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MECHANISMS OF CELL PROLIFERATION AND DIFFERENTIATION

# Comparison of Six Promoters for Transient Expression of Luciferase Reporter Gene in Cultured *Bombyx mori* Cells (BmN)

**Yong Huang[a, b], Jian Ping Wang[a], Fei Song[b], Xin Wang[b], and Xing Jia Shen[b]**

[a] *Animal Science and Technology College, He Nan University of Science and Technology, Luoyang City, Henan Province, 471003 P.R. China*

[b] *The Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang City, Jiangsu Province, 212018 P.R. China*

*shenxj63@yahoo.com.cn*

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**Abstract**—The Luciferase (*luc*) reporter gene was used to construct recombinant plasmids containing six different promoters, which are the cytoplasmic actin3 promoter (*A3*), the fibroin heavy chain promoter (*Fib-H*), the fibroin light chain promoter (*Fib-L*), a glycoprotein (*P25*) promoter, Sericin-1 promoter (*Ser-1*) and the *Bombyx mori* nuclear polyhedrosis virus immediate (BmNPV) early protein promoter 1(*IE-1*), respectively. These recombinant plasmids, which are pGL3-*A3-luc*, pGL3-*Fib-H-luc*, pGL3-*Fib-L-luc*, pGL3-*P25-luc*, pGL3-*Ser-1-luc* and pGL3-*IE-1-luc* had been constructed successfully by restriction enzyme digestion and PCR analysis, and then were transfected into BmN cells to measure the activity of the six promoters to drive *luc* reporter gene transient expression in cells. Transfection and transcriptional experiment indicated that except pGL3-*Fib-L-luc*, pGL3-*P25-luc*, pGL3-*Ser-1-luc*, others three kinds of recombinant plasmids all transfected BmN cells obviously. The promoters of *Fib-H*, *A3* and *IE-1* enhanced the transient expression activity of *luc* reporter gene in the BmN cells and their activity strengthened sequentially.

*Keywords*: *Bombyx mori* cells, promoter, *Luc* reporter gene, expression activity

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## INTRODUCTION

The silkworm (*Bombyx mori*) is a domestic insect. The previous studies showed that the BmNPV immediate early gene promoter 1(*IE-1*) and silk protein heavy chain gene (*Fib-H*) in the silkworm larvae could activated the expression of exogenous gene as well as the two promoter genes were also transfected in *Drosophila* and silkworm cells (Angelichio et al., 1991; Hua et al., 1994; Lu et al., 1996; Zhang et al., 2008). The silk protein light chain gene (*Fib-L*) promoter was used for research of transgenic silkworm and successfully drives the expression of target genes by homologous recombination and the *piggyBac* transposon-mediated transgenic silkworm (Tamura et al., 2000) The recombinant virus-mediated transient expression system driven the expression of EGFP gene by *A3* promoter and implying that *A3* promoter can be used to study gene expression and regulation of the infected viral tissue (Guo et al., 2005; Zhang et al., 2008). In addition, the expression of the sericin (Ser) gene and *P2*5 gene also were studied (Chevillard et al., 1986; Matsuno et al., 1989; Nony et al., 1995; Liu et al., 2006). So far, the promoter activity of the *P25* and *Ser-1* genes was few reported in BmN cells.

*Luc* reporter gene from the North American firefly can catalyze the oxidative carboxylation of the firefly and emitting photons which can be captured by photometer for quantitatively analysis. The pGL-Basic vector containing the *luc* reporter gene plasmid vector can use a tool since the detection of *luc* is fast, easy, good linearity and it is becoming the most widely used reporter gene (Gambhir et al., 1999; Naylor, 1999; Leclerc et al., 2000). In this study, the six recombinant expression plasmids were constructed containing *A3*, *Fib-H*, *Fib-L*, *P25*, *Ser*-1and *IE-1* promoters using pGL3-Basic vector. These plasmids were transfected in BmN cells and the activities are discussed by detecting *luc* transient expression level. The aim is to screen the promoters which are expressed high efficiency in *Bombyx mori* cells and provide experimental evidence for the establishment of transgenic silkworm technology platform and also provide technical support for further study of the miRNAs interacting with target genes.

## MATERIALS AND METHODS

### *1. Materials and Reagents*

The silkworm variety was maintained in the Sericulture Research Institute, Chinese Academy of Agri-

[1] The article is published in the original.

