Specific Targeted Integration of Kanamycin Resistance-Associated Nonselectable DNA in the Genome of the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Sophisticated genome manipulation requires the possibility to modify any intergenic or intragenic DNA sequence at will, without leaving large amounts of undesired vector DNA at the site of alteration. To this end, a series of vectors was developed from a previous gene knockout plasmid system to integrate nonselectable foreign DNA at any desired genomic location in yeast, with a minimum amount of residual plasmid DNA. These vectors have two mutated Flp *recognition targets (*FRT*) sequences flanking the* KanMX4 *gene and multiple sites for subcloning the DNA fragment to be integrated. The selectable marker can be recycled by Flp site-specific excision between the identical* FRT*s, thereby allowing the integration of further DNA fragments. With this system, the* NLS-tetR-GFP *and* DsRed *genes were successfully integrated at the* thr1 *locus, and the* RVB1 *gene was tagged at the C-terminus with the V5-epitope-6-histidine tag. This plasmid system provides for a new molecular tool to integrate any DNA fragment at any genome location in [*cir$^+$*] yeast strains. Moreover, the system can be extrapolated to other eukaryotic cells in which the* FLP/FRT *system functions efficiently.*

INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, the 2-µm FLP encodes a site-specific recombinase acting on two specific sites, termed the Flp recognition target (*FRT*), which are located at the center of the inverted repeats (1,2). It had been shown that the Flp protein interacts specifically with a 50-bp DNA sequence that includes three 13-bp repeats; the third of which is inverted with respect to the other two and separated from them by an 8-bp core sequence (3,4). Mutation in the *FRT* core sequence prevents recombination; however, identical mutations are well tolerated and do not affect site-specific recombination (5–7). Flp can also mediate intermolecular recombination between the two *FRT*s located on different plasmids (8,9), and it is known that DNA heterology between the *FRT* core region of the endogenous 2 µm and of a foreign plasmid does not permit site-specific recombination between them. The *FLP/FRT* system carrying different mutations in the *FRT* core region has been employed for the construction of recyclable vectors for multiple gene disruption in yeast (6,10). Within the recyclable marker methodology, several other systems are available to date for gene disruption and replacement (11–14) as well as for epitope tagging of chromosomal genes (15,16), but none is capable of integrating at a specific locus any desired DNA sequence that has no directly detectable phenotype. The *FLP/FRT* system has recently been improved to implement a new, advanced strategy for in vivo genomic DNA alterations. The new system, called specific targeted integration of kanamycin resistance-associated nonselectable DNA (STIK) allows the integration at any genomic location of DNA sequences that express no directly selectable phenotype, such as spacers, tags, nuclear localization signal (NLS) sequence, and any intergenic or otherwise heterologous sequence. The STIK system accomplishes this task by exploiting the temporary integration of the recyclable, positively selectable *KanMX4* marker. To demonstrate the usefulness of this system, the two new plasmids pGKGE and pXKXE were used to perform gene disruption and replacement of the *THR1* gene with the *NLS-tetR-GFP* and *DsRed* fluorescent markers, respectively, while the new plasmid pHKHE was used to tag the *RVB1* gene. After these alterations, the selectable marker could be excised, and thus, similarly, other DNA sequences could be integrated in the same strain.

MATERIALS AND METHODS

Strains, Media, and Culture Conditions

The *S. cerevisiae* strain used in this study is the disomic strain for chromosome VIII, Z140-51D: a (*thr1*/+,*CUP1*/+,*arg4-2*/+,*arg4-17*/+) *his5 ade2 trp1 trp5 leu1 [cir$^+$]*, and its derivatives, in which one or both of the *thr1* alleles were replaced with the fluorescent marker gene GFP: Z140-51G, a (*thr1/THR1::FRTG-NLS-tetR-GFP,CUP1/+, arg4-2/+,arg4-17/+) his5 ade2 trp1*

Table 1. Primers Used in This Work (5′→3′)

