Dual Luciferase Assay System for Rapid Assessment of Gene Expression in *Saccharomyces cerevisiae*

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A new reporter system has been developed for quantifying gene expression in the yeast *Saccharomyces cerevisiae*. The system relies on two different reporter genes, *Renilla* and firefly luciferase, to evaluate regulated gene expression. The gene encoding *Renilla* luciferase is fused to a constitutive promoter (*PGK1* or *SPT15*) and integrated into the yeast genome at the *CAN1* locus as a control for normalizing the assay. The firefly luciferase gene is fused to the test promoter and integrated into the yeast genome at the *ura3* or *leu2* locus. The dual luciferase assay is performed by sequentially measuring the firefly and *Renilla* luciferase activities of the same sample, with the results expressed as the ratio of firefly to *Renilla* luciferase activity (Fluc/Rluc). The yeast dual luciferase reporter (DLR) was characterized and shown to be very efficient, requiring approximately 1 minute to complete each assay, and has proven to yield data that accurately and reproducibly reflect promoter activity. A series of integrating plasmids were generated that contain either the firefly or *Renilla* luciferase gene preceded by a multicloning region in two different orientations and the three reading frames to make possible the generation of translational fusions. Additionally, each set of plasmids contains either the *URA3* or *LEU2* marker for genetic selection in yeast. A series of S288C-based yeast strains, including a two-hybrid strain, were developed to facilitate the use of the yeast DLR assay. This assay can be readily adapted to a high-throughput platform for studies requiring numerous measurements.

The ease of growth and genetic manipulation of the yeast *Saccharomyces cerevisiae* has made it a model organism that is used in a wide range of biological studies. Much of our knowledge about the regulation of eukaryotic gene expression can initially be traced to studies in yeast. For these investigations, reporter genes have been used extensively, with β-galactosidase, encoded by the *lacZ* gene of *Escherichia coli*, being the most common. For several reasons, β-galactosidase is a non-ideal reporter for the study of gene expression in yeast. Although yeast cells expressing β-galactosidase offer a visual assay for gene expression, with cells that express the enzyme displaying a blue color in the presence of the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, quantitative β-galactosidase assays are time-consuming and offer lower sensitivity than newer reporter systems. The normalization of β-galactosidase assays has routinely been achieved via quantifying total protein of the yeast lysate (26) or on the basis of the optical density (*A*490) of the yeast culture (11). The use of colorimetric assays to measure total protein requires significant time independent of the β-galactosidase assay, while the use of optical density measurements can yield highly variable results depending on the spectrophotometer and yeast strain. In addition, the long half-life of β-galactosidase (2, 23) requires that independent yeast cultures be harvested at approximately the same cell density to obtain consistent data. The long half-life of β-galactosidase also precludes its use for gene regulation studies that involve transient induction/repression of gene expression. Moreover, the lower sensitivity of β-galactosidase assays may require the use of *lacZ* fusions on 2μm episomes to obtain reliable measurements, and this can add to the experimental inaccuracy of the assay due to variability in the copy number of the plasmid.

In mammalian cells, dual reporters have been commonly used to improve the accuracy of transient-reporter assays when analyzing gene expression. For example, the use of deletion analyses to define the important *cis*-acting elements within a given promoter requires that promoter deletion plasmids be cotransfected (pairwise) with a control reporter to provide an internal control for normalizing the activity of the experimental reporter. One reporter system used in mammalian cells for these types of gene expression studies is the dual luciferase reporter (DLR) assay (31).

The DLR assay exploits the differing biochemical requirements for luminescence of the firefly (*Photinus pyralis*) and sea pansy (*Renilla reniformis*) luciferase proteins (31). This allows for the sequential quantitative measurement of both luciferase activities in a single protein extract. The use of luciferase as a gene reporter has increased in popularity for researchers working with bacterial and mammalian cells; however, appropriate vectors containing these luminescent reporters for yeast have been limited. Both the firefly and *Renilla* luciferase proteins have proven to be highly effective as gene reporters, because the assays are extremely sensitive, rapid, reproducible, and easy to perform (31). Moreover, both luciferases have been shown to have a linear range of activity covering at least 5 orders of magnitude (31). Firefly luciferase (Fluc) is a 61-kDa monomeric protein that does not require posttranslational processing for activity, and luminescence occurs via the oxidation of luciferin to oxyluciferin in a reaction requiring ATP,
Mg$^{2+}$, and O$_2$ (31). Renilla luciferase (Rluc) is a 36-kDa monomeric protein that is glycosylated in its natural host; however, this posttranslational modification is not required for its activity (31). The luminescence generated by Renilla luciferase utilizes O$_2$ and coelenterazine (31). The dissimilarity in the substrates for the two luciferases makes it possible to selectively distinguish between the luminescent reactions for each enzyme. Using the DLR reagents, the luminescence of the firefly luciferase can be measured by addition of the luciferin reagent, and this reaction is subsequently quenched while simultaneously activating the luminescence of the Renilla luciferase. Thus, one can sequentially measure the luminescence of both reporters in a single reaction tube.

We have adapted the DLR assay to yeast. The DLR assay offers several advantages for measuring gene expression in yeast. First, the sensitivity of the luciferase assay allows the reporter to be integrated into the genome and still provide measurable signals, alleviating the issue of copy number variation observed with episomal reporters. Second, the presence of a control reporter in the genome, expressed from a constitutive promoter, provides a control for experimental variations, such as cell number, pipetting errors, cell lysis efficiency, and assay efficiency. Finally, as a result of having an internally controlled assay system, the yeast DLR assay can be adapted to a high-throughput platform where numerous assays can be performed simultaneously.

In developing the yeast DLR assay, we have generated a large set of vectors and yeast strains for conveniently constructing reporter plasmids and subsequently assaying gene expression. In addition, a yeast strain containing the DLR system was generated for quantifying protein-protein interactions in a yeast two-hybrid screen. In performing these studies, it was discovered that the yeast DLR assay was even more rapid than initially anticipated, and its reproducibility is unmatched by other reporters available for studies in yeast. We have characterized the yeast DLR system and demonstrate the utility of the assay to rapidly evaluate the regulation of gene expression.

### MATERIALS AND METHODS

**Yeast strains and growth media.** *S. cerevisiae* strains used in this study were all isogenic derivatives of FY2 (36), except Y190, and are shown in Table 1. Yeast strains were grown on rich, omission synthetic medium, 5-fluoroorotic acid medium, prepared as previously described (12, 32, 33) and supplemented with 2% glucose, raffinose, or lactate as needed. The sporulation medium was 1.5% sodium, prepared as previously described (32, 33) and supplemented with 2% glucose, raffinose, or lactate as needed. The sporulation medium was 1.5% potassium acetate (pH 7.5) supplemented with uracil and the required amino acids. Canavanine medium was arginine omission synthetic medium (SC - Arg) with 60 mg/liter canavanine sulfate added.

