Chemistry of Natural Compounds, Vol. 34, No. 5, 1998

SYNTHESIS OF RECOMBINANT DNA WITH THE β-GALACTOSIDASE GENE PLACED UNDER THE CONTROL OF THE BACULOVIRUS PROMOTER OF THE POLYHEDRIN GENE

E. V. Uporova, Sh. Sh. Alimukhamedov, A. D. Lebedev, Yu, É. Ionova, A. Sh.Tashpulatov, and Sh. S. Azimova

UDC 575.27;577.21;579.25.5

A genetic-engineering construction designed for homologous recombination with viral DNA of the wild type has been obtained that is based on the vector pBK273 and contains the DNA sequence of the virus of Bombyx mori *nuclear polyhedrosis. The region of the reporter gene LacZ coding β-galactosidase has been inserted into this constructiom under the control of the strong promoter of the polyhedrin gene, which ensures a high level of expression, and this permits the creation of a model system for expressing proteins.*

One of the main problems of modern biotechnology is the production of recombinant proteins from genetic constructions with an inducible promoter ensuring the required level of expression.

In the production of biologically active proteins from cloned genes, the acquisition by the proteins of the correct spatial structure or the performance of the appropriate posttranslational modifications (glycosylation, phosphorylation, etc.) requires the expression of the genes in systems based on eukaryotic cells. There is a whole series of eukaryotic cell systems in which the expression of recombinant DNAs takes place fairly effectively — these are yeasts and mammalian cells [1] —, but the level of expression in them is insufficient for their wide use in biotechnology. A new direction in biotechnology has appeared comparatively recently — the use of the baculovirus/insect c ^{18} ystem for obtaining recombinant proteins.

For the highly effective expression of foreign genes in insect cells baculoviruses are used as viral vectors that are independent of helpers. A particular subtype of baculoviruses is required for this purpose — nuclear polyhedrosis viruses (NPVs). These viruses multiply only in certain cell lines. Moreover, it is possible to obtain large amounts of protein even in insect larvae [2].

Usually, in the construction of recombinant baculoviruses the kDNA of the selected gene is ligated with a vector molecule containing a fragment of viral DNA such that this gene proves to be under the control of a strong viral promoter — the promoter of the polyhedrin gene. Recombination between homologous sequences of the viral and the plasmid DNAs, leading to the substitution of the polyhedrin gene in viral DNA of the wild type in the plasmid sequence takes place in a cell culture. Since the polyhedrin gene is not necessary for the replication of the virus, the recombinant virus can multiply without the participation of a helper.

As a model for expression in a cell culture of the silkworm moth *Bombyx mori* we selected the *E. coli* lacZ gene, which codes for β-galactosidase. The expression of this protein can readily be determined by the visual screening of plaques of the recombinant baculovirus. Infected cells producing β-galactosidase will impart a blue coloration to the plaques when the chromogenic substrate X-gal is added to the medium.

The plasmid pBK 273 is a vector based on plasmid pUC19, which contains a fragment of the genome of *Bombyx mori* (the silkworm moth) having size of 7 tbp, promoter and terminator sequences of the polyhedrin gene without the coding part, and a polylinker containing unique sites for cloning Kpn I, Sac I, and EcoR I [3]. The introduction at any of these sites of foreign DNA coding the amino acid sequence of a protein places it under the control of the promoter of the polyhedrin gene. The plasmid DNA created in this way can be used to obtain a recombinant baculovirus.

Institute of Plant Genetics and Experimental Biology, Academy of Sciences of the Republic of Uzbekistan, Tashkentskaya Oblast, fax (3712) 64 22 30. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 684—687, September-October, 1998. Original article submitted February 6, 1998.

 0009-3130/98/3405-0620$20.00

To construct the recombinant plasmid we developed the cloning scheme of Fig. 1.