THR1ΔF1:	ATGGTTCGTGCCTTCAAAATTAAAGTTCCAGCTTCTTCCGAA AAATAGGCGTATCACGAG
THR1ΔR1:	GCTGTTCGACGCTAGCACCATCGTATGGCAGGCTCAGTAGTC GATGATAAGCTGTCAAAC
THR1ΔR2:	CGAACCTGTTGATAATTTCTTGAGAGATTTCTTCGAATTCTTA ACTGTGCCCTCCATGG
THR1F1:	GAGTCATCATCTCGAAAAG
THR1R1:	TTAGCATCAGAACGCAATGG
1-V5F:	AAGGTCAACAAAGATTTTAGAAACTTCCGCAAATTATTTG AAGGGCGAGCTTCGAGGTCAC
1-V5R:	TATTTTTATTTATGAAATGTGCTTTAGGCTTTCTTCACTGTCG ATGATAAGCTGTCAAAC
RVB1F2:	CAAGATGTTACCTTGCACGA
RVB1R2:	CAAGTAATAGCAGCAACAAC
K1:	CAATCGATAGATTGTCGCAC
K2:	TTATGCCTCTTCCGACCATC
Polylinker:	TACGTACGTACGCCGCGGCCGCGAATTC

trp5 leu1 [cir$^+$], and GFP plus *DsRed*: Z140-51GR, a (*thr1::RFP-FRTH/THR1::FRTG-NLS-tetR-GFP,CUP1/+, arg4-2/+,arg4-17/+) his5 ade2 trp1 trp5 leu1 [cir$^+$]*, respectively. Diploid strain DUPOT-SL: *gal2/+,leu2/leu2 ::FRTX,arg10/+,ade2/+,ura3-52/ura3-5[cir$^+$]* and haploid strain YPH250: a *ade2 leu2 lys2 his3 trp1 ura3 [cir$^+$]* were also used. Yeast peptone dextrose (YPD) and kanamycin-containing media were prepared as described (17). The yeast transformation protocol was used as described in the European Functional Analysis Network (EUROFAN) program manual (http://www.mips.biochem.mpg.de/proj/eurofan/eurofan_1/b0/home_requisites/guideline/exp-transformation.html). *E. coli* strain DH5α was used for plasmid propagation. *E. coli* cultures were grown in LB broth supplemented ampicillin as previously described (17).

Plasmids

The YIplac128 vector, harboring the *NLS-tetR-GFP* chimeric DNA fragment under the yeast *URA3* promoter and followed by the *ADH1* terminator, was obtained from Kim Nasmyth's laboratory (IMP, Vienna, Austria). The Living Colors® pDsRed1-N1 vector was obtained from BD Biosciences Clontech (Palo Alto, CA, USA). In the *DsRed* gene construct, the cytomegalovirus (*CMV*) promoter was replaced with the yeast *LEU2* promoter. Plasmid pTOPO-RUVB containing the *Bacillus subtilis RuvB* gene fused in frame with the V5His6x epitope was constructed in our laboratory using the pYES2.1 TOPO TA™ Cloning Kit (Invitrogen, Carlsbad, CA, USA). Plasmids pGKG, pXKX, and pHKH, bearing different mutations in the central core region of the *FRT* sequence and pWKW, having wild-type *FRT*s, were constructed in our laboratory (6). From these plasmids, the new series of vectors was obtained as described in the Results section, an example of which is shown in Figure 1A.

Primers

Table 1 lists the primers used in this study, and their details are as follows. THR1ΔF1: chimeric forward primer, having a 40-bp homologous sequence from the ATG start codon of the *THR1* gene and 20 bp homologous upstream of the 5′ *FRT*; THR1ΔR1: chimeric reverse primer, having 40 bp homologous

to the downstream sequence of *THR1* locus and 20 bp homologous downstream of the 3′ *FRT*; THR1ΔR2: chimeric reverse primer, having 40 bp homologous to the sequence of *THR1* locus, further upstream of the THR1ΔR1 primer, and 20 bp homologous downstream of the *FRT*; THR1F1: forward primer homologous to 100 bp upstream of the ATG of the *THR1* gene; THR1R1: reverse primer homologous to 300 bp downstream of the ATG of the *THR1* gene; 1-V5 F: chimeric forward primer, having 40 bp homologous to the sequence immediately upstream of the *RVB1* STOP codon and 20 bp homologous to the sequence upstream of the V5 epitope in pH-RuvBV5H6; 1-V5 R: chimeric reverse primer, having 40 bp homologous to the sequence immediately downstream of the *RVB1* STOP codon and 20 bp homologous to the sequence downstream of the *FRT*; RVB1F2: forward primer homologous to the sequence 533 bp upstream of the STOP codon of the *RVB1* gene; RVB1R2: reverse primer homologous to the sequence 364 bp downstream of the STOP codon of the *RVB1* gene; K1: reverse primer homologous to a 500-bp sequence downstream of the ATG of the *KanMX4* gene; and K2: forward primer homologous to the sequence 898 bp upstream of the STOP codon of the *KanMX4* gene.