**Table 1.** The up and down primers sequence for promoter amplification

| Reporter plasmids | Forward and reverse primer |
|---|---|
| pGL3-*A3-luc* | 5'-GGAGATCTCCGCTACGATATCATTATCATA-3' |
| | 5'-GTCCATGGCTTGAATTAGTATAGTATTATTAAATAA-3' |
| pGL3-*Fib-H-luc* | 5'-GGAGATCTGAGAATGTCTGGACAG-3' |
| | 5'-GTCCATGGTTGAGAGTTGGAACCG-3' |
| pGL3-*Fib-L-luc* | 5'-GGAGATCTCGACTCGCCAAGTTACGTC-3' |
| | 5'-GTCCATGGGTGGTCTGTTATGTGACC-3' |
| pGL3-*P25-luc* | 5'-GGAGATCTTAGATAATTCGGCATTGTG-3' |
| | 5'-GTCCATGGCATGTTGTTGCGCGAATAA-3' |
| pGL3-*Ser-1-luc* | 5'-GGAGATCTGCAGTATGTCGTGCTAA-3' |
| | 5'-GTCCATGGTGAGAGTTGGAACCGAAC-3' |
| pGL3-*IE-1-luc* | 5'-GGAGATCTGATTTGCAGTTCGGGA-3' |
| | 5'-GTCCATGGAGTCGTTTGGTTGTTCA-3' |

culture Science. The plasmid of pGL-Basic vector which containing *luc* reporter gene, and the pAc5.1 plasmid and the *Bombyx mori* cell line (BmN) were preserved in our laboratory. All the restriction enzymes, high fidelity *Taq* enzyme, T4 DNA ligase and other main reagents were purchased from TakaRa. The cell culture medium TC-100, fetal bovine serum (FBS) and Lipofectamine 2000 were from Invitrogen. *Luc* assay kit was purchased from Promega.

### *2. Construct of Reporter Plasmids*

Plasmids for promoter activity measurements were constructed using pGL3-basic vector. *A3*, *Fib-H*, *Fib-L*, *P25*, *Ser-1* promoter sequence was PCR from the silkworm DNA with the primers which contained *Bgl*II and *Nco*I sites (underlined) (Table 1). The *IE-1* gene promoter sequence was PCR form *Bombyx mori* nuclear polyhedrosis virus DNA, which also contained the same *Bgl*II and *Nco*I sites. The amplified fragment was treated with *Bgl*II and *Nco*I and then ligated into the vector pGL3-basic vector in the upstream of *luc* gene sequence to form the recombinant plasmids pGL3-*Fib-H-luc*, pGL3-*Fib-L-luc*, pGL3-*P25-luc*, pGL3-*Ser-1-luc* and pGL3-*IE-1-luc*.

### *3. Cell Culture and Transient Transfections*

The BmN cells were seeded into 12 $cm^2$ flasks at a density of about $5 \times 10^5$ cells/mL and cultured for 48 h. Before transfection, the medium was removed and the cells were washed three times by serum-free TC-100 medium. The cells were transfected with 100 μL transfection solution containing 5 μL Lipofectamine 2000 and 1 μg recombinant reporter plasmid DNA in 1 mL serum-free medium for 4–6 h. Then the serum-free medium was replaced by TC-100 medium supplemented with 10% fetal bovine serum, penicillin (200 μg/mL) and streptomycin (100 μg/mL) at 27°C with 5% $CO_2$. The 0.5 μg normalization plasmids pAc5.1 plasmid was accompanied with reporter plasmid for transfection. Cells transfected with pGL-basic vector served as the blank.

### *4. Luciferase Assay*

Three independent transfections with triplicates each time were performed, and cell lysates were collected 48 h after transfection, cells were washed with phosphate-buffered saline (PBS, pH 7.4) and treated with lysis buffer for *luc* assays. *Luc* activities were measured according to the manufacturer's instructions (Promega). Relative recombinant reporter plasmids activity for treated cells was obtained by normalization to non-promoter treated pGL Basic vector, respectively. *Luc* activity was normalized to a co-transfected plasmid pAc5.1 expressing β-galactosidase. Each experiment was repeated more than three times, and the averages were used in comparisons.

## RESULTS

### *1. Identification of Recombinant luc Expression Vectors*

The recombinant plasmids were constructed as follows (Figure 1). The 532 bp *A3*, 487 bp *Fib-H*, 602 bp *Fib-L*, 1233 bp *P25*, 566 bp *Ser-1* and 632 bp *IE-1* promoters sequence were digested by *Bgl*II and *Nco*I and subcloned to pGL3-basic vector. PCR products (Figure 2) and restriction enzyme digestion products were analyzed on agarose gel (Figure 3). The results showed that these promoters sequences were successfully cloned into upstream of the *luc* reporter gene.

