Additional Modules for Versatile and Economical PCR-based Gene Deletion and Modification in Saccharomyces cerevisiae

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An important recent advance in the functional analysis of Saccharomyces cerevisiae genes is the development of the one-step PCR-mediated technique for deletion and modification of chromosomal genes. This method allows very rapid gene manipulations without requiring plasmid clones of the gene of interest. We describe here a new set of plasmids that serve as templates for the PCR synthesis of fragments that allow a variety of gene modifications. Using as selectable marker the S. cerevisiae TRP1 gene or modules containing the heterologous Schizosaccharomyces pombe his5 + or Escherichia coli kan’ gene, these plasmids allow gene deletion, gene overexpression (using the regulatable GAL1 promoter), C- or N-terminal protein tagging [with GFP(S65T), GST, or the 3HA or 13Myc epitope], and partial N- or C-terminal deletions (with or without concomitant protein tagging). Because of the modular nature of the plasmids, they allow efficient and economical use of a small number of PCR primers for a wide variety of gene manipulations. Thus, these plasmids should further facilitate the rapid analysis of gene function in S. cerevisiae.

INTRODUCTION

In Saccharomyces cerevisiae, gene deletion and the modification of chromosomal genes by homologous recombination are now standard techniques. Among the useful gene modifications are placing a gene under control of a regulatable promoter for overexpression and/or protein-depletion studies and adding sequences encoding protein tags that allow facile protein detection and isolation. Recently, a PCR-mediated technique has been developed that allows single-step deletion and tagging of chromosomal genes (McElver and Weber, 1992; Baudin et al., 1993; Lorenz et al., 1995; Wach et al., 1994, 1997, 1998). In this method, the PCR primers used have 5'-ends (~40 nucleotides) that correspond to the desired target gene sequences and 3'-ends (~20 nucleotides) that anneal to and allow amplification of the selectable marker gene (and, if included in the template, of sequences encoding a tag). The amplified DNA is

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transformed directly into yeast, and homologous recombinants that carry the deleted or tagged target gene are identified. The method works best when the selectable marker itself cannot undergo homologous recombination with the host genome, and to this end heterologous marker modules based on the Escherichia coli kan^R gene (which confers resistance to G418/geneticin: Jiminez and Davies, 1980; Hadfield et al., 1990) or the Schizosaccharomyces pombe his5^+ gene (which complements S. cerevisiae his3 mutations) have been developed (Wach et al., 1994, 1997).

Tagging of a protein has many uses. The Aequorea victoria green fluorescent protein (GFP) is proving to be invaluable for localizing proteins, both for protein localization by immunofluorescence (Prasher, 1995; Heim et al., 1996; Niedenthal and Tsien, 1996; Prasher, 1995; Heim et al., 1996) and to this end heterologous marker modules based on the Escherichia coli kan^R gene (which confers resistance to G418/geneticin: Jiminez and Davies, 1980; Hadfield et al., 1990) or the Schizosaccharomyces pombe his5^+ gene (which complements S. cerevisiae his3 mutations) have been developed (Wach et al., 1994, 1997).

**MATERIALS AND METHODS**

**Strains, growth conditions, and DNA methods**

All S. cerevisiae strains were derived from YEF473 (a/α leu3-52/ura3-52 his3Δ200/his3Δ200 trplα-63/trplα-63 leu2-1/leu2-1 lys2-801/lys2-801) (Bi and Pringle, 1996). Yeast were grown at 30°C on YP solid medium, synthetic complete (SC) liquid or solid medium, or Y M-R rich liquid medium (Lillie and Pringle, 1980; Guthrie and Fink, 1991) containing 2% dextrose, 1% raffinose, or 1% raffinose plus various concentrations of galactose (as indicated).YPD-G418 plates contained YP medium with 2% dextrose and 200 μg/ml G418 (Life Technologies, Gaitersburg, M D). E. coli strain DH125 (Life Technologies) and standard media and methods (Ausubel et al., 1995) were used for plasmid manipulations. Yeast genomic DNA was isolated according to the method of Hoffman and Winston (1987). Plasmid DNA was isolated from E. coli and from agarose gels using Qiagen kits (Qiagen, Santa Clarita, CA). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Yeastmaker DNA (Clontech Laboratories, Palo Alto, CA) was used as carrier DNA in yeast transformations.