Standard Molecular Biology Techniques

Plasmid DNA was extracted from *E. coli* using the Wizard® Plus mini-preparation kit (Promega, Madison, WI, USA). Restriction enzymes and the DNA polymerase I Klenow fragment were obtained from New England Biolabs (Beverly, MA, USA) and used according to the manufacturer's recommendations. Restriction fragments were separated by gel electrophoresis and purified using the QIAquick® gel extraction kit (Qiagen, Valencia, CA, USA), as specified by the manufacturer.

Fluorescent Microscopy

The yeast strain was grown on G418 plates, and the cells were harvested and washed once with water. Approximately 10^7 cells were mounted on a glass slide and air-dried. Subsequently, 5 µL mounting medium (Vector Laboratories, Burlingame, CA, USA) were added over the dried culture. The green fluorescence was visualized using an Axiovert® 100M confocal microscope (Carl Zeiss Jena, Jena, Germany).

PCR Analysis

Yeast colony PCR. Yeast colonies picked from the plate were suspended in 50 µL sterile water and incubated with 5 µL Lyticase™ (Sigma, St. Louis, MO, USA) at 40 U/µL for 15 min at room temperature. The suspension was centrifuged at 400× *g* for 1 min, harvested, and the supernatant was discarded. Cells were denatured at 100°C for 10 min and placed on ice for 5 min. The pellet was then suspended in 40 µL sterile water, and 10 µL were used as a template for PCR. The reaction was performed following the standard EUROFAN B0 Program protocol. PCR for the amplification of replacement cassettes consisted of one cycle of 2 min at 94°C, followed by 20 cycles at 94°C for 30 s, 54°C for 30 s, and 68°C for 4 min. Subsequently, there were 10 cycles at 94°C for 30 s, 59°C for 30 s, and 68°C for 4 min, followed by one cycle of 68°C for 5 min. A mixture of *Taq* DNA polymerase and *Pfu* DNA polymerase (Promega) in a ratio of 1:1 was used for the amplification of the replacement cassettes. After amplification, 10 µL of the samples were run on a standard 1.2% agarose gel for size analysis.

Subcloning the *NLS-tetR-GFP*, *DsRed*, and *RUVB-V5His6x* DNA Fragment in the pGKGE, pXKXE, and pHKHE Vectors

To subclone *NLS-tetR-GFP* in the pGKGE vector, it was cut with the *Sna*BI restriction enzyme to create blunt ends. The 2.2-kb region bearing the *NLS-tetR-GFP* fragment was excised from the vector YIplac128 by

