

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
24 August 2006 (24.08.2006)

PCT

(10) International Publication Number
WO 2006/088307 A1

(51) International Patent Classification:
C12N 15/74 (2006.01)

(21) International Application Number:

PCT/KR2006/000521

(22) International Filing Date:

15 February 2006 (15.02.2006)

(25) Filing Language:

Korean

(26) Publication Language:

English

(30) Priority Data:

10-2005-0012892

16 February 2005 (16.02.2005) KR

(71) Applicant (for all designated States except US): **LG CHEM, LTD.** [KR/KR]; 20, Yeouido-dong, Yeongdeungpo-gu, Seoul 150-721 (KR).

(72) Inventors: **RHEE, Joowon**; 102-202 Samchang Apt., Sanseong-dong, Jung-gu, Daejeon 301-211 (KR). **CHO, Junhyeong**; 405-201 EXPO Apt., Jeonmin-dong, Yuseong-gu, Daejeon 305-762 (KR). **LEE, Sanghyun**; 7-207 LG CHEM Apt., 381-42 Doryong-dong, Yuseong-gu, Daejeon 305-340 (KR). **PARK, Ohjin**;

109-1206 Hanbit Apt., Eoeun-dong, Yuseong-gu, Daejeon 305-755 (KR). **PARK, Si Jae**; 108-1203 Chowon Apt., Mannyeon-dong, Seo-gu, Daejeon 302-740 (KR).

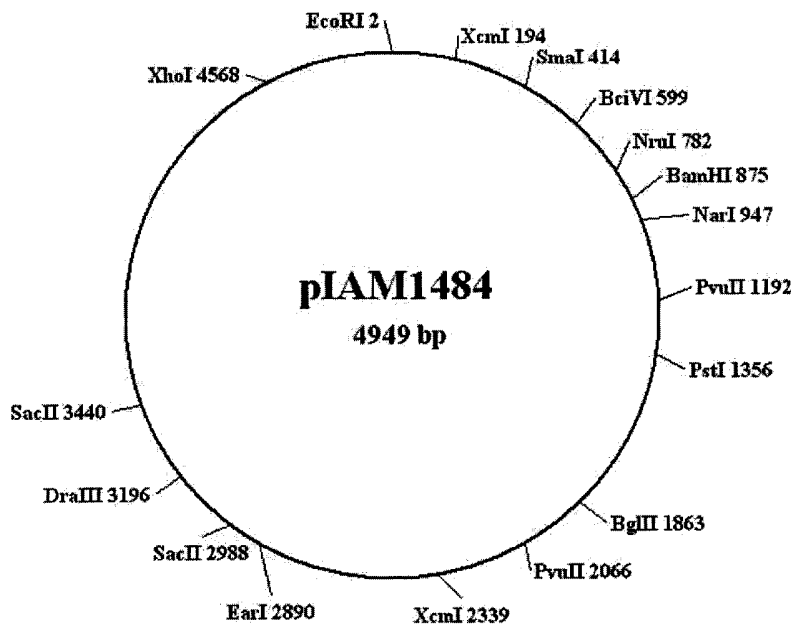
(74) Agent: **LEE, Cheo Young**; 11th Floor Won Bldg., 648-26, Yeoksam-dong, Gangnam-gu, Seoul 135-080 (KR).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: RHODOCOCCLUS - E. COLI SHUTTLE VECTOR



(57) Abstract: The present invention relates to a novel Rhodococcus-E. coli shuttle vector containing a replication origin of novel plasmid derived from Rhodococcus erythropolis IAM 1484 and a DNA replication origin derived from E. coli, which can replicated bidirectionally in E. coli and Rhodococcus, recombinant vector in which a target gene is operably linked to the shuttle vector, and a microorganism transformed with the recombinant vector. The shuttle vector according to the present invention has relatively small size, can be replicable in various kinds of the genus Rhodococcus microorganism and has high replication stability in a host cell. Therefore, it is useful for the cloning of various genes derived from Rhodococcus.

WO 2006/088307 A1

**Published:**

- with international search report
- with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

RHODOCOCCLUS-E. COLI SHUTTLE VECTOR

FIELD OF THE INVENTION

5

The present invention relates to a novel *Rhodococcus-E. coli* shuttle vector containing a replication origin of novel plasmid derived from *Rhodococcus erythropolis* IAM1484 and a DNA replication origin derived from *E. coli*, which can replicate bidirectionally in *E. coli* and *Rhodococcus*, a recombinant vector in which a target gene is operably linked to said *Rhodococcus-E. coli* shuttle vector, and a microorganism transformed with the recombinant vector.

BACKGROUND OF THE RELATED ART

15

The genus *Rhodococcus* microorganism produces biosurfactant (Philp, J.C. *et al.*, *Appl. Microbiol. Biotechnol.*, 59:318, 2002), and antibiotics (Hu, T.L., *Water Sci. Technol.*, 47:169, 2003), etc, as well as, biodegrades steroid compounds, bioconvert xenobiotic compound and nitril compounds (US 5,135,858) and degrades acrylic acid (US 5,998,180; US application no. 11/224,314). Since it has wide metabolism activity as the above, its importance has been emphasized gradually.

20

In light of its importance, genetic and physiologic research of the genus *Rhodococcus* microorganisms are widely belong conducted (Rahman, M.T. *et al.*, *Vet. Microbiol.*, 94:143, 2003), however, metabolism research of the genus *Rhodococcus* and similar microorganisms thereof are much restricted due to absence of proper host/vector system.(Finnerty, *Annu. Rev. Microbiol.*, 46:193, 1992). Accordingly, effective host/vector system regarding the genus *Rhodococcus* host are required to produce strains having high metabolism activity.

