The Journal of Microbiology, June 1997, p.149-151 Vol. 35, No. 2

A Plasmid Vector Facilitating Gene Expression in Both Yeast and Mammalian Cells

Tae Ho Lee

Department of Biology, Yonsei University, Seoul 120-749, Korea

(Received May 13, 1997 / Accepted June 4, 1997)

A plasmid vector with combined features of yeast shuttle vector and mammalian expression vector was constructed to facilitate expression of cloned gene in both cell-types. All necessary elements required for plasmid maintenance and selection in *E. coli*, yeast and mammalian cells were size-economically arranged in this plasmid. The human cytomegalovirus (CMV) immediate early promoter and yeast *GAL1* promoter were sequentially placed in front of the gene to be expressed. The synthetic splicing donor and acceptor sequences were inserted into the immediate upstream and downstream of the *GAL1* promoter, allowing the CMV promoter to direct the expression of a given gene in mammalian cell environment by splicing out the interfering *GAL1* promoter sequence. When the resulting vector containing *LacZ* as a test gene was introduced into yeast and mammalian cells, both cells efficiently produced β-galactosidase, demonstrating its dual host usage.

Key words: Expression plasmids, shuttle vectors, yeast, mammalian cells

Gene expression using heterologous hosts such as yeast and mammalian cells is used industrially as well as academically to produce large amounts of desired proteins. Most yeast or mammalian expression vectors are shuttle vectors, i.e., they contain replicators and genetic markers that allow their selection and maintenance either in *E. coli* or in the host of interest (yeast or mammalian cells). They also contain the host-specific promoter elements that direct efficient transcription of a given gene. Under some circumstances when the quality of the protein expressed in a particular host (e.g., yeast) is not desirable, the trial of the gene expression in an alternative host system, for example, mammalian cells could be taken into consideration. In this case the gene of interest has to be re-cloned into the vector that is specifically designed for mammalian cell expression. Although the re-cloning step does not require extensive work, it is rather inconvenient and time consuming, especially when the restriction enzyme sites accommodating the insertion of the gene into the mammalian expression vector are not available. To avoid such a step, I developed a plasmid expression vector (pYM 101) that allows the expression of a cloned gene in both yeast and mammalian cells. The pYM101 contains two replicators in addition to the pMB1 replication origin sequence for maintenance in *E. coli*: one is a yeast replicator derived from the 2 μm circle, a naturally occurring plasmid that is native to most lab strains of *Saccharomyces cerevisiae*, the other is a SV40 replication origin sequence that permits autonomous replication extrachromosomally in most mammalian cells producing SV40 large T antigens. As a dominant selection marker for the manipulation in *E. coli*, the suppressor F (supF) tRNA selection system was used instead of ampicillin resistance selection to reduce the plasmid size (3). Furthermore, the cytomegalovirus (CMV) immediate early promoter, which is constitutively active in most mammalian cells, and the yeast inducible *GAL1* promoter were placed sequentially in front of the gene to be expressed, in which the yeast *GAL1* promoter located at the immediate-upstream of the target gene is able to drive the expression of the gene directly in yeast. To allow the CMV promoter to direct the expression of the gene over the interfering *GAL1* promoter sequence, the synthetic splicing donor and acceptor sequence were inserted in the 5- and 3-end of *GAL1* promoter sequence, respectively, so that the *GAL1* promoter sequence could be spliced out in mammalian cell environment. The adapters for the splicing donor and acceptor sequence used in this study are as follows:

splicing acceptor:

```
AGCTCTTAAGTTTTTTTTTTTACAGTA
     GAATTCAAAAAAAAAAAATGTCATTCGA
```