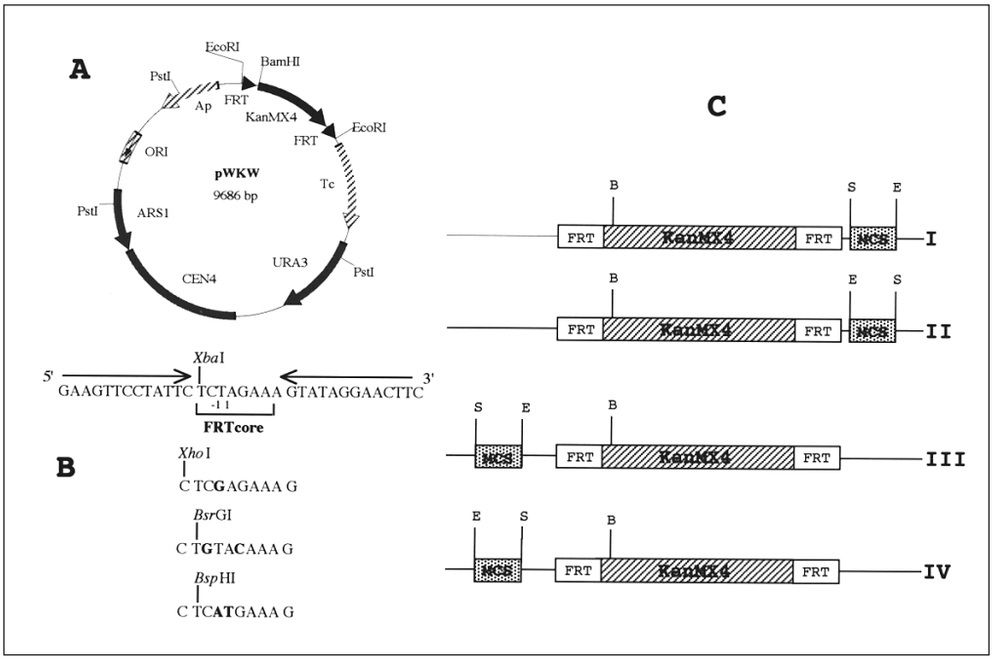

Figure 1. Schematic representation of the wild-type pWKW vector. Panel A shows the major plasmid elements and restriction sites. The different sequences of the *FRT* core present on the various vectors are indicated in panel B. The wild-type *FRT* sequence with restriction site *Xba*I, the *FRT* sequence with the mutation generating the *Xho*I site that generated the *Bsr*GI site, which generated the *Bsp*HI site, are present on the plasmids pWKW, pXKX, pGKG, and pHKH, respectively. In this vector, the MCS has been cloned in both orientations, in either *Eco*RI restriction site, giving rise to the four types of vectors (I, pHKGE; II, pXKXE; III, pHKHE; and IV, pWKWE) represented in panel C.

double digestion with restriction enzymes *Eco*RI and *Bam*HI. The recessed 5′ ends of this fragment were gap-filled using DNA polymerase I Klenow fragment. Lastly, this 2.2-kb fragment was further subcloned into the pGKGE vector (Figure 1C, I). Similarly, the *DsRed* fragment was subcloned into the pXKXE vector (Figure 1C, II).

To subclone *RuvB-V5His6x* in the pHKHE vector, pTOPO-RuvB was cut by double digestion with *Pvu*II and *Xba*I restriction enzymes. The recessed 5′ end of this fragment was gap-filled using the DNA polymerase I Klenow fragment. Finally, the 1.5-kb DNA fragment was subcloned into pHKHE *Sna*BI site (Figure 1C, III), which gave rise to the plasmid pH-RuvBV5His6x.

Immunochemical Techniques

The V5 epitope-tagged protein was detected using the mouse monoclonal Anti-V5 Antibody (Invitrogen). Whole protein extracts were prepared according to the protocol available (http://www.pmci.unimelb.edu.au/core_facilites/manual/mb460.asp). Western blot analysis was performed as previously described (17). The proteins were visualized on the blot membrane after Western blot hybridization using a stain with a solution of 0.1% Ponceau-S Red stain and 1% acetic acid for 1 min.

RESULTS

Construction of New Recyclable STIK Vectors for Genomic Integration of Nonselectable DNA Fragments

A series of vectors carrying the selectable marker *KanMX4* flanked by different *FRT* sequences such as pWKW, pGKG, pHKH, and pXKX were previously constructed in our laboratory (Figure 1A) (6,10). The vector pWKW has wild-type *FRT* sequences, whereas the other three vectors have different mutations in the *FRT* core region. Figure 1B shows the sequences of the wild-type and mutated *FRT*. These vectors were further modified by deleting one of the two *Eco*RI sites flanking the *FRT*s and subcloning a multiple cloning site (MCS) (*Sna*B1, *Spl*1, *Sac*II, *Not*I, and *Eco*RI) at the remaining *Eco*RI site. The position and orientation of the MCS were determined by DNA sequencing (CRIBI, University of Padova) (Figure 1C). These modified vectors were designated as pGKGE, pXKXE, pHKHE, and pWKWE, according to the core *FRT* sequence present and the *Eco*RI restriction site that was used for cloning (Figure 1C, I–IV, respectively). The MCS enables the subcloning of the desired sequence in the plasmid to be used as a template for the PCR amplification of the integration cassette. After the integration of the cassette, Flp-induced site-specific recombination occurs only between identical *FRT* sequences, thus allowing the excision of the selectable marker, while leaving one *FRT* scar sequence integrated in the DNA, together with the sequence of interest (Figure 2).