25

30

US 4,952,500 teaches an *E. coli*-*Rhodococcus* shuttle vector using circular plasmid obtained from the genus *Rhodococcus* H13-A. US patent No. 4,920,054 teaches a shuttle vector replicable in *Rhodococcus equi*, *Corynebacterium*, *Bacillus subtilis* and *Staphylococcus aureus* using replication origin of *Rhodococcus*. US 5,654,180
5 also teaches a shuttle vector containing replication origin derived from a replicable plasmid (pRC001, pRC002, pRC003, pRC004) in *Rhodococcus rhodochrous* and replication origin derived from replicable plasmid (pHSG299, pHSG298, pUC19, pUC18) in *E. coli*. KP 1999-048213 teaches a shuttle vector containing DNA replication origin derived from pEk of *E. coli*, DNA replication origin derived from
10 PCSP21197 of *Rhodococcus rhodochrous*, an ampicillin resistant gene as a selection marker expressed in *E. coli*, and a kanamycin resistant gene as a selection marker in *Rhodococcus rhodochrous*. Also, JP 8-056669 teaches shuttle vector which can be bidirectionally replicated in *Rhodococcus* and *E. coli*, containing DNA replication origin derived from *E. coli*, and DNA replication origin which can be replicated in
15 the genus *Rhodococcus* derived from plasmid (pNC500, pNC 903) from a strain belongs to nocardiform bacterium.

Besides, shuttle vectors using plasmids derived from *Rhodococcus fascians* (Desomer *et al.*, *J. Bacteriol.*, 170:2401, 1998; Desomer *et al.*, *Appl. Environ.*
20 *Microbiol.*, 56:2818, 1990), *Rhodococcus erythropolis* (JP 10248578; EP 757101; JP 09028379; US 5,705,386; De Mot *et al.*, *Microbiol.*, 146:3137, 1997), *Rhodococcus rhodochrous* (Kulakov *et al.*, *Plasmid*, 38:61, 1997), *Rhodococcus equi* (Zeng *et al.*, *Plasmid*, 38:180, 1997), *Rhodococcus* sp. (WO 89/07151; US 4,952,500; Vogt Singer *et al.*, *J. Bacteriol.*, 170:638, 1988; *Appl. Environ.*
25 *Microbiol.*, 64:4363, 1998) are reported.

In spite of the development of the above shuttle vectors, they are just theoretical research and development. So, till now, there is no commercially available tool which enables gene manipulation of *Rhodococcus* or microorganisms similar to

hodococcus. Because, replicase and replication protein enabling a replication of plasmid in host are not known much.

Some proteins used in preparation of an expression vector or shuttle vector has been
5 known (Denis-Larose *et al.*, *Appl. Environ. Microbiol.*, 64:4363, 1998; Billington, *et al.*, *J. Bacteriol.* 180:3233, 1998; Dasen, G..H. GI:3212128; and Mendes, *et al.*, GI:6523480), however, the numbers and their utilities are much restricted. Although the replication stability is very important in *Rhodococcus* shuttle vector, there is almost no shuttle vector having the excellent replication stability. In many cases,
10 antibiotics causing economic or safety problems in commercial utilization of microorganism must be used so that the replication stability of plasmid can be secured. However, the mechanism of maintaining for replication stability without antibiotics or related proteins thereof are not known specifically.

15 To solve these problems, a shuttle vector which conducts readily the cloning of other genes readily, contains strong replication proteins and has high replication stability in various hosts is required.

20 SUMMARY OF THE INVENTION

The present invention is devised so that the above problems are solved. An object of the present invention is to provide a shuttle vector having high replication stability without antibiotics, which enables replications in various hosts since it contains
25 gene sequence encoding an important proteins for replication of the plasmid.

Another object of the present invention is to provide a recombinant vector in which the target gene is operably linked to the *Rhodococcus-E. coli* shuttle vector and the genus *Rhodococcus* microorganism transformed with the recombinant vector.

To achieve the above objects, the present invention provides a shuttle vector containing DNA replication origin derived from *E. coli*, DNA replication origin derived from *Rhodococcus erythropolis*, an ampicillin resistant gene as a selection marker expressed in *E. coli*, and a kanamycin resistant gene as a selection marker in
5 *Rhodococcus*.

In the present invention, said DNA replication origin from *Rhodococcus erythropolis* is preferably derived from plasmid of *Rhodococcus erythropolis* IAM1484, and said plasmid is preferably pIAM1484.

10 In the present invention, the nucleotide sequence in said shuttle vector preferably has at least 95% homology to that in pJW1484 or SEQ ID NO:1, and said shuttle vector which has at least 95% homology to that of SEQ ID NO:1 is preferably pLG1484.

15 The present invention also provides a recombinant vector in which the target gene is operably linked to the shuttle vector and the genus *Rhodococcus* microorganism transformed with said recombinant vector.

20 The above and other features and embodiments of the present invention will be more fully apparent from the following detailed description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

25 FIG. 1 is a restriction enzyme map of plasmid pIAM1484 derived from *Rhodococcus erythropolis* IAM1484.

FIG. 2 is a process for preparing shuttle vector pJW1484 from plasmid pIAM1484
30 and pBluscript SK(-).

FIG. 3 is a process for preparing the shuttle vector pLG1484 according to the present invention.

