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Dai et al.

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(54) **ISOLATED FUNGAL PROMOTERS AND GENE TRANSCRIPTION TERMINATORS AND METHODS OF PROTEIN AND CHEMICAL PRODUCTION IN A FUNGUS**

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(65) **Prior Publication Data**

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(51) **Int. Cl.**

C07H 21/04	(2006.01)
C12Q 1/68	(2006.01)
C12N 15/00	(2006.01)
C12N 15/63	(2006.01)
C12N 1/00	(2006.01)

(52) **U.S. Cl.** **536/24.1**; 435/6; 435/69.1; 435/320.1

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

(56) **References Cited**

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Jefferson et al., European Molecular Biology Organization Journal, 1987, 3901-3907, vol. 6.

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(57) **ABSTRACT**

The present invention encompasses isolated gene regulatory elements and gene transcription terminators that are differentially expressed in a native fungus exhibiting a first morphology relative to the native fungus exhibiting a second morphology. The invention also encompasses a method of utilizing a fungus for protein or chemical production. A transformed fungus is produced by transforming a fungus with a recombinant polynucleotide molecule. The recombinant polynucleotide molecule contains an isolated polynucleotide sequence linked operably to another molecule comprising a coding region of a gene of interest. The gene regulatory element and gene transcription terminator may temporally and spatially regulate expression of particular genes for optimum production of compounds of interest in a transgenic fungus.

18 Claims, 16 Drawing Sheets

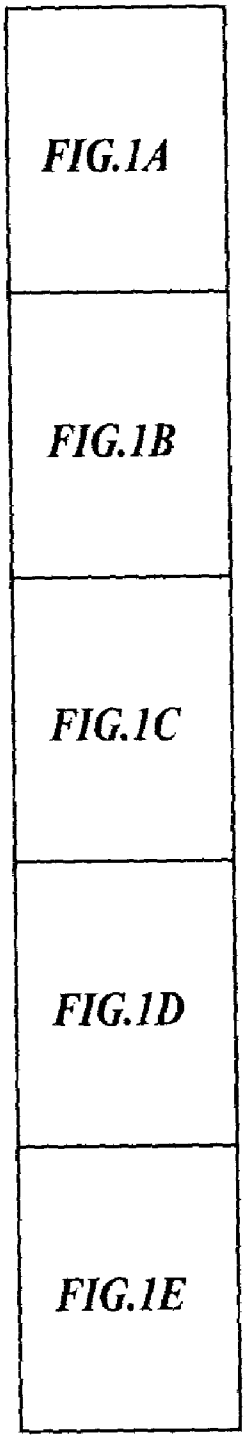

FIG.1

FIG.1A

```
  1 ATCCACAGCAGATGGATCATAAGCAGTCAGACTGCAGGTCAGGTATCGGA    50
  1                                                        0
 51 GTCCGAGACATTCGAACTAGTCTCCGACGCCACTGGAAAAATTCCTGCAC   100
  1                                                        0
101 TCGCCCACACGTGGTAAGCGATACGACTACATATTGTGTGGACAGAGGAA   150
  1                                                        0
151 TGTGGCCTCGAGCAGAGAAAGCTTGCCAACATGAAGATCACTGGCAGGCG   200
  1                                                        0
201 TGCTCATGAAAGCCATTCCGTGGGTTTTGTTTGGATAACCCGCAAGGTAC   250
  1                                                        0
251 ATACTCCGGGAGTGCTTGTCTCTTCAAGGTTCGCAGTATGACGGATCATC   300
  1                                                        0
301 TCCCTTGGTACGAAGGAAGGCATGTTATCAGTTATCGTGCCTTGTTAGTG   350
  1                                                        0
351 GCATTGGCAGTCGGAACGAGGGTCCACTAACCCAGTCAGGAACGAGGAAT   400
  1                                                        0
401 GAGCGACAGGAACCAGAGAATCTTCACCCAACATAGCGATGGATGATCTC   450
  1                                                        0
451 ATCGAGGACGTTGATCACCTCTCTCGCGGGGACTTTCAACGACGAACGGT   500
  1                                                        0
501 CAGTTTGCAGAATGAAACCCCCTTGACAATCTGTTGATCTGCGGCCAGTG   550
                                          ||||.|||||.|
  1                                       ctgcagccagcg    12

551 GGAAGAAA----GGAGGGAGTACGTGG---------------GTAGTAAC   581
    |||.||||    |||||||.|||||||               ||||||||
 13 ggaggaaaaaagggagggactacgtgggtagtagtagtagtagtagtaac    62

582 ATGACTTGTGTGTTTCTTGGTGTCTCTCCGTAGCAATTTAGGCGACCATC   631
    .||||        ||.||.||||.|||||||||||||||||||||.|||
 63 ctgac--------tttttgtgtttctccgtagcaatttaggcgactatc   104

632 CGATTACACGGGGGTGGAGACACCGGACAGGTTCCTTGGTGCCTTT----   677
    |||||||||||||||||.||||||||||||||||||||||||||||
105 cgattacacgggggtggggacaccggacaggttccttggtgcctttgga   154
```

FIG.1B

```
678 -------GGAGGACACGAGATGCGTTTAGTGCCTCTGGTCCCAATATTCG   720
           .|||||||||||||||..||..||||||||||||||||||||||
155 ctttaggagaggacacgagatggattgggtgcctctggtcccaatattcg   204

721 GAAGGTGGTAATTAAACTCTGTGCCTGGCCACTTCGGTGATTTAACGCTT   770
    ||| ||||||||||||||||||||||.||||||||||||| |||||||||
205 gaa-gtggtaattaaactctgtgcctgtccacttcggtga-ttaacgctt   252

771 CGGCCTCGTGGCGTGTCTATGTCTCATTTGTGTCAAACCAGGACGCACCG   820
    ||||||||||||||||||||||||||||||.||.||||||||..||||
253 cggcctcgtggcgtgtctatgtctcatttgtgccagaccaggactgaccg   302

821 GAAGCAGCTGGCAAGGCTCCGGAAGGCGAAGCCAATCAAGCACCACTCGA   870
    ||||.||||||||||||||||||||||||||||||||||||||||..|
303 gaagaagctggcaaggctccggaaggcgaagccaatcaagcaccacttta   352

871 TGAGGGGCACTGATCCATCCATTGTAAATTTTACATGAGGGTAATTTCCC   920
    |||||||||||||||||||||.|||||.||||||||||||||||||||
353 tgaggggcactgatccatccatcgtaaaatttacatgagggtaatttccc   402

921 AGGTAATTTGCCCTGC-GGCTATGTCATTGAGAATGGAAAAGTCTCCGGA   969
    |||||||||||||||| |||||||||||||||||||||||||||||||
403 aggtaatttgccctgcgggctatgtcattgagaatggaaaagtctccgga   452

970 TAATATTTGCCAAAAATGTGAGATGTGTGTGCGTG-------------TG   1006
    ||.|||||||||.||||||||||||||||.|.|.|              ..
453 tattatttgccagaaatgtgagatgtgtgagaggggaaaaaaaaaaaaa   502

1007 TGAAAACGCTCGAGCTTCTGGAAGTGAAACAAAAGCTGAAAGGA--AAGG   1054
     ..||||||||||||||||||||||||||| |||||||.|||||  ||||
503 aaaaaacgctcgagcttctggaagtgaaac-aaagctggaaggaggaagg   551

1055 AGGTG---GTGATGGCGA-TAATGGTGGTGGTGGTGGTGTTTGTTTG   1100
     ||..|   |.|..|.||| .||.||.|||  ...||.||||||||||||||
552 agagggacgagcagacgaggaaaggaggt--aaatgatggtgtttgtttg   599
```

FIG.1C

```
1101 TTTGTTTGCGCGCGAATCCCTTGCGGGCCAAGTTCCACCAAC-GACTTCT   1149
     |||     .|||||||||||||||||||||||||||||||||| .||||||
 600 ttt-----tgcgcgaatcccttgcgggccaagttccaccaacaaacttct    644

1150 CTTTCTACTGTGTCTCTTCGTACTCCGTCCAGCTGCTGCTAGCCATCAAC   1199
     |||||||     .|||||. |.| ||.||||.|           .||||
 645 ctttcta-----actcttt-ttc-ccttccatc------------acaac    675

1200 AACATCCTTCCTTCTCCGTTCTCGGGGTTCCTCCGTTGTTCCTGGCCTGG   1249
     ||||||||.||||||||||||||||||||||||||||||||.|.|||
 676 aacatcctcccttctccgttctcggggttcctccgttgttcctgacttgg    725

1250 TCTGACATAAGGTTATGATTGTTTCACATGTCCCACGGCTTCGCCGGCTT   1299
     ||||||||||||||||||||||||||.||||||||||||||||||||||
 726 tctgacataaggttatgattgtttcacttgtcccacggcttcgccggctt    775

1300 GGAGCTGAGACCCTCTTCTGAGTC-AATGGTACCATTTTGCCGAATTCGT   1348
     .||||||||||||||||||||||| ||||||||||||||||||..||||
 776 agagctgagaccctcttctgagtcaaatggtaccattttgccgatatcgt    825

1349 GGCTAGTTCTC-TATTTCTATGCTCTTGACTTTGGTACCGTTGGCATTAG   1397
     ||||||||||| ||.||.||.|||||.||.|.||||||||||||||||||
 826 ggctagttctcttacttttacgctctggattatggtaccgttggcattag    875

1398 TTTGATC-------TACTAATAAAGAGCCTAGTTTTAGGCGAATATACAC   1440
     |||||||       |||||||||..||||||||||||||||.||||||||
 876 tttgatctattccgtactaataacaagcctagttttaggcggatatacac    925

1441 TGTTACCCACCGGGTAGTATTCAGTAGCTAC--CCTCCCACTCCCC--AG   1486
     ||||||||| |.|||||.||||||||..|||  |||||||||.|.|  ||
 926 tgttaccca-caggtagcattcagtaaatacctcctcccactactcttag    974

1487 GCTCCCACGCTGAGAGCCTTGATTCGATGTCTCTCCTAAAATTGCTAGGC   1536
     ||||||||||.||||||||||||||||||||||||||||||||.||||
 975 gctcccacgctcagagccttgattcgatgtctctcctaaaattgccaggc   1024
```

FIG.1D

```
1537 TGTTAGCGCCCTGGCAGATGAACCCCCGCTCATCCCTCGTATATGCGG--   1584
     ||||||||||||||||||||||||||||||||||||||||||||.|||||
1025 tgttagcgccctggcagatgaacccccgctcatccctcgtatctgcggtc   1074

1585 --------TCTCAATTTCTGAGTGGCCCACGCCT-CCGAGTATCTTTGAG   1625
             |||||||||||||||||||||||||| ||||||||||||||
1075 tcaatttatctcaatttctgagtggcccacgcctcccgagtatctttgag   1124

1626 CACATCCACGATGGAGGGAGGCGATCCAAGCGGTCTAACAGCGGACTAAA   1675
     ||.|||||||||||||||..||||||||||||.||||||||||||||||
1125 catatccacgatggaggggagcgatccaagcggactaacagcggactaaa   1174

1676 CCGCTCTGTGTAAGCCAGTCAGAGAGTCATACTGGCTTGAGGTGACATCG   1725
     |||| | ||||||||||||||||||||||||||||||||||||||||||
1175 ccgc-c-gtgtaagccagtcagagagtcatactggcttgaggtgacatcg   1222

1726 CCAATTCATTTCACAAGGTTTAGTCGGGGGAGGGTAGGCCCCATACATTC   1775
     ||.|||||||||.||||||||||||||||.||||||||||||||||||||
1223 cctattcatttcgcaaggtttagtcggggaagggtaggccccatacattc   1272

1776 CACCGTTCTCAAAGTTTACCAGGCATTTCTCACACTAACCATGCAATAGT   1825
     |||||||||||||||||||||.|||.|||.|.||||||||||||||||||
1273 caccgttctcaaagtttaccagacatctcttagactaaccatgcaatagt   1322

1826 AGGTAACTAGCAGTAGTCTTGAACGCTGTTCCTGAGCAAGTTCCCAATCA   1875
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1323 aggtaactagcagtagtcttgaacgctgttcctgagcaagttcccaatca   1372

1876 GCAATT----TGAAAGAATAATTTCCTTTGACCCACCGGGTAAATGAGCC   1921
     ||||||    ||||||||||||||||.||||||||||||| |||||||||
1373 gcaatttgaatgaaagaataatttcccttgacccaccggg-aaatgagcc   1421
```

FIG.1E

```
1922 GCAGATTTGGCGATGTTGGGCTCGGAGCCTGGTAGGTAGTAGTGAATGTC   1971
     |||||||||||||||||||||||.|||||||||||||||.||||||||||||
1422 gcagatttggcgatgttgggcttggagcctggtaggttgtagtgaatgtc   1471

1972 ATCCCCTCCATAGGGGGGAATT--GGGAGGGGGGCTGTGAATGGACTTGT   2019
     |||||||||||||||||||||||  |.||||||||||||||||.||||||||
1472 atcccctccatagggggaattgagagagggggctgtgaagggacttgt   1521

2020 CCTACGCCTGTCGCATCCCCATCATTCATATACTTGAATG-TCTCTTCTC   2068
     ||||||||||||.||||||||||||||||||||||||||| ||..|||.|
1522 cctacgcctgtcacatccccatcattcatatacttgaatgttcctttccc   1571

2069 CCCCCTCCTCCTTCTCTTTCTCTCCTTCCCTTCTCACGATTTGACGTCCC   2118
     |||||||||||||||||||||||||.||||||||||||||||||||||||
1572 cccccctcctccttctctttctctcgttcccttctcacgatttgacgtccc   1621

2119 TCGCGTTTTCGCCCTCTCCCACGGTAGTCACTCCTTTGCACTACATACAC   2168
     ||||.||||||||||||||||||||||||||||||||||||||||||||||
1622 tcgcattttcgccctctcccacggtagtcactcctttgcactacatacac   1671

2169 GAAGTCTTACTTCCAGTCACTCTTTGAA-ACCACTTCTCAATATCCCTAC   2217
     |||||.||||||||||||||||||||| ..||||...||||||||||||
1672 gaagttttacttccagtcactctttgaattacactctccaatatccctac   1721

2218 CTCTTATCATTCTTTACTTCACGCACAAGACACGAAAGTgaacctgtaaa   2267
     ||..||||||||||||||.||||.||||||||||||||||||...|.|||
1722 ctactatcattctttacatcacacacaagacacgaaagtgaaatcgaaaa   1771

2268 aatg                                                2271
     ||||
1772 aatg                                                1775
```

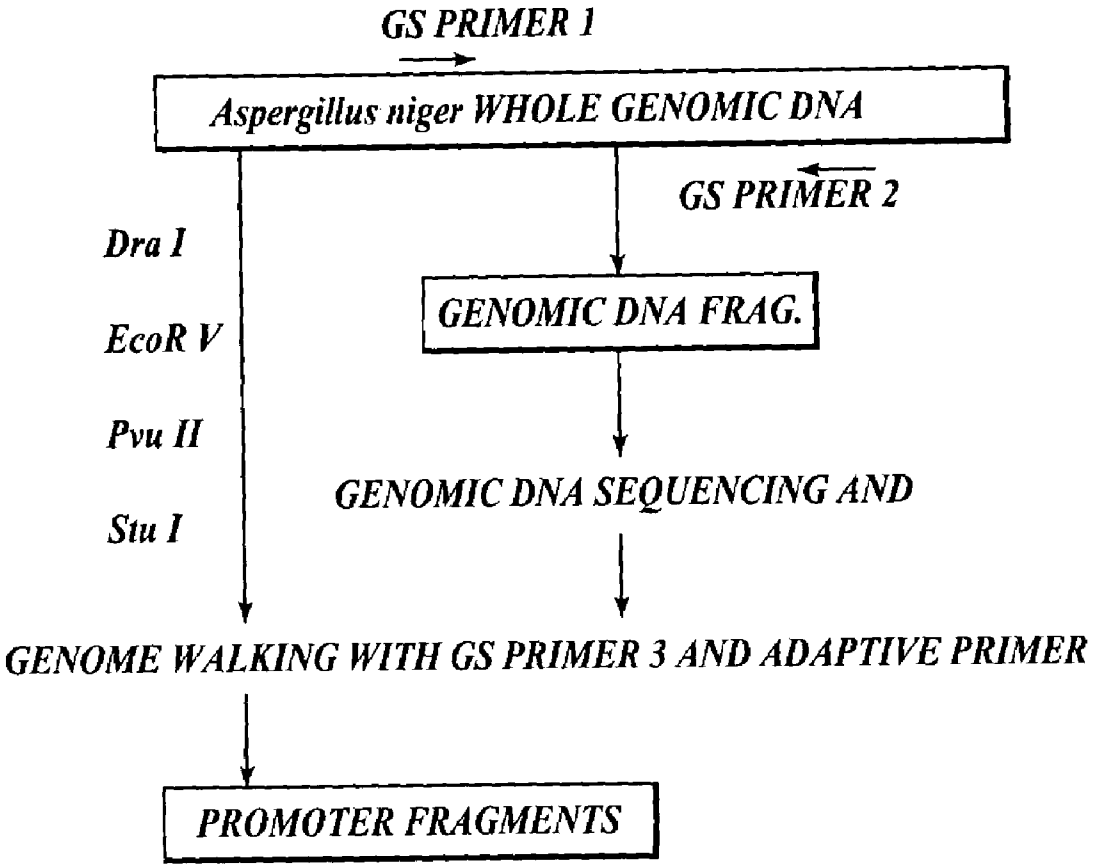
GS PRIMER 1
Aspergillus niger WHOLE GENOMIC DNA
GS PRIMER 2
Dra I
EcoR V
Pvu II
Stu I
GENOMIC DNA FRAG.
GENOMIC DNA SEQUENCING AND
GENOME WALKING WITH GS PRIMER 3 AND ADAPTIVE PRIMER
PROMOTER FRAGMENTS

