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SHORT COMMUNICATION

The Use of *HIS6* Gene as a Selectable Marker for Yeast Vector

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The yeast *Saccharomyces cerevisiae HIS6* gene has been shown to be functional as a selectable marker for selecting and maintaining a yeast vector in yeast *S. cerevisiae* host cells. The yeast *HIS6* gene encodes an enzyme involved in the yeast histidine biosynthesis. The yeast *HIS6* gene was cloned into a yeast expression vector. The resultant recombinant plasmid was introduced into yeast host cells defective in endogenous *HIS6* gene. The functionality of the *HIS6* gene as a selectable marker was tested by growing transformed cells on selective minimum medium lacking histidine supplementation.

Key words: *HIS6* gene, selectable marker, vector, transformation, yeast

INTRODUCTION

The use of yeast as a cloning host especially for overproduction of proteins of commercial value offers a number of advantages such as the ability to glycosylate proteins, absence of pyrogenic toxins, and the ability to clone a large piece of DNA. Currently, a number of different kinds of yeast vector have been routinely employed for gene cloning and expression in yeast cells. One of the important features of a yeast vector is the incorporation of selectable marker which allows selection of vector harboring-transformants. The currently commonly used yeast selectable markers include *LEU2*, *HIS3*, *URA3*, and *TRP1* genes (Old & Primrose 1989). The use of the yeast *HIS6* gene as a selectable marker has yet to be reported. The present study was intended to analyze the functionality of the yeast *HIS6* gene as a selectable marker for selecting and maintaining a yeast vector in yeast host cells.

The yeast *HIS6* gene encodes phosphoribosyl formimino-5-amino-1-phosphoribosyl-4-imidazolecarboxamide isomerase, the enzyme responsible for converting N'-[(5'-phosphoribosyl) formimino}-5-aminoimidazole-4-carboxamide ribonucleotide in the fourth step of the yeast histidine biosynthesis. The gene is functionally homologous to the *HISA* gene in *Escherichia coli* (Fani *et al*. 1997).

The yeast *HIS6* gene was first isolated by Fani and colleagues (1997) from a yeast genomic library by complementation analysis of the *his*A mutation of an *E. coli* strain. From library screening it was obtained three complementing clones denoted pRD2, pRD5, and pRD64 which contained a Sau3AI partial fragment of 7.7, 12.0, or 2.8 kb respectively. The DNA inserts in the three recombinant plasmids were subsequently found to be partially overlapping.

In order to locate more precisely the region able to complement the yeast *his6* mutation, the complementing fragment of plasmid pRD64 was further subcloned. It was found that a 1.5 kb fragment cloned in the pRD643 was the smallest fragment maintaining the complementing activity. Following nucleotide sequence analysis it was revealed that the fragment contains a 783 bp open reading frame (ORF). This ORF encodes the enzyme phosphoribosyl formimino-5-amino-1-phosphoribosyl-4-imidazolecarboxamide isomerase (5' Pro-FAR isomerase, EC 5.3.1.16) of 261 amino acids (molecular weight of 29,554 kDa) and was designated the *HIS6* gene (Fani *et al*. 1997).

MATERIALS AND METHODS

Materials. *Saccharomyces cerevisiae* strain M31 [*atp8*, *mit*$^{-}$, *his*6, *ade1*] a collection strain of the Department of Biochemistry and Molecular Biology, Monash University, has previously been described (Nagley *et al.* 1988). The plasmid pRD643 has been described (Fani *et al*. 1997). The yeast expression vector YEplac112 has been described previously (Gietz & Sugino 1988).

Molecular Cloning. Retrieving of the *HIS6* gene from plasmid pRD643 and its cloning into the yeast expression vector was carried out using standard methods (Sambrook *et al.* 1989).

Yeast Transformation. Introduction of recombinant plasmid vector into yeast strain M31 was done as described by Klebe *et al.* (1983).

RESULTS

Cloning the Yeast *HIS6* Gene Into a Yeast Expression Vector. In order to test the functionality of the yeast *HIS6* gene as a selectable marker for selecting and maintaining a yeast vector in yeast host cells, the *HIS6* gene was cloned

into a yeast expression vector and the resulted recombinant plasmid was introduced into a yeast *S. cerevisiae* strain lacking functional endogenous *HIS6* gene.

For these purposes, the *HIS6* gene contained in the plasmid pRD643 (kindly provided by Renato Fani; Fani *et al.* 1997) was retrieved and then cloned into the yeast expression vector YEplac112 (Gietz & Sugino 1988). Plasmid pRD643 is pGEM7Zf(+) (Promega) harboring a 1.5 kb *Sau*3AI/*Cla*I *his6* complementing fragment. To introduce the *HIS6* gene containing fragment into vector YEplac112, the *HIS6* fragment was retrieved using *Sac*I/*Pae*I restriction enzyme digestion. These sites exist as part of the multiple cloning sites of pGEM7Zf(+) flanking the insert in pRD643. The 1.5 fragment released was then purified and ligated into vector YEplac112 previously digested with *Sac*I/*Pae*I. The overall cloning strategy is diagrammatically illustrated in Figure 1.

