr
3665                 3670                 3675

cga  ctc ggg cac ggg tgagcgacac gaacgacaaa agacccaggc cgcacatcag   33432
Arg  Leu Gly His Gly
3680

cgcggcctgg gtccgatgag ccgccttcgg gattcgaacc cgagacctac gcattacgag  33492

tgcgttgctc tggccatctg agctaaggcg gcgtgctggg tgcacccatg gtgcgatcag  33552

cgacgtcggt aagtctacac agtttcgagg ggtggttcgt accggccccg gagcgggccg  33612

gggctgcctc cggtgggcgg ggcggctacg agcagcgtgt gccgtcggcg ggcggggtgc  33672

cgtcgaggag gtaggtgttg atggcggagt cgacgcaggc gctgccgcgg ccgtacgggg  33732

tgtggccgtc gccgtcgtag gtgaggaggc gggccgtggc gagctggccg gccagaccct  33792

cggcccagcg gtacggggtg gccgggtccc ggg                               33825


<210> SEQ ID NO 2
<211> LENGTH: 341
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 2

Leu Ile Ala Asn Arg Thr Leu Glu Leu Leu Ser Leu Leu Gln Thr Gln
1               5                  10                  15

Arg Glu Trp Thr Gly Asp Gly Leu Ala Glu Arg Leu Gly Val Ser Pro
            20                  25                  30

Arg Thr Val Arg Arg Asp Ile Asn Arg Leu Arg Glu Leu Gly Tyr Pro
        35                  40                  45
```

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```
Val Thr Ala Thr Lys Gly Pro Ser Gly Ser Tyr Arg Leu Ser Arg Gly
    50                  55                  60

Ala Arg Leu Pro Pro Leu Ile Val Asp Asp Glu Gln Ala Leu Ala Ile
65                  70                  75                  80

Ala Leu Ser Leu Gln Thr Ala Pro Ala Ser Val Thr Gly Met Gly Asp
                85                  90                  95

Ala Thr Lys Arg Ala Leu Asn Ser Ile Gln Glu Leu Leu Pro Pro His
            100                 105                 110

Leu Ala His Arg Leu Ala Thr Phe Ser Val Glu Gln Ile Glu Asn Ala
        115                 120                 125

Trp Glu Leu Ala Pro Pro Gln Val Asp Pro Ser Leu Leu Ala Gln Leu
    130                 135                 140

Ser Ser Ala Ala Gln Gln Arg Asp Leu Val Arg Phe Ser Tyr Arg Ser
145                 150                 155                 160

Ile His His Asp Ser Met Gln Asp Gly Glu Val Leu Ala Glu Pro His
                165                 170                 175

Arg Leu Val Val Trp Ser Gly Arg Trp Tyr Leu Val Ala Tyr Asp Gln
            180                 185                 190

Gln Arg Ser Ser Trp His Ala Tyr Arg Val Asp Arg Ile Lys Asp Leu
        195                 200                 205

Ala Pro Thr Ala Trp Arg Phe Gly Glu Arg Glu Gly Pro Asp Glu Asp
    210                 215                 220

Ile Thr Arg Phe Val Gln Asn Gln Pro Asp Arg Gly Asp Thr Pro Asp
225                 230                 235                 240

Thr Trp Pro Cys Trp Gly Thr Val Leu Met Glu Cys Pro Ala Ser Leu
                245                 250                 255

Val Ala Lys Trp Ala Pro Gly Val Ala Ser Phe Glu Ala Val Asp Asp
            260                 265                 270

Arg Val Thr Arg Ile Gln Met Gly Ala Trp Ser Trp Ser Ala Leu Ile
        275                 280                 285

Gly Phe Leu Ile Thr Phe Ser Cys Arg Phe Thr Val Glu Gly Pro Pro
    290                 295                 300

Glu Leu Val Ala Ala Ala Arg Arg Val Met Gly Leu Ile Asp Val Gly
305                 310                 315                 320

Ile Pro Ser His Asp Pro Leu Ala Glu Pro Ser Ser Arg Thr Pro Gly
                325                 330                 335

Pro Ser Ala Gly Arg
            340


<210> SEQ ID NO 3
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 3

Met Ile Arg Ile Ala Val Ile Leu Gly Ser Thr Arg Pro Gly Arg Arg
1               5                   10                  15

Gly Ala Val Val Ala Gln Trp Val Ala Glu Val Ala Ala Arg His Pro
            20                  25                  30

Ala Ala Val Met Gly Glu Ala Glu Phe Glu Leu Val Asp Leu Ala Glu
        35                  40                  45

Tyr Gly Leu Pro Leu Leu Asp Glu Pro Val Pro Ala Met Phe Gly Gln
    50                  55                  60

Tyr Gln Lys Glu Glu Thr Arg Arg Trp Ala Ala Ala Ile Gly Ser Phe
65                  70                  75                  80
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```
Asp Gly Phe Val Phe Val Thr Pro Glu Tyr Asn His Ser Val Pro Ala
                85                  90                  95

Ala Leu Lys Asn Ala Ile Asp His Leu Phe Ala Glu Trp Thr Asp Lys
            100                 105                 110

Ala Ala Gly Phe Val Ser Tyr Gly Val His Gly Gly Thr Arg Ala Val
        115                 120                 125

Glu His Leu Arg Leu Ala Leu Ala Glu Val Lys Val Ala Gly Val Arg
    130                 135                 140

Ser Gln Val Val Leu Ser Val Phe Asn Asp Phe Asp Tyr Thr Gly Cys
145                 150                 155                 160

Asp Met Thr Asp Pro Thr Ala Met Gly Arg Phe Thr Pro Gly Pro Gln
                165                 170                 175

Gln Glu Gln Thr Val Asn Thr Met Leu Asp Glu Val Val Ala Trp Ser
            180                 185                 190

Thr Ala Leu Lys Pro Leu Arg Thr Ala Ala Thr Ala Glu Ala Asp Gly
        195                 200                 205

Arg Ala Val Ser Val
    210


<210> SEQ ID NO 4
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 4

Met Thr Leu His Ala Ala Glu Ala Ile Pro Ser His Val Pro Val Leu
1               5                   10                  15

Val Val Gly Ala Gly Pro Thr Gly Leu Met Leu Gly Ala Glu Leu Ala
            20                  25                  30

Leu His Gly Ser Arg Pro Leu Val Ile Asp Ala Leu Pro Ser Pro Ser
        35                  40                  45

Gly Gln Ser Arg Ala Leu Gly Phe Thr Val Arg Thr Leu Glu Ile Phe
    50                  55                  60

Lys Gln Arg Gly Ile Leu Gly Arg Phe Gln Gly Leu Ala Pro Val Pro
65                  70                  75                  80

Gly Val His Phe Ala Gly Leu Ser Ile Lys Gly Asp His Leu Ser Ser
                85                  90                  95

Ser Met Arg Pro Ala Asn Gln Tyr Pro Gln Ser Lys Thr Glu Gln Val
            100                 105                 110

Leu Ala Ala Trp Ala Glu Glu Leu Gly Val Pro Val Arg Arg Pro Trp
        115                 120                 125

Thr Leu Thr Ser Met Glu Pro Thr Gly Thr Gly Tyr Arg Cys Val Leu
    130                 135                 140

Ser Gly Pro Ala Gly Gln Gln Thr Val Asp Ala Asp Tyr Val Val Gly
145                 150                 155                 160

Cys Asp Gly Ala Gly Ser Phe Val Arg Glu Ala Ile Gly Met Pro Thr
                165                 170                 175

Lys Arg Thr Pro Pro Ser Val Gln Met Leu Leu Gly Asp Leu Arg Gly
            180                 185                 190

Cys Gly Leu Pro Asp Glu Pro Phe Gly Val Lys His Glu Lys Gly Met
        195                 200                 205

Val Met Ser Ala Pro Leu Gly Asp Gly Thr Glu Arg Val Ile Val Cys
    210                 215                 220

Asp Phe Thr Gln Pro Met Arg Pro Gln Gly Thr Pro Val Thr His Asp
225                 230                 235                 240
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```
Glu Ile Lys Ala Ala Tyr Glu Gln Val Val Gly Ser Pro Leu Ala Asp
                245                 250                 255

Gly Glu Cys Leu Trp Ala Ser Ser Phe Ser Asp Ala Ser Ser Leu Val
            260                 265                 270

Glu Ser Tyr Arg Ser Gly Arg Ala Leu Leu Val Gly Asp Thr Ala His
        275                 280                 285

Thr His Leu Pro Ala Gly Gly Gln Gly Met Asn Val Ser Ile Gln Asp
    290                 295                 300

Ala Val Asn Val Gly Trp Lys Leu Ala Leu Val Ser Gln Gly Arg Ala
305                 310                 315                 320

Pro Asp Thr Leu Leu Asp Thr Tyr His Ala Glu Arg Tyr Pro Val Gly
                325                 330                 335

Arg Glu Leu Leu Leu Asn Thr Ala Ala Gln Gly Gln Val Phe Leu Arg
            340                 345                 350

Gly Pro Glu Val Asp Pro Leu Arg Glu Val Leu Arg Arg Leu Leu Asn
        355                 360                 365

Ile Arg Glu Val Ser Val Leu Leu Ala Asp Gly Val Ser Gly Leu Asp
    370                 375                 380

Ile Arg Tyr Asp Met Gly Leu Pro Glu Ala Pro Pro Pro Thr Gly Glu
385                 390                 395                 400

Arg Leu Pro Pro Asp Val Phe His Val Val Gly Thr Gly Gly Asp Ala
                405                 410                 415

Val Glu Glu Leu Arg His Gly Ala Ala Leu Leu Ile Val Pro Ser Pro
            420                 425                 430

Asp Ser Pro Ala Ser Ser Leu Val Ala Pro Trp Arg Asp Gln Val Arg
        435                 440                 445

Val Val His Ala Arg Pro Thr Asp Pro Asp Trp Gly Gly Glu Pro Ala
    450                 455                 460

Ala Ser Ser His Trp Phe Val Arg Pro Asp Gly His Ile Ala Trp Ala
465                 470                 475                 480

Gly Thr Glu Phe Ser Glu Leu Ser Ala Ser Leu Ser Arg Trp Leu Gly
                485                 490                 495

Gln Pro Ala Ala
            500


<210> SEQ ID NO 5
<211> LENGTH: 419
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 5

Met Ser Arg Arg Val Phe Ile Thr Gly Val Gly Val Val Ala Pro Gly
1               5                   10                  15

Ala Val Gly Arg Asp Pro Phe Trp Glu Leu Leu Thr Gln Gly Arg Thr
            20                  25                  30

Ala Thr Arg Arg Leu Ser Leu Cys Asp Pro Glu Pro Phe Arg Ser Gln
        35                  40                  45

Val Ala Ala Glu Ala Asp Phe Asp Ala Glu Ala Ala Gly Leu Ser Glu
    50                  55                  60

Arg Gln Ser Ala Glu Leu Asp Arg Ala Ala Gln Phe Ala Leu Val Ala
65                  70                  75                  80

Ala Arg Glu Ala Val Glu Asp Ala Ala Trp Ser Glu Thr Cys Pro Pro
                85                  90                  95

Glu Arg Ala Gly Val Ile Val Gly Ser Ala Val Gly Ala Thr Thr Lys
            100                 105                 110
```

