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Epitope Tagging of Yeast Genes using a PCR-based Strategy: More Tags and Improved Practical Routines

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Epitope tagging of proteins as a strategy for the analysis of function, interactions and the subcellular distribution of proteins has become widely used. In the yeast *Saccharomyces cerevisiae*, molecular biological techniques have been developed that use a simple PCR-based strategy to introduce epitope tags to chromosomal loci (Wach *et al*., 1994). To further employ the power of this strategy, a variety of novel tags was constructed. These tags were combined with different selectable marker genes, resulting in PCR amplificable modules. Only one set of primers is required for the amplification of any module. Furthermore, convenient laboratory techniques are described that facilitate the genetic manipulations of yeast strains, as well as the analysis of the epitope-tagged proteins. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS — yeast; *Saccharomyces cerevisiae*; epitope tagging; transformation

INTRODUCTION

The genome of the yeast *Saccharomyces cerevisiae* was the first eukaryotic genome that has been completely sequenced (Goffeau *et al*., 1996). This fact, together with its easy genetic manipulability, make bakers' yeast a highly versatile unicellular eukaryotic system to study basic cellular processes. In addition, PCR-based methods exist which allow gene targeting for either deletion–replacement or insertion of heterologous DNA at discrete loci on the genome, allowing a fast functional analysis of genes (Baudin *et al*., 1993; Wach *et al*., 1994). Several such modules have been described for either the deletion of a gene or its selective tagging at the C-terminus (Wach *et al*., 1997; Schneider *et al*., 1995).

Such an approach to targeted disruption or tagging of genes is particularly useful for systematic analysis of several genes in parallel. As many yeast genes have homologues in higher eukaryotes, many research groups not primarily focused on work with yeast cells became interested in studying the function of these genes in yeast. The success of this work largely depends on the availability of easily applicable methods to manipulate yeast strains.

Here we report additional modules for the C-terminal tagging of chromosomal genes. Our modules can be used with one common primer set. We further demonstrate the functionality of

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our modules, using the *SPC42* gene coding for a spindle pole component (Donaldson and Kilmartin, 1996). The additional aim of this study is to describe precisely basic protocols for the successful chromosomal tagging of yeast genes. The provided methods are based on existing procedures that have been optimized for this purpose.

MATERIALS AND METHODS

Plasmid construction

Standard techniques were used for DNA manipulations (Sambrook *et al*., 1989). The construction of the plasmids containing the modules is summarized in Table 1. All primers used are listed in Table 2. Annealed oligonucleotides were cloned into restriction sites, as described below ('linker tailing'). The *TRP1* gene from *Kluyveromyces lactis* (*klTRP1*) complements a *trp1* mutation of *Saccharomyces cerevisiae*. The *klTRP1* gene contains a terminator sequence from the adjacent open reading frame that is used to properly terminate gene fusions resulting with these modules. The *kanMX6* and *His3MX6* modules have been described previously (Wach *et al*., 1994, 1997).

PCR amplification of modules

For amplification of the modules using the S2-SPC42 and S3-SPC42 primers, standard PCR reactions (hot start) using Taq-polymerase (2 U Taq per 100 µl reaction) and Taq-buffer provided by various suppliers were used. The annealing temperature was 54°C, the elongation time was 1 min per 1 kb of DNA (for estimation of the length of the PCR products, see Table 1). Best results in terms of yield and reliability were obtained with the 'Expand™ Long Template PCR System' Kit (Boehringer-Mannheim). A fraction (2–5 µl) of the PCR reactions was analysed by agarose gel electrophoresis. The residual reaction was ethanol precipitated and the DNA was resuspended in 15 µl of water. From this, 3–5 µl were used to transform competent yeast cells.

Linker tailing

This method was employed to clone the oligonucleotide linkers that code for the TEV site or for the 7HIS tag into the respective restriction sites. It allowed the insertion of exactly one oligonucleotide linker of 15–35 bases in length into a restriction site with an efficiency of greater than 90%. Neither dephosphorylation of the vector nor phosphorylation of the oligonucleotides was required. The method was based on an idea originally described by Lathe *et al*. (1984).

Annealing of the linker A mixture of the two oligonucleotides (5 µM each) dissolved in water was heated to 85°C for 2 min and then incubated at room temperature for about 1–2 h. The annealed oligonucleotides were stored at − 20°C.