Following ligation, the reaction mixture was used to transform competent *E. coli* cells and transformants were selected on solid Luria Bertani plus ampicillin (LBA) medium. Putative positive clones harboring YEplac112::*HIS6* were randomly screened by isolating plasmid DNA followed by restriction enzyme digestion analysis using *Sac*I/*Pae*I restriction enzymes. The appearance of a band of about 1.5 kb in the agarose gel electrophoresis analysis (Figure 2) indicated the successful introduction of the *HIS6* gene into the yeast expression vector YEplac112.

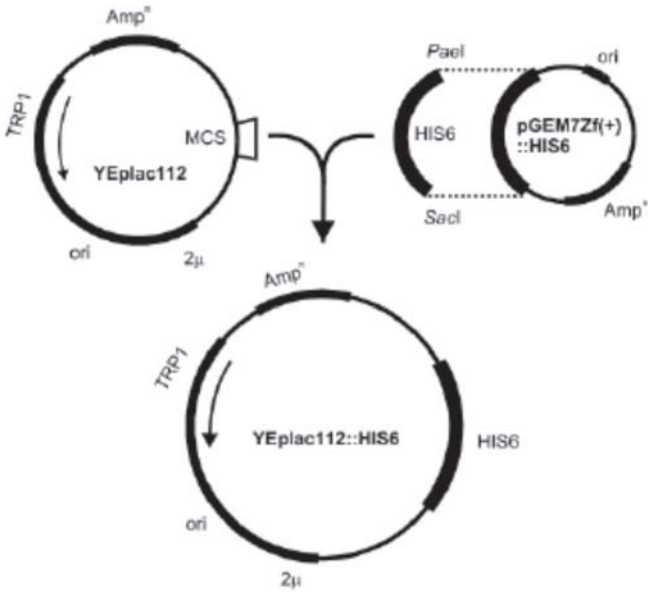

Figure 1. Construction of recombinant plasmid YEplac112::*HIS6*. A schematic diagram of introduction of the *HIS6* gene into the yeast expression vector YEplac112 is shown. A *Pae*I/*Sac*I fragment containing *HIS6* gene was generated by digesting plasmid pGEM7Zf(+)::HIS6 (pRD643) (Fani *et al*. 1997) with *Pae*I/*Sac*I restriction enzymes. Following recovery and purification, the 1.5 kb fragment was ligated into the plasmid YEplac112 that had been previously digested with the same enzymes. The resultant plasmid was denoted YEplac112::*HIS6*. *Pae*I = *Pae*I restriction site, *Sac*I = *Sac*I restriction site, *HIS6* = the *HIS6* gene, MCS = multiple cloning sites, Amp^R = ampicillin resistance gene, 2μ = yeast 2μ plasmid autonomous replication sequence, ori = pBR322 origin of replication, *TRP1* = yeast *TRP1* gene.

Functionality of the Yeast *HIS6* Gene as a Selectable Marker. In order to test the ability of the cloned *HIS6* gene to complement *his6* deficiency in yeast cells, the plasmid YEplac112::*HIS6* was introduced into M31 (*mit*$^-$, *ade1*, *his6*, *atp8*) cells, a yeast *S. cerevisiae* strain lacking functional *HIS6* gene due to mutation in the *HIS6* gene (Macreadie *et al.* 1983). The M31 cells therefore are unable to synthesize histidine *de novo*. Approximately 20 μg plasmid DNA was used to transform M31 using the method of Klebe *et al.* 1983. Transformants were selected on solid minimal medium without histidine supplementation. Since the M31 strain also has *ade1* genotype and the YEplac112 does not carry yeast *ADE1* sequences, adenine was included in the selective medium.

From the results shown in Figure 3, it was clear that the cloned *HIS6* gene was able to rescue the *his6* mutation of strain M31 since M31 cells transformed with plasmid YEplac112::*HIS6* were able to grow on minimal medium without histidine supplementation while the untransformed M31 cells were failed to display growth on the same medium. In order to confirm this observation, the introduced YEplac112::*HIS6* plasmid was recovered from M31 transformants using the method of Hoffman and Winston (1987). The plasmid DNA was extracted from a mid-logarithmic phase culture of M31 transformants in a buffer solution containing sodium dodecyl sulphate (SDS) and Triton X-100. Following addition of phenol/chloroform solution and glass bead the mixture was vigorously vortexed and the centrifuged. The plasmid DNA recovered from the top aqueous layer was amplified in *E. coli* cells. Following large scale DNA isolation from *E. coli*, the plasmid was introduced into "fresh" M31 cells. Following

