Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2186403 C 2006/01/24

(11)(21) 2 186 403

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1995/03/15
(87) Date publication PCT/PCT Publication Date: 1995/10/05
(45) Date de délivrance/Issue Date: 2006/01/24
(85) Entrée phase nationale/National Entry: 1996/09/24
(86) N° demande PCT/PCT Application No.: EP 1995/000958
(87) N° publication PCT/PCT Publication No.: 1995/026406
(30) Priorités/Priorities: 1994/03/25 (P 44 10 382.4) DE;
1994/06/15 (P 44 20 785.9) DE

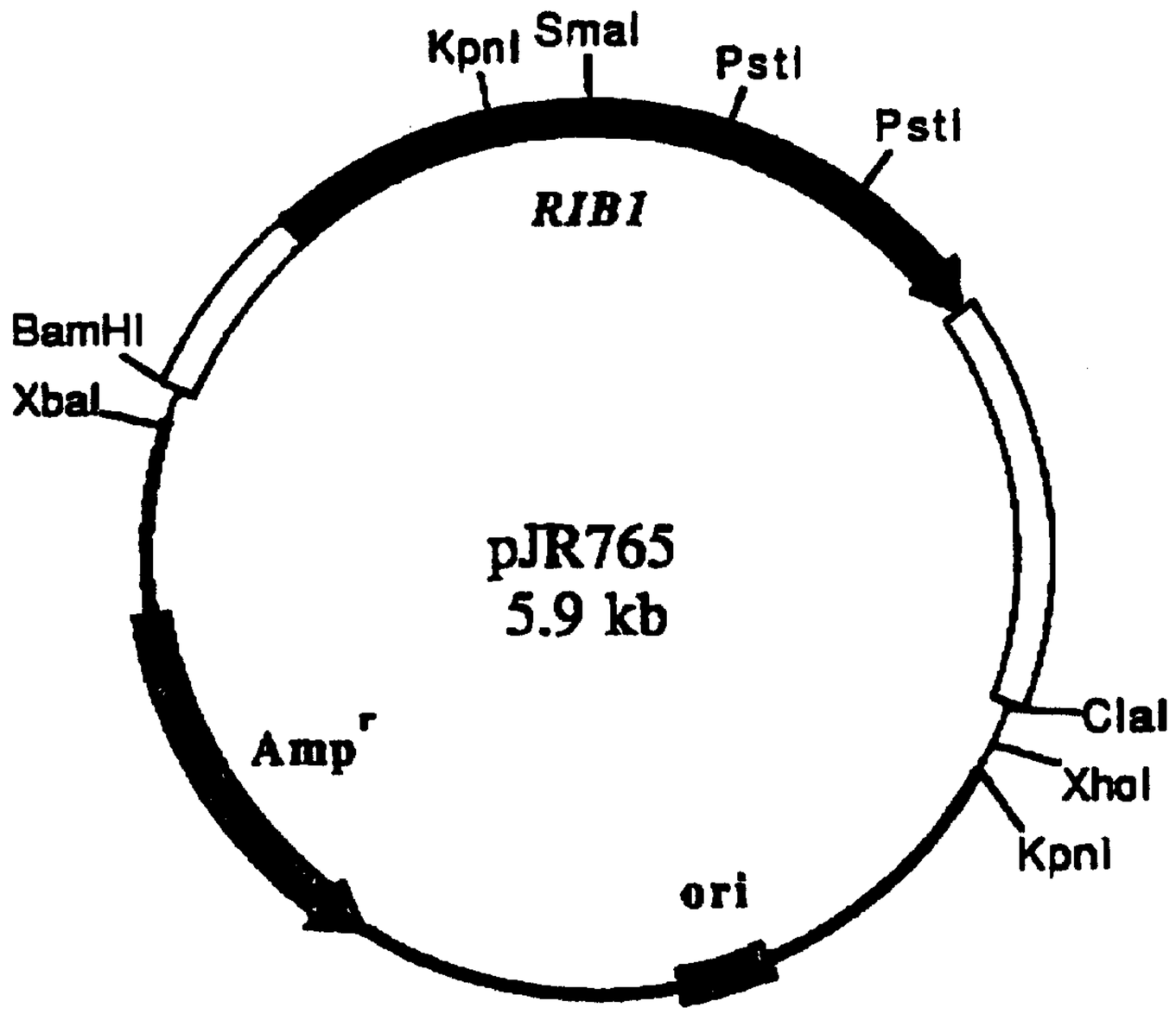
(51) Cl.Int.^{7}/Int.Cl.^{7} C12N 15/52, C12N 15/55, C12N 15/54, C12N 15/53, C12P 25/00

(72) Inventeurs/Inventors:
REVUELTA DOVAL, JOSE LUIS, ES;
BUITRAGO SERNA, MARIA JOSE, ES;
SANTOS GARCIA, MARIA ANGELES, ES

(73) Propriétaire/Owner:
BASF AKTIENGESELLSCHAFT, DE

(74) Agent: ROBIC

(54) Titre : BIOSYNTHESE DE LA RIBOFLAVINE DANS DES CHAMPIGNONS
(54) Title: RIBOFLAVIN BIOSYNTHESIS IN FUNGI



(57) Abrégé/Abstract:
The invention concerns the riboflavin-biosynthesis genes in the fungus Ashbya gossypii as well as a method of producing riboflavin using these genes and gene products.

PCT

WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 6 :
C12N 15/52, 15/53, 15/54, 15/55, 15/81, 1/19, C12P 25/00 // (C12N 1/19, C12R 1:865)

A2

(11) Internationale Veröffentlichungsnummer: WO 95/26406

(43) Internationales Veröffentlichungsdatum: 5. Oktober 1995 (05.10.95)

(21) Internationales Aktenzeichen: PCT/EP95/00958

(22) Internationales Anmeldedatum: 15. März 1995 (15.03.95)

(30) Prioritätsdaten:

P 44 10 382.4	25. März 1994 (25.03.94)	DE
P 44 20 785.9	15. Juni 1994 (15.06.94)	DE

(81) Bestimmungsstaaten: CA, CN, JP, RU, US, europäisches Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Veröffentlicht
Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.

(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: BASF AKTIENGESELLSCHAFT [DE/DE]; D-67056 Ludwigshafen (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: REVUELTA DOVAL, Jose, Luis [ES/ES]; Pza. La Parra, 4, E-37001 Salamanca (ES). BUITRAGO SERNA, Maria, Jose [ES/ES]; Avenida de los Cedros, 33, E-37004 Salamanca (ES). SANTOS GARCIA, Maria, Angeles [ES/ES]; Versalles, 7, E-37009 Santa Marta (ES).

(74) Gemeinsamer Vertreter: BASF AKTIENGESELLSCHAFT; D-67056 Ludwigshafen (DE).

(54) Title: RIBOFLAVIN SYNTHESIS IN FUNGI

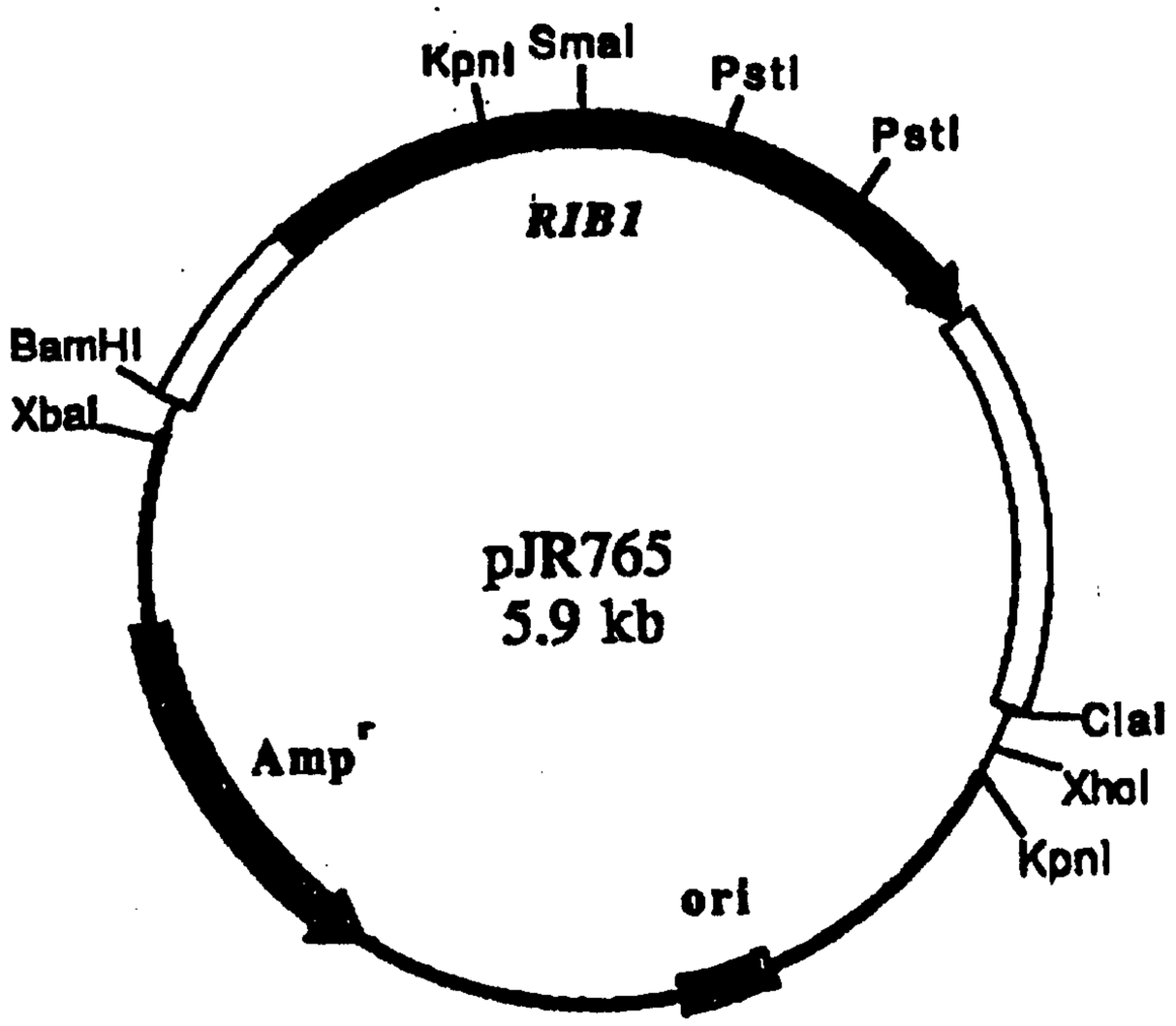
(54) Bezeichnung: RIBOFLAVIN-BIOSYNTHESE IN PILZEN

(57) Abstract

The invention concerns the riboflavin-biosynthesis genes in the fungus Ashbya gossypii as well as a method of producing riboflavin using these genes and gene products.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft die Gene für Riboflavin-Biosynthese in dem Pilz Ashbya gossypii sowie gentechnische Verfahren zur Herstellung von Riboflavin unter Verwendung dieser Gene und Genprodukte.



2186403

Riboflavin biosynthesis in fungi

The present invention relates to the genes for riboflavin biosynthesis in fungi, to the proteins encoded thereby and to genetic engineering processes for preparing riboflavin using these genes and gene products.

The preparation of riboflavin by fermentation of fungi such as Eremothecium ashbyii or Ashbya gossypii has been disclosed (The Merck Index, Windholz et al., eds. Merck & Co., page 1183 (1983)).

EP 405370 describes riboflavin-overproducing bacterial strains which have been obtained by transformation of the riboflavin biosynthesis genes from Bacillus subtilis.

Since the genetics of riboflavin biosynthesis in bacteria and eukaryotes differ, the abovementioned genes from Bacillus subtilis are unsuitable for a recombinant process for preparing riboflavin using eukaryotic producer organisms such as Ashbya gossypii.

The cloning of the riboflavin biosynthesis genes of the yeast Saccharomyces cerevisiae was described in a patent application filed at the German Patent Office on Nov. 19, 1992.

However, it was not possible to clone the Ashbya gossypii riboflavin biosynthesis genes using the S. cerevisiae rib genes by conventional hybridization methods; evidently the homology of the rib genes from S. cerevisiae and A. gossypii was not great enough for hybridization.

It is an object of the present invention to isolate the riboflavin biosynthesis genes from a eukaryote in order in this way to provide a recombinant process for preparing riboflavin in a eukaryotic producer organism.

We have found that this object is achieved by isolation of six genes (rib genes) found in the ascomycete Ashbya gossypii which code for enzymes of riboflavin biosynthesis starting from GTP.

The invention relates to the following DNA sequences:

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 2, or for an analog or derivative of the polypeptide shown in SEQ ID NO: 2, in which one or more amino acids have been deleted, added or replaced by other amino

acids, without essentially reducing the enzymatic action of the polypeptide.

More specifically, the invention relates to an isolated DNA sequence which codes for a polypeptide with the amino acid sequence (GTP cyclohydrolase II) depicted in SEQ ID NO: 2, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 2 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 2, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 2 hybridizes with the polynucleotide depicted in SEQ ID NO: 1 at 42°C in 5 x SSC in 50% formamide solution.

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 4, or for an analog or derivative of the polypeptide shown in SEQ ID NO: 4, in which one or more amino acids have been deleted, added or replaced by other amino acids, without essentially reducing the enzymatic action of the polypeptide.

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 6, or for an analog or derivative of the polypeptide shown in SEQ ID NO: 6, in which one or more amino acids have been deleted, added or replaced by other amino acids, without essentially reducing the enzymatic action of the polypeptide.

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 8, or for an analog or derivative of the polypeptide shown in SEQ ID NO: 8, in which one or more amino acids have been deleted, added or replaced by other amino acids, without essentially reducing the enzymatic action of the polypeptide.

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 10, or for an analog or deriva-

tive of the polypeptide shown in SEQ ID NO: 10, in which one or more amino acids have been deleted, added or replaced by other amino acids, without essentially reducing the enzymatic action of the polypeptide.

DNA sequences which code for a polypeptide with the amino-acid sequence depicted in SEQ ID NO: 12, or for an analog or derivative of the polypeptide shown in SEQ ID NO: 12, in which one or more amino acids have been deleted, added or replaced by other amino acids, without essentially reducing the enzymatic action of the polypeptide.

The genes and their gene products (polypeptides) are shown in the sequence listing with their primary structure and are assigned as follows:

SEQ ID NO: 1: rib 1 gene
SEQ ID NO: 2: rib 1 gene product (GTP cyclohydrolase II)
SEQ ID NO: 3: rib 2 gene
SEQ ID NO: 4: rib 2 gene product (DRAP deaminase)
SEQ ID NO: 5: rib 3 gene

SEQ ID NO: 6: rib 3 gene product (DBP synthase)
SEQ ID NO: 7: rib 4 gene
SEQ ID NO: 8: rib 4 gene product (DMRL synthase)
SEQ ID NO: 9: rib 5 gene
SEQ ID NO: 10: rib 5 gene product (riboflavin synthase)
SEQ ID NO: 11: rib 7 gene
SEQ ID NO: 12: rib 7 gene product (HTP reductase)

Guanosine triphosphate (GTP) is converted by GTP cyclohydrolase II (rib 1 gene product) into 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5-phosphate. This compound is subsequently reduced by the rib 7 gene product to 2,5-diaminoribitylamino-2,4(1H,3H)-pyrimidine 5-phosphate and then deaminated by the rib 2 gene product to 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione. Subsequently, in a reaction catalyzed by the rib 4 gene product, the C4 compound DBP is added on to result in 6,7-dimethyl-8-ribityllumazine (DMRL), from which riboflavin is produced in the reaction catalyzed by the rib 5 gene product. The C4 compound DBP (L-3,4-dihydroxy-2-butanone 4-phosphate) is formed from D-ribulose 5-phosphate in a reaction catalyzed by the rib 3 gene product.