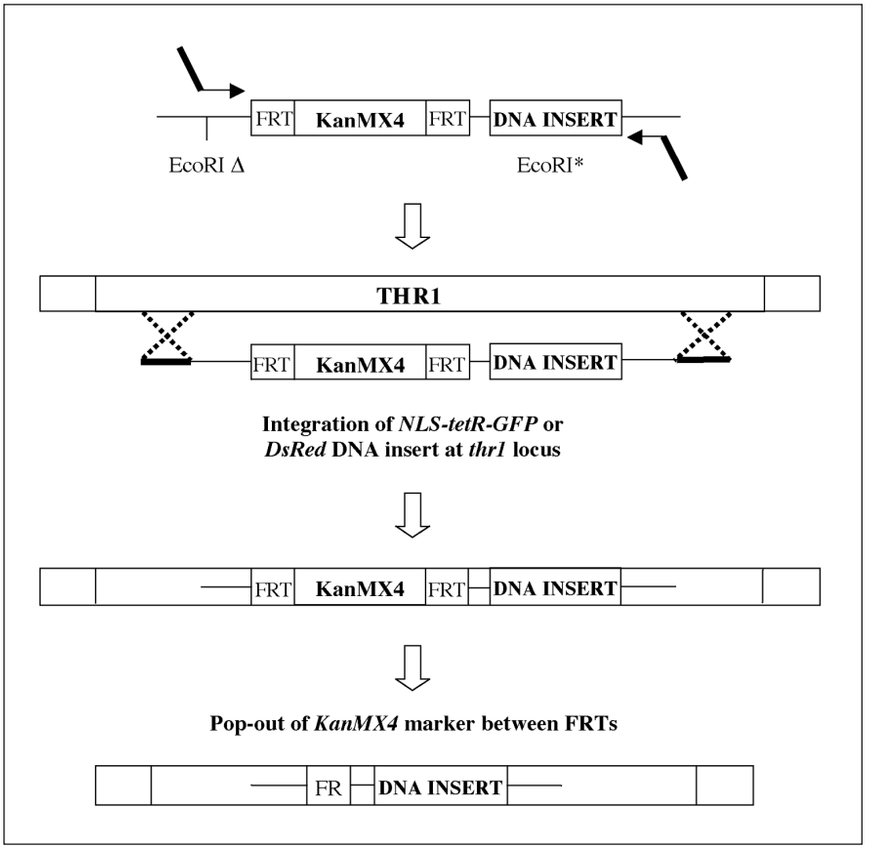

Figure 2. Schematic representation of the STIK-based integration of an *NLS-tetR-GFP* or *DsRed* fluorescent marker at the *thr1* locus on chromosome VIII. After the integration of the fluorescent marker and pop-out of *KanMX4*, the second fluorescent marker has been integrated in the same way into the second allele. EcoRIΔ and EcoRI*, remaining and deleted restriction site, respectively.

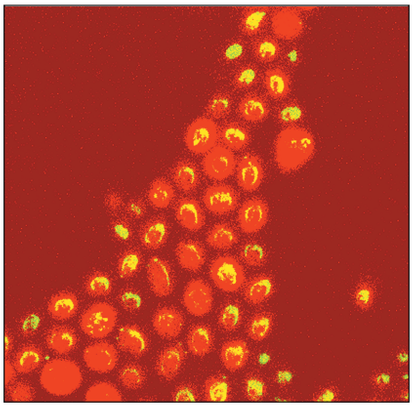

Figure 3. Confocal fluorescence microscopy of disomic cells (1n+1) of the yeast *S. cerevisiae* harboring *NLS-tetR-GFP* and *DsRed* fluorescent marker genes each integrated at the *thr1* locus on the two copies of chromosomes VIII. The green fluorescence is visible within the nucleus of the cells, while the red is present in the cytoplasm. Cells that have both disomic chromosomes exhibit both red and green fluorescence. Some cells have lost either the green or red fluorescence, indicating the loss of the related copy of the chromosome.