Ligation About 500 ng–2 µg of appropriately digested plasmid DNA (enzyme inactivated and ethanol precipitated) was ligated with a high molar excess (at least 200-fold) of linker (2–5 µl annealed linker) in a 20 µl ligation reaction (2 U T4-DNA-ligase, 1 mM ATP) at 16°C for 1–16 h.

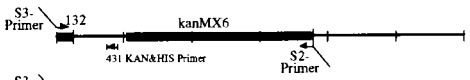
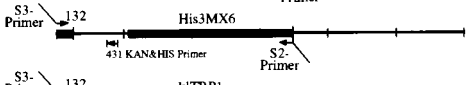
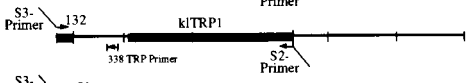
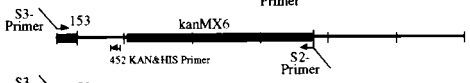
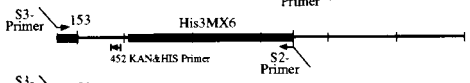
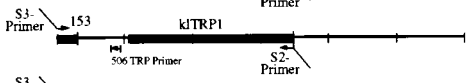
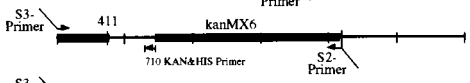
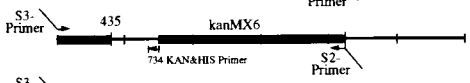
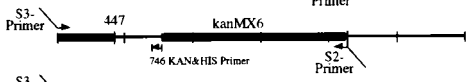
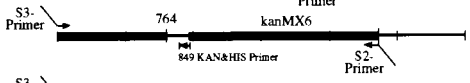
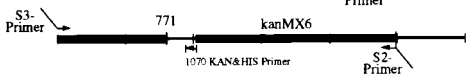
Annealing of the tailed linkers Excess linkers were removed by running the ligation reaction over a 0·7% low-melting agarose gel. The band containing the plasmid (which usually appears fuzzy and smeary) was cut out, while avoiding excess agarose. The small agarose block was placed in a tube and incubated at 80–85°C for 2–3 min. The temperature had to be sufficiently high to melt the linker pairs (from a ligated linker, only one oligonucleotide is covalently connected with the plasmid) but not high enough to completely melt the plasmid backbone. The sample was then quickly placed into a beaker containing 0·5 l of 70–75°C preheated water and this beaker was then placed into a water bath at 37°C for approximately 2 h to allow slow cooling and thereby the annealing of the linker tails. The still-liquid DNA–agarose mixture was then used for transformation into *E. coli* cells by electroporation.

E. coli *transformation* Electroporation competent cells (40 µl) of strain DH5α (or any other standard laboratory strain) were mixed with 2–3 µl of the 37°C DNA–agarose mixture. The DNA was quickly mixed with the cold *E. coli* cell suspension in order to avoid gelation of the agarose. Electroporation was continued as described (Sambrook *et al*., 1989). Routinely, many more than 1000 transformants were obtained. Plasmid DNA from four different transformants was isolated for restriction digestion and sequencing.

Yeast strains and growth conditions

The S288C-derived strain YPH499 (*MAT***a** *ura3-52 lys2-801*^{amber} *ade2-101*^{ochre} *trp1Δ63*

Table 1. Properties and construction of the different modules.

