Brazilian Journal of Microbiology (2009) 40: 778-781
ISSN 1517-8382

NEW VECTORS DERIVED FROM pUC18 FOR CLONING AND THERMAL-INDUCED EXPRESSION IN *Escherichia coli*

Mauro Aparecido Souza Xavier[1]; André Kipnis[2]; Fernando Araripe Gonçalves Torres[1]; Spartaco Astofi-Filho[3*]

[1]Universidade de Brasília, Brasília, DF, Brasil. [2]Universidade Federal de Goiás, Goiânia, GO, Brasil. [3]Universidade Federal do Amazonas, Manaus, AM, Brasil.

Submitted: June 26, 2008; Returned to authors for corrections: March 22, 2009; Approved: June 28, 2009.

ABSTRACT

We report the construction of two vectors for *Escherichia coli*: pUC72, for molecular cloning, and pPLT7, for thermal-induced expression. The main feature of pUC72 is a novel polylinker region that includes restriction sites for *Nde* I and *Nco* I which provide an ATG codon for proper translation initiation of expressed genes. Vector pPLT7 is ideal for thermo-inducible expression in host cells that carry the cI857 repressor gene. The use of pPLT7 was validated by the successful expression of the genes encoding carp and porcine growth hormones. These vectors provide novel cloning possibilities in addition to simple, non-expensive, high level expression of recombinant proteins in *E. coli*.

Key words: Heterologous expression, *Escherichia coli*, molecular cloning, induced expression.

Vectors pUC18 and pUC19 are small high-copy number plasmids that are widely used for cloning and manipulation of DNA fragments (9). One important feature of these plasmids is the presence of a multiple cloning site (MCS) within the coding region of the *lacZα* fragment which allows the detection of cells harboring recombinant plasmids by their inability to cleave the chromogenic substrate X-Gal (7). In order to facilitate the subcloning and transfer of cloned DNA sequences from pUC plasmids to other vectors we have modified the original MCS of pUC18 by introducing new restriction sites. First, the *Nde* I restriction site present in pUC18 was deleted after digestion of this plasmid with this enzyme following treatment with the Klenow enzyme and ligation with T4 DNA ligase. The resulting plasmid, named pUC28, was digested with *Xba* I and *Hind* III and then ligated to annealed synthetic oligonucleotides containing new restriction sites. The resulting vector, pUC72, contains the following novel restriction sites: *Bgl* II, *Nde* I and *Nco* I (Figure 1A). The reading frame of the *lacZα* fragment was retained allowing the screening of recombinant plasmids by the white/blue selection on plates containing X-Gal. In pUC72 we introduced the *Nco* I (5'-CCATGG-3') and *Nde* I (5'-CATATG-3') restriction sites because they contain a methionine codon (ATG) which can be used for proper translation initiation. This is a desirable feature not commonly found in pUC-derived vectors which allows inserts containing these sites at the 5′-ends to be readily cloned into this vector without the addition of extra amino acids at the N-terminus of the expressed protein. Furthermore, genes cloned into pUC72 are less likely to be

***Corresponding Author. Mailing address:** Universidade Federal do Amazonas, Centro de Apoio Multidisciplinar - Bloco G, Campus Universitário, Bairro Coroado, Manaus, AM, CEP 69077-000, Brazil.; E-mail: sastolfi@ufam.edu.br

translated because the cloning orientation of the insert does not allow in-frame fusion with the *lacZ*α gene. This is particular relevant when the cloned gene codes for toxic or environmentally dangerous proteins.

Most commercially available expression vectors require the synthetic IPTG inducer to promote transcription from the pLac promoter. These vectors are generally derived from pUC plasmids because of their high-copy number - a requirement for high level expression of recombinant proteins in *E. coli*. In this work we sought the construction of a bacterial expression vector based on the strong lambda phage pL promoter (3). First, a ~300 pb fragment containing the lambda phage pL promoter and phage T7 gene 10 Shine Dalgarno was amplified by PCR from pPLT4 (1) using primers O123 (5'-GAATTCCATATGTATATCTCCTTCTTAAAG-3') and O122 5'-GCGGAATTCCTACCAAACAATGCCCCCT-3'). The amplicon was digested with *Eco*R I and cloned into *Eco*R I-digested pUC28 resulting in vector pPLT7 (Figure 1B).