DNA for plasmid constructions and yeast transformation was generated by PCR using the Expand system (Boehringer Mannheim, Indianapolis, IN) and HotStart 100 tubes (Molecular Bio-Products, San Diego, CA). The lower mix (final volume, 25 μl) contained 2.5 μl of Expand buffer with 17.5 mM MgCl₂, 0.8 mM of each dNTP, 10 μg of BSA, and 2 μM of each primer. The upper mix (final volume, 75 μl) contained 7.5 μl of Expand buffer with 17.5 mM MgCl₂, 0.75 μl of the Expand enzyme mixture, and 0.1 μg of template plasmid DNA. Reactions were run for 20 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min/kb of the desired product at 68°C; the 20 cycles were followed by a 10-min extension at 68°C.

PCR screening of transformants for integration by homologous recombination was done in 50 μl reactions containing 2 μM of each primer, 2 mM MgSO₄, 5 μg of BSA, 0.2 mM of each dNTP, 0.5 μl (2.5 units) of Taq DNA polymerase (Promega, Madison, WI), and 1 μl (~0.5 μg) of yeast genomic DNA. PCR reactions were run for 36 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min/kb of the desired product at 68°C; the 36 cycles were followed by a 10-min extension at 68°C.

PCR screening of transformants for integration by homologous recombination was done in 50 μl reactions containing 2 μM of each primer, 2 mM MgSO₄, 5 μg of BSA, 0.2 mM of each dNTP, 0.5 μl (2.5 units) of Taq DNA polymerase (Promega, Madison, WI), and 1 μl (~0.5 μg) of yeast genomic DNA. PCR reactions were run for 36 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min/kb of the desired product at 68°C; the 36 cycles were followed by a 10-min extension at 68°C.

**Plasmid constructions**

Plasmids pFA6a-kanMX6, pFA6a-His3MX6, pFA6a-GFP(S65T)-kanMX6, and pFA6a-GFP(S65T)-His3MX6 have been described by Wach et al. (1997); plasmid pGTEP (Tyers et al., 1993; M. Tyers, G. Tokiwa, and B. Futcher, personal communication) contains sequences encoding three tandem repeats of the influenza virus hemagglutinin epitope (3HA); plasmid pCR2.1 15X Myc (containing sequences encoding 15 tandem repeats of the Myc epitope) was kindly provided by O. M ondesert and P. R ussell; plasmid pGEX-2T (Smith and Johnson, 1988) contains the...
Figure 1. Modules for use as PCR templates to generate fragments for gene manipulation. Gray boxes: selectable markers including the kanMX6 module (Wach et al., 1994), the S. cerevisiae TRP1 gene (see text), and the His3MX6 module including the S. pombe his5+ gene (Wach et al., 1997). Black boxes: protein-tagging modules consisting of the sequences encoding 3HA, 13Myc, GST, or GFP(S65T) together with the S. cerevisiae ADH1 terminator. Arrows within the boxes indicate directions of transcription. Arrows outside the boxes indicate forward (F) and reverse (R) PCR primers (not to scale; see Table 1); the bent portions represent the regions of the primers homologous to the yeast target sequences. PCR product sizes are indicated assuming that each primer includes exactly 40 nucleotides of homology to the target sequence. Restriction sites used for cloning are indicated; note that the AscI site at the junction of 13Myc and TADH1 sequences was lost during the construction of the pFA6a-13Myc plasmid series (see Materials and Methods). (A) Modules to be used for gene deletion, C-terminal protein tagging, or C-terminal protein truncation with or without protein tagging. (B) Modules to be used for placing a full-length or N-terminally truncated gene under control of the GAL1 promoter (white boxes) with or without concomitant protein tagging.
Schistosoma japonicum gene encoding GST; and plasmid pBM 272 (Johnston and D avis, 1984) contains the S. cerevisiae GAL1/10 promoter region. Other plasmids (Figure 1) were constructed as follows; in these descriptions, italics in oligonucleotide primer sequences indicate a restriction enzyme site, underlining indicates the complement of a stop codon, and bold face indicates the complement of a start codon. Sequencing of all PCR products immediately after their initial cloning identified no mutations resulting from the PCR (except in the case of the 13 M yc sequences; see below), and sequencing of insert junctions confirmed the preservation of the desired reading frames (where relevant).

