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# Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds

(*Saccharomyces cerevisiae*; plasmid; multicopy vector; polylinker; promoter; heterologous expression; cDNA cloning)

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SUMMARY

An expression system for *Saccharomyces cerevisiae* (*Sc*) has been developed which, depending on the chosen vector, allows the constitutive expression of proteins at different levels over a range of three orders of magnitude and in different genetic backgrounds. The expression system is comprised of cassettes composed of a weak *CYC1* promoter, the *ADH* promoter or the stronger *TEF* and *GPD* promoters, flanked by a cloning array and the *CYC1* terminator. The multiple cloning array based on pBIISK (Stratagene) provides six to nine unique restriction sites, which facilitates the cloning of genes and allows for the directed cloning of cDNAs by the widely used ZAP system (Stratagene). Expression cassettes were placed into both the centromeric and 2μ plasmids of the pRS series [Sikorski and Hieter, Genetics 122 (1989) 19–27; Christianson et al., Gene 110 (1992) 119–122] containing *HIS3*, *TRP1*, *LEU2* or *URA3* markers. The 32 expression vectors created by this strategy provide a powerful tool for the convenient cloning and the controlled expression of genes or cDNAs in nearly every genetic background of the currently used *Sc* strains.

## INTRODUCTION

The unicellular eukaryote *Saccharomyces cerevisiae* (*Sc*) has become a popular model system for molecular biology. An increasing number of gene products from *Sc* shows a significant structural and/or functional similarity to those of humans and other higher eukaryotes. Many mutants of *Sc* have been successfully used for the isolation of functional homologues from other species by heterologous complementation. Furthermore, *Sc* is now widely used for studying the interaction of proteins from various organisms by the two-hybrid-system (Fields and Song, 1989; Gyuris et al., 1993; Harper et al., 1993).

The analyses described above require the ectopic expression of genes or cDNAs at different levels under the control of heterologous promoters. Several expression vectors for yeast have been constructed for this purpose (Rose and Broach, 1990). Many of these vectors however suffer from certain disadvantages. They often do not provide a cloning array with multiple restriction sites. Most of them are derivatives of the large pBR322 plasmid lacking the versatility of current *E. coli* vectors. In addition, many of the yeast expression vectors consist of individual vector backbones and cloning arrays. As a consequence the exchange of selectable markers genes, which may be desirable for the expression of several different proteins or for the use in different genetic backgrounds, is usually

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Abbreviations: *ADH*, gene encoding alcohol dehydrogenase 1; bp, base pair(s); *CEN*, centromere; *CYC1*, gene encoding cytochrome-*c* oxidase, *GPD*, gene encoding glyceraldehyde-3-phosphate dehydrogenase; HA, hemagglutinin; kb, kilobase(s) or 1000 bp; 2μ, yeast plasmid; MCS, multiple cloning site; nt, nucleotide(s); *ori*, origin of replication; PCR, polymerase chain reaction; *Sc*, *Saccharomyces cerevisiae*; u, unit(s); *TEF*, gene encoding translation elongation factor 1α; *UAS*, upstream activation sequence(s).

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difficult. Furthermore these vectors are not ideally suitable for the comparison of transcription-level-dependent effects.

In this paper, we describe a series of compact vectors designed for the convenient cloning of genes and their controlled expression at different levels. Expression levels can be manipulated by changing either the strength of transcription or the copy number of recombinant gene. Along these lines, we have established an expression system composed of several promoters of different strength, a multiple cloning array and a terminator from the *CYC1* gene (Zaret and Sherman, 1982). These expression cassettes were introduced into low (*CEN/ARS*) or high copy number (2μ) plasmids carrying one of the four different selection markers of the pRS-series of vectors (Sikorski and Hieter, 1989; Christianson et al., 1992).

## EXPERIMENTAL AND DISCUSSION

### (a) Construction of the vectors

For the construction of a new series of yeast expression vectors, we chose four constitutive promoters of different strength derived from the genes encoding cytochrome-*c* oxidase (*CYC1*; Guarente et al., 1984), alcohol dehydrogenase 1 (*ADH* from the *ADH1* gene; the Hitzeman et al., 1981), translation elongation factor 1α (*TEF* from the *TEF2* gene; Schirmaier and Philippsen, 1984; Nagashima et al., 1986) and glyceraldehyde-3-phosphate dehydrogenase (*GPD*; Musti et al., 1982; Bitter et al., 1984). The wild-type *ADH* promoter is active when cells are grown in glucose media but can be repressed 2–10-fold on non-fermentable carbon sources (Beier and Young, 1982; Denis et al., 1983). The truncated version of the *CYC1* promoter generated here is very weak and no longer inducible due to a deletion of most of the *UAS2* sequence (Guarente et al., 1984).