The DNA sequences described in SEQ ID NO: 1, 3, 5, 7, 9, 11 code for the polypeptides described in SEQ ID NO: 2, 4, 6, 8, 10, 12.

DNA sequences, apart from those specified in the sequence listing, which are also suitable are those which, as a consequence of the degeneracy of the genetic code, have a different DNA sequence but code for the same polypeptide.

The invention also relates to those DNA sequences which code for a gene product (polypeptide) with a primary structure other than that detailed in the sequence listing as long as the gene product still has essentially the same biological properties as the gene product specified in the sequence listing. Biological properties mean, in particular, the enzymatic activities bringing about the biosynthesis of riboflavin.

Such modified gene products with essentially the same biological properties can be obtained by deletion or addition of one or more amino acids or peptides or by replacing amino acids by other amino acids, or can be isolated from organisms other than Ashbya gossypii.

The DNA sequences which code for the modified gene products are, as a rule, homologous to the extent of 80 percent or more with the DNA sequences shown in the sequence listing. Such DNA

sequences can be isolated starting from the DNA sequences described in SEQ ID NO: 1, 3, 5, 7, 9, 11, for example with conventional hybridization methods or the PCR technique from eukaryotes other than Ashbya gossypii. These DNA sequences hybridize under standard conditions with the DNA sequences described in SEQ ID NO: 1, 3, 5, 7, 9, 11.

Standard conditions mean, for example, temperatures from 42 to 58°C in an aqueous buffer solution with a concentration of from 0.1 to 1 x SSC (1 x SSC: 0.15 M NaCl, 15 mM sodium citrate pH 7.2). The experimental conditions for DNA hybridizations are described in textbooks of genetic engineering, for example in Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989.

The invention also relates to regulating sequences, in particular promoter sequences, which are located upstream in the 5' direction of the DNA sequences coding for the appropriate polypeptide. The regulating sequences are specified in the sequence listing and explained in detail below.

Regulating sequence for rib 1 gene:
SEQ ID NO: 1 nucleotide 1-242

Regulating sequence for rib 2 gene:
SEQ ID NO: 3 nucleotide 1-450

Regulating sequence for rib 3 gene:
SEQ ID NO: 5 nucleotide 1-314

Regulating sequence for rib 4 gene:
SEQ ID NO: 7 nucleotide 1-270

Regulating sequence for rib 5 gene:
SEQ ID NO: 9 nucleotide 1-524

Regulating sequence for rib 7 gene:
SEQ ID NO: 11 nucleotide 1-352

The regulating sequences can also be truncated in the 5' and/or 3' direction with negligible diminution in their functioning.

Essential for the regulating action are, as a rule, fragments of 30–100, preferably 40–70, nucleotides from the abovementioned sequence regions.

These regulating sequences can also be optimized in their functioning, by comparison with the natural sequences, by directed mutagenesis.

The regulating sequences according to the invention are suitable for overexpression of genes in Ashbya, in particular of genes responsible for riboflavin biosynthesis.

The invention also relates to expression vectors which contain one or more of the DNA sequences according to the invention. Such expression vectors are obtained by providing the DNA sequences according to the invention with suitable functional regulating signals. Such regulating signals are DNA sequences which are responsible for the expression, for example promoters, operators, enhancers, ribosome binding sites, and which are recognized and obeyed by the host organism.

It is also possible where appropriate for the expression vector to comprise other regulating signals which, for example, control replication or recombination of the recombinant DNA in the host organism.

The invention likewise relates to the host organisms transformed with the DNA sequences or expression vectors according to the invention. Eukaryotic organisms are preferably used as host organisms, particularly preferably those of the genus Saccharomyces, Candida, Pichia, Eremothecium or Ashbya. Particularly preferred species are Saccharomyces cerevisiae, Candida flaveri, Candida famata, Eremothecium ashbyii and Ashbya gossypii.

The invention also includes a recombinant process for preparing riboflavin in which the transformed host organisms according to the invention are cultured in a conventional way by fermentation, and the riboflavin produced during the fermentation is isolated from the fermentation medium and, where appropriate, purified.

The rib genes and gene products can be isolated and characterized as described in the example and in the sequence listing.

Example 1

Isolation of the Ashbya gossypii riboflavin biosynthesis genes (rib genes)

a. Construction of an Ashbya gossypii cDNA bank

RNA was completely extracted from the mycelium of the riboflavin-overproducing strain Ashbya gossypii ATCC 10195 in the late logarithmic phase of growth after cultivation on YEPD medium (Sherman et al., "Methods in yeast genetics", Cold Spring Harbor, New York, 1989).

Poly(A)$^+$ RNA was purified by adsorption on and elution from oligo(dT)-cellulose twice (Aviv and Leder, Proc. Natl. Acad. Sci. USA 69, 1972, 1408–1412). The cDNA was isolated by the general method of Gubler and Hoffmann (Gene 25, 1983, 263), and synthetic EcoRI adaptors were added on to the ends of the blunt-ended cDNA molecules. The cDNA fragments after cutting with EcoRI were subsequently phosphorylated using T4 polynucleotide kinase and cloned into the dephosphorylated vector pYEura3* which had been cut with EcoRI (Fig. 1). pYEura3 (Clonetech Laboratories, Inc., California) is a yeast expression vector which contains the galactose-inducible GAL1 and GAL10 promoters and URA, CEN4 and ARS1. These yeast elements permit the transformation and expression of cloned DNA fragments in yeast cells.

Aliquots from the ligation reaction were used to transform highly competent (Hanahan, DNA Cloning, ed. D.M. Glover; IRL Press, Oxford 1985, 109) E. coli XL1-Blue* (Bullock et al., Biotechniques 5 (1987) 376–378), and transformants were selected on the basis of their ampicillin resistance.

About 3 x 10^5 ampicillin-resistant cells were combined and amplified, and plasmid DNA was isolated therefrom (Birnboim and Doly, Nucleic Acids Res. 7, 1979, 1513).

b. Isolation of Ashbya gossypii cDNA clones which code for riboflavin-producing enzymes

cDNA clones from Ashbya gossypii which code for riboflavin-producing enzymes were isolated by functional complementation of Saccharomyces cerevisiae mutants involved in riboflavin biosynthesis.

The strains AJ88 (Mata leu2 his3 rib1::URA3 ura3–52), AJ115 (Matalpha leu2 inos1 rib2::URA3 ura3–52), AJ71 (Matalpha leu2 inos1 rib3::URA3 ura3–52), AJ106 (Matalpha leu2 inos1 rib4::URA3 ura3–52), AJ66 (Mata canR inos1 rib5::URA3 ura3–52) and AJ121 (Matalpha leu2 inos1 rib7::URA3 ura3–52) are mutated strains produced by destruction of one of the six genes (rib1 to rib5 and rib7) involved in riboflavin biosynthesis in Saccharomyces cerevisiae.

* trademarks

These strains were each transformed with 25 µg of cDNA from the Ashbya gossypii cDNA bank and plated on solid galactose-containing medium without riboflavin. After growth for approximately one week, rib$^+$ transformants were isolated from the culture dishes.

In each case one transformant from each transformed mutant (Rib1+, Rib2+, Rib3+, Rib4+, Rib5+ and Rib7+) was analyzed and it was found in all cases that the Rib$^+$ phenotype was expressed only in galactose medium but not in glucose medium.

These results demonstrate that the Rib+ phenotype was expressed under the control of the galactose-inducible GAL10 promoter located on the plasmid.

Plasmid DNA was isolated from the Rib1+, Rib2+, Rib3+, Rib4+, Rib5+ and Rib7+ transformants by transformation of E. coli and was called pJR715, pJR669, pJR788, pJR733, pJR681 and pJR827.

Partial sequencing of the cDNA inserts present in these plasmids confirmed that they code for proteins which are analogous to proteins of the rib gene products from Saccharomyces.

c. Isolation of Ashbya gossypii genomic clones which code for riboflavin-producing enzymes

In order to isolate the genomic copies of the riboflavin-producing genes of Ashbya gossypii, a genomic bank of Ashbya gossypii ATCC 10195 was constructed in the cosmid superCos1 (Stratagene Cloning Systems, California) and screened with ^{32}P-labeled probes which were derived from the cDNA copies of the rib1, rib2, rib3, rib4, rib5 and rib7 genes of Ashbya gossypii.

Cosmid clones with rib1, rib2, rib3, rib4, rib5 and rib7 DNA were isolated by colony hybridization (Grunstein and Hogness, Proc. Natl. Acad. Sci. USA 72, 1975, 3961–3965). Further Southern analyses of enzymatically cleaved cosmid DNA using the same rib–specific cDNA probes made it possible to identify defined restriction fragments which contained the rib1, rib2, rib3, rib4, rib5 and rib7 genes of Ashbya gossypii.

A BamHI–ClaI DNA fragment which was 3.1 kb in length and contained the complete rib1 gene of Ashbya gossypii coding for GTP cyclohydrolase II was found. This fragment was isolated from an agarose gel and cloned into the pBluescript KS (+) phagemid (Stratagene Cloning Systems) cut with BamHI and ClaI, and in this way provided the plasmid pJR765 (Fig. 2).

CA2186403

A DNA sequence (SEQ ID NO: 1) which was 1,329 bp in length and contained the rib1 open reading frame of 906 bp, 242 bp of the 5'—noncoding region and 181 bp of the 3'—noncoding region was obtained.

The complete Ashbya gossypii rib2 gene which codes for DRAP deaminase was found on an EcoRI—PstI fragment which was 3.0 kb in length and which, cloned into pBluescript KS (+), yielded the plasmid pJR758 (Fig. 3).

A region 2,627 bp in length from the EcoRI—PstI insert with the 1,830 bp open reading frame of rib2, 450 bp of the 5'—untranslated region and 347 bp of the 3'—untranslated region was sequenced (SEQ ID NO: 3).

The complete Ashbya gossypii rib 3 gene which codes for DBP synthase was found on a PstI—HindIII fragment which was 1.5 kb in length and, cloned into pBluescript KS (+), yielded the plasmid pJR790 (Fig. 4).

A region 1,082 bp in length from the PstI—HindIII insert with the 639 bp open reading frame of rib 3, 314 bp of the 5'—untranslated region and 129 bp of the 3'—untranslated region was sequenced (SEQ ID NO: 5).

The Ashbya gossypii rib4 gene which codes for DMRL synthase was found on a PstI—PstI fragment which was 3.2 kb in length and, cloned into pBluescript KS (+), yielded the plasmid pJR762 (Fig. 5).

A region 996 bp in length from the PstI—PstI insert with the 519 bp open reading frame of rib4, 270 bp of the 5'—untranslated region and 207 bp of the 3'—untranslated region was sequenced (SEQ ID NO: 7).

The complete Ashbya gossypii rib5 gene which codes for riboflavin synthase was found on a PstI—PstI fragment which was 2.5 kb in length and, cloned into pBluescript KS (+), yielded the plasmid pJR739 (Fig. 6).

A region 1,511 bp in length from the PstI—PstI insert with the 708 bp open reading frame of rib5, 524 bp of the 5'—untranslated region and 279 bp of the 3'—untranslated region was sequenced (SEQ ID NO: 9).

Finally, the Ashbya gossypii rib7 gene which codes for HTP reductase was found on an EcoRI–EcoRI fragment which was 4.1 kb in length and, cloned into pBluescript KS (+), yielded the plasmid pJR845 (Fig. 7).

A region 1,596 bp in length from the EcoRI–EcoRI insert with the 741 bp open reading frame of rib7, 352 bp of the 5'–untranslated region and 503 bp of the 3'–untranslated region was sequenced (SEQ ID NO: 11).

Example 2

mRNA analysis of the Ashbya gossypii rib genes

Northern analyses were carried out to identify the rib-specific transcripts. The total RNA was isolated from the Ashbya gossypii strain ATCC 10195 as described in Example 1. The RNA samples from the strain (5 µg) were fractionated by electrophoresis on 0.8% agarose formaldehyde gels together with RNA size markers and blotted in vacuo onto nylon membranes (Thomas, Proc. Natl. Acad. Sci. USA, 77, 1980, 5201–5205).

The nylon membranes were separately hybridized with ^{32}P–labeled rib-specific DNA probes at 42°C in 5 x SSC and in the presence of 50% formamide. The Ashbya gossypii rib1 gene is expressed as unique message of about 1,150 nucleotides, which was detected in both strains by an SmaI–SacI probe 0.7 kbp in length from the plasmid pJR765 (Fig. 8).

In a similar way, unique 1,900 nucleotide-long rib2, 900 nucleotide-long rib3, 800 nucleotide-long rib4, 1,050 nucleotide-long rib5 and 1,000 nucleotide-long rib7 transcripts were detected in the blots using an SmaI–SmaI fragment 0.5 kbp in length from pJR758, a HindIII–KpnI fragment 0.6 kbp in length from pJR790, an ScaI–HindIII fragment 0.5 kbp in length from pJR739 and a PstI–PstI fragment 0.3 kbp in length from pJR845 as specific probe.

Example 3

Expression of the Ashbya gossypii rib genes in Saccharomyces cerevisiae

It is possible as described in Example 1 to grow Saccharomyces cerevisiae mutants which have been well investigated and are defective in one stage of riboflavin biosynthesis on culture media without riboflavin if they harbor a plasmid which codes for

the complementing Ashbya enzymes. In order to test the function of the Ashbya gossypii rib gene products, flavin-producing enzyme activities were measured in cell-free extracts from S. cerevisiae mutants which harbored one of the expression plasmids pJR715, pJR669, pJR788, pJR733, pJR681 and pJR827.

These plasmids which are derived from pYEura3 and are described in Example 1 contain Ashbya gossypii rib-specific cDNA fragments under the control of the galactose-inducible GAL10 promoter.

Cell-free protein extracts from S. cerevisiae were obtained from cultures which had grown in liquid medium to an optical density of about 2 OD.

The cells were harvested, washed with cold 20 mM tris HCl, pH 7.5, and resuspended in the same buffer, which was supplemented with 1 mM phenylethylsulfonyl fluoride.

Cell lysates were prepared by vortexing in the presence of glass beads and centrifuging at 3,000 g and 4°C for 20 min.

GTP cyclohydrolase II, DRAP deaminase, DBP synthase, DMRL synthase, riboflavin synthase and HTP reductase enzyme activities were determined as described in the literature (Shavlovsky et al., Arch. Microbiol. 124, 1980, 255–259; Richter et al., J. Bacteriol. 175, 1993, 4045–4051; Klein and Bacher, Z. Naturforsch. 35b, 1980, 482–484; Richter et al., J. Bacteriol. 174, 1992, 4050–4056; Nielsen et al., J. Biol. Chem. 261, 1986, 3661; Plaut and Harvey, Methods Enzymol. 18B, 1971, 515–538; Hollander and Brown, Biochem. Biophys. Res. Commun. 89, 1979, 759–763; Shavlovski et al., Biochim. Biophys. Acta, 428, 1976, 611–618).

