

DESCRIPTION

NOVEL PLASMIDS AND UTILIZATION THEREOF

Technical Field

[0001] The present invention relates to novel plasmids derived from any of microorganisms belonging to the genus *Rhodococcus* (hereinafter referred to as "the genus *Rhodococcus*") and to utilization thereof. More specifically, the invention relates to plasmids or their partial DNA fragments (hereinafter also referred to simply as "DNA fragments"), and to shuttle vectors, vectors, transformants, aminoketone asymmetric reductase production methods and optically active aminoalcohol production methods which utilize them.

Background Art

[0002] The genus *Rhodococcus* is known to produce enzymes involved in nitrile metabolism and to produce enzymes which asymmetrically reduce aminoketones. In particular, *Rhodococcus erythropolis* is known to have very high aminoketone asymmetric reduction activity. Such microorganisms and enzymes act on α -aminoketones to high selectively produce optically active β -aminoalcohols at high yields (for example, Patent documents 1 and 5). Thus, it has long been desired to develop a host-vector system intended for mass production of useful enzymes and the like in the genus *Rhodococcus*. However, the development of vectors suitable for the genus *Rhodococcus* as hosts has lagged behind. Only a few strains of the genus *Rhodococcus* have been found with plasmids, namely *Rhodococcus* sp. H13-A (Non-patent document 1), *Rhodococcus rhodochrous* ATCC4276 (Patent document 2), *Rhodococcus*

rhodochrous ATCC4001 (Patent document 3) and *Rhodococcus erythropolis* IFO12320 (Patent document 4).

[0003] [Patent document 1] WO01/73100

[Patent document 2] Japanese Unexamined Patent
5 Publication HEI No. 4-148685

[Patent document 3] Japanese Unexamined Patent
Publication HEI No. 4-330287

[Patent document 4] Japanese Unexamined Patent
Publication HEI No. 9-28379

10 [Patent document 5] WO02/070714

[Non-patent document 1] J. Bacteriol., 170, 638, 1988

Disclosure of the Invention

Problems to be Solved by the Invention

[0004] As mentioned above, it has been desired to develop new
15 vectors for breeding and improve to industrially useful strains (mutant
strains) from the genus *Rhodococcus*. In particular, self-cloning
systems are preferred from the standpoint of safety of the recombinant
DNA microbes and their products which may be used as foods and
additives. It is an object of the present invention to provide novel
20 plasmids that can be used as vectors for such a host-vector system.

[0005] It is desirable to create recombinant microbes suitable for
industrial application from among *Rhodococcus erythropolis* which has
aminoketone asymmetric reduction activity. In particular, it is a first
object of the invention to provide novel plasmids or their partial DNA
25 fragments which can be used to create such recombinant microbes.

[0006] If a plasmid such as described above can be obtained, it

would become easy to construct a shuttle vector that is replicable even in other microbes. It is therefore a second object of the invention to provide nucleotide sequence data relating to DNA replication (replication region, etc.) necessary for construction of such a shuttle vector.

[0007] It is a third object of the invention to provide shuttle vectors that are replicable in both the genus *Rhodococcus* and *E. coli*.

[0008] It is a fourth object of the invention to apply the shuttle vectors to an aminoketone asymmetric reductase.

Means for Solving the Problems

[0009] The present inventors carefully screened plasmids for vector construction from among *Rhodococcus* strains, and as a result discovered several novel plasmids usable as vectors for host-vector systems.

[0010] Furthermore, the present inventors found that it is possible to construct shuttle vectors by transferring into the aforementioned plasmids a drug resistance gene and a gene region that is replicable in *E. coli*. As a result there were obtained nucleotide sequence data, plasmids and shuttle vectors that achieve the objects stated above, and the present invention has thereupon been completed.

[0011] Specifically, the present invention provides a DNA fragment, a DNA, a plasmid, a shuttle vector, a vector, a transformant, a method for production of an aminoketone asymmetric reductase, and a method for production of an optically active aminoalcohol, according to following (1) to (39).

(1) A DNA fragment having at least one nucleotide sequence selected

from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

(2) A plasmid or a partial DNA fragment thereof, characterized by comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

(3) A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22.

(4) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22.

(5) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22 and comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

(6) A DNA fragment having the nucleotide sequence set forth as SEQ

ID NO: 76.

(7) A plasmid or a partial DNA fragment thereof, characterized by comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

5 (8) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22,
10 comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, and comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

15 (9) A circular plasmid characterized by comprising a plasmid or a partial DNA fragment according to any one of (1) to (8), wherein the numbers of restriction endonuclease cleavage sites are *Bam*H I: 2, *Eco*R I: 2, *Kpn*I: 1, *Pvu* II: 1, *Sac*I: 1 and *Sma*I: 1, and the size is approximately 5.4 kbp.

20 (10) A plasmid having the nucleotide sequence set forth as SEQ ID NO: 73.

(11) A plasmid or a DNA fragment according to any one of (1) to (10), characterized by being derived from a bacterium belonging to the genus *Rhodococcus*.

25 (12) A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ

ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72.

(13) A plasmid or a partial DNA fragment thereof, characterized by comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72.

(14) A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 61, SEQ ID NO: 62 and SEQ ID NO: 69.

(15) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 61, SEQ ID NO: 62 and SEQ ID NO: 69.

(16) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 61, SEQ ID NO: 62 and SEQ ID NO: 69 and comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of

the nucleotide sequences set forth as SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72.

(17) A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 61, SEQ ID NO: 62 and SEQ ID NO: 69, comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, and comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

(18) A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 67 and SEQ ID NO: 47.

(19) A plasmid or a partial DNA fragment thereof, characterized by comprising a mobilization protein region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 67 and SEQ ID NO: 47.

(20) A DNA fragment having the nucleotide sequence set forth as SEQ ID NO: 75.

(21) A plasmid or a partial DNA fragment thereof, characterized by comprising a mobilization-related region having the nucleotide sequence set forth as SEQ ID NO: 75.

(22) A circular plasmid characterized by comprising a plasmid or DNA

fragment according to any one of (12) to (21), wherein the numbers of restriction endonuclease cleavage sites are *Bam*H I: 2, *Pvu* II: 4, *Sac* I: 3 and *Sma* I: 4, and the size is approximately 5.8 kbp.

(23) A plasmid having the nucleotide sequence set forth as SEQ ID NO: 74.

(24) A plasmid or a DNA fragment according to any one of (12) to (23), characterized by being derived from a bacterium belonging to the genus *Rhodococcus*.

(25) A DNA fragment having the nucleotide sequence set forth as SEQ ID NO: 77.

(26) A DNA fragment characterized by comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 77.

(27) A shuttle vector replicable in bacteria belonging to the genus *Rhodococcus* and *E. coli*, and comprising a plasmid or partial DNA fragment thereof according to any one of (1) to (26) and a DNA region replicable in *E. coli*.

(28) A vector characterized by being constructed using a shuttle vector according to (27).

(29) A vector characterized by comprising a plasmid or DNA fragment according to any one of (6), (7), (25) or (26).

(30) A vector according to (28) or (29), characterized by having inserted therein an aminoketone asymmetric reductase gene.

(31) A vector according to (30), characterized in that the aminoketone asymmetric reductase gene is a nucleic acid coding for a protein consisting the amino acid sequence set forth as SEQ ID NO: 78, or a nucleic acid that codes for a protein having the amino acid sequence set

forth as SEQ ID NO: 78 with a deletion, insertion, substitution or addition of one or a plurality of amino acids, and having aminoketone asymmetric reduction activity.

5 (32) A vector according to (30), characterized in that the aminoketone asymmetric reductase gene is a nucleic acid consisting the nucleotide sequence set forth as SEQ ID NO: 79, or a nucleic acid that hybridizes with nucleic acid having a nucleotide sequence complementary to the nucleotide set forth as SEQ ID NO: 79 under stringent conditions, and that codes for a protein having aminoketone asymmetric reduction
10 activity.

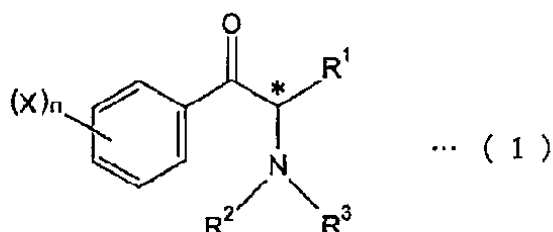
(33) A transformant containing a vector according to (28) or (29).

(34) A transformant containing a vector according to any one of (30) to (32).

(35) A method for production of an aminoketone asymmetric reductase,
15 which comprises a culturing step in which a transformant according to (34) is cultured in medium that allows growth of said transformant, and a purification step in which the aminoketone asymmetric reductase is purified from said transformant obtained in said culturing step.

20 (36) A method for production of an optically active aminoalcohol, wherein an aminoketone asymmetric reductase obtained by the production method of (35) is reacted with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1):

25 [Chemical Formula 1]



wherein X may be the same or different and represents at least one species selected from the group consisting of halogen, lower alkyl, hydroxyl optionally protected with a protecting group, nitro and sulfonyl;

n represents an integer of 0 to 3;

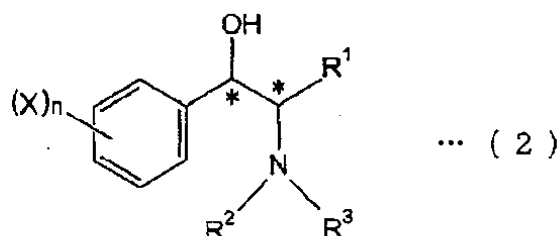
R¹ represents lower alkyl;

R² and R³ may be the same or different and represent at least one species selected from the group consisting of hydrogen and lower alkyl; and

* represents asymmetric carbon,

or a salt thereof, to produce an optically active aminoalcohol compound represented by the following general formula (2):

[Chemical Formula 2]

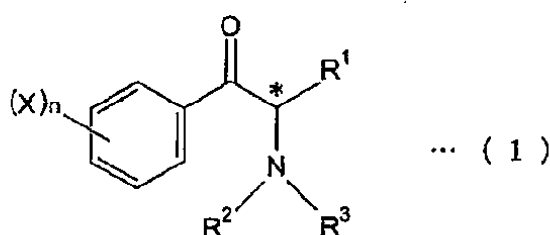


wherein X, n, R¹, R², R³ and * have the same definitions as above,

and having the desired optical activity.

(37) A method for production of an optically active aminoalcohol, wherein a transformant according to (34) is reacted with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1):

[Chemical Formula 3]



wherein X may be the same or different and represents at least one species selected from the group consisting of halogen, lower alkyl, hydroxyl optionally protected with a protecting group, nitro and sulfonyl;

n represents an integer of 0 to 3;

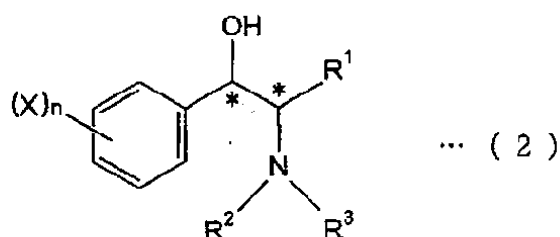
R¹ represents lower alkyl;

R² and R³ may be the same or different and represent at least one species selected from the group consisting of hydrogen and lower alkyl; and

* represents asymmetric carbon,

or a salt thereof, to produce an optically active aminoalcohol compound represented by the following general formula (2):

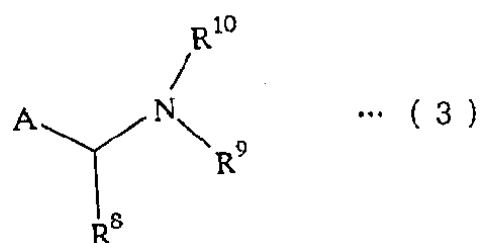
[Chemical Formula 4]



wherein X, n, R¹, R², R³ and * have the same definitions as above,
and having the desired optical activity.

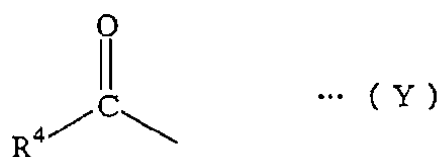
- 5 (38) A production method for an optically active aminoalcohol
according to (37), wherein the production method for the optically
active aminoalcohol is carried out with further addition of a compound
represented by the following general formula (3):

[Chemical Formula 5]

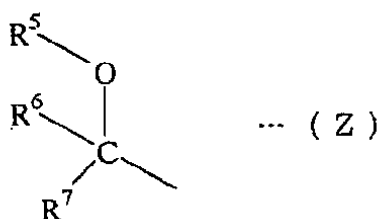


- 10 wherein A represents the following formula (Y) or (Z):

[Chemical Formula 6]



wherein R^4 represents hydrogen, optionally substituted C1-3 alkyl, a C5-10 hydrocarbon ring which is bonded to R^8 or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^8 ,
 5 [Chemical Formula 7]



wherein R^5 represents hydrogen, C1-3 alkyl or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^6 or R^9 ;

10 R^6 represents hydrogen, optionally substituted C1-3 alkyl, a C5-10 hydrocarbon ring which is bonded to R^8 or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^5 or R^9 ;

R^7 represents hydrogen or optionally substituted C1-6 alkyl;

15 R^8 represents hydrogen, carboxyl, optionally substituted C1-6 alkyl, a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms

which is bonded to R⁴ or a C5-10 hydrocarbon ring which is bonded to R⁶;

R⁹ represents hydrogen, optionally substituted C1-6 alkyl, optionally substituted C1-6 alkyloxycarbonyl, optionally substituted acyl or a 5-
5 to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁵ or R⁶; and

R¹⁰ represents hydrogen or optionally substituted C1-6 alkyl, or a pharmaceutically acceptable salt or solvate thereof, for production of an optically active aminoalcohol.

10 (39) A shuttle vector according to (27), having a nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 89 to SEQ ID NO: 100.

Effect of the Invention

[0012] The plasmids of the invention are novel plasmids
15 unknown to the prior art, and are valuable as vectors for host-vector systems belonging to the industrially useful the genus *Rhodococcus*. They are of particular utility in the creation of recombinant microbes capable of industrial asymmetric reduction of aminoketones. An example of asymmetric reduction of an aminoketone to which such
20 microbes may contribute is a reaction for production of d-(1S, 2S)-pseudoephedrine from 1-2-methylamino-1-phenyl-1-propanone.

[0013] The plasmids of the invention can coexist in single *Rhodococcus* cell and therefore can be used not only alone for their replicating function, but also as compatible plasmids. That is, by
25 inserting different protein (for example, enzyme) genes into the different plasmids, it is possible to express the proteins simultaneously

in the same cell.

[0014] The shuttle vectors of the invention are useful for creation of industrially useful recombinant microbes of the genus *Rhodococcus* and *Escherichia coli*.

5 [0015] The nucleotide sequence data relating to DNA replication obtained from the plasmids of the invention may serve as the basis for construction of the aforementioned shuttle vectors, and specifically they provide DNA fragments as constituent elements of the vectors.

Brief Description of the Drawings

10 [0016]

Fig. 1 is a restriction enzyme cleavage map of plasmid pRET1100.

Fig. 2 is a restriction enzyme cleavage map of plasmid pRET1000.

15 Fig. 3 is a summary illustration for construction of shuttle vector pRET1101.

Fig. 4 is a summary illustration for construction of shuttle vector pRET1102.

20 Fig. 5 is a summary illustration for construction of shuttle vector pRET1103.

Best Mode for Carrying Out the Invention

[0017] Preferred embodiments of the invention will now be explained.

25 [0018] The first plasmid of the invention is a plasmid isolated from the genus *Rhodococcus*, or a derivative thereof. Specifically, it may be isolated from, for example, *Rhodococcus erythropolis*

IAM1400, IAM1503, JCM2893 and JCM2894 strains, has a size of approximately 5.4 kbp and is a circular plasmid cleavable by the restriction enzymes shown in Table 1. The plasmids isolated from each of these strains are designated as pRET1100, pRET1300, pRET1500 and pRET1700, respectively. Plasmids of the invention may be prepared from these sample strains by publicly known methods (for example, boiling, alkali dissolution, cesium chloride density gradient ultracentrifugation: Lab Manual Idenshi Kogaku, 3rd Edition, Chapter 10, pp.55-59, Maruzen).

[0019] [Table 1]

Restriction enzyme	Number of cleavage sites	Fragment sizes (kbp)
<i>Bam</i> H I	2	0.4, 5.0
<i>Eco</i> R I	2	0.3, 5.1
<i>Kpn</i> I	1	5.4
<i>Pvu</i> II	1	5.4
<i>Sac</i> I	1	5.4
<i>Sma</i> I	1	5.4

[0020] Fig. 1 shows a restriction enzyme cleavage map for pRET1100. This plasmid was sequenced by a publicly known method (using a fluorescent automatic sequencer, for example) and its full nucleotide sequence was revealed to be 5444 bp set forth as SEQ ID NO: 73 of the Sequence Listing.

[0021] The second plasmid of the invention is also a plasmid isolated from the genus *Rhodococcus*, or its derivative. Specifically, it may be isolated from, for example, *Rhodococcus rhodnii* JCM3203, has a size of approximately 5.8 kbp and is a circular plasmid cleavable by the restriction enzymes shown in Table 2. This plasmid is

designated as pRET1000.

[0022] [Table 2]

Restriction enzyme	Number of cleavage sites	Fragment sizes (kbp)
<i>Bam</i> H I	2	2.0, 3.8
<i>Pvu</i> II	4	0.1, 1.4, 2.0, 2.3
<i>Sac</i> I	3	0.9, 1.0, 3.9
<i>Sma</i> I	4	0.1, 1.2, 1.6, 2.9

[0023] Fig. 2 shows a restriction enzyme cleavage map for pRET1000. This plasmid was also sequenced by a publicly known method and its full nucleotide sequence was revealed to be 5813 bp set forth as SEQ ID NO: 74 of the Sequence Listing.

[0024] The plasmids of the invention (natural- or wild-types) are circular plasmids that can also be defined by the restriction enzyme cleavage patterns shown in Tables 1 and 2. Thus, the present invention encompasses the following two types of circular plasmids.

(1) A circular plasmid derived from a *Rhodococcus* strain, characterized by having a size of approximately 5.4 kbp and possessing the following restriction enzyme cleavage sites: *Bam*H I:2, *Eco*R I:2, *Kpn* I:1, *Pvu* II:1, *Sac* I:1 and *Sma* I:1.

(2) A circular plasmid derived from a *Rhodococcus* strain, characterized by having a size of approximately 5.8 kbp and possessing the following restriction enzyme cleavage sites: *Bam*H I:2, *Pvu* II:4, *Sac* I:3 and *Sma* I:4.

[0025] As a result of analysis of the nucleotide sequences of plasmids pRET1100 and pRET1000 (i.e., SEQ ID NO: 73 and SEQ ID NO: 74), there is predicted the existence of a group of nucleotide sequences (open reading frames, hereinafter "orf") coding for proteins

for DNA replication or other functions.

[0026] In the relevant technical field, "DNA replication" refers to using DNA itself as template to form two double-stranded DNA molecules exactly identical to existing double-stranded DNA (parent DNA). The replication mechanism consists of three stages: initiation from the starting point of replication (replication origin), DNA chain elongation and termination. During replication, a portion of the DNA double strand is unraveled and new DNA strands are synthesized complementary to each single strand. The double strand is unraveled by DNA helicase and helix destabilizing proteins (also known as single-strand DNA-binding protein), and the unraveled portion is referred to as the replication fork. The template DNA in the direction from 3' to 5' toward the replication fork is the "leading strand", and the one in the direction from 5' to 3' is the "lagging strand". DNA polymerase extends the DNA strand in the direction from 5' to 3'. Therefore when the leading strand is the template, DNA is synthesized in the direction of the replication fork. However when the opposite lagging strand is the template, the DNA strand must be extended in the opposite direction from the replication fork. Consequently, replication of the lagging strand is accomplished in fragments of about 200 bases, known as Okazaki fragments. Every approximately 200 bases, RNA primer is used with DNA as template to synthesize 10 bases of RNA in the direction from 5' to 3'. From this RNA as primer, DNA polymerase synthesizes a DNA strand in the direction from 5' to 3' on the lagging strand as template. The replicated DNA fragment of approximately 200 bases then binds to the single-stranded DNA from which RNA is

removed. In this replication mechanism, several proteins including DNA helicase and helix-destabilizing protein work together to form the replicating machinery. Other proteins involved include DNA topoisomerase (which prevents twisting during the DNA replication), replication initiation proteins and replication termination proteins. The DNA replication mechanism is described in detail in, for example, "Saibou no Bunshiseibutsugaku [Molecular Biology of the Cell]", 3rd Edition, translated by Keiko Nakamura et al., pp.251-262, Kyoikusha, 1996.

[0027] Upon analysis of the nucleotide sequences of the plasmids pRET1100 and pRET1000, they were found to include sequences of AT-rich homologous or analogous repeats and a sequence thought to have a DNA secondary structure, i.e. a nucleotide sequence predicted to be a DNA replication region (a nucleotide sequence region recognized by proteins involved in DNA replication or a region including the DNA replication origin), in the vicinity of the aforementioned orf relating to DNA replication.

[0028] DNA replication requires a DNA replication region and a region coding for a protein involved in DNA replication (hereinafter referred to as "DNA replication-related protein"). According to the present invention it is possible to obtain data relating to the nucleotide sequences of these regions for both plasmids pRET1100 and pRET1000.

[0029] First, the nucleotide sequences set forth as SEQ ID NO: 35-37 were identified as DNA replication regions for plasmid pRET1100. As regions coding for proteins related to DNA replication

there were identified the nucleotide sequences set forth as SEQ ID NO: 1-3 (orf1), the nucleotide sequence set forth as SEQ ID NO: 4 (orf2), the nucleotide sequences set forth as SEQ ID NO: 5-16 (orf3), the nucleotide sequences set forth as SEQ ID NO: 17-21 (orf4), the nucleotide sequences set forth as SEQ ID NO: 22-26 (orf5), the nucleotide sequence set forth as SEQ ID NO: 27 or 28 (orf6), the nucleotide sequence set forth as SEQ ID NO: 29 or 30 (orf7), the nucleotide sequence set forth as SEQ ID NO: 31 or 32 (orf8), and the nucleotide sequence set forth as SEQ ID NO: 33 or 34 (orf9).

[0030] Construction of a plasmid capable of DNA replication from pRET1100 requires that the recombinant plasmid have at least one DNA replication region and at least one coding region (orf) for a DNA replication-related protein. Thus, the (recombinant) plasmids of the invention are characterized by comprising at least one DNA replication region and at least one coding region for a DNA replication-related protein. The coding region for a DNA replication-related protein preferably has a nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, 4, 14, 17 and 22.