To construct plasmid pFA 6a-TRP1, the ~920-bp PCR product obtained using pRS304 (Sikorski and Hieter, 1989) as template, forward primer 5'-AAAAAGATCTGCTACAATCTTGTACCGAGC-3' and reverse primer 5'-AAACTCTTACGCATCTGTGGGCGG-3' was digested with BglI and Pmel and ligated into BglI/Pmel-digested pFA 6a-kanMX 6, thus replacing the kanMX 6 module with the S. cerevisiae TRP1 gene. To construct plasmid pFA 6a-GFP(S65T)-TRP1, the BglI-Pmel fragment containing TRP1 from pFA 6a-TRP1 was ligated into BglI/Pmel-digested pFA 6a-GFP(S65T)-kanMX 6, thus replacing the kanMX 6 module with TRP1. To construct plasmid pFA 6a-3HA-kanMX 6, the PCR product obtained using pGTEP (see above) as template, forward primer 5'-AAAAATTAATTTAGTTCCCTCTATACTGTTG-3' and reverse primer 5'-AAAAAGGCGGCGCTCAAGACTGCTGTAATCTGGAAC-3' was digested with PacI and Ascl and ligated into PacI/Ascl-digested pFA 6a-GFP(S65T)-kanMX 6, thus replacing GFP(S65T) with a 125-bp fragment encoding a 3HA epitope followed by a stop codon introduced by the primer. Plasmids pFA 6a-3HA-TRP1 and pFA 6a-3HA-His3MX 6 were then created by digesting pFA 6a-3HA-kanMX 6 with BglI and Pmel and ligating in the corresponding fragments from pFA 6a-TRP1 and pFA 6a-His3MX 6, respectively.

To construct plasmid pFA 6a-13Myc-kanMX 6, the PCR product obtained using PCR Z.1 15X M yc (see above) as template, forward primer 5'-AAAAAATTAAAATTTAGTTCCCTCTATACTGTTG-3' and reverse primer 5'-AAAAAGGCGGCGCTCAAGACTGCTGTAATCTGGAAC-3' was cloned into pGEM-TA (Promega), yielding plasmid pGEM-TA/M yc. Sequence analysis of the insert in pGEM-TA/M yc revealed that the Pcl site at one end was intact and that the insert encoded 13 M yc epitopes (the DNA encoding two M yc epitopes had apparently been lost during the PCR reaction) followed by the stop codon introduced by the primer. However, the Ascl site had been altered during the PCR reaction. Thus, pFA 6a-13Myc-kanMX 6 was constructed by digestion of pGEM-TA/M yc with EcoRI (at a site present in the pGEM-TA sequences), followed by blunt-ending of the units using T4 DNA polymerase and then digestion with Pcl. The resulting 594-bp fragment was purified and ligated to pFA 6a-GFP(S65T)-kanMX 6 that had been digested with Ascl, made blunt ended as above, and then digested with Pcl. Plasmids pFA 6a-13Myc-kanMX 6 and pFA 6a-13Myc-His3MX 6 were constructed by replacing the BglII-Pmel fragment containing the kanMX 6 module of pFA 6a-13Myc-kanMX 6 with the corresponding fragments from pFA 6a-TRP1 and pFA 6a-His3MX 6, respectively.