#### Oligonucleotides

Oligonucleotides used in the present study are listed in Table 2.

**Construction of the luciferase reporter plasmids.** To construct the plasmids YlpRluc1U1, YlpRluc2U1, YlpRluc1U3, YlpRluc2L1, and YlpRluc2L3, the *Renilla* luciferase gene (Rluc) was amplified by PCR from the template pRL-CMV (Promega Corp., Madison, WI) using either the 5’ primer oDM2040, oDM2081, or oDM2082 to generate the three reading frames of the polylinker and the 3’ primer oDM2025. The 5’ primers incorporate a unique HindIII site into the amplified product, while oDM2024 adds a unique PvuII site at the 3’ end. Each of the three 0.9-kb Rluc products were individually digested with HindIII/PvuII and purified by agarose gel electrophoresis, and ligated with each of the Rluc PCR products. To generate YlpRluc1U1R, YlpRluc2UR, and YlpRluc3UR, the Rluc gene was amplified by PCR as described above, except the 5’ primers were oDM2085, oDM2086, and oDM2087, respectively, to generate the reading frames and oDM2041 was the 3’ primer. The 5’ primers incorporated a unique

| Strain | Genotype | Source |
|--------|----------|--------|
| FY837  | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 | F. Winston |
| FY250  | MATa ura3–52 leu2α-1 his3Δ-200 trp1Δ-63 | F. Winston |
| Y190   | MATa ura3–52 his3Δ-200 ade2–101 trp1–901 leu2–3,112 gal4Δ gal80Δ cyh2 LYS::GAL1::HIS3 MEL1 URA3::GAL1 intoxic | S. Eldredge |
| DMY185 | MATa ura3–52 his3Δ-200 ade2–101 trp1–901 leu2–3,112 gal4Δ gal80Δ cyh2 LYS::GAL1::HIS3 MEL1 URA3::GAL1:trp1Δ | This study |
| DMY190 | MATa ura3–52 his3Δ-200 ade2–101 trp1–901 leu2–3,112 gal4Δ gal80Δ cyh2 LYS::GAL1::HIS3 MEL1 URA3::GAL1:trp1Δ | This study |
| DMY191 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY192 | MATa ura3–52 his3Δ-200 ade2–101 trp1–901 leu2–3,112 gal4Δ gal80Δ cyh2 LYS::GAL1::HIS3 MEL1 URA3::GAL1:trp1Δ | This study |
| DMY194 | MATa ura3–52::Fluc-URA3 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY195 | MATa ura3–52::GAL1-Fluc-URA3 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY196 | MATa ura3–52::ADH1-Fluc-URA3 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY204 | MATa ura3–52::CYCI–26SA-Fluc-URA3 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY205 | MATa ura3–52::CYCI–12TA-Fluc-URA3 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY212 | MATa ura3–52 leu2α-1 his3Δ-200 can1Δ::PGK1-Rluc | This study |
| DMY213 | MATa ura3–52 leu2α-1 his3Δ-200 can1Δ::PGK1-Rluc | This study |
| DMY214 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY215 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY217 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY218 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY219 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::PGK1-Rluc | This study |
| DMY220 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::SPT15-Rluc | This study |
| DMY221 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::SPT15-Rluc | This study |
| DMY222 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::SPT15-Rluc | This study |
| DMY223 | MATa ura3–52 leu2α-1 his3Δ-200 lys2α-202 can1Δ::SPT15-Rluc | This study |
For the disruption of the CAN1 locus, the plasmid pDM505 was constructed by digestion of pCAM100 (20) with HindIII and the 1.4-kb CAN1 fragment, purified and ligated with HindIII-Blal sites into the plasmid vector pUC18. The plasmids pDM505, pDM506, and pDM507 were used in the construction of the CAN1 promoter reporter plasmid pDM508, which was digested with HindIII/PvuII.

**General plasmid constructions.** To generate the plasmid for targeting the reporter to the CAN1 locus, the CAN1 gene was amplified by PCR from S. cerevisiae FY250 genomic DNA using oligonucleotide primers oDM0236 and oDM0237, designed to incorporate unique 5' and 3' SstI and PstI restriction sites, respectively. The amplified CAN1 fragment was inserted into pUC18 by digestion with SstI/PstI and ligated to generate the plasmid pDM499. To generate the PGK1-Fluc reporter plasmid pDM501, the PGK1 promoter region was amplified from the plasmid pDB20 (5) with SstI/HindIII, and the 1.5-kb fragment was isolated and ligated with SstI/HindIII-Blal sites into the plasmid vector YIp357R, which was digested with HindIII/PvuII.

**TABLE 2. Oligonucleotide primers used in this study**

| Primer name | Sequence |
|-------------|----------|
| oDM0123 | 5'-GGGATGACTGAATTCACGGGCGGTTC-3' |
| oDM0124 | 5'-GGGAAAAGGGGCGCTTGTATCTCAGGGC-3' |
| oDM0236 | 5'-GGGGcagctGGTTGATGACCCAAAGGCTTG-3' |
| oDM0237 | 5'-GGGGcagctCCATCTTGCGTGAATAGCTTTG-3' |
| oDM0238 | 5'-GGGGcagctGAATCCTTTCAAAAGATGGGCTT-3' |
| oDM0239 | 5'-GGGGcagctTTTTTTAATTTTGTGAAAGT-3' |
| oDM0240 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0241 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0242 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0243 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0244 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0245 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0246 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0247 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0248 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |
| oDM0249 | 5'-GGGGcagctTTTATTTTATATTTGTTGTG-3' |

* Sequences in lowercase letters represent relevant restriction enzyme sites.