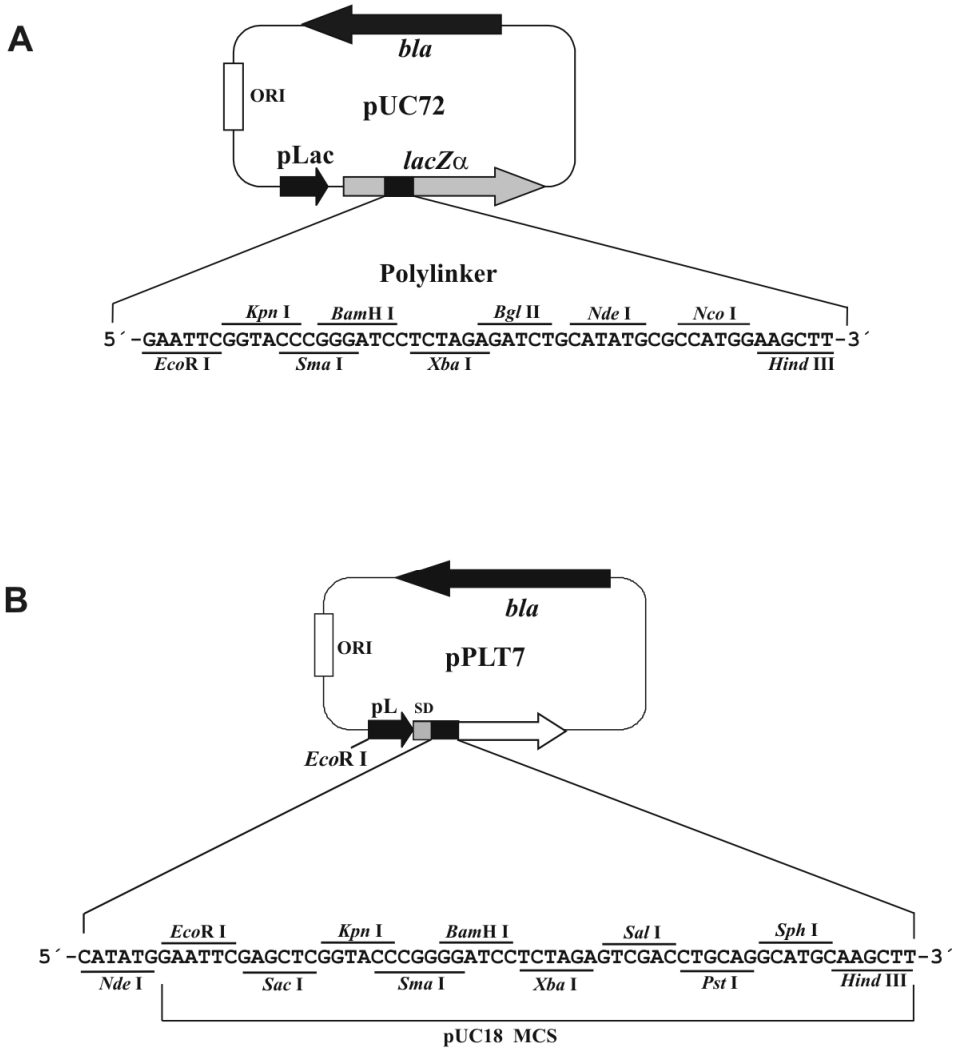

Figure 1. General features of pUC72 (A) and pPLT7 (B). Only relevant restrictions sites are shown. MCS = multiple cloning site; SD = Shine Dalgarno region.

The lambda phage pL promoter is negatively controlled by a repressor coded by the cI gene. A mutant form of cI (cI857) renders the repressor inactivate at temperatures above 37 °C. This behavior has been exploited in the establishment of an approach for controlled expression in *E. coli* based on growth temperature (2, 6, 8). The use of cI857 for regulated expression from the pL promoter is of great interest for industrial purposes since it offers an inexpensive and easy way to control the expression of recombinant proteins in *E. coli*. Recommended *E. coli* hosts for pPLT7 include N4830-1 and M5219 which contain the cI857 gene integrated in the genome (2, 4). The use of pPLT7 was tested in *E. coli* for the expression of two commercially important proteins: carp and porcine growth hormones. PCR-amplified fragments for both cDNA´s were cloned as *Nde* I-*Bam*H I fragments into pUC72 and then transferred to pPLT7 digested with the same enzymes (data not shown). The resulting plasmids were used to transform N4830-1 or M5219 *E. coli* cells. Host cells transformed with these plasmids were incubated at 30 ^{o}C for 4 hours before shifting to the induction temperature (40-42 ^{o}C). Samples (1 mL) were collected at different time points and after centrifugation pellets were washed with saline 0.9% and ressuspended in lysis buffer 2X (5). Figure 2 shows the protein expression profiles of two different *E. coli* strains transformed with pPLT7-derived constructs. In both cases, high level expression of recombinant carp and porcine growth hormones was achieved after a thermal shift and the heterologous proteins had the expected molecular mass (~22 kDa).

pUC-derived vectors have a spread use in molecular biology because they exhibit high copy number, genetic stability and multiple cloning sites for DNA manipulations. The plasmids described in this work provide novel additional features which may reveal useful for the manipulation of cloned DNA fragments and expression of recombinant proteins in *E. coli*.