Protein was quantified by the Peterson method (Anal. Biochem. 83, 1977, 346–356). As is evident from Tab. 1, the plasmid pJR715 brings about the expression of GTP cyclohydrolase II activity in the S. cerevisiae mutant AJ88. Furthermore, this activity is present only in cells which have grown on galactose medium, which indicates that the rib1 cDNA expression of Ashbya gossypii takes place under the control of the galactose-inducible GAL10 promoter.

These results therefore demonstrate that rib1 codes for GTP cyclohydrolase II in Ashbya gossypii. It was shown in a similar way that rib2 codes for DRAP deaminase, rib3 codes for DBP synthase, rib4 codes for DMRL synthase, rib5 codes for riboflavin synthase and rib7 codes for HTP reductase in this fungus.

Tab. 1

GTP cyclohydrolase II activity of the S. cerevisiae rib1 mutant AJ88 and its transformants.

Strain	Plasmid	GTP cyclohydrolase II U/mg protein **)	
		Glucose	Galactose
X 2180-1A*	-	0.48	0.34
AJ 88	-	n.d.	n.d.
AJ 88	pIR715	n.d.	21.60

n.d.:not detected

*) Wild-type

**) Units of GTP cyclohydrolase II activities
1U catalyzes the formation of 1 nmol of HTP per hour

Tab.2

DRAP deaminase activity of the S. cerevisiae rib2 mutant AJ115 and its transformants.

Strain	Plasmid	DRAP deaminase U/mg protein *)	
		Glucose	Galactose
X 2180-1A	-	0.45	0.38
AJ 115	-	n.d.	n.d.
AJ 115	pIR669	n.d.	53.22

n.d.:not detected

*) 1U catalyzes the formation of 1 nmol of ARAP per hour

Tab.3

DBP synthase activity of the S. cerevisiae rib3 mutant AJ71 and its transformants.

Strain	Plasmid	DBP synthase U/mg protein *)	
		Glucose	Galactose
X 2180-1A	-	0.80	0.75
AJ 71	-	n.d.	n.d.
AJ 71	pIR788	n.d.	25.19

n.d.:not detected

*) 1U catalyzes the formation of 1 nmol of DBP per hour

Tab.4

DMRL synthase activity of the S. cerevisiae rib4 mutant AJ106 and its transformants.

Strain	Plasmid	DMRL synthase U/mg protein *)	
		Glucose	Galactose
X 2180-1A	-	2.04	1.73
AJ 106	-	n.d.	n.d.
AJ 106	pIR733	n.d.	86.54

n.d.:not detected

*) 1U catalyzes the formation of 1 nmol of DMRL per hour

Tab.5

Riboflavin synthase activity of the S. cerevisiae rib5 mutant AJ66 and its transformants.

Strain	Plasmid	Riboflavin synthase U/mg protein *)	
		Glucose	Galactose
X 2180-1A	-	4.41	3.80
AJ 66	-	n.d.	n.d.
AJ 66	pIR681	n.d.	164.20

n.d.:not detected

*) 1U catalyzes the formation of 1 nmol of riboflavin per hour

Tab.6

HTP reductase activity of the S. cerevisiae rib7 mutant AJ121 and its transformants.

Strain	Plasmid	HTP reductase U/mg protein *)	
		Glucose	Galactose
X 2180-1A	-	1.86	2.54
AJ 121	-	n.d.	n.d.
AJ 121	pIR827	n.d.	46.21

n.d.:not detected

*) 1U catalyzes the formation of 1 nmol of DRAP per hour

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: BASF Aktiengesellschaft

(B) STREET: Carl-Bosch-Strasse 38

(C) CITY: Ludwigshafen

(E) COUNTRY: Federal Republic of Germany

(F) POSTAL CODE: D-67056

(G) TELEPHONE: 0621/6048526

(H) TELEFAX: 0621/6043123

(I) TELEX: 1762175170

(ii) TITLE OF APPLICATION: Riboflavin biosynthesis in fungi

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER-READABLE FORM:

(A) MEDIUM TYPE: floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1329 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..242
(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 243..1148
(ix) FEATURES:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1149..1329

