Note

Construction of the Transcriptional and Translational Signals-cloning Vector in *Saccharomyces cerevisiae*

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We have constructed a plasmid in which the γ -glutamyl-cysteine synthetase (GSH-I) structural gene from *E. coli* B was fused to a yeast (*Saccharomyces cerevisiae*) chromosomal DNA fragment. Introduction of the plasmid into yeast cells by transformation has resulted in the expression of high GSH-I activity followed by higher contents of glutathione (GSH).^{1,2)}

This suggested that the GSH-I structural gene (*AgshI*) fragment is useful for the cloning of transcriptional and translational signals from yeast chromosomal DNA. This paper deals with the construction of a transcriptional and translational signal-cloning vector in *S. cerevisiae*.

The strains used in this experiment were *E. coli* HB101³⁾ and *S. cerevisiae* YNN27.⁴⁾ The plasmid used was pGSR1518-4 (see Fig. 1), which can express the GSH-I activity in transformants due to the fused *gshI* placed on it.¹⁾ L-Medium (tryptone 10 g, yeast extract 5 g, NaCl 5 g

per liter, pH 7.0) was used for *E. coli*. SD-medium (Difco yeast nitrogen base 6.7 g, glucose 20 g, 1% adenine 0.1 ml, 1% tryptophan 0.4 ml per liter) and YPD-medium (yeast extract 10 g, peptone 10 g, glucose 20 g per liter) were used for the yeast. Transformation of *E. coli* was done by the method of Mandel and Higa.⁵⁾ The method of Ito *et al.*⁶⁾ was used for transformation of the yeast. Chromosomal and plasmid DNAs were prepared by the methods of Cryer *et al.*⁷⁾ and Birnboim and Doly,⁸⁾ respectively.

The promoter probe vector, pPG100, was constructed from pGSR1518-4 by digestion with *Bam*HI and the Klenow fragment of DNA polymerase I followed by ligation of the synthetic *Xho*I linker (pC-C-T-C-G-A-G-G) to the resulting blunt ends and deletion of the *Bam*HI-*Xho*I fragment which can act as a promoter in yeast (Fig. 1). Figure 2 shows the nucleotide sequence near the portion of the ligated junction on pPG100, which has a unique *Bam*HI site and a unique *Xho*I site adjacent on the upstream side to the *AgshI* segment.

To clone transcriptional and translational signals from yeast in pPG100, *S. cerevisiae* YNN27 chromosomal DNA (1.0 μ g) was cleaved with *Bam*HI (or *Xho*I), mixed with 0.2 μ g of the cleaved plasmid pPG100 with the same endonuclease and then ligated. Recombinant plasmid DNA was then introduced into *E. coli* HB101 by transformation (1×10^3 Ap^r transformants/ μ g DNA). Next, 10 μ g of the plasmid DNA recovered from these transformants was used to transform YNN27. Transformants (40 URA3 transformants/ μ g DNA) on the SD-medium were tested for the content of GSH by a method using nitroprusside.⁹⁾ Almost all transformant colonies remained white-pink, while approximately 1% of colonies became red immediately after treatment with ammonium hydroxide. Of these, four red transformants were selected, and the GSH-I activity and GSH content in each transformant were measured to find the level of the expression of *gshI* due to the cloned promoter fragment from the yeast chromosome. As shown in Table I, higher GSH-I activities and GSH contents were observed in the cell-free extracts of the four transformants than in the yeast YNN27 carrying pPG100 alone.

FIG. 1. Construction of the Promoter Probe Vector pPG100.

pPG100 was constructed from pGSR1518-4 by deletion of the DNA fragment between the unique *Bam*HI site and the unique *Xho*I site located in the first codon of *gshI*, followed by the ligation of synthetic *Xho*I linker.

TABLE I. GSH-I ACTIVITIES AND GSH CONTENTS IN TRANSFORMANTS CARRYING PLASMIDS WITH CHROMOSOMAL DNA FRAGMENTS

Plasmid	Restriction enzyme	Inserted DNA length (kb)	GSH-I activity ($\mu\text{mol/hr/mg}$)	GSH content (% on dry weight)
pPG100-1	<i>Bam</i> HI	6.5	50	0.5
pPG100-2	<i>Bam</i> HI	4.2	60	0.6
pPG100-3	<i>Xho</i> I	3.0	200	0.9
pPG100-4	<i>Xho</i> I	1.8	50	0.6
pPG100	(Control)	—	30	0.4

GSHI activity and GSH content were measured by the methods of Murata *et al.*¹²⁾ and Tietze,¹³⁾ respectively.

FIG. 2. The Nucleotide Sequence at the Site of *Bam*HI and *Xho*I.

Previous works on plasmid vectors for the detection and cloning of transcriptional and translational signals from yeast chromosomal DNA were reported by Casadaban *et al.*¹⁰⁾ and Rose *et al.*¹¹⁾ Here we show an additional promoter probe vector in yeast, using the *AgshI* segment from *E. coli*.

We believe this promoter probe vector can be used for the detection and functional analysis of promoters from yeast.

REFERENCES

- 1) Y. Ohtake, H. Tezuka, S. Yabuuchi, K. Watanabe, K. Murata and A. Kimura, Abstract of Papers, the Annual Meeting of the Agricultural Chemical Society of Japan, Kyoto, April, 1986, p. 18.
- 2) Y. Ohtake, H. Tezuka, S. Yabuuchi, K. Watanabe, K. Murata and A. Kimura, in preparation.
- 3) H. W. Boyer and D. Roulland-Dussoix, *J. Mol. Biol.*, **41**, 459 (1969).
- 4) D. T. Stinchcomb, C. Mann and R. W. Davis, *J. Mol. Biol.*, **158**, 157 (1982).
- 5) M. Mandel and A. Higa, *J. Mol. Biol.*, **53**, 154 (1970).
- 6) H. Ito, Y. Fukuda, K. Murata and A. Kimura, *J. Bacteriol.*, **153**, 163 (1983).
- 7) D. R. Cryer, R. Eccleshall and J. Marmur, in "Methods in Cell Biology," Vol. 12, ed. by D. M. Prescott, Academic Press, New York, 1975, p. 39.
- 8) H. C. Birnboim and L. Doly, *Nucleic Acid Res.*, **7**, 1513 (1979).
- 9) P. Apontowiel and W. Berends, *Biochim. Biophys. Acta*, **399**, 10 (1975).
- 10) M. J. Casadaban, J. Chou and S. N. Cohen, *J. Bacteriol.*, **143**, 971 (1980).
- 11) M. Rose, M. J. Casadaban and D. Botstein, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2460 (1981).
- 12) K. Murata and A. Kimura, *J. Gen. Microbiol.*, **128**, 1047 (1982).
- 13) F. Tietze, *Anal. Biochem.*, **27**, 502 (1969).