[0031] The region of the nucleotide sequence set forth as SEQ ID NO: 76 has been suggested as a promoter involved in expression of replication-related proteins, and the plasmids of the invention preferably comprise a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

[0032] For plasmid construction, the DNA fragments are appropriately selected based on the aforementioned nucleotide

sequence data. The present invention also encompasses derivatives or functional (DNA-replicating) fragments of the plasmids.

[0033] Next, the nucleotide sequences set forth as SEQ ID NO: 70-72 were identified as DNA replication regions for plasmid pRET1000. As regions coding for proteins related to DNA replication there were identified the nucleotide sequences set forth as SEQ ID NO: 38-41 (orf10), the nucleotide sequence set forth as SEQ ID NO: 42 or 43 (orf11), the nucleotide sequence set forth as SEQ ID NO: 44 (orf12), the nucleotide sequence set forth as SEQ ID NO: 45 or 46 (orf13), the nucleotide sequences set forth as SEQ ID NO: 48-50 (orf14), the nucleotide sequence set forth as SEQ ID NO: 51 or 52 (orf15), the nucleotide sequence set forth as SEQ ID NO: 53 or 54 (orf16), the nucleotide sequence set forth as SEQ ID NO: 55 (orf17), the nucleotide sequences set forth as SEQ ID NO: 56-60 (orf18), the nucleotide sequence set forth as SEQ ID NO: 61 (orf19), the nucleotide sequence set forth as SEQ ID NO: 62 (orf20), and the nucleotide sequences set forth as SEQ ID NO: 63-69 (orf21).

[0034] Construction of a plasmid capable of DNA replication from pRET1000 requires that the recombinant plasmid have at least one DNA replication region and at least one coding region (orf) for a DNA replication-related protein. Thus, the (recombinant) plasmids of the invention are characterized by comprising at least one DNA replication region and at least one coding region for a DNA replication-related protein. The coding region for a DNA replication-related protein preferably has a nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 40, 42,

44, 45, 53, 55, 56, 61, 62 and 69.

[0035] The regions with the nucleotide sequences set forth as SEQ ID NO: 67 and 47 are homologous with mobilization proteins, and have been implicated in mobilization. The region with the nucleotide sequence set forth as SEQ ID NO: 75 has been implicated in gene expression of mobilization protein and suggested as a recognition site for an expressed protein. Thus, the plasmids of the invention preferably include mobilization protein regions having the nucleotide sequences set forth as SEQ ID NO: 67 and 47, or include a region involved in mobilization having the nucleotide sequence set forth as SEQ ID NO: 75.

[0036] For plasmid construction, the DNA fragments are appropriately selected based on the aforementioned nucleotide sequence data. The present invention also encompasses derivatives or functional (DNA-replicating) fragments of the plasmids.

[0037] The plasmids or DNA fragments of the invention may also contain nucleotide sequences with a substitution, deletion or insertion of one or a plurality of nucleotides in a DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion thereof, so long as the function of each region is not impaired.

[0038] The shuttle vectors of the invention may be any which comprise a plasmid or DNA fragment having a DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, and a DNA region that is replicable in *E. coli*, and which are replicable in the genus

Rhodococcus and *E. coli*, such as those having the nucleotide sequences set forth as SEQ ID NO: 89 to 100. The shuttle vectors of the invention may also have nucleotide sequences with one or a plurality of nucleotide substitutions, deletions or insertions in the
5 aforementioned nucleotide sequences, so long as they are replicable in the genus *Rhodococcus* and *E. coli*.

[0039] The "plurality" referred to above will differ depending on the type of region, and specifically may be 2-1100, preferably 2-800, more preferably 2-300, even more preferably 2-100, yet more
10 preferably 2-20 and most preferably 2-10.

[0040] As a plasmid or DNA fragment having substantially the same nucleotide sequence as the aforementioned DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion
15 thereof, there may be mentioned specifically, a nucleotide sequence which hybridizes with a DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion thereof, under
20 stringent conditions. Here "stringent conditions" are conditions under which specific hybrids are formed and non-specific hybrids are not formed. While it is difficult to precisely quantify the conditions, one example is a set of conditions that permit hybridization of DNA with high homology, such as 80% or greater, preferably 90% or greater or more preferably 95% or greater homology, while not permitting
25 hybridization of DNA with lower homology. More specifically, there may be mentioned hybridization conditions with ordinary Southern

hybridization washing at 60°C, 1xSSC, 0.1% SDS or preferably Southern hybridization washing at 0.1xSSC, 0.1% SDS corresponding salt concentration. When a DNA fragment with a length of approximately 300 bp is used as a portion of the DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, the hybridization washing conditions may be 50°C, 2xSSC, 0.1% SDS.

[0041] The aforementioned plasmid or DNA fragment having substantially the same nucleotide sequence as the aforementioned DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion thereof, may be obtained by, for example, modification of a DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion thereof, by site-directed mutagenesis so as to have a substitution, deletion or insertion of nucleotides at a specific site. Such modified DNA may also be obtained by mutation treatment known in the prior art. As mutation treatments there may be mentioned methods of *in vitro* treatment of DNA including a DNA replication region, DNA replication-related protein coding region, promoter region, mobilization protein region or mobilization-related region, or a portion thereof, with hydroxylamine or the like, and methods of treating a microbe possessing the DNA above, such as the genus *Escherichia*, with ultraviolet rays or with a mutagenic agent ordinarily used for mutagenesis such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) or EMS.

[0042] Nucleotide substitutions, deletions or insertions as mentioned above include those found in naturally occurring mutants or variants due to differences in *Rhodococcus* strains.

[0043] A shuttle vector of the invention includes a DNA fragment (A) as the aforementioned plasmid or portion thereof, and a DNA region (B) which is replicable in *E. coli*. In some cases it is preferred for the shuttle vector to comprise a DNA region including a drug resistance gene. In the relevant technical field, a "shuttle vector" is a vector which comprises the DNA replication mechanism for two different cell types, and preferably also a drug resistance gene or the like as a selective marker, allowing its auto-replication in the two different cell types. The DNA fragment (A) as the aforementioned plasmid or portion thereof is a DNA region that is replicable in the genus *Rhodococcus*. The DNA region (B) which is replicable in *E. coli* may be a full plasmid or a portion thereof, so long as it can be replicated and amplified in *E. coli*. As such DNA regions that are replicable in *E. coli* there may be used, for example, the plasmids pUC18, pHSG299 and pHSG398.

[0044] When the shuttle vector of the invention includes a drug resistance gene, the preferred ones are ampicillin resistance gene, kanamycin resistance gene and chloramphenol resistance gene, but there are no particular restrictions on the manner of drug so long as the gene is expressed in the genus *Rhodococcus* and *E. coli* as hosts and confers drug resistance to the host cells, in order to allow verification of the presence of plasmids in the two genera based on resistance to the drug. Also, a plurality of such drug resistance genes may be used in

combination.

[0045] The shuttle vector preferably contains multiple cloning sites (multicloning sites), and the cloning sites and drug resistance gene may be induced from, for example, an *E. coli* plasmid. That is, a publicly known *E. coli* plasmid such as one listed above may be cleaved with an appropriate restriction endonuclease and a DNA region containing the cloning sites and drug resistance gene constructed and ligated with another DNA fragment (a DNA region which is replicable in the genus *Rhodococcus*).

[0046] As an illustration, outline of shuttle vector constructions is shown in Figs. 3 to 5. The shuttle vectors may be constructed by treating the aforementioned plasmids and *E. coli* plasmids with suitable restriction endonucleases and then ligating them. In this manner, the present inventors constructed 18 shuttle vectors (Table 5) using the *Rhodococcus* plasmids pRET1000, pRET1100 or pRET1200, and the *E. coli* plasmids pUC18, pHSG299 or pHSG398.

[0047] The shuttle vectors of the invention are replicable in the genus *Rhodococcus* and *E. coli* as hosts, and are industrially useful. The *Rhodococcus* and *E. coli* strains transformed by the shuttle vectors of the invention, as well as other microbial transformants, are useful in this way and such transformants are also encompassed by the scope of the invention.

[0048] A vector of the invention is characterized by being constructed using a shuttle vector of the invention. Specifically, it is a vector having target DNA inserted therein which is to be introduced into the shuttle vector of the invention. The DNA to be introduced and

the shuttle vector of the invention are treated with appropriate restriction endonucleases and then ligated them to construct the vector. The vector may then be used to obtain transformants having the desired DNA transferred therein.

5 [0049] As examples of DNA to be inserted there may be mentioned aminoketone asymmetric reductase genes and coenzyme-regenerating system enzyme genes. Aminoketone asymmetric reductase genes are genes coding for aminoketone asymmetric reductases as described in WO02/070714, and more specifically, DNA
10 coding for a protein comprising the amino acid sequence set forth as SEQ ID NO: 78 (aminoketone asymmetric reductase derived from *R. erythropolis* MAK-34), and particularly DNA comprising the nucleotide sequence set forth as SEQ ID NO: 79. The entirety of the content described in WO02/070714 is incorporated herein by reference.

15 [0050] An aminoketone asymmetric reductase is any having the properties described in WO02/070714, and includes a protein having the amino acid sequence set forth as SEQ ID NO: 78 of the Sequence Listing, as well as proteins having amino acid sequences obtained by deletion, insertion, substitution or addition of one or more amino acids
20 in the aforementioned amino acid sequence, and exhibiting aminoketone asymmetric reduction activity. Aminoketone asymmetric reduction activity is activity of producing an optically active aminoalcohol represented by general formula (2) above using an α -aminoketone represented by general formula (1) above as the substrate.

25 [0051] There are no particular restrictions on the methods of deletion, insertion, substitution and addition, and any publicly known

methods may be employed. For example, there may be mentioned the methods described in "Zoku Seikagaku Jikken Kouza 1, Idenshi Kenkyuhou II", edited by the Japanese Biochemical Society, p105 (Hirose, S.), Tokyo Kagaku Dojin (1986); "Shin Seikagaku Jikken Kouza 2, Kakusan III (Recombinant DNA Technology)", edited by the Japanese Biochemical Society, p.233 (Hirose, S.), Tokyo Kagaku Dojin (1992); R.Wu, L. Grossman ed., "Methods in Enzymology", Vol. 154, p.350 & p.367, Academic Press, New York (1987); R.Wu, L. Grossman, ed., "Methods in Enzymology", Vol. 100, p.457 & p.468, Academic Press, New York (1983); J.A. Wells et al., "Gene", Vol. 34, p.315 (1985); T. Grundstroem et al., "Nucleic Acids Res", Vol. 13, p.3305 (1985); J. Taylor et al., "Nucleic Acids Res.", Vol. 13, p.8765 (1985); R.Wu, ed., "Methods in Enzymology", Vol. 155, p.568, Academic Press, New York (1987); and A.R. Oliphant et al., "Gene", Vol.44, p.177 (1986). As specific examples, there may be mentioned the site-directed mutagenesis method (site-specific mutagenesis method) utilizing synthetic oligonucleotides, the Kunkel method, the dNTP[α S] method (Eckstein method), and the region-directed mutagenesis method using sulfurous acid or nitrous acid.

[0052] Sugar chains are attached to the majority of proteins, and substitution of one or a plurality of amino acids can modify the attachment of sugar chains. Thus, the aminoketone asymmetric reductases of the invention also include proteins having the amino acid sequence set forth as SEQ ID NO: 78 of the Sequence Listing and having modifications of sugar chains, so long as they exhibit the aforementioned aminoketone asymmetric reduction activity.

[0053] The aminoketone asymmetric reductases of the invention may also have modifications of their amino acid residues by chemical methods, or their derivatives may be enhanced by modification or partial degradation using peptidase enzymes such as pepsin, chymotrypsin, papain, bromelain, endopeptidase and exopeptidase.

[0054] When the aminoketone asymmetric reductases of the invention are produced by a gene recombinant method, a fusion protein may be expressed and then converted or processed into a protein having biological activity which is substantially equivalent to a natural aminoketone asymmetric reductase either *in vivo* or *ex vivo*. In this case, a fusion production method ordinarily employed for genetic engineering may be used, and the fusion protein may be purified by affinity chromatography or the like, utilizing the fused portion thereof. Modification and enhancement of protein structures may be carried out with reference to "Shin Seikagaku Jikken Kouza 1, Tanpakushitsu VII, Tanpakushitsu Kogaku", edited by the Japanese Biochemical Society, Tokyo Kagaku Dojin (1993), by the methods described therein, the methods described in literature cited therein, or methods which are essentially equivalent thereto.

[0055] The aminoketone asymmetric reductase of the invention may also differ from naturally occurring forms in the identities of one or more of the amino acid residues or in the positions of one or more of the amino acid residues. The present invention also encompasses deletion analogues with deletion of one or more (for example, 1-80, preferably 1-60, more preferably 1-40, even more preferably 1-20 and especially 1-10) amino acid residues, substitution analogues with

substitution of one or more (for example, 1-80, preferably 1-60, more preferably 1-40, even more preferably 1-20 and especially 1-10) amino acid residues or addition analogues with addition of one or more (for example, 1-80, preferably 1-60, more preferably 1-40, even more preferably 1-20 and especially 1-10) amino acid residues peculiar to natural aminoketone asymmetric reductases. Also encompassed are enzymes having the domain structure characteristic of natural aminoketone asymmetric reductases. There may also be mentioned isomers of the aminoketone asymmetric reductases.

[0056] So long as the domain structure characteristic of natural aminoketone asymmetric reductases is maintained, all mutants above are also encompassed among the aminoketone asymmetric reductases of the invention. In addition, it is assumed that enzymes having a primary structural conformation substantially equivalent to natural aminoketone asymmetric reductases of the invention, or a portion thereof, as well as enzymes having biological activity substantially equivalent to natural aminoketone asymmetric reductases, may also be included. Naturally occurring mutants may also be mentioned. The aminoketone asymmetric reductases of the invention may be separated and purified in the manner explained below. The present invention encompasses DNA fragments coding for the aforementioned polypeptides, polypeptides of aminoketone asymmetric reductases having all or some of the natural features, and DNA fragments coding for analogues or derivatives thereof. The nucleotides of the aminoketone asymmetric reductases may be modified (for example, with addition, deletions or substitutions), and such modified forms are

also encompassed by the invention.

[0057] An aminoketone asymmetric reductase gene according to the invention is a nucleic acid coding for any of the aforementioned aminoketone asymmetric reductases. As representative examples there may be mentioned nucleic acid coding for a protein having the amino acid sequence set forth as SEQ ID NO: 78 of the Sequence Listing, and especially nucleic acid having the nucleotide sequence set forth as SEQ ID NO: 79, but since several nucleotide sequences (codons) can code for each amino acid, there exist numerous nucleic acids coding for a protein having the amino acid sequence set forth as SEQ ID NO: 78. Thus, all such nucleic acids are also encompassed among the aminoketone asymmetric reductase genes of the invention. Here, "coding for a protein" means that, when the DNA consists of two strands, one of the two complementary strands has a nucleotide sequence coding for the protein, and therefore the nucleic acids of the invention include nucleic acids comprising nucleotide sequences directly coding for the amino acid sequence set forth as SEQ ID NO: 78 and nucleic acids comprising nucleotide sequences which are complementary thereto. In addition, the aminoketone asymmetric reductase genes of the invention may be nucleic acids which hybridize with nucleic acid comprising a nucleotide sequence complementary to SEQ ID NO: 79 under stringent conditions, and which code for proteins with aminoketone asymmetric reduction activity. Here, "stringent conditions" has the same definition as explained above.

[0058] The coenzyme-regenerating system enzyme gene may be one for various dehydrogenases, specifically, glucose dehydrogenase,

glucose-6-phosphate dehydrogenase, aldehyde dehydrogenases, alcohol dehydrogenases, organic acid dehydrogenases and amino acid dehydrogenases. More specifically, there may be suitably used acetaldehyde dehydrogenase, ethanol dehydrogenase, propanol dehydrogenase, glycerol dehydrogenase, formate dehydrogenase, acetate dehydrogenase, butyrate dehydrogenase, lactate dehydrogenase, maleate dehydrogenase and glutamate dehydrogenase.

[0059] A transformant according to the invention is characterized by comprising the aforementioned vector. The transformant is obtained by introducing the vector into host cells. The vector introduction method may be a publicly known method, such as the calcium phosphate method, lipofection, electroporation, microinjection or the like.

[0060] For example, a transformant of the invention comprising a vector having an aminoketone asymmetric reductase gene inserted therein has aminoketone asymmetric reduction activity, and may be applied for an aminoketone asymmetric reductase production method or optically active aminoalcohol production method as described below.

[0061] The method for production of an aminoketone asymmetric reductase of the invention is characterized by comprising a culturing step in which transformants containing a vector having an aminoketone asymmetric reductase gene inserted therein are cultured in medium which allows growth of the transformants, and a purification step in which the aminoketone asymmetric reductase is purified from the transformants obtained in the culturing step.

[0062] The method for culturing may be a publicly known

method with no particular restrictions so long as it permits growth of the cells used, and ordinarily a liquid medium containing a carbon source, nitrogen source and other nutrients is used. As carbon sources for the medium there may be used any of those that can be utilized by the cells. Specifically, there may be mentioned sugars such as glucose, fructose, sucrose, dextrin, starch and sorbitol, alcohols such as methanol, ethanol and glycerol, organic acids such as fumaric acid, citric acid, acetic acid and propionic acid, and their salts, hydrocarbons such as paraffin, and mixtures thereof. As nitrogen sources there may be used any of those that can be utilized by the cells. Specifically, there may be mentioned ammonium salts of inorganic acids such as ammonium chloride, ammonium sulfate and ammonium phosphate; ammonium salts of organic acids such as ammonium fumarate and ammonium citrate; nitric acid salts such as sodium nitrate and potassium nitrate; and inorganic or organic nitrogenous compounds such as meat extract, yeast extract, malt extract and peptone, as well as mixtures thereof. The medium may also contain appropriately added nutrient sources ordinarily used for culturing, such as inorganic salts, trace metal salts and vitamins. When necessary, there may also be added to the medium substances that promote cell growth and buffering substances effective for maintaining the pH of the medium.

[0063] The culturing of the cells may be carried out under conditions suitable for growth. Specifically, the medium pH may be 3-10, preferably 4-9, and the temperature may be 0-50°C, preferably 20-40°C. The cell culturing may be conducted either under aerobic or anaerobic conditions. The culturing time is preferably 10-150 hours,

but should be appropriately determined for the type of cells used.

[0064] The culture solution of the cells cultured in the manner described above is filtered or centrifuged and the cells are rinsed with water or buffer solution. The rinsed cells are suspended in a suitable amount of buffer solution for disruption of the cells. The method of disruption is not particularly restricted but as examples there may be mentioned mechanical disruption with a mortar, Dynamill, French press, ultrasonic cell disruptor or the like. The aminoketone asymmetric reductase in the cell-free extract obtained by filtration or centrifugation of the solid matter from the cell disruptate is recovered by an ordinary enzyme isolating method.

[0065] There are no particular restrictions on the method for isolation of the enzyme and any publicly known method may be employed, but as examples there may be mentioned purification by salting out such as ammonium sulfate precipitation; gel filtration methods using Sephadex and the like; ion-exchange chromatography methods using carriers with diethylaminoethyl groups or carboxymethyl groups; hydrophobic chromatography using carriers with hydrophobic groups such as butyl, octyl and phenyl; dye gel chromatography methods; electrophoresis methods; dialysis; ultrafiltration methods; affinity chromatography methods; high performance liquid chromatography methods and the like.

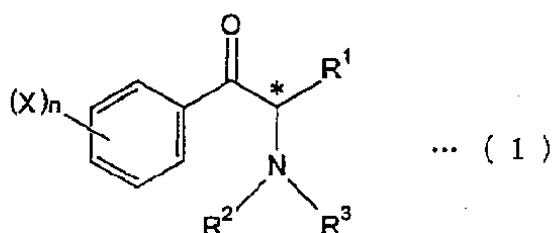
[0066] The enzyme may also be used as an immobilized enzyme. There are no particular restrictions on the method and any publicly known method may be employed, among which there may be mentioned immobilization of the enzyme or the enzyme-producing

cells, and the immobilization may be accomplished by a carrier bonding method such as a covalent bonding method or adsorption method, a crosslinking method, entrapment method or the like. A condensing agent such as glutaraldehyde, hexamethylene diisocyanate or hexamethylene diisothiocyanate may also be used if necessary. Other immobilizing methods include: a monomer method in which a monomer is gelled by polymerizing reaction; a prepolymer method in which molecules larger than monomers are polymerized; a polymer method in which a polymer is gelled; immobilization using polyacrylamide; immobilization using natural polymers such as alginic acid, collagen, gelatin, agar and κ -carrageenan; and immobilization using synthetic polymers such as photosetting resins and urethane polymers.

[0067] The enzyme purified in this manner is judged as having been adequately purified if a single band is confirmed in electrophoresis (SDS-PAGE, etc.).

[0068] A method for production of an optically active aminoalcohol according to the invention is characterized to produce an optically active aminoalcohol compound represented by the following general formula (2), which compound exhibits the desired optical activity, by reacting an aminoketone asymmetric reductase obtained by the production method of the invention with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1) or a salt thereof.

[Chemical Formula 8]



wherein X may be the same or different and represents at least one species selected from the group consisting of halogen, lower alkyl, hydroxyl optionally protected with a protecting group, nitro and sulfonyl;

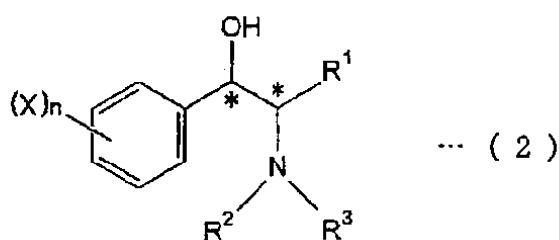
n represents an integer of 0 to 3;

R¹ represents lower alkyl;

R² and R³ may be the same or different and represent at least one species selected from the group consisting of hydrogen and lower alkyl; and

* represents asymmetric carbon.

[Chemical Formula 9]



wherein X, n, R¹, R², R³ and * have the same definitions as above.

[0069] First, the α -aminoketone compound represented by general formula (1) according to the invention will be explained.

[0070] The substituent X is as follows. As the aforementioned halogen there may be mentioned fluorine, chlorine, bromine and iodine.

[0071] As lower alkyl there are preferred C1-6 alkyl, among which there may be mentioned methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, hexyl and the like. These may have straight-chain or branched structures. As substituents they may contain halogens such as fluorine or chlorine, or hydroxyl, alkyl, amino, alkoxy and the like.

[0072] As protecting groups for hydroxyl optionally protected with a protecting group there may be mentioned groups that can be removed by treatment with water, groups that can be removed by acid or weak base treatment, groups that can be removed by hydrogenation or groups that can be removed with Lewis acid catalysts and thiourea, and such protecting groups include optionally substituted acyl, optionally substituted silyl, alkoxyalkyl, optionally substituted lower alkyl, benzyl, p-methoxybenzyl, 2,2,2-trichloroethoxycarbonyl, allyloxycarbonyl, trityl and the like.