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```
Leu Glu Glu Val Tyr Arg Gln Leu Ser Arg Asp Gly Ser Leu Trp Asp
        115                 120                 125

Val Ala Pro Asp Ser Pro Ala Glu Leu Tyr Ser Tyr Phe Val Pro Ser
    130                 135                 140

Ser Phe Ala Ser Gly Ile Ala His Asp Leu Gly Val Thr Gly Gln Ser
145                 150                 155                 160

Gly Val Val Ser Thr Gly Cys Thr Ser Gly Ile Asp Ser Val Gly Asn
                165                 170                 175

Ala Trp Glu Leu Ile Gln Ser Gly Ile Leu Asp Ser Ala Val Cys Gly
            180                 185                 190

Ala Thr Asp Ala Pro Ile Ser Pro Ile Thr Val Ala Cys Phe Asp Thr
        195                 200                 205

Ile Lys Ala Thr Ser Thr Tyr Asn Asp Thr Pro Glu Ser Ala Ser Arg
    210                 215                 220

Pro Phe Asp Ala Thr Arg Gly Gly Phe Val Leu Gly Glu Gly Ser Ala
225                 230                 235                 240

Met Phe Val Leu Glu Ser Glu Glu Ser Val His Arg Arg Gly Ala Arg
                245                 250                 255

Val Tyr Gly Glu Ile Arg Gly Tyr Ala Ser Arg Cys Asn Ala Tyr His
            260                 265                 270

Met Thr Gly Leu Lys Ala Asp Gly Arg Glu Leu Ala Glu Ala Val Val
        275                 280                 285

Ser Ala Leu Gly Gln Ala Gly Val Asp Pro Gly Arg Leu Asp Tyr Val
    290                 295                 300

Asn Ala His Gly Ser Gly Thr Lys Gln Asn Asp Arg His Glu Thr Ala
305                 310                 315                 320

Ala Leu Lys Ser Ser Leu Gly Pro Ala Ala His Asp Val Pro Ile Ser
                325                 330                 335

Ser Ile Lys Ser Met Ile Gly His Ser Leu Gly Ala Ile Gly Ser Leu
            340                 345                 350

Glu Ile Ala Ala Cys Ala Leu Ala Leu Arg Asp Asp Val Ile Pro Pro
        355                 360                 365

Thr Ala Asn Leu Thr Arg Pro Asp Pro Glu Leu Asp Leu Asp Tyr Val
    370                 375                 380

Pro Val His Ala Arg Lys Gln Pro Thr Asn Ser Val Leu Thr Thr Gly
385                 390                 395                 400

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

His His Ser


<210> SEQ ID NO 6
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 6

Met Ser Ala Arg Val Thr Met Asp Asp Leu Arg Arg Ala Leu Glu Glu
1               5                   10                  15

Gly Ser Gly Val Asp Glu Gly Val Asp Leu Asp Thr Asp Leu Glu Thr
            20                  25                  30

Met Ala Phe Ser Glu Leu Gly Tyr Asp Ser Leu Ala Val Leu Glu Thr
        35                  40                  45

Gly Leu Arg Leu Gly Arg Glu Asn Asp Ile Glu Leu Asp Asp Ser Val
    50                  55                  60
```

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```
Phe Ala Asp Leu Asp Thr Pro Gln Gln Met Leu Asp Ala Val Asn Asp
65                  70                  75                  80

Ala Leu Ala Arg Gln Ala Ala Ala Ser
                85
```

<210> SEQ ID NO 7
<211> LENGTH: 316
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 7

```
Met Ala Asp Pro Ala Arg Thr Asp Leu His Ser Ala Thr Ile Thr Gly
1               5                   10                  15

Ser Ala Asp Ala Val Tyr Arg Arg Leu Glu Asp Val Gly Gln Trp Ser
            20                  25                  30

Gln Met Phe Glu Pro Thr Ile His Gly Ala Glu Leu Ala Arg Asp Gly
        35                  40                  45

Asn Arg Gln Thr Ile Gln Leu Trp Ala Thr Ala Asn Gly Glu Pro Lys
    50                  55                  60

Ala Trp Val Ser Glu Arg Glu Leu Asp Pro Val Ala Arg Thr Ile Arg
65                  70                  75                  80

Phe Ala Gln Thr Val Thr Ser Ser Pro Val Ala Glu Met Ser Gly Ala
                85                  90                  95

Trp Gln Val Leu Pro Leu Ser Glu Asp Thr Cys Arg Val Glu Leu Thr
            100                 105                 110

His Thr Tyr Arg Ala Glu Asn Asp Ser Ala Glu Ser Leu Thr Trp Ile
        115                 120                 125

Ala Arg Ala Val Glu Thr Asn Ser Thr Lys Glu Leu Ser Ala Leu Lys
    130                 135                 140

Phe Ala Cys Glu Arg Asp Ala Asp Ser Glu Ala Ser Pro Phe Thr Phe
145                 150                 155                 160

Thr Asp Ala Val Asp Thr Thr Val Asp Pro Val Leu Leu Phe Ser Phe
                165                 170                 175

Leu Asp Arg Gly Glu Leu Trp Ala Gly Arg Leu Glu His Val Ala Glu
            180                 185                 190

Ala Glu Met Arg Glu Phe Ser Asp Gly Leu Gln Phe Leu Arg Met Arg
        195                 200                 205

Thr Arg Thr Pro Asp Gly Asp Thr His Val Thr Glu Ser Tyr Arg Val
    210                 215                 220

Ser Gln Ser Pro Ala Arg Leu Leu Tyr Lys Gln Val Thr Leu Pro Ala
225                 230                 235                 240

Leu Leu Ser Leu His Thr Gly Glu Trp Thr Ile Thr Pro Ala Gly Glu
                245                 250                 255

Ser Trp Arg Val Thr Ser Lys His Thr Val Ala Ile Asp Pro Asp Ala
            260                 265                 270

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

Leu Ala Arg Arg Asn Leu Gly Asn Asn Ser Leu Arg Thr Leu Glu Ala
    290                 295                 300

Ala Val Arg Trp Ala Gly Thr Ala Val Ser Gln Arg
305                 310                 315
```