Properties of the modules					Construction of the modules					
Schematic drawings of the modules	Length of PCR-prod. with S2- and S3-primers	Tag	Selection marker	Plasmid name	Origin of backbone	Origin of insert	PCR-primers	Template	Linker-oligos	Restriction sites used
	1830	3HA	*kanMX6*	pYM1	pFA6a-GFPS65T-kanMX6[7]	PCR	3HA-1 3HA-2	pGTEP[1]	—	BamHI/BssHII
	1674	3HA	*His3MX6*	pYM2	pYM1	pFA6a-His5MX6	—	—	—	NcoI/EcoRV
	1312	6HA	*k1TRP1*	pYM3	pFA6a-kanMX4[8]	PCR	T1-TRP1 T2-TRP1	pWZV90[2]	—	SalI/SacI
	1851	3Myc	*kanMX6*	pYM4	pFA6a-GFPS65T-kanMX6	PCR	3Myc-1 3Myc-2	pUC19-Myc[3]	—	BamHI/BssHII
	1695	3Myc	*His3MX6*	pYM5	pYM4	pFA6a	—	—	—	NcoI/EcoRV
	1480	9Myc	*k1TRP1*	pYM6	pFA6a-kanMX4	PCR	T1-TRP1 T2-TRP1	pWZV87[2]	—	SalI/SacI
	2109	ProA	*kanMX6*	pYM7	pFA6a-GFPS65T-kanMX6	PCR	ProA-3 ProA-4	pNSP1-4ZproA[4]	—	BamHI/BssHII
	2133	TEV-ProA	*kanMX6*	pYM8	pYM7	Oligo	—	—	TEV-1 TEV-2	BamH1
	2145	TEV-ProA-7HIS	*kanMX6*	pYM9	pYM8	Oligo	—	—	7HIS-1 7HIS-2	EcoRI (partial)/AscI
	1989	TEV-ProA-7HIS	*HIS3MX6*	pYM10	pYM9	pFA6a-His5MX6	—	—	—	NcoI/EcoRV
	2251	TEV-GST-6HIS	*kanMX4*	pYM11	pFA6a-kanMX4	PCR	TEV-GST TEV-6HIS	pGEX-5X-1[5]	—	BglII in BamHI/BssHII
	2469	yEGFP	*kanMX4*	pYM12[6]	pFA6a-kanMX4	PCR		yEGFP[9]	—	

This table gives an overview over the different modules with their respective selection marker for selection in yeast. All plasmids harbour the Amp^R gene and ori for amplification in *E. coli*. In the schematic drawings of the modules, positions of the S2 and S3 primer binding sites, and the positions of the primers that can be used for PCR verification of the correct integration (for *kanMX6* and *His3MX6* modules the KAN&HIS primer and for *klTRP1* modules the TRP primer (sequences are given in Table 2) are indicated. The table further lists the plasmids and their sources, the names of the primers, the templates and linker-oligos used to construct the respective plasmids, as well as the restriction sites used for subcloning.

[1]Tyers *et al.*, 1993; [2]W. Zachariae and K. Nasmyth, unpublished; [3]Schneider *et al.*, 1995; [4]Hurt, 1988; [5]Pharmacia; [6]P. Dumoulin and B. Winsor, manuscript in preparation; [7]Wach *et al.*, 1997; [8]Wach *et al.*, 1994; [9]Cormack *et al.*, 1997.

Table 2. Oligonucleotide used in this study.

Sequence	Name
5′-CTGCAGGTCGACTCCGGTTCTGCTGCTAGT-3′	P1-TRP1
5′-GAATTCGAGCTCGCCTCGAGGCCAGAAGAC-3′	P2-TRP1
5′-GATCCGAGAATCTTTATTTTCAGG-3′	TEV-1
5′-GATCCCTGAAAATAAAGATTCTCG-3′	TEV-2
5′-TGGGCCTCCATGTCGCTGG-3′	KAN&HIS primer
5′-GCTATTCATCCAGCAGGCCTC-3′	TRP primer
5′-GACAGATCTGAGAATCTTTATTTTCAGGGATCCCCTATACTAGGTTATTGG-3′	TEV-GST
5′-GACGCGCGCTCAATGGTGATGGTGATGGTGGTCAGTCACGATGCGGGCC-3′	GST-6HIS
5′-GGAGATCCGGAATCTTTTACCCATACGATGT-3′	3HA-1
5′-AAGTGGCGCGCCCTAGCACTGAGCAGCGTAATCTG-3′	3HA-2
5′-CCCGGGGATCCTCTAGAGG-3′	3MYC-1
5′-AAGTGGCGCGCCCTAGACTCTAGATGATCCGTTCAAG-3′	3MYC-2
5′-AATTCCCACCATCACCATCACCATCACTAGGG-3′	7His-1
5′-CGCGCCCTAGTGATGGTGATGGTGATGGTGGG-3′	7His-2
5′-GGAGAGGATCCCCTCAACAAAACAAAACCG-3′	PROA-3
5′-AAGTGGCGCGCCCTAAAGAGCCGCGGAATTCGC-3′	PROA-4
5′-AACGCTTTAAGAATGCGCCATACTCCTTAACTGCTTTTTAAATCAATCGATG AATTCGAGCTCG-3′	S2-SPC42
5′-AATAATATGTCAGAAACATTCGCAACTCCCACTCCCAATAATCGACGTACGC TGCAGGTCGAC-3′	S3-SPC42