splicing donor:

```
AGCTGGTTTAGTGAACCGCCAATAGCCGCCAAGGTGAGTG
     CCAAATCACTTGGCGGTTATCGGCGGTTCCACTCACTCAG
```

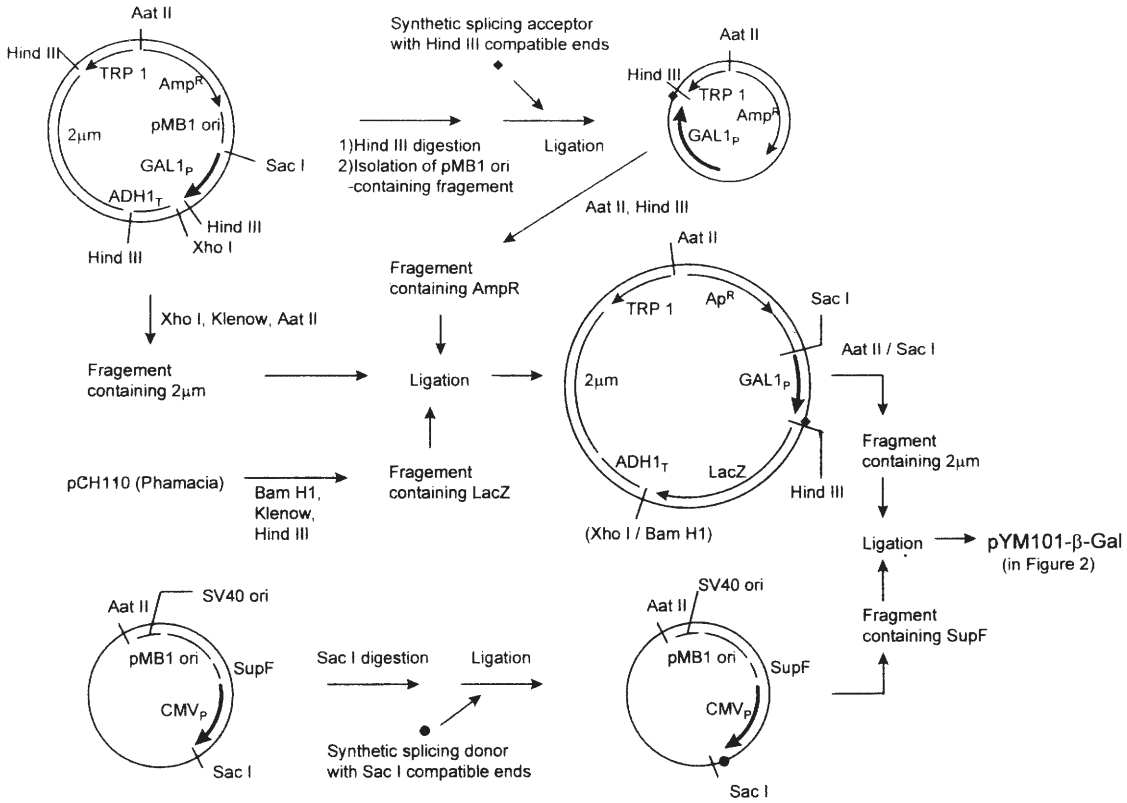

Fig. 1. Schematic diagram of plasmid construction. pYM101-β-Gal was assembled from various sequence elements which were derived from pJG4-5 (5), pCH110, and pCDM8 derivatives (3) as indicated. Abbreviations: SV40 ori, replication origin sequence of SV40 virus; pMB1 ori, *E. coli* replicator; supF, suppressor F tRNA gene; Amp^R, β-lactamase gene; CMV_P, human cytomegalovirus immediate early promoter; $GAL1_P$, *GAL1* promoter; $ADH1_T$, *ADH1* terminator; 2 μm, yeast replicator 2 μm circle.

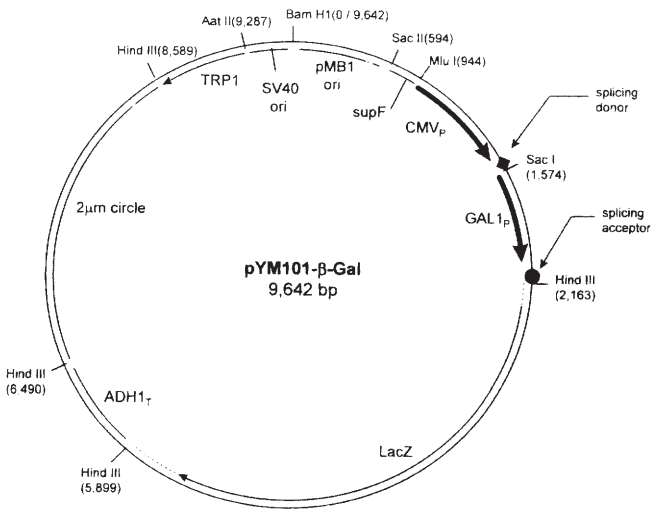

Fig. 2. Physical map of pYM101-β-Gal. Abbreviations used are the same as in Fig. 1.