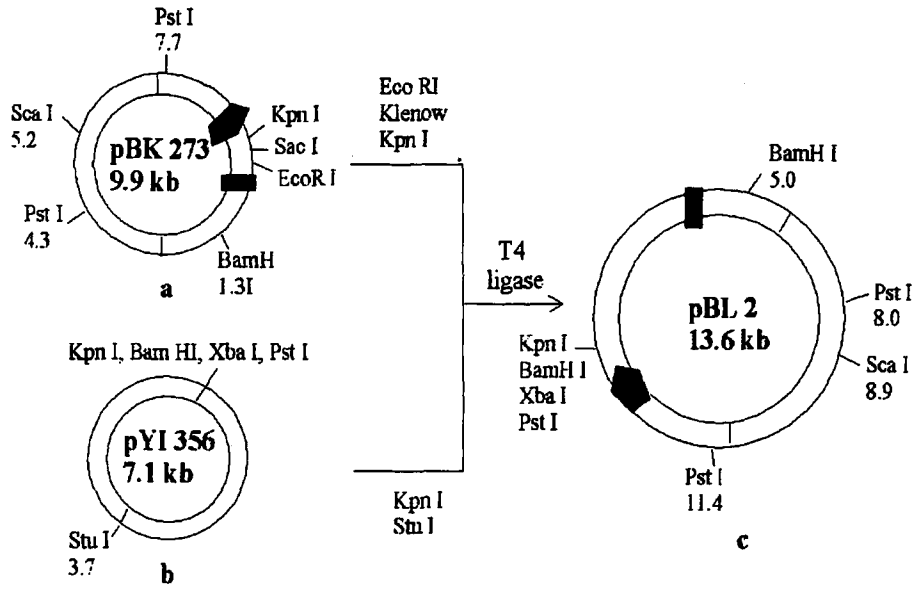

Fig. 1. Cloning scheme with physical maps of the plasmids: pBK 273 (*a*), pYI 356 (*b*), and pBL 2 (*c*).

Vector pBK 273 (Fig. 1, *a*) was prepared and cloned according to its physical map in the following way. The vector was linearized at the site of EcoR I restriction endonuclease. In the process, "sticky" ends were formed, which were filled in with the aid of a large fragment of the DNA of *E. coli* polymerase I (Klenow fragment) [4]. After this, restriction was conducted with Kpn I endonuclease. As a result, vectorial linear DNA with a sticky end at the Kpn I site and a flush end was obtained.

According to the physical map of plasmid pYI 356 (Fig. 1, *b*), the DNA fragment containing the lacZ gene was cleaved at restriction sites by nucleases Kpn I and Stu I. Since "sticky" ends and also flush ends were formed at the Kpn I sites in the prepared vector and the lacZ gene, the possibility appeared of directed ligation ensuring the correct orientation of the lacZ gene relative to the promoter in the recombinant plasmid (Fig. 1, *c*). A plasmid-free strain of *E. coli* was transformed by the genetic construction that had been obtained, and clones containing the recombinant DNA were selected by mass screening after the isolation of the DNA from them by alkaline lysis [4].

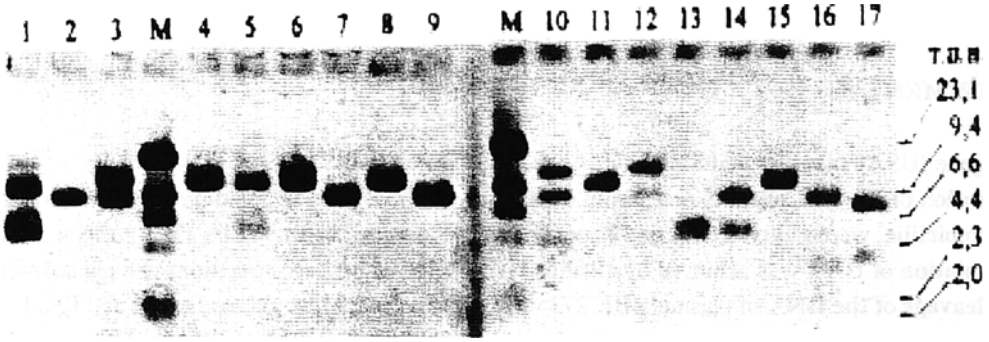

Fig. 2. Restriction analysis of the plasmids pBK 273 and pBL 2.

Plasmid pBK 273 treated with the following restrictases: *1*) native; *2*) Kpn I and EcoR I; *5*) Xba I; *7*) Kpn I; *9*) Sca I; *11*) Xba and Sca I; *13*) Kpn I and Sca I; *15*) BamH I; *17*) Pst I.