FIG.2

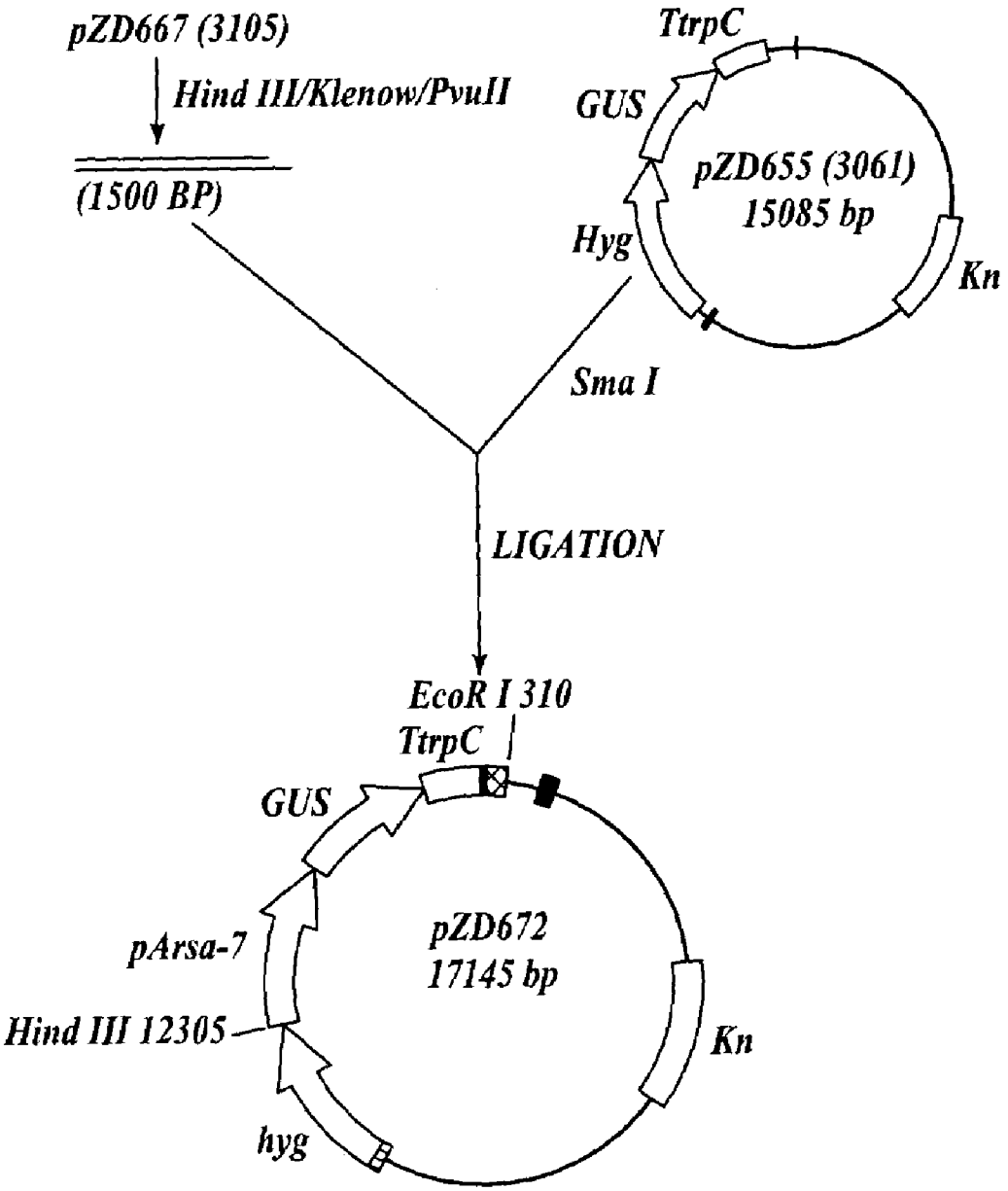
pZD667 (3105)
Hind III/Klenow/PvuII
(1500 BP)
TtrpC
GUS
pZD655 (3061)
15085 bp
Hyg
Kn
Sma I
LIGATION
EcoR I 310
TtrpC
GUS
pZD672
17145 bp
pArsa-7
Hind III 12305
Kn
hyg

FIG.3

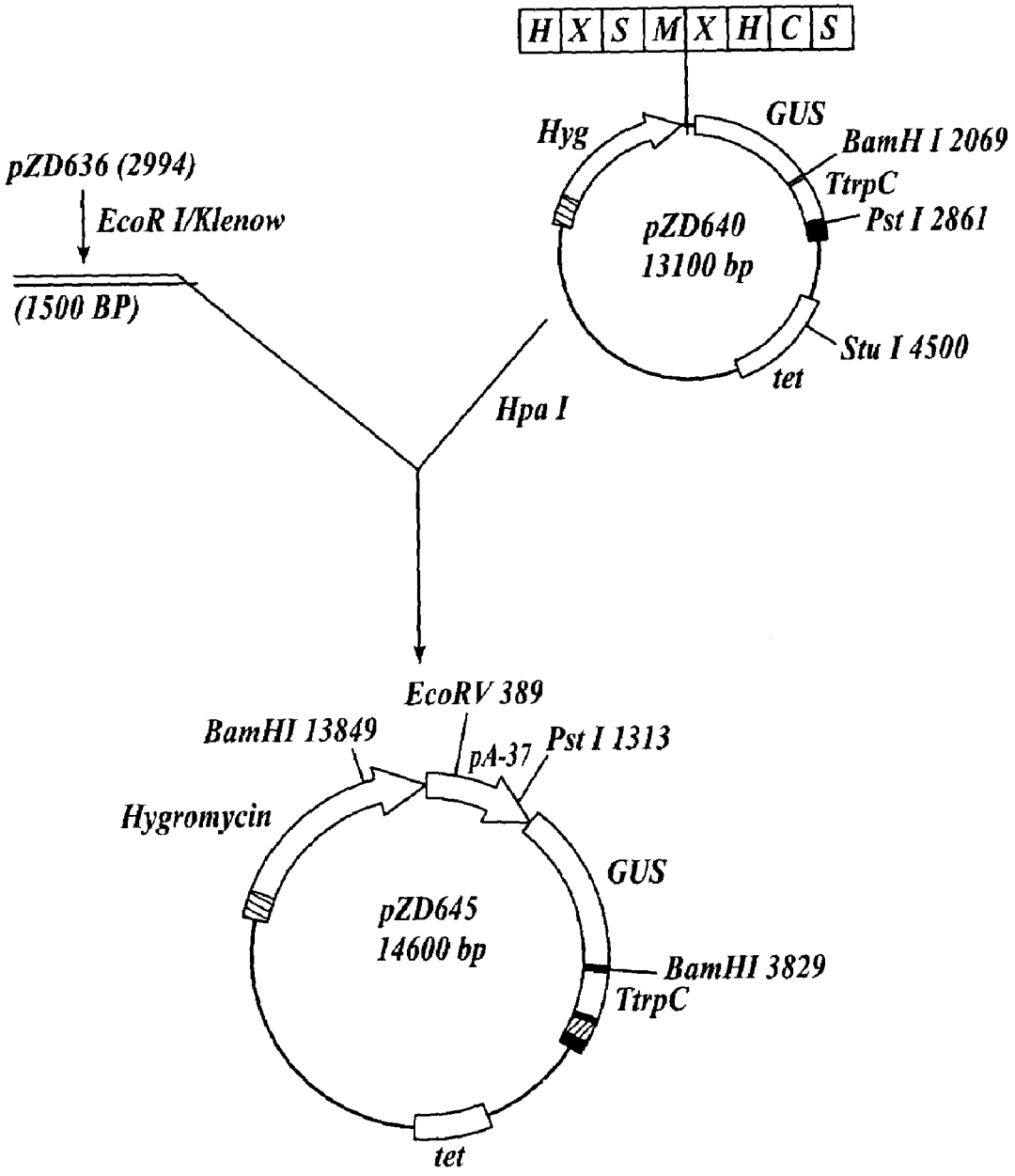
H X S M X H C S
Hyg
GUS
BamH I 2069
TtrpC
Pst I 2861
pZD640
13100 bp
Stu I 4500
tet
pZD636 (2994)
EcoR I/Klenow
(1500 BP)
Hpa I
EcoRV 389
BamHI 13849
pA-37
Pst I 1313
Hygromycin
GUS
pZD645
14600 bp
BamHI 3829
TtrpC
tet

FIG.4

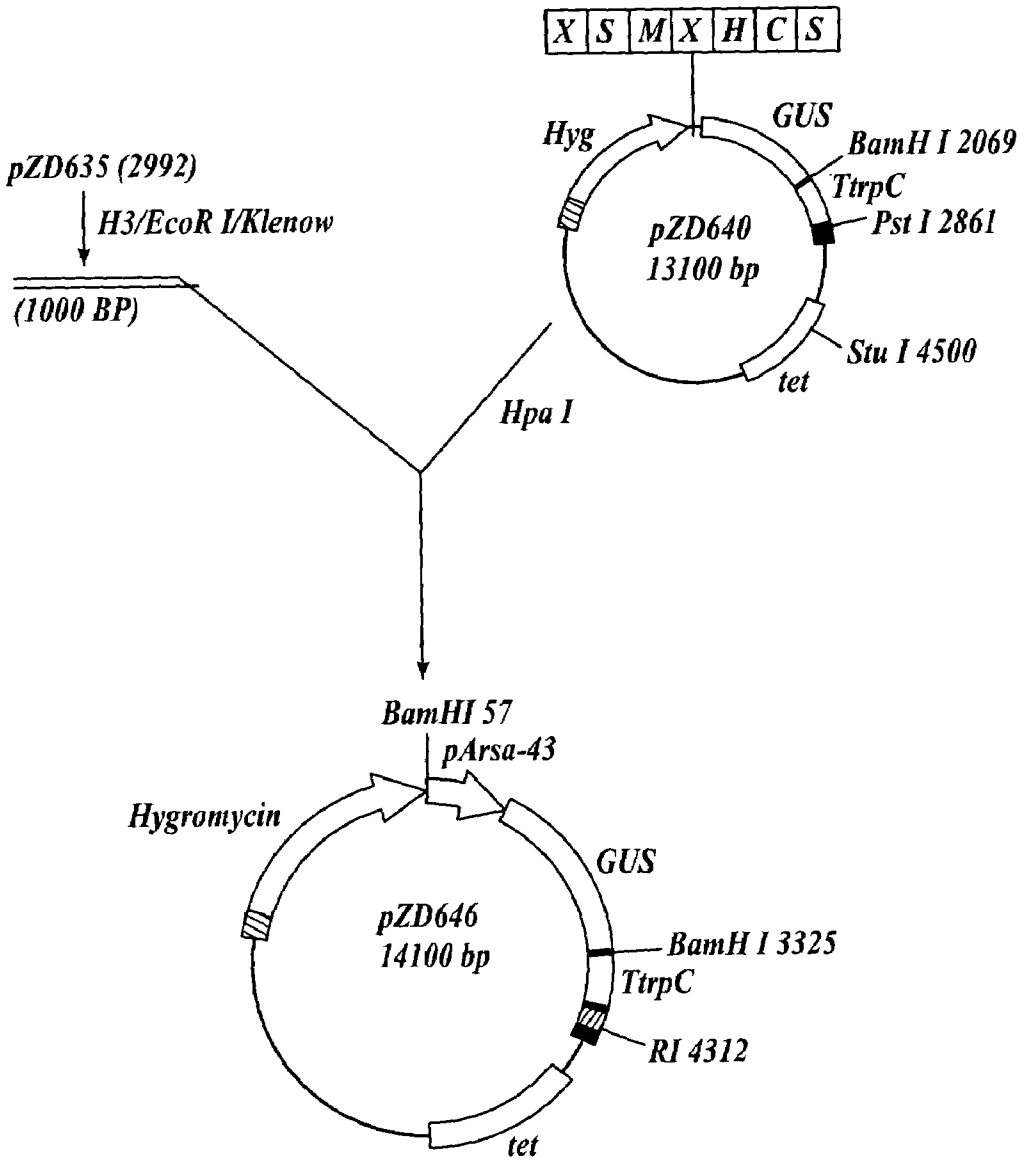
X S M X H C S
Hyg
GUS
BamH I 2069
TtrpC
Pst I 2861
pZD640
13100 bp
Stu I 4500
tet
pZD635 (2992)
H3/EcoR I/Klenow
(1000 BP)
Hpa I
BamHI 57
pArsa-43
Hygromycin
GUS
pZD646
14100 bp
BamH I 3325
TtrpC
RI 4312
tet

FIG.5

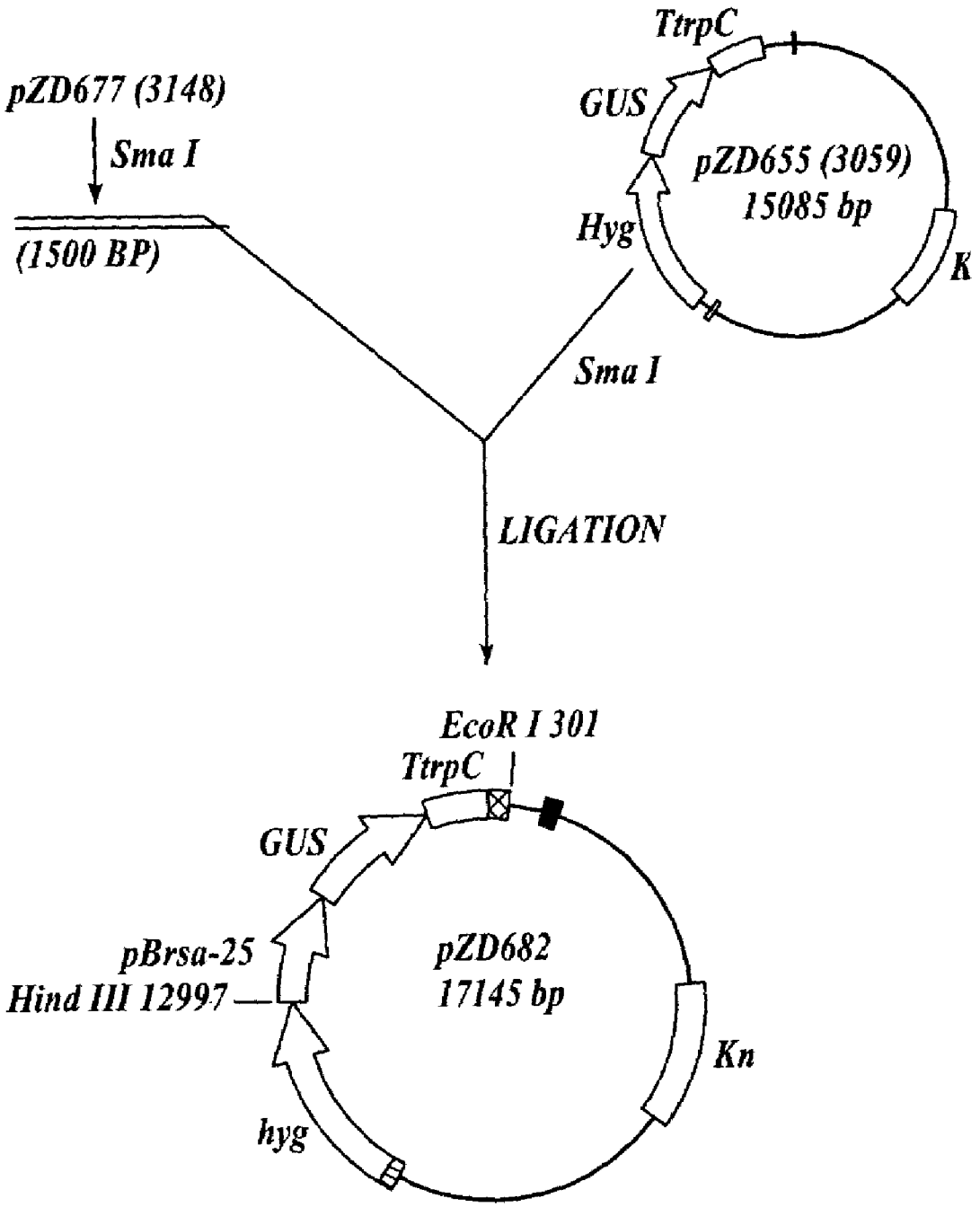
pZD677 (3148)
Sma I
(1500 BP)
TtrpC
GUS
pZD655 (3059)
15085 bp
Hyg
K
Sma I
LIGATION
EcoR I 301
TtrpC
GUS
pBrsa-25
Hind III 12997
pZD682
17145 bp
Kn
hyg