FIG. 4 is a graph showing the replication stability of preparing the shuttle vector
5 pLG1484 according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION AND, PREFERRED EMBODIMENT

10

In one aspect, the present invention is related to a novel *Rhodococcus-E. coli* shuttle vector replicable efficiently in *Rhodococcus* being used industrially as an important strain.

15 In the present invention, *Rhodococcus-E. coli* shuttle vector(pJW1484) is prepared using plasmid pIAM1484 isolated and purified from *Rhodococcus erythropolis* IAM1484 and plasmid pBluescript SK(-) widely used in *E. coli*. And, the only regions contributing to replication and stability are finally selected and shuttle vector (pLG1484) is prepared using them. Then, it is identified that the shuttle
20 vector pLG1484 is replicable in various microorganisms and its replication stability is high.

To prepare the shuttle vector according to present invention, first, cryptic plasmid was isolated from *Rhodococcus erythropolis* IAM1484. As a result of searching for
25 restriction site of plasmid, restriction enzyme *EcoRI* site is discovered. So, cryptic plasmid and *E. coli* plasmid, pBluescript SK(-) were digested with *EcoRI* to clone into *E. coli*. And, DNA sequence of cryptic plasmid part in the cloned plasmid was determined using transposon, then, replication origin and replicase region assumed as an important region regarding replication are identified to obtain the
30 plasmid(pJW1484) containing an incidently obtained kanamycin tolerant gene.

For good transfer to a host and convenience of gene manipulation, shuttle vector pLG1484 was prepared by deleting parts that are not involved in replication in pJW1484 sequence.

- 5 The inventors examined a host range of pLG1484 for many kinds of the genus *Rhodococcus* strains and replication stability from *Rhodococcus erythropolis* LG12 to identify that the shuttle vector of present invention contained strong replication proteins and had high replication stability in various hosts.
- 10 The shuttle vector according to the present invention is useful to introduce a target gene into the genus *Rhodococcus* microorganism. Therefore, in another aspect, the present invention is related to a recombinant vector in which the target gene is operably linked to the shuttle vector and the genus *Rhodococcus* microorganism transformed with said recombinant vector.
- 15 The introduction of the target gene into the shuttle vector according to the present invention is achieved by the conventional method. To facilitate the introduction of the target gene into the shuttle vector, synthetic oligonucleotide adaptor or linker according to method can be used.
- 20 Also, to increase the expression of the target gene, expression control sequence can be added. In the present invention, "expression control sequence" means DNA sequence essential for the expression of encoding sequence linked operably in a certain host. This control sequence contains a promoter for transcription, arbitrary
- 25 operator sequence for the control of transcription, a sequence encoding proper mRNA ribosome binding region, and a control sequence of termination of transcription and translation.

- Nucleic acid is linked operably to other nucleic acids in case of arrangement of
- 30 functional relationship. This can be a gene and control sequence(s) linked by the

method enabling the gene expression when proper target gene is combined with control sequence(s). For example, when DNA for a pre-sequence or secretion leader is expressed as pre-protein participating in the secretion of polypeptide, it is linked operably to DNA for a polypeptide; and, if a promoter or an enhancer effects the transcription of sequence, it is linked operably to encoding sequence; or, if ribosome binding region effects the transcription of sequence, it is operably linked to an encoding sequence; or, if a ribosome binding region is arranged to facilitate the translation, it is operably linked to an encoding sequence. Generally, "operably linked" means that linked DNA sequence is in contact and, in case of secretion leader, it is in contact and exists in reading frame. However, an enhancer does not need to be in contact. The linkage of these sequences is conducted by ligation in convenient restriction enzyme site. If restriction enzyme site does not exist, synthetic oligonucleotide adaptor or linker is used by conventional method.

The recombinant vector in which the shuttle vector and a target gene according to present invention are operably linked, can be transformed with a proper host cell by method. Preferred host cell is the genus *Rhodococcus* bacterium. In the present invention the genus *Rhodococcus* bacteria that can be used are not particularly limited, but, it can be generally selected from the group consisting of *Rhodococcus coprophilus*, *R. equi*, *R. erythropolis*, *R. fascians*, *R. globerula*, *R. rhodnii*, *R. rhodochrous*, *R. ruber* and *R. rubrum*.

Examples

Hereinafter, the present invention will be described in more detail by examples. It is to be understood, however, that these examples are given to more fully describe the present invention and are not construed to limit the present invention.

Example 1: Isolation of cryptic plasmid derived from *Rhodococcus erythropolis*

IAM1484

In this example, the cryptic plasmid was isolated from *Rhodococcus erythropolis* IAM1484 (*Rhodococcus erythropolis* IAM1484; ACTC 15961). First, 5ml of *Rhodococcus erythropolis* IAM1484 strain was cultured in YEPD medium (bactopeptone 2%, yeast extracts 1%, glucose 2%) in test tube at 30 °C and 200rpm overnight, then cell pellet was obtained by centrifugation of cultures. The obtained cell pellet was washed twice with 500 μ l of TE buffer (Tris 10mM, EDTA 1mM, pH 8) to obtain pellet by centrifugation. The pellet was suspended with 500 μ l of TE buffer, and 1mg of lysozyme was added and suspended sufficiently, and then it was kept at 37 °C for 1hr to weaken the cell wall, followed by isolating and purifying plasmid (pIAM1484; FIG. 1) by the process disclosed in Molecular Cloning, Cold Spring Harbor Laboratory, 1989.

