FILED
DEC 18 2001
DAVID W. DANIEL, CLERK
US DISTRICT COURT, EDNC
BY _____ DEP. CLERK

UNITED STATES DISTRICT COURT

EASTERN DISTRICT OF NORTH CAROLINA

SOUTHERN DIVISION

MONSANTO COMPANY	*	
Plaintiff	*	
	*	
	*	
VERSUS	*	**Case No.:** 7:01-CV-230-BR(1)
	*	
BOBBY O. GARRELL and TERRY GARRELL	*	
d/b/a GARRELL & GARRELL FARM	*	
PARTNERS	*	
Defendants	*	
	*	

* * * * * * * * * * * * * * * * * *

COMPLAINT

COMES NOW the plaintiff, Monsanto Company (hereinafter "Monsanto"), by and through its undersigned counsel, and for its Complaint at law against Bobby O. Garrell and Terry Garrell d/b/a Garrell & Garrell Farm Partners (hereinafter referred to as "the Garrells" or "defendants"), makes the following allegations:

PLAINTIFF

1. Monsanto is a company organized and existing under the laws of the State of Delaware with its principal place of business in St. Louis, Missouri. It is authorized to do business and is doing business in North Carolina and this judicial district.

DEFENDANT

2. The defendants, Bobby O. Garrell and Terry Garrell, are individuals who have obtained the age of majority and are residents and domiciliaries of Columbus County, North Carolina.

JURISDICTION AND VENUE

3. Subject matter jurisdictions is conferred upon this Court pursuant to 28 U.S.C. §1331, in that one or more of Monsanto's claims arise under the laws of the United States, as well as 28 U.S.C.§1338, granting district courts original jurisdiction over any civil action regarding patents.

4. Venue is proper in this judicial district pursuant to 28 U.S.C. §1400, in that the defendant resides in this judicial district and a substantial part of the events giving rise to this claim for patent infringement occurred in this judicial district.

GENERAL ALLEGATIONS

5. Monsanto is in the business of developing, manufacturing, licensing and selling agricultural biotechnology, agricultural chemicals and other agricultural products. After the investment of substantial time, expense and expertise, Monsanto developed a new plant biotechnology that involves the insertion into plants a gene that causes the plant to be resistant to glyphosate based herbicides such as Roundup Ultra®[1] or Touchdown®[2].

6. This new biotechnology has been utilized by Monsanto in soybeans. The genetically improved soybeans are marketed by Monsanto as Roundup Ready® [3] soybeans.

7. Roundup Ultra® and Touchdown® are non-selective herbicides which will cause severe injury or death to soybean varieties that do not contain the Roundup Ready® technology.

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[1] Roundup Ultra® is a registered trademark of Monsanto Company.

[2] Touchdown® is a registered trademark of Syngenta.

3 Roundup Ready® is a registered trademark of Monsanto Company.

8. Monsanto's Roundup Ready® technology is protected by U.S. Patent Numbers 5,633,435 and 5,352,605 which are attached hereto as Exhibits "A" and "B". These patents were issued and assigned to Monsanto prior to the events giving rise to this action.

9. Monsanto placed the required statutory notice that its Roundup Ready® technology was patented on the labeling of all bags containing Roundup Ready® soybean seed. In particular, each bag of Roundup Ready® soybean seed is marked with notice of U.S. Patent Nos. 5,633,435 and 5,352,605.

10. Monsanto licenses the use of Roundup Ready® seed technology to soybean producers at the retail marketing level through a limited use license agreement, commonly referred to as a Technology Agreement.

11. Under the terms of the Monsanto Technology Agreement, a purchaser/licensee is prohibited from saving, selling, reselling or otherwise transferring any seed produced from the purchased seed for use as planting seed. The only permissible use of the patent protected seed allowed by the Monsanto Technology Agreement is to market the crop derived therefrom as a grain commodity.

12. Authorized purchasers of Roundup Ready® soybeans are also required to pay a license fee (otherwise referred to as a "technology fee") for each commercial unit of seed purchased.

13. Monsanto does not authorize the planting of saved (bin run and/or brown bag) Roundup Ready® soybeans.

14. Defendants planted brown bag Roundup Ready® soybean seed in contravention of Monsanto's patent rights.

15. Upon information and belief, Defendants knowingly, intentionally and willfully planted saved Roundup Ready® soybeans without authorization in violation of Monsanto's patent rights in those soybeans.

COUNT ONE - PATENT INFRINGEMENT - PATENT NO. 5,633,435

16. Each and every allegation set forth in the above numbered paragraphs is hereby incorporated by reference just as if it was explicitly set forth hereunder.

17. On May 27, 1997, United States Patent No. 5,633,435 was duly and legally issued to plaintiff for an invention of Glyphosate-Tolerant-5-Enolpyruvylshikimate-3-Phosphate Synthesis and since that date, plaintiff has been and still is the owner of that patent. This invention is related to plant molecular biology, and it specifically covers, among other things, the progeny of Roundup Ready®soybeans and the applications of glyphosate to a glyphosate tolerant crop.

18. Monsanto placed the required statutory notice that its Roundup Ready® technology was protected by U.S. Patent No. 5,633,435 on the labeling of all bags containing Roundup Ready® soybean seed in compliance with 35 U.S.C. §287.

19. Defendants' conduct, as set forth above, constitutes the use of patented invention within the United States during the terms of Patent No. 5,633,435, all in violation of 35 U.S.C.§271. Accordingly, Monsanto has a right of civil action pursuant to 35 U.S.C. § 281.

20. Defendants have infringed and may still continue to infringe U.S. Patent No. 5,633,435 by making, selling, offering for sale, using or otherwise transferring Roundup Ready® soybeans embodying the patented invention without authorization from Monsanto, and will continue to do so unless enjoined by this Court.

21. Pursuant to 35 U.S.C. §283, Monsanto is entitled to injunctive relief in accordance with the principles of equity to prevent the infringement of rights secured by its patents.

22. Pursuant to 35 U.S.C. § 284, Monsanto is entitled to damages adequate to compensate for the infringement, although in no event less than a reasonable royalty, together with such interest and costs to be taxed to the infringer. Further, the damages should be trebled because the infringement was willful pursuant to 35 U.S.C. §284. Monsanto is also entitled to reimbursement of its attorney's fees pursuant to 35 U.S.C. §285 because this is an exceptional case.

COUNT TWO - PATENT INFRINGEMENT - PATENT NO. 5,352,605

23. Each and every allegation set forth in the above numbered paragraphs is hereby incorporated by reference just as if it were explicitly set forth hereunder.

24. On October 4, 1994, United States Patent No. 5,352,605 was duly and legally issued to plaintiff for an invention in Chimeric Genes for Transforming Plant Cells Using Viral Promoters, and since that date, plaintiff has been and still is the owner of that patent. This invention is in the field of genetic engineering and plant biology.

25. Monsanto placed the required statutory notice that its Roundup Ready® technology was protected by U.S. Patent No. 5,352,605 on the labeling of all bags containing Roundup Ready® soybean seed in compliance with 35 U.S.C. §287.

26. Defendants' conduct, as set forth above, constitutes the unauthorized use of a patented invention within the United States during the term of Patent No. 5,352,605, all in violation of 35 U.S.C. §271, and Monsanto therefore has a right of civil action against the defendants pursuant to 35 U.S.C. §281.

27. The defendants have and may still be infringing that patent by making, selling, offering for sale, using or otherwise transferring Roundup Ready® soybeans embodying the patented invention without authorization from Monsanto, and will continue to do so unless enjoined by this Court.

28. Pursuant to 35 U.S.C. §283, Monsanto is entitled to injunctive relief in accordance with the principles of equity to prevent the infringement of rights secured by its patents.

29. Pursuant to 35 U.S.C. §284, Monsanto is entitled to damages adequate to compensate for the infringement, although in no event less than a reasonable royalty, together with such interest and costs to be taxed to the infringer. Monsanto requests that these damages be trebled pursuant to 35 U.S.C. §284 in light of the defendant's knowing, willful, deliberate and conscious infringement of the patent right at issue.

30. The infringing activities of defendant bring this case within the ambit of the exceptional case contemplated by 35 U.S.C. §285, thus Monsanto requests the award of reasonable attorney's fees.

WHEREFORE, Monsanto Company prays that process and due form of law issue to Defendants herein, requiring them to appear and answer, all and singular, the allegation of this Complaint, and that after due proceedings are had, there be judgment in favor of Monsanto Company and against the defendants, providing the following remedies to Monsanto:

1. Entry of judgment for damages, together with interest and costs, to compensate Monsanto for the defendants' patent infringement;

2. Trebling of damages awarded Monsanto for the infringement of its patents together with reasonable attorney's fees;

3. Entry of an order prohibiting the defendants from planting, transferring or selling the infringing articles to a third party;

4. Entry of a permanent injunction to prevent defendants from using, cleaning or planting any of Monsanto's proprietary seed biotechnology without express written authorization from Monsanto; and

5. Such other relief as the Court may deem appropriate.

Respectfully submitted,

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ATTORNEYS FOR MONSANTO COMPANY

US005633435A

United States Patent [19]

Barry et al.

[11] Patent Number: 5,633,435

[45] Date of Patent: May 27, 1997

[54] GLYPHOSATE-TOLERANT 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASES

[75] Inventors: Gerard F. Barry, St. Louis; Ganesh M. Kishore, Chesterfield; Stephen R. Padgette, Grover; William C. Stallings, Glencoe, all of Mo.

[73] Assignee: Monsanto Company, St. Louis, Mo.

[21] Appl. No.: 306,863

[22] Filed: Sep. 13, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 749,611, Aug. 28, 1991, abandoned, which is a continuation-in-part of Ser. No. 576,537, Aug. 31, 1990, abandoned.

[51] Int. Cl.[6] A01H 4/00; C12N 15/82

[52] U.S. Cl. 800/205; 800/250; 800/DIG. 17; 800/DIG. 43; 800/DIG. 26; 536/23.2; 47/58; 435/320.1; 435/172.3; 435/413; 435/411; 435/414; 435/415; 435/418; 435/417; 435/416

[58] Field of Search ..... 800/205, DIG. 43, 800/DIG. 17, 250, DIG. 26; 536/23.2, 23.4, 23.7; 435/320.1, 172.1, 172.3

[56] References Cited

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5,310,667	5/1994	Eichholtz et al.	435/172.3

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0 193 259 9/1986 European Pat. Off. 800/205

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Comai, et al (1988) Journal of Biological Chemistry 263:15104–15109.

Fitzgibbon (Dec. 1988) Ph.D. Thesis University Microfilms International, 1989, pp. viii–ix, 18, 22–29, 32, 93, 96–108.

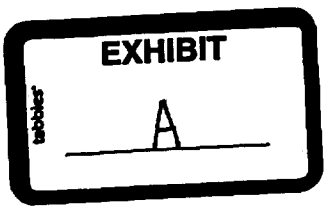
Primary Examiner—Che S. Chereskin

Attorney, Agent, or Firm—Dennis R. Hoerner, Jr.

[57] ABSTRACT

Genes encoding Class II EPSPS enzymes are disclosed. The genes are useful in producing transformed bacteria and plants which are tolerant to glyphosate herbicide. Class II EPSPS genes share little homology with known, Class I EPSPS genes, and do not hybridize to probes from Class I EPSPS's. The Class II EPSPS enzymes are characterized by being more kinetically efficient than Class I EPSPS's in the presence of glyphosate. Plants transformed with Class II EPSPS genes are also disclosed as well as a method for selectively controlling weeds in a planted transgenic crop field.

87 Claims, 70 Drawing Sheets



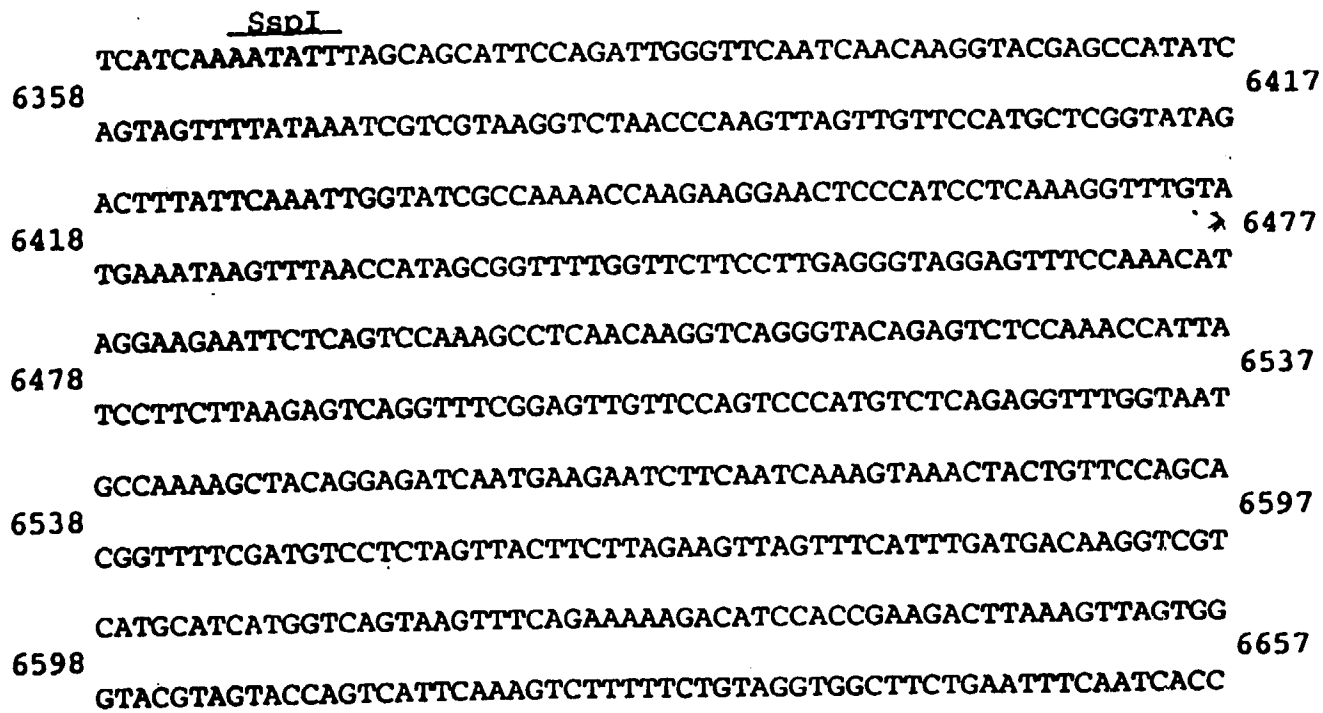


Figure 1A

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```
                                                    6717
GCATCTTTGAAAGTAATCTTGTCAACATCGAGCAGCTGGCTTGTGGGGACCAGACAAAAA
6658
CGTAGAAACTTTCATTAGAACAGTTGTAGCTCGTCGACCGAACACCCCTGGTCTGTTTTT
AGGAATGGTGCAGAATTGTTAGGCGCACCTACCAAAAGCATCTTTGCCTTTATTGCAAAG
                                                    6777
6718
TCCTTACCACGTCTTAACAATCCGCGTGGATGGTTTTCGTAGAAACGGAAATAACGTTTC
ATAAAGCAGATTCCTCTAGTACAAGTGGGGAACAAAATAACGTGGAAAAGAGCTGTCCTG
                                                    6837
6778
TATTTCGTCTAAGGAGATCATGTTCACCCCTTGTTTTATTGCACCTTTTCTCGACAGGAC
ACAGCCCACTCACTAATGCGTATGACGAACGCAGTGACGACCACACAAAGAATTCCCTCTA
                                                    6897
6838
TGTCGGGTGAGTGATTACGCATACTGCTTGCGTCACTGCTGGTGTTTTCTTAAGGGAGAT
                                    SspI
TATAAGAAGGCATTCATTCCCATTTGAAGGATCATCAGATACTAACCAATATTTCTC
                                                    6954
6898
ATATTCTTCCGTAAGTAAGGGTAAACTTCCTAGTAGTCTATGATTGGTTATAAAGAG
```

Figure 1B

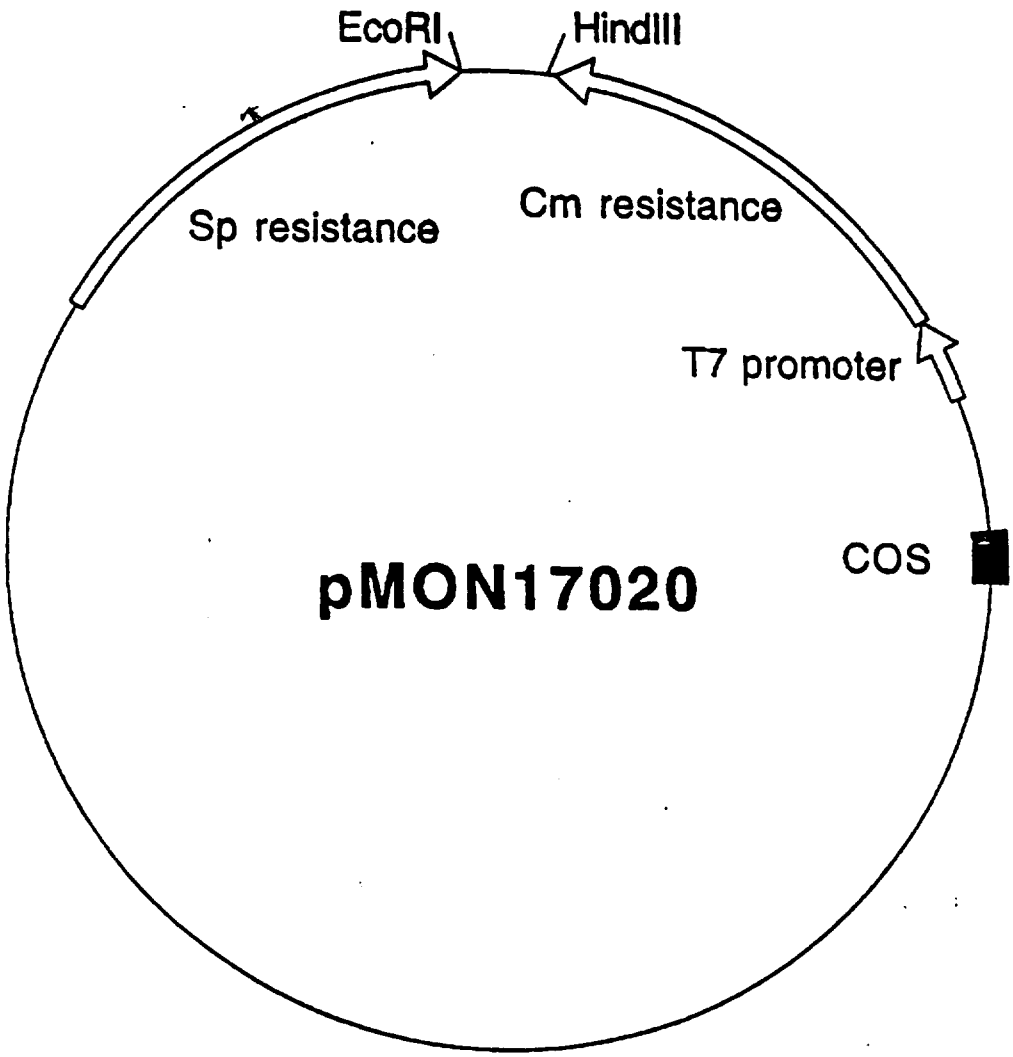
EcoRI
HindIII
Sp resistance
Cm resistance
T7 promoter
COS
pMON17020

Figure 2

```
AAGCCCGCGT TCTCTCCGGC GCTCCGCCCG GAGAGCCGTG GATAGATTAA GGAAGACGCC      60

C   ATG TCG CAC GGT GCA AGC AGC CGG CCC GCA ACC GCC CGC AAA TCC      106
    Met Ser His Gly Ala Ser Ser Arg Pro Ala Thr Ala Arg Lys Ser
      1               5                  10                  15

TCT GGC CTT TCC GGA ACC GTC CGC ATT CCC GGC GAC AAG TCG ATC TCC      154
Ser Gly Leu Ser Gly Thr Val Arg Ile Pro Gly Asp Lys Ser Ile Ser
                  20                  25                  30

CAC CGG TCC TTC ATG TTC GGC GGT CTC GCG AGC GGT GAA ACG CGC ATC      202
His Arg Ser Phe Met Phe Gly Gly Leu Ala Ser Gly Glu Thr Arg Ile
              35                  40                  45

ACC GGC CTT CTG GAA GGC GAG GAC GTC ATC AAT ACG GGC AAG GCC ATG      250
Thr Gly Leu Leu Glu Gly Glu Asp Val Ile Asn Thr Gly Lys Ala Met
          50                  55                  60

CAG GCC ATG GGC GCC AGG ATC CGT AAG GAA GGC GAC ACC TGG ATC ATC      298
Gln Ala Met Gly Ala Arg Ile Arg Lys Glu Gly Asp Thr Trp Ile Ile
      65                  70                  75

GAT GGC GTC GGC AAT GGC GGC CTC CTG GCG CCT GAG GCG CCG CTC GAT      346
Asp Gly Val Gly Asn Gly Gly Leu Leu Ala Pro Glu Ala Pro Leu Asp
  80                  85                  90                  95
```

Figure 3A

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TTC GGC AAT GCC GCC ACG GGC TGC CGC CTG ACC ATG GGC CTC GTC GGG      394
Phe Gly Asn Ala Ala Thr Gly Cys Arg Leu Thr Met Gly Leu Val Gly
          100                 105                 110

GTC TAC GAT TTC GAC AGC ACC TTC ATC GGC GAC GCC TCG CTC ACA AAG      442
Val Tyr Asp Phe Asp Ser Thr Phe Ile Gly Asp Ala Ser Leu Thr Lys
      115                 120                 125

CGC CCG ATG GGC CGC GTG TTG AAC CCG CTG CGC GAA ATG GGC GTG CAG      490
Arg Pro Met Gly Arg Val Leu Asn Pro Leu Arg Glu Met Gly Val Gln
  130                 135                 140

GTG AAA TCG GAA GAC GGT GAC CGT CTT CCC GTT ACC TTG CGC GGG CCG      538
Val Lys Ser Glu Asp Gly Asp Arg Leu Pro Val Thr Leu Arg Gly Pro
145                 150                 155

AAG ACG CCG ACG CCG ATC ACC TAC CGC GTG CCG ATG GCC TCC GCA CAG      586
Lys Thr Pro Thr Pro Ile Thr Tyr Arg Val Pro Met Ala Ser Ala Gln
160                 165                 170                 175

GTG AAG TCC GCC GTG CTG CTC GCC GGC CTC AAC ACG CCC GGC ATC ACG      634
Val Lys Ser Ala Val Leu Leu Ala Gly Leu Asn Thr Pro Gly Ile Thr
                180                 185                 190

ACG GTC ATC GAG CCG ATC ATG ACG CGC GAT CAT ACG GAA AAG ATG CTG      682
Thr Val Ile Glu Pro Ile Met Thr Arg Asp His Thr Glu Lys Met Leu
            195                 200                 205
```

Figure 3B

```
CAG GGC TTT GGC GCC AAC CTT ACC GTC GAG ACG GAT GCG GAC GGC GTG    730
Gln Gly Phe Gly Ala Asn Leu Thr Val Glu Thr Asp Ala Asp Gly Val
        210                 215                 220

CGC ACC ATC CGC CTG GAA GGC CGC GGC AAG CTC ACC GGC CAA GTC ATC    778
Arg Thr Ile Arg Leu Glu Gly Arg Gly Lys Leu Thr Gly Gln Val Ile
    225                 230                 235

GAC GTG CCG GGC GAC CCG TCC ACG GCC TTC CCG CTG GTT GCG GCC    826
Asp Val Pro Gly Asp Pro Ser Ser Thr Ala Phe Pro Leu Val Ala Ala
240                 245                 250                 255

CTG CTT GTT CCG GGC TCC GAC GTC ACC ATC CTC AAC GTG CTG ATG AAC    874
Leu Leu Val Pro Gly Ser Asp Val Thr Ile Leu Asn Val Leu Met Asn
                260                 265                 270

CCC ACC CGC ACC GGC CTC ATC CTG ACG CTG CAG GAA ATG GGC GCC GAC    922
Pro Thr Arg Thr Gly Leu Ile Leu Thr Leu Gln Glu Met Gly Ala Asp
            275                 280                 285

ATC GAA GTC ATC AAC CCG CGC CTT GCC GGC GGC GAA GAC GTG GCG GAC    970
Ile Glu Val Ile Asn Pro Arg Leu Ala Gly Gly Glu Asp Val Ala Asp
        290                 295                 300

CTG CGC GTT CGC TCC TCC ACG CTG AAG GGC GTC ACG GTG CCG GAA GAC    1018
Leu Arg Val Arg Ser Ser Thr Leu Lys Gly Val Thr Val Pro Glu Asp
    305                 310                 315
```

Figure 3C

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CGC GCG CCT TCG ATG ATC GAC GAA TAT CCG ATT CTC GCT GTC GCC GCC      1066
Arg Ala Pro Ser Met Ile Asp Glu Tyr Pro Ile Leu Ala Val Ala Ala
320                 325                 330                 335

GCC TTC GCG GAA GGG GCG ACC GTG ATG AAC GGT CTG GAA GAA CTC CGC      1114
Ala Phe Ala Glu Gly Ala Thr Val Met Asn Gly Leu Glu Glu Leu Arg
                340                 345                 350

GTC AAG GAA AGC GAC CGC CTC TCG GCC GTC GCC AAT GGC CTC AAG CTC      1162
Val Lys Glu Ser Asp Arg Leu Ser Ala Val Ala Asn Gly Leu Lys Leu
            355                 360                 365

AAT GGC GTG GAT TGC GAT GAG GGC GAG ACG TCG CTC GTC GTG CGC GGC      1210
Asn Gly Val Asp Cys Asp Glu Gly Glu Thr Ser Leu Val Val Arg Gly
        370                 375                 380

CGC CCT GAC GGC AAG GGG CTC GGC AAC GCC TCG GGC GCC GCC GTC GCC      1258
Arg Pro Asp Gly Lys Gly Leu Gly Asn Ala Ser Gly Ala Ala Val Ala
    385                 390                 395

ACC CAT CTC GAT CAC CGC ATC GCC ATG AGC TTC CTC GTC ATG GGC CTC      1306
Thr His Leu Asp His Arg Ile Ala Met Ser Phe Leu Val Met Gly Leu
400                 405                 410                 415

GTG TCG GAA AAC CCT GTC ACG GTG GAC GAT GCC ACG ATG ATC GCC ACG      1354
Val Ser Glu Asn Pro Val Thr Val Asp Asp Ala Thr Met Ile Ala Thr
                420                 425                 430
```

Figure 3D

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AGC TTC CCG GAG TTC ATG GAC CTG ATG GCC GGG CTG GGC GCG AAG ATC      1402
Ser Phe Pro Glu Phe Met Asp Leu Met Ala Gly Leu Gly Ala Lys Ile
            435                 440                 445

GAA CTC TCC GAT ACG AAG GCT GCC TGATGACCTT CACAATCGCC ATCGATGGTC    1456
Glu Leu Ser Asp Thr Lys Ala Ala
        450                 455

CCGCTGCGGC CGGCAAGGGG ACGCTCTCGC GCCGTATCGC GGAGGTCTAT GGCTTTCATC   1516

ATCTCGATAC GGGCCTGACC TATCGCGCCA CGGCCAAAGC GCTGCTCGAT CGCGGCCTGT   1576

CGCTTGATGA CGAGGCGGTT GCGGCCGATG TCGCCCGCAA TCTCGATCTT GCCGGGCTCG   1636

ACCGGTCGGT GCTGTCGGCC CATGCCATCG GCGAGGCGGC TTCGAAGATC GCGGTCATGC   1696

CCTCGGTGCG GCGGGCGCTG GTCGAGGCGC AGCGCAGCTT TGCGGCGCGT GAGCCGGGCA   1756

CGGTGCTGGA TGGACGCGAT ATCGGCACGG TGGTCTGCCC GGATGCGCCG GTGAAGCTCT   1816

ATGTCACCGC GTCACCGGAA GTGCGCGCGA AACGCCGCTA TGACGAAATC CTCGGCAATG   1876

GCGGGTTGGC CGATTACGGG ACGATCCTCG AGGATATCCG CCGCCGCGAC GAGCGGGACA   1936

TGGGTCGGGC GGACAGTCCT TTGAAGCCCG CCGACGATGC GCACTT                  1982
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Figure 3E

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                                                                    60
GTAGCCACAC ATAATTACTA TAGCTAGGAA GCCCGCTATC TCTCAATCCC GCGTGATCGC

                                                                   112
GCCAAAATGT GACTGTGAAA AATCC ATG TCC CAT TCT GCA TCC CCG AAA CCA
                            Met Ser His Ser Ala Ser Pro Lys Pro
                              1               5

                                                                   160
GCA ACC GCC CGC CGC TCG GAG GCA CTC ACG GGC GAA ATC CGC ATT CCG
Ala Thr Ala Arg Arg Ser Glu Ala Leu Thr Gly Glu Ile Arg Ile Pro
 10                  15                  20                  25

                                                                   208
GGC GAC AAG TCC ATC TCG CAT CGC TCC TTC ATG TTT GGC GGT CTC GCA
Gly Asp Lys Ser Ile Ser His Arg Ser Phe Met Phe Gly Gly Leu Ala
                 30                  35                  40

                                                                   256
TCG GGC GAA ACC CGC ATC ACC GGC CTT CTG GAA GGC GAG GAC GTC ATC
Ser Gly Glu Thr Arg Ile Thr Gly Leu Leu Glu Glu Gly Asp Val Ile
             45                  50                  55

                                                                   304
AAT ACA GGC CGC GCC ATG CAG GCC ATG GGC GCG AAA ATC CGT AAA GAG
Asn Thr Gly Arg Ala Met Gln Ala Met Gly Ala Lys Ile Arg Lys Glu
         60                  65                  70

                                                                   352
GGC GAT GTC TGG ATC ATC AAC GGC GTC GGC AAT GGC TGC CTG TTG CAG
Gly Asp Val Trp Ile Ile Asn Gly Val Gly Asn Gly Cys Leu Leu Gln
     75                  80                  85
```

Figure 4A

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CCC GAA GCT GCG CTC GAT TTC GGC AAT GCC GGA ACC GGC GCG CGC CTC    400
Pro Glu Ala Ala Leu Asp Phe Gly Asn Ala Gly Thr Gly Ala Arg Leu
 90                  95                 100                 105

ACC ATG GGC CTT GTC GGC ACC TAT GAC ATG AAG ACC TCC TTT ATC GGC    448
Thr Met Gly Leu Val Gly Thr Tyr Asp Met Lys Thr Ser Phe Ile Gly
                110                 115                 120

GAC GCC TCG CTG TCG AAG CGC CCG ATG GGC CGC GTG CTG AAC CCG TTG    496
Asp Ala Ser Leu Ser Lys Arg Pro Met Gly Arg Val Leu Asn Pro Leu
            125                 130                 135

CGC GAA ATG GGC GTT CAG GTG GAA GCA GCC GAT GGC GAC CGC ATG CCG    544
Arg Glu Met Gly Val Gln Val Glu Ala Ala Asp Gly Asp Arg Met Pro
        140                 145                 150

CTG ACG CTG ATC GGC CCG AAG ACG GCC AAT CCG ATC ACC TAT CGC GTG    592
Leu Thr Leu Ile Gly Pro Lys Thr Ala Asn Pro Ile Thr Tyr Arg Val
    155                 160                 165

CCG ATG GCC TCC GCG CAG GTA AAA TCC GCC GTG CTG CTC GCC GGT CTC    640
Pro Met Ala Ser Ala Gln Val Lys Ser Ala Val Leu Leu Ala Gly Leu
170                 175                 180                 185

AAC ACG CCG GGC GTC ACC ACC GTC ATC GAG CCG GTC ATG ACC CGC GAC    688
Asn Thr Pro Gly Val Thr Thr Val Ile Glu Pro Val Met Thr Arg Asp
                190                 195                 200
```

Figure 4B

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CAC ACC GAA AAG ATG CTG CAG GGC TTT GGC GCC GAC CTC ACG GTC GAG      736
His Thr Glu Lys Met Leu Gln Gly Phe Gly Ala Asp Leu Thr Val Glu
            205                 210                 215

ACC GAC AAG GAT GGC GTG CGC CAT ATC CGC ATC ACC GGC CAG GGC AAG      784
Thr Asp Lys Asp Gly Val Arg His Ile Arg Ile Thr Gly Gln Gly Lys
        220                 225                 230

CTT GTC GGC CAG ACC ATC GAC GTG CCG GGC GAT CCG TCA TCG ACC GCC      832
Leu Val Gly Gln Thr Ile Asp Val Pro Gly Asp Pro Ser Ser Thr Ala
    235                 240                 245

TTC CCG CTC GTT GCC GCC CTT CTG GTG GAA GGT TCC GAC GTC ACC ATC      880
Phe Pro Leu Val Ala Ala Leu Leu Val Glu Gly Ser Asp Val Thr Ile
250                 255                 260                 265

CGC AAC GTG CTG ATG AAC CCG ACC CGT ACC GGC CTC ATC CTC ACC TTG      928
Arg Asn Val Leu Met Asn Pro Thr Arg Thr Gly Leu Ile Leu Thr Leu
                270                 275                 280

CAG GAA ATG GGC GCC GAT ATC GAA GTG CTC AAT GCC CGT CTT GCA GGC      976
Gln Glu Met Gly Ala Asp Ile Glu Val Leu Asn Ala Arg Leu Ala Gly
            285                 290                 295

GGC GAA GAC GTC GCC GAT CTG CGC GTC AGG GCT TCG AAG CTC AAG GGC     1024
Gly Glu Asp Val Ala Asp Leu Arg Val Arg Ala Ser Lys Leu Lys Gly
        300                 305                 310
```

Figure 4C

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GTC GTC GTT CCG CCG GAA CGT GCG CCG TCG ATG ATC GAC GAA TAT CCG  1072
Val Val Val Pro Pro Glu Arg Ala Pro Ser Met Ile Asp Glu Tyr Pro
      315                 320                 325

GTC CTG GCG ATT GCC GCC TCC TTC GCG GAA GGC GAA ACC GTG ATG GAC  1120
Val Leu Ala Ile Ala Ala Ser Phe Ala Glu Gly Glu Thr Val Met Asp
  330                 335                 340                 345

GGG CTC GAC GAA CTG CGC GTC AAG GAA TCG GAT CGT CTG GCA GCG GTC  1168
Gly Leu Asp Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Val
                350                 355                 360

GCA CGC GGC CTT GAA GCC AAC GGC GTC GAT TGC ACC GAA GGC GAG ATG  1216
Ala Arg Gly Leu Glu Ala Asn Gly Val Asp Cys Thr Glu Gly Glu Met
            365                 370                 375

TCG CTG ACG GTT CGC GGC CGC CCC GAC GGC AAG GGA CTG GGC GGC GGC  1264
Ser Leu Thr Val Arg Gly Arg Pro Asp Gly Lys Gly Leu Gly Gly Gly
        380                 385                 390

ACG GTT GCA ACC CAT CTC GAT CAT CGT ATC GCG ATG AGC TTC CTC GTG  1312
Thr Val Ala Thr His Leu Asp His Arg Ile Ala Met Ser Phe Leu Val
    395                 400                 405

ATG GGC CTT GCG GCG GAA AAG CCG GTG ACG GTT GAC GAC AGT AAC ATG  1360
Met Gly Leu Ala Ala Glu Lys Pro Val Thr Val Asp Asp Ser Asn Met
410                 415                 420                 425
```

Figure 4D

```
ATC GCC ACG TCC TTC CCC GAA TTC ATG GAC ATG ATG CCG GGA TTG GGC      1408
Ile Ala Thr Ser Phe Pro Glu Phe Met Asp Met Met Pro Gly Leu Gly
                430                 435                 440

GCA AAG ATC GAG TTG AGC ATA CTC TAGTCACTCG ACAGCGAAAA TATTATTTGC    1462
Ala Lys Ile Glu Leu Ser Ile Leu
            445

GAGATTGGGC ATTATTACCG GTTGGTCTCA GCGGGGGTTT AATGTCCAAT CTTCCATACG   1522

TAACAGCATC AGGAAATATC AAAAAAGCTT TAGAAGGAAT TGCTAGAGCA GCGACGCCGC   1582

CTAAGCTTTC TCAAGACTTC GTTAAAACTG TACTGAAATC CCGGGGGGTC CGGGGATCAA   1642

ATGACTTCAT TTCTGAGAAA TTGGCCTCGC A                                   1673
```

Figure 4E

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```
                                                                    54
GTGATCGCGC CAAAATGTGA CTGTGAAAAA TCC ATG TCC CAT TCT GCA TCC CCG
                                  Met Ser His Ser Ala Ser Pro
                                   1               5
                                                                   102
AAA CCA GCA ACC GCC CGC CGC TCG GAG GCA CTC ACG GGC GAA ATC CGC
Lys Pro Ala Thr Ala Arg Arg Ser Glu Ala Leu Thr Gly Glu Ile Arg
      10                  15                  20
                                                                   150
ATT CCG GGC GAC AAG TCC ATC TCG CAT CGC TCC TTC ATG TTT GGC GGT
Ile Pro Gly Asp Lys Ser Ile Ser His Arg Ser Phe Met Phe Gly Gly
      25                  30                  35
                                                                   198
CTC GCA TCG GGC GAA ACC CGC ATC ACC GGC CTT CTG GAA GGC GAG GAC
Leu Ala Ser Gly Glu Thr Arg Ile Thr Gly Leu Leu Glu Gly Glu Asp
      40                  45                  50                  55
                                                                   246
GTC ATC AAT ACA GGC CGC GCC ATG CAG GCC ATG GGC GCG AAA ATC CGT
Val Ile Asn Thr Gly Arg Ala Met Gln Ala Met Gly Ala Lys Ile Arg
                  60                  65                  70
                                                                   294
AAA GAG GGC GAT GTC TGG ATC ATC AAC GGC GTC GGC AAT GGC TGC CTG
Lys Glu Gly Asp Val Trp Ile Ile Asn Gly Val Gly Asn Gly Cys Leu
                  75                  80                  85
                                                                   342
TTG CAG CCC GAA GCT GCG CTC GAT TTC GGC AAT GCC GGA ACC GGC GCG
Leu Gln Pro Glu Ala Ala Leu Asp Phe Gly Asn Ala Gly Thr Gly Ala
                  90                  95                  100
```

Figure 5A

```
CGC CTC ACC ATG GGC CTT GTC GGC ACC TAT GAC ATG AAG ACC TCC TTT      390
Arg Leu Thr Met Gly Leu Val Gly Thr Tyr Asp Met Lys Thr Ser Phe
    105                 110                 115

ATC GGC GAC GCC TCG CTG TCG AAG CGC CCG ATG GGC CGC GTG CTG AAC      438
Ile Gly Asp Ala Ser Leu Ser Lys Arg Pro Met Gly Arg Val Leu Asn
120                 125                 130                 135

CCG TTG CGC GAA ATG GGC GTT CAG GTG GAA GCA GCC GAT GGC GAC CGC      486
Pro Leu Arg Glu Met Gly Val Gln Val Glu Ala Ala Asp Gly Asp Arg
                140                 145                 150

ATG CCG CTG ACG CTG ATC GGC CCG AAG ACG GCC AAT CCG ATC ACC TAT      534
Met Pro Leu Thr Leu Ile Gly Pro Lys Thr Ala Asn Pro Ile Thr Tyr
            155                 160                 165

CGC GTG CCG ATG GCC TCC GCG CAG GTA AAA TCC GCC GTG CTG CTC GCC      582
Arg Val Pro Met Ala Ser Ala Gln Val Lys Ser Ala Val Leu Leu Ala
        170                 175                 180

GGT CTC AAC ACG CCG GGC GTC ACC ACC GTC ATC GAG CCG GTC ATG ACC      630
Gly Leu Asn Thr Pro Gly Val Thr Thr Val Ile Glu Pro Val Met Thr
    185                 190                 195

CGC GAC CAC ACC GAA AAG ATG CTG CAG GGC TTT GGC GCC GAC CTC ACG      678
Arg Asp His Thr Glu Lys Met Leu Gln Gly Phe Gly Ala Asp Leu Thr
200                 205                 210                 215
```

Figure 5B

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```
726
GTC GAG ACC GAC AAG GAT GGC GTG CGC CAT ATC CGC ATC ACC GGC CAG
Val Glu Thr Asp Lys Asp Gly Val Arg His Ile Arg Ile Thr Gly Gln
                220                 225                 230

774
GGC AAG CTT GTC GGC CAG ACC ATC GAC GTG CCG GGC GAT CCG TCA TCG
Gly Lys Leu Val Gly Gln Thr Ile Asp Val Pro Gly Asp Pro Ser Ser
            235                 240                 245

822
ACC GCC TTC CCG CTC GTT GCC GCC CTT CTG GTG GAA GGT TCC GAC GTC
Thr Ala Phe Pro Leu Val Ala Ala Leu Leu Val Glu Gly Ser Asp Val
        250                 255                 260

870
ACC ATC CGC AAC GTG CTG ATG AAC CCG ACC CGT ACC GGC CTC ATC CTC
Thr Ile Arg Asn Val Leu Met Asn Pro Thr Arg Thr Gly Leu Ile Leu
    265                 270                 275

918
ACC TTG CAG GAA ATG GGC GCC GAT ATC GAA GTG CTC AAT GCC CGT CTT
Thr Leu Gln Glu Met Gly Ala Asp Ile Glu Val Leu Asn Ala Arg Leu
280                 285                 290                 295

966
GCA GGC GAA GAC GTC GCC GAT CTG CGC GTC AGG GCT TCG AAG CTC
Ala Gly Glu Asp Val Ala Asp Leu Arg Val Arg Ala Ser Lys Leu
            300                 305                 310

1014
AAG GGC GTC GTT CCG CCG GAA CGT GCG CCG TCG ATG ATC GAC GAA
Lys Gly Val Val Pro Pro Glu Arg Ala Pro Ser Met Ile Asp Glu
        315                 320                 325
```

Figure 5C

```
TAT CCG GTC CTG GCG ATT GCC GCC TCC TTC GCG GAA GGC GAA ACC GTG   1062
Tyr Pro Val Leu Ala Ile Ala Ala Ser Phe Ala Glu Gly Glu Thr Val
            330                 335                 340

ATG GAC GGG CTC GAC GAA CTG CGC GTC AAG GAA TCG GAT CGT CTG GCA   1110
Met Asp Gly Leu Asp Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala
        345                 350                 355

GCG GTC GCA CGC GGC CTT GAA GCC AAC GGC GTC GAT TGC ACC GAA GGC   1158
Ala Val Ala Arg Gly Leu Glu Ala Asn Gly Val Asp Cys Thr Glu Gly
    360                 365                 370                 375

GAG ATG TCG CTG ACG GTT CGC GGC CGC CCC GAC GGC AAG GGA CTG GGC   1206
Glu Met Ser Leu Thr Val Arg Gly Arg Pro Asp Gly Lys Gly Leu Gly
                380                 385                 390

GGC GGC ACG GTT GCA ACC CAC CTC GAT CAT CGT ATC GCG ATG AGC TTC   1254
Gly Gly Thr Val Ala Thr His Leu Asp His Arg Ile Ala Met Ser Phe
            395                 400                 405

CTC GTG ATG GGC CTT GCG GCG GAA AAG CCG GTG ACG GTT GAC GAC AGT   1302
Leu Val Met Gly Leu Ala Ala Glu Lys Pro Val Thr Val Asp Asp Ser
        410                 415                 420

AAC ATG ATC GCC ACG TCC TTC CCC GAA TTC ATG GAC ATG ATG CCG GGA   1350
Asn Met Ile Ala Thr Ser Phe Pro Glu Phe Met Asp Met Met Pro Gly
    425                 430                 435
```

Figure 5D

```
                                                    1400
TTG GGC GCA AAG ATC GAG TTG AGC ATA CTC TAGTCACTCG ACAGCGAAAA
Leu Gly Ala Lys Ile Glu Leu Ser Ile Leu
440                 445

                                                    1460
TATTATTTGC GAGATTGGGC ATTATTACCG GTTGGTCTCA GCGGGGGTTT AATGTCCAAT

                                                    1500
CTTCCATACG TAACAGCATC AGGAAATATC AAAAAAGCTT
```

Figure 5E

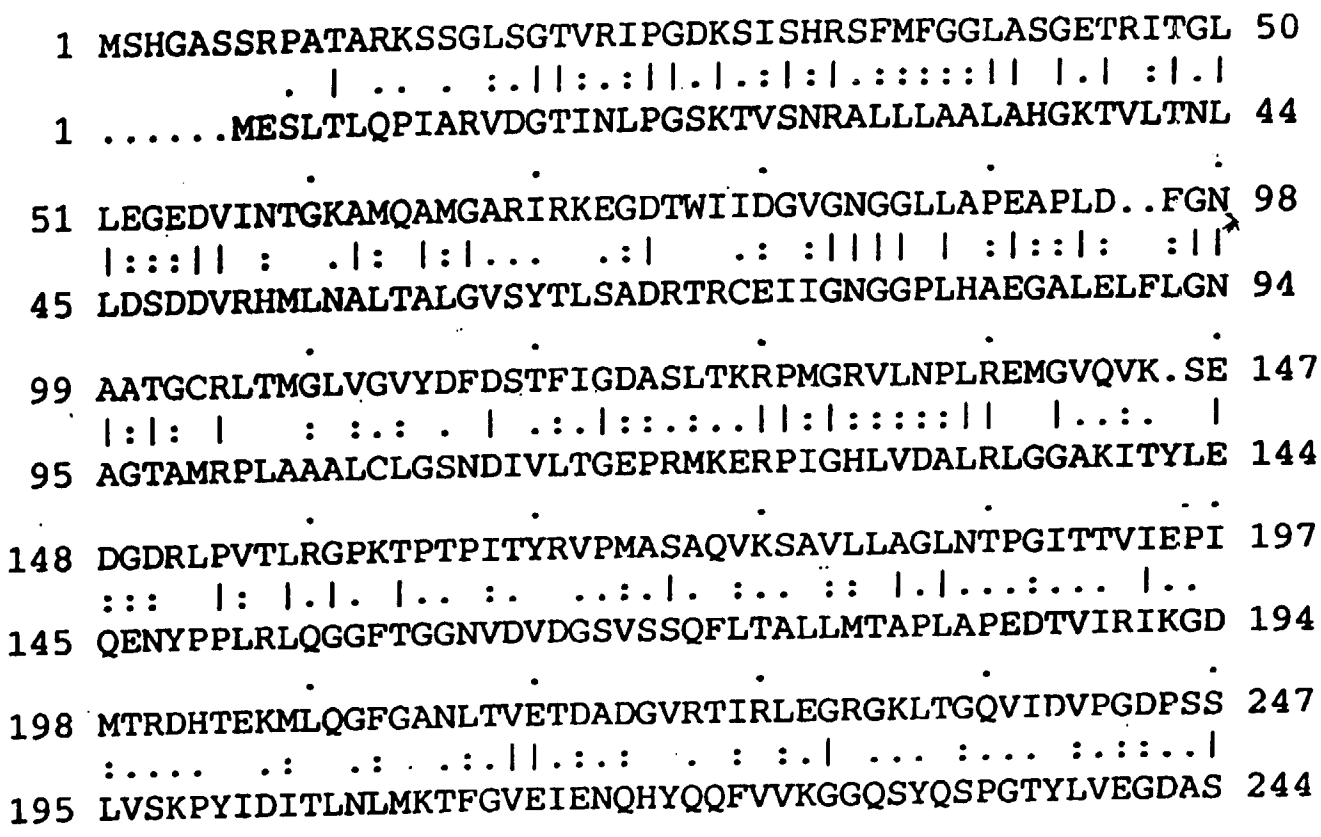

Figure 6A

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```
248 TAFPLVAALLVPGSDVTILNVLMNPTRTGLILT..LQEMGADIEVINPRL 295
    .|  ::||  :.|:.|.: .: .|. ..:: :.  |:.|||.|
245 SASYFLAAAAIKGGTVKVTGIGRNSMQGDIRFADVLEKMGATI..... 287

296 AGGEDVADLRVRSSTLKGVTVPEDRAPSMIDEYPILAVAAAFAEGATVMN 345
    . |:|   :.. .:.|.::.:. :: |  .|.  .:|.|| ||.|.| :.
288 CWGDDY..ISCTRGELNAIDMDMNHIP...DAAMTIATAALFAKGTTRLR 332

346 GLEELRVKESDRLSAVANGLKLNGVDCDEGETSLVVRGRPDGKGLGNASG 395
    .: ::|||.||| |:|.:|:  |.:.:||.. : : ..|:  .:
333 NIYNWRVKETDRLFAMATELRKVGAEVEEGHDYIRI.TPPEKLNF..... 376

396 AAVATHLDHRIAMSFLVMGLVSENPVTVDDATMIATSFPEFMDLMAGLGA 445
    |.:||. |||:||:| :::| |:.|||: |:. .|..||:::: :| ::.
377 AEIATYNDHRMAMCFSLVAL.SDTPVTILDPKCTAKTFPDYFEQLARISQ 425

446 KIELSDTKAA* 456

426 AA*..... 428
```

Figure 6B

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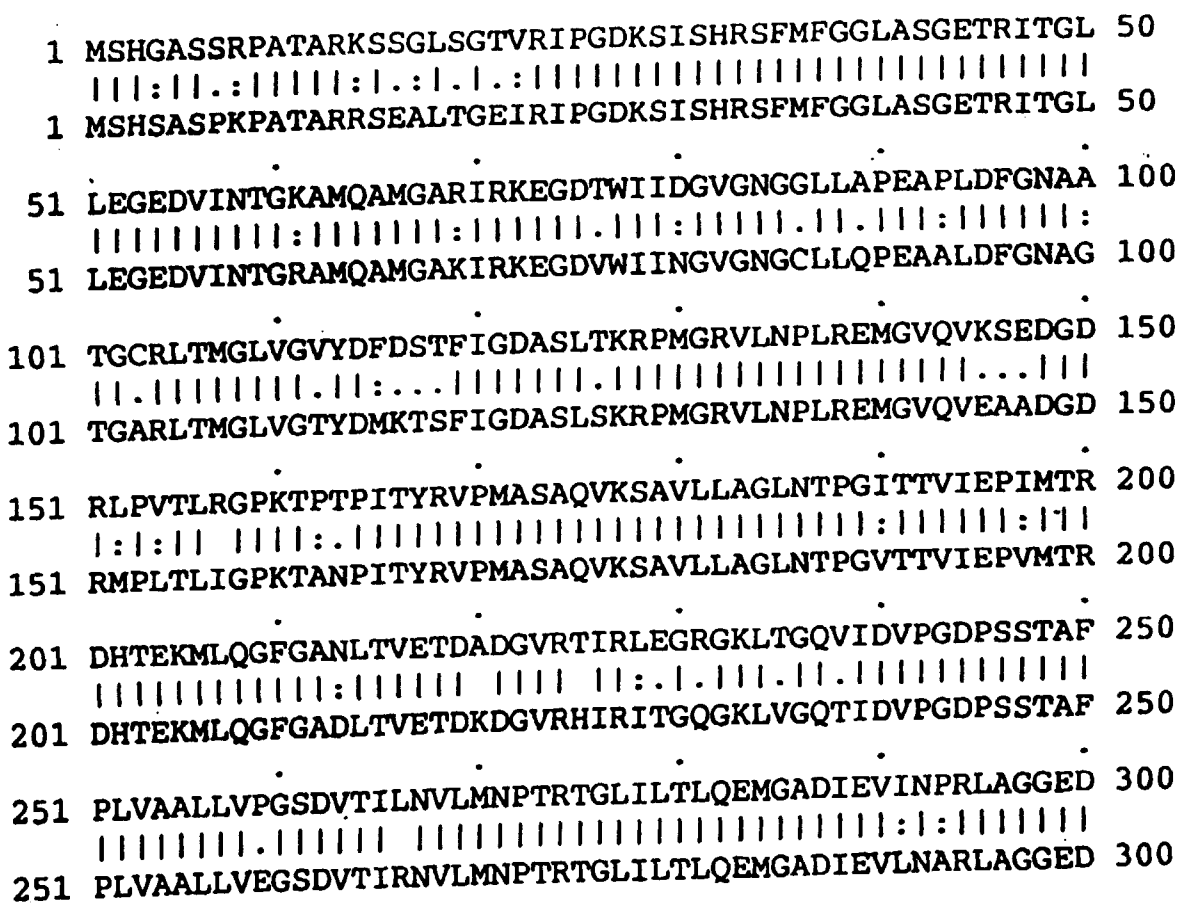
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1 MSHSASPKPATARRSEALTGEIRIPGDKSISHRSFMFGGLASGETRITGL 50
51 LEGEDVINTGKAMQAMGARIRKEGDTWIIDGVGNGGLLAPEAPLDFGNAA 100
51 LEGEDVINTGRAMQAMGAKIRKEGDVWIINGVGNGCLLQPEAALDFGNAG 100
101 TGCRLTMGLVGVYDFDSTFIGDASLTKRPMGRVLNPLREMGVQVKSEDGD 150
101 TGARLTMGLVGTYDMKTSFIGDASLSKRPMGRVLNPLREMGVQVEAADGD 150
151 RLPVTLRGPKTPTPITYRVPMASAQVKSAVLLAGLNTPGITTVIEPIMTR 200
151 RMPLTLIGPKTANPITYRVPMASAQVKSAVLLAGLNTPGVTTVIEPVMTR 200
201 DHTEKMLQGFGANLTVETDADGVRTIRLEGRGKLTGQVIDVPGDPSSTAF 250
201 DHTEKMLQGFGADLTVETDKDGVRHIRITGQGKLVGQTIDVPGDPSSTAF 250
251 PLVAALLVPGSDVTILNVLMNPTRTGLILTLQEMGADIEVINPRLAGGED 300
251 PLVAALLVEGSDVTIRNVLMNPTRTGLILTLQEMGADIEVLNARLAGGED 300

Figure 7A

```
301 VADLRVRSSTLKGVTVPEDRAPSMIDEYPILAVAAAFAEGATVMNGLEEL 350
    |||||||.|.||||.||.:|||||||||||:|||:||.||||.|||:|||
301 VADLRVRASKLKGVVVPPERAPSMIDEYPVLAIAASFAEGETVMDGLDEL 350

351 RVKESDRLSAVANGLKLNGVDCDEGETSLVVRGRPDGKGLGNASGAAVAT 400
    |||||||||.|||.||.. |||||.||| ||.|||||||||||||    |:.|||
351 RVKESDRLAAVARGLEANGVDCTEGEMSLTVRGRPDGKGLG...GGTVAT 397

401 HLDHRIAMSFLVMGLVSENPVTVDDATMIATSFPEFMDLMAGLGAKIELS 450
    |||||||||||||||||..|.|||||||..|||||||||||:|:|||||||||
398 HLDHRIAMSFLVMGLAAEKPVTVDDSNMIATSFPEFMDMMPGLGAKIELS 447

451 DTKAA* 456

448 IL.... 449
```

Figure 7B

```
CCATGGCTCA CGGTGCAAGC AGCCGTCCAG CAACTGCTCG TAAGTCCTCT GGTCTTTCTG   60

GAACCGTCCG TATTCCAGGT GACAAGTCTA TCTCCCACAG GTCCTTCATG TTTGGAGGTC  120

TCGCTAGCGG TGAAACTCGT ATCACCGGTC TTTTGGAAGG TGAAGATGTT ATCAACACTG  180

GTAAGGCTAT GCAAGCTATG GGTGCCAGAA TCCGTAAGGA AGGTGATACT TGGATCATTG  240

ATGGTGTTGG TAACGGTGGA CTCCTTGCTC CTGAGGCTCC TCTCGATTTC GGTAACGCTG  300

CAACTGGTTG CCGTTTGACT ATGGGTCTTG TTGGTGTTTA CGATTTCGAT AGCACTTTCA  360

TTGGTGACGC TTCTCTCACT AAGCGTCCAA TGGGTCGTGT GTTGAACCCA CTTCGCGAAA  420

TGGGTGTGCA GGTGAAGTCT GAAGACGGTG ATCGTCTTCC AGTTACCTTG CGTGGACCAA  480

AGACTCCAAC GCCAATCACC TACAGGGTAC CTATGGCTTC CGCTCAAGTG AAGTCCGCTG  540

TTCTGCTTGC TGGTCTCAAC ACCCCAGGTA TCACCACTGT TATCGAGCCA ATCATGACTC  600

GTGACCACAC TGAAAAGATG CTTCAAGGTT TTGGTGCTAA CCTTACCGTT GAGACTGATG  660

CTGACGGTGT GCGTACCATC CGTCTTGAAG GTCGTGGTAA GCTCACCGGT CAAGTGATTG  720

ATGTTCCAGG TGATCCATCC TCTACTGCTT TCCCATTGGT TGCTGCCTTG CTTGTTCCAG  780

GTTCCGACGT CACCATCCTT AACGTTTTGA TGAACCCAAC CCGTACTGGT CTCATCTTGA  840
```

Figure 8A

```
CTCTGCAGGA AATGGGTGCC GACATCGAAG TGATCAACCC ACGTCTTGCT GGTGGAGAAG    900
ACGTGGCTGA CTTGCGTGTT CGTTCTTCTA CTTTGAAGGG TGTTACTGTT CCAGAAGACC    960
GTGCTCCTTC TATGATCGAC GAGTATCCAA TTCTCGCTGT TGCAGCTGCA TTCGCTGAAG   1020
GTGCTACCGT TATGAACGGT TTGGAAGAAC TCCGTGTTAA GGAAAGCGAC CGTCTTTCTG   1080
CTGTCGCAAA CGGTCTCAAG CTCAACGGTG TTGATTGCGA TGAAGGTGAG ACTTCTCTCG   1140
TCGTGCGTGG TCGTCCTGAC GGTAAGGGTC TCGGTAACGC TTCTGGAGCA GCTGTCGCTA   1200
CCCACCTCGA TCACCGTATC GCTATGAGCT TCCTCGTTAT GGGTCTCGTT TCTGAAAACC   1260
CTGTTACTGT TGATGATGCT ACTATGATCG CTACTAGCTT CCCAGAGTTC ATGGATTTGA   1320
TGGCTGGTCT TGGAGCTAAG ATCGAACTCT CCGACACTAA GGCTGCTTGA TGAGCTC      1377
```

Figure 8B

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```
AGATCTATCG ATAAGCTTGA TGTAATTGGA GGAAGATCAA AATTTTCAAT CCCCATTCTT      60

CGATTGCTTC AATTGAAGTT TCTCCG ATG GCG CAA GTT AGC AGA ATC TGC AAT      113
                               Met Ala Gln Val Ser Arg Ile Cys Asn
                                 1               5

GGT GTG CAG AAC CCA TCT CTT ATC TCC AAT CTC TCG AAA TCC AGT CAA  ↗    161
Gly Val Gln Asn Pro Ser Leu Ile Ser Asn Leu Ser Lys Ser Ser Gln
 10                  15                  20                  25

CGC AAA TCT CCC TTA TCG GTT TCT CTG AAG ACG CAG CAG CAT CCA CGA      209
Arg Lys Ser Pro Leu Ser Val Ser Leu Lys Thr Gln Gln His Pro Arg
                 30                  35                  40

GCT TAT CCG ATT TCG TCG TCG TGG GGA TTG AAG AAG AGT GGG ATG ACG      257
Ala Tyr Pro Ile Ser Ser Ser Trp Gly Leu Lys Lys Ser Gly Met Thr
             45                  50                  55

TTA ATT GGC TCT GAG CTT CGT CCT CTT AAG GTC ATG TCT TCT GTT TCC      305
Leu Ile Gly Ser Glu Leu Arg Pro Leu Lys Val Met Ser Ser Val Ser
         60                  65                  70

ACG GCG TGC ATG C                                                   318
Thr Ala Cys Met
     75
```

Figure 9

```
AGATCTATCG ATAAGCTTGA TGTAATTGGA GGAAGATCAA AATTTTCAAT CCCCATTCTT   60

CGATTGCTTC AATTGAAGTT TCTCCG ATG GCG CAA GTT AGC AGA ATC TGC AAT   113
                             Met Ala Gln Val Ser Arg Ile Cys Asn
                               1               5

GGT GTG CAG AAC CCA TCT CTT ATC TCC AAT CTC TCG AAA TCC AGT CAA   161
Gly Val Gln Asn Pro Ser Leu Ile Ser Asn Leu Ser Lys Ser Ser Gln
  10              15                  20                  25

CGC AAA TCT CCC TTA TCG GTT TCT CTG AAG ACG CAG CAG CAT CCA CGA   209
Arg Lys Ser Pro Leu Ser Val Ser Leu Lys Thr Gln Gln His Pro Arg
              30                  35                  40

GCT TAT CCG ATT TCG TCG TGG GGA TTG AAG AAG AGT GGG ATG ACG       257
Ala Tyr Pro Ile Ser Ser Trp Gly Leu Lys Lys Ser Gly Met Thr
          45                  50                  55
```

Figure 10A

```
TTA ATT GGC TCT GAG CTT CGT CCT CTT AAG GTC ATG TCT TCT GTT TCC      305
Leu Ile Gly Ser Glu Leu Arg Pro Leu Lys Val Met Ser Ser Val Ser *
      60              65              70
ACG GCG GAG AAA GCG TCG GAG ATT GTA CTT CAA CCC ATT AGA GAA ATC      353
Thr Ala Glu Lys Ala Ser Glu Ile Val Leu Gln Pro Ile Arg Glu Ile
  75              80              85
TCC GGT CTT ATT AAG TTG CCT GGC TCC AAG TCT CTA TCA AAT AGA ATT      401
Ser Gly Leu Ile Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile
  90              95             100             105
C                                                                   402
```

Figure 10B

```
AGATCTTTCA AGA ATG GCA CAA ATT AAC AAC ATG GCT CAA GGG ATA CAA      49
           Met Ala Gln Ile Asn Asn Met Ala Gln Gly Ile Gln
            1               5                  10

ACC CTT AAT CCC AAT TCC AAT TTC CAT AAA CCC CAA GTT CCT AAA TGT     97
Thr Leu Asn Pro Asn Ser Asn Phe His Lys Pro Gln Val Pro Lys Ser
        15                  20                  25

TCA AGT TTT CTT GTT TTT GGA TCT AAA AAA CTG AAA AAT TCA GCA AAT    145
Ser Ser Phe Leu Val Phe Gly Ser Lys Lys Leu Lys Asn Ser Ala Asn
    30                  35                  40

TCT ATG TTG GTT TTG AAA AAA GAT TCA ATT TTT ATG CAA AAG TTT TGT    193
Ser Met Leu Val Leu Lys Lys Asp Ser Ile Phe Met Gln Lys Phe Cys
45                  50                  55                  60

TCC TTT AGG ATT TCA GCA TCA GTG GCT ACA GCC TGC ATG C              233
Ser Phe Arg Ile Ser Ala Ser Val Ala Thr Ala Cys Met
                65                  70
```

Figure 11

```
AGATCTGCTA GAAATAATTT TGTTTAACTT TAAGAAGGAG ATATATCC ATG GCA CAA      57
                                                     Met Ala Gln
                                                      1

ATT AAC AAC ATG GCT CAA GGG ATA CAA ACC CTT AAT CCC AAT TCC AAT      105
Ile Asn Asn Met Ala Gln Gly Ile Gln Thr Leu Asn Pro Asn Ser Asn
      5                  10                  15

TTC CAT AAA CCC CAA GTT CCT AAA TCT TCA AGT TTT CTT GTT TTT GGA      153
Phe His Lys Pro Gln Val Pro Lys Ser Ser Ser Phe Leu Val Phe Gly
 20                  25                  30                  35