Integration of the *NLS-tetR-GFP* and *DsRed* Sequence at the *THR1* Loci

To demonstrate that our system could be used for repeated targeted integration, we replaced both threonine (*thr1*) loci on chromosome VIII in *S. cerevisiae* strain Z140-51D disomic for that chromosome. Each *THR1* and *thr1* threonine allele was subsequently replaced with *NLS-tetR-GFP* and *DsRed* constructs, respectively. The *tetR* gene fused in frame with the simian virus 40 (SV40) *NLS* sequence at its 5′ end and *GFP* at its 3′ end was subcloned into the pGKGE plasmid (Figure 1C, I). This plasmid was then used as a template for the amplification of the replacement cassette using primers THR1ΔF1 and THR1ΔR1. Both the forward and reverse THR1ΔF1 and THR1ΔR1 chimeric primers (60 bp) were designed in such a way that 40 bp are homologous to the sequences upstream and downstream, respectively, within *THR1*. The remaining 20 bp are homologous to the sequence of the vector upstream of the 5′ *FRT* and to the subcloned *NLS-tetR-GFP* fragment, respectively (Figure 2). Yeast strain ZI40-51D was transformed with the PCR-amplified, linear 3.6-kb *NLS-tetR-GFP* cassette, and the transformants were selected on YPD plates supplemented with geneticin (G418). Eventually, the *THR1* gene was substituted with the *NLS-tetR-GFP* and two *FRT*s flanking the *KanMX4* gene. To confirm that the integration event occurred at the *THR1* locus, geneticin-resistant transformants were further analyzed by yeast colony PCR using primers THRF1, THRR1, and K1 (data not shown). Moreover, the new strain, designated as Z140-51G, harboring the substitution of *THR1* with the *NLS-tetR-GFP* cassette, fails to grow on minimal medium without threonine, as expected. The *KanMX4* marker was excised spontaneously by growing the G418-resistant, Z140-51G strain in the nonselective liquid medium (YPD) for several generations to relieve the selective pressure for the marker. Approximately one hundred cells per plate were plated onto solid YPD. After incubation, the colonies obtained were further replica-plated onto YPD containing G418 to screen for G418-sensitive colonies. The frequency of the G418-sensitive colonies was found to be of the order of 10^{-3}, which is similar to that reported for the former set of knockout plasmid vectors (6). The loss of the marker was further confirmed by yeast colony PCR analysis using primers THR1F1, THRR1, and K1. At this stage, the remaining *thr1* locus was similarly replaced with the *DsRed* sequence encoding the red protein. This was initially subcloned into the pXKXE vector (Figure 1C, II) that served as a template for the PCR amplification of the *DsRed* replacement cassette using primers THR1ΔF1 and THR1ΔR2. The cassette was then integrated at the remaining threonine locus, which created a new G418-resistant strain Z140-51GR. The integration event was again confirmed by PCR analysis using primers THR1F1, THRR1, and K1 (data not shown).

The green and red fluorescence thus expressed by the integrated *NLS-tetR-GFP* and *DsRed* genes was visualized using confocal microscopy (Figure 3). From the picture, one can see that the fluorescence is present in almost all of the cells. The green fluorescence is localized within the nucleus due to the presence of the SV40 virus NLS sequence in the tetR-GFP construct, while the DsRed protein stains the entire cell due to its absence. Thus, the *NLS-tetR-GFP* and *DsRed* genes were successfully integrated at both *thr1* loci.

Integration of the PCR-Tagging Cassette

To demonstrate the versatility of the STIK system for tagging chromosomal genes, we subcloned a DNA fragment encoding the V5His6x epitope into plasmid pHKHE (Figure 1C, III) to create plasmid pH-V5His6x, which was used as a template for the construction of the tagging cassette. The 65-bp forward (1-V5F) and reverse (1-V5R) chimeric primers required for the construction of the *RVB1* locus tagging cassette are reported in Table 1, and their position relative to target genomic sequence is shown in Figure 4.