[0073] The aforementioned acyl groups include acetyl, chloroacetyl, dichloroacetyl, pivaloyl, benzoyl, p-nitrobenzoyl and the like. They may contain hydroxyl, alkyl, alkoxy, nitro, halogen and the like as substituents. The aforementioned silyl groups include trimethylsilyl, t-butyldimethylsilyl, triarylsilyl and the like. They may contain alkyl, aryl, hydroxyl, alkoxy, nitro, halogen and the like as substituents. The aforementioned alkoxyalkyl groups include methoxymethyl, 2-methoxyethoxymethyl and the like. The aforementioned lower alkyl include C1-6 alkyl, among which there

may be mentioned methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, hexyl and the like. These may have straight-chain or branched structures. As substituents they may contain halogen such as fluorine or chlorine, or hydroxyl, alkyl, amino, alkoxy and the like.

[0074] X may be nitro or sulfonyl, and specifically there may be mentioned methylsulfonyl and the like.

[0075] The number "n" for X is an integer of 0-3, and is preferably 0.

[0076] R^1 in general formula (1) above represents lower alkyl. As lower alkyl there are preferred C1-6 alkyl, among which there may be mentioned methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, hexyl and the like. These may have straight-chain or branched structures.

[0077] Each of R^2 and R^3 represent hydrogen or lower alkyl. The lower alkyl include C1-6 alkyl, among which there may be mentioned methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, hexyl and the like. These may have straight-chain or branched structures.

[0078] As salts of the aforementioned α -aminoketone compounds there may be mentioned salts of inorganic acids such as hydrochloride, sulfate, nitrate, phosphate and carbonate, and salts of organic acids such as acetic acid and citric acid.

[0079] The α -aminoketone can be easily synthesized by halogenation (for example, bromination) of the α -carbon of a corresponding 1-phenylketone derivative, followed by replacement of

the halogen such as bromine with an amine (Ger. (East), 11, 332, Mar.12, 1956).

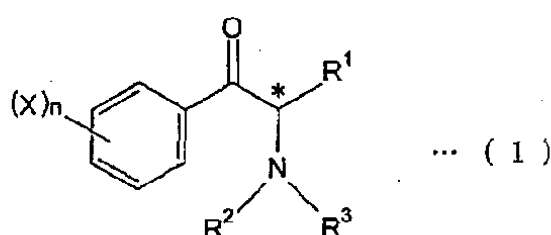
[0080] The optically active aminoalcohol represented by general formula (2) above according to the invention will now be explained. In
5 general formula (2), X, n, R¹, R², R³ and * have the same definitions as in general formula (1) above. As β -aminoalcohols having the desired optical activity there may be mentioned (1S, 2S)aminoalcohols. As specific examples of (1S, 2S)aminoalcohols there may be mentioned d-threo-2-methylamino-1-phenylpropanol (d-pseudoephedrine), d-threo-
10 2-dimethylamino-1-phenylpropanol (d-methylpseudoephedrine), (1S, 2S)- α -(1-aminoethyl)-benzyl alcohol (d-norpseudoephedrine), (1S, 2S)-1-(p-hydroxyphenyl)-2-methylamino-1-propanol, (1S, 2S)- α -(1-aminoethyl)-2,5-dimethoxy-benzyl alcohol, (1S, 2S)-1-(m-hydroxyphenyl)-2-amino-1-propanol, (1S, 2S)-1-(p-hydroxyphenyl)-2-
15 amino-1-propanol, (1S, 2S)-1-phenyl-2-ethylamino-1-propanol, (1S, 2S)-1-phenyl-2-amino-1-butanol, (1S, 2S)-1-phenyl-2-methylamino-1-butanol and the like.

[0081] The conditions for reaction of the aminoketone asymmetric reductase are not particularly restricted so long as an
20 optically active aminoalcohol represented by general formula (2) having the desired optical activity is produced, but since the enzyme optimum pH is 8.1 and the optimum temperature is 55°C, the reaction is preferably carried out under conditions of pH 7-9 and 30-65°C temperature.

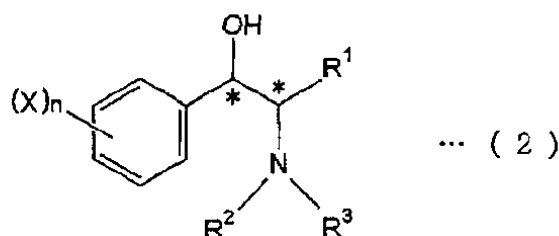
[0082] A method for production of an optically active
25 aminoalcohol according to the invention is also characterized to

produce an optically active aminoalcohol compound represented by the following general formula (2), which compound exhibits the desired optical activity, by reacting a transformant of the invention with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1) or a salt thereof.

[Chemical Formula 10]



[Chemical Formula 11]

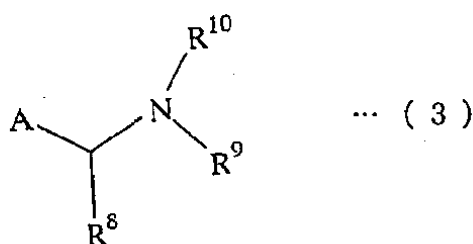


[0083] As the reaction conditions for the reaction described above, for example, the transformants shake cultured in liquid medium may be collected, an aqueous aminoketone solution (0.1-10% concentration) added to the obtained cells, and reaction conducted at a temperature of 20-40°C for a period of several hours to one day while regulating the pH to between 6-8. Upon completion of the reaction, the cells may be separated and the product in the reaction solution isolated to obtain an

optically active aminoalcohol. The reaction may be conducted in the same manner for treated transformant cells (dry cells or immobilized cells) or the enzyme or immobilized enzyme obtained from the transformants.

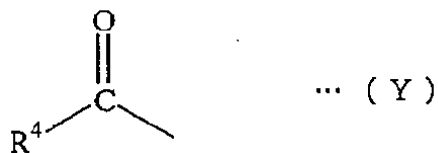
- 5 [0084] In the production method for an optically active aminoalcohol of the invention, the reaction may be carried out with further addition of a compound represented by the following general formula (3) or a pharmaceutically acceptable salt or solvate thereof, for more efficient production of the optically active aminoalcohol.

10 [Chemical Formula 12]



(wherein A represents the following formula (Y) or (Z))

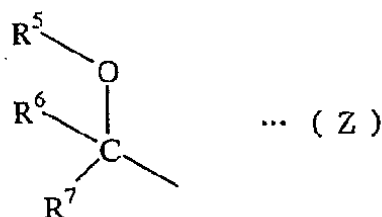
[Chemical Formula 13]



- 15 (wherein R⁴ represents hydrogen, optionally substituted C1-3 alkyl, a C5-10 hydrocarbon ring which is bonded to R⁸ or a 5- to 8-membered

heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁸)

[Chemical Formula 14]



5 (wherein R⁵ represents hydrogen, C1-3 alkyl or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁶ or R⁹, R⁶ represents hydrogen, optionally substituted C1-3 alkyl, a C5-
10 hydrocarbon ring which is bonded to R⁸ or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁵ or R⁹, and R⁷ represents hydrogen or optionally substituted C1-6 alkyl);
10 R⁸ represents hydrogen, carboxyl, optionally substituted C1-6 alkyl, a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁴ or a C5-10 hydrocarbon ring which is bonded to R⁶; R⁹ represents hydrogen, optionally substituted C1-6 alkyl,
15 optionally substituted C1-6 alkyloxycarbonyl, optionally substituted acyl or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R⁵ or R⁶; and R¹⁰ represents hydrogen or optionally substituted C1-6 alkyl)

[0085] In general formula (3) above, C1-3 alkyl may be straight-
20 chain or branched, and specifically there may be mentioned methyl, ethyl, n-propyl, isopropyl and the like. C1-6 alkyl may be straight-

chain or branched, and specifically there may be mentioned methyl, ethyl, n-propyl, isopropyl, n-butyl, i-butyl, s-butyl, t-butyl, pentyl, hexyl and the like. As C5-10 hydrocarbon rings there may be mentioned cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecanyl and the like.

[0086] As heteroatoms for the 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms there may be mentioned nitrogen, oxygen, sulfur and the like, among which nitrogen and oxygen are particularly preferred, and as 5- to 8-membered heterocyclic skeletons there may be mentioned pyrrolidine, piperidine, imidazolidine, piperazine, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, morpholine and the like.

[0087] As C1-6 alkyloxycarbonyl there may be mentioned methyloxycarbonyl, ethyloxycarbonyl, isopropyloxycarbonyl, isobutyloxycarbonyl, t-butyloxycarbonyl and the like. As acyl there may be mentioned formyl, acetyl, propionyl, butyryl, isobutyryl, pivaloyl, benzoyl, valeryl and the like. When the aforementioned C1-3 or C1-6 alkyl, C1-6 alkyloxycarbonyl or acyl have substituents there are no particular restrictions on the types, positions and numbers of substituents, and as examples of substituents there may be mentioned halogen such as fluorine and chlorine, hydroxyl, alkyl, carboxyl, amino, alkoxy, nitro, aryl and the like. As pharmaceutically acceptable salts there may be mentioned salts of inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid and phosphoric acid, salts of organic acids such as acetic acid and citric acid, salts of inorganic bases such as Na, K, Mg, Ca and ammonia, and salts of organic bases such as

triethylamine and cyclohexylamine.

[0088] As examples of compounds represented by general formula (3) above there may be mentioned 1-acetylamino-2-hydroxypropane, 1-methylamino-2-hydroxypropane, 1-amino-2-oxopropane, 1-amino-2-hydroxycyclopentane, 1-amino-2,3-dihydroxypropane, L-threonine, 4-amino-3-hydroxybutanoic acid, 1-amino-2-oxocyclohexane, morpholine, 3-hydroxypyrrolidine, 3-hydroxypiperidine, 2-aminomethyl-tetrahydrofuran, 1-(2-hydroxypropyl)amino-2-hydroxypropane, 1-t-butyloxycarbonylamino-2-hydroxypropane, 2-amino-3-hydroxybutane, DL-serine, 1-amino-2-hydroxypropane, 1-amino-2-hydroxybutane and 1-amino-2-hydroxycyclohexane. Compounds among these having asymmetric carbons may be optically active forms or racemic forms, unless otherwise specified.

[0089] Addition of such activity inducers to the medium can induce cellular activity and thus more efficiently promote production of the optically active β -aminoalcohol than when no such activity inducers are added. The activity inducers may be used alone, or several such activity inducers may be used in admixture. The amount of such activity inducers is preferably 0.01-10 wt% with respect to the medium.

[0090] The reaction method for production of the β -aminoalcohol of the invention is not particularly restricted so long as it is a method in which the cells or the cell-produced enzyme is reacted with an enantiomeric mixture of an α -aminoketone compound represented by general formula (1) above or its salt, to produce the corresponding

optically active β -aminoalcohol compound represented by general formula (2), and the reaction is initiated by mixing the cells rinsed with buffer solution or water with the α -aminoketone aqueous solution used as the starting material.

5 [0091] The reaction conditions may be selected within a range that does not impede production of the optically active β -aminoalcohol compound represented by general formula (2). The cell volume is preferably 1/100 to 1000-fold and more preferably 1/10 to 100-fold in terms of dry weight with respect to the racemic aminoketone. The
10 concentration of the racemic aminoketone substrate is preferably 0.01-20% and more preferably 0.1-10%. The pH of the reaction solution is preferably 5-9 and more preferably 6-8, and the reaction temperature is preferably 10-50°C and more preferably 20-40°C. The reaction time is preferably 5-150 hours, but this may be appropriately determined
15 depending on the cell type.

[0092] In order to more efficiently promote the reaction, there may be added sugars such as glucose, organic acids such as acetic acid and energy sources such as glycerol. These may be used alone or as mixtures. The amount of addition is preferably 1/100 to 10-fold with
20 respect to the substrate. Coenzymes and the like may also be added. As coenzymes there may be used nicotinamide adenine dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the like,
25 either alone or in mixtures, added in amounts of preferably 1/1000 to 1/5 with respect to the racemic aminoketone. In addition to such

coenzymes, there may be added coenzyme-regenerating enzymes such as glucose dehydrogenase, in amounts of 1/1000 to 1/5 with respect to the racemic aminoketone. Also, substrates for coenzyme-regenerating enzymes, such as glucose, may be added, in amounts of 1/100 to 10-fold with respect to the racemic aminoketone. There may also be used combinations of sugars such as glucose, organic acids such as acetic acid, energy sources such as glycerol, coenzymes, coenzyme-regenerating enzymes and coenzyme-regenerating enzyme substrates. These usually accumulate in the cells but if necessary they may be added to increase the reaction speed or yield, and therefore may be added as appropriate.

[0093] If the reaction solution is reacted with addition of the specific salts described above under the aforementioned conditions, racemization of the unreacted α -aminoketone isomers will be aided, thus more efficiently promoting conversion to the enantiomer which will serve as the substrate of the cells or cell-produced enzyme. This will tend to yield the target aminoalcohol from the starting material at a high yield of 50% or greater.

[0094] As salts that promote racemization of unreacted α -aminoketones there may be used weak acid salts such as acetate, tartarate, benzoate, citrate, malonate, phosphate, carbonate, paranitrophenol salt, sulfite and borate, but there are preferably used phosphate (for example, sodium dihydrogen phosphate, potassium dihydrogen phosphate, ammonium dihydrogen phosphate), carbonate (for example, sodium carbonate, sodium hydrogen carbonate, potassium carbonate, ammonium carbonate) and citrate (for example,

sodium citrate, potassium citrate, ammonium citrate). Mixtures thereof may also be used, with a buffer solution with a pH of 6.0-8.0 added to a final concentration of preferably 0.01-1 M. In the case of a phosphate, for example, sodium dihydrogen phosphate and sodium monohydrogen phosphate may be mixed in a proportion of between 9:1 and 5:95.

[0095] The optically active α -aminoalcohol produced by the reaction may be purified by ordinary separation and purification means. For example, the optically active β -aminoalcohol may be obtained directly from the reaction solution or after separation of the cells, by being subjected to a common purification process such as membrane separation, extraction with an organic solvent (for example, toluene, chloroform, etc.), column chromatography, vacuum concentration, distillation, crystallization, recrystallization or the like. The optical purity of the produced optically active β -aminoalcohol can be measured by high performance liquid chromatography (HPLC).

Examples

[0096] The present invention will now be explained in greater detail through examples, with the understanding that these examples in no way limit the technical scope of the invention.

[0097] (Example 1) Isolation and purification of plasmids

(1) Method

Rhodococcus strains were inoculated to 5 mL of GPY medium (1% glucose, 0.5% bactopectone, 0.3% yeast extract) and cultured with shaking at 25°C. After adding 250 μ L of a 100 mg/mL ampicillin solution in the logarithmic growth phase, culturing was continued at 25°C for 2 hours with shaking. The cells were harvested by

centrifugation (12 krpm, 5 min), and after removing off the supernatant, they were suspended in 1 mL of 50 mM Tris (pH 7.5), the cells were again harvested by centrifugation (12 krpm, 5 min) and the supernatant was removed off. They were then suspended in 250 μ L of a 10 mg/mL lysozyme solution dissolved in TE solution (10 mM Tris (pH 7.5), 1 mM EDTA), and the suspension was allowed to stand at 37°C for 30 minutes. Next, 100 μ L of 3 M sodium chloride and 25 μ L of 10% SDS were added and the mixture was allowed to stand at -20°C overnight. To the supernatant from centrifugation (12 krpm, 5 min) there were added 0.5 μ L each of 50 μ g/mL Proteinase K and 50 μ g/mL RNase A, and the mixture was allowed to stand at 37°C for 15 minutes. An equivalent of phenol/chloroform/isoamyl alcohol solution was added and centrifugation was performed (12 krpm, 5 min). A 2.5-fold amount of ethanol was added to the supernatant, the mixture was centrifuged (12 krpm, 5 min), and the precipitate was dissolved in 50 μ L of sterilized water. Confirmation of plasmids was accomplished by electrophoresis with 0.8% agarose gel and staining with ethidium bromide, followed by UV irradiation.

[0098] (2) Test bacteria strains and results

Throughout the examples, the presence or absence of plasmids was screened from available strains belonging to the genus *Rhodococcus* and its related genus *Mycobacterium* followed the method described in (1) above.

[0099] Table 3 shows the screened strains confirmed to contain plasmids. Specifically, *Rhodococcus erythropolis* (IAM1400, IAM1503, JCM2893, JCM2894 and JCM2895) and *Rhodococcus*

rhodnii (JCM3203) were confirmed to contain plasmids of approximately 5.4 kbp and 5.8 kbp, respectively. These plasmids were designated according to the names listed in Table 3: pRET1100, pRET1200, pRET1300, pRET1400, pRET1500, pRET1600, pRET1700, pRET1800, pRET0500, pRET1000 (see Table 3).

[0100] *R. erythropolis* IAM1400 and IAM1503 are described in "IAM Catalogue of Strains, Third Edition, 2004" published by the Institute of Molecular and Cellular Biosciences, The University of Tokyo, and are available from the institute. Also, *R. erythropolis* JCM2893, JCM2894 and JCM2895 and *R. rhodnii* JCM3203 are described in "JCM Catalogue of Strains, Eighth Edition 2002" published by RIKEN, Japan, and are available from the institute.

[0101] [Table 3]

Strain	No.	Size (kbp)	Name
<i>Rhodococcus erythropolis</i>	IAM 1400	5.4	pRET1100
		5.4	pRET1200
ditto	IAM 1503	5.4	pRET1300
		5.4	pRET1400
ditto	JCM 2893	5.4	pRET1500
		5.4	pRET1600
ditto	JCM 2894	5.4	pRET1700
		5.4	pRET1800
ditto	JCM 2895	5.4	pRET0500
<i>Rhodococcus rhodnii</i>	JCM 3203	5.8	pRET1000

[0102] (Example 2) Identification of restriction endonuclease sites

Various restriction endonucleases were used to determine restriction endonuclease sites, for classification of the plasmids shown in Table 3. Each plasmid was isolated by the method described in

Example 1, and then digested with *EcoR* I, *Hind* III, *Pvu* II, *Sca* I, *Sph* I, *Sma* I, *Sac* I, *Bam*H I and *Kpn* I, and electrophoresed on 0.8% agarose gel for confirmation of the DNA fragments. The size marker used was Loading Quick DNA size Marker λ /*EcoR* I+*Hind* III double digest (Toyobo). The numbers of sites cleaved by the restriction endonucleases and the sizes of the fragments were determined based on the size marker. The results are shown in Table 4.

[0103] [Table 4]

	<i>R. erythropolis</i>									<i>R. rhodnii</i>
	IAM 1400		IAM 1503		JCM 2893		JCM 2894		JCM 2895	JCM 3203
	pRET1100	pRET1200	pRET1300	pRET1400	pRET1500	pRET1600	pRET1700	pRET1800	pRET0500	pRET1000
<i>Bam</i> H I	2(0.4, 5.0)	1(5.4)	same as pRET1100	same as pRET1200	same as pRET1100	same as pRET1200	same as pRET1100	same as pRET1200	same as pRET1200	2(2.0, 3.8)
<i>Eco</i> R I	2(0.3, 5.1)	1(5.4)								0
<i>Hind</i> III	0	0								0
<i>Kpn</i> I	1(5.4)	0								0
<i>Pvu</i> II	1(5.4)	2(0.9, 4.5)								4(0.1, 1.4, 2.0, 2.3)
<i>Sac</i> I	1(5.4)	1(5.4)	pRET1100	pRET1200	pRET1100	pRET1200	pRET1100	pRET1200	pRET1200	3(0.9, 1.0, 3.9)
<i>Sca</i> I	0	0								0
<i>Sph</i> I	0	0								0
<i>Sma</i> I	1(5.4)	2(0.4, 0.5)								4(0.1, 1.2, 1.6, 2.9)

Values in parentheses indicate sizes (kbp)

[0104] Based on the analysis results shown above, the plasmids in Table 3 were classified into three types: plasmids possessing the same restriction endonuclease sites as pRET1100, plasmids possessing the same restriction endonuclease sites as pRET1200, and pRET1000.

[0105] (Example 3) Plasmid sequencing and homology search

As the plasmids were classified into three types, i.e. pRET1000, pRET1100 and pRET1200 based on the results of Example 2, it was attempted to sequence each of the plasmids.

[0106] First, the DNA fragments of the plasmids were cloned for determination of the nucleotide sequences. For *Rhodococcus*

erythropolis (IAM1400), the plasmids (pRET1100, pRET1200) were isolated and digested with *Sma* I and *Sac* I. Upon electrophoresis on 0.8% agarose gel, DNA fragments with sizes of approximately 0.5 kbp, approximately 1.7 kbp, approximately 3.7 kbp and approximately 4.9 kbp were confirmed. The respective DNA fragments were recovered from the agarose gel using a GFXTM PCR DNA and Gel Band Purification Kit (Amersham Bioscience) and used as insert DNA. Separately, pBluescript II KS(-) was used after digesting with *Sma* I alone or with *Sma* I and *Sac* I, as vector DNA. The insert DNA and vector DNA were ligated with Ligation High (Toyobo) and used to transform *E. coli* JM109. The obtained transformants were screened using a GFX Micro Plasmid Prep Kit (Amersham Bioscience) to obtain different clones.

[0107] For *Rhodococcus rhodnii* (JCM3203), the plasmid (pRET1000) was isolated and then digested with *Bam*H I. Upon electrophoresis on 0.8% agarose gel, DNA fragments with sizes of approximately 2.0 kbp and approximately 3.8 kbp were confirmed. The respective DNA fragments were recovered from the gel using the aforementioned Kit and used as insert DNA. The vector DNA used was pBluescript II KS(-) digested with *Bam*H I.

[0108] Determination of the nucleotide sequences of the plasmid inserts was accomplished by the primer walking method. The apparatus used was an ABI PRISMTM 310NT Genetic Analyzer, and the enzyme used was a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI).

[0109] First, P7 (M13 forward, Toyobo) and P8 (M13 reverse,

Toyobo) primers were used for partial decoding of the insert nucleotide sequences. Next, primers were designed within the decoded sequence (using the sequence analyzing software DNASIS Pro; Hitachi Software Corp.), and the designed primers (synthetic oligo DNA) were used for further decoding of the nucleotide sequence. This procedure was repeated until decoding of the entirety of each insert nucleotide sequence. Upon completion of the insert nucleotide sequence decoding, primers were designed for reaction from the ends of each insert to the vector direction in order to analyze how the inserts were linked, and PCR was conducted (using KOD -plus-), using the plasmid isolated from *Rhodococcus erythropolis* (IAM1400) as template. The PCR product was purified using a GFXTM PCR DNA and Gel Band Purification Kit, and sequencing was carried out using the same primers used for PCR, to analyze the arrangement of the inserts.