<210> SEQ ID NO 8
<211> LENGTH: 451
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

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<400> SEQUENCE: 8

```
Met Thr Glu Pro Glu Thr Ser Asp Val Leu Val Val Gly Ala Gly Pro
1               5                  10                  15

Ser Gly Leu Leu Leu Ala Gly Ile Leu Ala Gly Ala Gly Ala Arg Val
            20                  25                  30

Thr Val Leu Glu Ala Arg Asp Ala Pro Ser Pro Gln Thr Arg Ala Ser
        35                  40                  45

Thr Leu His Ala Arg Ala Arg Glu Ile Leu Asp His His Gly Val Glu
    50                  55                  60

Phe Ser Pro Glu Leu Pro Trp Ser Ala His Gly His Tyr Gly Gly Leu
65                  70                  75                  80

Arg Val Asp Leu Ser Arg Val Asp Ser Gly Arg Ala Gly Val Trp Lys
                85                  90                  95

Cys Pro Gln Pro Glu Leu Val Arg Thr Leu Thr Gly Trp Ala Arg Gly
            100                 105                 110

His Gly Ala Arg Leu Leu His Gly Glu His Val Glu Ser Val Arg Glu
        115                 120                 125

Gln Gly Gly Arg Cys Leu Val Arg Thr Arg Ala Gly Thr Thr Phe Ser
    130                 135                 140

Gly Thr Leu Leu Val Ala Ala Asp Gly Arg Arg Ser Thr Val Arg Ser
145                 150                 155                 160

Leu Leu Gly Ile Gly Cys Gly Gly Ala Pro Ala Thr Arg Val Leu Val
                165                 170                 175

Gln Ala Asp Val His Gly Asp Gly Leu Ala Gly Arg Arg Phe Glu Arg
            180                 185                 190

His Gly Arg Tyr Thr Val Thr Ala Ala Pro Ile Ser Pro Gly Ile Thr
        195                 200                 205

Arg Val Met Leu His Asp Pro Arg Trp Pro Ala Gly Glu Glu Arg Thr
    210                 215                 220

Leu Glu Asp Leu Arg Arg Ala Trp Lys Glu Ser Thr Gly Glu Thr Leu
225                 230                 235                 240

Pro Ala Glu Pro Ser Trp Ser Arg Thr Phe Ser Asp Asp Thr Thr Val
                245                 250                 255

Ala His Pro Leu Val Lys Gly Arg Val Val Leu Cys Gly Asp Ala Ala
            260                 265                 270

His Pro Phe Val Pro Ile Gly Gly Gln Ala Leu Asn Thr Ser Leu Met
        275                 280                 285

Asp Ala Glu Ala Leu Gly Trp Arg Val Leu Gly Tyr Leu Asp Asp Gly
    290                 295                 300

Asp Arg Gln Gly Leu Leu Asp Tyr Gln Asp Glu Arg Phe Ser Trp Leu
305                 310                 315                 320

Thr Val Leu Ala Gly Arg Leu Arg Ala Gln Ala Arg Leu Leu Phe Asp
                325                 330                 335

Thr Asp Ala Ala Ala Thr Glu Arg Lys Ala Leu Val Ala Ala Arg Leu
            340                 345                 350

Ala Gly Asp Ala Asp Tyr Arg Arg Arg Ile Ala Asp Ala Leu Ala Gly
        355                 360                 365

Val Asp Val Cys Tyr Leu Thr Pro Gly Gly Ala Val Arg Arg Arg Leu
    370                 375                 380

Ser Pro Ala Arg Leu Arg Glu Thr Gly Val Asn Pro Gly Ala Arg Arg
385                 390                 395                 400

Val Gln Arg Ala Leu Val Pro Asp Asp Gly Thr Arg Thr Asp Ala Trp
                405                 410                 415
```

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```
Ile Arg Pro Asp His His Trp Tyr Pro Val Ala Arg Asp Gly Ala Arg
            420                 425                 430

Gln Asp Trp Asp Asp Ala Val Arg Leu His Asp Asp Leu Glu Pro Glu
        435                 440                 445

Val Thr Arg
    450


<210> SEQ ID NO 9
<211> LENGTH: 339
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 9

Val Pro His Gln Ala Thr Gly Ala Ala Pro Asp Gly Gly Gly Ser Ala
1               5                   10                  15

Pro Arg Ser Leu Val Leu Met Leu Pro Gly Gln Gly Ser Gln Phe Ala
            20                  25                  30

Ala Met Gly Val Pro Leu Tyr Glu Ser Asp Ala Arg Phe Arg Lys Ala
        35                  40                  45

Leu Asp Asp Phe Phe Asp Ala Phe Gly Thr Gly Ala Glu Arg Leu Arg
    50                  55                  60

Arg Glu Trp Leu His Gly Ser Ala Gln Gly Ile Glu Arg Gly Ser Phe
65                  70                  75                  80

Ala Gln Pro Met Leu Phe Gly Leu Asp Tyr Ala Ala Gly Ala Val Trp
                85                  90                  95

Leu Glu Glu Leu Lys Gly Val Asp Val Thr Leu Val Gly His Ser Val
            100                 105                 110

Gly Glu Leu Ala Ala Ala Thr Leu Ala Gly Ala Phe Asp Leu Glu Leu
        115                 120                 125

Ala Gly Ala Leu Leu Ala Glu Arg Ala Arg Leu Leu Asp Ala Ala Pro
    130                 135                 140

Arg Gly Gly Met Ile Ala Cys Arg Ala Thr Glu Glu Ser Leu Arg Glu
145                 150                 155                 160

His Leu Asp Ala Leu Gly Gly Arg Ala Val Ile Ala Ala Glu Asn Ala
                165                 170                 175

Asp Asn Gln Cys Val Val Ser Cys Ala Glu Glu Asp Leu Pro Asp Thr
            180                 185                 190

Met Arg Tyr Leu Gly Ser His Gly Val Thr Cys Leu Arg Val Ala Ser
        195                 200                 205

Thr Glu Pro Phe His Ser Pro Leu Leu Ala Pro Ala Ala Ala Arg Phe
    210                 215                 220

Glu Glu Phe Leu Ala Arg Arg Gly His Arg Leu Ser Thr Thr Glu Leu
225                 230                 235                 240

Pro Met Val Ser Ala Tyr Ser Ala Arg Arg Ile Ser Gly Arg Glu Ile
                245                 250                 255

Met Pro Ala Ser Phe Trp Thr Arg Gln Met Ala Glu Lys Val Arg Phe
            260                 265                 270

Trp Glu Ala Leu Arg His Asn Phe Asp Ser Gly Pro Arg Thr Phe Val
        275                 280                 285

Glu Ile Gly Pro Gly Thr Val Leu Ser Leu Ala Ala Arg Arg Leu Pro
    290                 295                 300

Ser Val Arg Ala Arg Arg Ser Thr Val Ile Ser Thr Met Pro Arg His
305                 310                 315                 320
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Arg Pro His Pro Glu His Trp Glu Ser Ala Ile His Glu Val Ala Glu
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Glu Phe Cys


<210> SEQ ID NO 10
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 10

Met Gly Phe Ile Arg Phe Asp Val Leu Gly Pro Leu Arg Val Arg Cys
1               5                   10                  15

Asp Asp Thr Leu Leu Gln Leu Thr Gly Arg Lys Tyr Arg Thr Val Val
            20                  25                  30

Ser Tyr Leu Ala Leu Gln Pro Glu Tyr Ser Val Ala Ile Glu Asp Leu
        35                  40                  45

Val Arg Ala Ala Trp Ser Asp Lys Arg Pro Ser Ser Ala His His Gln
    50                  55                  60

Val Arg Lys Met Val Ser Ala Leu Arg Thr Ser Leu Asp Gln Asp Trp
65                  70                  75                  80

Asp Leu Val Ala Thr Ser Gln Asp Gly Tyr Met Leu Lys Leu Pro Pro
                85                  90                  95

Lys Gln Ser Asp Val Ser Glu Phe Cys Arg Leu Phe Asp Gln Val Met
            100                 105                 110