To construct plasmid pFA 6a-6a-GST-kanMX 6, the ~700-bp PCR product obtained using pGEX-2T (see above) as template, forward primer 5'-AAAAATTAATTTAGTTCCCTCTATACTGTTG-3' and reverse primer 5'-AAAAAGGCGGCGCTCAAGACTGCTGTAATCTGGAAC-3' was digested with PacI and Ascl and ligated into PacI/Ascl-digested pFA 6a-GFP(S65T)-kanMX 6, thus replacing GFP(S65T) with sequences encoding GST followed by a stop codon introduced by the primer. To construct plasmids pFA 6a-GST-TRP1 and pFA 6a-GST-His3MX 6, BglII-Pmel fragments from pFA 6a-TRP1 and pFA 6a-His3MX 6, respectively, were ligated into BglI/Pmel-digested pFA 6a-GST-kanMX 6.

To construct plasmid pFA 6a-kanMX 6-6PGAL1, the PCR product (containing the GAL1 promoter) obtained using pBM 272 (see above) as template, forward primer 5'-AAAAAGATCTGCTGTAATCTGGAAC-3' and reverse primer 5'-AAAAATTAATTTAGTTCCCTCTATACTGTTG-3' was digested with BglI and Pcl, and the resulting 569-bp fragment was ligated into BglI/Pcl-digested pFA 6a-kanMX 6. To construct plasmids pFA 6a-TRP1-PGAL1 and pFA 6a-His3MX 6-PGAL1, the BglI-Pcl fragment containing the PGAL1 promoter from pFA 6a-kanMX 6-PGAL1 was ligated into BglI/Pcl-digested pFA 6a-TRP1 and pFA 6a-His3MX 6, respectively.

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**PCR-based Gene Deletion and Modification**

Table 1. PCR primers used to amplify the transformation modules.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Purpose</th>
<th>Primer sequencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Deletion</td>
<td>5’-(gene-specific sequence) CCGATCCCCGGGTATTTA-3’b</td>
</tr>
<tr>
<td>R1</td>
<td>Deletion/C-terminal tagging</td>
<td>5’-(gene-specific sequence) GAATTCTAGCTTGGTAAAC-3’b,c</td>
</tr>
<tr>
<td>F2</td>
<td>C-terminal tagging</td>
<td>5’-(gene-specific sequence) CGGATCCCGGTATTTA-3’c</td>
</tr>
<tr>
<td>F3</td>
<td>C-terminal truncation (no tag)</td>
<td>5’-(gene-specific sequence) TGA GGCCGCGCCACTTCTA-3’c</td>
</tr>
<tr>
<td>F4</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt; introduction</td>
<td>5’-(gene-specific sequence) GAATTCTAGCTTGGTAAAC-3’d</td>
</tr>
<tr>
<td>R2</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt; (no tag)</td>
<td>5’-(gene-specific sequence) CATTTGAGATCCGCTTT-3’a</td>
</tr>
<tr>
<td>R3</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt; with 3HA tagging</td>
<td>5’-(gene-specific sequence) GCA CTG ACG GAC GTA ATC TG-3’f</td>
</tr>
<tr>
<td>R4</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt; with GST tagging</td>
<td>5’-(gene-specific sequence) ACG CGG AAC CAG ATC CGA TT-3’f</td>
</tr>
<tr>
<td>R5</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt; with GF(P565T) tagging</td>
<td>5’-(gene-specific sequence) TTT GTA TAG TTC ATC CAT GC-3’f</td>
</tr>
</tbody>
</table>

The primer combinations used for various manipulations and the orientations and locations of the forward (F) and reverse (R) primers relative to the plasmid templates are indicated in Figure 1. The reading frames for primers used to introduce protein tags are indicated by spacing in the sequences, and restriction enzyme sites included in the primers (see Figure 1) are indicated by italics: GGATCC, BamHI; GAATTCT, EcoRI; GTTAAAC, PmeI; TTTAATTA, PstI; GGCAGCCGC, AscI.

*For deletions, the gene-specific sequences of the forward primer are typically chosen to end just upstream of the start codon, whereas those of the reverse primer are chosen to end just downstream of the stop codon.

*For tagging of full-length proteins, the gene-specific sequences of the forward primer are chosen to end just upstream of the stop codon, preserving the reading frame of the tag, whereas those of the reverse primer are chosen to end just downstream of the stop codon. For C-terminal truncations, the gene-specific sequences of the forward primer are chosen depending on the desired location of the truncation. If the protein is to be truncated without tagging, primer F3 (which includes a stop codon; underlining) is used with one of the plasmids of Figure 1A that contains the AscI site at the junction of the tag and T<sub>ADH1</sub> sequences.