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
TTTCTGTCCG CATACTTCAT ATGCTCATCG CACATTGATA ATGTACATTC GAAAAATTTC      60
AAGATTAGCC TCCGTGAACA GCGATTTACC TTAGGCAAAA GTAACAAAAG GCTTTTCCGT     120
AGGTGCTTTG TCATTCAACA ATCCACGTCG GAATTGGCGA CTATATAGTG TAGGGCCCAT     180
AAAGCAGTAG TCGGTGTTGA TAGCTGTGTC AGACCAACTC TTTGTTAATT ACTGAAGCTG     240
AT ATG ACT GAA TAC ACA GTG CCA GAA GTG AGG TGT GTC GCA CGC GCG         287
   Met Thr Glu Tyr Thr Val Pro Glu Val Arg Cys Val Ala Arg Ala
     1               5                  10                  15
CGC ATA CCG ACG GTA CAG GGC ACC GAT GTC TTC CTC CAT CTA TAC CAC       335
Arg Ile Pro Thr Val Gln Gly Thr Asp Val Phe Leu His Leu Tyr His
                20                  25                  30
AAC TCG ATC GAC AGC AAG GAA CAC CTA GCG ATT GTC TTC GGC GAG AAC       383
Asn Ser Ile Asp Ser Lys Glu His Leu Ala Ile Val Phe Gly Glu Asn
            35                  40                  45
ATA CGC TCG CGG AGT CTG TTC CGG TAC CGG AAA GAC GAC ACG CAG CAG       431
Ile Arg Ser Arg Ser Leu Phe Arg Tyr Arg Lys Asp Asp Thr Gln Gln
        50                  55                  60
GCG CGG ATG GTG CGG GGC GCC TAC GTG GGC CAG CTG TAC CCC GGG CGG       479
Ala Arg Met Val Arg Gly Ala Tyr Val Gly Gln Leu Tyr Pro Gly Arg
    65                  70                  75
ACC GAG GCA GAC GCG GAT CGG CGT CAG GGC CTG GAG CTG CGG TTT GAT       527
Thr Glu Ala Asp Ala Asp Arg Arg Gln Gly Leu Glu Leu Arg Phe Asp
80                  85                  90                  95
GAG ACA GGG CAG CTG GTG GTG GAG CGG GCG ACG ACG TGG ACC AGG GAG       575
Glu Thr Gly Gln Leu Val Val Glu Arg Ala Thr Thr Trp Thr Arg Glu
                100                 105                 110
CCG ACA CTG GTG CGG CTG CAC TCG GAG TGT TAC ACG GGC GAG ACG GCG       623
Pro Thr Leu Val Arg Leu His Ser Glu Cys Tyr Thr Gly Glu Thr Ala
            115                 120                 125
TGG AGC GCG CGG TGC GAC TGC GGG GAG CAG TTC GAC CAG GCG GGT AAG       671
Trp Ser Ala Arg Cys Asp Cys Gly Glu Gln Phe Asp Gln Ala Gly Lys
        130                 135                 140
CTG ATG GCT GCG GCG ACA GAG GGC GAG GTG GTT GGC GGT GCG GGG CAC       719
Leu Met Ala Ala Ala Thr Glu Gly Glu Val Val Gly Gly Ala Gly His
    145                 150                 155
GGC GTG ATC GTG TAC CTG CGG CAG GAG GGC CGC GGC ATC GGG CTA GGC       767
Gly Val Ile Val Tyr Leu Arg Gln Glu Gly Arg Gly Ile Gly Leu Gly
160                 165                 170                 175
```

GAG AAG CTG AAG GCG TAC AAC CTG CAG GAC CTG GGC GCG GAC ACG GTG 815
Glu Lys Leu Lys Ala Tyr Asn Leu Gln Asp Leu Gly Ala Asp Thr Val
180 185 190

CAG GCG AAC GAG CTG CTC AAC CAC CCT GCG GAC GCG CGC GAC TTC TCG 863
Gln Ala Asn Glu Leu Leu Asn His Pro Ala Asp Ala Arg Asp Phe Ser
195 200 205

TTG GGG CGC GCA ATC CTA CTG GAC CTC GGT ATC GAG GAC ATC CGG TTG 911
Leu Gly Arg Ala Ile Leu Leu Asp Leu Gly Ile Glu Asp Ile Arg Leu
210 215 220

CTC ACG AAT AAC CCC GAC AAG GTG CAG CAG GTG CAC TGT CCG CCG GCG 959
Leu Thr Asn Asn Pro Asp Lys Val Gln Gln Val His Cys Pro Pro Ala
225 230 235

CTA CGC TGC ATC GAG CGG GTG CCC ATG GTG CCG CTT TCA TGG ACT CAG 1007
Leu Arg Cys Ile Glu Arg Val Pro Met Val Pro Leu Ser Trp Thr Gln
240 245 250 255

CCC ACA CAG GGC GTG CGC TCG CGC GAG CTG GAC GGC TAC CTG CGC GCC 1055
Pro Thr Gln Gly Val Arg Ser Arg Glu Leu Asp Gly Tyr Leu Arg Ala
260 265 270

AAG GTC GAG CGC ATG GGG CAC ATG CTG CAG CGG CCG CTG GTG CTG CAC 1103
Lys Val Glu Arg Met Gly His Met Leu Gln Arg Pro Leu Val Leu His
275 280 285

ACG TCT GCG GCG GCC GAG CTC CCC CGC GCC AAC ACA CAC ATA TAATCTTTGC 1155
Thr Ser Ala Ala Ala Glu Leu Pro Arg Ala Asn Thr His Ile
290 295 300

TATATTAAAA CTCTATAAAC GTATGCCACA CGGCGCCCGC GGGCTGCCAC ACGCTGCTCA 1215

CGGGCTGCCG AACAGTTCTA ACAAGTAATC GCGCGCCTCG CCAGTGATCG TGGCGAGCAC 1275

CTTGTCGTCC ATCATCACAT ATCCTCGGCT ACAGTCGTCG TTGAAGAGCG TGCA 1329

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 301 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Thr Glu Tyr Thr Val Pro Glu Val Arg Cys Val Ala Arg Ala Arg
1 5 10 15

Ile Pro Thr Val Gln Gly Thr Asp Val Phe Leu His Leu Tyr His Asn
20 25 30

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

Arg Ser Arg Ser Leu Phe Arg Tyr Arg Lys Asp Asp Thr Gln Gln Ala
50 55 60

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

Glu Ala Asp Ala Asp Arg Arg Gln Gly Leu Glu Leu Arg Phe Asp Glu
85 90 95

Thr Gly Gln Leu Val Val Glu Arg Ala Thr Thr Trp Thr Arg Glu Pro
100 105 110
Thr Leu Val Arg Leu His Ser Glu Cys Tyr Thr Gly Glu Thr Ala Trp
115 120 125
Ser Ala Arg Cys Asp Cys Gly Glu Gln Phe Asp Gln Ala Gly Lys Leu
130 135 140
Met Ala Ala Ala Thr Glu Gly Glu Val Val Gly Gly Ala Gly His Gly
145 150 155 160
Val Ile Val Tyr Leu Arg Gln Glu Gly Arg Gly Ile Gly Leu Gly Glu
165 170 175
Lys Leu Lys Ala Tyr Asn Leu Gln Asp Leu Gly Ala Asp Thr Val Gln
180 185 190
Ala Asn Glu Leu Leu Asn His Pro Ala Asp Ala Arg Asp Phe Ser Leu
195 200 205
Gly Arg Ala Ile Leu Leu Asp Leu Gly Ile Glu Asp Ile Arg Leu Leu
210 215 220
Thr Asn Asn Pro Asp Lys Val Gln Gln Val His Cys Pro Pro Ala Leu
225 230 235 240
Arg Cys Ile Glu Arg Val Pro Met Val Pro Leu Ser Trp Thr Gln Pro
245 250 255
Thr Gln Gly Val Arg Ser Arg Glu Leu Asp Gly Tyr Leu Arg Ala Lys
260 265 270
Val Glu Arg Met Gly His Met Leu Gln Arg Pro Leu Val Leu His Thr
275 280 285
Ser Ala Ala Ala Glu Leu Pro Arg Ala Asn Thr His Ile
290 295 300

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2627 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..450

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 451..2280

(ix) FEATURES:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 2281..2627

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CTGCAGGACA ATTTAAATTA CGATTACACG CGGCAGCCTT CTTGGTGCGA CAGGATTTTG      60
TACAAGAATG ACCCCAAGCG GGTAAGAGTT CATAGGTATG CCTCGATTGA TAGACGTTCC     120
ATTTTGAATT ATACTGATCA CGAACCCGTA ACGCTCGATG TCAGCGTTTC ATGCCATACA     180
CAATTTGTCC CAATGGCTAT GCAGAATATT TCCCCACAGA GCACCATGGA AATGTATGTG     240
GGAGACGTCA CAGATATACT ACTGATGTTG TTCTCCAGAG TATACTACGC CCCTACCATA     300
TTCGATCTTG TGGTATTGAC GATATTCCTC TGTTTGGTTT TACTGGCACT ATTCCGTTTG     360
ACGGTATAGC GCTATTCGTT CATAGTGACA CATGCGGCAC TAGCTATTCA GCGAATCCTT     420
TATAAACTGC TACTTAACGT TCGTAACACC ATG CTC AAA GGC GTT CCT GGC CTT       474
                                 Met Leu Lys Gly Val Pro Gly Leu
                                   1               5

CTT TTT AAG GAG ACG CAA CGT CAT CTG AAA CCC AGG CTG GTT AGG ATT       522
Leu Phe Lys Glu Thr Gln Arg His Leu Lys Pro Arg Leu Val Arg Ile
     10                  15                  20

ATG GAA AAC ACA TCG CAG GAT GAG AGT CGC AAA AGA CAG GTC GCT TCG       570
Met Glu Asn Thr Ser Gln Asp Glu Ser Arg Lys Arg Gln Val Ala Ser
 25                  30                  35                  40

AAC TTG AGC AGC GAT GCC GAT GAG GGC TCG CCG GCA GTT ACG AGG CCG       618
Asn Leu Ser Ser Asp Ala Asp Glu Gly Ser Pro Ala Val Thr Arg Pro
                 45                  50                  55

GTT AAA ATC ACC AAA CGC CTC AGG AAG AAG AAC CTC GGG ACA GGC GAG       666
Val Lys Ile Thr Lys Arg Leu Arg Lys Lys Asn Leu Gly Thr Gly Glu
             60                  65                  70

CTA CGG GAC AAA GCA GGA TTC AAG TTG AAG GTG CAA GAC GTG AGC AAA       714
Leu Arg Asp Lys Ala Gly Phe Lys Leu Lys Val Gln Asp Val Ser Lys
         75                  80                  85

AAC CGT CAC AGA CAG GTC GAT CCG GAA TAC GAA GTC GTG GTA GAT GGC       762
Asn Arg His Arg Gln Val Asp Pro Glu Tyr Glu Val Val Val Asp Gly
     90                  95                  100

CCG ATG CGC AAG ATC AAA CCG TAT TTC TTC ACA TAC AAG ACT TTC TGC       810
Pro Met Arg Lys Ile Lys Pro Tyr Phe Phe Thr Tyr Lys Thr Phe Cys
105                 110                 115                 120

AAG GAG CGC TGG AGA GAT CGG AAG TTG CTT GAT GTG TTT GTG GAT GAA       858
Lys Glu Arg Trp Arg Asp Arg Lys Leu Leu Asp Val Phe Val Asp Glu
                125                 130                 135

TTT CGG GAC CGC GAT AGG CCT TAC TAC GAG AAA GTC ATC GGT TCG GGT       906
Phe Arg Asp Arg Asp Arg Pro Tyr Tyr Glu Lys Val Ile Gly Ser Gly
            140                 145                 150

GGT GTG CTC CTG AAC GGT AAG TCA TCG ACG TTA GAT AGC GTA TTG CGT       954
Gly Val Leu Leu Asn Gly Lys Ser Ser Thr Leu Asp Ser Val Leu Arg
        155                 160                 165

AAT GGA GAC CTC ATT TCG CAC GAG CTG CAC CGT CAT GAG CCA CCG GTC      1002
Asn Gly Asp Leu Ile Ser His Glu Leu His Arg His Glu Pro Pro Val
    170                 175                 180

TCC TCT AGG CCG ATT AGG ACG GTG TAC GAA GAT GAT GAC ATC CTG GTG      1050
Ser Ser Arg Pro Ile Arg Thr Val Tyr Glu Asp Asp Asp Ile Leu Val
185                 190                 195                 200
```

```
ATT GAC AAG CCC AGC GGG ATT CCA GCC CAT CCC ACC GGG CGT TAC CGC      1098
Ile Asp Lys Pro Ser Gly Ile Pro Ala His Pro Thr Gly Arg Tyr Arg
                205                 210                 215
TTC AAC TCC ATT ACG AAA ATA CTT GAA AAA CAG CTT GGA TAC ACT GTT      1146
Phe Asn Ser Ile Thr Lys Ile Leu Glu Lys Gln Leu Gly Tyr Thr Val
            220                 225                 230
CAT CCA TGT AAC CGA CTG GAC CGC CTA ACC AGT GGC CTA ATG TTC TTG      1194
His Pro Cys Asn Arg Leu Asp Arg Leu Thr Ser Gly Leu Met Phe Leu
        235                 240                 245
GCA AAA ACT CCA AAG GGA GCC GAT GAG ATG GGT GAT CAG ATG AAG GCG      1242
Ala Lys Thr Pro Lys Gly Ala Asp Glu Met Gly Asp Gln Met Lys Ala
    250                 255                 260
CGC GAA GTG AAG AAA GAA TAT GTT GCC CGG GTT GTT GGG GAA TTT CCT      1290
Arg Glu Val Lys Lys Glu Tyr Val Ala Arg Val Val Gly Glu Phe Pro
265                 270                 275                 280
ATA GGT GAG ATA GTT GTG GAT ATG CCA CTG AAG ACT ATA GAG CCG AAG      1338
Ile Gly Glu Ile Val Val Asp Met Pro Leu Lys Thr Ile Glu Pro Lys
                285                 290                 295
CTT GCC CTA AAC ATG GTT TGC GAC CCG GAA GAC GAA GCG GGC AAG GGC      1386
Leu Ala Leu Asn Met Val Cys Asp Pro Glu Asp Glu Ala Gly Lys Gly
            300                 305                 310
GCT AAG ACG CAG TTC AAA AGA ATC AGC TAC GAT GGA CAA ACG AGC ATA      1434
Ala Lys Thr Gln Phe Lys Arg Ile Ser Tyr Asp Gly Gln Thr Ser Ile
        315                 320                 325
GTC AAG TGC CAA CCG TAC ACG GGC CGG ACG CAT CAG ATC CGT GTT CAC      1482
Val Lys Cys Gln Pro Tyr Thr Gly Arg Thr His Gln Ile Arg Val His
    330                 335                 340
TTG CAA TAC CTG GGC TTC CCA ATT GCC AAC GAT CCG ATT TAT TCC AAT      1530
Leu Gln Tyr Leu Gly Phe Pro Ile Ala Asn Asp Pro Ile Tyr Ser Asn
345                 350                 355                 360
CCG CAC ATA TGG GGC CCA AGT CTG GGC AAG GAA TGC AAA GCA GAC TAC      1578
Pro His Ile Trp Gly Pro Ser Leu Gly Lys Glu Cys Lys Ala Asp Tyr
                365                 370                 375
AAG GAG GTC ATC CAA AAA CTA AAC GAA ATT GGT AAG ACT AAA TCT GCG      1626
Lys Glu Val Ile Gln Lys Leu Asn Glu Ile Gly Lys Thr Lys Ser Ala
            380                 385                 390
GAA AGT TGG TAC CAT TCT GAT TCC CAA GGT GAA GTT TTC AAA GGG GAA      1674
Glu Ser Trp Tyr His Ser Asp Ser Gln Gly Glu Val Phe Lys Gly Glu
        395                 400                 405
CAA TGC GAT GAA TGT GGC ACC GAA CTG TAC ACT GAC CCG GGC CCG AAT      1722
Gln Cys Asp Glu Cys Gly Thr Glu Leu Tyr Thr Asp Pro Gly Pro Asn
    410                 415                 420
GAT CTT GAC TTA TGG TTG CAT GCA TAT CGG TAT GAA TCC ACT GAA CTG      1770
Asp Leu Asp Leu Trp Leu His Ala Tyr Arg Tyr Glu Ser Thr Glu Leu
425                 430                 435                 440
```

```
GAT GAG AAC GGT GCT AAA AAG CGG AGT TAC TCT ACT GCG TTT CCT GAG      1818
Asp Glu Asn Gly Ala Lys Lys Arg Ser Tyr Ser Thr Ala Phe Pro Glu
                445                 450                 455
TGG GCT CTT GAG CAG CAC GGC GAC TTC ATG CGG CTT GCC ATC GAA CAG      1866
Trp Ala Leu Glu Gln His Gly Asp Phe Met Arg Leu Ala Ile Glu Gln
            460                 465                 470
GCT AAG AAA TGC CCA CCC GCG AAG ACA TCA TTT AGC GTT GGT GCC GTG      1914
Ala Lys Lys Cys Pro Pro Ala Lys Thr Ser Phe Ser Val Gly Ala Val
        475                 480                 485
TTA GTT AAT GGG ACC GAG ATT TTG GCC ACT GGT TAC TCA CGG GAG CTG      1962
Leu Val Asn Gly Thr Glu Ile Leu Ala Thr Gly Tyr Ser Arg Glu Leu
    490                 495                 500
GAA GGC AAC ACG CAC GCT GAA CAA TGT GCA CTT CAA AAA TAT TTT GAA      2010
Glu Gly Asn Thr His Ala Glu Gln Cys Ala Leu Gln Lys Tyr Phe Glu
505                 510                 515                 520
CAA CAT AAA ACC GAC AAG GTT CCT ATT GGT ACA GTA ATA TAC ACG ACT      2058
Gln His Lys Thr Asp Lys Val Pro Ile Gly Thr Val Ile Tyr Thr Thr
                525                 530                 535
ATG GAG CCT TGT TCT CTC CGT CTC AGT GGT AAT AAA CCG TGT GTT GAG      2106
Met Glu Pro Cys Ser Leu Arg Leu Ser Gly Asn Lys Pro Cys Val Glu
            540                 545                 550
CGT ATA ATC TGC CAG CAG GGT AAT ATT ACT GCT GTT TTT GTT GGC GTA      2154
Arg Ile Ile Cys Gln Gln Gly Asn Ile Thr Ala Val Phe Val Gly Val
        555                 560                 565
CTT GAG CCA GAC AAC TTC GTG AAG AAC AAT ACA AGT CGT GCG CTA TTG      2202
Leu Glu Pro Asp Asn Phe Val Lys Asn Asn Thr Ser Arg Ala Leu Leu
    570                 575                 580
GAA CAA CAT GGT ATA GAC TAT ATT CTT GTC CCT GGG TTT CAA GAA GAA      2250
Glu Gln His Gly Ile Asp Tyr Ile Leu Val Pro Gly Phe Gln Glu Glu
585                 590                 595                 600
TGT ACT GAA GCC GCA TTG AAG GGT CAT TGATTTTGCT GCGAATTGTA            2297
Cys Thr Glu Ala Ala Leu Lys Gly His
                605                 610
GATGACTTAA AATATCGAGG CGTATAATTC GTCGCATTTT ATATAGTTAT CTATGTTTAC    2357
ATGACTGTTT AAGCTTGATC TATATTTCTC AAGTGAATTG CCACATATGT TGGTACGGTA    2417
ATAAATTAAT GAGGGAGTTT TGAAATTCGC AACCAATCTT ATATACGTTT GATGATATAA    2477
ACGGATTGAG ATTCATTAAG CTACCTGATT TTCGCTGAAC TGTTTGTTAT AGGTTTTTAC    2537
AGTAAGATAG TTCCTAAGTT TGTTTATTGT CCCCAGTCGG CCAATTGTTC CGGACTTATT    2597
ATTATTACCA TTAGTGGTGT TAGTAGTATT                                     2627
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 609 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CA2186403

Met Leu Lys Gly Val Pro Gly Leu Leu Phe Lys Glu Thr Gln Arg His
1 5 10 15
Leu Lys Pro Arg Leu Val Arg Ile Met Glu Asn Thr Ser Gln Asp Glu
20 25 30
Ser Arg Lys Arg Gln Val Ala Ser Asn Leu Ser Ser Asp Ala Asp Glu
35 40 45
Gly Ser Pro Ala Val Thr Arg Pro Val Lys Ile Thr Lys Arg Leu Arg
50 55 60
Lys Lys Asn Leu Gly Thr Gly Glu Leu Arg Asp Lys Ala Gly Phe Lys
65 70 75 80
Leu Lys Val Gln Asp Val Ser Lys Asn Arg His Arg Gln Val Asp Pro
85 90 95
Glu Tyr Glu Val Val Val Asp Gly Pro Met Arg Lys Ile Lys Pro Tyr
100 105 110
Phe Phe Thr Tyr Lys Thr Phe Cys Lys Glu Arg Trp Arg Asp Arg Lys
115 120 125
Leu Leu Asp Val Phe Val Asp Glu Phe Arg Asp Arg Asp Arg Pro Tyr
130 135 140
Tyr Glu Lys Val Ile Gly Ser Gly Gly Val Leu Leu Asn Gly Lys Ser
145 150 155 160
Ser Thr Leu Asp Ser Val Leu Arg Asn Gly Asp Leu Ile Ser His Glu
165 170 175
Leu His Arg His Glu Pro Pro Val Ser Ser Arg Pro Ile Arg Thr Val
180 185 190
Tyr Glu Asp Asp Asp Ile Leu Val Ile Asp Lys Pro Ser Gly Ile Pro
195 200 205
Ala His Pro Thr Gly Arg Tyr Arg Phe Asn Ser Ile Thr Lys Ile Leu
210 215 220
Glu Lys Gln Leu Gly Tyr Thr Val His Pro Cys Asn Arg Leu Asp Arg
225 230 235 240
Leu Thr Ser Gly Leu Met Phe Leu Ala Lys Thr Pro Lys Gly Ala Asp
245 250 255
Glu Met Gly Asp Gln Met Lys Ala Arg Glu Val Lys Lys Glu Tyr Val
260 265 270
Ala Arg Val Val Gly Glu Phe Pro Ile Gly Glu Ile Val Val Asp Met
275 280 285
Pro Leu Lys Thr Ile Glu Pro Lys Leu Ala Leu Asn Met Val Cys Asp
290 295 300
Pro Glu Asp Glu Ala Gly Lys Gly Ala Lys Thr Gln Phe Lys Arg Ile
305 310 315 320
Ser Tyr Asp Gly Gln Thr Ser Ile Val Lys Cys Gln Pro Tyr Thr Gly
325 330 335
Arg Thr His Gln Ile Arg Val His Leu Gln Tyr Leu Gly Phe Pro Ile
340 345 350
Ala Asn Asp Pro Ile Tyr Ser Asn Pro His Ile Trp Gly Pro Ser Leu
355 360 365

Gly Lys Glu Cys Lys Ala Asp Tyr Lys Glu Val Ile Gln Lys Leu Asn
370 375 380
Glu Ile Gly Lys Thr Lys Ser Ala Glu Ser Trp Tyr His Ser Asp Ser
385 390 395 400
Gln Gly Glu Val Phe Lys Gly Glu Gln Cys Asp Glu Cys Gly Thr Glu
405 410 415
Leu Tyr Thr Asp Pro Gly Pro Asn Asp Leu Asp Leu Trp Leu His Ala
420 425 430
Tyr Arg Tyr Glu Ser Thr Glu Leu Asp Glu Asn Gly Ala Lys Lys Arg
435 440 445
Ser Tyr Ser Thr Ala Phe Pro Glu Trp Ala Leu Glu Gln His Gly Asp
450 455 460
Phe Met Arg Leu Ala Ile Glu Gln Ala Lys Lys Cys Pro Pro Ala Lys
465 470 475 480
Thr Ser Phe Ser Val Gly Ala Val Leu Val Asn Gly Thr Glu Ile Leu
485 490 495
Ala Thr Gly Tyr Ser Arg Glu Leu Glu Gly Asn Thr His Ala Glu Gln
500 505 510
Cys Ala Leu Gln Lys Tyr Phe Glu Gln His Lys Thr Asp Lys Val Pro
515 520 525
Ile Gly Thr Val Ile Tyr Thr Thr Met Glu Pro Cys Ser Leu Arg Leu
530 535 540
Ser Gly Asn Lys Pro Cys Val Glu Arg Ile Ile Cys Gln Gln Gly Asn
545 550 555 560
Ile Thr Ala Val Phe Val Gly Val Leu Glu Pro Asp Asn Phe Val Lys
565 570 575
Asn Asn Thr Ser Arg Ala Leu Leu Glu Gln His Gly Ile Asp Tyr Ile
580 585 590
Leu Val Pro Gly Phe Gln Glu Glu Cys Thr Glu Ala Ala Leu Lys Gly
595 600 605
His

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1082 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..314

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 315..953
(ix) FEATURES:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 954..1082
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
CCCTTCTTGC ACGGTCGTTT CTGAAACTCT ACGATTATTG GAACAATGAG TAAGTCCTCA      60
AATGTACCAC CTATCTGTAG TTTACTATCG GATTTACTGG CTAAGAGCTG ACCTGTTAGG     120
CAAGTGAAAC ATATCACATC GCCAGCAGGT TGGGCTACCA AGGATAGTTG ATGACTTCCA     180
TCACCTATAA AAGCGGCTTG AGTGCTTTTG CAATGATTCT GTTCACATGA TGGACAAGAA     240
ATACGTACAA AAATTTCAAC GTTTTACAAG TTCCCAAGCT TAGTCAACTC ATCACCAACG     300
ACAAACCAAG CAAC ATG ACA AGC CCA TGC ACT GAT ATC GGT ACC GCT ATA        350
                Met Thr Ser Pro Cys Thr Asp Ile Gly Thr Ala Ile
                  1               5                  10
GAG CAG TTC AAG CAA AAT AAG ATG ATC ATC GTC ATG GAC CAC ATC TCG        398
Glu Gln Phe Lys Gln Asn Lys Met Ile Ile Val Met Asp His Ile Ser
            15                  20                  25
AGA GAA AAC GAG GCC GAT CTA ATA TGT GCA GCA GCG CAC ATG ACT GCC        446
Arg Glu Asn Glu Ala Asp Leu Ile Cys Ala Ala Ala His Met Thr Ala
        30                  35                  40
GAG CAA ATG GCA TTT ATG ATT CGG TAT TCC TCG GGC TAC GTT TGC GCT        494
Glu Gln Met Ala Phe Met Ile Arg Tyr Ser Ser Gly Tyr Val Cys Ala
    45                  50                  55                  60
CCA ATG ACC AAT GCG ATT GCC GAT AAG CTA GAC CTA CCG CTC ATG AAC        542
Pro Met Thr Asn Ala Ile Ala Asp Lys Leu Asp Leu Pro Leu Met Asn
                65                  70                  75
ACA TTG AAA TGC AAG GCT TTC TCC GAT GAC AGA CAC AGC ACT GCG TAT        590
Thr Leu Lys Cys Lys Ala Phe Ser Asp Asp Arg His Ser Thr Ala Tyr
            80                  85                  90
ACA ATC ACC TGT GAC TAT GCG CAC GGG ACG ACG ACA GGT ATC TCC GCA        638
Thr Ile Thr Cys Asp Tyr Ala His Gly Thr Thr Thr Gly Ile Ser Ala
        95                  100                 105
CGT GAC CGG GCG TTG ACC GTG AAT CAG TTG GCG AAC CCG GAG TCC AAG        686
Arg Asp Arg Ala Leu Thr Val Asn Gln Leu Ala Asn Pro Glu Ser Lys
    110                 115                 120
GCT ACC GAC TTC ACG AAG CCA GGC CAC ATT GTG CCA TTG CGT GCC CGT        734
Ala Thr Asp Phe Thr Lys Pro Gly His Ile Val Pro Leu Arg Ala Arg
125                 130                 135                 140
GAC GGC GGC GTG CTC GAG CGT GAC GGG CAC ACC GAA GCG GCG CTC GAC        782
Asp Gly Gly Val Leu Glu Arg Asp Gly His Thr Glu Ala Ala Leu Asp
                145                 150                 155
TTG TGC AGA CTA GCG GGT GTG CCA GAG GTC GCT GCT ATT TGT GAA TTA        830
Leu Cys Arg Leu Ala Gly Val Pro Glu Val Ala Ala Ile Cys Glu Leu
            160                 165                 170
```

