Punjab Univ. J. Zool., Vol. 24 (1-2), pp. 61-66, 2009

RECONSTRUCTION OF HIGH EXPRESSION VECTOR PK223-3

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Abstract: Vector pBR223-3 is reported as high expression vector having *tac* promoter. In this study linear pK223-3 was ligated with multiple cloning site of pUC19 cut at *Eco*RI and *Hind* III sites in order to recircularize it. Both vector and insert were double digested with enzymes, ligated, transformed and checked for the circular vector. Resulting Plasmid pKK223N was circular 4.5 Kb in size having *tac* promoter having two antibiotic resistant gene ampicillin and tetracycline.

Keywords: tac promotor, EcoRI, Hind III, ampicillin, tetracycline

INTRODUCTION

High Expression Vector pK223-3 was provided by Prof Dr Mohammad Akhtar from one of his colleagues at Southampton, UK in the form of linear plasmid (10ng) PstI/SalI precut. It was a 4585bp high expression vector with *tac* promoter and ampicillin and tetracycline antibiotic resistance sites. For cloning and expression in this vector a control should be a circular pBR223-3. Unfortunately this was not available now a day that's why the only choice remains was to design a strategy to re-circularize the open vector. In this study pK223-3 high expression vector was recovered and propagated by ligation with an *Eco*RI and *Hind* III cut insert and transformation using dH5α.

One can design linkers with multiple cloning sites and ligate with linear plasmid. But it was time consuming and expensive. Hence another strategy was designed to circularize the linear pBR223-3 vector in which Multiple Cloning Site (MCS) was cut from plasmid pUC19 and ligated with linear pBR223-3.

0079-8045/09/0061-0066 $ 03.00/0

MATERIALS AND METHODS

Restriction Digestion

Linear Plasmid and pUC19 was double digested with EcoRI and Hind III. Reaction was set as follows:

10X buffer	4 µl
EcoRI	1 µl (10 U)
Hind III	1 µl (10 U)
DNA	9 µl
Water	to make volume 40 ml.

Reaction was incubated at 37˚C for 1.5 hours. Enzymes were inactivated for 10 minutes at 70˚ C.

Ethanol Precipitation for short fragments

To 10µl of double digestion mix, 30µl of 95% ethanol and 1.6µl of 3M sodium acetate (0.12M) was mixed and placed on ice for 10-15 minutes. DNA short fragment was collected by centrifugation for 30 min at 4˚ C at 13000rpm. Invisible pellet (short fragment DNA) was washed with 80% ethanol to get rid of salt. It was centrifuged at 4˚ C for 5 min at 13000 rpm, air dried and dissolved in 20 µl of deionized water and used for ligation (Abbas, 2001).

Ligation

The digested pBR223-3 plasmids and pUC19 were added to ligation mixture in 1:3 ratio. The following ligation reaction was set up:

Ligase 10X buffer	3 µl
Vector	10 µl (10 pmol)
Insert	5 µl (30 pmol)
T4 DNA ligase	1 µl (3 units)
BSA	1 µl
Water	to make volume 30 ml.

The contents were mixed by finger flicking, centrifuged briefly and then incubated at 4 °C overnight.

Transformation

Ligated DNA was transformed in dH5α using heat shock method as follows: Ligation reaction (20 µl)was added to an eppendorf tube containing 50 µl of competent cells and stored on ice for 20 minutes then heat shocked at 42 °C for 2 minutes and added 1 ml of LB medium. The tube containing the transformation reaction was incubated at 37 °C for 1 hour in incubator shaker and reaction was plated onto LB-Ampicillin plates.

Miniplasmid Preparation

Plasmid preparation was performed according to Sambrook (1989) with some amendments. Overnight grown 1.5ml broth culture was centrifuged for 10 min at 4000rpm. Clear medium was discarded completely and the bacterial cell pellet was mixed with 100µl of Lysis solution I and placed at room temperature for 5 minutes. Freshly prepared Lysis solution II (200 µl) was added mixed with finger flicking and incubated on ice for 5 min. After this 150 µl of solution III was added, mixed by vortexing and placed on ice for 5 min.. I t was centrifuged for 15 min at 14000rpm to separate cell debris. To the clear supernatant 800µl of absolute ethanol was added and placed at room temperature for 2-5 min. Nucleic acid pellet was collected by centrifugation for 15 min at 14000rpm. Pellet was washed twice with 70% ethanol, air dried and dissolved in 30µl of TE. Plasmid DNA (5 µl) was used for restriction digestion.

