Studies on the regulation of gene expression have frequently used the β-galactosidase (β-gal) coding sequence (lacZ) from *Escherichia coli* as a reporter gene. A more recent application for lacZ gene fusions has been to detect and analyze protein-protein interactions using the yeast two-hybrid system (4). The chromogenic β-gal substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) is often used for the sensitive but only semiquantitative colorimetric detection of enzyme-producing yeast cell colonies growing on agar plates (1,7). However, for the quantitative determination of β-gal activity from liquid yeast cultures, colorimetric assays using o-nitrophenyl-β-D-galactopyranoside (ONPG; References 6,10 and 12) or chlorophenol red-β-D-galactopyranoside (CPRG; References 3 and 14) as chromogenic substrates are commonly used. These assays offer poor sensitivities with detection limits of approximately 1 ng and 100 pg of enzyme, respectively (3,9). Additionally, they are time-consuming, as it takes up to 4 h from cell extracts to final results.

It has been reported that as little as 2 fg of β-gal can be detected with an assay incorporating the chemiluminescent substrate 3-(4-methoxyspiro{1,2-dioxetane-3,2′-tricyclo[3,3,1,13,7]decan}-4-yl)phenyl-β-D-galactopyranoside (AMPGD; References 2 and 9). Besides an increase in sensitivity, this assay offers a superior dynamic range. The chemiluminescent signal ranges over four orders of magnitude, while the conventional quantitation methods have dynamic ranges of less than two orders (9). Furthermore, the AMPGD assay is easy to use and much more rapid to perform, leading from extracts to results in less than 1 h. Thus, for the quantitation of β-gal from transfected mammalian cells, chromogenic substrates have been widely replaced by AMPGD. In yeast however, the colorimetric assays are still the only established quantitation methods for β-gal.

In our laboratory, a chemiluminescent assay originally developed for the quantitation of β-gal from mammalian cells was modified (2,9) in a way that makes it applicable to yeast cells. It has been optimized for *Sacccharomyces cerevisiae* reporter strains that

**Table 1. Comparison of β-Gal Activities Obtained by Colorimetric and Chemiluminometric Quantitation Methods**

<table>
<thead>
<tr>
<th>Plasmid Expression</th>
<th>ONPG</th>
<th>AMPGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pAS2 GAL4(1–147)</td>
<td>–</td>
<td>3235</td>
</tr>
<tr>
<td>pCL1 GAL4(1–881)</td>
<td>1598</td>
<td>12 190 267</td>
</tr>
<tr>
<td>pSE1112 (pAS-SNF1) GAL4(1–147)</td>
<td>&lt;1</td>
<td>7872</td>
</tr>
<tr>
<td>pSE1111 (pACT-SNF4) GAL4(768–881)-SNF4</td>
<td>&lt;1</td>
<td>4202</td>
</tr>
<tr>
<td>pSE1112 + pSE1111 GAL4(1–147)-SNF1 GAL4(768–881)-SNF4</td>
<td>10</td>
<td>85 748</td>
</tr>
</tbody>
</table>

Plasmid constructs were transformed into *S. cerevisiae* Y190 (8) by a modified lithium acetate method (13). Transformants were grown on synthetic media lacking either tryptophan (pAS2 and pSE1112 transformants) or leucine (pSE1111 and pCL1 transformants) or both aa (pSE1111 and pSE1112 transformants). Colorimetric β-gal assays using ONPG (Sigma Chemical, München, Germany) as a substrate were performed as described (10), and results are displayed as units defined by Miller. Chemiluminometric assays using AMPGD were performed exactly as described in the text, and results are given as relative light units. All assays were done in triplicate from independent transformants, and enzyme activities represent the mean values from these experiments. Variations were typically ±20% or less among triplicates for both colorimetric and chemiluminometric assays.