his3Δ200 leu2Δ1) (Sikorski and Hieter, 1989) was used for our manipulation. YPD and synthetic drop-out media were prepared as described (Sherman, 1991). A refraction of 1 OD_{600} of cells grown in YPAD medium (YPD medium supplemented with 100 mg/ml adenine) to logarithmic growth phase corresponds to $4{\cdot}3 \times 10^7$ cells/ml for strain YPH499, if measured with an LKB Ultrospec Biochrome II (Pharmacia) spectrophotometer. Note that the OD_{600} to cells per ml correlation has to be determined individually for each spectrophotometer type because of the different path lengths. G418 (geneticin) plates were made by dissolving 200 mg/l of solid geneticin (Sigma, G-9516) in autoclaved YPD or YPAD medium containing 2% Bacto agar (Difco) after the medium has reached a temperature below 60°C.

Competent frozen yeast cells and yeast transformation procedure

The transformation protocol was based on the LiOAc method (Schiestl and Gietz, 1989). The following solutions were used:

- SORB: 100 mM LiOAc, 10 mM Tris–HCl pH 8 (from 1 M stock), 1 mM EDTA/NaOH pH 8 (from 0·5 M stock), 1 M sorbitol (special grade for molecular biology from Merck), adjusted with dilute acetic acid to pH 8, sterile filtered (can be stored at room temperature for several months, a volume of 0·5 l was usually prepared).
- PEG: 100 mM LiOAc, 10 mM Tris–HCl pH 8 (from 1 M stock), 1 mM EDTA/NaOH (from 0·5 M stock) pH 8, 40% PEG3350 (Sigma), sterile filtered (can be stored at 4°C for several months, a volume of 50–100 ml was usually prepared).
- Carrier DNA: Salmon sperm DNA (10 mg/ml, Gibco BRL) was denatured at 100°C for 10 min and cooled on ice. Such prepared carrier DNA can be stored at −20°C and repeatedly used.

Growth of yeast cells Yeast cells were inoculated from a fresh pre-culture and grown for at least 6–8 h or overnight to a density of 0·5–0·7 OD_{600} at 30°C (approximately 2×10^7 cells/ml) in YPAD medium.

Competent yeast cells Yeast cells were harvested by centrifugation (500 × *g*, 5 min, room temperature), washed once with 0·1–0·5 volumes sterile water (room temperature) and once with 0·1–0·2 volumes SORB (room temperature). SORB was removed by aspiration. The cells were finally resuspended in a total volume of 360 µl SORB per 50 ml

of cell culture and 40 µl of carrier DNA (0°C) was added. The cells were aliquoted into appropriate portions (e.g. 50 µl, at room temperature) and placed at − 80°C (no shock freezing).

Transformation Usually, 10 µl of competent cells were used for the transformation of plasmid DNA and 50 µl of cells for the transformation of a PCR product. The DNA was placed into a sterile 1·5 ml tube (maximal 2 µl plasmid DNA per 10 µl of cells), the competent cells were thawed and added. The suspension was mixed well before a six-fold volume of PEG was added. Cells were mixed throughout and incubated at room temperature for approximately 30 min. DMSO was added (1/9 volume to make a final concentration of approximately 10%). The cells were placed in a water bath at 42°C for 5–20 min (15 min works best with most strains). The cells were sedimented (2–3 min at 2000 rpm), the supernatant was removed (since it slows down growth on the plates) and the cells were resuspended in 100–200 µl of liquid medium (SC-TRP or SC-HIS; for *kanMX6* selection, see below).

Selection for transformants In cases where auxotrophy markers were used for selection of the plasmid or PCR product, cells were plated on to synthetic complete medium lacking the corresponding amino acid (*His3MX6*, SC-HIS plates; *klTRP1*, SC-TRP plates). In cases where PCR products containing the dominant resistance marker *kanMX6* were used, the cells were resuspended in approximately 3 ml YPAD, incubated on a shaker for 2–3 h at 23°C or 30°C, harvested and spread on a G418 plate (further details are given below).