Ser Gly Pro Leu Thr Ser Asp Asp Asp Leu Ser Ala Ala Tyr Ser Ala
        115                 120                 125

Leu Ala Leu Trp Arg Gly Arg Pro Cys Glu Gly Ser Glu Pro His Gly
    130                 135                 140

Gln Glu Arg Arg Ile Ser Gln Leu Val Glu Gln His Arg Val Leu Leu
145                 150                 155                 160

Asn Lys Thr Val Gln Gly Phe Gly Asp Arg Gly Arg Ser Asp Glu Leu
                165                 170                 175

Ala Ser Ile Leu His Val Ala Ser Lys Ile His Gly Gln Pro Val Thr
            180                 185                 190

Ala Arg Ser Gly Val Ala Val Pro Ala Pro Ala Val Ser Tyr Ala Gly
        195                 200                 205

Thr Thr Gln Val Pro Glu Pro Ser Gly Ser Thr Thr Pro Pro Pro Arg
    210                 215                 220

Pro Gly Ser Pro Val Gly Pro Arg Cys Leu Pro Arg Asp Leu Gln Asp
225                 230                 235                 240

Phe Gly Gly Arg Glu Arg Glu Ile Asn Glu Leu Gln Lys Leu Leu Thr
                245                 250                 255

Ala Glu Gly Pro His Pro Gln Leu Val Ala Thr Val His Gly Met Ser
            260                 265                 270

Gly Val Gly Lys Thr Ala Val Ala Val Arg Leu Ala His Arg Leu Ala
        275                 280                 285

His His Tyr Pro Asp Gly Gln Leu Phe Val Ser Leu Asp Gly Phe Ser
    290                 295                 300

Ser Ala Ser Thr Ala Thr Val Ser Asn Ala Leu Gly Ile Leu Leu Arg
305                 310                 315                 320

Gln Lys Gly Leu Ala Asp Glu Asp Ile Ser Pro Ser Glu Asp Gly Arg
                325                 330                 335

Leu Ala Gln Trp Arg Thr Ile Thr Ala Gly Gln Lys Leu Leu Val Val
            340                 345                 350
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Leu Asp Asp Val Cys Asp Ile Glu Gln Val Glu Pro Leu Ile Pro Pro
        355                 360                 365

Ser Ser Glu Ser Ala Cys Ile Ile Thr Ser Arg Ile Ile Leu Asn Gly
    370                 375                 380

Ile Asp Gly Ala His His Ile Ser Leu Glu Val Pro Asp Glu Asp Glu
385                 390                 395                 400

Cys Leu Glu Ile Leu Ser Cys Met Ile Gly Arg Arg Phe Asp Asp Glu
                405                 410                 415

Glu Thr Lys Asp Ala Arg Ala Leu Ile Gln Gln Cys Ala Asn Leu Pro
            420                 425                 430

Leu Ala Leu Arg Leu Ala Ala Ala Arg Ile Ser Thr Arg Asp Phe Leu
        435                 440                 445

Asn Leu Arg Glu Leu Ser Glu Gln Leu Ser Ser Ser Ala Ser Ile Phe
    450                 455                 460

Ser Glu Leu Glu Val Pro Gly Arg Ser Leu Val Gly Arg Leu Met Thr
465                 470                 475                 480

Ser Phe Thr Cys Leu Glu Asp Phe Asp His Asp Arg Tyr Leu Arg Leu
                485                 490                 495

Ser Leu Leu Pro Cys Pro Glu Ile Asp Glu Thr Ser Val Ala Ala Val
            500                 505                 510

Leu Gly Val Ser Thr Asp Trp Ala Arg Arg Ala Cys Arg Arg Phe Ala
        515                 520                 525

Asp Arg Ala Leu Leu Gln Arg Thr Arg Cys Gly Thr Tyr Arg Met His
    530                 535                 540

Pro Leu Leu Leu Gln Ala Ala Gln Leu Glu Ala Gln Lys Thr Ile Pro
545                 550                 555                 560

Phe Glu Glu Gln Arg Arg Leu Val Arg Ala Ala Phe Leu His Tyr Lys
                565                 570                 575

Ala Ser Asn Gly Leu Val Gly Ala Ser Arg Ile Ser Pro Ser Arg Val
            580                 585                 590

Pro Asp Gly His Val Val Leu Arg Thr Leu Thr Gln Ser Ala Lys Leu
        595                 600                 605

Ala Ala Arg Leu Gly Leu Gln Glu Glu Leu Ala Asp Leu Tyr Thr Ala
    610                 615                 620

Trp Lys Glu Leu Leu Pro Leu Val Leu Asp Arg Arg Gln Gln Glu Ala
625                 630                 635                 640

Val Gly Arg Arg Val Leu Ala Val Ser Gln His Leu Asp Arg Pro Ala
                645                 650                 655

Cys Glu Gly Ala Pro His Arg Arg Arg Pro Arg Gln Ala Arg Asp Met
            660                 665                 670

Leu Pro Glu Gly Gln Arg
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<210> SEQ ID NO 11
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 11

Met Asp Arg Val Leu Pro Tyr Ala Ala Gly Ser Glu Ala Leu Leu Ser
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Ser Arg Glu His Gly Pro Thr Val Ser Glu Arg Thr Val Ser Ala Gln
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Glu Ile Val Val Gly Gly Gly Gly Leu Leu Gly Arg His Ile Leu Gly
        35                  40                  45
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Val Leu Gly Asn Arg Leu Ser Arg Arg Val Arg Ile Pro Trp Asp Asp
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His Gly Arg Ala Cys Glu Gln Leu Tyr Ala Leu Gly Arg Asp Leu Ala
65                  70                  75                  80

Gln Gln Pro Ala Arg Trp Asn Leu Tyr Trp Cys Ala Gly Leu Ala Val
                85                  90                  95

Phe His Thr Pro Ala Glu Gln Val Glu Arg Glu Arg Leu Gln Val Ser
            100                 105                 110

Leu Leu Leu Ala Gly Ile Asn Asp Gly Leu Glu Arg Ser Gly Gly Pro
        115                 120                 125

Thr Gly Gly Ala Leu Phe Leu Ala Ser Ser Ala Gly Gly Ala Phe Ala
    130                 135                 140

Gly Ser Glu His Pro Pro Phe Thr Glu Phe Ser Pro Pro Val Pro Thr
145                 150                 155                 160

Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Glu Glu Glu Ala Glu Val
                165                 170                 175

Leu Ala Arg Arg Trp Arg Leu Pro Thr Val Ser Gly Arg Ile Thr Asn
            180                 185                 190

Leu Tyr Gly Pro Gly Gln Asn Leu Asp Lys Asn Gln Gly Leu Val Ser
        195                 200                 205

Ala Leu Val Lys Ala Gln Leu Thr Gly Glu Pro Leu Arg Leu Arg Ala
    210                 215                 220

Ala Leu Glu Thr Thr Arg Asp Tyr Ile Tyr Ala Arg Asp Cys Ala Arg
225                 230                 235                 240

Met Val Val Ser Ala Met Glu Thr Val Arg Ser Arg Thr Arg Gly Thr
                245                 250                 255

Asp Pro His Val Arg Lys Ile Phe Ser Ser Gly Arg Arg Leu Arg Ile
            260                 265                 270

Asp Glu Leu Leu Arg Ile Ala Glu Arg Leu Phe Asp Arg Pro Val Pro
        275                 280                 285

Val Val His Glu Pro Val Ala Gly Gly Ala Asn Val Asn Leu Ser Val
    290                 295                 300

Glu Ser Arg Val Trp Ala Asp Leu Glu Ser Ser Pro Phe Leu Ser Ile
305                 310                 315                 320

Glu Glu Gly Met Arg Ala Val Arg Ser Asp Leu Arg Tyr Arg Leu Gly
                325                 330                 335