TCT AAA AAA CTG AAA AAT TCA GCA AAT TCT ATG TTG GTT TTG AAA AAA      201
Ser Lys Lys Leu Lys Asn Ser Ala Asn Ser Met Leu Val Leu Lys Lys
                 40                  45                  50
```

Figure 12A

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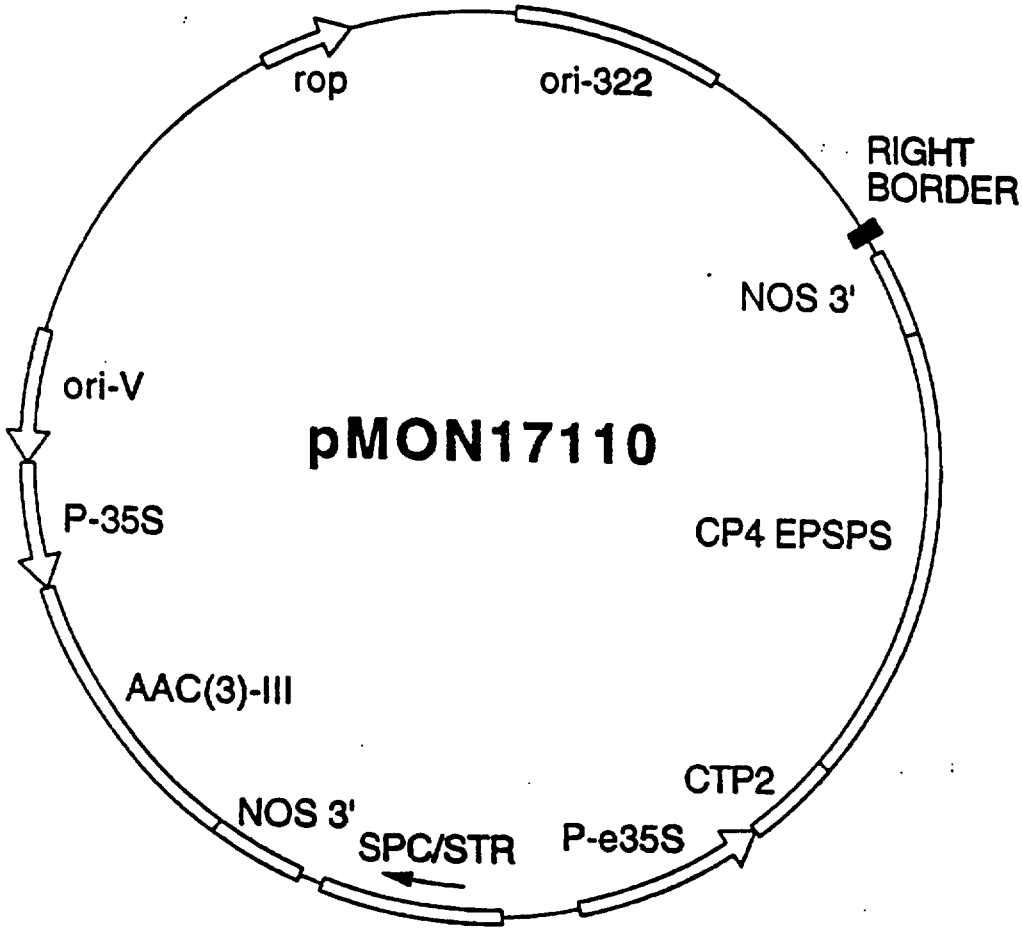
```
                                                                    249
GAT TCA ATT TTT ATG CAA AAG TTT TGT TCC TTT AGG ATT TCA GCA TCA
Asp Ser Ile Phe Met Gln Lys Phe Cys Ser Phe Arg Ile Ser Ala Ser
                55                  60                  65
                                                                    297
GTG GCT ACA GCA CAG AAG CCT TCT GAG ATA GTG TTG CAA CCC ATT AAA
Val Ala Thr Ala Gln Lys Pro Ser Glu Ile Val Leu Gln Pro Ile Lys
            70                  75                  80
                                                                    345
GAG ATT TCA GGC ACT GTT AAA TTG CCT GGC TCT AAA TCA TTA TCT AAT
Glu Ile Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn
        85                  90                  95
                                                                    352
AGA ATT C
Arg Ile
100
```

Figure 12B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RIGHT BORDER
NOS 3'
ori-V
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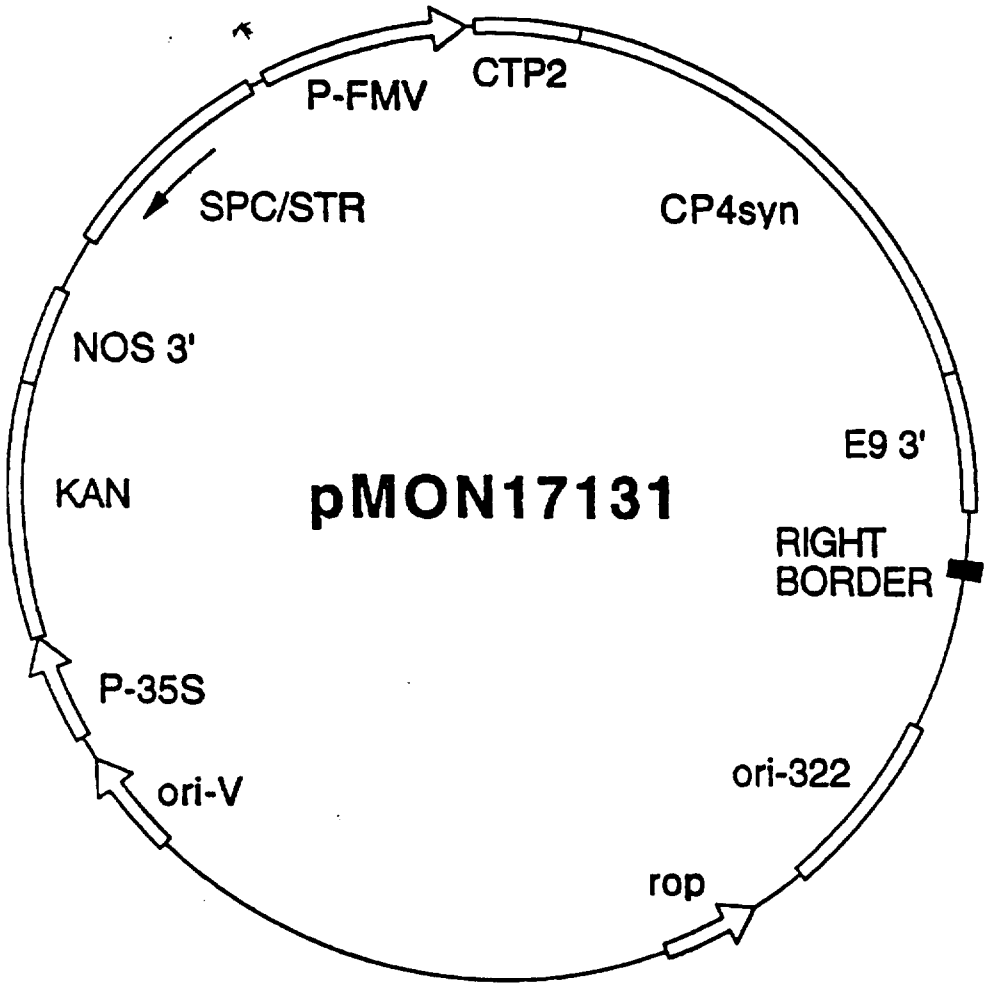
P-35S
CP4 EPSPS
AAC(3)-III
CTP2
NOS 3'
SPC/STR
P-e35S

Figure 13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CTP2
SPC/STR
CP4syn
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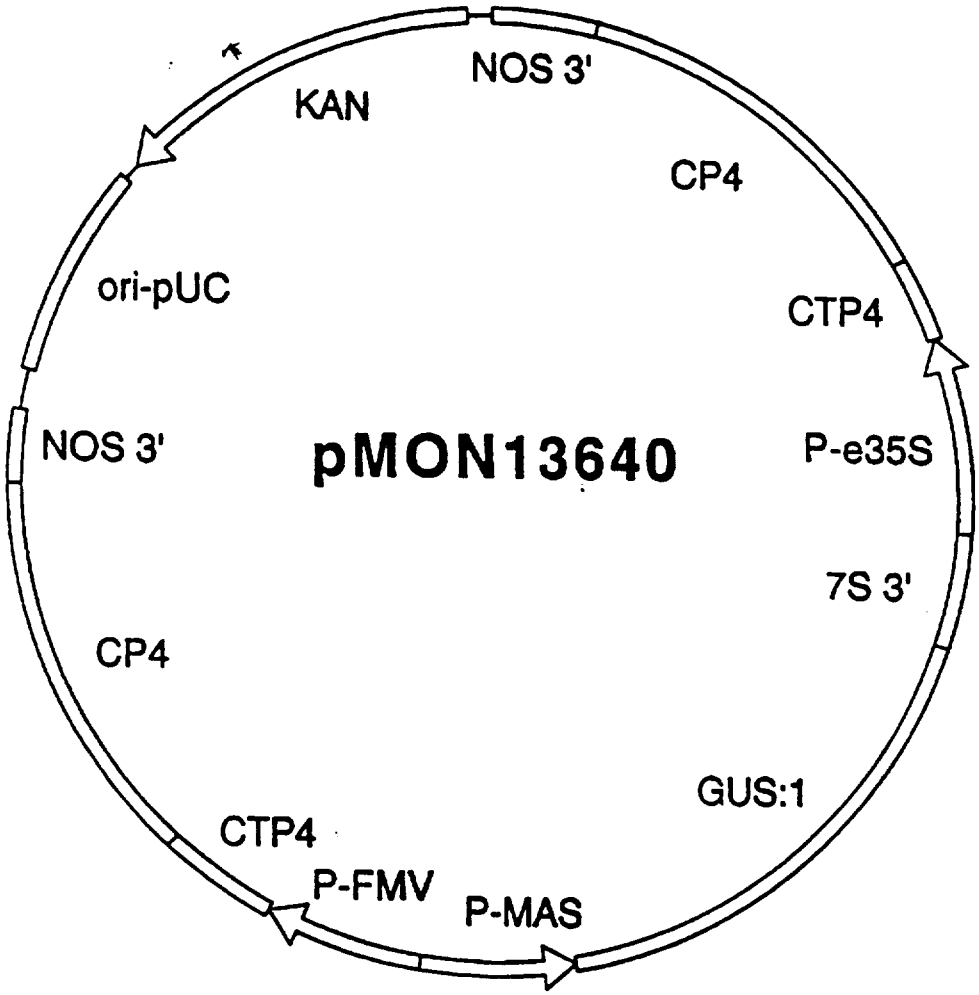
E9 3'
KAN
pMON17131
RIGHT BORDER
P-35S
ori-V
ori-322
rop

Figure 14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KAN
CP4
ori-pUC
CTP4
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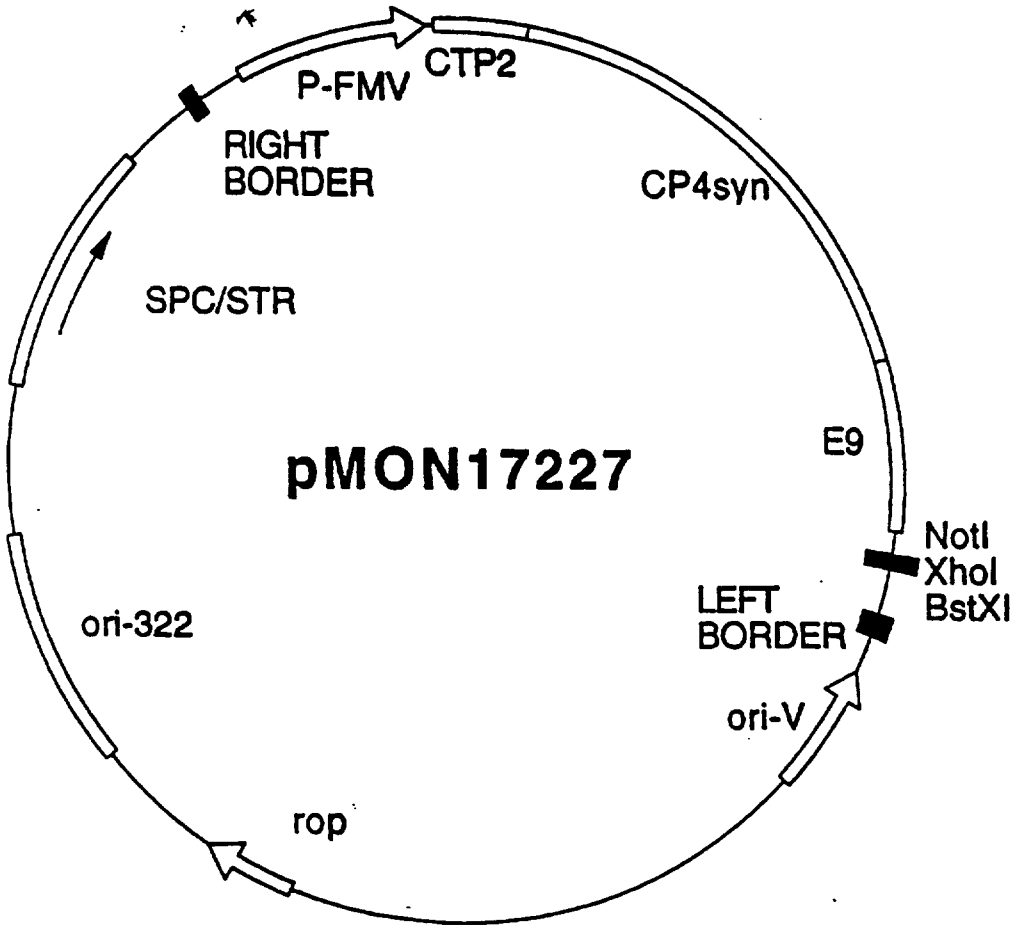
P-e35S
7S 3'
CP4
GUS:1
CTP4
P-FMV
P-MAS

Figure 15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CTP2
RIGHT
BORDER
CP4syn
SPC/STR
pMON17227
E9
NotI
XhoI
BstXI
LEFT
BORDER
ori-322
ori-V
rop

Figure 16

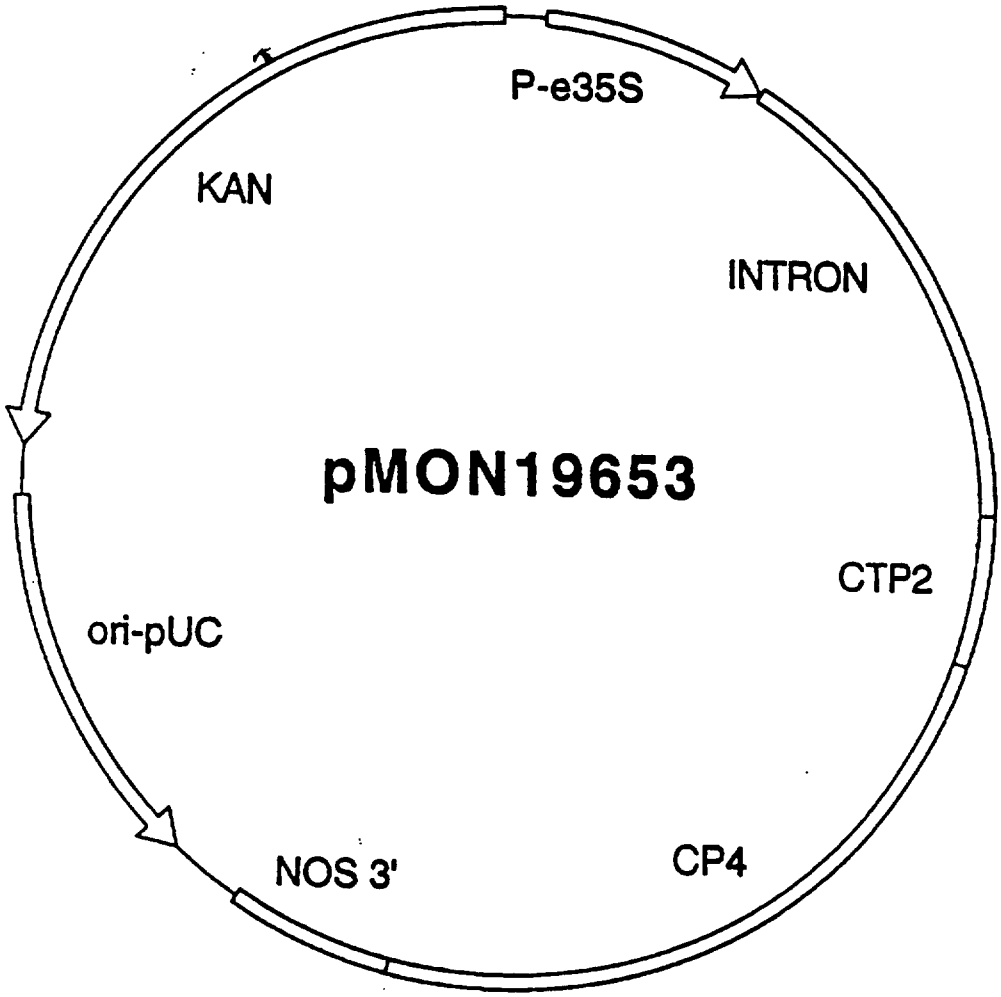

Figure 17

```
ATG AAA CGA GAT AAG GTG CAG ACC TTA CAT GGA GAA ATA CAT ATT CCC      48
Met Lys Arg Asp Lys Val Gln Thr Leu His Gly Glu Ile His Ile Pro
 1               5                  10                  15

GGT GAT AAA TCC ATT TCT CAC CGC TCT GTT ATG TTT GGC GCG CTA GCG      96
Gly Asp Lys Ser Ile Ser His Arg Ser Val Met Phe Gly Ala Leu Ala
            20                  25                  30

GCA GGC ACA ACA ACA GTT AAA AAC TTT CTG CCG GGA GCA GAT TGT CTG     144
Ala Gly Thr Thr Thr Val Lys Asn Phe Leu Pro Gly Ala Asp Cys Leu
        35                  40                  45

AGC ACG ATC GAT TGC TTT AGA AAA ATG GGT GTT CAC ATT GAG CAA AGC     192
Ser Thr Ile Asp Cys Phe Arg Lys Met Gly Val His Ile Glu Gln Ser
    50                  55                  60

AGC AGC GAT GTC GTG ATT CAC GGA AAA GGA ATC GAT GCC CTG AAA GAG     240
Ser Ser Asp Val Val Ile His Gly Lys Gly Ile Asp Ala Leu Lys Glu
65                  70                  75                  80

CCA GAA AGC CTT TTA GAT GTC GGA AAT TCA GGT ACA ACG ATT CGC CTG     288
Pro Glu Ser Leu Leu Asp Val Gly Asn Ser Gly Thr Thr Ile Arg Leu
                85                  90                  95

ATG CTC GGA ATA TTG GCG GGC CGT CCT TTT TAC AGC GCG GTA GCC GGA     336
Met Leu Gly Ile Leu Ala Gly Arg Pro Phe Tyr Ser Ala Val Ala Gly
            100                 105                 110
```

Figure 18A

```
GAT GAG AGC ATT GCG AAA CGC GCA ATG AAG CGT GTG ACT GAG CCT TTG   384
Asp Glu Ser Ile Ala Lys Arg Pro Met Lys Arg Val Thr Glu Pro Leu
        115                 120                 125

AAA AAA ATG GGG GCT AAA ATC GAC GGC AGA GCC GGC GGA GAG TTT ACA   432
Lys Lys Met Gly Ala Lys Ile Asp Gly Arg Ala Gly Gly Glu Phe Thr
    130                 135                 140

CCG CTG TCA GTG AGC GGC GCT TCA TTA AAA GGA ATT GAT TAT GTA TCA   480
Pro Leu Ser Val Ser Gly Ala Ser Leu Lys Gly Ile Asp Tyr Val Ser
145                 150                 155                 160

CCT GTT GCA AGC GCG CAA ATT AAA TCT GCT GTT TTG CTG GCC GGA TTA   528
Pro Val Ala Ser Ala Gln Ile Lys Ser Ala Val Leu Leu Ala Gly Leu
                165                 170                 175

CAG GCT GAG GGC ACA ACA ACT GTA ACA GAG CCC CAT AAA TCT CGG GAC   576
Gln Ala Glu Gly Thr Thr Thr Val Thr Glu Pro His Lys Ser Arg Asp
            180                 185                 190

CAC ACT GAG CGG ATG CTT TCT GCT TTT GGC GTT AAG CTT TCT GAA GAT   624
His Thr Glu Arg Met Leu Ser Ala Phe Gly Val Lys Leu Ser Glu Asp
        195                 200                 205

CAA ACG AGT GTT TCC ATT GCT GGT GGC CAG AAA CTG ACA ACA GCT GAT   672
Gln Thr Ser Val Ser Ile Ala Gly Gly Gln Lys Leu Thr Ala Ala Asp
    210                 215                 220
```

Figure 18B

```
ATT TTT GTT CCT GGA GAC ATT TCT TCA GCC GCG TTT TTC CTT GCT GCT    720
Ile Phe Val Pro Gly Asp Ile Ser Ser Ala Ala Phe Phe Leu Ala Ala
225                 230                 235                 240

GGC GCG ATG GTT CCA AAC AGC AGA ATT GTA TTG AAA AAC GTA GGT TTA    768
Gly Ala Met Val Pro Asn Ser Arg Ile Val Leu Lys Asn Val Gly Leu
                245                 250                 255

AAT CCG ACT CGG ACA GGT ATT ATT GAT GTC CTT CAA AAC ATG GGG GCA    816
Asn Pro Thr Arg Thr Gly Ile Ile Asp Val Leu Gln Asn Met Gly Ala
            260                 265                 270

AAA CTT GAA ATC AAA CCA TCT GCT GAT AGC GGT GCA GAG CCT TAT GGA    864
Lys Leu Glu Ile Lys Pro Ser Ala Asp Ser Gly Ala Glu Pro Tyr Gly
        275                 280                 285

GAT TTG ATT ATA GAA ACG TCA TCT CTA AAG GCA GTT GAA ATC GGA GGA    912
Asp Leu Ile Ile Glu Thr Ser Ser Leu Lys Ala Val Glu Ile Gly Gly
    290                 295                 300

GAT ATC ATT CCG CGT TTA ATT GAT GAG ATC CCT ATC ATC GCG CTT CTT    960
Asp Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro Ile Ile Ala Leu Leu
305                 310                 315                 320

GCG ACT CAG GCG GAA GGA ACC ACC GTT ATT AAG GAC GCG GCA GAG CTA   1008
Ala Thr Gln Ala Glu Gly Thr Thr Val Ile Lys Asp Ala Ala Glu Leu
                325                 330                 335
```

Figure 18C

```
AAA GTG AAA GAA ACA AAC CGT ATT GAT ACT GTT GTT TCT GAG CTT CGC    1056
Lys Val Lys Glu Thr Asn Arg Ile Asp Thr Val Val Ser Glu Leu Arg
            340                 345                 350

AAG CTG GGT GCT GAA ATT GAA CCG ACA GCA GAT GGA ATG AAG GTT TAT    1104
Lys Leu Gly Ala Glu Ile Glu Pro Thr Ala Asp Gly Met Lys Val Tyr
        355                 360                 365

GGC AAA CAA ACG TTG AAA GGC GGC GCT GCA GTG TCC AGC CAC GGA GAT    1152
Gly Lys Gln Thr Leu Lys Gly Gly Ala Ala Val Ser Ser His Gly Asp
    370                 375                 380

CAT CGA ATC GGA ATG CTT GGT ATT GCT TCC TGT ATA ACG GAG GAG    1200
His Arg Ile Gly Met Leu Gly Ile Ala Ser Cys Ile Thr Glu Glu
385                 390                 395                 400

CCG ATT GAA ATC GAG CAC ACG GAT GCC ATT CAC GTT TCT TAT CCA ACC    1248
Pro Ile Glu Ile Glu His Thr Asp Ala Ile His Val Ser Tyr Pro Thr
                405                 410                 415

TTC TTC GAG CAT TTA AAT AAG CTT TCG AAA AAA TCC TGA    1287
Phe Phe Glu His Leu Asn Lys Leu Ser Lys Lys Ser
            420                 425
```

Figure 18D

```
ATG GTA AAT GAA CAA ATC ATT GAT ATT TCA GGT CCG TTA AAG GGC GAA   48
Met Val Asn Glu Gln Ile Ile Asp Ile Ser Gly Pro Leu Lys Gly Glu
 1               5                  10                  15

ATA GAA GTG CCG GGC GAT AAG TCA ATG ACA CAC CGT GCA ATC ATG TTG   96
Ile Glu Val Pro Gly Asp Lys Ser Met Thr His Arg Ala Ile Met Leu
            20                  25                  30

GCG TCG CTA GCT GAA GGT GTA TCT ACT ATA TAT AAG CCA CTA CTT GGC  144
Ala Ser Leu Ala Glu Gly Val Ser Thr Ile Tyr Lys Pro Leu Leu Gly
        35                  40                  45

GAA GAT TGT CGT CGT ACG ATG GAC ATT TTC CGA CAC TTA GGT GTA GAA  192
Glu Asp Cys Arg Arg Thr Met Asp Ile Phe Arg His Leu Gly Val Glu
    50                  55                  60

ATC AAA GAA GAT GAT GAA AAA TTA GTT GTG ACT TCC CCA GGA TAT CAA  240
Ile Lys Glu Asp Asp Glu Lys Leu Val Val Thr Ser Pro Gly Tyr Gln
65                  70                  75                  80

GTT AAC ACG CCA CAT CAA GTA TTG TAT ACA GGT AAT TCT GGT ACG ACA  288
Val Asn Thr Pro His Gln Val Leu Tyr Thr Gly Asn Ser Gly Thr Thr
                85                  90                  95

ACA CGA TTA TTG GCA GGT TTG TTA AGT GGT TTA GGT AAT GAA AGT GTT  336
Thr Arg Leu Leu Ala Gly Leu Leu Ser Gly Leu Gly Asn Glu Ser Val
            100                 105                 110
```

Figure 19A

```
                                                                    384
TTG TCT GGC GAT GTT TCA ATT GGT AAA AGG CCA ATG GAT CGT GTC TTG
Leu Ser Gly Asp Val Ser Ile Gly Lys Arg Pro Met Asp Arg Val Leu
        115                 120                 125

                                                                    432
AGA CCA TTG AAA CTT ATG GAT GCG AAT ATT GAA GGT ATT GAA GAT AAT
Arg Pro Leu Lys Leu Met Asp Ala Asn Ile Glu Gly Ile Glu Asp Asn
    130                 135                 140

                                                                    480
TAT ACA CCA TTA ATT ATT AAG CCA TCT GTC ATA AAA GGT ATA AAT TAT
Tyr Thr Pro Leu Ile Ile Lys Pro Ser Val Ile Lys Gly Ile Asn Tyr
145                 150                 155                 160

                                                                    528
CAA ATG GAA GTT GCA AGT GCA CAA GTA AAA AGT GCC ATT TTA TTT GCA
Gln Met Glu Val Ala Ser Ala Gln Val Lys Ser Ala Ile Leu Phe Ala
                165                 170                 175

                                                                    576
AGT TTG TTT TCT AAG GAA CCG ACC ATC ATT AAA GAA TTA GAT GTA AGT
Ser Leu Phe Ser Lys Glu Pro Thr Ile Ile Lys Glu Leu Asp Val Ser
            180                 185                 190

                                                                    624
CGA AAT CAT ACT GAG ACG ATG TTC AAA CAT TTT AAT ATT CCA ATT GAA
Arg Asn His Thr Glu Thr Met Phe Lys His Phe Asn Ile Pro Ile Glu
        195                 200                 205

                                                                    672
GCA GAA GGG TTA TCA ATT AAT ACA ACC CCT GAA GCA ATT CGA TAC ATT
Ala Glu Gly Leu Ser Ile Asn Thr Thr Pro Glu Ala Ile Arg Tyr Ile
    210                 215                 220
```

Figure 19B

```
AAA CCT GCA GAT TTT CAT GTT CCT GGC GAT ATT TCA TCT GCA GCG TTC      720
Lys Pro Ala Asp Phe His Val Pro Gly Asp Ile Ser Ser Ala Ala Phe
225                 230                 235                 240

TTT ATT GTT GCA GCA CTT ATC ACA CCA GGA AGT GAT GTA ACA ATT CAT      768
Phe Ile Val Ala Ala Leu Ile Thr Pro Gly Ser Asp Val Thr Ile His
                245                 250                 255

AAT GTT GGA ATC AAT CAA ACA CGT TCA GGT ATT ATT GAT ATT GTT GAA      816
Asn Val Gly Ile Asn Gln Thr Arg Ser Gly Ile Ile Asp Ile Val Glu
            260                 265                 270

AAA ATG GGC GGT AAT ATC CAA CTT TTC AAT CAA ACA ACT GGT GCT GAA      864
Lys Met Gly Gly Asn Ile Gln Leu Phe Asn Gln Thr Thr Gly Ala Glu
        275                 280                 285

CCT ACT GCT TCT ATT CGT ATT CAA TAC ACA CCA ATG CTT CAA CCA ATA      912
Pro Thr Ala Ser Ile Arg Ile Gln Tyr Thr Pro Met Leu Gln Pro Ile
    290                 295                 300

ACA ATC GAA GGA GAA TTA GTT CCA AAA GCA ATT GAT GAA CTG CCT GTA      960
Thr Ile Glu Gly Glu Leu Val Pro Lys Ala Ile Asp Glu Leu Pro Val
305                 310                 315                 320

ATA GCA TTA CTT TGT ACA CAA GCA GTT GGC ACG AGT ACA ATT AAA GAT     1008
Ile Ala Leu Leu Cys Thr Gln Ala Val Gly Thr Ser Thr Ile Lys Asp
                325                 330                 335
```

Figure 19C

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```
GCC GAG GAA TTA AAA GTA AAA GAA ACA AAT AGA ATT GAT ACA ACG GCT  1056
Ala Glu Glu Leu Lys Val Lys Glu Thr Asn Arg Ile Asp Thr Thr Ala
        340                 345                 350

GAT ATG TTA AAC TTG TTA GGG TTT GAA TTA CAA CCA ACT AAT GAT GGA  1104
Asp Met Leu Asn Leu Leu Gly Phe Glu Leu Gln Pro Thr Asn Asp Gly
    355                 360                 365

TTG ATT ATT CAT CCG TCA GAA TTT AAA ACA AAT GCA ACA GAT ATT TTA  1152
Leu Ile Ile His Pro Ser Glu Phe Lys Thr Asn Ala Thr Asp Ile Leu
370                 375                 380

ACT GAT CAT CGA ATA GGA ATG ATG CTT GCA GTT GCT TGT GTA CTT TCA  1200
Thr Asp His Arg Ile Gly Met Met Leu Ala Val Ala Cys Val Leu Ser
385                 390                 395                 400

AGC GAG CCT GTC AAA ATC AAA CAA TTT GAT GCT GTA AAT GTA TCA TTT  1248
Ser Glu Pro Val Lys Ile Lys Gln Phe Asp Ala Val Asn Val Ser Phe
                405                 410                 415

CCA GGA TTT TTA CCA AAA CTA AAG CTT TTA CAA AAT GAG GGA TAA     1293
Pro Gly Phe Leu Pro Lys Leu Lys Leu Leu Gln Asn Glu Gly
            420                 425                 430
```

Figure 19D

	1				50
PG2982	....	....	....	MSHSASPKPA	TARRSEALTG
LBAA	....	....	....	MSHSASPKPA	TARRSEALTG
Agrobacterium CP4	....	....	....	MSHGASSRPA	TARKSSGLSG
B. subtilis	....	....	....	.........M	KRDKVQTLHG
S. aureus	....	....	....	.....MVNEQ	IIDISGPLKG
S. cerevisiae	....	....	....	......LVYP	FKDIPADQQK
A. nidulans	....	....	....	.......VHP	..GVAHSSNV
B. napus	....	....	....	.K....ASEI	VLQPIREISG
A. thaliana	....	....	....	.K....ASEI	VLQPIREISG
N. tabacum	....	....	....	.K....PNEI	VLQPIKDISG
L. esculentum	....	....	....	.K....PHEI	VLXPIKDISG
P. hybrida	....	....	....	.K....PSEI	VLQPIKEISG
Z. mays	....	....	....	....AGAEEI	VLQPIKEISG
S. gallinarum	....	....	....	......MESL	TLQPIARVDG
S. typhimurium	....	....	....	......MESL	TLQPIARVDG
S. typhi	....	....	....	......MESL	TLQPIARVDG
E. coli	....	....	....	......MESL	TLQPIARVDG
K. pneumoniae	....	....	....	......MESL	TLQPIARVDG
Y. entoercolitica	....	....	....	.....MLESL	TLHPIALING
H. influenzae	....	....	....	......MEKI	TLAPISAVEG
P. multocida	....	....	....	..MIKDATAI	TLNPISYIEG
A. salmonicida	....	....	....	.......NSL	RLEPISRVAG
B. pertussis	....	....	....	...MSGLAYL	DLPAARLARG
Consensus	----------	----------	----------	----------	----------

Figure 20A

	51				100
PG2982	EIRIPGDKSI	SHRSFMFGGL	ASGETRITGL	LEGEDVINTG	RAMQAM.GAK
LBAA	EIRIPGDKSI	SHRSFMFGGL	ASGETRITGL	LEGEDVINTG	RAMQAM.GAK
Agrobacterium CP4	TVRIPGDKSI	SHRSFMFGGL	ASGETRITGL	LEGEDVINTG	KAMQAM.GAR
B. subtilis	EIHIPGDKSI	SHRSVMFGAL	AAGTTTVKNF	LPGADCLSTI	DCFRKM.GVH
S. aureus	EIEVPGDKSM	THRAIMLASL	AEGVSTIYKP	LLGEDCRRTM	DIFRHL.GVE
S. cerevisiae	VVIPPGSKSI	SNRALILAAL	GEGQCKIKNL	LHSDDTKHML	TAVHELKGAT
A. nidulans	ICAPPGSKSI	SNRALVLAAL	GSGTCRIKNL	LHSDDTEVML	NALERLGAAT
B. napus	LIKLPGSKSL	SNRILLLAAL	SEGTTVVDNL	LNSDDINYML	DALKKL.GLN
A. thaliana	LIKLPGSKSL	SNRILLLAAL	SEGTTVVDNL	LNSDDINYML	DALKRL.GLN
N. tabacum	TVKLPGSKSL	SNRILLLAAL	SKGRTVVDNL	LSSDDIHYML	GALKTL.GLH
L. esculentum	TVKLPGSKSL	SNRILLLAAL	SEGRTVVDNL	LSSDDIHYML	GALKTL.GLH
P. hybrida	TVKLPGSKSL	SNRILLLAAL	SEGTTVVDNL	LSSDDIHYML	GALKTL.GLH
Z. mays	TVKLPGSKSL	SNRILLLAAL	SEGTTVVDNL	LNSEDVHYML	GALRTL.GLS
S. gallinarum	AINLPGSKSV	SNRALLLAAL	ACGKTVLTNL	LDSDDVRHML	NALSAL.GIN
S. typhimurium	AINLPGSKSV	SNRALLLAAL	PCGKTALTNL	LDSDDVRHML	NALSAL.GIN
S. typhi	AINLPGSKSV	SNRALLLAAL	ACGKTVLTNL	LDSDDVRHML	NALSAL.GIN
E. coli	TINLPGSKTV	SNRALLLAAL	AHGKTVLTNL	LDSDDVRHML	NALTAL.GVS
K. pneumoniae	TVNLPGSKSV	SNRALLLAAL	ARGTTVLTNL	LDSDDVRHML	NALSAL.GVH
Y. entoercolitica	TVNLPGSKSV	SNRALLLAAL	AEGTTQLNNL	LDSDDIRHML	NALQAL.GVK
H. influenzae	TINLPGSKSL	SNRALLLAAL	AKGTTKVTNL	LDSDDIRHML	NALKAL.GVR
P. multocida	EVRLPGSKSL	SNRALLLSAL	AKGKTTLTNL	LDSDDVRHML	NALKEL.GVT
A. salmonicida	EVNLPGSKSV	SNRALLLAAL	ARGTTRLTNL	LDSDDIRHML	AALTQL.GVK
B. pertussis	EVALPGSKSI	SNRVLLLAAL	AEGSTEITGL	LDSDDTRVML	AALRQL.GVS
Consensus	----PG-K--	--R------L	--G-------	L---D-----	----------

Figure 20B

	101				150
PG2982	IRKEGDVWII	NGVGNGCLLQ	P......EAA	LDFGNAGTGA	RLTMGLVGTY
LBAA	IRKEGDVWII	NGVGNGCLLQ	P......EAA	LDFGNAGTGA	RLTMGLVGTY
Agrobacterium CP4	IRKEGDTWII	DGVGNGGLLA	P......EAP	LDFGNAATGC	RLTMGLVGVY
B. subtilis	IEQSSSDVVI	HGKGIDALKE	P......ESL	LDVGNSGTTI	RLMLGILAGR
S. aureus	IKEDDEKLVV	TSPGYQ.VNT	P......HQV	LYTGNSGTTT	RLLAGLLSGL
S. cerevisiae	ISWEDNGETV	VVEGHGG...	.STLSACADP	LYLGNAGTAS	RFLTSLAALV
A. nidulans	FSWEEEGEVL	VVNGKGG...	..NLQASSSP	LYLGNAGTAS	RFLTTVATLA
B. napus	VERDSVNNRA	VVEGCGGIFP	ASLDSKSDIE	LYLGNAGTAM	RPLTAAVTAA
A. thaliana	VETDSENNRA	VVEGCGGIFP	ASIDSKSDIE	LYLGNAGTAM	RPLTAAVTAA
N. tabacum	VEDDNENQRA	IVEGCGGQFP	VGKKSEEEIQ	LFLGNAGTAM	RPLTAAVTVA
L. esculentum	VEDDNENQRA	IVEGCGGQFP	VGKKSEEEIQ	LFLGNAGTAM	RPLTAAVTVA
P. hybrida	VEEDSANQRA	VVEGCGGLFP	VGKESKEEIQ	LFLGNAGTAM	RPLTAAVTVA
Z. mays	VEADKAAKRA	VVVGCGGKFP	VE.DAKEEVQ	LFLGNAGTAM	RPLTAAVTAA
S. gallinarum	YTLSADRTRC	DITGNGGPLR	AP....GALE	LFLGNAGTAM	RPLAAALCL.
S. typhimurium	YTLSADRTRC	DITGNGGALR	AP....GALE	LFLGNAGTAM	RPLAAALCL.
S. typhi	YTLSADRTRC	DITGNGGPLR	AS....GTLE	LFLGNAGTAM	RPLAAALCL.
E. coli	YTLSADRTRC	EIIGNGGPLH	AE....GALE	LFLGNAGTAM	RPLAAALCL.
K. pneumoniae	YVLSSDRTRC	EVTGTGGPLQ	AG....SALE	LFLGNAGTAM	RPLAAALCL.
Y. entoercolitica	YRLSADRTRC	EVDGLGGKLV	AE....QPLE	LFLGNAGTAM	RPLAAALCL.
H. influenzae	YQLSDDKTIC	EIEGLGGAFN	IQ....DNLS	LFLGNAGTAM	RPLTAALCLK
P. multocida	YQLSEDKSVC	EIEGLGRAFE	WQ....SGLA	LFLGNAGTAM	RPLTAALCLS
A. salmonicida	YKLSADKTEC	TVHGLGRSFA	VS....APVN	LFLGNAGTAM	RPLCAALCL.
B. pertussis	VGEVAD..GC	VTIEGVARFP	TE.....QAE	LFLGNAGTAF	RPLTAALALM
Consensus	----------	----------	----------	L--GN--T--	R---------

Figure 20C

	151				200
PG2982	DM......KT	SFIGDASLSK	RPMGRVLNPL	REMGVQVEAA	DGDRMPLT..
LBAA	DM......KT	SFIGDASLSK	RPMGRVLNPL	REMGVQVEAA	DGDRMPLT..
Agrobacterium CP4	DF......DS	TFIGDASLTK	RPMGRVLNPL	REMGVQVKSE	DGDRLPVT..
B. subtilis	PF......YS	AVAGDESIAK	RPMKRVTEPL	KKMGAKIDGR	AGGEFTPL..
S. aureus	GN......ES	VLSGDVSIGK	RPMDRVLRPL	KLMDANIEG.	IEDNYTPL..
S. cerevisiae	NST.SSQKYI	VLTGNARMQQ	RPIAPLVDSL	RANGTKIEYL	NNEGSBPIKV
A. nidulans	NS..STVDSS	VLTGNNRMKQ	RPIGDLVDAL	TANVLPLNTS	KGRASLPLKI
B. napus	G....GNASY	VLDGVPRMRE	RPIGDLVVGL	KQLGADVECT	LGTNCPPVRV
A. thaliana	G....GNASY	VLDGVPRMRE	RPIGDLVVGL	KQLGADVECT	LGTNCPPVRV
N. tabacum	G....GHSRY	VLDGVPRMRE	RPIGDLVDGL	KQLGAEVDCF	LGTNCPPVRI
L. esculentum	G....GHSRY	VLDGVPRMRE	RPIGDLVDGL	KQLGAEVDCS	LGTNCPPVRI
P. hybrida	G....GNSRY	VLDGVPRMRE	RPISDLVDGL	KQLGAEVDCF	LGTKCPPVRI
Z. mays	G....GNATY	VLDGVPRMRE	RPIGDLVVGL	KQLGADVDCF	LGTDCPPVRV
S. gallinarum	.....GQNEI	VLTGEPRMKE	RPIGHLVDSL	RQGGANIDYL	EQENYPPLRL
S. typhimurium	.....GQNEI	VLTGEPRMKE	RPIGHLVDSL	RQGGANIDYL	EQENYPPLRL
S. typhi	.....GQNEI	VLTGEPRMKE	RPIGHLVDSL	RQGGANIDYL	EQENYPPLRL
E. coli	.....GSNDI	VLTGEPRMKE	RPIGHLVDAL	RLGGAKITYL	EQENYPPLRL
K. pneumoniae	.....GSNDI	VLTGEPRMKE	RPIGHLVDAL	RQGGAQIDYL	EQENYPPLRL
Y. entoercolitica	.....GKNDI	VLTGEPRMKE	RPIGHLVDAL	RQGGAQIDYL	EQENYRR.CI
H. influenzae	G.NHEV..EI	ILTGEPRMKE	RPILHLVDAL	RQAGADIRYL	ENEGYPPLAI
P. multocida	TPNREGKNEI	VLTGEPRMKE	RPIQHLVDAL	CQAGAEIQYL	EQEGYPPIAI
A. salmonicida	.....GSGEY	MLGGEPRMEE	RPIGHLVDCL	ALKGAHIQYL	KKDGYPPLVV
B. pertussis	G......GDY	RLSGVPRMHE	RPIGDLVDAL	RQFGAGIEYL	GQAGYPPLRI
Consensus	----------	---G------	RP-------L	----------	----------

Figure 20D

	201				250
PG2982	.....LIGPK	TANPITYRVP	MASAQVKSAV	LLAGLN....	....TPGVTT
LBAA	.....LIGPK	TANPITYRVP	MASAQVKSAV	LLAGLN....	....TPGVTT
Agrobacterium CP4	.....LRGPK	TPTPITYRVP	MASAQVKSAV	LLAGLN....	....TPGITT
B. subtilis	.....SVSGA	SLKGIDYVSP	VASAQIKSAV	LLAGLQ....	....AEGTTT
S. aureus	.....IIKPS	VIKGINYQME	VASAQVKSAI	LFASLF....	....SKEPTI
S. cerevisiae	YTDSVFKG..	...GRIELAA	TVSSQYVSSI	LMCAPYAE..	.EPVTLALVG
A. nidulans	AASGGFAG..	...GNINLAA	KVSSQYVSSL	LMCAPYAK..	.EPVTLRLVG
B. napus	NANGGLPG..	...GKVKLSG	SISSQYLTAL	LMAAP.LA..	.LGDVEIEII
A. thaliana	NANGGLPG..	...GKVKLSG	SISSQYLTAL	LMSAP.LA..	.LGDVEIEIV
N. tabacum	VSKGGLPG..	...GKVKLSG	SISSQYLTAL	LMAAP.LA..	.LGDVEIEII
L. esculentum	VSKGGLPG..	...GKVKLSG	SISSQYLTAL	LMAAP.LA..	.LGDVEIEII
P. hybrida	VSKGGLPG..	...GKVKLSG	SISSQYLTAL	LMAAP.LA..	.LGDVEIEII
Z. mays	NGIGGLPG..	...GKVKLSG	SISSQYLSAL	LMAAP.LP..	.LGDVEIEII
S. gallinarum	RG..GFIG..	...GDIEVDG	SVSSQFLTAL	LMTAP.LA..	.PKDTIIRVK
S. typhimurium	RG..GFTG..	...GDIEVDG	SVSSQFLTAL	LMTAP.LA..	.PKDTIIRVK
S. typhi	RG..GFIG..	...GDIEVDG	SVSSQFLTAL	LMTAP.LA..	.PEDTIIRVK
E. coli	QG..GFTG..	...GNVDVDG	SVSSQFLTAL	LMTAP.LA..	.PEDTVIRIK
K. pneumoniae	RG..GFTG..	...GDVEVDG	SVSSQFLTAL	LMASP.LA..	.PQDTVIAIK
Y. entoercolitica	AG..GFRG..	...GKLTVDG	SVSSQFLTAL	LMTAP.LA..	.EQDTEIQIQ
H. influenzae	RNK.GIKG..	...GKVKIDG	SISSQFLTAL	LMSAP.LA..	.ENDTEIEII
P. multocida	RNT.GLKG..	...GRIQIDG	SVSSQFLTAL	LMAAP.MA..	.EADTEIEII
A. salmonicida	DAK.GLWG..	...GDVHVDG	SVSSQFLTAF	LMAAPAMA..	.PVIPRIHIK
B. pertussis	GGGSIRVD..	...GPVRVEG	SVSSQFLTAL	LMAAPVLARR	SGQDITIEVV
Consensus	----------	----------	--S-Q-----	L---------	----------

Figure 20E

	251				300
PG2982	VIEPVMTRDH	TEKMLQGFGA	DLTVETDKDG	VRHIRITGQG	KLVGQ.TIDV
LBAA	VIEPVMTRDH	TEKMLQGFGA	DLTVETDKDG	VRHIRITGQG	KLVGQ.TIDV
Agrobacterium CP4	VIEPIMTRDH	TEKMLQGFGA	NLTVETDADG	VRTIRLEGRG	KLTGQ.VIDV
B. subtilis	VTEPHKSRDH	TERMLSAFGV	KLSEDQTS..	...VSIAGGQ	KLTAA.DIFV
S. aureus	IKELDVSRNH	TETMFKHFNI	PIEAEGLS..	..INTTPEAI	RYIKPADFHV
S. cerevisiae	GKPISKLYVD	MTIKMMEKFG	IN.VET.STT	EPYTYYIPKG	HYINPSEYVI
A. nidulans	GKPISQPYID	MTTAMMRSFG	ID..VQKSTT	EEHTYHIPQG	RYVNPAEYVI
B. napus	DKLISVPYVE	MTLKLMERFG	VS..AEHSDS	WDRFFVKGGQ	KYKSPGNAYV
A. thaliana	DKLISVPYVE	MTLKLMERFG	VS..VEHSDS	WDRFFVKGGQ	KYKSPGNAYV
N. tabacum	DKLISVPYVE	MTLKLMERFG	VS..VEHTSS	WDKFLVRGGQ	KYKSPGKAYV
L. esculentum	DKLISVPYVE	MTLKLMERFG	VF..VEHSSG	WDRFLVKGGQ	KYKSPGKAFV
P. hybrida	DKLISVPYVE	MTLKLMERFG	IS..VEHSSS	WDRFFVRGGQ	KYKSPGKAFV
Z. mays	DKLISIPYVE	MTLRLMERFG	VK..AEHSDS	WDRFYIKGGQ	KYKSPKNAYV
S. gallinarum	GELVSKPYID	ITLNLMKTFG	VE..IAN.HH	YQQFVVKGGQ	QYHSPGRYLV
S. typhimurium	GELVSKPYID	ITLNLMKTFG	VE..IAN.HH	YQQFVVKGGQ	QYHSPGRYLV
S. typhi	GELVSKPYID	ITLNLMKTFG	VE..IAN.HH	YQQFVVKGGQ	QYHSPGRYLV
E. coli	GELVSKPYID	ITLNLMKTFG	VE..IEN.QH	YQQFVVKGGQ	SYQSPGTYLV
K. pneumoniae	GDLVSKPYID	ITLNLMKTFG	VE..VEN.QA	YQRFIVRGNQ	QYQSPGDYLV
Y. entoercolitica	GELVSRPYID	ITLHLMKTFG	VE..VEN.QA	YQIFHIKGGQ	TYRSPGIYLV
H. influenzae	GELVSKPYID	ITLHLMKAFG	VD..VVH.EN	YQKFQVKGNQ	SYISPNKYLV
P. multocida	GELVSKPYID	ITLAMMRDFG	VK..VEN.HH	YQRFLVKGHQ	QYQSPHRFLV
A. salmonicida	GELVSKPYID	ITLKMMQTFG	VE..VEN.QA	YKLFYIKGNQ	SIVSPGDFLV
B. pertussis	GELISKPYIE	ITLHIMNSSG	VV..IEH.DN	WRAFTIARDA	VYRGPGRMAI
Consensus	----------	ITLNLMARFG	VS..V.RRDG	----------	----------

Figure 20F

```
                                                                  350
301
PG2982          PGDPSSTAFP LVAALLVEGS DVTIRNVLMN PTRTGL...I LTLQEMGADI
LBAA            PGDPSSTAFP LVAALLVEGS DVTIRNVLMN PTRTGL...I LTLQEMGADI
Agrobacterium CP4 PGDPSSTAFP LVAALLVPGS DVTILNVLMN PTRTGL...I LTLQEMGADI
B. subtilis     PGDISSAAFF LAAGAMVPNS RIVLKNVGLN PTRTGI...I DVLQNMGAKL
S. aureus       PGDISSAAFF IVAALITPGS DVTIHNVGIN QTRSGI...I DIVEKMGGNI
S. cerevisiae   ESDASSATYP LAFAA.MTGT TVTVPNIGFE SLQGDARFAR DVLKPMGCKI
A. nidulans     ESDASCATYP LAVAA.VTGT TCTVPNIGSA SLQGDARFAV EVLRPMGCTV
B. napus        EGDASSASYF LAGAA.ITGE TVTVEGCGTT SLQGDVKFA. EVLEKMGCKV
A. thaliana     EGDASSASYF LAGAA.ITGE TVTVEGCGTT SLQGDVKFA. EVLEKMGCKV
N. tabacum      EGDASSASYF LAGAA.VTGG TVTVEGCGTS SLQGDVKFA. EVLEKMGAEV
L. esculentum   EGDASSASYF LAGAA.VTGG TVTVEGCGTS SLQGDVKFA. EVLEKMGAEV
P. hybrida      EGDASSASYF LAGAA.VTGG TITVEGCGTN SLQGDVKFA. EVLEKMGAEV
Z. mays         EGDASSASYF LAGAA.ITGG TVTVEGCGTT SLQGDVKFA. EVLEMMGAKV
S. gallinarum   EGDASSASYF LAAGA.IKGG TVKVTGIGRK SMQGDIRFA. DVLEKMGATI
S. typhimurium  EGDASSASYF LAAGA.IKGG TVKVTGIGRK SMQGDIRFA. DVLHKMGATI
S. typhi        EGDASSASYF LAAGG.IKGG TVKVTGIGGK SMQGDIRFA. DVLEKMGATI
E. coli         EGDASSASYF LAAAA.IKGG TVKVTGIGRN SMQGDIRFA. DVLEKMGATI
K. pneumoniae   EGDASSASYF LAAGA.IKGG TVKVTGIGRN SVQGDIRFA. DVLEKMGATV
Y. entoercolitica EGDASSASYF LAAAA.IKGG TVRVTGIGKQ SVQGDTKFA. DVLEKMGAKI
H. influenzae   EGDASSASYF LAAGA.IK.G KVKVTGIGKN SIQGDRLFA. DVLEKMGAKI
P. multocida    EGDASSASYF LAAAA.IK.G KVKVTGVGKN SIQGDRLFA. DVLEKMGAHI
A. salmonicida  EGDASSASYF LAAGA.IK.G KVRVTGIGKH SI.GDIHFA. DVLERMGARI
B. pertussis    EGDASTASYF LALGA.IGGG PVRVTGVGED SIQGDVAFA. ATLAAMGADV
Consensus       --D-S------ ---------- ---------- ---------- -----MG---
```

Figure 20G

	351				400
PG2982	EVLNARLAGG	EDVADLRVR.	ASKLKGVVVP	PERAPSMIDE	YPVLAIAASF
LBAA	EVLNARLAGG	EDVADLRVR.	ASKLKGVVVP	PERAPSMIDE	YPVLAIAASF
Agrobacterium CP4	EVINPRLAGG	EDVADLRVR.	SSTLKGVTVP	EDRAPSMIDE	YPILAVAAAF
B. subtilis	EIKPSADSGA	EPYGDLIIE.	TSSLKAVEIG	GDIIPRLIDE	IPIIALLATQ
S. aureus	QL.FNQTTGA	EPTASIRIQY	TPMLQPITIE	GELVPKAIDE	LPVIALLCTQ
S. cerevisiae	....TQTATS	TTVSGPPV..	...GTLKPLK	HVDMEPMTDA	FLTACVVAAI
A. nidulans	....EQTETS	TTVTGPSD..	...GILRATS	KRGYGT.NDR	CVPRCFRTGS
B. napus	....SWTENS	VTVTGPSRDA	FGMRHLRAV.	DVNMNKMPDV	AMTLAVVALF
A. thaliana	....SWTENS	VTVTGPPRDA	FGMRHLRAI.	DVNMNKMPDV	AMTLAVVALF
N. tabacum	....TWTENS	VTVKGPPRNS	SGMKHLRAV.	DVNMNKMPDV	AMTLAVVALF
L. esculentum	....TWTENS	VTVKGPPRNS	SGMKHLRAI.	DVNMNKMPDV	AMTLAVVALF
P. hybrida	....TWTENS	VTVKGPPRSS	SGRKHLRAI.	DVNMNKMPDV	AMTLAVVALY
Z. mays	....TWTETS	VTVTGPPREP	FGRKHLKAI.	DVNMNKMPDV	AMTLAVVALF
S. gallinarum	....TWGDDF	I........A	CTRGELHAI.	DMDMNHIPDA	AMTIATTALF
S. typhimurium	....TWGDDF	I........A	CTRGELHAI.	DMDMNHIPDA	AMTIATTALF
S. typhi	....TWGDDF	I........A	CTRGELHAI.	DMDMNHIPDA	AMTIATTALF
E. coli	....CWGDDY	I........S	CTRGELNAI.	DMDMNHIPDA	AMTIATAALF
K. pneumoniae	....TWGEDY	I........A	CTRGELNAI.	DMDMNHIPDA	AMTIATAALF
Y. entoercolitica	....SWGDDY	I........E	CSRGELQGI.	DMDMNHIPDA	AMTIATTALF
H. influenzae	....TWGEDF	I........Q	AEHAELNGI.	DMDMNHIPDA	AMTIATTALF
P. multocida	....TWGDDF	I........Q	VEKGNLKGI.	DMDMNHIPDA	AMTIATTALF
A. salmonicida	....TWGDDF	I........E	AEQGPLHGV.	DMDMNHIPDV	GHDHSCQSHC
B. pertussis	....RYGPGW	IETRGVRVAE	GGR..LKAF.	DADFNLIPDA	AMTAATLALY
Consensus	----------	----------	----------	--------D-	----------

Figure 20H

	401				450
PG2982	AEG.......	ETVMDGLDEL	RVKESDRLAA	VARGLEANGV	DCTEGEMSLT
LBAA	AEG.......	ETVMDGLDEL	RVKESDRLAA	VARGLEANGV	DCTEGEMSLT
Agrobacterium CP4	AEG.......	ATVMNGLEEL	RVKESDRLSA	VANGLKLNGV	DCDEGETSLV
B. subtilis	AEG.......	TTVIKDAAEL	KVKETNRIDT	VVSELRKLGA	EIEPTADGMK
S. aureus	AVG.......	TSTIKDAEEL	KVKETNRIDT	TADMLNLLGF	ELQPTNDGLI
S. cerevisiae	SHDSDPNSAN	TTTIEGIANQ	RVKECNRILA	MATELAKFGV	KTTELPDGIQ
A. nidulans	HRPMEKSQTT	PPVSSGIANQ	RVKECNRIKA	MKDELAKFGV	ICREHDDGLE
B. napus	ADG.......	PTTIRDVASW	RVKETERMIA	ICTELRKLGA	TV.EEGSDYC
A. thaliana	ADG.......	PTTIRDVASW	RVKETERMIA	ICTELRKLGA	TV.EEGSDYC
N. tabacum	ADG.......	PTAIRDVASW	RVKETERMIA	ICTELRKLGA	TV.VEGSDYC
L. esculentum	ADG.......	PTTIRDVASW	RVKETERMIA	ICTELRKLGA	TV.VEGSDYC
P. hybrida	ADG.......	PTAIRDVASW	RVKETERMIA	ICTELRKLGA	TV.EEGPDYC
Z. mays	ADG.......	PTAIRDVASW	RVKETERMVA	IRTELTKLGA	SV.EEGPDYC
S. gallinarum	AKG.......	TTTLRNIYNW	RVKETDRLFA	MATELRKVGA	EV.EEGHDYI
S. typhimurium	AKG.......	TTTLRNIYNW	RVKETDRLFA	MATELRKVGA	EV.EEGHDYI
S. typhi	AKG.......	TTTLRNIYNW	RVKETDRLFA	MATELRKVGA	EV.EEGHDYI
E. coli	AKG.......	TTRLRNIYNW	RVKETDRLFA	MATELRKVGA	EV.EEGHDYI
K. pneumoniae	ARG.......	TTTLRNIYNW	RVKETDRLFA	MATELRKVGA	EV.EEGEDYI
Y. entoercolitica	ADG.......	PTVIRNIYNW	RVKETDRLSA	MATELRKVGA	EV.EEGQDYI
H. influenzae	SNG.......	ETVIRNIYNW	RVKETDRLTA	MATELRKVGA	EV.EEGEDFI
P. multocida	AEG.......	ETVIRNIYNW	RVKETDRLTA	MATELRKVGA	EV.EEGEDFI
A. salmonicida	LPR.......	VPPHSQHLQL	AVRD.DRCTP	CTHGHRRAQA	GVSEEGTTFI
B. pertussis	ADG.......	PCRLRNIGSW	RVKETDRIHA	MHTELEKLGA	GV.QSGADWL
Consensus	----------	----------	-V----R---	----------	----------

Figure 20I

	451				500
PG2982	VRGRPDGKGL	G...GG....	TVATHLDHRI	AMSFLVMGLA	.........A
LBAA	VRGRPDGKGL	G...GG....	TVATHLDHRI	AMSFLVMGLA	.........A
Agrobacterium CP4	VRGRPDGKGL	GNASGA....	AVATHLDHRI	AMSFLVMGLV	.........S
B. subtilis	VYGKQTLKG.	....GA....	AVSSHGDHRI	GMMLGIASCI	.........T
S. aureus	IHPSEFKTN.	....AT....	DI..LTDHRI	GMMLAVACVL	.........S
S. cerevisiae	VHGLNSIKDL	KVPSDSSGPV	GVCTYDDHRV	AMSFSLLAGM	VNSQNERDEV
A. nidulans	IDGIDR.SNL	RQPVG.....	GVFCYDDHRV	AFSFSVL.SL	VTPQ......
B. napus	VITP..PAKV	KPA.......	EIDTYDDHRM	AMAFSLAAC.	.........A
A. thaliana	VITP..PKKV	KTA.......	EIDTYDDHRM	AMAFSLAAC.	.........A
N. tabacum	IITP..PEKL	NVT.......	EIDTYDDHRM	AMAFSLAAC.	.........A
L. esculentum	IITP..PEKL	NVT.......	EIDTYDDHRM	AMAFSLAAC.	.........A
P. hybrida	IITP..PEKL	NVT.......	DIDTYDDHRM	AMAFSLAAC.	.........A
Z. mays	IITP..PEKL	NVT.......	AIDTYDDHRM	AMAFSLAAC.	.........A
S. gallinarum	RITP..PAKL	QHA.......	DIGTYNDHRM	AMCFSLVAL.	.........S
S. typhimurium	RITP..PAKL	QHA.......	DIGTYNDHRM	AMCFSLVAL.	.........S
S. typhi	RITP..PAKL	QHA.......	DIGTYNDHRM	AMCFSLVAL.	.........S
E. coli	RITP..PEKL	NFA.......	EIATYNDHRM	AMCFSLVAL.	.........S
K. pneumoniae	RITP..PLTL	QFA.......	EIGTYNDHRM	AMCFSLVAL.	.........S
Y. entoercolitica	RVVP..PAQL	IAA.......	EIGTYNDHRM	AMCFSLVAL.	.........S
H. influenzae	RIQPLALNQF	KHA.......	NIETYNDHRM	AMCFSLIAL.	.........S
P. multocida	RIQPLNLAQF	QHA.......	ELNI.HDHRM	AMCFALIAL.	.........S
A. salmonicida	TRDAADPAQA	RRD.......	R..HLQRSRI	AMCFSLVAL.	.........S
B. pertussis	EVAPPEPGGW	RDA.......	HIGTWDDHRM	AMCFLLAAF.	.........G
Consensus	----------	----------	--------R-	----------	----------

Figure 20J

	501			538
PG2982	EKPVTVDDSN	MIATSFPEFM	DMMPGLGAKI	ELSIL...
LBAA	EKPVTVDDSN	MIATSFPEFM	DMMPGLGAKI	ELSIL...
Agrobacterium CP4	ENPVTVDDAT	MIATSFPEFM	DLMAGLGAKI	ELSDTKAA
B. subtilis	EEPIEIEHTD	AIHVSYPTFF	EHLNKLSKKS	....
S. aureus	SEPVKIKQFD	AVNVSFPGFL	PKLKLLQNEG	....
S. cerevisiae	ANPVRILERH	CTGKTWPGWW	DVLH......	....
A. nidulans	..PTLILEKE	CVGKTWPGWW	DTLRQLFKV.	....
B. napus	DVPVTIKDPG	CTRKTFPDYF	QVLESITKH.	....
A. thaliana	DVPITINDSG	CTRKTFPDYF	QVLERITKH.	....
N. tabacum	DVPVTIKDPG	CTRKTFPNYF	DVLQQYSKH.	....
L. esculentum	DVPVTIKNPG	CTRKTFPDYF	EVLQKYSKH.	....
P. hybrida	DVPVTINDPG	CTRKTFPNYF	DVLQQYSKH.	....
Z. mays	EVPVTIRDPG	CTRKTFPDYF	DVLSTFVKN.	....
S. gallinarum	DTPVTILDPK	CTAKTFPDYF	EQLARMSTPA	....
S. typhimurium	DTPVTILDPK	CTAKTFPDYF	EQLARMSTPA	....
S. typhi	DTPVTILDPK	CTAKTFPDYF	EQLARMSTPA	....
E. coli	DTPVTILDPK	CTAKTFPDYF	EQLARISQAA	....
K. pneumoniae	DTPVTILDPK	CTAKTFPDYF	GQLARISTLA	....
Y. entoercolitica	DTPVTILDPK	CTAKTFPDYF	EQLARLSQIA	....
H. influenzae	NTPVTILDPK	CTAKTFPTFF	NEFE....KI	CLKN....
P. multocida	KTSVTILDPS	CTAKTFPTFL	ILFTLNTREV	AYR.....
A. salmonicida	DIAVTINDPG	CTSKTFPDYF	DKLASVSQAV	....
B. pertussis	PAAVRILDPG	CVSKTFPDYF	DVYAGLLAAR	D.......
Consensus	----------	------P---	----------	--------

Figure 20K

```
ACGGGCTGTA ACGGTAGTAG GGGTCCCGAG CACAAAAGCG GTGCCGGCAA GCAGAACTAA   60

TTTCCATGGG GAATAATGGT ATTTCATTGG TTTGGCCTCT GGTCTGGCAA TGGTTGCTAG  120

GCGATCGCCT GTTGAAATTA ACAAACTGTC GCCCTTCCAC TGACCATGGT AACGATGTTT  180

TTTACTTCCT TGACTAACCG AGGAAAATTT GGCGGGGGGC AGAAATGCCA ATACAATTTA  240

GCTTGGTCTT CCCTGCCCCT AATTTGTCCC CTCC ATG GCC TTG CTT TCC CTC CTC   292
                              Met Ala Leu Leu Ser Leu
                               1               5

AAC AAT CAT CAA TCC CAT CAA CGC TTA ACT GTT AAT CCC CCT GCC CAA      340
Asn Asn His Gln Ser His Gln Arg Leu Thr Val Asn Pro Pro Ala Gln
          10                  15                  20

GGG GTC GCT TTG ACT GGC CGC CTA AGG GTG CCG GGG GAT AAA TCC ATT     388
Gly Val Ala Leu Thr Gly Arg Leu Arg Val Pro Gly Asp Lys Ser Ile
      25                  30                  35

TCC CAT CGG GCC TTG ATG TTG GGG GCG ATC GCC ACC GGG GAA ACC ATT     436
Ser His Arg Ala Leu Met Leu Gly Ala Ile Ala Thr Gly Glu Thr Ile
  40                  45                  50

ATC GAA GGG CTA CTG TTG GGG GAA GAT CCC CGT AGT ACG GCC CAT TGC     484
Ile Glu Gly Leu Leu Leu Gly Glu Asp Pro Arg Ser Thr Ala His Cys
55                  60                  65                  70
```

Figure 21A

```
                                                                  532
TTT CGG GCC ATG GGA GCA GAA ATC AGC GAA CTA AAT TCA GAA AAA ATC
Phe Arg Ala Met Gly Ala Glu Ile Ser Glu Leu Asn Ser Glu Lys Ile
                75                  80                  85

                                                                  580
ATC GTT CAG GGT CGG GGT CTG GGA CAG TTG CAG GAA CCC AGT ACC GTT
Ile Val Gln Gly Arg Gly Leu Gly Gln Leu Gln Glu Pro Ser Thr Val
            90                  95                 100

                                                                  628
TTG GAT GCG GGG AAC TCT GGC ACC ACC ATG CGC TTA ATG TTG GGC TTG
Leu Asp Ala Gly Asn Ser Gly Thr Thr Met Arg Leu Met Leu Gly Leu
        105                 110                 115

                                                                  676
CTA GCC GGG CAA AAA GAT TGT TTA TTC ACC GTC ACC GGC GAT GAT TCC
Leu Ala Gly Gln Lys Asp Cys Leu Phe Thr Val Thr Gly Asp Asp Ser
    120                 125                 130

                                                                  724
CTC CGT CAC CGC CCC ATG TCC CGG GTA ATT CAA CCC TTG CAA CAA ATG
Leu Arg His Arg Pro Met Ser Arg Val Ile Gln Pro Leu Gln Gln Met
135                 140                 145                 150

                                                                  772
GGG GCA AAA ATT TGG GCC CGG AGT AAC GGC AAG TTT GCG CCG CTG GCA
Gly Ala Lys Ile Trp Ala Arg Ser Asn Gly Lys Phe Ala Pro Leu Ala
                155                 160                 165

                                                                  820
GTC CAG GGT AGC CAA TTA AAA CCG ATC CAT CAC TAC CAT TCC CCC ATT GCT
Val Gln Gly Ser Gln Leu Lys Pro Ile His Tyr His Ser Pro Ile Ala
            170                 175                 180
```

Figure 21B

```
                                                                    868
TCA GCC CAG GTA AAG TCC TGC CTG TTG CTA GCG GGG TTA ACC ACC GAG
Ser Ala Gln Val Lys Ser Cys Leu Leu Leu Ala Gly Leu Thr Thr Glu
            185                 190                 195

                                                                    916
GGG GAC ACC ACG GTT ACA GAA CCA GCT CTA TCC CGG GAT CAT AGC GAA
Gly Asp Thr Thr Val Thr Glu Pro Ala Leu Ser Arg Asp His Ser Glu
        200                 205                 210

                                                                    964
CGC ATG TTG CAG GCC TTT GGA GCC AAA TTA ACC ATT GAT CCA GTA ACC
Arg Met Leu Gln Ala Phe Gly Ala Lys Leu Thr Ile Asp Pro Val Thr
215                 220                 225                 230

                                                                   1012
CAT AGC GTC ACT GTC CAT GGC CCG GCC CAT TTA ACG GGG CAA CGG GTG
His Ser Val Thr Val His Gly Pro Ala His Leu Thr Gly Gln Arg Val
                235                 240                 245

                                                                   1060
GTG GTG CCA GGG GAC ATC AGC TCG GCG GCC TTT TGG TTA GTG GCG GCA
Val Val Pro Gly Asp Ile Ser Ser Ala Ala Phe Trp Leu Val Ala Ala
            250                 255                 260

                                                                   1108
TCC ATT TTG CCT GGA TCA GAA TTG TTG GTG GAA AAT GTA GGC ATT AAC
Ser Ile Leu Pro Gly Ser Glu Leu Leu Val Glu Asn Val Gly Ile Asn
        265                 270                 275

                                                                   1156
CCC ACC AGG ACA GGG GTG TTG GAA GTG TTG GCC CAG ATG GGG GCG GAC
Pro Thr Arg Thr Gly Val Leu Glu Val Leu Ala Gln Met Gly Ala Asp
    280                 285                 290
```

Figure 21C

```
ATT ACC CCG GAG AAT GAA CGA TTG GTA ACG GGG GAA CCG GTA GCA GAT   1204
Ile Thr Pro Glu Asn Glu Arg Leu Val Thr Gly Glu Pro Val Ala Asp
295                 300                 305                 310

CTG CGG GTT AGG GCA AGC CAT CTC CAG GGT TGC ACC TTC GGC GGC GAA   1252
Leu Arg Val Arg Ala Ser His Leu Gln Gly Cys Thr Phe Gly Gly Glu
                315                 320                 325

ATT ATT CCC CGA CTG ATT GAT GAA ATT CCC ATT TTG GCA GTG GCG GCG   1300
Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro Ile Leu Ala Val Ala Ala
            330                 335                 340

GCC TTT GCA GAG GGC ACT ACC CGC ATT GAA GAT GCC GCA GAA CTG AGG   1348
Ala Phe Ala Glu Gly Thr Thr Arg Ile Glu Asp Ala Ala Glu Leu Arg
        345                 350                 355

GTT AAA GAA AGC GAT CGC CTG GCG GCC ATT GCT TCG GAG TTG GGC AAA   1396
Val Lys Glu Ser Asp Arg Leu Ala Ala Ile Ala Ser Glu Leu Gly Lys
    360                 365                 370

ATG GGG GCC AAA GTC ACC GAA TTT GAT GAT GGC CTG GAA ATT CAA GGG   1444
Met Gly Ala Lys Val Thr Glu Phe Asp Asp Gly Leu Glu Ile Gln Gly
375                 380                 385                 390

GGA AGC CCG TTA CAA GGG GCC GAG GTG GAT AGC TTG ACG GAT CAT CGC   1492
Gly Ser Pro Leu Gln Gly Ala Glu Val Asp Ser Leu Thr Asp His Arg
                395                 400                 405
```

Figure 21D

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```
ATT GCC ATG GCG TTG GCG ATC GCC GCT TTA GGT AGT GGG GGG CAA ACA      1540
Ile Ala Met Ala Leu Ala Ile Ala Ala Leu Gly Ser Gly Gly Gln Thr
            410                 415                 420

ATT ATT AAC CGG GCG GAA GCG GCC GCC ATT TCC TAT CCA GAA TTT TTT      1588
Ile Ile Asn Arg Ala Glu Ala Ala Ala Ile Ser Tyr Pro Glu Phe Phe
        425                 430                 435

GGC ACG CTA GGG CAA GTT GCC CAA GGA TAAAGTTAGA AAAACTCCTG            1635
Gly Thr Leu Gly Gln Val Ala Gln Gly
    440                 445

GGCGGTTTGT AAATGTTTTA CCAAGGTAGT TTGGGGTAAA GGCCCCAGCA AGTGCTGCCA    1695

GGGTAATTTA TCCGCAATTG ACCAATCGGC ATGGACCGTA TCGTTCAAAC TGGGTAATTC    1755

TCCCTTTAAT TCCTTAAAAG CTCGCTTAAA ACTGCCCAAC GTATCTCCGT AATGGCGAGT    1815

GAGTAGAAGT AATGGGGCCA AACGGCGATC GCCACGGGAA ATTAAAGCCT GCATCACTGA    1875

CCACTTATAA CTTTCGGGA                                                 1894
```

Figure 21E

```
TTTAAAAACA ATGAGTTAAA AAATTATTTT TCTGGCACAC GCGCTTTTTT TGCATTTTTT      60

CTCCCATTTT TCCGGCACAA TAACGTTGGT TTTATAAAAG GAAATG ATG ATG ACG        115
                                                   Met Met Thr
                                                     1

AAT ATA TGG CAC ACC GCG CCC GTC TCT GCG CTT TCC GGC GAA ATA ACG  >     163
Asn Ile Trp His Thr Ala Pro Val Ser Ala Leu Ser Gly Glu Ile Thr
  5                  10                  15

ATA TGC GGC GAT AAA TCA ATG TCG CAT CGC GCC TTA TTA TTA GCA GCG       211
Ile Cys Gly Asp Lys Ser Met Ser His Arg Ala Leu Leu Leu Ala Ala
 20                  25                  30                  35

TTA GCA GAA GGA CAA ACG GAA ATC CGC GGC TTT TTA GCG TGC GCG GAT       259
Leu Ala Glu Gly Gln Thr Glu Ile Arg Gly Phe Leu Ala Cys Ala Asp
                 40                  45                  50

TGT TTG GCG ACG CGG CAA GCA TTG CGC GCA TTA GGC GTT GAT ATT CAA       307
Cys Leu Ala Thr Arg Gln Ala Leu Arg Ala Leu Gly Val Asp Ile Gln
            55                  60                  65