FIG.6

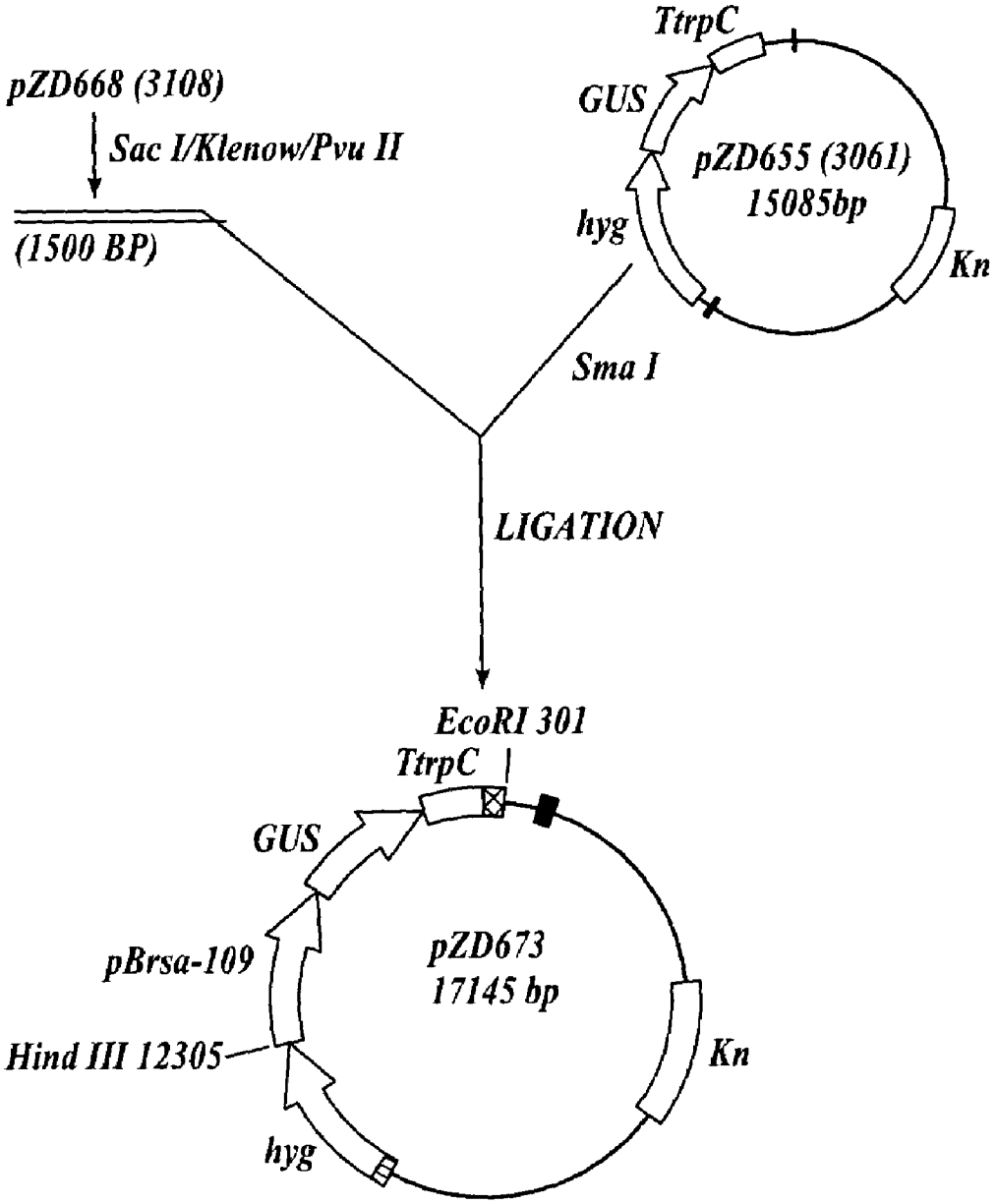
pZD668 (3108)
Sac I/Klenow/Pvu II
(1500 BP)
TtrpC
GUS
pZD655 (3061)
15085bp
hyg
Kn
Sma I
LIGATION
EcoRI 301
TtrpC
GUS
pBrsa-109
pZD673
17145 bp
Hind III 12305
Kn
hyg

FIG. 7

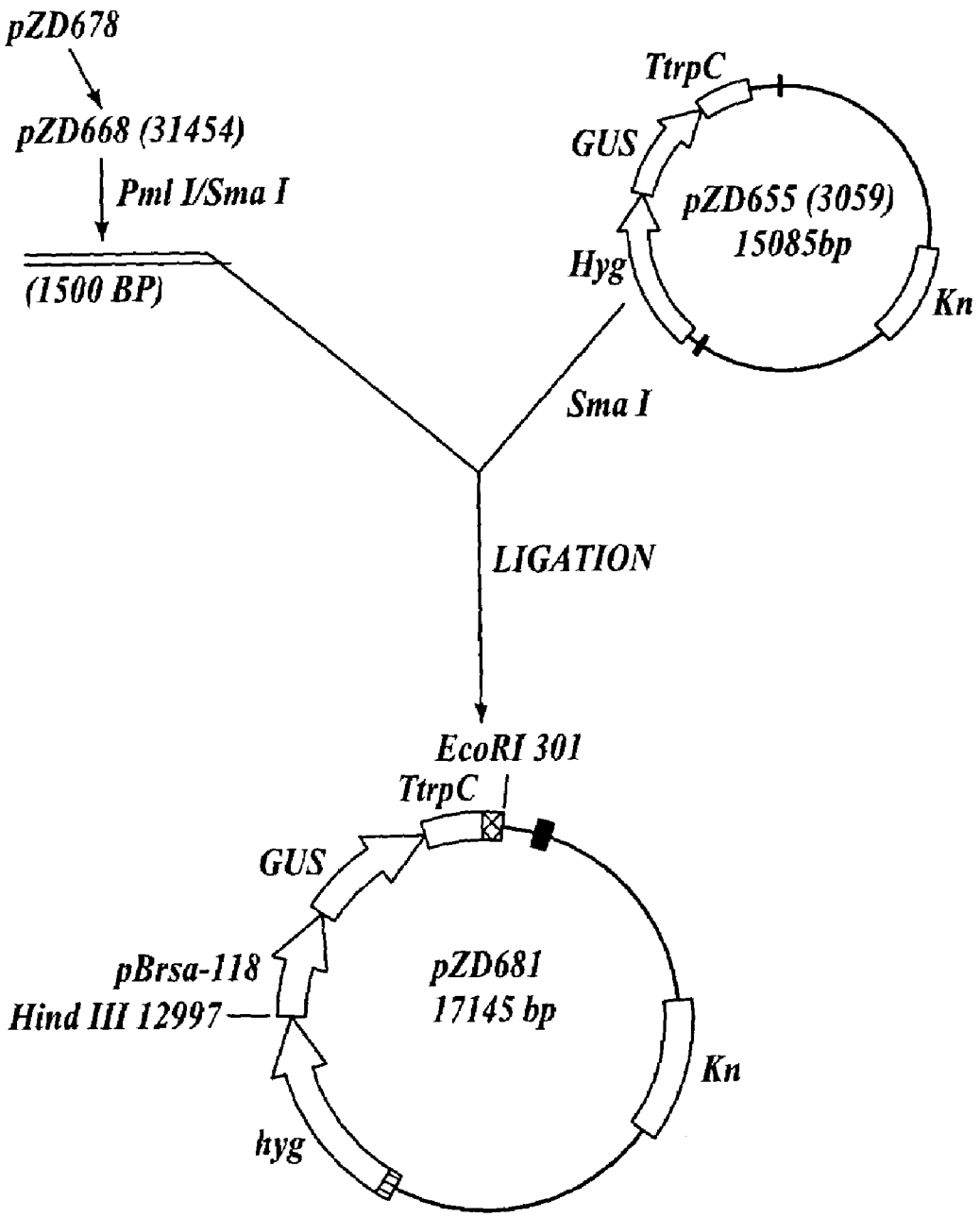
pZD678
pZD668 (31454)
Pml I/Sma I
(1500 BP)
TtrpC
GUS
pZD655 (3059)
15085bp
Hyg
Kn
Sma I
LIGATION
EcoRI 301
TtrpC
GUS
pBrsa-118
Hind III 12997
pZD681
17145 bp
Kn
hyg

FIG. 8

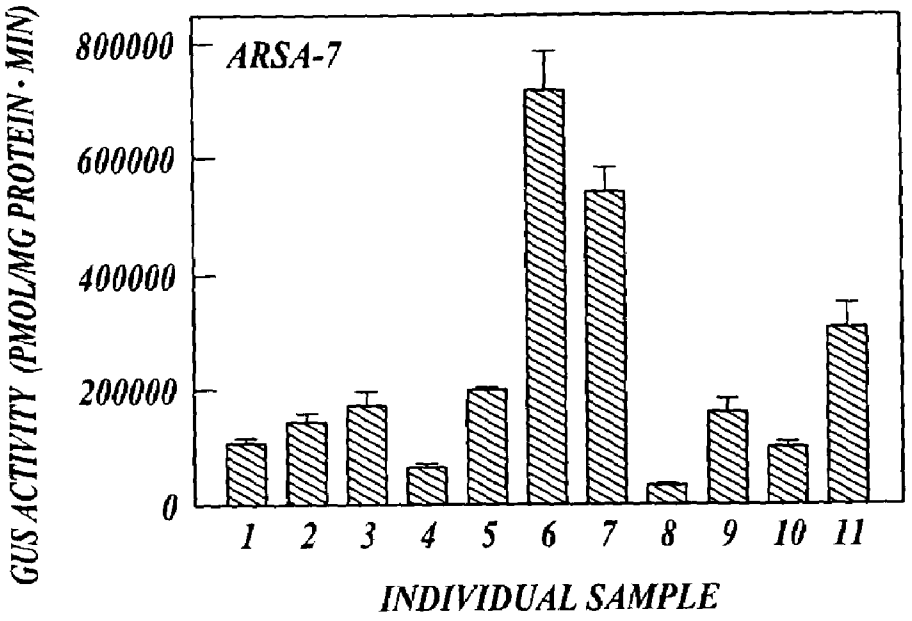
ARSA-7
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
800000
600000
400000
200000
0
1 2 3 4 5 6 7 8 9 10 11
INDIVIDUAL SAMPLE

FIG.9

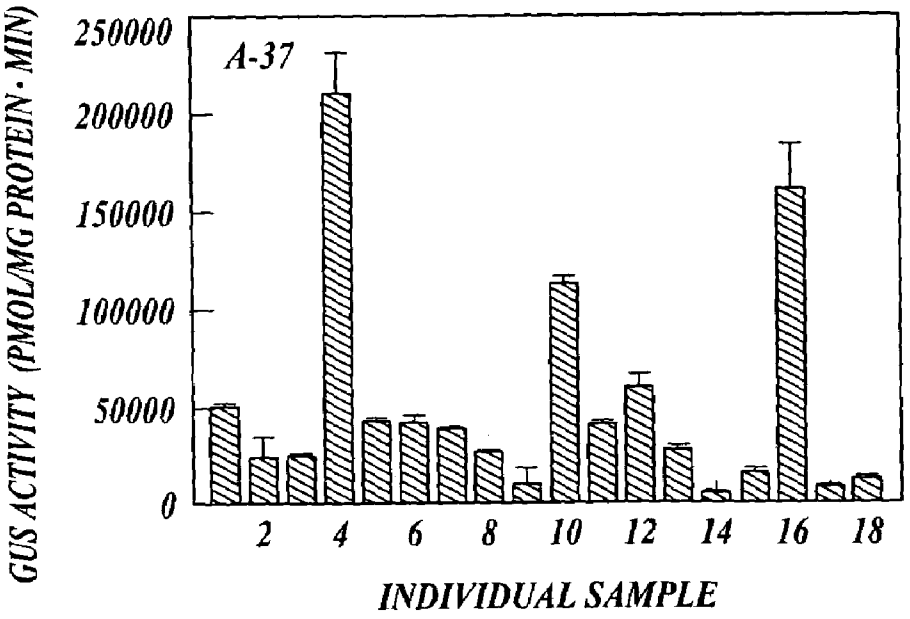
A-37
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
250000
200000
150000
100000
50000
0
2 4 6 8 10 12 14 16 18
INDIVIDUAL SAMPLE

FIG.10

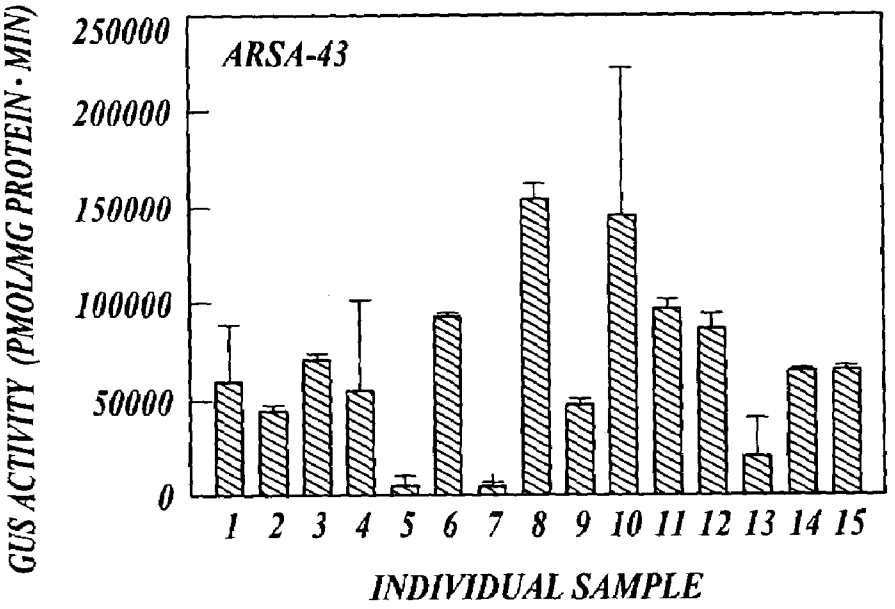
ARSA-43
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
250000
200000
150000
100000
50000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
INDIVIDUAL SAMPLE

FIG.11

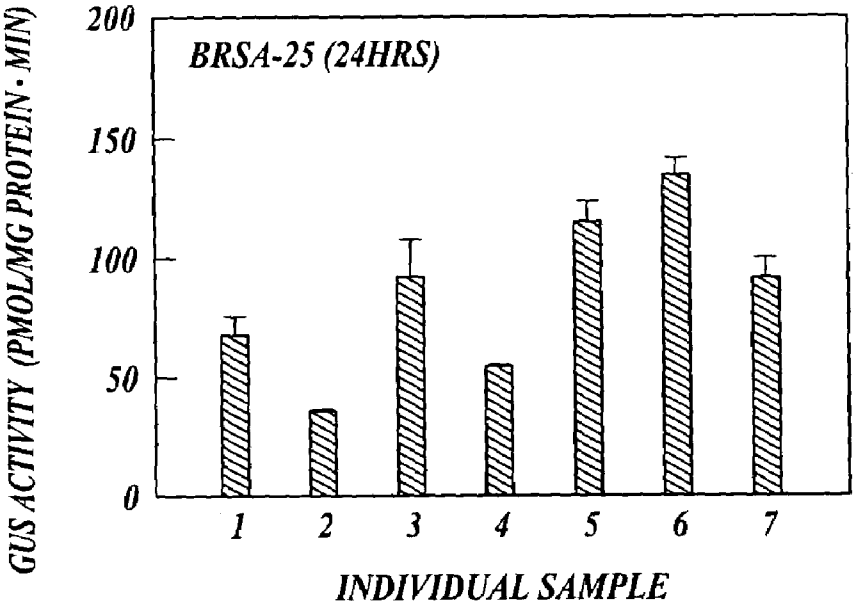
BRSA-25 (24HRS)
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
200
150
100
50
0
1 2 3 4 5 6 7
INDIVIDUAL SAMPLE

FIG.12

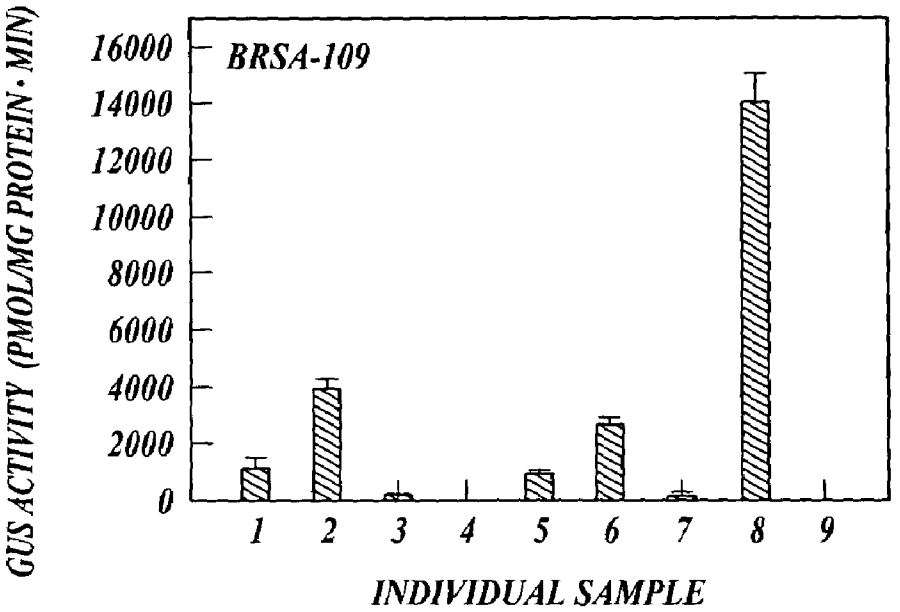
BRSA-109
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
16000
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
9
INDIVIDUAL SAMPLE

FIG.13

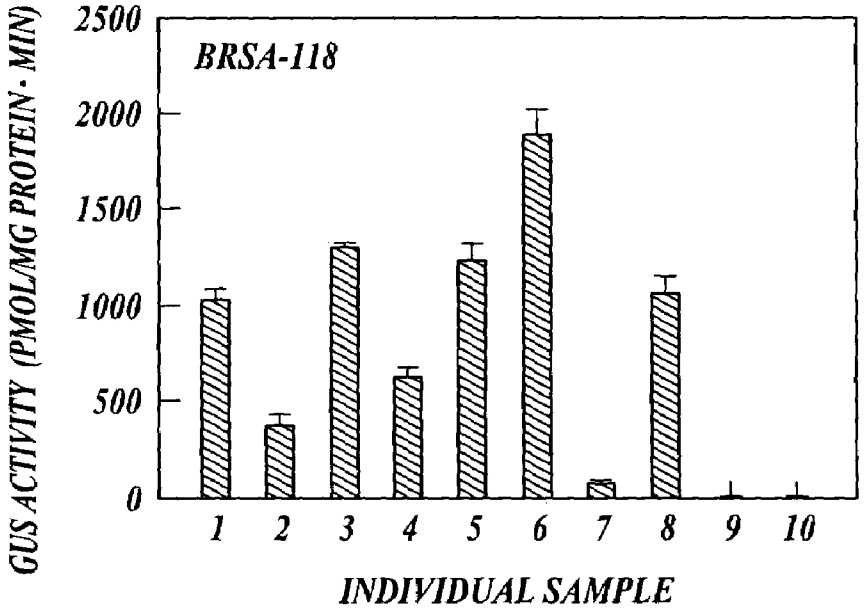
BRSA-118
GUS ACTIVITY (PMOL/MG PROTEIN · MIN)
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
10
INDIVIDUAL SAMPLE

FIG.14

ISOLATED FUNGAL PROMOTERS AND GENE TRANSCRIPTION TERMINATORS AND METHODS OF PROTEIN AND CHEMICAL PRODUCTION IN A FUNGUS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under contract DE-AC0676RLO-1830, awarded by the U.S. Department of Energy. The Government has certain rights in this invention.