Yeast cell lysis

This protocol is adapted from previously described methods (Wright *et al.*, 1989; Horvath and Riezman, 1994; Riezman *et al.*, 1983; Yaffe and Schatz, 1984). Cells were grown to the desired growth phase. A volume corresponding to 0·5–3·0 OD_{600} of cells was removed, and the cells were harvested and resuspended in 1 ml of cold water (for quick expression tests, a tooth pick full of cells from a fresh plate can be resuspended in 1 ml of water). The cell suspensions were mixed with 150 µl of 1·85 M NaOH, 7·5% β-mercaptoethanol (freshly prepared) and placed on ice for 15 min. Then 150 µl of 55% trichloroacetic acid (TCA) (w/v, stored in the dark) was added and the mixture was incubated for 10 min on ice. The cells were pelleted (10 min at 14 000 rpm, at 4°C or at room temperature). The supernatant was removed, the cells centrifuged briefly for a second time and all residual traces of TCA were aspirated off. The pellet was resuspended in 100 µl HU-buffer per OD_{600} of cells (HU-buffer: 8 M urea, 5% SDS, 200 mM Tris pH 6·8, 1 mM EDTA, with bromophenol blue as colouring and pH indicator, 1·5% DTT; the buffer is stored without DTT at − 20°C). Resuspension of the cells can be aided by using a sonicator bath. Denaturation of the proteins was performed for 10 min at 65°C (37°C for some membrane proteins), preferably on a vortex-mixer/heater. In cases where the buffer capacity of the HU-buffer was not high enough to neutralize the remaining traces of the trichloroacetic acid (yellow colour), 1–3 µl of 2 M Tris-base was added. After centrifugation (5 min at 14 000 rpm, room temperature) aliquots corresponding to 0·2–0·5 OD_{600} of cells were analysed by SDS–PAGE (Laemmli, 1970), followed by immunoblotting. For unknown reasons, TCA-treated proteins require a longer blotting transfer time than non-treated proteins (about 1·5–2-fold the normal time) (Wright *et al.*, 1989).

Immunological and other techniques

For the detection of epitope-tagged proteins, tag-specific antibodies were used: mouse monoclonal 9E10 antibody recognizes the Myc epitope (Boehringer-Ingelheim), mouse monoclonal 12CA5 (Boehringer-Mannheim) or new monoclonal antibodies from various other companies recognize the HA epitope. The anti-Spc42p antibodies have been described previously (Knop and Schiebel, 1997). The protein A tag (ProA tag) was detected using subsequent incubations with rabbit-anti goat/HRP (horseradish peroxidase) conjugates and goat-anti rabbit/HRP conjugates (both from Jackson Immuno Research Laboratories). This sandwich procedure allows a highly sensitive detection of the ProA tag. The functionality of the ProA tag is not affected by the TCA cell lysis procedure.

Yeast spindle pole bodies (SPBs) from *SPC42-TEV-ProA* cells were isolated as described (Rout and Kilmartin, 1990). To cleave the ProA moiety from Spc42p-TEV-ProA, isolated SPBs were incubated with recombinant TEV-protease (Gibco BRL) in the buffer provided by the supplier.

RESULTS

Recent papers have described modules that can be used to generate PCR products with flanking homologous sequences, allowing their targeted integration into the genome of yeast cells (Puig *et al.*, 1998; Longtine *et al.*, 1998; Wach *et al.*, 1997; Schneider *et al.*, 1995). This method has been found useful to investigate the function of yeast genes. We constructed a set of new modules (Table 1) with novel properties, thereby extending the list of available modules. Some modules contain multiple HA or Myc tags in combination with the recombinant heterologous *His3MX6* or the *kanMX6* marker (Wach *et al.*, 1994) or the *TRP1* gene from *Kluyveromyces lactis* (*klTRP1*). Other modules contain GST or ProA tags in combination with a histidine tag (HIS tag) and a cleavage site for the TEV-protease (Parks *et al.*, 1994). The GST tag binds to glutathione sepharose (Smith *et al.*, 1988) and the ProA tag, derived from protein A from *Staphylococcus aureus*, binds to IgGs sepharose. The 6 × HIS or 7 × HIS tags bind to nickel/NTA sepharose. These tags have been proved to be useful to purify protein complexes from yeast extract (Knop and Schiebel, 1997; Siegers *et al.*, 1998). One module contains the yEGFP variant of the green fluorescent protein that is optimized for expression in fungi (Cormack *et al.*, 1997). Together, these modules allow the application of a set of biochemical and cell biological methods, such as immunofluorescence, Western blotting, native and denaturing immunoprecipitation and complex isolation. Combinations of different tags in one strain allow the investigation of protein–protein interactions in immunoprecipitation experiments (see also below).