Both haploid YPH250 and diploid DUPOT-SL cells were transformed with the 1.75-kb PCR amplified linear tagging cassette, and transformants were selected on YPD plates supplemented with geneticin (G418). Cassette integration was confirmed by yeast colony PCR analysis using primers RVB1F2, RVB1R2, and K2 (data not shown). No further excision of the *KanMX4* marker was applied in this case. Whole cell proteins were visualized by staining the membrane with Ponceau-S Red stain (Figure 5, left panel). The transformants were assayed for correct expression of the desired Rvb1p-tagged variant by Western blot analysis (Figure 5, right panel). Figure 5 shows that the insertion of the DNA sequence tag was successful and did not alter the expression or the structure of the Rvb1 protein.

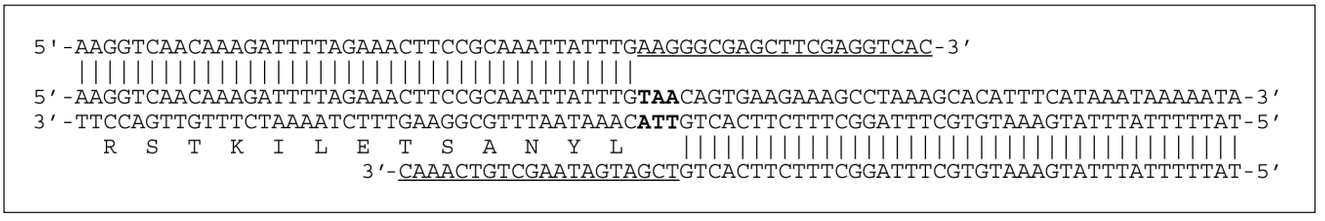

Figure 4. Position of the chimeric primers 1-V5F and 1-V5R used for the in-frame cloning of the V5His6x epitope at the 3′ end of *RVB1*. Forty base pairs of the forward and reverse primers (vertical lines) are homologous to the *RVB1* ORF sequence upstream, and within its terminator downstream, of the stop TAA codon (in bold), respectively. Additional 20 bases complementary to the subcloned V5His6x DNA fragment and to the sequence outside the *FRT*s (corresponding to the vector) are underlined.

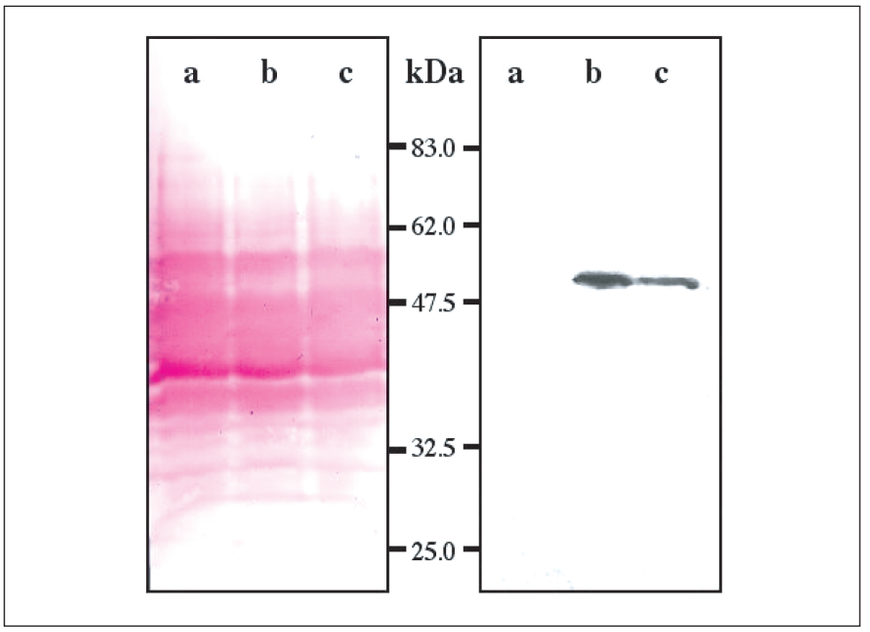

Figure 5. Whole cell proteins visualized by Ponceau-S Red staining (left panel) and Western blot analysis (right panel) from yeast strains: lane a, wild-type; lane b, RVB1-tagged YPH250; and lane c, *RVB1*-tagged DUPOT-SL.