AGA GAA AAA GAA ATA GTG ACG ATT CGC GGT GTG GGA TTT CTG GGT TTG       355
Arg Glu Lys Glu Ile Val Thr Ile Arg Gly Val Gly Phe Leu Gly Leu
        70                  75                  80
```

Figure 22A

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```
CAG CCG CCG AAA GCA CCG TTA AAT ATG CAA AAC AGT GGC ACT AGC ATG   403
Gln Pro Pro Lys Ala Pro Leu Asn Met Gln Asn Ser Gly Thr Ser Met
      85                  90                  95

CGT TTA TTG GCA GGA ATT TTG GCA GCG CAG CGC TTT GAG AGC GTG TTA   451
Arg Leu Leu Ala Gly Ile Leu Ala Ala Gln Arg Phe Glu Ser Val Leu
 100                 105                 110                 115

TGC GGC GAT GAA TCA TTA GAA AAA CGT CCG ATG CAG CGC ATT ATT ACG   499
Cys Gly Asp Glu Ser Leu Glu Lys Arg Pro Met Gln Arg Ile Ile Thr
                120                 125                 130

CCG CTT GTG CAA ATG GGG GCA AAA ATT GTC AGT CAC AGC AAT TTT ACG   547
Pro Leu Val Gln Met Gly Ala Lys Ile Val Ser His Ser Asn Phe Thr
            135                 140                 145

GCG CCG TTA CAT ATT TCA GGA CGC CCG CTG ACC GGC ATT GAT TAC GCG   595
Ala Pro Leu His Ile Ser Gly Arg Pro Leu Thr Gly Ile Asp Tyr Ala
        150                 155                 160

TTA CCG CTT CCC AGC GCG CAA TTA AAA AGT TGC CTT ATT TTG GCA GGA   643
Leu Pro Leu Pro Ser Ala Gln Leu Lys Ser Cys Leu Ile Leu Ala Gly
    165                 170                 175

TTA TTG GCT GAC GGT ACC ACG CGG CTG CAT ACT TGC GGC ATC AGT CGC   691
Leu Leu Ala Asp Gly Thr Thr Arg Leu His Thr Cys Gly Ile Ser Arg
180                 185                 190                 195
```

Figure 22B

```
                                                                    739
GAC CAC ACG GAA CGC ATG TTG CCG CTT TTT GGT GGC GCA CTT GAG ATC
Asp His Thr Glu Arg Met Leu Pro Leu Phe Gly Gly Ala Leu Glu Ile
            200                 205                 210

                                                                    787
AAG AAA GAG CAA ATA ATC GTC ACC GGT GGA CAA AAA TTG CAC GGT TGC
Lys Lys Glu Gln Ile Ile Val Thr Gly Gly Gln Lys Leu His Gly Cys
        215                 220                 225

                                                                    835
GTG CTT GAT ATT GTC GGC GAT TTG TCG GCG GCG GCG TTT TTT ATG GTT
Val Leu Asp Ile Val Gly Asp Leu Ser Ala Ala Ala Phe Phe Met Val
    230                 235                 240

                                                                    883
GCG GCT TTG ATT GCG CCG CGC GCG GAA GTC GTT ATT CGT AAT GTC GGC
Ala Ala Leu Ile Ala Pro Arg Ala Glu Val Val Ile Arg Asn Val Gly
245                 250                 255

                                                                    931
ATT AAT CCG ACG CGG GCG GCA ATC ATT ACT TTG TTG CAA AAA ATG GGC
Ile Asn Pro Thr Arg Ala Ala Ile Ile Thr Leu Leu Gln Lys Met Gly
260                 265                 270                 275

                                                                    979
GGA CGG ATT GAA TTG CAT CAT CAG CGC TTT TGG GGC GCC GAA CCG GTG
Gly Arg Ile Glu Leu His His Gln Arg Phe Trp Gly Ala Glu Pro Val
            280                 285                 290

                                                                   1027
GCA GAT ATT GTT GTT TAT CAT TCA AAA TTG CGC GGC ATT ACG GTG GCG
Ala Asp Ile Val Val Tyr His Ser Lys Leu Arg Gly Ile Thr Val Ala
        295                 300                 305
```

Figure 22C

```
CCG GAA TGG ATT GCC AAC GCG ATT GAT GAA TTG CCG ATT TTT TTT ATT      1075
Pro Glu Trp Ile Ala Asn Ala Ile Asp Glu Leu Pro Ile Phe Phe Ile
        310                 315                 320

GCG GCA GCT TGC GCG GAA GGG ACG ACT TTT GTG GGC AAT TTG TCA GAA      1123
Ala Ala Ala Cys Ala Glu Gly Thr Thr Phe Val Gly Asn Leu Ser Glu
    325                 330                 335

TTG CGT GTG AAA GAA TCG GAT CGT TTA GCG GCG ATG GCG CAA AAT TTA      1171
Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Met Ala Gln Asn Leu
340                 345                 350                 355

CAA ACT TTG GGC GTG GCG TGC GAC GTT GGC GCC GAT TTT ATT CAT ATA      1219
Gln Thr Leu Gly Val Ala Cys Asp Val Gly Ala Asp Phe Ile His Ile
                360                 365                 370

TAT GGA AGA AGC GAT CGG CAA TTT TTA CCG GCG CGG GTG AAC AGT TTT      1267
Tyr Gly Arg Ser Asp Arg Gln Phe Leu Pro Ala Arg Val Asn Ser Phe
            375                 380                 385

GGC GAT CAT CGG ATT GCG ATG AGT TTG GCG GTG GCA GGT GTG CGC GCG      1315
Gly Asp His Arg Ile Ala Met Ser Leu Ala Val Ala Gly Val Arg Ala
        390                 395                 400

GCA GGT GAA TTA TTG ATT GAT GAC GGC GCG GTG GCG GCG GTT TCT ATG      1363
Ala Gly Glu Leu Leu Ile Asp Asp Gly Ala Val Ala Ala Val Ser Met
    405                 410                 415
```

Figure 22D

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```
                                                          1411
CCG CAA TTT CGC GAT TTT GCC GCC GCA ATT GGT ATG AAT GTA GGA GAA
Pro Gln Phe Arg Asp Phe Ala Ala Ala Ile Gly Met Asn Val Gly Glu
420                 425                 430                 435

                                                          1465
AAA GAT GCG AAA AAT TGT CAC GAT TGATGGTCCT AGCGGTGTTG GAAAAGGCAC
Lys Asp Ala Lys Asn Cys His Asp
                440

                                                          1479
GGTGGCGCAA GCTT
```

Figure 22E

```
                          1                                          40
                PG2982    ........MS HSASPKPATA RRSEALTGEI RIPGDKSISH
                  LBAA    ........MS HSASPKPATA RRSEALTGEI RIPGDKSISH
      Agrobacterium CP4   ........MS HGASSRPATA RKSSGLSGTV RIPGDKSISH
Synechocystis sp. PCC6803 MALLSLNNHQ SHQRLTVNPP AQGVALTGRL RVPGDKSISH
            B. subtilis   .......... .......MKR DKVQTLHGEI HIPGDKSISH
             D. nodosus   .......... ..MMTNIWHT APVSALSGEI TICGDKSMSH
              S. aureus   .......... ...MVNEQII DISGPLKGEI EVPGDKSMTH
              Consensus   ---------- ---------- -----L-G-- -I-GDKS--H
                          41                                         80
                PG2982    RSFMFGGLAS GETRITGLLE GEDVINTGRA MQAMGAKI.R
                  LBAA    RSFMFGGLAS GETRITGLLE GEDVINTGRA MQAMGAKI.R
      Agrobacterium CP4   RSFMFGGLAS GETRITGLLE GEDVINTGKA MQAMGARI.R
Synechocystis sp. PCC6803 RALMLGAIAT GETIIEGLLL GEDPRSTAHC FRAMGAEISE
            B. subtilis   RSVMFGALAA GTTTVKNFLP GADCLSTIDC FRKMGVHI.E
             D. nodosus   RALLLAALAE GQTEIRGFLA CADCLATRQA LRALGVDI.Q
              S. aureus   RAIMLASLAE GVSTIYKPLL GEDCRRTMDI FRHLGVEI.K
              Consensus   R--MF---A- G---I---L- --D---T--- ---MG--I--
                          81                                        120
                PG2982    KEGDVWIING VGNGCLLQPE AALDFGNAGT GARLTMGLVG
                  LBAA    KEGDVWIING VGNGCLLQPE AALDFGNAGT GARLTMGLVG
      Agrobacterium CP4   KEGDTWIIDG VGNGGLLAPE APLDFGNAAT GCRLTMGLVG
Synechocystis sp. PCC6803 LNSEKIIVQG RGLGQLQEPS TVLDAGNSGT TMRLMLGLLA
            B. subtilis   QSSSDVVIHG KGIDALKEPE SLLDVGNSGT TIRLMLGILA
             D. nodosus   REKEIVTIRG VGFLGLQPPK APLNMQNSGT SMRLLAGILA
              S. aureus   EDDEKLVVTS PGYQ.VNTPH QVLYTGNSGT TTRLLAGLLS
              Consensus   -------I-- -G------P- --L---N--T --RL--G---
```

Figure 23A

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```
                          121                                                 160
                PG2982    TY.DMKTSFI GDASLSKRPM GRVLNPLREM GVQVEAADGD
                LBAA      TY.DMKTSFI GDASLSKRPM GRVLNPLREM GVQVEAADGD
      Agrobacterium CP4   VY.DFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
Synechocystis sp. PCC6803 GQKDCLFTVT GDDSLRHRPM SRVIQPLQQM GAKIWARSNG
             B. subtilis  G.RPFYSAVA GDESIAKRPM KRVTEPLKKM GAKIDGRAGG
               D. nodosus AQR.FESVLC GDESLEKRPM QRIITPLVQM GAKIVSHSNF
                S. aureus GLGN.ESVLS GDVSIGKRPM DRVLRPLKLM DANIEGIEDN
                Consensus ---------- GD-S---RPM -RV--PL--M ---I------
                          161                                                 200
                PG2982    RMPLTLIGPK TANPITYRVP MASAQVKSAV LLAGLNTPGV
                LBAA      RMPLTLIGPK TANPITYRVP MASAQVKSAV LLAGLNTPGV
      Agrobacterium CP4   RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI
Synechocystis sp. PCC6803 KFAPLAVQGS QLKPIHYHSP IASAQVKSCL LLAGLTTEGD
             B. subtilis  EFTPLSVSGA SLKGIDYVSP VASAQIKSAV LLAGLQAEGT
               D. nodosus T.APLHISGR PLTGIDYALP LPSAQLKSCL ILAGLLADGT
                S. aureus .YTPLIIKPS VIKGINYQME VASAQVKSAI LFASLFSKEP
                Consensus ---------- ----I-Y--- --SAQ-KS-- -LA-L-----
                          201                                                 240
                PG2982    TTVIEPVMTR DHTEKMLQGF .....GADLT VETDKDGVRH
                LBAA      TTVIEPVMTR DHTEKMLQGF .....GADLT VETDKDGVRH
      Agrobacterium CP4   TTVIEPIMTR DHTEKMLQGF .....GANLT VETDADGVRT
Synechocystis sp. PCC6803 TTVTEPALSR DHSERMLQAF .....GAKLT IDPVTHSV..
             B. subtilis  TTVTEPHKSR DHTERMLSAF .....GVKLS EDQT..SV..
               D. nodosus TRLHTCGISR DHTERMLPLF .....GGALE IKK..EQI..
                S. aureus TIIKELDVSR NHTETMFKHF NIPIEAEGLS INTTPEAIRY
                Consensus T--------R-H-E-ML--F --------L- -------V--
```

Figure 23B

	241			280
PG2982	IRITGQGKLV	GQTIDVPGDP	SSTAFPLVAA	LLVEGSDVTI
LBAA	IRITGQGKLV	GQTIDVPGDP	SSTAFPLVAA	LLVEGSDVTI
Agrobacterium CP4	IRLEGRGKLT	GQVIDVPGDP	SSTAFPLVAA	LLVPGSDVTI
Synechocystis sp. PCC6803	.TVHGPAHLT	GQRVVVPGDI	SSAAFWLVAA	SILPGSELLV
B. subtilis	.SIAGGQKLT	AADIFVPGDI	SSAAFFLAAG	AMVPNSRIVL
D. nodosus	.IVTGGQKLH	GCVLDIVGDL	SAAAFFMVAA	LIAPRAEVVI
S. aureus	IKPAD.....	...FHVPGDI	SSAAFFIVAA	LITPGSDVTI
Consensus	----------	-----V-GD-	S--AF---A-	----------

	281			320
PG2982	RNVLMNPTRT	GLILTLQEMG	ADIEVLNARL	AGGEDVADLR
LBAA	RNVLMNPTRT	GLILTLQEMG	ADIEVLNARL	AGGEDVADLR
Agrobacterium CP4	LNVLMNPTRT	GLILTLQEMG	ADIEVINPRL	AGGEDVADLR
Synechocystis sp. PCC6803	ENVGINPTRT	GVLEVLAQMG	ADITPENERL	VTGEPVADLR
B. subtilis	KNVGLNPTRT	GIIDVLQNMG	AKLEIKPSAD	SGAEPYGDLI
D. nodosus	RNVGINPTRA	AIITLLQKMG	GRIELHHQRF	WGAEPVADIV
S. aureus	HNVGINQTRS	GIIDIVEKMG	GNIQLFNQT.	TGAEPTASIR
Consensus	-NV--N-TR-	--------MG	----------	---E------

	321			360
PG2982	VR.ASKLKGV	VVPPERAPSM	IDEYPVLAIA	ASFAEGETVM
LBAA	VR.ASKLKGV	VVPPERAPSM	IDEYPVLAIA	ASFAEGETVM
Agrobacterium CP4	VR.SSTLKGV	TVPEDRAPSM	IDEYPILAVA	AAFAEGATVM
Synechocystis sp. PCC6803	VR.ASHLQGC	TFGGEIIPRL	IDEIPILAVA	AAFAEGTTRI
B. subtilis	IE.TSSLKAV	EIGGDIIPRL	IDEIPIIALL	ATQAEGTTVI
D. nodosus	VY.HSKLRGI	TVAPEWIANA	IDELPIFFIA	AACAEGTTFV
S. aureus	IQYTPMLQPI	TIEGELVPKA	IDELPVIALL	CTQAVGTSTI
Consensus	V-----L---	----E-----	IDE-PI----	---A-G----

Figure 23C

```
                          361                                          400
                 PG2982   DGLDELRVKE SDRLAAVARG LEANGVDCTE GEMSLTVRGR
                   LBAA   DGLDELRVKE SDRLAAVARG LEANGVDCTE GEMSLTVRGR
       Agrobacterium CP4  NGLEELRVKE SDRLSAVANG LKLNGVDCDE GETSLVVRGR
Synechocystis sp. PCC6803 EDAAELRVKE SDRLAAIASE LGKMGAKVTE FDDGLEIQGG
            B. subtilis   KDAAELKVKE TNRIDTVVSE LRKLGAEIEP TADGMKVYGK
            D. nodosus    GNLSELRVKE SDRLAAMAQN LQTLGVACDV GADFIHIYGR
             S. aureus    KDAEELKVKE TNRIDTTADM LNLLGFELQP TNDGLIIHPS
             Consensus    ----EL-VKE --R------- L---G----- ------V---
                          401                                          440
                 PG2982   PDGKGLG... GGTVATHLDH RIAMSFLVMG LAAEKPVTVD
                   LBAA   PDGKGLG... GGTVATHLDH RIAMSFLVMG LAAEKPVTVD
       Agrobacterium CP4  PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
Synechocystis sp. PCC6803 SPLQ...... GAEVDSLTDH RIAMALAIAA LGSGGQTIIN
            B. subtilis   QTLK.G.... GAAVSSHGDH RIGMMLGIAS CITEEPIEIE
            D. nodosus    SDRQFL.... PARVNSFGDH RIAMSLAVAG VRAAGELLID
             S. aureus    E.......FK TNATDILTDH RIGMMLAVAC VLSSEPVKIK
             Consensus    ---------- --------DH RI-M-L-V-- --------I-
                          441                              473
                 PG2982   DSNMIATSFP EFMDMMPGLG AKIELSIL.. ...
                   LBAA   DSNMIATSFP EFMDMMPGLG AKIELSIL.. ...
       Agrobacterium CP4  DATMIATSFP EFMDLMAGLG AKIELSDTKA A..
Synechocystis sp. PCC6803 RAEAAAISYP EFFGTLGQVA QG*....... ...
            B. subtilis   HTDAIHVSYP TFFEHLNKLS KKS....... ...
            D. nodosus    DGAVAAVSMP QFRDFAAAIG MNVGEKDAKN CHD
             S. aureus    QFDAVNVSFP GFLPKLKLLQ NEG....... ...
             Consensus    -------S-P -F-------- ---------- ---
```

Figure 23D

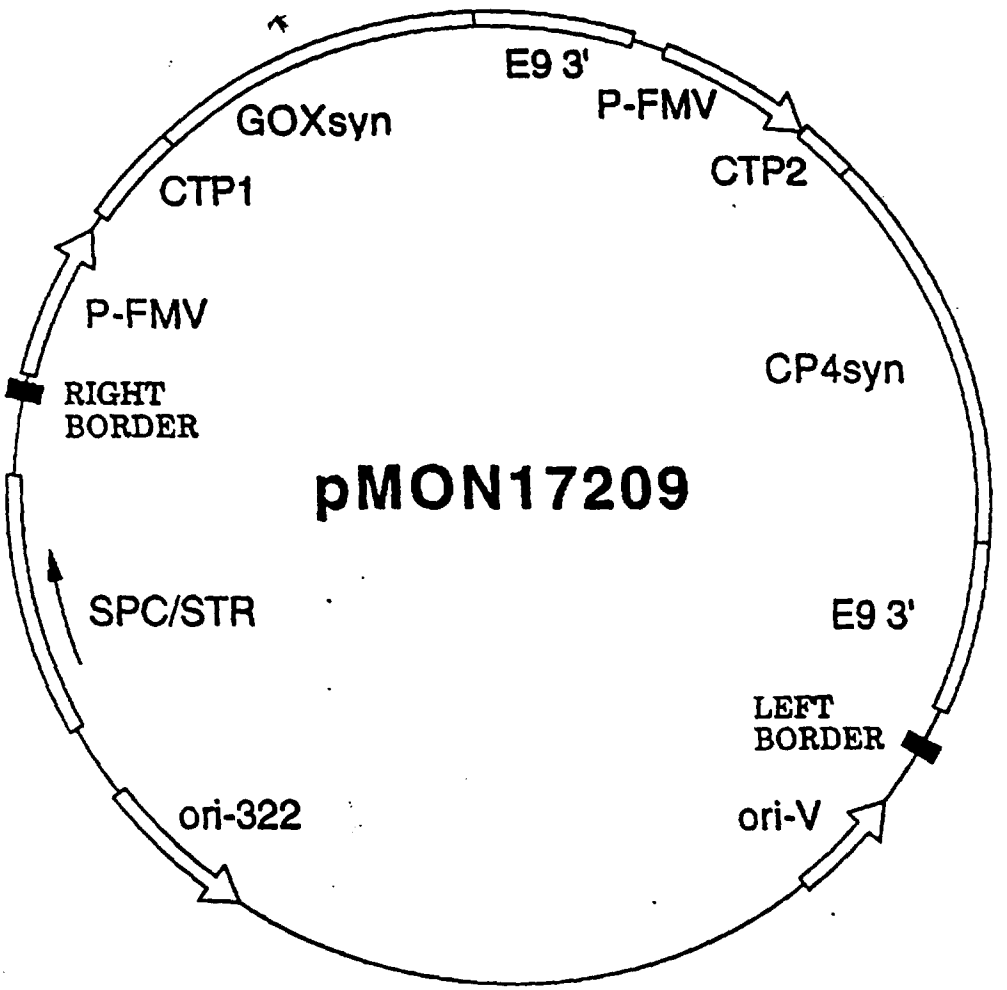
E9 3'
P-FMV
CTP2
CP4syn
E9 3'
LEFT BORDER
ori-V
ori-322
SPC/STR
RIGHT BORDER
P-FMV
CTP1
GOXsyn
pMON17209

Figure 24

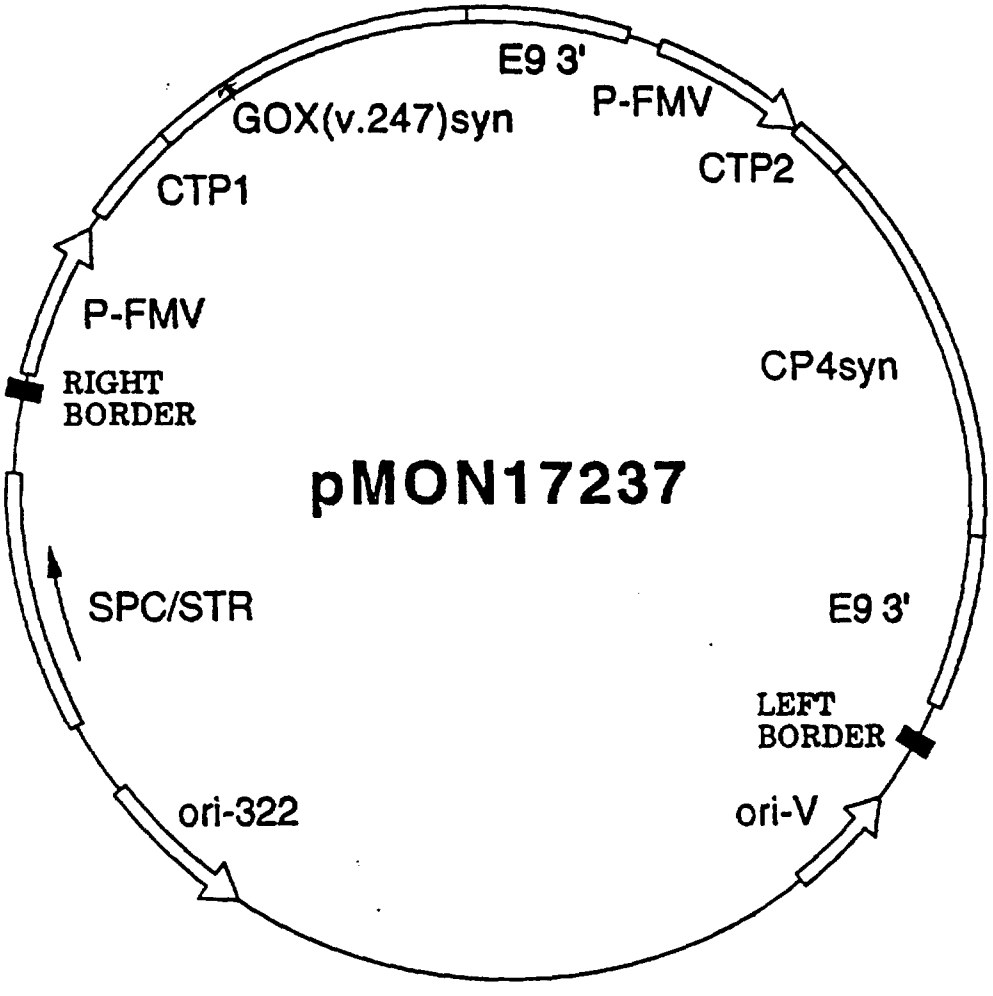
E9 3'
P-FMV
GOX(v.247)syn
CTP2
CTP1
P-FMV
CP4syn
RIGHT BORDER
pMON17237
SPC/STR
E9 3'
LEFT BORDER
ori-322
ori-V

Figure 25

GLYPHOSATE-TOLERANT 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASES

This is a continuation-in-part of a U.S. patent application Ser. No. 07/749,611, filed Aug. 28, 1991 now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/576,537, filed Aug. 31, 1990, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates in general to plant molecular biology and, more particularly, to a new class of glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases.

Recent advances in genetic engineering have provided the requisite tools to transform plants to contain foreign genes. It is now possible to produce plants which have unique characteristics of agronomic importance. Certainly, one such advantageous trait is more cost effective, environmentally compatible weed control via herbicide tolerance. Herbicide-tolerant plants may reduce the need for tillage to control weeds thereby effectively reducing soil erosion.

One herbicide which is the subject of much investigation in this regard is N-phosphonomethylglycine commonly referred to as glyphosate. Glyphosate inhibits the shikimic acid pathway which leads to the biosynthesis of aromatic compounds including amino acids, plant hormones and vitamins. Specifically, glyphosate curbs the conversion of phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid to 5-enolpyruvyl-3-phosphoshikimic acid by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (hereinafter referred to as EPSP synthase or EPSPS). For purposes of the present invention, the term "glyphosate" should be considered to include any herbicidally effective form of N-phosphonomethylglycine (including any salt thereof) and other forms which result in the production of the glyphosate anion in plants.

It has been shown that glyphosate-tolerant plants can be produced by inserting into the genome of the plant the capacity to produce a higher level of EPSP synthase in the chloroplast of the cell (Shah et al., 1986) which enzyme is preferably glyphosate-tolerant (Kishore et al. 1988). Variants of the wild-type EPSPS enzyme have been isolated which are glyphosate-tolerant as a result of alterations in the EPSPS amino acid coding sequence (Kishore and Shah, 1988; Schulz et al., 1984; Sost et al., 1984; Kishore et al., 1986). These variants typically have a higher K_i for glyphosate than the wild-type EPSPS enzyme which confers the glyphosate-tolerant phenotype, but these variants are also characterized by a high K_m for PEP which makes the enzyme kinetically less efficient (Kishore and Shah, 1988; Sost et al., 1984; Schulz et al., 1984; Kishore et al., 1986; Sost and Amrhein, 1990). For example, the apparent K_m for PEP and the apparent K_i for glyphosate for the native EPSPS from *E. coli* are 10 μM and 0.5 μM while for a glyphosate-tolerant isolate having a single amino acid substitution of an alanine for the glycine at position 96 these values are 220 μM and 4.0 mM, respectively. A number of glyphosate-tolerant plant variant EPSPS genes have been constructed by mutagenesis. Again, the glyphosate-tolerant EPSPS was impaired due to an increase in the K_m for PEP and a slight reduction of the V_{max} of the native plant enzyme (Kishore and Shah, 1988) thereby lowering the catalytic efficiency (V_{max}/K_m) of the enzyme. Since the kinetic constants of the variant enzymes are impaired with respect to PEP, it has been proposed that high levels of overproduction of the variant enzyme, 40–80 fold, would be required to maintain

normal catalytic activity in plants in the presence of glyphosate (Kishore et al., 1988).

While such variant EPSP synthases have proved useful in obtaining transgenic plants tolerant to glyphosate, it would be increasingly beneficial to obtain an EPSP synthase that is highly glyphosate-tolerant while still kinetically efficient such that the amount of the glyphosate-tolerant EPSPS needed to be produced to maintain normal catalytic activity in the plant is reduced or that improved tolerance be obtained with the same expression level.

Previous studies have shown that EPSPS enzymes from different sources vary widely with respect to their degree of sensitivity to inhibition by glyphosate. A study of plant and bacterial EPSPS enzyme activity as a function of glyphosate concentration showed that there was a very wide range in the degree of sensitivity to glyphosate. The degree of sensitivity showed no correlation with any genus or species tested (Schulz et al., 1985). Insensitivity to glyphosate inhibition of the activity of the EPSPS from the Pseudomonas sp. PG2982 has also been reported but with no details of the studies (Fitzgibbon, 1988). In general, while such natural tolerance has been reported, there is no report suggesting the kinetic superiority of the naturally occurring bacterial glyphosate-tolerant EPSPS enzymes over those of mutated EPSPS enzymes nor have any of the genes been characterized. Similarly, there are no reports on the expression of naturally glyphosate-tolerant EPSPS enzymes in plants to confer glyphosate tolerance.

For purposes of the present invention the term "mature EPSP synthase" relates to the EPSPS polypeptide without the N-terminal chloroplast transit peptide. It is now known that the precursor form of the EPSP synthase in plants (with the transit peptide) is expressed and upon delivery to the chloroplast, the transit peptide is cleaved yielding the mature EPSP synthase. All numbering of amino acid positions are given with respect to the mature EPSP synthase (without chloroplast transit peptide leader) to facilitate comparison of EPSPS sequences from sources which have chloroplast transit peptides (i.e., plants and fungi) to sources which do not utilize a chloroplast targeting signal (i.e., bacteria).

In the amino acid sequences which follow, the standard single letter or three letter nomenclature are used. All peptide structures represented in the following description are shown in conventional format in which the amino group at the N-terminus appears to the left and the carboxyl group at the C-terminus at the right. Likewise, amino acid nomenclature for the naturally occurring amino acids found in protein is as follows: alanine (Ala;A), asparagine (Asn;N), aspartic acid (Asp;D), arginine (Arg;R), cysteine (Cys;C), glutamic acid (Glu;E), glutamine (Gln;Q), glycine (Gly;G), histidine (His;H), isoleucine (Ile;I), leucine (Leu;L), lysine (Lys;K), methionine (Met;M), phenylalanine (Phe;F), proline (Pro;P), serine (Ser;S), threonine (Thr;T), tryptophan (Trp;W), tyrosine (Tyr;Y), and valine (Val;V). An "X" is used when the amino acid residue is unknown and parentheses designate that an unambiguous assignment is not possible and the amino acid designation within the parentheses is the most probable estimate based on known information.

The term "nonpolar" amino acids include alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and methionine. The term "uncharged polar" amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The term "charged polar" amino acids includes the "acidic" and "basic" amino acids. The term "acidic" amino acids includes aspartic acid and glutamic

acid. The term "basic" amino acid includes lysine, arginine and histidine. The term "polar" amino acids includes both "charged polar" and "uncharged polar" amino acids.

Deoxyribonucleic acid (DNA) is a polymer comprising four mononucleotide units, dAMP (2'-Deoxyadenosine-5-monophosphate), dGMP (2'-Deoxyguanosine-5-monophosphate), dCMP (2'-Deoxycytosine-5-monophosphate) and dTMP (2'-Deoxythymosine-5-monophosphate) linked in various sequences by 3',5'-phosphodiester bridges. The structural DNA consists of multiple nucleotide triplets called "codons" which code for the amino acids. The codons correspond to the various amino acids as follows: Arg (CGA, CGC, CGG, CGT, AGA, AGG); Leu (CTA, CTC, CTG, CTT, TTA, TTG); Ser (TCA, TCC, TCG, TCT, AGC, AGT); Thr (ACA, ACC, ACG, ACT); Pro (CCA, CCC, CCG, CCT); Ala (GCA, GCC, GCG, GCT); Gly (GGA, GGC, GGG, GGT); Ile (ATA, ATC, ATT); Val (GTA, GTC, GTG, GTT); Lys (AAA, AAG); Asn (AAC, AAT); Gln (CAA, CAG); His (CAC, CAT); Glu (GAA, GAG); Asp (GAC, GAT); Tyr (TAC, TAT); Cys (TGC, TGT); Phe (TTC, TTT); Met (ATG); and Trp (UGG). Moreover, due to the redundancy of the genetic code (i.e., more than one codon for all but two amino acids), there are many possible DNA sequences which may code for a particular amino acid sequence.

SUMMARY OF THE INVENTION

DNA molecules comprising DNA encoding kinetically efficient, glyphosate-tolerant EPSP synthases are disclosed. The EPSP synthases of the present invention reduce the amount of overproduction of the EPSPS enzyme in a transgenic plant necessary for the enzyme to maintain catalytic activity while still conferring glyphosate tolerance. The EPSP synthases described herein represent a new class of EPSPS enzymes, referred to hereinafter as Class II EPSPS enzymes. Class II EPSPS enzymes of the present invention usually share only between about 47% and 55% amino acid similarity or between about 22% and 30% amino acid identity to other known bacterial or plant EPSPS enzymes and exhibit tolerance to glyphosate while maintaining suitable K_m (PEP) ranges. Suitable ranges of K_m (PEP) for EPSPS for enzymes of the present invention are between 1–150 μM, with a more preferred range of between 1–35 μM, and a most preferred range between 2–25 μM. These kinetic constants are determined under the assay conditions specified hereinafter. An EPSPS of the present invention preferably has a K_i for glyphosate range of between 15–10000 μM. The K_i/K_m ratio should be between about 2–500, and more preferably between 25–500. The V_{max} of the purified enzyme should preferably be in the range of 2–100 units/mg (μmoles/minute.mg at 25° C.) and the K_m for shikimate-3-phosphate should preferably be in the range of 0.1 to 50 μM.

Genes coding for Class II EPSPS enzymes have been isolated from five (5) different bacteria: *Agrobacterium tumefaciens* sp. strain CP4, Achromobacter sp. strain LBAA, Pseudomonas sp. strain PG2982, *Bacillus subtilis*, and *Staphylococcus aureus*. The LBAA and PG2982 Class II EPSPS genes have been determined to be identical and the proteins encoded by these two genes are very similar to the CP4 protein and share approximately 84% amino acid identity with it. Class II EPSPS enzymes often may be distinguished from Class I EPSPS's by their inability to react with polyclonal antibodies prepared from Class I EPSPS enzymes under conditions where other Class I EPSPS enzymes would readily react with the Class I antibodies as well as the presence of certain unique regions of amino acid homology which are conserved in Class II EPSP synthases as discussed hereinafter.

Other Class II EPSPS enzymes can be readily isolated and identified by utilizing a nucleic acid probe from one of the Class II EPSPS genes disclosed herein using standard hybridization techniques. Such a probe from the CP4 strain has been prepared and utilized to isolate the Class II EPSPS genes from strains LBAA and PG2982. These genes may also optionally be adapted for enhanced expression in plants by known methodology. Such a probe has also been used to identify homologous genes in bacteria isolated de novo from soil.

The Class II EPSPS enzymes are preferably fused to a chloroplast transit peptide (CTP) to target the protein to the chloroplasts of the plant into which it may be introduced. Chimeric genes encoding this CTP-Class II EPSPS fusion protein may be prepared with an appropriate promoter and 3' polyadenylation site for introduction into a desired plant by standard methods.

To obtain the maximal tolerance to glyphosate herbicide it is preferable to transform the desired plant with a plant-expressible Class II EPSPS gene in conjunction with another plant-expressible gene which expresses a protein capable of degrading glyphosate such as a plant-expressible gene encoding a glyphosate oxidoreductase enzyme as described in PCT Application No. WO 92/00377, the disclosure of which is hereby incorporated by reference.

Therefore, in one aspect, the present invention provides a new class of EPSP synthases that exhibit a low K_m for phosphoenolpyruvate (PEP), a high V_{max}/K_m ratio, and a high K_i for glyphosate such that when introduced into a plant, the plant is made glyphosate-tolerant such that the catalytic activity of the enzyme and plant metabolism are maintained in a substantially normal state. For purposes of this discussion, a highly efficient EPSPS refers to its efficiency in the presence of glyphosate.

More particularly, the present invention provides EPSPS enzymes having a K_m for phosphoenolpyruvate (PEP) between 1–150 μM and a K_i(glyphosate)/K_m (PEP) ratio between 3–500, said enzymes having the sequence domains:

-R-X_1-H-X_2-E-(SEQ ID NO:37), in which
  X_1 is an uncharged polar or acidic amino acid,
  X_2 is serine or threonine; and
-G-D-K-X_3-(SEQ ID NO:38), in which
  X_3 is serine or threonine; and
-S-A-Q-X_4-K-(SEQ ID NO:39), in which
  X_4 is any amino acid; and
-N-X_5-T-R-(SEQ ID:40), in which
  X_5 is any amino acid.

Exemplary Class II EPSPS enzyme sequences are disclosed from seven sources: Agrobacterium sp. strain designated CP4, Achromobacter sp. strain LBAA, Pseudomonas sp. strain PG2982, *Bacillus subtilis* 1A2, *Staphylococcus aureus* (ATCC 35556), Synechocystis sp. PCC6803 and *Dichelobacter nodosus*.

In another aspect of the present invention, a double-stranded DNA molecule comprising DNA encoding a Class II EPSPS enzyme is disclosed. Exemplary Class II EPSPS enzyme DNA sequences are disclosed from seven sources: Agrobacterium sp. strain designated CP4, Achromobacter sp. strain LBAA, Pseudomonas sp. strain PG2982, *Bacillus subtilis* 1A2, *Staphylococcus aureus* (ATCC 35556), Synechocystis sp. PCC6803 and *Dichelobacter nodosus*.

In a further aspect of the present invention, nucleic acid probes from EPSPS Class II genes are presented that are suitable for use in screening for Class II EPSPS genes in

other sources by assaying for the ability of a DNA sequence from the other source to hybridize to the probe.

In yet another aspect of the present invention, a recombinant, double-stranded DNA molecule comprising in sequence:

a) a promoter which functions in plant cells to cause the production of an RNA sequence;

b) a structural DNA sequence that causes the production of an RNA sequence which encodes a Class II EPSPS enzyme having the sequence domains:

-R-X_1-H-X_2-E-(SEQ ID NO:37), in which

X_1 is an uncharged polar or acidic amino acid,

X_2 is serine or threonine; and

-G-D-K-X_3-(SEQ ID NO:38), in which

X_3 is serine or threonine; and

-S-A-Q-X_4-K-(SEQ ID NO:39), in which

X_4 is any amino acid; and

-N-X_5-T-R-(SEQ ID:40), in which

X_5 is any amino acid; and

c) a 3' nontranslated region which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the EPSP synthase polypeptide to enhance the glyphosate tolerance of a plant cell transformed with said DNA molecule.

In still yet another aspect of the present invention, transgenic plants and transformed plant cells are disclosed that are made glyphosate-tolerant by the introduction of the above-described plant-expressible Class II EPSPS DNA molecule into the plant's genome.

In still another aspect of the present invention, a method for selectively controlling weeds in a crop field is presented by planting crop seeds or crop plants transformed with a plant-expressible Class II EPSPS DNA molecule to confer glyphosate tolerance to the plants which allows for glyphosate containing herbicides to be applied to the crop to selectively kill the glyphosate sensitive weeds, but not the crops.

Other and further objects, advantages and aspects of the invention will become apparent from the accompanying drawing figures and the description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A, 1B, show the DNA sequence (SEQ ID NO:1) for the full-length promoter of figwort mosaic virus (FMV35S).

FIG. 2 shows the cosmid cloning vector pMON17020.

FIG. 3A, 3B, 3C, 3D and 3E show the structural DNA sequence (SEQ ID NO:2) for the Class II EPSPS gene from bacterial isolate Agrobacterium sp. strain CP4 and the deduced amino acid sequence (SEQ ID NO:3).

FIG. 4A, 4B, 4C, 4D and 4E show the structural DNA sequence (SEQ ID NO:4) for the Class II EPSPS gene from the bacterial isolate Achromobacter sp. strain LBAA and the deduced amino acid sequence (SEQ ID NO:5).

FIG. 5A, 5B, 5C, 5D and 5E show the structural DNA sequence (SEQ ID NO:6) for the Class II EPSPS gene from the bacterial isolate Pseudomonas sp. strain PG2982 and the deduced amino acid sequence (SEQ ID NO:7).

FIG. 6A and 6B show the Bestfit comparison of the CP4 EPSPS amino acid sequence (SEQ ID NO:3) with that for the *E. coli* EPSPS (SEQ ID NO:8).

FIG. 7A and 7B show the Bestfit comparison of the CP4 EPSPS amino acid sequence (SEQ ID NO:3) with that for the LBAA EPSPS (SEQ ID NO:5).

FIG. 8A and 8B show the structural DNA sequence (SEQ ID NO:9) for the synthetic CP4 Class II EPSPS gene.

FIG. 9 shows the DNA sequence (SEQ ID NO:10) of the chloroplast transit peptide (CTP) and encoded amino acid sequence (SEQ ID NO:11) derived from the *Arabidopsis thaliana* EPSPS CTP and containing a SphI restriction site at the chloroplast processing site, hereinafter referred to as CTP2.

FIG. 10A and 10B show the DNA sequence (SEQ ID NO:12) of the chloroplast transit peptide and encoded amino acid sequence (SEQ ID NO:13) derived from the *Arabidopsis thaliana* EPSPS gene and containing an EcoRI restriction site within the mature region of the EPSPS, hereinafter referred to as CTP3.

FIG. 11 shows the DNA sequence (SEQ ID NO:14) of the chloroplast transit peptide and encoded amino acid sequence (SEQ ID NO:15) derived from the *Petunia hybrida* EPSPS CTP and containing a SphI restriction site at the chloroplast processing site and in which the amino acids at the processing site are changed to -Cys-Met-, hereinafter referred to as CTP4.

FIG. 12A and 12B show the DNA sequence (SEQ ID NO:16) of the chloroplast transit peptide and encoded amino acid sequence (SEQ ID NO:17) derived from the *Petunia hybrida* EPSPS gene with the naturally occurring EcoRI site in the mature region of the EPSPS gene, hereinafter referred to as CTP5.

FIG. 13 shows a plasmid map of CP4 plant transformation/expression vector pMON17110.

FIG. 14 shows a plasmid map of CP4 synthetic EPSPS gene plant transformation/expression vector pMON17131.

FIG. 15 shows a plasmid map of CP4 EPSPS free DNA plant transformation expression vector pMON13640.

FIG. 16 shows a plasmid map of CP4 plant transformation/direct selection vector pMON17227.

FIG. 17 shows a plasmid map of CP4 plant transformation/expression vector pMON19653.

FIG. 18A, 18B, 18C and 18D show the structural DNA sequence (SEQ ID NO:41) for the Class II EPSPS gene from the bacterial isolate *Bacillus subtilis* and the deduced amino acid sequence (SEQ ID NO:42).

FIG. 19A, 19B, 19C and 19D show the structural DNA sequence (SEQ ID NO:43) for the Class II EPSPS gene from the bacterial isolate *Staphylococcus aureus* and the deduced amino acid sequence (SEQ ID NO:44).

FIG. 20A, 20B, 20C, 20D, 20E, 20F, 20G, 20H, 20I, 20J and 20K show the Bestfit comparison of the representative Class II EPSPS amino acid sequences Pseudomonas sp. strain PG2982 (SEQ ID NO:7), Achromobacter sp. strain LBAA (SEQ ID NO:5), Agrobacterium sp. strain designated CP4 (SEQ ID NO:3), *Bacillus subtilis* (SEQ ID NO:42), and *Staphylococcus aureus* (SEQ ID NO:44) with that for representative Class I EPSPS amino acid sequences [*Saccharomyces cerevisiae* (SEQ ID NO:49), *Aspergillus nidulans* (SEQ ID NO:50), *Brassica napus* (SEQ ID NO:51), *Arabidopsis thaliana* (SEQ ID NO:52), *Nicotina tobacum* (SEQ ID NO:53), *L. esculentum* (SEQ ID NO:54), *Petunia hybrida* (SEQ ID NO:55), *Zea mays* (SEQ ID NO:56), *Salmonella gallinarum* (SEQ ID NO:57), *Salmonella typhimurium* (SEQ ID NO:58), *Salmonella typhi* (SEQ ID NO:65), *E. coli* (SEQ ID NO:8), *K. pneumoniae* (SEQ ID NO:59), *Y. enterocolitica* (SEQ ID NO:60), *H. influenzae* (SEQ ID NO:61), *P. multocida* (SEQ ID NO:62), *Aeromonas salmonicida* (SEQ ID NO:63), *Bacillus pertussis* (SEQ ID NO:64)] and illustrates the conserved regions among

Class II EPSPS sequences which are unique to Class II EPSPS sequences. To aid in a comparison of the EPSPS sequences, only mature EPSPS sequences were compared. That is, the sequence corresponding to the chloroplast transit peptide, if present in a subject EPSPS, was removed prior to making the sequence alignment.

FIG. 21A, 21B, 21C, 21D and 21E show the structural DNA sequence (SEQ ID NO:66) for the Class II EPSPS gene from the bacterial isolate Synechocystis sp. PCC6803 and the deduced amino acid sequence (SEQ ID NO:67).

FIG. 22A, 22B, 22C, 22D and 22E show the structural DNA sequence (SEQ ID NO:68) for the Class II EPSPS gene from the bacterial isolate *Dichelobacter nodosus* and the deduced amino acid sequence (SEQ ID NO:69).

FIG. 23A, 23B, 23C and 22D show the Bestfit comparison of the representative Class II EPSPS amino acid sequences Pseudomonas sp. strain PG2982 (SEQ ID NO:7), Achromobacter sp. strain LBAA (SEQ ID NO:5), Agrobacterium sp. strain designated CP4 (SEQ ID NO:3), Synechocystis sp. PCC6803 (SEQ ID NO:67), *Bacillus subtilis* (SEQ ID NO:42), *Dichelobacter nodosus* (SEQ ID NO:69) and *Staphylococcus aureus* (SEQ ID NO:44).

FIG. 24 a plasmid map of canola plant transformation/expression vector pMON17209.

FIG. 25 a plasmid map of canola plant transformation/expression vector pMON17237.

STATEMENT OF THE INVENTION

The expression of a plant gene which exists in double-stranded DNA form involves synthesis of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' non-translated region which adds polyadenylate nucleotides to the 3' end of the RNA.

Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA, and to initiate the transcription into mRNA using one of the DNA strands as a template to make a corresponding complementary strand of RNA. A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of Agrobacterium tumefaciens), the cauliflower mosaic virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide) and the full-length transcript promoter from the figwort mosaic virus (FMV35S), promoters from the maize ubiquitin and rice actin genes. All of these promoters have been used to create various types of DNA constructs which have been expressed in plants; see, e.g., PCT publication WO 84/02913 (Rogers et al., Monsanto).

Promoters which are known or found to cause transcription of DNA in plant cells can be used in the present invention. Such promoters may be obtained from a variety of sources such as plants and plant DNA viruses and include, but are not limited to, the CaMV35S and FMV35S promoters and promoters isolated from plant genes such as ssRUBISCO genes and the maize ubiquitin and rice actin genes. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of a Class II EPSPS to render the plant substantially tolerant to glyphosate herbicides. The amount of Class II EPSPS needed to induce the desired tolerance may vary with the plant species. It is preferred that the promoters utilized have relatively high expression in all meristematic tissues in addition to other tissues inasmuch as it is now known that glyphosate is translocated and accumulated in this type of plant tissue. Alternatively, a combination of chimeric genes can be used to cumulatively result in the necessary overall expression level of the selected Class II EPSPS enzyme to result in the glyphosate-tolerant phenotype.

The mRNA produced by a DNA construct of the present invention also contains a 5' non-translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs, as presented in the following examples, wherein the non-translated region is derived from both the 5' non-translated sequence that accompanies the promoter sequence and part of the 5' non-translated region of the virus coat protein gene. Rather, the non-translated leader sequence can be derived from an unrelated promoter or coding sequence as discussed above.

Preferred promoters for use in the present invention the full-length transcript (SEQ ID NO:1) promoter from the figwort mosaic virus (FMV35S) and the full-length transcript (35S) promoter from cauliflower mosaic virus (CaMV), including the enhanced CaMV35S promoter (Kay et al. 1987). The FMV35S promoter functions as strong and uniform promoter with particularly good expression in meristematic tissue for chimeric genes inserted into plants, particularly dicotyledons. The resulting transgenic plant in general expresses the protein encoded by the inserted gene at a higher and more uniform level throughout the tissues and cells of the transformed plant than the same gene driven by an enhanced CaMV35S promoter. Referring to FIG. 1, the DNA sequence (SEQ ID NO:1) of the FMV35S promoter is located between nucleotides 6368 and 6930 of the FMV genome. A 5' non-translated leader sequence is preferably coupled with the promoter. The leader sequence can be from the FMV35S genome itself or can be from a source other than FMV35S.

For expression of heterologous genes in monocotyledonous plants the use of an intron has been found to enhance expression of the heterologous gene. While one may use any of a number of introns which have been isolated from plant genes, the use of the first intron from the maize heat shock 70 gene is preferred.

The 3' non-translated region of the chimeric plant gene contains a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the viral RNA. Examples of suitable 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylated signal of Agrobacterium tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene, and (2) plant genes like the soybean storage protein genes and the small subunit of the ribulose-1,5-bisphosphate carboxylase (ssRUBISCO) gene. An example of a preferred 3' region is that from the ssRUBISCO gene from pea (E9), described in greater detail below.

The DNA constructs of the present invention also contain a structural coding sequence in double-stranded DNA form which encodes a glyphosate-tolerant, highly efficient Class II EPSPS enzyme.

Identification of glyphosate-tolerant, highly efficient EPSPS enzymes

In an attempt to identify and isolate glyphosate-tolerant, highly efficient EPSPS enzymes, kinetic analysis of the EPSPS enzymes from a number of bacteria exhibiting tolerance to glyphosate or that had been isolated from suitable sources was undertaken. It was discovered that in some cases the EPSPS enzymes showed no tolerance to inhibition by glyphosate and it was concluded that the tolerance phenotype of the bacterium was due to an impermeability to glyphosate or other factors. In a number of cases, however, microorganisms were identified whose EPSPS enzyme showed a greater degree of tolerance to inhibition by glyphosate and that displayed a low K_m for PEP when compared to that previously reported for other microbial and plant sources. The EPSPS enzymes from these microorganisms were then subjected to further study and analysis.

Table I displays the data obtained for the EPSPS enzymes identified and isolated as a result of the above described analysis. Table I includes data for three identified Class II EPSPS enzymes that were observed to have a high tolerance to inhibition to glyphosate and a low K_m for PEP as well as data for the native Petunia EPSPS and a glyphosate-tolerant variant of the Petunia EPSPS referred to as GA101. The GA101 variant is so named because it exhibits the substitution of an alanine residue for a glycine residue at position 101 (with respect to Petunia). When the change introduced into the Petunia EPSPS (GA101) was introduced into a number of other EPSPS enzymes, similar changes in kinetics were observed, an elevation of the K_i for glyphosate and of the K_m for PEP.

TABLE I

Kinetic characterization of EPSPS enzymes

ENZYME SOURCE	K_m PEP (μM)	K_i Glyphosate (μM)	K_i/K_m
Petunia	5	0.4	0.08
Petunia GA101	200	2000	10
PG2982	2.1–3.1[1]	25–82	~8–40
LBAA	~7.3–9[2]	60 (est)[7]	~7.9
CP4	12[3]	2720	227
B. subtilis 1A2	13[4]	440	33.8
S. aureus	5[5]	200	40

[1]Range of PEP tested = 1–40 μM
[2]Range of PEP tested = 5–80 μM
[3]Range of PEP tested = 1.5–40 μM
[4]Range of PEP tested = 1–60 μM
[5]Range of PEP tested = 1–80 μM
[7](est) = estimated

The Agrobacterium sp. strain CP4 was initially identified by its ability to grow on glyphosate as a carbon source (10 mM) in the presence of 1 mM phosphate. The strain CP4 was identified from a collection obtained from a fixed-bed immobilized cell column that employed Manville R-635 diatomaceous earth beads. The column had been run for three months on a waste-water feed from a glyphosate production plant. The column contained 50 mg/ml glyphosate and NH_3 as NH_4Cl. Total organic carbon was 300 mg/ml and BOD's (Biological Oxygen Demand—a measure of "soft" carbon availability) were less than 30 mg/ml. This treatment column has been described (Heitkamp et al., 1990). Dworkin-Foster minimal salts medium containing glyphosate at 10 mM and with phosphate at 1 mM was used to select for microbes from a wash of this column that were capable of growing on glyphosate as sole carbon source. Dworkin-Foster minimal medium was made up by combining in 1 liter (with autoclaved H_2O), 1 ml each of A, B and C and 10 ml of D (as per below) and thiamine HCl (5 mg).

A.	D-F Salts (1000X stock; per 100 ml; autoclaved):		
	H_3BO_3	1 mg	
	$MnSO_4.7\ H_2O$	1 mg	
	$ZnSO_4.7\ H_2O$	12.5 mg	
	$CuSO_4.5\ H_2O$	8 mg	
	$NaMoO_3.3\ H_2O$	1.7 mg	
B.	$FeSO_4.7\ H_2O$ (1000X Stock; per 100 ml; autoclaved)		0.1 g
C.	$MgSO_4.7\ H_2O$ (1000X Stock; per 100 ml; autoclaved)		20 g
D.	$(NH_4)_2SO_4$ (100X stock; per 100 ml; autoclaved)		20 g

Yeast Extract (YE; Difco) was added to a final concentration of 0.01 or 0.001%. The strain CP4 was also grown on media composed of D–F salts, amended as described above, containing glucose, gluconate and citrate (each at 0.1%) as carbon sources and with inorganic phosphate (0.2–1.0 mM) as the phosphorous source.

Other Class II EPSPS containing microorganisms were identified as Achromobacter sp. strain LBAA (Hallas et al., 1988), Pseudomonas sp. strain PG2982 (Moore et al., 1983; Fitzgibbon 1988), *Bacillus subtilis* 1A2 (Heinzer et al., 1984) and *Staphylococcus aureus* (O'Connell et al., 1993). It had been reported previously, from measurements in crude lysates, that the EPSPS enzyme from strain PG2982 was less sensitive to inhibition to glyphosate than that of *E. coli*, but there has been no report of the details of this lack of sensitivity and there has been no report on the K_m for PEP for this enzyme or of the DNA sequence for the gene for this enzyme (Fitzgibbon, 1988; Fitzgibbon and Braymer, 1990). Relationship of the Class II EPSPS to those previously studied

All EPSPS proteins studied to date have shown a remarkable degree of homology. For example, bacterial and plant EPSPS's are about 54% identical and with similarity as high as 80%. Within bacterial EPSPS's and plant EPSPS's themselves the degree of identity and similarity is much greater (see Table II).

TABLE II

Comparison between exemplary Class I EPSPS protein sequences[1]

	similarity	identity
E. coli vs. *S. typhimurium*	93	88
P. hybrida vs. *E. coli*	72	55
P. hybrida vs. *L. esculentum*	95	88

[1]The EPSPS sequences compared here were obtained from the following references: *E. coli*, Rogers et al., 1983; *S. typhimurium*, Stalker et al., 1985; *Petunia hybrida*, Shah et al., 1986; and tomato (*L. esculentum*), Gasser et al., 1988.

When crude extracts of CP4 and LBAA bacteria (50 μg protein) were probed using rabbit anti-EPSPS antibody (Padgette et al., 1987) to the Petunia EPSPS protein in a Western analysis, no positive signal could be detected, even with extended exposure times (Protein A—^{125}I development system) and under conditions where the control EPSPS (Petunia EPSPS, 20 ng; a Class I EPSPS) was readily detected. The presence of EPSPS activity in these extracts was confirmed by enzyme assay. This surprising result, indicating a lack of similarity between the EPSPS's from these bacterial isolates and those previously studied, coupled with the combination of a low K_m for PEP and a high K_i for glyphosate, illustrates that these new EPSPS enzymes are different from known EPSPS enzymes (now referred to as Class I EPSPS).

Glyphosate-tolerant Enzymes in Microbial Isolates

For clarity and brevity of disclosure, the following description of the isolation of genes encoding Class II EPSPS enzymes is directed to the isolation of such a gene from a bacterial isolate. Those skilled in the art will recognize that the same or similar strategy can be utilized to isolate such genes from other microbial isolates, plant or fungal sources.

Cloning of the Agrobacterium sp. strain CP4 EPSPS Gene(s) in *E. coli*

Having established the existence of a suitable EPSPS in Agrobacterium sp. strain CP4, two parallel approaches were undertaken to clone the gene: cloning based on the expected phenotype for a glyphosate-tolerant EPSPS; and purification of the enzyme to provide material to raise antibodies and to obtain amino acid sequences from the protein to facilitate the verification of clones. Cloning and genetic techniques, unless otherwise indicated, are generally those described in Maniatis et al., 1982 or Sambrook et al., 1987. The cloning strategy was as follows: introduction of a cosmid bank of strain Agrobacterium sp. strain CP4 into *E. coli* and selection for the EPSPS gene by selection for growth on inhibitory concentrations of glyphosate.

Chromosomal DNA was prepared from strain Agrobacterium sp. strain CP4 as follows: The cell pellet from a 200 ml L-Broth (Miller, 1972), late log phase culture of Agrobacterium sp. strain CP4 was resuspended in 10 ml of Solution I; 50 mM Glucose, 10 mM EDTA, 25 mM Tris -Cl, pH 8.0 (Birnboim and Doly, 1979). SDS was added to a final concentration of 1% and the suspension was subjected to three freeze-thaw cycles, each consisting of immersion in dry ice for 15 minutes and in water at 70° C. for 10 minutes. The lysate was then extracted four times with equal volumes of phenol:chloroform (1:1; phenol saturated with TE; TE=10 mM Tris pH8.0; 1.0 mM EDTA) and the phases separated by centrifugation (15000 g; 10 minutes). The ethanol-precipitable material was pelleted from the supernatant by brief centrifugation (8000 g; 5 minutes) following addition of two volumes of ethanol. The pellet was resuspended in 5 ml TE and dialyzed for 16 hours at 4° C. against 2 liters TE. This preparation yielded a 5 ml DNA solution of 552 μg/ml.

Partially-restricted DNA was prepared as follows. Three 100 μg aliquot samples of CP4 DNA were treated for 1 hour at 37° C. with restriction endonuclease HindIII at rates of 4, 2 and 1 enzyme unit/μg DNA, respectively. The DNA samples were pooled, made 0.25 mM with EDTA and extracted with an equal volume of phenol:chloroform. Following the addition of sodium acetate and ethanol, the DNA was precipitated with two volumes of ethanol and pelleted by centrifugation (12000 g; 10 minutes). The dried DNA pellet was resuspended in 500 μl TE and layered on a 10–40% Sucrose gradient (in 5% increments of 5.5 ml each) in 0.5M NaCl, 50 mM Tris pH8.0, 5 mM EDTA. Following centrifugation for 20 hours at 26,000 rpm in a SW28 rotor, the tubes were punctured and ~1.5 ml fractions collected. Samples (20 μl) of each second fraction were run on 0.7% agarose gel and the size of the DNA determined by comparison with linearized lambda DNA and HindIII-digested lambda DNA standards. Fractions containing DNA of 25–35 kb fragments were pooled, desalted on AMICON10 columns (7000 rpm; 20° C.; 45 minutes) and concentrated by precipitation. This procedure yielded 15 μg of CP4 DNA of the required size. A cosmid bank was constructed using the vector pMON17020. This vector, a map of which is presented in FIG. 2, is based on the pBR327 replicon and contains the spectinomycin/streptomycin (Sp^r;spc) resistance gene from Tn7 (Fling et al., 1985), the chloramphenicol resistance gene (Cm^r;cat) from Tn9 (Alton et al., 1979), the gene10 promoter region from phage T7 (Dunn et al., 1983), and the 1.6 kb BglII phage lambda cos fragment from pHC79 (Hohn and Collins, 1980). A number of cloning sites are located downstream of the cat gene. Since the predominant block to the expression of genes from other microbial sources in *E. coli* appears to be at the level of transcription, the use of the T7 promoter and supplying the T7 polymerase in trans from the pGP1-2 plasmid (Tabor and Richardson, 1985), enables the expression of large DNA segments of foreign DNA, even those containing RNA polymerase transcription termination sequences. The expression of the spc gene is impaired by transcription from the T7 promoter such that only Cm^r can be selected in strains containing pGP1-2. The use of antibiotic resistances such as Cm resistance which do not employ a membrane component is preferred due to the observation that high level expression of resistance genes that involve a membrane component, i.e. β-lactamase and Amp resistance, give rise to a glyphosate-tolerant phenotype. Presumably, this is due to the exclusion of glyphosate from the cell by the membrane localized resistance protein. It is also preferred that the selectable marker be oriented in the same direction as the T7 promoter.

The vector was then cut with HindIII and treated with calf alkaline phosphatase (CAP) in preparation for cloning. Vector and target sequences were ligated by combining the following:

Vector DNA (HindIII/CAP)	3 μg
Size fractionated CP4 HindIII fragments	1.5 μg
10X ligation buffer	2.2 μl
T4 DNA ligase (New England Biolabs) (400 U/μl)	1.0 μl

and adding H_2O to 22.0 μl. This mixture was incubated for 18 hours at 16° C. 10X ligation buffer is 250 mM Tris-HCl, pH 8.0; 100 mM $MgCl_2$; 100 mM Dithiothreitol; 2 mM Spermidine. The ligated DNA (5 μl) was packaged into lambda phage particles (Stratagene; Gigapack Gold) using the manufacturer's procedure.

A sample (200 μl) of *E. coli* HB101 (Boyer and Rolland-Dussoix, 1973) containing the T7 polymerase expression plasmid pGP1-2 (Tabor and Richardson, 1985) and grown overnight in L-Broth (with maltose at 0.2% and kanamycin at 50/μg/ml) was infected with 50 μl of the packaged DNA. Transformants were selected at 30° C. on M9 (Miller, 1972) agar containing kanamycin (50 μg/ml), chloramphenicol (25 μg/ml), L-proline (50 μg/ml), L-leucine (50 μg/ml) and B1 (5 μg/ml), and with glyphosate at 3.0 mM. Aliquot samples were also plated on the same media lacking glyphosate to titer the packaged cosmids. Cosmid transformants were isolated on this latter medium at a rate of ~5×10^5 per μg CP4 HindIII DNA after 3 days at 30° C. Colonies arose on the glyphosate agar from day 3 until day 15 with a final rate of ~1 per 200 cosmids. DNA was prepared from 14 glyphosate-tolerant clones and, following verification of this phenotype, was transformed into *E. coli* GB100/pGP1-2 (*E. coli* GB100 is an aroA derivative of MM294 [Talmadge and Gilbert, 1980]) and tested for complementation for growth in the absence of added aromatic amino acids and aminobenzoic acids. Other aroA strains such as SR481 (Bachman et al., 1980; Padgette et al., 1987), could be used and would be suitable for this experiment. The use of GB100 is merely exemplary and should not be viewed in a limiting sense. This aroA strain usually requires that growth media be supplemented with L-phenylalanine, L-tyrosine and L-tryptophan each at 100 μg/ml and with para-hydroxybenzoic acid, 2,3-dihydroxybenzoic acid and para-aminobenzoic acid

each at 5 μg/ml for growth in minimal media. Of the fourteen cosmids tested only one showed complementation of the aroA- phenotype. Transformants of this cosmid, pMON17076, showed weak but uniform growth on the unsupplemented minimal media after 10 days.

The proteins encoded by the cosmids were determined in vivo using a T7 expression system (Tabor and Richardson, 1985). Cultures of *E. coli* containing pGP1-2 (Tabor and Richardson, 1985) and test and control cosmids were grown at 30° C. in L-broth (2 ml) with chloramphenicol and kanamycin (25 and 50 μg/ml, respectively) to a Klett reading of ~50. An aliquot was removed and the cells collected by centrifugation, washed with M9 salts (Miller, 1972) and resuspended in 1 ml M9 medium containing glucose at 0.2%, thiamine at 20 μg/ml and containing the 18 amino acids at 0.01% (minus cysteine and methionine). Following incubation at 30° C. for 90 minutes, the cultures were transferred to a 42° C. water bath and held there for 15 minutes. Rifampicin (Sigma) was added to 200 μg/ml and the cultures held at 42° C. for 10 additional minutes and then transferred to 30° C. for 20 minutes. Samples were pulsed with 10 μCi of ^{35}S-methionine for 5 minutes at 30° C. The cells were collected by centrifugation and suspended in 60–120 μl cracking buffer (60 mM Tris-HCl 6.8, 1% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue). Aliquot samples were electrophoresed on 12.5% SDS-PAGE and following soaking for 60 minutes in 10 volumes of Acetic Acid-Methanol-water (10:30:60), the gel was soaked in ENLIGHTNING™ (DUPONT) following manufacturer's directions, dried, and exposed at –70° C. to X-Ray film. Proteins of about 45 kd in size, labeled with ^{35}S-methionine, were detected in number of the cosmids, including pMON17076.

Purification of EPSPS from Agrobacterium sp. strain CP4

All protein purification procedures were carried out at 3°–5° C. EPSPS enzyme assays were performed using either the phosphate release or radioactive HPLC method, as previously described in Padgette et al., 1987, using 1 mM phosphoenol pyruvate (PEP, Boehringer) and 2 mM shikimate-3-phosphate (S3P) substrate concentrations. For radioactive HPLC assays, ^{14}-CPEP (Amersham) was utilized. S3P was synthesized as previously described in Wibbenmeyer et al. 1988. N-terminal amino acid sequencing was performed by loading samples onto a Polybrene precycled filter in aliquots while drying. Automated Edman degradation chemistry was used to determine the N-terminal protein sequence, using an Applied Biosystems Model 470A gas phase sequencer (Hunkapiller et al., 1983) with an Applied Biosystems 120A PTH analyzer.

Five 10-liter fermentations were carried out on a spontaneous "smooth" isolate of strain CP4 that displayed less clumping when grown in liquid culture. This reduced clumping and smooth colony morphology may be due to reduced polysaccharide production by this isolate. In the following section dealing with the purification of the EPSPS enzyme, CP4 refers to the "smooth" isolate—CP4-S1. The cells from the three batches showing the highest specific activities were pooled. Cell paste of Agrobacterium sp. CP4 (300 g) was washed twice with 0.5 L of 0.9% saline and collected by centrifugation (30 minutes, 8000 rpm in a GS3 Sorvall rotor). The cell pellet was suspended in 0.9 L extraction buffer (100 mM TrisCl, 1 mM EDTA, 1 mM BAM (Benzamidine), 5 mM DTT, 10% glycerol, pH 7.5) and lysed by 2 passes through a Manton Gaulin cell. The resulting solution was centrifuged (30 minutes, 8000 rpm) and the supernatant was treated with 0.21 L of 1.5% protamine sulfate (in 100 mM TrisCl, pH 7.5, 0.2% w/v final protamine sulfate concentration). After stirring for 1 hour, the mixture was centrifuged (50 minutes, 8000 rpm) and the resulting supernatant treated with solid ammonium sulfate to 40% saturation and stirred for 1 hour. After centrifugation (50 minutes, 8000 rpm), the resulting supernatant was treated with solid ammonium sulfate to 70% saturation, stirred for 50 minutes, and the insoluble protein was collected by centrifugation (1 hour, 8000 rpm). This 40–70% ammonium sulfate fraction was then dissolved in extraction buffer to give a final volume of 0.2 L, and dialyzed twice (Spectrum 10,000 MW cutoff dialysis tubing) against 2 L of extraction buffer for a total of 12 hours.

To the resulting dialyzed 40–70% ammonium sulfate fraction (0.29 L) was added solid ammonium sulfate to give a final concentration of 1M. This material was loaded (2 ml/min) onto a column (5 cm×15 cm, 295 ml) packed with phenyl Sepharose CL-4B (Pharmacia) resin equilibrated with extraction buffer containing 1M ammonium sulfate, and washed with the same buffer (1.5 L, 2 ml/min). EPSPS was eluted with a linear gradient of extraction buffer going from 1M to 0.00M ammonium sulfate (total volume of 1.5 L, 2 ml/min). Fractions were collected (20 ml) and assayed for EPSPS activity by the phosphate release assay. The fractions with the highest EPSPS activity (fractions 36–50) were pooled and dialyzed against 3×2 L (18 hours) of 10 mM TrisCl, 25 mM KCl, 1 mM EDTA, 5 mM DTT, 10% glycerol, pH 7.8.

The dialyzed EPSPS extract (350 ml) was loaded (5 ml/min) onto a column (2.4 cm×30 cm, 136 ml) packed with Q-Sepharose Fast Flow (Pharmacia) resin equilibrated with 10 mM TrisCl, 25 mM KCl, 5 mM DTT, 10% glycerol, pH 7.8 (Q Sepharose buffer), and washed with 1 L of the same buffer. EPSPS was eluted with a linear gradient of Q Sepharose buffer going from 0.025M to 0.40M KCl (total volume of 1.4 L, 5 ml/min). Fractions were collected (15 ml) and assayed for EPSPS activity by the phosphate release assay. The fractions with the highest EPSPS activity (fractions 47–60) were pooled and the protein was precipitated by adding solid ammonium sulfate to 80% saturation and stirring for 1 hour. The precipitated protein was collected by centrifugation (20 minutes, 12000 rpm in a GSA Sorvall rotor), dissolved in Q Sepharose buffer (total volume of 14 ml), and dialyzed against the same buffer (2×1 L, 18 hours).

The resulting dialyzed partially purified EPSPS extract (19 ml) was loaded (1.7 ml/min) onto a Mono Q 10/10 column (Pharmacia) equilibrated with Q Sepharose buffer, and washed with the same buffer (35 ml). EPSPS was eluted with a linear gradient of 0.025M to 0.35M KCl (total volume of 119 ml, 1.7 ml/min). Fractions were collected (1.7 ml) and assayed for EPSPS activity by the phosphate release assay. The fractions with the highest EPSPS activity (fractions 30–37) were pooled (6 ml).

The Mono Q pool was made 1M in ammonium sulfate by the addition of solid ammonium sulfate and 2 ml aliquots were chromatographed on a Phenyl Superose 5/5 column (Pharmacia) equilibrated with 100 mM TrisCl, 5 mM DTT, 1M ammonium sulfate, 10% glycerol, pH 7.5 (Phenyl Superose buffer). Samples were loaded (1 ml/min), washed with Phenyl Superose buffer (10 ml), and eluted with a linear gradient of Phenyl Superose buffer going from 1M to 0.00M ammonium sulfate (total volume of 60 ml, 1 ml/min). Fractions were collected (1 ml) and assayed for EPSPS activity by the phosphate release assay. The fractions from each run with the highest EPSPS activity (fractions ~36–40) were pooled together (10 ml, 2.5 mg protein). For N-terminal amino acid sequence determination, a portion of

one fraction (#39 from run 1) was dialyzed against 50 mM $NaHCO_3$ (2×1 L). The resulting pure EPSPS sample (0.9 ml, 77 μg protein) was found to exhibit a single N-terminal amino acid sequence of:

XH(G)ASSRPATARKSS(G)LX(G)(T)V(R)PG(D)(K)(M) (SEQ ID NO:18).