In order to demonstrate the functionality of the modules, we used them to tag the *SPC42* gene (Donaldson and Kilmartin, 1996). The primers required to target the PCR-products to the desired chromosomal location are outlined in Figure 1 using *SPC42* as an example. The homologous sequences have to be at least 45 bases in length, with an additional 19 bases (S2: 5′-ATC GAT GAA TTC GAG CTC G-3′) or 18 bases (S3: 5′-CGT ACG CTG CAG GTC GAC-3′) complementary to vector sequences (see Materials and Methods). Frozen competent yeast cells were transformed with the PCR-amplified modules. Transformants were selected either on G418-plates (*kanMX6* marker), or on SC-plates lacking either histidine (SC-HIS, *His3MX6*-marker) or tryptophane (SC-TRP, *klTRP1*-marker). The selection of transformants on G418 plates usually resulted in many transient transformants (2000–20 000) and fewer (10–100) stable transformants. However, if such plates were replica-plated on to fresh G418-plates, the stable transformants become readily visible after 1 day of incubation (Wach *et al.*, 1994). On SC-TRP or SC-HIS plates, no transient transformants were detected. In all cases, the amount of transformants depended on the length of the homologous sequences and on the transformation competence of the yeast cells. When we optimized the transformation protocol using a standard yeast plasmid (pRS315; Sikorski and Hieter, 1989), up to 1×10^6 transformants were obtained per 1 µg of plasmid-DNA or 20–100 transformants per 5 µg of PCR-amplified cassette with 45 bp homology region, using 50 µl frozen cells (data not shown).

Routinely, three transformants were assayed for the expression of the desired *SPC42*-tagged variant (Figure 2A) by Western blotting. Cells were grown on YPAD plates as squares for one day and then an amount corresponding to the head of a match (about 3 OD_{600} of cells) was lysed using the protocol described in Materials and Methods. This protocol, together with urea (8 M) in the sample buffer extracts, efficiently extracts proteins from yeast cells grown on various media and circumvents proteolytic degradation problems, often resulting from proteinase B (PrB) activation in Laemmli SDS sample buffer (unpublished observations). Analysis of three transformants by immunoblotting using antibodies specific for the tags (Figure 2A) or for Spc42p (data not shown) revealed that for all modules tested, at least two transformants expressed a tagged Spc42p protein. We observed with the 9Myc or the 6HA modules, that not all transformants expressed the full-length 9Myc-tagged or 6HA-tagged Spc42p-proteins (Figure 2B). This most probably resulted from rearrangements occurring as a consequence of the direct repeat nature of the multiple tags, leading to the loss of some tags. Besides functional analysis, the correct integration of the modules at the desired locus can be verified using PCR and genomic yeast DNA (Longtine *et al.*, 1998). The positions of primers that bind in the cassettes are indicated in Table 1. Their sequences are shown in Table 2.

To test the functionality of the TEV–protease cleavage site, we isolated SPBs of the *SPC42-TEV-ProA-7HIS* strain. Incubation of the SPBs with