[0110] The results of sequencing showed that pRET1100 consisted of 5444 bp, with a G+C content of 59%. The full determined nucleotide sequence is set forth as SEQ ID NO: 73 of the Sequence Listing. Plasmid pRET1200 consisted of 5421 bp and had a G+C content of 62%. Plasmid pRET1000 consisted of 5813 bp and had a G+C content of 67%. The full determined nucleotide sequence is set forth as SEQ ID NO: 74 of the Sequence Listing.

[0111] A homology search for the determined nucleotide sequences using DNASIS Pro revealed that pRET1000 and pRET1100 were novel plasmids. On the other hand, pRET1200 had approximately 99.6% homology with pN30 (GenBank accession no. AF312210) (calculated based on pRET1200).

[0112] For pRET1000 and pRET1100, comparison was made with publicly known plasmids based on the determined nucleotide sequences, using DNASIS Pro. As a result, neither of the plasmids were found to have completely matching restriction endonuclease sites with other plasmids.

[0113] (Example 4) Nucleotide sequence analysis

The results of analysis of the nucleotide sequences of pRET1100 and pRET1000 are shown below.

[0114] The following orfs were found in pRET1100:

orf1 (SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3) consisting of the nucleotide sequence from bases 202, 238 or 337 to 480 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf2 (SEQ ID NO: 4) consisting of the nucleotide sequence from bases 477 to 758 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf3 (SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 16) consisting of the nucleotide sequence from bases 862, 1294, 1450, 1462, 1486, 1489, 1513, 1630, 1645, 1687, 2224 or 2227 to 2409 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf4 (SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20 or SEQ ID NO: 21) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 1875, 1734, 1701, 1674 or 1581 to 1444 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf5 (SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 2828, 2792, 2747, 2594 or 2540 to 2406 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf6 (SEQ ID NO: 27 or SEQ ID NO: 28) consisting of the nucleotide sequence from bases 2971 or 3049 to 3306 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf7 (SEQ ID NO: 29 or SEQ ID NO: 30) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 3577 or 3571 to 3053 of the nucleotide sequence set forth as SEQ ID NO: 73;

orf8 (SEQ ID NO: 31 or SEQ ID NO: 32) consisting of the nucleotide sequence from bases 3339 or 3648 to 3902 of the nucleotide sequence set forth as SEQ ID NO: 73; and

orf9 (SEQ ID NO: 33 or SEQ ID NO: 34) consisting of the nucleotide sequence from bases 4366 or 4477 to 5034 of the nucleotide sequence set forth as SEQ ID NO: 73.

[0115] The following orfs were found in pRET1000:

orf10 (SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40 or SEQ ID NO: 41) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 3350, 3251, 2945 or 2849 to 2412 of the nucleotide sequence set forth as SEQ ID NO: 74;

orf11 (SEQ ID NO: 42 or SEQ ID NO: 43) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 2365 or 2332 to 2159 of the nucleotide sequence set forth as SEQ

ID NO: 74;

orf12 (SEQ ID NO: 44) consisting of the nucleotide sequence from bases 3197 to 3526 of the nucleotide sequence set forth as SEQ ID NO: 74;

5 orf13 (SEQ ID NO: 45 or SEQ ID NO: 46) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 4035 or 3996 to 3679 of the nucleotide sequence set forth as SEQ ID NO: 74;

10 orf14 (SEQ ID NO: 48, SEQ ID NO: 49 or SEQ ID NO: 50) consisting of the nucleotide sequence from bases 4621, 4654 or 4666 to 4830 of the nucleotide sequence set forth as SEQ ID NO: 74;

15 orf15 (SEQ ID NO: 51 or SEQ ID NO: 52) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 5161 or 5062 to 4709 of the nucleotide sequence set forth as SEQ ID NO: 74;

orf16 (SEQ ID NO: 53 or SEQ ID NO: 54) consisting of the nucleotide sequence from bases 2331 or 2334 to 2618 of the nucleotide sequence set forth as SEQ ID NO: 74;

20 orf17 (SEQ ID NO: 55) consisting of the nucleotide sequence from bases 2907 to 3242 of the nucleotide sequence set forth as SEQ ID NO: 74;

25 orf18 (SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60) consisting of the nucleotide sequence from bases 1650, 1689, 1713, 1827 or 1875 to 2162 of the nucleotide sequence set forth as SEQ ID NO: 74;

orf19 (SEQ ID NO: 61) consisting of the nucleotide sequence

from bases 1906 to 2169 of the nucleotide sequence set forth as SEQ ID NO: 74;

orf20 (SEQ ID NO: 62) consisting of the nucleotide sequence complementary to the nucleotide sequence from bases 810 to 553 of the nucleotide sequence set forth as SEQ ID NO: 74;

orf21 (SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69) consisting of the nucleotide sequence from bases 117, 147, 306, 456, 5144, 5276 or 5534 to 656 of the nucleotide sequence set forth as SEQ ID NO: 74.

[0116] The DNA replication region of pRET1100 is the region represented by the nucleotide sequence set forth as SEQ ID NO: 35 (from bases 2410 to 3200), the nucleotide sequence set forth as SEQ ID NO: 36 (from bases 1000 to 1500) or the nucleotide sequence set forth as SEQ ID NO: 37 (from bases 5000 to 500). The DNA replication region of pRET1000 is the region represented by the nucleotide sequence set forth as SEQ ID NO: 70 (from bases 3355 to 3507), the nucleotide sequence set forth as SEQ ID NO: 71 (from bases 4290 to 4350) or the nucleotide sequence set forth as SEQ ID NO: 72 (from bases 3570 to 3894).

[0117] The region of the nucleotide sequence from bases 5144 to 656 (SEQ ID NO: 67) and the region of the nucleotide sequence from bases 4381 to 4830 (SEQ ID NO: 47) of the nucleotide sequence of pRET1000 (SEQ ID NO: 74) are homologous with mobilization proteins, suggesting that they are involved in mobilization.

[0118] A DNA secondary structure is predicted for the region of

the nucleotide sequence from bases 4260 to 4339 (SEQ ID NO: 75) of the nucleotide sequence of pRET1000 (SEQ ID NO: 74), and it is presumably involved in expression of the mobilization protein gene or is the recognition site of the expressed protein.

5 [0119] On the other hand, it was suggested that the region of the nucleotide sequence from bases 761 to 868 (SEQ ID NO: 76) of the nucleotide sequence of pRET1100 (SEQ ID NO: 73) is a promoter involved in expression of a protein related to replication.

[0120] (Example 5) Construction of shuttle vectors

10 For construction of a shuttle vector between *Rhodococcus* strains and *E. coli*, the *Rhodococcus* plasmids pRET1000, pRET1100 and pRET1200 and the *E. coli* plasmids pUC18, pHSG299 and pHSG398 were used for the following experiment.

[0121] First, DNA fragments were prepared from *R. erythropolis* plasmids. Specifically, plasmids pRET1100 and pRET1200 were
15 obtained from *R. erythropolis* (IAM1400), and then *Alw44* I was used for digestion of pRET1100 at 37°C for 2 hours and Blunting High (Toyobo) was used for blunting of the ends, while *Bsp*LU11 I was used for digestion of pRET1200 at 48°C for 2 hours and Blunting High
20 (Toyobo) was used for blunting of the ends, to obtain DNA fragments of *R. erythropolis* plasmid. Each of the DNA fragments was dissolved in TE solution.

[0122] For pRET1000, plasmid pRET1000 was obtained from *R. rhodnii* (JCM3203), and then *Drd* I was used for digestion of
25 pRET1000 at 37°C for 2 hours and Blunting High was used for blunting of the ends, to obtain pRET1000 DNA fragments, which were

dissolved in TE solution.

[0123] Next, DNA fragments were prepared from the *E. coli* plasmids. Specifically, pUC18 (containing the ampicillin-resistance gene (Amp^r)) was digested with *Sma* I at 30°C for 2 hours, and
5 pHSG299 (containing the kanamycin-resistance gene (Km^r)) and pHSG398 (containing the chloramphenicol-resistance gene (Cm^r)) were digested with *Hinc* II at 37°C for 2 hours to obtain DNA fragments of *E. coli* plasmid, which were dissolved in TE.

[0124] After ligating the DNA fragments from the *Rhodococcus*
10 and *E. coli* plasmids prepared in the manner described above, they were used for transformation in *E. coli* DH5 α , which were plated on LB (1% tryptophan, 0.5% yeast extract, 1% sodium chloride; pH 7.2) agar medium containing 100 μ g/mL kanamycin, 100 μ g/mL ampicillin or 30 μ g/mL chloramphenicol, coated with 30 μ L of 0.1 M IPTG (isopropyl- β -galactoside) and 4% X-gal (5-bromo-4-chloro-3-indole- β -D-galactopyranoside) and allowed to stand at 30°C for 60 hours. White colonies were selected from among the appearing colonies, and were
15 cultured with shaking in LB liquid medium containing 100 μ g/mL kanamycin, 100 μ g/mL ampicillin or 30 μ g/mL chloramphenicol, at 30°C for 60 hours. The DNA was purified from the obtained culture solution using a GFXTM Micro Plasmid Prep Kit (Amersham Bioscience, with purification under the manufacturer's specified conditions). The obtained DNA was confirmed by electrophoresis on 0.8% agarose gel. The obtained shuttle vectors are shown in Table 5,
20 and the methods for constructing each of the shuttle vectors using pRET1100 are shown in Figs. 3 to 5.
25

[0125] [Table 5]

Constructed shuttle vectors	Origin	
	<i>Rhodococcus</i>	<i>E. coli</i>
pRET1001, pRET1001Rv	pRET1000	pUC18
pRET1002, pRET1002Rv	pRET1000	pHSG299
pRET1003, pRET1003Rv	pRET1000	pHSG398
pRET1101, pRET1101Rv	pRET1100	pUC18
pRET1102, pRET1102Rv	pRET1100	pHSG299
pRET1103, pRET1103Rv	pRET1100	pHSG398
pRET1201, pRET1201Rv	pRET1200	pUC18
pRET1202, pRET1202Rv	pRET1200	pHSG299
pRET1203, pRET1203Rv	pRET1200	pHSG398

[0126] The shuttle vectors constructed with pRET1100 and pUC18, pHSG299 or pHSG398 were designated respectively as pRET1101 (SEQ ID NO: 89), pRET1102 (SEQ ID NO: 90) or pRET1103 (SEQ ID NO: 91), respectively. Of the shuttle vectors, pRET1101 exhibits ampicillin resistance, pRET1102 exhibits kanamycin resistance and pRET1103 exhibits chloramphenicol resistance. Also, the shuttle vectors pRET1101 to 1103 wherein the *E. coli* gene and pRET1100 were linked in reverse (Rv) were designated respectively as pRET1101Rv (SEQ ID NO: 92), pRET1102Rv (SEQ ID NO: 93) and pRET1103Rv (SEQ ID NO: 94).

[0127] Similarly, the shuttle vectors constructed using pRET1000 and pRET1200 were designated as pRET1001-pRET1003 (SEQ ID NO: 95-SEQ ID NO: 97) and pRET1001Rv-pRET1003Rv (SEQ ID NO: 98-SEQ ID NO: 100), and as pRET1201-pRET1203 and pRET1201Rv-pRET1203Rv (Table 5).

[0128] (Example 6) Examining method of transformation to *R. erythropolis*

The *Rhodococcus-E. coli* shuttle vectors obtained in Example 5 were used for transformation of *R. erythropolis* MAK-34 strain (MAK-34; deposited at the National Institute of Bioscience and Human-Technology, National Institute of Advanced Industrial Science and Technology, Ministry of Economy, Trade and Industry, (currently: International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology) on February 15, 2001 as FERM BP-7451). Electroporation was investigated as the method of gene transfer.

[0129] First, *R. erythropolis* MAK-34 strain was inoculated to 5 mL of GPY medium and cultured with shaking at 30°C for 36 hours. After seeding 1 mL of culture solution in 100 mL of LB medium, culturing was continued at 200 rpm at 30°C for 10 hours. The cultured cells were harvested by centrifugation (12 krpm, 5 min, 4°C) and the harvested cells were rinsed twice with ultrapurified water. The rinsed cells were harvested by centrifugation (12 krpm, 5 min, 4°C) and suspended in 2.4 mL of a 10% glycerol solution. The suspension was dispensed into 300 µl portions and frozen at -80°C as competent cells.

[0130] A 90 µL portion of the prepared competent cells and a 5 µL portion of the shuttle vector (pRET1001, pRET1002, pRET1003, pRET1101, pRET1102, pRET1103, pRET1201, pRET1202 or pRET1203) were mixed on ice. The mixed solution was gently poured into a 0.1 cm cuvette which had been cooled on ice, and was set in a Gene Pulser II Electroporation System (BIO-RAD). After pulsing at 20 kV/cm, 400 Ω, 25 µF, the mixed solution was added with 300 µL of LB medium immediately and was allowed to stand at 25°C for 3 hours.

[0131] A portion of the cell suspension was plated on an antibiotic-containing LB plate (100 µg/mL kanamycin, 100 µg/mL ampicillin or 30 µg/mL chloramphenicol). As a result, colonies were obtained when using pRET1002, pRET1102 and pRET1202 containing the kanamycin resistance gene. In order to confirm that the obtained colonies contained the plasmids, the plasmids were isolated and all were verified to contain the shuttle vector.

[0132] This suggested that *R. erythropolis* can be transformed by electroporation and that pRET1002, pRET1102 and pRET1202 function as shuttle vectors.

[0133] (Example 7) Obtaining aminoketone asymmetric reductase gene (*mak* gene)

The *mak* gene was isolated from *R. erythropolis* MAK-34 strain for insertion of the *mak* gene into the shuttle vector shown in Fig. 5.

[0134] First, genomic DNA was obtained from *R. erythropolis* MAK-34 strain. After inoculating *R. erythropolis* MAK-34 strain to 5 mL of GPY medium, culturing with shaking was performed at 30°C for 48 hours, and then the culture solution was seeded in 100 mL of GPY medium and subcultured at 200 rpm at 30°C for 10 hours. The genomic DNA was obtained using a Genomic DNA Buffer set and Genomic-tip 500/G (QIAGEN).

[0135] The obtained genomic DNA was used as template for PCR using KOD -plus-. The primers used were MAKF1 (5'-GAATCTTCTCGTTGATGCAGATCAGGTC-3'; SEQ ID NO: 80) and MAKR2 (5'-CTGACTCCGTAGTGTCTGCCAGTTC-3'; SEQ ID NO: 81), for PCR at an annealing temperature of 68°C and

extension reaction for 1 minute and 50 seconds. The obtained PCR product was subjected to phenol/chloroform treatment and ethanol precipitation, and then mixed with pUC18 that had been digested with *Sma* I for 2 hours at 30°C, and ligated therewith using Ligation High. Competent High (Toyobo) was used for transformation of *E. coli* DH5 α , which was then plated on LB agar medium (containing 100 μ g/mL ampicillin) that had been coated with 30 μ L of 0.1 M IPTG and 4% X-gal, and was allowed to stand at 30°C for 60 hours. White colonies were selected from among the appearing colonies, and were cultured with shaking in LB liquid medium containing 100 μ g/mL ampicillin at 30°C for 60 hours. The DNA was purified from the obtained culture solution using a GFXTM Micro Plasmid Prep Kit. The obtained DNA was confirmed by electrophoresis on 0.8% agarose gel. The obtained clone was designated as pMAK-1.

[0136] (Example 8) Construction of expression vector - 1

A promoter and aminoketone asymmetric reductase gene (*mak* gene) were inserted into the shuttle vector shown in Table 5.

[0137] First, an expression vector (without exogenous promoter) containing approximately 400 bp upstream from the *mak* gene was constructed.

[0138] pMAK-1 was digested with *Sma* I at 30°C for 2 hours, and then with *Pst* I at 37°C for 2 hours. The solution was supplied for 0.8% agarose gel electrophoresis. The DNA size marker used was Loading Quick DNA size Marker λ /*Eco*R I+*Hind* III double digest. After electrophoresis, an approximately 1.4 kbp DNA fragment was purified using a GFXTM PCR DNA and Gel Band Purification Kit, and

used as the insert DNA. On the other hand, the vector used was pRET1102 digested with *Hinc* II and *Pst* I at 37°C for 2 hours. The DNA fragments were ligated with Ligation High and Competent High was used for transformation of *E. coli* DH5 α . The cells were plated on
5 LB agar medium containing 100 μ g/mL kanamycin and allowed to stand at 30°C for 60 hours.

[0139] The appearing colonies were cultured with shaking on LB liquid medium containing 100 μ g/mL kanamycin at 30°C for 60 hours. The DNA was purified from the obtained cultured medium using a
10 GFX™ Micro Plasmid Prep Kit. The obtained DNA was confirmed by 0.8% agarose gel electrophoresis.

[0140] For screening, the obtained DNA without restriction endonuclease treatment and the DNA after digestion with *Pst* I at 37°C for 2 hours were subjected to 0.8% agarose gel electrophoresis, and the
15 target plasmid was obtained based on the size of the DNA. The size marker used was Loading Quick DNA size Marker λ EcoR I+*Hind* III double digest, pRET1102 and pRET1102 that had been digested with *Pst* I at 37°C for 2 hours. The plasmid obtained in this manner was designated as pRET1104.

20 [0141] (Example 9) Construction of expression vector - 2

The shuttle vectors were reduced, since reduction of shuttle vectors is effective for expression vector enhancement, gene modification, transformation efficiency improvement and replication in cells.

25 [0142] First, shuttle vector pRET1102 was reduced. After digesting pRET1102 with *Bam*H I and *Hinc* II for 2 hours, it was

electrophoresed on 0.8% agarose gel and an approximately 2.7 kbp DNA fragment was recovered using a GFXTM PCR DNA and Gel Band Purification Kit to prepare a pRET1102 DNA fragment. The size marker used was Loading Quick DNA size Marker λ EcoR I+Hind III double digest.

[0143] Separately, a DNA fragment replicable in *E. coli* was prepared by digesting pHSG299 with *Bam*H I and *Hinc* II for 2 hours, subjecting it to 0.8% agarose gel electrophoresis, and recovering an approximately 2.7 kbp DNA fragment using a GFXTM PCR DNA and Gel Band Purification Kit.

[0144] The DNA fragments were ligated with Ligation High and Competent High was used for transformation of *E. coli* JM109 cells, which were then plated on LB agar medium, containing 100 μ g/mL kanamycin, that had been coated with 30 μ L of 0.1 M IPTG and 4% X-gal, and was allowed to stand at 30°C for 48 hours.

[0145] White colonies were selected from among the appearing colonies, and were cultured with shaking in LB liquid medium containing 100 μ g/mL kanamycin at 30°C for 48 hours. The DNA was purified from the obtained culture solution using a GFXTM Micro Plasmid Prep Kit. The reduced shuttle vector of pRET1102 obtained in this manner was designated as pRET1123 (approximately 5.3 kbp).

[0146] Next, shuttle vector pRET1202 was reduced. The *Rhodococcus*-derived DNA fragment was prepared by digesting pRET1202 with *Eco*R I for 2 hours and then with *Dra* III for 2 hours, using Blunting High for blunting of the ends, performing 0.8% agarose gel electrophoresis, and then recovering an approximately 3.7 kbp

DNA fragment using a GFXTM PCR DNA and Gel Band Purification Kit. The size marker used was Loading Quick DNA size Marker λ EcoR I+Hind III double digest. The DNA fragment was inserted at the Hinc II site of pHSG299. After ligation, Competent High was used for transformation of *E. coli* DH5 α , which was then plated on LB agar medium, containing 100 μ g/mL kanamycin, that had been coated with 30 μ L of 0.1 M IPTG and 4% X-gal, and was allowed to stand at 30°C for 72 hours. White colonies were selected from among the appearing colonies, and were cultured with shaking in LB liquid medium containing 100 μ g/mL kanamycin at 30°C for 72 hours. The DNA was purified from the obtained culture solution using a GFXTM Micro Plasmid Prep Kit. When the plasmid obtained by screening was digested with *Sac* I, *Bam*H I, *Pst* I or *Eco*R I for 2 hours, all of the clones had approximately 500 bp clipped at the side of *Eco*R I site of the *Rhodococcus*-derived region. The plasmid was designated as pRET1204 (approximately 5.9 kbp). It was not possible to obtain a clone with no clipping of the genus *Rhodococcus* replication region.

[0147] The shuttle vector pRET1002 was reduced in a similar manner to obtain pRET1006 (approximately 4.9 kbp).

[0148] *R. erythropolis* was transformed with these three reduced plasmids, pRET1006, pRET1123 and pRET1204, and upon confirming the presence or absence of shuttle vector by the method described in Example 6, all the shuttle vectors were detected in the transformed cells. This suggested that the three reduced plasmids pRET1006, pRET1123 and pRET1204 are replicated in *R. erythropolis*.

[0149] (Example 10) Construction of expression vector - 3

An expression vector was constructed by having the *mak* gene inserted into the shuttle vector constructed in Example 9.

[0150] The *Pst* I site of pRET1123 constructed in Example 9 was deleted for cloning of the promoter in the single step. After digesting pRET1123 with *Pst* I for 2 hours, Blunting High was used for blunting of the ends and Ligation High was used for ligation. The solution was used to transform *E. coli* JM109 using Competent High, and culturing was performed on an LB plate containing 100 µg/mL kanamycin at 30°C for 36 hours. The formed colonies were inoculated on LB liquid medium containing 100 µg/mL kanamycin and cultured at 30°C for 24 hours, and then the DNA was purified using a GFX™ Micro Plasmid Prep Kit to obtain pRET1132.

[0151] The obtained pRET1132 was digested with *Pst* I for 1 hour and then electrophoresed on 0.8% agarose, which resulted in confirming lack of cleavage of pRET1132 by *Pst* I. As controls there were used pRET1123 and pRET1132 not digested with *Pst* I, and pRET1123 digested with *Pst* I.

[0152] (Example 11) Construction of expression vector - 4

A clone was constructed having a promoter and the *mak* gene inserted in the aforementioned shuttle vector.