DISCUSSION

The objective of this work was to construct a series of plasmid vectors that could be used to integrate any DNA sequence of interest, including those nonselectable phenotypically, to a desired coding or noncoding genomic target location using the *FLP/FRT*-mediated recombination system and the *KanMX4* as a selectable marker in *S. cerevisiae*. A series of PCR-template vectors bearing different mutations in the core region of the *FRT*s was previously reported from our laboratory (6,10). These vectors enable one to perform multiple gene disruption and knockout using a recyclable selectable marker. In our new STIK system, the PCR-amplified *FRT-KanMX4-FRT* DNA fragment directs the integration to the genomic location of homology, allows its own positive selection, and then it is lost by *FLP/FRT*-mediated site-specific excision. This leaves the DNA sequence of interest integrated at the selected chromosomal location, together with a 54-bp DNA "scar" consisting of 20 bp homologous to the plasmid template, which can be either 5′-TCGATGATAAGCTGTCAAAC-3′ or 5′-AAAAATAGGCGTATCACGAG-3′, depending on the *Eco*RI site that was used for polylinker insertion and 34 bp of the mutated *FRT* employed (6). Recombination between wild-type and mutated scars, as well as between different *FRT* scars is not favored; therefore, their presence is not inducing any genomic instability due to internal recombination. The most salient feature of our work is that our newly constructed series of PCR template vectors, pGKGE, pHKHE, pXKXE, and pWKWE, overcomes the limitation laid down by the impossibility of performing the targeted integration of DNA fragments for which there is no direct selection possible. Therefore, this system provides a broad horizon of possibilities to integrate any nonhomologous DNA sequence at the desired chromosomal location, without leaving relevant exogenous vector sequences that could interfere with the experiment. All the above vectors can be used in [*cir*$^+$] strains; however, pWKWE can only be used in [*cir*°] strains due to the presence of wild-type *FRT*s in this vector that could recombine with the *FRT*s of the endogenous 2-µm circle. The extrapolation of this system to [*cir*°] strains involves the presence of the endogenous *FLP* site-specific recombinase

gene in the vectors themselves. The existing plasmids, pGFKF, pHFKH, pXFKX from our previous work (6), carrying the endogenous *FLP* gene, can be used as substrate for the construction of additional vectors to be employed in [*cir*°] strains.

To validate our work, the *NLS-tetR-GFP* and *DsRed* DNA fragments were subcloned in the pGKGE and pXKXE vectors, respectively, and the entire region was PCR amplified along with the two *FRT*s and *KanMX4*. These cassettes were integrated in the ZI40-51D strain at the *thr1* locus on chromosome VIII, and the phenotypic expression of the fluorescent markers was observed by confocal microscopy (Figure 3). Moreover, to demonstrate the usefulness of this system for protein tagging, we constructed a module that contains the V5 epitope in combination with a 6His-tag. This module allows for the expression of tagged proteins under their own regulatory elements and, subsequently, their immediate use for a large set of biochemical and cell biology tests (14). We tagged the essential *RVB1* gene in haploid and diploid genetic background using the YPH250 and DUPOT-SL strain, respectively. Both strains correctly expressed the C-terminal tagged Rvb1 protein, as demonstrated by Western blot analysis (Figure 5), although in this experiment, the selectable *KanMX4* marker was not popped out. This also confirms that the presence of the 3′ *FRT* in the construct can act as an effective transcription terminator as previously reported (7) because the original terminator sequence was disrupted in the tagging process.

Our STIK plasmid system can be used for the construction of multiple deletions, replacement of endogenous regulatory elements, and tagging of gene products with high efficiency, using the common *KanMX4* as a recyclable selectable marker. Furthermore, this plasmid system can be easily extrapolated to other eukaryotic cells like *Schizosaccharomyces pombe, Drosophila melanogaster*, maize, *Xenopus laevis* embryos, and human cells, in which the *FLP*/*FRT* site-specific recombination has been demonstrated to function effectively (16,18–21). In these cases, an inducible *FLP* recombinase gene should be cotransfected together with the new vector.

This new STIK system will greatly benefit, in particular, those experiments of cell biology in which fewer marker genes are available for DNA sequence integration.

To this end, we have made constructions of the plasmid series containing the GFP variant blue fluorescent protein (BFP) and the herpes simplex virus (HSV) epitope tag. We will also be available to insert other commonly used tags, such as GST, if requested.

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