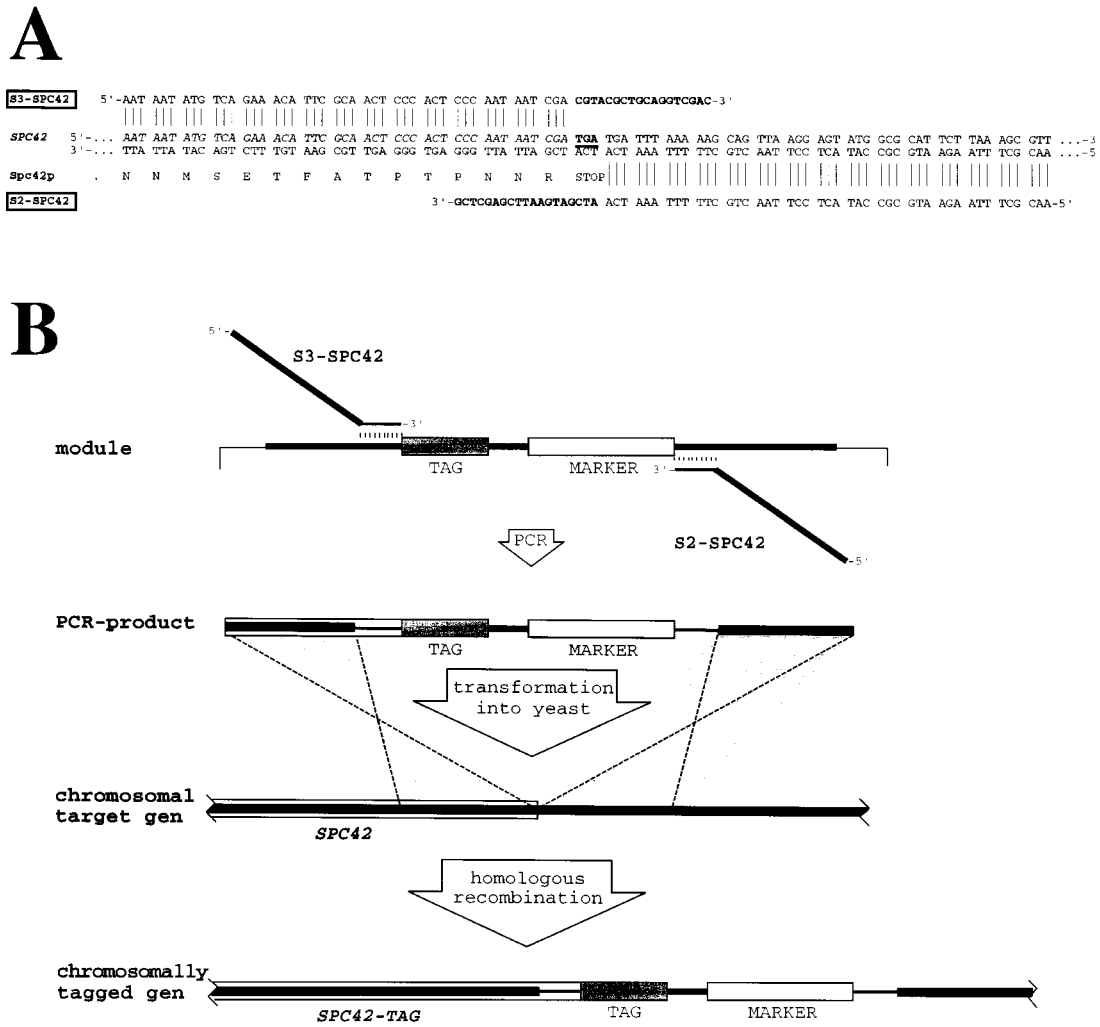


Figure 1. (A) General strategy used to construct S2 and S3 primers required for C-terminal tagging of chromosomal ORFs. The example shown here is for the *SPC42* gene. **Bold**: annealing sequences of the S2-SPC42 and S3-SPC42 primers for the pYM-modules. Bold+underlined: the STOP codon of the *SPC42* gene. (B) The different steps leading to an epitope tagged yeast gene. In a first step, the desired module is PCR amplified using S2- and S3-primers. The PCR product is then transformed into yeast cells. Both ends of the PCR product contain homologous sequences (which derive from the S2 and S3 primers) that target the DNA to be introduced at the desired chromosomal location via homologous recombination. Correct transformants now express an in-frame fusion of the target gene with the epitope tag. Terminators provided by the modules substitute for the original terminators.

recombinant TEV–protease demonstrated that the TEV-cleavage site is functional (Figure 2B).

When we performed subsequent rounds of tagging using *kanMX6* and *His3MX6* modules, we realized that a *His3MX6* module can integrate into a previously integrated *kanMX6* module, thus replacing the *kanMX6* marker, and vice versa. This was not the case with the *klTRP1* modules, as they have much less sequence homology with the other modules. Assaying such transformants for the presence of both markers (*His3MX6* and *kanMX6*) allowed the easy detection of the correct double-tagged cells.