The remaining Phenyl Superose EPSPS pool was dialyzed against 50 mM TrisCl, 2 mM DTT, 10 mM KCl, 10% glycerol, pH 7.5 (2×1 L). An aliquot (0.55 ml, 0.61 mg protein) was loaded (1 ml/min) onto a Mono Q 5/5 column (Pharmacia) equilibrated with Q Sepharose buffer, washed with the same buffer (5 ml), and eluted with a linear gradient of Q Sepharose buffer going from 0–0.14M KCl in 10 minutes, then holding at 0.14M KCl (1 ml/min). Fractions were collected (1 ml) and assayed for EPSPS activity by the phosphate release assay and were subjected to SDS-PAGE (10–15%, Phast System, Pharmacia, with silver staining) to determine protein purity. Fractions exhibiting a single band of protein by SDS-PAGE (22–25, 222 μg) were pooled and dialyzed against 100 mM ammonium bicarbonate, pH 8.1 (2×1 L, 9 hours).

Trypsinolysis and peptide sequencing of Agrobacterium sp strain CP4 EPSPS

To the resulting pure Agrobacterium sp. strain CP4 EPSPS (111 μg) was added 3 μg of trypsin (Calbiochem), and the trypsinolysis reaction was allowed to proceed for 16 hours at 37° C. The tryptic digest was then chromatographed (1 ml/min) on a C18 reverse phase HPLC column (Vydac) as previously described in Padgette et al., 1988 for *E. coli* EPSPS. For all peptide purifications, 0.1% trifluoroacetic acid (TFA, Pierce) was designated buffer "RP-A" and 0.1% TFA in acetonitrile was buffer "RP-B". The gradient used for elution of the trypsinized Agrobacterium sp. CP4 EPSPS was: 0–8 minutes, 0% RP-B; 8–28 minutes, 0–15% RP-B; 28–40 minutes, 15–21% RP-B; 40–68 minutes, 21–49% RP-B; 68–72 minutes, 49–75% RP-B; 72–74 minutes, 75–100% RP-B. Fractions were collected (1 ml) and, based on the elution profile at 210 nm, at least 70 distinct peptides were produced from the trypsinized EPSPS. Fractions 40–70 were evaporated to dryness and redissolved in 150 μl each of 10% acetonitrile, 0.1% trifluoroacetic acid.

The fraction 61 peptide was further purified on the C18 column by the gradient: 0–5 minutes, 0% RP-B; 5–10 minutes, 0–38% RP-B; 10–30 minutes, 38–45% B. Fractions were collected based on the UV signal at 210 nm. A large peptide peak in fraction 24 eluted at 42% RP-B and was dried down, resuspended as described above, and rechromatographed on the C18 column with the gradient: 0–5 minutes, 0% RP-B; 5–12 min, 0–38% RP-B; 12–15 min, 38–39% RP-B; 15–18 minutes, 39% RP-B; 18–20 minutes, 39–41% RP-B; 20–24 minutes, 41% RP-B; 24–28 minutes, 42% RP-B. The peptide in fraction 25, eluting at 41% RP-B and designated peptide 61–24–25, was subjected to N-terminal amino acid sequencing, and the following sequence was determined:

APSM(I)(D)EYPLAV (SEQ ID NO:19)

The CP4 EPSPS fraction 53 tryptic peptide was further purified by C18 HPLC by the gradient 0% B (5 minutes), 0–30% B (5–17 minutes), 30–40% B (17–37 minutes). The peptide in fraction 28, eluting at 34% B and designated peptide 53–28, was subjected to N-terminal amino acid sequencing, and the following sequence was determined:

ITGLLEGEDVINTGK (SEQ ID NO:20).

In order to verify the CP4 EPSPS cosmid clone, a number of oligonucleotide probes were designed on the basis of the sequence of two of the tryptic sequences from the CP4 enzyme (Table III). The probe identified as MID was very low degeneracy and was used for initial screening. The probes identified as EDV-C and EDV-T were based on the same amino acid sequences and differ in one position (underlined in Table III below) and were used as confirmatory probes, with a positive to be expected only from one of these two probes. In the oligonucleotides below, alternate acceptable nucleotides at a particular position are designated by a "/" such as A/C/T.

TABLE III

Selected CP4 EPSPS peptide sequences and DNA probes

PEPTIDE 61-24-25 APSM(I)(D)EYPLAV	(SEQ ID NO:19)
Probe MID; 17-mer; mixed probe; 24-fold degenerate ATGATA/CTGAC/TGAG/AIAC/TCC	(SEQ ID NO:21)
PEPTIDE 53-28 ITGLLEGEDVINTGK	(SEQ ID NO:20)
Probe EDV-C; 17-mer; mixed probe; 48-fold degenerate GAA/GGAC/TGTAC/G/TATA/C/TAAC/AC	(SEQ ID NO:22)
Probe EDV-T; 17-mer; mixed probe; 48-fold degenerate GAA/GGAC/TGTAC/G/TATA/C/TA/TAC	(SEQ ID NO:23)

The probes were labeled using gamma-^{32}P-ATP and polynucleotide kinase. DNA from fourteen of the cosmids described above was restricted with EcoRI, transferred to membrane and probed with the oligonucleotide probes. The conditions used were as follows: prehybridization was carried out in 6× SSC, 10× Denhardt's for 2–18 hour periods at 60° C., and hybridization was for 48–72 hours in 6× SSC, 10× Denhardt's, 100 μg/ml tRNA at 10° C. below the T_d for the probe. The T_d of the probe was approximated by the formula 2° C.×(A+T)+4° C.×(G+C). The filters were then washed three times with 6× SSC for ten minutes each at room temperature, dried and autoradiographed. Using the MID probe, an ~9.9 kb fragment in the pMON17076 cosmid gave the only positive signal. This cosmid DNA was then probed with the EDV-C (SEQ ID NO:22) and EDV-T (SEQ ID NO:23) probes separately and again this ~9.9 kb band gave a signal and only with the EDV-T probe.

The combined data on the glyphosate-tolerant phenotype, the complementation of the *E. coli* aroA- phenotype, the expression of a ~45 Kd protein, and the hybridization to two probes derived from the CP4 EPSPS amino acid sequence strongly suggested that the pMON17076 cosmid contained the EPSPS gene.

Localization and subcloning of the CP4 EPSPS gene

The CP4 EPSPS gene was further localized as follows: a number of additional Southern analyses were carried out on different restriction digests of pMON17076 using the MID (SEQ ID NO:21) and EDV-T (SEQ ID NO:23) probes separately. Based on these analyses and on subsequent detailed restriction mapping of the pBlueScript (Stratagene) subclones of the ~9.9 kb fragment from pMON17076, a 3.8 kb EcoRI-SalI fragment was identified to which both probes hybridized. This analysis also showed that MID (SEQ ID NO:21) and EDV-T (SEQ ID NO:23) probes hybridized to different sides of BamHI, ClaI, and SacII sites. This 3.8 kb fragment was cloned in both orientations in pBlueScript to form pMON17081 and pMON17082. The phenotypes imparted to *E. coli* by these clones were then determined. Glyphosate tolerance was determined following transformation into *E. coli* MM294 containing pGP1–2 (pBlueScript also contains a T7 promoter) on M9 agar media containing glyphosate at 3 mM. Both pMON17081 and pMON17082 showed glyphosate-tolerant colonies at three days at 30° C.

at about half the size of the controls on the same media lacking glyphosate. This result suggested that the 3.8 kb fragment contained an intact EPSPS gene. The apparent lack of orientation-dependence of this phenotype could be explained by the presence of the T7 promoter at one side of the cloning sites and the lac promoter at the other. The aroA phenotype was determined in transformants of *E. coli* GB100 on M9 agar media lacking aromatic supplements. In this experiment, carried out with and without the Plac inducer IPTG, pMON17082 showed much greater growth than pMON17081, suggesting that the EPSPS gene was expressed from the SalI site towards the EcoRI site.

Nucleotide sequencing was begun from a number of restriction site ends, including the BamHI site discussed above. Sequences encoding protein sequences that closely matched the N-terminus protein sequence and that for the tryptic fragment 53-28 (SEQ ID NO:20) (the basis of the EDV-T probe) (SEQ ID NO:23) were localized to the SalI side of this BamHI site. These data provided conclusive evidence for the cloning of the CP4 EPSPS gene and for the direction of transcription of this gene. These data coupled with the restriction mapping data also indicated that the complete gene was located on an ~2.3 kb XhoI fragment and this fragment was subcloned into pBlueScript. The nucleotide sequence of almost 2 kb of this fragment was determined by a combination of sequencing from cloned restriction fragments and by the use of specific primers to extend the sequence. The nucleotide sequence of the CP4 EPSPS gene and flanking regions is shown in FIG. 3 (SEQ ID NO:2). The sequence corresponding to peptide 61-24-25 (SEQ ID NO:19) was also located. The sequence was determined using both the SEQUENASE™ kit from IBI (International Biotechnologies Inc.) and the T7 sequencing/ Deaza Kit from Pharmacia.

That the cloned gene encoded the EPSPS activity purified from the Agrobacterium sp. strain CP4 was verified in the following manner: By a series of site directed mutageneses, BglII and NcoI sites were placed at the N-terminus with the fMet contained within the NcoI recognition sequence, the first internal NcoI site was removed (the second internal NcoI site was removed later), and a SacI site was placed after the stop codons. At a later stage the internal NotI site was also removed by site-directed mutagenesis. The following list includes the primers for the site-directed mutagenesis (addition or removal of restriction sites) of the CP4 EPSPS gene. Mutagenesis was carried out by the procedures of Kunkel et al. (1987), essentially as described in Sambrook et al. (1989).

PRIMER Bgl4: (addition of BglII and NcoI sites to N-terminus)
CGTGGATAGATCTAGGAAGACAACCATGGCTCACGGTC
(SEQ ID NO:24)

PRIMER Sph2 (addition of SphI site to N-terminus)
GGATAGATTAAGGAAGACGCGCATGCTTCACGGTGCAAGCAGCC
(SEQ ID NO:25)

PRIMER S1 (addition of SacI site immediately after stop codons)
GGCTGCCTGATGAGCTCCACAATGGCCATCGATGG
(SEQ ID NO:26)

PRIMER N1 (removal of internal NotI recognition site)
CGTCGCTCGTCGTGGGTGGCCGCCCTGACGGC
(SEQ ID NO:27)

PRIMER Nco1 (removal of first internal NcoI recognition site)
CGGGCAAGGCCATGCAGGCTATGGGCGCC
(SEQ ID NO:28)

-continued

PRIMER Nco2 (removal of second internal NcoI recognition site)
CGGGCTGGGGCCTGACTATGGGCCTCGTCGG
(SEQ ID NO:29)

This CP4 EPSPS gene was then cloned as a NcoI-BamHI N-terminal fragment plus a BamHI-SacI C-terminal fragment into a PrecA-gene10L expression vector similar to those described (Wong et al., 1988; Olins et al., 1988) to form pMON17101. The K_m for PEP and the K_i for glyphosate were determined for the EPSPS activity in crude lysates of pMON17101/ GB100 transformants following induction with nalidixic acid (Wong et al., 1988) and found to be the same as that determined for the purified and crude enzyme preparations from Agrobacterium sp. strain CP4.

Characterization of the EPSPS gene from Achromobacter sp. strain LBAA and from Pseudomonas sp. strain PG2982

A cosmid bank of partially HindIII-restricted LBAA DNA was constructed in *E. coli* MM294 in the vector pHC79 (Hohn and Collins, 1980). This bank was probed with a full length CP4 EPSPS gene probe by colony hybridization and positive clones were identified at a rate of ~1 per 400 cosmids. The LBAA EPSPS gene was further localized in these cosmids by Southern analysis. The gene was located on an ~2.8 kb XhoI fragment and by a series of sequencing steps, both from restriction fragment ends and by using the oligonucleotide primers from the sequencing of the CP4 EPSPS gene, the nucleotide sequence of the LBAA EPSPS gene was completed and is presented in FIG. 4 (SEQ ID NO:4).

The EPSPS gene from PG2982 was also cloned. The EPSPS protein was purified, essentially as described for the CP4 enzyme, with the following differences: Following the Sepharose CL-4B column, the fractions with the highest EPSPS activity were pooled and the protein precipitated by adding solid ammonium sulfate to 85% saturation and stirring for 1 hour. The precipitated protein was collected by centrifugation, resuspended in Q Sepharose buffer and following dialysis against the same buffer was loaded onto the column (as for the CP4 enzyme). After purification on the Q Sepharose column, ~40 mg of protein in 100 mM Tris pH 7.8, 10% glycerol, 1 mM EDTA, 1 mM DTT, and 1M ammonium sulfate, was loaded onto a Phenyl Superose (Pharmacia) column. The column was eluted at 1.0 ml/minutes with a 40 ml gradient from 1.0M to 0.00M ammonium sulfate in the above buffer.

Approximately 1.0 mg of protein from the active fractions of the Phenyl Superose 10/10 column was loaded onto a Pharmacia Mono P 5/10 Chromatofocusing column with a flow rate of 0.75 ml/minutes. The starting buffer was 25 mM bis-Tris at pH 6.3, and the column was eluted with 39 ml of Polybuffer 74, pH 4.0. Approximately 50 μg of the peak fraction from the Chromatofocusing column was dialyzed into 25 mM ammonium bicarbonate. This sample was then used to determine the N-terminal amino acid sequence.

The N-terminal sequence obtained was:

XHHASPEDATARRH (where X=an unidentified residue) (SEQ ID NO:30)

A number of degenerate oligonucleotide probes were designed based on this sequence and used to probe a library of PG2982 partial-HindIII DNA in the cosmid pHC79 (Hohn and Collins, 1980) by colony hybridization under nonstringent conditions. Final washing conditions were 15 minutes with 1× SSC, 0.1% SDS at 55° C. One probe with the sequence GCGGTBGCSGGYTTSGG (where B=C, G, or T; S=C or G, and Y=C or T) (SEQ ID NO:31) identified a set of cosmid clones.

The cosmid set identified in this way was made up of cosmids of diverse HindIII fragments. However, when this set was probed with the CP4 EPSPS gene probe, a cosmid containing the PG2982 EPSPS gene was identified (designated as cosmid 9C1 originally and later as pMON20107). By a series of restriction mappings and Southern analysis this gene was localized to a ~2.8 kb XhoI fragment and the nucleotide sequence of this gene was determined. This DNA sequence (SEQ ID NO:6) is shown in FIG. 5. There are no nucleotide differences between the EPSPS gene sequences from LBAA (SEQ ID NO:4) and PG2982 (SEQ ID NO:6). The kinetic parameters of the two enzymes are within the range of experimental error.

A gene from PG2982 that imparts glyphosate tolerance in *E. coli* has been sequenced (Fitzgibbon, 1988; Fitzgibbon and Braymer, 1990). The sequence of the PG2982 EPSPS Class II gene shows no homology to the previously reported sequence suggesting that the glyphosate-tolerant phenotype of the previous work is not related to EPSPS.

Characterization of the EPSPS from *Bacillus subtilis*

Bacillus subtilis 1A2 (prototroph) was obtained from the Bacillus Genetic Stock Center at Ohio State University. Standard EPSPS assay reactions contained crude bacterial extract with, 1 mM phosphoenolpyruvate (PEP), 2 mM shikimate-3-phosphate (S3P), 0.1 mM ammonium molybdate, 5 mM potassium fluoride, and 50 mM HEPES, pH 7.0 at 25° C. One unit (U) of EPSPS activity is defined as one μmol EPSP formed per minute under these conditions. For kinetic determinations, reactions contained crude bacterial, 2 mM S3P, varying concentrations of PEP, and 50 mM HEPES, pH 7.0 at 25° C. The EPSPS specific activity was found to be 0.003 U/mg. When the assays were performed in the presence of 1 mM glyphosate, 100% of the EPSPS activity was retained. The $appK_m$(PEP) of the *B. subtilis* EPSPS was determined by measuring the reaction velocity at varying concentrations of PEP. The results were analyzed graphically by the hyperbolic, Lineweaver-Burk and Eadie-Hofstee plots, which yielded $appK_m$(PEP) values of 15.3 μM, 10.8 μM and 12.2 μM, respectively. These three data treatments are in good agreement, and yield an average value for $appK_m$(PEP) of 13 μM. The $appK_i$(glyphosate) was estimated by determining the reaction rates of *B. subtilis* 1A2 EPSPS in the presence of several concentrations of glyphosate, at a PEP concentration of 2 μM. These results were compared to the calculated V_{max} of the EPSPS, and making the assumption that glyphosate is a competitive inhibitor versus PEP for *B. subtilis* EPSPS, as it is for all other characterized EPSPSs, an $appK_i$(glyphosate) was determined graphically. The $appK_i$(glyphosate) was found to be 0.44 mM.

The EPSPS expressed from the *B. subtilis* aroE gene described by Henner et al. (1986) was also studied. The source of the *B. subtilis* aroE (EPSPS) gene was the *E. coli* plasmid-bearing strain ECE13 (original code=MM294[p trp100]; Henner, et al., 1984; obtained from the Bacillus Genetic Stock Center at Ohio State University; the culture genotype is [pBR322 trp100] Ap [in MM294] [pBR322::6 kb insert with trpFBA-hisH]). Two strategies were taken to express the enzyme in *E. coli* GB100 (aroA-): 1) the gene was isolated by PCR and cloned into an overexpression vector, and 2) the gene was subcloned into an overexpression vector. For the PCR cloning of the *B. subtilis* aroE from ECE13, two oligonucleotides were synthesized which incorporated two restriction enzyme recognition sites (NdeI and EcoRI) to the sequences of the following oligonucleotides:

(SEQ ID NO:45)
CCAACATATGAAACCAGATAAGCGTGCAG

(SEQ ID NO:46)
CCAATTCAAACTTCAGGATCTTGAGATAGAAAATG

The other approach to the isolation of the *B. subtilis* aroE gene, subcloning from ECE13 into pUC118, was performed as follows:

(i) Cut ECE13 and pUC with XmaI and SphI.

(ii) Isolate 1700bp aroE fragment and 2600bp pUC118 vector fragment.

(iii) Ligate fragments and transform into GB100.

The subclone was designated pMON21133 and the PCR-derived clone was named pMON21132. Clones from both approaches were first confirmed for complementation of the aroA mutation in *E. coli* GB100. The cultures exhibited EPSPS specific activities of 0.044 U/mg and 0.71 U/mg for the subclone (pMON21133) and PCR-derived clone (pMON21132) enzymes, respectively. These specific activities reflect the expected types of expression levels of the two vectors. The *B. subtilis* EPSPS was found to be 88% and 100% resistant to inhibition by 1 mM glyphosate under these conditions for the subcloned (pMON21133) and PCR-derived (pMON21132) enzymes, respectively. The $appK_m$ (PEP) and the $appK_i$(glyphosate) of the subcloned *B. subtilis* EPSPS (pMON21133) were determined as described above. The data were analyzed graphically by the same methods used for the 1A2 isolate, and the results obtained were comparable to those reported above for *B. subtilis* 1A2 culture.

Characterization of the EPSPS gene from *Staphylococcus aureus*

The kinetic properties of the *S. aureus* EPSPS expressed in *E. coli* were determined, including the specific activity, the $appK_m$(PEP), and the $appK_i$(glyphosate). The *S. aureus* EPSPS gene has been previously described (O'Cormell et al., 1993)

The strategy taken for the cloning of the *S. aureus* EPSPS was polymerase chain reaction (PCR), utilizing the known nucleotide sequence of the *S. aureus* aroA gene encoding EPSPS (O'Cormell et al., 1993). The *S. aureus* culture (ATCC 35556) was fermented in an M2 facility in three 250 mL shake flasks containing 55 mL of TYE (tryptone 5 g/L, yeast extract 3 g/L, pH 6.8). The three flasks were inoculated with 1.5 mL each of a suspension made from freeze dried ATCC 35556 *S. aureus* cells in 90 mL of PBS (phosphate-buffered saline) buffer. Flasks were incubated at 30° C. for 5 days while shaking at 250 rpm. The resulting cells were lysed (boiled in TE [tris/EDTA] buffer for 8 minutes) and the DNA utilized for PCR reactions. The EPSPS gene was amplified using PCR and engineered into an *E. coli* expression vector as follows:

(i) two oligonucleotides were synthesized which incorporated two restriction enzyme recognition sites (NcoI and SacI) to the sequences of the oligonucleotides:

(SEQ ID NO:47)
GGGGCCATGGTAAATGAACAAATCATTG

(SEQ ID NO:48)
GGGGGAGCTCATTATCCCTCATTTTGTAAAAGC

(ii) The purified, PCR-amplified aroA gene from *S. aureus* was digested using NcoI and SacI enzymes.

(iii) DNA of pMON 5723, which contains a pRecA bacterial promoter and Gene10 leader sequence (Olins et al., 1988) was digested NcoI and SacI and the 3.5 kb digestion product was purified.

(iv) The *S. aureus* PCR product and the NcoI / SacI pMON 5723 fragment were ligated and transformed into *E. coli* JM101 competent cells.

(v) Two spectinomycin-resistant *E. coli* JM101 clones from above (SA#2 and SA#3) were purified and transformed into a competent aroA- *E. coli* strain, GB100

For complementation experiments SAGB#2 and SAGB#3 were utilized, which correspond to SA#2 and SA#3, respectively, transformed into *E. coli* GB100. In addition, *E. coli* GB100 (negative control) and pMON 9563 (wt petunia EPSPS, positive control) were tested for AroA complementation. The organisms were grown in minimal media plus and minus aromatic amino acids. Later analyses showed that the SA#2 and SA#3 clones were identical, and they were assigned the plasmid identifier pMON21139.

SAGB#2 in *E. coli* GB100 (pMON21139) was also grown in M9 minimal media and induced with nalidixic acid. A negative control, *E. coli* GB100, was grown under identical conditions except the media was supplemented with aromatic amino acids. The cells were harvested, washed with 0.9% NaCl, and frozen at −80° C., for extraction and EPSPS analysis.

The frozen pMON21139 *E. coli* GB100 cell pellet from above was extracted and assayed for EPSPS activity as previously described. EPSPS assays were performed using 1 mM phosphoenolpyruvate (PEP), 2 mM shikimate-3-phosphate (S3P), 0. 1 mM ammonium molybdate, 5 mM potassium fluoride, pH 7.0, 25° C. The total assay volume was 50μL, which contained 10 μL of the undiluted desalted extract.

The results indicate that the two clones contain a functional aroA/EPSPS gene since they were able to grow in minimal media which contained no aromatic amino acids. As expected, the GB100 culture did not grow on minimal medium without aromatic amino acids (since no functional EPSPS is present), and the pMON9563 did confer growth in minimal media. These results demonstrated the successful cloning of a functional EPSPS gene from *S. aureus*. Both clones tested were identical, and the *E. coli* expression vector was designated pMON21139.

The plasmid pMON21139 in *E. coli* GB100 was grown in M9 minimal media and was induced with nalidixic acid to induce EPSPS expression driven from the RecA promoter. A desalted extract of the intracellular protein was analyzed for EPSPS activity, yielding an EPSPS specific activity of 0.005 μmol/min mg. Under these assay conditions; the *S. aureus* EPSPS activity was completely resistant to inhibition by 1 mM glyphosate. Previous analysis had shown that *E. coli* GB100 is devoid of EPSPS activity.

The $appK_m$(PEP) of the *S. aureus* EPSPS was determined by measuring the reaction velocity of the enzyme (in crude bacterial extracts) at varying concentrations of PEP. The results were analyzed graphically using several standard kinetic plotting methods. Data analysis using the hyperbolic, Lineweaver-Burke, and Eadie-Hofstee methods yielded $appK_m$(PEP) constants of 7.5, 4.8, and 4.0 μM, respectively. These three data treatments are in good agreement, and yield an average value for $appK_m$(PEP) of 5 μM.

Further information of the glyphosate tolerance of *S. aureus* EPSPS was obtained by determining the reaction rates of the enzyme in the presence of several concentrations of glyphosate, at a PEP concentration of 2 μM. These results were compared to the calculated maximal velocity of the EPSPS, and making the assumption that glyphosate is a competitive inhibitor versus PEP for *S. aureus* EPSPS, as it is for all other characterized EPSPSs, an $appK_i$(glyphosate) was determined graphically. The $appK_i$(glyphosate) for *S. aureus* EPSPS estimated using this method was found to be 0.20 mM.

The EPSPS from *S. aureus* was found to be glyphosate-tolerant, with an $appK_i$(glyphosate) of approximately 0.2 mM. In addition, the $appK_m$(PEP) for the enzyme is approximately 5 μM, yielding a $appK_i$(glyphosate)/$appK_m$(PEP) of 40.

Alternative Isolation Protocols for Other Class II EPSPS Structural Genes

A number of Class II genes have been isolated and described here. While the cloning of the gene from CP4 was difficult due to the low degree of similarity between the Class I and Class II enzymes and genes, the identification of the other genes was greatly facilitated by the use of this first gene as a probe. In the cloning of the LBAA EPSPS gene, the CP4 gene probe allowed the rapid identification of cosmid clones and the localization of the intact gene to a small restriction fragment and some of the CP4 sequencing primers were also used to sequence the LBAA (and PG2982) EPSPS gene(s). The CP4 gene probe was also used to confirm the PG2982 gene clone. The high degree of similarity of the Class II EPSPS genes may be used to identify and clone additional genes in much the same way that Class I EPSPS gene probes have been used to clone other Class I genes. An example of the latter was in the cloning of the *A. thaliana* EPSPS gene using the *P. hybrida* gene as a probe (Klee et al., 1987).

Glyphosate-tolerant EPSPS activity has been reported previously for EPSP synthases from a number of sources. These enzymes have not been characterized to any extent in most cases. The use of Class I and Class II EPSPS gene probes or antibody probes provide a rapid means of initially screening for the nature of the EPSPS and provide tools for the rapid cloning and characterization of the genes for such enzymes.

Two of the three genes described were isolated from bacteria that were isolated from a glyphosate treatment facility (Strains CP4 and LBAA). The third (PG2982) was from a bacterium that had been isolated from a culture collection strain. This latter isolation confirms that exposure to glyphosate is not a prerequisite for the isolation of high glyphosate-tolerant EPSPS enzymes and that the screening of collections of bacteria could yield additional isolates. It is possible to enrich for glyphosate degrading or glyphosate resistant microbial populations (Quinn et al., 1988; Talbot et al., 1984) in cases where it was felt that enrichment for such microorganisms would enhance the isolation frequency of Class II EPSPS microorganisms. Additional bacteria containing class II EPSPS genes have also been identified. A bacterium called C 12, isolated from the same treatment column beads as CP4 (see above) but in a medium in which glyphosate was supplied as both the carbon and phosphorus source, was shown by Southern analysis to hybridize with a probe consisting of the CP4 EPSPS coding sequence. This result, in conjunction with that for strain LBAA, suggests that this enrichment method facilitates the identification of Class II EPSPS isolates. New bacterial isolates containing Class II EPSPS genes have also been identified from environments other than glyphosate waste treatment facilities. An inoculum was prepared by extracting soil (from a recently harvested soybean field in Jerseyville, Ill.) and a population of bacteria selected by growth at 28° C. in Dworkin-Foster medium containing glyphosate at 10 mM as a source of carbon (and with cycloheximide at 100 μg/ml to prevent the growth of fungi). Upon plating on L-agar media, five colony types were identified. Chromosomal DNA was prepared from 2ml L-broth cultures of these isolates and the presence of a Class II EPSPS gene was probed using a the CP4 EPSPS coding sequence probe by Southern analysis

under stringent hybridization and washing conditions. One of the soil isolates, S2, was positive by this screen.

Class II EPSPS enzymes are identifiable by an elevated K_i for glyphosate and thus the genes for these will impart a glyphosate tolerance phenotype in heterologous hosts. Expression of the gene from recombinant plasmids or phage may be achieved through the use of a variety of expression promoters and include the T7 promoter and polymerase. The T7 promoter and polymerase system has been shown to work in a wide range of bacterial (and mammalian) hosts and offers the advantage of expression of many proteins that may be present on large cloned fragments. Tolerance to growth on glyphosate may be shown on minimal growth media. In some cases, other genes or conditions that may give glyphosate tolerance have been observed, including over expression of beta-lactamase, the *fgrA* gene (Fitzgibbon and Braymer, 1990), or the gene for glyphosate oxidoreductase (PCT Pub. No. WO92/00377). These are easily distinguished from Class II EPSPS by the absence of EPSPS enzyme activity.

The EPSPS protein is expressed from the *aroA* gene (also called *aroE* in some genera, for example, in Bacillus) and mutants in this gene have been produced in a wide variety of bacteria. Determining the identity of the donor organism (bacterium) aids in the isolation of Class II EPSPS gene—such identification may be accomplished by standard microbiological methods and could include Gram stain reaction, growth, color of culture, and gas or acid production on different substrates, gas chromatography analysis of methylesters of the fatty acids in the membranes of the microorganism, and determination of the GC % of the genome. The identity of the donor provides information that may be used to more easily isolate the EPSPS gene. An AroA- host more closely related to the donor organism could be employed to clone the EPSPS gene by complementation but this is not essential since complementation of the *E. coli* AroA mutant by the CP4 EPSPS gene was observed. In addition, the information on the GC content the genome may be used in choosing nucleotide probes—donor sources with high GC % would preferably use the CP4 EPSPS gene or sequences as probes and those donors with low GC would preferably employ those from *Bacillus subtilis*, for example. Relationships between different EPSPS genes

The deduced amino acid sequences of a number of Class I and the Class II EPSPS enzymes were compared using the Bestfit computer program provided in the UWGCG package (Devereux et al. 1984). The degree of similarity and identity as determined using this program is reported. The degree of similarity/identity determined within Class I and Class II protein sequences is remarkably high, for instance, comparing *E. coli* with *S. typhimurium* (similarity/identity=93%/88%) and even comparing *E. coli* with a plant EPSPS (*Petunia hybrida*; 72%/55%). These data are shown in Table IV. The comparison of sequences between Class I and Class II, however, shows a much lower degree of relatedness between the Classes (similarity/identity=50–53%/23–30%). The display of the Bestfit analysis for the *E. coli* (SEQ ID NO:8) and CP4 (SEQ ID NO:3) sequences shows the positions of the conserved residues and is presented in FIG. 6. Previous analyses of EPSPS sequences had noted the high degree of conservation of sequences of the enzymes and the almost invariance of sequences in two regions—the "20–35" and "95–107" regions (Gasser et al., 1988; numbered according to the Petunia EPSPS sequence)—and these regions are less conserved in the case of CP4 and LBAA when compared to Class I bacterial and plant EPSPS sequences (see FIG. 6 for a comparison of the *E. coli* and CP4 EPSPS sequences with the *E. coli* sequence appearing as the top sequence in the Figure). The corresponding sequences in the CP4 Class II EPSPS are:

PGDEKSISHRSFMFGGL (SEQ ID NO:32)
and
LDPGNAATGCRLT (SEQ ID NO:33)

These comparisons show that the overall relatedness of Class I and Class II is EPSPS proteins is low and that sequences in putative conserved regions have also diverged considerably.

In the CP4 EPSPS an alanine residue is present at the "glycine101" position. The replacement of the conserved glycine (from the "95–107" region) by an alanine results in an elevated K_i for glyphosate and in an elevation in the K_m for PEP in Class I EPSPS. In the case of the CP4 EPSPS, which contains an alanine at this position, the K_m for PEP is in the low range, indicating that the Class II enzymes differ in many aspects from the EPSPS enzymes heretofore characterized.

Within the Class II isolates, the degree of similarity/identity is as high as that noted for that within Class I (Table IVA). FIG. 7 displays the Bestfit computer program alignment of the CP4 (SEQ ID NO:3) and LBAA (SEQ ID NO:5) EPSPS deduced amino acid sequences with the CP4 sequence appearing as the top sequence in the Figure. The symbols used in FIGS. 6 and 7 are the standard symbols used in the Bestfit computer program to designate degrees of similarity and identity.

TABLE IVA [1,2]

Comparison of relatedness of EPSPS protein sequences

Comparison between Class I and Class II EPSPS protein sequences	similarity	identity
S. cerevisiae vs. CP4	54	30
A. nidulans vs. CP4	50	25
B. napus vs. CP4	47	22
A. thaliana vs. CP4	48	22
N. tabacum vs. CP4	50	24
L. esculentum vs. CP4	50	24
P. hybrida vs. CP4	50	23
Z. mays vs. CP4	48	24
S. gallinarum vs. CP4	51	25
S. typhimurium vs. CP4	51	25
S. typhi vs. CP4	51	25
K. pneumoniae vs. CP4	56	28
Y. enterocolitica vs. CP4	53	25
H. influenzae vs. CP4	53	27
P. multocida vs. CP4	53	30
A. salmonicida vs. CP4	53	25
H. pertussis vs. CP4	53	27
E. coli vs. CP4	52	26
E. coli vs. LBAA	52	26
E. coli vs. *B. subtilis*	53	29
E. coli vs. *D. radiodurans*	53	32
E. coli vs. *E. aurora*	53	29
E.coli vs. *Synechocystis* sp. PCC6803	53	30
Comparison between Class I EPSPS protein sequences	**similarity**	**identity**
E. coli vs. *S. typhimurium*	98	88
P. hybrida vs. *E. coli*	72	55
Comparison between Class II EPSPS protein sequences	**similarity**	**identity**
D. radiodurans vs. CP4	62	43
LBAA vs. CP4	50	83
PG2892 vs. CP4	50	83
E. aurora vs. CP4	38	34

TABLE IVA [1,2]-continued

B. subtilis vs. CP4	39	41
Synechocystis sp. PCC6803 vs. CP4	42	45

[1] The EPSPS sequences compared here were obtained from the following references: *E. coli*, Rogers et al., 1983; *S. typhimurium*, Stalker et al., 1985; *Petunia hybrida*, Shah et al., 1986; *Lycopersicon*, Maskell et al., 1988; *S. cerevisiae*, Duncan et al., 1987, *Synechocystis sp.* PCC6803, Dalla Chiesa et al., 1994 and *D. nodosum*, Alibhai et al., 1994.

[2] "GAP" Program, Genetics Computer Group, (1991), Program Manual for the GCG Package, Version 7, April 1991, 575 Science Drive, Madison, Wisconsin, USA 53711

The relative locations of the major conserved sequences among Class II EPSP synthases which distinguishes this group from the Class I EPSP synthases is listed below in Table IVB.

TABLE IVB

	Location of Conserved Sequences in Class II EPSP Synthase			
Source	Seq. 1[1]	Seq. 2[2]	Seq. 3[3]	Seq. 4[4]
CP4				
start	200	26	173	271
end	204	29	177	274
LBAA				
start	200	26	173	271
end	204	29	177	274
PO2982				
start	200	26	173	273
end	204	29	177	276
B. subtilis				
start	190	17	164	257
end	194	20	168	260
S. aureus				
start	198	21	166	261
end	197	24	170	264
Synechocystis sp. PCC6808				
start	210	34	183	278
end	214	38	187	281
D. nodosus				
start	195	22	168	261
end	199	25	172	264
min. start	190	17	164	257
max. end	214	38	187	281

[1] -R-X_1-H-X_2-E-(SEQ ID NO:37)
[2] -G-D-K-X_3-(SEQ ID NO:38)
[3] -S-A-Q-X_4-K-(SEQ ID NO:39)
[4] -N-X_5-T-R-(SEQ ID NO:40)

The domains of EPSP synthase sequence identified in this application were determined to be those important for maintenance of glyphosate resistance and productive binding of PEP. The information used in identifying these domains included sequence alignments of numerous glyphosate-sensitive EPSPS molecules and the three-dimensional x-ray structures of *E. coli* EPSPS (Stallings, et al. 1991) and CP4 EPSPS. The structures are representative of a glyphosate-sensitive (i.e., Class I) enzyme, and a naturally-occuring glyphosate-tolerant (i.e., Class II) enzyme of the present invention. These exemplary molecules were superposed three-dimensionally and the results displayed on a computer graphics terminal. Inspection of the display allowed for structure-based fine-tuning of the sequence alignments of glyphosate-sensitive and glyphosate-resistant EPSPS molecules. The new sequence alignments were examined to determine differences between Class I and Class II EPSPS enzymes. Seven regions were identified and these regions were located in the x-ray structure of CP4 EPSPS which also contained a bound analog of the intermediate which forms catalytically between PEP and S3P.

The structure of the CP4 EPSPS with the bound intermediate analog was displayed on a computer graphics terminal and the seven sequence segments were examined. Important residues for glyphosate binding were identified as well as those residues which stabilized the conformations of those important residues; adjoining residues were considered necessary for maintenance of correct three-dimensional structural motifs in the context of glyphosate- sensitive EPSPS molecules. Three of the seven domains were determined not to be important for glyphosate tolerance and maintenance of productive PEP binding. The following four primary domains were determined to be characteristic of Class II EPSPS enzymes of the present invention:

-R-XrH-X_1-E(SEQ ID NO:37), in which
X_1 is an uncharged polar or acidic amino acid,
X_2 is serine or threonine,
The Arginine (R) reside at position 1 is important because the positive charge of its guanidium group destabilizes the binding of glyphosate. The Histidine (H) residue at position 3 stabilizes the Arginine (R) residue at position 4 of SEQ ID NO:40. The Glutamic Acid (E) residue at position 5 stabilizes the Lysine (K) residue at position 5 of SEQ ID NO:39.

-G-D-K-X_3(SEQ ID NO:38), in which
X_3 is serine or threonine,
The Aspartic acid (D) residue at position 2 stabilizes the Arginine (R) residue at position 4 of SEQ ID NO:40. The Lysine (K) residue at position 3 is important because for productive PEP binding.

-S-A-Q-X_4-K(SEQ ID NO:39), in which
X_4 is any amino acid,
The Alanine (A) residue at position 2 stabilizes the Arginine (R) residue at position 1 of SEQ ID NO:37. The Serine (S) residue at position 1 and the Glutamine (Q) residue at position 3 are important for productive S3P binding.

-N-X_5-T-R(SEQ ID NO:40) in which
X_5 is any amino acid,
The Asparagine (N) residue at position 1 and the Threonine (T) residue at position 3 stabilize residue X_1 at position 2 of SEQ ID NO:37. The Arginine (R) residue at position 4 is important because the positive charge of its guanidium group destabilizes the binding of glyphosate.

Since the above sequences are only representative of the Class II EPSPSs which would be included within the generic structure of this group of EPSP synthases, the above sequences may be found within a subject EPSP synthase molecule within slightly more expanded regions. It is believed that the above-described conserved sequences would likely be found in the following regions of the mature EPSP synthases molecule:

-R-X_1-H-X_2-E-(SEQ ID NO:37) located between amino acids 175 and 230 of the mature EPSP synthase sequence;

-G-D-K-X_3-(SEQ ID NO:38) located between amino acids 5 and 55 of the mature EPSP synthase sequence;

-S-A-Q-X_4-K-(SEQ ID NO:39) located between amino acids 150 and 200 of the mature EPSP synthase sequence; and

-N-X_g-T-R-(SEQ ID NO:40) located between amino acids 245 and 295 of the mature EPSPS synthase sequence.

One difference that may be noted between the deduced amino acid sequences of the CP4 and LBAA EPSPS proteins is at position 100 where an Alanine is found in the case of the CP4 enzyme and a Glycine is found in the case of the LBAA enzyme. In the Class I EPSPS enzymes a Glycine is usually found in the equivalent position, i.e Glycine96 in *E. coli* and *K. pneumoniae* and Glycine101 in Petunia. In the case of these three enzymes it has been reported that converting that Glycine to an Alanine results in an elevation of the appKi for glyphosate and a concomitant elevation in the appKm for PEP (Kishore et al., 1986; Kishore and Shah, 1988; Sost and Amrhein, 1990), which, as discussed above, makes the enzyme less efficient especially under conditions of lower PEP concentrations. The Glycine100 of the LBAA EPSPS was converted to an Alanine and both the appKm for PEP and the appKi for glyphosate were determined for the variant. The Glycine100Alanine change was introduced by mutagenesis using the following primer:

CGGCAATGCCGGCCACCGGCGGCCGCC (SEQ ID NO:34)

and both the wild type and variant genes were expressed in *E. coli* in a RecA promoter expression vector (pMON17201 and pMON17264, respectively) and the appKm's and appKi's determined in crude lysates. The data indicate that the appKi(glyphosate) for the G100A variant is elevated about 16-fold (Table V). This result is in agreement with the observation of the importance of this G-A change in raising the appKi(glyphosate) in the Class I EPSPS enzymes. However, in contrast to the results in the Class I G-A variants, the appKm(PEP) in the Class II (LBAA) G-A variant is unaltered. This provides yet another distinction between the Class II and Class I EPSPS enzymes.

TABLE V

	appKm(PEP)	appKi(glyphosate)
Lysate prepared from:		
E. coli/pMON17201 (wild type)	5.3 μM	28 μM*
E. coli/pMON17264 (G100A variant)	5.5 μM	459 μM†

*range of PEP: 2–40 μM.
†range of glyphosate: 0–350 μM; range of glyphosate: 0–5000 μM.

The LBAA G100A variant, by virtue of its superior kinetic properties, should be capable of imparting improved in planta glyphosate tolerance.

Modification and Resynthesis of the Agrobacterium sp. strain CP4 EPSPS Gene Sequence

The EPSPS gene from Agrobacterium sp. strain CP4 contains sequences that could be inimical to high expression of the gene in plants. These sequences include potential polyadenylation sites that are often and A+T rich, a higher G+C % than that frequently found in plant genes (63% versus ~50%), concentrated stretches of G and C residues, and codons that are not used frequently in plant genes. The high G+C % in the CP4 EPSPS gene has a number of potential consequences including the following: a higher usage of G or C than that found in plant genes in the third position in codons, and the potential to form strong hair-pin structures that may affect expression or stability of the RNA. The reduction in the G+C content of the CP4 EPSPS gene, the disruption of stretches of G's and C's, the elimination of potential polyadenylation sequences, and improvements in the codon usage to that used more frequently in plant genes, could result in higher expression of the CP4 EPSPS gene in plants.

A synthetic CP4 gene was designed to change as completely as possible those inimical sequences discussed above. In summary, the gene sequence was redesigned to eliminate as much as possible the following sequences or sequence features (while avoiding the introduction of unnecessary restriction sites): stretches of G's and C's of 5 or greater; and A+T rich regions (predominantly) that could function as polyadenylation sites or potential RNA destabilization region. The sequence of this gene is shown in FIG. 8 (SEQ ID NO:9). This coding sequence was expressed in *E. coli* from the RecA promoter and assayed for EPSPS activity and compared with that from the native CP4 EPSPS gene. The apparent Km for PEP for the native and synthetic genes was 11.8 and 12.7, respectively, indicating that the enzyme expressed from the synthetic gene was unaltered. The N-terminus of the coding sequence was mutagenized to place an SphI site at the ATG to permit the construction of the CTP2-CP4 synthetic fusion for chloroplast import. The following primer was used to accomplish this mutagenesis:

GGACCGCTGCTTGCACCGTGAAGCATGCTDAAGCTTGGCGTAATCATGG. (SEQ ID NO:35)

Expression of Chloroplast Directed CP4 EPSPS

The glyphosate target in plants, the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme, is located in the chloroplast. Many chloroplast-localized proteins, including EPSPS, are expressed from nuclear genes as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP) that is removed during the import steps. Examples of other such chloroplast proteins include the small subunit (SSU) of Ribulose-1,5-bisphosphate carboxylase (RUBISCO), Ferredoxin, Ferredoxin oxidoreductase, the Light-harvesting-complex protein I and protein II, and Thioredoxin F. It has been demonstrated in vivo and in vitro that non-chloroplast proteins may be targeted to the chloroplast by use of protein fusions with a CTP and that a CTP sequence is sufficient to target a protein to the chloroplast.

A CTP-CP4 EPSPS fusion was constructed between the *Arabidopsis thaliana* EPSPS CTP (Klee et al., 1987) and the CP4 EPSPS coding sequences. The Arabidopsis CTP was engineered by site-directed mutagenesis to place a SphI restriction site at the CTP processing site. This mutagenesis replaced the Glu-Lys at this location with Cys-Met. The sequence of this CTP, designated as CTP2 (SEQ ID NO:10), is shown in FIG. 9. The N-terminus of the CP4 EPSPS gene was modified to place a SphI site that spans the Met codon. The second codon was converted to one for leucine in this step also. This change had no apparent effect on the in vivo activity of CP4 EPSPS in *E. coli* as judged by rate of complementation of the aroA allele. This modified N-terminus was then combined with the SacI C-terminus and cloned downstream of the CTP2 sequences. The CTP2-CP4 EPSPS fusion was cloned into pBlueScript KS(+). This vector may be transcribed in vitro using the T7 polymerase and the RNA translated with ^{35}S-Methionine to provide material that may be evaluated for import into chloroplasts isolated from *Lactuca sativa* using the methods described hereinafter (della-Cioppa et al., 1986, 1987). This template was transcribed in vitro using T7 polymerase and the ^{35}S-methionine-labeled CTP2-CP4 EPSPS material was shown

to import into chloroplasts with an efficiency comparable to that for the control Petunia EPSPS (control-^{35}S labeled PreEPSPS [pMON6140; della-Cioppa et al., 1986]).

In another example the Arabidopsis EPSPS CTP, designated as CTP3, was fused to the CP4 EPSPS through an EcoRI site. The sequence of this CTP3 (SEQ ID NO:12) is shown in FIG. 10. An EcoRI site was introduced into the Arabidopsis EPSPS mature region around amino acid 27, replacing the sequence -Arg-Ala-Leu-Leu- with -Arg-Ile-Leu-Leu- in the process. The primer of the following sequence was used to modify the N-terminus of the CP4 EPSPS gene to add an EcoRI site to effect the fusion to the

CTP3:GGAAGACCCCCACGAATTCACCGTGCAAGCAGCCGG
(SEQ ID NO:36) (the EcoRI site is underlined.

This CTP3-CP4 EPSPS fusion was also cloned into the pBlueScript vector and the T7 expressed fusion was found to also import into chloroplasts with an efficiency comparable to that for the control Petunia EPSPS (pMON6140).

A related series of CTPs, designated as CTP4 (SphI) and CTP5 (EcoRI), based on the Petunia EPSPS CTP and gene were also fused to the SphI- and EcoRI-modified CP4 EPSPS gene sequences. The SphI site was added by site-directed mutagenesis to place this restriction site (and change the amino acid sequence to -Cys-Met-) at the chloroplast processing site. All of the CTP-CP4 EPSPS fusions were shown to import into chloroplasts with approximately equal efficiency. The CTP4 (SEQ ID NO:14) and CTP5 (SEQ ID NO:16) sequences are shown in FIGS. 11 and 12.

A CTP2-LBAA EPSPS fusion was also constructed following the modification of the N-terminus of the LBAA EPSPS gene by the addition of a SphI site. This fusion was also found to be imported efficiently into chloroplasts.

By similar approaches, the CTP2-CP4 EPSPS and the CTP4-CP4 EPSPS fusion have also been shown to import efficiently into chloroplasts prepared from the leaf sheaths of corn. These results indicate that these CTP-CP4 fusions could also provide useful genes to impart glyphosate tolerance in monocot species.

The use of CTP2 or CTP4 is preferred because these transit peptide constructions yield mature EPSPS enzymes upon import into the chloroplast which are closer in composition to the native EPSPSs not containing a transit peptide signal. Those skilled in the art will recognize that various chimeric constructs can be made which utilize the functionality of a particular CTP to import a Class II EPSPS enzyme into the plant cell chloroplast. The chloroplast import of the Class II EPSPS can be determined using the following assay.

Chloroplast Uptake Assay

Intact chloroplasts are isolated from lettuce (*Lactuca sativa*, var. longifolia) by centrifugation in Percoll/ficoll gradients as modified from Bartlett et al., (1982). The final pellet of intact chloroplasts is suspended in 0.5 ml of sterile 330 mM sorbitol in 50 mM Hepes-KOH, pH 7.7, assayed for chlorophyll (Arnon, 1949), and adjusted to the final chlorophyll concentration of 4 mg/ml (using sorbitol/Hepes). The yield of intact chloroplasts from a single head of lettuce is 3–6 mg chlorophyll.

A typical 300 µl uptake experiment contained 5 mM ATP, 8.3 mM unlabeled methionine, 322 mM sorbitol, 58.3 mM Hepes-KOH (pH 8.0), 50 µl reticulocyte lysate translation products, and intact chloroplasts from *L. sativa* (200 µg chlorophyll). The uptake mixture is gently rocked at room temperature (in 10×75 mm glass tubes) directly in front of a fiber optic illuminator set at maximum light intensity (150 Watt bulb). Aliquot samples of the uptake mix (about 50 µl) are removed at various times and fractionated over 100 µl silicone-oil gradients (in 150 µl polyethylene tubes) by centrifugation at 11,000× g for 30 seconds. Under these conditions, the intact chloroplasts form a pellet under the silicone-oil layer and the incubation medium (containing the reticulocyte lysate) floats on the surface. After centrifugation, the silicone-oil gradients are immediately frozen in dry ice. The chloroplast pellet is then resuspended in 50–100 µl of lysis buffer (10 mM Hepes-KOH pH 7.5, 1 mM PMSF, 1 mM benzamidine, 5 mM e-amino-n-caproic acid, and 30 µg/ml aprotinin) and centrifuged at 15,000× g for 20 minutes to pellet the thylakoid membranes. The clear supernatant (stromal proteins) from this spin, and an aliquot of the reticulocyte lysate incubation medium from each uptake experiment, are mixed with an equal volume of 2×SDS-PAGE sample buffer for electrophoresis (Laemmli, 1970).

SDS-PAGE is carried out according to Laemmli (1970) in 3–17% (w/v) acrylamide slab gels (60 mm×1.5 mm) with 3% (w/v) acrylamide stacking gels (5 mm×1.5 mm). The gel is fixed for 20–30 min in a solution with 40% methanol and 10% acetic acid. Then, the gel is soaked in EN^{3}HANCE™ (DuPont) for 20–30 minutes, followed by drying the gel on a gel dryer. The gel is imaged by autoradiography, using an intensifying screen and an overnight exposure to determine whether the CP4 EPSPS is imported into the isolated chloroplasts.

Plant Transformation

Plants which can be made glyphosate-tolerant by practice of the present invention include, but are not limited to, soybean, cotton, corn, canola, oil seed rape, flax, sugarbeet, sunflower, potato, tobacco, tomato, wheat, rice, alfalfa and lettuce as well as various tree, nut and vine species.

A double-stranded DNA molecule of the present invention ("chimeric gene") can be inserted into the genome of a plant by any suitable method. Suitable plant transformation vectors include those derived from a Ti plasmid of Agrobacterium tumefaciens, as well as those disclosed, e.g., by Herrera-Estrella (1983), Bevan (1984), Klee (1985) and EPO publication 120,516 (Schilperoort et al.). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of Agrobacterium, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen.

Class II EPSPS Plant transformation vectors

Class II EPSPS DNA sequences may be engineered into vectors capable of transforming plants by using known techniques. The following description is meant to be illustrative and not to be read in a limiting sense. One of ordinary skill in the art would know that other plasmids, vectors, markers, promoters, etc. would be used with suitable results. The CTP2-CP4 EPSPS fusion was cloned as a BglII-EcoRI fragment into the plant vector pMON979 (described below) to form pMON17110, a map of which is presented in FIG. 13. In this vector the CP4 gene is expressed from the enhanced CaMV35S promoter (E35S; Kay et al. 1987). A FMV35S promoter construct (pMON17116) was completed in the following way: The SalI-NotI and the NotI-BglII fragments from pMON979 containing the Spc/AAC(3)-III/orIV and the pBR322/Right Border/NOS 3'/CP4 EPSPS gene segment from pMON17110 were ligated with the XhoI-BglII FMV35S promoter fragment from pMON981. These vectors were introduced into tobacco, cotton and canola.

A series of vectors was also completed in the vector pMON977 in which the CP4 EPSPS gene, the CTP2-CP4 EPSPS fusion, and the CTP3-CP4 fusion were cloned as BglII-SacI fragments to form pMON17124, pMON17119, and pMON17120, respectively. These plasmids were introduced into tobacco. A pMON977 derivative containing the CTP2-LBAA EPSPS gene was also completed (pMON17206) and introduced into tobacco.

The pMON979 plant transformation/expression vector was derived from pMON886 (described below) by replacing the neomycin phosphotransferase typeII (KAN) gene in pMON886 with the 0.89 kb fragment containing the bacterial gentamicin-3-N-acetyltransferase type III (AAC(3)-III) gene (Hayford et al., 1988). The chimeric P-35S/AA(3)-III/NOS 3' gene encodes gentamicin resistance which permits selection of transformed plant cells. pMON979 also contains a 0.95 kb expression cassette consisting of the enhanced CaMV 35S promoter (Kay et al., 1987), several unique restriction sites, and the NOS 3' end (P-En-CaMV35S/NOS 3'). The rest of the pMON979 DNA segments are exactly the same as in pMON886.

Plasmid pMON886 is made up of the following segments of DNA. The first is a 0.93 kb AvaI to engineered-EcoRV fragment isolated from transposon Tn7 that encodes bacterial spectinomycin/streptomycin resistance (Spc/Str), which is a determinant for selection in *E. coli* and *Agrobacterium tumefaciens*. This is joined to the 1.61 kb segment of DNA encoding a chimeric kanamycin resistance which permits selection of transformed plant cells. The chimeric gene (P-35S/KAN/NOS 3') consists of the cauliflower mosaic virus (CaMV) 35S promoter, the neomycin phosphotransferase typeII (KAN) gene, and the 3'-nontranslated region of the nopaline synthase gene (NOS 3') (Fraley et al., 1983). The next segment is the 0.75 kb oriV containing the origin of replication from the RK2 plasmid. It is joined to the 3.1 kb SalI to PvuI segment of pBR322 (ori322) which provides the origin of replication for maintenance in *E. coli* and the bom site for the conjugational transfer into the *Agrobacterium tumefaciens* cells. The next segment is the 0.36 kb PvuI to BclI from pTiT37 that carries the nopaline-type T-DNA right border (Fraley et al., 1985).

The pMON977 vector is the same as pMON981 except for the presence of the P-En-CaMV35S promoter in place of the FMV35S promoter (see below).

The pMON981 plasmid contains the following DNA segments: the 0.93 kb fragment isolated from transposon Tn7 encoding bacterial spectinomycin/streptomycin resistance [Spc/Str; a determinant for selection in *E. coli* and *Agrobacterium tumefaciens* (Fling et al., 1985)]; the chimeric kanamycin resistance gene engineered for plant expression to allow selection of the transformed tissue, consisting of the 0.35 kb cauliflower mosaic virus 35S promoter (P-35S) (Odell et al., 1985), the 0.83 kb neomycin phosphotransferase typeII gene (KAN), and the 0.26 kb 3'-nontranslated region of the nopaline synthase gene (NOS 3') (Fraley et al., 1983); the 0.75 kb origin of replication from the RK2 plasmid (oriV) (Stalker et al., 1981); the 3.1 kb SalI to PvuI segment of pBR322 which provides the origin of replication for maintenance in *E. coli* (ori-322) and the bom site for the conjugational transfer into the *Agrobacterium tumefaciens* cells, and the 0.36 kb PvuI to BclI fragment from the pTiT37 plasmid containing the nopaline-type T-DNA right border region (Fraley et al., 1985). The expression cassette consists of the 0.6 kb 35S promoter from the figwort mosaic virus (P-FMV35S) (Gowda et al., 1989) and the 0.7 kb 3' non-translated region of the pea rbcS-E9 gene (E9 3') (Coruzzi et al., 1984, and Morelli et al., 1985). The 0.6 kb SspI fragment containing the FMV35S promoter (FIG. 1) was engineered to place suitable cloning sites downstream of the transcriptional start site. The CTP2-CP4syn gene fusion was introduced into plant expression vectors (including pMON981, to form pMON17131; FIG. 14) and transformed into tobacco, canola, potato, tomato, sugarbeet, cotton, lettuce, cucumber, oil seed rape, poplar, and Arabidopsis.

The plant vector containing the Class II EPSPS gene may be mobilized into any suitable Agrobacterium strain for transformation of the desired plant species. The plant vector may be mobilized into an ABI Agrobacterium strain. A suitable ABI strain is the A208 *Agrobacterium tumefaciens* carrying the disarmed Ti plasmid pTiC58 (pMP90RK) (Koncz and Schell, 1986). The Ti plasmid does not carry the T-DNA phytohormone genes and the strain is therefore unable to cause the crown gall disease. Mating of the plant vector into ABI was done by the triparental conjugation system using the helper plasmid pRK2013 (Ditta et al., 1980). When the plant tissue is incubated with the ABI::plant vector conjugate, the vector is transferred to the plant cells by the vir functions encoded by the disarmed pTiC58 plasmid. The vector opens at the T-DNA right border region, and the entire plant vector sequence may be inserted into the host plant chromosome. The pTiC58 Ti plasmid does not transfer to the plant cells but remains in the Agrobacterium.

Class II EPSPS free DNA vectors

Class II EPSPS genes may also be introduced into plants through direct delivery methods. A number of direct delivery vectors were completed for the CP4 EPSPS gene. The vector pMON13640, a map of which is presented in FIG. 15, is described here. The plasmid vector is based on a pUC plasmid (Vieira and Messing, 1987) containing, in this case, the nptII gene (kanamycin resistance; KAN) from Tn903 to provide a selectable marker in *E. coli*. The CTP4-EPSPS gene fusion is expressed from the P-FMV35S promoter and contains the NOS 3' polyadenylation sequence fragment and from a second cassette consisting of the E35S promoter, the CTP4-CP4 gene fusion and the NOS 3' sequences. The scoreable GUS marker gene (Jefferson et al., 1987) is expressed from the mannopine synthase promoter (P-MAS; Velten et al., 1984) and the soybean 7S storage protein gene 3' sequences (Schuler et al., 1982). Similar plasmids could also be made in which CTP-CP4 EPSPS fusions are expressed from the enhanced CaMV35S promoter or other plant promoters. Other vectors could be made that are suitable for free DNA delivery into plants and such are within the skill of the art and contemplated to be within the scope of this disclosure.

Plastid transformation:

While transformation of the nuclear genome of plants is much more developed at this time, a rapidly advancing alternative is the transformation of plant organelles. The transformation of plastids of land plants and the regeneration of stable transformants has been demonstrated (Svab et al., 1990; Maliga et al., 1993). Transformants are selected, following double cross-over events into the plastid genome, on the basis of resistance to spectinomycin conferred through rRNA changes or through the introduction of an aminoglycoside 3"-adenyltransferase gene (Svab et al., 1990; Svab and Maliga, 1993), or resistance to kanamycin through the neomycin phosphotransferase NptII (Carrer et al., 1993). DNA is introduced by biolistic means (Svab et al., 1990; Maliga et al., 1993) or by using polyethylene glycol (O'Neill et al., 1993). This transformation route results in the production of 500–10,000 copies of the introduced sequence per cell and high levels of expression of the

introduced gene have been reported (Carrer et al., 1993; Maliga et al., 1993). The use of plastid transformation offers the advantages of not requiring the chloroplast transit peptide signal sequence to result in the localization of the heterologous Class II EPSPS in the chloroplast and the potential to have many copies of the heterologous plant-expressible Class II EPSPS gene in each plant cell since at least one copy of the gene would be in each plastid of the cell.

Plant Regeneration

When expression of the Class II EPSPS gene is achieved in transformed cells (or protoplasts), the cells (or protoplasts) are regenerated into whole plants. Choice of methodology for the regeneration step is not critical, with suitable protocols being available for hosts from Leguminosae (alfalfa, soybean, clover, etc.), Umbelliferae (carrot, celery, parsnip), Cruciferae (cabbage, radish, rapeseed, etc.), Cucurbitaceae (melons and cucumber), Gramineae (wheat, rice, corn, etc.), Solanaceae (potato, tobacco, tomato, peppers), various floral crops as well as various trees such as poplar or apple, nut crops or vine plants such as grapes. See, e.g., Ammirato, 1984; Shimamoto, 1989; Fromm, 1990; Vasil, 1990.

The following examples are provided to better elucidate the practice of the present invention and should not be interpreted in any way to limit the scope of the present invention. Those skilled in the art will recognize that various modifications, truncations, etc. can be made to the methods and genes described herein while not departing from the spirit and scope of the present invention.

In the examples that follow, EPSPS activity in plants is assayed by the following method. Tissue samples were collected and immediately frozen in liquid nitrogen. One gram of young leaf tissue was frozen in a mortar with liquid nitrogen and ground to a fine powder with a pestle. The powder was then transferred to a second mortar, extraction buffer was added (1 ml/gram), and the sample was ground for an additional 45 seconds. The extraction buffer for canola consists of 100 mM Tris, 1 mM EDTA, 10% glycerol, 5 mM DTT, 1 mM BAM, 5 mM ascorbate, 1.0 mg/ml BSA, pH 7.5 (4° C.). The extraction buffer for tobacco consists of 100 mM Tris, 10 mM EDTA, 35 mM KCl, 20% glycerol, 5 mM DTT, 1 mM BAM, 5 mM ascorbate, 1.0 mg/ml BSA, pH 7.5 (4° C.). The mixture was transferred to a microfuge tube and centrifuged for 5 minutes. The resulting supernatants were desalted on spin G-50 (Pharmacia) columns, previously equilibrated with extraction buffer (without BSA), in 0.25 ml aliquots. The desalted extracts were assayed for EPSP synthase activity by radioactive HPLC assay. Protein concentrations in samples were determined by the BioRad microprotein assay with BSA as the standard.

Protein concentrations were determined using the BioRad Microprotein method. BSA was used to generate a standard curve ranging from 2–24 μg. Either 800 μl of standard or diluted sample was mixed with 200 μl of concentrated BioRad Bradford reagent. The samples were vortexed and read at A(595) after ~5 minutes and compared to the standard curve.

EPSPS enzyme assays contained HEPES (50 mM), shikimate-3-phosphate (2 mM), NH_4 molybdate (0.1 mM) and KF (5 mM), with or without glyphosate (0.5 or 1.0 mM). The assay mix (30 μl) and plant extract (10 μl) were preincubated for 1 minute at 25° C. and the reactions were initiated by adding ^{14}C-PEP (1 mM). The reactions were quenched after 3 minutes with 50 μl of 90% EtOH/0.1M HOAc, pH 4.5. The samples were spun at 6000 rpm and the resulting supernatants were analyzed for ^{14}C-EPSP production by HPLC. Percent resistant EPSPS is calculated from the EPSPS activities with and without glyphosate.

The percent conversion of ^{14}C labeled PEP to ^{14}C EPSP was determined by HPLC radioassay using a C18 guard column (Brownlee) and an AX_{100} HPLC column (0.4×25 cm, Synchropak) with 0.28M isocratic potassium phosphate eluant, pH 6.5, at 1 ml/min. Initial velocities were calculated by multiplying fractional turnover per unit time by the initial concentration of the labeled substrate (1 mM). The assay was linear with time up to ~3 minutes and 30% turnover to EPSPS. Samples were diluted with 10 mM Tris, 10% glycerol, 10 mM DTT, pH 7.5 (4° C.) if necessary to obtain results within the linear range.

In these assays DL-dithiothreitol (DTT), benzamidine (BAM), and bovine serum albumin (BSA, essentially globulin free) were obtained from Sigma. Phosphoenolpyruvate (PEP) was from Boehringer Mannheim and phosphoenol-[1-^{14}C]pyruvate (28 mCi/mmol) was from Amersham.

EXAMPLES

Example 1

Transformed tobacco plants have been generated with a number of the Class II EPSPS gene vectors containing the CP4 EPSPS DNA sequence as described above with suitable expression of the EPSPS. These transformed plants exhibit glyphosate tolerance imparted by the Class II CP4 EPSPS.

Transformation of tobacco employs the tobacco leaf disc transformation protocol which utilizes healthy leaf tissue about 1 month old. After a 15–20 minutes surface sterilization with 10% Clorox plus a surfactant, the leaves are rinsed 3 times in sterile water. Using a sterile paper punch, leaf discs are punched and placed upside down on MS104 media (MS salts 4.3 g/l, sucrose 30 g/l, B5 vitamins 500×2 ml/l, NAA 0.1 mg/l, and BA 1.0 mg/l) for a 1 day preculture.

The discs are then inoculated with an overnight culture of a disarmed Agrobacterium ABI strain containing the subject vector that had been diluted 1/5 (i.e.: about 0.6 OD). The inoculation is done by placing the discs in centrifuge tubes with the culture. After 30 to 60 seconds, the liquid is drained off and the discs were blotted between sterile filter paper. The discs are then placed upside down on MS104 feeder plates with a filter disc to co-culture.

After 2–3 days of co-culture, the discs are transferred, still upside down, to selection plates with MS104 media. After 2–3 weeks, callus tissue formed, and individual clumps are separated from the leaf discs. Shoots are cleanly cut from the callus when they are large enough to be distinguished from stems. The shoots are placed on hormone-free rooting media (MSO: MS salts 4.3 g/l, sucrose 30 g/l, and B5 vitamins 500×2 ml/l) with selection for the appropriate antibiotic resistance. Root formation occurred in 1–2 weeks. Any leaf callus assays are preferably done on rooted shoots while still sterile. Rooted shoots are then placed in soil and kept in a high humidity environment (i.e.: plastic containers or bags). The shoots are hardened off by gradually exposing them to ambient humidity conditions.

Expression of CP4 EPSPS protein in transformed plants

Tobacco cells were transformed with a number of plant vectors containing the native CP4 EPSPS gene, and using different promoters and/or CTP's. Preliminary evidence for expression of the gene was given by the ability of the leaf tissue from antibiotic selected transformed shoots to recallus on glyphosate. In some cases, glyphosate-tolerant callus was selected directly following transformation. The level of expression of the CP4 EPSPS was determined by the level

of glyphosate-tolerant EPSPS activity (assayed in the presence of 0.5 mM glyphosate) or by Western blot analysis using a goat anti-CP4 EPSPS antibody. The Western blots were quantitated by densitometer tracing and comparison to a standard curve established using purified CP4 EPSPS. These data are presented as % soluble leaf protein. The data from a number of transformed plant lines and transformation vectors are presented in Table VI below.

TABLE VI

Expression of CP4 EPSPS in transformed tobacco tissue

Vector	Plant #	CP4 EPSPS ** (% leaf protein)
pMON17110	25313	0.02
pMON17110	25329	0.04
pMON17116	25095	0.02
pMON17119	25106	0.09
pMON17119	25762	0.09
pMON17119	25767	0.03

**Glyphosate-tolerant EPSPS activity was also demonstrated in leaf extracts for these plants.

Glyphosate tolerance has also been demonstrated at the whole plant level in transformed tobacco plants. In tobacco, R_o transformants of CTP2-CP4 EPSPS were sprayed at 0.4 lb/acre (0.448 kg/hectare), a rate sufficient to kill control non-transformed tobacco plants corresponding to a rating of 3, 1 and 0 at days 7, 14 and 28, respectively, and were analyzed vegetatively and reproductively (Table VII).

TABLE VII

Glyphosate tolerance in R_o tobacco CP4 transformants*

	Score**			
	Vegetative			
Vector/Plant #	day 7	day 14	day 28	Fertile
pMON17110/25313	6	4	2	no
pMON17110/25329	9	10	10	yes
pMON17119/25106	9	9	10	yes

*Spray rate = 0.4 lb/acre (0.448 kg/hectare)

**Plants are evaluated on a numerical scoring system of 0–10 where a vegetative score of 10 represents no damage relative to nonsprayed controls and 0 represents a dead plant. Reproductive scores (Fertile) are determined at 28 days after spraying and are evaluated as to whether or not the plant is fertile.

Example 2A

Canola plants were transformed with the pMON17110, pMON17116, and pMON17131 vectors and a number of plant lines of the transformed canola were obtained which exhibit glyphosate tolerance.

Plant Material

Seedlings of *Brassica napus* cv Westar were established in 2 inch (~5 cm) pots containing Metro Mix 350. They were grown in a growth chamber at 24° C., 16/8 hour photoperiod, light intensity of 400 $uEm^{-2}sec^{-1}$ (HID lamps). They were fertilized with Peters 20-10-20 General Purpose Special. After 2½ weeks they were transplanted to 6 inch (~15 cm) pots and grown in a growth chamber at 15°/10° C. day/night temperature, 16/8 hour photoperiod, light intensity of 800 $uEm^{-2}sec^{-1}$ (HID lamps). They were fertilized with Peters 15-30-15 Hi-Phos Special.

Transformation/Selection/Regeneration

Four terminal internodes from plants just prior to bolting or in the process of bolting but before flowering were removed and surfaced sterilized in 70% v/v ethanol for 1 minute, 2% w/v sodium hypochlorite for 20 minutes and rinsed 3 times with sterile deionized water. Stems with leaves attached could be refrigerated in moist plastic bags for up to 72 hours prior to sterilization. Six to seven stem segments were cut into 5 mm discs with a Redco Vegetable Slicer 200 maintaining orientation of basal end.

The Agrobacterium was grown overnight on a rotator at 24° C. in 2 mls of Luria Broth containing 50 mg/l kanamycin, 24 mg/l chloramphenicol and 100 mg/l spectinomycin. A 1:10 dilution was made in MS (Murashige and Skoog) media giving approximately 9×10^8 cells per ml. This was confirmed with optical density readings at 660 nm. The stem discs (explants) were inoculated with 1.0 ml of Agrobacterium and the excess was aspirated from the explants.