Plasmid pBL2, treated with the following restrictases: *3*) native; *4*) Xba I; *6*) Kpn I; *8*) Sca I; *10*) Xba I and Sca I; *12*) Kpn I and Sca I; *14*) BamH I; *16*) Pst I; M) phage λ DNA treated with restrictase Hind III.

Figure 2 shows the results of the comparative restriction mapping of the recombinant plasmid pBL 2 and of the initial vectorial plasmid pBK 273 at the Xba I, Kpn I, Sca I, BamH I, and Pst I sites. After electrophoresis, DNA fragments were revealed the masses of which (Table 1) corresponded to theoretical calculations from the physical maps of these plasmids. This shows the correct orientation of the lacZ gene relative to the promoter in plasmid pBL 2.

TABLE 1. Theoretical Calculations of the Masses of the DNA Fragments from the Maps of the Plasmids after Cleavage with Restrictases

No	DNA	Restrictases	DNA fragments, t.b.p.
1	pBK 273	-	native
2	pBK 273	Kpn I; EcoR	9.9
3	pBL 2	-	native
4	pBL 2	Xba I	13.6
5	pBK 273	Xba I	native
6	pBL 2	Kpn I	13.6
7	pBK 273	Kpn I	9.9
8	pBL 2	Sca I	13.6
9	pBK 273	Sca I	9.9
10	pBL 2	Xba I; Sca I	8.9; 4.7
11	pBK 273	Xba I; Sca I	9.9
12	pBL 2	Kpn I; Sca I	8.9; 4.7
13	pBK 273	Kpn I; Sca I	5.2; 4.7
14	pBL 2	BamH I	8.6; 5.0
15	pBK 273	BamH I	9.9
16	pBL 2	Pst I	8.0; 3.4; 2.2
17	pBK 273	Pst I	6.5; 3.4
M	phage λ	Hind III	23.1; 9.4; 6.6; 4.4; 2.3; 2.0; 0.5

Thus, the production of recombinant DNA (pBL 2) containing the lacZ gene under the control of the promoter of the polyhedrin gene has been demonstrated. The prerequisites have thereby been created for obtaining from *Bombyx mori* baculoviruses a model system for expressing proteins required for medicine and agriculture in biotechnological amounts.

EXPERIMENTAL

Stratagene (USA) enzymes and Sigma (USA) reagents were used.

Cultivation of *E. coli* Cells. *E. coli* strains containing plasmids pBK 273 and pYI 356, which possess the genome for resistance to ampicillin, were cultivated on 1xYT medium in the presence of ampicillin (30 μg/ml) at 37°C [5].

The isolation of DNA was achieved by alkaline lysis, followed by deproteination with phenol—chloroform [4].

The cleavage of the DNA of plasmid pBK 273 was performed with restrictases EcoR I and Kpn I; the DNA of plasmid pYI 356 was cleaved with restrictases Kpn I and Stu I, as described in [4].

The Klenow Reaction. After cleavage with restrictase EcoR I, plasmid pBK 273 was treated with the Klenow fragment of *E. coli* DNA polymerase I, dATP and dTTP being added to fill up the shortened 3′-ends [4].

Synthesis of the Vector pBK 273 with the LacZ genome. Ligation of the vector with the fragment was conducted in the minimum volume (10 μl) in a molar ratio of 1:6, respectively, with the aid of phage T4 DNA ligase. The reaction took 14 h at 12°C [4].

Transformation of *E. coli* Cells. Competent cells of the DH5-α plasmid-free strain of *E. coli*, obtained by the method using $CaCl_2$ [4], were transformed with the ligase mixture. The efficiency of transformation amounted to 10^6 clones/μg of vectorial DNA.

Identification of the DNA of the Recombinant Clones. The molecular mass of the isolated plasmid DNA was determined electrophoretically [4]. Clones containing DNA with a molecular mass of approximately 14 t.b.p. were investigated by restriction analysis. The DNAs of the recombinant clones and of pBK 273 were analyzed after single and double cleavage by the restrictases Xba I, Sca I, Kpn I, BamH I, Pst I, Sca I and Kpn I, and Xba I and Sca I [4]. The molecular masses of the fragments obtained were determined after electrophoresis in 0.7% agarose gel.

As the molecular mass marker we used phage λ DNA that had been treated with restrictase Hind III (see Fig. 2).

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