GTA AGC GAA AGG GAC GTC GGG CTG ATG ATG ACT TTG GAT GAG TGT ATA 878
Val Ser Glu Arg Asp Val Gly Leu Met Met Thr Leu Asp Glu Cys Ile
175 180 185
GAA TTC AGC AAG AAG CAC GGT CTT GCC CTC ATC ACC GTG CAT GAC CTG 926
Glu Phe Ser Lys Lys His Gly Leu Ala Leu Ile Thr Val His Asp Leu
190 195 200
AAG GCT GCA GTT GCC GCC AAG CAG TAGACGGCAA CGAGTTCTTT AAGTCGGTGT 980
Lys Ala Ala Val Ala Ala Lys Gln
205 210
TCATTTATGT AATATACCAT TTCATCGAAA AAGTCAAATG GTATGAACTA GATTTATCAA 1040
TAGTATCTAA GAGTTATGGT ATTCGCAAAA GCTTATCGAT AC 1082

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Thr Ser Pro Cys Thr Asp Ile Gly Thr Ala Ile Glu Gln Phe Lys
1 5 10 15
Gln Asn Lys Met Ile Ile Val Met Asp His Ile Ser Arg Glu Asn Glu
20 25 30
Ala Asp Leu Ile Cys Ala Ala Ala His Met Thr Ala Glu Gln Met Ala
35 40 45
Phe Met Ile Arg Tyr Ser Ser Gly Tyr Val Cys Ala Pro Met Thr Asn
50 55 60
Ala Ile Ala Asp Lys Leu Asp Leu Pro Leu Met Asn Thr Leu Lys Cys
65 70 75 80
Lys Ala Phe Ser Asp Asp Arg His Ser Thr Ala Tyr Thr Ile Thr Cys
85 90 95
Asp Tyr Ala His Gly Thr Thr Thr Gly Ile Ser Ala Arg Asp Arg Ala
100 105 110
Leu Thr Val Asn Gln Leu Ala Asn Pro Glu Ser Lys Ala Thr Asp Phe
115 120 125
Thr Lys Pro Gly His Ile Val Pro Leu Arg Ala Arg Asp Gly Gly Val
130 135 140
Leu Glu Arg Asp Gly His Thr Glu Ala Ala Leu Asp Leu Cys Arg Leu
145 150 155 160
Ala Gly Val Pro Glu Val Ala Ala Ile Cys Glu Leu Val Ser Glu Arg
165 170 175
Asp Val Gly Leu Met Met Thr Leu Asp Glu Cys Ile Glu Phe Ser Lys
180 185 190
Lys His Gly Leu Ala Leu Ile Thr Val His Asp Leu Lys Ala Ala Val
195 200 205
Ala Ala Lys Gln
210

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 996 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..270

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 271..789

(ix) FEATURES:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 790..996

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

```
TGGTATAATG ATACAGGAAG TGAAAATCCG AAAGGTTCAG ACGATGAAAA GAGTTTGAGA      60
CGCATCAATG ATCAGCTTTG AGCTATATGT AAGTCTATTA ATTGATTACT AATAGCAATT     120
TATGGTATCC TCTGTTCTGC ATATCGACGG TTCTCACGTG ATGATCAGCT TGAGGCTTCG     180
CGGATAAAGT TCCATCGATT ACTATAAAAC CATCACATTA AACGTTCACT ATAGGCATAC     240
ACACAGACTA AGTTCAAGTT AGCAGTGACA ATG ATT AAG GGA TTA GGC GAA GTT      294
                                 Met Ile Lys Gly Leu Gly Glu Val
                                   1               5
GAT CAA ACC TAC GAT GCG AGC TCT GTC GAG GTT GGC ATT GTC CAC GCG       342
Asp Gln Thr Tyr Asp Ala Ser Ser Val Glu Val Gly Ile Val His Ala
     10                  15                  20
AGA TGG AAC AAG ACT GTC ATT GAC GCT CTC GAC CAA GGT GCA ATT GAG       390
Arg Trp Asn Lys Thr Val Ile Asp Ala Leu Asp Gln Gly Ala Ile Glu
 25                  30                  35                  40
AAA CTG CTT GCT ATG GGA GTG AAG GAG AAG AAT ATC ACT GTA AGC ACC       438
Lys Leu Leu Ala Met Gly Val Lys Glu Lys Asn Ile Thr Val Ser Thr
                 45                  50                  55
GTT CCA GGT GCG TTT GAA CTA CCA TTT GGC ACT CAG CGG TTT GCC GAG       486
Val Pro Gly Ala Phe Glu Leu Pro Phe Gly Thr Gln Arg Phe Ala Glu
             60                  65                  70
CTG ACC AAG GCA AGT GGC AAG CAT TTG GAC GTG GTC ATC CCA ATT GGA       534
Leu Thr Lys Ala Ser Gly Lys His Leu Asp Val Val Ile Pro Ile Gly
         75                  80                  85
GTC CTG ATC AAA GGC GAC TCA ATG CAC TTT GAA TAT ATA TCA GAC TCT       582
Val Leu Ile Lys Gly Asp Ser Met His Phe Glu Tyr Ile Ser Asp Ser
     90                  95                  100
```

```
GTG ACT CAT GCC TTA ATG AAC CTA CAG AAG AAG ATT CGT CTT CCT GTC        630
Val Thr His Ala Leu Met Asn Leu Gln Lys Lys Ile Arg Leu Pro Val
105                 110                 115                 120
ATT TTT GGT TTG CTA ACG TGT CTA ACA GAG GAA CAA GCG TTG ACA CGT        678
Ile Phe Gly Leu Leu Thr Cys Leu Thr Glu Glu Gln Ala Leu Thr Arg
                125                 130                 135
GCA GGC CTC GGT GAA TCT GAA GGC AAG CAC AAC CAC GGT GAA GAC TGG        726
Ala Gly Leu Gly Glu Ser Glu Gly Lys His Asn His Gly Glu Asp Trp
            140                 145                 150
GGT GCT GCT GCC GTG GAG ATG GCT GTA AAG TTT GGC CCA CGC GCC GAA        774
Gly Ala Ala Ala Val Glu Met Ala Val Lys Phe Gly Pro Arg Ala Glu
        155                 160                 165
CAA ATG AAG AAG TGAATATTAA AAAATCACTA CTTAAAATTA ACGTTTTTAT            826
Gln Met Lys Lys
    170
TATGTCTATA TCAAATTCTT ACGTGATAAC TTTTGATTTC GCTTCCTGGA TTGGCGCAAG      886
GCCTCCCTGT GTCGCAGTTT TTGTTCACGG GTCCACACAG CTCTGTTTTC CCAGAACATA      946
TCCTCCCAGC CGGCGAACCG GTTAGACGCT TCTGCTGGCG TTCTTATTTT                 996
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 172 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Ile Lys Gly Leu Gly Glu Val Asp Gln Thr Tyr Asp Ala Ser Ser
 1               5                  10                  15
Val Glu Val Gly Ile Val His Ala Arg Trp Asn Lys Thr Val Ile Asp
            20                  25                  30
Ala Leu Asp Gln Gly Ala Ile Glu Lys Leu Leu Ala Met Gly Val Lys
        35                  40                  45
Glu Lys Asn Ile Thr Val Ser Thr Val Pro Gly Ala Phe Glu Leu Pro
    50                  55                  60
Phe Gly Thr Gln Arg Phe Ala Glu Leu Thr Lys Ala Ser Gly Lys His
65                  70                  75                  80
Leu Asp Val Val Ile Pro Ile Gly Val Leu Ile Lys Gly Asp Ser Met
                85                  90                  95
His Phe Glu Tyr Ile Ser Asp Ser Val Thr His Ala Leu Met Asn Leu
            100                 105                 110
Gln Lys Lys Ile Arg Leu Pro Val Ile Phe Gly Leu Leu Thr Cys Leu
        115                 120                 125
Thr Glu Glu Gln Ala Leu Thr Arg Ala Gly Leu Gly Glu Ser Glu Gly
    130                 135                 140
Lys His Asn His Gly Glu Asp Trp Gly Ala Ala Ala Val Glu Met Ala
145                 150                 155                 160
Val Lys Phe Gly Pro Arg Ala Glu Gln Met Lys Lys
                165                 170
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1511 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..524

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 525..1232

(ix) FEATURES:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 1233..1511

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
TGTATTCAAC CTGGAGGATA ACGAAATTTC CATGGCGCGG GCGATACCAA CCCACAGGAG      60
CCAGATATAA GACCAATCCC GGCGGGTGTG CCAGCCGCCA TCAGAGACAG CGGGCCAGCA     120
AGGCATGTGA AGTCAAAAGG CGCCAGCTCC TTATCCGCTC CCGCACAAGC AGGACCGGCA     180
TATCCCGATG AGCGCGCCAG CACCCAGACG CTACACCACC ATTCGAAGTA GACTTTAAAA     240
GAGCGCTTTC CAGCTTCTCA GGCAGTTAGC TCTACGACAA AGGAACCAAG TGATTTTCCC     300
GATAGACGCG ACTTGCTCAA CGATGTTTCT GTGACCAGCG CAAGGAGAGA TAGTCCTAAA     360
GTATAATCAG ATAGTTAGTC GTATCTTCTA GTTTTATTAG TCAGCTACAT GGCGAACCGC     420
CATTTCCTTA TGCATGTCTT ACGAGTTTAA AAAGCTCGCG GTAGCAGAAA AGAAGATGCA     480
TAGATGGCAT ACCGAAGCCT ATATCGCCCA TAGAAGTTGA TAGG ATG TTT ACC GGT      536
                                                  Met Phe Thr Gly
                                                    1

ATA GTG GAA CAC ATT GGC ACT GTT GCT GAG TAC TTG GAG AAC GAT GCC       584
Ile Val Glu His Ile Gly Thr Val Ala Glu Tyr Leu Glu Asn Asp Ala
  5                  10                  15                  20

AGC GAG GCA GGC GGC AAC GGT GTG TCA GTC CTT ATC AAG GAT GCG GCT       632
Ser Glu Ala Gly Gly Asn Gly Val Ser Val Leu Ile Lys Asp Ala Ala
                 25                  30                  35

CCG ATA CTG GCG GAT TGC CAC ATC GGT GAC TCG ATT GCA TGC AAT GGT       680
Pro Ile Leu Ala Asp Cys His Ile Gly Asp Ser Ile Ala Cys Asn Gly
             40                  45                  50

ATC TGC CTG ACG GTG ACG GAG TTC ACG GCC GAT AGC TTC AAG GTC GGG       728
Ile Cys Leu Thr Val Thr Glu Phe Thr Ala Asp Ser Phe Lys Val Gly
         55                  60                  65

ATC GCA CCA GAA ACA GTT TAT CGG ACG GAA GTC AGC AGC TGG AAA GCT       776
Ile Ala Pro Glu Thr Val Tyr Arg Thr Glu Val Ser Ser Trp Lys Ala
     70                  75                  80
```

```
GGC TCC AAG ATC AAC CTA GAA AGG GCC ATC TCG GAC GAC AGG CGC TAC        824
Gly Ser Lys Ile Asn Leu Glu Arg Ala Ile Ser Asp Asp Arg Arg Tyr
 85                  90                  95                 100
GGC GGG CAC TAC GTG CAG GGC CAC GTC GAC TCG GTG GCC TCT ATT GTA        872
Gly Gly His Tyr Val Gln Gly His Val Asp Ser Val Ala Ser Ile Val
                105                 110                 115
TCC AGA GAG CAC GAC GGG AAC TCT ATC AAC TTT AAG TTT AAA CTG CGC        920
Ser Arg Glu His Asp Gly Asn Ser Ile Asn Phe Lys Phe Lys Leu Arg
            120                 125                 130
GAT CAA GAG TAC GAG AAG TAC GTA GTA GAA AAG GGT TTT GTG GCG ATC        968
Asp Gln Glu Tyr Glu Lys Tyr Val Val Glu Lys Gly Phe Val Ala Ile
        135                 140                 145
GAC GGT GTG TCG CTG ACT GTA AGC AAG ATG GAT CCA GAT GGC TGT TTC       1016
Asp Gly Val Ser Leu Thr Val Ser Lys Met Asp Pro Asp Gly Cys Phe
    150                 155                 160
TAC ATC TCG ATG ATT GCA CAC ACG CAG ACC GCT GTA GCC CTT CCA CTG       1064
Tyr Ile Ser Met Ile Ala His Thr Gln Thr Ala Val Ala Leu Pro Leu
165                 170                 175                 180
AAG CCG GAC GGT GCC CTC GTG AAC ATA GAA ACG GAT GTT AAC GGC AAG       1112
Lys Pro Asp Gly Ala Leu Val Asn Ile Glu Thr Asp Val Asn Gly Lys
                185                 190                 195
CTA GTA GAG AAG CAG GTT GCA CAG TAC CTG AAT GCG CAG CTG GAA GGT       1160
Leu Val Glu Lys Gln Val Ala Gln Tyr Leu Asn Ala Gln Leu Glu Gly
            200                 205                 210
GAG AGC TCG CCA TTG CAG CGC GTG CTC GAA AGG ATT ATT GAA TCC AAG       1208
Glu Ser Ser Pro Leu Gln Arg Val Leu Glu Arg Ile Ile Glu Ser Lys
        215                 220                 225
CTT GCT AGC ATC TCA AAT AAG TGATTATATT ATCTTGGGTG CTGTATATCT          1259
Leu Ala Ser Ile Ser Asn Lys
    230                 235
TATGTATGTC TTACGACTGT GAATCAGAGG GGTGGCAGCT GGAACACCAG CGACACACCT     1319
TCGTCTCCCG CGGTGATCAG CCTTCTGTTT TCCTCAAGTA GTACAAAGTC TAGGACACCC     1379
TGTTGTGGCC AACGCAAACA TGGAGCTGCT GCCCGTTACG CACGTCGAAC TCGTAGACCT     1439
TGCCGTCAAT GCACGAGGCG AACAGGTGGA AACCGGTGGT CTTGTCAAAC CGCCAGCTTC     1499
GTGACCGAGT CC                                                        1511
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 235 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Met Phe Thr Gly Ile Val Glu His Ile Gly Thr Val Ala Glu Tyr Leu
 1               5                  10                  15
Glu Asn Asp Ala Ser Glu Ala Gly Gly Asn Gly Val Ser Val Leu Ile
            20                  25                  30
```