Agarose Gel Electrophoresis

Plasmid DNA and digested DNA was analyzed on agarose gel electrophoresis as described in Sambrook (1989).

RESULTS AND DISCUSSION

Vector pBR223-3 is reported as high expression vector having tac promoter that is regulated by lac repressor and is independent of cAMP, its regulation is mediated by the crp gene product. We got linear pKK223-3 PstI/SalI precut in very low concentration (10ng in 10µl) hence it was first cut with EcoRI and Hind III and ligated with a 0.5 kb EcoRI/HindIII cut insert in order to recover and manipulate it (Abbas and Shakoori, 2005).

M	2	3	4	5	6	7	8

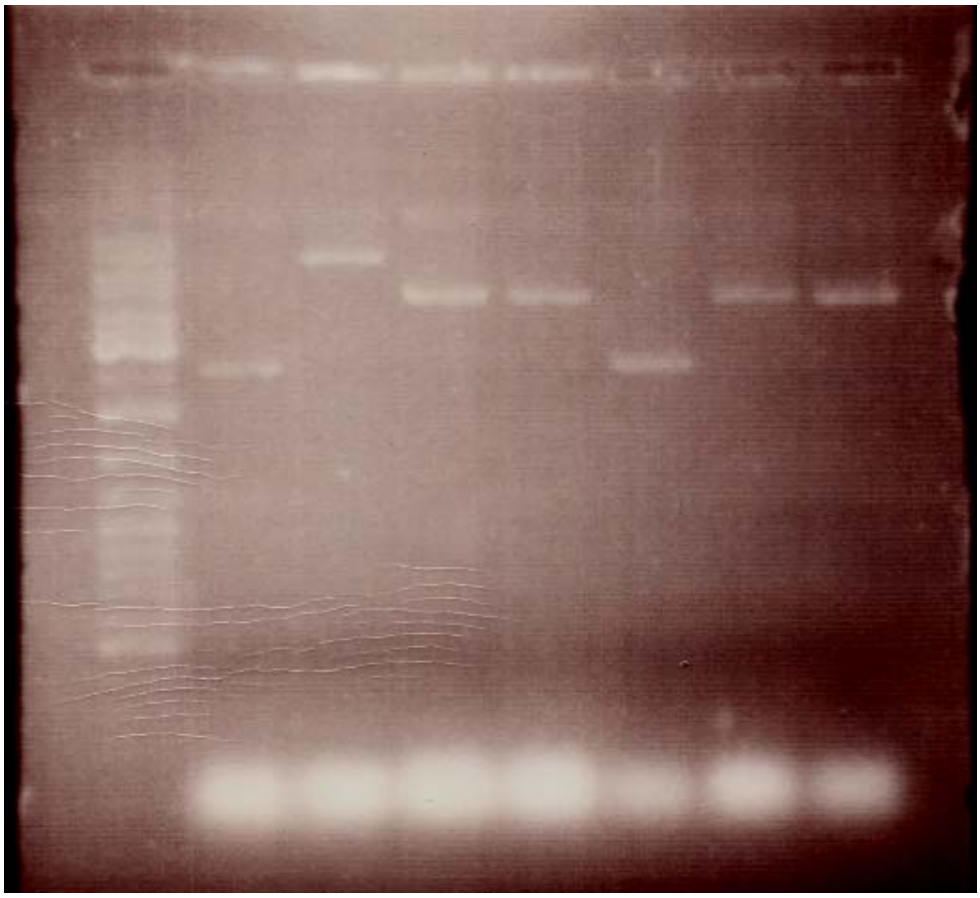

Fig. 1 Restriction analysis of Re-circularized Plasmid Lane1 marker, Lane 2 & 6 cicular pUC19, Lane3 Ligated pUC19 and pKK223-3 at EcoRI & Hind III site, Lane 4, 5, 7 & 8 showed circular pKK223N

For good ligation vector insert ratio is very important (Glover and Hames 2002). The ratio was calculated in pmol ends. Linear pKK223-3 and pUC19 was cut with EcoRI and Hind III, ligated using T4 DNA ligase and transformed in dH5α using heat shock method (Ausubel, et al., 1987 and Howe 2006).