EcoRI site on the 5' end of the PCR-amplified product. The amplified Fluc genes were digested with EcoRI/PvuII; purified by agarose gel electrophoresis, and ligated with the vector YIp357R (UR43) (25), which was digested with EcoRI/PvuII to remove lacZ. To construct the plasmids YIpFlucL1R, YIpFlucL2R, and YIpFlucL3R, Fluc was excised from YIpFluc1R, YIpFluc2R, and YIpFluc3R, respectively, with HindIII/PvuII and ligated into the vector YIp367R (LEU2) (25), which was digested with HindIII/PvuII. A similar strategy was employed to generate the firefly luciferase (Fluc) reporter constructs. To construct YIpFluc1U1, YIpFluc1U2, YIpFluc1U3, YIpFluc1L1, YIpFluc1L2, and YIpFluc1L3, the firefly luciferase gene (Fluc) was amplified by PCR from the plasmid template pGL3 (Promega Corp., Madison, WI) using the 5' primer oDM0242, oDM0243, or oDM0244 and the 3' primer oDM0245. The 1.7-kb Fluc PCR products were digested with HindIII/PvuII and ligated into either YIp358 or YIp368 digested with HindIII/PvuII. To generate YIpFluc1U1R, YIpFluc1U2R, and YIpFluc1UR, the Fluc gene was amplified by PCR with the 5' primers oDM0246, oDM0247, and oDM0248 respectively, and the 3' primer oDM0249. The 1.7-kb Fluc PCR products were digested with HindIII/PvuII and ligated with the vector YIp357R that had been digested with EcoRI/PvuII. YIpFluc1L1R, YIpFluc1L2R, and YIpFluc1L3R were constructed by excising Fluc from YIpFluc1U1R, YIpFluc1U2R, and YIpFluc1UR, respectively, with HindIII/PvuII, and ligating the purified Fluc fragment into YIp367R, which was digested with HindIII/PvuII.
liquid yeast extract-peptone-dextrose (YPD) medium to assay for Renilla luciferase activity. To verify the correct genomic structure of the disrupted can1 locus, Southern blot analysis was performed on each isolate having Renilla luciferase activity. Yeast strain DMY220 containing the SPT15-Rluc reporter disrupting the CAN1 locus was generated by transformation of FY837 with pDM549 that was digested with EcoRI/PstI, and the selection and confirmation of the correct recombination event were as described above for DMY191. Using DMY191 and DMY220, additional strains containing various auxotrophic markers and differing mating types were generated by standard genetic crosses to FY250. The mating type and auxotrophies were scored for each segregant, and Renilla luciferase activity was verified for each of the strains shown in Table 1. All of the Fluc reporter constructs containing the URA3 marker were integrated at the ara3-52 locus by digestion with StuI. To generate the two-hybrid strain DMY192, strain Y190 was grown on YPD medium and subsequently plated on 5-fluoroorotic acid medium containing canavanine. Several independent colonies were purified from each transformation and inoculated to liquid YPD medium to assay for luciferase activity. To verify the disruption of the CAN1 locus, Southern blot analysis was performed on each isolate having Renilla luciferase activity. DMY190 was subsequently transformed with the GAL1-Fluc reporter pDM505 that was linearized with StuI to direct integration at the ara3-52 locus, creating the intermediate strain DMY185. To generate the strain DMY190, containing the PGK1-Fluc reporter disrupting CAN1, DMY185 was transformed with pDM504 that was digested with SstI/PstI to release the can1-PGK1-Fluc-can1 disruption fragment. Transformants were selected on SC–Arg medium containing canavanine. Several independent colonies were purified from each transformation and inoculated to liquid YPD medium to assay for luciferase activity. To verify the disruption of the CAN1 locus, Southern blot analysis was performed on each isolate having Renilla luciferase activity. DMY190 was subsequently transformed with the GAL1-Fluc reporter pDM505 that was linearized with StuI to direct integration at the ara3-52 locus.

Southern blot analysis. For preparation of S. cerevisiae genomic DNA, cells were grown to saturation in YPD medium at 30°C. Genomic DNA was isolated for Southern blot analysis using the glass bead-phenol protocol as previously described (16). The DNA was digested with HindIII, resolved by electrophoresis on 0.7% agarose gels, and transferred to GeneScreen Plus membranes (NEN Life Sciences Products, Boston, MA) as described elsewhere (29). The probe for the CAN1 locus was a 1.1-kb HindIII/PstI fragment of the gene obtained by digestion of pDM499. The probes were radioactively labeled with [α-32P]dCTP (6,000 Ci/mmol; Amersham Biosciences) using the random primer labeling kit (U.S. Biochemicals, Cleveland, OH) according to the manufacturer’s instructions. Hybridizations and washes were performed at 65°C using previously described protocols (29).

Dual luciferase assays. Dual luciferase assays were performed with the dual luciferase reporter assay system (Promega Corp., Madison, WI). All reagents were prepared as described by the manufacturer. The 5× passive lysis buffer was supplied by the manufacturer and used for cell lysis as described below in Results. Briefly, cells were grown to exponential phase in the appropriate medium, and 5 to 10 μl of cells was removed directly from culture and transferred to 100 μl of 1× passive lysis buffer. After allowing lysis for 10 to 15 s, a 10-μl aliquot of cell lysate containing luciferase measurement buffer (10 mM Tris-HCl, pH 7.5, and a Turner Design TD-20/20 luminometer. The following steps were used for luminescence measurements: 100 μl of the firefly luciferase reagent (LARII) was added to the test sample, with a 10-s equilibration time and measurement of lucinescence with a 10-s integration time, followed by addition of 100 μl of the Renilla luciferase reagent and firefly quenching (Stop & Glo), 10-s equilibration time, and measurement of luminescence with a 10-s integration time. The data are represented as the ratio of firefly to Renilla luciferase activity (Fluc/Rluc). Since the Fluc/Rluc ratio was typically less than 1, the ratio was multiplied by 1,000 to give values that were whole numbers.

Northern blot analysis. S. cerevisiae strains were grown to an optical density at 600 nm (OD600) of ~1.0 in rich medium (YPD) containing either 2% glucose, raffinose, or lactate as the carbon source. The cells were harvested by centrifugation, and total RNA was prepared by the glass bead-acid phenol method as previously described (1). Approximately 30 μg of each total RNA sample was loaded, separated by formaldehyde-1% agarose gel electrophoresis, and transferred to GeneScreen Plus membranes (DuPont-NEN Research Products) according to the manufacturer’s protocol. The membranes were hybridized and washed under standard high-stringency conditions (29). The CYC1 probe used for hybridization was obtained by PCR amplification of the CYC1 coding region using oligonucleotide primers dODM123 and dODM124 and S. cerevisiae CYC1 250 genomic DNA as template. The PCR product was purified by agarose gel electrophoresis and subsequently radiolabeled with [α-32P]dCTP (Amersham) by use of a random primer labeling kit (U.S. Biochemicals, Cleveland, OH) according to the manufacturer’s protocol. The transcript levels were quantified on a Molecular Dynamics PhosphorImager.

Requests for strains and plasmids. The yeast strains and plasmids described in this work are available upon request from the corresponding author (D.S.M.) at the contact information shown or at http://biology.uark.edu/dmcnabb/dmcnabb.html.