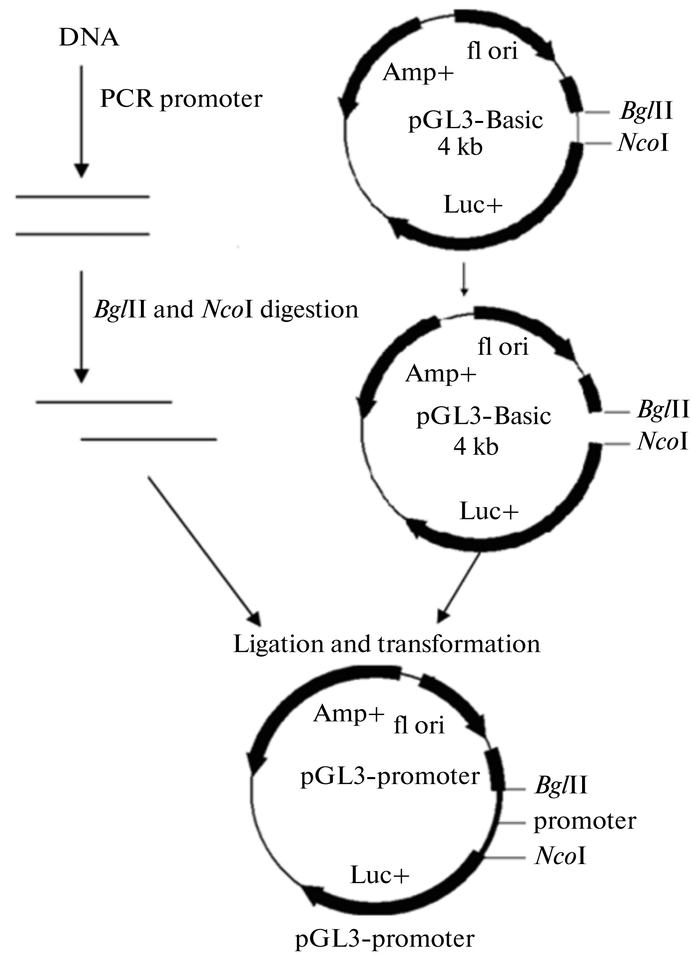


**Fig. 1.** Construction of recombinant promoter plasmid.

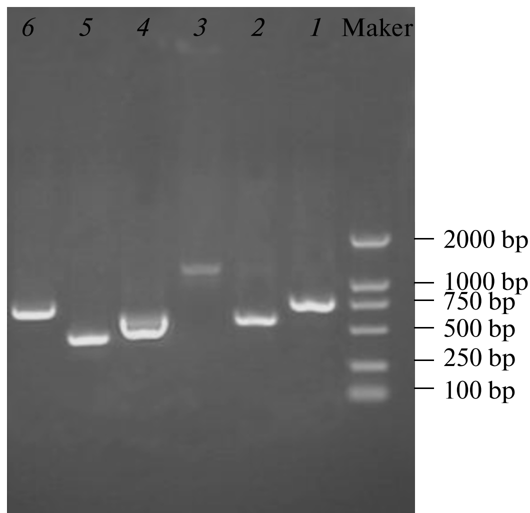


**Fig. 2.** The amplication resilt of promoter by PCR. Marker DL2000; 1, *IE-1*; 2, *Ser-1;* 3, *P25;* 4, *A3;* 5, *Fib-H*; 6, *Fib-L*.

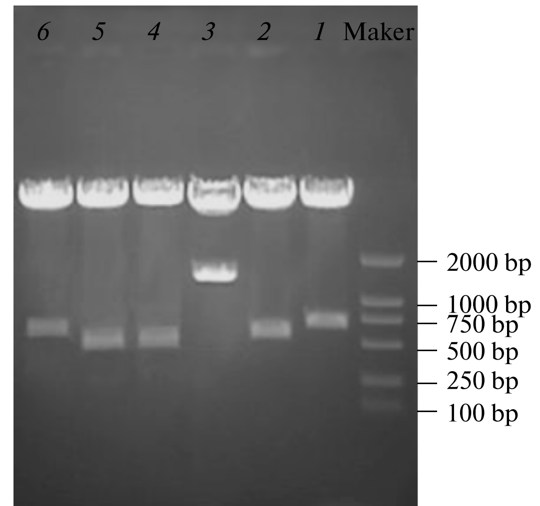


**Fig. 3.** Double enzyme digestion of recombinant plasmid. Marker DL2000; 1, *IE-1;* 2, *Ser-1*; 3, *P25*; 4, *A3*; 5, *Fib-H*; 6, *Fib-L*.