Example 2: Cloning of cryptic plasmid

To simplify the handling of cryptic plasmid, pIAM1484 isolated in Example 1 was combined with replication origin (ori) in *E. coli* and pBluescript SK(-) having an ampicillin resistant gene (FIG. 2). The precise location replication origin of cryptic plasmid was not known, however, one *Eco*RI restriction enzyme site existed in the present invention, so that one linear plasmid could be secured in the case of digesting cryptic plasmid with *Eco*RI restriction enzyme. And, it was ligated with pBluescript SK(-) plasmid which was digested with *Eco*RI restriction enzyme and was dephosphorylated. Then, about 8kb of plasmid was obtained by introduction of *E. coli*.

Example 3: Preparation of pJW1484

The whole sequence of cryptic plasmid was determined using EZ::TNTM <KAN-2> Insertion Kits of Epicentri corp. as disclosed in the prior art (Goryshin, I.Y. and Reznikoff, W.S., *J. Biol. Chem.*, 273: 7367, 1998). The colonies in which transposon was added at 500bp intervals in cryptic plasmid region were selected,

and they were transformed into *Rhodococcus erythropolis* LG12, respectively. Then, important region for replication was assumed using success or failure of transformation.

- 5 As a result, colony which could not be transformed was plasmids in which transposon is added at 300bp, 800bp, and 1300bp, respectively from start region of cryptic plasmid. By Blast search, 706~1877bp region of 1~2000bp region was assumed as a binding protein region and replicase replicating plasmid directly and 1~706bp region was identified as replication origin. The plasmid in which
10 transposon containing a kanamycin tolerant gene is located at 2kb from *EcoRI* site of cryptic plasmid was isolated, and this is named pJW1484 (FIG. 2).

- 1 μ g of isolated pJW1484 was introduced to *Rhodococcus erythropolis* LG12 (KCTC 18102P) using cuvet for electroporation, having 1mm gap at 1250 Volt,
15 25 μ F in Gene Pulser II System of Biorad.

Example 4: Preparation of pLG1484

- To minimize the size of a shuttle vector, sequences unrelated to replication of *E.*
20 *coli* and *Rhodococcus* was deleted among sequences of pJW1484 plasmid prepared in Example 3, and the assumed region related to replication and antibiotics resistance in *Rhodococcus* was selected. In other words, PCR was preformed using pJW1484 as a template with primers, SEQ ID NOs 2 and 3 to obtain about 3.3kb of DNA PCR products. The 5' of the primer was modified with phosphate.

- 25 Cryptic forward (forward primer) (SEQ ID NO: 2):

5-P-GACACATTTTCGACCGAAGGACATC-3

Kan reverse (reverse primer) (SEQ ID NO: 3):

5-P-CACGGTTGATGAGAGCTTTGTTGTAG-3

Also, PCR was performed using pJW1484 as a template with primers, SEQ ID NOs 4 and 5 to obtain the region related to replication and antibiotic tolerance in *E. coli*. As a result, 2.8kb of DNA fragment was obtained.

M13 forward RC (forward primer) (SEQ ID: 4):

5 5-ACTGGCCGTCGTTTTAC-3

M13 reverse RC (reverse primer) (SEQ ID: 5):

5-CATGGTCATAGCTGTTTCC-3

Each PCR product was blunt-ended using T4 DNA polymerase, ligated using T4 DNA ligase, and introduced to *E. coli*. The cloned 6153bp of plasmid was named pLG1484 (FIG. 3) and deposited to international deposition institute, KCTC on January 21, 2005. DNA sequence of pLG1484 is depicted in SEQ ID NO: 1.

Example 5: Replication stability of pLG1484

15

After *Rhodococcus erythropolis* LG12 cells containing pLG1484 prepared in Example 4 was cultured in YEPD medium containing 40 μ g/mL of kanamycin until late exponential phase, the cells in 1:100 dilution were inoculated in 50mL of liquid YEPD medium without antibiotics and cultured at 30°C. 24 hours later, the cells were again diluted 100times and inoculated in 50mL of liquid YEPD medium without antibiotics. As repeated, cells of each step are plated on solid YEPD medium without antibiotics, and generation time is measured by colony counting.

After 100 colonies produced from the solid media were inoculated in solid YEPD medium without antibiotics and cultured for 24hours, each colony was inoculated in solid YEPD medium containing 40 μ g/mL of kanamycin. Then, the replication stability of pLG1484 was examined by measuring the number of kanamycin-sensitive colonies among the 100 colonies (FIG. 4).

As a result, as illustrated in FIG. 4, *Rhodococcus erythropolis* LG12 cells containing pLG1484 according to the present invention showed excellent replication stability without selection by antibiotics. About 0.22% of whole cells per one reproduction in medium excluding antibiotics were cells without shuttle vector pLG1484. As compared to the report that the pMVS301 plasmid in *Rhodococcus* sp. AS-50-1 (Vogt Singer & Finnerty, *J. Bacteriol.*, 170:638, 1998) and pK4 plasmid in *Rhodococcus rhodochrous* ATCC 12674 (*Proc. Natl. Acad. Sci. USA*, 93(20):10572, 1996) were deactivated at the rate of 1~1.5%, it was identified that pLG1484 according to the present invention has high replication stability.