His Gly
```

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<210> SEQ ID NO 12
<211> LENGTH: 33825
<212> TYPE: DNA
<213> ORGANISM: Streptomyces griseoflavus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (21413)..(21736)
<223> OTHER INFORMATION: ORF16 shown on this duplicate of SEQ ID NO:1
      due to sequence overlap with ORF17
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (22992)..(24164)
<223> OTHER INFORMATION: ORF18 shown on this duplicate of SEQ ID NO:1
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<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (24449)..(25225)
<223> OTHER INFORMATION: ORF20 shown on this duplicate of SEQ ID NO:1
      due to sequence overlap with ORF19
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<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (27552)..(28481)
<223> OTHER INFORMATION: ORF23 shown on this duplicate of SEQ ID NO:1
due to sequence overlap with ORF22

<400> SEQUENCE: 12

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                55                  60                  65

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Lys Ser Asp Pro Arg Phe Ile Arg Val Ser Asn Asp Leu Arg Pro Tyr
            70                  75                  80

atc gag gcc tac gcc ccg gac tgg caa tca ccg aag gac gcc atg gca    21706
Ile Glu Ala Tyr Ala Pro Asp Trp Gln Ser Pro Lys Asp Ala Met Ala
        85                  90                  95

gag cgc ttc tat cac tgg agt tcg aaa cga tgagccgcag ggtcttcatc      21756
Glu Arg Phe Tyr His Trp Ser Ser Lys Arg
    100                 105

accggggtcg gtgtcgtcgc gccgggagcc gtcggacgtg acccctttctg ggagctgctg  21816

acccaagggc gcacggccac ccgccggctc agcctctgcg acccggagcc cttccggtcc  21876

caggtggccg cggaggccga cttcgacgcc gaggcggcgg ggctgtcgga gcggcagtcc  21936

gcggaactgg accgggcggc gcagttcgcc ctggtcgccg cccgtgaagc ggtcgaggac  21996

gcggcatggt ccgagacatg tcctcccgaa cgcgccggag tgatcgtggg ttcggccgtc  22056

ggagccacga ccaagctcga ggaggtctac cggcagctca gccgtgacgg ctccctctgg  22116

gacgtggccc ccgactcccc cgccgagctg tactcgtact tcgtgcccag ctcgttcgcc  22176

tccggcatcg cacacgacct cggcgtcacg gggcagagcg gcgtcgtgtc gaccgggtgc  22236

acctccggga tcgactccgt cggcaacgcc tgggaactga tccagagcgg catcctggac  22296

tccgccgtct gcggtgccac cgacgccccc atctcgccca tcaccgtcgc ctgcttcgac  22356

acgatcaagg cgacatcgac gtacaacgac accccggaga gcgcctcacg gccgttcgac  22416

gccacacggg gcggcttcgt cctcggcgag ggcagcgcga tgttcgtcct cgaatcggag  22476

gaatccgtcc accgtcgcgg cgcacgcgtc tacggcgaga tccgcggcta cgcgagccgc  22536

tgcaacgcct accacatgac cggtctcaag gccgacggac gcgagctggc ggaggccgtc  22596

gtctccgctc tcggccaggc aggcgtggac ccgggccggc tcgactacgt caacgcccac  22656

ggcagcggca cgaagcagaa cgaccgccac gagaccgccg cgctgaagtc gtccctcgga  22716

cccgccgccc acgacgtgcc gatcagttcg atcaagtcga tgatcggcca ttcgctgggc  22776

gccatcgggt cgttggagat cgccgcctgc gccctggcgc tgcgggacga cgtgatcccg  22836

ccgacggcca atctcacccg gccggatccg gaactcgatc tggactacgt gccggtccac  22896

gcgcgcaagc agccgaccaa cagcgtgctc acgaccggaa gcggcttcgg tgggtttcag  22956

agcgccatgg ttctcacgga cccggagcat cactc atg acc gca cac atc acc     23009
                                    Met Thr Ala His Ile Thr
                                        110
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ggc atc gac atc gtc tcc ccg ctg ggc ctg tcc cgc gag gaa cac tgg    23057
Gly Ile Asp Ile Val Ser Pro Leu Gly Leu Ser Arg Glu Glu His Trp
115                 120                 125                 130

aag gcc ctc ctc gac gga tgc agc ggt ctg agg gcg acg cag tcg ttc    23105
Lys Ala Leu Leu Asp Gly Cys Ser Gly Leu Arg Ala Thr Gln Ser Phe
                135                 140                 145

gac tcc agc agg tac gac aac ccc atc agc ggg gag gtg ccc cac ttc    23153
Asp Ser Ser Arg Tyr Asp Asn Pro Ile Ser Gly Glu Val Pro His Phe
            150                 155                 160

gcc ccg gag ggc ctg ccc aag cgg ctg ctg ccg gcc acc gac cgg atg    23201
Ala Pro Glu Gly Leu Pro Lys Arg Leu Leu Pro Ala Thr Asp Arg Met
        165                 170                 175

acc cag atg tcg ctg gtc gcc gcg gcg ggg gcg ttc gac gac agc ggt    23249
Thr Gln Met Ser Leu Val Ala Ala Ala Gly Ala Phe Asp Asp Ser Gly
    180                 185                 190

gtc gac acg agc cgg gtc gac ccc ctc gga gtc ggt gtc atg acg gcg    23297
Val Asp Thr Ser Arg Val Asp Pro Leu Gly Val Gly Val Met Thr Ala
195                 200                 205                 210

tcc acc gcg ggg ggt tac gcg ttc ggg cag aag gag ctg cag aac ctg    23345
Ser Thr Ala Gly Gly Tyr Ala Phe Gly Gln Lys Glu Leu Gln Asn Leu
                215                 220                 225

tgg tcc aag ggg ccc agg tac gtc agc acc cat cag tcc tac gcc tgg    23393
Trp Ser Lys Gly Pro Arg Tyr Val Ser Thr His Gln Ser Tyr Ala Trp
            230                 235                 240

ttc tac gcg gtc aac acc ggt cag atc tcc atc cgg cac ggc tgc cag    23441
Phe Tyr Ala Val Asn Thr Gly Gln Ile Ser Ile Arg His Gly Cys Gln
        245                 250                 255

ggc cac agc gga gtg atc gtc gcg gac gac gcc ggc ggg ctc gac gcg    23489
Gly His Ser Gly Val Ile Val Ala Asp Asp Ala Gly Gly Leu Asp Ala
    260                 265                 270

atc tcc ttc gcc gcc cgc cgt ctg gcg cgc ggc aac cgc gtc atg ctc    23537
Ile Ser Phe Ala Ala Arg Arg Leu Ala Arg Gly Asn Arg Val Met Leu
275                 280                 285                 290

acc ggg tcg gtg gac agc acg atg tgt ccc tgg ggg cgg gtc gcg cac    23585
Thr Gly Ser Val Asp Ser Thr Met Cys Pro Trp Gly Arg Val Ala His
                295                 300                 305

acc tcg acc ggc atg ctc tcg gca tcc acc gac gcg cgg gcc gcg tac    23633
Thr Ser Thr Gly Met Leu Ser Ala Ser Thr Asp Ala Arg Ala Ala Tyr
            310                 315                 320

ctt ccg ttc gac gca cga gcc aac ggg tgg gtc aac ggc gaa ggc ggc    23681
Leu Pro Phe Asp Ala Arg Ala Asn Gly Trp Val Asn Gly Glu Gly Gly
        325                 330                 335

gcg cac ctc gtg ctg cag acc cac agt gac ggc cgc tac gcg gcg gtg    23729
Ala His Leu Val Leu Gln Thr His Ser Asp Gly Arg Tyr Ala Ala Val
    340                 345                 350

ctc ggt cac ggt gcg acc atg gac gat ccc cgc gcc gcc ccg ggc acg    23777
Leu Gly His Gly Ala Thr Met Asp Asp Pro Arg Ala Ala Pro Gly Thr
355                 360                 365                 370

ggc ctc gtc cgg gcg atc cac ctc gcg ctc ggc gcg gcg cgg ctg cgc    23825
Gly Leu Val Arg Ala Ile His Leu Ala Leu Gly Ala Ala Arg Leu Arg
                375                 380                 385

ccc ggc gac atc agt gtg gtg ttc gcc gac gcg gcc ggc acc cgg gag    23873
Pro Gly Asp Ile Ser Val Val Phe Ala Asp Ala Ala Gly Thr Arg Glu
            390                 395                 400

gcg gac acc gcc gag gcc gcc gcc ctc gcc gag gtc ttc ggg ccg gat    23921
Ala Asp Thr Ala Glu Ala Ala Ala Leu Ala Glu Val Phe Gly Pro Asp
        405                 410                 415

tcc gtc ccc gtc acc gcg ccc aag gcg gcg acc ggc cgg atg ggc tgc    23969
Ser Val Pro Val Thr Ala Pro Lys Ala Ala Thr Gly Arg Met Gly Cys
    420                 425                 430
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ggg acg gcc gca ctc gac gtc gcg acg gcg gtg ctc gcc ctc cgc gac    24017
Gly Thr Ala Ala Leu Asp Val Ala Thr Ala Val Leu Ala Leu Arg Asp
435                 440                 445                 450

cag acg att ccc ccc acc gtc aac gtc cag gcc gac gcg tcc ctg ggg    24065
Gln Thr Ile Pro Pro Thr Val Asn Val Gln Ala Asp Ala Ser Leu Gly
                455                 460                 465

gtc aac ctg tgc agc gtc gcc aca cac cac ccc ctc acc aac gtc ctg    24113
Val Asn Leu Cys Ser Val Ala Thr His His Pro Leu Thr Asn Val Leu
            470                 475                 480

gtc ctg gcc cgg ggc gtc ggt ggg ttc aac tcg gcc ctg atc gtc ggg    24161
Val Leu Ala Arg Gly Val Gly Gly Phe Asn Ser Ala Leu Ile Val Gly
        485                 490                 495

aaa tgagagaagg agcaaggaat gtccgcacgc gtcaccatgg acgatctcag         24214
Lys

gcgagccctc gaggagggct ccggtgtcga cgagggcgtc gatcttgaca ccgacctcga  24274

aaccatggcg ttctccgagc tggggtacga ctccctggcg gtgctggaga ccggcctgcg  24334

cctcggccgc gagaacgaca tcgagctcga cgactcggtg ttcgccgacc tcgacacgcc  24394

tcagcagatg ctggacgcgg tcaacgatgc cctcgcgcgt caggcggcgg catc gtg    24451
                                                            Val
                                                            500

acc tct ccc cgt cat gcc ctg gtc acc ggc ggt tcc agc ggc ata gga    24499
Thr Ser Pro Arg His Ala Leu Val Thr Gly Gly Ser Ser Gly Ile Gly
                505                 510                 515

aag tcc gtc gca cgg cgc ctg gcc tcg gcc ggc cac acc gtc acg atc    24547
Lys Ser Val Ala Arg Arg Leu Ala Ser Ala Gly His Thr Val Thr Ile