TECHNICAL FIELD

The invention pertains to isolated polynucleotide molecules of gene regulatory elements in filamentous fungi. More specifically, the present invention relates to isolation of filamentous fungal promoters and gene transcription terminators, construction of recombinant polynucleotide constructs, and methods for protein and chemical production in a fungus.

BACKGROUND

Fungi are increasingly important in the production of many commercially-useful products. For example, filamentous fungi currently produce a number of metabolites on the industrial scale including antibiotics such as penicillins and cephalosporins, and organic acids such as citric and fumaric acids. Filamentous fungi are also used for the industrial production of enzymes such as proteases and lipases.

Utilization of a filamentous fungus species for production of a desired compound often involves growing submerged cultures of the fungus. Filamentous fungi can exhibit numerous morphologies in submerged cultures, including pelleted and "filamented" morphologies. When fungi in culture exhibit a filamented morphology, the presence of the filaments can increase the viscosity of the culture medium. The increased viscosity can affect the mass transfer and aeration properties of the culture, cause mixing problems in a bioreactor, and result in decreased productivity.

Alternatively, filamentous fungi can exhibit a pelleted morphology. In contrast to cultures of fungi exhibiting a filamented morphology, fungi cultures exhibiting a pelleted morphology can have relatively low viscosities and require substantially less power for mixing and aeration of the culture. Productivity for many compounds, for example citric acid, itaconic acid, statins, penicillins, and various enzymes, can be enhanced by utilizing fungus exhibiting a pelleted morphology. However, in certain fungal species, production of chemicals, for example peptic enzymes or fumaric acid, can be enhanced by utilizing a fungus exhibiting a filamented morphology. Typical practices in fungus-assisted chemical/protein production do not deliberately control the morphology of the fungus.

During fungal-morphology formation, a series of genes are up regulated or down regulated. To achieve optimal production of chemicals and/or proteins of interest, one can utilize the promoters and transcription terminators that exhibit strong constitutive expression of those genes. Concurrently, one can utilize induced gene expression at specific culture conditions and key stages in the cell's development to maximize gene expression and minimize adverse effects on fungal growth that may be associated with the enhanced production of certain chemicals and/or proteins. Thus a need exists for isolated fungal promoters and transcription terminators for regulation of gene expression in a fungus as well as methods for promoting enhanced production of desired chemicals and proteins.

SUMMARY

In view of the foregoing and other problems, disadvantages, and drawbacks of traditional chemical and protein production in a fungus, the present invention has been devised. The invention encompasses isolated polynucleotide molecules comprising polynucleotide sequences that regulate the expression of genes that are differentially expressed in a native fungus exhibiting a pelleted morphology relative to a filamented morphology. In one aspect, the invention encompasses promoters that possess strong, constitutive activity in genes that are differentially expressed in native fungi exhibiting a pelleted morphology relative to a filamented morphology. The invention also encompasses inducible gene promoters that, for example, initiate expression at certain developmental stages in the native fungus. In another aspect, the invention encompasses transcription terminators from genes that are differentially expressed in native fungi exhibiting the pelleted morphology relative to native fungi exhibiting the filamented morphology.

One object of the present invention is to introduce new genetic material into eukaryotic organisms such as filamentous fungi to establish new strains for use in production of chemicals and/or proteins.

Another object of the present invention is to regulate the morphology formation in filamentous fungi.

A further object of the present invention encompasses a method for constitutive production of a compound, such as in chemical and protein production utilizing a transformed host cell.

A still further object of the present invention encompasses a method of induced production of a compound from a transformed host cell.

Another object of the present invention is to use the isolated *Aspergillus niger* (*A. niger*) promoters to regulate expression of foreign genes as well as reintroduced native genes for chemical or protein production.

DESCRIPTION OF DRAWINGS

Preferred embodiments of the invention are described below with reference to the following accompanying drawings.

FIGS. **1**A-**1**E compare the isolated nucleotide sequences for the promoter region of the *A. niger* Balu-42 gene, SEQ ID NO:50 (top sequence), and for the promoter region of the *Aspergillus kawachii* cwpB gene for a hypothetical protein.

FIG. **2** is an illustration of the procedure for promoter and transcription terminator sequence isolation by genome walking.

FIG. **3** is a schematic illustrating a plasmid vector pZD672, which contains the promoter region of the pelleted-associated Arsa-7 gene (SEQ ID NO:46) and the β-glucoronidase (GUS) reporter gene, for *Agrobacterium*-mediated transformation in *A. niger.*

FIG. **4** is a schematic illustrating a plasmid vector pZD645, which contains the promoter region of the pelleted-associated A-37 gene (SEQ ID NO:47) and the GUS reporter gene, for *Agrobacterium*-mediated transformation in *A. niger.*

FIG. **5** is a schematic illustrating a plasmid vector pZD646, which contains the promoter region of the pelleted-associated Arsa-43 gene (SEQ ID NO:48) and the GUS reporter gene, for *Agrobacterium*-mediated transformation in *A. niger.*

FIG. **6** is a schematic illustrating a plasmid vector pZD682, which contains the promoter region of the filamented-associated Brsa-25 gene (SEQ ID NO:51) and the GUS reporter gene, for *Agrobacterium*-mediated transformation in *A. niger.*

FIG. **7** is a schematic illustrating a plasmid vector pZD673, which contains the promoter region of the filamented-asso-

ciated Brsa-109 gene (SEQ ID NO:53) and the GUS reporter gene, for *Agrobacterium*-mediated transformation in *A. niger*.

FIG. **8** is a schematic illustrating a plasmid vector pZD681, which contains the promoter region of the filamented-associated Brsa-118 gene (SEQ ID NO:54) and the GUS reporter gene, for *Agrobacterium*-mediated transformation in *A. niger*.

FIG. **9** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the pelleted-associated Arsa-7 gene (SEQ ID NO:46) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

FIG. **10** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the pelleted-associated A-37 gene (SEQ ID NO:47) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

FIG. **11** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the pelleted-associated Arsa-43 gene (SEQ ID NO:48) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

FIG. **12** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the filamented-associated Brsa-25 gene (SEQ ID NO:51) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

FIG. **13** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the filamented-associated Brsa-109 gene (SEQ ID NO:53) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

FIG. **14** is a plot of the promoter activity for a number of individual *A. niger* strains transformed with the promoter region of the filamented-associated Brsa-118 gene (SEQ ID NO:54) and the GUS reporter gene. The promoter activity is determined via GUS activity assays and is expressed as pmol MU/mg protein/min.

DETAILED DESCRIPTION

For a clear and concise understanding of the specification and claims, including the scope given to such terms, the following definitions are provided:

The filamentous fungi of the present invention are eukaryotic microorganisms and include all filamentous forms of the subdivision Eumycotina. A vegetative mycelium composed of chitin, cellulose, and other complex polysaccharides characterizes these fungi. The filamentous fungi of the present invention are morphologically, physiologically, and genetically distinct from yeasts. Vegetative growth by filamentous fungi is by hyphal elongation while carbon catabolism is obligately aerobic. Various species of filamentous fungi from the three major fungal groups may be used as expression hosts including Basidiomycetes, Ascomycetes, and Zygomycetes. An exemplary member of the Basidiomycetes group is *Phanerochaete chrysosporium*. Exemplary members of the group of Ascomycetes and Imperfect Fungus include *Aspergillus niger, Aspergillus oryzae, Aspergillus terreus, Emericella nidulans, Neurospora crassa, Fusarium oxysporum, Penicillium chrysogenum*, and *Trichoderma reesei*. Exemplary members of the Zygomycetes group include but are not limited to *Rhizomucor miehei* and *Rhizopus oryzae*.

As used herein, the terms filamented and pelleted can refer to the morphology of filamentous fungi. Thus, filamentous fungi can be characterized by having a filamented morphology or a pelleted morphology.

As used herein, a morphology-enhanced promoter can refer to a DNA sequence that, when operably linked to a gene, can exhibit enhanced promoter activity and increased transcription of that gene in a specific morphology compared to some or all other morphologies in an organism. For example, a pelleted-enhanced promoter is a DNA sequence that directs a relatively higher level of transcription for genes associated with a pelleted morphology. An analogous term can be applied to transcription terminators.

A cloning vector is a DNA molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences and marker genes can be inserted in a determinable fashion without loss of an essential biological function of the vector. The marker gene aids in the identification and selection of cells transformed with the cloning vector. Marker genes can typically include genes that provide tetracycline, kanamycin, or ampicillin resistance.

A transgene expression vector can mean a DNA molecule comprising a foreign gene that the host cell expresses. Typically, certain regulatory elements, which include constitutive or inducible promoters, morphology-specific regulatory elements and enhancers, and transcription terminators control expression of the gene. Such a gene is said to be "operably linked to" the regulatory elements.

A recombinant host can be any prokaryotic or eukaryotic cell that contains one or more recombinant DNA molecules, whether or not the DNA is genomically integrated. This term also includes those prokaryotic or eukaryotic cells that have been genetically engineered to contain the cloned gene(s) in the chromosome or genome of the host cell.

A transgenic fungal strain is a fungal strain having one or more fungal cells that contain a foreign gene. In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A DNA molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA.

Constitutive can refer to continuous expression of a gene without any regulation. When used in conjunction with a particular morphology, it can also refer to expression of a gene under all conditions for that morphology.

Homology can refer to the degree of similarity between sequences of nucleic acids or amino acids with regard to positional identity. It can also refer to the concept of similar functional properties among different nucleotide or amino acid sequences.

Foreign gene as used herein can refer to genes from other organisms as well as native genes that are re-introduced to the organism.

Heterologous can refer to aspects, for example, gene expression or proteins, that derive from or relate to different organisms.

The present invention encompasses nine promoters and seven transcription terminators discovered in a fungal strain, *Aspergillus niger* (*A. niger*), which is a citric-acid-producing organism. The nucleotide sequences for the pelleted-enhanced promoters for the Arsa-7, A-37, Arsa-43, and A-90 genes as well as the filamented-enhanced promoters for the Brsa-25, Brsa-47, Brsa-109, and Brsa-118 genes are set forth in SEQ ID NOs. 46-49 and 51-54, respectively. The nucleotide sequence for the promoter for the Balu-42 gene is set forth in SEQ ID NO:50 and has a 66.9% identity to the promoter region of *Aspergillus kawachii* cwpB gene for a hypothetical protein, as shown in FIGS. **1**A-**1**E. The length of filamented-enhanced gene promoter Balu-42 is 2271 base

pairs. Based on a Basic Local Alignment Search Tool (BLAST) search, the remaining promoters show no homology to any known promoters in the GeneBank database, the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) fungi nucleotide database, or the genome database of *A. nidulans, N. crassa*, and *M. grisea*.

The nucleotide sequences for the three filamented-enhanced transcription terminators for the Brsa-25, Brsa-47, and Brsa-118 genes, as well as the four pelleted-enhanced transcription terminators for the Arsa-7, A-37, Arsa-43, and A-90 genes are set forth in SEQ ID NOs. 59-61 and 55-58, respectively. These transcription terminators do not show any significant similarity to known sequences in the GeneBank database, the EMBL-EBI fungi nucleotide database, or the genome database of *A. nidulans, N. crassa*, and *M. grisea*. The genes associated with the 16 regulatory elements encompassed by the resent invention are described in published U.S. patent application Ser. No. 10/442,017, titled "Isolated Polynucleotides and Methods of Promoting a Morphology in a Fungus" by Lasure et al., the contents of which are herein incorporated by reference.

The actual promoter fragments and transcription terminators comprising the polynucleotide sequences set forth in SEQ ID NOs. 46-61 were obtained from *A. niger* strain number 11414 at the American Type Culture Collection (ATCC11414). Culture samples of *A. niger* (filamented morphology) were harvested two days after inducement. The samples were centrifuged to form culture pellets, which were frozen with liquid nitrogen and stored at −80° C. for total genome DNA extraction. Total genomic DNA of *A. niger* was extracted by the cetyltrimethylamrnonium bromide (CTAB) method.

Genome walking served as an effective means for isolating the desired nucleotide sequences. Briefly, the technique consists of digesting genomic DNA with restriction endonuclease Dra I, EcoR V, Puv II, or Stu I and linking the respective fragments with an adaptor oligonucleotide to form four genome walking libraries named DraI, EcoRV, PvuII, or StuI library, respectively. A gene-specific primer (GSP) and an adaptor primer provided by the manufacturer of the GENOMEWALKER™ kits (Clontech Laboratories, Inc., Palo Alto, Calif.) were used to isolate the gene-specific promoter or transcription terminator fragments. The genomic DNA sequence was determined by sequencing the DNA polymerase chain reaction (PCR) products. One GSP was designed for promoter isolation and another one for gene transcription terminator isolation.

Once identified, each of the promoters and transcription terminators described above can be operably linked to additional DNA segments to form DNA constructs. A first DNA segment comprising at least a portion of a functional promoter sequence encompassed by the present invention (SEQ ID NO:46-54) can be operably linked to a second DNA segment comprising a DNA sequence coding a protein of interest. For example, the second DNA segment may comprise a GUS reporter gene or it may comprise a coding sequence that is differentially expressed in a native fungus exhibiting a pelleted morphology relative to the native fungus exhibiting a filamented morphology. Alternatively, the second DNA segment can comprise a sequence encoding a protein of interest which is not natively expressed in fungus, or which does not exhibit morphology-based differential expression in native fungus. Specific examples of proteins of interest include, but are not limited to cellulases, amyglucosidases, amylases, lipases, microbial rennets, xylanases, galactosidases, mannanases, glucanases, phytases, monoclonal antibodies, bovin serium albumin and human blood coagulation-associated proteins. Furthermore, the 3'-end of the second DNA segment in the construct can be operably linked to a third DNA segment comprising a transcription terminator. In a preferred embodiment, the third DNA segment comprises at least a portion of a transcription terminator encompassed by the present invention (SEQ ID NO:55-61).

The present invention can also encompass a vector. A non-limiting example of such a vector can be one that will produce a fungus carrying the DNA sequence of interest, and can comprise, though at low efficiency, a naked piece of DNA capable of conferring the properties of this invention. Another example of a vector includes a transgene expression vector for the fungal strain, *Aspergillus niger*, which utilizes one of the native promoters to regulate the expression of a β-glucoronidase (GUS) reporter gene in *A. niger*. Further, this vector can be used as a chromosomal integration vector for other foreign gene expression in *A. niger*.

Additional examples of vectors can comprise the DNA construct as described above as well as lactate dehydrogenase cDNA from *Rhizopus oryzae* for lactic acid production in *Aspergillus niger*, genes of cellulases from *Trichoderma reesei*, cDNA of hen egg-white lysozyme (HEWL), and cDNA of single chain Fv (scFv) antibody fragments. The DNA fragments, which comprise the coding sequences of any of genes of interest, can be inserted between the 5'-end and the 3'-end of a promoter and a transcription terminator, respectively, of the present invention.

The constructs and vectors as described above can utilize promoter sequences of the present invention having strong, constitutive activity or inducible gene promoters that, for example, initiate expression at certain developmental stages in the native fungus. Examples of developmental stages can include, but are not limited to vegetative, sexual, pelleted morphology formation, and filamentous morphology formation. The early pelleted morphology formation stage can occur approximately 6 to 12 hrs after inoculation of spores into culture medium. Late pelleted morphology formation stage can occur, for example, 3 days after inoculation of spores into the culture medium

The particular method of transformation typically guides selection of an appropriate vector, or whether to even use a vector. For example, a heterologous nucleic acid sequence can be introduced into a fungal cell utilizing *Agrobacterium tumefaciens* containing a Ti plasmid. When using an *A. tumefaciens* culture as a transformation vehicle, it can be most advantageous to use a non-oncogenic strain of the *Agrobacterium* as the vector carrier so that normal non-oncogenic differentiation of the transformed cells is possible. It can also be preferable to have the *Agrobacterium* harbor a binary Ti plasmid system. The binary system comprises 1) a first Ti plasmid having a virulence region that is essential for the introduction of transfer-DNA (T-DNA) into fungi, and 2) a chimeric plasmid. The chimeric plasmid contains at least one border region of the T-DNA region of a wild-type Ti plasmid flanking the nucleic acid to be transferred. Binary Ti plasmid systems have proven to be effective in transforming fungal cells. Such a binary system can be preferred because it typically does not require integration into the Ti plasmid in *Agrobacterium*.

Methods involving the use of *Agrobacterium* include, but are not limited to :1) co-cultivation of *Agrobacterium* with fungal spores; 2) transformation of fungal cells or tissues with *Agrobacterium*; and 3) transformation of fungal protoplasts with *Agrobacterium*.

The construct described herein can also be introduced into a fungal cell chemically through contact between the cell and the construct. For example, nucleic acid may be transferred into fungal cells using polyethylene glycol/$CaCl_2$-mediated genetic material uptake by the fungal cell. Alternatively, the nucleic acid can be introduced into fungal cells by electroporation. In this technique, fungal protoplasts are electroporated in the presence of vectors or nucleic acids containing the

relevant nucleic acid sequences. Electroporated fungal protoplasts can reform the cell wall, divide and form fungal tissues. Selection of the transformed fungal cells with the transformed gene can then be accomplished using phenotypic markers. The nucleic acid can also be introduced into fungal cells by microprojectile particle bombardment (biolistic) transformation. The nucleic acid can be coated on particles for nucleic acid delivery by rupture discs. The particles can comprise tungsten (M5) while the rupture discs can be, for example, 1100-psi rupture discs. The optimal distance between the rupture disc and the tungsten particle carrier and between the launch assembly and target cells can be adjusted to suit different fungal cells.