Lys Asp Ala Ala Pro Ile Leu Ala Asp Cys His Ile Gly Asp Ser Ile
35 40 45
Ala Cys Asn Gly Ile Cys Leu Thr Val Thr Glu Phe Thr Ala Asp Ser
50 55 60
Phe Lys Val Gly Ile Ala Pro Glu Thr Val Tyr Arg Thr Glu Val Ser
65 70 75 80
Ser Trp Lys Ala Gly Ser Lys Ile Asn Leu Glu Arg Ala Ile Ser Asp
85 90 95
Asp Arg Arg Tyr Gly Gly His Tyr Val Gln Gly His Val Asp Ser Val
100 105 110
Ala Ser Ile Val Ser Arg Glu His Asp Gly Asn Ser Ile Asn Phe Lys
115 120 125
Phe Lys Leu Arg Asp Gln Glu Tyr Glu Lys Tyr Val Val Glu Lys Gly
130 135 140
Phe Val Ala Ile Asp Gly Val Ser Leu Thr Val Ser Lys Met Asp Pro
145 150 155 160
Asp Gly Cys Phe Tyr Ile Ser Met Ile Ala His Thr Gln Thr Ala Val
165 170 175
Ala Leu Pro Leu Lys Pro Asp Gly Ala Leu Val Asn Ile Glu Thr Asp
180 185 190
Val Asn Gly Lys Leu Val Glu Lys Gln Val Ala Gln Tyr Leu Asn Ala
195 200 205
Gln Leu Glu Gly Glu Ser Ser Pro Leu Gln Arg Val Leu Glu Arg Ile
210 215 220
Ile Glu Ser Lys Leu Ala Ser Ile Ser Asn Lys
225 230 235

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1596 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ashbya gossypii

(ix) FEATURES:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..352

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 353..1093

(ix) FEATURES:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 1094..1596

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
AGAAGAAGCG CAGGCGCCAG TCCGAGCTGG AGGAGAACGA GGCGGCGCGG TTGACGAACA      60
GCGCGCTGCC CATGGACGAT GCGGGTATAC AGACGGCGGG TATACAGACG GCGGGTGGTG     120
CCGAGAGAGG CACCAGGCCG GCTTCCTCCA GCGATGCAAG GAAGAGAAGG GGACCAGAGG     180
CGAAGTTCAA GCCATCTAAG GTACAGAAGC CCCAATTGAA GCGAACTGCA TCGTCCCGGG     240
CGGATGAGAA CGAGTTCTCG ATATTATAGA GGCCCCCGTT TCGAGTGATT GGCGTCAAAA     300
ACGGCTATCT GCCTTCGTCC GCCCCCACCA CCCTCGGGAA CACTGGCAAA CC ATG          355
                                                          Met
                                                           1

GCG CTA ATA CCA CTT TCT CAA GAT CTG GCT GAT ATA CTA GCA CCG TAC          403
Ala Leu Ile Pro Leu Ser Gln Asp Leu Ala Asp Ile Leu Ala Pro Tyr
          5                  10                  15

TTA CCG ACA CCA CCG GAC TCA TCC GCA CGC CTG CCG TTT GTC ACG CTG          451
Leu Pro Thr Pro Pro Asp Ser Ser Ala Arg Leu Pro Phe Val Thr Leu
     20                  25                  30

ACG TAT GCG CAG TCC CTA GAT GCT CGT ATC GCG AAG CAA AAG GGT GAA          499
Thr Tyr Ala Gln Ser Leu Asp Ala Arg Ile Ala Lys Gln Lys Gly Glu
35                  40                  45

AGG ACG GTT ATT TCG CAT GAG GAG ACC AAG ACA ATG ACG CAT TAT CTA          547
Arg Thr Val Ile Ser His Glu Glu Thr Lys Thr Met Thr His Tyr Leu
50                  55                  60                  65

CGC TAC CAT CAT AGC GGC ATC CTG ATT GGC TCG GGC ACA GCC CTT GCG          595
Arg Tyr His His Ser Gly Ile Leu Ile Gly Ser Gly Thr Ala Leu Ala
                70                  75                  80

GAC GAC CCG GAT CTC AAT TGC CGG TGG ACA CCT GCA GCG GAC GGG GCG          643
Asp Asp Pro Asp Leu Asn Cys Arg Trp Thr Pro Ala Ala Asp Gly Ala
          85                  90                  95

GAT TGC ACC GAA CAG TCT TCA CCA CGA CCC ATT ATC TTG GAT GTT CGG          691
Asp Cys Thr Glu Gln Ser Ser Pro Arg Pro Ile Ile Leu Asp Val Arg
     100                 105                 110

GGC AGA TGG AGA TAC CGC GGG TCC AAA ATA GAG TAT CTG CAT AAC CTT          739
Gly Arg Trp Arg Tyr Arg Gly Ser Lys Ile Glu Tyr Leu His Asn Leu
115                 120                 125

GGC AAG GGG AAG GCG CCC ATA GTG GTC ACG GGG GGT GAG CCG GAG GTC          787
Gly Lys Gly Lys Ala Pro Ile Val Val Thr Gly Gly Glu Pro Glu Val
130                 135                 140                 145

CGC GAA CTA GGC GTC AGT TAC CTG CAG CTG GGT GTC GAC GAG GGT GGC          835
Arg Glu Leu Gly Val Ser Tyr Leu Gln Leu Gly Val Asp Glu Gly Gly
                150                 155                 160

CGC TTG AAT TGG GGC GAG TTG TTT GAG CGA CTC TAT TCT GAG CAC CAC          883
Arg Leu Asn Trp Gly Glu Leu Phe Glu Arg Leu Tyr Ser Glu His His
          165                 170                 175

CTG GAA AGT GTC ATG GTC GAA GGC GGC GCG GAG GTG CTC AAC CAG CTG          931
Leu Glu Ser Val Met Val Glu Gly Gly Ala Glu Val Leu Asn Gln Leu
     180                 185                 190

CTG CTG CGC CCA GAT ATT GTG GAC AGT CTG GTG ATC ACG ATA GGA TCC          979
Leu Leu Arg Pro Asp Ile Val Asp Ser Leu Val Ile Thr Ile Gly Ser
195                 200                 205
```