**Figure 1. Dose-response curves for the AMPGD and ONPG quantitation methods.** The yeast strain Y190 carrying the pCL1 plasmid was grown under conditions described in the Table 1 legend. Different amounts of cells were subjected to β-gal quantitation using the chemiluminometric or colorimetric method as described in the text. Relative enzyme activities are given as relative light units for the AMPGD assay or arbitrary units (absorbance change at 420 nm per minute × 10⁸) for the ONPG assay and represent the log mean for duplicate samples.
are commonly used for the analysis of protein-protein interactions using the two-hybrid system (e.g., Y190) (8). This strain carries a GAL1lacZ fusion gene where β-gal production is under the control of the GAL4 upstream activating sequence. We have tested the chemiluminimetric assay using plasmids that encode the entire 881 amino acids (aa) of the GAL4 protein (pCL1; CLONTECH Laboratories, Heidelberg, Germany) and the 147 amino-terminal residues that represent the GAL4 DNA-binding domain (pAS2; Reference 8). Additionally, we used constructs encoding the two yeast proteins SNF1 and SNF4 fused to GAL4(1–147) or the GAL4-activating region II (aa 768–881), respectively. These proteins have been previously shown to interact in the yeast two-hybrid system (4). Upon comparing the enzyme activities obtained from assays with ONPG and AMPGD, results (Table 1) indicated that the chemiluminimetric method is fully applicable to yeast cells. We further performed dose-response experiments confirming the increased sensitivity and extended dynamic range of the AMPGD assay compared with the standard ONPG method in the yeast system (Figure 1).

For the chemiluminimetric quantitation of β-gal, yeast cells were grown in 5 mL of the appropriate synthetic selective medium to late logarithmic phase (5 x 10^7 to 2 x 10^8 cells/mL), and the optical densities at 600 nm of the cultures were determined. For each sample, the optical density equivalent to 5 x 10^5 cells was transferred to a reaction tube and pelletted by centrifugation in a tabletop centrifuge (30 s at 20 000 x g). After aspiration of the supernatant, cells were washed with 1 mL of phosphate-buffered saline without calcium and magnesium ions and pelleted once more. The supernatant was removed completely. Then, 100 µL of a lysis solution (100 mM potassium phosphate, pH 7.8, 0.2% Triton® X-100, 1 mM dithiothreitol) were added to each sample, and the tubes were incubated on a tabletop shaker (e.g., Model 5432 Mixer; Eppendorf, Madison, WI, USA) for 20 min at 4°C. Then cells were further permeabilized by two cycles of freezing and thawing. This treatment leads to permeabilized but not completely lysed cells. Alternatively, cells can be completely broken open (e.g., by the use of glass beads). However, in our hands, complete cell breakage did not result in increased activities, indicating that all of the intracellular β-gal becomes available by the permeabilizing procedure given above. After the last freeze-thaw cycle, 2–20 µL of individual cell extracts were mixed with 200 µL of reaction buffer (100 mM sodium phosphate, pH 8.0, 1 mM magnesium chloride) containing 100 µg/mL AMPGD (Galacton™; Tropix, Bedford, MA, USA) in luminometer cuvettes. The amount of cell extract required varies depending on the amount of β-gal expression. After incubation for 15 min at room temperature, the cuvettes were placed in a Lumat LB 9501 Luminometer (Berthold Analytical, Baden-Baden, Germany) in luminometer cuvettes. The amount of cell extract required varies depending on the amount of β-gal expression. After incubation for 15 min at room temperature, the cuvettes were placed in a Lumat LB 9501 Luminometer (Berthold Analytical, Baden-Baden, Germany). Alternatively, a liquid scintillation counter can be used; however, sensitivity could be lower (5,11). Following injection of 300 µL Light Emission Accelerator (Tropix) and a 3-s delay, photon emission of the samples was counted for 5 s.

We think that our chemiluminimetric β-gal assay is extremely useful for studies on gene expression and two-hybrid applications in yeast. Being, to our knowledge, the most sensitive assay for the quantitation of β-gal available, detection of very weak protein-protein interactions should be possible. Because it allows more accurate discrimination between small differences in enzyme concentrations, the increased dynamic range is especially favorable for mutational analyses within the two-hybrid system, where protein domains or single amino acids critical for an interaction are to be defined.

REFERENCES


We thank Stephen J. Elledge for providing the yeast strain Y190 and the plasmids pSE1111, pSE1112 and pAS2. Address correspondence to Michael Nevels, Institut für Medizinische Mikrobiologie und Hygiene, Franz-Josef-Strauss-Allee 11, Universität Regensburg, D-93053 Regensburg, Germany. Internet: michael.nevels@klinik.uni-regensburg.de.

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