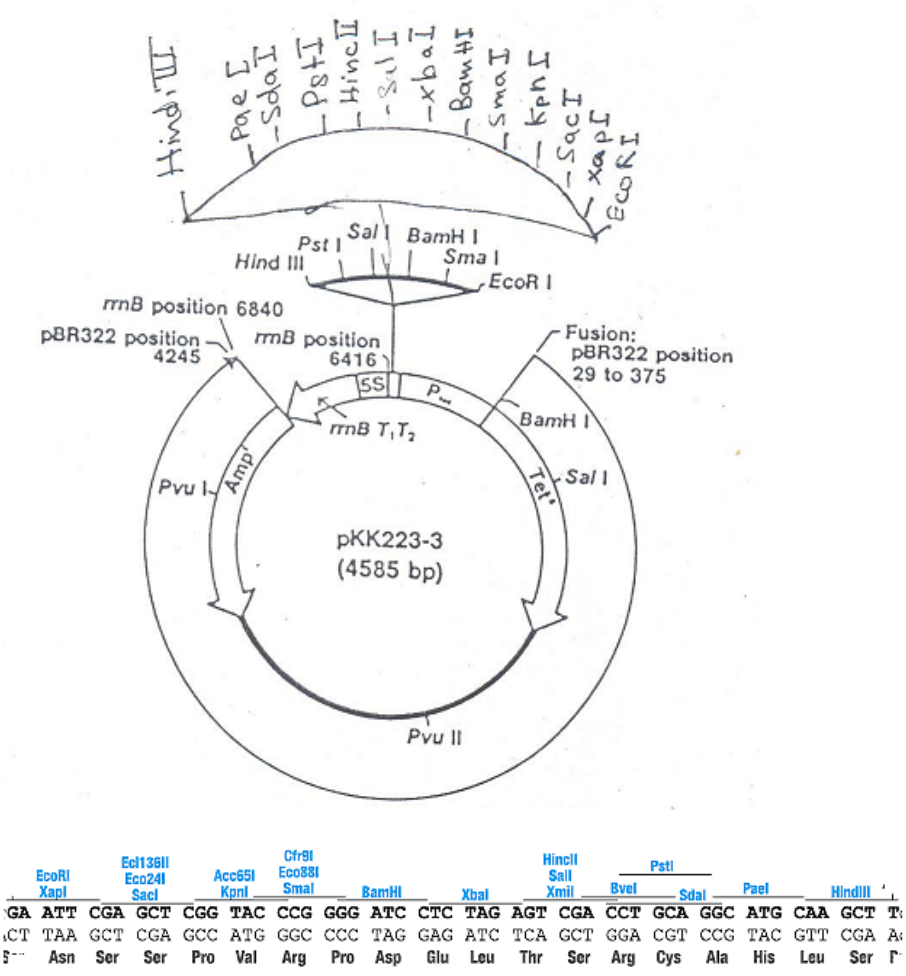

Fig. 2 Map of pKK223N (Reconstructed pKK223-3)

Transformed colonies were used for mini plasmid preparation, double digested with EcoRI and Hind III and visualized on Agarose gel. Figure 1 showed the results of restriction digestion plasmid DNA. Results showed that three kinds of plasmids were obtained 1st product was pUC19, 2.8Kb in size (Lane 2 and 6), 2nd. Combination of pUC19 and pBR223-3 7.3 kb size (Lane 3) and 3rd product was recircularized pBR223-3 4.5 Kb size Lane (4, 5, 7 and 8). Third plasmid was our desired circular pBR223-3

that was named as pBR223-3N as shown in Figure 2. This high expression vector will be used to express foreign gene in *E. coli*.

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(Received: 20 March, 2009; Revised: 20 July, 2009)