The vectors described above can be used to facilitate the expression and/or secretion of heterologous proteins in fungal fermentation culture. Fungal cells comprising a transgene expression vector that allows high-level expression of a protein product of interest can be placed and maintained into fungal fermentation cultures and induced using appropriate agents. The protein of interest can be mammalian proteins, plant proteins, fungal proteins, or bacterial proteins, including, but not limited to, human blood factor proteins, plant proteases, fungal cellulases and hemicellulases, and thermally-stable DNA polymerases of bacteria, respectively. The result can be high-level production of the desired heterologous protein. Techniques for isolating the heterologous proteins can include, but are not limited to fractional precipitation, various chromatographies, and ultracentrifugation. In some cases, the proteins produced by the transgenic fungal cells are not the desired product, but are used rather to enhance production of another chemical. In such instances, the transgenic fungal cells of the present invention can be allowed to produce proteins, for example, enzymes, that enhance production of the desired chemical. Chemicals of interest can include, but are not limited to acids and statins. Examples of acids can include aconitic acid, citric acid, fumaric acid, itaconic acid, malic acid, succinic acid, oxalic acid, gluconic acid, and lactic acid. Examples of statins can include lovastatin and compactin

By combining the technology of the present invention with production methods described herein as well as those that are well-established (e.g., fungal fermentation and product recovery), chemical compounds and recombinant proteins can be efficiently and economically produced for the biopharmaceutical, industrial processing, animal health, and bioremediation industries. The following examples are given to illustrate the present invention. It should be understood that the invention is not to be limited to the specific conditions or details described in these examples.

EXAMPLE 1

Isolation of Fungal Promoters and Transcription Terminators

The *A. niger* (ATCC11414) cells were grown in a liquid flask culture with non-citric acid production media containing 1000 ppb Mn^{2+}, 140 g/l glucose, 3.1 g/l NH_4NO_3, 0.15 g/l KH_2PO_4, 0.15 g/l NaCl, 2.2 g/l $MgSO_4.7H_2O$, 6.6 mg/l $ZnSO_4.7H_2O$, and 0.1 mg/l $FeCl_3$ adjusted to pH 2.0 with 4 M H_2SO_4. The biomass was then harvested by centrifugation and the genomic DNA was isolated by the CTAB method. Based on cDNA sequences of *A. niger* genes identified in U.S. patent application Ser. No. 10/442,017, two sets of gene-specific primers, GSP-1 (SEQ. ID NO.:1-9) and GSP-2 (SEQ. ID NO.:10-18) at 5'-end and 3'-end, respectively, were designed, synthesized, and used to isolate genomic DNA fragments of a specific gene by genomic PCR. The DNA sequences of specific genome DNA fragments were determined by conventional DNA sequencing. As shown in FIG. **2**, the genomic DNA sequence was used as a source DNA sequence to design additional primers (SEQ. ID NO.:19-34), designated generally as gene specific primers-3 (GSP-3) for isolation of fungal promoters or transcription terminators via genomic PCR. Table 1 lists the sequences for each of the gene specific primers as well as the adaptor primers.

The genomic DNA was first digested separately with restriction endonucleases Dra I, EcoR V, Pvu II, or Stu I. This digestion generated a series of genomic DNA fragments with blunt ends. After generation of the blunt-end fragments, a GENOMEWALKER™ adaptor oligonucleotide of 48-base pairs was linked to the ends of genomic DNA fragments to generate four separate genome-walking libraries. The libraries were designated as Dra I, EcoR V, Pvu II, and Stu I, respectively. The genome-walking libraries were used as genomic DNA templates for genomic PCR with adaptor primer 1 (SEQ. ID NO. 35) or 2 (SEQ. ID NO.:36) and the appropriate GSP-3 fragment (SEQ ID NO:19-34). The PCR fragments were separated by low melting point agarose gel electrophoresis and isolated by gelase digestion and a microcon centrifugal device. The PCR fragment was then inserted into the pGEM-Teasy vector for DNA replication and DNA sequencing. The PCR fragments were aligned with known genome DNA sequences using the BLAST 2 program to verify the identity of the newly isolated promoter or transcription terminator fragment.

TABLE 1

Oligonucleotides (GSP-1, GSP-2, GSP-3, and adaptor primers) used for promoter and transcription terminator isolation

SEQ ID NO:	Gene	Oligonucleotide name	Oligonucleotide
Gene specific primer (GSP-1) used for genome DNA isolation			
1	Balu-42	FP-35 (Balu42-5P)	5'-CCA CGG TAG TCA CTC CTT TGC ACT A-3'
2	Brsa-25	FP-37 (Brsa25-5P)	5'-CCT CTA TTC TGT CTC CCT TCG GCG AT-3'
3	Brsa-47	FP-51 (Brs47-P5)	5'-GCA ATC GTC TTC CCG TCG TTC A-3'
4	Brsa-109	FP-55 (Brs109-P5)	5'-GTC TGT CGT GGT GTC GTA TCA AAT G-3'
5	Brsa-118	FP-39 (Brsa118-5P)	5'-CTC CTT CTT CCC CCC CAT ACA TCA-3'
6	Arsa-7	FP-47 (Arsa-7-P5)	5'-GCT GTG CTT CGT ACC TTC ATT TCG-3'
7	A-37	FP-43 (A37-5P)	5'-GCC ATC TAT CAA CAC GAG AGA AAA C-3'

TABLE 1-continued

Oligonucleotides (GSP-1, GSP-2, GSP-3, and adaptor primers) used for promoter and transcription terminator isolation

SEQ ID NO:	Gene	Oligonucleotide name	Oligonucleotide
8	Arsa-43	FP-95 (Arsa43-5P)	5'-TGC AGA TCT TCG TTA AGA CCC TCA C-3'
9	A-90	FP-57 (A90-5P)	5'-CTC TCC CAC CTC CCC AGC CTT TCC T-3'
Gene specific primer (GSP-2) used for genome DNA isolation			
10	Balu-42	FP-36 (Balu42-3P)	5'-GAG TCG ACG AAT CGA ATC GAA TCG-3'
11	Brsa-25	FP-38 (Brsa25-3P)	5'-GAC ACC ATC ACA GAC ATA TAC AGA GA-3'
12	Brsa-47	FP-52 (Brs47-P3)	5'-CAA AGA GTG GCT GTA GTT GGC T-3'
13	Brsa-109	FP-56 (Brs109-P3)	5'-GTG CCC ATC AGA AGT GAA CCA AGA-3'
14	Brsa-118	FP-40 (Brsa118-3P)	5'-GCA TTC CAG CTC CTG TCT GGA CAA-3'
15	Arsa-7	FP-48 (Arsa-7-P3)	5'-CAC AAG CGT CCA ATC CAT CAC A-3'
16	A-37	FP-44 (A35-3P)	5'-GAG ATC GAC AAG GTA ACA TTC CAG AA-3'
17	Arsa-43	FP-96 (Arsa43-3P)	5'-GCG GAG GAC AAG ATG GAG AGT AGA C-3'
18	A-90	FP-58 (A90-3P)	5'-CCA AGG TAA AGC AGA TCT AAT GG-3'
Gene specific primer (GSP-3) used for promoter isolation			
19	Balu-42	FP-79 (Balu-42R)	5'-ACT TTC GTG TCT TGT GCG TGA AGT AA-3'
20	Brsa-25	FP-81 (Brsa-25R)	5'-GGT TTC TTT ATC CTG TCC GTA TGC TG-3'
21	Brsa-47	FP-85 (Brsa-47R)	5'-GAC GGT TTA TAT TCG ACC ACG CCT CA-3'
22	Brsa-109	FP-87 (Brsa-109R)	5'-GCT AGT GGC CTT CAT TGT TGT ATG AG-3'
23	Brsa-118	FP-89 (Brsa-118R)	5'-TGA ATG TGT AAA AGG AGG AGG GGT AA-3
24	Arsa-7	FP-91 (Arsa-7R)	5'-AGT AAG GCG AAA TGA AGG TAC GAA GC-3'
25	A-37	FP-93 (A-37R)	5'-CAG CAG CAG ACA TTG TGA TGT GAT AG-2
26	Arsa-43	FP-99 (Arsa-43R)	5'-GAT GCC CTC CTT ATC CTG GAT CTT G-3'
27	A-90	FP-105 (A-90R)	5'-GCG GTC AGA AGA GAC TTG AAG GAG AC-3'
Gene specific primer (GSP-3) used for transcriptional terminator isolation			
28	Brsa-25	FP-82 (Brsa-25L)	5'-CTG TGG AGT AGA TGG GCA CTC TTG AT-3'
29	Brsa-47	FP-86 (Brsa-47L)	5'-CAC CCA CCT AGT AAT GCT TAG CCA TC-3'
30	Brsa-118	FP-90 (Brsa-118L)	5'-TTT GTG GTT CGC CTT AAT AGA GCT TG-3'
31	Arsa-7	FP-92 (Arsa-7L)	5'-ATC ATC TGA CGC TGA TGC AAT AGT TC-3'
32	A-37	FP-94 (A-37L)	5'-GGA CAT GGA CAT GGA TAT GAG TTT GA-3'
33	Arsa-43	FP-100 (Arsa-43L)	5'-CTT TAG CAC GGC TCA TCT ACG GTT G-3'
34	A-90	FP-104 (A-90L)	5'-TTG AGC TCG AGT GGA AAG GTG TAC G-3'
35		Adaptor primer-1	5'-GTA ATA CGA CTC ACT ATA GGG C-3'
36		Adaptor primter-2	5'-ACT ATA GGG CAC GCG TGG T-3'
Gene specific primer used for deletion of ATG-transcription start site at the promoter fragment's 3'-end			
37	Arsa-7	FP-135 (pArsa-7-412H5)	5'-TCA AGC TTC TGC TCC AAC GCG CTA TCA AAT CGA A-3'C-3'
38	Arsa-7	FP-136 (pArsa-7-2040P3)	5'-CAC AGC TGA TTG AAA GAA TAG AGA GTG ATG GAG TTG -3'
39	A-37	FP-125 (A-37-P-Xba-RI)	5'-CGG AAT TCT CTA GAG TGA TGT GGA TAG GGA TGG GAA TAA G-3'
40	Arsa-43	FP124 (Arsa-43-P-Cla-H3)	5'-CCA AGC TTA TCG ATG TTG TAG AAG CGC AGT TAA TGG TGT ATG -3'
41	Brsa-25	FP-152 (Brsa25-1677Sma)	5'-ATC CCG GGT AAA GCA AGG CGA ATG ACG AAG ACA -3'
42	Brsa-109	FP-137 (PBrsa-109-23S5)	5'-CAG AGC TCC TCC TGT CTG AGT GTT GTC TCA -3'
43	Brsa-109	FP-138 (pBrsa-109-1835P3)	5'-CTC AGC TGT TGT ATG AGA GGT GTA TAT GTA TGT -3'
44	Brsa-118	FP-155 (Brsal 18-1502pml)	5'-GCA CGT GAA TGT GTA AAA GGA GGA GGG GTA -3'
45		T-7 primer	5'-TAA TAC GAC TCA CTA TAG GG-3'

EXAMPLE 2

This example describes the steps taken to prepare different fungal promoters fused in front of a GUS reporter gene with the 3'-TtripC transcription terminator. Use of the constructs produced according to this example demonstrates the function of different promoters and their potential use in the production of different proteins and chemicals via various fungi.

Since the GUS reporter gene contains its own ATG-translation start site, in the transgene expression vector, introduction of a proper restriction endonuclease site at the 3'-end of the promoter was preceded by removal of the ATG-translation start site from all the promoter fragments being prepared for function analysis. PCR fragments were cloned into the pGEM-Teasy vector and the presence of the promoter fragment was confirmed by restriction endonuclease digestion. The promoter fragment released by restriction endonucleases was inserted into binary vectors pZD640 or pZD655 for *Agrobacterium*-mediated transformation. The method of construction for specific vectors for *Agrobacterium*-mediated transformation is described as follows:

The PCR fragment containing the promoter for the pelleted-associated Arsa-7 gene (SEQ ID NO:46) was first isolated via genome walking with gene specific primer FP-91 (SEQ ID NO:24) and subsequently cloned into pGEM-Teasy vector to form pZD611. The plasmid DNA was then sequenced to confirm the newly isolated fragments. In order to remove the ATG-transcription start site at the promoter fragment's 3'-end, pZD611 was used for a template for PCR with primer FP-135 (SEQ ID NO 37) and FP-136 (SEQ ID NO:38). Referring to FIG. **3**, the PCR product was inserted into pGEM-Teasy to form pZD667. Then the Arsa-7 promoter fragment (SEQ ID NO:46) was excised by Hind III and Pvu II and treated with DNA polymerase I-large fragment. The promoter fragment was finally inserted into the restriction endonuclease Sma I site of pZD655 in front of GUS reporter gene to form pZD672.

Similarly, the pelleted-enhanced A-37 gene promoter (SEQ ID NO:47) was first isolated from the genomic DNA using GENOMEWALKER™ kits and gene-specific primer FP-93 (SEQ ID NO:25), which was inserted into pGEM-Teasy to form pZD612. The A-37 promoter fragment (SEQ ID NO:47) was then prepared by PCR with primer FP-125 (SEQ ID NO:39) and T-7 (SEQ ID NO:45) and inserted into a PCR 4 TOPO™ vector (Invitrogen Corporation, Carlsbad, Calif.) to form pZD636. Referring to FIG. **4**, the promoter fragment in pZD636 was excised with restriction endonuclease EcoR I and treated with DNA polymerase I-large fragment. Finally, the promoter fragment was inserted into pZD640 to form pZD645.

The pelleted-enhanced Arsa-43 gene promoter (SEQ ID NO:48) was first isolated from the genomic DNA using GENOMEWALKER™ kits and gene specific primer FP-99 (SEQ ID NO:26). The promoter was subsequently inserted into pGEM-Teasy vector to form pZD614. The ATG-sequence at the 3'-end of the Arsa-43 promoter fragment (SEQ ID NO:48) was then removed by PCR using FP-124 (SEQ ID NO:40) and reverse primers. Referring to FIG. **5**, the remaining fragment was cloned into the PCR-4-TOPO™ vector to generate pZD635. The Arsa-43 promoter (SEQ ID NO:48) was excised with restriction endonuclease Hind III and EcoR I, which was treated with DNA polymerase I, large fragment. Finally, the fragment was inserted in front of the GUS reporter gene at restriction endonuclease Hpa I site to form pZD646.

The filamented-enhanced Brsa-25 gene promoter (SEQ ID NO:51) was isolated using GENOMEWALKER™ kits and gene specific primer FP-81 (SEQ ID NO: 20). The isolated Brsa-25 promoter was then cloned into pGEM-Teasy vector to form pZD619. The promoter DNA fragment was confirmed by DNA sequencing. The ATG-sequence at the 3'-end of the promoter was removed and a restriction endonuclease site Sma I was added to the same end by PCR using gene specific primer FP-152 (SEQ ID NO:41) and a T-7 (SEQ ID NO:45) primer, which was further cloned into a pGEM-Teasy vector to form pZD677. Referring to FIG. **6**, the promoter fragment was excised with restriction endonuclease Sma I and cloned into pZD655 to form pZD682.

The filamented-enhanced, Brsa-109 gene promoter (SEQ ID NO:53) was isolated with GENOMEWALKER™ kits and gene specific primer FP-87 (SEQ ID NO: 22). The isolated Brsa-109 promoter was subsequently cloned into pGEM-Teasy vector to form pZD613. The ATG at the 3'-end of the promoter was removed and the restriction endonuclease Pvu II was introduced at the same end of the promoter fragment by PCR with gene specific primers FP-137 (SEQ ID NO:42) and FP-138 (SEQ ID NO:43). The promoter fragment was then inserted into pGEM-Teasy vector to form pZD668. Referring to FIG. **7**, the promoter was isolated with Sac I and Pvu II, treated with DNA polymerase I-large fragment, and cloned into pZD655 in front of the GUS reporter gene to form pZD673.

The filamented-enhanced Brsa-118 gene promoter (SEQ ID NO:54) was isolated with GENOMEWALKER™ kits and gene specific primer FP-89 (SEQ ID NO: 23). The isolated Brsa-118 promoter was subsequently cloned into a PCR-4-Blunt-TOPO™ vector to form pZD610. The ATG at the 3'-end of the promoter was removed and the restriction endonuclease Pml I was introduced at the same end of the promoter by PCR with gene specific primer FP-155 (SEQ ID NO:44) and T-7 primer (SEQ ID NO: 45). The promoter fragment was inserted into pGEM-Teasy vector to form pZD678. Referring to FIG. **8**, the promoter was isolated out with Pml I and Sma I and cloned into pZD655 in front of the GUS reporter gene to form pZD681.

EXAMPLE 3

This example describes the methodology used for *Agrobacterium*-mediated transformation and colorimetric GUS assays of the GUS reporter gene under the control of the different *A. niger* gene promoters. Application of this system enables one to study the function of the sequences inserted in front of the reporter gene in terms of transcriptional activity.

Escherichia coli DH5α was used as the recombinant host for routine cloning experiments. The *Agrobacterium tumefaciens* strain AGL0 served as the host for the binary vectors and in the transformation of *A. niger.*

Transformation of the constructs carrying backbone binary vector pZD640 or 655 into *Agrobacterium tumefaciens* strain AGL0 was conducted by the freeze-and-thaw method as described by Ebert et al. in the *Proceedings of the National Academy of Sciences USA,* 84:5745-5749 (1987), the content of which is incorporated herein by reference. Plasmid DNA from the transformed *Agrobacterium* clones was isolated and digested with various restriction endonucleases and analyzed in agarose gel electrophoresis to confirm transformation of each construct. Fungal spore transformation was performed as described in the article by Dai et al., titled Identification of genes associated with morphology in *Aspergillus niger* by using suppression subtractive hybridization (Applied Environmental Microbiology 70: 2474-2485 (2004)), the content of which is incorporated herein by reference. At least 30 independently transformed fungal strains were selected for each promoter construct described in Example 2. Transformed colonies were removed from the agar selective media, which contained minimal medium (see J. W. Bennett and L. L. Lasure eds., *More Gene Manipulations in Fungi*, Academic Press Inc, San Diego, pp 441-458.) with 200 μg ml^{-1} hygromycin and 200 μg ml^{-1} cefotaxime, and then grown under sterile but equivalent conditions for spore production.