Promoters were cloned as PCR-generated *Sac*I-*Xba*I fragments into a vector based on the plasmid pRS416 (Sikorski and Hieter, 1989) which already carried a PCR-generated *Xho*I-*Kpn*I fragment of the *CYC1*-terminator (Fig. 1). From the resulting plasmids, termed p416prom, the different expression cassettes (Fig. 1B) were cloned into the centromeric and 2μ plasmids (p41Xprom and p42Xprom; Fig. 1A) of the pRS series carrying a *HIS3*, *TRP1*, *LEU2* or *URA3* marker gene (p4X3, p4X4, p4X5 or p4X6 in Fig. 1A). The MCS of these plasmids based on pBIISK (Stratagene, La Jolla, CA, USA) provides 6 to 9 unique cloning sites depending on the plasmid backbone (Table I). This MCS allows the construction of cDNA libraries by the directional cloning of cDNAs generated by the widely used ZAP system (Stratagene). Since the polylinker does not provide an ATG start codon only inserts carrying their own start codon will be expressed. In case a useful cloning site is not unique due to a second site in the marker gene, the insert can first be cloned into a vector lacking this site and then be transferred into the vector carrying the desired selectable marker gene.

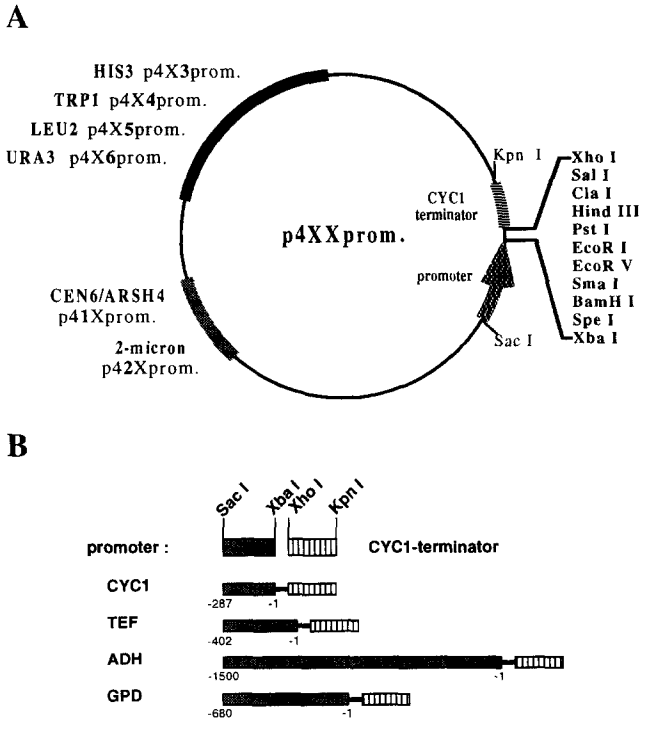


Fig. 1. Structure of the expression vectors. (**A**) Schematic map of the plasmids. The nomenclature is based on the plasmids described by Christianson et al. (1992). For example, plasmid p424ADH carries the *ADH* promoter and is based on pRS424 carrying the *TRP1* gene and the 2μ *ori*. Shown are the restriction sites of the MCS (bold) between the promoter (arrow) and the terminator. Unique sites depending on the plasmid backbone are shown in Table I. (**B**) Maps of the several promoters (shaded boxes) and the *CYC1* terminator (striped box) used for the expression cassettes. Numbers below the boxes represent the regions of the promoters in bp cloned by PCR relative to the start codon (+1 would be A of the ATG codon).

TABLE I

MCS for expression vectors

| Plasmid[a] | Marker gene | Restriction sites (in MCS)[b] |
|---|---|---|
| p4X3 | *HIS3* | ***Xba*I *Spe*I *Bam*HI *Sma*I *Eco*RV *Eco*RI** *Pst*I *Hin*dIII ***Cla*I *Sal*I *Xho*I** |
| p4X4 | *TRP1* | *Xba*I ***Spe*I *Bam*HI *Sma*I** *Eco*RV ***Eco*RI** ***Pst*I** *Hin*dIII ***Cla*I *Sal*I *Xho*I** |
| p4X5 | *LEU2* | ***Xba*I *Spe*I *Bam*HI *Sma*I** *Eco*RV *Eco*RI ***Pst*I *Hin*dIII** *Cla*I ***Sal*I *Xho*I** |
| p4X6 | *URA3* | ***Xba*I *Spe*I *Bam*HI *Sma*I** *Eco*RV ***Eco*RI** *Pst*I ***Hin*dIII *Cla*I *Sal*I *Xho*I** |

[a] See Fig. 1.

[b] Unique restriction sites are shown in boldface. The *Xba*I site is only unique in the centrometric p41X vectors. The *Xho*I site is not unique in the p4XX**CYC1** vectors (see Fig. 1).

**(b) Expression rates of the vectors**

In order to compare the expression rates achieved by the different vectors, a *lacZ* gene was cloned as a *XbaI-BamHI* cassette downstream of the promoters in the plasmids carrying the *URA3* marker. The reporter constructs were transformed into the strain YPH499 (Sikorski and Hieter, 1989) and the β-galactosidase activity was determined as described (Ausubel et al., 1991). As can be seen in Fig. 2, expression levels varied by approx. three orders of magnitudes, with the lowest level seen with the centromeric *CYC1* construct (0.9 units), and the highest level found with the 2μ *GPD* construct (935 units).