The explants were placed basal side down in petri plates containing 1/10× standard MS salts, B5 vitamins, 3% sucrose, 0.8% agar, pH 5.7, 1.0 mg/l 6-benzyladenine (BA). The plates were layered with 1.5 ml of media containing MS salts, B5 vitamins, 3% sucrose, pH 5.7, 4.0 mg/l p-chlorophenoxyacetic acid, 0.005 mg/l kinetin and covered with sterile filter paper.

Following a 2 to 3 day co-culture, the explants were transferred to deep dish petri plates containing MS salts, B5 vitamins, 3% sucrose, 0.8% agar, pH 5.7, 1 mg/l BA, 500 mg/l carbenicillin, 50 mg/l cefotaxime, 200 mg/l kanamycin or 175 mg/l gentamicin for selection. Seven explants were placed on each plate. After 3 weeks they were transferred to fresh media, 5 explants per plate. The explants were cultured in a growth room at 25° C., continuous light (Cool White).

Expression Assay

After 3 weeks shoots were excised from the explants. Leaf recallusing assays were initiated to confirm modification of R_o shoots. Three tiny pieces of leaf tissue were placed on recallusing media containing MS salts, B5 vitamins, 3% sucrose, 0.8% agar, pH 5.7, 5.0 mg/l BA, 0.5 mg/l naphthalene acetic acid (NAA), 500 mg/l carbenicillin, 50 mg/l cefotaxime and 200 mg/l kanamycin or gentamicin or 0.5 mM glyphosate. The leaf assays were incubated in a growth room under the same conditions as explant culture. After 3 weeks the leaf recallusing assays were scored for herbicide tolerance (callus or green leaf tissue) or sensitivity (bleaching).

Transplantation

At the time of excision, the shoot stems were dipped in Rootone® and placed in 2 inch (~5 cm) pots containing Metro-Mix 350 and placed in a closed humid environment. They were placed in a growth chamber at 24° C., 16/8 hour photoperiod, 400 $uEm^{-1}sec^{-2}$ (HID lamps) for a hardening-off period of approximately 3 weeks.

The seed harvested from R_o plants is R_1 seed which gives rise to R_1 plants. To evaluate the glyphosate tolerance of an R_o plant, its progeny are evaluated. Because an R_o plant is assumed to be hemizygous at each insert location, selfing results in maximum genotypic segregation in the R_1. Because each insert acts as a dominant allele, in the absence of linkage and assuming only one hemizygous insert is required for tolerance expression, one insert would segregate 3:1, two inserts, 15:1, three inserts 63:1, etc. Therefore, relatively few R_1 plants need be grown to find at least one resistant phenotype.

Seed from an R_o plant is harvested, threshed, and dried before planting in a glyphosate spray test. Various techniques have been used to grow the plants for R_1 spray evaluations. Tests are conducted in both greenhouses and growth chambers. Two planting systems are used; ~10 cm pots or plant trays containing 32 or 36 cells. Soil used for

planting is either Metro 350 plus three types of slow release fertilizer or plant Metro 350. Irrigation is either overhead in greenhouses or sub-irrigation in growth chambers. Fertilizer is applied as required in irrigation water. Temperature regimes appropriate for canola were maintained. A sixteen hour photoperiod was maintained. At the onset of flowering, plants are transplanted to ~15 cm pots for seed production.

A spray "batch" consists of several sets of R_1 progenies all sprayed on the same date. Some batches may also include evaluations of other than R_1 plants. Each batch also includes sprayed and unsprayed non-transgenic genotypes representing the genotypes in the particular batch which were putatively transformed. Also included in a batch is one or more non-segregating transformed genotypes previously identified as having some resistance.

Two-six plants from each individual R_0 progeny are not sprayed and serve as controls to compare and measure the glyphosate tolerance, as well as to assess any variability not induced by the glyphosate. When the other plants reach the 2–4 leaf stage, usually 10 to 20 days after planting, glyphosate is applied at rates varying from 0.28 to 1.12 kg/ha, depending on objectives of the study. Low rate technology using low volumes has been adopted. A laboratory track sprayer has been calibrated to deliver a rate equivalent to field conditions.

A scale of 0 to 10 is used to rate the sprayed plants for vegetative resistance. The scale is relative to the unsprayed plants from the same R_0 plant. A 0 is death, while a 10 represents no visible difference from the unsprayed plant. A higher number between 0 and 10 represents progressively less damage as compared to the unsprayed plant. Plants are scored at 7, 14, and 28 days after treatment (DAT), or until bolting, and a line is given the average score of the sprayed plants within an R_0 plant family.

Six integers are used to qualitatively describe the degree of reproductive damage from glyphosate:

0: No floral bud development

2: Floral buds present, but aborted prior to opening

4: Flowers open, but no anthers, or anthers fail to extrude past petals

6: Sterile anthers

8: Partially sterile anthers

10: Fully fertile flowers

Plants are scored using this scale at or shortly after initiation of flowering, depending on the rate of floral structure development.

Expression of EPSPS in Canola

After the 3 week period, the transformed canola plants were assayed for the presence of glyphosate-tolerant EPSPS activity (assayed in the presence of glyphosate at 0.5 mM). The results are shown in Table VIII.

TABLE VIII

Expression of CP4 EPSPS in transformed Canola plants

	Plant #	% resistant EPSPS activity of Leaf extract (at 0.5 mM glyphosate)
Vector Control		0
pMON17110	41	47
pMON17110	52	28
pMON17110	71	82
pMON17110	104	75
pMON17110	172	84
pMON17110	177	85

TABLE VIII-continued

Expression of CP4 EPSPS in transformed Canola plants

	Plant #	% resistant EPSPS activity of Leaf extract (at 0.5 mM glyphosate)
pMON17110	252	29*
pMON17110	350	49
pMON17116	40	25
pMON17116	99	87
pMON17116	175	94
pMON17116	176	43
pMON17116	182	18
pMON17116	252	69
pMON17116	298	44*
pMON17116	332	89
pMON17116	383	97
pMON17116	395	52

*assayed in the presence of 1.0 mM glyphosate

R_1 transformants of canola were then grown in a growth chamber and sprayed with glyphosate at 0.56 kg/ha (kilogram/hectare) and rated vegetatively. These results are shown in Table IXA–IXC. It is to be noted that expression of glyphosate resistant EPSPS in all tissues is preferred to observe optimal glyphosate tolerance phenotype in these transgenic plants. In the Tables below, only expression results obtained with leaf tissue are described.

TABLE IXA

Glyphosate tolerance in Class II EPSPS canola R_1 transformants (pMON17110 = P-E35S; pMON17116 = P-FMV35S; R1 plants; Spray rate = 0.56 kg/ha)

Vector/Plant No.	% resistant EPSPS*	Vegetative Score** day 7	Vegetative Score** day 14
Control Water	0	5	3
pMON17110/41	47	6	7
pMON17110/71	82	6	7
pMON17110/177	85	9	10
pMON17116/40	25	8	9
pMON17116/99	87	9	10
pMON17116/175	94	9	10
pMON17116/176	43	6	3
pMON17116/182	18	9	10
pMON17116/383	97	9	10

TABLE IXB

Glyphosate tolerance in Class II EPSPS canola R_1 transformants (pMON17131 = P-FWV35S; R1 plants; Spray rate = 0.84 kg/ha)

Vector/Plant No.	Vegetative score** day 14	Reproductive score day 28
17131/78	10	10
17131/102	9	10
17131/115	9	10
17131/136	9	10
17131/157	9	10
17131/169	10	10
17131/255	10	10
control Water	1	0

TABLE IXC

Glyphosate tolerance in Class I EPSPS canola transformants (P-ES35S; R2 Plants; Spray rate = 0.28 kg/ha)

Vector/Plant No.	% resistant EPSPS*	Vegetative Score** day 7	Vegetative Score** day 14
Control Westar	0	4	2
pMON17715	96	5	6
pMON17744	95	8	8
pMON17796	86	6	4
pMON17818	81	7	8
pMON17885	57	7	6

*% resistant EPSPS activity in the presence of 0.5 mM glyphosate
**A vegetative score of 10 indicates no damage, a score of 0 is given to a dead plant.

The data obtained for the Class II EPSPS transformants may be compared to glyphosate-tolerant Class I EPSP transformants in which the same promoter is used to express the EPSPS genes and in which the level of glyphosate-tolerant EPSPS activity was comparable for the two types of transformants. A comparison of the data of pMON17110 [in Table IXA] and pMON17131 [Table IXB] with that for pMON899 [in Table IXC; the Class I gene in pMON899 is that from *A. thaliana* (Klee et al., 1987) in which the glycine at position 101 was changed to an alanine] illustrates that the Class II EPSPS is at least as good as that of the Class I EPSPS. An improvement in vegetative tolerance of Class II EPSPS is apparent when one takes into account that the Class II plants were sprayed at twice the rate and were tested as R_1 plants.

Example 2B

The construction of two plant transformation vectors and the transformation procedures used to produce glyphosate-tolerant canola plants are described in this example The vectors, pMON17209 and pMON17237, were used to generate transgenic glyphosate-tolerant canola lines. The vectors each contain the gene encoding the 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) from Agrobacterium sp. strain CP4. The vectors also contain either the gox gene encoding the glyphosate oxidoreductase enzyme (GOX) from Achromobacter sp. strain LBAA (Barry et al., 1992) or the gene encoding a variant of GOX (GOX v.247) which displays improved catalytic properties. These enzymes convert glyphosate to aminomethylphosphonic acid and glyoxylate and protect the plant from damage by the metabolic inactivation of glyphosate. The combined result of providing an alternative, resistant EPSPS enzyme and the metabolism of glyphosate produces transgenic plants with enhanced tolerance to glyphosate

Molecular biology techniques. In general, standard molecular biology and microbial genetics approaches were employed (Maniatis et al., 1982). Site-directed mutageneses were carried out as described by Kunkel et al. (1987). Plant-prefered genes were synthesized and the sequence confirmed.

Plant transformation vectors. The following describes the general features of the plant transformation vectors that were modified to form vectors pMON17209 and pMON17237. The Agrobacterium mediated plant transformation vectors contain the following well-characterized DNA segments which are required for replication and function of the plasmids (Rogers and Klee, 1987; Klee and Rogers, 1989).

The first segment is the 0.45 kb ClaI-DraI fragment from the pTi15955 octopine Ti plasmid which contains the T-DNA left border region (Barker et al., 1983). It is joined to the 0.75 kb origin of replication (oriV) derived from the broad-host range plasmid RK2 (Stalker et al., 1981). The next segment is the 3.1 kb SalI-PvuI segment of pBR_{322} which provides the origin of replication for maintenance in *E. coli* and the bom site for the conjugational transfer into the *Agrobacterium tumefaciens* cells (Bolivar et al., 1977). This is fused to the 0.93 kb fragment isolated from transposon Tn7 which encodes bacterial spectinomycin and streptomycin resistance (Fling et al., 1985), a determinant for the selection of the plasmids in *E. coli* and Agrobacterium. It is fused to the 0.36 kb PvuI-BclI fragment from the pTiT37 plasmid which contains the nopaline-type T-DNA right border region (Fraley et al., 1985). Several chimeric genes engineered for plant expression can be introduced between the Ti right and left border regions of the vector. In addition to the elements described above, this vector also includes the 35S promoter/NPTII/NOS 3' cassette to enable selection of transformed plant tissues on kanamycin (Klee and Rogers, 1989; Fraley et al., 1983; and Odell, et al., 1985) within the borders. An "empty" expression cassette is also present between the borders and consists of the enhanced E35S promoter (Kay et al., 1987), the 3' region from the small subunit of RUBP carboxylase of pea (E9) (Coruzzi et al., 1984; Morelli et al., 1986), and a number of restriction enzyme sites that may be used for the cloning of DNA sequences for expression in plants. The plant transformation system based on *Agrobacterium tumefaciens* delivery has been reviewed (Klee and Rogers, 1989; Fraley et al., 1986). The Agrobacterium mediated transfer and integration of the vector T-DNA into the plant chromosome results in the expression of the chimeric genes conferring the desired phenotype in plants.

Bacterial Inoculum. The binary vectors are mobilized into *Agrobacterium tumefaciens* strain ABI by the triparental conjugation system using the helper plasmid pRK2013 (Ditta et al., 1980). The ABI strain contains the disarmed pTiC58 plasmid pMP90RK (Koncz and Schell, 1986) in the chloramphenicol resistant derivative of the *Agrobacterium tumefaciens* strain A208.

Transformation procedure. Agrobacterium inocula were grown overnight at 28° C. in 2 ml of LBSCK (LBSCK is made as follows: LB liquid medium [1 liter volume]=10 g NaCl; 5 g Yeast Extract;10 g tryptone; pH 7.0, and autoclave for 22 minutes. After autoclaving, add spectinomycin (50 mg/ml stock)—2 ml, kanamycin (50 mg/ml stock)—1 ml. and chloramphenicol (25 mg/ml stock)—1 mL). One day prior to inoculation, the Agrobacterium was subcultured by inoculating 200 µl into 2 ml of fresh LBSCK and grown overnight. For inoculation of plant material, the culture was diluted with MSO liquid medium to an A_{660} range of 0.2–0.4.

Seedlings of *Brassica napus* cv. Westar were grown in Metro Mix 350 (Humanert Seed Co., St. Louis, Mo.) in a growth chamber with a day/night temperature of 15°/10° C., relative humidity of 50%, 16h/8h photoperiod, and at a light intensity of 500 µmol m^{-2} sec^{-1}. The plants were watered daily (via sub-irrigation) and fertilized every other day with Peter's 15:30:15 (Fogelsville, Pa.).

In general, all media recipes and the transformation protocol follow those in Fry et. al. (1987). Five to six week-old Westar plants were harvested when the plants had bolted (but prior to flowering), the leaves and buds were removed, and the 4–5 inches of stem below the flower buds were used as the explant tissue source. Following sterilization with 70% ethanol for 1 min and 35% Clorox for 20 min,

the stems were rinsed three times with sterile water and cut into 5 mm-long segments (the orientation of the basal end of the stem segments was noted). The plant material was incubated for 5 minutes with the diluted Agrobacterium culture at a rate of 5 ml of culture per 5 stems. The suspension of bacteria was removed by aspiration and the explants were placed basal side down—for an optimal shoot regeneration response—onto co-culture plates (½ MSO solid medium with a 1.5 ml TXD (tobacco xanthi diploid) liquid medium overlay and covered with a sterile 8.5 cm filter paper). Fifty-to-sixty stem explants were placed onto each co-culture plate.

After a 2 day co-culture period, stem explants were moved onto MS medium containing 750 mg/l carbenicillin, 50 mg/l cefotaxime, and 1 mg/l BAP (benzylaminopurine) for 3 days. The stem explants were then placed for two periods of three weeks each, again basal side down and with 5 explants per plate, onto an MS/0.1 mM glyphosate, selection medium (also containing carbenicillin, cefotaxime, and BAP (The glyphosate stock [0.5M] is prepared as described in the following: 8.45 g glyphosate [analytical grade] is dissolved in 50 ml deionized water, adding KOH pellets to dissolve the glyphosate, and the volume is brought to 100 ml following adjusting the pH to 5.7. The solution is filter-sterilized and stored at 4° C.). After 6 weeks on this glyphosate selection medium, green, normally developing shoots were excised from the stem explants and were placed onto fresh MS medium containing 750 mg/l carbenicillin, 50 mg/l cefotaxime, and 1 mg/l BAP, for further shoot development. When the shoots were 2–3 inches tall, a fresh cut at the end of the stem was made, the cut end was dipped in Root-tone, and the shoot was placed in Metro Mix 350 soil and allowed to harden-off for 2–3 weeks.

Construction of Canola transformation vector pMON17209. The EPSPS gene was isolated originally from Agrobacterium sp. strain CP4 and expresses a highly tolerant enzyme. The original gene contains sequences that could be inimical to high expression of the gene in some plants. These sequences include potential polyadenylation sites that are often A+T rich, a higher G+C % than that frequently found in dicotyledonous plant genes (63% versus ~50%), concentrated stretches of G and C residues, and codons that may not used frequently in dicotyledonous plant genes. The high G+C % in the CP4 EPSPS gene could also result in the formation of strong hairpin structures that may affect expression or stability of the RNA. A plant preferred version of the gene was synthesized and used for these vectors. This coding sequence was expressed in *E. coli* from a PRecA-gene10L vector (Olins et al., 1988) and the EPSPS activity was compared with that from the native CP4 EPSPS gene. The $appK_m$ for PEP for the native and synthetic genes was 11.8 μM and 12.7 μM, respectively, indicating that the enzyme expressed from the synthetic gene was unaltered. The N-terminus of the coding sequence was then mutagenized to place an SphI site (GCATGC) at the ATG to permit the construction of the CTP2-CP4 synthetic fusion for chloroplast import. This change had no apparent effect on the in vivo activity of CP4 EPSPS in *E. coli* as judged by complementation of the aroA mutant. A CTP-CP4 EPSPS fusion was constructed between the *Arabidopsis thaliana* EPSPS CTP (Klee et al., 1987) and the CP4 EPSPS coding sequences. The Arabidopsis CTP was engineered by site-directed mutagenesis to place a SphI restriction site at the CTP processing site. This mutagenesis replaced the Gln-Lys at this location with Cys-Met. The CTP2-CP4 EPSPS fusion was tested for import into chloroplasts isolated from *Lactuca sativa* using the methods described previously (della-Cioppa et al., 1986; 1987).

The GOX gene that encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) was cloned originally from Achromobacter sp. strain LBAA (Hallas et al., 1988; Barry et al., 1992). The gox gene from strain LBAA was also resynthesized in a plant-preferred sequence version and in which many of the restriction sites were removed (PCT Appln. No. WO 92/00377). The GOX protein is targeted to the plastids by a fusion between the C-terminus of a CTP and the N-terminus of GOX. A CTP, derived from the SSU1A gene from *Arabidopsis thaliana* (Timko et al., 1988) was used. This CTP (CTP1) was constructed by a combination of site-directed mutageneses. The CTP1 is made up of the SSU1A CTP (amino acids 1–55), the first 23 amino acids of the mature SSU1A protein (56–78), a serine residue (amino acid 79), a new segment that repeats amino acids 50 to 56 from the CTP and the first two from the mature protein (amino acids 80–87), and an alanine and methionine residue (amino acid 88 and 89). An NcoI restriction site is located at the 3' end (spans the Met89 codon) to facilitate the construction of precise fusions to the 5' of GOX. At a later stage, a BglII site was introduced upstream of the N-terminus of the SSU1A sequences to facilitate the introduction of the fusions into plant transformation vectors. A fusion was assembled between CTP1 and the synthetic GOX gene.

The CP4 EPSPS and GOX genes were combined to form pMON17209 as described in the following. The CTP2-CP4 EPSPS fusion was assembled and inserted between the constitutive FMV35S promoter (Gowda et al., 1989; Richins et al., 1987) and the E9 3' region (Coruzzi et al., 1984; Morelli et al., 1985) in a pUC vector (Yanisch-Perron et al., 1985; Vieira and Messing, 1987) to form pMON17190; this completed element may then be moved easily as a NotI-NotI fragment to other vectors. The CTP1-GOX fusion was also assembled in a pUC vector with the FMV35S promoter. This element was then moved as a HindIII-BamHI fragment into the plant transformation vector pMON10098 and joined to the E9 3' region in the process. The resultant vector pMON17193 has a single NotI site into which the FMV 35S/CTP2-CP4 EPSPS/E9 3' element from pMON17190 was cloned to form pMON17194. The kanamycin plant transformation selection cassette (Fraley et al., 1985) was then deleted from pMON17194, by cutting with XhoI and re-ligating, to form the pMON17209 vector (FIG. 24).

Construction of Canola transformation vector pMON17237. The GOX enzyme has an apparent Km for glyphosate [$appK_m$(glyphosate)] of ~25 mM. In an effort to improve the effectiveness of the glyphosate metabolic rate in plants, a variant of GOX has been identified in which the $appK_m$(glyphosate) has been reduced approximately 10-fold; this variant is referred to as GOX v.247 and the sequence differences between it and the original plant-preferred GOX are illustrated in PCT Appln. No. WO 92/00377. The GOX v.247 coding sequence was combined with CTP1 and assembled with the FMV35S promoter and the E9 3' by cloning into the pMON17227 plant transformation vector to form pMON17241. In this vector, effectively, the CP4 EPSPS was replaced by GOX v.247. The pMON17227 vector had been constructed by replacing the CTP1-GOX sequences in pMON17193 with those for the CTP2-CP4 EPSPS, to form pMON17199 and followed by deleting the kanamycin cassette (as described above for pMON17209). The pMON17237 vector (FIG. 25) was then completed by cloning the FMV35S/CTP2-CP4 EPSPS/E9 3' element as a NotI-NotI fragment into pMON17241.

Example 3

Soybean plants were transformed with the pMON13640 (FIG. 15) vector and a number of plant lines of the transformed soybean were obtained which exhibit glyphosate tolerance.

Soybean plants are transformed with pMON13640 by the method of microprojectile injection using particle gun technology as described in Christou et al. (1988). The seed harvested from R_0 plants is R_1 seed which gives rise to R_1 plants. To evaluate the glyphosate tolerance of an R_0 plant, its progeny are evaluated. Because an R_0 plant is assumed to be hemizygous at each insert location, selfing results in maximum genotypic segregation in the R_1. Because each insert acts as a dominant allele, in the absence of linkage and assuming only one hemizygous insert is required for tolerance expression, one insert would segregate 3:1, two inserts, 15:1, three inserts 63:1, etc. Therefore, relatively few R_1 plants need be grown to find at least one resistant phenotype.

Seed from an R_0 soybean plant is harvested, and dried before planting in a glyphosate spray test. Seeds are planted into 4 inch (~5 cm) square pots containing Metro 350. Twenty seedlings from each R_0 plant is considered adequate for testing. Plants are maintained and grown in a greenhouse environment. A 12.5–14 hour photoperiod and temperatures of 30° C. day and 24° C. night is regulated. Water soluble Peters Pete Lite fertilizer is applied as needed.

A spray "batch" consists of several sets of R_1 progenies all sprayed on the same date. Some batches may also include evaluations of other than R_1 plants. Each batch also includes sprayed and unsprayed non-transgenic genotypes representing the genotypes in the particular batch which were putatively transformed. Also included in a batch is one or more non-segregating transformed genotypes previously identified as having some resistance.

One to two plants from each individual R_0 progeny are not sprayed and serve as controls to compare and measure the glyphosate tolerance, as well as to assess any variability not induced by the glyphosate. When the other plants reach the first trifoliate leaf stage, usually 2–3 weeks after planting, glyphosate is applied at a rate equivalent of 128 oz./acre (8.895 kg/ha) of Roundup®. A laboratory track sprayer has been calibrated to deliver a rate equivalent to those conditions.

A vegetative score of 0 to 10 is used. The score is relative to the unsprayed progenies from the same R_0 plant. A 0 is death, while a 10 represents no visible difference from the unsprayed plant. A higher number between 0 and 10 represents progressively less damage as compared to the unsprayed plant. Plants are scored at 7, 14, and 28 days after treatment (DAT). The data from the analysis of one set of transformed and control soybean plants are described on Table X and show that the CP4 EPSPS gene imparts glyphosate tolerance in soybean also.

TABLE X

Glyphosate tolerance in Class II EPSPS soybean transformants
(P-E35S, P-FMV35S; RO plants; Spray rate = 128 oz./acre)

Vector/Plant No.	Vegetative score day 7	day 14	day 28
13640/40-11	5	6	7
13640/40-3	9	10	10
13640/40-7	4	7	7
control A5403 2	1	0	
control A5403 1	1	0	

Example 4

The CP4 EPSPS gene may be used to select transformed plant material directly on media containing glyphosate. The ability to select and to identify transformed plant material depends, in most cases, on the use of a dominant selectable marker gene to enable the preferential and continued growth of the transformed tissues in the presence of a normally inhibitory substance. Antibiotic resistance and herbicide tolerance genes have been used almost exclusively as such dominant selectable marker genes in the presence of the corresponding antibiotic or herbicide. The nptII/kanamycin selection scheme is probably the most frequently used. It has been demonstrated that CP4 EPSPS is also a useful and perhaps superior selectable marker/selection scheme for producing and identifying transformed plants.

A plant transformation vector that may be used in this scheme is pMON17227 (FIG. 16). This plasmid resembles many of the other plasmids described infra and is essentially composed of the previously described bacterial replicon system that enables this plasmid to replicate in *E. coli* and to be introduced into and to replicate in Agrobacterium, the bacterial selectable marker gene (Spc/Str), and located between the T-DNA right border and left border is the CTP2-CP4 synthetic gene in the FMV35S promoter-E9 3' cassette. This plasmid also has single sites for a number of restriction enzymes, located within the borders and outside of the expression cassette. This makes it possible to easily add other genes and genetic elements to the vector for introduction into plants.

The protocol for direct selection of transformed plants on glyphosate is outlined for tobacco. Explants are prepared for pre-culture as in the standard procedure as described in Example 1: surface sterilization of leaves from 1 month old tobacco plants (15 minutes in 10% clorox+surfactant; 3× dH_2O washes); explants are cut in 0.5×0.5 cm squares, removing leaf edges, mid-rib, tip, and petiole end for uniform tissue type; explants are placed in single layer, upside down, on MS104 plates+2 ml 4COO5K media to moisten surface; pre-culture 1–2 days. Explants are inoculated using overnight culture of Agrobacterium containing the plant transformation plasmid that is adjusted to a titer of 1.2×10^8 bacteria/ml with 4COO5K media. Explants are placed into a centrifuge tube, the Agrobacterium suspension is added and the mixture of bacteria and explants is "Vortexed" on maximum setting for 25 seconds to ensure even penetration of bacteria. The bacteria are poured off and the explants are blotted between layers of dry sterile filter paper to remove excess bacteria. The blotted explants are placed upside down on MS104 plates+2 ml 4COO5K media+filter disc. Co-culture is 2–3 days. The explants are transferred to MS104+Carbenicillin 1000 mg/l+cefotaxime 100 mg/l for 3 days (delayed phase). The explants are then transferred to MS104+glyphosate 0.05 mM+Carbenicillin 1000 mg/l+cefotaxime 100 mg/l for selection phase. At 4–6 weeks shoots are cut from callus and placed on MSO+Carbenicillin 500 mg/l rooting media. Roots form in 3–5 days, at which time leaf pieces can be taken from rooted plates to confirm glyphosate tolerance and that the material is transformed.

The presence of the CP4 EPSPS protein in these transformed tissues has been confirmed by immunoblot analysis of leaf discs. The data from one experiment with pMON17227 is presented in the following: 139 shoots formed on glyphosate from 400 explants inoculated with Agrobacterium ABI/pMON17227; 97 of these were positive on recallusing on glyphosate. These data indicate a transformation rate of 24 per 100 explants, which makes this a

highly efficient and time saving transformation procedure for plants. Similar transformation frequencies have been obtained with pMON17131 and direct selection of transformants on glyphosate with the CP4 EPSPS genes has also been shown in other plant species, including, Arabidopsis, soybean, corn, wheat, potato, tomato, cotton, lettuce, and sugarbeet.

The pMON17227 plasmid contains single restriction enzyme recognition cleavage sites (NotI, XhoI, and BstXI) between the CP4 glyphosate selection region and the left border of the vector for the cloning of additional genes and to facilitate the introduction of these genes into plants.

Example 5A

The CP4 EPSPS gene has also been introduced into Black Mexican Sweet (BMS) corn cells with expression of the protein and glyphosate resistance detected in callus.

The backbone for this plasmid was a derivative of the high copy plasmid pUC119 (Viera and Messing, 1987). The 1.3 Kb PspI-DraI pUC119 fragment containing the origin of replication was fused to the 1.3 Kb SmaI-HindIII filled fragment from pKC7 (Rao and Rogers, 1979) which contains the neomycin phosphotransferase type II gene to confer bacterial kanamycin resistance. This plasmid was used to construct a monocot expression cassette vector containing the 0.6 kb cauliflower mosaic virus (CaMV) 35S RNA promoter with a duplication of the −90 to −300 region (Kay et al., 1987), an 0.8 kb fragment containing an intron from a maize gene in the 5' untranslated leader region, followed by a polylinker and the 3' termination sequences from the nopaline synthase (NOS) gene (Fraley et al., 1983). A 1.7 Kb fragment containing the 300 bp chloroplast transit peptide from the Arabidopsis EPSP synthase fused in frame to the 1.4 Kb coding sequence for the bacterial CP4 EPSP synthase was inserted into the monocot expression cassette in the polylinker between the intron and the NOS termination sequence to form the plasmid pMON19653 (FIG. 17).

pMON19653 DNA was introduced into Black Mexican Sweet (BMS) cells by co-bombardment with EC9, a plasmid containing a sulfonylurea-resistant form of the maize acetolactate synthase gene. 2.5 mg of each plasmid was coated onto tungsten particles and introduced into log-phase BMS cells using a PDS-1000 particle gun essentially as described (Klein et al., 1989). Transformants are selected on MS medium containing 20 ppb chlorsulfuron. After initial selection on chlorsulfuron, the calli can be assayed directly by Western blot. Glyphosate tolerance can be assessed by transferring the calli to medium containing 5mM glyphosate. As shown in Table XI, CP4 EPSPS confers glyphosate tolerance to corn callus.

TABLE XI

Expression of CP4 in BMS Corn Callus - pMON 19653

Line	CP4 expression (% extract protein)
284	0.0098
287	0.096
290	0.061
295	0.073
299	0.113
309	0.042
313	0.009

To measure CP4 EPSPS expression in corn callus, the following procedure was used: BMS callus (3 g wet weight) was dried on filter paper (Whatman#1) under vacuum, reweighed, and extraction buffer (500 µl/g dry weight; 100 mM Tris, 1 mM EDTA, 10% glycerol) was added. The tissue was homogenized with a Wheaton overhead stirrer for 30 seconds at 2.8 power setting. After centrifugation (3 minutes, Eppendorf microfuge), the supernatant was removed and the protein was quantitated (BioRad Protein Assay). Samples (50 µg/well) were loaded on an SDS PAGE gel (Jule, 3–17%) along with CP4 EPSPS standard (10 ng), electrophoresed, and transferred to nitrocellulose similarly to a previously described method (Padgette, 1987). The nitrocellulose blot was probed with goat anti-CP4 EPSPS IgG, and developed with I-125 Protein G. The radioactive blot was visualized by autoradiography. Results were quantitated by densitometry on an LKB UltraScan XL laser densitomer and are tabulated below in Table X.

TABLE XII

Glyphosate resistance in BMS Corn Callus using pMON 19653

Vector	Experiment	# chlorsulfuron-resistant lines	# cross-resistant to Glyphosate
19653	253	120	81/120 = 67.5%
19653	254	80	37/80 = 46%
EC9 control	253/254	8	0/8 = 0%

Improvements in the expression of Class II EPSPS could also be achieved by expressing the gene using stronger plant promoters, using better 3' polyadenylation signal sequences, optimizing the sequences around the initiation codon for ribosome loading and translation initiation, or by combination of these or other expression or regulatory sequences or factors.

Example 5B

The plant- expressible genes encoding the CP4 EPSPS and a glyphosate oxidoreductasease enzyme (PCT Pub. No. WO92/00377) were introduced into embryogenic corn callus through particle bombardment. Plasmid DNA was prepared using standard procedures (Ausubel et al., 1987), cesium-chloride purified, and re-suspended at 1 mg/ml in TE buffer. DNA was precipitated onto M10 tungsten or 1.0 µg gold particles (BioRad) using a calcium chloride/spermidine precipitation protocol, essentially as described by Klein et al. (1987). The PDS1000® gunpowder gun (BioRad) was used. Callus tissue was obtained by isolating 1–2 mm long immature embryos from the "HI-II" genotype (Armstrong et al., 1991), or HI-II X B73 crosses, onto a modified N6 medium (Armstrong and Green, 1985; Songstad et al., 1991). Embryogenic callus ("type-II"; Armstrong and Green, 1985) initiated from these embryos was maintained by subculturing at two week intervals, and was bombarded when less than two months old. Each plate of callus tissue was bombarded from 1 to 3 times with either tungsten or gold particles coated with the plasmid DNA(s) of interest. Callus was transferred to a modified N6 medium containing an appropriate selective agent (either glyphosate, or one or more of the antibiotics kanamycin, G418, or paromomycin) 1–8 days following bombardment, and then re-transferred to fresh selection media at 2–3 week intervals. Glyphosate-resistant calli first appeared approximately 6–12 weeks post-bombardment. These resistant calli were propagated on selection medium, and samples were taken for assays gene expression. Plant regeneration from resistant calli was accomplished essentially as described by Petersen et al. (1992).

In some cases, both gene(s) were covalently linked together on the same plasmid DNA molecule. In other instances, the genes were present on separate plasmids, but were introduced into the same plant through a process termed "co-transformation". The 1 mg/ml plasmid preparations of interest were mixed together in an equal ratio, by volume, and then precipitated onto the tungsten or gold particles. At a high frequency, as described in the literature (e.g., Schocher et al., 1986), the different plasmid molecules integrate into the genome of the same plant cell. Generally the integration is into the same chromosomal location in the plant cell, presumably due to recombination of the plasmids prior to integration. Less frequently, the different plasmids integrate into separate chromosomal locations. In either case, there is integration of both DNA molecules into the same plant cell, and any plants produced from that cell.

Transgenic corn plants were produced as described above which contained a plant-expressible CP4 gene and a plant-expressible gene encoding a glyphosate oxidoreductase enzyme.

The plant-expressible CP4 gene comprised a structural DNA sequence encoding a CTP2/CP4 EPSPS fusion protein. The CTP2/CP4 EPSPS is a gene fusion composed of the N-terminal 0.23 Kb chloroplast transit peptide sequence from the *Arabidopsis thaliana* EPSPS gene (Klee et al. 1987, referred to herein as CTP2), and the C-terminal 1.36 Kb 5-enolpyruvylshikimate-3-phosphate synthase gene (CP4) from an Agrobacterium species. Plant expression of the gene fusion produces a pre-protein which is rapidly imported into chloroplasts where the CTP is cleaved and degraded (della-Cioppa et al., 1986) releasing the mature CP4 protein.

The plant-expressible gene expressing a glyphosate oxidoreductase enzyme comprised a structual DNA sequence comprising CTP1/GOXsyn gene fusion composed of the N-terminal 0.26 Kb chloroplast transit peptide sequence derived from the *Arabidopsis thaliana* SSU 1a gene (Timko et al., 1988 referred to herein as CTP1), and the C-terminal 1.3 Kb synthetic gene sequence encoding a glyphosate oxidoreductase enzyme (GOXsyn, as described in PCT Pub. No. WO92/00377 previously incorporated by reference). The GOXsyn gene encodes the enzyme glyphosate oxidoreductase from an Achromobacter sp. strain LBAA which catalyzes the conversion of glyphosate to herbicidally inactive products, aminomethylphosphonate and glyoxylate. Plant expression of the gene fusion produces a pre-protein which is rapidly imported into chloroplasts where the CTP is cleaved and degraded (della-Cioppa et al., 1986) releasing the mature GOX protein.

Both of the above described genes also include the following regulatory sequences for plant expression: (i) a promoter region comprising a 0.6 Kb 35S cauliflower mosaic virus (CaMV) promoter (Odell et al., 1985) with the duplicated enhancer region (Kay et al., 1987) which also contains a 0.8 Kb fragment containing the first intron from the maize heat shock protein 70 gene (Shah et al., 1985 and PCT Pub. No. WO93/19189, the disclosure of which is hereby incorporated by reference); and (ii) a 3' non-translated region comprising a 0.3 Kb fragment of the 3' non-translated region of the nopaline synthase gene (Fraley et al., 1983 and Depicker, et al., 1982) which functions to direct polyadenylation of the mRNA.

The above described transgenic corn plants exhibit tolerance to glyphosate herbicide in greenhouse and field trials.

Example 6

The LBAA Class II EPSPS gene has been introduced into plants and also imparts glyphosate tolerance. Data on tobacco transformed with pMON17206 (infra) are presented in Table XIII.

TABLE XIII

Tobacco Glyphosate Spray Test
(pMON17206: E35S - CTP2-LBAA EPSPS: 0.4 lbs/a)

Line	7 Day Rating
33338	9
34586	9
33328	9
34606	9
33377	9
34611	10
34607	10
34601	9
34589	9
Sansom (Control)	4

From the foregoing, it will be recognized that this invention is one well adapted to attain all the ends and objects hereinabove set forth together with advantages which are obvious and which are inherent to the invention. It will be further understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims. Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matter herein set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1 1 1) NUMBER OF SEQUENCES: 69

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 597 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: DNA (genomic)

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

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AGGAAGAATT CTCAGTCCAA AGCCTCAACA AGGTCAGGGT ACAGAGTCTC CAAACCATTA     180
GCCAAAAGCT ACAGGAGATC AATGAAGAAT CTTCAATCAA AGTAAACTAC TGTTCCAGCA     240
CATGCATCAT GGTCAGTAAG TTTCAGAAAA AGACATCCAC CGAAGACTTA AAGTTAGTGG     300
GCATCTTTGA AAGTAATCTT GTCAACATCG AGCAGCTGGC TTGTGGGGAC CAGACAAAAA     360
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(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(1 1) MOLECULE TYPE: DNA (genomic)

(1 x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 48..1436

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   1               5                  10                  15

TCT GGC CTT TCC GGA ACC GTC CGC ATT CCC GGC GAC AAG TCG ATC TCC        154
Ser Gly Leu Ser Gly Thr Val Arg Ile Pro Gly Asp Lys Ser Ile Ser
            20                  25                  30

CAC CGG TCC TTC ATG TTC GGC GGT CTC GCG AGC GGT GAA ACG CGC ATC        202
His Arg Ser Phe Met Phe Gly Gly Leu Ala Ser Gly Glu Thr Arg Ile
        35                  40                  45

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Thr Gly Leu Leu Glu Gly Glu Asp Val Ile Asn Thr Gly Lys Ala Met
    50                  55                  60

CAG GCC ATG GGC GCC AGG ATC CGT AAG GAA GGC GAC ACC TGG ATC ATC        298
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65                  70                  75

GAT GGC GTC GGC AAT GGC GGC CTC CTG GCG CCT GAG GCG CCG CTC GAT        346
Asp Gly Val Gly Asn Gly Gly Leu Leu Ala Pro Glu Ala Pro Leu Asp
80                  85                  90                  95

TTC GGC AAT GCC GCC ACG GGC TGC CGC CTG ACC ATG GGC CTC GTC GGG        394
Phe Gly Asn Ala Ala Thr Gly Cys Arg Leu Thr Met Gly Leu Val Gly
                100                 105                 110

GTC TAC GAT TTC GAC AGC ACC TTC ATC GGC GAC GCC TCG CTC ACA AAG        442
Val Tyr Asp Phe Asp Ser Thr Phe Ile Gly Asp Ala Ser Leu Thr Lys
            115                 120                 125

CGC CCG ATG GGC CGC GTG TTG AAC CCG CTG CGC GAA ATG GGC GTG CAG        490
Arg Pro Met Gly Arg Val Leu Asn Pro Leu Arg Glu Met Gly Val Gln
        130                 135                 140

GTG AAA TCG GAA GAC GGT GAC CGT CTT CCC GTT ACC TTG CGC GGG CCG        538
Val Lys Ser Glu Asp Gly Asp Arg Leu Pro Val Thr Leu Arg Gly Pro
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AAG ACG CCG ACG CCG ATC ACC TAC CGC GTG CCG ATG GCC TCC GCA CAG        586
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160                 165                 170                 175

GTG AAG TCC GCC GTG CTG CTC GCC GGC CTC AAC ACG CCC GGC ATC ACG        634
Val Lys Ser Ala Val Leu Leu Ala Gly Leu Asn Thr Pro Gly Ile Thr
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ACG GTC ATC GAG CCG ATC ATG ACG CGC GAT CAT ACG GAA AAG ATG CTG        682
Thr Val Ile Glu Pro Ile Met Thr Arg Asp His Thr Glu Lys Met Leu
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Gln Gly Phe Gly Ala Asn Leu Thr Val Glu Thr Asp Ala Asp Gly Val
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Arg Thr Ile Arg Leu Glu Gly Arg Gly Lys Leu Thr Gly Gln Val Ile
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240                 245                 250                 255

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CCC ACC CGC ACC GGC CTC ATC CTG ACG CTG CAG GAA ATG GGC GCC GAC        922
Pro Thr Arg Thr Gly Leu Ile Leu Thr Leu Gln Glu Met Gly Ala Asp
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ATC GAA GTC ATC AAC CCG CGC CTT GCC GGC GGC GAA GAC GTG GCG GAC        970
Ile Glu Val Ile Asn Pro Arg Leu Ala Gly Gly Glu Asp Val Ala Asp
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    305                 310                 315

CGC GCG CCT TCG ATG ATC GAC GAA TAT CCG ATT CTC GCT GTC GCC GCC      1066
Arg Ala Pro Ser Met Ile Asp Glu Tyr Pro Ile Leu Ala Val Ala Ala
320                 325                 330                 335

GCC TTC GCG GAA GGG GCG ACC GTG ATG AAC GGT CTG GAA GAA CTC CGC      1114
Ala Phe Ala Glu Gly Ala Thr Val Met Asn Gly Leu Glu Glu Leu Arg
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Val Lys Glu Ser Asp Arg Leu Ser Ala Val Ala Asn Gly Leu Lys Leu
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AAT GGC GTG GAT TGC GAT GAG GGC GAG ACG TCG CTC GTC GTG CGC GGC      1210
Asn Gly Val Asp Cys Asp Glu Gly Glu Thr Ser Leu Val Val Arg Gly
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CGC CCT GAC GGC AAG GGG CTC GGC AAC GCC TCG GGC GCC GCC GTC GCC      1258
Arg Pro Asp Gly Lys Gly Leu Gly Asn Ala Ser Gly Ala Ala Val Ala
    385                 390                 395

ACC CAT CTC GAT CAC CGC ATC GCC ATG AGC TTC CTC GTC ATG GGC CTC      1306
Thr His Leu Asp His Arg Ile Ala Met Ser Phe Leu Val Met Gly Leu
400                 405                 410                 415

GTG TCG GAA AAC CCT GTC ACG GTG GAC GAT GCC ACG ATG ATC GCC ACG      1354
Val Ser Glu Asn Pro Val Thr Val Asp Asp Ala Thr Met Ile Ala Thr
                420                 425                 430

AGC TTC CCG GAG TTC ATG GAC CTG ATG GCC GGG CTG GGC GCG AAG ATC      1402
Ser Phe Pro Glu Phe Met Asp Leu Met Ala Gly Leu Gly Ala Lys Ile
            435                 440                 445

GAA CTC TCC GAT ACG AAG GCT GCC TGATGACCTT CACAATCGCC ATCGATGGTC     1456
Glu Leu Ser Asp Thr Lys Ala Ala
        450                 455

CCGCTGCGGC CGGCAAGGGG ACGCTCTCGC GCCGTATCGC GGAGGTCTAT GGCTTTCATC    1516

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(2) INFORMATION FOR SEQ ID NO:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 459 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:

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                 85                  90                  95

Gly Asn Ala Ala Thr Gly Cys Arg Leu Thr Met Gly Leu Val Gly Val
            100                 105                 110

Tyr Asp Phe Asp Ser Thr Phe Ile Gly Asp Ala Ser Leu Thr Lys Arg
        115                 120                 125

Pro Met Gly Arg Val Leu Asn Pro Leu Arg Glu Met Gly Val Gln Val
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Lys Ser Glu Asp Gly Asp Arg Leu Pro Val Thr Leu Arg Gly Pro Lys
145                 150                 155                 160

Thr Pro Thr Pro Ile Thr Tyr Arg Val Pro Met Ala Ser Ala Gln Val
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Val Ile Glu Pro Ile Met Thr Arg Asp His Thr Glu Lys Met Leu Gln
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225                 230                 235                 240

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Leu Val Pro Gly Ser Asp Val Thr Ile Leu Asn Val Leu Met Asn Pro
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Lys Glu Ser Asp Arg Leu Ser Ala Val Ala Asn Gly Leu Lys Leu Asn
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Gly Val Asp Cys Asp Glu Gly Glu Thr Ser Leu Val Val Arg Gly Arg
    370                 375                 380

Pro Asp Gly Lys Gly Leu Gly Asn Ala Ser Gly Ala Ala Val Ala Thr
385                 390                 395                 400

His Leu Asp His Arg Ile Ala Met Ser Phe Leu Val Met Gly Leu Val
                405                 410                 415

Ser Glu Asn Pro Val Thr Val Asp Asp Ala Thr Met Ile Ala Thr Ser
            420                 425                 430

Phe Pro Glu Phe Met Asp Leu Met Ala Gly Leu Gly Ala Lys Ile Glu
        435                 440                 445

Leu Ser Asp Thr Lys Ala Ala
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1073 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 86..1432

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

```
GTAGCCACAC ATAATTACTA TAGCTAGGAA GCCCGCTATC TCTCAATCCC GCGTGATCGC      60

GCCAAAATGT GACTGTGAAA AATCC ATG TCC CAT TCT GCA TCC CCG AAA CCA      112
                            Met Ser His Ser Ala Ser Pro Lys Pro
                              1               5

GCA ACC GCC CGC CGC TCG GAG GCA CTC ACG GGC GAA ATC CGC ATT CCG      160
Ala Thr Ala Arg Arg Ser Glu Ala Leu Thr Gly Glu Ile Arg Ile Pro
 10                  15                  20                  25

GGC GAC AAG TCC ATC TCG CAT CGC TCC TTC ATG TTT GGC GGT CTC GCA      208
Gly Asp Lys Ser Ile Ser His Arg Ser Phe Met Phe Gly Gly Leu Ala
                 30                  35                  40

TCG GGC GAA ACC CGC ATC ACC GGC CTT CTG GAA GGC GAG GAC GTC ATC      256
Ser Gly Glu Thr Arg Ile Thr Gly Leu Leu Glu Gly Glu Asp Val Ile
             45                  50                  55

AAT ACA GGC CGC GCC ATG CAG GCC ATG GGC GCG AAA ATC CGT AAA GAG      304
Asn Thr Gly Arg Ala Met Gln Ala Met Gly Ala Lys Ile Arg Lys Glu
         60                  65                  70

GGC GAT GTC TGG ATC ATC AAC GGC GTC GGC AAT GGC TGC CTG TTG CAG      352
Gly Asp Val Trp Ile Ile Asn Gly Val Gly Asn Gly Cys Leu Leu Gln
     75                  80                  85

CCC GAA GCT GCG CTC GAT TTC GGC AAT GCC GGA ACC GGC GCG CGC CTC      400
Pro Glu Ala Ala Leu Asp Phe Gly Asn Ala Gly Thr Gly Ala Arg Leu
 90                  95                 100                 105

ACC ATG GGC CTT GTC GGC ACC TAT GAC ATG AAG ACC TCC TTT ATC GGC      448
Thr Met Gly Leu Val Gly Thr Tyr Asp Met Lys Thr Ser Phe Ile Gly
                110                 115                 120

GAC GCC TCG CTG TCG AAG CGC CCG ATG GGC CGC GTG CTG AAC CCG TTG      496
Asp Ala Ser Leu Ser Lys Arg Pro Met Gly Arg Val Leu Asn Pro Leu
            125                 130                 135

CGC GAA ATG GGC GTT CAG GTG GAA GCA GCC GAT GGC GAC CGC ATG CCG      544
Arg Glu Met Gly Val Gln Val Glu Ala Ala Asp Gly Asp Arg Met Pro
        140                 145                 150

CTG ACG CTG ATC GGC CCG AAG ACG GCC AAT CCG ATC ACC TAT CGC GTG      592
Leu Thr Leu Ile Gly Pro Lys Thr Ala Asn Pro Ile Thr Tyr Arg Val
    155                 160                 165

CCG ATG GCC TCC GCG CAG GTA AAA TCC GCC GTG CTG CTC GCC GGT CTC      640
Pro Met Ala Ser Ala Gln Val Lys Ser Ala Val Leu Leu Ala Gly Leu
170                 175                 180                 185

AAC ACG CCG GGC GTC ACC ACC GTC ATC GAG CCG GTC ATG ACC CGC GAC      688
Asn Thr Pro Gly Val Thr Thr Val Ile Glu Pro Val Met Thr Arg Asp
                190                 195                 200

CAC ACC GAA AAG ATG CTG CAG GGC TTT GGC GCC GAC CTC ACG GTC GAG      736
His Thr Glu Lys Met Leu Gln Gly Phe Gly Ala Asp Leu Thr Val Glu
            205                 210                 215

ACC GAC AAG GAT GGC GTG CGC CAT ATC CGC ATC ACC GGC CAG GGC AAG      784
Thr Asp Lys Asp Gly Val Arg His Ile Arg Ile Thr Gly Gln Gly Lys
        220                 225                 230

CTT GTC GGC CAG ACC ATC GAC GTG CCG GGC GAT CCG TCA TCG ACC GCC      832
Leu Val Gly Gln Thr Ile Asp Val Pro Gly Asp Pro Ser Ser Thr Ala
```

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```
    235                 240                 245

TTC CCG CTC GTT GCC GCC CTT CTG GTG GAA GGT TCC GAC GTC ACC ATC      888
Phe Pro Leu Val Ala Ala Leu Leu Val Glu Gly Ser Asp Val Thr Ile
250                 255                 260                 265

CGC AAC GTG CTG ATG AAC CCG ACC CGT ACC GGC CTC ATC CTC ACC TTG      936
Arg Asn Val Leu Met Asn Pro Thr Arg Thr Gly Leu Ile Leu Thr Leu
                270                 275                 280

CAG GAA ATG GGC GCC GAT ATC GAA GTG CTC AAT GCC CGT CTT GCA GGC      984
Gln Glu Met Gly Ala Asp Ile Glu Val Leu Asn Ala Arg Leu Ala Gly
            285                 290                 295

GGC GAA GAC GTC GCC GAT CTG CGC GTC AGG GCT TCG AAG CTC AAG GGC     1032
Gly Glu Asp Val Ala Asp Leu Arg Val Arg Ala Ser Lys Leu Lys Gly
        300                 305                 310

GTC GTC GTT CCG CCG GAA CGT GCG CCG TCG ATG ATC GAC GAA TAT CCG     1080
Val Val Val Pro Pro Glu Arg Ala Pro Ser Met Ile Asp Glu Tyr Pro
    315                 320                 325

GTC CTG GCG ATT GCC GCC TCC TTC GCG GAA GGC GAA ACC GTG ATG GAC     1128
Val Leu Ala Ile Ala Ala Ser Phe Ala Glu Gly Glu Thr Val Met Asp
330                 335                 340                 345

GGG CTC GAC GAA CTG CGC GTC AAG GAA TCG GAT CGT CTG GCA GCG GTC     1176
Gly Leu Asp Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Val
                350                 355                 360

GCA CGC GGC CTT GAA GCC AAC GGC GTC GAT TGC ACC GAA GGC GAG ATG     1224
Ala Arg Gly Leu Glu Ala Asn Gly Val Asp Cys Thr Glu Gly Glu Met
            365                 370                 375

TCG CTG ACG GTT CGC GGC CGC CCC GAC GGC AAG GGA CTG GGC GGC GGC     1272
Ser Leu Thr Val Arg Gly Arg Pro Asp Gly Lys Gly Leu Gly Gly Gly
        380                 385                 390

ACG GTT GCA ACC CAT CTC GAT CAT CGT ATC GCG ATG AGC TTC CTC GTG     1320
Thr Val Ala Thr His Leu Asp His Arg Ile Ala Met Ser Phe Leu Val
    395                 400                 405

ATG GGC CTT GCG GCG GAA AAG CCG GTG ACG GTT GAC GAC AGT AAC ATG     1368
Met Gly Leu Ala Ala Glu Lys Pro Val Thr Val Asp Asp Ser Asn Met
410                 415                 420                 425

ATC GCC ACG TCC TTC CCC GAA TTC ATG GAC ATG ATG CCG GGA TTG GGC     1416
Ile Ala Thr Ser Phe Pro Glu Phe Met Asp Met Met Pro Gly Leu Gly
                430                 435                 440

GCA AAG ATC GAG TTG AGC ATA CTC TAGTCACTCG ACAGCGAAAA TATTATTTGC     1464
Ala Lys Ile Glu Leu Ser Ile Leu
            445

GAGATTGGGC ATTATTACCG GTTGGTCTCA GCGGGGGTTT AATGTCCAAT CTTCCATACG    1524

TAACAGCATC AGGAAATATC AAAAAAGCTT TAGAAGGAAT TGCTAGAGCA GCGACGCCGC    1584

CTAAGCTTTC TCAAGACTTC GTTAAAACTG TACTGAAATC CCGGGGGGTC CGGGGATCAA    1644

ATGACTTCAT TTCTGAGAAA TTGGCCTCGC A                                   1675
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 448 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ser His Ser Ala Ser Pro Lys Pro Ala Thr Ala Arg Arg Ser Glu
 1               5                  10                  15

Ala Leu Thr Gly Glu Ile Arg Ile Pro Gly Asp Lys Ser Ile Ser His
            20                  25                  30
```

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```
Arg Ser Phe Met Phe Gly Gly Leu Ala Ser Gly Glu Thr Arg Ile Thr
            35                  40                  45

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

Ala Met Gly Ala Lys Ile Arg Lys Glu Gly Asp Val Trp Ile Ile Asn
    65                  70                  75                  80

Gly Val Gly Asn Gly Cys Leu Leu Glu Pro Glu Ala Ala Leu Asp Phe
                85                  90                  95

Gly Asn Ala Gly Thr Gly Ala Arg Leu Thr Met Gly Leu Val Gly Thr
            100                 105                 110

Tyr Asp Met Lys Thr Ser Phe Ile Gly Asp Ala Ser Leu Ser Lys Arg
        115                 120                 125

Pro Met Gly Arg Val Leu Asn Pro Leu Arg Glu Met Gly Val Glu Val
    130                 135                 140

Glu Ala Ala Asp Gly Asp Arg Met Pro Leu Thr Leu Ile Gly Pro Lys
145                 150                 155                 160

Thr Ala Asn Pro Ile Thr Tyr Arg Val Pro Met Ala Ser Ala Glu Val
                165                 170                 175

Lys Ser Ala Val Leu Leu Ala Gly Leu Asn Thr Pro Gly Val Thr Thr
            180                 185                 190

Val Ile Glu Pro Val Met Thr Arg Asp His Thr Glu Lys Met Leu Glu
        195                 200                 205

Gly Phe Gly Ala Asp Leu Thr Val Glu Thr Asp Lys Asp Gly Val Arg
    210                 215                 220

His Ile Arg Ile Thr Gly Glu Gly Lys Leu Val Gly Glu Thr Ile Asp
225                 230                 235                 240

Val Pro Gly Asp Pro Ser Ser Thr Ala Phe Pro Leu Val Ala Ala Leu
                245                 250                 255

Leu Val Glu Gly Ser Asp Val Thr Ile Arg Asn Val Leu Met Asn Pro
            260                 265                 270

Thr Arg Thr Gly Leu Ile Leu Thr Leu Glu Glu Met Gly Ala Asp Ile
        275                 280                 285

Glu Val Leu Asn Ala Arg Leu Ala Gly Gly Glu Asp Val Ala Asp Leu
    290                 295                 300

Arg Val Arg Ala Ser Lys Leu Lys Gly Val Val Val Pro Pro Glu Arg
305                 310                 315                 320

Ala Pro Ser Met Ile Asp Glu Tyr Pro Val Leu Ala Ile Ala Ala Ser
                325                 330                 335

Phe Ala Glu Gly Glu Thr Val Met Asp Gly Leu Asp Glu Leu Arg Val
            340                 345                 350

Lys Glu Ser Asp Arg Leu Ala Ala Val Ala Arg Gly Leu Glu Ala Asn
        355                 360                 365

Gly Val Asp Cys Thr Glu Gly Glu Met Ser Leu Thr Val Arg Gly Arg
    370                 375                 380

Pro Asp Gly Lys Gly Leu Gly Gly Gly Thr Val Ala Thr His Leu Asp
385                 390                 395                 400

His Arg Ile Ala Met Ser Phe Leu Val Met Gly Leu Ala Ala Glu Lys
                405                 410                 415

Pro Val Thr Val Asp Asp Ser Asn Met Ile Ala Thr Ser Phe Pro Glu
            420                 425                 430

Phe Met Asp Met Met Pro Gly Leu Gly Ala Lys Ile Glu Leu Ser Ile
        435                 440                 445

Leu
```

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 54..1389

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

```
GTGATCGCGC CAAAATGTGA CTGTGAAAAA TCC ATG TCC CAT TCT GCA TCC CCG      54
                                      Met Ser His Ser Ala Ser Pro
                                        1               5

AAA CCA GCA ACC GCC CGC CGC TCG GAG GCA CTC ACG GGC GAA ATC CGC     102
Lys Pro Ala Thr Ala Arg Arg Ser Glu Ala Leu Thr Gly Glu Ile Arg
            10                  15                  20

ATT CCG GGC GAC AAG TCC ATC TCG CAT CGC TCC TTC ATG TTT GGC GGT     150
Ile Pro Gly Asp Lys Ser Ile Ser His Arg Ser Phe Met Phe Gly Gly
        25                  30                  35

CTC GCA TCG GGC GAA ACC CGC ATC ACC GGC CTT CTG GAA GGC GAG GAC     198
Leu Ala Ser Gly Glu Thr Arg Ile Thr Gly Leu Leu Glu Gly Glu Asp
    40                  45                  50                  55

GTC ATC AAT ACA GGC CGC GCC ATG CAG GCC ATG GGC GCG AAA ATC CGT     246
Val Ile Asn Thr Gly Arg Ala Met Gln Ala Met Gly Ala Lys Ile Arg
                60                  65                  70

AAA GAG GGC GAT GTC TGG ATC ATC AAC GGC GTC GGC AAT GGC TGC CTG     294
Lys Glu Gly Asp Val Trp Ile Ile Asn Gly Val Gly Asn Gly Cys Leu
            75                  80                  85

TTG CAG CCC GAA GCT GCG CTC GAT TTC GGC AAT GCC GGA ACC GGC GCG     342
Leu Gln Pro Glu Ala Ala Leu Asp Phe Gly Asn Ala Gly Thr Gly Ala
        90                  95                  100

CGC CTC ACC ATG GGC CTT GTC GGC ACC TAT GAC ATG AAG ACC TCC TTT     390
Arg Leu Thr Met Gly Leu Val Gly Thr Tyr Asp Met Lys Thr Ser Phe
    105                 110                 115

ATC GGC GAC GCC TCG CTG TCG AAG CGC CCG ATG GGC CGC GTG CTG AAC     438
Ile Gly Asp Ala Ser Leu Ser Lys Arg Pro Met Gly Arg Val Leu Asn
120                 125                 130                 135

CCG TTG CGC GAA ATG GGC GTT CAG GTG GAA GCA GCC GAT GGC GAC CGC     486
Pro Leu Arg Glu Met Gly Val Gln Val Glu Ala Ala Asp Gly Asp Arg
                140                 145                 150

ATG CCG CTG ACG CTG ATC GGC CCG AAG ACG GCC AAT CCG ATC ACC TAT     534
Met Pro Leu Thr Leu Ile Gly Pro Lys Thr Ala Asn Pro Ile Thr Tyr
            155                 160                 165

CGC GTG CCG ATG GCC TCC GCG CAG GTA AAA TCC GCC GTG CTG CTC GCC     582
Arg Val Pro Met Ala Ser Ala Gln Val Lys Ser Ala Val Leu Leu Ala
        170                 175                 180

GGT CTC AAC ACG CCG GGC GTC ACC ACC GTC ATC GAG CCG GTC ATG ACC     630
Gly Leu Asn Thr Pro Gly Val Thr Thr Val Ile Glu Pro Val Met Thr
    185                 190                 195

CGC GAC CAC ACC GAA AAG ATG CTG CAG GGC TTT GGC GCC GAC CTC ACG     678
Arg Asp His Thr Glu Lys Met Leu Gln Gly Phe Gly Ala Asp Leu Thr
200                 205                 210                 215

GTC GAG ACC GAC AAG GAT GGC GTG CGC CAT ATC CGC ATC ACC GGC CAG     726
Val Glu Thr Asp Lys Asp Gly Val Arg His Ile Arg Ile Thr Gly Gln
                220                 225                 230

GGC AAG CTT GTC GGC CAG ACC ATC GAC GTG CCG GGC GAT CCG TCA TCG     774
Gly Lys Leu Val Gly Gln Thr Ile Asp Val Pro Gly Asp Pro Ser Ser
```

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```
                335                 340                 345

ACC GCC TTC CCG CTC GTT GCC GCC CTT CTG GTG GAA GGT TCC GAC GTC        832
Thr Ala Phe Pro Leu Val Ala Ala Leu Leu Val Glu Gly Ser Asp Val
        350                 355                 360

ACC ATC CGC AAC GTG CTG ATG AAC CCG ACC CGT ACC GGC CTC ATC CTC        870
Thr Ile Arg Asn Val Leu Met Asn Pro Thr Arg Thr Gly Leu Ile Leu
    365                 370                 375

ACC TTG CAG GAA ATG GGC GCC GAT ATC GAA GTG CTC AAT GCC CGT CTT        918
Thr Leu Gln Glu Met Gly Ala Asp Ile Glu Val Leu Asn Ala Arg Leu
380                 385                 390                 395

GCA GGC GGC GAA GAC GTC GCC GAT CTG CGC GTC AGG GCT TCG AAG CTC        966
Ala Gly Gly Glu Asp Val Ala Asp Leu Arg Val Arg Ala Ser Lys Leu
                400                 405                 410

AAG GGC GTC GTC GTT CCG CCG GAA CGT GCG CCG TCG ATG ATC GAC GAA       1014
Lys Gly Val Val Val Pro Pro Glu Arg Ala Pro Ser Met Ile Asp Glu
            415                 420                 425

TAT CCG GTC CTG GCG ATT GCC GCC TCC TTC GCG GAA GGC GAA ACC GTG       1062
Tyr Pro Val Leu Ala Ile Ala Ala Ser Phe Ala Glu Gly Glu Thr Val
        430                 435                 440

ATG GAC GGG CTC GAC GAA CTG CGC GTC AAG GAA TCG GAT CGT CTG GCA       1110
Met Asp Gly Leu Asp Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala
    445                 450                 455

GCG GTC GCA CGC GGC CTT GAA GCC AAC GGC GTC GAT TGC ACC GAA GGC       1158
Ala Val Ala Arg Gly Leu Glu Ala Asn Gly Val Asp Cys Thr Glu Gly
460                 465                 470                 475

GAG ATG TCG CTG ACG GTT CGC GGC CGC CCC GAC GGC AAG GGA CTG GGC       1206
Glu Met Ser Leu Thr Val Arg Gly Arg Pro Asp Gly Lys Gly Leu Gly
                480                 485                 490

GGC GGC ACG GTT GCA ACC CAT CTC GAT CAT CGT ATC GCG ATG AGC TTC       1254
Gly Gly Thr Val Ala Thr His Leu Asp His Arg Ile Ala Met Ser Phe
            495                 500                 505

CTC GTG ATG GGC CTT GCG GCG GAA AAG CCG GTG ACG GTT GAC GAC AGT       1302
Leu Val Met Gly Leu Ala Ala Glu Lys Pro Val Thr Val Asp Asp Ser
        510                 515                 520

AAC ATG ATC GCC ACG TCC TTC CCC GAA TTC ATG GAC ATG ATG CCG GGA       1350
Asn Met Ile Ala Thr Ser Phe Pro Glu Phe Met Asp Met Met Pro Gly
    525                 530                 535

TTG GGC GCA AAG ATC GAG TTG AGC ATA CTC TAGTCACTCG ACAGCGAAAA        1400
Leu Gly Ala Lys Ile Glu Leu Ser Ile Leu
540                 545

TATTATTTGC GAGATTGGGC ATTATTACCG GTTGGTCTCA GCGGGGGTTT AATGTCCAAT    1460

CTTCCATACG TAACAGCATC AGGAAATATC AAAAAAGCTT                          1500
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Ser His Ser Ala Ser Pro Lys Pro Ala Thr Ala Arg Arg Ser Glu
  1               5                  10                  15

Ala Leu Thr Gly Glu Ile Arg Ile Pro Gly Asp Lys Ser Ile Ser His
            20                  25                  30

Arg Ser Phe Met Phe Gly Gly Leu Ala Ser Gly Glu Thr Arg Ile Thr
        35                  40                  45

Gly Leu Leu Glu Gly Glu Asp Val Ile Asn Thr Gly Arg Ala Met Gln
```

```
     50                  55                  60
Ala Met Gly Ala Lys Ile Arg Lys Glu Gly Asp Val Trp Ile Ile Asn
 65                  70                  75                  80
Gly Val Gly Asn Gly Cys Leu Leu Glu Pro Glu Ala Ala Leu Asp Phe
                 85                  90                  95
Gly Asn Ala Gly Thr Gly Ala Arg Leu Thr Met Gly Leu Val Gly Thr
            100                 105                 110
Tyr Asp Met Lys Thr Ser Phe Ile Gly Asp Ala Ser Leu Ser Lys Arg
        115                 120                 125
Pro Met Gly Arg Val Leu Asn Pro Leu Arg Glu Met Gly Val Gln Val
    130                 135                 140
Gln Ala Ala Asp Gly Asp Arg Met Pro Leu Thr Leu Ile Gly Pro Lys
145                 150                 155                 160
Thr Ala Asn Pro Ile Thr Tyr Arg Val Pro Met Ala Ser Ala Gln Val
                165                 170                 175
Lys Ser Ala Val Leu Leu Ala Gly Leu Asn Thr Pro Gly Val Thr Thr
            180                 185                 190
Val Ile Glu Pro Val Met Thr Arg Asp His Thr Glu Lys Met Leu Gln
        195                 200                 205
Gly Phe Gly Ala Asp Leu Thr Val Glu Thr Asp Lys Asp Gly Val Arg
    210                 215                 220
His Ile Arg Ile Thr Gly Gln Gly Lys Leu Val Gly Gln Thr Ile Asp
225                 230                 235                 240
Val Pro Gly Asp Pro Ser Ser Thr Ala Phe Pro Leu Val Ala Ala Leu
                245                 250                 255
Leu Val Glu Gly Ser Asp Val Thr Ile Arg Asn Val Leu Met Asn Pro
            260                 265                 270
Thr Arg Thr Gly Leu Ile Leu Thr Leu Gln Glu Met Gly Ala Asp Ile
        275                 280                 285
Glu Val Leu Asn Ala Arg Leu Ala Gly Gly Glu Asp Val Ala Asp Leu
    290                 295                 300
Arg Val Arg Ala Ser Lys Leu Lys Gly Val Val Val Pro Pro Glu Arg
305                 310                 315                 320
Ala Pro Ser Met Ile Asp Glu Tyr Pro Val Leu Ala Ile Ala Ala Ser
                325                 330                 335
Phe Ala Glu Gly Glu Thr Val Met Asp Gly Leu Asp Glu Leu Arg Val
            340                 345                 350
Lys Glu Ser Asp Arg Leu Ala Ala Val Ala Arg Gly Leu Glu Ala Asn
        355                 360                 365
Gly Val Asp Cys Thr Glu Gly Glu Met Ser Leu Thr Val Arg Gly Arg
    370                 375                 380
Pro Asp Gly Lys Gly Leu Gly Gly Gly Thr Val Ala Thr His Leu Asp
385                 390                 395                 400
His Arg Ile Ala Met Ser Phe Leu Val Met Gly Leu Ala Ala Glu Lys
                405                 410                 415
Pro Val Thr Val Asp Asp Ser Asn Met Ile Ala Thr Ser Phe Pro Glu
            420                 425                 430
Phe Met Asp Met Met Pro Gly Leu Gly Ala Lys Ile Glu Leu Ser Ile
        435                 440                 445
Leu
```

(2) INFORMATION FOR SEQ ID NO:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 453 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Ser Leu Thr Leu Gln Pro Ile Ala Arg Val Asp Gly Thr Ile Asn Leu
 1               5                  10                  15

Pro Gly Ser Lys Thr Val Ser Asn Arg Ala Leu Leu Leu Ala Ala Leu
            20                  25                  30

Ala His Gly Lys Thr Val Leu Thr Asn Leu Leu Asp Ser Asp Asp Val
        35                  40                  45

Arg His Met Leu Asn Ala Leu Thr Ala Leu Gly Val Ser Tyr Thr Leu
    50                  55                  60

Ser Ala Asp Arg Thr Arg Cys Glu Ile Ile Gly Asn Gly Gly Pro Leu
65                  70                  75                  80

His Ala Glu Gly Ala Leu Glu Leu Phe Leu Gly Asn Ala Gly Thr Ala
                85                  90                  95

Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Ser Asn Asp Ile Val
            100                 105                 110

Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His Leu Val
        115                 120                 125

Asp Ala Leu Arg Leu Gly Gly Ala Lys Ile Thr Tyr Leu Glu Glu Glu
    130                 135                 140

Asn Tyr Pro Pro Leu Arg Leu Glu Gly Gly Phe Thr Gly Gly Asn Val
145                 150                 155                 160

Asp Val Asp Gly Ser Val Ser Ser Gln Phe Leu Thr Ala Leu Leu Met
                165                 170                 175

Thr Ala Pro Leu Ala Pro Glu Asp Thr Val Ile Arg Ile Lys Gly Asp
            180                 185                 190

Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu Asn Leu Met Lys Thr
        195                 200                 205

Phe Gly Val Glu Ile Glu Asn Gln His Tyr Gln Gln Phe Val Val Lys
    210                 215                 220

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

Ala Ser Ser Ala Ser Tyr Phe Leu Ala Ala Ala Ala Ile Lys Gly Gly
                245                 250                 255

Thr Val Lys Val Thr Gly Ile Gly Arg Asn Ser Met Gln Gly Asp Ile
            260                 265                 270

Arg Phe Ala Asp Val Leu Glu Lys Met Gly Ala Thr Ile Cys Trp Gly
        275                 280                 285

Asp Asp Tyr Ile Ser Cys Thr Arg Gly Glu Leu Asn Ala Ile Asp Met
    290                 295                 300

Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr Ala Ala
305                 310                 315                 320

Leu Phe Ala Lys Gly Thr Thr Arg Leu Arg Asn Ile Tyr Asn Trp Arg
                325                 330                 335

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

Val Gly Ala Glu Val Glu Glu Gly His Asp Tyr Ile Arg Ile Thr Pro
        355                 360                 365

Pro Glu Lys Leu Asn Phe Ala Glu Ile Ala Thr Tyr Asn Asp His Arg
    370                 375                 380
```