[0153] A clone was constructed having a *Pst* I site upstream from the *mak* gene, for insertion of a promoter. The procedure was carried out in the following manner to obtain a clone having His-Tag added to the C-terminus of the aminoketone asymmetric reductase. PCR was conducted with KOD -plus- using the pMAK-1 obtained in Example 7

as template, MAKPstF (5'-

GACCACTGCAGATCAATCAACTCTGATGAGGTCC-3'; SEQ ID
 NO: 82) and MAKHisBglIIR (5'-

CGCTTAGATCTCAGTTCGCCGAGCGCCATCGCCG-3'; SEQ ID
 NO: 83) as primers, with an annealing temperature of 68°C and

extension reaction for 1 minute and 50 seconds. A PCR fragment
 (insert) produced by digesting the obtained PCR product with *Bgl* II at

37°C for 2 hours was ligated with pQE70 (digested with *Sph* I at 37°C
 for 2 hours, blunted with Blunting High and digested with *Bgl* II at

37°C for 2 hours) using Ligation High, and then Competent High was
 used for transformation of *E. coli* DH5α cells, which were plated on LB

agar medium containing 100 µg/mL ampicillin and allowed to stand at
 30°C for 60 hours. The appearing colonies were cultured with shaking

on LB liquid medium containing 100 µg/mL ampicillin at 30°C for 60
 hours. The DNA was purified using a GFXTM Micro Plasmid Prep Kit.

The obtained DNA was confirmed by 0.8% agarose gel electrophoresis.

[0154] For screening, the DNA without restriction endonuclease
 treatment and the DNA after digestion with *Pst* I and *Bgl* II at 37°C for

2 hours were subjected to 0.8% agarose gel electrophoresis, and the
 target plasmid was obtained based on the size of the DNA. The

plasmid obtained in this manner was designated as pMAK-2. The size
 marker used was Loading Quick DNA size Marker λ/*Eco*R I+*Hind* III

double digest, pQE70, and pQE70 that had been digested with *Bgl* II at
 37°C for 2 hours.

[0155] A clone was constructed by inserting the pRET1200 *repA*
 promoter (obtained by PCR amplification using as template a clone of

pRET1204 wherein the orientation of *repA* encoded by the

Rhodococcus-derived DNA fragment was in the same orientation as the kanamycin resistance gene encoded by pHSG299, and using as primers P1200rep-Pst5195 (5'-AGCCGCTGCAGAAGCAACACCGCATCCGCCCATTG-3'; SEQ ID NO: 84) and P7 (5'-CGCCAGGGTTTTCCCAGTCACGAC-3'; SEQ ID NO: 85), with an annealing temperature of 60°C and extension reaction for 1 minute, followed by digestion with *EcoR* I and *Pst* I at 37°C for 2 hours) at the *EcoR* I-*Pst* I site of pMAK-2 (designated as pMAK-19).

[0156] Next, PCR was conducted with KOD -plus- using as template pMAK-19 and as primers pQE70F1 (5'-GGCGTATCACGAGGCCCTTTCGTCTTCACC-3'; SEQ ID NO: 86) and pQE70R1135Bm (5'-GGTTGGATCCGTCATCACCGAAACGCGCGAGGCAG-3'; SEQ ID NO: 87), with an annealing temperature of 60°C and extension reaction for 3 minutes. The PCR product was purified from the reaction solution by using a GFXTM PCR DNA and Gel Band Purification Kit and after digestion of the purified PCR product with *EcoR* I and *BamH* I for 2 hours, it was electrophoresed on 0.8% agarose gel and the DNA fragment was purified by using a GFXTM PCR DNA and Gel Band Purification Kit. The DNA fragment was used as an insert DNA.

[0157] Separately, a vector to be used as the expression shuttle vector was obtained by digesting pRET1132 with *EcoR* I and *BamH* I for 2 hours, subjecting the DNA fragment to 0.8% agarose gel electrophoresis and purifying the DNA fragment by using a GFXTM

PCR DNA and Gel Band Purification Kit. After mixing the insert DNA and vector, Ligation High was used for ligating them and Competent High was used for transformation of *E. coli* JM109 cells, which were plated on an LB plate containing 100 µg/mL kanamycin.

5 The obtained colonies were cultured on LB liquid medium containing 100 µg/mL kanamycin, and then the plasmid DNA was recovered by using a GFXTM Micro Plasmid Prep Kit and subjected to 0.8% agarose gel electrophoresis for screening. The size markers used were Loading Quick DNA size Marker λ/*EcoR* I+*Hind* III double digest and
10 pRET1132. The obtained expression vector was designated as pRET1133.

[0158] Also, pMAK-19 was digested with *EcoR* I and *Hind* III at 37°C for 2 hours, blunted with Blunting High and subjected to 0.8% agarose gel electrophoresis, and the approximately 1.6 kbp DNA
15 fragment was purified by using a GFXTM PCR DNA and Gel Band Purification Kit. The clone having this fragment inserted at the *Hinc* II site of pRET1102 was designated as pRET1114.

[0159] The pRET1133 promoter was also modified. The *mak* gene-expressing promoter encoded in pRET1133 is the *repA* gene
20 promoter of pRET1200 and has a length of approximately 800 bp, and a plasmid was constructed by having approximately 200 bp clipped off from this promoter. The promoter used for the cloning was prepared by PCR. Plasmid pRET1200 was used as template, P1204rep-Ec2958 (5'-CGCGGAATTCGACCACCACGCACGCACACCGCAC-3'; SEQ
25 ID NO: 88) and P1200rep-Pst5195 (5'-AGCCGCTGCAGAAGCAACACCGCATCCGCCCATTG-3'; SEQ

ID NO: 84) were used as primers, and KOD -plus- was used as the PCR enzyme for PCR at an annealing temperature of 60°C and extension reaction for 50 seconds. The PCR product was purified by using a GFXTM PCR DNA and Gel Band Purification Kit, digested with the restriction endonucleases *EcoR* I and *Pst* I for 2 hours, and subjected to 1.6% agarose gel electrophoresis, and the DNA fragment was purified by using a GFXTM PCR DNA and Gel Band Purification Kit. The DNA fragment was used as the insert DNA. The nucleotide sequence of the promoter region in the DNA fragment is set forth as SEQ ID NO: 77.

[0160] Separately, for the vector, pRET1133 was digested with restriction endonucleases *EcoR* I and *Pst* I for 2 hours and subjected to 0.8% agarose gel electrophoresis, and an approximately 7.2 kbp DNA fragment was purified by using a GFXTM PCR DNA and Gel Band Purification Kit. The size marker used was Loading Quick DNA size Marker λ /*EcoR* I+*Hind* III double digest.

[0161] The insert DNA and vector obtained in this manner were ligated by using Ligation High, and Competent High was used for transformation of *E. coli* JM109 cells, which were plated on an LB plate containing 100 μ g/mL kanamycin. The obtained colonies were cultured on LB liquid medium containing 100 μ g/mL kanamycin, and then the plasmid DNA was recovered by using a GFXTM Micro Plasmid Prep Kit and subjected to 0.8% agarose gel electrophoresis for screening. The size markers used were Loading Quick DNA size Marker λ /*EcoR* I+*Hind* III double digest and pRET1133.

[0162] Also, after digesting the obtained DNA with restriction

endonucleases *EcoR*.I and *Pst* I for 2 hours, it was subjected to 1.6% agarose gel electrophoresis and a DNA fragment corresponding to the approximately 600 bp insert DNA was confirmed. The size marker used was a 100 bp DNA Ladder. The expression vector obtained in this manner was designated as pRET1138.

[0163] (Example 12) Preparation of recombinant *R. erythropolis* and measurement of enzyme activity

The aforementioned expression vectors pRET1102, pRET1104, pRET1114 and pRET1138 were used for transformation of *R. erythropolis* MAK-34 strain and *R. erythropolis* JCM2895 (provided by RIKEN Japan), and the enzyme activity was measured. The aminoketone asymmetric reductase purified from MAK-34 strain has the abilities to react with 1-2-methylamino-1-phenyl-1-propanone as described in International Patent Publication WO02/070714, and to produce d-(1S, 2S)-pseudoephedrine. It was also reacted with 1-2-dimethylaminopropiophenone, 1-amino-2-butanone, etc. and production of each corresponding β -aminoalcohol was confirmed.

[0164] The activity assay was conducted by preparing a reaction solution with a cell density O.D. = 5, 2% glucose and 0.2 M sodium phosphate buffer (pH 6.0), and 3% (1S, 2S)-2-(N-ethylamino)-1-phenyl-1-propanol (EAM) was contained in the reaction as substrate. A synthesis method for EAM is described in J. Am. Chem. Soc., Vol. 50, pp.2287-2292, 1928. The reaction solution was incubated with shaking at 30°C for 16 hours. Confirmation of (1S, 2S)-2-(N-ethylamino)-1-phenyl-1-propanol (EPE), which was β -aminoalcohol as the reaction product, was accomplished by HPLC. The column used

was an Inertsil Ph-3 3.0 × 75 mm, the eluent was aqueous 7% acetonitrile and 0.05 M sodium phosphate buffer (pH 6.0), and the detection was carried out with UV (220 nm).

[0165] The results of the activity assay carried out in this manner are shown in Table 6. The pRET1104-introduced recombinant cells lacking the exogenous promoter region exhibited about the same activity as the pRET1102-introduced recombinant cells lacking the *mak* gene used as the control, and no recombinant enzyme expression was found.

[0166] With transformation of pRET1114 into MAK-34 strain, high specific activity was found compared to pRET1104. This indicated that the pRET1200 *repA* promoter region inserted into the vector functions as a promoter.

[0167] With transformation of pRET1138, the specific activity of the recombinant *R. erythropolis* MAK-34 strain was 37.7 µg/h·mL/O.D. while the specific activity of the recombinant *R. erythropolis* JCM2895 was 34.9 µg/h·mL/O.D., and therefore expression of the enzyme in *R. erythropolis* strain was confirmed.

[0168] [Table 6]

Vector	MAK-34	JCM2895
pRET1102	1.0	1.0
pRET1104	0.7	2.0
pRET1114	17.2	not tested
pRET1138	37.7	34.9

Specific activity (units: µg/h·mL/O.D.)

[0169] (Example 13) Purification of enzyme

The recombinant cells obtained in Example 12 were cultured at

30°C for 4 days in 100 mL of LB medium containing 100 µg/mL kanamycin, the cells were harvested by centrifugation at 12,000 rpm for 5 minutes and the protein having His-tag was purified with The QIAexpressionist Kit (Qiagen). Specifically, the cells were disrupted
5 by ultrasonic treatment, the supernatant was obtained by centrifugation, and the protein was purified with a nickel chelate column. Upon applying the obtained protein to SDS-PAGE, a band of protein, which molecular weight is approximately 28,000, was observed. This molecular weight is roughly equivalent to the molecular weight of the
10 aminoketone asymmetric reductase described in International Patent Publication WO02/070714, thus indicating that the aminoketone asymmetric reductase was produced in the recombinant *Rhodococcus* strains.

[0170] (Example 14) Enzymatic production of β-aminoalcohol

15 A 0.5 mL portion of reaction solution containing the purified enzyme (0.5 µg/mL) obtained in Example 13, 5 mM NADPH, 120 mM Tris-HCl (pH 7.5) and 5 mM EAM was reacted at 37°C for 16 hours. The substrate and product (EPE) were analyzed by HPLC. The column used was an Inertsil Ph-3 3.0 × 75 mm, the eluent was aqueous 7%
20 acetonitrile and 0.05 M sodium phosphate buffer (pH 6.0), and the detection was carried out with UV (220 nm). The results confirmed production of EPE.

[0171] Similarly, the purified enzyme or the crude enzyme extract obtained from the recombinant cells cultured as described in Example
25 13 was reacted with 1-2-dimethylaminopropiophenone and 1-amino-2-butanone, etc. and production of the corresponding β-aminoalcohols

was confirmed.

Industrial Applicability

[0172] As explained above, the plasmids and shuttle vectors of the invention are derived from *Rhodococcus* strains (especially *Rhodococcus erythropolis* and *Rhodococcus rhodnii*), and when utilized them for modification of the same bacteria by recombination, they allow creation of bacterial strains that more efficiently produce aminoketone asymmetric reductases. They also permit mass production of useful enzymes including aminoketone asymmetric reductases in transformants.

CLAIMS

1. A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

5 2. A plasmid or a partial DNA fragment thereof, characterized by comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

10 3. A DNA fragment having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22.

15 4. A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22.

20 5. A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22 and comprising a DNA replication region having at least one nucleotide
25 sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID

NO: 37.

6. A DNA fragment having the nucleotide sequence set forth as SEQ ID NO: 76.

5 7. A plasmid or a partial DNA fragment thereof, characterized by comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

10 8. A plasmid or a partial DNA fragment thereof, characterized by comprising a coding region for a DNA replication-related protein having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 22, comprising a DNA replication region having at least one nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, and comprising a promoter region having the nucleotide sequence set forth as SEQ ID NO: 76.

15 9. A circular plasmid characterized by comprising a plasmid or a partial DNA fragment according to any one of claims 1 to 8, wherein the numbers of restriction endonuclease cleavage sites are *Bam*H I: 2, *Eco*R I: 2, *Kpn* I: 1, *Pvu* II: 1, *Sac* I: 1 and *Sma* I: 1, and the size is approximately 5.4 kbp.

20 10. A plasmid having the nucleotide sequence set forth as SEQ ID NO: 73.

25 11. A plasmid or a DNA fragment according to any one of claims 1 to 10, characterized by being derived from a bacterium belonging to the genus *Rhodococcus*.

12. A shuttle vector replicable in bacteria belonging to the genus *Rhodococcus* and *E. coli*, and comprising a plasmid or partial DNA fragment thereof according to any one of claims 1 to 11 and a DNA region replicable in *E. coli*.

5 13. A vector characterized by being constructed using a shuttle vector according to claim 12.

14. A vector characterized by comprising a plasmid or DNA fragment according to claim 6 or 7.

10 15. A vector according to claim 13 or 14, characterized by having inserted therein an aminoketone asymmetric reductase gene.

15 16. A vector according to claim 15, characterized in that the aminoketone asymmetric reductase gene is a nucleic acid coding for a protein consisting the amino acid sequence set forth as SEQ ID NO: 78, or a nucleic acid that codes for a protein having the amino acid sequence set forth as SEQ ID NO: 78 with a deletion, insertion, substitution or addition of one or a plurality of amino acids, and having aminoketone asymmetric reduction activity.

20 17. A vector according to claim 15, characterized in that the aminoketone asymmetric reductase gene is a nucleic acid consisting the nucleotide sequence set forth as SEQ ID NO: 79, or a nucleic acid that hybridizes with nucleic acid having a nucleotide sequence complementary to the nucleotide set forth as SEQ ID NO: 79 under stringent conditions, and that codes for a protein having aminoketone asymmetric reduction activity.

25 18. A transformant containing a vector according to claim 13 or 14.

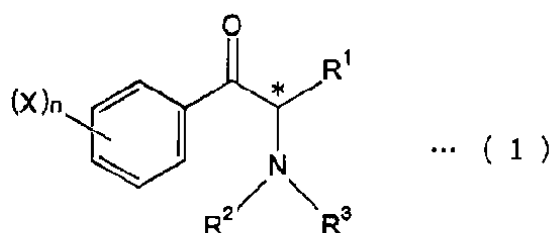
19. A transformant containing a vector according to any one of claims 15 to 17.

20. A method for production of an aminoketone asymmetric reductase, which comprises a culturing step in which a transformant according to claim 19 is cultured in medium that allows growth of said transformant, and

a purification step in which the aminoketone asymmetric reductase is purified from said transformant obtained in said culturing step.

21. A method for production of an optically active aminoalcohol, wherein an aminoketone asymmetric reductase obtained by the production method of claim 20 is reacted with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1):

[Chemical Formula 1]



wherein X may be the same or different and represents at least one species selected from the group consisting of halogen, lower alkyl, hydroxyl optionally protected with a protecting group, nitro and sulfonyl;

n represents an integer of 0 to 3;

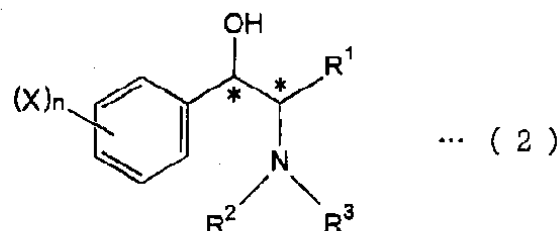
R^1 represents lower alkyl;

R^2 and R^3 may be the same or different and represent at least one species selected from the group consisting of hydrogen and lower alkyl; and

5 * represents asymmetric carbon,

or a salt thereof, to produce an optically active aminoalcohol compound represented by the following general formula (2):

[Chemical Formula 2]

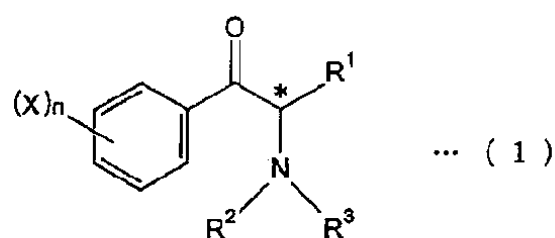


10 wherein X, n, R^1 , R^2 , R^3 and * have the same definitions as above, and having the desired optical activity.

22. A method for production of an optically active aminoalcohol, wherein a transformant according to claim 19 is reacted with an enantiomeric mixture of an α -aminoketone compound represented by the following general formula (1):

15

[Chemical Formula 3]



wherein X may be the same or different and represents at least one species selected from the group consisting of halogen, lower alkyl, hydroxyl optionally protected with a protecting group, nitro and sulfonyl;

n represents an integer of 0 to 3;

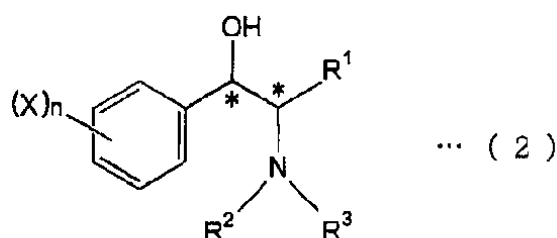
R¹ represents lower alkyl;

R² and R³ may be the same or different and represent at least one species selected from the group consisting of hydrogen and lower alkyl; and

* represents asymmetric carbon,

or a salt thereof, to produce an optically active aminoalcohol compound represented by the following general formula (2):

[Chemical Formula 4]

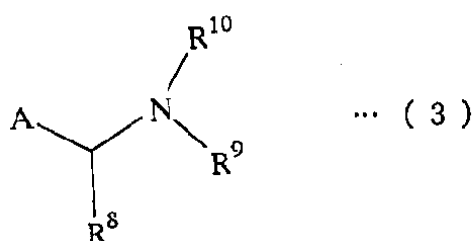


wherein X, n, R¹, R², R³ and * have the same definitions as above,

and having the desired optical activity.

23. A production method for an optically active aminoalcohol according to claim 22, wherein the production method for the optically active aminoalcohol is carried out with further addition of a compound represented by the following general formula (3):

[Chemical Formula 5]



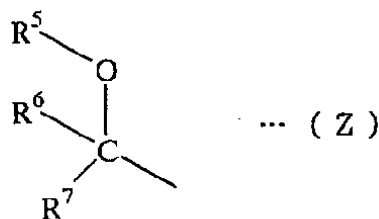
wherein A represents the following formula (Y) or (Z):

[Chemical Formula 6]



wherein R^4 represents hydrogen, optionally substituted C1-3 alkyl, a C5-10 hydrocarbon ring which is bonded to R^8 or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^8 ,

[Chemical Formula 7]



wherein R^5 represents hydrogen, C1-3 alkyl or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^6 or R^9 ;

5 R^6 represents hydrogen, optionally substituted C1-3 alkyl, a C5-10 hydrocarbon ring which is bonded to R^8 or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^5 or R^9 ;

R^7 represents hydrogen or optionally substituted C1-6 alkyl;

10 R^8 represents hydrogen, carboxyl, optionally substituted C1-6 alkyl, a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^4 or a C5-10 hydrocarbon ring which is bonded to R^6 ;

15 R^9 represents hydrogen, optionally substituted C1-6 alkyl, optionally substituted C1-6 alkyloxycarbonyl, optionally substituted acyl or a 5- to 8-membered heterocyclic skeleton containing 1-3 heteroatoms which is bonded to R^5 or R^6 ; and

20 R^{10} represents hydrogen or optionally substituted C1-6 alkyl, or a pharmaceutically acceptable salt or solvate thereof, for production of an optically active aminoalcohol.