RESULTS

Design and construction of the yeast dual luciferase assay system. As outlined in the introduction, our goal was to adapt the dual luciferase assay to S. cerevisiae and to determine whether it would be advantageous over the commonly used β-galactosidase assay in evaluating gene expression in yeast. The initial objective was to generate yeast strains containing a constitutively expressed Rluc gene integrated into the genome to serve as the recipient strain for experimental Fluc fusion reporters. The constitutively expressed Rluc would provide an internal control for evaluating the level of Fluc expression, analogous to ACT1 as a normalization control for Northern blotting. We chose to place the Rluc reporter at the CAN1 locus due to the ease of genetic selection (Can’), which could be used subsequently to generate additional yeast strains by standard genetic techniques with the Can’ providing a convenient phenotypic assay for the Rluc reporter. Using the parent strain FY837 (36), we generated strains DMY191 and DMY220 as described in Materials and Methods. These strains contain the Rluc gene fused to either the PGK1 or SPT15 promoter, respectively, disrupting the CAN1 locus. To identify transformants that contained the Rluc reporter versus spontaneous can1 mutants, each Can’ colony was screened for Renilla luciferase activity, and the Rluc+ strains were subsequently verified by Southern blot analysis to contain either the PGK1-Rluc or SPT15-Rluc fusion at the can1Δ locus (data not shown). Using these strains, additional yeast strains of each mating type and with various auxotrophic markers were generated by mating with FY250, and the segregants from the crosses were screened for the markers, mating type, and Can’ and verified to have Renilla luciferase activity (Table 1).

We next generated a set of integration vectors containing either the Fluc or Rluc gene with a polylinker containing multiple restriction sites for promoter cloning and different selectable markers for targeting integration. In addition, some investigators may prefer translational versus transcriptional fusions; therefore, the vectors were constructed with the three possible reading frames to make translational fusions more convenient. We considered generating these plasmids with an ARS/CEN element, which would be more convenient for rapidly generating the yeast strains. The difficulty we perceived with the ARS/CEN plasmids was the requirement to maintain selection, limiting the spectrum of media one could use for study. In addition, slight variations in copy number with the luciferase reporter plasmids might lead to increased variability among independent samples. Thus, we chose to generate plasmids for integration into the genome. As the starting plasmids for construction of the luciferase vectors, we used YIp358, YIp368, YIp357R, and YIp367R, which are integrating lacZ reporter vectors that have been widely used (25). Starting with these four vectors offer several advantages in building the yeast DLR system, including (i) the availability of both LEU2 and URA3 selectable markers for integration; (ii) two different orientations of the restriction site polylinker for greater flexibility for cloning; (iii) the lacZ gene could be conveniently excised and replaced with either Fluc or Rluc; and (iv) most of
the restriction sites in the polylinker were unique to the vector, adding to the ease of cloning. Thus, we generated a series of 12 Fluc reporter plasmids and 12 Rluc reporter plasmids as outlined in Materials and Methods (Fig. 1). Although the yeast strains described above may preclude the need for the Rluc reporters, we generated the 12 Rluc vectors to provide the flexibility of introducing the yeast DLR system into any strain background by simply cloning a control promoter into the Rluc vector and subsequently integrating the vector at either the leu2 or ura3 locus and the Fluc experimental reporter at the other locus. Moreover, investigators performing gene reporter studies that may be impacted by the can1 null allele in our strains would be able to use the alternative DLR assay setup. Thus, the only requirement for using the yeast DLR assay

FIG. 1. Schematic diagram of the firefly and Renilla luciferase reporter plasmids. A. Fluc and Rluc plasmids with their corresponding sizes (bp), containing either the URA3 or LEU2 selectable marker, are shown, with 1, 2, and 3 indicating the three different reading frames of the multicloning polylinker as depicted in panel B. Relevant restriction sites are shown, including those commonly used to target integration to the ura3 or leu2 locus. The plasmids containing the polylinker in the reverse orientation are not shown; however, they are identical to those shown except for the orientation of the polylinker. B. Sequence of the polylinker region, with the restriction enzyme sites shown (underlined) in the forward (1, 2, and 3) and reverse (1R, 2R, and 3R) orientations. The 1, 2, and 3 and 1R, 2R, and 3R to the left of the diagram depict the reading frames. The variations in reading frames occur after the HindIII and EcoRI sites of the forward and reverse polylinker, respectively. An example of the nomenclature for plasmid names is as follows: YipFlucU1, yeast integration plasmid containing firefly luciferase with the URA3 selectable marker in reading frame 1. The vectors ending with R indicate the reverse orientation of the multicloning polylinker.
would be the availability of both the leu2 and ura3 markers in the strain of choice.

**Stability of firefly and Renilla luciferase activities in vitro.** During the initial screening procedures, we observed that yeast cells suspended in 1× passive lysis buffer (Promega Corp.) for 15 to 30 s yielded significant and reproducible firefly and Renilla luciferase activities without mechanical lysis procedures. Moreover, we tested the passive versus mechanical lysis (glass beads) methods and observed that both methods yielded similar results (data not shown). The passive lysis buffer was designed to yield optimum firefly and Renilla luciferase activities when used for mammalian cell lysis. Thus, the luciferase activity observed in yeast is presumably due to the disruption of the plasma membrane integrity, similar to the chloroform permeabilizing method employed in performing β-galactosidase assays with yeast (11). Nevertheless, the simplicity of the passive lysis method was further explored, since it would significantly reduce the time and effort needed to prepare cells for the assay.

To further evaluate the lysis method and the in vitro stability of firefly and Renilla luciferases, the ADH1-Fluc reporter, pDM513, was integrated at the ura3-52 locus of strain DMY191 to generate strain DMY196. DMY196 was subsequently grown in YPD medium at 30°C to exponential phase, an aliquot of cells was suspended in 1× passive lysis buffer and allowed to incubate for various times at room temperature, and the firefly and Renilla luciferase activities were subsequently determined (Fig. 2A). The Renilla luciferase activity is stable for an extended period after addition of passive lysis buffer. In contrast, the firefly luciferase activity was more sensitive to the time of incubation in lysis buffer, with its activity reduced by as much as 50% after 10 min in 1× passive lysis buffer. A semilogarithmic graph of the data (Fig. 2A) shows that the degradation of firefly luciferase follows first-order kinetics ($t_{1/2} = 10$ min), suggesting a single event is sufficient to cause the activity loss (data not shown). This is in contrast to the stability of firefly luciferase in mammalian cells (CHO cells), where the enzyme activity is stable for up to 6 hours at room temperature (31), and suggests that a component of the yeast extract may be responsible for the instability. The knowledge of this loss in activity is important, since the Fluc/Rluc ratio could be significantly altered if cells remain in lysis buffer for a prolonged and variable period before measurement. However, since the yeast DLR assay can be completed in less than a minute from cell lysis to measurement of luminescence, this does not present a significant issue, but it does indicate that each individual assay should be performed sequentially from lysis to measurement rather than lysing several samples simultaneously and subsequently performing the luminescence measurements.

