mouse genotyping by the three-primer multiple PCR. However, both of the smaller PCR products of 1068 (specific for the wild-type allele of mGluR4) and 1170 bp (specific for the mGluR4 transgene) were visible. Thus, all three allele combinations were clearly distinguishable on agarose gels stained with ethidium bromide. DNA from wild-type mice give a single smaller band (1068 bp; see Figure 2B, lanes 4, 5, 6 and 10). All transgenic homozygous mice showed the 1170-bp product (Figure 2B, lanes 2 and 9), and the heterozygous mice showed both PCR products (Figure 2B, lanes 1, 3 and 8). The results of the three-primer multiple PCR genotyping of mGluR4 mutant mice were confirmed by Southern blot and immunoblot analysis (data not shown).

The results presented here confirm that multiple primer PCR can be applied to the genotyping of transgenic mice. The three-primer protocol outlined above increases the reliability of a genotyping screen because, unlike conventional two-primer PCR, three-primer PCR will give a PCR product regardless of the genotype. Our results indicate that three-primer multiple PCR provides a very useful alternative for genotyping transgenic mouse and ES cells lines.

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An In Vitro Assay of β-Galactosidase from Yeast

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The quantification of β-galactosidase in yeast cells harboring a *lac*Z reporter gene linked to the promoter gene of interest is an important and commonly used procedure in many laboratories working with yeast cells. In particular, with the burgeoning use of interaction cloning strategies as typified by the yeast two-hybrid system, this assay has become indispensable for the accurate characterization of truly interacting clones and for subsequently determining their interaction domains.

In general, two methods are commonly used to lyse the yeast cells of interest prior to performing a β-galactosidase quantification reaction (8). One method (7) relies on the shearing and crude lysis of yeast cells induced by sequential rounds of vortex mixing of the cells with glass beads. Activity is then subsequently normalized by the amount of protein assayed. The second method (5) utilizes a permeabilization step in which the yeast cells are first rendered permeable to the enzymatic substrate and the activity is subsequently normalized by an estimate of the number of yeast cells assayed.

We have used both methods extensively and have found that for the accurate comparison of β-galactosidase activity from yeast two-hybrid strains such as Y153 (3) or RH6IIE (4) having different genetic backgrounds, the first method gives the most consistent and reproducible results, thus permitting an accurate ranking of the relative strengths of interaction among different clones.

While lysis of cells by glass bead vortex mixing is the preferred method, it has a drawback of being a very tedious and time-consuming procedure, particularly when larger numbers of samples have to be analyzed.

We therefore sought an alternative procedure that would result in an effective and rapid lysis of the yeast cell wall and that would still permit an accurate quantification of β-galactosidase activity.

We have found that adapting a common freeze/thawing lysis technique for cultured mammalian cells gives results as accurate and reproducible as those obtained with the glass beads lysis method and, in addition, results in considerable reductions in both the time and trouble required to perform this assay.

Briefly, yeast cell cultures (5 mL) were grown in selective medium with vigorous shaking at 30°C until an optical density $(OD)_{595}$ of 1.0–1.5 was reached. Cells were then centrifuged for 5 min at 2500× *g*. The supernatants were discarded and the cell pellets were resuspended in 250 µL of 250 mM Tris-HCl, pH 8.0 and 12.5 µL 100 mM phenylmethylsulfonyl fluoride (PMSF) stock solution (Bachem, Buchs, St. Gallen, Switzerland). The pellets were then transferred to microcentrifuge tubes. The tubes were rapidly frozen in liquid nitrogen for 10 s and immediately transferred to a 37°C water bath for 90 s. This procedure was then repeated two more times. After the last thawing cycle in the 37°C water bath, the cells were briefly vortex mixed and then centrifuged for 5 min at 12000× *g* at 4°C. Twenty-five microliters of the supernatant were subsequently taken for protein concentration determination using the Bradford dye binding assay (2). Between 25–100 µL of the supernatant were then added directly to 0.9–0.975 mL of Z buffer (6) to make a total volume of 1 mL. Then it was incubated at 28°C for 5 min. Then 0.2 mL of 4 mg/mL *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Sigma Chemical, Buchs, St. Gallen, Switzerland) stock solution was added and the reactions proceeded as described (8).

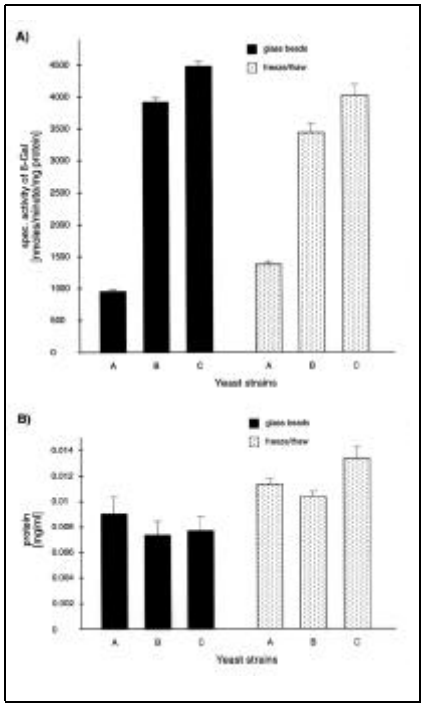

Figure 1. β-galactosidase activity and protein concentrations obtained with different lysis protocols. A) β-galactosidase specific activity normalized by protein content for the yeast strain Y153 which contains two copies of an integrated 4× GAL4 binding site *lac*Z reporter gene and the following activator plasmids: A. GAL4-MybCT (2µ, *trp1*); B. GAL4-VP16FA (ARS-CEN, *trp1)* (1); C. GAL4-VP16FA (2µ, *trp1*) (1). B) Protein concentrations of lysed yeast cells. Yeast strains are the same as for A. Standard deviations derived from experiments performed in triplicate are depicted on each column.

We quantified the β-galactosidase activity from three different yeast strains, each harboring a different GAL4 chimeric transactivator plasmid to induce transcription from the integrated GAL4-*lac*Z reporter plasmid (parental yeast strain Y153, *MAT***a** *leu2-3, 112, ura3-52, trp1-901, his3-D200, ade2-101, gal4D gal80D URA 3::GAL-lacZ, LYS2::GAL-HIS3* (3). We grew 30-mL cultures and then divided them into six 5-mL samples, three of which were processed by the glass beads method and three by the rapid freeze/thaw technique, enabling a direct comparison of the reproducibility of the two methods. The results are depicted in Figure 1: Panel A demonstrates that the freeze/thaw technique yields β-galactosidase activity levels highly comparable to those obtained by the glass beads lysis method. Panel B confirms that the freeze/thaw technique liberates comparable amounts of total protein from yeast cells when compared to the glass beads lysis method.

In addition, we routinely perform plasmid miniprep isolations from freeze/thaw lysed cells, by taking 250 µL of the isolated crude extract and performing sequential phenol, phenol/chloroform and chloroform extractions, followed by an ethanol/salt precipitation step.

This technique should prove useful for those researchers who require fast and reproducible β-galactosidase activity levels from a number of different yeast strains simultaneously.

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