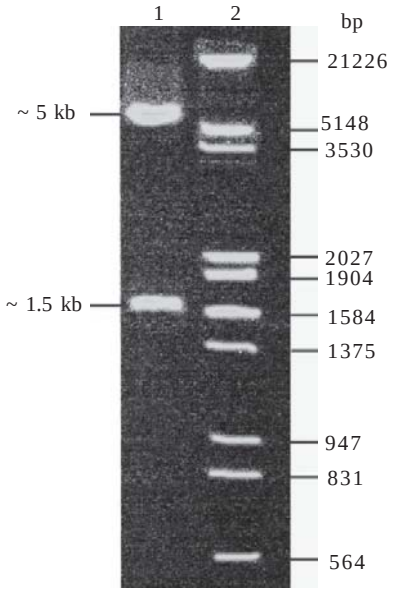

Figure 2. Restriction enzyme digestion analysis of plasmid YEplac112::*HIS6*. Restriction digestion analysis of a putative recombinant plasmid YEplac112 harboring the *HIS6* gene is presented. Lane 1: *Pae*I/*Sac*I digest of putative YEplac112::*HIS6*. The upper band in this line is the vector YEplac112 (about 5 kb). The 1.5 kb band is that expected if the insert into YEplac112 is as expected (the *HIS6* gene). Lane 2: lambda DNA digested with *Hind*III/*Eco*RI restriction enzymes. The size of each fragment is indicated.

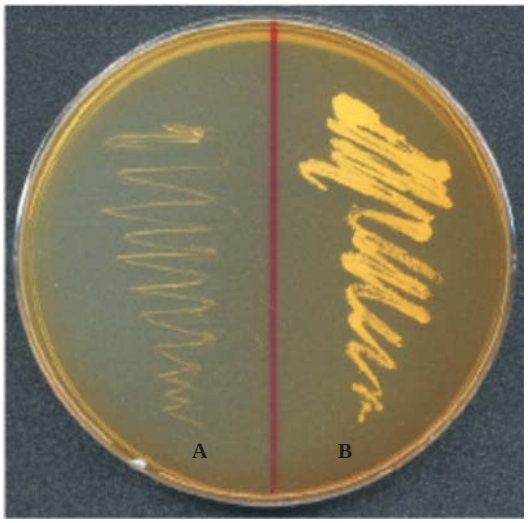

Figure 3. Functional analysis of the yeast *HIS6* gene in strain M31. The functionality of the yeast *HIS6* gene in strain M31 was tested by transforming M31 cells with YEplac112::*HIS6*. The transformants were then grown on selective minimal plus adenine (min + ADE) medium and incubated at 28 °C for 3 days. Panel A: untransformed M31 cells. Panel B: M31 cells transformed with YEplac112::*HIS6*.

plating on solid minimal medium lacking histidine it was found that the "second round" transformants were able to grow on this medium indicating that the presence of functional *HIS6* gene in these cells. The yeast *HIS6* gene selectable marker, therefore, was shown to be satisfactory for selecting and maintaining a yeast vector in host strain M31.

DISCUSSION

The present study has added the repertoire of selectable markers for construction of yeast vector. In addition, the present study has allowed a more extensive used of yeast strains with *his6* auxotrophy as host cells for various studies involving gene expression in yeast. The M31 strain, for example, has routinely been employed in the study of structure, function, and assembly aspects of subunit 8 of yeast *S. cerevisiae* mitochondrial ATP synthase (Cox *et al*. 1992; Artika 2007). The availability of HIS6 marker in addition to the routinely employed ADE1 marker has permitted the maintenance in M31 strain of two different yeast expression vectors each utilizing a different promoter and harboring a different variant of subunit 8 gene. This system therefore has allowed the expression of each variant to be independently regulated. This dual control expression strategy is of particular important in the *in vivo* study of assembly of defective subunit 8 variants.

The availability of HIS6 marker to allow the development of a dual control expression system for subunit 8 variants in M31 cells has been sought since one of the problems in testing the assembly of nonfunctional or functionally impaired variants of subunit 8 proteins is the tendency of the *mit*$^-$ mutant (M31) host cells to turn into petite cells. It has been observed that yeast cells with nonfunctional mitochondria are genetically unstable and they have a tendency to lose an introduced plasmid (Gray 1996). In the dual control expression system, the functional (wildtype) subunit 8 gene is expressed under a conditional promoter system and the expression of the subunit 8 variant gene is under the control of a different promoter system. The expression of the functional subunit 8 gene is intended to ensure the maintenance of stable *mit*$^-$ yeast cells during cell propagation. It is under conditional expression control so that its expression can be turned off when the expression of the variant is turned on, and then the assembly behavior of the variant can be accurately determined. One of the advantages of using *HIS6* gene as selectable marker is that it allows the use of yeast mutant strains with unique *his6* mutation as host cells. In addition, the availability of HIS6 marker opens up the opportunity to develop new series of yeast expression vector by incorporating HIS6 marker. This will extend the availability and choices of yeast expression system for various types of studies and commercial purposes.

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