Given the instability of the firefly luciferase activity after cell lysis, we then assessed the stability of the enzyme activities after addition of their respective substrates, luciferin and coelenterazine. For these studies strain DMY196 was grown in YPD medium at 30°C to exponential phase, an aliquot of cells was placed in 1× passive lysis buffer for 15 seconds, and the luminescence was measured for 10 seconds at 30-second intervals for 6 min. In both assays, the DLR reagents were used in such a manner as to simulate a normal assay, with only the luciferin reagent added to measure firefly luciferase activity or, alternatively, the luciferin and coelenterazine reagents both added to measure Renilla luciferase activity. We observed that the stabilities of the firefly and Renilla luciferase activities were the inverse of those observed after cell lysis, with the firefly luciferase being quite stable even beyond the 6 min of the assay while the Renilla luciferase activity had dropped approximately 50% by 90 seconds (Fig. 2B). A semilogarithmic graph of these data for Renilla luciferase revealed first-order kinetics of activity loss (data not shown). Our results are consistent with previous observations in mammalian cells (CHO cells) reported by Sherf et al. (31), who demonstrated the Renilla luciferase activity decays by ~20% after 12 seconds. These data suggest that the firefly luciferase is more stable in the presence of its substrate, while the Renilla luciferase activity is rapidly lost in the yeast lysate. The instability of the Renilla luciferase activity was of particular concern, because the assay requires a ~30-second interval after luciferin addition before

![FIG. 2. Stability of firefly and Renilla luciferase activities in vitro.](image-url)
the coelenterazine reagent can be added. If the addition of the luciferin reagent was causing the instability, then Renilla luciferase activity could be reduced by at least 20% before the coelenterazine is added to a sample, and the variability in timing between luciferin and coelenterazine addition could yield a significant experimental error.

To address this issue, strain DMY196 was grown in YPD medium at 30°C to exponential phase, an aliquot of cells was removed to passive lysis buffer for 15 seconds, and luciferase activity was measured. To evaluate whether the luciferin reagent was the source of the instability, the firefly luciferase activity was determined immediately and the samples were subsequently incubated at room temperature for various times before addition of the coelenterazine reagent and the measurement of the Renilla luciferase activity. The Fluc/Rluc values (x10^-3) at 0, 30, 60, and 90 seconds after addition of the luciferin reagent were 4,180, 3,940, 5,310, and 3,850, respectively. The mean value for these readings was 4,320 (±675), a 16% standard deviation, indicating that the luciferin reagent does not promote the instability of the Renilla luciferase. Therefore, we hypothesized that the coelenterazine reagent destabilizes the enzyme, or the amount of Renilla luciferase in the reaction mixture causes rapid substrate depletion. To distinguish between these possibilities, an assay was performed in which the Renilla luciferase activity was measured at 30-second intervals until the activity had dropped by 90%. At this point, fresh coelenterazine reagent was added, and the measurements continued. In the presence of fresh substrate, the enzyme activity continued to decrease, suggesting that the substrate was not limiting (data not shown). Thus, we conclude that the coelenterazine reagent or the Stop & Glo used to quench the firefly luciferase activity must destabilize the Renilla luciferase activity in yeast lysates. Nevertheless, if the 10-s measurements of Renilla luciferase activity are performed immediately after the addition of the Renilla substrate to a sample, reliable data are obtained.

Sensitivity and linearity of the luciferase assay. Three important criteria for any reporter assay are the background, sensitivity, and linearity. To evaluate background luminescence resulting from the spontaneous oxidation of luciferin or coelenterazine with components of a yeast lysate, we used the data obtained from the luciferase assays performed when identifying strains containing PGK1-Rluc disrupting the CAN1 locus. As mentioned previously, we obtained a number of Can’ strains that were negative for either luciferase activity; nevertheless, these strains were screened for both firefly and Renilla luciferase activities and provided an excellent data set for background endogenous luciferase activity. For 14 different Can’ isolates that lacked Renilla luciferase activity, the background activity for firefly luciferase activity was 0.03 (±0.01) units and the Renilla luciferase activity was 0.87 (±0.11). Thus, for both luciferases, the background activity was negligible.

The other important criteria for the reporters are sensitivity and linearity. To evaluate these in the context of expression in yeast, the GAL1-Fluc reporter pDM505 was integrated at the ura3-52 locus of strain DMY191. The strain, designated DMY195, was subsequently grown to exponential phase in YP-galactose medium at 30°C. The cell numbers were then quantified by hemocytometer, and serial dilutions were prepared such that between 100 and 40,000 cells were used per assay for both firefly and Renilla luciferase activities. As anticipated, both the firefly and Renilla luciferase activities increased linearly with increasing amounts of cells (Fig. 3A and B), and the relationship of firefly to Renilla luciferase activity correlated perfectly (Fig. 3C). Surprisingly, the sensitivity of the assay was such that both luciferase activities could be detected reliably with as few as 100 yeast cells; however, the error between replicates was slightly higher at lower cell concentrations. Importantly, the Fluc/Rluc ratio was determined at each cell concentration (100, 200, 400, 800, 1,000, 2,000, 5,000, 10,000, 20,000, and 40,000 cells), with three replicates per cell concentration (30 total samples), and the combined mean value was 149 (±7) (Fig. 3D). Thus, the sensitivity, reproducibility, and linearity of the reporter assay were judged to be excellent. Although the instability of the firefly luciferase in the cell lysis buffer and the instability of the Renilla luminescence after the addition of substrate reagent might raise concerns about the reproducibility of the Fluc/Rluc ratios, these data demonstrate that those theoretical concerns do not raise any practical problems with the assay.