Example 6: Host range of pLG1484

Rhodococcus globerulla (KCCM 40036), *Rhodococcus rhodochrous* (KCCM 40120), *Rhodococcus equi* (KCCM 12541) and *Rhodococcus rubber* (KCCM 41053) obtained from KCCM (Korea Culture Center of Microorganism) were transformed with pLG1484, respectively, so that host range of *Rhodococcus-E. coli* shuttle vector pLG1484 was identified. After each cell was cultured, mixed with 1 μ g of recombinant plasmid pLG1484 and conducted transformation using cuvet for electroporation, having 1mm gap in the condition of 1250 Volt, 25 μ F. The transformant was determined by acquisition of resistance to 50 μ g/mL of kanamycin.

Table 1

Host cells	Transformants/1 μ g pLG1484DNA
<i>R. erythropolis</i> LG12	4.3 x 10 ⁴
<i>R. equi</i> KCCM 12541	3.2 x 10 ⁵
<i>R. globerulla</i> KCCM 40036	2.6 x 10 ⁴
<i>R. rhodochrous</i> KCCM 40120	0
<i>R. rubber</i> KCCM 41053	0

As a result, as illustrated in Table 1, pLG1484 according to the present invention has broad host range because it can be replicated in *Rhodococcus equi*, *Rhodococcus*

globerulla, and *Rhodococcus erythropolis* etc. whose genealogy are in the far distance. Transformation efficiency of *Rhodococcus equi* was 7 times greater than that of *Rhodococcus erythropolis*, and that of *Rhodococcus globerulla* was half of that of *Rhodococcus erythropolis*. And there was no transformation in case of
5 *Rhodococcus Rhodochrous* and *Rhodococcus Rubber*. From these results, it is identified that the shuttle vector pLG1484 according to the present invention has broad host range compared with pAN12 (WO 02/055709A2), pDA71 (Dabbs E.R. *et al.*, *Plasmid*, 23:42, 1990), and pFAJ2600 (Demot *et al.*, *Microbiol.*, 146:3137, 1997) which are already developed shuttle vectors for *Rhodococcus*.

10

While the present invention has been described in detail with reference to the specific features, it will be apparent to those skilled in the art that this description is only for a preferred embodiment and does not limit the scope of the present invention. Thus, the substantial scope of the present invention will be defined by
15 the appended claims and equivalents thereof.

INDUSTRIAL APPLICABILITY

20 As described more in detail, the present invention has an effect to provide a shuttle vector which can replicate bidirectionally in *E. coli* and *Rhodococcus*, and a recombinant vector in which the target gene is operably linked to the shuttle vector, and a microorganism transformed with the recombinant vector. The shuttle vector according to the present invention has many advantages upon gene manipulation
25 due to relatively small size and contains strong replication proteins. Also, it secures stability in fermentation process or biotransformation process because it can be replicated sufficiently in medium without antibiotics due to its high replication stability in *Rhodococcus*. In addition, it has a broad host range for many kinds of the genus *Rhodococcus*, so it is useful for the cloning of target genes derived from
30 the genus *Rhodococcus* and various cells.

THE CLAIMS

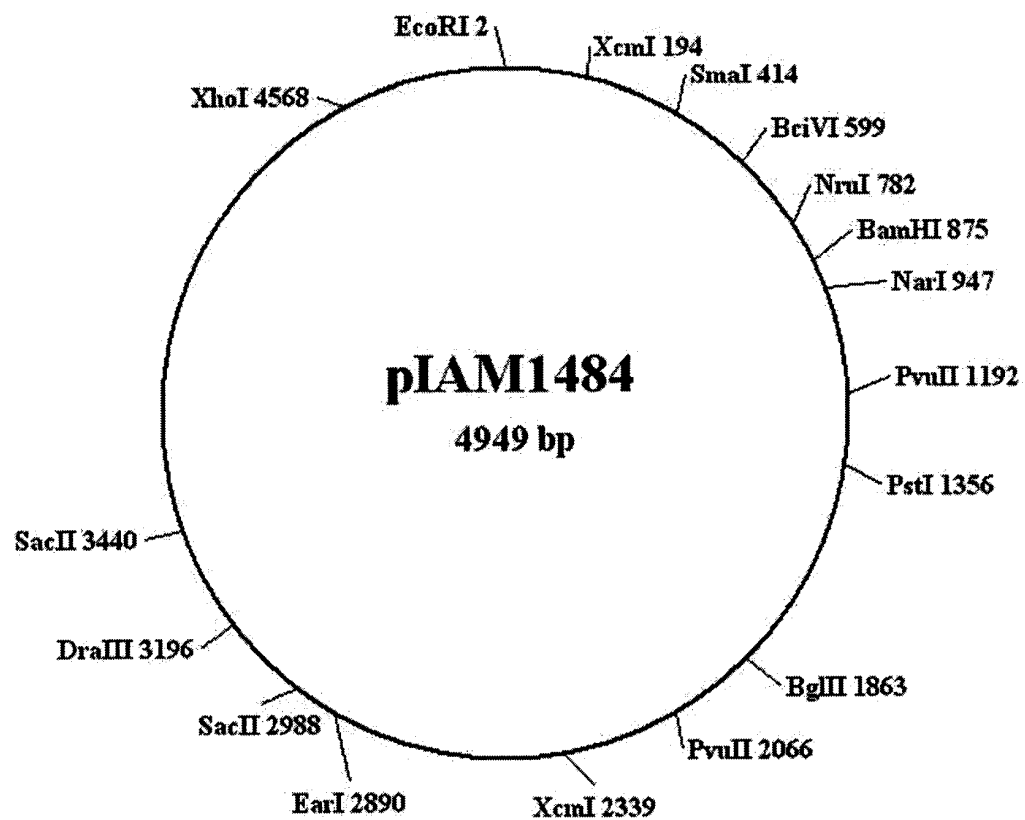
What is claimed is:

- 5 1. A *Rhodococcus-E. coli* shuttle vector comprising a DNA replication origin derived from *Rhodococcus erythropolis*, a DNA replication origin derived from *E. coli*, an ampicillin resistant gene as a selection marker expressed in *E. coli*, and a kanamycin resistant gene as a selection marker in the genus *Rhodococcus*.
- 10 2. The *Rhodococcus-E. coli* vector according to claim 1, wherein the DNA replication origin derived from *Rhodococcus erythropolis* is derived from plasmid of *Rhodococcus erythropolis* IAM1484.
3. The *Rhodococcus-E. coli* shuttle vector according to claim 2, wherein the
15 plasmid is pIAM1484.
4. The *Rhodococcus-E. coli* shuttle vector according to claim 1, wherein the shuttle vector is pJW1484.
- 20 5. The *Rhodococcus-E. coli* shuttle vector according to claim 1, wherein the shuttle vector having at least 95% homology to a nucleotide sequence depicted in SEQ ID NO:1.
6. The *Rhodococcus-E. coli* shuttle vector according to claim 5, wherein the shuttle
25 vector is pLG1484.
7. A recombinant vector, in which a target gene is operably linked to the pJW1484 shuttle vector of claim 4.
- 30 8. A microorganism transformed with the recombinant vector of claim 7.

9. The transformed microorganism according to claim 8, wherein the microorganism is the genus *Rhodococcus*.
10. The transformed microorganism according to claim 9, wherein the genus
5 *Rhodococcus* microorganism is selected from the group consisting of *Rhodococcus*
coprophilus, *R. equi*, *R. erythropolis*, *R. fascians*, *R. globerula*, *R. rhodnii*, *R.*
rhodochrous, *R. ruber* and *R. rubrum*.
11. A recombinant vector, in which a target gene is operably linked to the pLG1484
10 shuttle vector of claim 6.
12. A microorganism transformed with the recombinant vector of claim 11.
13. The transformed microorganism according to claim 12, wherein the
15 microorganism is the genus *Rhodococcus*.
14. The transformed microorganism according to claim 13, wherein the genus
Rhodococcus microorganism is selected from the group consisting of *Rhodococcus*
coprophilus, *R. equi*, *R. erythropolis*, *R. fascians*, *R. globerula*, *R. rhodnii*, *R.*
20 *rhodochrous*, *R. ruber* and *R. rubrum*.