            520                 525                 530

tgc ggt cgt gac tcc gaa agg ctc cag cag gcc gcc aag gaa ctg tcg    24595
Cys Gly Arg Asp Ser Glu Arg Leu Gln Gln Ala Ala Lys Glu Leu Ser
        535                 540                 545

gag cag ggt gca ccc gtc acc tcg ctg atc gcc gac gtc agc aag ccc    24643
Glu Gln Gly Ala Pro Val Thr Ser Leu Ile Ala Asp Val Ser Lys Pro
    550                 555                 560

cgc cag gtg ggc gat ctg gtc cgc gag gcc gtg gag acg aac ggt ccc    24691
Arg Gln Val Gly Asp Leu Val Arg Glu Ala Val Glu Thr Asn Gly Pro
565                 570                 575                 580

ctc ggg atc ctc gtc aac aac gcg ggc agg aac gga ggc ggc cgg acc    24739
Leu Gly Ile Leu Val Asn Asn Ala Gly Arg Asn Gly Gly Gly Arg Thr
                585                 590                 595

gcg gag ctg agc gac gag ctg tgg cgg gag gta ctg agc acc aac ctc    24787
Ala Glu Leu Ser Asp Glu Leu Trp Arg Glu Val Leu Ser Thr Asn Leu
            600                 605                 610

gac agc gtt ttc tac gtc acg cgg gag gtg ctg gcc cgt ggc ggc atc    24835
Asp Ser Val Phe Tyr Val Thr Arg Glu Val Leu Ala Arg Gly Gly Ile
        615                 620                 625

ggc gag gtg gac cac gcc cgg atc atc aac atc gcc tcc acc gcg ggg    24883
Gly Glu Val Asp His Ala Arg Ile Ile Asn Ile Ala Ser Thr Ala Gly
    630                 635                 640

aag cag gga gtt ctg ctg gcc gcc ccg tac tcc gcc tcc aag cac ggt    24931
Lys Gln Gly Val Leu Leu Ala Ala Pro Tyr Ser Ala Ser Lys His Gly
645                 650                 655                 660

gtc gtc ggc ttc acc aag gcg gtg ggc aag gag ctg gcc cct cag ggg    24979
Val Val Gly Phe Thr Lys Ala Val Gly Lys Glu Leu Ala Pro Gln Gly
                665                 670                 675

atc acc gtg aac gcc gtc tgc ccg ggc tac gtg gag acc ccg atg gcc    25027
Ile Thr Val Asn Ala Val Cys Pro Gly Tyr Val Glu Thr Pro Met Ala
            680                 685                 690
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tca cgg gtc cgg cag gcc tac gca gac gcc tgg gag acc acg gag gcc    25075
Ser Arg Val Arg Gln Ala Tyr Ala Asp Ala Trp Glu Thr Thr Glu Ala
        695                 700                 705

gag gtg ctg tcc gcc ttc gag gcg aag atc ccg ctc ggc cgg tac agc    25123
Glu Val Leu Ser Ala Phe Glu Ala Lys Ile Pro Leu Gly Arg Tyr Ser
    710                 715                 720

acg ccc gac gag gtc gcc tcg ctg gtc gag tac ctc acg acc gaa gga    25171
Thr Pro Asp Glu Val Ala Ser Leu Val Glu Tyr Leu Thr Thr Glu Gly
725                 730                 735                 740

gcc gcc tcg atc acg gct cag gcg ttc aac gtg tgc ggc ggc ctc ggc    25219
Ala Ala Ser Ile Thr Ala Gln Ala Phe Asn Val Cys Gly Gly Leu Gly
                745                 750                 755

aac ttc taggagatga ttcacatggc cgatccggct cgcacagacc tgcactccgc     25275
Asn Phe

cacgatcacc ggcagcgccg acgcggtgta ccgccgtctg gaggacgtcg ggcagtggtc  25335

ccagatgttc gaaccgacca tccacggcgc ggaactggcc cgggacggga acaggcagac  25395

gatccagctg tgggccaccg ccaacggaga acccaaggcc tgggtctccg agcgtgagct  25455

cgaccccgtc gcgcgcacca tccgcttcgc gcagaccgtc acctcctcgc ccgtcgccga  25515

gatgtccggc gcgtggcagg tgctgcccct gtccgaggac acctgccggg tcgaactcac  25575

gcacacctac cgtgcggaga acgactcggc ggagtcgctc acatggatcg cccgagccgt  25635

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cagcgaggcc agtcccttca ccttcaccga tgcggtggac accacggtcg accccgtcct  25755

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caggactggg acgacgcggt gcgcctccac gacgacttgg aacccgaggt gacgcg gtg  27554
                                                                Val

aga gcg ttc ctg ttc ccc ggt cag ggg acc cag aag atc ggc atg ggc    27602
Arg Ala Phe Leu Phe Pro Gly Gln Gly Thr Gln Lys Ile Gly Met Gly
760                 765                 770                 775

acc tac ctg cga gaa cgg tac ccc cac ctg atc gcg ccg ttg tgg cgg    27650
Thr Tyr Leu Arg Glu Arg Tyr Pro His Leu Ile Ala Pro Leu Trp Arg
                780                 785                 790

gag gcg gac gac gtc ctg ggt ttc ccc ctc acc cgc ctc tgc gag gaa    27698
Glu Ala Asp Asp Val Leu Gly Phe Pro Leu Thr Arg Leu Cys Glu Glu
            795                 800                 805

ggc ccc ggc gag aag ctc cgc cac atg ccg gtc acc cag ccc gcc gtc    27746
Gly Pro Gly Glu Lys Leu Arg His Met Pro Val Thr Gln Pro Ala Val
        810                 815                 820

ttc ctg tgc agt tac gcc gcg ctc gtc gcc gcg cag gcg aac ggc gcg    27794
Phe Leu Cys Ser Tyr Ala Ala Leu Val Ala Ala Gln Ala Asn Gly Ala
    825                 830                 835

gag ccg gac gtc atc gcg ggc cac agt ctg ggc gag tac tcg gcg ctg    27842
Glu Pro Asp Val Ile Ala Gly His Ser Leu Gly Glu Tyr Ser Ala Leu
840                 845                 850                 855

gcg gcg gcc ggc gtc ctc acc tgg cag gag gtc ctt cag ctc gtc cac    27890
Ala Ala Ala Gly Val Leu Thr Trp Gln Glu Val Leu Gln Leu Val His
                860                 865                 870

cgc cgc ggt cag ctc atg gcg gag gtg cag cac aag gtg gac ggg aag    27938
Arg Arg Gly Gln Leu Met Ala Glu Val Gln His Lys Val Asp Gly Lys
            875                 880                 885

atg gcg gcc gtc atc ggt ctc gcc atc ggg cag gtc gag gag atc tgc    27986
Met Ala Ala Val Ile Gly Leu Ala Ile Gly Gln Val Glu Glu Ile Cys
        890                 895                 900

gag cag gtg cgg tcc gag acc ggt gag gtg gtc gag gtg gcc aac cac    28034
Glu Gln Val Arg Ser Glu Thr Gly Glu Val Val Glu Val Ala Asn His
    905                 910                 915

aac gag ccc ctc cag gtc gtc gtc tcc ggc cag tgc gct gcc ata gac    28082
Asn Glu Pro Leu Gln Val Val Val Ser Gly Gln Cys Ala Ala Ile Asp
920                 925                 930                 935

ctc ctg gtc cag cgc gtc gcg acg gcg acc gac gtc cgc acg tcc gtc    28130
Leu Leu Val Gln Arg Val Ala Thr Ala Thr Asp Val Arg Thr Ser Val
                940                 945                 950

ctg agg atc ggt ggc ccg gcc cac tcc agt ctc atg ggc agc gtc gcg    28178
Leu Arg Ile Gly Gly Pro Ala His Ser Ser Leu Met Gly Ser Val Ala
            955                 960                 965

ggg gac ttc gtg gag tac ctc cgg cgc ttc gac ttc tgc acg ccc aag    28226
Gly Asp Phe Val Glu Tyr Leu Arg Arg Phe Asp Phe Cys Thr Pro Lys
        970                 975                 980

acg atg ctg atc tcc ggg tcg acc gcc gag ccc tac gcg agt gcg gag    28274
Thr Met Leu Ile Ser Gly Ser Thr Ala Glu Pro Tyr Ala Ser Ala Glu
    985                 990                 995
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gag  atc agg cac cag ctc  ggc agg cag ctg gtg  cac cgg gtg cgg     28319
Glu  Ile Arg His Gln Leu  Gly Arg Gln Leu Val  His Arg Val Arg
1000                1005                1010

tgg  gtg gac gtg atg gcg  cag ctc gag agg ctg  ggg gtc gca cag     28364
Trp  Val Asp Val Met Ala  Gln Leu Glu Arg Leu  Gly Val Ala Gln
1015                1020                1025

acc  tgg gag ctg ggg ccg  ggc aag gtc ctc tcg  gga ttc gta cag     28409
Thr  Trp Glu Leu Gly Pro  Gly Lys Val Leu Ser  Gly Phe Val Gln
1030                1035                1040

cgg  tcg ctg cct cag gtg  cgg acg tac cgc gcg  aat gat ctg ccg     28454
Arg  Ser Leu Pro Gln Val  Arg Thr Tyr Arg Ala  Asn Asp Leu Pro
1045                1050                1055

tcc  ttc ctg gcc ggc gtg  acg ggc tgg tgagccggtg aagcacgcag        28501
Ser  Phe Leu Ala Gly Val  Thr Gly Trp
1060                1065

tgccgcatca ggcaaccggc gccgctcccg acggaggggg gtccgcgccc cggtccctcg  28561

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ggcgtgggta aaaccgccgt cgccgtccgc ctggcgcaca gactagccca tcactatccg 31081

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gaagacggcc gcctcgcaca atggcggacc atcaccgccg gacagaagct gctcgtcgtg 31261

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ttcgacgacg aggagacgaa ggacgcccgc gcgctgatcc agcagtgcgc caatctgccg 31501

ctggcactcc gtctcgccgc cgcccggata tcgacgcgcg acttcctgaa cctccgggaa 31561

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agtctggtcg gccggctcat gacgtccttc acgtgcctgg aggacttcga tcacgaccgg 31681

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cgtgctgggc aatcggctca gccggcgggt acgcatcccg tgggacgacc acggccgcgc 32581

ctgtgagcag ctctacgcgc tgggcaggga cctggctcag cagccggcgc gctggaacct 32641

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cgtcgtccac gagccggtgg cgggaggggc gaacgtcaac ctctcggtcg agtcccgggt  33301

atgggcggac ctcgaatcgt ccccttcct cagcatcgag gaagggatgc gcgccgtccg  33361

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gccgtacggg gtgtggccgt cgccgtcgta ggtgaggagg cgggccgtgg cgagctggcc  33781

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```

<210> SEQ ID NO 13
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 13