Growth phase sensitivity of the yeast DLR assay. As noted in the introduction, the half-life of β-galactosidase in yeast cells requires that independent yeast cultures be harvested at approximately the same growth phase to obtain consistent data. To determine whether the yeast DLR assay was sensitive to growth phase, strain DMY196, containing the constitutively expressed ADH1-Fluc reporter, was grown in YPD medium to various optical densities, and the firefly and Renilla luciferase activities were measured. As shown in Fig. 4, the Fluc/Rluc ratio was similar between OD600 values ranging from 0.3 through 1.5, with a combined mean value of 282 (±51); thus, the standard deviation was within 18%. Therefore, the yeast DLR assay is not completely independent of growth phase, but it is much less sensitive than β-galactosidase to the stage of growth. In practice, it is time-consuming to grow differing yeast strains that demonstrate varying growth kinetics to tightly controlled cell densities. The ability to perform the assay over a broader range of growth densities while still yielding acceptable errors is of considerable practical benefit in the ease of performance of gene expression studies. As a practical point, it is not necessary to measure the actual cell density as long as the cells are within the range of 0.3 to 1.5, since the PGK1- or SPT15-Rluc reporter will allow one to normalize each individual culture.

Stability of firefly luciferase activity in vivo. As noted in the introduction, β-galactosidase is very stable in yeast, with a half-life reported to be as long as ~20 h (2). To determine the half-life of firefly luciferase, strain DMY195 was grown overnight in YPD medium and subsequently inoculated to YP-Gal and grown for 4 h at 30°C, a period of time previously shown to allow sufficient GAL1 induction (data not shown). The cells were subsequently shifted to YPD, causing repression of the GAL1 promoter, and luciferase assays were performed on aliquots of the culture every 30 min thereafter for 3 h. The zero-hour time point was assayed immediately after transfer to YPD medium, the Fluc/Rluc activity ratio at this point was defined as 100%, and the Fluc/Rluc ratios of subsequent samples were determined in an identical manner. Three independent experiments were performed, and a semilogarithmic plot of the data shows first-order kinetics of activity loss with a
half-life of 96 (±7) minutes (Fig. 5). Thus, firefly luciferase has a significantly shorter in vivo half-life than β-galactosidase; therefore, it is more amenable to transient gene regulation studies.

**Analysis of gene regulation using the dual luciferase assay.**

The data obtained thus far suggest that the yeast DLR assay would be an accurate and reliable reporter system. To demonstrate the utility of the system, the inducible expression of the well-characterized CYC1 promoter (from the gene encoding iso-1-cytochrome c) was examined. The firefly luciferase reporter plasmids pDM529 and pDM531, containing the CYC1 (10) and CYC1 (9) promoters, respectively, were integrated at the ura3-52 locus of DMY191 to generate strains DMY206 and DMY204, respectively. In addition, to evaluate the background activity, the promoterless vector YIpFlucU1 was also integrated at the ura3-52 locus to generate strain DMY194. The strains were grown to exponential phase in YPD, YP-raffinose, or YP-lactate and assayed for luciferase activity. As shown in Fig. 6A, the CYC1 and CYC1 promoters were expressed at low levels on glucose and induced approximately 15-fold on the nonfermentable carbon source lactate. This is consistent with the levels of CYC1 mRNA induction observed by Northern blot analysis (Fig. 6B), suggesting the system reliably recapitulates gene expression. Importantly, the YIpFlucU1 vector gave no significant firefly luciferase activity on any of the media tested. It should be noted that CYC1 and CYC1 promoters display similar levels of expression and induction on the three different carbon sources, even though the CYC1 promoter contains the additional binding site for the transcriptional activator Hap1p.

**FIG. 3.** Sensitivity and linearity of the luciferase assay. A and B. Yeast strain DMY195 containing the GAL1-Fluc and PGK1-Rluc reporters was grown in YP-galactose medium to exponential phase. The cell numbers were quantified by hemocytometer, and dilutions were prepared such that the indicated number of cells was assayed. The data represent the means of three replicate assays performed at each dilution. C. Firefly versus Renilla luciferase activity is linear over a 400-fold range of cell concentrations analyzed. The data plotted are from panels A and B. D. Fluc/Rluc ratio is independent of the number of cells assayed. The data plotted are those shown in panels A and B. In each panel, the bars indicate 1 standard deviation and the correlation coefficient is indicated (\( r^2 \)).

**FIG. 4.** Effect of growth phase at the time of cell lysis on the yeast DLR assay. Strain DMY196 containing the ADH1-Fluc and PGK1-Rluc reporters was grown at 30°C in YPD medium to the various OD600 readings shown. At approximately each optical density, an aliquot of cells was removed, and the firefly and Renilla luciferase activities were measured. The data represent the means of two independent experiments with three replicate assays at each OD600 value. The bars indicate 1 standard deviation.
In the S288C strain background used in these studies, the HAP1 gene contains a transposon insertion that abolishes its ability to contribute to activated transcription (7); thus, we observe no major difference between the two CYC1 reporters in transcriptional induction. These data are supported by the Northern blot data (Fig. 6B), which were obtained using total RNA isolated from strain FY250, one of the parental strains used in the construction of the yeast DLR system.

Two-hybrid reporter using the yeast DLR assay. Given the ease, convenience, and reliability of the yeast DLR assay, this system was ideally suited for studying two-hybrid interactions. Moreover, with the recent efforts directed toward mapping the complete proteome interactions, a two-hybrid strain easily amenable to a high-throughput platform would be a timely molecular tool. Thus, a strain designated DMY192, containing the PGK1-Rluc reporter introduced at the can1 locus and a GAL1-Fluc reporter integrated at the ura3-52 locus, was generated as outlined in Materials and Methods. To verify the activity of the GAL1-Fluc reporter in DMY192, plasmid pDB20L (vector), pMA441 (Gal4p DNA-binding domain), or pCL1 (Gal4p) was introduced into the strain, transformants were grown to exponential phase (OD 600 of ~0.8) in SC-Leu-Ura medium, and luciferase activity was determined. The Gal1-Fluc reporter had essentially no activity in the presence of the vector (1.2 ± 0.2 units), and the Gal4p DNA-binding domain induced the reporter only slightly (10 ± 0.4 units), whereas in the presence of the full-length Gal4p, the Fluc/Rluc ratio (2,606 ± 86 units) was induced over 2,000-fold compared to vector. Thus, the Gal1-Fluc reporter is tightly regulated in the two-hybrid strain.

To evaluate whether the yeast strain DMY192 can reliably detect protein-protein interactions via luciferase assays, a directed two-hybrid experiment was performed using the plasmids pAS2-1 (Gal4p DBD only), pACT2 (Gal4p activation domain only), pTD1-1 (Gal4p AD-SV40 large T antigen), pLAM5'-1 (Gal4p DBD-lamin), and pVA3-1 (Gal4p DBD-p53). These plasmids are supplied as controls with the BD Biosciences-Clontech (Mountain View, CA) two-hybrid system and provide a useful means of assessing the luciferase activity in the two-hybrid strain. The plasmids were introduced into DMY192 in pairwise combinations, transformants containing

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FIG. 5. Half-life of firefly luciferase in vivo. Yeast strain DMY195 containing the GAL1-Fluc and PGK1-Rluc reporters was grown in YP-galactose for 4 h at 30°C, the culture was subsequently transferred to YPD medium, and incubation continued at 30°C. At the indicated times (in minutes), cells were removed and firefly and Renilla luciferase activities were measured. The Fluc/Rluc ratio at time zero was set at 100%, and subsequent measurements were expressed as a percentage of the original value. The data shown represent the means of three independent experiments, with the bars showing 1 standard deviation, and the correlation coefficient is 0.995.