```
AAG TTC CTG GGC TCA CTA GGT GTT GCG GTC TCA CCA GCT GAG GAG GTG      1027
Lys Phe Leu Gly Ser Leu Gly Val Ala Val Ser Pro Ala Glu Glu Val
210                 215                 220                 225
AAC CTA GAG CAT GTG AAC TGG TGG CAC GGA ACA AGT GAC AGT GTT TTG      1075
Asn Leu Glu His Val Asn Trp Trp His Gly Thr Ser Asp Ser Val Leu
                230                 235                 240
TGC GGC CGG CTC GCA TAGCGGTTAT GACTGGTCTA CTAGTTAAAA CTATTTACTC      1130
Cys Gly Arg Leu Ala
            245
CTATACATAT TGCGTCACAT AGCGTTTATC CCCCTCGCCA ACCGCCTCGT GCCGTTGGAA    1190
ACACGGCGGC CGGGGGACCT CAAGCGCTCC GCATCGACTA GTTTAATTTA CAAACAGATT    1250
CTGTAACTTG CGTAACGGCC AGAGGTCTCT GACTTTCTGA TAATCTTCAC CACCTCACCT    1310
CGCTTCAACC CCAGGTATAA TGCAACTTGG ATCCATCCTC TGGATTCTAG GTAACTGAGA    1370
TTCCTTTAAC CTGTATCTCT TCAACAACTC CTTCTTTTCT TCGTCGCTGA GTTTGATATG    1430
TTTTGGCACA AGCTCATGGT GCGTGATATT TACCACCAAA GCTGTTTCGT TGAAAGTCTC    1490
AATTGTAGCA GGAGCGACGG AGGGAAGCAG TTTCAACGCG CTGGGCGTTA TGCCGTTCTG    1550
ATATATGAAA ATACCCGTCT GGAAGTTCTT CTCGCCAATG TGGATC                   1596
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 246 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Met Ala Leu Ile Pro Leu Ser Gln Asp Leu Ala Asp Ile Leu Ala Pro
 1               5                  10                  15
Tyr Leu Pro Thr Pro Pro Asp Ser Ser Ala Arg Leu Pro Phe Val Thr
            20                  25                  30
Leu Thr Tyr Ala Gln Ser Leu Asp Ala Arg Ile Ala Lys Gln Lys Gly
        35                  40                  45
Glu Arg Thr Val Ile Ser His Glu Glu Thr Lys Thr Met Thr His Tyr
    50                  55                  60
Leu Arg Tyr His His Ser Gly Ile Leu Ile Gly Ser Gly Thr Ala Leu
65                  70                  75                  80
Ala Asp Asp Pro Asp Leu Asn Cys Arg Trp Thr Pro Ala Ala Asp Gly
                85                  90                  95
Ala Asp Cys Thr Glu Gln Ser Ser Pro Arg Pro Ile Ile Leu Asp Val
            100                 105                 110
Arg Gly Arg Trp Arg Tyr Arg Gly Ser Lys Ile Glu Tyr Leu His Asn
        115                 120                 125
Leu Gly Lys Gly Lys Ala Pro Ile Val Val Thr Gly Gly Glu Pro Glu
    130                 135                 140
Val Arg Glu Leu Gly Val Ser Tyr Leu Gln Leu Gly Val Asp Glu Gly
145                 150                 155                 160
Gly Arg Leu Asn Trp Gly Glu Leu Phe Glu Arg Leu Tyr Ser Glu His
                165                 170                 175
```

His Leu Glu Ser Val Met Val Glu Gly Gly Ala Glu Val Leu Asn Gln
180 185 190
Leu Leu Leu Arg Pro Asp Ile Val Asp Ser Leu Val Ile Thr Ile Gly
195 200 205
Ser Lys Phe Leu Gly Ser Leu Gly Val Ala Val Ser Pro Ala Glu Glu
210 215 220
Val Asn Leu Glu His Val Asn Trp Trp His Gly Thr Ser Asp Ser Val
225 230 235 240
Leu Cys Gly Arg Leu Ala
245

CLAIMS

1. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (GTP cyclohydrolase II) depicted in SEQ ID NO: 2, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 2 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 2, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 2 hybridizes with the polynucleotide depicted in SEQ ID NO: 1 at 42°C in 5 x SSC in 50% formamide solution.

2. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (DRAP deaminase) depicted in SEQ ID NO: 4, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 4 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 4, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 4 hybridizes with the polynucleotide depicted in SEQ ID NO: 3 at 42°C in 5 x SSC in 50% formamide solution.

3. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (DBP synthase) depicted in SEQ ID NO: 6, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 6 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 6, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 6 hybridizes with the polynucleotide depicted in SEQ ID NO: 5 at 42°C in 5 x SSC in 50% formamide solution.

4. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (DMRL synthase) depicted in SEQ ID NO: 8, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 8 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 8, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 8 hybridizes with the polynucleotide depicted in SEQ ID NO: 7 at 42°C in 5 x SSC in 50% formamide solution.

5. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (riboflavin synthase) depicted in SEQ ID NO: 10, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 10 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 10, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO:10 hybridizes with the polynucleotide depicted in SEQ ID NO: 9 at 42°C in 5 x SSC in 50% formamide solution.

6. An isolated DNA sequence which codes for a polypeptide with the amino acid sequence (HTP reductase) depicted in SEO ID NO: 12, or for an analog or derivative of the polypeptide depicted in SEQ ID NO: 12 having essentially the same enzymatic activity as the polypeptide depicted in SEQ ID NO: 12, with the proviso that the DNA sequence of the analog or derivative of the polypeptide depicted in SEQ ID NO: 12 hybridizes with the polynucleotide depicted in SEQ ID NO: 11 at 42°C in 5 x SSC in 50% formamide solution.

7. An expression vector containing one or more DNA sequences as defined in any one of claims 1 to 6.

8. A host microorganism which has been transformed with an expression vector as defined in claim 7.

9. A recombinant method of producing riboflavin, comprising the steps of:

1) constructing the expression vector defined in claim 7;

2) transforming a compatible host with said recombinant vector such that the DNA sequence coding for the polypeptide can be expressed by the host;

3) culturing the transformed host in a suitable growth medium to produce said riboflavin; and

4) recovering said riboflavin from the growth medium.

Fig. 1

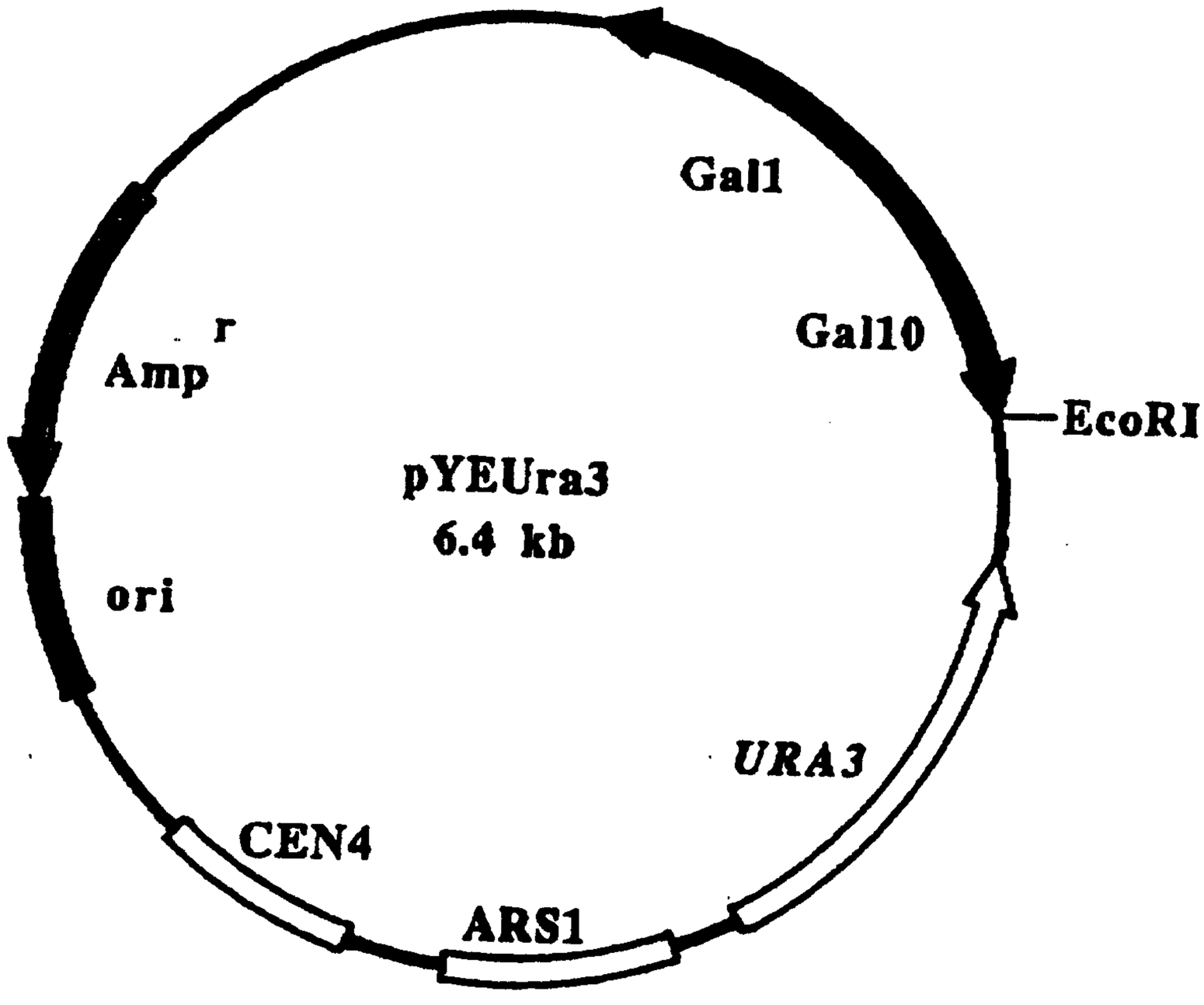


Fig. 2

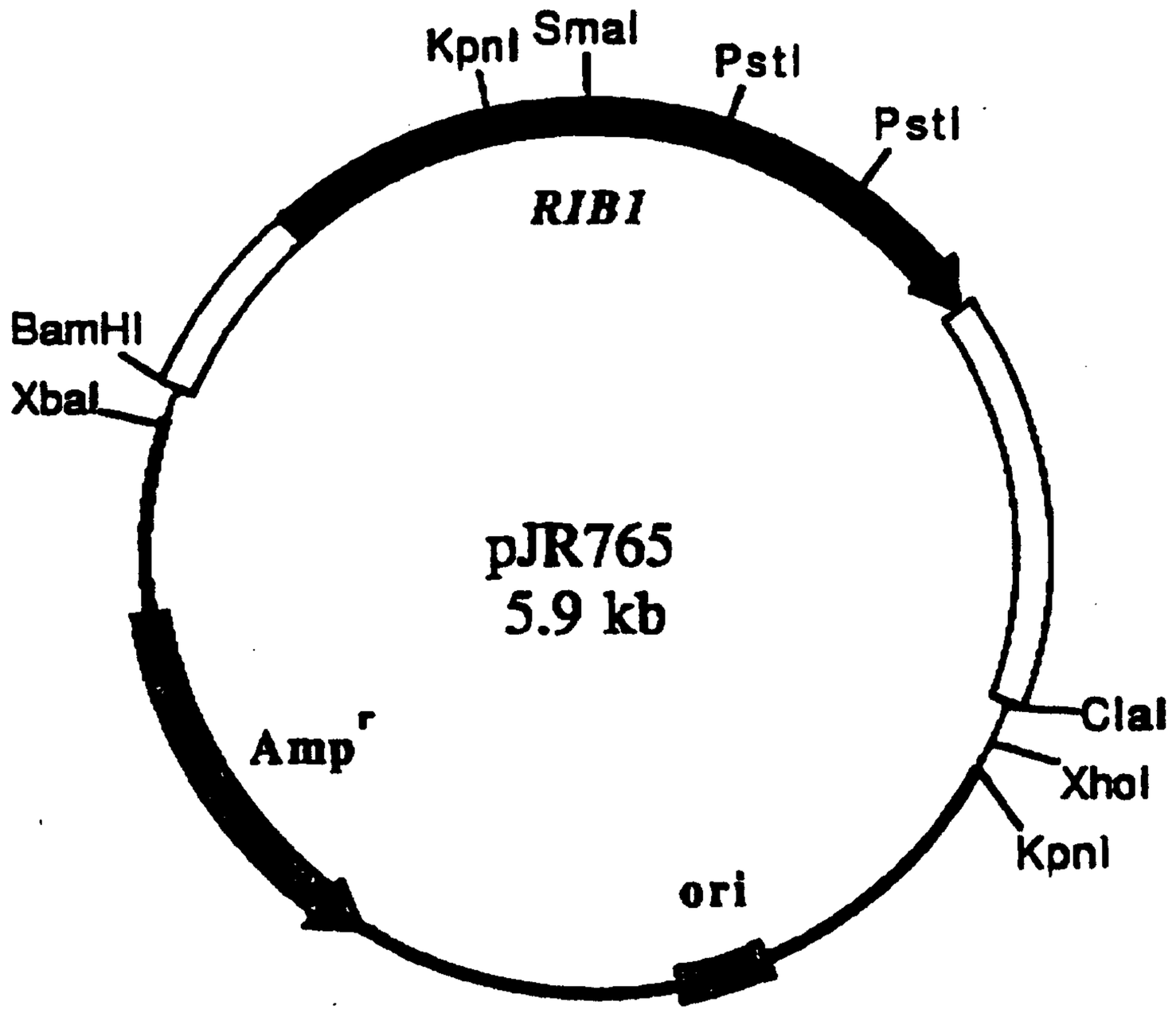

KpnI
SmaI
PstI
PstI
RIB1
BamHI
XbaI
pJR765
5.9 kb
ClaI
XhoI
KpnI
Amp^r
ori

Fig. 3

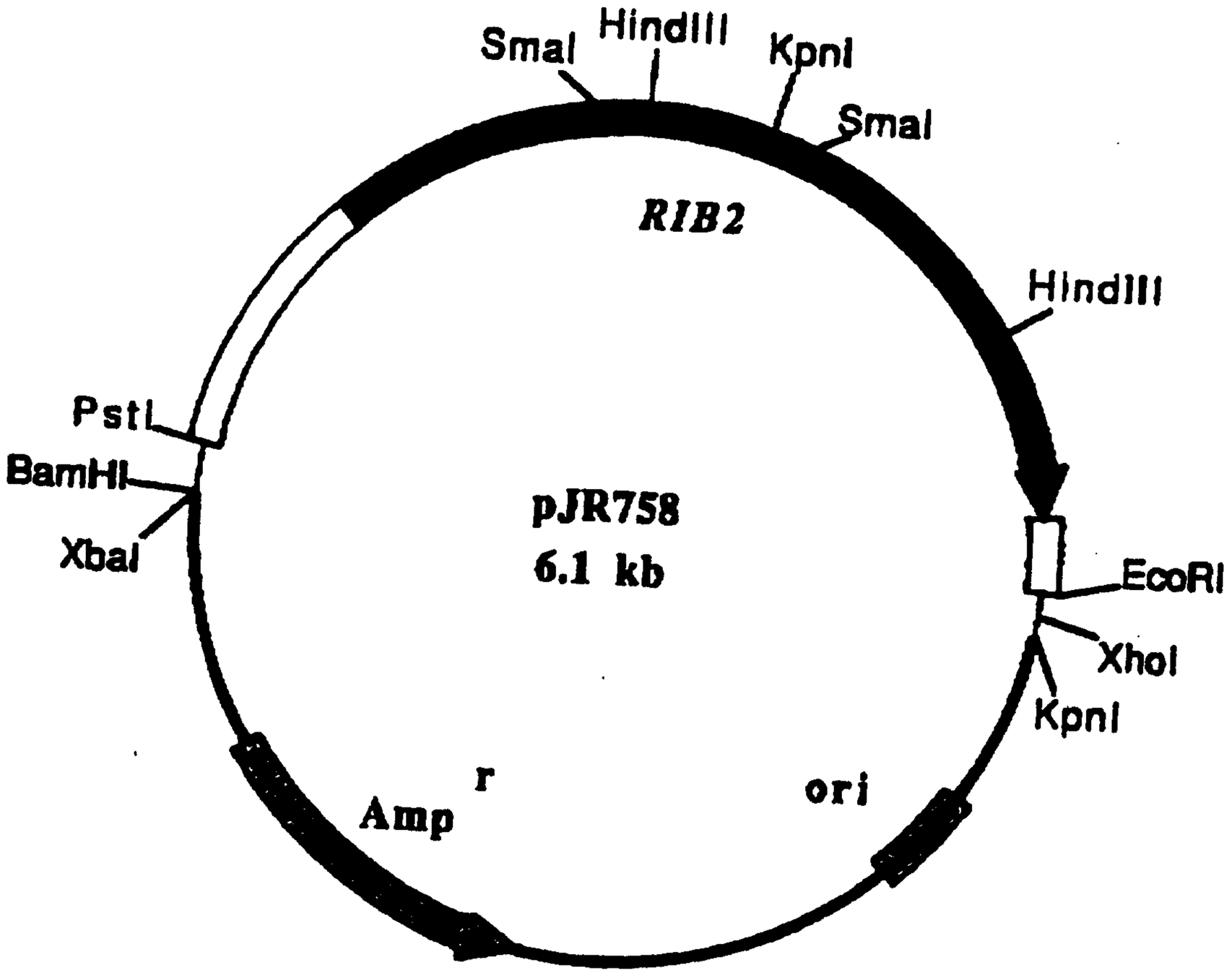

SmaI
HindIII
KpnI
SmaI
RIB2
HindIII
PstI
BamHI
XbaI
pJR758
6.1 kb
EcoRI
XhoI
KpnI
Amp r
ori

Fig. 4

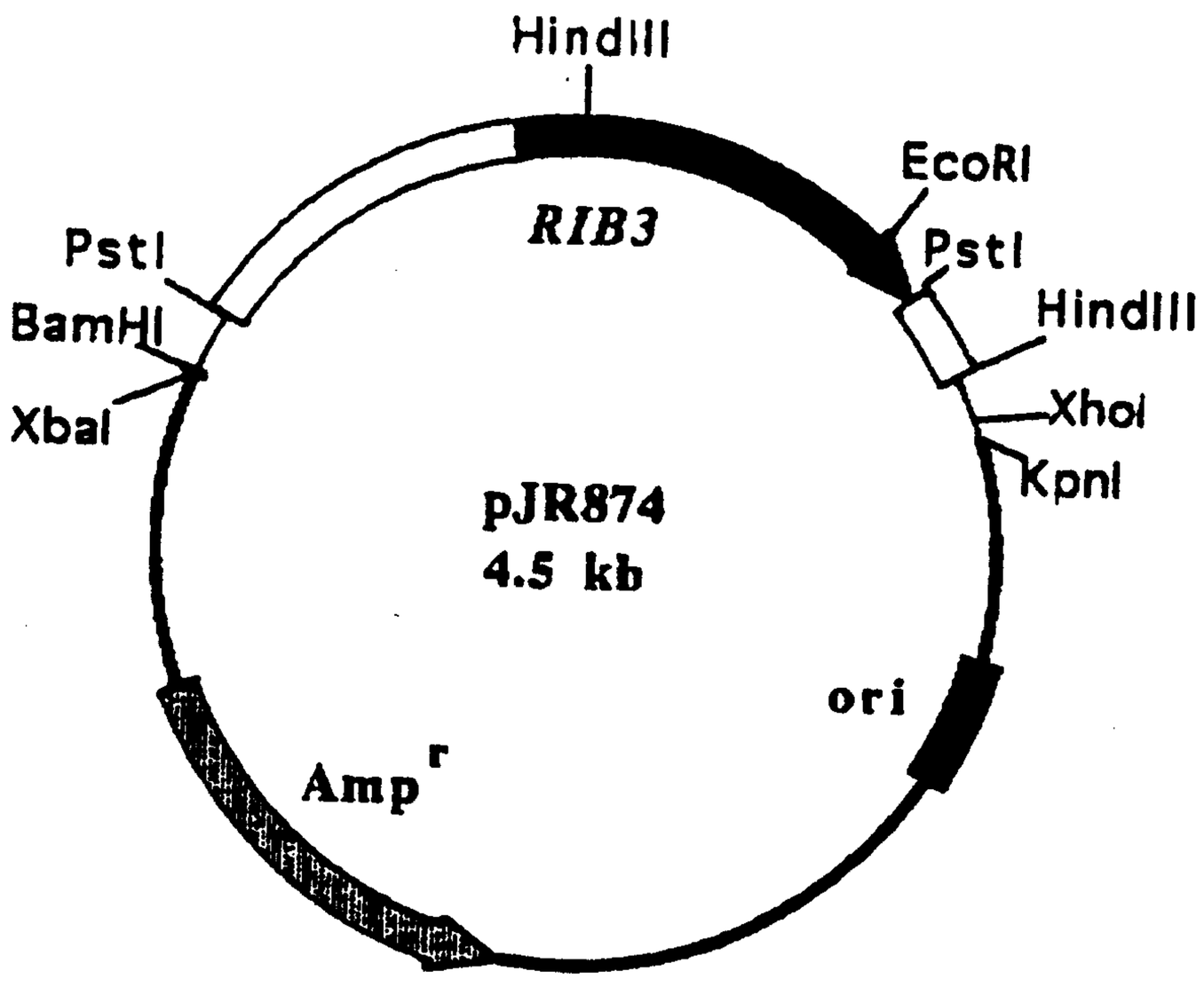

HindIII
EcoRI
RIB3
PstI
PstI
BamHI
HindIII
XbaI
XhoI
KpnI
pJR874
4.5 kb
ori
Amp r

Fig. 5

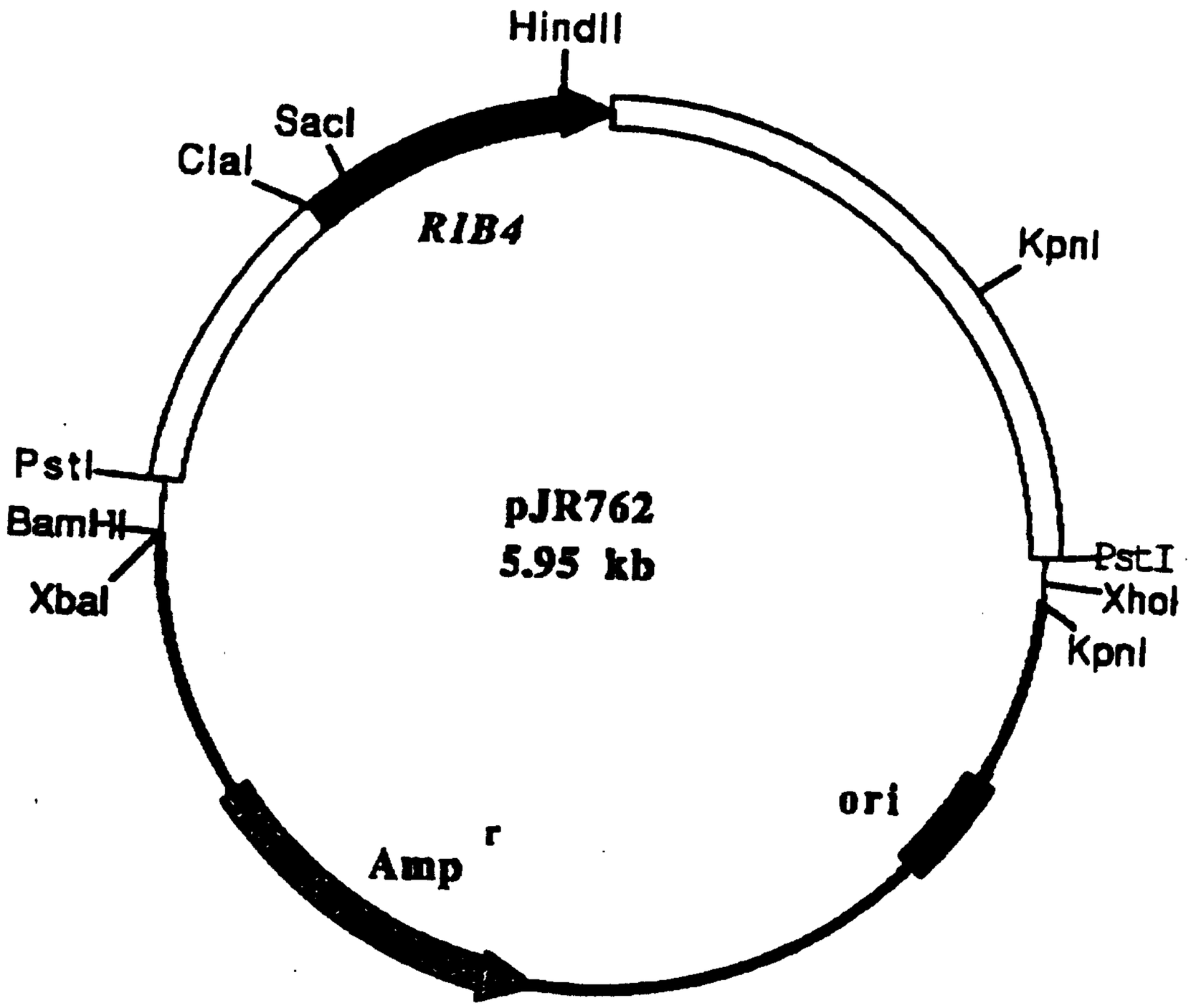

HindII
SacI
ClaI
RIB4
KpnI
PstI
BamHI
XbaI
pJR762
5.95 kb
PstI
XhoI
KpnI
ori
Amp r

Fig. 6

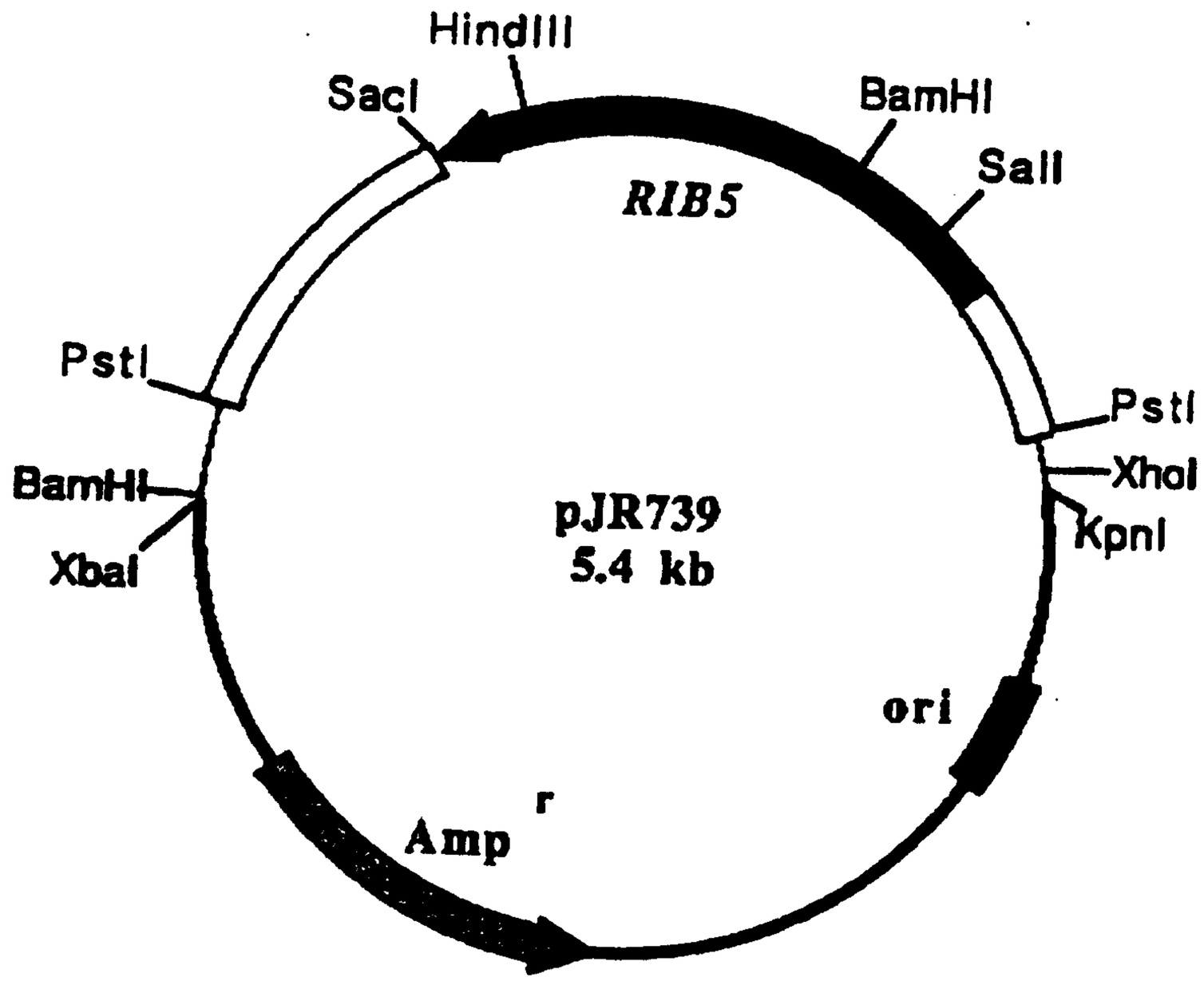
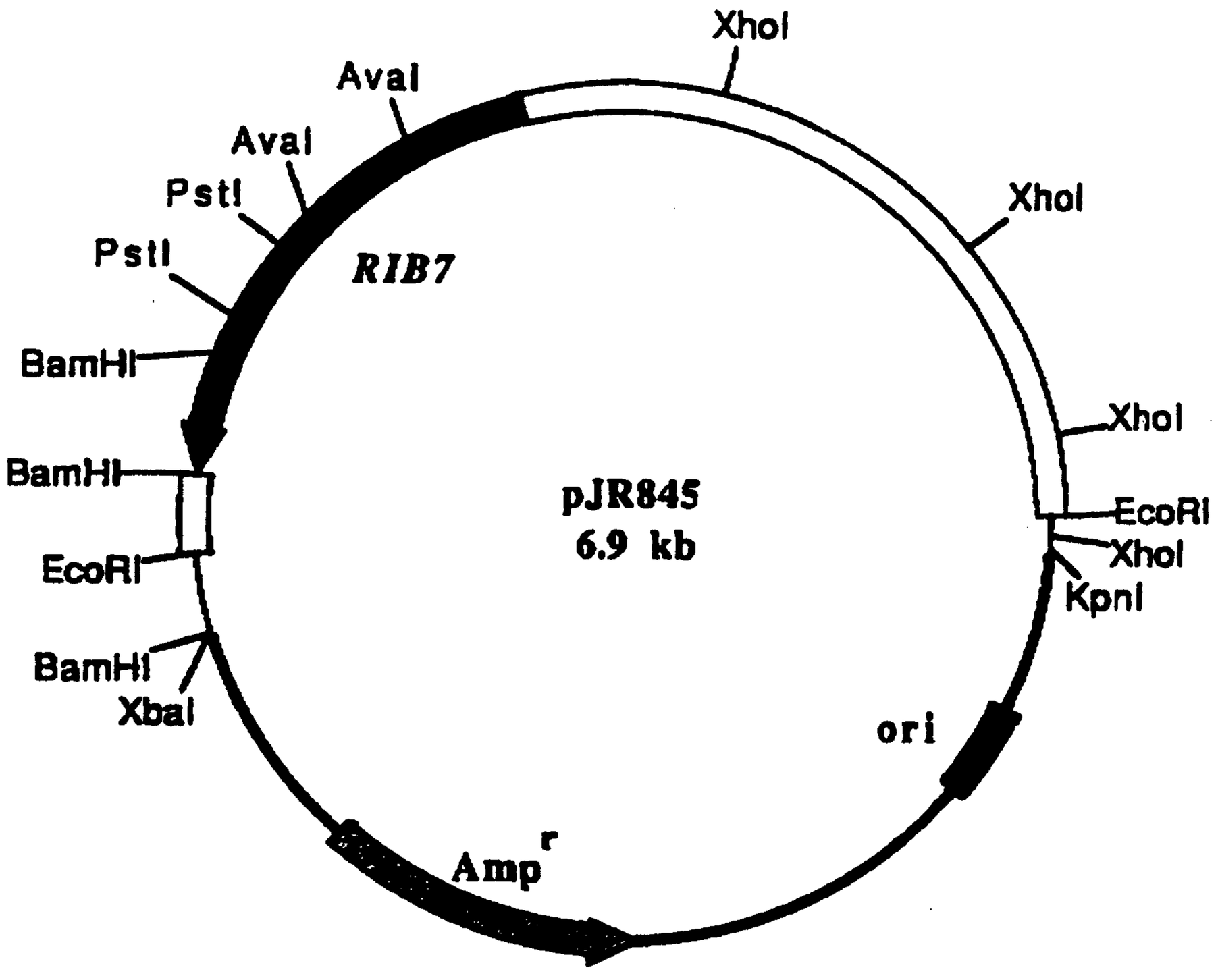
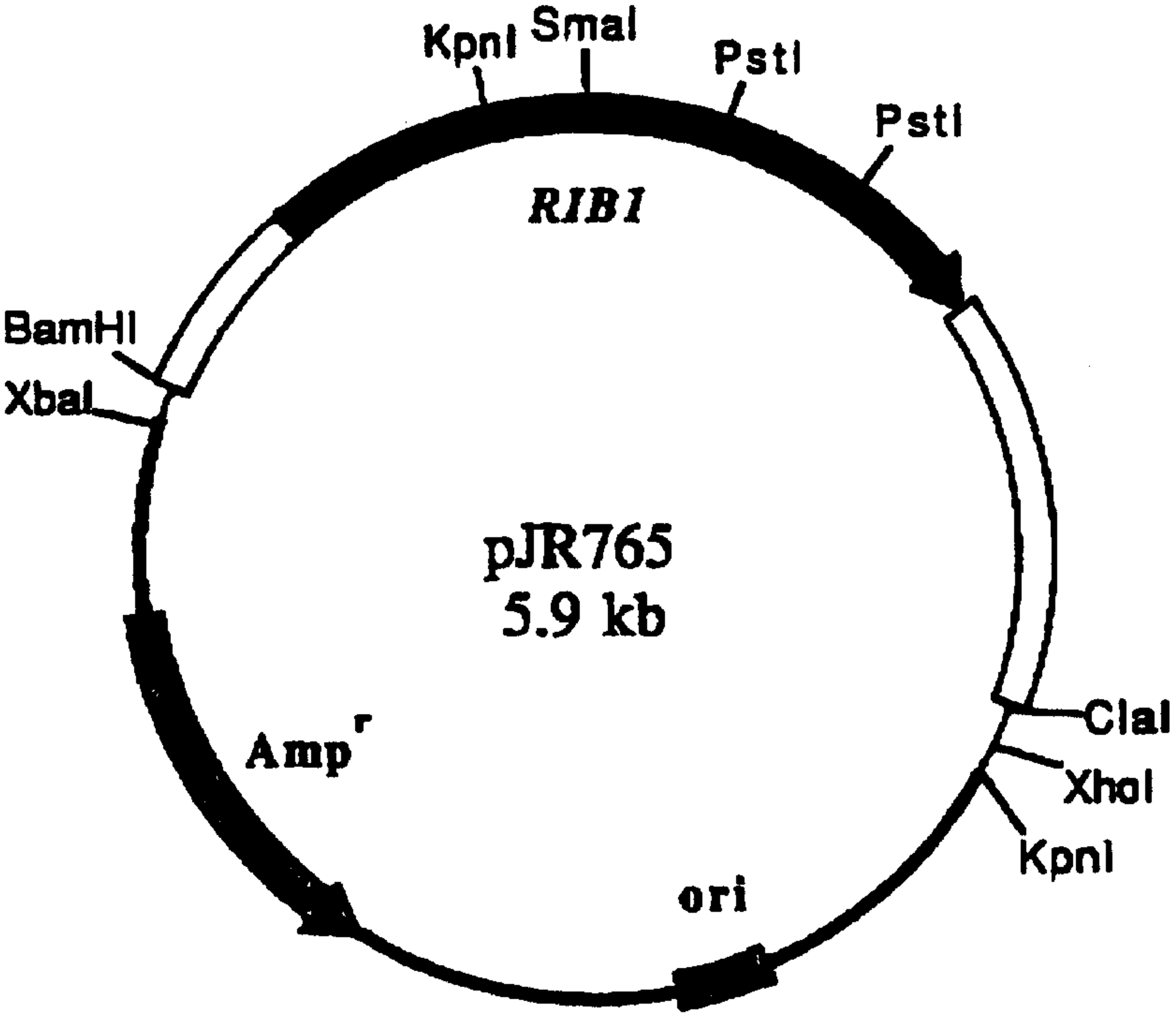

HindIII
SacI
BamHI
SalI
RIB5
PstI
PstI
BamHI
XhoI
XbaI
KpnI
pJR739
5.4 kb
ori
Amp r

Fig. 7


XhoI
AvaI
AvaI
PstI
PstI
RIB7
BamHI
BamHI
EcoRI
BamHI
XbaI
pJR845
6.9 kb
XhoI
XhoI
EcoRI
XhoI
KpnI
ori
Amp r


KpnI
SmaI
PstI
PstI
RIB1
BamHI
XbaI
pJR765
5.9 kb
Amp^r
ori
ClaI
XhoI
KpnI