The spores were enumerated and then cultured in a proper culture medium at a temperature of 30° C. and a mixing speed of 250 rpm for 2 days. Finally, the biomass was harvested for a GUS activity assay. Fluorometric quantitation of GUS activity was performed according to Jefferson et al. in the *European Molecular Biology Organization Journal,* 6:3901-3907 (1987), the content of which is herein incorporated by reference.

Biomasses of independent transgenic fungal strains were harvested from fresh test-tube cultures by centrifugation at various times ranging between one and three days. Extraction was performed by sonicating on ice five times for ten seconds each using a lysis buffer (50 mM sodium phosphate, pH 7.0, 10 mM EDTA, 0.1% TritonX-100, 0.1% sarkosyl and 10 mM β-mercaptoethanol).

Protein concentrations were determined by the BIO-RAD™ reagent protein assay (Bio-Rad Laboratories, Hercules, Calif.) according to the Bradford method. The GUS activity assay involved incubating approximately 5-10 μg of protein in the presence of 1 mM 4-methylumbelliferyl β-D-glucuronide in 100 μl of lysis buffer at 37° C. Samples from each reaction were taken at 0, 10, 20 and 40 minutes. The enzyme reaction was quenched in 0.2 M sodium carbonate (Na_2CO_3). The standard curve for 4-methylumbelliferon at 50, 100, 150, 200, 250, 300, 350 and 400 nM concentrations was generated with a FL600 Fluorescent Microplate Reader. The amount of 4-methylumbelliferyl β-D-glucuronide converted to 4-methylumbelliferon (MU) by GUS enzyme was determined with FL600 Fluorescent Microplate Reader and the MU standard curve. The GUS enzyme activity is expressed as pmol MU per mg protein min.

Referring to FIG. **9**, the expression of the GUS gene with the Arsa-7 promoter (SEQ ID NO:46) was at a high level and gradually increased under pelleted culture conditions. It remained at barely detectable levels for the first three days of growth in filamented culture conditions and then rapidly increased after three days of growth. The plot shows the activity of pelleted-enhanced Arsa-7 gene promoter (SEQ ID NO:46) in the protein extract of two days old individual transformant under pelleted growth conditions. The promoter activity is expressed at pmol MU/mg protein/min. The promoter activity in most of transgenic strains is about 200,000 pmol MU/mg protein/min. Transgenic strain No. 7 has the strongest activity among the 11 strains. The promoter activity is about four times higher than the hybrid Mac promoters that consist of the B-domain of 35S cauliflower mosaic virus promoter and the manopine synthase promoter of *Agrobacterium tumefaciens*. This activity appears to be the strongest one used in plant transgene expression. It is about 20 times higher than the yeast α-amylase promoter.

Referring to FIG. **10**, the A-37 promoter (SEQ ID NO:47) activity is still higher than the yeast α-amylase and is comparable to that of the hybrid MAC promoter. The plot shows the activity of the pelleted enhanced A-37 gene promoter (SEQ ID NO: 47) in the protein extract of two days old individual transformant under pelleted growth conditions. The GUS activity of most transformants was around 50,000 pmol MU/mg protein/min, while transgenic strains 4 and 16 were about 150,000 to 200,000 pmol MU/mg protein/min. The data show that the A-37 promoter (SEQ ID NO:47) has high constitutive expression levels at pelleted culture conditions. Expression was low during the first day of growth prior to the rapid increase thereafter to the end of growth.

Referring to FIG. **11**, the Arsa-43 promoter (SEQ ID NO:48) is a polyubiquitin gene that is constitutively expressed at pelleted culture conditions. However, under filamented growth conditions its expression was low during the first day of growth, and thereafter increased rapidly to steady states for the rest of the filamented growth. Again, the plot shows the activity of the pelleted-enhanced Arsa-43 gene promoter (SEQ ID NO:48) in the protein extract of two days old individual transformant under pelleted growth conditions. For comparison purposes, the GUS activity of most transformants is around 5,000 to 10,000 pmol MU/mg protein/min.

FIG. **12** shows the activity of filamented associated gene Brsa-25 promoter (SEQ ID NO:51) in the protein extract of two days old individual transformant under filamentous growth conditions. The GUS activity of most transformants is around 50 to 100 pmol MU/mg protein/min. The Brsa-25 promoter is filamented specific and functions temporally. Its transcription increases rapidly at the first day culture and decreases to low levels at two and three day cultures. Thereafter, its transcription augments to the level of first cultures.

Referring to FIG. **13**, the Brsa-109 promoter (SEQ ID NO:53) is constitutive and filamented-specific. The plot shows the activity of the filamented-enhanced Brsa-109 gene promoter in the protein extract of two days old individual transformant under filamentous growth conditions. The GUS activity of most of the transformants was around 1000 to 4000 pmol MU/mg protein/min, except transformant clone 8, which had an activity level over 14000 pmol MU/mg protein/min. The Brsa-109 gene promoter (SEQ ID NO:53) can be used for the expression of genes of interest in filamented growth conditions.

Referring to FIG. **14**, the Brsa-118 promoter (SEQ ID NO:54) is temporally dependent and filamented specific, similar to the promoter of the Brsa-25 promoter (SEQ ID NO:53). The plot shows the activity of the filamented-enhanced Brsa-118 gene promoter in the protein extract of two day old individual transformant under filamentous growth conditions. The GUS activity of most transformants was around 500 to 2000 pmol MU/mg protein/min. This promoter can be used for expression of genes of interest in different developmental stages.

EXAMPLE 4

This example describes the necessary steps taken to prepare different fungal transcription terminators and insert them into the host vector pGEM-Teasy for plasmid DNA preparation. The DNA was sequenced and aligned against known DNA fragments to confirm the newly isolated transcription terminators. The transcription terminators can be used for heterologous gene expression in fungi.

The transcription terminator of the pelleted-associated Arsa-7 gene (SEQ ID NO:55) was isolated with GENOM-EWALKER™ kits and gene specific primer FP-92 (SEQ ID NO:31). The genome walking libraries in Example 2 were used as template DNAs for genomic PCR with adaptor primer 1 (SEQ ID NO:35) and FP-92 primers (SEQ ID NO:31). The DNA fragments were cloned into pGEM-Teasy vector to form pZD621. The DNA sequence of the Arsa-7 gene transcription terminator (SEQ ID NO: 55) in pZD621 was determined and aligned with the known genomic DNA sequence of the Arsa-7 gene to confirm the newly isolated fragments.

The transcription terminator of the pelleted-associated A-37 gene (SEQ ID NO:56) was isolated with GENOM-EWALKER™ kits and gene specific primer FP-94 (SEQ ID NO:32). The genome walking libraries in Example 2 were used as template DNAs for genomic PCR with adaptor primer 2 (SEQ ID NO:36) and FP-92 primers (SEQ ID NO:31). The DNA fragments were cloned into pGEM-Teasy vector to form pZD622. The DNA sequence of the A-37 gene transcription terminator (SEQ ID NO: 56) in pZD622 was determined and aligned with the known genomic DNA sequence of the A-37 gene to confirm the newly isolated fragments.

The transcription terminator of the pelleted-associated Arsa-43 gene (SEQ ID NO:57) was isolated with GENOM-EWALKER™ kits and gene specific primer FP-100 (SEQ ID

NO:33). The genome walking libraries in example 2 were used as template DNAs for genomic PCR with adaptor primer 1 (SEQ ID NO:35) and FP-100 primers (SEQ ID NO:33). The DNA fragments were cloned into pGEM-Teasy vector to form pZD615. The DNA sequence of the Arsa-43 transcription terminator (SEQ ID NO: 57) in pZD615 was determined and aligned with the known genomic DNA sequence of the Arsa-43 gene to confirm the newly isolated fragments.

The transcription terminator of pelleted-associated A-90 gene (SEQ ID NO:58) was isolated with GENOMEWALKER™ kits and gene specific primer FP-104 (SEQ ID NO:34). The genome walking libraries in example 2 were used as template DNAs for genomic PCR with adaptor primer 1 (SEQ ID NO:35) and FP-104 primers (SEQ ID NO:34). The DNA fragments were cloned into pGEM-Teasy vectors to form pZD617. The DNA sequence of the A-90 gene transcription terminator (SEQ ID NO:

58) in pZD617 was determined and aligned with the known genomic DNA sequence of the A-90 gene to confirm the newly isolated fragments.

The transcription terminator of filamented-associated Brsa-25 gene (SEQ ID NO:59) was isolated with GENOMEWALKER™ kits and gene specific primer FP-82 (SEQ ID NO:28). The genome walking libraries in example 2 were used as template DNAs for genomic PCR with adaptor primer 2 (SEQ ID NO:36) and FP-82 primers (SEQ ID NO:28). The DNA fragments were cloned into pGEM-Teasy vectors to form pZD620. The DNA sequence of the Brsa-25 gene transcription terminator (SEQ ID NO: 59) in pZD620 was determined and aligned with the known genomic DNA sequence of the Brsa-25 gene to confirm the newly isolated fragments.

The transcription terminator of filamented-associated gene Brsa-47 (SEQ ID NO:60) was isolated with GENOMEWALKER™ kits and gene specific primer FP-86 (SEQ ID NO:29). The genome walking libraries in example 2 were used as template DNAs for genomic PCR with adaptor primer 1 (SEQ ID NO:35) and FP-86 primers (SEQ ID NO:29). The DNA fragments were cloned into pGEM-Teasy vectors to form pZD626. The DNA sequence of the Brsa-47 gene transcription terminator (SEQ ID NO: 60) in pZD626 was determined and aligned with the known genomic DNA sequence of the Brsa-47 gene to confirm the newly isolated fragments.

The transcription terminator of filamented-associated Brsa-118 gene (SEQ ID NO:61) was isolated with GENOMEWALKER™ kits and gene specific primer FP-90 (SEQ ID NO:30). The genome walking libraries in example 2 were used as template DNAs for genomic PCR with adaptor primer 1 (SEQ ID NO:35) and FP-90 primers (SEQ ID NO:30). The DNA fragments were cloned into pGEM-Teasy vectors to form pZD627. The DNA sequence of Brsa-118 gene transcription terminator (SEQ ID NO: 61) in pZD627 was determined and aligned with the known genomic DNA sequence of the Brsa-118 gene to confirm the newly isolated fragments.

While a number of embodiments of the present invention have been shown and described, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. The appended claims, therefore, are intended to cover all such changes and modifications as they fall within the true spirit and scope of the invention.

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<210> SEQ ID NO 42
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 42

cagagctcct cctgtctgag tgttgtctca 30

<210> SEQ ID NO 43
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

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<400> SEQUENCE: 43

ctcagctgtt gtatgagagg tgtatatgta tgt 33

<210> SEQ ID NO 44
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 44

gcacgtgaat gtgtaaaagg aggaggggta 30

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 45

taatacgact cactataggg 20

<210> SEQ ID NO 46
<211> LENGTH: 2043
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 46

aaacctaatg aaaataacat gaatctcagg attataccca tatcgactgt atcgcatcct 60

tctcattttg gccccttga ctcgcaatca tcgggagccc acgagtccgc tgcgggacgc 120

cggctcgctc ccaatccttt gccccagggg tcaaatgagc agtcctctat gacagtggga 180

agccacgccg gtcaggccaa atattgagag tcgagagtta gttatctgat ttcgtcaagc 240

ctgccgatcg cgcagaaact ggtagcgacc taggaccggt ggaccgccag ttaggagggg 300

actgcgaggg tgcgaagata aagtgaaaca tcccgatcgg ataaatgggc cgtgcagacg 360

ggggaccaat cagcttacgc agcccagggg atctgcatag ggccacgcca gctgctccaa 420

cgcgctatca aatcgaaggc ttgaaacgaa cagatgccat aatccgacag ccgtttgttt 480

cattcagagt agctcgctag catggtgacg actggtccag gccccatttg tcgtcatctt 540

gggccattcc atccatcacc ttcaactctg ccatgcagga aaccatggat agcctagcaa 600

aaccccggca tggacagatg ccagcgaaga cttccaccct acactagggt cccctcaggg 660

tcccattcct gttaatcccc ctattattgg gtccaccttg tgagctcccc caactttgac 720

ggggaaagct ctattccgag ttcggctaca acgttcccag cgagggcatc atgtaaaacc 780

tcataaaaac gactttttct gatggatagg cagtgcaggt agaggaatga ctttcccccca 840

cagtgattat catgtttgtc ctgaccatag cttgcaggat gatctgtaag cgggagagga 900

ttatgctgca cgtagaggac actgaccaca acttctgttc ctcgagatgg acccacccaa 960

ataacgtaga gtcaaggacc cgccatcgtt gggcccccaa gaacacacca gagctgacta 1020

gccttccgct tagttagcac tacgacctgt cgactgtcag tgtcgagagt cgagactggg 1080

ctgacccacc aacttggaac cgccacagcg gcaggggaca gcttgatcga ggacgtcagc 1140

tccctggcac gctggttgcc attggataga gattatcaac cagttgaatt catccaccga 1200

cgatctgagg cactttttga ggctttccca gtgagtccac tgagtttggg tggacgatgg 1260

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gtagagagac aaccagacga agcattacca agggactcat gacggaaccg caaatagacc   1320
accaacaaca gccgcagcca ggatcaagcc acctccaaga ggcagggggg ccaaggagag   1380
ggacagtcga gtcatctatt ctgaataggc gatgaagaga tgaaacgctg gagtgtcggc   1440
tggcctgtga ctgcttccag ggcgagccgc gcacgtgggg ccgccacaga cagccagcca   1500
gacttcttcc cttctcttcc tatccatcaa tagcatcctc tacctacata ctcccttctc   1560
acagatccaa ctaccggctt catgcttagc cgacccacag aagcccagca ggtacgttca   1620
aaccctattt tgcatcagcc ctgcccctga gccactctac cacccccac aagcgccggg   1680
tctgccgatc cgtgcggttc ttgcatgtcc agcataacct gatctattgc tgacagtcga   1740
cgtctcagat gcaggcgagc cgaattcggt gacaacagtg catgacgaat gcttggttct   1800
ttccacgcat ctcaccagat ggatggaggt catcatcgct gggtcactgc cgacccagcg   1860
cttggagagc gccttataaa agcctccctt gccccagcca ggatcattcc tcattcagct   1920
caaattctct ttcctttgat ctcaactacc attccttaag aagctgtgct tcgtaccttc   1980
atttcgcctt actttttttc tgcttactac tacaactcca tcactctcta ttctttcaat   2040
atg                                                                 2043
```

<210> SEQ ID NO 47
<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 47

```
ctgacgttga cattgaacgc tccatcaacc aaggagttgg gggtatcgag tttcatccct     60
atggggccaa acaattagca atgcttaatg cttgtgcaga aaataagcac accgggaatg    120
cgaggaaccg acagcctgca ttaaactgtg cttcggaaat tttatttccc tcctctgaga    180
cagtctcatt gatccttctg aaaatttatc cgatgggatt tccacagcac gctgtgctgc    240
ctatcgtgga tgctcgcacg gaagtttcta tatcctgatc gttaagcagg atcatgttct    300
atatttatca ctcatggctg agctgctctt agacatatca accgcactaa aatggcgatc    360
agagcaacaa ttaacaatgc cgaggatatc gatttgtcgc ctgatttttg agtaagtttg    420
tctattttta ctcggtgaaa cagcgtctca gccaggaacc gttacgagta cgacacagcc    480
aacacgggcc ataacaaggc gagcccctga tcctcggggc ccccgtcgtt cacgagccga    540
ggtgatcgct aagcgggact cgcggatcaa caggcgcgac cgtgtttcag atgtcaccat    600
gacaaggtgg ctatgagtaa ttccaggcga cgtgccgtgt gttagtgcca tgcggcaatg    660
atgggccgcc aagagtagtt ggggatgcag tggagagaga gagagacaac tcatacccag    720
atttgattca ttacttcagt acgtgcagac atgacatctg ataaaaccta ttccgaccga    780
gtcgtggttc tagaccggcg ggcttggcga ccgcaacacg acctttgcgt acattccaga    840
cgacgaaaca ctgcatcagg cacgggcatg ccggatcgag cgagaccctt gacagaattt    900
ggggcggccc ctgatgatgt gcggcctcaa agcgtcatca cccatttcaa cctgccagga    960
acagcaacgt tggagccaat cgcggatgca aatctggctg cctagaatag caattgccac   1020
ggcctcagcc cccgtgattg cgcggcccaa caggccttcc attggctgaa accccggata   1080
agccttgggt tttgtgcagt agtggaagct tggcaagtta ctgagccaat catattcctc   1140
taattcctcc aaggagggtg ggggcctgct aacgtcacgg acctgcttcc attgccttcc   1200
cctctgcccg tccttccatc ccagcccggt cggccgcgtc acagacccgg ctggaaaggc   1260
aacaaggctc gcaacctcat gcccatcatt ggctggtcct gcgtgatgct gcaggtcagc   1320
```

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ttccaaactc agtcgcccat gctgaccttt ttttatgcag ccgggctgct tctttcattt   1380

ataggtcccc gtctggcatg tgtcttccct tccaacttcc cgactcactc cacctttttct   1440

catctgtcat ctgtacctag attccttctt atatcttatc cgtggttcct tcttttctgg   1500

ccaagatctt agccatctat caacacgaga gaaaacttat tcccatccct atccacatca   1560

caatg                                                               1565
```