```
Leu Phe Ser Ser Leu Ile Val Ala Arg Met Asp Thr Gly His Ala Glu
1               5                   10                  15

Ala Val Ala Asp Val Phe Ala Gly Phe Asp Ala Thr Asp Met Pro Ala
            20                  25                  30

Arg Met Gly Thr Arg Arg Arg Glu Leu Phe Arg Tyr Arg Gly Leu Tyr
        35                  40                  45

Phe His Leu Gln Asp Phe Glu Thr Pro Asp Gly Thr Glu Ala Val Glu
    50                  55                  60

Ala Ala Lys Ser Asp Pro Arg Phe Ile Arg Val Ser Asn Asp Leu Arg
65                  70                  75                  80

Pro Tyr Ile Glu Ala Tyr Ala Pro Asp Trp Gln Ser Pro Lys Asp Ala
                85                  90                  95

Met Ala Glu Arg Phe Tyr His Trp Ser Ser Lys Arg
            100                 105
```

<210> SEQ ID NO 14
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

-continued

<400> SEQUENCE: 14

```
Met Thr Ala His Ile Thr Gly Ile Asp Ile Val Ser Pro Leu Gly Leu
1               5                   10                  15

Ser Arg Glu Glu His Trp Lys Ala Leu Leu Asp Gly Cys Ser Gly Leu
            20                  25                  30

Arg Ala Thr Gln Ser Phe Asp Ser Ser Arg Tyr Asp Asn Pro Ile Ser
        35                  40                  45

Gly Glu Val Pro His Phe Ala Pro Glu Gly Leu Pro Lys Arg Leu Leu
    50                  55                  60

Pro Ala Thr Asp Arg Met Thr Gln Met Ser Leu Val Ala Ala Ala Gly
65                  70                  75                  80

Ala Phe Asp Asp Ser Gly Val Asp Thr Ser Arg Val Asp Pro Leu Gly
                85                  90                  95

Val Gly Val Met Thr Ala Ser Thr Ala Gly Gly Tyr Ala Phe Gly Gln
            100                 105                 110

Lys Glu Leu Gln Asn Leu Trp Ser Lys Gly Pro Arg Tyr Val Ser Thr
        115                 120                 125

His Gln Ser Tyr Ala Trp Phe Tyr Ala Val Asn Thr Gly Gln Ile Ser
    130                 135                 140

Ile Arg His Gly Cys Gln Gly His Ser Gly Val Ile Val Ala Asp Asp
145                 150                 155                 160

Ala Gly Gly Leu Asp Ala Ile Ser Phe Ala Ala Arg Arg Leu Ala Arg
                165                 170                 175

Gly Asn Arg Val Met Leu Thr Gly Ser Val Asp Ser Thr Met Cys Pro
            180                 185                 190

Trp Gly Arg Val Ala His Thr Ser Thr Gly Met Leu Ser Ala Ser Thr
        195                 200                 205

Asp Ala Arg Ala Ala Tyr Leu Pro Phe Asp Ala Arg Ala Asn Gly Trp
    210                 215                 220

Val Asn Gly Glu Gly Gly Ala His Leu Val Leu Gln Thr His Ser Asp
225                 230                 235                 240

Gly Arg Tyr Ala Ala Val Leu Gly His Gly Ala Thr Met Asp Asp Pro
                245                 250                 255

Arg Ala Ala Pro Gly Thr Gly Leu Val Arg Ala Ile His Leu Ala Leu
            260                 265                 270

Gly Ala Ala Arg Leu Arg Pro Gly Asp Ile Ser Val Val Phe Ala Asp
        275                 280                 285

Ala Ala Gly Thr Arg Glu Ala Asp Thr Ala Glu Ala Ala Ala Leu Ala
    290                 295                 300

Glu Val Phe Gly Pro Asp Ser Val Pro Val Thr Ala Pro Lys Ala Ala
305                 310                 315                 320

Thr Gly Arg Met Gly Cys Gly Thr Ala Ala Leu Asp Val Ala Thr Ala
                325                 330                 335

Val Leu Ala Leu Arg Asp Gln Thr Ile Pro Pro Thr Val Asn Val Gln
            340                 345                 350

Ala Asp Ala Ser Leu Gly Val Asn Leu Cys Ser Val Ala Thr His His
        355                 360                 365