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```
Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro Val Thr
385                 390                 395                 400

Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr Phe Glu
                405                 410                 415

Gln Leu Ala Arg Ile Ser Gln
            420
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

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

```
CCATGGCTCA CGGTGCAAGC AGCCGTCCAG CAACTGCTCG TAAGTCCTCT GGTCTTTCTG      60
GAACCGTCCG TATTCCAGGT GACAAGTCTA TCTCCCACAG GTCCTTCATG TTTGGAGGTC     120
TCGCTAGCGG TGAAACTCGT ATCACCGGTC TTTTGGAAGG TGAAGATGTT ATCAACACTG     180
GTAAGGCTAT GCAAGCTATG GGTGCCAGAA TCCGTAAGGA AGGTGATACT TGGATCATTG     240
ATGGTGTTGG TAACGGTGGA CTCCTTGCTC CTGAGGCTCC TCTCGATTTC GGTAACGCTG     300
CAACTGGTTG CCGTTTGACT ATGGGTCTTG TTGGTGTTTA CGATTTCGAT AGCACTTTCA     360
TTGGTGACGC TTCTCTCACT AAGCGTCCAA TGGGTCGTGT GTTGAACCCA CTTCGCGAAA     420
TGGGTGTGCA GGTGAAGTCT GAAGACGGTG ATCGTCTTCC AGTTACCTTG CGTGGACCAA     480
AGACTCCAAC GCCAATCACC TACAGGGTAC CTATGGCTTC CGCTCAAGTG AAGTCCGCTG     540
TTCTGCTTGC TGGTCTCAAC ACCCCAGGTA TCACCACTGT TATCGAGCCA ATCATGACTC     600
GTGACCACAC TGAAAAGATG CTTCAAGGTT TTGGTGCTAA CCTTACCGTT GAGACTGATG     660
CTGACGGTGT GCGTACCATC CGTCTTGAAG GTCGTGGTAA GCTCACCGGT CAAGTGATTG     720
ATGTTCCAGG TGATCCATCC TCTACTGCTT TCCCATTGGT TGCTGCCTTG CTTGTTCCAG     780
GTTCCGACGT CACCATCCTT AACGTTTTGA TGAACCCAAC CGGTACTGGT CTCATCTTGA     840
CTCTGCAGGA AATGGGTGCC GACATCGAAG TGATCAACCC ACGTCTTGCT GGTGGAGAAG     900
ACGTGGCTGA CTTGCGTGTT CGTTCTTCTA CTTTGAAGGG TGTTACTGTT CCAGAAGACC     960
GTGCTCCTTC TATGATCGAC GAGTATCCAA TTCTCGCTGT TGCAGCTGCA TTCGCTGAAG    1020
GTGCTACCGT TATGAACGGT TTGGAAGAAC TCCGTGTTAA GGAAAGCGAC CGTCTTTCTG    1080
CTGTCGCAAA CGGTCTCAAG CTCAACGGTG TTGATTGCGA TGAAGGTGAG ACTTCTCTCG    1140
TCGTGCGTGG TCGTCCTGAC GGTAAGGGTC TCGGTAACGC TTCTGGAGCA GCTGTCGCTA    1200
CCCACCTCGA TCACCGTATC GCTATGAGCT TCCTCGTTAT GGGTCTCGTT TCTGAAAACC    1260
CTGTTACTGT TGATGATGCT ACTATGATCG CTACTAGCTT CCCAGAGTTC ATGGATTTGA    1320
TGGCTGGTCT TGGAGCTAAG ATCGAACTCT CCGACACTAA GGCTGCTTGA TGAGCTC      1377
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

-continued

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 87..317

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

```
AGATCTATCG ATAAGCTTGA TGTAATTGGA GGAAGATCAA AATTTTCAAT CCCCATTCTT      60

CGATTGCTTC AATTGAAGTT TCTCCG ATG GCG CAA GTT AGC AGA ATC TGC AAT      113
                             Met Ala Gln Val Ser Arg Ile Cys Asn
                               1               5

GGT GTG CAG AAC CCA TCT CTT ATC TCC AAT CTC TCG AAA TCC AGT CAA      161
Gly Val Gln Asn Pro Ser Leu Ile Ser Asn Leu Ser Lys Ser Ser Gln
 10                  15                  20                  25

CGC AAA TCT CCC TTA TCG GTT TCT CTG AAG ACG CAG CAG CAT CCA CGA      209
Arg Lys Ser Pro Leu Ser Val Ser Leu Lys Thr Gln Gln His Pro Arg
                 30                  35                  40

GCT TAT CCG ATT TCG TCG TCG TGG GGA TTG AAG AAG AGT GGG ATG ACG      257
Ala Tyr Pro Ile Ser Ser Ser Trp Gly Leu Lys Lys Ser Gly Met Thr
             45                  50                  55

TTA ATT GGC TCT GAG CTT CGT CCT CTT AAG GTC ATG TCT TCT GTT TCC      305
Leu Ile Gly Ser Glu Leu Arg Pro Leu Lys Val Met Ser Ser Val Ser
         60                  65                  70

ACG GCG TGC ATG C                                                   318
Thr Ala Cys Met
     75
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Met Ala Gln Val Ser Arg Ile Cys Asn Gly Val Gln Asn Pro Ser Leu
 1               5                  10                  15

Ile Ser Asn Leu Ser Lys Ser Ser Gln Arg Lys Ser Pro Leu Ser Val
            20                  25                  30

Ser Leu Lys Thr Gln Gln His Pro Arg Ala Tyr Pro Ile Ser Ser Ser
        35                  40                  45

Trp Gly Leu Lys Lys Ser Gly Met Thr Leu Ile Gly Ser Glu Leu Arg
    50                  55                  60

Pro Leu Lys Val Met Ser Ser Val Ser Thr Ala Cys Met
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 87..401

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

```
AGATCTATCG ATAAGCTTGA TGTAATTGGA GGAAGATCAA AATTTTCAAT CCCCATTCTT      60

CGATTGCTTC AATTGAAGTT TCTCCG ATG GCG CAA GTT AGC AGA ATC TGC AAT      113
```

-continued

```
                                Met Ala Gln Val Ser Arg Ile Cys Asn
                                 1               5

GGT GTG CAG AAC CCA TCT CTT ATC TCC AAT CTC TCG AAA TCC AGT CAA      161
Gly Val Gln Asn Pro Ser Leu Ile Ser Asn Leu Ser Lys Ser Ser Gln
 10                  15                  20                  25

CGC AAA TCT CCC TTA TCG GTT TCT CTG AAG ACG CAG CAG CAT CCA CGA      209
Arg Lys Ser Pro Leu Ser Val Ser Leu Lys Thr Gln Gln His Pro Arg
                 30                  35                  40

GCT TAT CCG ATT TCG TCG TCG TGG GGA TTG AAG AAG AGT GGG ATG ACG      257
Ala Tyr Pro Ile Ser Ser Ser Trp Gly Leu Lys Lys Ser Gly Met Thr
             45                  50                  55

TTA ATT GGC TCT GAG CTT CGT CCT CTT AAG GTC ATG TCT TCT GTT TCC      305
Leu Ile Gly Ser Glu Leu Arg Pro Leu Lys Val Met Ser Ser Val Ser
         60                  65                  70

ACG GCG GAG AAA GCG TCG GAG ATT GTA CTT CAA CCC ATT AGA GAA ATC      353
Thr Ala Glu Lys Ala Ser Glu Ile Val Leu Gln Pro Ile Arg Glu Ile
     75                  80                  85

TCC GGT CTT ATT AAG TTG CCT GGC TCC AAG TCT CTA TCA AAT AGA ATT      401
Ser Gly Leu Ile Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile
 90                  95                 100                 105

C                                                                   402
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Met Ala Gln Val Ser Arg Ile Cys Asn Gly Val Gln Asn Pro Ser Leu
 1               5                  10                  15

Ile Ser Asn Leu Ser Lys Ser Ser Gln Arg Lys Ser Pro Leu Ser Val
            20                  25                  30

Ser Leu Lys Thr Gln Gln His Pro Arg Ala Tyr Pro Ile Ser Ser Ser
        35                  40                  45

Trp Gly Leu Lys Lys Ser Gly Met Thr Leu Ile Gly Ser Glu Leu Arg
    50                  55                  60

Pro Leu Lys Val Met Ser Ser Val Ser Thr Ala Glu Lys Ala Ser Glu
65                  70                  75                  80

Ile Val Leu Gln Pro Ile Arg Glu Ile Ser Gly Leu Ile Lys Leu Pro
                85                  90                  95

Gly Ser Lys Ser Leu Ser Asn Arg Ile
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..232

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

-continued

```
AGATCTTTCA AGA ATG GCA CAA ATT AAC AAC ATG GCT CAA GGG ATA CAA          49
                Met Ala Gln Ile Asn Asn Met Ala Gln Gly Ile Gln
                  1               5                  10

ACC CTT AAT CCC AAT TCC AAT TTC CAT AAA CCC CAA GTT CCT AAA TCT          97
Thr Leu Asn Pro Asn Ser Asn Phe His Lys Pro Gln Val Pro Lys Ser
            15                  20                  25

TCA AGT TTT CTT GTT TTT GGA TCT AAA AAA CTG AAA AAT TCA GCA AAT         145
Ser Ser Phe Leu Val Phe Gly Ser Lys Lys Leu Lys Asn Ser Ala Asn
        30                  35                  40

TCT ATG TTG GTT TTG AAA AAA GAT TCA ATT TTT ATG CAA AAG TTT TGT         193
Ser Met Leu Val Leu Lys Lys Asp Ser Ile Phe Met Gln Lys Phe Cys
    45                  50                  55                  60

TCC TTT AGG ATT TCA GCA TCA GTG GCT ACA GCC TGC ATG C                   233
Ser Phe Arg Ile Ser Ala Ser Val Ala Thr Ala Cys Met
                    65                  70
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Met Ala Gln Ile Asn Asn Met Ala Gln Gly Ile Gln Thr Leu Asn Pro
  1               5                  10                  15

Asn Ser Asn Phe His Lys Pro Gln Val Pro Lys Ser Ser Ser Phe Leu
            20                  25                  30

Val Phe Gly Ser Lys Lys Leu Lys Asn Ser Ala Asn Ser Met Leu Val
        35                  40                  45

Leu Lys Lys Asp Ser Ile Phe Met Gln Lys Phe Cys Ser Phe Arg Ile
    50                  55                  60

Ser Ala Ser Val Ala Thr Ala Cys Met
65                  70
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 352 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 48..351

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
AGATCTGCTA GAAATAATTT TGTTTAACTT TAAGAAGGAG ATATATCC ATG GCA CAA        57
                                                    Met Ala Gln
                                                      1

ATT AAC AAC ATG GCT CAA GGG ATA CAA ACC CTT AAT CCC AAT TCC AAT          105
Ile Asn Asn Met Ala Gln Gly Ile Gln Thr Leu Asn Pro Asn Ser Asn
    5                  10                  15

TTC CAT AAA CCC CAA GTT CCT AAA TCT TCA AGT TTT CTT GTT TTT GGA          153
Phe His Lys Pro Gln Val Pro Lys Ser Ser Ser Phe Leu Val Phe Gly
20                  25                  30                  35

TCT AAA AAA CTG AAA AAT TCA GCA AAT TCT ATG TTG GTT TTG AAA AAA          201
Ser Lys Lys Leu Lys Asn Ser Ala Asn Ser Met Leu Val Leu Lys Lys
```

-continued

```
                    40                  45                  50

GAT TCA ATT TTT ATG CAA AAG TTT TGT TCC TTT AGG ATT TCA GCA TCA      249
Asp Ser Ile Phe Met Gln Lys Phe Cys Ser Phe Arg Ile Ser Ala Ser
                55                  60                  65

GTG GCT ACA GCA CAG AAG CCT TCT GAG ATA GTG TTG CAA CCC ATT AAA      297
Val Ala Thr Ala Gln Lys Pro Ser Glu Ile Val Leu Gln Pro Ile Lys
            70                  75                  80

GAG ATT TCA GGC ACT GTT AAA TTG CCT GGC TCT AAA TCA TTA TCT AAT      345
Glu Ile Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn
        85                  90                  95

AGA ATT C                                                           351
Arg Ile
100
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Ala Gln Ile Asn Asn Met Ala Gln Gly Ile Gln Thr Leu Asn Pro
  1               5                  10                  15

Asn Ser Asn Phe His Lys Pro Gln Val Pro Lys Ser Ser Ser Phe Leu
            20                  25                  30

Val Phe Gly Ser Lys Lys Leu Lys Asn Ser Ala Asn Ser Met Leu Val
        35                  40                  45

Leu Lys Lys Asp Ser Ile Phe Met Gln Lys Phe Cys Ser Phe Arg Ile
    50                  55                  60

Ser Ala Ser Val Ala Thr Ala Gln Lys Pro Ser Glu Ile Val Leu Gln
65                  70                  75                  80

Pro Ile Lys Glu Ile Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser
                85                  90                  95

Leu Ser Asn Arg Ile
            100
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Xaa His Gly Ala Ser Ser Arg Pro Ala Thr Ala Arg Lys Ser Ser Gly
1               5                  10                  15

Leu Xaa Gly Thr Val Arg Ile Pro Gly Asp Lys Met
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala Pro Ser Met Ile Asp Glu Tyr Pro Ile Leu Ala Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ile Thr Gly Leu Leu Glu Gly Glu Asp Val Ile Asn Thr Gly Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGATGGAYG ARTAYCC 17

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GARGAYGTNA THAACAC 17

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GARGAYGTNA THAATAC 17

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid

-continued

(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGTGGATAGA TCTAGGAAGA CAACCATGGC TCACGGTC 38

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGATAGATTA AGGAAGACGC GCATGCTTCA CGGTGCAAGC AGCC 44

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGCTGCCTGA TGAGCTCCAC AATCGCCATC GATGG 35

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGTCGCTCGT CGTGCGTGGC CGCCCTGACG GC 33

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGGGCAAGGC CATGCAGGCT ATGGGCGCC 29

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid

-continued

(A) DESCRIPTION: Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGGGCTGCCG CCTGACTATG GGCCTCGTCG G 31

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Xaa His Ser Ala Ser Pro Lys Pro Ala Thr Ala Arg Arg Ser Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCGGTBGCSG GYTTSGG 17

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Pro Gly Asp Lys Ser Ile Ser His Arg Ser Phe Met Phe Gly Gly Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Leu Asp Phe Gly Asn Ala Ala Thr Gly Cys Arg Leu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

-continued

(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGCAATGCC GCCACCGGCG CGCGCC 26

(2) INFORMATION FOR SEQ ID NO:35:

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

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGACGGCTGC TTGCACCGTG AAGCATGCTT AAGCTTGGCG TAATCATGG 49

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGAAGACGCC CAGAATTCAC GGTGCAAGCA GCCGG 35

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa at position 2 is Gly, Ser, Thr, Cys, Tyr, Asn, Gln, Asp, or Glu"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa at position 4 is Ser or Thr"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Arg Xaa His Xaa Glu
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa at position 4 is Ser or Thr"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Asp Lys Xaa
1

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa at position 4 is Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ser Ala Glu Xaa Lys
1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa at position 2 is Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asn Xaa Thr Arg
1

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1287 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1287

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
ATG AAA CGA GAT AAG GTG CAG ACC TTA CAT GGA GAA ATA CAT ATT CCC      48
Met Lys Arg Asp Lys Val Gln Thr Leu His Gly Glu Ile His Ile Pro
 1               5                  10                  15

GGT GAT AAA TCC ATT TCT CAC CGC TCT GTT ATG TTT GGC GCG CTA GCG      96
Gly Asp Lys Ser Ile Ser His Arg Ser Val Met Phe Gly Ala Leu Ala
            20                  25                  30

GCA GGC ACA ACA ACA GTT AAA AAC TTT CTG CCG GGA GCA GAT TGT CTG     144
Ala Gly Thr Thr Thr Val Lys Asn Phe Leu Pro Gly Ala Asp Cys Leu
        35                  40                  45

AGC ACG ATC GAT TGC TTT AGA AAA ATG GGT GTT CAC ATT GAG CAA AGC     192
```

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```
Ser Thr Ile Asp Cys Phe Arg Lys Met Gly Val His Ile Glu Glu Ser
 50                  55                  60

AGC AGC GAT GTC GTG ATT CAC GGA AAA GGA ATC GAT GCC CTG AAA GAG      240
Ser Ser Asp Val Val Ile His Gly Lys Gly Ile Asp Ala Leu Lys Glu
 65                  70                  75                  80

CCA GAA AGC CTT TTA GAT GTC GGA AAT TCA GGT ACA ACG ATT CGC CTG      288
Pro Glu Ser Leu Leu Asp Val Gly Asn Ser Gly Thr Thr Ile Arg Leu
                 85                  90                  95

ATG CTC GGA ATA TTG GCG GGC CGT CCT TTT TAC AGC GCG GTA GCC GGA      336
Met Leu Gly Ile Leu Ala Gly Arg Pro Phe Tyr Ser Ala Val Ala Gly
            100                 105                 110

GAT GAG AGC ATT GCG AAA CGC CCA ATG AAG CGT GTG ACT GAG CCT TTG      384
Asp Glu Ser Ile Ala Lys Arg Pro Met Lys Arg Val Thr Glu Pro Leu
        115                 120                 125

AAA AAA ATG GGG GCT AAA ATC GAC GGC AGA GCC GGC GGA GAG TTT ACA      432
Lys Lys Met Gly Ala Lys Ile Asp Gly Arg Ala Gly Gly Glu Phe Thr
    130                 135                 140

CCG CTG TCA GTG AGC GGC GCT TCA TTA AAA GGA ATT GAT TAT GTA TCA      480
Pro Leu Ser Val Ser Gly Ala Ser Leu Lys Gly Ile Asp Tyr Val Ser
145                 150                 155                 160

CCT GTT GCA AGC GCG CAA ATT AAA TCT GCT GTT TTG CTG GCC GGA TTA      528
Pro Val Ala Ser Ala Gln Ile Lys Ser Ala Val Leu Leu Ala Gly Leu
                165                 170                 175

CAG GCT GAG GGC ACA ACA ACT GTA ACA GAG CCC CAT AAA TCT CGG GAC      576
Gln Ala Glu Gly Thr Thr Thr Val Thr Glu Pro His Lys Ser Arg Asp
            180                 185                 190

CAC ACT GAG CGG ATG CTT TCT GCT TTT GGC GTT AAG CTT TCT GAA GAT      624
His Thr Glu Arg Met Leu Ser Ala Phe Gly Val Lys Leu Ser Glu Asp
        195                 200                 205

CAA ACG AGT GTT TCC ATT GCT GGT GGC CAG AAA CTG ACA GCT GCT GAT      672
Gln Thr Ser Val Ser Ile Ala Gly Gly Gln Lys Leu Thr Ala Ala Asp
    210                 215                 220

ATT TTT GTT CCT GGA GAC ATT TCT TCA GCC GCG TTT TTC CTT GCT GCT      720
Ile Phe Val Pro Gly Asp Ile Ser Ser Ala Ala Phe Phe Leu Ala Ala
225                 230                 235                 240

GGC GCG ATG GTT CCA AAC AGC AGA ATT GTA TTG AAA AAC GTA GGT TTA      768
Gly Ala Met Val Pro Asn Ser Arg Ile Val Leu Lys Asn Val Gly Leu
                245                 250                 255

AAT CCG ACT CGG ACA GGT ATT ATT GAT GTC CTT CAA AAC ATG GGG GCA      816
Asn Pro Thr Arg Thr Gly Ile Ile Asp Val Leu Gln Asn Met Gly Ala
            260                 265                 270

AAA CTT GAA ATC AAA CCA TCT GCT GAT AGC GGT GCA GAG CCT TAT GGA      864
Lys Leu Glu Ile Lys Pro Ser Ala Asp Ser Gly Ala Glu Pro Tyr Gly
        275                 280                 285

GAT TTG ATT ATA GAA ACG TCA TCT CTA AAG GCA GTT GAA ATC GGA GGA      912
Asp Leu Ile Ile Glu Thr Ser Ser Leu Lys Ala Val Glu Ile Gly Gly
    290                 295                 300

GAT ATC ATT CCG CGT TTA ATT GAT GAG ATC CCT ATC ATC GCG CTT CTT      960
Asp Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro Ile Ile Ala Leu Leu
305                 310                 315                 320

GCG ACT CAG GCG GAA GGA ACC ACC GTT ATT AAG GAC GCG GCA GAG CTA     1008
Ala Thr Gln Ala Glu Gly Thr Thr Val Ile Lys Asp Ala Ala Glu Leu
                325                 330                 335

AAA GTG AAA GAA ACA AAC CGT ATT GAT ACT GTT GTT TCT GAG CTT CGC     1056
Lys Val Lys Glu Thr Asn Arg Ile Asp Thr Val Val Ser Glu Leu Arg
            340                 345                 350

AAG CTG GGT GCT GAA ATT GAA CCG ACA GCA GAT GGA ATG AAG GTT TAT     1104
Lys Leu Gly Ala Glu Ile Glu Pro Thr Ala Asp Gly Met Lys Val Tyr
        355                 360                 365

GGC AAA CAA ACG TTG AAA GGC GGC GCT GCA GTG TCC AGC CAC GGA GAT     1152
```

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```
Gly Lys Gln Thr Leu Lys Gly Gly Ala Ala Val Ser Ser His Gly Asp
    370                 375                 380

CAT CGA ATC GGA ATG ATG CTT GGT ATT GCT TCC TGT ATA ACG GAG GAG      1200
His Arg Ile Gly Met Met Leu Gly Ile Ala Ser Cys Ile Thr Glu Glu
385                 390                 395                 400

CCG ATT GAA ATC GAG CAC ACG GAT GCC ATT CAC GTT TCT TAT CCA ACC      1248
Pro Ile Glu Ile Glu His Thr Asp Ala Ile His Val Ser Tyr Pro Thr
                405                 410                 415

TTC TTC GAG CAT TTA AAT AAG CTT TCG AAA AAA TCC TGA                  1287
Phe Phe Glu His Leu Asn Lys Leu Ser Lys Lys Ser
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Met Lys Arg Asp Lys Val Gln Thr Leu His Gly Glu Ile His Ile Pro
  1               5                  10                  15

Gly Asp Lys Ser Ile Ser His Arg Ser Val Met Phe Gly Ala Leu Ala
            20                  25                  30

Ala Gly Thr Thr Thr Val Lys Asn Phe Leu Pro Gly Ala Asp Cys Leu
        35                  40                  45

Ser Thr Ile Asp Cys Phe Arg Lys Met Gly Val His Ile Glu Gln Ser
    50                  55                  60

Ser Ser Asp Val Val Ile His Gly Lys Gly Ile Asp Ala Leu Lys Glu
65                  70                  75                  80

Pro Glu Ser Leu Leu Asp Val Gly Asn Ser Gly Thr Thr Ile Arg Leu
                85                  90                  95

Met Leu Gly Ile Leu Ala Gly Arg Pro Phe Tyr Ser Ala Val Ala Gly
            100                 105                 110

Asp Glu Ser Ile Ala Lys Arg Pro Met Lys Arg Val Thr Glu Pro Leu
        115                 120                 125

Lys Lys Met Gly Ala Lys Ile Asp Gly Arg Ala Gly Gly Glu Phe Thr
    130                 135                 140

Pro Leu Ser Val Ser Gly Ala Ser Leu Lys Gly Ile Asp Tyr Val Ser
145                 150                 155                 160

Pro Val Ala Ser Ala Gln Ile Lys Ser Ala Val Leu Leu Ala Gly Leu
                165                 170                 175

Gln Ala Glu Gly Thr Thr Thr Val Thr Glu Pro His Lys Ser Arg Asp
            180                 185                 190

His Thr Glu Arg Met Leu Ser Ala Phe Gly Val Lys Leu Ser Glu Asp
        195                 200                 205

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

Ile Phe Val Pro Gly Asp Ile Ser Ser Ala Ala Phe Phe Leu Ala Ala
225                 230                 235                 240

Gly Ala Met Val Pro Asn Ser Arg Ile Val Leu Lys Asn Val Gly Leu
                245                 250                 255

Asn Pro Thr Arg Thr Gly Ile Ile Asp Val Leu Gln Asn Met Gly Ala
            260                 265                 270

Lys Leu Glu Ile Lys Pro Ser Ala Asp Ser Gly Ala Glu Pro Tyr Gly
        275                 280                 285
```

-continued

```
Asp Leu Ile Ile Glu Thr Ser Ser Leu Lys Ala Val Glu Ile Gly Gly
     290                 295                 300

Asp Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro Ile Ile Ala Leu Leu
305                 310                 315                 320

Ala Thr Glu Ala Glu Gly Thr Thr Val Ile Lys Asp Ala Ala Glu Leu
                325                 330                 335

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

Lys Leu Gly Ala Glu Ile Glu Pro Thr Ala Asp Gly Met Lys Val Tyr
        355                 360                 365

Gly Lys Gln Thr Leu Lys Gly Gly Ala Ala Val Ser Ser His Gly Asp
    370                 375                 380

His Arg Ile Gly Met Met Leu Gly Ile Ala Ser Cys Ile Thr Glu Glu
385                 390                 395                 400

Pro Ile Glu Ile Glu His Thr Asp Ala Ile His Val Ser Tyr Pro Thr
                405                 410                 415

Phe Phe Glu His Leu Asn Lys Leu Ser Lys Lys Ser
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1293 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1293

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

```
ATG GTA AAT GAA CAA ATC ATT GAT ATT TCA GGT CCG TTA AAG GGC GAA      48
Met Val Asn Glu Gln Ile Ile Asp Ile Ser Gly Pro Leu Lys Gly Glu
 1               5                  10                  15

ATA GAA GTG CCG GGC GAT AAG TCA ATG ACA CAC CGT GCA ATC ATG TTG      96
Ile Glu Val Pro Gly Asp Lys Ser Met Thr His Arg Ala Ile Met Leu
            20                  25                  30

GCG TCG CTA GCT GAA GGT GTA TCT ACT ATA TAT AAG CCA CTA CTT GGC     144
Ala Ser Leu Ala Glu Gly Val Ser Thr Ile Tyr Lys Pro Leu Leu Gly
        35                  40                  45

GAA GAT TGT CGT CGT ACG ATG GAC ATT TTC CGA CAC TTA GGT GTA GAA     192
Glu Asp Cys Arg Arg Thr Met Asp Ile Phe Arg His Leu Gly Val Glu
    50                  55                  60

ATC AAA GAA GAT GAT GAA AAA TTA GTT GTG ACT TCC CCA GGA TAT CAA     240
Ile Lys Glu Asp Asp Glu Lys Leu Val Val Thr Ser Pro Gly Tyr Gln
65                  70                  75                  80

GTT AAC ACG CCA CAT CAA GTA TTG TAT ACA GGT AAT TCT GGT ACG ACA     288
Val Asn Thr Pro His Gln Val Leu Tyr Thr Gly Asn Ser Gly Thr Thr
                85                  90                  95

ACA CGA TTA TTG GCA GGT TTG TTA AGT GGT TTA GGT AAT GAA AGT GTT     336
Thr Arg Leu Leu Ala Gly Leu Leu Ser Gly Leu Gly Asn Glu Ser Val
            100                 105                 110

TTG TCT GGC GAT GTT TCA ATT GGT AAA AGG CCA ATG GAT CGT GTC TTG     384
Leu Ser Gly Asp Val Ser Ile Gly Lys Arg Pro Met Asp Arg Val Leu
        115                 120                 125

AGA CCA TTG AAA CTT ATG GAT GCG AAT ATT GAA GGT ATT GAA GAT AAT     432
Arg Pro Leu Lys Leu Met Asp Ala Asn Ile Glu Gly Ile Glu Asp Asn
    130                 135                 140
```

-continued

```
TAT ACA CCA TTA ATT ATT AAG CCA TCT GTC ATA AAA GGT ATA AAT TAT      480
Tyr Thr Pro Leu Ile Ile Lys Pro Ser Val Ile Lys Gly Ile Asn Tyr
145                 150                 155                 160

CAA ATG GAA GTT GCA AGT GCA CAA GTA AAA AGT GCC ATT TTA TTT GCA      528
Gln Met Glu Val Ala Ser Ala Gln Val Lys Ser Ala Ile Leu Phe Ala
                165                 170                 175

AGT TTG TTT TCT AAG GAA CCG ACC ATC ATT AAA GAA TTA GAT GTA AGT      576
Ser Leu Phe Ser Lys Glu Pro Thr Ile Ile Lys Glu Leu Asp Val Ser
            180                 185                 190

CGA AAT CAT ACT GAG ACG ATG TTC AAA CAT TTT AAT ATT CCA ATT GAA      624
Arg Asn His Thr Glu Thr Met Phe Lys His Phe Asn Ile Pro Ile Glu
        195                 200                 205

GCA GAA GGG TTA TCA ATT AAT ACA ACC CCT GAA GCA ATT CGA TAC ATT      672
Ala Glu Gly Leu Ser Ile Asn Thr Thr Pro Glu Ala Ile Arg Tyr Ile
    210                 215                 220

AAA CCT GCA GAT TTT CAT GTT CCT GGC GAT ATT TCA TCT GCA GCG TTC      720
Lys Pro Ala Asp Phe His Val Pro Gly Asp Ile Ser Ser Ala Ala Phe
225                 230                 235                 240

TTT ATT GTT GCA GCA CTT ATC ACA CCA GGA AGT GAT GTA ACA ATT CAT      768
Phe Ile Val Ala Ala Leu Ile Thr Pro Gly Ser Asp Val Thr Ile His
                245                 250                 255

AAT GTT GGA ATC AAT CAA ACA CGT TCA GGT ATT ATT GAT ATT GTT GAA      816
Asn Val Gly Ile Asn Gln Thr Arg Ser Gly Ile Ile Asp Ile Val Glu
            260                 265                 270

AAA ATG GGC GGT AAT ATC CAA CTT TTC AAT CAA ACA ACT GGT GCT GAA      864
Lys Met Gly Gly Asn Ile Gln Leu Phe Asn Gln Thr Thr Gly Ala Glu
        275                 280                 285

CCT ACT GCT TCT ATT CGT ATT CAA TAC ACA CCA ATG CTT CAA CCA ATA      912
Pro Thr Ala Ser Ile Arg Ile Gln Tyr Thr Pro Met Leu Gln Pro Ile
    290                 295                 300

ACA ATC GAA GGA GAA TTA GTT CCA AAA GCA ATT GAT GAA CTG CCT GTA      960
Thr Ile Glu Gly Glu Leu Val Pro Lys Ala Ile Asp Glu Leu Pro Val
305                 310                 315                 320

ATA GCA TTA CTT TGT ACA CAA GCA GTT GGC ACG AGT ACA ATT AAA GAT     1008
Ile Ala Leu Leu Cys Thr Gln Ala Val Gly Thr Ser Thr Ile Lys Asp
                325                 330                 335

GCC GAG GAA TTA AAA GTA AAA GAA ACA AAT AGA ATT GAT ACA ACG GCT     1056
Ala Glu Glu Leu Lys Val Lys Glu Thr Asn Arg Ile Asp Thr Thr Ala
            340                 345                 350

GAT ATG TTA AAC TTG TTA GGG TTT GAA TTA CAA CCA ACT AAT GAT GGA     1104
Asp Met Leu Asn Leu Leu Gly Phe Glu Leu Gln Pro Thr Asn Asp Gly
        355                 360                 365

TTG ATT ATT CAT CCG TCA GAA TTT AAA ACA AAT GCA ACA GAT ATT TTA     1152
Leu Ile Ile His Pro Ser Glu Phe Lys Thr Asn Ala Thr Asp Ile Leu
    370                 375                 380

ACT GAT CAT CGA ATA GGA ATG ATG CTT GCA GTT GCT TGT GTA CTT TCA     1200
Thr Asp His Arg Ile Gly Met Met Leu Ala Val Ala Cys Val Leu Ser
385                 390                 395                 400

AGC GAG CCT GTC AAA ATC AAA CAA TTT GAT GCT GTA AAT GTA TCA TTT     1248
Ser Glu Pro Val Lys Ile Lys Gln Phe Asp Ala Val Asn Val Ser Phe
                405                 410                 415

CCA GGA TTT TTA CCA AAA CTA AAG CTT TTA CAA AAT GAG GGA TAA        1293
Pro Gly Phe Leu Pro Lys Leu Lys Leu Leu Gln Asn Glu Gly
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 430 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Met Val Asn Glu Glu Ile Ile Asp Ile Ser Gly Pro Leu Lys Gly Glu
 1               5                  10                  15

Ile Glu Val Pro Gly Asp Lys Ser Met Thr His Arg Ala Ile Met Leu
            20                  25                  30

Ala Ser Leu Ala Glu Gly Val Ser Thr Ile Tyr Lys Pro Leu Leu Gly
        35                  40                  45

Glu Asp Cys Arg Arg Thr Met Asp Ile Phe Arg His Leu Gly Val Glu
    50                  55                  60

Ile Lys Glu Asp Asp Glu Lys Leu Val Val Thr Ser Pro Gly Tyr Glu
65                  70                  75                  80

Val Asn Thr Pro His Glu Val Leu Tyr Thr Gly Asn Ser Gly Thr Thr
                85                  90                  95

Thr Arg Leu Leu Ala Gly Leu Leu Ser Gly Leu Gly Asn Glu Ser Val
            100                 105                 110

Leu Ser Gly Asp Val Ser Ile Gly Lys Arg Pro Met Asp Arg Val Leu
        115                 120                 125

Arg Pro Leu Lys Leu Met Asp Ala Asn Ile Glu Gly Ile Glu Asp Asn
    130                 135                 140

Tyr Thr Pro Leu Ile Ile Lys Pro Ser Val Ile Lys Gly Ile Asn Tyr
145                 150                 155                 160

Glu Met Glu Val Ala Ser Ala Glu Val Lys Ser Ala Ile Leu Phe Ala
                165                 170                 175

Ser Leu Phe Ser Lys Glu Pro Thr Ile Ile Lys Glu Leu Asp Val Ser
            180                 185                 190

Arg Asn His Thr Glu Thr Met Phe Lys His Phe Asn Ile Pro Ile Glu
        195                 200                 205

Ala Glu Gly Leu Ser Ile Asn Thr Thr Pro Glu Ala Ile Arg Tyr Ile
    210                 215                 220

Lys Pro Ala Asp Phe His Val Pro Gly Asp Ile Ser Ser Ala Ala Phe
225                 230                 235                 240

Phe Ile Val Ala Ala Leu Ile Thr Pro Gly Ser Asp Val Thr Ile His
                245                 250                 255

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

Lys Met Gly Gly Asn Ile Glu Leu Phe Asn Glu Thr Thr Gly Ala Glu
        275                 280                 285

Pro Thr Ala Ser Ile Arg Ile Glu Tyr Thr Pro Met Leu Glu Pro Ile
    290                 295                 300

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

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

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

Asp Met Leu Asn Leu Leu Gly Phe Glu Leu Glu Pro Thr Asn Asp Gly
        355                 360                 365

Leu Ile Ile His Pro Ser Glu Phe Lys Thr Asn Ala Thr Asp Ile Leu
    370                 375                 380

Thr Asp His Arg Ile Gly Met Met Leu Ala Val Ala Cys Val Leu Ser
385                 390                 395                 400
```

-continued

```
Ser Glu Pro Val Lys Ile Lys Gly Phe Asp Ala Val Asn Val Ser Phe
                405                 410                 415

Pro Gly Phe Leu Pro Lys Leu Lys Leu Leu Gly Asn Glu Gly
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
GGAACATATG AAACGAGATA AGGTGCAG                                    28
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
GGAATTCAAA CTTCAGGATC TTGAGATAGA AAATG                            35
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
GGGGCCATGG TAAATGAACA AATCATTG                                    28
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Other nucleic acid
(A) DESCRIPTION: Synthetic DNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
GGGGGAGCTC ATTATCCCTC ATTTTGTAAA AGC                              33
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

-continued

(2) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Thr Asp Glu Thr Leu Val Tyr Pro Phe Lys Asp Ile Pro Ala Asp
1               5                  10                  15

Glu Glu Lys Val Val Ile Pro Pro Gly Ser Lys Ser Ile Ser Asn Arg
            20                  25                  30

Ala Leu Ile Leu Met Ala Leu Gly Glu Gly Glu Cys Lys Ile Lys Asn
        35                  40                  45

Leu Leu His Ser Asp Asp Thr Lys His Met Leu Thr Ala Val His Glu
    50                  55                  60

Leu Lys Gly Ala Thr Ile Ser Trp Glu Asp Asn Gly Glu Thr Val Val
65                  70                  75                  80

Val Glu Gly His Gly Gly Ser Thr Leu Ser Ala Cys Ala Asp Pro Leu
                85                  90                  95

Tyr Leu Gly Asn Ala Gly Thr Ala Ser Arg Phe Leu Thr Ser Leu Ala
            100                 105                 110

Ala Leu Val Asn Ser Thr Ser Ser Glu Lys Tyr Ile Val Leu Thr Gly
        115                 120                 125

Asn Ala Arg Met Glu Glu Arg Pro Ile Ala Pro Leu Val Asp Ser Leu
    130                 135                 140

Arg Ala Asn Gly Thr Lys Ile Glu Tyr Leu Asn Asn Glu Gly Ser Leu
145                 150                 155                 160

Pro Ile Lys Val Tyr Thr Asp Ser Val Phe Lys Gly Gly Arg Ile Glu
                165                 170                 175

Leu Ala Ala Thr Val Ser Ser Glu Tyr Val Ser Ser Ile Leu Met Cys
            180                 185                 190

Ala Pro Tyr Ala Glu Glu Pro Val Thr Leu Ala Leu Val Gly Gly Lys
        195                 200                 205

Pro Ile Ser Lys Leu Tyr Val Asp Met Thr Ile Lys Met Met Glu Lys
    210                 215                 220

Phe Gly Ile Asn Val Glu Thr Ser Thr Thr Glu Pro Tyr Thr Tyr Tyr
225                 230                 235                 240

Ile Pro Lys Gly His Tyr Ile Asn Pro Ser Glu Tyr Val Ile Glu Ser
                245                 250                 255

Asp Ala Ser Ser Ala Thr Tyr Pro Leu Ala Phe Ala Ala Met Thr Gly
            260                 265                 270

Thr Thr Val Thr Val Pro Asn Ile Gly Phe Glu Ser Leu Glu Gly Asp
        275                 280                 285

Ala Arg Phe Ala Arg Asp Val Leu Lys Pro Met Gly Cys Lys Ile Thr
    290                 295                 300

Glu Thr Ala Thr Ser Thr Thr Val Ser Gly Pro Pro Val Gly Thr Leu
305                 310                 315                 320

Lys Pro Leu Lys His Val Asp Met Glu Pro Met Thr Asp Ala Phe Leu
                325                 330                 335

Thr Ala Cys Val Val Ala Ala Ile Ser His Asp Ser Asp Pro Asn Ser
            340                 345                 350

Ala Asn Thr Thr Thr Ile Glu Gly Ile Ala Asn Glu Arg Val Lys Glu
        355                 360                 365

Cys Asn Arg Ile Leu Ala Met Ala Thr Glu Leu Ala Lys Phe Gly Val
    370                 375                 380

Lys Thr Thr Glu Leu Pro Asp Gly Ile Glu Val His Gly Leu Asn Ser
385                 390                 395                 400

Ile Lys Asp Leu Lys Val Pro Ser Asp Ser Ser Gly Pro Val Gly Val
                405                 410                 415
```

-continued

```
Cys Thr Tyr Asp Asp His Arg Val Ala Met Ser Phe Ser Leu Leu Ala
            420                 425                 430

Gly Met Val Asn Ser Gln Asn Gln Arg Asp Glu Val Ala Asn Pro Val
        435                 440                 445

Arg Ile Leu Glu Arg His Cys Thr Gly Lys Thr Trp Pro Gly Trp Trp
    450                 455                 460

Asp Val Leu His Ser Glu Leu Gly Ala Lys Leu Asp Gly Ala Glu Pro
465                 470                 475                 480
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 460 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Leu Ala Pro Ser Ile Glu Val His Pro Gly Val Ala His Ser Ser Asn
1               5                  10                  15

Val Ile Cys Ala Pro Pro Gly Ser Lys Ser Ile Ser Asn Arg Ala Leu
            20                  25                  30

Val Leu Ala Ala Leu Gly Ser Gly Thr Cys Arg Ile Lys Asn Leu Leu
        35                  40                  45

His Ser Asp Asp Thr Glu Val Met Leu Asn Ala Leu Glu Arg Leu Gly
    50                  55                  60

Ala Ala Thr Phe Ser Trp Glu Glu Glu Gly Glu Val Leu Val Val Asn
65                  70                  75                  80

Gly Lys Gly Gly Asn Leu Gln Ala Ser Ser Ser Pro Leu Tyr Leu Gly
                85                  90                  95

Asn Ala Gly Thr Ala Ser Arg Phe Leu Thr Thr Val Ala Thr Leu Ala
            100                 105                 110

Asn Ser Ser Thr Val Asp Ser Ser Val Leu Thr Gly Asn Asn Arg Met
        115                 120                 125

Lys Gln Arg Pro Ile Gly Asp Leu Val Asp Ala Leu Thr Ala Asn Val
    130                 135                 140

Leu Pro Leu Asn Thr Ser Lys Gly Arg Ala Ser Leu Pro Leu Lys Ile
145                 150                 155                 160

Ala Ala Ser Gly Gly Phe Ala Gly Gly Asn Ile Asn Leu Ala Ala Lys
                165                 170                 175

Val Ser Ser Gln Tyr Val Ser Ser Leu Leu Met Cys Ala Pro Tyr Ala
            180                 185                 190

Lys Glu Pro Val Thr Leu Arg Leu Val Gly Gly Lys Pro Ile Ser Gln
        195                 200                 205

Pro Tyr Ile Asp Met Thr Thr Ala Met Met Arg Ser Phe Gly Ile Asp
    210                 215                 220

Val Gln Lys Ser Thr Thr Gln Gln His Thr Tyr His Ile Pro Gln Gly
225                 230                 235                 240

Arg Tyr Val Asn Pro Ala Glu Tyr Val Ile Glu Ser Asp Ala Ser Cys
                245                 250                 255

Ala Thr Tyr Pro Leu Ala Val Ala Ala Val Thr Gly Thr Thr Cys Thr
            260                 265                 270

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

Val Glu Val Leu Arg Pro Met Gly Cys Thr Val Gln Gln Thr Glu Thr
    290                 295                 300
```

-continued

```
Ser Thr Thr Val Thr Gly Pro Ser Asp Gly Ile Leu Arg Ala Thr Ser
305                 310                 315                 320

Lys Arg Gly Tyr Gly Thr Asn Asp Arg Cys Val Pro Arg Cys Phe Arg
                325                 330                 335

Thr Gly Ser His Arg Pro Met Gln Lys Ser Gln Thr Thr Pro Pro Val
            340                 345                 350

Ser Ser Gly Ile Ala Asn Gln Arg Val Lys Gln Cys Asn Arg Ile Lys
        355                 360                 365

Ala Met Lys Asp Gln Leu Ala Lys Phe Gly Val Ile Cys Arg Gln His
    370                 375                 380

Asp Asp Gly Leu Gln Ile Asp Gly Ile Asp Arg Ser Asn Leu Arg Gln
385                 390                 395                 400

Pro Val Gly Gly Val Phe Cys Tyr Asp Asp His Arg Val Ala Phe Ser
                405                 410                 415

Phe Ser Val Leu Ser Leu Val Thr Pro Gln Pro Thr Leu Ile Leu Gln
            420                 425                 430

Lys Gln Cys Val Gly Lys Thr Trp Pro Gly Trp Trp Asp Thr Leu Arg
        435                 440                 445

Gln Leu Phe Lys Val Lys Leu Gln Gly Lys Gln Leu
    450                 455                 460
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Lys Ala Ser Gln Ile Val Leu Gln Pro Ile Arg Gln Ile Ser Gly Leu
1               5                  10                  15

Ile Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu
            20                  25                  30

Ala Ala Leu Ser Gln Gly Thr Thr Val Val Asp Asn Leu Leu Asn Ser
        35                  40                  45

Asp Asp Ile Asn Tyr Met Leu Asp Ala Leu Lys Lys Leu Gly Leu Asn
    50                  55                  60

Val Glu Arg Asp Ser Val Asn Asn Arg Ala Val Val Glu Gly Cys Gly
65                  70                  75                  80

Gly Ile Phe Pro Ala Ser Leu Asp Ser Lys Ser Asp Ile Gln Leu Tyr
                85                  90                  95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
            100                 105                 110

Ala Ala Gly Gly Asn Ala Ser Tyr Val Leu Asp Gly Val Pro Arg Met
        115                 120                 125

Arg Gln Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
    130                 135                 140

Ala Asp Val Gln Cys Thr Leu Gly Thr Asn Cys Pro Pro Val Arg Val
145                 150                 155                 160

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

Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ala Ala Pro Leu Ala
            180                 185                 190

Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Val Pro
```

-continued

```
            195                 200                 205

Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val Ser Ala
    210                 215                 220

Glu His Ser Asp Ser Trp Asp Arg Phe Phe Val Lys Gly Gly Glu Lys
225                 230                 235                 240

Tyr Lys Ser Pro Gly Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Glu Thr Val Thr Val
            260                 265                 270

Glu Gly Cys Gly Thr Thr Ser Leu Glu Gly Asp Val Lys Phe Ala Glu
        275                 280                 285

Val Leu Glu Lys Met Gly Cys Lys Val Ser Trp Thr Glu Asn Ser Val
    290                 295                 300

Thr Val Thr Gly Pro Ser Arg Asp Ala Phe Gly Met Arg His Leu Arg
305                 310                 315                 320

Ala Val Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
                325                 330                 335

Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Thr Ile Arg Asp Val
            340                 345                 350

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr
        355                 360                 365

Glu Leu Arg Lys Leu Gly Ala Thr Val Glu Glu Gly Ser Asp Tyr Cys
    370                 375                 380

Val Ile Thr Pro Pro Ala Lys Val Lys Pro Ala Glu Ile Asp Thr Tyr
385                 390                 395                 400

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp
                405                 410                 415

Val Pro Val Thr Ile Lys Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro
            420                 425                 430

Asp Tyr Phe Glu Val Leu Glu Ser Ile Thr Lys His
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Lys Ala Ser Glu Ile Val Leu Glu Pro Ile Arg Glu Ile Ser Gly Leu
1               5                  10                  15

Ile Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu
            20                  25                  30

Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn Ser
        35                  40                  45

Asp Asp Ile Asn Tyr Met Leu Asp Ala Leu Lys Arg Leu Gly Leu Asn
    50                  55                  60

Val Glu Thr Asp Ser Glu Asn Asn Arg Ala Val Val Glu Gly Cys Gly
65                  70                  75                  80

Gly Ile Phe Pro Ala Ser Ile Asp Ser Lys Ser Asp Ile Glu Leu Tyr
                85                  90                  95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
            100                 105                 110
```

-continued

```
Ala Ala Gly Gly Asn Ala Ser Tyr Val Leu Asp Gly Val Pro Arg Met
        115                 120                 125

Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
    130                 135                 140

Ala Asp Val Glu Cys Thr Leu Gly Thr Asn Cys Pro Pro Val Arg Val
145                 150                 155                 160

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

Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ser Ala Pro Leu Ala
            180                 185                 190

Leu Gly Asp Val Glu Ile Glu Ile Val Asp Lys Leu Ile Ser Val Pro
        195                 200                 205

Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val Ser Val
    210                 215                 220

Glu His Ser Asp Ser Trp Asp Arg Phe Phe Val Lys Gly Gly Gln Lys
225                 230                 235                 240

Tyr Lys Ser Pro Gly Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Cys Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Glu Thr Val Thr Val
            260                 265                 270

Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
        275                 280                 285

Val Leu Glu Lys Met Gly Cys Lys Val Ser Trp Thr Glu Asn Ser Val
    290                 295                 300

Thr Val Thr Gly Pro Pro Arg Asp Ala Phe Gly Met Arg His Leu Arg
305                 310                 315                 320

Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
                325                 330                 335

Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Thr Ile Arg Asp Val
            340                 345                 350

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr
        355                 360                 365

Glu Leu Arg Lys Leu Gly Ala Thr Val Glu Glu Gly Ser Asp Tyr Cys
    370                 375                 380

Val Ile Thr Pro Pro Lys Lys Val Lys Thr Ala Glu Ile Asp Thr Tyr
385                 390                 395                 400

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp
                405                 410                 415

Val Pro Ile Thr Ile Asn Asp Ser Gly Cys Thr Arg Lys Thr Phe Pro
            420                 425                 430

Asp Tyr Phe Glu Val Leu Glu Arg Ile Thr Lys His
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Lys Pro Asn Glu Ile Val Leu Glu Pro Ile Lys Asp Ile Ser Gly Thr
1               5                   10                  15

Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu
            20                  25                  30
```

-continued

```
Ala Ala Leu Ser Lys Gly Arg Thr Val Val Asp Asn Leu Leu Ser Ser
            35                  40                  45

Asp Asp Ile His Tyr Met Leu Gly Ala Leu Lys Thr Leu Gly Leu His
        50                  55                  60

Val Glu Asp Asp Asn Glu Asn Glu Arg Ala Ile Val Glu Gly Cys Gly
65                  70                  75                  80

Gly Glu Phe Pro Val Gly Lys Lys Ser Glu Glu Glu Ile Glu Leu Phe
                85                  90                  95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
            100                 105                 110

Val Ala Gly Gly His Ser Arg Tyr Val Leu Asp Gly Val Pro Arg Met
        115                 120                 125

Arg Glu Arg Pro Ile Gly Asp Leu Val Asp Gly Leu Lys Gln Leu Gly
    130                 135                 140

Ala Glu Val Asp Cys Phe Leu Gly Thr Asn Cys Pro Pro Val Arg Ile
145                 150                 155                 160

Val Ser Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
                165                 170                 175

Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ala Ala Pro Leu Ala
            180                 185                 190

Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Val Pro
        195                 200                 205

Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val Ser Val
    210                 215                 220

Glu His Thr Ser Ser Trp Asp Lys Phe Leu Val Arg Gly Gly Gln Lys
225                 230                 235                 240

Tyr Lys Ser Pro Gly Lys Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Ser Tyr Phe Leu Ala Gly Ala Ala Val Thr Gly Gly Thr Val Thr Val
            260                 265                 270

Gln Gly Cys Gly Thr Ser Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
        275                 280                 285

Val Leu Glu Lys Met Gly Ala Glu Val Thr Trp Thr Glu Asn Ser Val
    290                 295                 300

Thr Val Lys Gly Pro Pro Arg Asn Ser Ser Gly Met Lys His Leu Arg
305                 310                 315                 320

Ala Val Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
                325                 330                 335

Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
            340                 345                 350

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr
        355                 360                 365

Glu Leu Arg Lys Leu Gly Ala Thr Val Val Glu Gly Ser Asp Tyr Cys
    370                 375                 380

Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Glu Ile Asp Thr Tyr
385                 390                 395                 400

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp
                405                 410                 415

Val Pro Val Thr Ile Lys Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro
            420                 425                 430

Asn Tyr Phe Asp Val Leu Glu Gln Tyr Ser Lys His
        435                 440
```

-continued

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Lys Pro His Glu Ile Val Leu Xaa Pro Ile Lys Asp Ile Ser Gly Thr
1               5                  10                  15

Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu
            20                  25                  30

Ala Ala Leu Ser Glu Gly Arg Thr Val Val Asp Asn Leu Leu Ser Ser
        35                  40                  45

Asp Asp Ile His Tyr Met Leu Gly Ala Leu Lys Thr Leu Gly Leu His
    50                  55                  60

Val Glu Asp Asp Asn Glu Asn Gln Arg Ala Ile Val Glu Gly Cys Gly
65                  70                  75                  80

Gly Gln Phe Pro Val Gly Lys Lys Ser Glu Glu Glu Ile Gln Leu Phe
                85                  90                  95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
            100                 105                 110

Val Ala Gly Gly His Ser Arg Tyr Val Leu Asp Gly Val Pro Arg Met
        115                 120                 125

Arg Glu Arg Pro Ile Gly Asp Leu Val Asp Gly Leu Lys Gln Leu Gly
    130                 135                 140

Ala Glu Val Asp Cys Ser Leu Gly Thr Asn Cys Pro Pro Val Arg Ile
145                 150                 155                 160

Val Ser Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
                165                 170                 175

Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ala Ala Pro Leu Ala
            180                 185                 190

Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Val Pro
        195                 200                 205

Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val Phe Val
    210                 215                 220

Glu His Ser Ser Gly Trp Asp Arg Phe Leu Val Lys Gly Gly Gln Lys
225                 230                 235                 240

Tyr Lys Ser Pro Gly Lys Ala Phe Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Ser Tyr Phe Leu Ala Gly Ala Ala Val Thr Gly Gly Thr Val Thr Val
            260                 265                 270

Glu Gly Cys Gly Thr Ser Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
        275                 280                 285

Val Leu Glu Lys Met Gly Ala Glu Val Thr Trp Thr Glu Asn Ser Val
    290                 295                 300

Thr Val Lys Gly Pro Pro Arg Asn Ser Ser Gly Met Lys His Leu Arg
305                 310                 315                 320

Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
                325                 330                 335

Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Thr Ile Arg Asp Val
            340                 345                 350

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr
        355                 360                 365
```

-continued

```
Glu Leu Arg Lys Leu Gly Ala Thr Val Val Gln Gly Ser Asp Tyr Cys
    370                 375                 380

Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Glu Ile Asp Thr Tyr
385                 390                 395                 400

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp
                405                 410                 415

Val Pro Val Thr Ile Lys Asn Pro Gly Cys Thr Arg Lys Thr Phe Pro
            420                 425                 430

Asp Tyr Phe Glu Val Leu Glu Lys Tyr Ser Lys His
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Lys Pro Ser Glu Ile Val Leu Glu Pro Ile Lys Glu Ile Ser Gly Thr
1               5                  10                  15

Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu
            20                  25                  30

Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Ser Ser
        35                  40                  45

Asp Asp Ile His Tyr Met Leu Gly Ala Leu Lys Thr Leu Gly Leu His
    50                  55                  60

Val Glu Glu Asp Ser Ala Asn Glu Arg Ala Val Val Glu Gly Cys Gly
65                  70                  75                  80

Gly Leu Phe Pro Val Gly Lys Glu Ser Lys Glu Glu Ile Glu Leu Phe
                85                  90                  95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
            100                 105                 110

Val Ala Gly Gly Asn Ser Arg Tyr Val Leu Asp Gly Val Pro Arg Met
        115                 120                 125

Arg Glu Arg Pro Ile Ser Asp Leu Val Asp Gly Leu Lys Gln Leu Gly
    130                 135                 140

Ala Glu Val Asp Cys Phe Leu Gly Thr Lys Cys Pro Pro Val Arg Ile
145                 150                 155                 160

Val Ser Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
                165                 170                 175

Ile Ser Ser Gln Tyr Leu Thr Ala Leu Leu Met Ala Ala Pro Leu Ala
            180                 185                 190

Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Val Pro
        195                 200                 205

Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Ile Ser Val
    210                 215                 220

Glu His Ser Ser Ser Ser Trp Asp Arg Phe Phe Val Arg Gly Gly Gln Lys
225                 230                 235                 240

Tyr Lys Ser Pro Gly Lys Ala Phe Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Ser Tyr Phe Leu Ala Gly Ala Ala Val Thr Gly Gly Thr Ile Thr Val
            260                 265                 270

Glu Gly Cys Gly Thr Asn Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
        275                 280                 285
```

Val Leu Glu Lys Met Gly Ala Glu Val Thr Trp Thr Glu Asn Ser Val
390 395 400

Thr Val Lys Gly Pro Pro Arg Ser Ser Ser Gly Arg Lys His Leu Arg
405 410 415 420

Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
425 430 435

Ala Val Val Ala Leu Tyr Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
440 445 450

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr
455 460 465

Glu Leu Arg Lys Leu Gly Ala Thr Val Glu Glu Gly Pro Asp Tyr Cys
470 475 480

Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Asp Ile Asp Thr Tyr
485 490 495 500

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp
505 510 515

Val Pro Val Thr Ile Asn Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro
520 525 530

Asn Tyr Phe Asp Val Leu Glu Ser Tyr Ser Lys His
535 540 545

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile Ser Gly
1 5 10 15

Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu
20 25 30

Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn
35 40 45

Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu Gly Leu
50 55 60

Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Ala Val Val Val Gly Cys
65 70 75 80

Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln Leu Phe
85 90 95

Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
100 105 110

Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro Arg Met
115 120 125

Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
130 135 140

Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val Arg Val
145 150 155 160

Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
165 170 175

Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro Leu Pro
180 185 190

Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Ile Pro

-continued

```
            195                 200                 205

Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val Lys Ala
    210                 215                 220

Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly Glu Lys
225                 230                 235                 240

Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
                245                 250                 255

Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val Thr Val
            260                 265                 270

Glu Gly Cys Gly Thr Thr Ser Leu Glu Gly Asp Val Lys Phe Ala Glu
        275                 280                 285

Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Glu Thr Ser Val
    290                 295                 300

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

Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
                325                 330                 335

Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
            340                 345                 350

Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Val Ala Ile Arg Thr
        355                 360                 365

Glu Leu Thr Lys Leu Gly Ala Ser Val Glu Glu Gly Pro Asp Tyr Cys
    370                 375                 380

Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Ala Ile Asp Thr Tyr
385                 390                 395                 400

Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Glu
                405                 410                 415

Val Pro Val Thr Ile Arg Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro
            420                 425                 430

Asp Tyr Phe Asp Val Leu Ser Thr Phe Val Lys Asn
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

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

Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Cys Gly Lys Thr Val Leu Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45

Asp Val Arg His Met Leu Asn Ala Leu Ser Ala Leu Gly Ile Asn Tyr
    50                  55                  60

Thr Leu Ser Ala Asp Arg Thr Arg Cys Asp Ile Thr Gly Asn Gly Gly
65                  70                  75                  80

Pro Leu Arg Ala Pro Gly Ala Leu Glu Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Gln Asn Glu
            100                 105                 110
```

-continued

```
Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His
        115                 120                 125

Leu Val Asp Ser Leu Arg Glu Gly Gly Ala Asn Ile Asp Tyr Leu Glu
    130                 135                 140

Glu Glu Asn Tyr Pro Pro Leu Arg Leu Arg Gly Gly Phe Ile Gly Gly
145                 150                 155                 160

Asp Ile Glu Val Asp Gly Ser Val Ser Ser Glu Phe Leu Thr Ala Leu
                165                 170                 175

Leu Met Thr Ala Pro Leu Ala Pro Lys Asp Thr Ile Ile Arg Val Lys
            180                 185                 190

Gly Glu Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu Asn Leu Met
        195                 200                 205

Lys Thr Phe Gly Val Glu Ile Ala Asn His His Tyr Glu Glu Phe Val
    210                 215                 220

Val Lys Gly Gly Gln Glu Tyr His Ser Pro Gly Arg Tyr Leu Val Glu
225                 230                 235                 240

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

Gly Gly Thr Val Lys Val Thr Gly Ile Gly Arg Lys Ser Met Gln Gly
            260                 265                 270

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

Trp Gly Asp Asp Phe Ile Ala Cys Thr Arg Gly Glu Leu His Ala Ile
    290                 295                 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305                 310                 315                 320

Thr Ala Leu Phe Ala Lys Gly Thr Thr Thr Leu Arg Asn Ile Tyr Asn
                325                 330                 335

Trp Arg Val Lys Glu Thr Asp Arg Leu Phe Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly His Asp Tyr Ile Arg Ile
        355                 360                 365

Thr Pro Pro Ala Lys Leu Gln His Ala Asp Ile Gly Thr Tyr Asn Asp
    370                 375                 380

His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
                405                 410                 415

Phe Glu Glu Leu Ala Arg Met Ser Thr Pro Ala
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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

Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Cys Gly Lys Thr Val Leu Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45
```

-continued

```
Asp Val Arg His Met Leu Asn Ala Leu Ser Ala Leu Gly Ile Asn Tyr
 50                  55                  60

Thr Leu Ser Ala Asp Arg Thr Arg Cys Asp Ile Thr Gly Asn Gly Gly
65                  70                  75                  80

Pro Leu Arg Ala Ser Gly Thr Leu Glu Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Gln Asn Glu
            100                 105                 110

Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His
        115                 120                 125

Leu Val Asp Ser Leu Arg Gln Gly Gly Ala Asn Ile Asp Tyr Leu Glu
    130                 135                 140

Gln Glu Asn Tyr Pro Pro Leu Arg Leu Arg Gly Gly Phe Ile Gly Gly
145                 150                 155                 160

Asp Ile Glu Val Asp Gly Ser Val Ser Ser Gln Phe Leu Thr Ala Leu
                165                 170                 175

Leu Met Thr Ala Pro Leu Ala Pro Glu Asp Thr Ile Ile Arg Val Lys
            180                 185                 190

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

Lys Thr Phe Gly Val Glu Ile Ala Asn His His Tyr Gln Gln Phe Val
    210                 215                 220

Val Lys Gly Gly Gln Gln Tyr His Ser Pro Gly Arg Tyr Leu Val Glu
225                 230                 235                 240

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

Gly Gly Thr Val Lys Val Thr Gly Ile Gly Gly Lys Ser Met Gln Gly
            260                 265                 270

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

Trp Gly Asp Asp Phe Ile Ala Cys Thr Arg Gly Glu Leu His Ala Ile
    290                 295                 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305                 310                 315                 320

Thr Ala Leu Phe Ala Lys Gly Thr Thr Thr Leu Arg Asn Ile Tyr Asn
                325                 330                 335

Trp Arg Val Lys Glu Thr Asp Arg Leu Phe Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly His Asp Tyr Ile Arg Ile
        355                 360                 365

Thr Pro Pro Ala Lys Leu Gln His Ala Asp Ile Gly Thr Tyr Asn Asp
    370                 375                 380

His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
                405                 410                 415

Phe Glu Gln Leu Ala Arg Met Ser Thr Pro Ala
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:69:

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

Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Arg Gly Thr Thr Val Leu Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45

Asp Val Arg His Met Leu Asn Ala Leu Ser Ala Leu Gly Val His Tyr
    50                  55                  60

Val Leu Ser Ser Asp Arg Thr Arg Cys Glu Val Thr Gly Thr Gly Gly
65                  70                  75                  80

Pro Leu Glu Ala Gly Ser Ala Leu Glu Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Ser Asn Asp
            100                 105                 110

Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His
        115                 120                 125

Leu Val Asp Ala Leu Arg Gln Gly Gly Ala Glu Ile Asp Tyr Leu Glu
    130                 135                 140

Glu Glu Asn Tyr Pro Pro Leu Arg Leu Arg Gly Gly Phe Thr Gly Gly
145                 150                 155                 160

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

Leu Met Ala Ser Pro Leu Ala Pro Glu Asp Thr Val Ile Ala Ile Lys
            180                 185                 190

Gly Glu Leu Val Ser Arg Pro Tyr Ile Asp Ile Thr Leu His Leu Met
        195                 200                 205

Lys Thr Phe Gly Val Glu Val Glu Asn Glu Asn Tyr Glu Arg Phe Ile
    210                 215                 220

Val Arg Gly Asn Gln Glu Tyr Glu Ser Pro Gly Asp Tyr Leu Val Glu
225                 230                 235                 240

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

Gly Gly Thr Val Lys Val Thr Gly Ile Gly Arg Asn Ser Val Gln Gly
            260                 265                 270

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

Trp Gly Glu Asp Tyr Ile Ala Cys Thr Arg Gly Glu Leu Asn Ala Ile
    290                 295                 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305                 310                 315                 320

Ala Ala Leu Phe Ala Arg Gly Thr Thr Thr Leu Arg Asn Ile Tyr Asn
                325                 330                 335

Trp Arg Val Lys Glu Thr Asp Arg Leu Phe Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly Glu Asp Tyr Ile Arg Ile
        355                 360                 365

Thr Pro Pro Leu Thr Leu Gln Phe Ala Glu Ile Gly Thr Tyr Asn Asp
    370                 375                 380

His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400
```

-continued

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
405 410 415

Phe Gly Gln Leu Ala Arg Ile Ser Thr Leu Ala
420 425

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:48:

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

Val Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu
20 25 30

Ala Ala Leu Ala Gln Gly Thr Thr Gln Leu Asn Asn Leu Leu Asp Ser
35 40 45

Asp Asp Ile Arg His Met Leu Asn Ala Leu Gln Ala Leu Gly Val Lys
50 55 60

Tyr Arg Leu Ser Ala Asp Arg Thr Arg Cys Glu Val Asp Gly Leu Gly
65 70 75 80

Gly Lys Leu Val Ala Glu Gln Pro Leu Glu Leu Phe Leu Gly Asn Ala
85 90 95

Gly Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Lys Asn
100 105 110

Asp Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly
115 120 125

His Leu Val Asp Ala Leu Arg Gln Gly Gly Ala Glu Ile Asp Tyr Leu
130 135 140

Glu Glu Glu Asn Tyr Arg Arg Cys Ile Ala Gly Gly Phe Arg Gly Gly
145 150 155 160

Lys Leu Thr Val Asp Gly Ser Val Ser Ser Gln Phe Leu Thr Ala Leu
165 170 175

Leu Met Thr Ala Pro Leu Ala Glu Glu Asp Thr Glu Ile Glu Ile Glu
180 185 190

Gly Glu Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu His Leu Met
195 200 205

Lys Ala Phe Gly Val Asp Val Val His Glu Asn Tyr Glu Ile Phe His
210 215 220

Ile Lys Gly Gly Gln Thr Tyr Arg Ser Pro Gly Ile Tyr Leu Val Glu
225 230 235 240

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

Gly Gly Thr Val Arg Val Thr Gly Ile Gly Lys Glu Ser Val Glu Gly
260 265 270

Asp Thr Lys Phe Ala Asp Val Leu Glu Lys Met Gly Ala Lys Ile Ser
275 280 285

Trp Gly Asp Asp Tyr Ile Glu Cys Ser Arg Gly Glu Leu Glu Gly Ile
290 295 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305 310 315 320

Thr Ala Leu Phe Ala Asp Gly Pro Thr Val Ile Arg Asn Ile Tyr Asn
325 330 335

```
Trp Arg Val Lys Glu Thr Asp Arg Leu Ser Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly Glu Asp Tyr Ile Arg Val
        355                 360                 365

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

His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
                405                 410                 415

Phe Glu Glu Leu Ala Arg Leu Ser Glu Ile Ala
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:61:

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

Asn Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Lys Gly Thr Thr Lys Val Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45

Asp Ile Arg His Met Leu Asn Ala Leu Lys Ala Leu Gly Val Arg Tyr
    50                  55                  60

Glu Leu Ser Asp Asp Lys Thr Ile Cys Glu Ile Glu Gly Leu Gly Gly
65                  70                  75                  80

Ala Phe Asn Ile Glu Asp Asn Leu Ser Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Thr Ala Ala Leu Cys Leu Lys Gly Asn His
            100                 105                 110

Glu Val Glu Ile Ile Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro
        115                 120                 125

Ile Leu His Leu Val Asp Ala Leu Arg Glu Ala Gly Ala Asp Ile Arg
    130                 135                 140

Tyr Leu Glu Asn Glu Gly Tyr Pro Pro Leu Ala Ile Arg Asn Lys Gly
145                 150                 155                 160

Ile Lys Gly Gly Lys Val Lys Ile Asp Gly Ser Ile Ser Ser Gln Phe
                165                 170                 175

Leu Thr Ala Leu Leu Met Ser Ala Pro Leu Ala Glu Asn Asp Thr Glu
            180                 185                 190

Ile Glu Ile Ile Gly Glu Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr
        195                 200                 205

Leu Ala Met Met Arg Asp Phe Gly Val Lys Val Glu Asn His His Tyr
    210                 215                 220

Gln Lys Phe Glu Val Lys Gly Asn Gln Ser Tyr Ile Ser Pro Asn Lys
225                 230                 235                 240

Tyr Leu Val Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Ala
                245                 250                 255

Gly Ala Ile Lys Gly Lys Val Lys Val Thr Gly Ile Gly Lys Asn Ser
```

-continued

```
                360                 365                 370

Ile Glu Gly Asp Arg Leu Phe Ala Asp Val Leu Glu Lys Met Gly Ala
            375                 380                 385