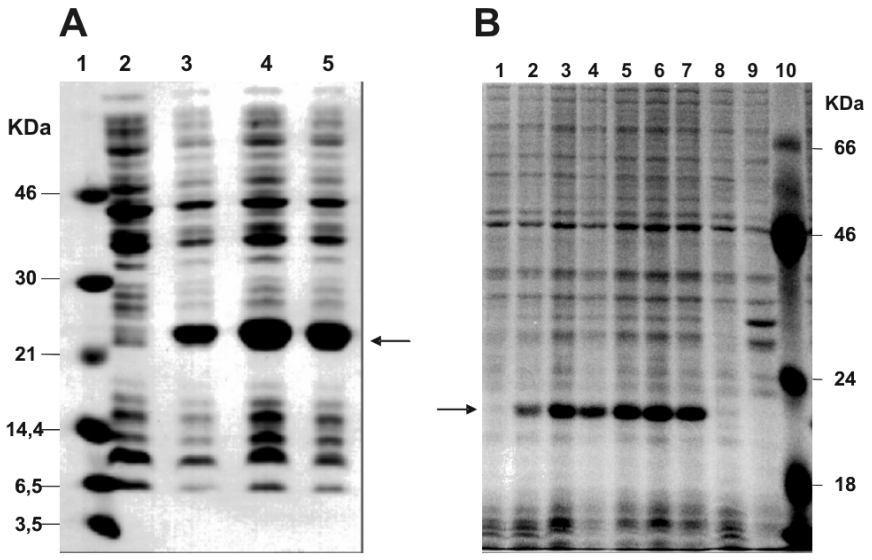

Figure 2. Protein expression profiles of *E. coli* cells harboring pPLT7 with cDNA sequences for carp (cGH) and porcine (pGH) growth hormones. (A) SDS-PAGE 17.5%. Lane 1, MW marker; lane 2, *E. coli* N4830-1 cells harboring cGH before thermal induction; lanes 3-5, samples collected 1, 2 and 3 hours after thermal induction at 42 ^{o}C, respectively. (B) SDS-PAGE 12.5 %. Lane 1: *E. coli* M5219 cells harboring pGH before thermal induction; lanes 2-7, samples collected after intervals of 30 minutes until 3 hours after thermal induction at 40 ^{o}C; lanes 8 and 9, *E. coli* M5219 transformed with pPLT7 before and after thermal induction (40 ^{o}C), respectively; lane 10, MW marker. The arrows indicate the position of induced proteins (~22 kDa).

ACKNOWLEDGMENTS

André Kipnis was sponsored with a fellowship from CNPq (Brazil).

REFERENCES

1. Astolfi-Filho, S.; Lima, B.D.; Thiemann, J.E.; de Sousa, H.R.T.; Vilela, L. (2000). Vector for expression of heterologous protein and methods for extracting recombinant protein and for purifying isolated recombinant insulin. U.S. Pat. 6.281.329.
2. Chao, Y.P.; Wen, C.S.; Wang, J.Y. (2004). A facile and efficient method to achieve LacZ overproduction by the expression vector carrying the thermoregulated promoter and plasmid copy number. *Biotechnol. Prog*. 20:420-425.
3. Deuschele, U.; Kammeru, W.; Gentz, R.; Bujard, H. (1986). Promoters of *Escherichia coli*: a hierarchy of *in vivo* strength indicates alternated structures. *EMBO J*., 5:2987-2994.
4. Gottesman, M.E.; Adhya, S.; Das, A. (1980). Transcription antitermination by bacteriophage lambda N gene product. *J. Mol. Biol*., 140:57-75.
5. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227:680-683.
6. Remaut, E.; Stanssens, P.; Fiers, W. (1981). Plasmid vectors for high-efficiency expression controlled by the P_L promoter of coliphage lambda. *Gene*, 15:81-93.
7. Ullmann, A.; Jacob, F.; Monod, J. (1967). Characterization by *in vivo* complementation of a peptide corresponding to an operator proximal segment of the β-galactosidase structural gene of *Escherichia coli*. *J. Mol. Biol*., 24:339-343.
8. Wang, H.; McConnell, D.J.; O´Mahony, D.O. (1990). An efficient temperature-inducible vector incorporating the T7 gene 10 translation initiation leader region. *Nucleic Acids Res*., 18:1070.
9. Yanish-Perron, C.; Vieira, J.; Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, 33:103-119.