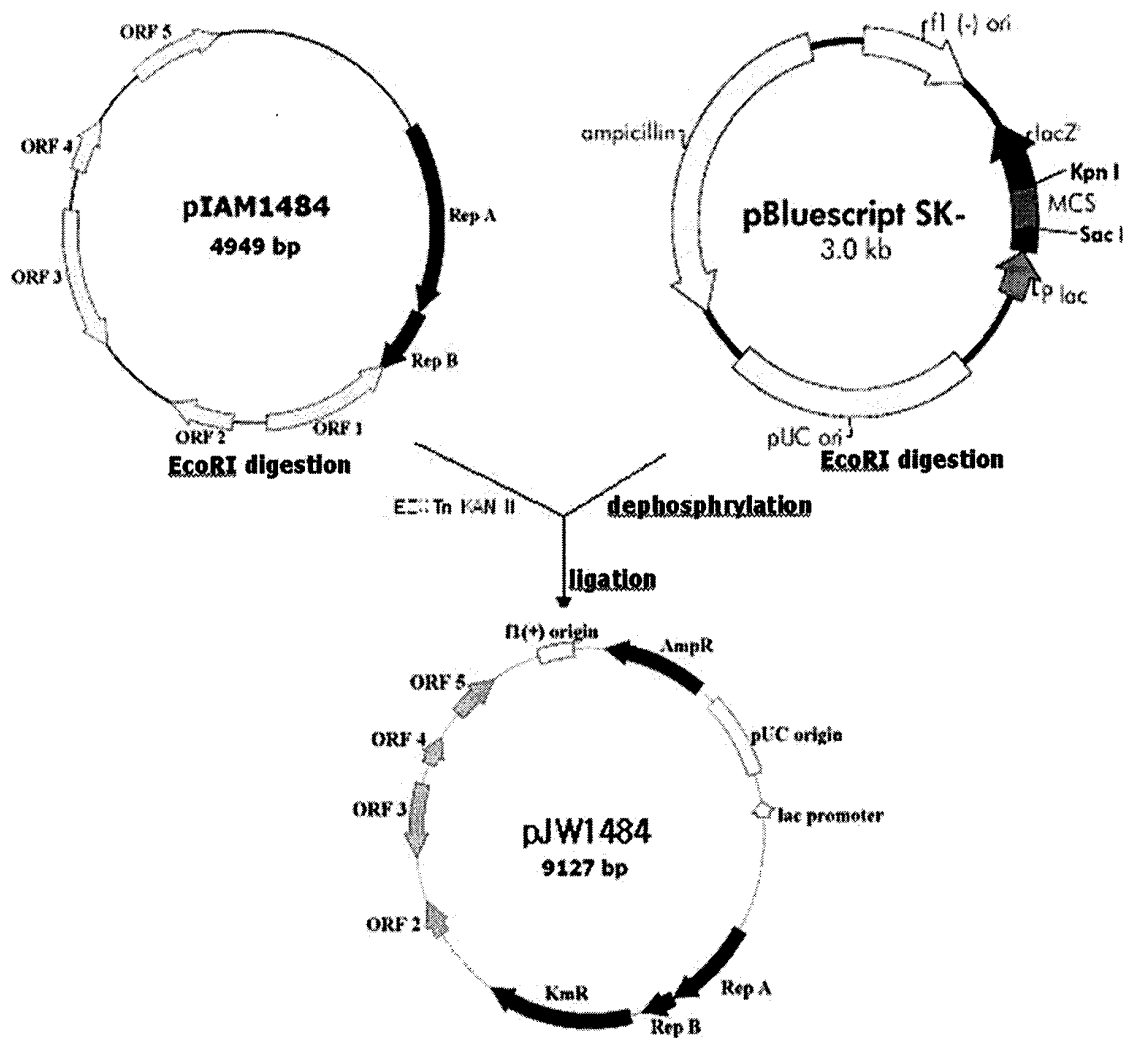
1/4

FIG. 1



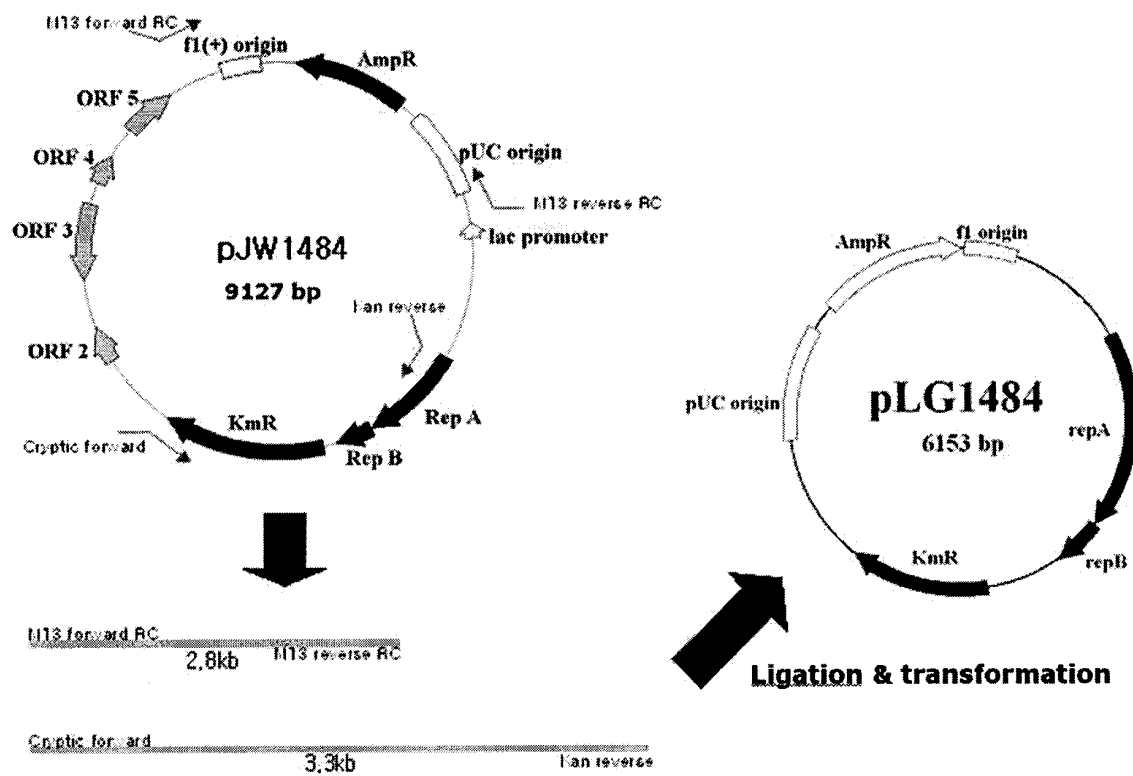
2/4

FIG. 2



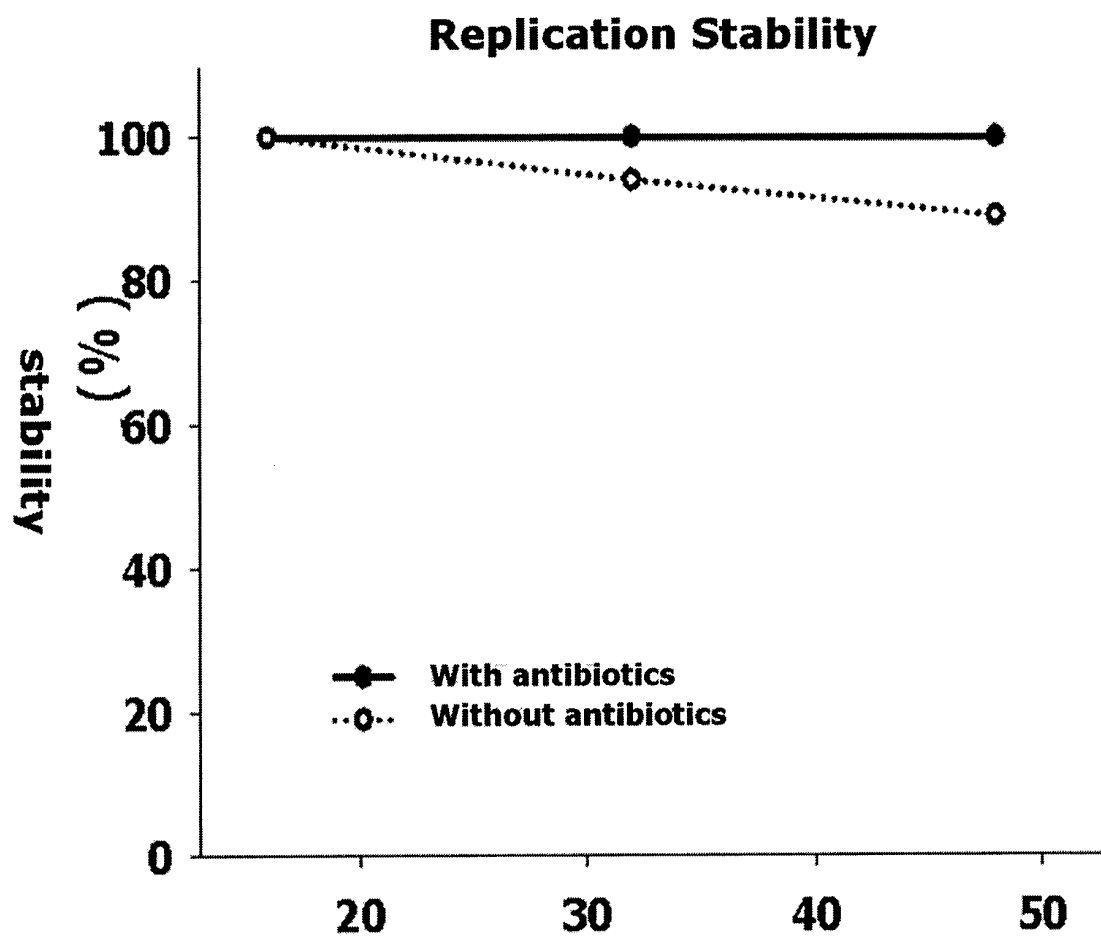
3/4

FIG. 3



4/4

FIG. 4



PCT

Print Out (Original in Electronic Form)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT-SAFE Version 3.51.001.176 MT/FOP 20060101/0.20.4rc.2.7
0-2	International Application No.	PCT/KR2006/000521
0-3	Applicant's or agent's file reference	PP-B0172

1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	paragraph number	15
1-3	Identification of deposit	
1-3-1	Name of depositary institution	KCTC Korean Collection for Type Cultures
1-3-2	Address of depositary institution	52, Oun-dong, Yusong-Ku, Taejon 305-333, Republic of Korea
1-3-3	Date of deposit	21 January 2005 (21.01.2005)
1-3-4	Accession Number	KCTC 10770BP
1-5	Designated States for Which Indications are Made	AP: (BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW)EA: (AM AZ BY KG KZ MD RU TJ TM)EP: (AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR)OA: (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG)AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KN KP KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/000521**A. CLASSIFICATION OF SUBJECT MATTER***C12N 15/74(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 C12N 15/74, C12N 15/70, C12N 15/09, C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and Applications for Inventions since 1975

Korean Utility Models and Applications for Utility Models since 1975

Japanese Utility Models and Applications for Utility Models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS, WPI, USPTO, PAJ, NCBI, CAPLUS(STN), INSPECT "shuttle vector, Rhodococcus, E. coli, Escherichia coli, origin of replication, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 20040115661 A1 (Bramucci, et al., US) 17.06.2004	1