The expression levels obtained with centromeric and 2μ vectors respectively, varied between 2.6-fold for the *CYC1* promoter and 30-fold for the ADH promoter. This roughly corresponds to the difference in copy number of 1 copy per cell for a centromeric plasmid and 10–30 copies per cell for a 2μ plasmid, as determined for the pRS series of plasmids (Christianson et al., 1992). The lower increase of expression observed with the *GPD* and *TEF* promoters (fivefold) in the 2μ relative to the centromeric plasmids might be due to insufficient levels of transcription factors in cells transformed with the high copy number 2μ plasmids. In the case of the *CYC1* promoter the complete lack of an *UAS* sequence might explain the relative low increase of expression from a 2μ plasmid.

Another strong advantage of the system presented here is based on the observation that all of the described vectors show considerable levels of expression in *E. coli* (data not shown). After cloning of cDNAs into these vectors, we frequently use this feature to check *E. coli* transformants for the expression of proteins of correct size by Western blotting. This is a convenient way to select appropriate clones for subsequent transformation into yeast cells. Comparison of the proteins made in *Sc* and *E. coli* often gives a first hint with respect to post-translational modifications. The moderate strength of expression from the yeast promoters in *E. coli* should not lead to a toxic effect observed for the strong over-expression of many eukaryotic sequences in *E. coli*.

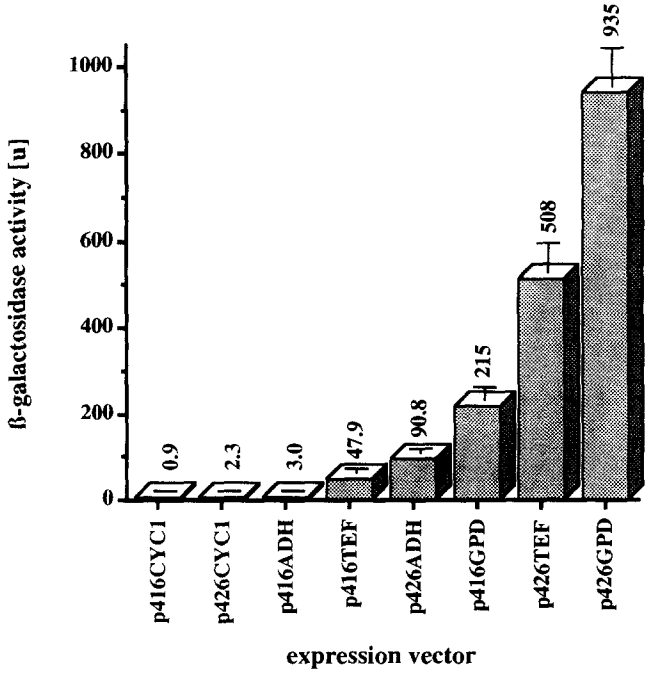


Fig. 2. Expression levels of the different vectors. The β-galactosidase activities of the promoter/*lacZ* fusions (see Fig. 1) in the plasmids p416 or p426 were determined as described by Ausubel et al. (1991). Values represent an average of five independent colonies tested. The standard deviation is given as a bar at the top of the columns.

**(c) Conclusions**

We have cloned a total of 32 yeast expression vectors that allow for the constitutive expression of heterologous proteins at various levels over a range of three orders of magnitude. The large multiple cloning array provides up to nine unique restriction sites for the convenient cloning of the desired genes or cDNAs. The different marker genes of the vectors facilitate the expression analysis in different genetic backgrounds. They also provide a powerful tool to study the co-expression in the same cell of up to four different genes at various levels. Cells carrying up to for different centromeric plasmids stably maintain the vectors and we did not observe recombination between the plasmids as well as interference between the promoters on expression level.

Many of the vectors described here have already been used successfully in our lab for the over-expression and co-expression of several yeast transcription factors (GCN4, YAP1 and YAP2; M.F. and R.M., data not shown) and cell cycle genes (SWI4, SWI6 and G1 cyclins; D.M., M.F. and R.M., data not shown). They also have been used for the heterologous expression of various murine and human cDNAs (Sewing et al., 1994) and fusion proteins (Jooss et al., 1994). In addition, based on this vector system, we constructed a new series of plasmids for the expression of proteins fused to the bacterial repressor LexA or the transactivation domain of the VP16 protein in a two hybrid system (J. Zwicker, M.F. and R.M., data not shown). We also generated analogous vectors that direct the expression of respective proteins with a N-terminal 9-amino-acid-long epitope from the influenza virus HA protein, thus allowing for the simple detection of the expressed protein by a high affinity monoclonal antibody raised against the HA epitope which is commercially available (Hiss Diagnostics).

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