Lys Ile Thr Trp Gly Glu Asp Phe Ile Glu Ala Glu His Ala Glu Leu
        390                 395                 400

Asn Gly Ile Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr
405                 410                 415

Ile Ala Thr Thr Ala Leu Phe Ser Asn Gly Glu Thr Val Ile Arg Asn
            420                 425                 430

Ile Tyr Asn Trp Arg Val Lys Glu Thr Asp Arg Leu Thr Ala Met Ala
        435                 440                 445

Thr Glu Leu Arg Lys Val Gly Ala Glu Val Glu Glu Gly Glu Asp Phe
    450                 455                 460

Ile Arg Ile Glu Pro Leu Ala Leu Asn Glu Phe Lys His Ala Asn Ile
465                 470                 475                 480

Glu Thr Tyr Asn Asp His Arg Met Ala Met Cys Phe Ser Leu Ile Ala
                485                 490                 495

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

Thr Phe Pro Thr Phe Phe Asn Glu Phe Glu Lys Ile Cys Leu Lys Asn
        515                 520                 525
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Val Ile Lys Asp Ala Thr Ala Ile Thr Leu Asn Pro Ile Ser Tyr Ile
1               5                  10                  15

Glu Gly Glu Val Arg Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ala
            20                  25                  30

Leu Leu Leu Ser Ala Leu Ala Lys Gly Lys Thr Thr Leu Thr Asn Leu
        35                  40                  45

Leu Asp Ser Asp Asp Val Arg His Met Leu Asn Ala Leu Lys Glu Leu
    50                  55                  60

Gly Val Thr Tyr Glu Leu Ser Glu Asp Lys Ser Val Cys Glu Ile Glu
65                  70                  75                  80

Gly Leu Gly Arg Ala Phe Glu Trp Glu Ser Gly Leu Ala Leu Phe Leu
                85                  90                  95

Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Leu Cys Leu
            100                 105                 110

Ser Thr Pro Asn Arg Glu Gly Lys Asn Glu Ile Val Leu Thr Gly Glu
        115                 120                 125

Pro Arg Met Lys Glu Arg Pro Ile Glu His Leu Val Asp Ala Leu Cys
    130                 135                 140

Glu Ala Gly Ala Glu Ile Glu Tyr Leu Glu Glu Glu Gly Tyr Pro Pro
145                 150                 155                 160

Ile Ala Ile Arg Asn Thr Gly Leu Lys Gly Gly Arg Ile Glu Ile Asp
                165                 170                 175

Gly Ser Val Ser Ser Glu Phe Leu Thr Ala Leu Leu Met Ala Ala Pro
            180                 185                 190
```

-continued

```
Met Ala Glu Ala Asp Thr Glu Ile Glu Ile Ile Gly Glu Leu Val Ser
            195                 200                 205

Lys Pro Tyr Ile Asp Ile Thr Leu Lys Met Met Glu Thr Phe Gly Val
        210                 215                 220

Glu Val Glu Asn Glu Ala Tyr Glu Arg Phe Leu Val Lys Gly His Glu
225                 230                 235                 240

Glu Tyr Glu Ser Pro His Arg Phe Leu Val Glu Gly Asp Ala Ser Ser
                245                 250                 255

Ala Ser Tyr Phe Leu Ala Ala Ala Ala Ile Lys Gly Lys Val Lys Val
            260                 265                 270

Thr Gly Val Gly Lys Asn Ser Ile Glu Gly Asp Arg Leu Phe Ala Asp
        275                 280                 285

Val Leu Glu Lys Met Gly Ala His Ile Thr Trp Gly Asp Asp Phe Ile
    290                 295                 300

Glu Val Glu Lys Gly Asn Leu Lys Gly Ile Asp Met Asp Met Asn His
305                 310                 315                 320

Ile Pro Asp Ala Ala Met Thr Ile Ala Thr Thr Ala Leu Phe Ala Glu
                325                 330                 335

Gly Glu Thr Val Ile Arg Asn Ile Tyr Asn Trp Arg Val Lys Glu Thr
            340                 345                 350

Asp Arg Leu Thr Ala Met Ala Thr Glu Leu Arg Lys Val Gly Ala Glu
        355                 360                 365

Val Glu Glu Gly Glu Asp Phe Ile Arg Ile Glu Pro Leu Asn Leu Ala
    370                 375                 380

Glu Phe Glu His Ala Glu Leu Asn Ile His Asp His Arg Met Ala Met
385                 390                 395                 400

Cys Phe Ala Leu Ile Ala Leu Ser Lys Thr Ser Val Thr Ile Leu Asp
                405                 410                 415

Pro Ser Cys Thr Ala Lys Thr Phe Pro Thr Phe Leu Ile Leu Phe Thr
            420                 425                 430

Leu Asn Thr Arg Glu Val Ala Tyr Arg
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 456 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

```
Asn Ser Leu Arg Leu Glu Pro Ile Ser Arg Val Ala Gly Glu Val Asn
1               5                   10                  15

Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala Ala
            20                  25                  30

Leu Ala Arg Gly Thr Thr Arg Leu Thr Asn Leu Leu Asp Ser Asp Asp
        35                  40                  45

Ile Arg His Met Leu Ala Ala Leu Thr Gln Leu Gly Val Lys Tyr Lys
    50                  55                  60

Leu Ser Ala Asp Lys Thr Glu Cys Thr Val His Gly Leu Gly Arg Ser
65                  70                  75                  80

Phe Ala Val Ser Ala Pro Val Asn Leu Phe Leu Gly Asn Ala Gly Thr
                85                  90                  95

Ala Met Arg Pro Leu Cys Ala Ala Leu Cys Leu Gly Ser Gly Glu Tyr
            100                 105                 110
```

-continued

```
Met Leu Gly Gly Gln Pro Arg Met Gln Glu Arg Pro Ile Gly His Leu
        115                 120                 125

Val Asp Cys Leu Ala Leu Lys Gly Ala His Ile Gln Tyr Leu Lys Lys
    130                 135                 140

Asp Gly Tyr Pro Pro Leu Val Val Asp Ala Lys Gly Leu Trp Gly Gly
145                 150                 155                 160

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

Leu Met Ala Ala Pro Ala Met Ala Pro Val Ile Pro Arg Ile His Ile
            180                 185                 190

Lys Gly Gln Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu His Ile
        195                 200                 205

Met Asn Ser Ser Gly Val Val Ile Glu His Asp Asn Tyr Lys Leu Phe
    210                 215                 220

Tyr Ile Lys Gly Asn Gln Ser Ile Val Ser Pro Gly Asp Phe Leu Val
225                 230                 235                 240

Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Ala Gly Ala Ile
                245                 250                 255

Lys Gly Lys Val Arg Val Thr Gly Ile Gly Lys His Ser Ile Gly Asp
            260                 265                 270

Ile His Phe Ala Asp Val Leu Glu Arg Met Gly Ala Arg Ile Thr Trp
        275                 280                 285

Gly Asp Asp Phe Ile Glu Ala Glu Gln Gly Pro Leu His Gly Val Asp
    290                 295                 300

Met Asp Met Asn His Ile Pro Asp Val Gly His Asp His Ser Gly Gln
305                 310                 315                 320

Ser His Cys Leu Pro Arg Val Pro Pro His Ser Gln His Leu Gln Leu
                325                 330                 335

Ala Val Arg Asp Asp Arg Cys Thr Pro Cys Thr His Gly His Arg Arg
            340                 345                 350

Ala Gln Ala Gly Val Ser Glu Glu Gly Thr Thr Phe Ile Thr Arg Asp
        355                 360                 365

Ala Ala Asp Pro Ala Gln Ala Arg Arg Asp Arg His Leu Gln Arg Ser
    370                 375                 380

Arg Ile Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Ile Ala Val
385                 390                 395                 400

Thr Ile Asn Asp Pro Gly Cys Thr Ser Lys Thr Phe Pro Asp Tyr Phe
                405                 410                 415

Asp Lys Leu Ala Ser Val Ser Gln Ala Val
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 443 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:64:

```
Met Ser Gly Leu Ala Tyr Leu Asp Leu Pro Ala Ala Arg Leu Ala Arg
1               5                  10                  15

Gly Glu Val Ala Leu Pro Gly Ser Lys Ser Ile Ser Asn Arg Val Leu
            20                  25                  30

Leu Leu Ala Ala Leu Ala Glu Gly Ser Thr Glu Ile Thr Gly Leu Leu
```

-continued

```
                    35                  40                  45
Asp Ser Asp Asp Thr Arg Val Met Leu Ala Ala Leu Arg Gln Leu Gly
            50                  55                  60
Val Ser Val Gly Glu Val Ala Asp Gly Cys Val Thr Ile Glu Gly Val
        65                  70                  75                  80
Ala Arg Phe Pro Thr Gln Gln Ala Glu Leu Phe Leu Gly Asn Ala Gly
                    85                  90                  95
Thr Ala Phe Arg Pro Leu Thr Ala Ala Leu Ala Leu Met Gly Gly Asp
                100                 105                 110
Tyr Arg Leu Ser Gly Val Pro Arg Met His Glu Arg Pro Ile Gly Asp
            115                 120                 125
Leu Val Asp Ala Leu Arg Gln Phe Gly Ala Gly Ile Glu Tyr Leu Gly
        130                 135                 140
Gln Ala Gly Tyr Pro Pro Leu Arg Ile Gly Gly Gly Ser Ile Arg Val
    145                 150                 155                 160
Asp Gly Pro Val Arg Val Glu Gly Ser Val Ser Ser Gln Phe Leu Thr
                165                 170                 175
Ala Leu Leu Met Ala Ala Pro Val Leu Ala Arg Arg Ser Gly Gln Asp
            180                 185                 190
Ile Thr Ile Glu Val Val Gly Gln Leu Ile Ser Lys Pro Tyr Ile Glu
        195                 200                 205
Ile Thr Leu Asn Leu Met Ala Arg Phe Gly Val Ser Val Arg Arg Asp
    210                 215                 220
Gly Trp Arg Ala Phe Thr Ile Ala Arg Asp Ala Val Tyr Arg Gly Pro
225                 230                 235                 240
Gly Arg Met Ala Ile Glu Gly Asp Ala Ser Thr Ala Ser Tyr Phe Leu
                245                 250                 255
Ala Leu Gly Ala Ile Gly Gly Gly Pro Val Arg Val Thr Gly Val Gly
            260                 265                 270
Glu Asp Ser Ile Gln Gly Asp Val Ala Phe Ala Ala Thr Leu Ala Ala
        275                 280                 285
Met Gly Ala Asp Val Arg Tyr Gly Pro Gly Trp Ile Glu Thr Arg Gly
    290                 295                 300
Val Arg Val Ala Glu Gly Gly Arg Leu Lys Ala Phe Asp Ala Asp Phe
305                 310                 315                 320
Asn Leu Ile Pro Asp Ala Ala Met Thr Ala Ala Thr Leu Ala Leu Tyr
                325                 330                 335
Ala Asp Gly Pro Cys Arg Leu Arg Asn Ile Gly Ser Trp Arg Val Lys
            340                 345                 350
Glu Thr Asp Arg Ile His Ala Met His Thr Glu Leu Glu Lys Leu Gly
        355                 360                 365
Ala Gly Val Glu Ser Gly Ala Asp Trp Leu Glu Val Ala Pro Pro Glu
    370                 375                 380
Pro Gly Gly Trp Arg Asp Ala His Ile Gly Thr Trp Asp Asp His Arg
385                 390                 395                 400
Met Ala Met Cys Phe Leu Leu Ala Ala Phe Gly Pro Ala Ala Val Arg
                405                 410                 415
Ile Leu Asp Pro Gly Cys Val Ser Lys Thr Phe Pro Asp Tyr Phe Asp
            420                 425                 430
Val Tyr Ala Gly Leu Leu Ala Ala Arg Asp
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:6:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

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

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

Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Cys Gly Lys Thr Val Leu Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45

Asp Val Arg His Met Leu Asn Ala Leu Ser Ala Leu Gly Ile Asn Tyr
    50                  55                  60

Thr Leu Ser Ala Asp Arg Thr Arg Cys Asp Ile Thr Gly Asn Gly Gly
65                  70                  75                  80

Pro Leu Arg Ala Ser Gly Thr Leu Gln Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Gln Asn Gln
            100                 105                 110

Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His
        115                 120                 125

Leu Val Asp Ser Leu Arg Gln Gly Gly Ala Asn Ile Asp Tyr Leu Glu
    130                 135                 140

Glu Glu Asn Tyr Pro Pro Leu Arg Leu Arg Gly Gly Phe Ile Gly Gly
145                 150                 155                 160

Asp Ile Glu Val Asp Gly Ser Val Ser Ser Gln Phe Leu Thr Ala Leu
                165                 170                 175

Leu Met Thr Ala Pro Leu Ala Pro Glu Asp Thr Ile Ile Arg Val Lys
            180                 185                 190

Gly Glu Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu Asn Leu Met
        195                 200                 205

Lys Thr Phe Gly Val Glu Ile Ala Asn His His Tyr Gln Gln Phe Val
    210                 215                 220

Val Lys Gly Gly Gln Gln Tyr His Ser Pro Gly Arg Tyr Leu Val Glu
225                 230                 235                 240

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

Gly Gly Thr Val Lys Val Thr Gly Ile Gly Gly Lys Ser Met Gln Gly
            260                 265                 270

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

Trp Gly Asp Asp Phe Ile Ala Cys Thr Arg Gly Glu Leu His Ala Ile
    290                 295                 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305                 310                 315                 320

Thr Ala Leu Phe Ala Lys Gly Thr Thr Thr Leu Arg Asn Ile Tyr Asn
                325                 330                 335

Trp Arg Val Lys Glu Thr Asp Arg Leu Phe Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly His Asp Tyr Ile Arg Ile
        355                 360                 365

Thr Pro Pro Ala Lys Leu Gln His Ala Asp Ile Gly Thr Tyr Asn Asp
    370                 375                 380
```

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```
His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
                405                 410                 415

Phe Glu Glu Leu Ala Arg Met Ser Thr Pro Ala
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 275..1618

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```
ACGGGCTGTA ACGGTAGTAG GGGTCCCGAG CACAAAAGCG GTGCCGGCAA GCAGAACTAA      60

TTTCCATGGG GAATAATGGT ATTTCATTGG TTTGGCCTCT GGTCTGGCAA TGGTTGCTAG     120

GCGATCGCCT GTTGAAATTA ACAAACTGTC GCCCTTCCAC TGACCATGGT AACGATGTTT     180

TTTACTTCCT TGACTAACCG AGGAAAATTT GGCGGGGGGC AGAAATGCCA ATACAATTTA     240

GCTTGGTCTT CCCTGCCCCT AATTTGTCCC CTGC ATG GCC TTG CTT TCC CTC          292
                                        Met Ala Leu Leu Ser Leu
                                          1               5

AAC AAT CAT CAA TCC CAT CAA CGC TTA ACT GTT AAT CCC CCT GCC CAA        340
Asn Asn His Gln Ser His Gln Arg Leu Thr Val Asn Pro Pro Ala Gln
            10                  15                  20

GGG GTC GCT TTG ACT GGC CGC CTA AGG GTG CCG GGG GAT AAA TCC ATT       388
Gly Val Ala Leu Thr Gly Arg Leu Arg Val Pro Gly Asp Lys Ser Ile
        25                  30                  35

TCC CAT CGG GCC TTG ATG TTG GGG GCG ATC GCC ACC GGG GAA ACC ATT       436
Ser His Arg Ala Leu Met Leu Gly Ala Ile Ala Thr Gly Glu Thr Ile
    40                  45                  50

ATC GAA GGG CTA CTG TTG GGG GAA GAT CCC CGT AGT ACG GCC CAT TGC       484
Ile Glu Gly Leu Leu Leu Gly Glu Asp Pro Arg Ser Thr Ala His Cys
55                  60                  65                  70

TTT CGG GCC ATG GGA GCA GAA ATC AGC GAA CTA AAT TCA GAA AAA ATC       532
Phe Arg Ala Met Gly Ala Glu Ile Ser Glu Leu Asn Ser Glu Lys Ile
                75                  80                  85

ATC GTT CAG GGT CGG GGT CTG GGA CAG TTG CAG GAA CCC AGT ACC GTT       580
Ile Val Gln Gly Arg Gly Leu Gly Gln Leu Gln Glu Pro Ser Thr Val
            90                  95                  100

TTG GAT GCG GGG AAC TCT GGC ACC ACC ATG CGC TTA ATG TTG GGC TTG       628
Leu Asp Ala Gly Asn Ser Gly Thr Thr Met Arg Leu Met Leu Gly Leu
        105                 110                 115

CTA GCC GGG CAA AAA GAT TGT TTA TTC ACC GTC ACC GGC GAT GAT TCC       676
Leu Ala Gly Gln Lys Asp Cys Leu Phe Thr Val Thr Gly Asp Asp Ser
    120                 125                 130

CTC CGT CAC CGC CCC ATG TCC CGG GTA ATT CAA CCC TTG CAA CAA ATG       724
Leu Arg His Arg Pro Met Ser Arg Val Ile Gln Pro Leu Gln Gln Met
135                 140                 145                 150

GGG GCA AAA ATT TGG GCC CGG AGT AAC GGC AAG TTT GCG CCG CTG GCA       772
Gly Ala Lys Ile Trp Ala Arg Ser Asn Gly Lys Phe Ala Pro Leu Ala
                155                 160                 165
```

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```
GTC CAG GGT AGC CAA TTA AAA CCG ATC CAT TAC CAT TCC CCC ATT GCT        620
Val Gln Gly Ser Gln Leu Lys Pro Ile His Tyr His Ser Pro Ile Ala
            170                 175                 180

TCA GCC CAG GTA AAG TCC TGC CTG TTG CTA GCG GGG TTA ACC ACC GAG        868
Ser Ala Gln Val Lys Ser Cys Leu Leu Leu Ala Gly Leu Thr Thr Glu
        185                 190                 195

GGG GAC ACC ACG GTT ACA GAA CCA GCT CTA TCC CGG GAT CAT AGC GAA        916
Gly Asp Thr Thr Val Thr Glu Pro Ala Leu Ser Arg Asp His Ser Glu
    200                 205                 210

CGC ATG TTG CAG GCC TTT GGA GCC AAA TTA ACC ATT GAT CCA GTA ACC        964
Arg Met Leu Gln Ala Phe Gly Ala Lys Leu Thr Ile Asp Pro Val Thr
215                 220                 225                 230

CAT AGC GTC ACT GTC CAT GGC CCG GCC CAT TTA ACG GGG CAA CGG GTG       1012
His Ser Val Thr Val His Gly Pro Ala His Leu Thr Gly Gln Arg Val
                235                 240                 245

GTG GTG CCA GGG GAC ATC AGC TCG GCG GCC TTT TGG TTA GTG GCG GCA       1060
Val Val Pro Gly Asp Ile Ser Ser Ala Ala Phe Trp Leu Val Ala Ala
            250                 255                 260

TCC ATT TTG CCT GGA TCA GAA TTG TTG GTG GAA AAT GTA GGC ATT AAC       1108
Ser Ile Leu Pro Gly Ser Glu Leu Leu Val Glu Asn Val Gly Ile Asn
        265                 270                 275

CCC ACC AGG ACA GGG GTG TTG GAA GTG TTG GCC CAG ATG GGG GCG GAC       1156
Pro Thr Arg Thr Gly Val Leu Glu Val Leu Ala Gln Met Gly Ala Asp
    280                 285                 290

ATT ACC CCG GAG AAT GAA CGA TTG GTA ACG GGG GAA CCG GTA GCA GAT       1204
Ile Thr Pro Glu Asn Glu Arg Leu Val Thr Gly Glu Pro Val Ala Asp
295                 300                 305                 310

CTG CGG GTT AGG GCA AGC CAT CTC CAG GGT TGC ACC TTC GGC GGC GAA       1252
Leu Arg Val Arg Ala Ser His Leu Gln Gly Cys Thr Phe Gly Gly Glu
                315                 320                 325

ATT ATT CCC CGA CTG ATT GAT GAA ATT CCC ATT TTG GCA GTG GCG GCG       1300
Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro Ile Leu Ala Val Ala Ala
            330                 335                 340

GCC TTT GCA GAG GGC ACT ACC CGC ATT GAA GAT GCC GCA GAA CTG AGG       1348
Ala Phe Ala Glu Gly Thr Thr Arg Ile Glu Asp Ala Ala Glu Leu Arg
        345                 350                 355

GTT AAA GAA AGC GAT CGC CTG GCG GCC ATT GCT TCG GAG TTG GGC AAA       1396
Val Lys Glu Ser Asp Arg Leu Ala Ala Ile Ala Ser Glu Leu Gly Lys
    360                 365                 370

ATG GGG GCC AAA GTC ACC GAA TTT GAT GAT GGC CTG GAA ATT CAA GGG       1444
Met Gly Ala Lys Val Thr Glu Phe Asp Asp Gly Leu Glu Ile Glu Gly
375                 380                 385                 390

GGA AGC CCG TTA CAA GGG GCC GAG GTG GAT AGC TTG ACG GAT CAT CGC       1492
Gly Ser Pro Leu Gln Gly Ala Glu Val Asp Ser Leu Thr Asp His Arg
                395                 400                 405

ATT GCC ATG GCG TTG GCG ATC GCC GCT TTA GGT AGT GGG GGG CAA ACA       1540
Ile Ala Met Ala Leu Ala Ile Ala Ala Leu Gly Ser Gly Gly Gln Thr
            410                 415                 420

ATT ATT AAC CGG GCG GAA GCG GCC GCC ATT TCC TAT CCA GAA TTT TTT       1588
Ile Ile Asn Arg Ala Glu Ala Ala Ala Ile Ser Tyr Pro Glu Phe Phe
        425                 430                 435

GGC ACG CTA GGG CAA GTT GCC CAA GGA TAAAGTTAGA AAAACTCCTG             1635
Gly Thr Leu Gly Gln Val Ala Gln Gly
    440                 445

GGCGGTTTGT AAATGTTTTA CCAAGGTAGT TTGGGGTAAA GGCCCCAGCA AGTGCTGCCA     1695

GGGTAATTTA TCCGCAATTG ACCAATCGGC ATGGACCGTA TCGTTCAAAC TGGGTAATTC     1755

TCCCTTTAAT TCCTTAAAAG CTCGCTTAAA ACTGCCCAAC GTATCTCCGT AATGGCGAGT     1815

GAGTAGAAGT AATGGGGCCA AACGGCGATC GCCACGGGAA ATTAAAGCCT GCATCACTGA     1875
```

-continued

CCACTTATAA CTTTCGGGA 1894

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:67:

```
Met Ala Leu Leu Ser Leu Asn Asn His Gln Ser His Gln Arg Leu Thr
 1               5                  10                  15

Val Asn Pro Pro Ala Gln Gly Val Ala Leu Thr Gly Arg Leu Arg Val
            20                  25                  30

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

Ala Thr Gly Glu Thr Ile Ile Glu Gly Leu Leu Leu Gly Glu Asp Pro
    50                  55                  60

Arg Ser Thr Ala His Cys Phe Arg Ala Met Gly Ala Glu Ile Ser Glu
65                  70                  75                  80

Leu Asn Ser Glu Lys Ile Ile Val Gln Gly Arg Gly Leu Gly Gln Leu
                85                  90                  95

Gln Glu Pro Ser Thr Val Leu Asp Ala Gly Asn Ser Gly Thr Thr Met
            100                 105                 110

Arg Leu Met Leu Gly Leu Leu Ala Gly Gln Lys Asp Cys Leu Phe Thr
        115                 120                 125

Val Thr Gly Asp Asp Ser Leu Arg His Arg Pro Met Ser Arg Val Ile
    130                 135                 140

Gln Pro Leu Gln Gln Met Gly Ala Lys Ile Trp Ala Arg Ser Asn Gly
145                 150                 155                 160

Lys Phe Ala Pro Leu Ala Val Gln Gly Ser Gln Leu Lys Pro Ile His
                165                 170                 175

Tyr His Ser Pro Ile Ala Ser Ala Gln Val Lys Ser Cys Leu Leu Leu
            180                 185                 190

Ala Gly Leu Thr Thr Gln Gly Asp Thr Thr Val Thr Glu Pro Ala Leu
        195                 200                 205

Ser Arg Asp His Ser Glu Arg Met Leu Gln Ala Phe Gly Ala Lys Leu
    210                 215                 220

Thr Ile Asp Pro Val Thr His Ser Val Thr Val His Gly Pro Ala His
225                 230                 235                 240

Leu Thr Gly Gln Arg Val Val Val Pro Gly Asp Ile Ser Ser Ala Ala
                245                 250                 255

Phe Trp Leu Val Ala Ala Ser Ile Leu Pro Gly Ser Gln Leu Leu Val
            260                 265                 270

Gln Asn Val Gly Ile Asn Pro Thr Arg Thr Gly Val Leu Gln Val Leu
        275                 280                 285

Ala Gln Met Gly Ala Asp Ile Thr Pro Glu Asn Glu Arg Leu Val Thr
    290                 295                 300

Gly Gln Pro Val Ala Asp Leu Arg Val Arg Ala Ser His Leu Gln Gly
305                 310                 315                 320

Cys Thr Phe Gly Gly Gln Ile Ile Pro Arg Leu Ile Asp Glu Ile Pro
                325                 330                 335

Ile Leu Ala Val Ala Ala Ala Phe Ala Glu Gly Thr Thr Arg Ile Glu
            340                 345                 350
```

-continued

```
Asp Ala Ala Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Ile
        355                 360                 365

Ala Ser Glu Leu Gly Lys Met Gly Ala Lys Val Thr Glu Phe Asp Asp
    370                 375                 380

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

Ser Leu Thr Asp His Arg Ile Ala Met Ala Leu Ala Ile Ala Ala Leu
                405                 410                 415

Gly Ser Gly Gly Glu Thr Ile Ile Asn Arg Ala Glu Ala Ala Ala Ile
            420                 425                 430

Ser Tyr Pro Glu Phe Phe Gly Thr Leu Gly Glu Val Ala Glu Gly
        435                 440                 445
```

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1479 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 107..1438

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```
TTTAAAAACA ATGAGTTAAA AAATTATTTT TCTGGCACAC GCGCTTTTTT TGCATTTTTT      60

CTCCCATTTT TCCGGCACAA TAACGTTGGT TTTATAAAAG GAAATG ATG ATG ACG        115
                                                  Met Met Thr
                                                   1

AAT ATA TGG CAC ACC GCG CCC GTC TCT GCG CTT TCC GGC GAA ATA ACG        163
Asn Ile Trp His Thr Ala Pro Val Ser Ala Leu Ser Gly Glu Ile Thr
  5                  10                  15

ATA TGC GGC GAT AAA TCA ATG TCG CAT CGC GCC TTA TTA TTA GCA GCG        211
Ile Cys Gly Asp Lys Ser Met Ser His Arg Ala Leu Leu Leu Ala Ala
 20                  25                  30                  35

TTA GCA GAA GGA CAA ACG GAA ATC CGC GGC TTT TTA GCG TGC GCG GAT        259
Leu Ala Glu Gly Gln Thr Glu Ile Arg Gly Phe Leu Ala Cys Ala Asp
                 40                  45                  50

TGT TTG GCG ACG CGG CAA GCA TTG CGC GCA TTA GGC GTT GAT ATT CAA        307
Cys Leu Ala Thr Arg Gln Ala Leu Arg Ala Leu Gly Val Asp Ile Gln
             55                  60                  65

AGA GAA AAA GAA ATA GTG ACG ATT CGC GGT GTG GGA TTT CTG GGT TTG        355
Arg Glu Lys Glu Ile Val Thr Ile Arg Gly Val Gly Phe Leu Gly Leu
         70                  75                  80

CAG CCG CCG AAA GCA CCG TTA AAT ATG CAA AAC AGT GGC ACT AGC ATG        403
Gln Pro Pro Lys Ala Pro Leu Asn Met Gln Asn Ser Gly Thr Ser Met
     85                  90                  95

CGT TTA TTG GCA GGA ATT TTG GCA GCG CAG CGC TTT GAG AGC GTG TTA        451
Arg Leu Leu Ala Gly Ile Leu Ala Ala Gln Arg Phe Glu Ser Val Leu
100                 105                 110                 115

TGC GGC GAT GAA TCA TTA GAA AAA CGT CCG ATG CAG CGC ATT ATT ACG        499
Cys Gly Asp Glu Ser Leu Glu Lys Arg Pro Met Gln Arg Ile Ile Thr
                120                 125                 130

CCG CTT GTG CAA ATG GGG GCA AAA ATT GTC AGT CAC AGC AAT TTT ACG        547
Pro Leu Val Gln Met Gly Ala Lys Ile Val Ser His Ser Asn Phe Thr
            135                 140                 145

GCG CCG TTA CAT ATT TCA GGA CGC CCG CTG ACC GGC ATT GAT TAC GCG        595
Ala Pro Leu His Ile Ser Gly Arg Pro Leu Thr Gly Ile Asp Tyr Ala
```

-continued

```
                150                 155                 160

TTA CCG CTT CCC AGC GCG CAA TTA AAA AGT TGC CTT ATT TTG GCA GGA      643
Leu Pro Leu Pro Ser Ala Gln Leu Lys Ser Cys Leu Ile Leu Ala Gly
    165                 170                 175

TTA TTG GCT GAC GGT ACC ACG CGG CTG CAT ACT TGC GGC ATC AGT CGC      691
Leu Leu Ala Asp Gly Thr Thr Arg Leu His Thr Cys Gly Ile Ser Arg
180                 185                 190                 195

GAC CAC ACG GAA CGC ATG TTG CCG CTT TTT GGT GGC GCA CTT GAG ATC      739
Asp His Thr Glu Arg Met Leu Pro Leu Phe Gly Gly Ala Leu Glu Ile
                200                 205                 210

AAG AAA GAG CAA ATA ATC GTC ACC GGT GGA CAA AAA TTG CAC GGT TGC      787
Lys Lys Glu Gln Ile Ile Val Thr Gly Gly Gln Lys Leu His Gly Cys
            215                 220                 225

GTG CTT GAT ATT GTC GGC GAT TTG TCG GCG GCG GCG TTT TTT ATG GTT      835
Val Leu Asp Ile Val Gly Asp Leu Ser Ala Ala Ala Phe Phe Met Val
        230                 235                 240

GCG GCT TTG ATT GCG CCG CGC GCG GAA GTC GTT ATT CGT AAT GTC GGC      883
Ala Ala Leu Ile Ala Pro Arg Ala Glu Val Val Ile Arg Asn Val Gly
    245                 250                 255

ATT AAT CCG ACG CGG GCG GCA ATC ATT ACT TTG TTG CAA AAA ATG GGC      931
Ile Asn Pro Thr Arg Ala Ala Ile Ile Thr Leu Leu Gln Lys Met Gly
260                 265                 270                 275

GGA CGC ATT GAA TTG CAT CAT CAG CGC TTT TGG GGC GCC GAA CCG GTG      979
Gly Arg Ile Glu Leu His His Gln Arg Phe Trp Gly Ala Glu Pro Val
                280                 285                 290

GCA GAT ATT GTT GTT TAT CAT TCA AAA TTG CGC GGC ATT ACG GTG GCG     1027
Ala Asp Ile Val Val Tyr His Ser Lys Leu Arg Gly Ile Thr Val Ala
            295                 300                 305

CCG GAA TGG ATT GCC AAC GCG ATT GAT GAA TTG CCG ATT TTT TTT ATT     1075
Pro Glu Trp Ile Ala Asn Ala Ile Asp Glu Leu Pro Ile Phe Phe Ile
        310                 315                 320

GCG GCA GCT TGC GCG GAA GGG ACG ACT TTT GTG GGC AAT TTG TCA GAA     1123
Ala Ala Ala Cys Ala Glu Gly Thr Thr Phe Val Gly Asn Leu Ser Glu
    325                 330                 335

TTG CGT GTG AAA GAA TCG GAT CGT TTA GCG GCG ATG GCG CAA AAT TTA     1171
Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Met Ala Gln Asn Leu
340                 345                 350                 355

CAA ACT TTG GGC GTG GCG TGC GAC GTT GGC GCC GAT TTT ATT CAT ATA     1219
Gln Thr Leu Gly Val Ala Cys Asp Val Gly Ala Asp Phe Ile His Ile
                360                 365                 370

TAT GGA AGA AGC GAT CGG CAA TTT TTA CCG GCG CGG GTG AAC AGT TTT     1267
Tyr Gly Arg Ser Asp Arg Gln Phe Leu Pro Ala Arg Val Asn Ser Phe
            375                 380                 385

GGC GAT CAT CGG ATT GCG ATG AGT TTG GCG GTG GCA GGT GTG CGC GCG     1315
Gly Asp His Arg Ile Ala Met Ser Leu Ala Val Ala Gly Val Arg Ala
        390                 395                 400

GCA GGT GAA TTA TTG ATT GAT GAC GGC GCG GTG GCG GCG GTT TCT ATG     1363
Ala Gly Glu Leu Leu Ile Asp Asp Gly Ala Val Ala Ala Val Ser Met
    405                 410                 415

CCG CAA TTT CGC GAT TTT GCC GCC GCA ATT GGT ATG AAT GTA GGA GAA     1411
Pro Gln Phe Arg Asp Phe Ala Ala Ala Ile Gly Met Asn Val Gly Glu
420                 425                 430                 435

AAA GAT GCG AAA AAT TGT CAC GAT TGATGGTCCT AGCGGTGTTG GAAAAGGCAC    1465
Lys Asp Ala Lys Asn Cys His Asp
                440

GGTGGCGCAA GCTT                                                    1479
```

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 443 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:49:

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

Glu Ile Thr Ile Cys Gly Asp Lys Ser Met Ser His Arg Ala Leu Leu
            20                  25                  30

Leu Ala Ala Leu Ala Glu Gly Gln Thr Glu Ile Arg Gly Phe Leu Ala
        35                  40                  45

Cys Ala Asp Cys Leu Ala Thr Arg Gln Ala Leu Arg Ala Leu Gly Val
    50                  55                  60

Asp Ile Gln Arg Glu Lys Glu Ile Val Thr Ile Arg Gly Val Gly Phe
65                  70                  75                  80

Leu Gly Leu Gln Pro Pro Lys Ala Pro Leu Asn Met Gln Asn Ser Gly
                85                  90                  95

Thr Ser Met Arg Leu Leu Ala Gly Ile Leu Ala Ala Gln Arg Phe Glu
            100                 105                 110

Ser Val Leu Cys Gly Asp Glu Ser Leu Glu Lys Arg Pro Met Gln Arg
        115                 120                 125

Ile Ile Thr Pro Leu Val Glu Met Gly Ala Lys Ile Val Ser His Ser
    130                 135                 140

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

Asp Tyr Ala Leu Pro Leu Pro Ser Ala Gln Leu Lys Ser Cys Leu Ile
                165                 170                 175

Leu Ala Gly Leu Leu Ala Asp Gly Thr Thr Arg Leu His Thr Cys Gly
            180                 185                 190

Ile Ser Arg Asp His Thr Glu Arg Met Leu Pro Leu Phe Gly Gly Ala
        195                 200                 205

Leu Glu Ile Lys Lys Glu Gln Ile Ile Val Thr Gly Gly Gln Lys Leu
    210                 215                 220

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

Phe Met Val Ala Ala Leu Ile Ala Pro Arg Ala Glu Val Val Ile Arg
                245                 250                 255

Asn Val Gly Ile Asn Pro Thr Arg Ala Ala Ile Ile Thr Leu Leu Gln
            260                 265                 270

Lys Met Gly Gly Arg Ile Glu Leu His His Gln Arg Phe Trp Gly Ala
        275                 280                 285

Glu Pro Val Ala Asp Ile Val Val Tyr His Ser Lys Leu Arg Gly Ile
    290                 295                 300

Thr Val Ala Pro Glu Trp Ile Ala Asn Ala Ile Asp Glu Leu Pro Ile
305                 310                 315                 320

Phe Phe Ile Ala Ala Ala Cys Ala Glu Gly Thr Thr Phe Val Gly Asn
                325                 330                 335

Leu Ser Glu Leu Arg Val Lys Glu Ser Asp Arg Leu Ala Ala Met Ala
            340                 345                 350

Gln Asn Leu Gln Thr Leu Gly Val Ala Cys Asp Val Gly Ala Asp Phe
        355                 360                 365

Ile His Ile Tyr Gly Arg Ser Asp Arg Gln Phe Leu Pro Ala Arg Val
    370                 375                 380
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-continued

Asn	Ser	Phe	Gly	Asp	His	Arg	Ile	Ala	Met	Ser	Leu	Ala	Val	Ala	Gly
385					390					395					400
Val	Arg	Ala	Ala	Gly	Glu	Leu	Leu	Ile	Asp	Asp	Gly	Ala	Val	Ala	Ala
				405					410					415	
Val	Ser	Met	Pro	Gln	Phe	Arg	Asp	Phe	Ala	Ala	Ala	Ile	Gly	Met	Asn
			420					425					430		
Val	Gly	Glu	Lys	Asp	Ala	Lys	Asn	Cys	His	Asp					
		435					440								

We claim:

1. An isolated DNA molecule which encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

2. A DNA molecule of claim 1 having the sequence of SEQ ID NO:2.

3. A DNA molecule of claim 1 having the sequence of SEQ ID NO:9.

4. A recombinant, double-stranded DNA molecule comprising in sequence:

a) a promoter which functions in plant cells to cause the production of an RNA sequence;

b) a structural DNA sequence that causes the production of an RNA sequence which encodes a EPSPS enzyme having the sequence domains:

-R-X_1-H-X_2-E-(SEQ ID NO:37), in which

X_1 is G, S, T, C, Y, N, Q, D or E;

X_2 is S or T; and

-G-D-K-X_3-(SEQ ID NO:38), in which

X_3 is S or T; and

-S-A-Q-X_4-K-(SEQ ID NO:39), in which

X_4 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X_5-T-R-(SEQ ID NO:40), in which

X_5 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

c) a 3' non-translated region which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the encoded EPSPS enzyme to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule.

5. A DNA molecule of claim 4 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

6. A DNA molecule of claim 4 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

7. A DNA molecule of claim 6 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

8. A DNA molecule of claim 5 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

9. A DNA molecule of claim 8 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

10. A DNA molecule of claim 8 in which the EPSPS sequence is SEQ ID NO:3.

11. A DNA molecule of claim 10 in which the promoter is a plant DNA virus promoter.

12. A DNA molecule of claim 11 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

13. A DNA molecule of claim 10 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.

14. A DNA molecule of claim 13 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

15. A method of producing genetically transformed plants which are tolerant toward glyphosate herbicide, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant, double-stranded DNA molecule comprising:

i) a promoter which functions in plant cells to cause the production of an RNA sequence,

ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R-X_1H-X_2-E-(SEQ ID NO:37), in which

X_1 is G, S, T, C, Y, N, Q, D or E;

X_2 is S or T; and

-G-D-K-X_3-(SEQ ID NO:38), in which

X_3 is S or T; and

-S-A-Q-X_4-K-(SEQ ID NO:39), in which

X_4 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X_5-T-R-(SEQ ID NO:40), in which

X_5 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the polypeptide to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule;

b) obtaining a transformed plant cell; and

c) regenerating from the transformed plant cell a genetically transformed plant which has increased tolerance to glyphosate herbicide.

16. A method of claim 15 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

17. A method of claim 16 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

18. A method of claim 15 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

19. A method of claim 18 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

20. A method of claim 19 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

21. A method of claim 19 in which the EPSPS enzyme is that set forth in SEQ ID NO:3.

22. A method of claim 21 in which the promoter is from a plant DNA virus.

23. A method of claim 22 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

24. A glyphosate-tolerant plant cell comprising a DNA molecule of claims 5, 8 or 10.

25. A glyphosate-tolerant plant cell of claim 24 in which the promoter is a plant DNA virus promoter.

26. A glyphosate-tolerant plant cell of claim 25 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

27. A glyphosate-tolerant plant cell of claim 24 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

28. A glyphosate-tolerate plant comprising plant cells of claim 27.

29. A glyphosate-tolerant plant of claim 28 in which the promoter is from a DNA plant virus promoter.

30. A glyphosate-tolerant plant of claim 29 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

31. A glyphosate-tolerant plant of claim 30 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

32. A method for selectively controlling weeds in a field containing a crop having planted crop seeds or plants comprising the steps of:

a) planting the crop seeds or plants which are glyphosate-tolerant as a result of a recombinant double-stranded DNA molecule being inserted into the crop seed or plant, the DNA molecule having:

i) a promoter which functions in plant cells to cause the production of an RNA sequence,

ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R-X_1-H-X_2-E-(SEQ ID NO:37), in which
X_1 is G, S, T, C, Y, N, Q, D or E;
X_2 is S or T; and

-G-D-K-X_3-(SEQ ID NO:38), in which
X_3 is S or T; and

-S-A-Q-X_4-K-(SEQ ID NO:39), in which
X_4 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X_5-T-R-(SEQ ID NO:40), in which
X_5 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the EPSPS enzyme to enhance the glyphosate tolerance of the crop plant transformed with the DNA molecule; and

b) applying to the crop and weeds in the field a sufficient amount of glyphosate herbicide to control the weeds without significantly affecting the crop.

33. A method of claim 32 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

34. A method of claim 33 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

35. A method of claim 32 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

36. A method of claim 35 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

37. A method of claim 36 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

38. A method of claim 36 in which the DNA molecule encodes an EPSPS enzyme as set forth in SEQ ID NO:3.

39. A method of claim 38 in which the DNA molecule further comprises a promoter selected from the group consisting of the CAMV35S and FMV35S promoters.

40. A method of claim 39 in which the crop plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

41. A DNA molecule of claim 5 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17.

42. A DNA molecule of claim 41 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

43. A DNA molecule of claim 5 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.

44. A DNA molecule of claim 43 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14.

45. A DNA molecule of claim 41 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

46. A DNA molecule of claim 42 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

47. A DNA molecule of claim 43 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

48. A DNA molecule of claim 44 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

49. A DNA molecule of claim 45 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

50. A DNA molecule of claim 46 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

51. A DNA molecule of claim 47 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

52. A DNA molecule of claim 48 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

53. A DNA molecule of claim 49 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

54. A DNA molecule of claim 50 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

55. A DNA molecule of claim 51 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

56. A DNA molecule of claim 52 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

57. A DNA molecule of claim 53 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

58. A DNA molecule of claim 54 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

59. A DNA molecule of claim 55 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

60. A DNA molecule of claim 56 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

61. A DNA molecule of claim 49 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

62. A DNA molecule of claim 50 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

63. A DNA molecule of claim 51 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

64. A DNA molecule of claim 52 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

65. A DNA molecule of claim 61 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

66. A DNA molecule of claim 62 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

67. A DNA molecule of claim 63 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

68. A DNA molecule of claim 64 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

69. A glyphosate-tolerant plant cell of claim 25 in which:

(a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;

(b) the structural DNA sequence encodes:

(i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and

(ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and

(c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

70. A glyphosate-tolerant plant cell of claim 69 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

71. A glyphosate-tolerant plant cell of claim 69 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and

(b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

72. A glyphosate-tolerant plant cell of claim 71 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

73. A glyphosate-tolerant plant cell of claim 71 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

74. A glyphosate-tolerant plant comprising a DNA molecule of claims 5, 8 or 10 in which:

(a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;

(b) the structural DNA sequence encodes:

(i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and

(ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and

(c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

75. A glyphosate-tolerant plant of claim 74 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

76. A glyphosate-tolerant plant of claim 75 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and

(b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

77. A glyphosate-tolerant plant of claim 76 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

78. A glyphosate-tolerant plant of claim 77 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalypcus, apple, lettuce, peas, lentils, grape and turf grasses.

79. A seed of a glyphosate-tolerant plant of claim 28.

80. A seed of a glyphosate-tolerant plant of claim 31.

81. A seed of a glyphosate-tolerant plant of claim 75.

82. A seed of a glyphosate-tolerant plant of claim 76.

83. A seed of a glyphosate-tolerant plant of claim 77.

84. A seed of a glyphosate-tolerant plant of claim 78.

85. A seed of a glyphosate-tolerant plant of claim 79.

86. A transgenic soybean plant which contains a heterologous gene which encodes an EPSPS enzyme having a K_m for phosphoenolpyruvate (PEP) between 1 and 150 μM and a K_i(glyphosate)/K_m(PEP) ratio between about 2 and 500, said plant exhibiting tolerance to N-phosphonomethylglycine herbicide at a rate of 1 lb/acre without significant yield reduction due to herbicide application.

87. Seed of a soybean plant of claim 86.

* * * * *

US005352605A

United States Patent [19]

Fraley et al.

[11] Patent Number: 5,352,605

[45] Date of Patent: Oct. 4, 1994

[54] CHIMERIC GENES FOR TRANSFORMING PLANT CELLS USING VIRAL PROMOTERS

[75] Inventors: Robert T. Fraley, Ballwin; Robert B. Horsch; Stephen G. Rogers, both of St. Louis, all of Mo.

[73] Assignee: Monsanto Company, St. Louis, Mo.

[21] Appl. No.: 146,621

[22] Filed: Oct. 28, 1993

Related U.S. Application Data

[63] Continuation of Ser. No. 625,637, Dec. 7, 1990, abandoned, which is a continuation of Ser. No. 931,492, Nov. 17, 1986, abandoned, which is a continuation-in-part of Ser. No. 485,568, Apr. 15, 1983, abandoned, which is a continuation-in-part of Ser. No. 458,414, Jan. 17, 1983, abandoned.

[51] Int. Cl.[5] C12N 5/00; C12N 15/00; C07H 21/04

[52] U.S. Cl. 435/240.4; 435/172.3; 435/320.1; 536/23.2; 536/24.1

[58] Field of Search 536/23.2, 24.1; 435/172.3, 240.4, 320.1; 800/205

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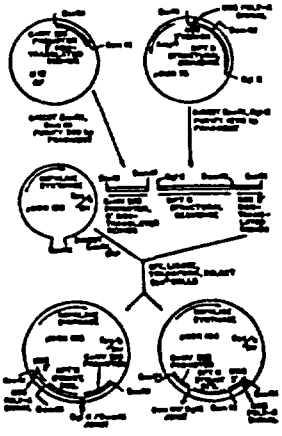
Primary Examiner—David T. Fox
Attorney, Agent, or Firm—Lawrence M. Lavin, Jr.; Dennis R. Hoerner, Jr.; Howard C. Stanley

[57] ABSTRACT

In one aspect the present invention relates to the use of viral promoters in the expression of chimeric genes in plant cells. In another aspect this invention relates to chimeric genes which are capable of being expressed in plant cells, which utilize promoter regions derived from viruses which are capable of infecting plant cells. One such virus comprises the cauliflower mosaic virus (CaMV). Two different promoter regions have been derived from the CaMV genome and ligated to heterologous coding sequences to form chimeric genes. These chimeric genes have been shown to be expressed in plant cells. This invention also relates to plant cells, plant tissue, and differentiated plants which contain and express the chimeric genes of this invention.

19 Claims, 10 Drawing Sheets



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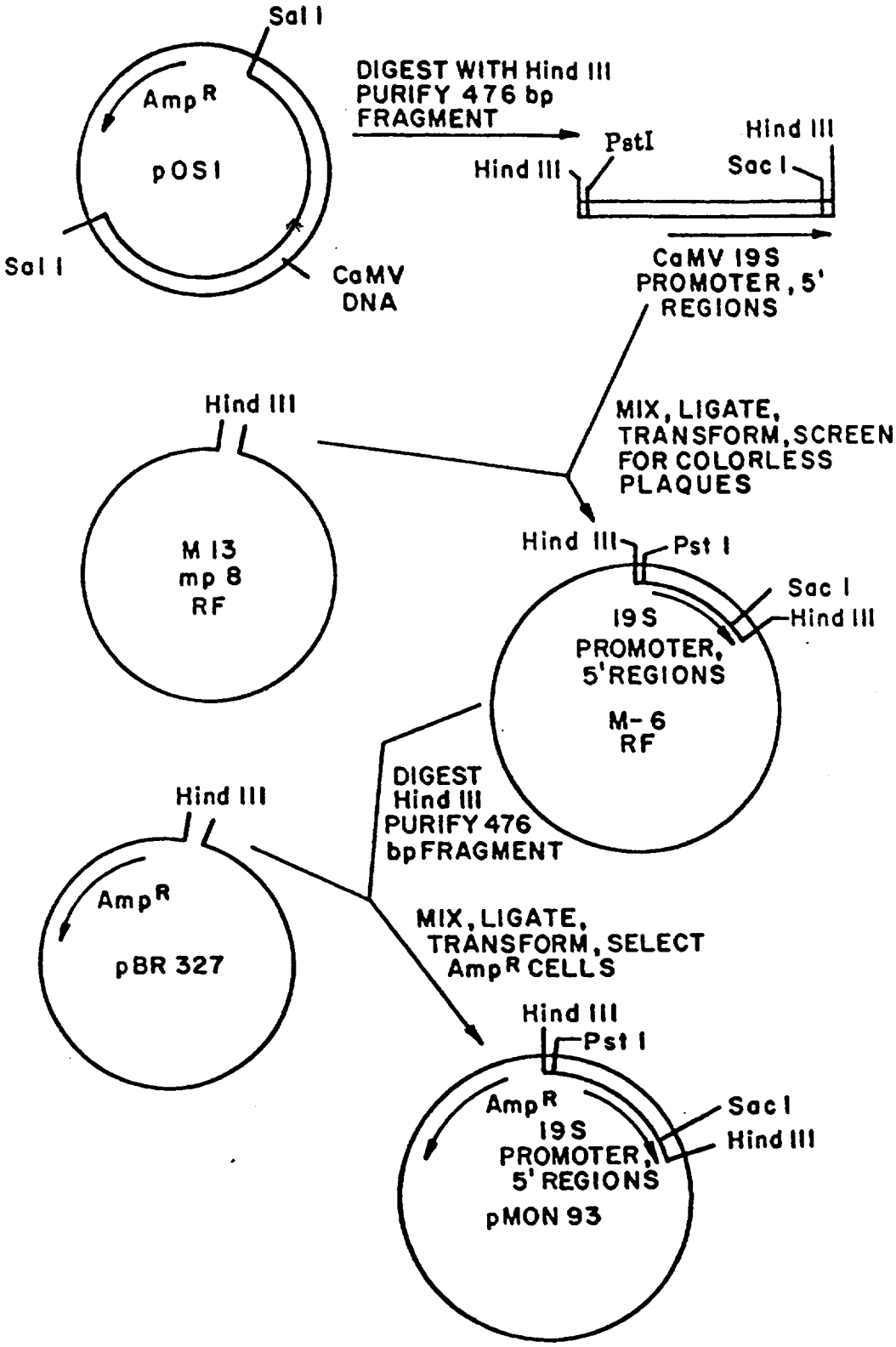
Sal I
Amp R
pOS1
Sal I
CaMV DNA
DIGEST WITH Hind III
PURIFY 476 bp FRAGMENT
PstI
Hind III
Hind III
Sac I
CaMV 19S PROMOTER, 5' REGIONS
Hind III
M 13 mp 8 RF
MIX, LIGATE, TRANSFORM, SCREEN FOR COLORLESS PLAQUES
Hind III
Pst I
Sac I
Hind III
19S PROMOTER, 5'REGIONS
M-6 RF
DIGEST Hind III PURIFY 476 bpFRAGMENT
Hind III
Amp R
pBR 327
MIX, LIGATE, TRANSFORM, SELECT Amp R CELLS
Hind III
Pst I
Amp R
19S PROMOTER, 5' REGIONS
pMON 93
Sac I
Hind III

Figure 1

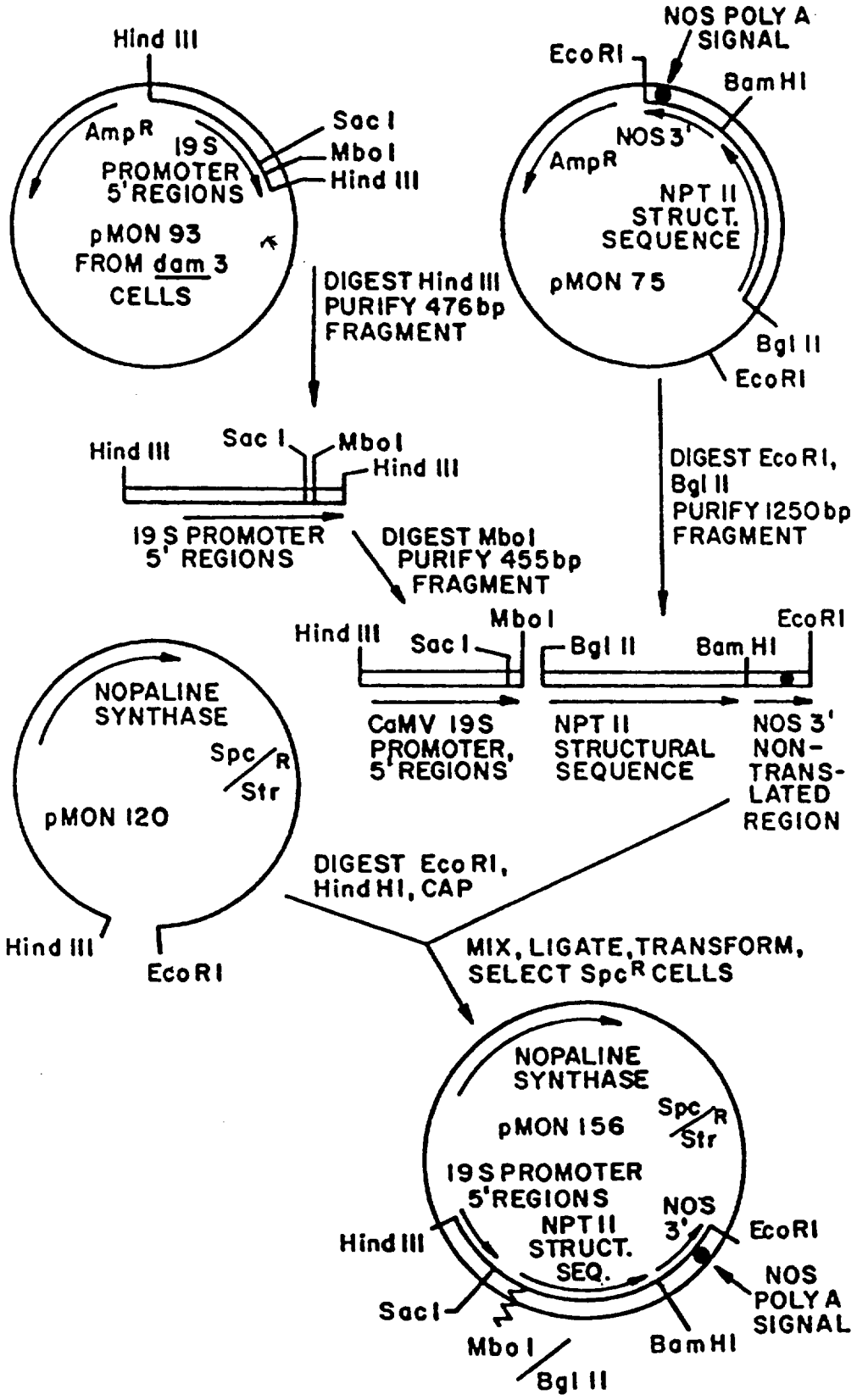
Hind III
Sac I
MboI
Hind III
AmpR
19S PROMOTER 5'REGIONS
pMON 93 FROM dam 3 CELLS
DIGEST Hind III PURIFY 476bp FRAGMENT
NOS POLY A SIGNAL
EcoRI
BamHI
NOS 3'
AmpR
NPT II STRUCT. SEQUENCE
pMON 75
Bgl II
EcoRI
Hind III
Sac I
MboI
Hind III
19S PROMOTER 5' REGIONS
DIGEST MboI PURIFY 455bp FRAGMENT
DIGEST EcoRI, Bgl II PURIFY 1250bp FRAGMENT
Hind III
Sac I
MboI
Bgl II
BamHI
EcoRI
CaMV 19S PROMOTER, 5'REGIONS
NPT II STRUCTURAL SEQUENCE
NOS 3' NON-TRANS-LATED REGION
NOPALINE SYNTHASE
Spc/Str R
pMON 120
Hind III
EcoRI
DIGEST EcoRI, HindHI, CAP
MIX, LIGATE, TRANSFORM, SELECT SpcR CELLS
NOPALINE SYNTHASE
pMON 156
Spc/Str R
19S PROMOTER 5'REGIONS
NPT II STRUCT. SEQ.
NOS 3'
EcoRI
NOS POLY A SIGNAL
Hind III
Sac I
MboI
Bgl II
BamHI

Figure 2

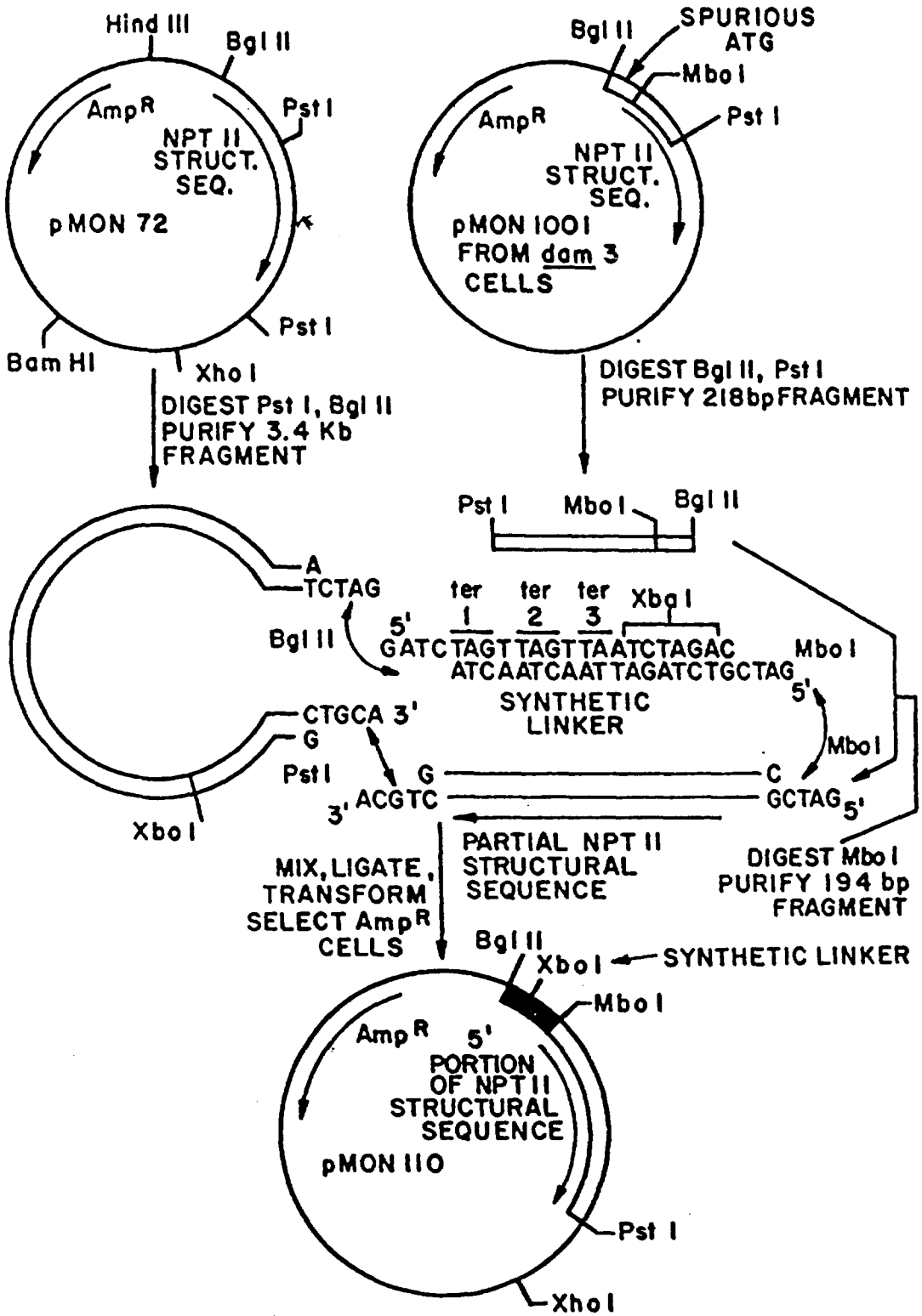
Hind III
Bgl II
Pst I
AmpR
NPT II STRUCT. SEQ.
pMON 72
Pst I
Bam HI
Xho I
DIGEST Pst I, Bgl II
PURIFY 3.4 Kb FRAGMENT
Bgl II
SPURIOUS ATG
Mbo I
AmpR
Pst I
NPT II STRUCT. SEQ.
pMON 1001 FROM dam 3 CELLS
DIGEST Bgl II, Pst I
PURIFY 218bp FRAGMENT
Pst I
Mbo I
Bgl II
A
TCTAG
Bgl II
5'
ter 1
ter 2
ter 3
Xbo I
GATC TAGT TAGT TAA TCTAGAC
ATC AATC AATT AGATCTGCTAG
Mbo I
5'
SYNTHETIC LINKER
CTGCA 3'
G
Pst I
G
C
3'
ACGTC
GCTAG 5'
Mbo I
Xbo I
PARTIAL NPT II STRUCTURAL SEQUENCE
MIX, LIGATE, TRANSFORM SELECT AmpR CELLS
DIGEST Mbo I PURIFY 194 bp FRAGMENT
Bgl II
Xbo I
SYNTHETIC LINKER
Mbo I
AmpR
5'
PORTION OF NPT II STRUCTURAL SEQUENCE
pMON 110
Pst I
Xho I

Figure 3

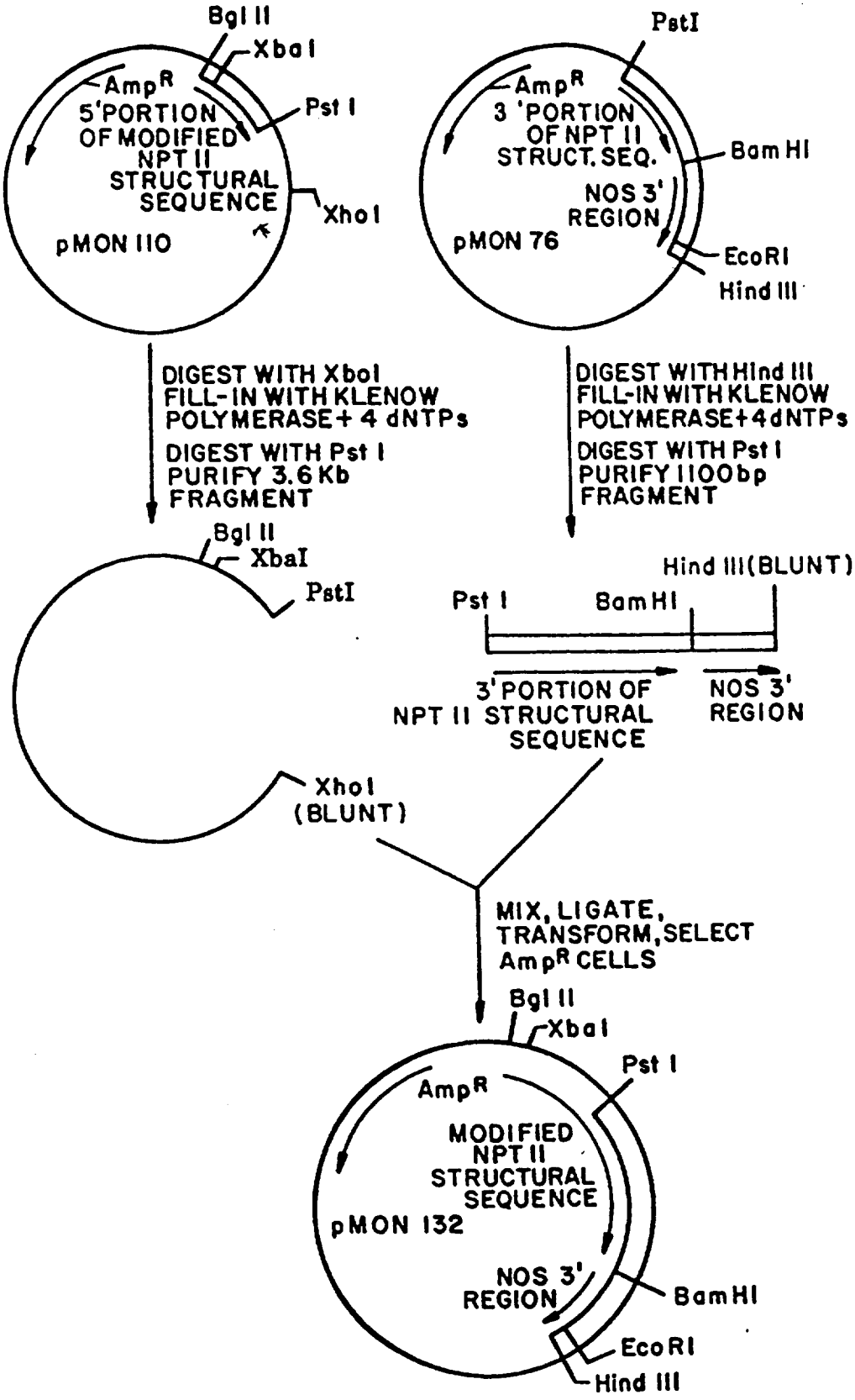
Bgl II
Xba I
AmpR
5'PORTION OF MODIFIED NPT II STRUCTURAL SEQUENCE
Pst I
Xho I
pMON 110
PstI
AmpR
3 'PORTION OF NPT II STRUCT. SEQ.
Bam HI
NOS 3' REGION
pMON 76
EcoRI
Hind III
DIGEST WITH Xbo I
FILL-IN WITH KLENOW POLYMERASE + 4 dNTPs
DIGEST WITH Pst I
PURIFY 3.6 Kb FRAGMENT
DIGEST WITH Hind III
FILL-IN WITH KLENOW POLYMERASE + 4 dNTPs
DIGEST WITH Pst I
PURIFY 1100 bp FRAGMENT
Bgl II
XbaI
PstI
Xho I (BLUNT)
Hind III(BLUNT)
Pst I
BamHI
3'PORTION OF NPT II STRUCTURAL SEQUENCE
NOS 3' REGION
MIX, LIGATE, TRANSFORM, SELECT AmpR CELLS
Bgl II
Xba I
Pst I
AmpR
MODIFIED NPT II STRUCTURAL SEQUENCE
pMON 132
NOS 3' REGION
BamHI
EcoRI
Hind III

Figure 4

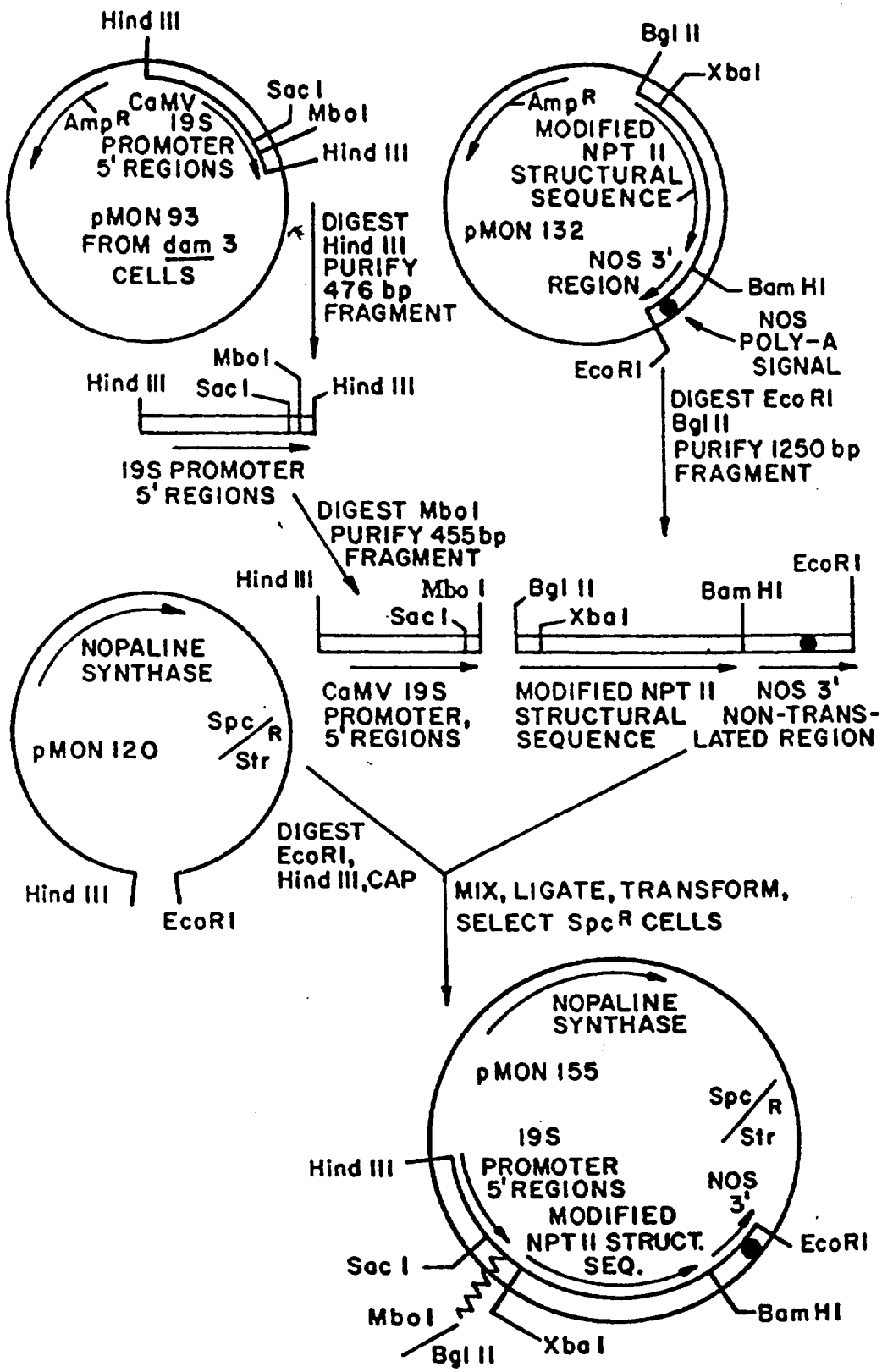
Hind III
Sac I
Mbo I
Amp R
CaMV 19S PROMOTER 5' REGIONS
Hind III
pMON 93 FROM dam 3 CELLS
DIGEST Hind III PURIFY 476 bp FRAGMENT
Mbo I
Hind III
Sac I
Hind III
19S PROMOTER 5' REGIONS
Bgl II
Xba I
Amp R
MODIFIED NPT II STRUCTURAL SEQUENCE
pMON 132
NOS 3' REGION
Bam HI
NOS POLY-A SIGNAL
Eco RI
DIGEST Eco RI Bgl II PURIFY 1250 bp FRAGMENT
DIGEST Mbo I PURIFY 455bp FRAGMENT
Hind III
Mbo I
Sac I
Bgl II
Xba I
Bam HI
Eco RI
CaMV 19S PROMOTER, 5' REGIONS
MODIFIED NPT II STRUCTURAL SEQUENCE
NOS 3' NON-TRANSLATED REGION
NOPALINE SYNTHASE
pMON 120
Spc R
Str
Hind III
Eco RI
DIGEST Eco RI, Hind III, CAP
MIX, LIGATE, TRANSFORM, SELECT Spc R CELLS
NOPALINE SYNTHASE
pMON 155
Spc R
Str
19S PROMOTER 5' REGIONS
Hind III
NOS 3'
MODIFIED NPT II STRUCT. SEQ.
Eco RI
Sac I
Mbo I
Bgl II
Xba I
Bam HI