DISCUSSION

The gene that codes for the essential spindle pole body component Spc42p, *SPC42* (Donaldson and

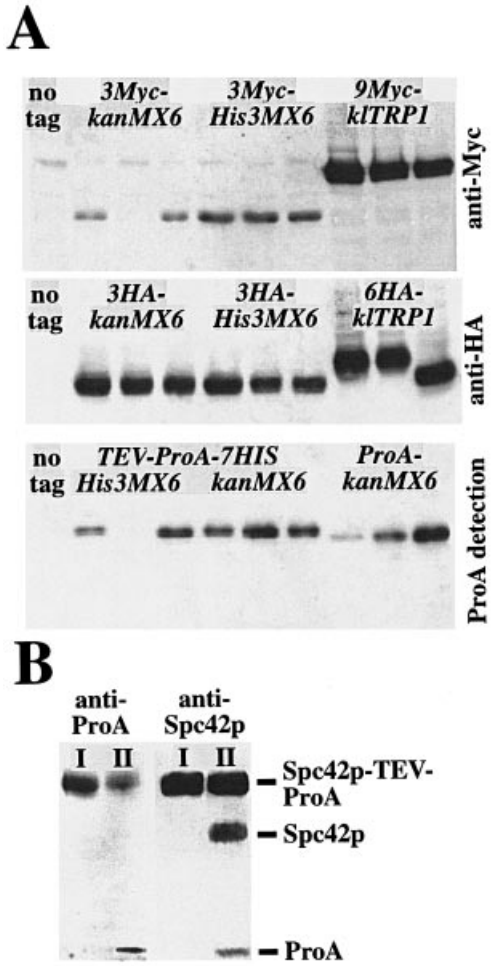


Figure 2. (A) Western blots showing tagged Spc42p variants using the different modules. Cells of strain YPH499 were transformed with PCR-products of the indicated modules using S2-SPC42 and S3-SPC42 primers. For Western blotting, cells were grown on YPAD plates for 1 day and then lysed as described. (B) Isolated SPBs of a strain expressing *SPC42-TEV-ProA* were incubated with TEV-proteinase. Lanes I, before incubation, Lanes II, after 4 h at 4°C. Antibodies as described in Materials and Methods. Only a fraction of Spc42p-TEV-ProA is cleaved by the TEV-proteinase, most probably because some Spc42p-TEV-ProA is buried inside the large SPB structure.

Kilmartin, 1996) was chosen as a chromosomal target to demonstrate the functionality of the constructed modules, because fusion of relatively large proteins to the C-terminus of Spc42p does not influence its *in vivo* function. If a tag does not abolish the essential function of the protein (as is the case for Spc42p), haploid strains can be used directly. In cases where genes of unknown function or essential genes are being investigated, it is recommended to tag the gene in a diploid strain. This strain can then be sporulated, allowing testing for viability of the haploid tagged cells. We observed that up to 80% of the genes that we have tested could be epitope tagged with the modules without affecting their function *in vivo*. However, this frequency may vary, depending on the classes of proteins under investigation. In one case, we observed a tag-dependent mislocalization of the protein, but the essential function of the protein was not affected (not shown). Consequently, some precautions have to be taken when a new gene is being investigated by this method. First, criteria that allow to judge the functionality should be applied. Second, one has to be aware that an epitope-tagged protein is always different from its wild-type counterpart, providing the chance for artifacts. Therefore, independent proof of results obtained with tagged derivatives is always desirable.

We investigated the functionality of the modules as well as the described methods in other genetic backgrounds, such as in W303 and SK-1 derived strains. In both cases we obtained similar results for *SPC42* with respect to efficiency and functionality.

While this manuscript was in preparation, reports of another set of modules (GST, MYC, HA, ProA and GFP), using the heterologous *kanMX6*, *His3MX6*, *klURA3* markers or the *S. cerevisiae TRP1* gene, were published (Longtine *et al.*, 1998; Puig *et al.*, 1998). Our modules complement this set and offer additional advantages: (a) all our modules contain heterologous selection markers, allowing the creation of double- and triple-tag strains with high efficiency; (b) we used the *klTRP1* marker first in such an approach and it proved to be very useful; (c) two tags (ProA/7HIS and GST/6HIS) were combined, allowing the purification of proteins via two affinity steps; (d) the TEV protease cleavage site was introduced in some of the tags, which was useful for the release of bound protein complexes from the affinity resin and for the restoration of the nearly native protein; (e) all modules can be amplified by the same set of primers. Taken together, we have described some new modules that are useful for C-terminal epitope tagging of yeast genes. The ease of use of these modules, together with easily applicable routine techniques, makes then useful for studying many yeast genes in parallel.

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NOTE

Plasmid DNA containing these modules and their sequences are available from Dr E. Schiebel (eschiebe@udcf.gla.ac.uk).

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