<210> SEQ ID NO 48
<211> LENGTH: 982
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 48

```
ctggagagga tccccttccc ccatcttccg ataagggatg cccccaactc acacgtcatc     60

gccgttgctg ccgccgcaag gccagttgtc gcattactcc ctgatcacca ccagtttgcc    120

tggtgagagg atacgaacaa ttatgagcaa ttcttcggag tagcaacgag tattttcacc    180

gggagtttca acgggttcta tttcaggaac acggctgcgg tctggattgg gtcgggctga    240

gataccgact ggtggcgtca gtggcgggta cggacggagt cgtcctggcc gctcgtagac    300

actcccccgg actgatatca ggccccggca actggcttcg tctcactcca gggcatcagg    360

agtgcctacc acatgggttc aggctttgcc ccgtcgtcta agtttgcagg acaaaatttt    420

cgtatgcgtt accactcttt cctttcagca accattccgt agtgaaaacc caataatagg    480

tggctgccgt gggagcctga gtcaacccaa ccagaacctt tctagtagat tctcccccaa    540

gcgcttcagc aacgaagcgt attggagaac caaatgacgc agaccaagcg gattccggtg    600

caatagccgg atggcaaggg aatcccccag gaggtgccag aagcgtcgcc cgaaaggtac    660

ttcgtctgac aggctaacac cgctcgggct aaggtccctg ctgctctttt ccctttattg    720

cgacttaacc tctaagccat tcccttgcat cacgttatct cactgaccga cctctgacta    780

aggcgcttcg cctccgccgc ctcccctcat tcacctcctc tcctgactgc ttaagccttc    840

tcttccttcc tctcactacc aaccctcctt catccctcat acctctcatc ctaccactca    900

cctttcgcgc atcgccatct gcgatcctcc ccacaacact ccacctagat acatacacca    960

ttaactgcgc ttctacaaca tg                                             982
```

<210> SEQ ID NO 49
<211> LENGTH: 748
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 49

```
atacacctcc attaaagtag ggggaataag tcggatacat ccactggacc gatcaactgc     60

aggtatccgc accgctgcag gaacaaccac cgcaaggtta ccccccggacg cttgctgtcc    120

agtcactgcc aaccgccagg cacacgggct gaataatggg cgtcaatatt tctctgtccc    180

actgtccctg agcgacacac ggtacccgcc cgatgacgtt ccatgggtcg gccgcggtga    240

ggatgcaggg gggtcaggaa cgctccgacg caggcaatca gagggggtcc gccgaacaat    300

ggaaaaagca acgattagtg actagttcga ctatactcat gcaagagcaa aaaagaacct    360

tcctcttgtg gagacctgat tggtcggaac caaattggcg cctagaaaaa gcacccagcc    420

ctaacttggt tctgcaactg ccactccccg ttgttgggcg tctatataac cgccctcttt    480

cccctccctg tctcctcttc gaaactcttc ttcctcgcct agatcttcct ctcccacctc    540
```

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```
cccagccttt ccttctttgc acctgtgccg tgcacggtcg agccattcct ccattctttg    600

aacatattgc ctggctccga gtagtctagc atccactcct tgcaagagca ctttgagaga    660

accggtcttc tcatactcaa aagttataca tacacatcac ttctctccga acaaaaccga    720

acagaattcg aagaacacat acacaatg                                       748
```

<210> SEQ ID NO 50
<211> LENGTH: 2271
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 50

```
atccacagca gatggatcat aagcagtcag actgcaggtc aggtatcgga gtccgagaca     60

ttcgaactag tctccgacgc cactggaaaa attcctgcac tcgcccacac gtggtaagcg    120

atacgactac atattgtgtg gacagaggaa tgtggcctcg agcagagaaa gcttgccaac    180

atgaagatca ctggcaggcg tgctcatgaa agccattccg tgggttttgt ttggataacc    240

cgcaaggtac atactccggg agtgcttgtc tcttcaaggt tcgcagtatg acggatcatc    300

tcccttggta cgaaggaagg catgttatca gttatcgtgc cttgttagtg gcattggcag    360

tcggaacgag ggtccactaa cccagtcagg aacgaggaat gagcgacagg aaccagagaa    420

tcttcaccca acatagcgat ggatgatctc atcgaggacg ttgatcacct ctctcgcggg    480

gactttcaac gacgaacggt cagtttgcag aatgaaaccc ccttgacaat ctgttgatct    540

gcggccagtg ggaagaaagg agggagtacg tgggtagtaa catgacttgt gtgtttcttg    600

gtgtctctcc gtagcaattt aggcgaccat ccgattacac gggggtggag acaccggaca    660

ggttccttgg tgcctttgga ggacacgaga tgcgtttagt gcctctggtc ccaatattcg    720

gaaggtggta attaaactct gtgcctggcc acttcggtga tttaacgctt cggcctcgtg    780

gcgtgtctat gtctcatttg tgtcaaacca ggacgcaccg gaagcagctg gcaaggctcc    840

ggaaggcgaa gccaatcaag caccactcga tgaggggcac tgatccatcc attgtaaatt    900

ttacatgagg gtaatttccc aggtaatttg ccctgcggct atgtcattga gaatggaaaa    960

gtctccggat aatatttgcc aaaaatgtga gatgtgtgtg cgtgtgtgaa aacgctcgag   1020

cttctggaag tgaaacaaaa gctgaaagga aaggaggtgg tgatggcgat aatggtggtg   1080

gtggtggtgg tgtttgtttg tttgtttgcg cgcgaatccc ttgcgggcca agttccacca   1140

acgacttctc tttctactgt gtctcttcgt actccgtcca gctgctgcta gccatcaaca   1200

acatccttcc ttctccgttc tcggggttcc tccgttgttc ctggcctggt ctgacataag   1260

gttatgattg tttcacatgt cccacggctt cgccggcttg gagctgagac cctcttctga   1320

gtcaatggta ccattttgcc gaattcgtgg ctagttctct atttctatgc tcttgacttt   1380

ggtaccgttg gcattagttt gatctactaa taaagagcct agttttaggc gaatatacac   1440

tgttacccac cgggtagtat tcagtagcta ccctcccact ccccaggctc ccacgctgag   1500

agccttgatt cgatgtctct cctaaaattg ctaggctgtt agcgccctgg cagatgaacc   1560

cccgctcatc cctcgtatat gcggtctcaa tttctgagtg gcccacgcct ccgagtatct   1620

ttgagcacat ccacgatgga gggaggcgat ccaagcggtc taacagcgga ctaaaccgct   1680

ctgtgtaagc cagtcagaga gtcatactgg cttgaggtga catcgccaat tcatttcaca   1740

aggtttagtc gggggagggt aggccccata cattccaccg ttctcaaagt ttaccaggca   1800

tttctcacac taaccatgca atagtaggta actagcagta gtcttgaacg ctgttcctga   1860

gcaagttccc aatcagcaat ttgaaagaat aatttccttt gacccaccgg gtaaatgagc   1920
```

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```
cgcagatttg gcgatgttgg gctcggagcc tggtaggtag tagtgaatgt catcccctcc   1980

atagggggga attgggaggg gggctgtgaa tggacttgtc ctacgcctgt cgcatcccca   2040

tcattcatat acttgaatgt ctcttctccc ccctcctcct tctctttctc tccttccctt   2100

ctcacgattt gacgtccctc gcgttttcgc cctctcccac ggtagtcact cctttgcact   2160

acatacacga agtcttactt ccagtcactc tttgaaacca cttctcaata tccctacctc   2220

ttatcattct ttacttcacg cacaagacac gaaagtgaac ctgtaaaaat g            2271
```

<210> SEQ ID NO 51
<211> LENGTH: 1680
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 51

```
atcctgaggt tgccaccatc aagtgcttgg tctgtttcca aggtacacat attctgcgta     60

gtggactaga acatccactt actacgtcgt tggagccgat gcggaggcca agcttcgtgc    120

ttgggaaaca agcaacaggc caagcaacga gtggatgggt cttggagcca ctgagcggtc    180

atgccgtcca tgggactggc ctcgatagta gaaggtcctt ctgataagcc cgtgtgcaca    240

gggcctcccg ggtttccggc tagtccatgc cacaggtttt tcttcactcc tttccctcac    300

ccctggccac ccatctgagg ttcatccaat cggtgacccc cgagaatgtc tccgcgctac    360

catagtagta tctagcactc ttagctactc ataagcgaca agtctttttg ggtttcgtgc    420

cgctatggct ggaaccatta attccggacg taggagcgtt gcagtcaggt agattgttgt    480

gtaagggaaa ttggtccatg aaaatcggca aaattgatgg gaagcaagac aagggagcgc    540

tagtgcagcg gtcgcatggt cacggttccc ccatccactc ggttctccgt cggcaatttc    600

tgtctttcct ttccctttg tcgttccctt cactttattg gggttattta ttgattctgg    660

taataatatt cgctcttatc ttcccccaac cgtcacgaaa atgggccttg gtccgatgtg    720

tgtgcatcca caaccgacca cccacaccac tacctcgtcc tcctccttcc ctataggcca    780

acattgcctt acggtgtatc ggacggtgct ccagatcgaa atttgcgatc caataagtcc    840

cctgcagaca ctaatcaagg tcaggtctca ttgggcgcga taacgtgctt cggccaggca    900

atcacactca tgaccatatt cttgctcatc ctcatcctca tccacatcat atcatcagga    960

tttcagtaag gtcagcagca tccgactcca gccgcagcaa gcctgtgacc ctggtctagt   1020

ctgcaattct ccgaacaaac gagcgtgttg acggtggagt ctcctggttc gtggcaagcc   1080

gttcgtgcag cccacggtta tctggtgtgt taccctccta aatcagttaa ccaagacgcc   1140

acccctcctt cggaccttcg acagatgctc cagaagacct cgatgtgcca atcaagttcc   1200

tgactagcgg tgatggcctc ctcaaagtgg ggagatgcag accgtttaag tttccactgg   1260

accgtcaatg gcattctgaa tgggtgccca ccgtggctcg aacatcgctg ctactggcgt   1320

tgattaaatt gcatcgataa ccagtgctgg atcagctcat actgacggcg ataatgtaga   1380

tactagccca cagtaatcca tcggattccg cctgctaatt ccgctcctcc cattcccatt   1440

tgtccctttg gttattagct agtggacgtt atctcccccg tcagcctccc attgactggt   1500

ctggcaccca ctaccagcta ctctgtagtc tcgcgccccc gtcgcgtgct tgctgcttgg   1560

cccttcttaa gcaccgccga tcccacctcc cccagttctg gatctttgca cccctcaagt   1620

tcgtcctcta ttctgtctcc cttcggcgat tgtcttcgtc attcgccttg ctttaccatg   1680
```

<210> SEQ ID NO 52

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<211> LENGTH: 2344
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 52

taatacgact cactataggg cacgcgtggt cgacggcccg ggctggtctg gcagatatat 60

gtttagaaac tgtccgaatt tcgagaacag acggccggtc acacagagac gtcgtaatcc 120

ctcgactgcc ctcacgcatc ctggtggacc gattgtccgg gtgacgcaga tcgaaacctt 180

ctgctgattc ttagctgtgt agcgtccagg aaccggtgac ctccgtacca tggctcggtt 240

tcaatagtac ccggcagtcg gcgacgcgca ttgctctcaa gattgagtag gatacctaag 300

gattgtaaac catgatgaca ttctttgtgc gtagtcgagg ttcaatctca tgatctggcg 360

gacgacaggc catgggtacc tgcctacgga ctatgcacga ctgctgtctt gtgtccgatt 420

ggcggacaat atccctcccc tagcagtact ctgtagtgcc gcagtgtgca gtaatgtact 480

ggtgtaatgc tccacgcaag ctctggatac ataccactat atcctaaacg caaaaccttt 540

gaatagaacc acttcttttg gatgatggat cccacatggt ctgactatat attctgctgc 600

gcgtcaagcg gctatctcac tgtctgacac tgagtcgctc gtcgtcagcc catggcgttt 660

gagtcggtta gttttgcttg ccgaaggtct agccgagtct ctgcccgaat gtttcccgcc 720

ctccgccaat cccacggccg atggacagcc tcaggctgcc ttccagccca tggatgccgt 780

gttgcctgag gaccttgcag cgggcgctat cacatgattg tgtcacagca agcaatgagg 840

agcagatcat gattagtgta cttagcttga accctactac taaattgcac acagtcattg 900

gaacaccaca cacagtgcaa tggtggggac aagcgccaaa tagactcgtc tccttttcac 960

aatccaggca gcagtcctgt tgggccgttg tgcacgcatt accgatggaa tagtccaggg 1020

gtcgtgatcc taccacggct cgtctgccga gctctccgct gctcccctgc ccacacacca 1080

cgagcttcct gtcgagcttg cttgcccgtg gcaattctga ttcgttctga tgcattatat 1140

ctcatgacta ttcttctcct atgaagtagc ctcctggcat atattctgca atattaactg 1200

gcacaagtct cgcttcagtc tggtgtcagc gtcggcaatc aactcctcat tatcgcgatt 1260

cgcgggcgga gccccgcgac tccgactgcc tgctagtaac cgacccacca tcgatgatgg 1320

atggagccca ggccacattc cgtcccgggc caggggggtc cggtgccagt ccttgagttc 1380

aactgtcttc gtcccatctt taggacaccg ctgctgggct tcttcctggg gataatcatg 1440

gcacccatga ttctatctcg ccgttcgtgg gctagcggca ggccaatgcc gggaacggca 1500

cagcgggcct ttatcgagac actgccaggc tatggcagag ttgtatagcg gaatggccat 1560

tttgagctgg aaggaataga ttcaaggtac tcgagagtca caatccgtaa gccacattca 1620

ctccgtatca ttatctagcc tctcattcac cagtcaaact catgagtgtc cggtagacat 1680

aggcacgatc tgctcaccgc aattgtcatt tgtgggatgt gctggacata cttggccatt 1740

tacgctttta cgcgggcgct cggaagtcaa cacctcgcta gacaatccct gaagcctgtc 1800

atttgccagg aaggtggact agtgcactgt tgagctggtt gggggtgcgg agcagtttgg 1860

atccggatac ggtcagcaac gtgacccgcc gtataagtac cgctccctcc tcgctttccc 1920

tcgacccttc ctccttctac cacccatcaa taccttcagt tcgttagcaa tcgtcttccc 1980

gtcgttcaat tcaacttctg atcacactct ctgaggcgtg gtcgaatata aaccgtcaaa 2040

attttcgcca cacttcttaa ctcgtaagtt ccccaccatt ctccgcggtc tcccgacgat 2100

ggctgcccct atcccagtgc tccaggaaca gcaattggac cttcctccaa gcaatcccac 2160

tttccagggt cttcttatgg ctaatctgtc tcctttcctt taggcggcac cacccgttca 2220

-continued

```
acggccggcg ctcatccaac cgtggtgggg caccggacta cgcattatac gtccagtaaa   2280

caactcgcag tctgaacact cgtattatct gtctcgcacc ccaatctgtc aactgtgaac   2340

aatg                                                                2344
```

