

FIRST EFFECTIVE SERIES OF CLONING VECTORS FOR TRANSFORMING DIFFERENT SPECIES OF AMYCOLATOPSIS

Biotech Consortium of India (BCIL) is seeking companies interested in commercializing a technology used for A Process for the development of cloning vector. Scientist at the University of Delhi have developed a series of cloning vector called as pRL series to facilitate genetic manipulations of a various species of Amycolatopsis such as *A. mediterranei* and *A. orientalis* and a process for the preparation, efficient transformation through electroporation and selection using different antibiotic pressures of the said series of cloning vectors.

Introduction:

It is known that several species and strains of the genus Amycolatopsis synthesize compounds which are produced on an industrial scale including extracellular enzymes such as amylases, cellulases and proteases as well as secondary metabolites such as antibiotics and other pharmacologically active molecules. *A. mediterranei* and *A. orientalis* are known to produce rifamycins and vancomycins respectively. A recently introduced glycopeptide antibiotic Balhimycin which exhibits antibacterial activity against methicillin resistant *Staphylococcus aureus* strains has been isolated from Amycolatopsis sp. Y-86 21022DSM 5908.

In addition, species of Amycolatopsis along with other closely related genera of the order Actinomycetales form the third major group of bacteria in terms of sales of antibiotic production. These bacteria until recently were not accessible for recombinant DNA techniques although methods of gene cloning have been developed for several species of Streptomyces which also belongs to the same order (Actinomycetales). This was mainly due to the lack of any plasmid, suitable for vector development in Amycolatopsis. Furthermore, standard transformation procedures as used in Streptomyces spp. are not applicable to these organisms and conjugation was the only technique for the introduction of DNA into these organisms.

This invention has, independent of Streptomyces cloning vectors, brought within the scope of Recombinant DNA technology, several industrially important actinomycetes.



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Salient Features:

Prof. Rup Lal, University of Delhi, South Campus succeeded in developing an effective series of cloning vectors with suitable marker genes and method of electroporation for the efficient transformation of the different species and strains of *A. mediterranei*. **U.S. Patent has been granted for the technology titled as ‘Cloning vector and a process for preparation thereof’ (US Patent No. 5,985,560).**

The process primarily requires the generation of unstable plasmid (concatamer) in *E. coli* and then transformation of these unstable plasmids in *A. mediterranei* through electroporation and selection of transformants containing the cloning vector under different antibiotic pressure. The DNA molecule comprising the gene whose expression is to be obtained in cells of an Amycolatopsis species is inserted into the cloning vector, the gene being operably linked to DNA transcription regulatory elements which direct transcription in cells of an Amycolatopsis species. The vector comprising the gene to be expressed is introduced into cells of an Amycolatopsis species resulting in the expression of the desired gene. The various vectors of the series that have been developed and their features vectors are listed below:

pRL Series of Vectors

	Vector	Size	Features
	(Selection markers and origin of replication)		
1.	pRL50	18.7 kb	two copies each of km/neo, .alpha.-amy, pA-rep, and pBR-ori, and one copy of ermE.
2.	pRL60	10.2 kb	km/neo, ermE, .alpha.-amy, pA-rep, and pBR-ori.
3.	pRL80	15.2 kb	two copies each of km/neo, pA-rep, am, and pBR-ori, and one copy of ermE
4.	pRL81	8.5 kb	am, km/neo, ermE, pA-rep, and pBR-ori.
5.	pRL82	6.8 kb	am, km/neo, pA-rep, and pBR-ori.
6.	pRL51	5.0 kb	km/neo, pA-rep and pBR-ori.
7.	pRL53	7.1 kb	km/neo, ermE, pA-rep, and pBR-ori.



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Thus, it is evident that the pRL series has several salient features which make it a potential tool for the genetic manipulations of the various strains of *Amycolatopsis*, the most important ones have been highlighted below:

1. Plasmid size ranges from 5 kb to 18.7 kb.
2. Each cloning vector has two origins of replication-pBR-ori, pA-rep.
3. Each cloning vector has any of the combination of the markers: .alpha.- amylase, ermE, km and amp.
4. A wide range of markers including .alpha.-amy, ermE and am function effectively in *A. mediterranei* and related strains.
5. alpha.-amylase can be used as morphological marker for the selection of transformants.
6. Cloning in .alpha.-amylase gene can lead to the direct selection of recombinant clones after iodine staining in *Amycolatopsis mediterranei*.

All these cloning vectors can be transformed through electroporation in different strains of *A. mediterranei*. The transformation protocol through electroporation for different strains of *A. mediterranei* has also been optimized. By using optimum conditions a transformation efficiency of 1×10^5 transformants/.mu.g of DNA can be obtained. These plasmids have a very broad host range as they are functional in almost all species of *Amycolatopsis* tested so far. The species of *Amycolatopsis* producing a variety of rifamycins that have been tried and tested are given at **Annex 1**.

The features stated above make pRL series an ideal series of cloning vectors which can be suitably used in the cloning, characterization and manipulation of genes involved in the synthesis of antibiotics, restriction enzyme and amylases, proteases or other useful products from these organisms, avoiding the major disadvantages associated in the previously known gene cloning methods for the same as described earlier.



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Host Range Of pRL1-Series Of Cloning Vectors

S.No	Organisms	Strain	Characteristic
1.	A. mediterranei	*DSM 40773	Rifamycin B
2.	A. mediterranei	DSM 43304	Rifamycin SV
3.	A. mediterranei	DSM 46095	Rifamycin SV
4.	A. mediterranei	DSM 46096	Rifamycin derivatives
5.	A. mediterranei	**MTCC 14	Rifamycin B
6.	A. mediterranei	MTCC-17	Rifamycin SV
7.	A. mediterranei	W-2800 ans-12	Rifamycin W
8.	A. mediterranei	T-208 ans-11	Rifamycin W (purine pur-20 auxotroph)
9.	A. mediterranei	T-206 ans-13 leu-1 str.2	Protorifamycin (leucine auxotroph, streptomycin resistance)
10	A. mediterranei	F.sub.1/24 ans-13	Protorifamycin
11	A. mediterranei	T-195 ans-13	Protorifamycin thi-8 (thiamine auxotroph)
12	A. mediterranei	S2802 ans-4	Rifa S (histidine his-3 auxotroph)
13	A. mediterranei	S2804 ans-4	Rifa S (serine ser-5 auxotroph)
14	A. orientalis	DSM 40040	Vancomycin

About BCIL:

BCIL was incorporated as public limited company in 1990 under the Indian Companies Act 1956. It is promoted by the Department of Biotechnology, Government of India and is financed by several all India financial institutions, venture capital funds and the corporate sector. BCIL has been actively involved in technology transfer, project consultancy, fund syndication, information dissemination, and manpower training & placement related to biotechnology over the last decade and half. BCIL has transferred more than 15 technologies in the last 5 years using its expertise in facilitating licensing agreements that allow healthy and productive cooperation between the inventor and the licensee.