*For N-terminal truncations, the gene-specific sequences are chosen from the region where the truncation is desired. In each case, the primer must maintain the reading frame of the start codon provided in the primer.

*For N-terminal tagging of full-length proteins, the gene-specific sequences are chosen to correspond to the N-terminal codons of the target gene; they may or may not include its start codon but must maintain the indicated reading frame. For N-terminal truncation with protein tagging, the gene-specific sequences are chosen from the region where the truncation is desired, maintaining the indicated reading frame. Each tag requires a unique primer that corresponds to the region just upstream of the stop codon present in the DNA encoding the protein tags.

GFP(S65T) under control of the GAL1 promoter, pFA6a-kanMX6-P<sub>GAL1</sub>, pFA6a-TRP1-P<sub>GAL1</sub>, and pFA6a-His3MX6-P<sub>GAL1</sub> were digested with P<sub>acI</sub> and BamHI and ligated to P<sub>acI</sub>-BglII fragments carrying sequences encoding the tags from pFA6a-3H<sub>A</sub>-kanMX6, pFA6a-G<sub>S</sub>ST-kanMX6, and pFA6a-GFP(P565T)-kanMX6.

Transformation of yeast and screening for homologous integration at the target gene

PCR was performed using one of the plasmids shown in Figure 1 as template and appropriate target-gene-specific primer pairs designed as indicated in Figure 1 and Table 1. The products from six to eight PCR reactions were pooled, extracted once with phenol:chloroform:isoamyl alcohol (25:24:1), precipitated, and resuspended in 10 μl of water. This concentrated DNA was transformed into S. cerevisiae cells using a lithium acetate protocol (Gietz et al., 1992). G418-resistant transformants (containing the kanMX6 module) were selected essentially as described previously (Wach et al., 1994; Wach, 1996). Briefly, the transformed cells were washed once with 1 ml of water, resuspended in 200 μl of water, and spread on two YPD plates (100 μl per plate). These plates were incubated at 30°C for 2–3 days and replica-plated to YPD-G418 plates. To identify stable transformants, the YPD-G418 plates...
were incubated at 30°C for 2–3 days and then replica-plated to fresh YPD-G418 plates, and G418-resistant colonies were picked and streaked on YPD-G418 plates. Trp⁺ and His⁺ transformants were selected by standard procedures (Guthrie and Fink, 1991). To identify transformants in which the module had indeed integrated by homologous recombination with the target gene sequences, genomic DNA was prepared and used as the template in PCR reactions (see above) using one primer that annealed within the transformation module and a second primer that annealed to the target gene locus outside the region altered. A PCR product of the expected size confirmed homologous integration; all transformants segregated 2:2 for the selectable marker.

Morphological observations

Differential interference contrast and epifluorescence microscopy were performed using a Nikon Microphot SA microscope. Cells were prepared for immunofluorescence as described by Pringle et al. (1991). Monoclonal anti-HA epitope (HA.11) and monoclonal anti-c-Myc epitope (9E10) antibodies were purchased from Berkeley Antibody Company (Richmond, CA). FITC and rhodamine-labeled secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA).

Plasmid requests

Send plasmid requests to Mark Longtine (fax: (+1) 919/962 0320; e-mail: mlunc@isis.unc.edu). Investigators planning to use one or more of the plasmids for commercial purposes should state this in their requests. To receive a DNA Strider file of the plasmid sequences, send a Macintosh-formatted disk. For plasmids containing the GFP(S65T) allele, a Howard Hughes Medical Institute material transfer agreement must be signed. To obtain this document, contact Roger Y. Tsien, Howard Hughes Medical Institute, Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92037–0647 (fax: (+1) 619/534 5270) and state that you want to obtain the pFA-series plasmids with GFP(S65T) registered to A. Wach and P. Philippens. A copy of the material transfer agreement must be received before plasmids containing GFP(S65T) can be shipped.