**Table 2.** Luciferase activity of the six reporter plasmids in BmN cells

| Reporter | pGL3-*IE-1-luc* | pGL3-*A3-luc* | pGL3-*Fib-H-luc* | pGL3-*Ser-1-luc* | pGL3-*Fib-L-luc* | pGL3-*P25-luc* |
|---|---|---|---|---|---|---|
| Luciferase activities, c.p.m per μg | 5426354 ± 66054.1919 | 13879 ± 971.48 | 12572 ± 192.27 | 5310 ± 80.43 | 4700 ± 60.18 | 1020 ± 10.66 |

Note: The data shown in the table are the average of three separate experiments after being normalized by blank control, β-galactosidase activites and total protein of cell extract. *Luc* activity of pGL-Basic transfected BmN cells (blank control) was 57 c.p.m ± 8.34 c.p.m.

The sequence of the PCR products and resulting plasmids were confirmed by sequencing performed by a commercial service provider (TakaRa).

### *2. Transient Expression Analysis in BmN Cells*

The six constructed reporter plasmids were separately transfected into BmN cells at the same time. The promoters of *P25*, *Fib-L*, *Ser-1*, *Fib-H*, *A3* and *IE-1* enhanced the transient expression activity of *luc* reporter gene in the BmN cells and their activity strengthened sequentially (Table 2). *Luc* activities suggested that the combination of six promoters all drive the transient expression of luc in BmN cells. The results showed that the promoters of *P25*, *Fib-L*, *Fib-H*, *Ser-1*, *A3* and *IE-1* enhanced the transient expression activity of *luc* reporter gene in the BmN cells and their activity strengthened sequentially. Compare with others five promoters, the activity of *P25* promoter are the lowest. The activity of the *IE-1* promoter was the highest and was approximately 50 thousand-fold higher than the *P25* promoter. The transfection results also showed that the luc activity of pGL-Basic is the most weakest and enzyme activity was only 57 photon counts per minute (c.p.m). The results implied that pGL-Basic freeing promoter in the silkworm Bm cells was nearly no expressed.

## DISCUSSION

At present, the cell transient transfection technology is the perfect. Previous studies for promoter are frequently performed in transiently transfected cells before further the analysis of *cis*-regulatory elements, which gives reproducible results and detect readily expression of *luc* reporter gene (Shen et al., 2004; Tang et al., 2005). But different cell lines will significantly affect the transfectional efficiency (Elnitski and Hardison, 1999). The ten times repeated experiments were carried out and the four times of them is effective indicating the use of cationic liposome-mediated method can mediate exogenous plasmid into the suspension cells.

In the recombinant six promoter plasmids, *IE-1* promoter has the highest expression of *luc* activity, *A3* and *Fib-H* have intermediate expression of *luc* activity, the remaining *P25* promoter activity is the lowest in BmN cells excepting for *Fib-L* and *Ser-1* promoter. It may be due to the length of the cloned promoter fragment or the source of the cell lines which affect the promoter activity. BmN cells, established from the ovarian tissue of silkworm, maybe have non-omnipotence. It is worthy of investigating further whether the cell strains is related to the promoter activity expression in transient transfection. Based on our experimental results, *IE*-1, *A3* and *Fib-H* promoters should be chose firstly in vitro using *Bombyx mori* cells lines.

The construction of these plasmids provides a good tool in our study. It can be used as positive control for gene promoter analysis and compared with results of transfection of recombinant plasmid to determine the transcriptional affect of different *cis*-acting element. We might insert promoter elements of the specific region into upstream or downstream sequence of the pGL3-Basic vector to further analyze biological role of promoter by *luc* expression changes and found the core promoter sequence which may play a key role in the transcription process. Meanwhile, we can comprehend the role of the specific trans-acting factors which may regulate the level of expression. There are many papers which have been reported to clone the 3'-UTR of the target gene of interest immediately downstream of the *luc* sequence contained in the reporter plasmid and verify the role of miRNAs genes (Pais et al., 2010; Lewis et al., 2003; Kuhn et al., 2008; Lee et al., 2008). The construction of promoter plasmid not only provide tools to test gene expression at the cellular level but also aid us to constructed different reporter gene plasmid for different types of cells, which were transfected with the reporter gene plasmid.

## ACKNOWLEDGMENTS

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