<210> SEQ ID NO 53
<211> LENGTH: 1838
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 53

```
cctcgatccc actctcccca ccctcctcct gtctgagtgt tgtctcatat acctcacccc     60

cagcgaagcc agcttggtag tccgccactt cacagaaacc atctttccgg cgaccacgcc    120

ggtagggttg gttctgtatg agcccatccg gccagacgat gcgttcgggc gcacgatggt    180

ggcgaatcta gccacgaggg ggattcagtt gcagacattg caggagtatg cgtcgctggg    240

ggcgcagcga cggaggttac gggagatggg attggacggg gggcaggcgg cagcagatgt    300

agatttcatc tgggaaaggt gggtgagtga gcgggagaag gaacgggtgg cagggttgga    360

gatgttggat gagatggagg agtggcagtt attggcaaga cattattgtg ttgcatgggg    420

gtggagggat gttcctggtg gagtgtttga gggatggagg gagatggaag ggcaggagga    480

gtaagagtat agtaagtata gtagtagtgt gcgtcatacg tgatggctag ctagctagtt    540

agtttgttgt acctaagtag ttccctagac tatcatataa ttattatata tgaatgaaaa    600

tccacgttga acgcttcgga gcgcacaggc caaggggcga gaaagaggag aagaagtgga    660

atcctgatgg aaaaaatggc agtccaagca acgtgcatgc aaccacctca ggacccctgt    720

ctattgaatt attattattg acaattattg cctgatttca tggtactcga cagggtataa    780

tggcagtttg cctagcaatc atttcattcg tgcaatcaaa cctgacggta gatacgagcg    840

aacgagcaag cgagagacag actaaacata gactattgcc taagctagca ctgtagctcc    900

ccgcctctgt gtacaattgg cttgcgtctc cccctttttc aaactgccaa agtccgtgcc    960

ggtcagtgac tgagactagc tcagaggcag aggcactgac tcgattgaac tcgagcacta   1020

ctttctggct tatactggaa taggatgctg atccggtctg gacgtgtgct gatcgtgatg   1080

tcttgactgg gagaggaagg gagtggttga gagtttgtcc cgtgtcatat ttcgtagagt   1140

tgagttgact tgacagcagg caataattat agatttgagc tggataagat ttaacagaaa   1200

tttctgtata ctctctatcc cccctctctg tgtctatact gtatcctttg cgtgagtgat   1260

cccaccaagt atggaagagt gtctcaaagg gtccacggac cccttatcca tccatcagga   1320

acagtacggt aacctacact attccactat ccccaaagaa gtaatctacg gggggtattc   1380

catgaactgc cgcagtgcaa aggccgctga ttggcgtgac cccctgagcg ggtcatgcct   1440

gattgggatc gaagctttaa ggctatccac attgggtaac ccggggagag catcactttc   1500

aggctactag cagtagacta gtagtcttct ctagtcctgc tggctggtgg ttgtgggttt   1560

ctcttttctc ttgtcttttc cctcgttctt ctctctttct tctcttcttt ctttctctgc   1620

ttcggtccag tctctcgttc ttgtctttac tgaccctagt ctttcgtttc gcgtggtctg   1680

tcgtggtgtc gtatcaaatg attattatta tcttctaacc tatccctctg cctatttgct   1740

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```

<210> SEQ ID NO 54

-continued

<211> LENGTH: 1506
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 54

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catggtggaa gggaaaagtg tggaaagagg gagaagacga ggggcatgcg acactcaaag 120

cctacggatc gggcaagacc atcagacaag cctatctatc ctgtgtggtc tattagatat 180

gccatctgat ccgaaacaat aacccgtaaa aagatactcc gagtccagac ggagttttcc 240

tcgcaagcag gtttgtcgtc atgggcaatc aatggccggg acgcagggga gagacaaagg 300

ataccaggga acgcatccca ttgccgtgtt aaagtgcttg gcatcccggg gacagagggg 360

tacattgcgg gtttacatca tgtgtctgca gttaagctgg attgtgtaag tagggtaata 420

ttattgcagc agtgagctcc aaaaagtacg gtggtgtgga gattgaatcc tcacgttcat 480

atcggtcagt gtgggagagt aacatgggcc gatgttgatc gagggcgggt gtatagttag 540

ggtgaatgcc atatcacaga acatggcggc aagagccggt gaaaaggaaa aggcaaaaaa 600

gaatcatcca cccggagcaa gatgagctgt cggtaagacc acttggagct aggttgtgca 660

atgatgcgtt gggtgtgaga gctgtggttg gaggcagccg tatcctgctc ccccgttttc 720

gggacatagg atgaagagta cggcgtatac cagatcctgg acaccatcag attttctccc 780

tctcaacaat tgtggaaatt aggaggtgga tcgttctgag ttgggagtcc tgtccggtga 840

aacttcccat ccacaatttc gaccccttttt cttctccccg tcatgggggga gaaatggtgt 900

atcgtcgaaa gaagtttgtt gatatgatgc gccgtgactt cgatcaccca aagaccatct 960

atactataga tctgaggcgg cgtgactgcg agaacaccgg cgggacaacc tcaggcaccc 1020

cagggcaggc cagggcgccg accaaccaca gcttgcagac tgagccagac aggcccacca 1080

ggccacgcac tagaagcaca ctaaaaaagt agctgatccg taagtattgt ctggctgcat 1140

aggaacgggg gccgacccag ttcgttgctt tttttttttc tttttttctt ttgcctccgg 1200

ccgatggtca gtgaccacct gggaaaccgt tcgcccgctg gtctcggggg ggatcctcta 1260

gtatatcgtg agcttcacta cttatactct cctctttcac cttctctcaa gctccttttt 1320

tctttctctc tcctccaaca aatttttctc ctcttacttt taatcatttt cttttattct 1380

ccttcttccc ccccatacat catactctcc gcaatagctc tctttcttga gtgttttgtg 1440

tcttaaactc tactgtccca ctttccgctt aatacttacc cctcctcctt ttacacattc 1500

accatg 1506

<210> SEQ ID NO 55
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<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 55

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ggatgaaggt gtgatggatt ggacgcttgt gtacattaat cactcaataa ccagtcattc 120

ctttgaccag atctggtaga aagacagaat tcggctgaaa ccttctctga atgtagtcca 180

tcgcgtagca ctattatgtc ccacttgctc taccatcatc gctaattctt gggattgatc 240

gtctcacttc cccatggaat atatactacc tggctcattt gatgagcccc actcctgttc 300

ttgatatata ccatgtaact tccaaattct gacggcgtag attctcctag actatttgaa 360

gtaaaataat agccatatgc tcacaaatcc aggcaacaat atcagatcat ggcaatagca 420

-continued

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tcattcaggc tgatgtatta cgccaattag tagtggtagt agtagtagta gtagtagtag    480

tagtagtagt agtagtagta gcacgcatct tcaatatcaa aataaataag ctcattcttt    540

ttggtgtcca ctataaagcg atagtactgt atcgaacctc caacacagat ctatagcacg    600

acctccccgc gattgaaaat atatacatga cacaccagtc atgaccccaa gtaaaatacc    660

actgctcgca gtaggaacta aatcttcctt acctgccccc catctactcc ttccacgcgc    720

acatcatgac tactaatatc cccctcagac cccaaatcac tctcacttct ctcaacttcc    780

atcccctcac cctgaaccac tttcggcctg cacggaaaac aatcaatcag catatcccac    840

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tgcaacctcc acgtcgccac cgtcgaaata atcaaaatca cagacataat cgtcaccgta    960

ataaaccctt ccggtactgc ggtgcatcca cctgctgcca caccaccagc ggcaaccagg   1020

cttgaaacac gtacgccatt tcgttcatgc tgcccacgac tagcgcgcgc tcttcgttgt   1080

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agggatggga tgggggggagg gggttggcac c                                 1171
```

<210> SEQ ID NO 56
<211> LENGTH: 878
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 56

```
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ttgggttcgg cgctggacga cgtatggata tggacatgga catggatatg agtttgattt    120

gattgagcgt gtacattact tcactgggta tgcttctgga atgttacctt gtcgatctct    180

tatttcatac tcctccatct ggggtttacc gacacccggt attcccaatc aaaactaact    240

gcagttcaca ccgatcgaca ctactgaatt gcatcgcacc tcgttccaag gatatcctcg    300

cttccagaga aacaaactac gccctcgcag ctctaacctc tcttggcacg cccgtattga    360

ctggccgacc agcaggcgaa ggcttggccg tatatatact ttatccggtc ctcggcctcc    420

gacccactgc ttgcctctat ccggatataa gcatccactt caccaagacg ctatccgcca    480

ctacagcatg ctttgggata atgtcccact caattgccac tcctactcac cgtataggtc    540

ttcttcgctt ggctgaagat ataagtttcc aggcaactat acttggctga tcttggcatg    600

ttcgaggaag atggaagggg cataggttac ggggttactg agtaacgggt ggaaaggagg    660

gagaattggg ttgttgttta aatgtctggt gggagccggg gggtgttgaa gttggaattt    720

gatcgttata gtcgcccgtt tgatactagt cgctctttta tacgttcact ttgtttgttg    780

gctaccatga agctgtctct ggctgttggg gcagccctga tgggatctgc tctggcagtg    840

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```

<210> SEQ ID NO 57
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<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 57

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tggcgttggg acggttgttt tcatcggttt ttatgatacg gataaattgg gcatacctta    120

gggtcaccat cttccatggt gccttgcgtc attcttttac ctaggaatca attcaataat    180
```

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catattccac ctgatatcta ccgctttttt tttgtagatt tagtaggaac ttgaggtaga    240

ccgttacacg ctttcagaga cgccgtcaac gtgcagttca agtcgctatc aaccacctta    300

cataacaaca tgagacgata tcaaatagga actgaatagc ttcaacttca tctacctgta    360

aattatcgaa caagtaacag acatcgagcg tgagtcaccg tcttcgccac ccgtatctcg    420

gcacgtgact gataccgtcc caaggcggcg tgggacagga aagtagcttc cattcatgaa    480

gctgacccag gagagcagtt gcaggcctgt agcacgctgg agatgtgagc atcagtcgtg    540

atgccctcct acttctacca cattgcgatc gaattatttg ctcgcccgca ctctgacctc    600

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gaatctctac tcccgttcca gaagcgccgg cactcaccgt gggcacatcg atcttctcgt    720

catcaagcgc atgggaaaca cccacgcccc tcacccaaca ctttcgccga aacccacggc    780

tcagactcta caaccagcgt tgcggacctc cgtacccctc acctctacac gacttcgccg    840

acaagcgcat ccgacttcga gcgcgacctc aggcttgata aagtatccat cgagtgcatt    900

gacatgatcc catcggagca ggacacaact gctgcgaagc ggacgggctg gaagaatgca    960

aaggagaatc ccatcgcgac cgggatcggc acggatatct tgggaggact acgcacgaaa   1020

ggcaaataca taccgctaga ccagagtacg tcggagagtg tttggggaat cgtgcatctt   1080

taccgagatg ctcaggagac gccgttcttg acagaagagg actacccctc gtacttgaaa   1140

ggttcggcag                                                          1150
```

<210> SEQ ID NO 58
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<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 58

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agggaaagag agacggacaa ccccatttta cccacccact ttactttcca atatattaca    180

attccaattt gaatcaaatt caaatccaaa tctaaattaa attaaattaa acacaacaca    240

tcacaaactt cctacacaaa aattgtaata atcaaatcat aaatcacatt caccaaacct    300

gaccctctcc acaagtccac atactaatct ttccccccta aacacactat tgggccccgt    360

tccacggatc ccccgaccca gaaagcagca acattggtcc ttgctcctcc ttaccccgtt    420

gacttctaca tatccatgga caacacacag cacctgccgt gttcattggc cggaaggcta    480

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gagttgagga cgtgttttaa gtaatttctt gattgagagt gagagagata agtactagtg    660

gggttcggct tggttccaat ggctcggtaa attgggcctt cgtcagtgag tgactctacg    720

tagtagataa tgtagagtct ggagagtctg gatttttttcc tcgcgtttct tggctggctt    780

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<210> SEQ ID NO 59
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<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

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<400> SEQUENCE: 59

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tctgtgatgg tgtcattatc atgtatggta cgacatggat gtgattttaa tgttaatgct 180

atgatcttct atcattattt ctaaatccac ttatatcctg tgtctacgtt atcaaccgtt 240

ctccactcat ttcccctctt atttgccact accggcttct tgccattcca cttgctgaat 300

cgccctagcc cgcgcttgag cagagccagc actgacatca ttgctctgta cagaggcctg 360

cccccagtca tcctcgaacc gaatatccgc cacagtctta gccgcatcat ccacctcaac 420

cag 423

<210> SEQ ID NO 60
<211> LENGTH: 635
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 60

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gtcagccaac tacagccact ctttgaatat cagtgactac cattcgtatc accattcttc 120

tcatatctct ctcattgtat atcactatca cttcgtatac cacacatccg tagatatcta 180

tgcgtcttcc ccaaatccga ataagcattc aacgccaact gccctcccaa atgcgcaaac 240

agaatattcc cctgaatctc ccccctcttg accaaatcca tcatccccgc aaaactcttc 300

ccctcataca ccggatccgt aataaacgcc tccgcttcgc cgcaaactca atcgcctccc 360

acgtcctctt atccggcact ccatacacac ccccatgcca ctcctcccac aactccacat 420

cctcctccga tacctcctcc ctccccaacc caatcctctc cgccgtctcc ttcgcaatcc 480

gcaacacctg ctccctcgtc tcctttccgt ggcactcgca tcaatcccca ccaccctcgt 540

cttccccctc ccatttccag cctctcgatc caacttctcc aacaacttac cagcccgggc 600

cgtcgaccac gcgtgcccta tagtgagtcg tatta 635

<210> SEQ ID NO 61
<211> LENGTH: 800
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 61

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cttagacggt ttaactagat tcccttcagc atgcgcattg tttatttgtg gttcgcctta 120

atagagcttg ggggcagcgg aatgctccta ccaatttccg ggtctgcttt tctcctttac 180

attggttctt aatgtttcat acgttgttca tgtatcctcc tagggaggag accttctctt 240

gtccagacag gagctggaat gcaattatat aagacgatga ccaataattc cagactcatc 300

aagagtcaga aagaagagtc atgaaaggac aatgattata atatggctaa tcaagatgta 360

taacttgaat aactgtctgt agtctttctc tgttttctcg accggattgt tggttgctta 420

ctgtagcata ccttgtcatg tgacatgggt gcaaagagtg gcgtgttctt cctgcacctt 480

caccccgttg caagttgcac tggttgaccc aagcgcctaa gtgacaggaa aatggatagg 540

tagacatcct gctaggttca gggacttatc ggtgggcgtg aaaccaggca tgaccaagaa 600

tagcagcagt ttgagctaca aggacgctct attgttttac ttcacgccga ctccgtttag 660

-continued

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agtatctgtc agtctctgtc tgacccatct acagccaaac ctcgtcacac aataagcact    720

caagttcatc taagatgact gtgattggtc cagaccagcc cgggccgtcg accacgcgtg    780

ccctatagtg agacgtatta                                                800
```

We claim:

1. An isolated polynucleotide molecule comprising a polynucleotide sequence that regulates expression of a gene comprising at least one of SEQ ID NOs 46 and 55, wherein said gene is differentially expressed in a native fungus exhibiting a pellet morphology relative to said native fungus exhibiting a filament morphology and wherein said isolated polynucleotide molecule is combined with a molecule comprising a coding region of a foreign gene for heterologous gene expression.

2. The isolated polynucleotide molecule as recited in claim **1**, wherein said expression is constitutive in said native fungus exhibiting said pellet morphology.

3. The isolated polynucleotide molecule as recited in claim **1**, wherein said expression initiates at a developmental stage in said native fungus exhibiting said filament morphology.

4. The isolated polynucleotide molecule as recited in claim **1**, wherein said expression is constitutive in said native fungus exhibiting said filament morphology.

5. The isolated polynucleotide molecule as recited in claim **1**, wherein said native fungus is *Aspergillus niger*.

6. An isolated polynucleotide molecule comprising a polynucleotide transcription terminator SEQ ID NO. 55 wherein said isolated polynucleotide molecule is combined with a molecule comprising a foreign gene for heterologous gene expression.

7. The isolated polynucleotide molecule as recited in claim **6**, wherein said native fungus is *Aspergillus niger*.

8. An isolated polynucleotide molecule comprising a promoter functional in fungi, wherein said promoter comprises at least of SEQ ID NO.:46.

9. An isolated polynucleotide molecule comprising a transcription terminator, wherein said transcription terminator is a functional terminator comprising SEQ ID NO.:55.

10. A DNA construct comprising the following elements operably linked in the direction of transcription:

a. a first DNA segment comprising SEQ ID NO.:46,

b. a second DNA segment comprising a sequence coding a protein of interest, the second DNA segment being heterologous relative to the functional promoter; and

c. a third DNA segment comprising a functional transcription terminator.

11. The DNA construct as recited in claim **10**, wherein said transcription terminator comprises SEQ ID NO.:55.

12. The DNA construct as recited in claim **10**, wherein said second DNA segment comprises a coding sequence that is differentially expressed in a native fungus exhibiting a pellet morphology relative to said native fungus exhibiting a filament morphology.

13. A vector comprising the DNA construct of claim **10**.

14. The vector as recited in claim **13**, wherein said second DNA segment comprises a coding sequence for GUS.

15. A transformed host cell comprising the DNA construct of claim **10**.

16. The transformed host cell as recited in claim **15**, wherein said transformed host cell constitutively expresses said second DNA segment.

17. The transformed host cell as recited in claim **15**, wherein expression of said second DNA segment by said transformed host cell is temporally-regulated or spatially-regulated.

18. The transformed host cell comprising the DNA construct of claim **15**, wherein said functional transcription terminator is SEQ ID NO.:55.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,449,569 B2
APPLICATION NO. : 10/920625
DATED : November 11, 2008
INVENTOR(S) : Dai et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Delete drawing sheet 11/16 and replace with the drawing sheet 11/16 attached.

Column 5, Line 38 – Replace "DraI, EcoRV, PuvII, or StuI" with --Dra I, EcoR V, Puv II, or Stu I--.

Signed and Sealed this

Twenty-first Day of April, 2009

JOHN DOLL
Acting Director of the United States Patent and Trademark Office

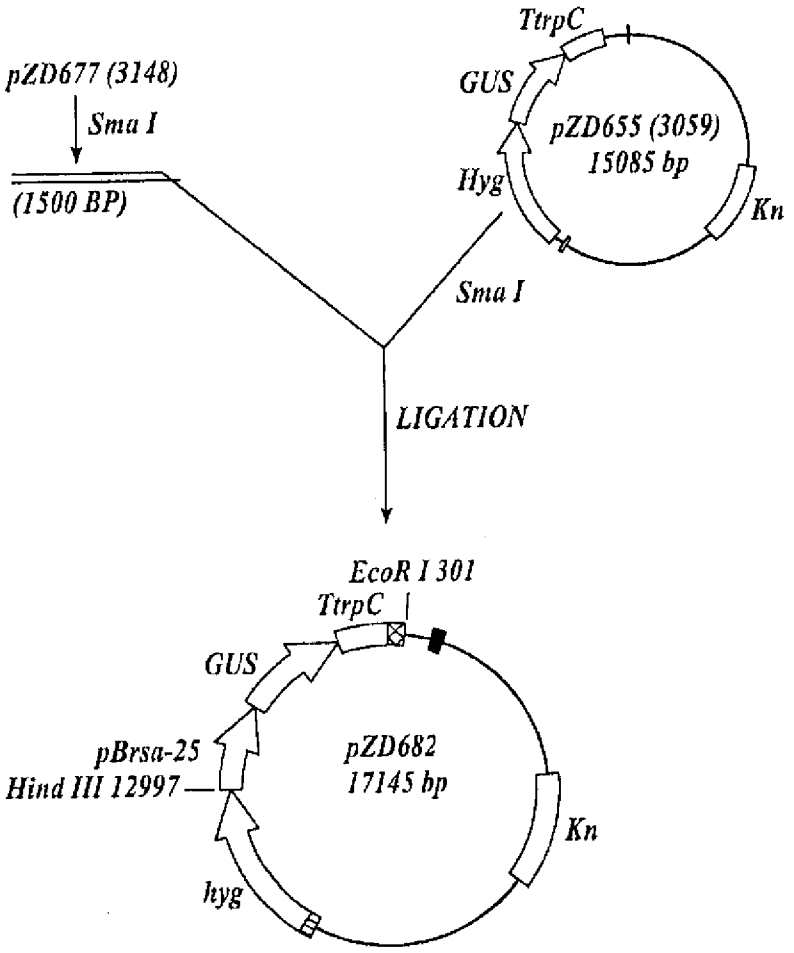
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FIG. 6