SEQUENCE LISTING

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<120> Novel plasmids and their uses

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 ttgoggggg tagccgcca ggggacgtg totgacgat cagcgaagcg gactggcag 120
 ggcggcagc gcctgggtt gttacatct gccacaactg tccgtgaatc tctgacagct 180
 cctgaaacgc ctggtcaggg ccttgcggaa tccgtgacgc ctgatgattt ttggtctcat 240
 tcgttcccc gcgtgacga tgtacgggc gcagctgctt ctttccagto ggtggotaa 300
 tgggatgggc gtgagggtcc gaggcgcgt ttctgtgtc gcctggcgt tgtccgctt 360
 gaggtttgtg atctcgacgc ccgcgaacga acgctgaac gtgcgtatct ggtgctcgg 420
 gctcgggtgg atatggcggc tgccaggcat aaotgccgt acgacttoga cgtggaagat 480
 gaagagtgg cggaaactgc ttctctgcaa ggctcgagg acgacgacat tgggggctgg 540
 tctcgggaga gggaaatagt gggctggtc gtctgttctc ggtcacggat gatcttgcga 600
 atggcagaac tgcactgggc tccatgatg gatttgcgg gcatttctgc gatggtgacc 660
 ctcacctatc cgggggactg gttacggtt gccccaccg gcgtgaggt caaaaaacat 720
 ctccagacgt tcttcaaacg gttcaacgg gcctggggca ttgootggat ggggtgcgtg 780
 aaaatggagt tccaaagcgc aggcgctccg cattttcacc tgtacatggt cctctctcat 840
 gggaaaggcag gagactogcg gaagctcgg catgatgctg agctcttgaa atgggagata 900
 gcacgtgcag aggtgaaga ccaggtcgc aggcgtatt tccgggaagc tocaagcgat 960
 ggattgaagt ttgtccgtg gttttctgc gtgtggccg acgtcgtaga tcatccggac 1020
 cccaaggaaa aagaaaagca cgtcagtgcc ggcactggag tggactacgc ggagggcacg 1080
 cgagggtcag atccgaaaag gcttgcgggt tactttacca agcatggaac ctttgcgcac 1140
 aaggaaatato agcaogtagt tctgtctcaa tggcagaaaa cgggtgcggg acctggcagg 1200

```

ttotgggget acogcggttt gtgcgcggcc acggtgccca cagagatttc ctgggatgag 1260
tacctgottt tatctcgac gttggaaga ttgtcagcgc gaacgaagat ctgggaccgc 1320
gttttacgag gcggtagcgc cggccacaga tggactaagg cgatgatgcg acgcacggtt 1380
accoggcacc gcttgacct cgtgaccggt gagattctgg gcacgaagac ggggaaggtt 1440
cgggcgccag tgaagaggtt tgtccggaat togggatacc tglgtgtaaa tgacgggccc 1500
gcactggctc gaacctcag cgtcttcgt acaagctgcc tgagctag 1548

```

```

<210> 6
<211> 1116
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 1294bp to 2409bp pRET1100

```

```

<400> 6
atggcggtg ccagggataa ctgcgcgtac gacttcgacg tggacgatga agagttggcg 60
gaaotggott ctctgaagg octcgaggac gacgacattg gggcctggto tgcggagagg 120
gaaatagtgg gctggtctgc tcttctcgg tcacggatga tcttgcaat ggcagaacto 180
gactgggctc ccatgatgga ttgcccggc attctcgga tggtagacct cacttatccg 240
ggggaotggc ttacggttgc cccacccggc gctgaggta aaaaacatct ccagacgttc 300
ttcaaacggt tcaaacgggc ctggggcatt gcctggatgg gtgctggaa aatggagtto 360
caaagccgag gcgtccgca ttttcacctg tacatggtcc ctctcatgg gaaggcagga 420
gactcgcgga agctcgggca tgatgctgag ctcttgaat gggagatago aogtgcagag 480
ggtgaagacc caggtcgcag gccgtatttc cgggaagctc caagcgatgg attgaagttt 540
ogtoogtggc tttctcggtt gtgggcgcac gtcttagatc atccggaccc caaggaaaaa 600
gaaaagcaog tcagtgoogg cactggagtg gactacgagg agggcacgcg agggtcagat 660
ccgaaaaggc ttgcggtgta cttctccaag catggaacct ttgcgcgcaa ggaatatcag 720
cacgtagtto ctgctcaatg gcagaaaaog ggtgogggac otggcaggtt ctggggctac 780
cgcggtttgt cgcggccac ggtgcgcacc gagatttctt gggatgagta cctgotttta 840
totcgcaagt tgcgacgatt gtcagcgcga acgaagatct gggacccggc tttacgaggo 900
ggtagcggcg gccacagatg gactaaggcg atgatgcgac gcacggttac ccggcaccgc 960
ttggacctcg tgacoggtga gattctgggc acgaagaogc ggaaggttcg ggcgcagtg 1020
aagaggtttg tccggaactt gggataaotg tgtgtaaat acgggcccgc actggctcga 1080
acctcagcc gtcttctgac aagctgctg agctag 1116

```

```

<210> 7
<211> 960
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 1450bp to 2409bp pRET1100

```

```

<400> 7
atgatcttgc gaatggcaga actcgactgg gtcccatga tggatttgc gggoatttct 60

```

```

gcgatggtga cctcaccta tccgggggac tggcttacgg ttgccccoac oggogotgag 120
gtcaaaaaac atctccagac gttcttcaaa cggttccaac gggcctgggg cattgcotgg 180
atgggtggtt ggaatatgga gttccaaagc agaggcgctc cgcattttca cctgtacatg 240
gtccctcctc atgggaaggc aggagactcg cggaaagtgo ggoatgatgc tgagctottg 300
aaatgggaga tagcacgtgc agagggtgaa gaccaggtgc gcaggccgta ttccgggaa 360
gtccaagcg atggattgaa gttctgtcgg tggottttotg oggtgtgggc cgacgtctga 420
gatcatccgg accccaagga aaaagaaaag cactcagtg ccggcactgg agtggactac 480
gcggagggca cgcgagggtc agatccgaaa aggtttgcgg tgtacttctc caagcatgga 540
acctttgcgg acaaggaata tcagcacgta gttcctgtct aatggcagaa aaogggtgog 600
ggacctggca ggttctgggg ctaccgcggt ttgtcgccgg ccacggctgc caccgagatt 660
toctgggatg agtacctgct tttatctcgc acgttgcgac gattgtcagc gcgaacgaag 720
atctgggacc oggtttacg aggcggtagc ggcgccaca gatggactaa ggcgatgatg 780
cgacgcacgg ttaccggca ccgcttggac ctctgacgc gtgagattct ggccacgaag 840
acgcggaagg ttoggcgcc agtgaagagg tttgtoggga cttcgggata cctgtgtgtc 900
aatgacgggc ccgactggc tgaaccctc agccgtcttc gtacaagctg cctgagctag 960

```

```

<210> 8
<211> 948
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1462bp to 2409bp pRET1100

```

```

<400> 8
atggcagaac tcgactgggc tcccatgatg gatttgcgg gcattcctgc gatggtgacc 60
ctcacctatc cgggggactg gottacggtt gccccacgc gcgctgaggt caaaaaacat 120
ctccagacgt tottcaaacg gttccaacgg gcctggggca ttgcctggat ggggtcgtgg 180
aaaatggagt tccaaagcg aggcgctcgg cattttcacc tgtacatggt cccicctcat 240
gggaaggcag gagactcgc gaagctcgg catgatgtgc agctcttgaa atgggagata 300
goacgtgcag aggggtgaaga ccaggtcgc aggcgtatt tccgggaago tocaagogat 360
ggattgaagt ttgtccgtg gttttctcgc gtgtgggcgc acgtcgtaga tcacccggac 420
cccaaggaaa aagaaaagca cgtcagtgcc ggcactggag tggactacgc ggagggcacg 480
cgagggtcag atccgaaaag gtttgcggtg tacttctcca agcatggaac ctttgcgcac 540
aaggaaatato agcacgtagt tctgtctcaa tggcagaaaa cgggtgcggg acctggcagg 600
ttotggggot acocgggtt gtogcggcc aoggtgcaa cagagatttc ctgggatgag 660
tacctgcttt tatctcgac gttgcgacga ttgtcagcgc gaacgaagat ctgggacccg 720
gctttacgag gcggtagcgg cggccacaga tggactaagg cgatgatgcg acgcacggtt 780
accggcacc gottggacct cgtgacgggt gagattctgg gcacgaagac gcggaagggt 840
cggcgccag tgaagaggtt tgcgggact tcgggatacc tgtgtgtcaa tgacgggccc 900
goactggcto gaaccotoag cgttcttgtt acaagctgcc tgagctag 948

```

<210> 9
 <211> 924
 <212> DNA
 <213> *Rhodococcus erythropolis*

<220>
 <221> misc_feature
 <223> 1486bp to 2409bp pRET1100

<400> 9
 atgatggatt tgcggggcat tctgcatg gtgaacctoa cctatccggg ggaotggott 60
 acggttgccc ccacggggc tgaggtcaaa aaacatctcc agacgttctt caaacggttc 120
 caacggggcct ggggcattgc ctggatgggt gcgtggaaaa tggagttcca aagccgaggc 180
 gctccgcatt ttcacctgta catggtccct cctcatggga aggcaggaga ctccggaag 240
 ctgcccgatg atgctgagct cttgaaatgg gagatagcac gtgcagaggg tgaagacca 300
 ggtccgaggc cgtatttccg ggaagctoa agcatggat tgaagtttg tccgtggctt 360
 totgcggtgt gggcggacgt cgtagatcat ccggaccoca agaaaaaga aaagcacgtc 420
 agtgcgggca ctggagtga ctacggcgag ggcaogcgag ggtcagatcc gaaaaggctt 480
 gcggtgtact toccaagca tggaaacctt gcgcacaagg aatatacga cgtagttcct 540
 gctcaatggc agaaaacggg tgcgggaact ggcaggttct ggggctaccg cgtttgtcg 600
 ooggocagg ctgcaccca gatttcttg gatgagtaac tgcctttatc tcgcacgttg 660
 cgacgattgt cagcggaac gaagatctgg gaccgggtt tacgaggcgg tagcggcggc 720
 cacagatgga ctaaggcgat gatgcgagc accgttacc ggcaocgtt ggacctogtg 780
 accggtgaga ttctgggac gaagacggg aaggttcggg cgcagtgaa gaggtttgto 840
 cggacttgg gatactgtg tctaatgac gggccgcac tggctogaac cctcagccgt 900
 ottogtaaa gtgcctgag ctac 924

<210> 10
 <211> 921
 <212> DNA
 <213> *Rhodococcus erythropolis*

<220>
 <221> misc_feature
 <223> 1489bp to 2409bp pRET1100

<400> 10
 atggatttgc cgggcattcc tgcatgggtg aacctaacct atccggggga ctggcttacg 60
 gttgccccca cggcgctga ggtaaaaaa catctcaga cgttcttcaa acggttccaa 120
 cgggcctggg gcattgcctg gatgggtgog tggaaatgg agttccaaag ccgaggcgct 180
 ccgcattttc acctgtacat ggtccctcct catgggaagg caggagactc gcggaagctg 240
 cggcatgatg ctgagctctt gaaatgggag atagcacgtg cagagggtga agaccaggt 300
 cgcaggccgt atttccggga agtccaagc gatggattga agttcgtcc gtggctttct 360
 gcggtgtggg ccgacgtcgt agatcatccg gacccaagg aaaaagaaaa goacgtcagt 420
 gccggcactg gattggacta ccgggagggc acccgagggt cagatccgaa aaggcttgcg 480
 gtgtacttct ccaagcatgg aacctttgoc gacaaggaat atcagcacgt agttcctgct 540
 caatggcaga aaacgggtgc gggacctggc aggttctggg gtaccgcgg tttgtcccg 600

```

gccacggctg ccaccgagat ttotgggat gagtaoctgo ttttatctcg cacgttgca 660
cgattgtcag cggaacgaa gatctgggac cggctttac gaggcggtag cggcggccac 720
agatggacta aggogatgat gcgacgcag gttaccggc accgcttga cctcgtgacc 780
ggtgagattc tgggcacgaa gacgggaag gttcgggogo cagtgaagag gtttgtccg 840
acttcgggat acctgtgtgt caatgacggg cccgcactgg ctogaacct cagccgtott 900
cgtacaagct gootgagota g 921

```

```

<210> 11
<211> 897
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 1513bp to 2409bp pRET1100

```

```

<400> 11
atggtgaccc tcacctatcc gggggactgg cttaacggtt cccccacgg cgctgaggtc 60
aaaaaacatc tccagacgtt otcaaaagg ttcaaaggg cctggggcat tgootggatg 120
ggtgcgtgga aaatggagtt ccaaagcga ggccgtccgc attttcacct gtacatggtc 180
cctcctcatg ggaaggcagg agactcggg aagctcggc atgatgtga gctottgaaa 240
tgggagatag cacgtgcaga ggtgaagac ccaggtcgca ggccgtattt cggggaagot 300
ccaagcgatg gattgaagtt togtcgtgg cttctcggg tgtggccga cgtcgtatg 360
catcgggacc ccaaggaaaa agaaaagcac gtcagtccg gcactggagt ggactacgcg 420
gagggcacgc gaggtcaga tccgaaaagg cttcgggtgt acttctccaa gcatggaacc 480
tttgccgaca aggaatatca gcaogtagtt cctgtcaat ggcagaaaac ggtgcccga 540
cctggcaggt tctggggcta ccgcggttg togcgggca cggctgccac cgagatttc 600
tgggatgagt acctgctttt atctcgacg ttgcgacgat tgcagcgcg aacgaagatc 660
tgggacccgg ctttacgagg cggtagcggc ggccacagat ggactaaggc gatgatgca 720
cgcacggtta cccggcaccg cttggacctc gtagccgtg agattctgg cacgaagac 780
cggaaaggtt cggcggcagt gaagaggttt gtcggactt cgggatacct gigtgtcaat 840
gacgggcccg cactggctcg aacctcago cgtcttogta caagctgcct gagctag 897

```

```

<210> 12
<211> 780
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 1630bp to 2409bp pRET1100

```

```

<400> 12
atgggtgcgt ggaaaatgga gttccaaagc agaggcgtc cgcattttca cctgtacatg 60
gtcctctctc atgggaaggc aggagactcg cggaagctgc ggcattgatc tgagctcttg 120
aaatgggaga tagcactgc agagggtgaa gaccacagtc gcaggccgta tttcgggaa 180
gtcccaagcg atggattgaa gtttgttcg tggctttctg cgggtgtggc cgcactgta 240

```

```

gatcatccgg accccaagga aaaagaaaag caogtoagt cgggcactgg agtggactac 300
goggagggca gcgaggggtc agatcogaaa aggtttgogg tgtacttoto caagcatgga 360
acctttgcog acaaggaata tcagcacgta gttcctgtct aatggcagaa aacgggtgcg 420
ggacctggca ggttctgggg ctaccgggtt ttgtgccgg ccacggctgc caccagatt 480
tcctgggatg agtacctgct tttatctcgc acgttgcgac gattgtcago gogaacgaag 540
atctgggao oegctttacg agggcgtagc ggogggccaca gatggactaa ggcgatgat 600
ogacgcacgg tiaccoggca ccgcttgac ctctlgacg gtgagattct gggcacgaag 660
acgcggaagg ttccggcgcc agtgaagagg tttgtccga cttcgggata cctgtgtgtc 720
aatgacgggc ccgcactggc tcgaacctc agccgtcttc gtacaagtg ootgagctag 780

```

```

<210> 13
<211> 765
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1645bp to 2409bp pRET1100

```

```

<400> 13
atggagttcc aaagocgagg ogctcgcac tttcacctgt acatggctcc tctcatggg 60
aaggcaggag actcgcggaa gctgggcat gatgotgago tottgaaatg ggagatagca 120
ogtgcagagg gtgaagacc aggtcgcagg ccgtatttc ggaagctcc aagcatgga 180
ttgaagtctt gtcctgggt ttctgggtg tggcgacg tcgtagatca tccggacccc 240
aagaaaaag aaaagcacgt cagtgcggc actggagtgg actacggga gggcacgga 300
gggtcagatc cgaagggct tgcgtgtac ttctccaagc atggaacctt tgcgacaag 360
gaatacagc acgtagtcc tgcctaatg cagaaaacgg gtgogggacc tggcaggttc 420
tgggctacc gcggtttgt gcgggccac gctgccacg agatttootg ggatgagtag 480
ctgcttttat ctgcacgtt gcacgatig tcagcgcaa cgaagatctg ggaccggct 540
ttacgaggcg gtagcgggg ccacagatg actaaggga tgatgcgac caoggttacc 600
cggcacctgt tggacctgt gacgggtgag attctggga cgaagacgc gaaggttcgg 660
gcgcagtg agaggtttgt ccggacttgg gataacctgt gtgtcaatga cgggcccga 720
ctggctcgaa ccctcagccg tcttcgtaca agctgcctga gotag 765

```

```

<210> 14
<211> 723
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1687bp to 2409bp pRET1100

```

```

<400> 14
atggtccctc ctcatgggaa ggcaggagac tocggaago tgcggcatga tgotgagctc 60
ttgaaatggg agatagcacg tgcagagggt gaagaccag gtgcaggcc gtatttcgg 120
gaagctocaa gcgatggatt gaagtctgt ccgtggcttt ctgcggtgtg ggcgacgtc 180
gtagatcatc cggaccccaa ggaagaaag aagcacgtca gtccggcac tggagtggac 240

```

```

tacggcggagg gcacgcgagg gtcagatccg aaaaggcttg cgggtgtactt ctccaagcat 300
ggaacctttg ccgacaagga atatcagcac gtagttcctg ctcaatggca gaaaacgggt 360
gcgggacctg gcaggttctg gggctacccg ggtttgtcgc oggccaoggo tgcacocag 420
atttcctggg atgagtaacct gcttttatct cgcacgttgc gacgattgic agcgcgaacg 480
aagatctggg acccggtttt acgaggcggt agcggcgccc acagatggac taaggcgatg 540
atgcgaogca cggttaccgc gcacgccttg gacctctga ccggtgagat totgggcacg 600
aagacogga agtttcgggc gccagtgaag aggtttgtcc ggacttcggg atacctgtgt 660
gtcaatgacg ggcccgcaact ggtcgaacc ctcagccgto ttctacaag ctgcctgagc 720
tag 723

```

```

<210> 15
<211> 186
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 2224bp to 2409bp pRET1100

```

```

<400> 15
atgatgcgac gcacggttac ccggcacccg tiggacctcg tgaccggtga gattctgggc 60
acgaagacgc ggaaggttcg ggcgccagtg aagaggtttg tccggacttc gggataacctg 120
tgtgtcaatg acgggcccgc actggctoga accctcagcc gtctctgtac aagctgcctg 180
agctag 186

```

```

<210> 16
<211> 183
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 2227bp to 2409bp pRET1100

```

```

<400> 16
atgcgaogca cggttaccgc gcacgccttg gacctctga ccggtgagat totgggcacg 60
aagacogga agtttcgggc gccagtgaag aggtttgtcc ggacttcggg atacctgtgt 120
gtcaatgacg ggcccgcaact ggtcgaacc ctcagccgto ttctacaag ctgcctgagc 180
tag 183

```

```

<210> 17
<211> 432
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 1875bp to 1444bp pRET1100

```

```

<400> 17
atgatctacg acgtcggccc acaccgcaga aagccaogga cgaaacttca atccatcgct 60
tggagcttcc cggaaatacg gcctgcgacc tgggtcttca ccctctgcac gtgotatoto 120

```

```

ccatttcaag agctcagcat catgccgcag ctccgcgcag tctctgcoct tcccatgagg 180
agggaccatg tacagggtgaa aatgcggago gcctgggott tggaactcca tttccacgc 240
acccatccag gcaatgcccc aggcgcgttg gaaccgtttg aagaacgtct ggagatgttt 300
tttgacctca gcgcgcgttg gggcaaccgt aagccagtc cccgatagg tgagggtcac 360
catgcagga atgcccggca aatccatcat gggagcccag tcgagttctg ccattcgcaa 420
gatcatccgt ga 432

```

```

<210> 18
<211> 291
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1734bp to 1444bp pRET1100

```

```

<400> 18
atgccgcagc ttccgcgcag ctctgcoct cccatgagga gggaccatgt acagggtgaa 60
atgcggagcg cctcggtttt ggaactccat ttccacgc cccatccagg caatgcccc 120
ggcccggttg aaccgtttga agaacgtctg gagatgtttt ttgacctcag gcgcgcgtgg 180
ggcaaccgta agccagtc cccgatagg gaggtcac atgcaggaa tgcccgcaa 240
atccatcatg ggagcccagt cgagttctgc cattcgcaag atcatccgtg a 291

```

```

<210> 19
<211> 258
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1701bp to 1444bp pRET1100

```

```

<400> 19
atgaggagg accatgtaca ggtgaaaatg cggagcgcoct cggotttga actccatttt 60
ccacgcaccc atccaggcaa tgcccaggc cgttggaac cgtttgaaga aogtotggag 120
atgttttttg acctcagcgc cgttgggggo aaccgtaagc cagtccccg gatagggtgag 180
ggtcaccatc gcaggaatgc cggcaaatc catcatggga gccagtcga gttotgcat 240
tcgcaagatc atccgtga 258

```

```

<210> 20
<211> 231
<212> DNA
<213> Rhodococcus erythropolis

<220>
<221> misc_feature
<223> 1674bp to 1444bp pRET1100

```

```

<400> 20
atgcggagcg cctcggtttt ggaactccat ttccacgc cccatccagg caatgcccc 60
ggcccggttg aaccgtttga agaacgtctg gagatgtttt ttgacctcag gcgcgcgtgg 120
ggcaaccgta agccagtc cccgatagg gaggtcac atgcaggaa tgcccgcaa 180
atccatcatg ggagcccagt cgagttctgc cattcgcaag atcatccgtg a 231

```

<210> 21
 <211> 138
 <212> DNA
 <213> *Rhodococcus erythropolis*

<220>
 <221> misc_feature
 <223> 1581bp to 1444bp pRET1100

<400> 21
 atgttttttg acctcagcgc cgggtgggggc aaccgtaagc cagtcccccg gatagggtgag 60
 ggtcaccatc gcaggaatgc cgggcaaacc catcatggga gccagtcga gttotgcoat 120
 tcgcaagatc atcgtga 138

<210> 22
 <211> 423
 <212> DNA
 <213> *Rhodococcus erythropolis*

<220>
 <221> misc_feature
 <223> 2828bp to 2406bp pRET1100

<400> 22
 atggtgggag ggcaaacctc ccaatagctc tcagttatga atgaagacag agacaacato 60
 atcgccaggt tccgctcga aatgctcgc tcaatcagg atgcaattca tttagoogca 120
 ctctccgca acgacgaaaa cgttatgcc gcaacagaag acaatcgacc cgtgcggaca 180
 caactatcgc aacaacagca gttgtcctg accgagctga cattggcga ccacatggaa 240
 aagctcgcgc gggagcacct cgtttacct gcgacagag cgcgggagat gaattgcacc 300
 tgggtagaga taggtcagtc gttgggtctc tctcccacg gagcgcagca ggcacacacc 360
 agaagccgac caaaacccgc catccagca aagacaaagc cgaaggcgt tccgctcgtc 420
 tag 423

<210> 23
 <211> 387
 <212> DNA
 <213> *Rhodococcus erythropolis*

<220>
 <221> misc_feature
 <223> 2792bp to 2406bp pRET1100

<400> 23
 atgaatgaag acagagacaa catcatcgcc aggttcgcgc tcgaaatgct ccgctcaato 60
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 gaagacaato gaccctgtgc gacacaaacta togaacaac agcaggttgt cctgaccgag 180
 ctgacattgg ccgaccacat ggaaaagctc gcgcgggagc acctogttta cctagccgac 240
 agagcgcggg agatgaattg caoctgggta gagataggtc agtcgttggg tctctctccc 300
 caccgagcgc agcagcgcat caccagaagc cgcacaaaac ccgcatcca gcaaaagaca 360
 aagccgaaag gcgttcgcgc cgtctag 387

<210> 24

<211> 342
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 2747bp to 2406bp pRET1100

<400> 24
 atgctccgct caatcgagga tgcaattcat ttagccgcac tctccgcgaa cgacgaaaac 60
 cgttatgccg caacagaaga caatcgaccc gtgoggaoao aaotatogca acaacagcag 120
 gttgtcctga ccgagctgac attggcgcac cacatggaaa agctcgcgcg ggagcacctc 180
 gtttacctag ccgacagagc gggggagatg aattgcacct gggtagagat aggtcagtcg 240
 ttgggtctct ctcgccacgg agcgcagcag cgcacaccca gaagccgcc cccaaacccgc 300
 atccagcaaa agacaaagcc gaaaggcgtt ccgcgcgtct ag 342

<210> 25
 <211> 189
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 2594bp to 2406bp pRET1100

<400> 25
 atggaaaagc tcgcgcggga gcacctcgtt tacctagccg acagagcgcg ggagatgaat 60
 tgcacctggg tagagatagg tcagtctgtg ggtctctctc cccacggagc gcagcagcgc 120
 atcaccagaa gcgcgccaaa acccgccatc cagcaaaaga caaagccgaa aggogttoog 180
 cgcgtctag 189

<210> 26
 <211> 135
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 2540bp to 2406bp pRET1100

<400> 26
 atgaattgca cctgggtaga gataggtcag tcgttgggtc tctctcccca cggagcgcag 60
 cagcgcacga ccagaagccg cccaaaaacc gccatccagc aaaagacaaa gcogaaaggc 120
 gttccgcgcg tctag 135