The overall plasmid construction schemes as well as physical map of the pYM101 containing the reporter *LacZ* gene (pYM101-β-Gal) are depicted in Fig. 1 and 2, respectively. To test its dual host usage, at first the pYM101-β-Gal was introduced into *Saccharomyces cerevisiae EGY48(MATa trp1 ura3, his3 LEU2::pLexAop6-LEU2)*(5) by using the litium acetate transformation method (4). Yeast colonies developed in tryptophan-deficient medium were selected and grown in complete minimal dropout medium (CM)(1.7 g yeast nitrogen base without amino acid, 5 g $(NH_4)_2SO_4$, 1.3 g amino acids dropout power) containing 2% glucose as a carbon source (5). The expression of β-galactosidase from the transformants was induced by shifting the culture to the CM medium containing 2% galactose. The β-galactosidase activity was measured as described elsewhere (4). The result revealed that β-galactosidase was efficiently induced at least 1,000 fold by galactose (data not shown). To visualize the expression of β-galactosidase, the selected colonies were streaked on CM-glucose or CM-galactose plate containing a chromogenic substrate X-GAL. As shown in Fig. 3A, blue-colored cells had developed on the galactose-containing plate. The utility of pYM101-β-Gal in mammalian cells was also examined in transient transfection system using COS-m6 cells. Typically, COS-m6 cells were grown to 70% confluency in a well of 6-well plate, and washed with DMEM prior to treatment with

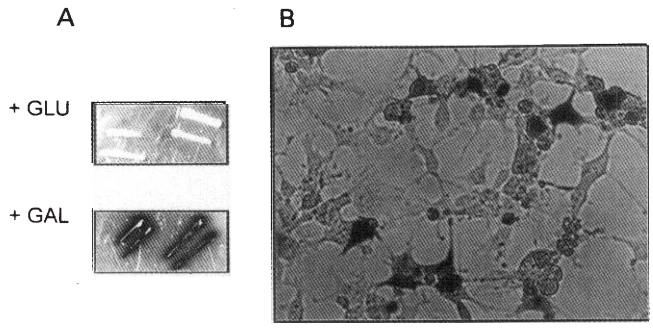

Fig. 3. Expression of β-galactosidase in pYM101-β-Gal transformed *Saccharomyces cerevisiae* in the presence of 2% galactose (A) and in COS-m6 cells transiently transfected with pYM101-β-Gal (B). Cells were processed for staining of β-galactosidase activity as described in the text. Blue-stained cells appeared as dark-colored in the black-and-white prints.

transfection mixture containing 2 μg of pYM101-β-Gal and 10 μl of lipofectamine in 1 ml of serum-free DMEM. The transfection mixture was discarded 5 hr later, and fresh DMEM containing 10% fetal bovine serum was added to the transfectants. After a 16-hr incubation, the expression of β-galactosidase of the transfectants was directly visualized by histochemical staining of β-galactodidase activity (1). For the staining, cells were fixed with 0.5% glutaradehyde for 15 min followed by extensive washing in PBS with 5 mM $MgCl_2$. Cells were stained with PBS containing 20 mM $K_3Fe(CN)_6$, 20 mM $K_4Fe(CN)_6 \cdot 3H_2O$, 1 mM $MgCl_2$, and 1 mg/ml X-GAL until a suitable color developed(4-6 hr) and were examined by phase contrast microscopy. As shown in Fig. 3B, cells expressing β-galactosidase were stained with blue color, indicating the efficient expression of β-galactosidase. Together with the results from the expression experiments in yeast, the expression of β-galactosidase in mammalian cells demonstrates that the newly designed plasmid pYM101 can be used routinely for heterologous gene expression in both yeast and mammalian cells. This vector could be further improved by introducing multiple cloning sites between the splicing acceptor site and *ADH1* terminator sequence to accommodate cloning of versatile genes.

Acknowledgment

This work was supported by a grant (96-043-06-01-3) from Korea Science and Engineering Foundation

References

1. **Kim, C.H., Y.-H. Song, K. Park, Y. Oh, and T.H. Lee.** 1995. Induction of cell death by myristylated death domain of p55 TNF receptor is not abolished by lpr^{cg}-like point mutation in death domain. *J. Inflamm.* **45**, 312-322.
2. **Schiestl R.H., and R.D. Gietz.** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* **16**, 339-346.
3. **Seed, B.** 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologus to its receptor CD2. *Nature* **329**, 840-842.
4. **Yocum, R.R., S. Hanley, R. West, Jr., M., and Ptashine.** 1984. Use of lacZ fusion to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **4**, 1985-1998.
5. **Zervos, A.S., J. Gyuris, and R. Brent.** 1993. Mix1. A protein that specifically interacts with Max to bind Myc-Max recognition sites. *cell* **72**, 3123-3129.