Y	- see [0005]~[0018], [0073]~[0075], [0077] & [0101]; Figs 1-3; claims	2-14
X	US 20030044807 A1 (Bramucci, et al., US) 06 Mar 2003	1
Y	- see abstract, Figs. and claims (no patent family)	2-14
Y	Appl Environ Microbiol. Vol.70(9):5557-68 (Nakashima, N., et al., JP) Sep 2004 - see abstract, Fig. 5, Table 1 and right col. of p. 5561 (pTip-QT1: GenBank accession No. AB127592.1)	2-14
A	WO 04033633 A2 (Embiosis Pharm. US) 22 Apr 2004 - see abstract, Figs. and claims	1-14
A	US 4920054 A (Kozlowski et al., CA) 24 Apr 1990 - see abstract, Figs. and claims (no patent family)	1-14

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 MAY 2006 (22.05.2006)

Date of mailing of the international search report

23 MAY 2006 (23.05.2006)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

SHIN, Weon Hye

Telephone No. 82-42-481-5591



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2006/000521

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4952500 A (Fimmerty, et al., US) 28 Aug 1990 - see abstract, Figs. and claims (cited in application)	1-14
A	JP 08056669 A2 (Sekiyu Sanyo, JP) 05 Mar 1996 - see abstract, Figs. and claims (cited in application)	1-14
A	KR 1019990048213 A (KAIST, KR) 05 Jul 1999 - see abstract, Figs. and claims (cited in application)	1-14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2006/000521

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



on paper



in electronic form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in electronic form



furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2006/000521

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 20040115661 A1	17 Jun 2004	CA 2426597 AA DE 60113448 C0 EP 1337645 A2 JP 16517623 T2 KR 1020030052242 A WO 2002055709 A2	18 Jul 2002 20 Oct 2005 27 Aug 2003 17 Jun 2004 26 Jun 2003 18 Jul 2002
WO 04033633 A2	22 Apr 2004	AU 2003275416 A1	04 May 2004
US 4952500 A	28 Aug 1990	WO 8907151 A1	10 Aug 1989