Quantitation of β -Galactosidase from Yeast Cells Using a Chemiluminescent Substrate

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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[3,3,1,1^{3,7}]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 estab-

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

Plasmid	Expression	β -Gal Activities	
		ONPG	AMPGD
—	—	<1	3235
pAS2	GAL4(1–147)	<1	7872
pCL1	GAL4(1–881)	1598	12 190 267
pSE1112 (pAS-SNF1)	GAL4(1–147)-SNF1	<1	3717
pSE1111 (pACT-SNF4)	GAL4(768–881)-SNF4	<1	4202
pSE1112 + pSE1111	GAL4(1–147)-SNF1 GAL4(768–881)-SNF4	10	85 748

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 $\pm 20\%$ or less among triplicates for both colorimetric and chemiluminometric assays.

lished quantitation methods for β -gal.

In our laboratory, a chemiluminometric assay originally developed for the quantitation of β -gal from mam-

malian cells was modified (2,9) in a way that makes it applicable to yeast cells. It has been optimized for *Saccharomyces cerevisiae* reporter strains that

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 $\times 10^8$) for the ONPG assay and represent the log mean for duplicate samples.

Benchmarks

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 chemiluminometric 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 chemiluminometric 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 chemiluminometric quantitation of β -gal, yeast cells were grown in 5 mL of the appropriate synthetic selective medium to late logarithmic phase (5×10^7 to 2×10^8 cells/mL), and the optical densities at 600 nm of the cultures were determined. For each sample, the culture volume equivalent to 5×10^7 cells was transferred to a reaction tube and pelleted by centrifugation in a tabletop centrifuge (30 s at 20 000 $\times$ 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 μ L 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 μ L of individual cell extracts were mixed with 200 μ L of reaction buffer (100 mM sodium phosphate, pH 8.0, 1 mM magnesium chloride) containing 100 μ g/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, Nashua, NH, USA). Alternatively, a liquid scintillation counter can be used; however, sensitivity could be lower (5,11). Following injection of 300 μ L Light Emission Accelerator (Tropix) and a 3-s delay, photon emission of the samples was counted for 5 s.

We think that our chemiluminometric 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.

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