Pro Leu Thr Asn Val Leu Val Leu Ala Arg Gly Val Gly Gly Phe Asn
    370                 375                 380

Ser Ala Leu Ile Val Gly Lys
385                 390
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-continued

<210> SEQ ID NO 15
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 15

Val Thr Ser Pro Arg His Ala Leu Val Thr Gly Gly Ser Ser Gly Ile
1 5 10 15

Gly Lys Ser Val Ala Arg Arg Leu Ala Ser Ala Gly His Thr Val Thr
20 25 30

Ile Cys Gly Arg Asp Ser Glu Arg Leu Gln Gln Ala Ala Lys Glu Leu
35 40 45

Ser Glu Gln Gly Ala Pro Val Thr Ser Leu Ile Ala Asp Val Ser Lys
50 55 60

Pro Arg Gln Val Gly Asp Leu Val Arg Glu Ala Val Glu Thr Asn Gly
65 70 75 80

Pro Leu Gly Ile Leu Val Asn Asn Ala Gly Arg Asn Gly Gly Gly Arg
85 90 95

Thr Ala Glu Leu Ser Asp Glu Leu Trp Arg Glu Val Leu Ser Thr Asn
100 105 110

Leu Asp Ser Val Phe Tyr Val Thr Arg Glu Val Leu Ala Arg Gly Gly
115 120 125

Ile Gly Glu Val Asp His Ala Arg Ile Ile Asn Ile Ala Ser Thr Ala
130 135 140

Gly Lys Gln Gly Val Leu Leu Ala Ala Pro Tyr Ser Ala Ser Lys His
145 150 155 160

Gly Val Val Gly Phe Thr Lys Ala Val Gly Lys Glu Leu Ala Pro Gln
165 170 175

Gly Ile Thr Val Asn Ala Val Cys Pro Gly Tyr Val Glu Thr Pro Met
180 185 190

Ala Ser Arg Val Arg Gln Ala Tyr Ala Asp Ala Trp Glu Thr Thr Glu
195 200 205

Ala Glu Val Leu Ser Ala Phe Glu Ala Lys Ile Pro Leu Gly Arg Tyr
210 215 220

Ser Thr Pro Asp Glu Val Ala Ser Leu Val Glu Tyr Leu Thr Thr Glu
225 230 235 240

Gly Ala Ala Ser Ile Thr Ala Gln Ala Phe Asn Val Cys Gly Gly Leu
245 250 255

Gly Asn Phe

<210> SEQ ID NO 16
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Streptomyces griseoflavus

<400> SEQUENCE: 16

Val Arg Ala Phe Leu Phe Pro Gly Gln Gly Thr Gln Lys Ile Gly Met
1 5 10 15

Gly Thr Tyr Leu Arg Glu Arg Tyr Pro His Leu Ile Ala Pro Leu Trp
20 25 30

Arg Glu Ala Asp Asp Val Leu Gly Phe Pro Leu Thr Arg Leu Cys Glu
35 40 45

Glu Gly Pro Gly Glu Lys Leu Arg His Met Pro Val Thr Gln Pro Ala
50 55 60

Val Phe Leu Cys Ser Tyr Ala Ala Leu Val Ala Ala Gln Ala Asn Gly
65 70 75 80

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Ala Glu Pro Asp Val Ile Ala Gly His Ser Leu Gly Glu Tyr Ser Ala
                85                  90                  95

Leu Ala Ala Ala Gly Val Leu Thr Trp Gln Glu Val Leu Gln Leu Val
            100                 105                 110

His Arg Arg Gly Gln Leu Met Ala Glu Val Gln His Lys Val Asp Gly
        115                 120                 125

Lys Met Ala Ala Val Ile Gly Leu Ala Ile Gly Gln Val Glu Glu Ile
    130                 135                 140

Cys Glu Gln Val Arg Ser Glu Thr Gly Glu Val Val Glu Val Ala Asn
145                 150                 155                 160

His Asn Glu Pro Leu Gln Val Val Val Ser Gly Gln Cys Ala Ala Ile
                165                 170                 175

Asp Leu Leu Val Gln Arg Val Ala Thr Ala Thr Asp Val Arg Thr Ser
            180                 185                 190

Val Leu Arg Ile Gly Gly Pro Ala His Ser Ser Leu Met Gly Ser Val
        195                 200                 205

Ala Gly Asp Phe Val Glu Tyr Leu Arg Arg Phe Asp Phe Cys Thr Pro
    210                 215                 220

Lys Thr Met Leu Ile Ser Gly Ser Thr Ala Glu Pro Tyr Ala Ser Ala
225                 230                 235                 240

Glu Glu Ile Arg His Gln Leu Gly Arg Gln Leu Val His Arg Val Arg
                245                 250                 255

Trp Val Asp Val Met Ala Gln Leu Glu Arg Leu Gly Val Ala Gln Thr
            260                 265                 270

Trp Glu Leu Gly Pro Gly Lys Val Leu Ser Gly Phe Val Gln Arg Ser
        275                 280                 285

Leu Pro Gln Val Arg Thr Tyr Arg Ala Asn Asp Leu Pro Ser Phe Leu
    290                 295                 300

Ala Gly Val Thr Gly Trp
305                 310
```

What is claimed:

1. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of:

a) a gilOI polynucleotide sequence that remains hybridized to the entirety of the full length complement of bases 19892 to 21391 of SEQ ID NO: 1 under stringent conditions of washing twice with 2 molar SSC buffer at a temperature of 45° C. and then twice with 0.1 molar SSC buffer at a temperature of 68° C., and encodes a polypeptide having FAD-dependent oxygenase activity;

b) a gilOII polynucleotide sequence that remains hybridized to the entirety of the full length complement of bases 11513 to 12196 of SEQ ID NO: 1 under stringent conditions of washing twice with 2 molar SSC buffer at a temperature of 45° C. and then twice with 0.1 molar SSC buffer at a temperature of 68° C., and encodes a post-poly-ketide synthase (post-PKS) tailoring polypeptide having oxygenase activity;

c) a gilOIII polynucleotide sequence that remains hybridized to the entirety of the full length complement of bases 6576 to 7769 of SEQ ID NO: 1 under stringent conditions of washing twice with 2 molar SSC buffer at a temperature of 45° C. and then twice with 0.1 molar SSC buffer at a temperature of 68° C., and encodes a post-PKS tailoring polypeptide having oxygenase activity; and

d) a gilOIV polynucleotide sequence that remains hybridized to the entirety of the full length complement of bases 26200 to 27552 of SEQ ID NO: 1 under stringent conditions of washing twice with 2 molar SSC buffer at a temperature of 45° C. and then twice with 0.1 molar SSC buffer at a temperature of 68° C., and encodes a polypeptide having FAD-dependent oxygenase activity.

2. An isolated nucleic acid molecule comprising at least one polynucleotide sequence selected from the group consisting of:

a) a full length gilOI polynucleotide sequence that encodes the GilOI polypeptide encoded by bases 19892 to 21391 of SEQ ID NO: 1; wherein the polypeptide has FAD-dependent oxygenase activity;

b) a full length gilOII polynucleotide sequence that encodes the GilII polypeptide encoded by the reverse complement of bases 11513 to 12196 of SEQ ID NO: 1; wherein the polypeptide has oxygenase activity;

c) a full length gilOIII polynucleotide sequence that encodes the GilOIII polypeptide encoded by the reverse complement of bases 6576 to 7769 of SEQ ID NO: 1; wherein the polypeptide has oxygenase activity; and

d) a full length giOIV polynucleotide sequence that encodes the GilIV polypeptide encoded by bases 26200 to 27552 of SEQ ID NO: 1; wherein the polypeptide has FAD-dependent oxygenase activity.

3. The nucleic acid molecule of either claim **1** or claim **2** wherein the nucleic acid molecule encodes the gilOI, gilOII, gilOIII and gilOIV oxygenases.

4. The nucleic acid molecule of either claim **1** or claim **2** wherein the nucleic acid molecule encodes the gilOI and gilOIV.

5. A recombinant DNA expression vector comprising the nucleic acid molecule of either claim **1** or claim **2**, wherein the nucleic acid is operably linked to expression control sequences.

6. A host cell comprising a recombinant DNA expression vector comprising the nucleic acid molecule of either claim **1** or claim **2**, wherein the nucleic acid is operably linked to expression control sequences.

7. A bacterial host cell comprising a recombinant DNA expression vector comprising the nucleic acid molecule of either claim **1** or claim **2**, wherein the nucleic acid is operably linked to expression control sequences.

* * * * *