<210> 27
 <211> 336
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 2971bp to 3306bp pRET1100

<400> 27
 atggcttga aagctgctgg caacgtgatt cctgattcct ccgcgtacga gtaccgggcg 60
 gttcaggctg agccgaagat ggicagaaaa gaccgggaag acccgaaactc tgagcagttc 120

cagaagcaga aggaacggcac gccggtgtgg togatcgaot goattcgggt cgaacgggca 180
 tcaggcaaca aggaatcgt gaccgtgacg gtccggacg tgatggaacc ggatgttgcg 240
 gggccggtgg agttotooga gatgattgco ggtttctggg ttctgcgcag tggttcgggc 300
 atgtggtttt cggcaagcgc cgtcgtttct ctctga 336

<210> 28
 <211> 258
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 3049bp to 3306bp pRET1100

<400> 28
 atggtcagaa aagaccggga agaccgaa totgagcagt tccagaagca gaaggacggc 60
 accgccgtgt ggtcagatga ctgcattcgg gtccacggg caccaggcaa caaggcaatc 120
 gtgacctga cggttccgga cgtgatgaa ccggtgttg cggggccggt ggagttctcc 180
 gagatattg ccggtttctg ggtttccgc agtggttcgg goatgtggtt ttccgcaagc 240
 gccgtcgtt ctctctga 258

<210> 29
 <211> 525
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 3577bp to 3053bp pRET1100

<400> 29
 atgtcagatgt actgcctcc gctgaacggc ccagctctt ccgagagag aacgaggcac 60
 ccggcaacgt ccgagaacac cccgttttcc caattcggat cggccggcac tctcagcggc 120
 acagcttcgg actgtgaac atoactgaac acgttcgccg ctgccaacc tgcgcaacc 180
 agcacaacaa cgagcacgag ggcaccaca ccagcgcaa cgccttttcc ttggacatt 240
 tccgaacctt tcgaggggag acgatcagc atcagagaga agcagcggcg ctgcccga 300
 accacatgcc cgaaccactg cgcgaaccc agaaaccggc aatcatctcg gagaactcca 360
 ccggccccc aacatccgt tccatcagc ccggaaccgt caccgtcagc attgccttgt 420
 tgcctgatgc ccggtcagc cgaatgcagt cgtcagaca caccggcgtg ccgtccttct 480
 gttctggaa ctgctcagag ttgggtctt ccgggtctt tctga 525

<210> 30
 <211> 519
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 3571bp to 3053bp pRET1100

<400> 30
 atgtactgcc ctccgtgaa cggcccccagc tcttcggag agagaacgag gcacccggca 60

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acgtccgaga acaccccggt ttcccacttc ggcctggccg gcactctcag cggcaacagct 120
toggactgtg aacgatoact gaacacgttc gccgcttgcc aacctgccgc aaccagcaca 180
aacacgagca cgagggcacc cacaccagc gcaacgcctt ttcctttgga catttcgaa 240
ccttccgagg ggcgacgato agcgatcaga gagaagcgac ggcgcttgcc gaaaaccaca 300
tgcccgaaac actgcgcgaa acccagaaac cggcaatcat ctccgagaaac tccaccggcc 360
ccgcaacata cggttccata acgtccgaa cgcacacgtt caccattgcc ttgttgctg 420
atgcccggtc gaccogaatg cagtcgatcg accacacggc cgtgccgtcc ttctgttct 480
ggaactgctc agagttcggg tcttcgggt cttttctga 519

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<210> 31
<211> 564
<212> DNA
<213> Rhodococcus erythropolis

```

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<220>
<221> misc_feature
<223> 3339bp to 3902bp pRET1100

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<400> 31
atgtccaaag gaaaaggcgt tgcgctgggt gtgggtgccg tcgtgctcgt gtttgtgctg 60
gttgccgagc gtggcgaagc ggcgaacgtg ttcagtgcac gttcacagtc cgaagctgtg 120
ccgtcgagag tgcggcgcca tccgaagtgg gaaaacgggg tgtctctgga cgttgccggg 180
tgctcgttcc tctctccgga agagctgggg ccgttcagcg gagggcagta catcgacata 240
gtgaggccag ttgagccgga gaggttggag ccgcactggg tgaggtcgcc tgagtgcgtt 300
tggcgctcga tgaatgtctc tgacctgttg gtttctgctc ttccagagtc caccogtccc 360
cccggcgatt tcgttcgttc gtggaaagtg gcgagtgatg attactgcta tgagggtgat 420
aaccgcgaag gcctgcactc tcgtatgcgc gtttgggtct ctgcaaaaaa ctggtggtgc 480
acagaacccg tactcgatcc gctcgttcgt cgtgtgagg tctttcctgc aaggcaaata 540
gttgtgcggg aagggttttc gtga 564

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```

<210> 32
<211> 255
<212> DNA
<213> Rhodococcus erythropolis

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```

<220>
<221> misc_feature
<223> 3648bp to 3902bp pRET1100

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<400> 32
atgaatgtct ctgacctgtt ggtttctgct cttccagagt ccaccogtcc ccccgcgat 60
ttcgttcgtt cgtggaaagt ggcgagtgat gattactgot atgagggtga taaccgcaa 120
ggctgcactt ctgctatgcc ggtttgggtc totgcaaaaa actggtggtg cacagaaccc 180
gtactcgata cgtcgttcg tcgctgtgag gtctttctc caaggcaaat cgttgtgccg 240
gaagggttt cgtga 255

```

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<210> 33
<211> 669
<212> DNA
<213> Rhodococcus erythropolis

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<220>
 <221> misc_feature
 <223> 4366bp to 5034bp pRET1100

<400> 33
 atgggcaccc caagcccaag taaccgctgg tgcgctggat atttcggcgg tggctctgtg 60
 agcggggaga agcggcacag cgaggccggc ccggtagaaa tcatttttt gatgctggca 120
 gtcaggcggg gggactacat cgtogcgtg actgoggto togoggtogg gttottogog 180
 gtgcgggttg agggtttttg gttcttggtc gtgcagtcg tcgctgcacc ggcgtggttg 240
 tttctgcgcg actgggaatc gaagcggagg gccgtacggg tctttgaacg ggcattggaag 300
 gggacacctg aatcccccg tattgtcttc tcccttgcc tctgaacgt ggcggggtct 360
 ctgcggaggt tgaggaggt tgaactggt tcggggatac gcacactcgt gtttttttg 420
 cggccgggag tcaactgcga gagtttgag aaagttagc ctgcgtgga agacgcgatg 480
 gggggtcacc gtcgcaagt agagaaggc gcccccgac aggtccggt cagagtatt 540
 gatgaggatt cgtgaagac gccgctgat ggggatggg cgaagatgt tctgtggaa 600
 gaggatacgt tcgacggtc tccggcgag acgcatcct ggttcgagca agaggggccc 660
 gcatcatga 669

<210> 34
 <211> 558
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 4477bp to 5034bp pRET1100

<400> 34
 atgctggcag tcaggcggg ggaactacac gtgcgctga ctgcggttct cgcggtcggg 60
 ttcttcggc tcgcggttga ggtttctg ttctgtgtog tcgcagtcac cgtgcaccg 120
 gcgtggtggt ttctgcgga ctgggaatog aagcggagg ccgtacgggt ctttgaacgg 180
 gcatggaagg ggaacctga atccccggg attgtctct ccttgccct gtogaacgtg 240
 ggggggtctc tgccgaggt gaggaagtt gaaactggt cggggatac cactctgtg 300
 tttttttgc gccccggagt cactgcgag agctttgaga aagttcgcc tgogctggca 360
 gacgcgatgg ggggtcacc ctgccaagta gagaagggt cccccggaca ggtccgctc 420
 agagtattg atgaggatc gatgaagac ccgctgatg cgggatggc gaaagatgt 480
 gtgctggaag aggatacgt cgaaggctt cggggcgaga cggatcctg gttcgagaa 540
 gaggggcgg catcatga 558

<210> 35
 <211> 791
 <212> DNA
 <213> Rhodococcus erythropolis

<220>
 <221> misc_feature
 <223> 2410bp to 3200bp pRET1100

<400> 35

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aogogoggaa ogootttogg ottttgtcttt tgotggatgg cgggttttgg goggettctg 60
gtgatgcgct gctgogctcc gtggggagag agacccaacg actgacctat ctctaccocag 120
gtgcaattca tctcccgccg tctgtoggct aggtaaacga ggtgctcccg cggagcttt 180
tccatgtggt cggccaatgt cagctoggto agggacaacct gctgttgttg cgatagtgtg 240
gtccgcacgg gtgatitgtc ttctgttgcg gcataacggt tttcgtcggt cggcgagagt 300
goggetaaat gaattgcctc ctgatitgag cggagcattt cgaacoggaa cctggcgatg 360
atgttgtoto tgtcttcatt cataactgaa gcgtattggg agtgttgccc toccaacctg 420
tgtgccaatg cagggtgtaa ctgagtcaca gtttctcaat agactccaag ttgtgatcc 480
ttttactccc aaaatggggc atgatgtgtg cgtgcctogg ttcagggggc aaagtctgac 540
acctcgaaag aaggcctcga catggctttg aaagctgctg gcaacgtgat tctgtattcc 600
tccggtacg agtacogggc gtttcaggto gagccgaaga tggtcagaaa agaccoggaa 660
gaccogaact ctgagcagtt ccagaagcag aaggacggca cggcggtgtg gtgatogao 720
tgcattcggg togaccgggc atcaggcaac aaggcaatcg tgaccgtgac ggttcoggac 780
gtgatggaa c 791

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<210> 36
<211> 501
<212> DNA
<213> Rhodococcus erythropolis

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```

<220>
<221> misc_feature
<223> 1000bp to 1500bp pRET1100

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```

<400> 36
cttgttacat ctgccacaac tgtccgtgaa tctctgccag ctctgaaac cgtggtcag 60
ggccttgcgg aatccgtgao ogotgatgat ttttggctc attcgttccc ccgogctgac 120
gatgtacgog ggcagctgc ttcttccag tgggtggota actgggatgg cgtgaggggt 180
ccgaggccgc gtttcttgt cgcgcctggc gttgtccgt tggaggtttg tgatctogca 240
cgcccgaaac gaaacggctga acgtgcgtat ctggctgctc gggtcgggt ggatatggcg 300
gtgcccaggo ataactogcc gtaccacttc gacgtggaag atgaagagtt ggcggaactg 360
gottototgo aaggcotoga ggacgaagac attgggggct ggtctcgga gagggaata 420
gtgggtggt ctgctcgttc tgggtcagg atgatcttgc gaatggcaga actcgactgg 480
gtcccatga tggatttgc g 501

```

```

<210> 37
<211> 945
<212> DNA
<213> Rhodococcus erythropolis

```

```

<220>
<221> misc_feature
<223> 5000bp to 500bp pRET1100

```

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<400> 37
gatcctggtt cgagcaagag ggccggcat catgagaaa tggcgggag tatctcgat 60
toctatcgt ctggcgct ctctagtcagg ggaagacgtt ggattogac tgcctcgga 120
cgccgtcac atgccatgc agggcaaac ccgatccgc aaaagtcagg cgactacaa 180

```

```

cgtgttagct caggcagcag cgaacgcggc ggttcgagtc gtagggtcgg acccgacaca 240
cgtactcctg gagcccttca aacatcgagg ggtgtccgag cttacgtgg ttccgggact 300
gaatgcgcag gccacgggtg acatgctggg ctgggtcaag cgtgagtctg atcgtcgcat 360
cgaccagatg tggcccttgc gtaccgacaa gttttccgag ttccggggctt cgttcccgct 420
gatactcgtc gtgctcgaag agtttcccg gatactcgag ggggcagcgg acgaagacgc 480
cgcgttaggc cgaacacctg ccgagcgtct cgcacccgcg atttcggcct acgtgcgtca 540
gatagcagcg cagtcggcaa agcgtggaat tcgccttctc ctgctctcgc aacgagcggg 600
ggcctcgatc attggcggaa atgcgcgttc gaatttoggg gtcaagatga ctctgagggt 660
ggacgaaccg gagtgggtga gaatgcttca tccgagcgtc tccccggaag actgigccct 720
ggtcgagacc ttcaagcctg gtacgtcctt ttccgagaag ccaggagaag gccggcagat 780
tatgcgatgc gacttttgcg ggcagtacgg gagatatgcg cgagccatcg agtcttcgga 840
tctgcgtttt ctgcgcaccc tcagcaaga ccaggcccaa cgcgaattct tcgtgagga 900
gttcgggtgtg gtggatcgtt catgactgga ccacaggaga gaaag 945

```

```

<210> 38
<211> 939
<212> DNA
<213> Rhodococcus rhodni i

```

```

<220>
<221> misc_feature
<223> 3350bp to 2412bp pRET1000

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<400> 38
atggttgagg tggagagca cacaggcggc gcctgggaac agctgtggct accgctgtgg 60
ccactggcaa ccgacgattt octogaoggo gtatacogga tgcggcgatc agacgcactg 120
gatcgccgct acatcgagtc gaacccgcag gcattgagca acctgctcgt cgtggacgtt 180
gaccacccgg accgcgcgtc gcgggcgctg tcggcggcgg ggaatcatcc tctgcggaac 240
gcgatcgttg agaaccccg taacgggcac gcacacgctg tgtggcgctt ggacagagcg 300
ttcaccgcga ccgagtcgcg ccgtcgttaag ccgtcgcctt atgcggcgcg cgtcacggaa 360
ggcctccggc gogcogtcaa gggggacaag ggcatttcgg gcctgatgac caagaacccg 420
actcacgggt actgggacac ccattggctg cacaccgagc ggcgatccct cgcggagctc 480
gaggcggaac tcggcatcca catgccgcca accgcgtggc ggcaaaccgg atcgcgccgt 540
gagaaccoga tcggcctcgg ccgaaactgc ggcctgttcg aaaccgcacg cacttgggcc 600
tacgcgaaa tcgccttcca ctggggcgac ccgacgggcc tcggggcgcc gatctatgog 660
gaagccgca acatcaacgc cacttcagg aacccggta caggcaggcc cgtccactg 720
ccagcaagcg agctacgcgc cgtcggggcc tcattaccc gctggatcac tacaagtc 780
cggatgtggg ccgacggccc tctgtctac gaggccacat tcctgccat acaagccgca 840
cgcggtcgca agatgagtga gaagaagcgc gaggcaaac ggaaacgagc gacgaagtc 900
gaccggaacg cattgtggga ggcagaccgt gggcgctga 939

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```

<210> 39
<211> 840
<212> DNA

```

<213> *Rhodococcus rhodnii*

<220>

<221> misc_feature

<223> 3251bp to 2412bp pRET1000

<400> 39

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atgcggcgat cagacgcact ggatcgccgc tacatcgagt cgaaccgcga ggcattgago    60
aacctgctcg tcgtggaagt tgaccacccg gacgcgcgcg tgcgggcgct gtcggcgcc    120
gggaatcacc ctctgccgaa cgcgatcgtg gagaaccccc gtaacgggga ogcacaogot    180
gtgtggcgcg tggcagagcc gttaacccgc accgagtaag ccgctcgtaa gccgctcgcc    240
tatgcggcgc cgcgcacga aggcctccgg cgcgcgcgtc aggggggaaa gggtattog    300
ggcctgatga ccaagaaccc gactcacggt gactgggaca ccattggct gcacaccgag    360
cggcgatccc tcgcgagct caggcgga ctcggcatcc acatgcgcc aacgcgctgg    420
cggcaaaccc gatcgccgc tgagaaccc atgggcctcg gcgaaactg cgcctgttc    480
gaaaccgcac gcacctgggc ctaccgcga atccgcttc actggggcga ccgaccggc    540
ctcggggcgc cgatctatgc ggaagccgc cagatcaac ccacgttcag gaaccggtc    600
acaggcaggg ccgatccact gccagcaagc gagctacgc ccgtcgccgc ctccattac    660
cgctggatca ctacaaagtc ccggatgtgg gccgacggcc ctgctgtota caggccaca    720
ttcatcgoca tacaagccgc accggctgc aagatgagt agaagaagc caggcacaac    780
cggaaacgag cgacgaaggt cgaccggaac gcattgtggg aggcagaccg tggcgctga    840

```

<210> 40

<211> 534

<212> DNA

<213> *Rhodococcus rhodnii*

<220>

<221> misc_feature

<223> 2945bp to 2412bp pRET1000

<400> 40

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atgaccaaga acccgactca cggtagctgg gaaacccatt ggtgcacac cgagcggcga    60
tccctcgccg agctcgaggg ggaactcggc atccacatgc cgccaacgcg ctggcggcaa    120
aaccgatcgc gccgtgagaa ccgatcggc ctcggcgaa actgcgcct gttcgaaccc    180
gcacgcacct gggcctaccg cgaatccgc ttccactggg gcgaccgcac cggcctcggg    240
gcccgatct atcggaagc ogaacagat aacgccact tcaggaaccc ggtcacaggc    300
aggcccgatc cactgccagc aagcgagata ogcgccgtc cggcctccat taccgctgg    360
atactacaa agtcocgat gtggcgacac gccctgtg tctacgaggg caccatcaco    420
gccatacaag ccgcacgcgg tcgcaagatg agtgagaaga agcgcgaggg aaaccggaaa    480
cgagcgacga aggtcgaccg gaacgcattg tgggagggc accgtggggc ctga    534

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<210> 41

<211> 438

<212> DNA

<213> *Rhodococcus rhodnii*

<220>

<221> misc_feature

<223> 2849bp to 2412bp pRET1000

<400> 41
 atgccgccaa cgcgctggcg gcaaaccoga togcgcggtg agaaccgat cggcctcggc 60
 cgaaactcgc cccgtgttoga aaccgcacgc acctgggcct accgcgaaat ccgtttcac 120
 tggggcgacc cgaccggcct cggggccgog atctatgcgg aagccgcaca gatcaacgcc 180
 aogttoagga acccggtcac aggcaggccc gatccactgc cagcaagoga gctacgcgcc 240
 gtgcggcct ccattaccgc ctggatcact acaaagtccc ggatgtgggc cgaaggccct 300
 gctgtctacg aggcacatt catcgccata caagccgcac goggtogcaa gatgagtgag 360
 aagaagcgcg aggcacaacc gaaacgagcg aogaagtog acoggaacgc attgtgggag 420
 gcagaccgtg ggcgctga 438

<210> 42
 <211> 207
 <212> DNA
 <213> *Rhodococcus rhodnii*

<220>
 <221> misc_feature
 <223> 2365bp to 2159bp pRET1000

<400> 42
 atgggggcct ccacgcgcac gatccagcgc atcatggcgc agccgcggga ccagttcctc 60
 gcacgggcag ccgagaaccg togcggggcc gtcgagctgc gcgagcaggc cctgaagtac 120
 cgcgagatcg ccgaggagat gggaatctcc accggaacgg tgggaaagct cctgcacgac 180
 gcacgcaagt acgcggtcag ctccatag 207

<210> 43
 <211> 174
 <212> DNA
 <213> *Rhodococcus rhodnii*

<220>
 <221> misc_feature
 <223> 2332bp to 2159bp pRET1000

<400> 43
 atggccgagc cgcgggacca gttoctgca ogggcagccg agaaccgtcg ccgggcgctc 60
 gagctgcgcg agcagggcct gaagtaccgc gagatcgccg aggagatggg aatctccacc 120
 ggaacggtgg gaaagctoot gcacgacgca cgcaagtacg cggtcagctc ctac 174

<210> 44
 <211> 330
 <212> DNA
 <213> *Rhodococcus rhodnii*

<220>
 <221> misc_feature
 <223> 3197bp to 3526bp pRET1000

<400> 44
 atgcctgcgg gticgactcg atgtagcggc gatccagtgc gtotgatogo cgcacccggt 60
 agacgcgctg gaggaatcg togtttgcca gtggccacag cggtagccac agctgttccc 120
 aggcgcggcc tgtgtgtctt tccaccgcaa ccatggggaa cactctaca cacaagatcg 180

atttattccg gtaacacaag ccagccaagt cagatgtttc ggtttctgga gcggtcctcc 240
 agacettiga gatccgctcc agaaacgtcc acaaattatt gggttacgtc gaaccaagcc 300
 ttatcaggta tcccgggggtt cgggggggtga 330

<210> 45
 <211> 357
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 4035bp to 3679bp pRET1000

<400> 45
 atgggggtggt tattgottgt tgcgtcgggg gccgtggcga tgggtggcgg tgtggtctta 60
 ccgcgcgggg atcgtctcgg gccggcacca ggatttcctt ggtcttggtt ggtgttccca 120
 tccacgtgca ttgccatcgc tccgcgggtg ggtgtcttgc ottggcccca agcgggttacc 180
 ggcaacgggga gctactggtg ggatccgcc agcgcgagct caccgacctt gcagttcctg 240
 tcaaaogago agtaocggcg cctcgtgaca ctgcgcgggt tgcagggggc gctaccggtg 300
 gtgtccctcg tgggaagcgg attgtgcgtg tgggcctggc gtcgacgcgg ctctctga 357

<210> 46
 <211> 318
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 3996bp to 3679bp pRET1000

<400> 46
 atggtggcgg gtgtggtctt accgcgcggg gatcgtctcg gcccggcacc aggatttccc 60
 tggttctggg tgggtgtccc atccacgtgc attgccatcg ctgcgcgggt ggggtgtctc 120
 gotttggccc aagcgggttac cggcacgggg agctactggt gggatccgcc cagcgcgago 180
 toaccgacct tgcagttcct gtcacacgag cagtaccggc gootcgtgac actgcgcggg 240
 ttgcaggggg cgtaccggtt ggtgtccctc gtgggaagcg gattgtgcgt gtgggcttgg 300
 cgtcgaogoo gottctga 318

<210> 47
 <211> 450
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 4381bp to 4830bp pRET1000

<400> 47
 atggcgcgtg acgtgcacg tgaacacggc oggaccgagg tccgcgcgcg tgcctcgcgg 60
 gccgcgtacg cggccccggc gaagcgcacc ogcaccgtgg cggtgoggtt gacgatggg 120
 gaggaggcgg cgtggatcga cgcgcgcgtg gccgatggcc accggcagct cggggcgttg 180
 gtgcgtgagc gggcgggtggc cggctatctc ggggaagtcg gcccgagac cggcagtgg 240
 atgtcggcgg aggcggccgc ggaggtcgcc gcgatgcggc agcagatgac gaaggtgggg 300

```

aacaacctga accagatcgc gagggcgatc aacgcggggc aggtgccgtc gcagatggcc 360
gagtcacctgc agaaggggtg gctggagagg tgggggcagg agttggggcg gatggcggat 420
cggctcgacg cgctcgacga ccagggtga 450

```

```

<210> 48
<211> 210
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 4621bp to 4830bp pRET1000

```