Figure 5

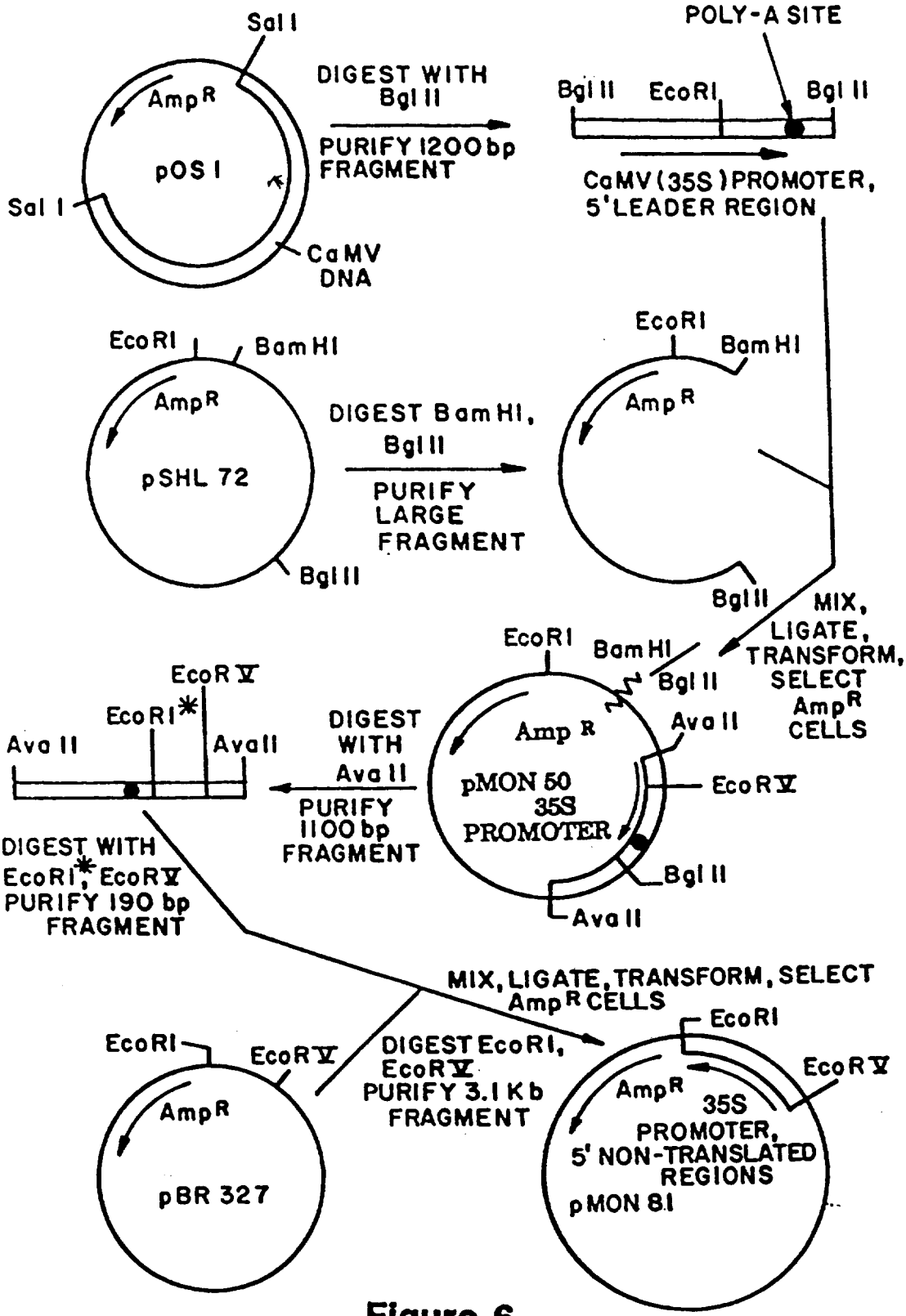
Sal I
AmpR
pOS I
Sal I
CaMV DNA
DIGEST WITH Bgl II
PURIFY 1200 bp FRAGMENT
POLY-A SITE
Bgl II
EcoRI
Bgl II
CaMV (35S) PROMOTER, 5'LEADER REGION
EcoRI
BamHI
AmpR
pSHL 72
Bgl II
DIGEST BamHI, Bgl II
PURIFY LARGE FRAGMENT
EcoRI
BamHI
AmpR
Bgl II
MIX, LIGATE, TRANSFORM, SELECT AmpR CELLS
EcoRI
BamHI
Bgl II
AmpR
pMON 50 35S PROMOTER
Ava II
EcoRV
Bgl II
Ava II
DIGEST WITH Ava II
PURIFY 1100 bp FRAGMENT
EcoRV
EcoRI*
Ava II
Ava II
DIGEST WITH EcoRI*, EcoRV
PURIFY 190 bp FRAGMENT
MIX, LIGATE, TRANSFORM, SELECT AmpR CELLS
EcoRI
EcoRV
AmpR
pBR 327
DIGEST EcoRI, EcoRV
PURIFY 3.1 Kb FRAGMENT
EcoRI
EcoRV
AmpR
35S PROMOTER, 5' NON-TRANSLATED REGIONS
pMON 81

Figure 6

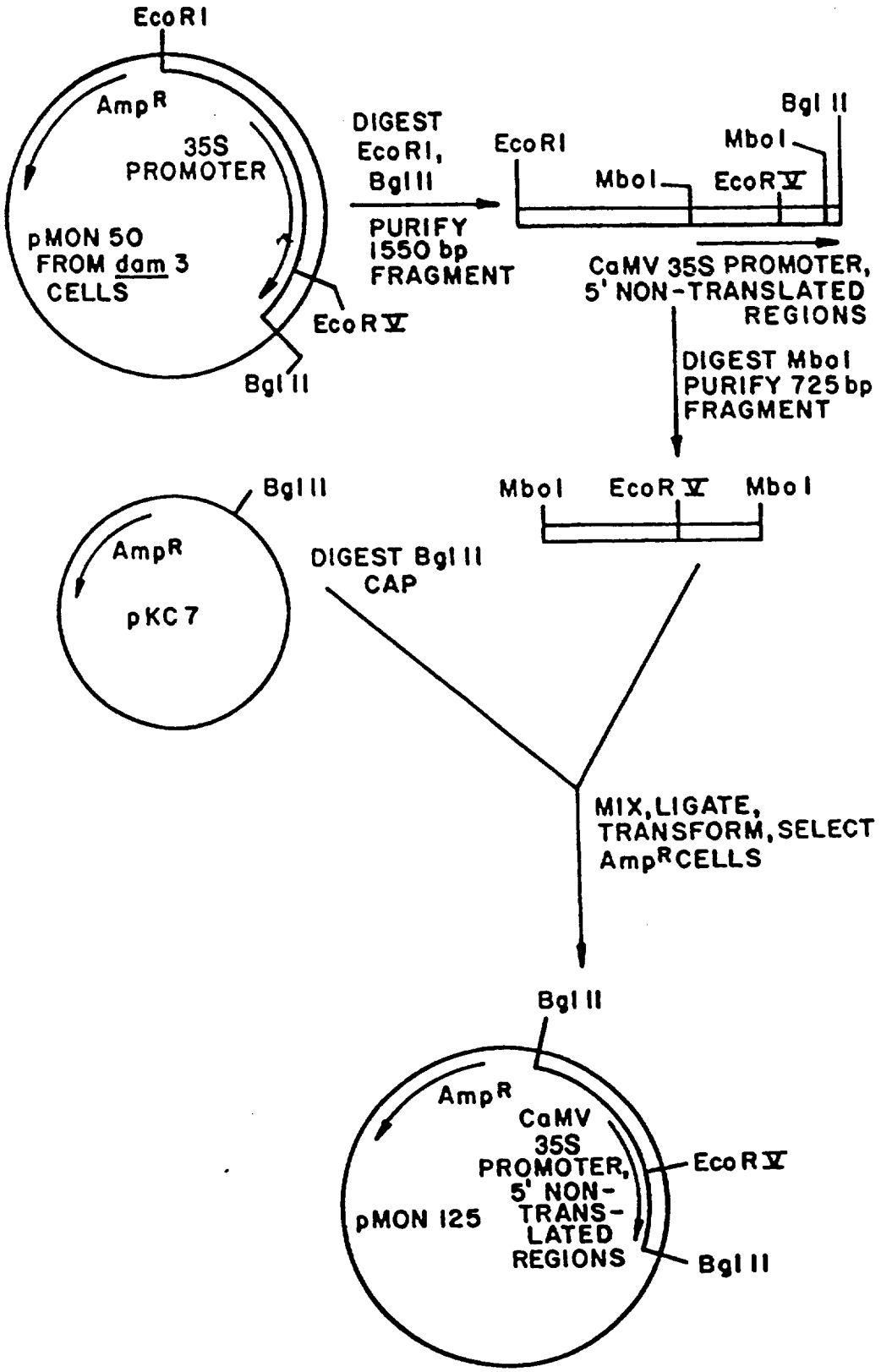
EcoRI
AmpR
35S PROMOTER
pMON 50 FROM dam 3 CELLS
EcoRV
BglII
DIGEST EcoRI, BglII
PURIFY 1550 bp FRAGMENT
EcoRI
MboI
MboI
EcoRV
BglII
CaMV 35S PROMOTER, 5' NON-TRANSLATED REGIONS
DIGEST MboI PURIFY 725 bp FRAGMENT
BglII
AmpR
pKC7
DIGEST BglII CAP
MboI
EcoRV
MboI
MIX, LIGATE, TRANSFORM, SELECT AmpR CELLS
BglII
AmpR
CaMV 35S PROMOTER, 5' NON-TRANSLATED REGIONS
pMON 125
EcoRV
BglII

Figure 7

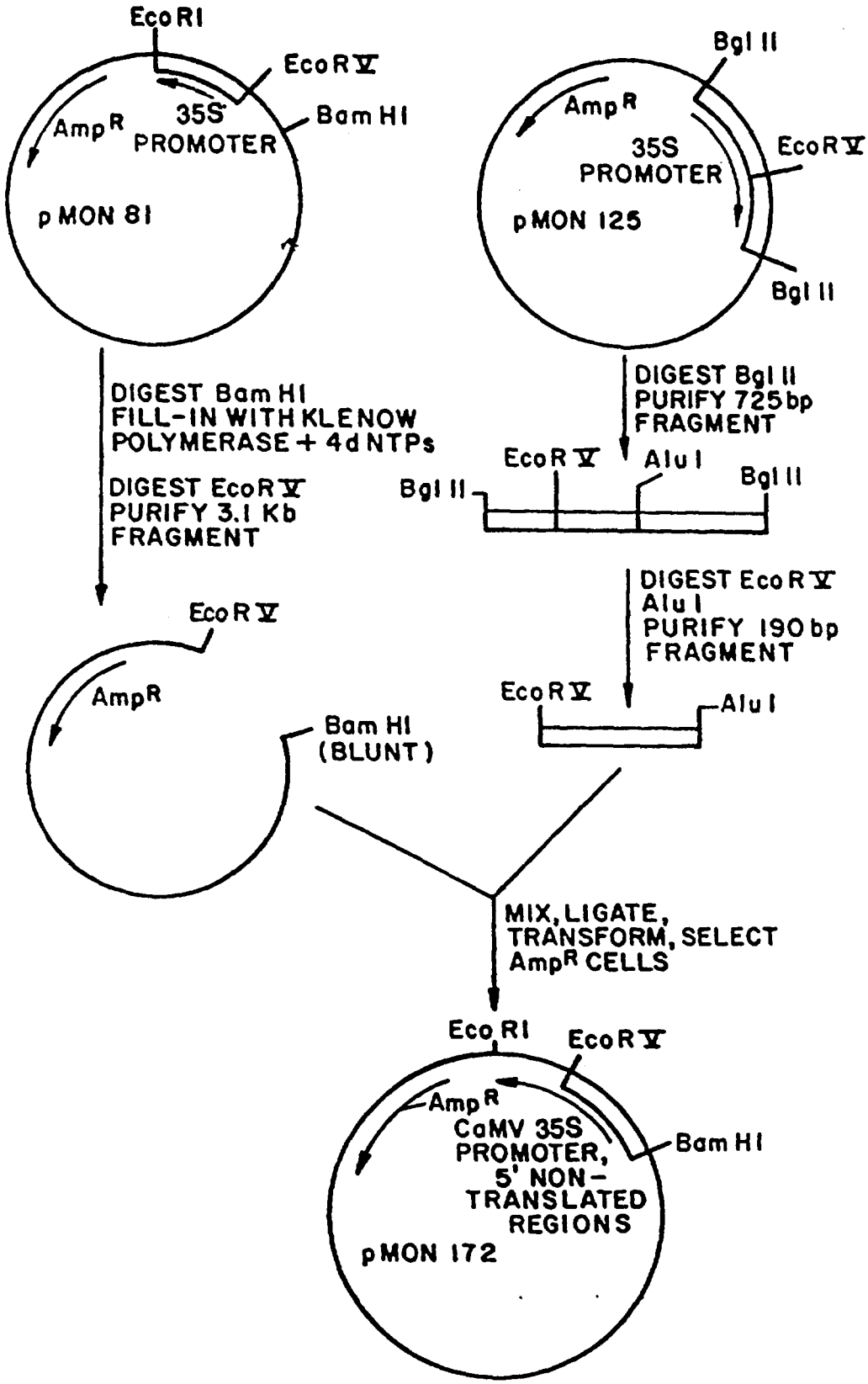
EcoRI
EcoRV
Amp^R
35S PROMOTER
BamHI
pMON 81
Bgl II
Amp^R
35S PROMOTER
EcoRV
pMON 125
Bgl II
DIGEST BamHI
FILL-IN WITH KLENOW
POLYMERASE + 4d NTPs
DIGEST EcoRV
PURIFY 3.1 Kb
FRAGMENT
DIGEST Bgl II
PURIFY 725bp
FRAGMENT
EcoRV
AluI
Bgl II
Bgl II
DIGEST EcoRV
AluI
PURIFY 190 bp
FRAGMENT
EcoRV
AmpR
BamHI
(BLUNT)
EcoRV
AluI
MIX, LIGATE,
TRANSFORM, SELECT
Amp^R CELLS
EcoRI
EcoRV
Amp^R
CaMV 35S
PROMOTER,
5' NON-
TRANSLATED
REGIONS
BamHI
pMON 172

Figure 8

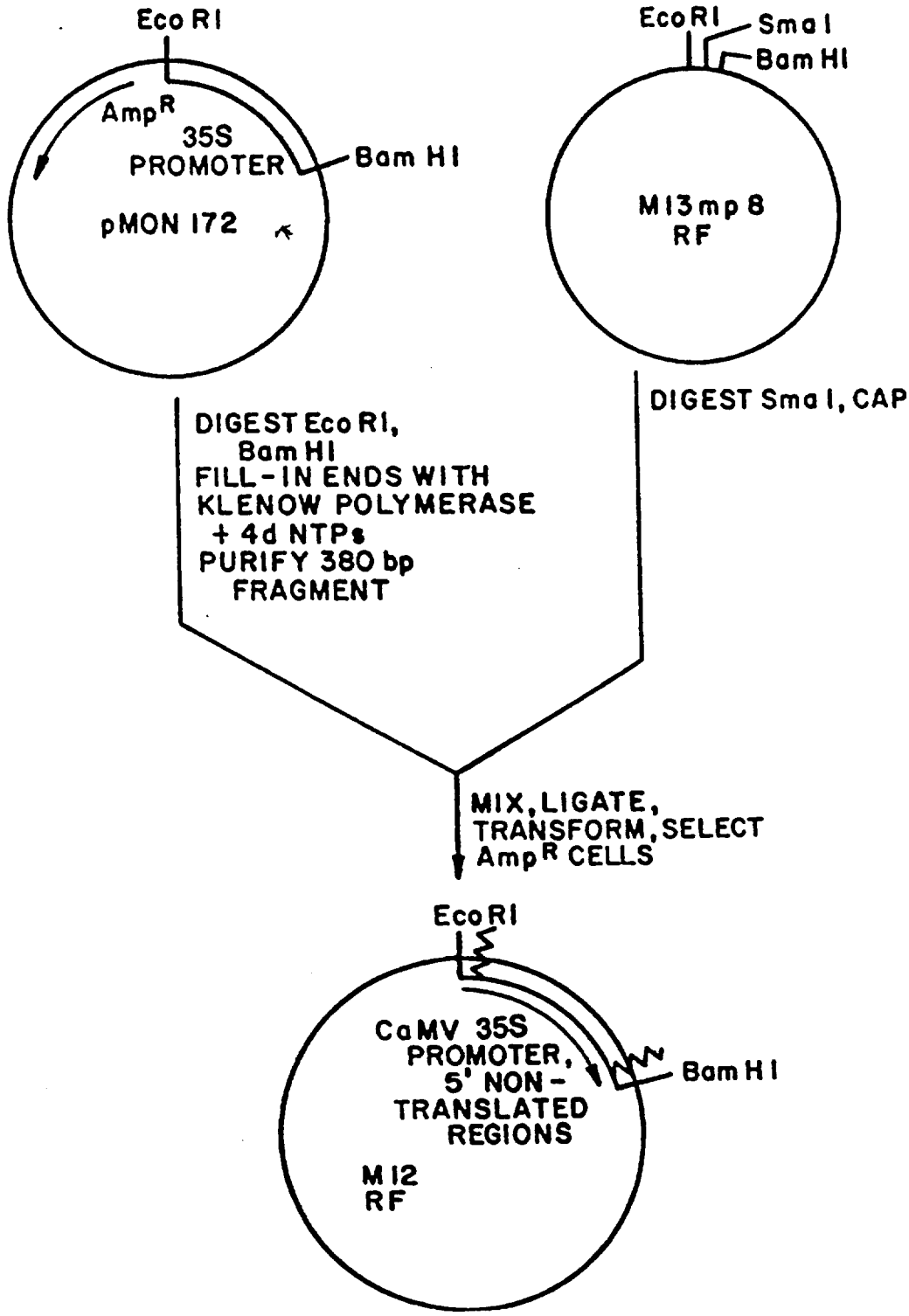
Eco RI
Amp^R
35S
PROMOTER
Bam HI
pMON 172
EcoRI
Sma I
Bam HI
M13mp 8
RF
DIGEST Eco RI,
Bam HI
FILL-IN ENDS WITH
KLENOW POLYMERASE
+ 4d NTPs
PURIFY 380 bp
FRAGMENT
DIGEST Sma I, CAP
MIX, LIGATE,
TRANSFORM, SELECT
Amp^R CELLS
EcoRI
CaMV 35S
PROMOTER,
5' NON-
TRANSLATED
REGIONS
Bam HI
M12
RF

Figure 9

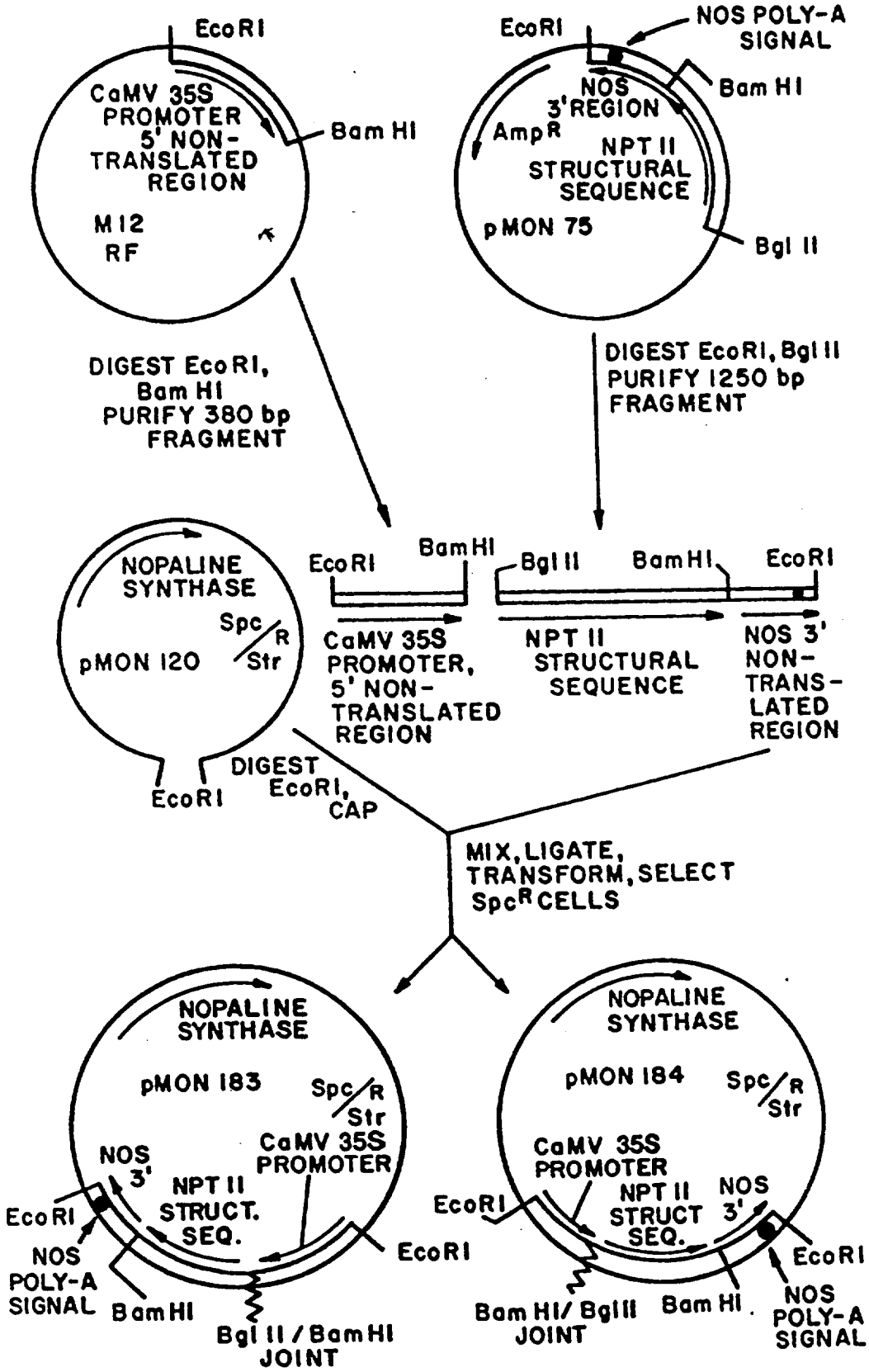
EcoRI
CaMV 35S PROMOTER 5' NON-TRANSLATED REGION
Bam HI
M12 RF
DIGEST EcoRI, Bam HI PURIFY 380 bp FRAGMENT
EcoRI
NOS POLY-A SIGNAL
NOS 3'REGION
Bam HI
AmpR
NPT II STRUCTURAL SEQUENCE
pMON 75
Bgl II
DIGEST EcoRI, Bgl II PURIFY 1250 bp FRAGMENT
NOPALINE SYNTHASE
Spc/Str R
pMON 120
EcoRI
DIGEST EcoRI, CAP
EcoRI
Bam HI
CaMV 35S PROMOTER, 5' NON-TRANSLATED REGION
Bgl II
Bam HI
EcoRI
NPT II STRUCTURAL SEQUENCE
NOS 3' NON-TRANS-LATED REGION
MIX, LIGATE, TRANSFORM, SELECT SpcR CELLS
NOPALINE SYNTHASE
pMON 183
Spc/Str R
NOS 3'
CaMV 35S PROMOTER
NPT II STRUCT. SEQ.
EcoRI
NOS POLY-A SIGNAL
Bam HI
EcoRI
Bgl II / Bam HI JOINT
NOPALINE SYNTHASE
pMON 184
Spc/Str R
CaMV 35S PROMOTER
EcoRI
NPT II STRUCT SEQ.
NOS 3'
EcoRI
Bam HI/ Bgl II JOINT
Bam HI
NOS POLY-A SIGNAL

Figure 10

CHIMERIC GENES FOR TRANSFORMING PLANT CELLS USING VIRAL PROMOTERS

RELATED APPLICATIONS

This is a File Wrapper continuation of application Ser. No. 07/625,637, filed Dec. 7, 1990, now abandoned, which is a continuation of U.S. Ser. No. 06/931,492, filed Nov. 17, 1986, now abandoned, which is a continuation-in-part of U.S. Ser. No. 06/485,568, filed Apr. 15, 1983, now abandoned, which is a continuation-in-part of U.S. Ser. No. 06/458,414, filed Jan. 17, 1983, now abandoned.

TECHNICAL FIELD

This invention is in the fields of genetic engineering and plant biology.

BACKGROUND ART

A virus is a microorganism comprising single or double stranded nucleic acid (DNA or RMA) contained within a protein (and possibly lipid) shell called a "capsid" or "coat". A virus is smaller than a cell, and it does not contain most of the components and substances necessary to conduct most biochemical processes. Instead, a virus infects a cell and uses the cellular processes to reproduce itself.

The following is a simplified description of how a DNA-containing virus infects a cell; RNA viruses will be disregarded in this introduction for the sake of clarity. First, a virus attaches to or enters a cell, normally called a "host" cell. The DNA from the virus (and possibly the entire viral particle) enters the host cell where it usually operates as a plasmid (a loop of extrachromosomal DNA). The viral DNA is transcribed into messenger RNA, which is translated into one or more polypeptides. Some of these polypeptides are assembled into new capsids, while others act as enzymes to catalyze various biochemical reactions. The viral DNA is also replicated and assembled with the capsid polypeptides to form new viral particles. These viral particles may be released gradually by the host cell, or they may cause the host cell to lyse and release them. The released viral particles subsequently infect new host cells. For more background information on viruses see, e.g., Stryer, 1981 and Matthews, 1970 (note: all references cited herein, other than patents, are listed with citations after the examples).

As used herein, the term "virus" includes phages and viroids, as well as replicative intermediates. As used herein, the phrases "viral nucleic acid" and DNA or RNA derived from a virus" are construed broadly to include any DNA or RNA that is obtained or derived from the nucleic acid of a virus. For example, a DNA strand created by using a viral RNA strand as a template, or by chemical synthesis to create a known sequence of bases determined by analyzing viral DNA, would be regarded as viral nucleic acid.

The host range of any virus (i.e., the variety of cells that a type of virus is capable of infecting) is limited. Some viruses are capable of efficient infection of only certain types of bacteria; other viruses can infect only plants, and may be limited to certain genera; some viruses can infect only mammalian cells. Viral infection of a cell requires more than mere entry of the viral DNA or RNA into the host cell; viral particles must be reproduced within the cell. Through various assays, those skilled in the art can readily determine whether any particular type of virus is capable of infecting any particular genus, species, or strain of cells. As used herein, the term "plant virus" is used to designate a virus which is capable of infecting one or more types of plant cells, regardless of whether it can infect other types of cells.

With the possible exception of viroids (which are poorly understood at present), every viral particle must contain at least one gene which can be "expressed" in infected host cells. The expression of a gene requires that a segment of DNA or RNA must be transcribed into or function as a strand of messenger RNA (mRNA), and the mRNA must be translated into a polypeptide. Most viruses have about 5 to 10 different genes, all of which are expressed in a suitable host cell.

In order to be expressed in a cell, a gene must have a promoter which is recognized by certain enzymes in the cell. Gene promoters are discussed in some detail in the parent application Ser. No. 458,414 cited above, the contents of which are incorporated herein by reference. Those skilled in the art recognize that the expression of a particular gene to yield a polypeptide is dependent upon two distinct cellular processes. A region of the 5' end of the gene called the promoter, initiates transcription of the gene to produce a mRNA transcript. The mRNA is then translated at the ribosomes of the cell to yield an encoded polypeptide. Therefore, it is evident that although the promoter may function properly, ultimate expression of the polypeptide depends at least in part on post-transcriptional processing of the mRNA transcript.

Promoters from viral genes have been utilized in a variety of genetic engineering applications. For example, chimeric genes have been constructed using various structural sequences (also called coding sequences) taken from bacterial genes, coupled to promoters taken from viruses which can infect mammalian cell(the most commonly used mammalian viruses are designated as Simian Virus 40 (SV40) and Herpes Simplex Virus (HSV)). These chimeric genes have been used to transform mammalian cells. See, e.g., Mulligan et al 1979; Southern and Berg 1982. In addition, chimeric genes using promoters taken from viruses which can infect bacterial cells have been used to transform bacterial cells; see, e.g., the phage lambda P_L promoter discussed in Maniatis et al, 1982.

Several researchers have theorized that it might be possible to utilize plant viruses as vectors for transforming plant cells. See, e.g., Hohn et al, 1982. In general, a "vector" is a DNA molecule useful for transferring one or more genes into a cell. Usually, a desired gene is inserted into a vector, and the vector is then used to infect the host cell.

Several researchers have theorized that it might be possible to create chimeric genes which are capable of being expressed in plant cells, by using promoters derived from plant virus genes. See, e.g., Hohn et al, 1982, at page 216.

However, despite the efforts of numerous research teams, prior to this invention no one had succeeded in (1) creating a chimeric gene comprising a plant virus promoter coupled to a heterologous structural sequence and (2) demonstrating the expression of such a gene in any type of plant cell.

CAULIFLOWER MOSAIC VIRUS (CaMV)

The entire DNA sequence of CaMV has been published. Gardner et al, 1981; Hohn et al, 1982. In its most

common form, the CaMV genome is about 8000 bp long. However, various naturally occurring infective mutants which have deleted about 500 bp have been discovered; see Howarth et al 1981. The entire CaMV genome is transcribed into a single mRNA, termed the "full-length transcript" having a sedimentation coefficient of about 35S. The promoter for the full-length mRNA (hereinafter referred to as "CaMV(35S)") is located in the large intergenic region about 1 kb counterclockwise from Gap 1 (see Guilley et al, 1982).

CaMV is believed to generate at least eight proteins; the corresponding genes are designated as Genes I through VIII. Gene VI is transcribed into mRNA with a sedimentation coefficient of 19S. The 19S mRNA is translated into a protein designated as P66, which is an inclusion body protein. The 19S mRNA is promoted by the 19S promoter, located about 2.5 kb counterclockwise from Gap 1.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to the use of viral promoters in the expression of chimeric genes in plant cells. In another aspect this invention relates to chimeric genes which are capable of being expressed in plant cells, which utilize promoter regions derived from viruses which are capable of infecting plant cells. One such virus comprises the cauliflower mosaic virus (CaMV). Two different promoter regions have been derived from the CaMV genome and ligated to heterologous coding sequences to form chimeric genes. These chimeric genes have been proven to be expressed in plant cells. This invention also relates to plant cells, plant tissue (including seeds and propagules), and differentiated plants which have been transformed to contain viral promoters and express the chimeric genes of this invention, and to polypeptides that are generated in plant cells by the chimeric genes of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The figures herein are schematic representations; they have not been drawn to scale.

FIG. 1 represents the creation and structure of plasmid pMON93.

FIG. 2 represents the creation and structure of plasmid pMON156.

FIG. 3 represents the creation and structure of plasmid pMON110.

FIG. 4 represents the creation and structure of plasmid pMON132.

FIG. 5 represents the creation and structure of plasmid pMON155.

FIG. 6 represents the creation and structure of plasmid pMON81.

FIG. 7 represents the creation and structure of plasmid pMON125.

FIG. 8 represents the creation and structure of plasmid pMON172.

FIG. 9 represents the creation and structure of phage M12.

FIG. 10 represents the creation and structure of plasmids pMON183 and pMON184.

DETAILED DESCRIPTION OF THE INVENTION

In one preferred embodiment of this invention, a chimeric gene was created which contained the following elements:

1. a promoter region and a 5' non-translated region derived from the CaMV (19S) gene, which codes for the P66 protein;
2. a partial coding sequence from the CaMV (19S) gene, including an ATG start codon and several internal ATG sequences, all of which were in the same frame as a TGA termination sequence immediately inside the desired ATG start codon of the NPTII gene;
3. a structural sequence derived from a neomycin phosphotransferase II (NPTII) gene; this sequence was preceded by a spurious ATG sequence, which was in the same reading frame as a TGA sequence within the NPTII structural sequence; and,
4. a 3' non-translated region, including a poly-adenylation signal, derived from a nopaline synthase (NOS) gene.

This chimeric gene, referred to herein as the CaMV(19S)-NPTII-NOS gene, was inserted into plasmid pMON120 (described in the parent application, Ser. No. 458,414; ATCC accession number 39263) to create a plasmid designated as pMON156. Plasmid pMON156 was inserted into an *Agrobacterium tumefaciens* cell, where it formed a co-integrate Ti plasmid by means of a single crossover event with a Ti plasmid in the *A. tumefaciens* cell, using a method described in the parent application. The chimeric gene in the co-integrate plasmid was within a modified T-DNA region in the Ti plasmid, surrounded by left and right T-DNA borders. *A. tumefaciens* cells containing the co-integrate Ti plasmids with the CaMV(19S)-NPTII-NOS genes were used to infect plant cells, using a method described in the parent application. Some of the plant cells were genetically transformed, causing them to become resistant to an antibiotic (kanamycin) at concentrations which are toxic to untransformed plant cells.

A similar chimeric gene was created and assembled in a plasmid designated as pMON155. This chimeric gene resembled the gene in pMON156, with two exceptions:

1. an oligonucleotide linker having stop codons in all three reading frames was inserted between the CaMV(19S) partial structural sequence and the NPTII structural sequence; and,
2. the spurious ATG sequence on the 5' side of the NPTII structural sequence was deleted.

The construction of this chimeric gene is described in Example 2. This gene was inserted into *A. tumefaciens* cells and subsequently into plant cells. Its level of expression was apparently higher than the expression of the similar gene in pMON156, as assayed by growth on higher concentrations of kanamycin.

CREATION OF PLASMIDS pMON183 and 184; CaMV(35S)

In an alternate preferred embodiment of this invention, a chimeric gene was created comprising

(1) a promoter region which causes transcription of the 35S mRNA of cauliflower mosaic virus, CaMV(35S);

(2) a structural sequence which codes for NPTII; and

(3) a nopaline synthase (NOS) 3' non-translated region.

The assembly of this chimeric gene is described in Example 3. This gene was inserted into plant cells and it caused them to become resistant to kanamycin.

Petunia plants cannot normally be infected by CaMV. Those skilled in the art may determine through routine experimentation whether any particular plant

viral promoter (such as the CaMV promoter) will function at satisfactory levels in any particular type of plant cell, including plant cells that are outside of the normal host range of the virus from which the promoter was derived.

It is possible to regenerate genetically transformed plant cells into differentiated plants. One method for such regeneration was described in U.S. patent application entited "Genetically Transformed Plants", Ser. No. 458,402, now abandoned. That application was filed simultaneously with, and incorporated by reference into, the parent application of this invention. The methods of application Ser. No. 458,402, now abandoned, may be used to create differentiated plants (and their progeny) which contain and express chimeric genes having plant virus promoters.

It is possible to extract polypeptides generated in plant cells by chimeric genes of this invention from the plant cells, and to purify such extracted polypeptides to a useful degree of purity, using methods and substances known to those skilled in the art.

Those skilled in the art will recognize, or may ascertain using no more than routine experimentation, numerous equivalents to the specific embodiments described herein. Such equivalents are within the scope of this invention, and are covered by the claims below.

EXAMPLES

Example 1: Creation and Use of pMON156

Plasmids which contained CaMV DNA were a gift to Monsanto Company from Dr. R. J. Shepherd, University of California, Davis. To the best of Applicants' knowledge and belief, these plasmids (designated as pOS1) were obtained by inserting the entire genome of a CaMV strain designated as CM4-184 (Howarth et al, 1981) into the Sal I restriction site of a pBR322 plasmid (Bolivar et al, 1978). *E. coli* cells transformed with pOS1 were resistant to ampicillin (Amp^R) and sensitive to tetracycline (Tet^S).

Various strains of CaMV suitable for isolation of CaMV DNA which can be used in this invention are publicly available; see, e.g., ATCC Catalogue of Strains II, p. 387 (3rd edition, 1981).

pOS1 DNA was cleaved with HindIII. Three small fragments were purified after electrophoresis on an 0.8% agarose gel using NA-45 membrane (Schleicher and Schuell, Keene NH). The smallest fragment, about 500 bp in size, contains the 19S promoter. This fragment was further purified on a 6% acrylamide gel. After various manipulations which did not change the sequence of this fragment (shown in FIG. 1), it was digested with MboI to created 455 bp HindIII-MboI fragment. This fragment was mixed with a 1250 bp fragment obtained by digesting pMON75 (described and shown in FIG. 9 of the parent application Ser. No. 458,414, now abandoned,) with BglII and EcoRI. This fragment contains the NPTII structural sequence and the NOS 3' non-translated region. The two fragments were ligated by their compatible MboI and BglII overhangs to create a fragment containing the CaMV(19S)-NPTII-NOS chimeric gene. This fragment was inserted into pMON120 (described and shown in FIG. 10 of the parent application, Ser. No. 458,414, now abandoned; ATCC accession number 39263) which had been cleaved with HindIII and EcoRI. The resulting plasmid was designated as pMON156, as shown in FIG. 2.

Plasmid pMON156 was inserted into *E. coli* cells and subsequently into *A. tumefaciens* cells where it formed a co-integrate Ti plasmid having the CaMV(19S)-NPTII-NOS chimeric gene surrounded by T-DNA borders. *A. tumefaciens* cells containing the co-integrate plasmids were co-cultivated with petunia cells. The foregoing methods are described in detail in a separate application, entitled "Plasmids for Transforming Plant Cells" Ser. No. 458,411, now abandoned, which was filed simultaneously with and incorporated by reference into parent application, Ser. No. 458,414, now abandoned.

The co-cultivated petunia cells were cultured on media containing kanamycin, an antibiotic which is toxic to petunia cells. Kanamycin is inactivated by the enzyme NPTII, which does not normally exist in plant cells. Some of the co-cultivated petunia cells survived and produced colonies on media containing up to 50 ug/ml kanamycin. This indicated that the CaMV(19S)-NPTII-NOS genes were expressed in petunia cells. These results were confirmed by Southern blot analysis of transformed plant cell DNA.

Example 2: Creation of pMON155

Plasmid pMON72 was obtained by inserting a 1.8 kb HindIII-BamHI fragment from bacterial transposon Tn5 (which contains an NPTII structural sequence) into a PstI- pBR327 plasmid digested with HindIII and BamHI. This plasmid was digested with BglII and PstI to remove the NPTII structural sequence.

Plasmid pMON1001 (described and shown in FIG. 6 of the parent application) from dam- cells was digested with BglII and PstI to obtain a 218 bp fragment with a partial NPTII structural sequence. This fragment was digested with MboI to obtain a 194 bp fragment.

A triple ligation was performed using (a) the large PstI-BglII fragment of pMON72; (b) PstI-MboI fragment from pMON1001; and (c) a synthetic linker with BglII and MboI ends having stop codons in all three reading frames. After transformation of *E. coli* cells and selection for ampicillin resistant colonies, plasmid DNA from Amp^R colonies was analyzed. A colony containing a plasmid with the desired structure was identified. This plasmid was designated pMON110, as shown on FIG. 3.

In order to add the 3' end of the NPTII structural sequence to the 5' portion in pMON110, pMON110 was treated with XhoI. The resulting overhanging end was filled in to create a blunt end by treatment with Klenow polymerase and the four deoxy-nucleotide triphosphates (dNTP's), A, T, C, and G. The Klenow polymerase was inactivated by heat, the fragment was digested with PstI, and a 3.6 kb fragment was purified. Plasmid pMON76 (described and shown in FIG. 9 of the parent application) was digested with HindIII, filled in to create a blunt end with Klenow polymerase and the four dNTP's, and digested with PstI. An 1100 bp fragment was purified, which contained part of the NPTII structural sequence, and a nopaline synthase (NOS) 3' non-translated region. This fragment was ligated with the 3.6 kb fragment from pMON110. The mixture was used to transform *E. coli* cells; Amp R cells were selected, and a colony having a plasmid with the desired structure was identified. This plasmid was designated pMON132, as shown on FIG. 4. Plasmid pMON93 (shown on FIG. 1) was digested with HindIII, and a 476 bp fragment was isolated. This fragment was digested with MboI, and a 455 bp HindIII-MboI fragment was purified which contained the CaMV (19S) promoter region, and 5' non-translated region.

Plasmid pMON132 was digested with EcoRI and BglII to obtain a 1250 bp fragment with (1) the synthetic linker equipped with stop codons in all three reading frames; (2) the NPTII structural sequence; and (3) the NOS 3' non-translated region. These two fragments were joined together through the compatible MboI nd BglII ends to create a CaMV (19S)-NPTII-NOS chimeric gene.

This gene was inserted into pMON120, which was digested with HindIII and EcoRI, to create plasmid pMON155, as shown in FIG. 5.

Plasmid pMON155 was inserted into *A. tumefaciens* GV3111 cells containing a Ti plasmid, pTiB6S3. The pMON155 plasmid formed a cointegrate plasmid with the Ti plasmid by means of a single crossover event. Cells which contain this co-integrate plasmid have been deposited with the American Type Culture Center, and have been assigned ATCC accession number 39336. A fragment which contains the chimeric gene of this invention can be obtained by digesting the co-integrate plasmid with HindIII and EcoRI, and purifying the 1.7 kb fragment. These cells have been used to transform petunia cells, allowing the petunia cells to grow on media containing at least 100 ug/ml kanamycin.

Example 3: Creation of pMON183 and 184

Plasmid pOS1 (described in Example 1) was digested with BglII, and a 1200 bp fragment was purified. This fragment contained the 35S promoter region and part of the 5' non-translated region. It was inserted into plasmid pSHL72 which had been digested with BamHI and BglII (pSHL72 is functionally equivalent to pAGO60, described in Colbere-Garapin et al, 1981). The resulting plasmid was designated as pMON50, as shown on FIG. 6.

The cloned BglII fragment contains a region of DNA that acts as a polyadenylation site for the 35S RNA transcript. This polyadenylation region was removed as follows: pMON50 was digested with AvaII and an 1100 bp fragment was purified. This fragment was digested with EcoRI* and EcoRV. The resulting 190 bp EcoRV-EcoRI* fragment was purified and inserted into plasmid pBR327, which had been digested with EcoRI* and EcoRV. The resulting plasmid, pMON81, contains the CaMV 35S promoter on a 190 bp EcoRV-EcoRI* fragment, as shown in FIG. 6.

To make certain the entire promoter region of CaMV(35S) was present in pMON81, a region adjacent to the 5' (EcoRV) end of the fragment was inserted into pMON81 in the following way. Plasmid pMON50 prepared from dam- cells was digested with EcoRI and BglII and the resultant 1550 bp fragment was purified and digested with MboI. The resulting 725 bp MboI fragment was purified and inserted into the unique BglII site of plasmid pKC7 (Rao and Rogers, 1979) to give plasmid pMON125, as shown in FIG. 7. The sequence of bases adjacent to the two MboI ends regenerates BglII sites and allows the 725 bp fragment to be excised with BglII.

To generate a fragment carrying the 35S promoter, the 725 bp BglII fragment was purified from pMON125 and was subsequently digested with EcoRV and AluI to yield a 190 bp fragment. Plasmid pMON81 was digested with BamHI, treated with Klenow polymerase and digested with EcoRV. The 3.1 kb EcoRV-BamHI(-blunt) fragment was purified, mixed with the 190 bp EcoRV-AluI fragment and treated with DNA ligase. Following transformation and selection of ampicillin-resistant cells, plasmid pMON172 was obtained which carries the CaMV(35S) promoter sequence on a 380 bp BamHI-EcoRI fragment, as shown on FIG. 8. This fragment does not carry the polyadenylation region for the 35S RNA. Ligation of the AluI end to the filled-in BamHI site regenerates the BamHI site.

To rearrange the restriction endonuclease sites adjacent to the CaMV(35S) promoter, the 380 bp BamHI-EcoRI fragment was purified from pMON172, treated with Klenow polymerase, and inserted into the unique smaI site of phage M13 mp8. One recombinant phage, M12, carried the 380 bp fragment in the orientation shown on FIG. 9. The replicative form DNA from this phage carries the 35S promoter fragment on an EcoRI(-5')-BamHI(3') fragment, illustrated below.

```
   EcoRI
1  |                                                                  70
GAATTCCCGATCc TATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAGGTGGCACTACAAATGCCAT
71                                                                  140
CATTGCGATAAAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCAC
141                                                                 210
CCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAT
                                                          TATA
211                                                         |       280
CTCCACTGACGTAAGGGATGACGCACAATCCACTATACCTTCGCAAGACCCTTCCTCTATATAAGGAAGT
                        5'mRNA
281                     |                                           350
TCATTTCATTTGGAGAGGACACGCTGAAATCACCAGTCTCTCTCTACAAATCTATCTCTCTCTATTTTCT
          Extra Translational Initiator                 BamHI
351       |                                             |
CCATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTGGGGATCC
```

Plasmids carrying a chimeric gene CaMV(35S) promoter region-NPTII structural sequence-NOS 3' non-translated region) were assembled as follows. The 380 bp EcoRI-BamHI CaMV(35S) promoter fragment was purified from phage M12 RF DNA and mixed with the 1250 bp BglII-EcoRI NPTII-NOS fragment from pMON75. Joining of these two fragments through their compatible BamHI and BglII ends results in a 1.6 kb CaMV(35S)-NPTII-NOS chimeric gene. This gene was inserted into pMON120 at the EcoRI site in both orientations. The resultant plasmids, pMON183 and 184, appear in FIG. 10. These plasmids differ only in the direction of the chimeric gene orientation.

These plasmids were used to transform petunia cells, as described in Example 1. The transformed cells are capable of growth on media containing 100 ug/ml kanamycin.

COMPARISON OF CaMV(35S) AND NOS PROMOTERS

Chimeric genes carrying the nopaline synthase (NOS) promoter or the cauliflower mosaic virus full-length transcript promoter (CaMV(35S)) were con-

structed. In both cases, the promoters, which contain their respective 5' non-translated regions were joined to a NPTII coding sequence in which the bacterial 5' leader had been modified so that a spurious ATG translational initiation signal (Southern and Berg, 1982) has been removed.

Plasmid pMON200 is a derivative of previously described intermediate vector pMON120 (ATCC accession number 39263). pMON200 contains a modified chimeric nopaline synthase-neomycin phosphotransferasenopaline synthase gene (NOS/NPTII/NOS) which confers kanamycin (Km^R) resistance to the transformed plant. The modified chimeric Km^R gene lacks an upstream ATG codon present in the bacterial leader sequence and a synthetic multilinker with unique HindIII, XhoI, BglII, XbaI, ClaI and EcoRI restriction sites.

Plasmid pMON273 is a derivative of pMON200 in which the nopaline synthase promoter of the chimeric NOS-NPTII-NOS gene has been replaced with the CaMV(35S) promoter.

The CaMV(35S) promoter fragment was isolated from plasmid pOS-1, a derivative of pBR322 carrying the entire genome of CM4-184 as a SalI insert (Howarth et al., 1981). The CM4-184 strain is a naturally occurring deletion mutant of strain CM1841. The nucleotide sequence of the CM1841 (Gardner et al., 1981) and Cabb-S (Franck et al., 1980) strains of CaMV have been published as well as some partial sequence for a different CM4-184 clone (Dudley et al., 1982). The nucleotide sequences of the 35S promoter regions of these three isolates are essentially identical. In the following the nucleotide numbers reflects the sequence of Gardner et al. (1981). The 35S promoter was isolated as an AluI (n 7143)-EcoRI* (n 7517) fragment which was inserted first into pBR322 cleaved with BamHI, treated with the Klenow fragment of DNA polymerase I and then cleaved with EcoRI. The promoter fragment was then excised from pBR322 with BamHI and EcoRI, treated with Klenow polymerase and inserted into the SmaI site of M13 mp8 so that the EcoRI site of the mp8 multilinker was at the 5' end of the promoter fragment. Site directed mutagenesis (Zoller and Smith, 1982) was then used to introduce a G at nucleotide 7464 to create a BglII site. The 35S promoter fragment was then excised from the M13 as a 330 bp EcoRI-BglII site. The 35S promoter fragment was then excised from the M13 as a 330 bp EcoRI-BglII fragment which contains the 35S promoter, 30 nucleotides of the 5' non-translated leader but does not contain any of the CaMV translational initiators nor the 35S transcript polyadenylation signal that is located 180 nucleotides downstream from the start of transcription (Covey et al., 1981; Guilley et al., 1982). The CaMV(35S) promoter sequence described above is listed below.

pMON273 CaMV 35S Promoter and 5' Leader

```
      EcoRI
1     |                                                          70
GAATTCCCGATCe TATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAGGTGGCACTACAAATGCCAT
71                                                               140
CATTGCGATAAAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCAC
141                                                              210
CCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAT
                                                      TATA
211                                                      |       280
CTCCACTGACGTAAGGGATGACGCACAATCCACTATACCTTCGCAAGACCCTTCCTCTATATAAGGAAGT
                          5'mRNA                  BglII
281                       |                       |   334
TCATTTCATTTGGAGAGGACACGCTGAAATCACCAGTCTCTCTCTACAAGATCT
```

The 35S promoter fragment was joined to a 1.3 kb BglII-EcoRI fragment containing the Tn5 neomycin phosphotransferase II coding sequence modified so that the translational initiator signal in the bacterial leader sequence had been removed and the NOS 3' non-translated region and inserted into pMON120 to give pMON273.

These plasmids were transferred in *E. coli* strain JM101 and then mated into *Agrobacterium tumefaciens* strain GV3111 carrying the disarmed pTiB6S3-SE plasmid as described by Fraley et al. (1983).

Plant Transformation

Cocultivation of Petunia protoplasts with *A. tumefaciens*, selection of kanamycin resistant transformed callus and regeneration of transgenic plants was carried out as described in Fraley et al. (1984).

Preparation of DNAs

Plant DNA was extracted by grinding the frozen tissue in extraction buffer (50 mM TRIS-HCl pH 8.0, 50 mM EDTA, 50 mM NaCl, 400 ul/ml EtBr, 2% sarcosyl). Following low speed centrifugation, cesium chloride was added to the supernatant (0.85 gm/ml). The CsCl gradients were centrifuged at 150,000×g for 48 hours. The ethidium bromide was extracted with isopropanol, the DNA was dialyzed, and ethanol precipitated.

Southern Hybridization Analysis

10 ug of each plant DNA was digested, with BamHI for pMON200 plant DNAs and EcoRI for pMON273 plant DNAs. The fragments were separated by electrophoresis on a 0.8% agarose gel and transferred to nitrocellulose (Southern, 1975). The blots were hybridized (50% formamide, 3xSSC, 5X denhardt's, 0.1% SDS and 20 ug/ml tRNA) with nick-translated pMON273 plasmid DNA for 48–60 hours at 42° C.

Preparation of RNA from Plant Tissue

Plant leaves were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The frozen tissue was added to a 1:1 mixture of grinding buffer and PCE (1% Tri-iso-propylnaphtalenesulfonic acid, 6% p-Aminosalicylic acid, 100 mM NaCl, 1% SDS and 50 mM 2-mercaptoethanol; PCI [phenol: chloroform: isoamyl alcohol (24:24:1)] and homogenized immediately with a polytron. The crude homogenate was mixed for 10 min and the phases separated by centrifugation. The aqueous phase then was re-extracted with an equal volume of PCI. The aqueous phase was ethanol precipitated with one tenth volume of 3M NaAcetate and 2.5 volumes of ethanol. The nucleic acid pellet was resuspended in water. An equal volume of 4M lithium chloride LiCl was added and the mix was placed on ice for 1 hour or overnight. Following cen-

trifugation, the pellet was resuspended in water the LiCl precipitation repeated 3 times. The final LiCl pellet was resuspended in water and ethanol precipitated.

Poly (A) containing RNA was isolated by passing total RNA over an Oligo d(T) cellulose Type III (Collaborative Research) column. Quantitation of the poly (A) containing RNA involved annealing an aliquot of the RNA to radio-labeled poly U [(uridylate 5,6-3H)-polyuridylic acid] (New England Nuclear), followed by RNase A treatment (10 ug per ml for 30 minutes at 37° C.). The reaction mix was spotted on DE-81 filter paper, washed 4× with 0.5M NaPhosphate (pH 7.5) and counted. Globin poly (A) containing RNA (BRL) was used as a standard.

Northern Hybridization Analysis

5 ug of poly (A) RNA from each plant source was treated with glyoxal and dimethysulfoxide (Maniatis, 1982). The RNAs were electrophoresed in 1.5% agarose gels (0.01M NaH_2HPO_4, pH 6.5) for 7 hours at 60 volts. The glyoxylated RNAs were electro-blotted (25 mM $NaH_2PO_4NaHPO_4$, pH 6.5) for 16 hours at 125 amps from the gel to GeneScreen® (New England Nuclear). The filters were hybridized as per manufacturer's instructions (50% formamide, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.02% ficoll, 5XSSC, 1.0% SDS, 100 u/ml tRNA and probe) for 48–60 hours at 42° C. with constant shaking. The nick-translated DNAs used as probes were the 1.3 kb BglII/EcoRI NPTII fragment purified from the pMON273 plasmid for detecting the NPTII transcript, and the petunia small subunit gene as an internal standard for comparing the amount of RNA per lane. The membranes were washed 2×100 ml of 2XSSC at room temperature for 5 minutes, 2×100 ml of 2XSSC/1.0% SDS at 65° C. for 30 minutes. The membranes were exposed to XAR-5 film with a DuPont intensifying screen at −80° C.

Neomycin Phosphotransferase Assay

The gel overlay assay was used to determine the steady state level of NPTII enzyme activity in each plant. Several parameters were investigated for optimizing the sensitivity of the assay in plant tissue. Early observations showed that the level of NPTII activity varied between leaves from different positions on the same plant. This variability was minimized when the plant extract was made from pooled tissue. A paper hole punch was used to collect 15 disks from both young and old leaves. Grinding the plant tissue in the presence of micro-beads (Ferro Corp) rather than glass beads increased the plant protein yield 4-fold.

To optimize detection of low levels of NPTII activity a saturation curve was prepared with 10–85 ug/lane of plant protein. For the pMON200 (NOS) plants, NPTII activity was not detectable at less than 50 ug/lane of total protein (2 hour exposure) while activity was detectable at 20 ug/lane for the pMON273 plants. There was a non-linear increase in NPTII activity for pMON200 NOS plants between 40 and 50 ug of protein per lane. This suggested that the total amount of protein may affect the stability of the NPTII enzyme. Supplementing plant cell extracts with 30–45 ug per lane of bovine serum albumin (BSA), resulted in a linear response; NPTII activity increased proportionately as plant protein levels increased. The addition of BSA appears to stabilize the enzyme, resulting in a 20-fold increase in the sensitivity of the assay. Experiments indicate that 25 ug/lane of pMON273 plant protein and 70 ug/lane of pMON200 plant protein was within the linear range of the assay in the presence of BSA. Elimination of SDS from the extraction buffer resulted in a 2-fold increase in assay sensitivity. Leaf disks were pooled from each plant for the assay. The tissue was homogenized with a glass rod in a microfuge tube with 150–200 ul of extraction buffer (20% glycerol, 10% β-mercaptoethanol, 125 mM Tris-HCl pH 6.8, 100 ug/ml bromophenol blue and 0.2% SDS). Following centrifugation in a microfuge for 20 minutes, total protein was determined using the Bradford assay. 25 ug of pMON273/3111SE plant protein or 70 ug of pMON200/3111SE plant protein, supplemented with BSA, was loaded on a native polyacrylamide gel as previously described. The polyacrylamide gel was equilibrated for 30 minutes in water and then 30 minutes in reaction buffer (67 mM TRIS-maleate pH 7.1, 43 mM $MgCl_2$, 400 mM NH_4Cl), transferred onto a glass plate, and overlaid with a 1.5% agarose gel. The overlay gel contained the neomycin phosphotransferase substrates: 450 uCi [γ-^{32}] ATP and 27 ug/ml neomycin sulfate (Sigma). After 1 hour at room temperature a sheet of Whatman P81 paper, two sheets of Whatman 3MM paper, a stack of paper towels and a weight were put on top of the agarose gel. The phosphorylated neomycin is positively charged and binds to the P81 phosphocellulose ion exchange paper. After blotting overnight, the P81 paper was washed 3× in 80° C. water, followed by 7 room temperature washes. The paper was air dried and exposed to XAR-5 film. Activity was quantitated by counting the ^{32}P-radioactivity in the NPTII spot. The NPTII transcript levels and enzyme activities in two sets of transgenic petunia plants were compared. In one set of plants (pMON273) the NPTII coding sequence is preceded by the CaMV(35S) promoter and leader sequences, in the other set of plants (PMON200) the NPTII coding region is preceded by the nopaline synthase promoter and leader sequences. The data indicates the pMON273 plants contain about a 30 fold greater level of NPTII transcript than the pMON200 plants, see Table I below.

TABLE I

QUANTITATION OF NPTII TRANSCRIPT LEVELS AND NPTII ACTIVITY IN pMON273 AND pMON200 PLANTS

Plant Number	Relative NPTII Transcript[a]	Relative NPTII Activity[b]
pMON 273		
3272	682	113
3271	519	1148
3349	547	447
3350	383	650
3343	627	1539
Average	551	779
pMON 200		
2782	0	0.22
2505	0	5.8
2822	0	0
2813	34	19
2818	0	1.0
3612	45	0.33
2823	97	23
Average	19	7
	~30-fold	~110-fold

TABLE I-continued

QUANTITATION OF NPTII TRANSCRIPT LEVELS AND NPTII ACTIVITY IN pMON273 AND pMON200 PLANTS

Plant Number	Relative NPTII Transcript[a]	Relative NPTII Activity[b]
	difference	difference

[a]Numbers derived from silver grain quantitation of autoradiogram. The RNA per lane was determined by filter hybridization to a petunia small subunit gene. The NPTII transcript values obtained with the NPTII probe were normalized for the amount of RNA in each lane.

[b]Numbers represent quantitation of NPT assay. Values were obtained by scintillation counting of 32-P-NPTII spots on the PE-81 paper used in the NPT assay as previously described. Values have been adjusted for the different amounts of protein loaded on the gels (25 ug) for pMON273 and 70 ug for pMON200 plants).

Consistent with this observation is the finding that the pMON273 leaf extracts have higher NPTII enzyme activity than the pMON200 leaf extracts. In several of the transgenic plants, there is a substantial variation in both RNA and enzyme levels which cannot be accounted for by the slight difference in gene copy number. Such "position effects" have been reported in transgenic mice and fruit flies and have not yet been adequately explained at the molecular level. Although, there is not a clear correlation between insert copy number and level of chimeric gene expression, the fact that 4 of the 7 pMON200 transgenic plants contain 2 copies of the NOS-NPTII-NOS gene would suggest that the differential expression of the CaMV(35S) promoter is actually slightly underestimated in these studies.

The constructs described in this comparative example have identical coding regions and 3' non-translated regions, indicating that the differences in the steady state transcript levels of these chimeric genes is a result of the 5' sequences.

COMPARISON OF CaMV19S AND CaMV(35S) PROMOTERS

Chimeric genes were prepared comprising either the CaMV19S or CaMV(35S) promoters. As in the above example, the promoters contained their respective 5' non-translated regions and were joined to a NPTII coding sequence in which the bacterial 5' leader had been modified to remove a spurious ATG translational initiation signal. The constructs tested were pMON203 and pMON204 containing the CaMV19S/NPTII/NOS gene and pMON273 containing the CaMV(35S)/NPTII/NOS gene.

Construction of pMON203

The CaMV 19S promoter fragment was isolated from plasmid pOS-1,a derivative of pBR322 carrying the entire genome of CM4-184 as a SalI insert (Howarth et al., 1981). The CM4-184 strain is a naturally occurring deletion mutant of strain CM1841. The references to nucleotide numbers in the following discussion are those for the sequence of CM1841 (Gardner et al., 1981). A 476 bp fragment extending from the HindIII site at bp 5372 to the HindIII site at bp 5848 was cloned into M13 mp8 for site directed mutagenesis (Zoller and Smith, 1982) to insert an XbaI (5'-TCTAGA) site immediately 5' of the first ATG translational initiation signal in the 19S transcript (Dudley et al., 1982). The resulting 400 bp HindIII-XbaI fragment was isolated and joined to the 1.3 kb XbaI-EcoRI fragment of pMON273 which carries the neomycin phosphotransferase II (NPTI') coding sequence modified so that the extra ATG translational initiation signal in the bacterial leader had been removed and the nopaline synthase 3' nontransiated region (NOS). The resulting 1.7 kb HindIII-EcoRI fragment was inserted into pMON120 between the EcoRI and HindIII sites to give pMON203. The complete sequence of the 19S promoter-NPTII leader is given below.

```
    HindIII
1|                                                              70
AAGCTTTAAAGCTGCAGAAAGGAATTACCACAGCAATGACAAAGAGACATTGGCGGTAATAAATACTATA
71                                                              140
AAGAAATTCAGTATTTATCTAACTCCTGTTCATTTTCTGATTAGGACAGATAATACTCATTTCAAGAGTT
141                                                             210
TTGTTAACCTTAATTACAAAGGAGATTCAAAACTTGGAAGAAACATCAGATGGCAAGCATGGCTTAGCCA
211                                                             280
CTATTCGTTTGATGTTGAACATATTAAAGGAACCGACAACCACTTTGCGGACTTCCTTTCAAGAGAATTC
281                                                             350
AATAAGGTTAATTCCTAATTGAAATCCGAAGATAAGATTCCCACACACTTGTGGCTGATATCAAAAAGGC
          TATA                                    5'  mRNA
351       |                                        |||     402
TACTACCTATATAAACACATCTCTGGAGACTGAGAAAATCAGACCTCCAAGC
      XbaI                  NPTII Initiator Signal
      |                     |
TCTAGACGATCGTTTCGC ATG
```

Construction of pMON204

The 400 bp HindIII-XbaI fragment containing the CaMV19S promoter was joined to a synthetic linker with the sequence:

```
          XbaI                BglII
           |                    |
5'-TCTAGACTCCTTACAACAGATCT
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to add a BglII site to the 3' end of the promoter fragment. The HindIII-BglII fragment was joined to the 1.3 kb BglII-EcoRI fragment of pMON128 that contains the natural, unmodified NPTII coding sequence joined to the NOS 3' nontranslated signals and inserted into the EcoRI and HindIII sites of pMON120. The resulting plasmid is pMON204. The CaMV 19S promoter signals in this plasmid are identical to those in pMON203. The only difference is the sequence of the 5' nontranslated leader sequence which in pMON204 contains the extra ATG signal found in the bacterial leader of NPTII and contains extra bases from the synthetic linker and bacterial leader sequence.

Petunia leaf discs were transformed and plants regenerated as described above. The gel overlay assay was used to determine NPTII levels in transformants.

Quantitation was done by scintillation counting of ^{32}P-neomycin, the end product of neomycin phosphotransferase activity. The average NPTII enzyme level determined for CaMV(35S) (pMON273) plants was 3.6 times higher than that determined for CaMV(19S) (pMON203 & 204) plants.

QUANTITATION OF NPTII ACTIVITY LEVELS IN pMON203, pMON204, AND pMON273 PLANTS

Construct	Plant Number	Relative NPTII Activity[a]	Average	
pMON203	4283	499,064	398,134	
pMON203	4248	297,204		356,203
pMON204	4275	367,580	314,273	
pMON204	4280	260,966		
pMON273	3350	1,000,674	1,302,731	
pMON273	3271	1,604,788		
	$\frac{35_S}{19_S}$	$\frac{1,302,721}{356,203} \approx 3.6$		

[a]Numbers represent quantitation of NPT assay. Values were obtained by scintillation counting of ^{32}P-NPTII spots on the PE-81 paper used in the NPT assay as previously described.

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We claim:

1. A chimeric gene which is expressed in plant cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter isolated from CaMV protein-encoding DNA sequences and a CaMV (19S) promoter isolated from CaMV protein-encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.

2. A chimeric gene of claim 1 in which the promoter is the CaMV(35S) promoter.

3. A chimeric gene of claim 1 in which the promoter is the CaMV(19S) promoter.

4. A plant cell which comprises a chimeric gene that contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter and a CaMV (19S) promoter, wherein said promoter is isolated from CaMV protein-encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.

5. A plant cell of claim 4 in which the promoter is the CaMV(35S) promoter.

6. A plant cell of claim 4 in which the promoter is the CaMV(19S) promoter.

7. An intermediate plant transformation plasmid which comprises a region of homology to an *Agrobacterium tumefaciens* vector, a T-DNA border region from *Agrobacterium tumefaciens* and a chimeric gene, wherein the chimeric gene is located between the T-DNA border and the region of homology, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.

8. A plant transformation vector which comprises a disarmed plant tumor inducing plasmid of *Agrobacterium tumefaciens* and a chimeric gene, wherein the chimeric gene contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.

9. A plant transformation vector of claim 8 in which the promoter is the CaMV(35S) promoter.

10. A plant transformation vector of claim 8 in which the promoter is the CaMV(19S) promoter.

11. The chimeric gene of claim 1 comprising in the 5' to 3' direction:
(1) the CaMV(35S) promoter,
(2) a structural sequence encoding neomycin phosphotransferase II, and
(3) a 3' non-translated polyadenylation sequence of nopaline synthase.

12. The chimeric gene of claim 1 comprising in the 5' to 3' direction:
(1) the CaMV(19S) promoter,
(2) a structural sequence encoding neomycin phosphotransferase II, and
(3) a 3' non-translated polyadenylation sequence of nopaline synthase.

13. A DNA construct comprising:
(A) a CaMV promoter selected from the group consisting of (1) a CaMV 35S promoter isolated from CaMV protein-encoding DNA sequences and (2) a CaMV 19S promoter isolated from CaMV protein-encoding DNA sequences, and
(B) a DNA sequence of interest heterologous to (A), wherein (B) is under the regulatory control of (A) when said construct is transcribed in a plant cell.

14. A chimeric gene which is transcribed and translated in plant cells, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of:
a) a CaMV 35S promoter region free of CaMV protein-encoding DNA sequences and
b) a CaMV 19S promoter region free of CaMV protein-encoding DNA sequences,
and a DNA sequence which is heterologous with respect to the promoter.

15. A chimeric gene which is expressed in plants cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter region free of CaMV protein-encoding DNA sequences and a CaMV(19S) promoter

region free of CaMV protein-encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter.

16. A chimeric gene which is transcribed in plants cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter free of CaMV protein-encoding DNA sequences and a CaMV(19S) promoter free of CaMV protein-encoding DNA sequences, a DNA sequence which is heterologous with respect to the promoter and a 3' non-translated polyadenylation signal sequence.

17. A plant cell which comprises a chimeric gene where said chimeric gene comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, wherein said promoter is free of CaMV protein-encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter and a 3' non-translated polyadenylation signal sequence.

18. An intermediate plasmid of claim 7 in which the promoter is the CaMV(19S) promoter.

19. An intermediate plasmid of claim 7 in which the promoter is the CaMV(35S) promoter.

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