RESULTS AND DISCUSSION

PCR template plasmids for gene deletion and gene tagging

Wach et al. (1994, 1997) have described a set of plasmids that can be used as templates in PCR reactions to generate DNA fragments that can be used for targeted modification of chromosomal genes. These plasmids use either the kanMX6 module (which confers resistance to G418) or the His3MX6 module (containing the S. pombe his5+ gene, which complements S. cerevisiae his3 mutations) as selectable marker and allow either gene deletion or generation of fusion genes encoding proteins with GFP (wild-type or the S65T mutant version) fused to their C-termini. In the work reported here, we have expanded this collection of plasmids in three ways.

First, we have created plasmids that allow use of the S. cerevisiae TRP1 gene as the selectable marker for gene deletion and the generation of C-terminal GFP(S65T) fusions (Figure 1A). A disadvantage of using the TRP1 marker in this method is that it can recombine efficiently with the endogenous trp1 locus; thus it is only practical for use in strains that contain TRP1 deletions such as trp1Δ-63 (Sikorski and Hieter, 1989). However, because the PCR products obtained using the kanMX6 and His3MX6 modules are homologous for long regions at their ends due to the shared A. gossypii TEF promoter and terminator sequences (Figure 1A), transformation of a strain containing one of these modules with a PCR product containing the other (to modify a second gene) may result in recombination with the previously integrated selectable marker rather than with sequences at the new target gene. Because the TRP1-containing PCR products have only ~20 bp of homology with either the kanMX6 or the His3MX6 module, this problem is avoided.

Second, we have created plasmids that allow use of any of the three selectable markers to generate fusion genes encoding proteins fused at their C-termini to a triple HA epitope, a 13Myc epitope, or GST (Figure 1A). These protein tags have all been widely used; commercially available monoclonal antibodies directed against the HA or Myc epitope (see Materials and Methods) work well for immunofluorescence, Western-blot analysis, and immunoprecipitation, and reagents for isolating GST-fusion proteins are also commercially available. In addition, we have had success in localizing GST-fusion proteins in yeast by

immunofluorescence using anti-GST antibodies (Bi and Pringle, 1996; Longtine et al., 1998). Appropriate design of the forward primer allows the same plasmids to be used for C-terminal protein truncation with or without the inclusion of a protein tag (see primers F2 and F3 in Figure 1A and Table 1).

Third, to allow the regulated expression and/or overexpression of full-length or N-terminally truncated proteins with or without N-terminal 3HA, GST, or GFP(S65T) tags, as well as of tagged or untagged full-length or C-terminally truncated proteins, we constructed a set of plasmids in which each of the selectable markers is cloned upstream of the GAL1 promoter (P GAL1) with or without associated tag sequences (Figure 1B). A proper design of the reverse primer (see Table 1) allows the generation of the N-terminal truncations.

Validation of the system

The system described here has been tested by manipulating several genes using the plasmids shown in Figure 1 as templates, primers designed as described in Table 1 (see also Figure 1), and the procedures described in Materials and Methods. For example, we tagged the 3’ end of GIN4 (Longtine et al., 1998) with 3HA sequences using the module from plasmid pFA6a-3HA-kanMX6. The normal cell morphology of the resulting strains indicated that Gin4p-3HA was functional, and it localized normally to the mother-bud neck (Longtine et al., 1998), as shown by immunofluorescence using anti-HA antibodies (Figure 2A).

To test the GAL1 promoter and TRP1 selectable-marker constructs, we then replaced the GIN4 promoter of one of the GIN4–3HA:kanMX6 strains by the module from plasmid pFA6a-TRP1-P GAL1. In the resulting strains, the expression of Gin4p-3HA was regulated by the carbon source, as expected. During growth on glucose, very few cells had detectable Gin4p-3HA (Figure 2B); rarely, weak Gin4p-3HA staining was visible (Figure 2B, arrowhead). The addition of galactose to cultures growing on raffinose rapidly induced the expression of Gin4p-3HA (Figure 2C), and long-term overexpression by growth on galactose resulted in abnormal cell morphologies (Figure 2D) like those observed after overexpression of normal Gin4p (Longtine et al., 1998). Similar results were obtained when the expression of a Myo1p-GFP fusion protein, which localizes to the mother-bud neck (Longtine et al., 1998; E. Bi and J. R. Pringle, unpublished results), was placed under control of the GAL1 promoter using the module from plasmid pFA6a-His3MX6-P GAL1 (Figure 2F, G). Interestingly, additional patches of Myo1p-GFP signal, which were often at or near the bud tips, were observed in many of the galactose-induced cells.

