IMPROVED DIFFERENTIATION OF β-gal+ COLONIES ON X-GAL PLATES CONTAINING
MYCOLOGICAL PEPTONE.
Ronan O'Kennedy and John W. Patching*
Department of Microbiology, University College Galway, Ireland.

SUMMARY.
Inclusion of mycological peptone in the base medium of X-gal plates facilitates the detection of isolates of *Saccharomyces cerevisiae* producing β-galactosidase due to improved colour development. Substitution of tryptone with mycological peptone in a standard *Escherichia coli* medium (LB) also facilitates colour development on X-gal plates and obviates the need for an inducer. The addition of mycological peptone to the base medium of X-gal plates offers a cost-effective method to improve the detection of the β-galactosidase gene in recombinant strains of *S. cerevisiae* and *E. coli*.

INTRODUCTION.
Heterologous β-galactosidase has long been employed as a standard marker for genetic analyses of *Saccharomyces cerevisiae* (Caunt et al., 1989; Guarente and Ptashne, 1981; Impoolsup et al., 1989). Significant differences may occur in the degree of colour development on X-gal-containing plates which depends on the formulation of the base medium. A suboptimal pH and nutrient rich medium components lead to decreased colour development and thus decreased sensitivity of plasmid stability determinations (Wang and Dasilva, 1993). In this paper we show much improved colour development by simple substitution of mycological peptone for bacteriological peptone or tryptone in yeast and bacterial media.

MATERIALS AND METHODS.
Strains and plasmids. The plasmid pLG669-β was maintained in *S. cerevisiae* strain CG379 (cir+ ura3 trp1 ade1 leu2 his3). The plasmid contained the yeast URA3 gene and a section of the *Escherichia coli* lacZ gene, under the control of the *Saccharomyces* CYC1 promoter. This promoter is derepressed under conditions of glucose limitation or growth on non-fermentable carbon sources (Guarente and Ptashne, 1981). The cloning vector pCR-Script™ SK+ (Stratagene, UK) was maintained in *Escherichia coli* JM109 (mcrA recA1 endA1 gyrA96 thi-1 hsdR17 (rK- mK+) supE44 relA1 Δ(lac-proAB)] [F' traD36 proAB lacIqZAM15].

Preparation of X-gal plates. Four types of yeast media were evaluated. Synthetic defined (SD) medium contained yeast nitrogen base without amino acids (6.7 g/l; Difco). Wickerham's Defined Medium (WDM) was as described by Wickerham (1946). Amino acids required for the growth of *S. cerevisiae* CG379 (Leu, His, Trp, Ura, Ade) were added to defined media (SD and WDM) at the concentrations recommended by Guthrie and Fink (1991). Defined media plates were prepared with Agar Bacteriological No.1 (20 g/l; Oxoid). Undefined YP medium contained bacteriological peptone (20 g/l; Oxoid) and yeast extract (10 g/l; Oxoid). Modified YP medium (YMP) contained mycological peptone (20 g/l; Oxoid) and yeast extract (10 g/l; Oxoid). Undefined YP and YMP plates were prepared with Agar Technical No.3 (20 g/l; Oxoid). All yeast growth media were made up in 0.1 M succinic acid buffer (pH 7.0) and supplemented with raffinose (20 g/l; Sigma) or glycerol (4% v/v; BDH, UK) as carbon sources after autoclaving.
E. coli growth medium (LB) contained tryptone (10g/l; Oxoid), NaCl (10g/l; BDH) and yeast extract (5g/l). In modified LB medium (LBM), tryptone was replaced by mycological peptone (10g/l). LB and LBM plates were prepared with Agar Technical No.3 (15g/l). After autoclaving, all plate media were supplemented with 40mg/l X-Gal (5-bromo-4-chloro-3-indolyl-galactoside; Sigma) from a stock solution dissolved in N,N-dimethylformamide (40g/l; stock stored at -20°C).

Experimental procedure. A sample of a glucose-limited chemostat culture of yeast grown on WDM medium (0.5g/l D-glucose) was diluted to give between 100 and 200 colony forming units (c.f.u.) per plate, and plated out in triplicate on to X-gal media (SD, WDM, YP and YMP). Plates were incubated for 48-72 hrs at 30°C. Overnight batch cultures of E. coli JM109 pCR-Script™ SK+ transformants were grown on LB containing 50µg/ml Ampicillin. Cultures of E. coli JM109 were grown on LB. These cultures were then plated out onto X-gal media (LB and LBM). Plates were incubated for 12-16hrs at 37°C.

The statistical significances of numerical results were determined using Student's t-test at a 95% confidence level. Where necessary, p values are noted in the text.

RESULTS AND DISCUSSION.

Figure 1 shows the appearance of X-gal plates prepared with various base media (SD, WDM, YP and YMP) and inoculated with the yeast. Raffinose or glycerol were used as the main carbon source so as to induce plasmid-borne β-galactosidase expression. No growth was observed on SD or WDM defined media containing glycerol. Little or no colour development was observed on either SD or WDM where raffinose was the carbon source (Fig.1; C and F). Colour development on YP plates was adequate for differentiating β-galactosidase-producing colonies but blue/white colour differences were difficult to discern (Fig.1; B and E).

Figure 1. The appearance of mixed colonies (β-gal+ and β-gal−) of Saccharomyces cerevisiae grown on X-gal plates containing various media bases: YMP medium with 2% Raffinose (A) or 4% Glycerol (D); YP medium with 2% Raffinose (B) or 4% Glycerol (E); SD medium with 2% Raffinose (C); WDM medium with 2% Raffinose (F).