FIG. 6. Analysis of CYC1 promoter activity using the dual luciferase assay. A. Yeast strains DMY194, DMY204, and DMY206 containing the Fluc reporters shown and the PGK1-Rluc control reporter were grown in the indicated media to exponential phase. Cells were lysed in 1× passive lysis buffer, and firefly and Renilla luciferase activities were determined. The data shown represent the means of three experiments, with the bars showing 1 standard deviation. B. Yeast strain FY250 was grown in rich medium containing the indicated carbon source to an OD600 of ~1.0. Total RNA was isolated, and 30 µg of RNA was resolved by formaldehyde-agarose gel electrophoresis and analyzed by Northern blotting using a probe for CYC1 mRNA as indicated, with rRNA shown as a loading control.
cultures. It should be noted that the copy number variations may exist between independent
the interacting proteins are expressed from 2 had previously observed; however, this may reflect the fact that
ificity of the luciferase signal. The standard deviation between
libility of the luciferase two-hybrid strain. Importantly, none of
expected protein-protein interaction and demonstrates the va-
large T antigen and the murine p53 (Fig. 7). This was the
SC-Leu-Trp medium, and luciferase assays were performed.
Two-hybrid strain DMY192 containing the plasmids encoding the in-
dicated proteins (described in Materials and Methods) was grown to
exponential phase in SC-Leu-Trp medium and subsequently assayed
for firefly and Renilla luciferase activities. The data represent the
means of three independent assays, with the bars indicating 1 standard
deviation.

FIG. 7. Dual luciferase assay for yeast two-hybrid interactions. Two-hybrid strain DMY192 containing the plasmids encoding the indicated proteins (described in Materials and Methods) was grown to exponential phase in SC-Leu-Trp medium and subsequently assayed for firefly and Renilla luciferase activities. The data represent the means of three independent assays, with the bars indicating 1 standard deviation.

each pair of plasmids were grown to exponential phase in
SC-Leu-Trp medium, and luciferase assays were performed. The only positive two-hybrid interaction was between the SV40 large T antigen and the murine p53 (Fig. 7). This was the expected protein-protein interaction and demonstrates the validity of the luciferase two-hybrid strain. Importantly, none of the other plasmid combinations gave a signal above that observed with the vectors, pAS2-1 and pACT2, showing the specificity of the luciferase signal. The standard deviation between individual cultures for each plasmid pair was higher than we had previously observed; however, this may reflect the fact that the interacting proteins are expressed from 2μm plasmids and the copy number variations may exist between independent cultures. It should be noted that GAL1_UAS_HIS3_TATA_HIS3 is present in DMY192 for the selection of protein-protein interactions, and the luciferase assays could be used subsequently to verify and quantify the strength of the interactions.

DISCUSSION

In developing the yeast DLR assay, we envisioned several important advantages of this system over the traditional β-galactosidase assays. First, the sensitivity of the luciferase assay allows the reporters to be integrated into the genome, alleviating the difficulty of copy number variation seen with episomal reporters. Second, the presence of the control reporter, expressed from a constitutive promoter, permits normalization for experimental variations such as cell number, pipetting volume errors, cell lysis efficiency, and assay efficiency. Third, the assay can be performed rapidly. Finally, the yeast DLR assay could be adapted for high-throughput applications.

There have been few reports describing the use of luciferase as a reporter in S. cerevisiae. Firefly luciferase has been utilized in studies on transcriptional and translational fidelity (30), while Renilla luciferase has been used for transcriptional regulation studies in Candida albicans (34). There are examples of the dual luciferase assay being used in yeast as a bicistronic mRNA reporter to study translation events such as ribosomal frameshifting or termination (14, 19, 20, 27, 28, 35). The system described herein was designed to study transcriptional regulation; however, we can envision many other uses for the dual reporting system.

We have used the yeast DLR assay to delimit the cis-acting elements necessary for transcriptional induction of the HAP4 promoter in S. cerevisiae. We have generated a series of HAP4 promoter deletions fused to firefly luciferase and used the yeast DLR assay to identify the important cis-acting elements responsible for its derepression in the absence of glucose by examining luciferase activity with strains grown in rich media containing glucose, raffinose, or lactate as the sole carbon source. To this end, we have delimited two regions within the HAP4 promoter that appear to be important for appropriate regulation, and we have also shown that a putative Mig1p-binding site within the promoter is not functionally relevant for the glucose repression (data not shown). Importantly for this discussion, the data were highly reproducible, with standard deviations of ~10% or less. Moreover, the different deletion constructs that did not remove relevant cis-acting elements yielded similar Fluc/Rluc ratios. Therefore, the instability of the firefly and Renilla luciferase activities described in Results, while puzzling, does not present a practical problem, provided the assays are performed sequentially and in a timely manner, as outlined. We can foresee problems with the yeast DLR assay with reporter studies involving components of the basal transcriptional machinery, since mutations that confer defects in the basal machinery may affect the activity of both promoters. Nevertheless, such problems can be identified and overcome by using only one reporter and normalizing to total protein or cell number.

The yeast DLR assay should be adaptable to a high-throughput platform for studies or screens that can be assessed via a transcriptional response. Such a high-throughput system would be relatively inexpensive for screening medicinal compounds in an effort to narrow down a small group of lead candidates to be further evaluated in an appropriate mammalian system. Another high-throughput application of the yeast DLR assay would be as a biosensor for screening the toxicity of compounds. The firefly luciferase reporter has been tested as a biosensor for toxic compounds (17), but the addition of the Renilla luciferase for normalization would refine the quantification. The only potential caveat to the high-throughput studies would be the instability of firefly luciferase after yeast cell lysis (t1/2 = 10 min) and of the Renilla luciferase after addition of the coelenterazine reagent (t1/2 = 90 s). A high-throughput platform would need to be designed to work within these parameters. Alternatively, newer substrate reagents designed for high-throughput applications, termed the Dual-Glo luciferase assay system, are available from Promega Corp., and these reagents yield an extended stability of the luminescence signal for both the firefly and Renilla luciferases. We have not tested these reagents in yeast; however, they may resolve the Renilla luciferase decay problem.