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relations using a small number of PCR primers. The
our hands, it has usually been >75% (ranging from
nation at the desired target site is typically high; in

overexpression (using the regulatable
markers, the plasmids allow gene deletion, gene
of doing double staining. Because high-quality
protein tagging for localization studies is the ease
with an N-terminal tag. A particular attraction of
and retain function when tagged at their C-termini,
and proteins that are not functional with one
C-terminal tag are often functional with another or
with an N-terminal tag. A particular attraction of
protein tagging for localization studies is the ease
of doing double staining. Because high-quality
mouse monoclonal antibodies to the HA and
Myc epitopes are available (see Materials and
Methods), a protein tagged with one of these
epitopes should generally be localizable in combi-
nation with a protein for which a non-mouse anti-
body is available. Moreover, the commercial avail-
ability of rabbit antibodies to both HA (Berkeley
Antibody Company) and GST (Molecular Probes,
Eugene OR; see also Bi and Pringle, 1996) allows
double staining of two tagged proteins.

The versatility of this system for PCR-mediated
gene modifications is far from exhausted. In
particular, it could clearly be extended by the
addition of other selectable markers (e.g., see
Langle-Rouault and Jacobs, 1995; van den Berg
and Steensma, 1997), additional promoters (e.g.,
see Niedenthal et al., 1996), and/or other protein
tags [including new GFP variants with altered
emission spectra (Heim and Tsien, 1996) that
could perhaps be used in combination with
GFP(S65T)-tagged proteins to allow double
labeling in living cells]. The approach can also be
used to introduce a reporter gene for assays of
promoter function (e.g., see Niedenthal et al.,
1996; Wach et al., 1998). A further useful variation
of this methodology allows the PCR-mediated
introduction of site-directed mutations into
chromosomal genes (Langle-Rouault and Jacobs,
1995). Finally, transformation of yeast with PCR-
generated fragments can also be used to introduce
sequences encoding a protein tag into a gene
carried on a resident plasmid (A. McKenzie and
J. R. Pringle, unpublished results); the plasmid
carrying the tagged gene can be isolated by elec-
troporation into E. coli, selecting for kanamycin
resistance, and then retransformed into yeast.

Conclusions

In this paper, we describe a set of plasmids useful
as templates for PCR-mediated gene modifications
in S. cerevisiae. Using any of three selectable
markers, the plasmids allow gene deletion, gene
overexpression (using the regulatable GAL1
promoter), C- or N-terminal protein tagging [with
GFP(S65T), GST, or the 3HA or 13Myc epitope],
and partial N- or C-terminal deletions (with or
without concomitant protein tagging). Because of
the modular nature of the plasmids (Figure 1;
Table 1), they allow a wide variety of gene manipu-
lations using a small number of PCR primers. The
efficiency of integration by homologous recombi-
nation at the desired target site is typically high; in
our hands, it has usually been >75% (ranging from
~20% to >95%).

The tagging of proteins often provides a rapid
and effective route to their localization and isola-
tion. Most proteins appear to localize normally and
retain function when tagged at their C-termini,
and proteins that are not functional with one
C-terminal tag are often functional with another or
with an N-terminal tag. A particular attraction of
protein tagging for localization studies is the ease
of doing double staining. Because high-quality
mouse monoclonal antibodies to the HA and
Myc epitopes are available (see Materials and
Methods), a protein tagged with one of these

(960)

References

Ausbub, F. M., Brent, R., Kingston, R. E., et al. (Eds)
Wiley and Sons, New York.


