bination-repaired plasmid topologies with the latest transformation technologies (1), it should be possible to routinely obtain transformation frequencies in the range of $10^8$–$10^9$ per microgram of plasmid DNA. Such frequencies will greatly aid in library screening strategies that entail very large numbers of transformed yeast cells.

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Improved β-Glucuronidase Reporter for Mammalian Gene Expression Analysis


Reporter gene assays have become essential for the study of genes and their corresponding regulatory elements. β-glucuronidase (GUS) is one of the most widely used reporter genes in plant genetic research and is used to a lesser extent in mammalian cells. The gene that encodes β-glucuronidase, gusA, was originally isolated from *Escherichia coli* by Jefferson et al. (2). GUS chemiluminescent assays are easy and non-isotopic. They allow the detection of amounts as low as 60 fg, with linear measurements over six orders of magnitude of enzyme concentration (1). The use of GUS as a reporter gene in plant genetics has been so successful largely because of the lack of endogenous GUS activity.

Like β-galactosidase (β-gal), GUS expression can be localized at the single cell level by histochemical staining, in the case of GUS, using 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-gluc) as a substrate. Also like β-gal, GUS activity is endogenous to animal cells. However, for using GUS as a reporter gene in animal cells, the substrate, X-gluc, has to go through a two-step reaction to produce the final colored precipitate, “X”. First, X-gluc is cleaved by GUS, and then the cleavage product, “X”, undergoes oxidative dimerization to produce CIBr-Indigo. CIBr-Indigo is extremely insoluble and precipitates immediately, resulting in cellular localization (8). Thus, by concentrating GUS in the nucleus of the cells, the staining should be able to detect cells that express amounts of GUS previously undetectable. Ideally, this
would increase the sensitivity of the GUS histochemical assay, which is essential for the determination of cell and tissue specificity. X-gluc histochemical staining of COS-7 cells was significantly enhanced with the pCEP4GUS2 construct (Figure 2A). Immunostaining was performed to determine the effectiveness of the nuclear localization sequence to localize the GUS enzyme in transfected cells. Two days posttransfection, cells were fixed and stained with a primary anti-GUS antibody (Molecular Probes, Eugene, Oregon, USA) and a fluorescein isothiocyanate (FITC) conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Cells were observed by fluorescent microscopy (Figure 2B).

GUS immunostaining was clearly localized to the nucleus in the cells transfected with pCEP4GUS2. Cells transfected with pCEP4GUS showed GUS immunostaining throughout the cytoplasm without any localized foci. This does not prove that the nuclear localization of the reporter enzyme, GUS, directly results in the improved sensitivity of the X-gluc histochemical assay that was observed. Nevertheless, it does confirm that the incorporation of the viral nuclear localization sequence can encourage nuclear localization of the GUS gene product.

In summary, the effectiveness of the GUS reporter gene has been enhanced with three modifications to the 5′ untranslated region and the coding region. Reporter gene activity is increased greater than fourfold in transiently transfected COS-7 cells, as determined by chemiluminescence and X-gluc histochemical assays. The incorporation of the nuclear localization region from the SV40 large T antigen

![Figure 1. An improved GUS construct for mammalian expression.](image)
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Figure 2. Histochemical staining of GUS and nuclear localization of GUS with pCEP4GUS2. (A) X-gluc staining of COS-7 cells transfected with (i) pCEP4GUS2, (ii) pCEP4GUS and (iii) pCEP4β-gal, as a negative control. Colors were modified slightly to enhance the color contrast. (B) Fluorescent micrographs of COS-7 cells immunostained with anti-GUS antibody following transient transfection with (i) pCEP4GUS2, (ii) pCEP4GUS and (iii) pCEP4β-gal.

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