Our overall goal was to build a system with the flexibility to generate dual luciferase reporters using our strains or other laboratory strains. With the available vectors, researchers can conveniently set up the yeast DLR assay using any promoter pair for the firefly and Renilla luciferase fusions to create a dual luciferase reporter system that fits their particular needs.
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REFERENCES

1. Ausubel, F. M., R. Brent, R. E. Kingston, D. B. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1994. Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.

2. Bachmair, A., D. Finley, and A. Varshavsky. 1986. In vivo half-life of a protein is a function of its amino-terminal residue. Science 234:179–186.

3. Bartel, P. L., C.-T. Chien, R. Sternberg, and S. Fields. 1993. Using the two-hybrid system to detect protein-protein interactions. p. 153–179. In D. A. Hartley (ed.), Cellular interactions in development: a practical approach. Oxford University Press, Oxford, England.

4. Fields, S., and O. Song. 1989. A novel genetic system to detect protein-protein interactions. Nature 340:245–246.

5. Fikes, J. D., D. M. Becker, F. Winston, and L. Guarente. 1990. Striking conservation of TFID in Schizosaccharomyces pombe and Saccharomyces cerevisiae. Nature 346:291–294.

6. Flick, J. S., and M. Johnston. 1990. Two systems of glucose repression of the GAL1 promoter in Saccharomyces cerevisiae. Mol. Cell. Biol. 10:4757–4760.

7. Gaisne, M., A. M. Becam, J. Verdiere, and C. J. Herbert. 1994. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the CYC1 gene of S. cerevisiae. Cell 76:503–511.

8. Guarente, L., T. Mason. 1983. Heme regulates transcription of the CYC1 gene of S. cerevisiae via an upstream activation site. Cell 32:1279–1286.

9. Guarente, L., and M. Ptashne. 1981. Fusion of Escherichia coli lacZ to the cytochrome c gene of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 78:2199–2203.

10. Guthrie, C., and G. R. Fink (ed.). 1991. Guide to yeast genetics and molecular biology. Academic Press, San Diego, Calif.

11. Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell 75:791–803.

12. Harger, J. W., and J. D. Dinman. 2003. An in vivo dual-luciferase assay system for studying translational recoding in the yeast Saccharomyces cerevisiae. Mol. Genet. Genomics 269:443–451.

13. Harper, J. W., R. G. Adami, N. Wei, K. Keyomarsi, and S. J. Elledge. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:605–616.

14. Hoffmann, C. S., and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. Gene 57:267–272.

15. Hollis, R. P., K. Killham, and L. A. Glover. 2000. Design and application of a biosensor for monitoring toxicity of compounds to cuskayotes. Appl. Environ. Microbiol. 66:1676–1679.

16. Iwabuchi, K., B. Li, P. Bartel, and S. Fields. 1993. Use of the two-hybrid system to identify the domain of p53 involved in oligomerization. Oncogene 8:1093–1096.

17. Jacobs, J. L., and D. J. Dinman. 2004. Systemic analysis of bicistronic reporter assay data. Nucleic Acids Res. 32:e160. [Online.] doi:10.1093/nar/gnh157.

18. Keeling, K. M., J. Lanier, M. Du, J. Salas-Marco, L. Gao, A. Kamenjak-Angeletti, and D. M. Bedwell. 2004. Leaky termination at premature stop codons antagonizes nonsense-mediated mRNA decay in S. cerevisiae. RNA 10:701–703.

19. Li, B., and S. Fields. 1993. Identification of mutations in p53 that affect its binding to SV40 large T antigen by using the yeast two-hybrid system. FASEB J. 7:957–963.

20. Lin, L. S., J. Elledge, C. A. Peterson, E. S. Bale, and R. J. Legeri. 1994. Specific association between the human DNA repair proteins XPA and ERCC1. Proc. Natl. Acad. Sci. USA 91:5012–5016.

21. Lin, S., and I. Zabin. 1972. Beta-galactosidase. Rates of synthesis and degradation of incomplete chains. J. Biol. Chem. 247:2205–2211.

22. Ma, J., and M. Ptashne. 1987. Deletion analysis of GAL4 defines two transcriptional activating segments. Cell 48:847–853.

23. Myers, A. M., A. Trzagoloff, D. M. Kinney, and C. J. Lusty. 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of lacZ fusions. Gene 45:299–310.

24. Rose, M., M. J. Casadaban, and D. Botstein. 1981. Yeast genes fused to beta-galactosidase in Escherichia coli can be expressed normally in yeast. Proc. Natl. Acad. Sci. USA 78:2460–2464.

25. Salas-Marco, J., and D. M. Bedwell. 2004. GTP hydrolysis by eRF3 facilitates stop codon decoding during eukaryotic translation termination. Mol. Cell. Biol. 24:7769–7778.

26. Salas-Marco, J., and D. M. Bedwell. 2005. Discrimination between defects in elongation fidelity and termination efficiency provides mechanistic insights into translational readthrough. J. Mol. Biol. 348:811–815.

27. Sambrook, J., E. G. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

28. Shaw, R. J., N. D. Bonawitz, and D. Reines. 2002. Use of an in vivo reporter assay to test for transcriptional and translational fidelity in yeast. J. Biol. Chem. 277:24420–24426.

29. Sherf, A. S., L. Navarro, R.R. Hannah, and K. V. Wood. 1996. Dual-luciferase reporter assay: an advanced co-reporter technology integrating firefly and Renilla luciferase assays. Promega Notes 57:2–8. [Online.] http://www.promega.com/notes/57/5753a/5753a.html.

30. Sherman, F. 1991. Getting started with yeast. Methods Enzymol. 194:3–21.

31. Sikorski, R. S., and J. D. Boeke. 1991. In vitro mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. Methods Enzymol. 194:302–318.

32. Srikanta, R., A. Klapach, W. W. Lorenz, L. K. Tsai, L. A. Laughlin, J. A. Gorman, and D. R. Soll. 1996. The sea pansy Renilla reniformis luciferase serves as a sensitive bioluminescent reporter for differential gene expression in Candida albicans. J. Bacteriol. 178:121–129.

33. Verge, V., M. Vonlanthen, J.-M. Masson, H. Trachsel, and M. Altmann. 2004. Localization of a promoter in the putative internal ribosome entry site of the Saccharomyces cerevisiae TIF4631 gene. RNA 10:277–286.

34. Winston, F., C. Dollard, and S. L. Ricupero-Hovasse. 1995. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast 11:53–55.