```

<400> 48
atgtcggcgg aggcggccgc ggaggtcgcg gcgatggggo agcagatgac gaaggtgggg 60
aacaacctga accagatcgc gagggcgatc aacgcggggc aggtgccgtc gcagatggcc 120
gagtcacctgc agaaggggtg gctggagagg tgggggcagg agttggggcg gatggcggat 180
cggctcgacg cgctcgacga ccagggtga 210

```

```

<210> 49
<211> 177
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 4654bp to 4830bp pRET1000

```

```

<400> 49
atgggcgacg agatgacgaa ggtggggaac aacctgaacc agatcgcgag ggcatcaaac 60
gccgggcagg tgccgtcgca gatggcggag tccctgcaga aggggtggct ggagaggtgg 120
gggcaggagt tggggcggat ggccgcatcg ctgcgcgcgc tcgacgacca gggctga 177

```

```

<210> 50
<211> 165
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 4666bp to 4830bp pRET1000

```

```

<400> 50
atgacgaagg tggggaacaa cctgaaccag atcgcgaggg cgatcaacgc cgggcagggtg 60
ccgtcgacga ttgcgagtc cctgcagaag ggggtggctgg agaggtgggg gcaggagtgg 120
gggcgggatg cggatcgggt cgacgcgctc gacgaccagg gctga 165

```

```

<210> 51
<211> 453
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 5161bp to 4709bp pRET1000

```

```

<400> 51
atgactctcg aagcccatcc gctggggac cgtctgcgcg atgtccgga actcggatc 60
ggtcagccgc cgatecccg ggcgcaccg cagcgagcaa tgccacaccg gottaccac 120
ccgcgcgttc gtgcggcgg ccgctcgaa gtccgcccc caccggctcg ggtttttggo 180
ggtagctgc accgatccg cgtacaccg ccgcgggca atcagccgc cgcctcggg 240
gggtagctg tgccgggtg ccttcccg ccgtgcaga tacgcgcga aaccctcgg 300
gtcgtgcgc gtgtgatct tcgcatcac gtcagccctg gtcgtcgagc gcgtcgagc 360
gatccgcat ccgccccaac tctgcccc accctccag ccacccctt tcaggggact 420
cggccatctg cgaaggcac tggccggct tga 453

```

```

<210> 52
<211> 354
<212> DNA
<213> Rhodococcus rhodnii

<220>
<221> misc_feature
<223> 5062bp to 4709bp pRET1000

```

```

<400> 52
atgccacacc ggcttaacca ccgogogtt cgtggggcg gcccgctga agtccgcgc 60
ccaccgggic ggttttttg cgtgacctg caccgatcc cgcacaccg tcccgccgc 120
aatcagccgc ccgcctcgg tcggtagct gtgcgggtg gccttcccg gcccgtcag 180
atacgcgc aaacctctg gtcgtgcgc cgtgtgatc ttgcgatac cgtcagccg 240
ggtgtcgag cgcgtcgag cgtccgcca tccgccccaa ctctgcccc cactctcca 300
gccacccctt ctgcaggac tcggccatct gcgaaggcac ctgccggcg ttga 354

```

```

<210> 53
<211> 288
<212> DNA
<213> Rhodococcus rhodnii

<220>
<221> misc_feature
<223> 2331bp to 2618bp pRET1000

```

```

<400> 53
atgatgcgt ggatgtgc cgtggaggc ccatcttct cggccagct cgcagctgc 60
tgcttcggc ggatggctc ttacgcgc accgtctgc tccacaatg cgttcgggc 120
gaccttcgt ctcgttttc gttttgcct cgccttctt tcaatcatc tgcgacgcg 180
tgccgttgt atgggatga atgtggctc gtagacaga ggcgcgtcg ccacatccg 240
ggactttgta gtgatcagc ggtaatga gcccggaag gcgctag 288

```

```

<210> 54
<211> 285
<212> DNA
<213> Rhodococcus rhodnii

<220>
<221> misc_feature
<223> 2334bp to 2618bp pRET1000

```

```

<400> 54
atgogctgga tegtgcgct ggaggccccc atcttctcgg ccagctogcg agctgtctgc 60
ttggggcgga tgggtcgttc aggccccacg gtctgcctcc cacaatgcgt tccggtcgac 120
cttctcgtct cgtttccggt ttgcctcgcg cttcttctca ctcattctgc gaccgcgtgc 180
ggcttgtatg gcgatgaatg tggcctcgta gacagcaggg ccgtcggccc acatccggga 240
ctttgtagtg atccagcggg taatggaggo cgcgacggcg cgtag 285

```

```

<210> 55
<211> 336
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 2907bp to 3242bp pRET1000

```

```

<400> 55
atgggtgtcc cagtcaccgt gagtcgggtt ctigtctatc aggcccgat agcccttgtc 60
cccttggaag gogggccgga ggccttcggt gacggcggcc gcataggcga gcggcttacg 120
acgggcgtac tcgtgcgggg tgaacggctc tgccagcgcc caccacagct gtgcgtgccc 180
gttacggggg ttctccacga tcgcgttcgg cagaggatga ttcccgcccg ccgacagcgc 240
ccgcagcgcg gcgtccgggt ggtcaacgtc cagcagcagc aggttctca atgcctgcgg 300
gttcgaatcg atgtagcggc gatccagtgc gtctga 336

```

```

<210> 56
<211> 513
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 1650bp to 2162bp pRET1000

```

```

<400> 56
atgcggattg aactagtcca ttgggggaac gatgaactga tgacggggga tcgtgacctc 60
cccatgctga ccatgcgga ggoggtggac gcgacgcaga ccagtgcagc cagatcaag 120
cgccgcctgc ggtggggcgc gttcccgaa gcggtccgca ctgcgcagcg gaagtggatg 180
attccctcgt gtgacctatc agggcaggg ctgagaccag ggaaaatgga gaaacctgac 240
ccgtgaacc cticaaatga ccgggtccgt gacctggcag ctgagaacgc cgagctccgt 300
cagccctgg ccgtggcga agocctggcc agcgaacgca atcggatcat cgacgtgcag 360
caacagatgc tcggatgct cgaagcccg ccggtgtcgg ccctggagcc cggggcgggt 420
ccagtgggg gtccgcgcgc gcccgcccg gccgcgatg gtccggcagc tacgggcgcg 480
ctggcccgga tacgtgcag gttctcggc tag 513

```

```

<210> 57
<211> 474
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 1689bp to 2162bp pRET1000

```

```

<400> 57
atgaccgggg atcgtgacct acccatgctg accatggccg agggcgtgga cgcgacgcag 60
accagtgaga gcaogataaa gcgcgcctg cgtcgggcg cgttcocgaa cgcggtccgc 120
actgcgcagc ggaagtggat gattccctc ggtgacctat cagcggcagg gctgagacca 180
gggaaaatgg cgaacctga cccggtgacc cttcaaatg accgggtccg tgacctggca 240
gctgagaacg ccgagctccg tcagcgctg googtggccg aagccctggc cagogaacgc 300
aatcggatca tcgacgtgca gcaacagatg ctccggatgc tcgaagcccg gccggtgtcg 360
gcootggagc ccgcggcggt tccagtggcg ggtccgcgcg cgcgcgtccc gccgcgcgat 420
ggtcgggcag ctacgggcgc cctggcccg atacgtcgac ggcttctcgg ctac 474

```

```

<210> 58
<211> 450
<212> DNA
<213> Rhodococcus rhodnii

<220>
<221> misc_feature
<223> 1713bp to 2162bp pRET1000

```

```

<400> 58
atgtgacca tcgcgaggo ggtggacgc aocgagacca gtgagagcac gatcaagcgc 60
cgctgcggt cgggcgcgtt ccggaacgc gtcgcactg ccgacgggaa gtggatgatt 120
ccctcgggt acctatcagc ggcaggcctg agaccaggga aaatggcgaa acctgaccgc 180
gtgaccctt caaatgacgc ggtccgtgac ctggcagctg agaacgcga gtcctcag 240
cgctggccg tggcgaagc cctggccagc gaacgcaatc ggatcatcga cgtgcagcaa 300
cagatgtcc ggatgtcga agcccggcg gtgtcggccc tggagccgc gccggttcca 360
gtggcgggtc cgcgcgcgc cgtccggcc gccgatggtc gggcagctac gggcgccctg 420
gcccggatac gtgcaggct tctcggctag 450

```

```

<210> 59
<211> 336
<212> DNA
<213> Rhodococcus rhodnii

<220>
<221> misc_feature
<223> 1827bp to 2162bp pRET1000

```

```

<400> 59
atgattcccc tcggtagct atcagcggca gggctgagac cagggaaaat ggcgaaacct 60
gacccggtga ccccttcaaa tgaccgggtc cgtgacctgg cagctgagaa cgcgagctc 120
cgtcagcgc tggcgtggc cgaagccctg gccagogaac goaatoggat catcgacgtg 180
cagcaacaga tgctcggat gctcgaagcc cggccggtgt cggccctgga gcccgggcg 240
gttcagtgg cggctoogcc gcgcgcgtc ccggccgcg atggtcgggc agctacgggc 300
gccctggccc ggatacgtc acggttctc ggctag 336

```

```

<210> 60
<211> 288
<212> DNA
<213> Rhodococcus rhodnii

```

<220>
 <221> misc_feature
 <223> 1875bp to 2162bp pRET1000

<400> 60
 atggcgaaac ctgaccoggt gaccccttca aatgaccggg tccgtgacct ggcagctgag 60
 aacgcagagc tccgtcagcg cctggccgtg gccgaagccc tggccagoga acgcaatcgg 120
 atcatcgacg tgcagcaaca gatgctcggg atgctogaag ccgggcccgt gtccggccctg 180
 gagcccgagg cggttccagt ggggggtccg ccgcccggcg tcccggccgc cgatggctcg 240
 gcagctacgg gcgccttggc cgggataagt cgaaggcttc tcggctag 288

<210> 61
 <211> 264
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 1906bp to 2169bp pRET1000

<400> 61
 atgaccgggt ccgtgacctg gcagctgaga acgcccagct ccgtcagcgc ctggccgtgg 60
 ccgaagccct ggcagagcaa cgcaatcgga tcatcgactg gcagcaacag atgctccgga 120
 tgctcgaagc ccggccgggtg toggccctgg agcccggcgc ggttccagtg gggggtccgc 180
 cgcgcgccgt ccggccggcc gatggtcggg cagctacggg cgccttggcc cggatacgtc 240
 gacggcttct cggctaggag ctga 264

<210> 62
 <211> 258
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 810bp to 553bp pRET1000

<400> 62
 atgtatggg aggtatgca ctttggcgcg ttatgtacgc atcctgggca cctggggcac 60
 gaccgacctt ctacgatcg atggtgttct tggacatgct tggccaggcc tgcgtctgtt 120
 ccctaagctc caagaaagcc ttctcgtct ctgctcagc tccattccg gattctcgac 180
 ctcggtcgcg gccgggtggc tgatacccg gggcggactg cggcatggtt ggtccctggc 240
 ggcgggcggg gggtttga 258

<210> 63
 <211> 540
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 117bp to 656bp pRET1000

<400> 63
 atgggaggcc acccgacacc gctacgggac atgctcgcg ccaggagca gcgcgggaag 60

```

ccgtggactc cggagcagaa acgccagtac gcgaccgcaa aagcccaagc agaacgcgc 120
gcgaaggcca aggacgccgc gaaatggacc gaggtcgccg gcggcggcta ccagcgggac 180
gtgcgcggga tgaacctgcg actgtgggtg gctgaggacg gcgcctggtc gatcaacctc 240
aagaaggacc ccgaccgcca gtacgccga ggtcaggccg acaccgtcgc gcaggcccaa 300
gccgcggcca cggccacagc gaaaacgcag gcccaggcga tgtggaagca ggtcccgccc 360
gacaagcgca ccgagtcago caccagagcg gtccggcgcg tgatcgcgga tctcaccccc 420
accaaaccgc ccgaggtcaa acccccggcc cgcgcgcagg gaccaaccat gccgcagtcg 480
gccccggggt atcagccacc cggccgcgac cgaggtcgag aatccggaat gggactgtga 540

```

```

<210> 64
<211> 510
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 147bp to 656bp pRET1000

```

```

<400> 64
atgctcgccg ccagggagca gcgccggaag ccgtggactc cggagcagaa acgccagtac 60
gcgaccgcaa aagcccaagc agaaogcgcc gcgaaggcca aggacgccgc gaaatggacc 120
gaggtcgccg gcggcggcta ccagcgggac gtgcgcggga tgaacctgcg actgtgggtg 180
gctgaggacg gcgcctggtc gatcaacctc aagaaggacc ccgaccgcca gtacgccga 240
ggtcaggccg acaccgtcgc gcaggcccaa gcgcgggcca cggccacagc gaaaacgcag 300
gcccaggcga tgtggaagca ggtcccgccc gacaagcgca ccgagtcago caccagagcg 360
gtccggcgcg tgatcgcgga tctcaccccc accaaaccgc ccgaggtcaa acccccggcc 420
cgcgcgcagg gaccaaccat gccgcagtcg gccccggggt atcagccacc cggcgcgcgac 480
cgaggtcgag aatccggaat gggactgtga 510

```

```

<210> 65
<211> 351
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 306bp to 656bp pRET1000

```

```

<400> 65
atgaacctgc gactgtgggt ggctgaggac ggccgctggc cgatcacctc gaagaaggac 60
ccgaccgccc agtacgccgc aggtcaggcc gacaccgtcg cgcaggccca agccgcggcc 120
acggccacag cgaaaacgca ggcccaggcg atgtggaagc aggtcccgcc cgacaagcgc 180
accgagtcag ccaccagagc ggtccggcgc gtgatcgccg atctcaccgc caccaaacc 240
gcgcagggtc aaaccccgcc cgcgcgcagg ggaccaacca tgccgcagtc ggcgccgggg 300
taccagccac ccggcccgca ccgaggtcga gaatccgga tgggactgtg a 351

```

```

<210> 66
<211> 201
<212> DNA
<213> Rhodococcus rhodnii

```

<220>
 <221> misc_feature
 <223> 456bp to 656bp pRET1000

<400> 66
 atgtggaagc aggtcccgcc cgacaagcgc accgagtcag ccaccagagc ggtccggcgc 60
 gtgatcgagg atctcaccoc caccaaaccc gccgaggcca aaccccgccg ccgccgccag 120
 ggaccaacca tgcgcagtc ggccccgggg tatcagccac ccggccgcga ccgaggtoga 180
 gaatccggaa tgggactgtg a 201

<210> 67
 <211> 1326
 <212> DNA
 <213> Rhodococcus rhodnii

<220>
 <221> misc_feature
 <223> 5144bp to 656bp pRET1000

<400> 67
 atgggttcg agagtcaccc ggggtggcg gtgcggcagc acgacgacca catccacctg 60
 gtgtgtccc ggttcgattt tcaggcgctg acctggaaga acagcaacga ccggtggaag 120
 gtcttcgagg tgatgcgcga ggtcgaacgc gcgcacggcc tgatcgaggt ggcgagcccg 180
 gagcggggcc gttggccggca agccagcagc ggcgagcaac gcgcgcgctt gcggaccggc 240
 aaggtggcgc agcgggacgg totgagggaa attgtgacg ccgcccgcga catcgccgca 300
 ggccagggtg tggggcgctt cgaagtggcg ctcttacaga acccgattac oagagtgcag 360
 gtgcggcgca acgtgcgaa gacgggcggg atgaatggct acagcttcaa cctgcccggc 420
 taactgacg ccgcggggga gccgatctgg ttgccggcct ccaaaactga ccggggtttg 480
 tccttgtcac agctggaaaa gacgtgacc agaccgcgc ccgaccgctt ccgcggcgag 540
 gagacgtgc ccggaagcg gctcgagcg gccgcgcgtt gggagcagcg ccgcgcgag 600
 gtgcggcgcg agcagttcgc agctgcccgc tgggagcagg ccgcgcgaa tgttggtgag 660
 accgcccggc ggtaccgccc cgaacagtc gccgacagc agtgggaagc ggtgaacgag 720
 gcgttgacca gccaaagacc ggccgaggag caggctgccg aggcagcgcg ggtgcctcc 780
 gtgtcatgg gaggccaccc gacaccgcta cgggacatgc tcgccgccca ggagcagcgc 840
 ccgaagccgt ggactccgga gcagaaacgc cagtacgga ccgcaaaagc ccaagcagaa 900
 ccgcgcgga aggccaagga ccgcgcgaaa tggaccgagg tcgccggcg ccgctaccag 960
 cgggacgtgc gcgggatgaa cctgcgactg tgggtggctg aggcggcgcg ctggtcgatc 1020
 aactcgaaga aggaacccga ccgccagtc gccgcaggtc aggcgcgac ccgtcgcgag 1080
 gcccaagccg ccgccacggc cacagcgaaa acgcaggccc aggcgatgtg gaagcaggtc 1140
 ccggccgaca agcgcaccga gtcagccacc agagcggtcc ggcgcgtgat ccgggatctc 1200
 accccacca aaccgcgga ggtcaaaccc ccggcccgcc gccagggacc aacctgcgc 1260
 cagtcggccc cggggtatca gccaccggc ccgcacgag gtgcgaaac ccgaatggga 1320
 ctgtga 1326

<210> 68

<211> 1194
 <212> DNA
 <213> *Rhodococcus rhodnii*

<220>
 <221> misc_feature
 <223> 5276bp to 656bp pRET1000

<400> 68
 atgcgcgagg tcgaacgcgc gcacggcctg atcgaggtag cgagcccgga gcgggcccgt 60
 ggccggcaag ccagcagcgg cgagcaacgc cgcgcggtgc ggacoggcaa ggtggcgcag 120
 cgggacggtc tgagggaat ttgacggcc gcccgcgaca tcgcgcgagg ccagggtgtg 180
 gggcgcttog aagtggogct cgtacagaac ccgattaccg gagtgcaggt gcggcgcaac 240
 gtcgcgaaga cgggcgggat gaatggctac agcttcaacc tgcggcgcta cgtcgacgcc 300
 gccggggagc cgtctgtgtt gccggcctcc aaactcgacc ggggtttgtc ctggtcacag 360
 ctggaaaaga cgtcgaccag acccgcccg gcacggcctc ccggcgagga gacggtgccg 420
 cggaaogcgc tcgagcgcgc cgcgcggtg gacgagcgc gccgcgaggt cggcgcgag 480
 cagttcgag ctgcgcgctg ggagcaggcc cgcgcgaatg ttggtgagac ggcggcgcg 540
 atccgcgcgc aacagtcgcg ggacacgaag tggaaagcag tgaacgaggo gttgaacago 600
 caagaccggg ccgaggagca ggttcgcgag gcagcgcggg tcgcctccgc tgtcatggga 660
 ggccacccga caccgctacg ggacatgctc gccgccagg agcagcgcgc gaagccgtgg 720
 actccggagc agaaacgcga gtacgcgacc gcaaaagccc aagcagaacg cgcgcggaag 780
 gccaaagcgc ccgcgaaatg gacgaggtc gccggcgcg gctaccagcg gacgtgcgc 840
 gggatgaacc tgcgactgtg ggtggctgag gacggcgctt ggtogatcac ctogaagaag 900
 gaccccgacc gccagtagc cgcaggtcag gccgacacc tcgcgcaggo ccaagccgcg 960
 gcoacggcca cagcgaaaac gcaggcccag gcgatgtgga agcaggtccc ggccgacaag 1020
 cgcacogagt cagcoaccag agcgtcccg cgcgtgatcg cggatctcac cccacccaaa 1080
 ccgcgcgagg tcaaaccccc ggcccgccgc cagggaacca ccatgccgca gtcggccccg 1140
 gggatatcgc caccggcgcg gcacogaggt cgagaatccg gaatgggact gtga 1194

<210> 69
 <211> 936
 <212> DNA
 <213> *Rhodococcus rhodnii*

<220>
 <221> misc_feature
 <223> 5534bp to 656bp pRET1000

<400> 69
 atgaatggt acagcttcaa cctgcccggc taactcgacg ccgcccggga gccgatctgg 60
 ttgcggcct ccaaaactga ccggggtttg toctggtcac agctggaaaa gaogtgacc 120
 agaccccgcc cggaccgcct cgcgcgcgag gagacggtag cgcggaagcg gctcgagcgc 180
 gccgcgcgt gggagcagcg ccgcgcgag gtggcgcgag agcagttcgc agctgccgcg 240
 tgggagcagg ccgcgcgcaa tgttggtgag acggccgggc ggatccgcgc cgaacagtc 300
 gccgacacga agtggaaagca ggtgaacgag gcgttgacca gccaaagacc gcccgaggag 360
 caggctgccg aggcagcgcg ggtcgccctc cgtgtcatgg gaggcoacco gacaccgcta 420

```

ogggacatgc tcgccgcccga ggagcagcgc cggagccgt ggactcggga gcagaaaogo 480
cagtacgcga ccgcaaaagc ccaagcagaa cgcgcgcgga aggccaaaggc cgcgcgcgaaa 540
tggaccgagg tcgccggcgg cggotacag cgggaogtgc gcgggatgaa cctgcgactg 600
tgggtggcig aggcaggcgc ctggtcgatc acctcgaaga aggaccccg cgcgcgtao 660
gcccaggtgc aggcgcgac cgtcgcgcag gcccaagccg cgcgcacggc cacagcgaaa 720
acgcaggccc aggcgatgtg gaagcaggto cgcgcgcgaa agcgcacgga gtcagccacc 780
agagcgggtc ggcgctgat cgcggatctc acccccacca aaccgcgcga ggtcaaacc 840
cgcgcgcgc gccagggacc aaccatgcc cagtcggccc cggggtatca gccaccgcgc 900
cgcgcacgag gtcgagaatc cggaatggga ctgtga 936

```

```

<210> 70
<211> 153
<212> DNA
<213> Rhodococcus rhodnii

```

```

<220>
<221> misc_feature
<223> 3355bp to 3507bp pRET1000

```

```

<400> 70
aacacactca cacacaagat cgatttattc cggtaacgaa cgcgcgcgaa gtcagatgtt 60
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```

```

<210> 71
<211> 61
<212> DNA
<213> Rhodococcus rhodnii

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```

<220>
<221> misc_feature
<223> 4290bp to 4350bp pRET1000

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<400> 71
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<210> 72
<211> 325
<212> DNA
<213> Rhodococcus rhodnii

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<220>
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<223> 3570bp to 3894bp pRET1000

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325

<210> 73

<211> 5444

<212> DNA

<213> Rhodococcus erythropolis

<220>

<221> misc_feature

<223> pRET1100 Full Length

<400> 73

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<213> Rhodococcus rhodni

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<221> misc_feature
<223> pRET1000 Full Length

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<210> 78
 <211> 259
 <212> PRT
 <213> Rhodococcus erythropolis

<400> 78

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